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**Development and evaluation of a
non-chemical method with
microwave source for disinfection
of legumes**

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Preface

Scientific papers published or submitted by the author in collaboration with supervisors and other scientists are listed in the following.

1. Taheri, S., Brodie, G., Jacob, M. V., & Antunes, E. (2018). **Dielectric properties of chickpea, red and green lentil in the microwave frequency range as a function of temperature and moisture content.** *Journal of Microwave Power and Electromagnetic Energy*, 52(3), 198-214. [Doi:10.1080/08327823.2018.1452550](https://doi.org/10.1080/08327823.2018.1452550)
2. Taheri, S., Brodie, G. I., & Gupta, D. (2019). **Heat uniformity study and viability of red lentil at different moisture contents after low dose microwave treatment.** *Transactions of the ASABE*, 62(2), 281-288. Doi: <https://doi.org/10.13031/trans.13002>
3. Taheri, S., Brodie, G. I., Gupta, D., & Dadu, R. H. R. (2019). **Effect of microwave radiation on internal inoculum of Ascochyta Blight in lentil seeds at different seed moisture contents.** *Transactions of the ASAE*, 62(1), 33-43. Doi: <https://doi.org/10.13031/trans.13088>
4. Taheri, S., Brodie, G., & Gupta, D. (2019). **Effectiveness of a microwave fluidised bed dryer in eradication of seed-borne Botrytis grey mould of lentil.** *Paper presented at the 17th International Conference on Microwave and High-frequency heating, Valencia, Spain.*, <http://dx.doi.org/10.4995/Ampere2019.2019.9635>
5. Taheri, S., Brodie, G., & Gupta, D. (2020). **Microwave fluidised bed drying of red lentil seeds: Drying kinetics and reduction of Botrytis grey mould pathogen.** *Food and Bioproducts Processing*, 119, 390-401. Doi: <https://doi.org/10.1016/j.fbp.2019.11.001>
6. Taheri, S., Brodie, G., & Gupta, D. (2020). **Fluidisation of lentil seeds during microwave drying and disinfection could prevent detrimental impacts on their chemical and biochemical characteristics.** *LWT - Food Science and Technology*, 109534. Doi: <https://doi.org/10.1016/j.lwt.2020.109534>
7. Taheri, S., Brodie, G. I., & Gupta, D., Jacob, M. V. **Afterglow of atmospheric non-thermal plasma for treatment of lentil seeds contaminated with botrytis grey mould;** *Under revision following peer review for "Innovative food science and emerging technologies"*
8. Taheri, S., Brodie, G. I., & Gupta, D. **Review: application of microwave technology in grain treatment;** *Under review "Food reviews international"*

Conference presentations:

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2. Taheri, S., & Brodie, G. (2018). **Evaluating control of *Ascochyta lentis* in lentil seeds by means of microwave processing**; Paper presented & published in the proceedings of the *52nd International Microwave Power Institute Symposium, Long Beach, California.*
3. Taheri, S., Brodie, G. I., & Gupta, D. (2019). **Effectiveness of a microwave fluidised bed dryer in eradication of seed-borne *Botrytis Grey* mould of lentil**; oral presentation; 17th international conference on Microwave and High-frequency heating, *AMPERE 2019, Valencia, Spain, 9-12 September*
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5. Taheri, S., Brodie, G. I., & Gupta, D., Jacob, M.V. (2019). **Disinfection of lentil seeds from its seed borne *Botrytis grey* mould (BGM) using non-thermal plasma**; oral presentation; *Australasian Microwave and RF symposium, Parkville, Victoria. 22nd November 2019*

Declaration

This is to certify that

- The thesis consists of my sole original work for PhD degree except that informed in the preface
- Due acknowledgement is presented in the next section to all other materials
- The thesis is less than 100,000 words in length including tables, figures, bibliography, and appendices.

Saeedeh Taheri

May 2020

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Abstract

Lentil is one of the important pulse crops in the world; however, its production is threatened by economically important diseases. Two of the devastating seed-borne diseases of this crop, across the world and in Australia, are *Ascochyta* blight (AB), caused by *Ascochyta lentis*, and *Botrytis* grey mould (BGM), caused by *Botrytis cinerea* and *Botrytis fabae*. Lack of genetically diverse sources of disease resistance in cultivated lentil varieties has increased dependence on fungicides to control these diseases. However, still, huge production losses are recorded worldwide as overuse of fungicides has resulted in the pathogen adaptation by mutations leading to their resistance and a reduction of fungicides' efficiency. Therefore, there is a dire need for the development of an environmentally friendly physical method to be implemented in the integrated disease management scheme in pulse crops, including lentil.

Microwave radiation is a sustainable and environmentally safe technology for food and agricultural processing. Its use for thermal treatment has shown great potential for sterilisation of food material as well as eradication of pests and pathogens from agricultural commodities. However, its potential in the grain industry and the control of related pathogens have not been fully explored, probably due to the concerns related to the resulting grain quality. Additionally, the indirect use of microwave, as a source for processing gas to produce non-thermal plasma followed by treatment of the seeds with the plasma, has recently gained a lot of attention. Therefore, the present study aimed to develop a thermal or non-thermal process, based on microwave radiation, to disinfect lentil seeds from its seed-borne pathogens.

The potential of a microwave multimode cavity with a turntable for the eradication of AB of lentil seeds at different seed moisture contents, microwave powers, and exposure times was evaluated. This process was assumed to be like the industrial-scale microwave system, with a conveyor belt, in terms of heat uniformity and efficiency of pathogen control. It was proven that the pathogen could be removed faster at higher seed moisture content; however, the seed viability was also more susceptible to microwave radiation at the higher seed moisture content (19% wb). Infected seed percentage (IS%) reduced from 17% to 8.9%, at a seed moisture content of 9.1%, by applying a 100% power level (max. 1100W) for 51 s without a significant seed germination loss. Nevertheless, seed vigour (measured by electrical conductivity) was negatively affected by these process parameters. Investigation of heat uniformity in this process showed that the difference between hot and cold spots could

reach as high as 50°C, which may account for the seed vigour loss before completing the eradication of the pathogen.

To improve heat-uniformity, a microwave fluidised bed (MFB) was designed and manufactured. The efficiency of this process, in the eradication of seed-borne BGM of lentil seeds, was firstly evaluated by investigation of the effective factors, including microwave power, exposure time, air temperature and seed moisture content with responses of IS%, seed germination, seed vigour, seeds' bed temperature, and moisture loss. The results showed that a 30% reduction in IS% could be achieved by applying microwave power of 300 W and air temperature of 60°C or microwave power of 400 W and air temperature of 50°C for 10 min at the seed moisture content of 18%, without a significant reduction in seed viability and vigour. The temperature history, measured at two points in the fluidised bed using fibre optic probes, showed that the difference between hot and cold spot did not exceed 5°C. It confirmed that there was a very good heat uniformity in the microwave fluidised bed process.

Evaluation of the effect of factors in the previous study showed that exposure time was an effective factor in reducing the IS%, while it did not have a significant effect on seed germination. To determine whether more reduction of the IS% could be achieved at prolonged exposure time, IS% reduction was examined in a drying experiment in the MFB dryer at the same process parameters as the previous experiment. The drying kinetics and time-saving, compared with the conventional fluidised bed, were also studied. The results confirmed that the same reduction of the IS% occurred, and therefore increasing exposure time did not improve the reduction of the infected seeds; however, it proved to be a very effective drying process (more than 54% reduction in the drying time). It means that the greatest reduction in infected seeds occurred during the first 10 min of the process. The reason for not having more reduction in IS% after a specific time could be the very fast desiccation of the pathogen during the treatments, which increased its thermal resistance and subsequently the minimum power, or temperature required for its eradication.

The seeds, which were dried in the previous experiment, were evaluated for any quality changes by examining their antioxidant enzyme activities, cooking quality, and their macronutrient using Fourier transform infrared (FTIR) micro spectroscopy. It was concluded that there was no significant quality deterioration in the most efficient process parameters (400 W & 50°C). In conclusion, there is great potential for microwave disinfection of lentil seeds under the condition where the hot spot is controlled so as not to harm the seed quality. It could be achieved by microwave-assisted drying with at least a

54% reduction in the drying time compared with conventional fluidised bed drying. One hundred percent eradication might not be achieved, but it can be considered as part of an integrated disease management process in lentil crops, with the ultimate goal of reducing initial fungicide application for seed treatment and later applications for the crop. However, there might be concerns over the heat tolerance of different grains and different batches of the same grain, which was the reason for considering the non-thermal treatment with microwave plasma.

Finally, the non-thermal process of plasma, created from a microwave source, was examined for its potential in the eradication of BGM from lentil seeds. Non-thermal plasma is the fourth state of the matter produced by energizing a gas and consists of various reactive species including atoms, ions, and radicals, which is a very promising non-chemical method of seed disinfection with a growth enhancement effect. Two modes of treatment were considered: direct argon plasma at low power (400 W); and treatment with the afterglow of the air or air/argon plasma (10 s and 100 s) followed by holding the seeds in the trapped processed gas for 5 min to 24 h. There was a 41% reduction in the infected seed percentage (IS%) after 100 s of treatment with the afterglow of air plasma following by 24 h holding and a 32.3% reduction occurred when they were treated by 30% air/70% argon plasma for 10 s and 60 min holding time. No significant change was observed in antioxidant enzyme activities of the treated seeds, except for triggering of catalase activity after 24 h of holding time. However, lentil seed's colour changed towards redness and yellowness after prolonged treatment time (400 s) or holding time (24 h). Therefore, the afterglow of microwave plasma or plasma processed air (PPA) could be further explored as a great tool for the disinfection of grains, without any negative effect on their viability.

Overall, both MFB and the afterglow flow of microwave plasma were able to reduce the seed-borne BGM of lentil seeds without any adverse effect on the seed viability. MFB would be useful when drying and disinfection are simultaneously desirable as the disinfection was just achievable at high seed moisture content with very fast drying of the seeds. However, the disinfection of dry lentil seeds was possible using the treatment with the afterglow of the plasma. Therefore, plasma treatment could be further explored for the treatment of other grains and their related pathogens if the dry seeds need to be disinfected.

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Chapter 1. General Introduction

Legumes are important sources of protein and are consumed as staples in many developing countries. Consumption of legumes has also increased by 4% in Australia in the three years, from 2015 to 2018 ([Locke, 2018](#)), which is an upward trend due to the awareness of their health benefits and movement toward a plant-based diet. In 2018, Australia and New Zealand produced a total of about 2.76 M tonnes of pulses while there were 24.6 M tonnes of importation by developing countries from the producing countries (FAOSTAT, 2019). Lentil is one of the important pulse crops in Australia, with an annual production of 0.25 Mt in 2018 (FAOSTAT, 2019). A large part of the lentil produced in Australia is exported to the Indian subcontinent and Mediterranean regions, with an export value of US \$502 million in 2017 (FAOSTAT, 2019).

However, pulse production in Australia has been threatened by many biotic and abiotic stresses including seed-borne pathogens, which cause huge losses in the field and storage. The total loss of Australian pulse crops, due to various diseases such as Ascochyta Blight and Botrytis grey mould, was estimated to be \$74 million per year or 14.7% of the gross value of their production ([Murray, 2012](#)). Many of these diseases are seed-borne with no or little external symptoms ([Lindbeck et al., 2009](#)), which necessitate seed testing and defining a standard threshold for each pathogen to consider the seeds to be healthy and safe for sowing purposes.

Among the pulse crops, lentil suffers from some expensive diseases, such as Ascochyta blight (AB) and Botrytis grey mould (BGM) and their losses cost \$2.7 million per year or 4.3% of the annual value of lentil crop in Australia ([GRDC, 2018b](#)). One of the considerations to combat fungal diseases is utilising resistant crop varieties. However, a change in the reaction of resistant cultivars and their infection was observed, which was attributed to a change in the pathogen's aggressiveness ([Davidson et al., 2016](#)). The mentioned loss would, therefore, be much higher if the current considerations for integrated disease management including fungicide application were not in place ([Murray, 2012](#)). At the same time, overuse of chemical fungicides and fumigants could cause pathogen adaptation leading to mutations and development of resistance, which ends in the loss of fungicides efficiency ([Hahn, 2014](#)) and the demand for investing in the development of new disinfection methods.

Physical methods of seed treatment such as applying hot air or hot water, which are effective against BGM ([Burgess, 1997](#)), have been considered for a very long time. Nonetheless, these traditional methods demand high energy, and drying the seeds is

required after treatment by moist heat. Therefore, new technologies such as irradiation ([Aziz et al., 2004](#); [Calado et al., 2014](#)), cold plasma ([Adhikari et al., 2020](#)), microwave and radiofrequency ([Macana & Baik, 2017](#); [Yadav et al., 2014](#)) for seed treatment have recently gained popularity among researchers.

Microwave energy has been considered for a long time as a fast and efficient non-chemical method of processing in the food and agricultural industries ([Lozano et al., 1986](#); [Ponne et al., 1996](#)). One of its applications is seed treatment to eliminate pests and pathogens, which still raises the concern of negative impacts on the host's quality, and unfortunately due to a lack of information on its effect on a wide range of pathogens and the seeds, it has not been industrialised worldwide in agriculture and specifically in the grain industry. One of the important concerns with microwave radiation is its non-uniformity in heating ([Vadivambal & Jayas, 2010](#)), which could also be the reason for host quality loss issues before a complete pathogen eradication can be achieved. Moreover, microwaves can be applied using an indirect treatment of the seeds via creating a non-thermal plasma, without increasing the temperature of the seed. Therefore, this work investigated the possibility of controlling seed-borne pathogens of lentil, namely AB and BGM, employing direct microwave radiation, with improved heat uniformity, and an indirect source of treatment to create non-thermal plasma.

The objectives of the present study were to:

- 1- Evaluate the heat uniformity and the efficiency of elimination of lentil seed-borne pathogens in a multi-mode microwave cavity with a turntable, which was assumed to be the same as an industrial microwave with conveyor belt in terms of heat uniformity.
- 2- Design and fabricate a microwave heating system with a good heat uniformity for the treatment of lentil seeds with the maximum pathogen control and minimum seed quality deterioration.
- 3- Determine the best process parameters, such as seed moisture content, microwave power, and the exposure time, for lentil seed treatment in a microwave thermal process.
- 4- Define the non-thermal effect of the microwave-induced cold plasma on the lentil seed-borne pathogens.

A literature review on the application of microwave energy in the grain industry is presented in Chapter 2 and the potential, challenges and future works are discussed. It is demonstrated that microwave radiation has many potential applications such as insect/fungi control in grain seeds, which is a focus of the present study. The main challenge, which is still a setback of industrialization, is the non-uniformity of microwave heating. It leads to a very high difference between hot and cold spot temperatures, which damages the seed quality before complete control of the insect/fungi in the host grain. Therefore, solving the problem of non-uniformity of heating is hypothesized to prevent food quality deterioration. In the second part of the literature review, the application of microwave for non-thermal treatment of grains by treating the seeds indirectly using a microwave-induced plasma is discussed.

As the first step of experimental work, in Chapter 3, the dielectric properties (real and imaginary parts of permittivity) of key legumes such as lentil and chickpea were measured and modelled over a frequency range that includes three key ISM (Industrial Scientific Medical) frequencies, at different seed moisture contents and temperatures. This information was required to study the heat uniformity and design of a proper process for grain treatment before doing any experiment to save time and money. The information also helped to decide the penetration depth of microwave in the legume seeds, and therefore the proper thickness of the selected legume seeds to be processed at the working microwave frequency, temperature range, and moisture content.

To fulfill objective 1, heat uniformity, seed viability change and a reduction of an important seed-borne pathogen of lentil (*Ascochyta lentis*) were evaluated in a multimode microwave cavity at different seed moisture contents, microwave powers and exposure times in Chapter 4 and Chapter 5. A turntable was used in this experiment to make the temperature distribution more uniform and have a similar condition of seed movement on a conveyor belt, which is usually used in the industrial equipment.

Working on objectives 2 and 3, it was decided that one of the methods to have uniform microwave radiation in discrete materials, such as grains, was to agitate the material at the same time as irradiation. They could be mechanically or pneumatically agitated, while all the considerations for microwave leakage or proper materials for the microwave cavity and moving parts should be considered. Therefore, as the next step to evaluate a more uniform microwave heating of lentil seeds, Chapter 6 and Chapter 7 explore a microwave fluidised bed, which was designed and built. A preliminary experiment was carried out to explore the effect of factors (such as microwave power, air temperature and exposure time) and find

the process parameters. From then on, Botrytis grey mould (BGM) of lentil seeds was used as a surrogate pathogen, as obtaining lentil seeds which were naturally infected with BGM or AB in the required volume was challenging. Therefore, with the aim of artificial inoculation of the seeds, BGM was more reasonable to be used as this pathogen causes surface infection and it was easier to artificially inoculate lentil seeds.

Chapter 8 goes on with an experiment of drying lentil seeds and the evaluation of BGM reduction as a part of the drying process. Drying kinetics of lentil seeds were also studied in the microwave fluidised bed dryer. Subsequently, the chemical and biochemical changes in the lentil seeds, after these treatments, were examined in Chapter 9. The chemical changes were tested by FTIR micro spectroscopy and the biochemical tests were carried out by measuring antioxidant enzyme activities. The hardness of soaked and cooked seeds was also evaluated with a texture analyser to study the effect of the microwave fluidised bed dryer on the texture of lentil.

To fulfill objective 4, the potential of cold plasma for treatment of the lentil seeds, contaminated with BGM, was explored in Chapter 10. Two possible modes of treatments, including a direct treatment with argon gas plasma and an indirect treatment with air or a combination of air and argon plasma, were applied. In the indirect treatments, plasma processed gas was trapped in a bottle containing the seeds, and the bottle was sealed to investigate the effect of the long-lived reactive species on the contaminated seeds. The quality change of the seeds was also assessed by examining seed germination, vigour, antioxidant enzyme activity, and colour.

Chapter 2. Literature review

2.1. Part one (microwave thermal treatment): Application of microwave technology for grain treatment, an overview

Under review in "Food reviews international"

2.1.1. Abstract

Microwave radiation as an emerging technology has shown great potential in food and agriculture. Microwave as a source for thermal treatment of grains will be discussed in this review. The applications of microwave heating for fungi control (disinfection), insect control (disinfestation), drying, modification of seed quality and seed germination enhancement will be reviewed, and challenges of industrialization will be discussed. The possibility of development of one process to achieve a combination of the mentioned applications will also be discussed.

2.1.2. Introduction

The increasing world population needs much more food than ever before. With diminishing resources in the world, the food and agriculture industry must devise new strategies to meet the demand. One way of responding to the growing demand for food is to reduce waste of agriculture products pre- and post-farmgate. Food loss and wastage in the world consists of approximately 1.3B tonnes of food or one-third of edible food produced for consumption ([FAO, 2017](#)). Reduction of this loss would mean providing more food for the fast-growing world population, which will need 60% more food by 2050 ([Alexandratos & Bruinsma, 2012](#)).

Microwave is a type of electromagnetic energy with a frequency range of 300MHz to 300GHz with the most commonly used frequencies of 915MHz and 2450MHz. It has been tried for different applications in food and agriculture and has shown a great potential for drying, sterilization, disinfestation, enzyme extraction, waste management, and many others. Utilizing microwave's thermal and non-thermal potential in food and agriculture industry could be a step towards replacing conventional methods with a more environmentally friendly technology.

Despite many advantages of electromagnetic energy, it has not been commercialized in some sections of food and agricultural industry. One of these sections is the grain industry, which has many potential applications of microwave energy. Therefore, the aims of this review are to summarize the principles and applications of dielectric heating (with a focus

on microwaves) for the treatment of grains with the aim of safe storage and reduction of post-harvest grain losses, food security, increasing the value for end-users, and finally to discuss the challenges and future works for taking advantage of microwave radiation in this sector.

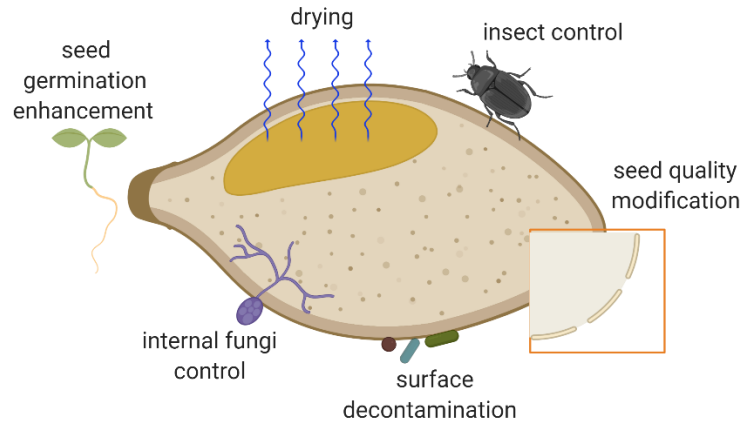


Figure 2.1 Summary of the applications of microwave heating for grain seeds (created in Biorender.com)

2.1.3. Microwave heating principles and dielectric properties

Microwave energy is converted to heat in a dielectric material by affecting their polar molecules. Dipolar molecules start to align with the oscillating electromagnetic field and rotate in proportion to the applied frequency of the electromagnetic fields. These movements cause intermolecular friction and heating of the material. Microwave heating is a volumetric process and it can be modeled as an internal heat source in the heat balance equation of a system and the dissipated microwave power itself can be described by equation (2.1)

$$P = 2\pi f \epsilon_0 \epsilon'' E^2 V \quad (2.1)$$

Where P is dissipated microwave power (W); f is electromagnetic field frequency; ϵ_0 is permittivity of free space (F m^{-1}); ϵ'' is loss factor of the material; E is electric field strength (V m^{-1}) and V is the volume of the material (m^3).

For treating a material by means of the microwave, the dielectric properties of the workload should be known. Dielectric properties regulate the behavior of the material, exposed to electromagnetic energy, and are expressed using the relative complex permittivity, $\epsilon = \epsilon' - j\epsilon''$. The real part (ϵ') is called the dielectric constant and the imaginary part (ϵ'') is the loss factor. The dielectric constant expresses the potential of a material to store energy and the loss factor shows the loss of electromagnetic energy in the form of heat. These properties depend on the frequency, temperature, moisture content and the composition of the material. They also depend on the bulk density for particles ([Nelson, 1991](#)).

Penetration depth, which is defined by equation (2.2), is expressed as the depth in a material where microwave power drops to $1/e$ ($e=2.718$) or 36.8% of its initial value. In this equation, c is the speed of light in free space and equals to 3×10^8 m s⁻¹ and f is frequency (Hz). Penetration depth is negatively correlated with frequency and thus at higher frequencies, energy penetrates less in the material and more surface heating occurs. The wavelength is another important factor, which is calculated by equation (2.3) using the dielectric properties. By knowing penetration depth and wavelength, heat uniformity of a particular geometry during microwave treatment can be predicted ([Bhattacharya & Basak, 2017](#); [Brodie, 2008](#)).

$$d_p = \frac{c}{2\pi f \sqrt{2\varepsilon' [\sqrt{1 + (\varepsilon''/\varepsilon')^2} - 1]}} \quad (2.2)$$

$$\lambda_m = \frac{c\sqrt{2}}{f \sqrt{\varepsilon'^2 + \varepsilon''^2 + \varepsilon'}} \quad (2.3)$$

Methods of measurement of dielectric properties of some grains and the models developed for them are summarised in Table 2.1. By knowing dielectric properties, it is possible to define penetration depth and predict heat uniformity or determine the proper thickness of the grains for achieving good heat uniformity at a specific frequency. It is also possible to predict if there could be any selective heating between the materials and the pests by comparing their dielectric constant and loss factor. However, it appeared to be more beneficial to develop a model, which relates dielectric properties to the moisture content of the material and processing temperature at a specific frequency. This model should be used in simulations of the process to find the electric field and temperature distribution and define proper process parameters as well as the best design for the electromagnetic applicator.

Finally, as grains (and seeds in general), are discrete particles, it is difficult to measure their exact penetration depth. Most studies have tried to measure dielectric properties of ground samples, which are usually less dense than the seeds and contain some air inside. [Guo et al. \(2008\)](#) suggested that the ground sample should be compressed to reach the true density of the seeds to overcome this problem. However, the particles are a mixture of seeds and air with known bulk density. The other point is that heat transfer in the sample is by conduction through contact points from one particle to the next, which can make the simulation of the process more complicated as the seeds can have more than one contact points in reality.

Table 2.1 Dielectric properties of some grains *

Grain	MC%	T (°C)	Frequency (MHz)	Method	Main result	Reference
Chickpea	7.9-20.9	20-90	10-1800	Open-ended coaxial-line probe & impedance analyser	$\epsilon''/\rho = 0.4552 \epsilon'/\rho - 0.8046$ (f=1800MHz)	(Guo et al., 2010)
Green pea	10.8-21.6	20-90	10-1800	Open-ended coaxial-line probe & impedance analyser	$\epsilon''/\rho = 0.4410 \epsilon'/\rho - 0.6241$ (f=1800MHz)	
Lentil	8.4-21.5	20-90	10-1800	Open-ended coaxial-line probe & impedance analyser	$\epsilon''/\rho = 0.4609 \epsilon'/\rho - 0.6347$ (f=1800MHz)	
Soybean	8.9-19.9	20-90	10-1800	Open-ended coaxial-line probe & impedance analyser	$\epsilon''/\rho = 0.5048 \epsilon'/\rho - 0.8869$ (f=1800MHz)	
Mung bean, Black eyed pea	10.2-22.3 8.8-20.9	20-60	10-1800	Open-ended coaxial probe and impedance analyzer	Differential heating between insects and legume at RF frequencies	(Jiao et al., 2011)
Cereal grains			5000-15000	Development of models for existing data	$\epsilon' = \epsilon'_0 + a \times \log f + b \times MC$ $\epsilon'' = \epsilon''_0 + c \times f + d \times MC$ for each of them a, b, c, d is defined	(Nelson & Trabelsi, 2011)
Common bean	4-13.5		3000 - 10600	Free space transmission	ϵ' remained constant & ϵ'' decreased with frequency	(Torrealba-Meléndez et al., 2014)
Barley, Corn, Sorghum, Wheat		20-60	915,2450, 5800	Free space transmission	ϵ' slightly increased & ϵ'' remained constant over the range of temperature	(Torrealba-Meléndez et al., 2015)
Common bean	8.8-12.3	20-60	800-2500	Free space transmission	ϵ' and ϵ'' decreased by increasing f , increased by increasing T, ϵ' increased and ϵ'' remained constant by increasing MC	(Torrealba-Meléndez et al., 2016)
Chickpea	8-20	24-85	700-7000	Open-ended coaxial probe	$\epsilon' = 60.76 - 6.05 \log f + 0.165 T$ $\epsilon'' = 2.707 - 6.985 \times 10^{-11} f + 0.001578 T - 2.049 \times 10^{-12} f T + 0.000456 T^2$ (MC = 19.8%)	(Taheri et al., 2018)
Red lentil	8-20		700-7000	Open-ended coaxial probe	$\frac{\epsilon''}{\rho} = 0.3994 \frac{\epsilon'}{\rho} - 0.9185$ (f=1800MHz)	(Taheri et al., 2018)

* MC: moisture content; T: temperature; ρ : grain's true density; f: frequency;

2.1.4. Microwave drying for safe storage of grains

Grains are usually harvested at relatively high moisture contents to prevent any physical damage during post-harvest practices. Weather condition is another reason which forces farmers to harvest grains earlier before they can dry out. Storage of the seeds at high moisture contents, depending on the storage temperature, leads to negative changes in seed physiological and biochemical properties and has a high risk of insects and fungi invasion. Therefore, artificial drying is one of the common post-harvest practices to remove excess

seed moisture for safe storage of grains and has benefits including long-time seed storage with no damage to seed quality, continuous supply of the grains throughout the year, maintenance of seed viability until the next sowing season and enabling early harvest to prevent physical damages ([Bala, 2016](#)). It should also be noted that safe storage seed moisture contents are different among different grains as well as different climate and storage conditions.

The conventional non-chemical method of artificial drying of grains includes applying airflow at a specific temperature to remove the excess moisture of the seeds. This can be done by batch or continuous flow of the grains in different patterns such as co-current, countercurrent or crossflow ([Jayas & Ghosh, 2006](#)). However, this conventional method of drying has the drawback of low thermal efficiency and very long time required for drying, which is the reason why grain drying is still reliant on desiccants. It has turned the researcher's attention to exploring new technologies in grain drying such as infrared and dielectric heating as faster and more energy-efficient methods. Some of these technologies, such as microwave drying, are still in the phase of research and development and one of the reasons is that the effect of the process on the quality attributes of the grains has not been fully discovered, though it is well documented that by adding microwave to conventional method of air drying, drying time decreases dramatically.

Soybean seed drying in microwave-assisted dryers was reported several times in references. [Shivhare et al. \(1993\)](#) studied drying of soybean seeds by a combination of microwave radiation and air at 30°C passing through the soybean bed and concluded that microwave at 0.13 W g⁻¹ could be applied to dry soybean seeds without any seed viability loss. [Wang et al. \(2009\)](#) designed a new hybrid microwave dryer for soybean drying. They used a rotary drum dryer inside a microwave oven with convective air passing through the soybean seeds and showed that cracking ratio of the soybean seeds was the lowest at the drum speed of 15-20 rpm. It increased with increasing airflow and microwave power. Drying rate, in their process, was reported to increase dramatically by increasing microwave power. Simulation and validation of soybean drying in a microwave-assisted fluidised bed dryer were investigated by [Zare&Ranjbaran \(2012\)](#). They confirmed that time saving of 83.39% to 98.07% and an energy saving of 82.07% to 95.22% could be achieved for drying of soybeans from the moisture content of 18.32% to 12% (db) by adding microwave to a conventional fluidised bed dryer. Soybean drying kinetics in a microwave fluidised bed dryer and their possible quality changes were later examined by [Khoshtaghaza et al. \(2015\)](#). They stated that addition of microwave heating reduced the

soybean cracking rate and increased the rehydration rate compared to conventional fluidised bed dryer.

Lentil seeds drying in a microwave oven was investigated at different microwave powers between 300-800 W and it was concluded that the least energy consumption could be achieved at the microwave power of 550W ([Iik et al., 2011](#)). It was also observed that there was no change in the crude protein of the seeds after drying, while crude oil content increased at all power levels. Drying kinetics of lentil seeds were later studied in a combined convective- infrared-microwave dryer ([Chayjian & Radmard, 2016](#)). Lentil's drying rate increased by increasing air temperature, infrared radiation, and microwave power

Microwave drying of rice was another field, which has shown a great potential to be applied in the rice industry. Microwave drying of a thin layer of rice was firstly evaluated in a microwave-vacuum dryer ([Wadsworth & Koltun, 1986](#)) and it was reported that there was no change in cooking and physicochemical characteristics of dried rice. Later [Kaasova et al. \(2002\)](#) compared conventional and microwave oven drying of rice and concluded that low power microwave did not affect total starch content of the rice but increasing microwave power increasingly damaged starch. However, they observed that the damaged starch was minimum when the initial moisture content of the rice was less than 23%. [Sangdao et al. \(2010\)](#) proposed a new applicator, comprising of perpendicular waveguides on a concentric cavity, for continuous microwave fluidised bed drying of paddy with improved heating uniformity. They suggested that this system had an efficiency of 61.5% and with a maximum capacity of 3.1 kg h⁻¹, its energy consumption and cost were 5.2 kJ and US\$47.7 ton⁻¹ respectively. [Horrungsiwat et al. \(2016\)](#) compared microwave hot air and steam drying of paddy rice and stated that both processes could be used for rice drying with increasing rice antioxidant activity. They concluded that microwave at 4-6 W g⁻¹ and steam at 400°C could be used for rice drying without a change in sensorial attributes, though they slightly increased rice hardness. Deep bed rice drying has been recently investigated in an industrial microwave with a frequency of 915 MHz ([Smith, 2017](#)), and it was suggested that drying rice from 23% to 17% could be done by applying microwave specific energy of 400 kJ kg⁻¹ without any significant difference in head rice yield compared to gentle drying with air at room temperature. It was concluded that microbial populations decreased, and milling quality of rice was optimum by employing this level of energy.

Drying of wheat and corn seeds, using the microwave and microwave-assisted technology, was examined by several authors. Drying of corn at initial seed moisture contents of 9.6%-32.5% (db, dry base) in a microwave oven with different power levels between 140 to 700

W (total energy of 84 kJ) was reported to have lower viscosity of flour suspension, which was concluded to be due to changes in protein and starch structure ([Velu et al., 2006a](#)). It was added that milling energy reduced as a result of this drying process. Corn drying, with an initial moisture content of 18.3%-42.3% (db) at the microwave power of 245 W, was indicated to increase seed stress crack and reduce seed germination, but applying 70W of microwave power for kernel drying preserved germination ([Song et al., 2013](#)). Seeds' viability, with higher initial moisture contents, was also reported to be more susceptible to higher microwave power. Winter wheat drying at initial seed moisture contents of 15%, 20%, and 25% was possible in a microwave oven at a power of 245 W with less than 2min of exposure time and without seed viability loss. However, higher powers (490 and 910 W) could reduce seed germination and was not advisable for drying of wheat seeds ([Hemis et al., 2012](#)).

There have been some suggestions about the apparatus which could be used for grain drying in the field or in storage ([Bensussan & Azam, 1982](#)). [Snaper \(2003\)](#) patented an apparatus consisting of microwave magnetrons and convective air for grain drying. They recovered heat from the internal combustion engine of the harvester machine to warm up the air and make the process more cost-effective. This apparatus could be used on-line during harvesting of the crop and before transferring them to the storage.

It can be concluded from the literature that microwave-assisted hot air is a promising, fast, and efficient method for grain drying. Microwave speeds up the drying process and hot air, either by blowing over the seeds or by fluidising them, helps to make the microwave heating uniform. However, there is still a lack of data in the literature on the quality assessment of most grains, especially pulses, after drying with this method. This could have been a setback to commercialize the process as an artificial method of grain drying. Also, there have not been enough investigations on optimizing the process parameters such as proper initial moisture content, microwave power, air temperature and air speed in the references. The other point is that, although there are sufficient data on the study of drying and drying kinetics of important grains using microwave and microwave-assisted processing, there have been few investigations of simultaneous drying and disinfection/ disinfestation of grains ([Pande et al., 2012](#); [Smith & Atungulu, 2018](#)).

2.1.5. Seed germination enhancement

The use of good quality seeds is a key factor in agricultural production. High quality seeds have high germination and growth potential as well as being free from pests and pathogens. There are several non-chemical methods for seed vigour enhancement, which can be

generally divided into three groups including physical, physiological and biological ([Irfan Afzal, 2016](#)). Among physical treatments, ionizing irradiation and microwave are the most promising methods ([Araujo et al., 2016](#)).

There are few works which reported the potential of microwave radiation in seed germination enhancement. Germination and vigour of lentil seeds at high moisture content increased after 30 s exposure to microwave power of 450 W ([Aladjadjiyan, 2010](#); [Taheri et al., 2019c](#)). [Tylkowska et al. \(2010\)](#) observed that treatment of 50 bean seeds with 9.5% moisture content in a microwave oven (650 W) for up to 120 s, increased germination capacities but had no effect on the final germination. [Jakubowski \(2015\)](#) reported the increase of germination, vigour, and fresh plant mass of bean seeds after 10 second exposure to microwave treatment at 100 W. However, a 60-s exposure to this microwave power had adverse effects on plant mass. [Kontar et al. \(2015\)](#) investigated the effect of microwave pre-treatment of grains and vegetable seeds. They stated that microwave processing at 1.8 kW kg⁻¹ for 15 s or 0.9 kW kg⁻¹ for 45 s can enhance grain crops' yield and germination of watermelon seeds. They also concluded that microwave treatment helps to form the Hydrogen and Hydroxyl ions, which leads to maltose hydrolysis and formation of glucose and increase in germination. They additionally observed that this information transmits from activated to dormant seeds.

Generally, to design a microwave process with the aim of seed enhancement, absorbed power by the seeds and the final temperature as well as moisture content of them should be taken into account. For each seed, depends on their size and characteristics of the seed coat, a specific level of microwave power and final temperature can break the dormancy and increase viability. However, after a threshold, embryo will be negatively affected, which lead to viability loss and seed damage. Unfortunately, there is not enough information about this potential of microwave in literature to draw a firm conclusion about the possibility of seed vigour enhancement.

2.1.6. Modification of seed quality for end-users

A summary of the application of microwave heating for seed quality modification is illustrated in Figure 2.2. Legumes are efficient sources of protein, as they need less input energy per kilogram of produced protein compared to meat ([Food & Nations, 2013](#)). However, they contain some antinutritional compounds, which can cause digestive problems. These compounds include protein types such as lectin or agglutinins, trypsin inhibitor, chymotrypsin inhibitor and non- protein types such as alkaloids, phytic acid and phenolic compounds (tannins, saponins) ([Roy et al., 2010](#)). The effect of microwave

treatment on antinutritional compounds of legumes was studied by [Hernandez-Infante et al. \(1998\)](#). In this study, microwave cooking was compared with conventional cooking in water to explore their effects on the reduction of trypsin inhibitor and hemagglutinins. They observed that microwave heating was effective in reducing the antinutritional factors of all legumes except for common bean and its efficiency was the same as conventional cooking. [Zhong et al. \(2015\)](#) compared microwave, radiofrequency, and high-pressure processing in reducing antinutritional compounds and protein digestibility of soybeans. For this experiment 250 g black soybean, which were previously soaked in water, cooked in vacuum microwave and RF (27 MHz) at respectively 1 kW and 6 kW powers for 30 min. They stated that microwave and RF did not have significant effect on total essential amino acids but were more effective in reducing antinutrients. They also reported that microwave and high pressure were more efficient than RF in enhancing protein digestibility.

Poor cooking quality of legumes is another problem related to its consumption. Microwave radiation has shown good potential in improvement of cooking quality of grains. [Marconi et al. \(2000\)](#) showed that microwave heating not only reduced cooking time of legumes, but also it decreased cooking loss compared to conventional boiling in water. Less vitamin and mineral loss, as a result of microwave cooking compared to conventional cooking, was also reported by other researchers ([Alajaji & El-Adawy, 2006](#); [El-Adawy, 2002](#)). However, [Ertas \(2013\)](#) stated that ultrasound was more effective than microwave cooking in reducing mineral loss.

Microwave pre-treatment of grains and legumes can be an efficient way to reduce the hardness of the seeds, which facilitates the dehulling process of grains ([Oomah et al., 2014](#)) and reduction of their cooking time ([Divekar et al., 2017](#)) . [López-Perea et al. \(2008\)](#) investigated the effect of microwave irradiation at 1450 W, on 100 g of barley seeds for 4-25 s. They reported that microwaving barley seeds for 4 sec, which raised the temperature to about 35°C, decreased hardness and lead to having higher and better quality malt extract from the barley seeds while 8 s of treatment increased their hardness. [Velu et al. \(2006b\)](#) reported that microwave drying of maize in a domestic oven (390 W, 3-16 min) improved its milling properties and decreased its flour's viscosity, which could be the result of change in protein and starch structure. [Divekar et al. \(2017\)](#) showed that there were some fissures on the seed coat after microwave treatment of pulses, which could be the result of vaporization of moisture content from the cotyledon and the moisture loss from starch granules. These made some holes on the seed coat, leading to more water uptake and reducing cooking time. However, they reported significant change in protein secondary structure as a result of this treatment. The same result for reduction of cooking time and

increasing dehulling and milling efficiency of mung bean after microwave treatment was reported ([Purohit et al., 2013a](#)).

Microwave processing is able to increase phenolic content and antioxidant activities of legumes. [Randhir&Shetty \(2004\)](#) suggested that microwave treatment of fava bean could increase phenolic content and its antioxidant activity during germination. They exposed fava bean to microwave for 15 to 60 s and reported changes from day one to day eight of germination. According to their results, at first day of germination antioxidant activity of microwave processed seeds were higher than control and after 7 days, sprouts of those seeds treated for 30 s, showed the most antioxidant activity as well as producing the largest amount of phenolic contents. It was concluded that this high antioxidant activity was related to the high amount of phenolic compound, which was induced by microwave treatment. Enhancing phenolic contents and antioxidant activity of chickpea, roasted at 450- 900 W for 5-15 min in microwave, was also reported ([Logihalli et al., 2017](#)).

Modification of grain's starch is another potential application of microwave heating as it was confirmed for lentil's starch ([González & Pérez, 2002](#)). The Isolated lentil's starch was processed by microwave power of 650 W, at 85°C for 6 min and it was concluded that this process was able to decrease starch retrogradation, which made it more beneficial to be used in the baking industry as high retrogradation of legume starch is an inhibitory factor for their application in bakery formulations.

Conclusively, microwave treatment is a promising method of modulating the characteristics of legumes and grains to make them more nutritional or pave the way for having more efficient food processing. It is also helpful to eliminate those problematic properties of legume, which are preventive to make them as an alternative source of protein. However, there is still ambiguity about the effect of this process on the protein secondary structure as well as the mechanism of these changes, which can be further studied.

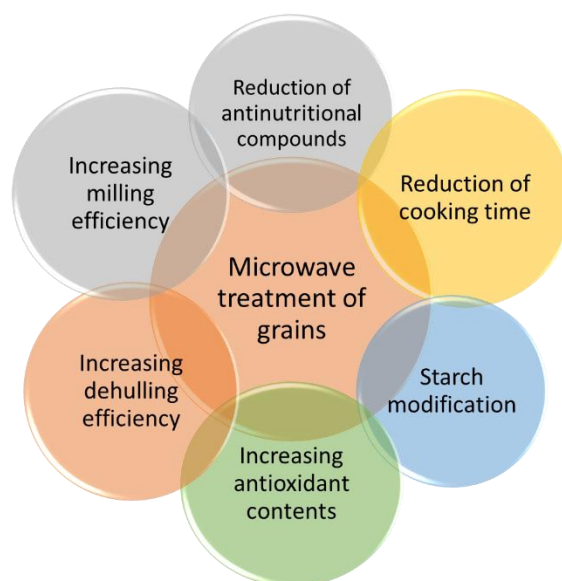


Figure 2.2 Microwave heating applications for grain quality modification

2.1.7. Disinfestation/ disinfection of grains by microwave heating

One of the most important contributions to annual world food loss, which mostly occurs in developing countries, is postharvest losses of grain crops, including cereals and legumes, due to contamination or physical damage during several operations after harvest. However, in many cases, the contamination of the crops starts from the field, where they grow and include insects, fungi and bacteria among which insects can cause more than 50% losses.

Decontamination of grains (disinfestation, disinfection) is still heavily reliant on chemical fungicides, herbicides, and insecticides. These chemicals are used for either preventing or controlling biotic stresses during post-harvest storage of grains. According to FAOSTAT (2017), countries around the world spent more than US\$29B on different pesticides for crop protection in 2016. However, there are many concerns regarding this huge usage of pesticides, including the chemical residues in the grains, adverse effects on the environment and development of chemical resistance by pests and pathogens ([Waard et al., 1993](#)). For instance, there have been several reports that new and stronger resistances against Phosphine are emerging among insects and much effort has been devoted to understanding the impact of these resistances ([Athanassiou & Arthur, 2018](#)). Therefore, replacing chemicals with natural or physical methods with little impact on the environment and low risk of resistance development seems to be a necessity. Among these safe alternatives is thermal treatment including dielectric heating using the microwave.

Thermotherapy is one of the oldest methods of eradication of micro and macro-organisms from grains, which can decrease the reliance on fungicides as well as protect the environment. There are some reviews on thermotherapy for pest control in grains ([Fallik &](#)

[Lurie, 2007](#); [Grondeau & Samson, 1994](#); [Hansen et al., 2011](#)). Nevertheless, conventional ways of thermotherapy, such as hot air and hot water, have the drawbacks of long processing times or drying of the seeds after treatment ([Grondeau & Samson, 1994](#)). As a result of these drawbacks, high-temperature short-time treatment, which can be provided by dielectric heating has been considered ([Tang et al., 2000](#)).

Advantages of dielectric heating over conventional heating for grain processing has been explained by many researchers. Firstly, it provides selective and volumetric heating mechanisms ([Brodie, 2011](#)) and intrinsically heats the pests, like insects, at a higher rate than the host due to their higher moisture content and higher dielectric properties than the host materials ([Nelson, 1996](#)). This difference in absorbing energy will be more obvious at lower frequencies, which help to remove the pests before affecting the grain quality. Secondly, dielectric processing tends to heat inside and outside of the commodity at the same time, while the mechanism of conventional heat treatment is convection from the surface and then conduction inside the sample. Hence, in dielectric heating, it is more probable that the pests, inside the product, could be eradicated before damaging seed quality. Finally, dielectric heating is much faster than conventional heating, which makes it a better choice for industrial processing. More details of research on insect and pathogen control using microwave heating are provided below.

2.1.7.1. Insect control (disinfestation)

Insect pests are the most important threat to stored products. They not only spoil the products directly but also could be a way of transferring fungal contamination. The cost of post-harvest loss due to insect infestation contributes considerably to the overall annual world food loss, which is approximately 1.3B tonnes of food or one-third of edible food produced for consumption ([FAO, 2017](#)). Additionally, the cost of insect disinfestation, as well as the development of new chemicals due to insects' resistance to the previous insecticides, are very high ([Nayak & Daglish, 2018](#)), which has necessitated investing in research and development of insect pest control, especially non- chemical methods, in stored grains.

Electromagnetic radiation is among the non-chemical methods of insect control, which have recently gained a lot of attention among researchers. When comparing dielectric properties of dry grains and their related insects, it can be concluded that the real and imaginary parts of permittivity at all stages of insect growth are higher than their hosts ([Nelson, 1991](#)). This could probably be due to the higher moisture content of the insects compared with the commodities, which leads to faster heating of the insect to a higher temperature than the

host. However, this difference in absorbed electromagnetic energy is higher at lower frequencies (RF compared to microwave) and thus lower frequencies have been recommended for this purpose ([Nelson, 1996](#); [Wang & Tang, 2001](#)).

A summary of the insect control in grains, using microwave energy, is represented in Table 2.2. There are a number of thorough reviews on RF and microwave insect control ([Macana & Baik, 2017](#); [Nelson, 1996](#); [Yadav et al., 2014](#)). Based on the literature, microwave has a good potential to eradicate the major insect pests of important grain crops including bruchids in legumes and rice weevil in cereal grains. However, the impact of these processes on all aspects of grain quality, as well as the proper types of process and process parameters, such as frequency, power, and exposure time, needs to be explored more. The process and process parameters need to be optimized to have more energy efficiency, more heating uniformity, and least quality change. It might demand a combination of microwave heating with other conventional methods if the cost of operation is reasonable. There has recently been an investigation of hot air assisted microwave treatment of chickpea and green gram seeds, at a seed moisture content of 7.5%-9.5%, to eradicate *Callosobruchus maculatus* adults ([Mohapatra et al., 2018](#)). It was stated that complete insect mortality for 500 g of grain sample (1cm depth) could be achieved at the microwave power of 2900W, air temperature of 60°C and exposure time of 6min. Although these process parameters were reported to reduce cooking time, they also reduced grain seeds germination. However, for having better results for all quality aspects of the host, utilizing lower microwave power combined with hot air could be recommended.

Table 2.2 Insect control in grains by microwave heating *

legume/grain	Initial MC% (wb)	Frequency (MHz)	Specific power (W g ⁻¹)	T (°C)	Time (s)	pest	Quality change	reference
Wheat	14,16, 18	2450	4, 6, 8, 10		28, 56	Insect (Tribolium castaneum, Cryptolestes ferrugineus, and Sitophilus granarius)	100% mortality of insect occurred at 500 W & 28 s but reduced G	(Vadivambal et al., 2007)
Rice	13-14	2450	0.085-0.68	55	90-720	Rice weevils		(Zhao et al., 2007)
Barley	14-18	2450	4-10	42.6, 53.7, 66.9, 73 (14%MC, 28 s) 57.3, 75.5, 91.2(14% MC, 56s)	28, 56	Tribolium castaneum (Coleoptera: Tenebrionidae)	Reduced α-amylase, diastatic power, soluble protein, viscosity at 500 W ,28 s but no	(Vadivambal et al., 2008)

				for up to 4 W g ⁻¹			change at 400 W g ⁻¹ , 56s; Both were effective on insects	
Barley		2450	14.5		4-8		Decreased hardness & increased malting quality after 4 s; 8 s exposure had an adverse effect	(López-Perea et al., 2008)
Chickpea, Green pea, Lentil	6.19- 6.54	27	2	60	600		Reduced moisture content & total weight	(Wang et al., 2010)
Chickpea		2450	0.7, 1.4, 2.1, 2.8, 3.5	40-80	300, 240, 200, 160, 100	Insect adult (<i>Callosobruchus</i> <i>chinensis</i>)	Reduced G & viability but achieved 100% insect mortality	(Singh et al., 2012)
Green gram		2450	0.7, 1.4, 2.1, 2.8, 3.5	40-80	300, 240, 160, 120, 70	Insect adult (<i>Callosobruchus</i> <i>chinensis</i>)	Reduced G & viability	(Singh et al., 2012)
Pigeon pea		2450	0.7, 1.4, 2.1, 2.8, 3.5	40-80	280, 240, 180, 140, 90	Insect adult (<i>Callosobruchus</i> <i>chinensis</i>)	Reduced G & viability	(Singh et al., 2012)
Lentil	6.9	27	0.938	60	600	Cowpea weevil	No change in MC, G, and color	(Jiao et al., 2012)
Green gram		2450	808 W		80	<i>Callosobruchus</i> sp.		(Pande et al., 2012)
Mung bean	12	2450	8	68.1	28	Cowpea weevil (<i>Callosobruchus</i> <i>maculatus</i>)	Reduced G by increasing power (200- 400W); 200W, 42s did not change G & MC	(Purohit et al., 2013b)
Common bean Soybean Chickpea Wheat, Corn		2450	700 W		60, 120, 180	-	Reduced mineral & protein content	(Ertas, 2013)
Cowpea	10.67 %	2450	~20		120- 600	Insect 1-2 day old (<i>Callosobruchus</i> <i>maculatus</i>)	Reduced G, emergence, protein, fat & fiber	(Echereobia et al., 2014)
Green gram	8	2450	1.5-13		50, 60, 90, 110, 120	Pulse bruchid	Reduced G and MC; moisture loss was minimum for the 1cm	(Mohapatra et al., 2014)

								thickness of grains	
Weak Beans	wheat	12.2/12.9	2450	1000 W (for 4cm thickness of the material)	70	75	Infesting fauna	Did not affect G & quality	(Lamberti et al., 2015)
	Cowpea		2450	1.5		5 - 25	Insect adult (Callosobruchus maculatus (F.) (Coleoptera: Chrysomelidae: Bruchidae)	25s exposure time was effective on insect eradication, but reduced germination	(Ahmady et al., 2016)
	Cowpea		2450	~48		30-150	larvae cowpea weevil Callosobruchus maculatus (Fabricius) (Bruchidae)	120 and 150s were effective on insect eradication, but reduced germination	(Silva Fontes et al., 2016)

* T: temperature, MC: moisture content, G: germination

2.1.7.2. Fungi and bacteria control (disinfection)

Fungal contamination is a serious problem of stored products around the world. Not only do they spoil the grains, reduce germination and viability, and degrade nutritious compounds, but some species can also produce toxic components like Mycotoxins, which are poisonous for both humans and animals. Today, 25% of cereals are not rendered fit for human consumption in the world due to mycotoxin contamination ([Hojnik et al., 2017](#)).

There are two types of grain fungi: field and storage fungi. Field fungi are those that invade the plants and the seeds before harvesting when the moisture content is very high. They survive at a water activity of more than 0.9 and are mostly those, which cause diseases in plants and seeds. Field fungi in grains, which include *Alternaria*, *Cladosporium*, *Helminthosporium*, and *Fusarium*, gradually die when the moisture content is below 13% ([Christensen & Kaufmann, 1969](#)). However, their spores can remain in the seeds and cause diseases later when the conditions are favorable again. Storage fungi usually attack the grains after harvesting. They cannot grow at a water activity below 0.65 at 20-25 °C ([Christensen & Kaufmann, 1969](#)), which is equivalent to a moisture content of 12.5-13.5% in wheat and rice. *Aspergillus* and *Penicillium* are the most important storage fungi and can spoil the grains by producing Mycotoxins and reducing their germination and nutritional values.

Therefore, moisture content and corresponding water activity has a great role in the survival of the fungi in grain seeds and likewise on their susceptibility to heat and electromagnetic energy ([Forsberg et al., 2002](#); [Jiao et al., 2016](#); [Taheri et al., 2019d](#)). On the other hand, grain seed viability is also more vulnerable at higher moisture contents when

treated at the same temperature or electromagnetic power. This contradiction, however, could be solved by optimizing the process parameters such as power, exposure time, seed moisture content and relative humidity of the process environment. For example, microwave treatment of winter wheat at 15% moisture content for the eradication of *Fusarium culmorum* decreased seed germination before eliminating the fungi while injecting steam during microwave processing, which was also able to make the heat more uniform, could control the pathogen without any reduction in germination ([Dieter von & Wolfgang, 2001](#)). Steam injection raised the relative humidity around the grain seeds and increased fungal susceptibility to electromagnetic heating. Although in some references it was concluded that at higher moisture contents lethality of fungi was higher with the same power and time ([Atmaca et al., 1995](#); [Reddy et al., 1998](#)), they cannot be used to draw any conclusions about the pathogen's susceptibility at higher seed moisture contents. The reason is that seeds with higher moisture contents absorb more electromagnetic energy at the same microwave power and exposure time due to their higher dielectric properties ([Taberi et al., 2018](#)) and thus seeds' temperature raises to a higher value, which can be the reason for faster or easier pathogen elimination. As a result, the final temperature of the host material should be considered as an important process parameter along with the grain moisture content when the aim of microwave treatment is pathogen control.

Dielectric heating application to eradicate or reduce some of the field and storage fungi in the grains are summarized in Table 2.3. What is obvious from the literature (Table 2.3) is that higher electromagnetic power, more exposure time and higher seed moisture contents lead to higher temperatures, which could be the reason for seed viability loss at these conditions. On the other hand, each pathogen can be eradicated at a specific temperature and relative humidity (or seed moisture content/ water activity). By considering just the thermal effect of the microwave, this temperature can be reached by absorbance of a specific amount of microwave energy, which corresponds to specific microwave power and exposure time. To investigate grain pathogen control utilising microwave energy, it seems more reasonable to find the threshold of pathogen susceptibility to heat (temperature) at different seed moisture content or relative humidity followed by reaching that temperature using the proper microwave power and exposure time and controlling the grain temperature so as not to exceed the limitation for the grain seed's quality. It is also worth investigating which of the higher power and lower exposure time or lower power and longer exposure time is best to reach the target temperature, and which would be more beneficial regarding host quality, pathogen control, and energy efficiency. In spite of the attempts to show dielectric heating's potential in grain pathogen control, which is

summarized in Table 2.3, there are not any information about important see-borne diseases of pulses or how much of the pathogens which could be eradicated without a significant effect on the pulse quality. There should also be more investigation on the combination of the microwave with conventional methods of disinfection.

*Table 2.3 Summary of seed fungal and bacterial infection control by microwave heating**

Grain	Initial MC%(wb)	Frequency (MHz)	Specific power (W g ⁻¹)	T°C	Time (s)	Pathogen	Quality change	Reference
Sorghum grain	12,14,16	1250	1.5, 3, 6	30-40; 90-110	30, 60	Fungi (Eurotium spp., Aspergillus candidus, A. niger and Penicillium spp.)	6 W g ⁻¹ , 60s dramatically reduced seed viability	(Atmaca et al., 1995)
Soybean, Common bean		2450	1420 W		0-420	Fungal Spores	Reduced G	(Cavalcante & Muchovej, 1993)
Wheat	8, 14, 20	2450	0.3,0.4,0.5, 0.6		20, 30, 40, 50 s min ⁻¹	Fusarium graminearum	Reduced pathogens from 36% to 7%, reduced G from 100 to 85% & seed vigour to 80% of control	(Reddy et al., 1998)
Soybean		2450			30, 45, 60	Inner fungi	30s was effective against fungi & didn't affect viability and vigour	(Reddy et al., 2000)
Common bean	9.5	2450	650 W/ 200 seeds	30.5-62.5	15-120	Fungus (Alternaria alternata, Fusarium spp. and Penicillium spp.)	was effective against Penicillium spp. & Improved seed vigour	(Tylkowska et al., 2010)
Wheat	12,15,18	2600, 5700, 9700	100, 100, 80 kW		600 - 1200	Seed-borne fungi (Phaeosphaeria nodorum, Pyrenophora tritici-repentis and Fusarium spp.).	Caused abnormally germinated seeds & was just effective against Tilletia caries	(GAURILČIKIENĖ et al., 2013)
Wheat	10- 40	2450	800 W/ 204 seeds		15, 30, 45	Fungi (saprophyte, Fusarium spp., Microdochium nivale)	Reduced seed viability at high MC	(Knox et al., 2013)

Dry bean	7.1-8.6	2450	1100 W/ 150 seeds		40	Bacterial blight (Xanthomonas axonopodis pv. phaseoli)	Reduced seed emergence up to 7%; little control of the disease	(Friesen et al., 2014a)
Dry bean	6.6-10.6	2450	1100 W/ 150 seeds		40	Bacterial blight (Colletotrichu m lindemuthianu m)	Decreased disease symptoms by 17-23% in combination with chemicals	(Friesen et al., 2014b)
Wheat Corn	12, 15	27	13.3	65, 70	600	Aspergillus flavus	Didn't affect seed vigour, reduced fungi up to 3-4 log at 15%MC & 2-3 log at 12% MC	(liao et al., 2016)
Brown rice, Barley	7.33, 4.98	2450	70		10-50	Aspergillus flavus & Aspergillus parasiticus	20 s exposure didn't affect grain quality with more than 90% reduction in pathogen	(Lee et al., 2017)
Red lentil	9, 16, 19	2450	4, 9.7	62- 99	19-127	Ascochyta lentis	Reduced infected seeds from 17% to 9% at 9% MC with no G loss	(Taheri et al., 2019d)

* T: temperature, MC: moisture content, G: germination

2.1.8. Challenges of microwave processing

2.1.8.1. Quality change

One of the most important concerns when exposing grains to microwave heating would be any negative change in qualities such as seed germination and vigour. Thus, to design a proper thermal treatment, the sensitivity of pests and hosts need to be evaluated first and if the pests are more sensitive than the commodity, the process is worth being considered. All agricultural products have a specific quality curve when exposing them to a thermal process. These curves depend on many factors such as properties of the materials or the process parameters. What is crucial in obtaining the curves of quality- temperature/time is examining the proper quality attributes related to the process and the host.

Dielectric heating could be considered as a thermal process for quality evaluation of the treated material. However, this process is not isothermal and usually, temperature rises during the exposure time and thus frequency, input power and exposure time have been commonly considered as key factors affecting the process and quality of the material. For example, applying microwave radiation at frequencies of 2.6, 5.7, 9.3 GHz decreased seedling vigour and increased the number of abnormally germinated seeds in wheat ([GAURILČIKIENĖ et al., 2013](#)). Soybeans, which were treated by microwave at 2450MHz for removing internal fungi, did not face any quality change after 30 seconds of exposure time, but the germination and structure of the seed cell walls were altered after 45 seconds ([Reddy et al., 2000](#)). Common bean germination improved after a 120-s exposure to microwave with the power of 650 W ([Tylkowska et al., 2010](#)) but exposure to 1420 W for 7 min reduced germination ([Cavalcante & Muchovej, 1993](#)). This could stem from the total energy absorbed by the samples and the final temperature of the seeds, which should also be considered in dielectric heating. The quality change of the grains as a result of considered process parameters in different kinds of grains are represented in Table 2.2 and Table 2.3.

Optimization of process parameters is a strategy to find the effective point of the process with maximum pest/pathogen reduction and minimum adverse effect on the quality of the materials. Optimization, however, has been rarely utilized by researchers to report the quality change of grains after microwave treatments. There was a study done by [Pande et al. \(2012\)](#) who optimized the power level and exposure time during microwave drying of green gram. The optimum point found through Response Surface Methodology (RSM) was microwave power of 808 W and exposure time of the 80 s, which resulted in 99.5% mortality of *Callosobruchus sp.* and seed moisture content of 8.9%. They concluded that at

this optimum condition, *in vitro* protein digestibility, Zn and Fe contents increased while there was no change in Mg, Mn, Cu, K; and Ca decreased slightly.

The sensitivity of different types of grains to electromagnetic heating is another issue which should be taken into account by thoroughly investigating the effect of process parameters on the quality attributes of different types of the grains after finding the proper process parameters for drying, disinfection or disinfestation. For instance, while studying the quality attributes of two different types of bean (Bayo and Negro) after microwave treatments with the aim of insect control ([Sosa-Morales et al., 2017](#)) it was revealed that there was no change in germination of Negro whereas Bayo's germination decreased at higher power levels (370, 510 and 950 W). It is interesting to note that the final temperature in all the treatments was 48.9 °C. In another study, dry beans, with a moisture content of 18%, were treated at the power level of 1100 W for eliminating bacterial blight (*Colletotrichum lindemuthianum*) and there was less than 10% reduction in germination and viability ([Friesen et al., 2014b](#)). Nevertheless, the latter's result can not be compared with the other two types of beans in the previous research as the seed moisture content and applied microwave power were different. Different cowpea cultivars were also reported to have different germination percentage after treating with the same microwave power and exposure time ([Silva Fontes et al., 2016](#)). The difference in susceptibility of the seed among different cultivars could be an obstacle to generalizing the obtained results for dielectric heating process parameters from a specific grain crop.

Moisture content is a very important factor which needs to be considered when designing a process of thermal treatment. It was reported that by increasing the moisture content of wheat and sorghum grains, adverse effects of microwave treatment on germination and vigour could increase ([Atmaca et al., 1995](#); [GAURILČIKIENĖ et al., 2013](#)). Germination reduced after Microwave treatment at the final temperature of 40°C for chickpea, green gram and pigeon pea ([Singh et al., 2012](#)), but it did not change during RF treatment at above 60°C for chickpea, green pea and lentil ([Wang et al., 2010](#)). One reason for this difference in the results could be different moisture contents of the seeds. Although there was some evidence that insect mortality was more at higher seed moisture contents while treating with microwave ([Vadivambal et al., 2007](#)), treating the dry form of the seeds was mostly preferred (Table 2.2 and Table 2.3), probably due to less risk of seed quality alteration.

Moreover, for determination of the effect of microwave processing on quality, the seed size is also of great importance. In thermal treatments, large seeds like soybean could lose their viability before a complete eradication of the pathogen and so their processing for the

eradication of pathogens seemed to be more difficult ([Grondeau & Samson, 1994](#)). Therefore, seeds with larger dimensions should not be exposed to higher power levels or longer exposure times, which would result in higher temperatures.

In general, having considered microwave processing for pest and pathogen control or grain drying, a thorough examination of the seed quality needs to be carried out at the optimized process parameters. These quality attributes, which differ among different grains and different end usage, are summarized by [Jayas&Ghosh \(2006\)](#). A possible solution for considering quality change of grains as a result of microwave treatment could be grouping them based on the parameters which affect the proper process parameters such as microwave power and exposure time. These grouping could be based on the seed's size, initial moisture content, seed coat thickness, protein/ lipid/starch contents or physical and dielectric properties of grains. After the grouping, the process parameters could be defined for each to facilitate the industrialization of the process.

One possible explanation for the drastic quality change of the grains before complete control of pests and pathogens could be non-uniformity of heat distribution during microwave processing. [Manickavasagan et al. \(2007\)](#) investigated the effect of microwave power and exposure time on wheat seed germination at different moisture contents in a continuous microwave dryer. They stated that germination of the seeds in hot spots was lower than those in other regions and it decreased with increasing power level and moisture contents. They concluded that drying wheat, even at a power level of 100 W, had adverse effects on germination in the hot spots. However, there are some solutions to overcome this problem and make sure that the temperature difference between hot and cold spots is minimum and pests could be eradicated before any adverse effect on the seed quality. These possible solutions will be further discussed in the next section.

2.1.8.2. Heat uniformity

Microwave radiation is intrinsically non- uniform in heating the materials which cause the existence of cold and hot spots in food materials. For drying grains by means of microwave heating, differences between cold and hot spots reached 59.2, 72.8 and 78.9 °C for Canola, Wheat, and Barley respectively ([Manickavasagan et al., 2006](#)). This non-uniformity, which was expressed as differences between maximum and minimum temperatures, was also observed in a microwave dryer with moving grains at different seed moisture contents on a conveyor belt ([Vadivambal et al., 2009](#)). What is obvious is that this non-uniformity of temperature is a major problem which can lead to significant grain viability loss or quality deterioration before completing the microwave processing.

The position of hot and cold spots directly depends on the shape and size of the material. Hot spots tend to be at the center for small cylinders and spheres with low loss factor. By increasing the dimensions and loss factor, the heating will move from the core to the surface ([Brodie, 2008](#); [REMMEN et al., 1996](#)). Some recent studies were carried out on the role of the material's thickness in the dissipation of microwave energy ([Bhattacharya & Basak, 2017](#); [Pu & Sun, 2016](#)). It was indicated that in thin materials (thickness: 4-6 mm), regardless of the shape, the starting point of heating is the geometrical center of the sample, while in thick materials in which the ratio of thickness to penetration depth is equal to or more than three, sample shape and location inside the microwave cavity affected this location.

Despite the mentioned problems, some solutions have been suggested for the prediction of temperature distribution and boosting its uniformity during irradiation. [Lorenson et al. \(1991\)](#) disclosed a method to increase heat uniformity by controlling the thickness of the product which controlled the irradiation modes. [Ho&Yam \(1992\)](#) tried different patterns of metal shielding around a cylindrical food model and concluded that heat uniformity could be improved by some special patterns without reducing the amount of absorbed power. For ready meals, it is stated that the heating uniformity is mostly affected by geometry, packaging and more importantly by the placement of the sample (location) in the cavity ([Ryynänen & Ohlsson, 1996](#)). [Peyre et al. \(1997\)](#) investigated the impact of changes in dielectric properties on heating patterns of foods in a microwave oven. They showed that materials with low (like ice) or moderate loss factors could be considered as a dielectric cavity and their size significantly affected the heating patterns inside them. Moderate loss materials; however, created more modes resulting in a more uniform heating pattern. [Ni et al. \(1999\)](#) showed that heat uniformity improved and the total moisture loss decreased by increasing the surface area for a specific volume. [Lee et al. \(2002\)](#) worked on the optimization of the heat/cold cycle for improving heat uniformity. In this method, microwave power was employed to heat the sample, followed by a holding time, which let the heat transfer inside the food by conduction. This heat/ hold cycles caused more even temperature distribution. [Zhang et al. \(2004\)](#) designed a container in which the frozen food was reheated uniformly when irradiated by microwave.

The more uniform temperature distribution was reported to be achieved by lower microwave power and longer exposure time ([Vadivambal & Jayas, 2010](#)) as well as shielding with aluminum foil ([Ho & Yam, 1992](#)). According to [Hong et al. \(2016\)](#), for the temperature below 200°C, less energy input will be required at lower powers. Therefore, at low power not only is the temperature more uniform but also less energy is needed. [Law et al. \(2016\)](#)

observed that loosely packed oil palm kernels had more heat uniformity than when tightly packed. When the particles were tightly packed, the maximum temperature occurred at the contact points while it was inside the kernels for loosely packed samples.

Regarding the effect of surrounding materials in microwave heating, using a turntable composed of different materials was found to improve the heat uniformity by 26-47% ([Ye et al., 2017](#)). The materials used in this research were Polyethylene (PE), Alumina, and Aluminium. The highest uniformity was achieved with the combination of PE and Alumina.

Generally, the following list of suggestions from the literature can increase heat uniformity in microwave processing:

- Mode stirrer and turntable
- Optimally designing the waveguide and the cavity
- Manipulating the heat cycle, using lower power & longer time
- Pulsed microwave instead of continuous radiation
- Designing a special packaging
- Using metal shielding for the material
- Controlling the geometry, size, depth, and the location of the material
- The combination of the microwave with other heating methods
- Fluidisation with air or mechanical mixing of particulate materials
- Surface temperature controlling

However, not all these solutions are applicable in microwave processing of grains. In this regard, combining microwave with other conventional methods might be considered a better tool for industrializing grain processing. It is possible to combine microwave with other kinds of irradiation like Gamma and Infra-red or processing with a partial vacuum so as to have more uniform heating as well as making the process more effective ([Yadav et al., 2014](#)). For drying purposes, it is beneficial to combine hot air with microwave irradiation in order to achieve volumetric, faster, uniform and energy-efficient heating ([Gowen et al., 2006](#); [Pu & Sun, 2017](#); [Sanga et al., 2002](#)). Fluidisation of particulate materials with air during microwave treatment was also recommended to have uniform temperature distribution ([Feng & Tang, 1998](#)). However, the susceptibility of the grains to cracking needs to be considered in this process. Controlling the surface temperature of the particulate materials by a continuous temperature monitoring was another recommendation to have uniform heating and preserving the grain quality ([Poongploy et al., 2018](#); [Xu et al., 2018](#)).

Process parameters such as microwave power, exposure time and seed moisture content are other factors which can be considered to study heat uniformity while designing a microwave process for grains. Non-uniformity, expressed as the difference between hot and cold spot, was reported to increase by increasing microwave power and exposure time during treatment of grains in a continuous microwave dryer ([Manickavasagan et al., 2006](#); [Vadivambal et al., 2009](#)) and remained constant when the microwave power is larger than a specific amount depending on the treated material ([Zhang et al., 2018](#)). However, increasing grain moisture content could lead to an increase or no change in the difference between the maximum and the minimum temperature ([Manickavasagan et al., 2006](#); [Taheri et al., 2019c](#); [Vadivambal et al., 2009](#)).

An issue raised here is that the definition of heat uniformity is not consistent among researchers in the field of microwave grain processing. As just mentioned, some researchers expressed the difference between hot and cold spot temperatures as an indicator of heat uniformity, while others calculated a Heat Uniformity Index (HUI) ([Wang et al., 2010](#); [Wang et al., 2005](#)). HUI has been expressed as the increase in the standard deviation of material temperature divided by the increase in average material temperature during the treatment time. Temperature can be measured by fibre optic probes in several points of the product (in the whole volume of the product) or the infrared image (surface temperature of the product) can be taken before and after the treatment following by the extraction of the temperature values in different points of the image. Coefficient of Variation (COV) was also considered for expressing heat uniformity of microwave processing ([Zhang et al., 2018](#)). The lower the HUI or COV is, the more uniform the temperature distribution will be.

Nevertheless, temperature difference and HUI are not always positively correlated. [Taheri et al. \(2019c\)](#) explained that the difference between the maximum and minimum surface temperature of a single layer of lentil seeds treated in a microwave oven increased by increasing moisture content and input microwave power, while HUI (or temperature uniformity index, TUI) decreased. It means by increasing maximum-minimum temperature difference, the heat uniformity in the whole material does not necessarily get worse. Conclusively, it seems more reasonable to consider both HUI and the difference between maximum and minimum temperature of the whole material for the study of heat uniformity in microwave processing.

To date, the industrial equipment, which has been designed for grain processing, is continuous treatment on a conveyor belt and receiving the radiation from the top through multiple waveguides. This process can be coupled by low-speed hot air flow on or across

the bed of the grain to increase the heating uniformity, and in the case of drying, to facilitate moisture removal from the grain's surface. Other lab-scale microwave processing which has been developed for grain treatments with improved heat uniformity are represented in Figure 2.3. There are also several other systems which have been developed for in-field treatment of grains ([Bensussan & Azam, 1982](#); [Snaper, 2003](#)).

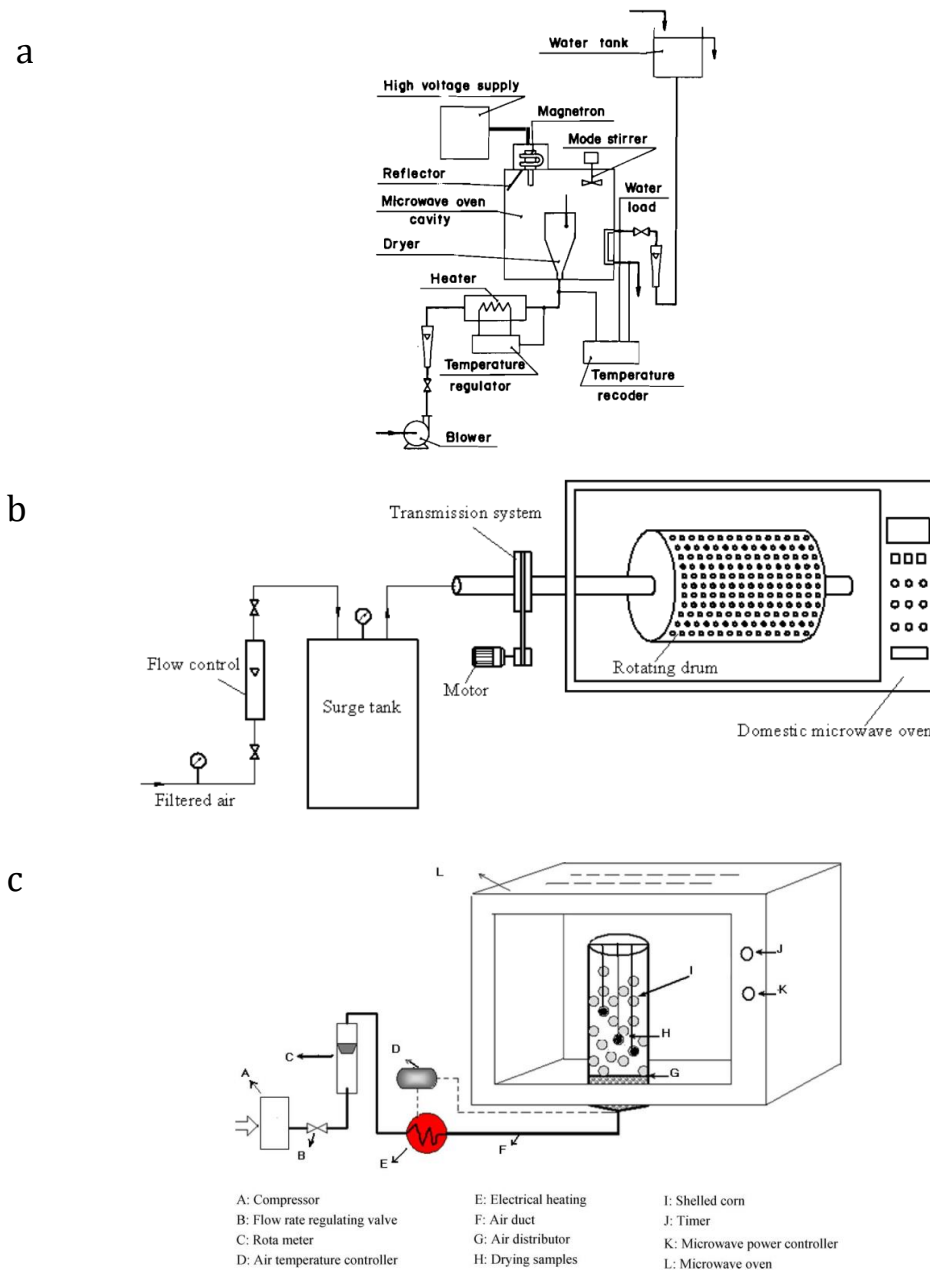


Figure 2.3 Schematic of three lab scale apparatus used for microwave processing of grains with improved heat uniformity; (a) Microwave spouted bed for drying particulate materials, ([Kudra, 1989](#)) (b) Microwave rotary process for soybean drying ([Wang et al., 2009](#)), (c) Microwave fluidised bed for drying shelled corn ([Momenzadeh et al., 2011](#))

2.1.9. Conclusion and future work

Safe storage and safe post-storage usage of grains for sowing and end-users puposes demand that they are free of pest and pathogens, at a safe seed moisture content and have

high and desirable quality attributes. All these targets could be achieved by microwave processing as their effectiveness have been evaluated separately. Now the question needs to be focused on how these targets could be achieved in one single process with electromagnetic processing. Insects have been eradicated at a temperature below 60°C, while fungi and bacteria might tolerate higher temperatures, depending on the availability of water. Therefore, partial control of both pests and pathogens as well as the possibility of quality modifications during grain drying for the reduction of the reliance on chemicals could be examined in future studies. However, it might seem a very challenging work to find an overlap between seed germination enhancement with the other applications, as it could be achievable at a very low microwave power or exposure time, which would not be sufficient for other purposes.

Two major problems related to microwave treatment are highly non-uniform heating and any negative effect on the product. Non-uniformity can be solved by a combination of microwave processing with other methods or designing a proper cavity and process in which temperature could be controlled, as stated for drying purpose, by fluidising grain bed using hot air or mechanical agitation. Surface temperature control by continuously changing the microwave power during the treatments is another strategy which could help in this regard. By improving heat uniformity and controlling surface temperature, the hot spot temperature could be controlled in order not to increase beyond the product's tolerable limitation and therefore, any negative effect on the seed quality attributes could also be prevented.

The other important fact for utilising microwave treatment is to optimize the process parameters to have the most energy efficiency. These parameters include power, time, and moisture content of the sample. By finding the critical points, the process of microwave heating can be combined with other conventional physical ways to obtain complete pest and pathogen control of stored commodities as well as reducing their moisture content for safe storage and have the best quality for end-users. This combination could be designed in a way to overcome the weakness of each method as well as with the aim of reducing the usage of chemical treatments. There is also a lack of proper models which relate the quality attributes of grains to power density or average temperature during dielectric heating. The models could be useful to find process parameters in all types of applicators and generalize the process.

2.2. Part two: Microwave driven cold plasma for disinfection of grains

2.2.1. Introduction

Pre-storage thermal treatment of grains and fresh produce is a non-chemical solution for reducing postharvest decay. These treatments are best to decrease the reliance on fungicides as well as protecting the environment. However, most thermal treatments affect the quality attributes of the commodity before completion of disinfection. Therefore, non-thermal methods of processing are gaining more popularity for pasteurisation and sterilisation in the food and agricultural industries.

Cold plasma is one of the emerging technologies that appears to be effective against many microorganisms. The attractiveness of plasma in the food industry is its short treatment time and non-thermal conditions, as well as leaving no residues in the food. In non-thermal plasma, the temperature of the electrons, which are responsible for the reactions, reaches 10,000-100,000 K (1-10eV) while the gas and ion temperatures remain at room temperature ([Petitpas et al., 2007](#)). Plasma treatment not only has a disinfection role in seeds and grains but also has great potential to enhance their germination rate ([Ohta, 2016](#)), water uptake and cooking quality ([Lee et al., 2015](#)).

The thermal effect of the microwave, as microwave heating, was reviewed in the previous section. However, microwave has the potential to be the source of non-thermal plasma, which itself has proven to have fungicidal effects. Therefore, the effect of cold plasma on microorganisms and their hosts (mainly grains) will be reviewed in this section, followed by a specific discussion on microwave-driven cold plasma for this application.

2.2.2. Plasma disinfection

There are many studies on the inhibitory effects of cold plasma on food-related microorganisms. For example, [Park et al. \(2007\)](#) used a microwave driven cold plasma (power of 1 kW) through a quartz tube with argon gas at 100 l min⁻¹ and atmospheric pressure to control methicillin-resistant *Staphylococcus aureus* and *E coli*. They showed that cold plasma ruptured the cell wall of the bacteria and reduced their size by releasing the cell contents. [Avramidis et al. \(2010\)](#) also reported that air plasma treatment of mycelia of *Fusarium culmorum* and *Ascochyta pinodella* for 180 and 360 s caused some cracks on their walls and the cytoplasm leaked out, which finally flattened and killed the mycelium. They concluded that when the vegetative cells are not accumulated on each other, their growth could be inhibited within a very short time (60 s). However, they utilised compressed air as the plasma gas and the system they used was a Dielectric barrier discharge (DBD) system, with a power of 560 W and a peak voltage of 20 kV.

Having studied the effect of cold plasma on the natural flora of chickpea, [Mitra et al. \(2014\)](#) indicated that apart from exposure time, size and shape as well as the surface area of the seeds could have a significant impact on the reduction of surface microorganisms by plasma treatment and suggested that the efficiency of the treatment could increase by shaking the samples. They showed that microbial inactivation by this method was better described by the Weibull model with no shoulder and tail than the first-order linear model.

In terms of different microorganisms, it is generally concluded that the elimination of fungi is much more difficult than bacteria. In one type of plasma source and gas like corona discharge, it takes 2-4 min to inactivate bacteria while the time needed for fungi could increase to 20-30 min, which could be due to more complex structures of fungi ([Scholtz et al., 2015](#)). It can also be seen in other studies, which are summarised in Table 2.4. For example, [Zahoranová et al. \(2018\)](#) showed that naturally occurring bacteria in maize were eliminated after 60 s, while a treatment time of 180 s was required to remove all the naturally occurring fungi.

Considering the host's quality, plasma can increase the wettability and decrease the hydrophobicity of the biological materials by etching the surface ([Surowsky et al., 2016](#)) or by oxidizing and reducing the lipid content on the surface ([Zahoranová et al., 2018](#)). This effect has been reported to be one of the reasons for increasing seed germination and their seedling growth, which itself is an attractive application of cold plasma but is not in the scope of this review.

A summary of grain treatment and any possible change in the host quality, utilising different apparatus of plasma, is represented in Table 2.4.

2.2.2.1. Inactivation mechanism

The microorganism inactivation, which also depends on the type of gas and relative humidity, is related to UV radiation and high energy ions and radicles such as atomic oxygen (O), hydroxyl radical (OH•), ozone (O₃), hydrogen peroxide (H₂O₂) and peroxyxynitrite ([Hojnik et al., 2017](#)). The responsible phenomenon in a microwave driven plasma, with Argon as the discharge gas, is reported to be mostly UV radiation. However, when the humid gas or combination of Argon and Oxygen is used in the process, some radicles are also responsible for the disinfection ([Guo et al., 2015](#)). Working at pressure, which is lower than atmospheric, could also make UV radiation a dominant reason for the disinfection ([Niemira, 2012](#)). The mechanisms of damage to microorganisms have been reported to be rupturing of their cell walls by charged particles and oxidation by the reactive species or UV ([Guo et al., 2015](#)).

Therefore, the mechanism of inactivation of the microorganisms can be decided by properly selecting the plasma gas and operating pressure.

2.2.2.2. *Effect of process parameters*

The inactivation of microorganisms depends on many parameters, such as plasma source, type of exposure to cold plasma, input power and voltage, type and speed of the gas, the water content of the gas, the specimen, the specimens' surface characteristics, the microbiological species and concentration, the exposure time, and the food shape ([Mohapatra et al., 2017](#)). Regarding the position of the food, there are three types of exposure to plasma: far from the generation point, which is indirect treatment or exposure to late afterglow; close to the generation point or exposure to early afterglow; and inside the plasma field ([Niemira, 2012](#)). The remote or indirect treatment is more flexible, but the reactive species are fewer (secondary chemicals) as the life span of most reactive species finish before reaching the sample. The second category, which is direct treatment, has a higher concentration of active components. However, this type can have an adverse effect on the host quality. In the final group, samples are placed between two electrodes and exposed to the highest amount of active and antimicrobial species.

Having compared the direct and indirect exposures, [Fridman et al. \(2007\)](#) showed that direct plasma could be a better surface sterilizer than indirect plasma in a DBD (dielectric barrier discharge), which could be the result of exposure to more effective species. However, [Hertwig et al. \(2015\)](#) indicated that remote microwave driven plasma treatment was more effective than a plasma jet for the disinfection of black peppercorns. This difference could be the result of different apparatus of plasma with different process parameters, which makes it difficult to decide whether direct or indirect exposure is more effective.

[Lu et al. \(2014\)](#) compared the effect of different process parameters like gas mixture, voltage, and type of exposure on decontamination efficiency of *E. coli* and *L. monocytogenes* and observed that indirect exposure in a DBD plasma with a gas containing CO₂ could be more effective than the direct treatment of the microorganisms. They concluded that this effect depends on the type of gas mixture. In their study, they hypothesized that possible recombination of the species, like ozone with longer shelf life, could occur and some other reactive species could form, which have more bactericidal impacts. They also mentioned that the gas containing more oxygen and less nitrogen was more efficient in bacterial eradication, due to production of more effective species like OH•, O³ and O. An interesting finding of their research was that at very low exposure time, there was leakage from

bacterial cells that was the result of membrane injury, but it had no effect on their DNA, which could lead to their recovery.

Concerning input power and voltage, the effect of process parameters in a fluidised bed cold plasma was investigated by [Dasan et al. \(2017\)](#). They reported that the eradication efficiency of spores of *Aspergillus flavus* and *Aspergillus parasiticus* on hazelnuts increased by increasing the voltage and frequency and by decreasing the diameter of the reactor. They also concluded that dry air was more efficient than nitrogen as the discharge gas. [Zhou et al. \(2011\)](#) used a dielectric discharge barrier to enhance tomato seed germination and concluded that the effect of plasma at different voltages is different on tomato yield. They indicated that there is an optimum voltage at which the maximum yield could be obtained and the Gaussian distribution curve could be used to describe the relation between voltage and fruit yield. [Lu et al. \(2014\)](#) also indicated that the efficiency of eradication of *Escherichia coli* and *Listeria monocytogenes* was higher at a higher applied voltage in a DBD plasma, both for direct and indirect exposure of the samples. This fact stemmed from more energy and so the production of more antimicrobial reactive species. [Butscher et al. \(2015\)](#) investigated the effect of increasing power and percentage of oxygen in Argon gas in a fluidised bed plasma for decontamination of wheat seeds. They observed that by increasing power, the plasma would be intensified, and the plasma zone was longer in the tube. However, by considering effective exposure time, increasing power did not have any beneficial effect. They also concluded that increasing the percentage of oxygen in the gas increased the efficiency of spore inactivation. Comparing different gasses in a Diffuse Coplanar Surface Barrier Discharge DCSBD was shown that using Oxygen as the working gas was more effective than Nitrogen or air in reducing fungi on hazelnuts ([Mošovská et al., 2019](#)). It was proven that plasma created from oxygen contained mostly Ozon while air generated plasma consisted of reactive species made of N and O and Nitrogen-created plasma was mostly UV-A.

With regard to the host surface, [Ziuzina et al. \(2014\)](#) reported that indirect cold plasma inactivation of bacteria from complex surfaces like strawberry was much more difficult than from the surface of cherry tomato. Having measured the lower concentration of ozone in strawberry packages, they suggested that more pores on the surface could cause ozone to be decomposed, which was the reason for less effectiveness of the plasma.

Relative humidity (RH) of the plasma gas is another important factor affecting the antimicrobial efficiency of the plasma. The importance of this factor was studied on the inactivation of bacterial spores ([Patil et al., 2014](#)). It was observed that air with more RH

was more effective at eliminating the spores and presenting more humidity in the air led to producing more effective particles like $\text{OH}^\cdot$ and H_2O_2 that sped up the disinfection process. Additionally, high RH could cause spores swelling which caused more damage to them.

Generally, the effect of all the process parameters should be considered while considering a plasma process for grain treatment. It is beneficial to find the optimized parameters for the best outcomes in the case of cost and efficiency of disinfection as well as host quality attributes. Plasma dose definition is one topic which has been recently argued ([Adhikari et al., 2020](#)). This is because different plasma sources and process parameters have been applied by researchers which makes it difficult to compare. Therefore, the definition and standardisation of the plasma dose is now required, which needs to be considered by researchers who use different apparatus for creating plasma.

2.2.3. Microwave plasma discharge

Non-thermal or cold plasma can be created by different sources from high voltage to high frequencies. They include DBD (dielectric barrier discharge), Corona discharge, atmospheric pressure plasma jet (APPJ) and microwave discharge. High voltage sources have recently gained more attention in food and agricultural research, probably due to the lower plasma temperature and the feasibility of creating the plasma in atmospheric air, which reduces the costs to a large extent.

In a microwave plasma discharge, which is a high frequency source, the plasma applicator is an important part. It defines the efficiency of plasma generation as well as the minimum and maximum powers which can be absorbed by the working gas. It also defines the size and uniformity of the plasma. There are different types of microwave plasma generators with different applications having been reviewed by [Lebedev \(2010\)](#). The attractiveness of a microwave plasma is that it can be operated without using any electrode and its ability to generate very high-density plasma (high density of reactive species). However, a magnetron as a microwave generator has the drawback of a short working life or instability when generating microwaves. This problem is going to be overcome by replacing magnetron generators with a solid-state source, which has more flexibility and stability at lower powers.

Table 2.4 Treatment of grains using cold plasma with the aim of disinfection

Material	Gas	Pressure	Temperature	Source	Pathogens	Time	Quality change	Efficiency of decontamination	Reference
Wheat, bean, chickpea, soybean, barley, oat, rye, lentil, corn	Air or SF6	Vacuum	Room		<i>Aspergillus spp. and Penicillium spp</i>	5-20 min	No change in germination & nutritive components (like gluten)	3-log reduction after 15 min	(Selcuk et al., 2008)
-	Air (3 m s ⁻¹)	Atmospheric	<45 °C	DBD-high voltage pulse generator (13 kHz)-p=560 W	<i>Ascochyta pinodella, Fusarium culmorum</i>	60,180,360 s	-	100% inhibition after 360 s; heating at 55°C for 720 s did not remove the fungi	(Avramidis et al., 2010)
Blue lupine, catgut, honey clover, and soy				5.28 MHz-RF-0.6 W cm ⁻³	<i>Fusarium</i> , black spot, <i>Stemphiliopsis</i> , <i>Anthraco</i>	10-15 min	10-20% increase in germination- more than 15min decrease in G	3-15% decrease in <i>Fusarium</i> , black spot, <i>Stemphiliopsis</i> - no effect on <i>Anthraco</i>	(Filatova et al., 2011)
Wheat, spring barley, soy, field pea	Air	40 Pa (vacuum)	Max. 310 °C	RF-5.28MHz-0.1-0.7 W cm ⁻³	Fungi	2,5, 7, 10, 15, 20 min	An increase of 4-7% in seed germination	6-14% reduction	(Filatova et al., 2012)
Rice and lemon	Air	Atmospheric		Five discharge electrodes and a UV lamp, 7-10 kV	Mould and <i>E. coli</i>	20 min for lemon and 90 min for rice seeds	No significant damage to the host	Complete eradication of the mould, combination of plasma and UV was more effective	(Hayashi et al., 2014)
Rice seeds	Air	Atmospheric	<45 °C	DBD- 3 W, 30 kV	Seed-borne <i>Gibberella fujikuroi</i>	120 s	No effect on the seedling emergence and height	More than 92%	(Jo et al., 2014)
Chickpea	Air		Max. 5 °C above ambient T	An electrode based on the surface micro-discharge (SMD)	Natural flora	2-5 min	A strong improvement in the seed germination (89.2 %), speed of germination (7.1±0.1 seeds d ⁻¹), and seed vigour, after 3 min (optimum 1 min)	1-2 log reduction	(Mitra et al., 2014)
Barley and corn	Air	15 Pa		Glow discharge, 100-200 W	Background fungi	20 min	No change of germination and	25% reduction in the number of fungi in barley	(Braşoveanu et al., 2015)

Wheat	Argon (15 nl min ⁻¹) with oxygen (5-10%)	5 mbar	Thermal-max. surface T: 90 °C	RF-13.56 MHz-700-900 W	<i>Bacillus amyloliquefaciens</i> endospores	30 s	seedling length, an increase in corn fresh weight No effect on flour and baking quality	seeds and 40% reduction in corn seeds 2 log reduction- spore inactivation is more with more O ₂	(Butscher et al., 2015)
Wheat	Air	Atmospheric	Low temperature	Cylindrical electrodes, 8 kV	Natural fungi	3- 30 s	No significant effect on seed germination and vigour	Significant reduction of all the present fungi after 10 s	(Kordas et al., 2015)
Barley and wheat	1-air- 100 SCCM 2- air 8 bars	1-low pressure- 100 Pa 2- atmospheric		1-pulsed MW 2- gliding arc discharge-50 Hz	Mycotoxins, namely deoxynivalenol (DON), deoxynivalenol-3-glucoside (D3G), and trichothecene mycotoxins (T-2).	1-5 min	1- An increase in germination 2- A reduction in germination no nutritive change	1 more effective than 2	(Kriz et al., 2015)
Wheat	Pulsed Argon (2.8 n l min ⁻¹)	Atmospheric		Pulse frequency (5-15 kHz) and pulse voltage (6-10 kV)	Endospores of <i>Geobacillus stearothermophilus</i> (as a model)	60 min	No change in gluten	3 log reduction (1 log after 10 min)	(Butscher et al., 2016)
Maize	Air & N ₂ (3000 l h ⁻¹)	Atmospheric	Non-thermal	5-10 kV; 18-25 kHz (at a maximum power of 655 W)- nuzzle with 4 mm	<i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i> spores	1- 5 min	-	5.48 and 5.20 log reduction	(Dasan et al., 2016)
Rice	Air/ argon (2.5 l min ⁻¹)	Atmospheric	<30°C	Hybrid cold-discharge plasma with 10×10 twin-tip electrodes, 14 kV	Natural fungi	1 min	An enhancement of seed wettability and germination	No fungi growth on the treated seedlings after 14 d while the untreated seedlings were invaded by fungi	(Khamseen et al., 2016)
Wheat	Air	Atmospheric	Cold	100 W cm ⁻³ (400 W)	<i>Fusarium nivale</i> , <i>F. culmorum</i> , <i>Trichothecium roseum</i> , <i>Aspergillus flavus</i> , <i>A. clavatus</i> .	10-80 s for seeds & 30-300 s for microbes	20-50 s: an increase in germination rate, dry weight, and vigour of seedlings	<i>Fusarium</i> spp. most sensitive; <i>Fusarium nivale</i> > <i>F. culmorum</i> > <i>Trichothecium roseum</i> > <i>Aspergillus flavus</i> > <i>A. clavatus</i> .	(Zahoranová et al., 2016)

Rice	Humid air (16 l min ⁻¹ + 91 µ l min ⁻¹ water)	Atmospheric		Surface discharge electrode, 20 kV, a 10 cm distance between the seeds and the end of quartz tube	<i>Fusarium fujikuroi</i> , <i>Burkholderia</i> <i>plantarii</i>	10 min (2 min interval shaking)	No adverse effect on the seed germination and seedling growth	A reduction of 18.1% (fungal blight) and 38.6% (bacterial blight) in the disease severity	(Ochi et al., 2017)
Rice culture	Air-130 s l min ⁻¹ ; 330 K	Atmospheric	Max. 60 °C	4 W cm ⁻² ; 38 kV	Mycotoxins produced by <i>Fusarium</i> <i>verticillioides</i> , <i>Fusarium</i> <i>avenaceum</i> , <i>Aspergillus nidulans</i> and <i>Fusarium</i> <i>graminearum</i> (FB1, EnnB, ST and ZEN)	60 s	-	Removed completely when pure, 2log reduction after 30 s	(ten Bosch et al., 2017)
Wheat and barley model media	Air	Atmospheric		DBD, 80 kV	Native microflora, pathogenic bacteria and fungi: <i>E. coli</i> , <i>Bacillus</i> and <i>Lactobacillus</i> , <i>B.</i> <i>atrophaeus</i> endospores	30 min	-	Maximum of 4.4 log reduction, more reduction on the hydrophobic surface than hydrophilic	(Los et al., 2017)
Wheat and barley	Air	Atmospheric		DBD, 80 kV	Native microflora, pathogenic bacteria	5 and 20 min with 2 or 24 h retention time	No change in germination after 5 min, but a reduction after 20 min	Resistance to plasma treatment: <i>E. coli</i> > <i>P.</i> <i>verrucosum</i> (spores) > <i>B.</i> <i>atrophaeus</i> (vegetative cells) > <i>B. atrophaeus</i> (endospores)	(Los et al., 2018)
Soybean	O ₂ / N ₂ (6 n l min ⁻¹)	Atmospheric		Quasi- stationary DBD, 25 kV, 65 or 85 W	Seed-borne <i>Diaporthe</i> / <i>Phomopsis</i> complex	1, 2, 3 min	29% reduction in catalase activity, 30% increase in glutathione content	49-81% reduction in the number of infected seed	(Pérez Pizá et al., 2018)
Pine seeds		Atmospheric		Diffuse Coplanar Surface Barrier Discharge (DCSBD)	<i>Fusarium</i> <i>circinatum</i>	5 – 300 s	Dramatic reduction of germination at 60 s or more	50% reduction after 10 s treatment	

Maize and barley	Argon/ N ₂ -O ₂ (500 and 1000 SCCM)	2-8 mbar	<40°C	Afterglow of microwave discharge, 25 W	<i>Fusarium graminearum</i> and <i>Fusarium verticillioides</i>	3 min (barley), 4 min (maize)	An improvement in the germination of contaminated seeds	With a mixture of gases, infected seeds reduced to below 10 or 40% and seed germination increased to 80%	(Szóke et al., 2018)
Maize	Air	Atmospheric		DCSBD, 20 kV, 400 W	Natural microbiota or <i>A. flavus</i> , <i>A. alternata</i> , <i>F. culmorum</i>	30 – 300 s	A decrease in water contact angle, removal of the lipid from the seed surface, no effect on seed germination up to 120 s	Complete elimination of natural bacteria and <i>F. culmorum</i> after 60 s and natural filamentous fungi after 180 s; <i>A. flavus</i> reduced to 10% and <i>A. alternata</i> reduced to 30% (from 100%) after 120 s	(Zahoranová et al., 2018)

2.2.4. Other forms of seed treatments using plasma

Plasma activated water (PAW) and plasma processed air (PPA) has been recently explored as potential tools for seed and fresh produce treatment. The idea of PPA is producing reactive species of ROS and RNS using microwave as the plasma generators and air as the plasma gas. This process was described by [Schnabel et al. \(2015\)](#) for fresh produce decontamination. PPA is also known as remote or indirect plasma processing. It was introduced by [Schnabel et al. \(2012\)](#) as an indirect method of treatment for microbial decontamination of *Brassica napus* seeds, which was shown to be more efficient than dielectric barrier discharge (DBD) treatment. They used a microwave source and ignited the plasma for 7 s with a power of 1.2 kW with 20 l min⁻¹ air as working gas following by the different holding times in the bottle in which PPA was captured.

PAW can be created by treating water with non-thermal plasma. The treated water contains ROS and RNS and has been proven to have great potential for the disinfection of food and agricultural commodities ([Thirumdas et al., 2018](#)). Processing of water, using cold plasma, could reduce the pH of the water due to the formation of reactive species from nitrogen and oxygen, and a part of the antimicrobial effect could stem from its acidic condition ([Los et al., 2020](#)). However, a high level of nitrite and nitrate also plays a crucial role in the disinfection of fresh produce ([Zhou et al., 2018](#)). PAW was shown to have great potential for the disinfection of fresh produces such as strawberries ([Ma et al., 2015](#)) and baby spinach leaves ([Risa Vaka et al., 2019](#)). It was also able to reduce *E. coli* of mung bean seeds by 6 log CFU ml⁻¹ after 6 h of treatment without any change in the seed germination ([Darmanin et al., 2020](#)).

Beside the disinfecting role, PAW has the potential to enhance seed germination and plant growth. [Zhang et al. \(2017b\)](#) demonstrated that PAW enhanced lentil crop growth when it was utilized for their irrigation. They also showed that activated tap water contains much more Nitrate than the activated demineralized water, while they contain the same amount of Hydrogen peroxide. Deionized water activated by N₂(10 l min⁻¹)- O₂ (0.4 l min⁻¹) microwave plasma contained hydrogen peroxide, nitrite and nitrate ions as well as NO ([Kang et al., 2019](#)). However, NO was confirmed to react continuously with the dissolved oxygen content of the water, which reduced its concentration over time.

Therefore, these two new technologies could be considered to compare with thermal or non-thermal (plasma) treatments of grain seeds, with the aim of seed-borne pathogen

control. The comparisons still need to be carried out with regard to efficiency of pathogen control, any quality changes in the host and the fixed and operating costs.

2.2.5. Conclusion

Considering the non-thermal treatment of the grains with a microwave source, microwave driven cold plasma is an emerging technology that should be explored further. This new technology has shown great potential for the crops' growth enhancement and seed sanitation. However, there is still a lack of data about its effect on many fungal diseases of grain crops as well as the host quality. PAW and PPA are also two new technologies that have been introduced recently to be considered in fresh produce and grains with a very good potential of sanitization and growth enhancement. These technologies are still in the phase of research and development and by providing enough information on all the aspects of the concerns, such as sensorial effect or long-term effects on the pathogens, it could enter the phase of industrialisation.

Chapter 3. Dielectric properties of Kabuli chickpea, red and green lentil in the microwave frequency range as a function of temperature and moisture content

Taheri, S., Brodie, G., Jacob, M. V., & Antunes, E. (2018). Dielectric properties of Kabuli chickpea, red and green lentil in the microwave frequency range as a function of temperature and moisture content. *Journal of Microwave Power and Electromagnetic Energy*, 52(3), 198-214. <https://doi.org/10.1080/08327823.2018.1452550>

3.1. Abstract

Dielectric properties are essential for applications in: thermal processes using microwave and radio frequency; predicting heat uniformity in dielectric heating; and developing moisture measurement equipment based on electromagnetic waves. While the dielectric properties of many legumes have been studied, their response as a function of moisture content and temperature at microwave frequencies has not been well explored. In this study, dielectric properties of Kabuli chickpea, green and red lentil were measured, using an open-ended coaxial probe, at four moisture contents between 8 to 20% wet base (wb), temperatures up to 80°C and in the microwave frequency range of 700-7000 MHz, which include three allowed frequencies of 915, 2450, 5800 in the Industrial, Scientific and Medical (ISM) microwave range. According to the results, at frequencies above 3000 MHz, there is a negative linear relation between loss factor and frequency and the linearity was more at higher moisture contents. Due to the linear correlation of the complex-plane plot, legumes densities could be predicted from their dielectric properties at 1800 MHz. At 2450 MHz, penetration depths at room temperature were below 5 cm and 15 cm for Kabuli chickpea kernels and powdered lentil seeds, respectively. Therefore, Kabuli chickpeas need to be treated in one layer during microwave processing for more heat uniformity.

3.2. Introduction

Legumes are effective source of protein, as they need less input per kilogram of produced protein compared to meat ([Food & Nations, 2013](#)). Kabuli chickpea (*Cicer arietinum L.*) and lentil are the most cultivated legumes in the world after bean and pea. Among the seed borne pathogens of lentil, *Ascochyta lentis* (which causes *Ascochyta blight*) and *Botrytis cinerea*, are the most problematic. This needs special treatment and cultivation to be prevented ([Chen et al., 2011](#)).

It is believed that seed borne inoculum can be controlled by hot water and dry heat treatment, though the germination of the seeds is negatively affected. As a result, the current ways of disinfection include applying chemicals like pesticides and fungicides. These chemicals, despite elimination of volatile mercurial, leave some concerns about human health and environmental impacts. There are also many pest species, which develop resistance toward chemicals and not only cause losses in production but also lead to investment in development of new chemicals to control them. Finally, consumers' awareness and their demands for chemical free products, have led to exploration of non-chemical methods in food and agricultural processing, such as microwave and radio frequency treatment.

There have been several research papers that introduce microwave as an alternative pest control in sorghum grains ([Atmaca et al., 1995](#)), wheat ([Knox et al., 2013](#); [Reddy et al., 1998](#); [Vadivambal et al., 2007](#)), dry bean ([Friesen, 2014](#)), brown rice and barley ([Lee et al., 2017](#)) and green grams ([Pande et al., 2012](#)). However, for developing a proper process of microwave treatment, the dielectric properties of the grains and legumes are required. These properties determine the behaviour of the material in the electromagnetic field and define the maximum thickness of the material, which can be treated at each frequency.

Dielectric properties are also of great importance because of their direct relation with moisture contents, which lead to developing microwave sensors for fast measurement of moisture content ([Abegaonkar et al., 1999](#); [Ahmady et al., 2016](#); [Silva Fontes et al., 2016](#); [Soltani & Alimardani, 2011a, 2011b](#); [Trabelsi et al., 1998a](#); [Trabelsi et al., 1998b](#); [Trabelsi et al., 2008](#)).

Dielectric properties of materials are usually expressed by relative complex permittivity, $\epsilon = \epsilon' - j\epsilon''$, where the real part (ϵ') is the dielectric constant and the imaginary part (ϵ'') is the loss factor. The dielectric constant shows the ability of a material to store energy and the loss factor represents the loss of electromagnetic energy in the material in the form of heat. These properties depend on the frequency, temperature, moisture content and the composition of the material. They also depend on the bulk density for particles ([Nelson, 1991](#)).

There are several methods of measuring dielectric properties, including: transmission line; coaxial probe (suitable for some solids, liquids or powders); cavity perturbation; and free space transmission (suitable for thin flat faced materials) ([Wee et al., 2009](#)). Open-ended coaxial probes and impedance analysers have been used several times for measuring the

dielectric properties of Kabuli chickpea, green pea, lentil and soybean ([Guo et al., 2010](#)), mung bean; black eyed pea ([Jiao et al., 2011](#)), and Macadamia nut kernel ([Wang et al., 2013](#)) over the frequency range of 10 - 1800 (MHz). Because it is very difficult to make a flat surface, which can have a good contact with the probe on the seeds, these researchers used compressed ground samples.

There are also some relationships between complex permittivity of the particles and its powder or granular form. Some of the reported relationships are listed below ([Nelson, 1991](#)):

$$\varepsilon_2 = \left[\frac{\frac{1}{\varepsilon^{\frac{2}{3}}} + v_2 - 1}{v_2} \right]^{\frac{1}{3}} \quad (3.1)$$

$$\varepsilon_2 = \frac{\varepsilon[3v_2 + 2(\varepsilon - 1)]}{3v_2\varepsilon - (\varepsilon - 1)} \quad (3.2)$$

$$\varepsilon_2 = \frac{1 - v_2 - \varepsilon^{2/3}}{1 - v_2 - \varepsilon^{-1/3}} \quad (3.3)$$

$$\varepsilon_2 = \frac{v_2(\varepsilon+2)+2(\varepsilon-1)}{v_2(\varepsilon+2)-(\varepsilon-1)} \quad (3.4)$$

$$\varepsilon_2 = \exp \left[\frac{\ln \varepsilon}{v_2} \right] \quad (3.5)$$

where ε_2 is the complex permittivity of the particles, ε is the complex permittivity of the mixture of particles and air and v_2 is the volume fraction of the particles, which can be calculated from ρ (bulk density of mixture) and ρ_2 (true density of particles) as $v_2 = \frac{\rho}{\rho_2}$.

Nelson, S. and S. Trabelsi (2011) developed equations (3.6) and (3.7) to relate dielectric properties of some grain seeds to the moisture content and frequency. Their equations are:

$$\varepsilon' = \varepsilon'_0 + a \log(f) + b M \quad (3.6)$$

$$\varepsilon'' = \varepsilon''_0 + c f + d M \quad (3.7)$$

where ε'_0 , ε''_0 , a , b , c , and d , are constant; M is moisture content and f is frequency. According to these equations, for the frequency range of 5-15 GHz, the dielectric constant has a linear relation with moisture content and logarithmic relationship to frequency, while the loss factor has a linear relation with both moisture and frequency. However, they did not consider temperature dependency of the dielectric properties.

Dielectric properties of peanut kernels were simulated with quadratic and cubic regression models at frequencies 27, 40, 915 and 2450 MHz and verified by experimental data. These models relate the dielectric properties to temperature and moisture content and are useful for simulation of dielectric heating processes ([Zhang et al., 2016](#)).

Some data exist for dielectric properties of chickpea and lentil at radio frequencies up to 1800 MHz ([Guo et al., 2010](#)). Nevertheless, there is no published data, which relate the dielectric properties of Kabuli chickpea and lentil to moisture content at microwave frequencies, which can be used for dielectric heating purposes. Temperature and moisture content change with time and position during dielectric heating. Thus, knowledge of the relationship between dielectric properties, moisture content and temperature would help design a proper process of dielectric heating and predict the temperature distribution.

The objectives of this research are to: 1) measure the dielectric properties of Kabuli chickpea, green and red lentil over the range of microwave frequency (700-7000 MHz); 2) define the relationship between dielectric properties, frequency, temperature and moisture content; and 3) calculate the penetration depth over this frequency range to evaluate the heat uniformity and determine the proper thickness of the legumes which can be treated by microwave energy.

3.3. Materials and Methods

3.3.1. Materials

Samples of Kabuli chickpea, green lentil and red lentil were purchased from a local supermarket. They were inspected for any foreign material or damaged seeds and kept in polyethylene bags at 5°C until testing. The initial thickness, length and width of Kabuli chickpeas were 8.15 ± 0.36 mm, 9.97 ± 0.46 mm, 8.03 ± 0.36 mm, respectively. The lengths of green lentil and red lentil are 6.5 ± 0.25 mm and 4.3 ± 0.33 mm and their thicknesses were 2.37 ± 0.16 mm and 2.24 ± 0.17 mm, respectively.

3.3.2. Sample preparation

To analyse the change of dielectric properties with moisture content, four moisture contents for each legume were prepared. Minimum and maximum moisture contents were based on the moisture contents of dried and freshly harvested samples, respectively.

To reach the final moisture content a mass of distilled water (m_w (g)), as determined by equation (3.8) , was poured intermittently on the sample with mass m_s (g) (about 200 g), which were placed in polyethylene bags.

$$m_w = m_s * \frac{MC_f - MC_i}{100 - MC_f} \quad (3.8)$$

(MC_f : final moisture content; MC_i : initial moisture content(%wb))

The polyethylene bags were sealed and mixed thoroughly at least twice a day while keeping refrigerated at 5°C, to ensure the moisture was uniformly distributed among the seeds. The samples were kept in the refrigerator for at least one week to achieve equilibrium and uniformity of moisture content. Before doing any tests, samples were taken from the refrigerator and kept at room temperature for about 2 hours.

3.3.3. Measurement of moisture content and true density

To measure the moisture contents, according to AOAC 925.10, with some modification, 2-3 g of powdered samples of Kabuli chickpeas, ground using an electrical coffee grinder, and whole seeds of lentil were dried in an air oven at 130°C until reaching constant weight. Moisture contents were calculated from the initial and the final weights of the samples. Moisture contents are the mean of replicate measurements.

For density measurement, the liquid displacement method was applied ([Johnny et al., 2015](#)). Toluene was used as the displacement liquid because it is absorbed by legumes much less than water and it can fill all parts of irregular surface of the seeds. Measurements were done in duplicate using a 25 ml pycnometer.

3.3.4. Measurement of dielectric properties

An open-ended coaxial probe (Agilent 85070E, 200 MHz to 50 GHz, Performance Probe 85070-60010) with network analyser (E8364B PNA network analyser) with frequency range of 10 MHz - 50 GHz was used for dielectric properties measurement. An external computer with Agilent software was used to control the network analyser. The probe and connecting cable to analyser were maintained fixed during measurements to avoid any errors. The probe kit is shown in Figure 3.1.

Before measurement, the network analyser was calibrated with open air, water and a short (a metal sheet). To confirm the calibration, the dielectric properties of water, which are well known at room temperature, were measured and after making these measurements match the correct curves of water, the measurements on the grain samples were carried out.

Dielectric properties of samples were measured at frequencies between 700 – 7000 MHz that include three allowed frequencies of 915, 2450, 5800 in the microwave ISM range. They were measured in duplicate and the mean values were calculated from these two replicates.

For the dielectric properties' measurement using the open-ended coaxial probe, a sample with a flat surface is required. When the flat surface of a solid comes to contact with the probe, the reflected signal is measured and will be related to relative permittivity. For chickpea, the seeds were split in half and then polished, to reach a flat surface. Lentil seeds are very small and it was not possible to make good contact with the probe; so they were ground using a coffee grinder and the powder was compressed in a cylinder (Guo et al., 2008). A polyethylene container with diameter of 1cm, in which the probe could enter, was used to hold the powdered samples during the experiment. For temperatures that were higher than room temperature, the containers were kept in a water bath in a small beaker and a digital thermometer was used to check the temperature. The thermometer probe was placed inside the control solid sample to ensure that the whole sample reached the desired temperature. During the time to reach the temperature (which was less than 3 min for 45°C), the container's lid was closed to ensure no moisture loss occurred.

The flat pieces of legumes were sealed in aluminium foil and placed on a plate heater. After reaching the desired temperature, the foil was opened at one end and the probe was placed on the flat surface with the foil wrapped around the probe, while the other side was still on the heater.

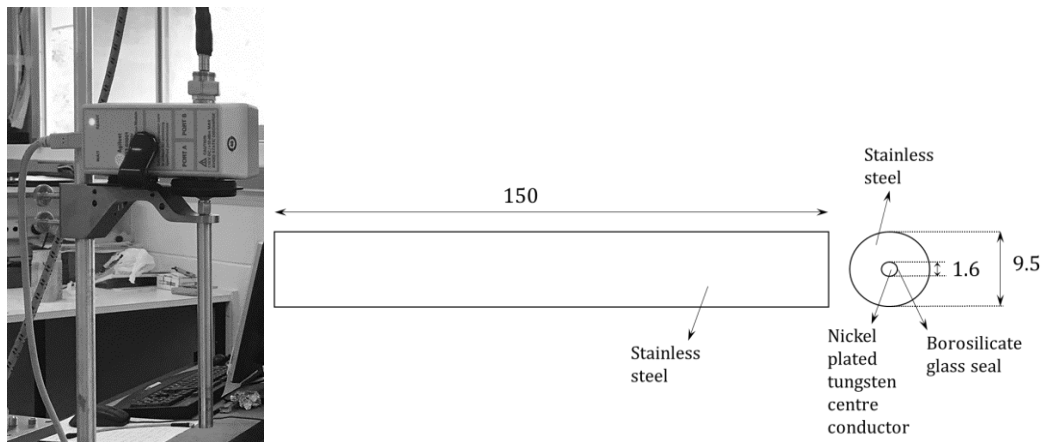


Figure 3.1 Dielectric probe kit (photo), the dimensions(mm) and materials of the probe (schematic)

3.3.5. Penetration depth and wavelength

Penetration depth (d_p) is defined as the depth in a material where microwave or RF power drops to $1/e$ ($e=2.718$) or 36.8% of its initial value and is calculated by equation (3.9) from the dielectric properties; where c is the speed of light in space, which equals to $3 \times 10^8 \text{ m s}^{-1}$ and f is frequency (Hz). Penetration depth has an inverse relation with frequency and so at higher frequencies, energy penetrates less in the material and more surface heating occurs.

Wavelength of the microwave and RF inside the material is another important factor, which can be calculated by equation (3.10) using the dielectric properties. By knowing penetration depth and wavelength, heat uniformity of a particular geometry during microwave treatment can be predicted ([Bhattacharya & Basak, 2017](#); [Brodie, 2008](#)).

$$d_p = \frac{c}{2\pi f \sqrt{2\varepsilon' [\sqrt{1 + (\varepsilon''/\varepsilon')^2} - 1]}} \quad (3.9)$$

$$\lambda_m = \frac{c\sqrt{2}}{f \sqrt{\varepsilon'^2 + \varepsilon''^2 + \varepsilon'}} \quad (3.10)$$

3.3.6. Cole- Cole chart

A model was developed by Debye ([Debye, 1929](#)) to describe the polarisation behaviour of dipoles in a pure polar material as equation (3.11).

$$\varepsilon = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + i\omega\tau} \quad (3.11)$$

Where ε is Complex dielectric permittivity; ε_∞ is permittivity at very high frequency; ε_s is static permittivity; ω and τ are angular frequency ($2\pi f$) and relaxation time, respectively.

By separating the model into real and imaginary parts and plotting dielectric constant vs. loss factor for a pure, polar material, a semicircle is obtained with one relaxation time ([Cole & Cole, 1941](#)). However, most materials are not purely polar, and they have several relaxation times, which leads to having a power of less than one for " $i\omega\tau$ ".

3.4. Results and discussion

3.4.1. Relation between density and moisture content

Figure 3.2 shows the relationship between chickpea kernel density and their moisture content. They were obtained by fitting a second order polynomial model to the data of ε' and ε'' against the true densities of chickpea seeds. Density decreases with increase of moisture content. The same trends were reported for chickpea ([Konak et al., 2002](#)) and lentil ([Amin et al., 2004](#)). This is due to the increasing proportion of water in the sample, which is less dense than the kernels.

For Kabuli chickpea and green lentil, the fitting models are according to equations (3.12) and (3.13), respectively.

$$\rho = \frac{1.09}{M} + 1.239; (R^2 = 0.995 \text{ for } M: 11.8 - 19.8) \quad (3.12)$$

$$\rho = \frac{1.7555}{M^{0.099}}; (R^2 = 0.9965 \text{ for } M: 8 - 19.8) \quad (3.13)$$

The same relation for chickpea was introduced by ([Guo et al., 2008](#)). A linear relation between density and moisture contents of lentil was found before ([Amin et al., 2004](#)).

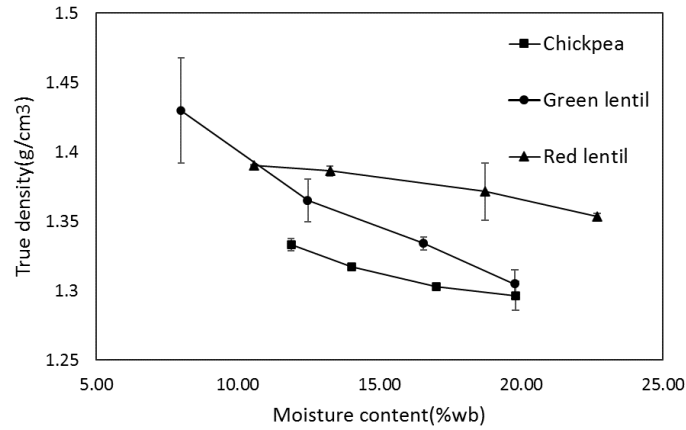


Figure 3.2 True density of legumes (g cm^{-3}) at four moisture contents

3.4.2. Dielectric properties of Kabuli chickpea

Figure 3.3 shows the dielectric constant and loss factor of Kabuli chickpea at room temperature and different moisture contents in the frequency range 700-7000 MHz. Dielectric constants decrease with increasing frequency and decreasing moisture content and has a linear relation with log frequency at each moisture content. [Nelson&Trabelsi \(2011\)](#) indicated the same relation for the frequency ranges 5-15 GHz, as mentioned in equation (3.6). A negative linear correlation exists between loss factor of Kabuli chickpea and frequencies above 3000 MHz for all moisture contents. However, they increase with frequency from 700 to around 2000 MHz and then again decrease to 7000 MHz. The same maximum point for loss factor at frequencies around 2450-4000 MHz was reported for peanuts at 10% moisture content ([Zhang et al., 2016](#)). [Wang et al. \(2003\)](#) concluded that the maximum points are due to bound water relaxation.

Figure 3.3 also indicate that the dielectric properties of Kabuli chickpea have a sudden rise after 17% moisture content, which can be due to transition from bound water to free water within the seed's cells. At low frequencies, ionic conductivity is a dominant mechanism of electric field dispersion while at microwave frequencies dipolar losses are more important. As a result, at these frequencies, water absorption is predominant and there is usually a sudden increase in dielectric properties after a transition in water content ([Cloude, 2010](#)).

Figure 3.4 illustrates the Cole-Cole chart of Kabuli chickpea at room temperature and different moisture content. It shows that the relaxation frequency for Kabuli chickpea at room temperature occurs between 1-3 GHz. Based on the Cole-Cole graph of Kabuli chickpea, a model was developed to describe the complex permittivity of Kabuli chickpea at room temperature according to equation (3.14). The coefficients for Kabuli chickpea at different moisture contents are presented in Table 3.1. Figure 3.5 shows the data obtained for Kabuli chickpea and the fitted model. This model helps when developing moisture-measuring sensors based on electromagnetic field at room temperature.

$$\varepsilon = \varepsilon_{\infty} + \frac{\varepsilon_s - \varepsilon_{\infty}}{1 + (j \frac{f}{f_0})^{1-\alpha}} \quad (3.14)$$

Table 3.1 Coefficients of equation (3.14)

Moisture Content (%)	ε_{∞}	ε_s	f_0 (GHz)	α
11.8	3.16	7.64	1.5	0.46
14.0	2.75	9.85	1.0	0.52
17.0	2.84	11.16	1.0	0.48
19.8	4.32	13.18	2.0	0.29

According to Figure 3.4, at higher moisture contents, the relaxation occurs at higher frequencies. It is because at higher moisture contents, free water is more abundant, compared to bound water and according to [Metaxas&Meredith \(1983\)](#) free water's relaxation will be at higher frequencies than bound water's. This means that at higher frequencies, the contribution of free water relaxation is becoming dominant.

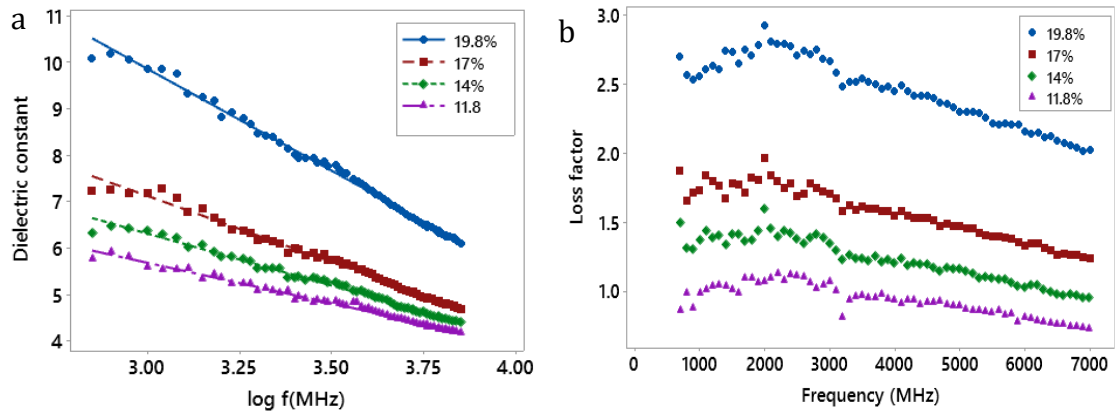


Figure 3.3 (a) Dielectric constant and (b) Loss factor of Kabuli chickpea at room temperature and different seed moisture contents; f : frequency.

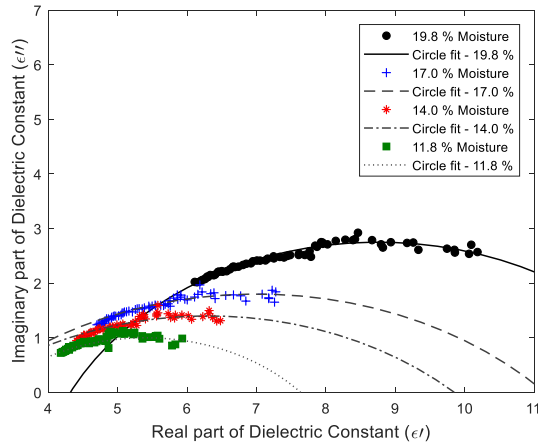


Figure 3.4 Cole-Cole chart for Kabuli chickpea at room T and different MC

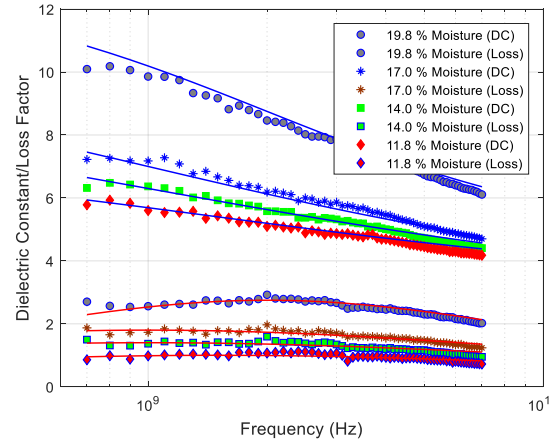


Figure 3.5 Dielectric properties of Kabuli chickpea at room temperature and different moisture contents, lines are fitted model

3.4.2.1. Density dependence

Density is one of the important factors that the dielectric properties are dependent upon. Dielectric properties of Kabuli chickpea have a quadratic relationship with density, as shown in Table 3.2. The same quadratic relation was found for dielectric properties of fishmeal (Nelson, 1991). These equations together with the relation between density and moisture content (equation (3.12)) are useful for simulation of microwave assisted drying of chickpea kernels.

Table 3.2 Dielectric properties of Kabuli chickpea at two temperatures as functions of density at frequency of 2450MHz

Temperature (°C)	Equation	R^2
24	$\epsilon'' = 2419 - 3645\rho + 1374\rho^2$	92.6
	$\epsilon' = 4466 - 6737\rho + 2543\rho^2$	87.5
45	$\epsilon'' = 3341 - 5033\rho + 1896\rho^2$	99.7
	$\epsilon' = 5712 - 8603\rho + 3242\rho^2$	99.6

3.4.2.2. Regression Model for Kabuli chickpea at different temperature

Table 3.3 shows the regression equations for dielectric constant and loss factor as a function of frequency and temperature for Kabuli chickpea at a moisture content of 19.8%. These models indicate that the dielectric constant has a linear relationship with temperature (24-80°C) and logarithmic relationship with frequency (700-7000 MHz) and loss factor has a linear relationship with frequency and with the square of the temperature. Figure 3.6 represents the relation between dielectric constants and loss factors with frequency and temperature. It is observed that at each frequency, the dielectric constant and loss factor increase with increasing temperature and the rate of increasing is almost the same for all frequencies. This could be because of decreasing viscosity and more ionic conduction (Wang

[et al., 2003](#)) or more free water molecules appear at higher temperature and so more dipole rotations will occur. However, change of dielectric properties with temperature at microwave frequencies, is much less than those at radio frequencies. [Guo et al. \(2008\)](#) reported a change from 0.81 to 248 for the loss factor of chickpea flour at 27 MHz within temperature ranged from 20 to 90°C, while according to present data, at 2450 MHz, the loss factor of Kabuli chickpea kernels increased from about 2.5 to 4.5 over almost the same temperature range. When the change of loss factor is so high with temperature, thermal runaway occurs, and local temperature difference will be high. It is because of more absorption of microwave energy with higher loss factor and so more temperature rise. Therefore, with the aim of drying one-layer of Kabuli chickpeas, microwave seems to provide more heat uniformity, less local heating, and so more final quality of the kernels.

[Zhang et al. \(2016\)](#) found cubic relationships between dielectric properties of peanut kernels and their temperature as well as moisture content at a frequency of 2450 MHz. [Li et al. \(2017\)](#) developed quartic equations to relate dielectric properties of almond kernels with temperature and moisture content at specific frequencies. These models are applicable to use in simulation of microwave processing of Kabuli chickpea.

Table 3.3 Regression model for Loss factor and dielectric constant of Kabuli chickpea at 19.8% moisture content (f = frequency Hz; T = temperature °C), frequency range of 700-7000MHz and temperature range of 24-80°C.

Dielectric properties	SSE*	R ²	RMSE*
$\epsilon' = 60.76 (\pm 3) - 6.054 (\pm 0.3) \log f + 0.1649 (\pm 0.0036) T$	243.7	0.96	0.7796
$\epsilon'' = 2.707 (\pm 0.24) - 6.985 \times 10^{-11} (\pm 3.914 \times 10^{-11}) f + 0.001578 (\pm 0.0085) T - 2.049 \times 10^{-12} (\pm 0.68 \times 10^{-12}) f T + 0.0004563 (\pm 0.00077) T^2$	28.96	0.92	0.2694

* SSE: sum of squared errors, RMSE: root mean squared error, R: coefficient of determination

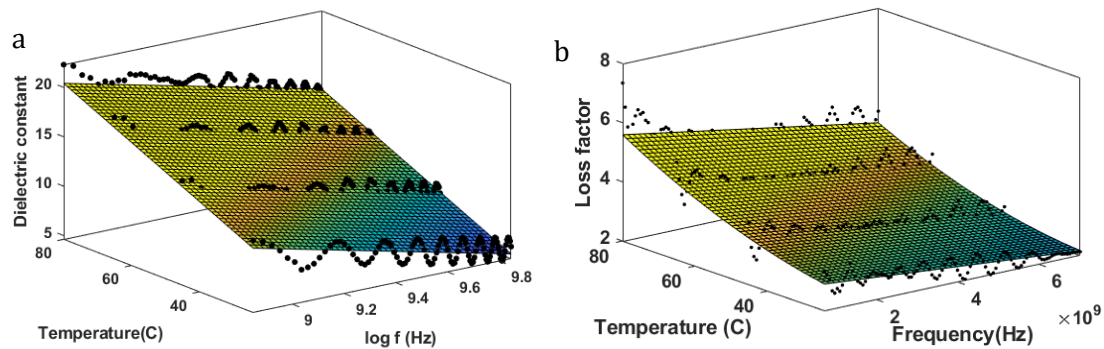


Figure 3.6 (a) Dielectric constants and (b) Loss factors of Kabuli chickpea at 19.8% MC at different temperatures (°C) and frequencies(Hz).

3.4.3. Dielectric properties of powdered green and red Lentil

Dielectric properties of powdered green lentil are shown in Figure 3.7. Dielectric constants decrease with increasing frequency while the loss factors are almost constant for each moisture content. Data at higher moisture contents are not consistent, which could be due to failure to have good contact with the probe.

Figure 3.8 illustrates the dielectric constant and loss factor of powdered red lentil at room temperature. The dielectric constants of red lentil have a linear relationship with the logarithm of frequency whereas the loss factors are approximately constant within the frequency range of 700-7000 MHz with slight decrease with frequency at a moisture content of 19%. Nevertheless, like Kabuli chickpea, the change of dielectric constants and loss factors with moisture contents are considerable.

One of the important applications of dielectric properties is to develop microwave sensors for measuring moisture contents of seeds. Because changes in bulk density can directly affect the dielectric properties, density independent relationships between loss factor and dielectric constant are required. [Trabelsi et al. \(1998a\)](#) introduced the complex-plane plot, which can solve this problem. According to this graph, $\frac{\epsilon''}{\rho}$ and $\frac{\epsilon'}{\rho}$ could have linear relationships at a specific frequency for different moisture contents and temperatures. Here, true density is used for plotting the graph as the whole relationship must not be different according to the relationship of the true and bulk density with volume fraction: $v_2 = \frac{\rho}{\rho_2}$.

The complex-plane plot of red lentil is represented in Figure 3.9 at frequency 1800 MHz. these data are fitted to the linear equation (3.15). These equations could be used in development of a moisture sensor based on microwave. The linearity is more at 1800 MHz than any other frequencies within the frequency range and the slope of the plot increase with increasing frequency (data not shown). Increasing slopes of argand diagrams as frequency increases are also reported by [Torrealba-Meléndez et al. \(2016\)](#), [Trabelsi et al. \(1998b\)](#) and [Trabelsi et al. \(2013\)](#) at microwave frequencies. However, in plots represented by [Guo et al. \(2010\)](#) and [Torrealba-Meléndez et al. \(2014\)](#), the slope decreased with frequency.

$$\frac{\epsilon''}{\rho} = 0.3994 \frac{\epsilon'}{\rho} - 0.9185 \quad (R^2 = 0.9779, \text{ at } 1800\text{MHz}); \rho: \text{density (g cm}^{-3}\text{)} \quad (3.15)$$

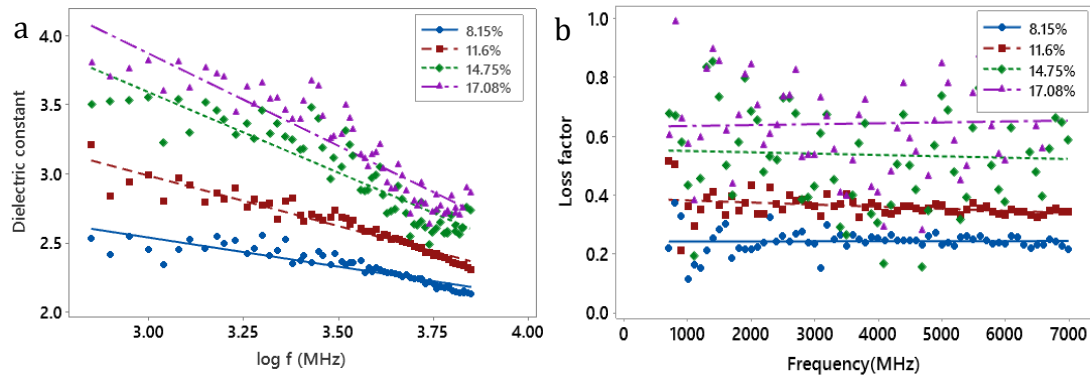


Figure 3.7 (a) Dielectric constants and (b) Loss factors of green lentil (powder) at room temperature and different moisture contents

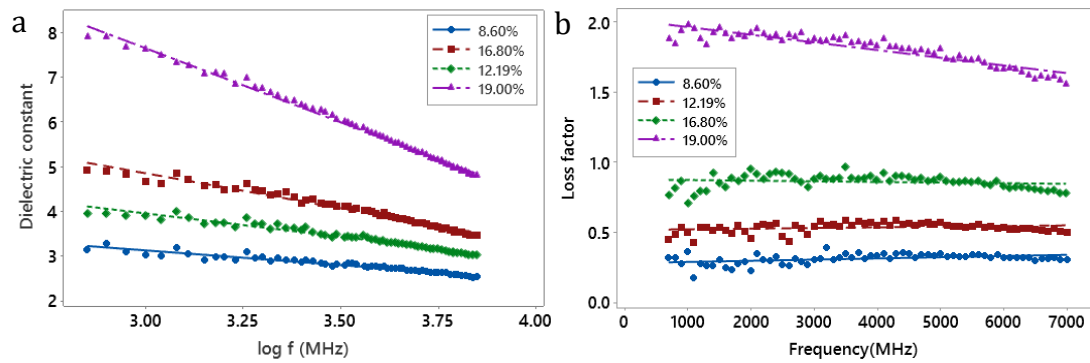


Figure 3.8 (a) Dielectric constants and (b) Loss factors of powdered red Lentil at different moisture content at room temperature

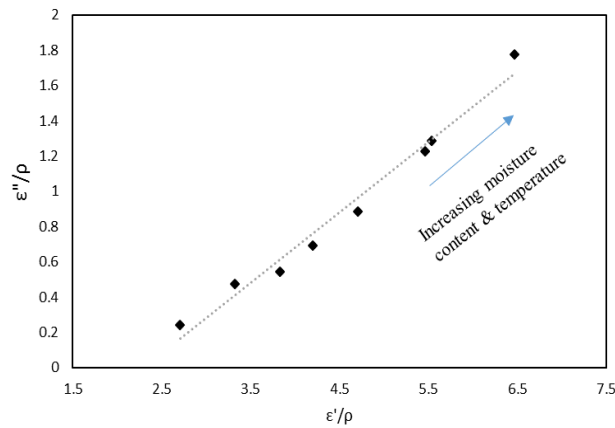


Figure 3.9 Complex-plane plot for powdered red lentil at 1800MHz

3.4.4. Penetration depth

Figure 3.10 shows the effects of temperature on penetration depth. Penetration depth decreases with increasing temperature, which is because of increasing dielectric properties. The same trend was achieved for chickpea at lower frequencies by (Guo et al., 2010). Penetration depths in Kabuli chickpea kernels at four frequencies and two temperatures are represented in Table 3.4.

These penetration depths are smaller than those obtained by [Guo et al. \(2010\)](#) at a frequency of 915 MHz. This could be due to different materials used for the measurements. Previous data was based on compressed powdered samples while in this study the kernels were polished to obtain a flat surface. Therefore, there was no impact of the seeds' coat on the measurements. Because the composition of the coat and cotyledons are different ([Chavan et al., 1987](#)), and dielectric properties depend on the chemical composition ([Nelson, 1991](#)), the dielectric properties and penetration depths are different for cotyledon and mixtures of cotyledon and seed coat. Besides, by calculating the dielectric properties from equations (3.1) to (3.5), the data for a bulk of seeds will be obtained and the penetration depths are much higher.

For powdered green and red lentil, penetration depths were calculated from dielectric properties measured at room temperature, according to equation (3.9). Fitted data to power equations are presented in Table 3.5 and Table 3.6. According to equation (3.5) and from the dielectric properties, bulk densities and volume fraction of the powder, the complex permittivity and penetration depths of lentil were calculated. Fitted power equations are shown in Table 3.5 and Table 3.6. In all cases, calculated penetration depths of seeds are less than those found for the powdered form. This is because whole seeds have a higher density than the powdered grain samples.

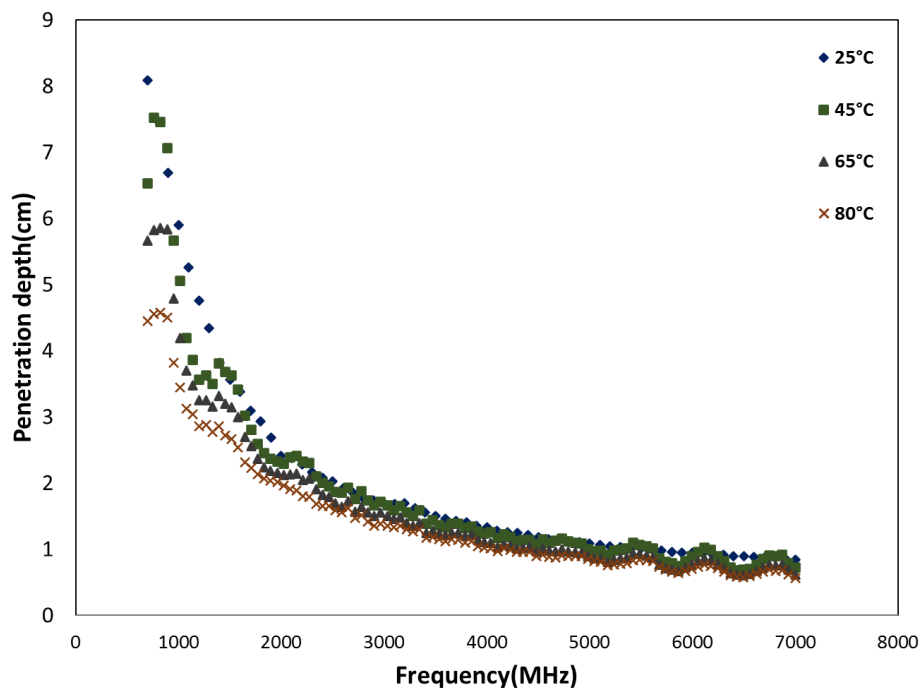


Figure 3.10 Penetration depth (cm) of Kabuli chickpea with 19.8% moisture content at different temperatures (°C)

Table 3.4 Penetration depth in Kabuli chickpea (cm) at different frequencies and two temperatures

moisture (g 100g ⁻¹ wb)	T (°C)	700MHz	900MHz	2450MHz	5800MHz
11.8	24	19.18	14.61	3.92	2.05
	45	17.74	10.00	3.48	1.70
14.0	24	11.50	10.34	3.25	1.66
	45	11.69	10.36	3.45	1.62
17.0	24	9.88	8.33	2.76	1.33
	45	9.25	6.71	2.30	1.01
19.8	24	8.08	6.68	2.05	0.96
	45	7.46	5.72	1.99	0.85

Table 3.5 Penetration depth of green lentil (cm) at room temperature and frequency range of 700-7000 MHz

MC% (wb)	Bulk density of compressed powder (g cm ⁻³)	Volume fraction	D _p of powdered green lentil(cm)	D _p of lentil calculated from powder(cm)	R ²
8.15	0.96	0.72	$57543*f^{-1.079}$	$28730*f^{-1.048}$	0.93
11.61	1.03	0.79	$24407*f^{-1.016}$	$13860*f^{-0.987}$	0.97
14.75	0.90	0.70	$2092*f^{-1.03}$	$5269*f^{-0.988}$	0.73
17.08	0.93	0.73	$10016*f^{-0.921}$	$6566*f^{-0.9648}$	0.79

Table 3.6 Penetration depth of red lentil (cm) at room temperature and frequency range of 700-7000MHz

MC% (wb)	Bulk density of compressed powder (g cm ⁻³)	Volume fraction	D _p of powdered red lentil(cm)	D _p of red lentil calculated from powder(cm)	R ²
8.60	1.15	0.89	$86786*f^{-1.15}$	$114100*f^{-1.2}$	0.975
12.19	1.01	0.80	$39962*f^{-1.108}$	$30760*f^{-1.124}$	0.992
16.80	0.98	0.799	$22672*f^{-1.087}$	$20320*f^{-1.126}$	0.993
19.00	1.08	0.89	$8436*f^{-1.033}$	$8267*f^{-1.06}$	0.997

3.5. Conclusion

Dielectric properties of Kabuli chickpea, green and red lentil at four moisture contents and two temperatures (24, 45 °C) were measured using an open-ended coaxial probe. The measurements were conducted in the frequency range of 700-7000 MHz which include three approved ISM microwave frequencies (915, 2450 and 5800 MHz). A model was developed to predict complex dielectric properties of Kabuli chickpea at room temperature

at different moisture contents. This model is useful in simulation of microwave processing of Kabuli chickpea for drying or disinfection when the simulation is based on one separate kernel like microwave spouted bed or microwave processing of a single layer on a conveyor belt. Loss factors of Kabuli chickpea have a peak in the mentioned frequency range while loss factors of lentil decrease slightly with frequency. The peak in the Kabuli chickpea curve could be because of both bound and free water relaxation. The minimum penetration depth in a Kabuli chickpea kernel at 2450 MHz is 1.5 cm for the highest moisture content and temperature, which indicates Kabuli chickpea, should be processed in one or two layers to have better heat uniformity. For Kabuli chickpea and red lentil, there was a linear relation between dielectric constants and logarithm of frequency. For Kabuli chickpea, the dielectric constant and loss factor increase with increasing temperature at all frequencies and moisture contents. These results are useful for development of microwave processing of legumes, which is a fast, efficient thermal method, and for development of moisture measurement devices based on electromagnetic waves. Finally, because of less change of loss factor with temperature at microwave frequencies, drying of one-layer of legumes seems to have better result at these frequencies than radio waves.

The data obtained in this chapter were used in the simulation of the electric field in lentil and Kabuli chickpea seeds for the study of heat uniformity in chapter 6. The data were also useful to define the depth of the seeds which can be treated with microwave energy during the experiments reported in the coming chapters.

Chapter 4. Heat uniformity study and viability of red lentil at different moisture contents after low dose microwave treatment

Taheri, S., Brodie, G., & Gupta, D. (2019). Heat Uniformity Study and Viability of Red Lentil at Different Seed Moisture Contents after Low-Dose Microwave Treatment. *Transactions of the ASABE*, 62(2), 281-288. Doi: <https://doi.org/10.13031/trans.13002>

4.1. Abstract

Microwave processing has recently gained a lot of attention for seed treatment to eradicate pests and pathogens. However, there are lack of data on the effect of microwave on legume's viability as well as heat uniformity after microwave treatment. To clarify this matter, 50 g of red lentil at four moisture contents (MC) between 9-21% wet base (wb), were treated in a microwave oven with full output power of 1100 W (producer information) or 3.33 W g⁻¹ (based on calorimetric method) at power levels (PL) of 30%, 40% and 50% and exposure times of 30 s and 60 s. Final temperature distribution was captured using an infrared thermal camera and absorbed power (W g⁻¹) and energy (J g⁻¹) were calculated using thermal properties of lentil. Lentil's viability was negatively affected at absorbed energy of higher than 70 J g⁻¹ at any moisture contents. For high moisture contents, more than 70 J g⁻¹ were absorbed at power levels 40% and 50% after 60 s exposure time. However, viability of dry lentil was not affected after exposure to power level of 50% for 60 s, which raised the average temperature to 55°C. Germination and vigour of lentil at 21% MC treated with power level 30% for 30 s increased by 8.6% and 20% and conductivity after 2 months storage decreased by 15%, which shows the stimulating effect of microwave on the lentil seeds.

4.2. Introduction

Microwave treatment is applying electromagnetic energy at frequencies in the range of 300 MHz to 300 GHz and wavelengths between 100 cm and 0.1 cm and the most common frequencies are 2450 and 915 MHz with wavelengths of 12 and 32 cm. Microwave penetration into an object is described by penetration depth (dp) where the microwave energy reduces to 37% (1/e) of its initial value. Penetration depth depends on the frequency and dielectric properties of the material. The dielectric properties are complex values made up of two components, namely the dielectric constant (the real part) and the loss factor (the imaginary part). These properties, which define the ability of the material to absorb and

turn the microwave energy into heat, are themselves functions of moisture content (or density), composition of the material, and temperature. Microwave treatment is usually considered as a thermal process and its effect is defined as an internal heat source in energy balance equations, as outlined below:

$$\rho C_p \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) + P_{MW} \quad (4.1)$$

$$P_{MW} = 2\pi f \varepsilon_0 \varepsilon'' E^2 \quad (4.2)$$

Where

- P_{MW} = microwave power dissipation ($W\ m^{-3}$).
- f = electromagnetic field frequency (Hz).
- ε_0 = permittivity of free space ($F\ m^{-1}$).
- ε'' = loss factor of the material.
- E = electric field strength ($V\ m^{-1}$).

Lentil is one of the important pulse crops which, together with chickpea, are the most cultivated pulses in the world after pea and bean ([Chen et al., 2011](#)). Microwave processing has been considered for insect control ([Lamberti, 2015](#); [Singh et al., 2012](#)), pathogen control ([Friesen et al., 2014b](#); [Taheri & Brodie, 2018](#)), drying for safe storage ([Iik et al., 2011](#)) and improvement in cooking quality ([Divekar et al., 2017](#); [Sosa-Morales et al., 2017](#)) of pulses. It is also reported that microwave pre-sowing treatment at low power and short time has stimulating effects on seeds' germination and viability ([Aladjadjiyan, 2010](#); [Monteiro et al., 2008](#); [Tylkowska et al., 2010](#)) and increases net income and profitability in the field ([Kontar et al., 2015](#)). However, there is still lack of data on heat uniformity of lentil seeds after microwave treatment as well as the effect of microwave treatment on the viability of the seeds at different moisture contents.

The objectives of this study were: to determine the effect of low dosage of microwave treatments on lentil's viability at different moisture content; to define temperature distribution and heat uniformity at different moisture contents of lentil after microwave treatments; to relate viability loss to the amount of absorbed microwave power and final average temperatures; and to determine if there is any stimulating effect of microwave energy on the seeds.

4.3. Materials and methods

4.3.1. Legume samples

Red lentil (with brown coat) were purchased from a local supermarket in Melbourne, Victoria. The length and thickness of red lentil, measured with a digital calliper, were 4.3 ± 0.33 and 2.24 ± 0.17 mm respectively and their 1000-seed weight was 33.068 ± 0.14 g. To reach different moisture contents, a known amount of water was added to the samples followed by storing in a refrigerator in sealed polyethylene bags. Lentil in bags were mixed thoroughly several times during one week of storage to make sure that all the water was absorbed by the seeds. After one week, moisture contents were determined by drying whole kernels of lentil at 130°C for 20 h (Tang & Sokhansanj, 1991). Four moisture contents of 9, 13, 17 and 21% (wb) were prepared. Moisture content of 13% is the safe storage moisture content for lentil while moisture content of the seeds should not be below 15% at the time of harvest to prevent mechanical damage (<http://www.pulseaus.com.au>). Dry form (9%) was also selected to be studied because microwave treatment with the aim of insect control is preferred to be done on dry seeds to prevent seed viability loss. The reason for evaluation of the highest moisture content (21%) was that there are some evidence that fungi are more susceptible to microwave at high seed moisture content (More et al., 1992), so it can be beneficial when the aim of microwave treatment is fungi control in lentil seeds.

4.3.2. Microwave treatment

A domestic microwave oven (SANYO, EM-S8588V, 1100 W) with frequency of 2450 MHz and 10 power levels (PL: 10%, 20%, ..., 100%) was used for this experiment by selecting the power level using the specified button of the inverter oven. The dimensions of the cavity ($W \times H \times D$) were $335 \times 330 \times 230$ mm and the working capacity was 34 L. Output power of the microwave oven, measured by calorimetric method at full power (Brodie et al., 2009), was 3.33 W g^{-1} of water. To do this measurement, 150 ml distilled water in a 200-ml beaker at room temperature was placed in the middle of the glass turntable in the microwave oven and heated at full power for 60s. Any change in the water mass was recorded and the absorbed power was calculated from the difference in the temperature and the weight loss using the heat capacity and latent heat of water. The measurements were carried out three times and the same beaker with water was kept at room temperature as a control to record its temperature and weight change. For each treatment of red lentil, about 50 g of the seeds were spread on a glass Petri dish with inner diameter of 138 mm and thickness of 2 mm. Thickness of the lentil layer in the container was less than 0.5 cm, while penetration depth of microwave at 2450 MHz in red lentil at high moisture content is more than 2 cm (Taheri

[et al., 2018](#)), it was assumed that radiation penetrates deep enough in to the seeds and temperature can be considered uniform along the vertical axis of the seeds. Plates were placed in the centre of the turntable and after each treatment, surface temperature distribution was immediately captured. The samples were thoroughly mixed to reach uniform temperature before cooling to room temperature by forced air at 24°C. When the samples cooled, their weight was measured on a four-digit balance (resolution of 0.0001 g and accuracy of ±1 mg) and loss of moisture content was calculated by comparing the final and initial weights. Treatments were done in triplicate.

The microwave oven had 10 power levels from 10 to 100% among which three power level of 30%, 40%, 50% and two exposure time of 30 and 60 s were chosen to treat the red lentil samples. These process parameters were chosen to have a range of average temperature between 30 to 80 °C with long and short exposure times.

4.3.3. *Temperature distribution*

After each microwave treatment, plates of samples were placed out immediately and thermal photos were taken, in less than 20 s, using an infrared thermal camera with thermal sensitivity of less than 0.1°C and IR resolution of 80 × 60 pixels (Model: FLIR C2 Education Kit, spectral range: 7.5-14 μm , FLIR Systems). Thermal photos were processed by ThermoCAM Researcher 2010 software (FLIR Systems, AB, Sweden). Using the area tool, the area with seeds was selected and then the results of maximum, minimum and average temperature, as well as standard deviation, were extracted. Temperature uniformity index was calculated according to equation (4.3) :

$$TUI = \frac{STD}{T_{av} - T_{in}} \quad (4.3)$$

where

- TUI = temperature uniformity index.
- STD = standard deviation.
- T_{av} = average temperature in the selected area.
- T_{in} = initial temperature of the sample.

The lower the TUI is, the more uniform the temperature will be. For accurate measurement of samples' temperatures, emissivity of the samples, distance of camera, air temperature and relative humidity should be defined in the camera settings. Air temperature and relative humidity were set as 20°C and 50% respectively and distance of camera was 0.3 m.

Emissivity of lentil was measured according to the following procedure. Nine grams of lentil seeds at moisture contents of 9%, 13%, 17% and 21% were put in a closed polyethylene container then placed in water baths at different temperatures. At each temperature, containers were left to reach equilibrium with the water's temperature. Surface temperature of the lentil was measured using a digital thermometer to make sure they were at the same temperature as the water bath. At this point the lid of the container was opened and thermal images were taken immediately. Emissivity was calculated using software ThermaCAM Researcher by knowing the actual temperature which was measured by the thermometer.

Due to small size of red lentil, it was assumed that the temperature was uniform inside the seeds, which would be equal to the surface temperature. The other assumption was that energy absorbed by the lentil was used to raise their temperature to the final average temperature and then the whole evaporation occurred at this final temperature. With these assumptions, average power absorbed from the microwave oven was calculated as below:

$$Q = \frac{m_g C_p (T_{av} - T_i) + m_w H_{fg}}{m_g \times t} \text{ (W g}^{-1}\text{)} \quad (4.4)$$

where m_g , m_w , T_{av} , T_i and t are sample initial mass (g), water loss (g), average temperature, initial temperature and exposure time (s) respectively. H_{fg} and C_p (Latent heat and Heat capacity) were calculated according to equations (4.5) ([Ranjbaran & Zare, 2013](#)) and (4.6) ([Tang et al., 1991b](#)) respectively. Both values were calculated based on final average temperature and initial moisture content.

$$H_{fg} = 2503 - 2.386T \text{ (J g}^{-1}\text{)} \quad (4.5)$$

$$C_p = 0.5773 + 0.00709T + \frac{MC}{1000} (62.2 - 91.4(MC/100)) \text{ (J g}^{-1} \text{K}^{-1}\text{)} \quad (4.6)$$

4.3.4. Quality tests

4.3.4.1. Germination and vigour

Germination and viability tests were done to evaluate the quality change of the seeds after each treatment. Germination tests were done at 23-25 °C, in 9 cm petri dishes by placing the seeds on Whatman filter paper number 1 and pouring 4 ml distilled water in each plate followed by placing the lids on the plates and leaving them in the laboratory space. For each treatment 20 seeds were placed in two petri dishes and each test was done in triplicate. Germinations were checked after 2 and 5 days, and germination energy was considered as

the germinated seeds after 2 days divided by the total number of seeds per replicate for lentil.

To measure vigour of lentil seeds, after 7 days from germination, seedlings were weighed and then dried at 50°C for 48 h. After this time, the dry weight of the seedlings was measured and then roots, and shoots were separated from the seeds and their weights were also measured separately using a four-digit scale (resolution of 0.0001 g and accuracy of ± 1 mg). Vigour index of the seeds was calculated according to equation (4.7) ([Vashisth & Nagarajan, 2008](#)).

$$\text{vigour index} = \text{germination\%} \times (\text{root dry weight} + \text{shoot dry weight}) \quad (4.7)$$

4.3.4.2. Conductivity test

Measuring conductivity of the soaking water of the seeds reveals how much seed mineral content leaches into the water across the seed cell's membrane. The more the leachate, the less the viability and vigour of the seeds will be. The conductivity test was done according to International Rules for seed testing (ISTA). Following each treatment, 100 seeds of lentil were counted and accurately weighed, then placed in 250 ml-Erlenmeyer flasks. Distilled water and seeds were left in room temperature (20 ± 2 °C) overnight to reach equilibrium. After this time, 100 ml of distilled water was poured in each container to cover the seeds and provide enough depth for seed soaking. Containers were covered by aluminium foil and after $24 \text{ h} \pm 15 \text{ min}$ electrical conductivity of the soaking water was measured using a DiST4 conductivity meter (HI98304, HANNA instruments, Australia) with resolution of 0.01 mS cm^{-1} and accuracy of $\pm 2\%$ F.S. at 25°C. The result was calculated according to equation (4.8). The test was carried out in triplicate.

$$\begin{aligned} &\text{Conductivity } (\mu\text{S cm}^{-1} \text{ g}^{-1}) \\ &= \frac{\text{conductivity reading } (\mu\text{S cm}^{-1}) - \text{background reading}}{\text{weight of seeds}} \quad (4.8) \end{aligned}$$

4.3.5. Statistical analysis

The significant difference between the results was analysed using one-way ANOVA and Fisher LSD pairwise at 95% confidence level in Minitab 18. Data are the means of three or four replicates. The significant effect of factors was assessed by general factorial regression.

4.4. Results and discussion

4.4.1. Heat uniformity

Emissivity of the samples are required when using infrared imaging to study the temperature distribution. Measured emissivity of red lentil with brown coat at two moisture content of 9 and 13% are shown in Table 4.1. Due to rapid evaporation, measuring emissivity at higher moisture contents, especially at higher temperatures, were not possible using the water bath method. According to the results of Table 4.1, an average of 0.9 was considered to process all the images.

Table 4.1 Emissivity of lentil seeds, data are mean $\pm$ SD, n=3

Moisture content% (wb)	Temperature	Emissivity(e)
9	50	0.956 $\pm$ 0.006
	60	0.913 $\pm$ 0.009
	70	0.866 $\pm$ 0.047
13	50	0.959 $\pm$ 0.012
	60	0.885 $\pm$ 0.030
	70	0.879 $\pm$ 0.033

Photographs, captured from the samples after microwave treatments, showed differences in ΔT and uniformity between different processes as well as different moisture contents. According to Figure 4.1(a), $\Delta T = (T_{\max} - T_{\min})$ generally increased with increasing power level and exposure time. This is in agreement with the results reported by [Manickavasagan et al. \(2006\)](#) for grains. However, increases in ΔT do not have a direct relation with temperature uniformity index. Thermal images of red lentil treated at 40% PL for 30 s, for two MC's of 21% (a) and 9% (b), are shown in Figure 4.2. It can be observed that at 21% MC, a bigger area is covered by higher temperature. Temperature uniformity indexes for different moisture contents of red lentil treated at different power levels and exposure time are shown in Figure 4.1(b). The less the uniformity index, the more uniform the temperature distribution will be. According to the result of general factorial regression and effects Pareto plot, power level, exposure time and moisture content as well as the combination of power-moisture content significantly affected temperature uniformity index (TUI). The results show that, at all power-time combinations except for power-time 50%-30s, lentil with 21% MC had the least temperature uniformity index (more heat uniformity) which was significantly lower than 13% MC. At power-time of 50%-30s there was no significant difference among TUIs of different moisture contents. Generally, heat uniformity improves by increasing power levels and exposure times at all moisture contents. [Manickavasagan et al. \(2006\)](#) did not find any difference between heat uniformity of different moisture

contents of grains. They used an industrial microwave dryer at 100 to 500 W to treat 50g of wheat, canola and barely at different moisture contents. [Huang et al. \(2015\)](#) reported that simulated temperature uniformity index increases with increasing moisture content of soybean and it is the opposite of the results obtained here. It could be because the term of moisture evaporation is not usually considered in the simulations in thermal balance equation. At low moisture contents, where moisture loss can be ignored, temperature distribution could be found by solving just the thermal balance. However, at higher moisture contents (here higher than 13%), both mass and heat transfer equations should be considered with Maxwell equations to find power and temperature distribution. [Brodie \(2007\)](#) indicated that simultaneous heat and mass transfer should be considered during microwave treatment of moist wood and represented analytical solution to solve the equations together.

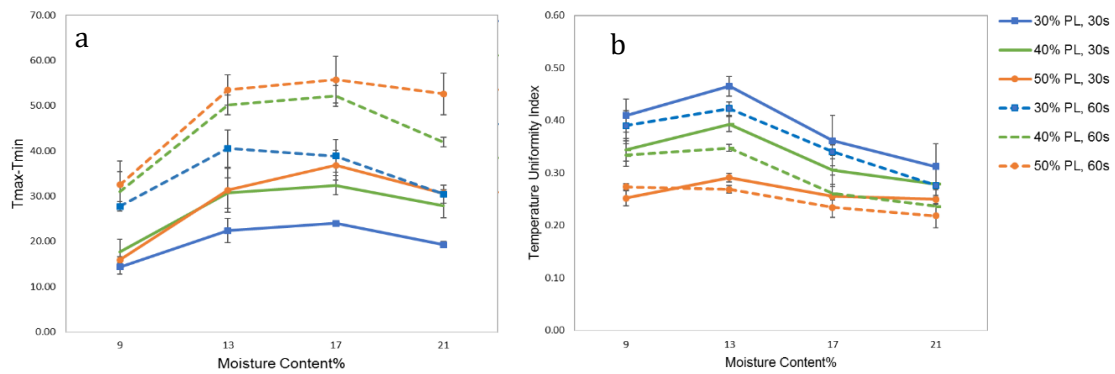


Figure 4.1 (a) Difference between hot and cold spot and (b) Temperature uniformity index of red lentil with different moisture contents, after microwave treatment at different power levels and exposure times

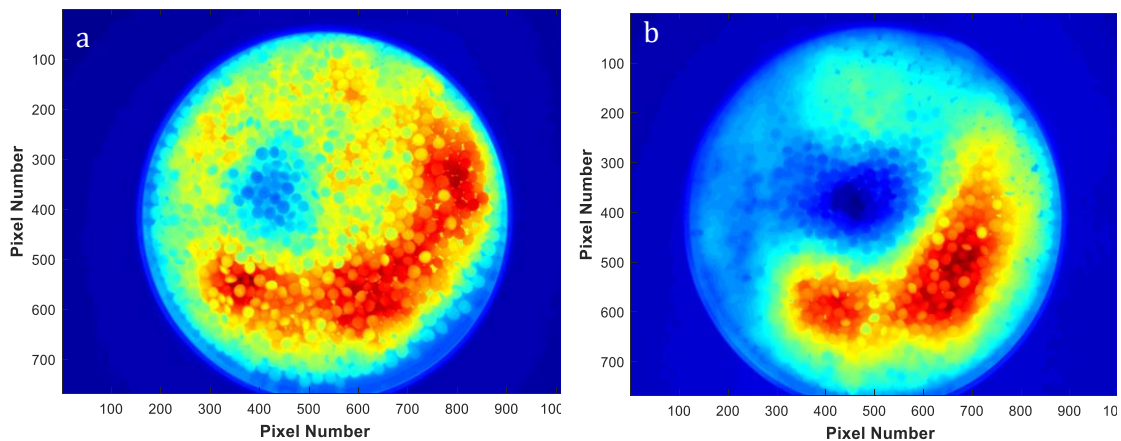


Figure 4.2 Temperature distribution ($^{\circ}\text{C}$) of red lentil with moisture content of (a) 21% and (b) 9%, after microwave treatment of 40%PL and 30 s (images by infrared thermal camera with IR resolution of 1024×768 pixels, Model: FLIR T1050sc 28)

4.4.2. Physical change

Moisture loss, maximum and average temperatures of red lentil after microwave treatments are shown in Figure 4.3 and Figure 4.4 respectively. Generally, for each moisture content, maximum and average temperature increased with increasing power level and exposure time and moisture loss followed the same rule. However, by increasing initial moisture content, moisture loss was higher, which can be the result of more free water being available at higher moisture contents and higher temperatures of the samples. According to Figure 4.4, maximum and average temperatures increased with increasing initial moisture contents up to a moisture content of 17% and after that temperature decreased. This point (moisture content of 17%) could be the critical moisture content, at which moisture cooling is higher and more absorbed energy is spent on evaporation than raising the temperature.

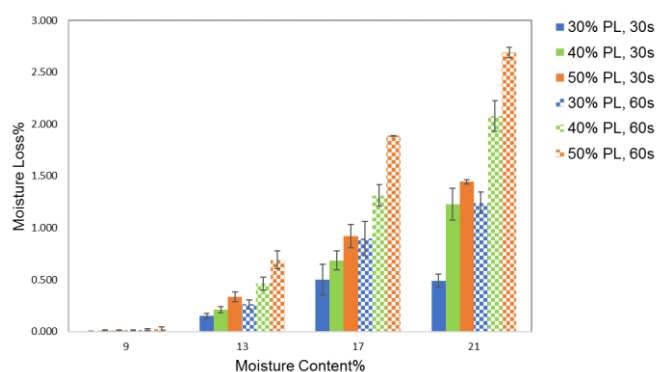


Figure 4.3 Effect of different microwave powers and exposure times on moisture loss in different initial moisture contents of red lentil, bars are standard deviations

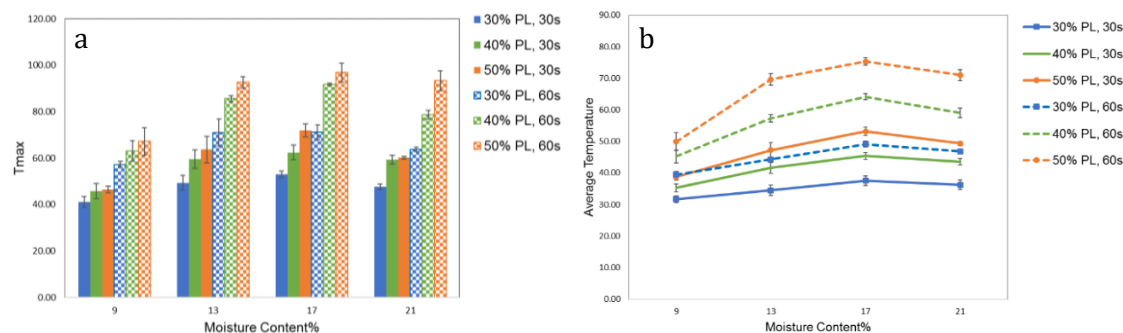


Figure 4.4 (a) Maximum and (b) Average temperature of lentil seeds after microwave treatment at different power levels and exposure times, error bars are one standard deviations from the mean

Germination energy (germination after 2 days), root dry weights and average absorbed powers are represented in Table 4.2. At 9.4% moisture content (MC), germination energy and root dry weights did not change significantly after microwave treatments. For 13.3% moisture, germination energy reduced at power level 50% and exposure time of 60 s, at which the average temperature and maximum surface temperatures are 70 and 92°C, 66

respectively. Mean absorbed power for this treatment, according to equation (4.4), is 1.53 W g^{-1} (91.8 J g^{-1}). For both moisture contents of 17 and 21%, high power levels of 40% and 50% and high exposure time of 60 s were lethal and lead to the reduction in seed viability to below 60%. These treatments increased average temperatures of the seeds to 64°C and 75°C for 17% and to 60°C and 71°C for 21% moisture contents. Based on calculation from surface temperatures, seeds at 17% MC absorbed 1.74 and 2.39 W g^{-1} at power levels 40% and 50% and at 21% MC they absorbed 2.02 and 2.62 W g^{-1} at these two power levels for 60 s exposure time.

Germination rate after 5 days and vigour of the red lentil seeds are also shown in Figure 4.5. As can be seen, both final germination and vigour reduced significantly when seeds were exposed to 50% PL for 60 s at moisture contents of 13, 17 and 21%. A power level of 40% for 60 s was also destructive for the last two moisture contents. [Vadivambal et al. \(2007\)](#), indicated that output power of 500 W is lethal for 50 g of wheat at moisture contents above 14% even at 28 s exposure time.

Results of conductivity, which is another way of expressing seed vigour, is shown in Figure 4.6. These results are in line with the results of germination and vigour index of lentil seeds. The bars with a star sign have significantly more conductivity compared to their control at the same moisture content. As it can be seen, power (%)–time(s) combination of 50–60 is lethal at moisture contents above 13% and 40%–60 is lethal for moistures above 17%.

If seed viability change is considered as a function of total absorbed energy (J g^{-1}), there will be one single plot for all the moisture contents (Figure 4.7). This will allow to have one model to predict seed viability from the amount of absorbed energy independent of seed moisture content, microwave power or treatment time. Fitting curves for lentil seeds at all moisture contents are represented in Figure 4.7. For plotting conductivity, the results of seeds at 21% MC were not considered due to the big difference of conductivity of the control sample. It is probably due to very short shelf life of seeds at this moisture content, which caused very high amount of leachate from the seeds after storage time. According to these results, by increasing total absorbed energy, germination decreases, and conductivity increases. The other point is that lentil seeds, at any moisture content, germination rate starts decreasing with a very fast slope after the point 70 J g^{-1} .

Table 4.2 Effect of microwave treatment on germination energy and root dry weight of different moisture contents of red lentil, data are mean $\pm$ SD, n=3

Moisture content (% wb)	Power level%	Time (s)	Absorbed Power (w g ⁻¹)	Germination energy% (2day)	Root dry weight (mg)
9.4	30	30	0.34	98.3 $\pm$ 1.67	76.6 $\pm$ 7.3
		60		88.3 $\pm$ 4.4	69.4 $\pm$ 5
	40	30	0.50	85 $\pm$ 10	63.6 $\pm$ 12.1
		60		90 $\pm$ 2.9	68.5 $\pm$ 10.6
	50	30	0.64	96.7 $\pm$ 3.3	75.7 $\pm$ 3.5
		60		83.3 $\pm$ 9.3	67.2 $\pm$ 11.4
	control	0	0	91.7 $\pm$ 4.4	67.4 $\pm$ 7
13.3	30	30	0.63	91.7 $\pm$ 1.7	62.1 $\pm$ 4.9
		60		75 $\pm$ 8.7	59.8 $\pm$ 3.6
	40	30	1.07	90 $\pm$ 5	73 $\pm$ 7.4
		60		75 $\pm$ 2.9	58.5 $\pm$ 4.4
	50	30	1.53	90 $\pm$ 2.9	74 $\pm$ 2.8
		60		68.3 $\pm$ 1.7*	45.1 $\pm$ 2.3*
	control	0	0	91.7 $\pm$ 1.7	68.3 $\pm$ 3.7
17	30	30	1.11	95 $\pm$ 2.9	72.9 $\pm$ 8.2
		60		73.3 $\pm$ 6.7	62.6 $\pm$ 5.5
	40	30	1.74	86.7 $\pm$ 7.3	62.5 $\pm$ 4.9
		60		55 $\pm$ 0**	38.6 $\pm$ 1.8**
	50	30	2.39	81.7 $\pm$ 8.8	69.2 $\pm$ 4.5
		60		23.3 $\pm$ 1.7**	14.9 $\pm$ 2.5**
	control	0	0	83.3 $\pm$ 3.3	61.8 $\pm$ 6.1
21	30	30	1.13	95 $\pm$ 2.9	66.4 $\pm$ 3.5
		60		80 $\pm$ 5.7	56.7 $\pm$ 6.4
	40	30	2.02	78.3 $\pm$ 4.4	64.8 $\pm$ 2.6
		60		46.7 $\pm$ 8.3***	36.8 $\pm$ 6.4***
	50	30	2.62	75 $\pm$ 7.6	56.5 $\pm$ 1.6
		60		12.5 $\pm$ 2.5***	38.5 $\pm$ 3.8***
	control	0	0	78.3 $\pm$ 6	61 $\pm$ 8.7

*, **, *** are significantly different from their control samples in the same column at 5% level by Fisher LSD Method

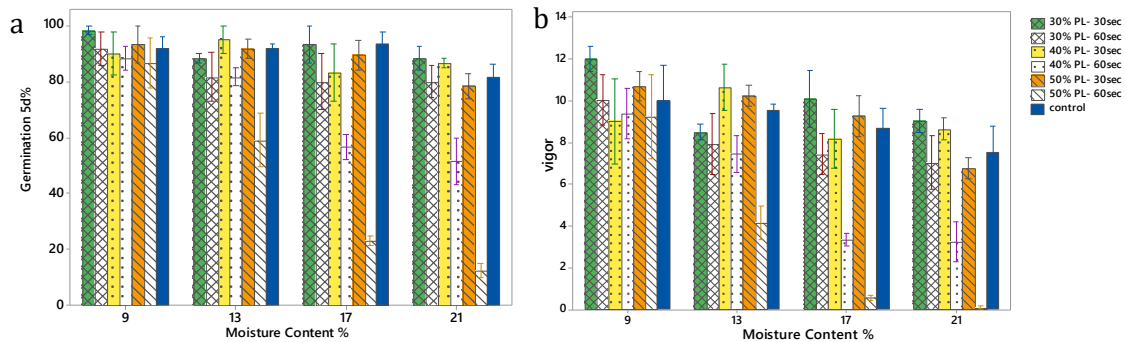


Figure 4.5 (a) Germination rate % and (b) Vigour of different moisture contents of red lentil after microwave treatments, bars are one standard error from the mean

4.4.3. Seed enhancement effect of microwave

Furthermore, from the data of Table 4.2, Figure 4.5 and Figure 4.6, it can be concluded that at the highest moisture content, absorbing 1.1 W g⁻¹ of microwave power (power level of 3 in this microwave oven) for the duration of 30 seconds, has stimulating effect on lentil seeds by increasing their germination and vigour from 81% and 7.5 to 88% and 9 respectively. This treatment is also able to increase the viability of lentil seeds at high moisture content

according to the conductivity data in Figure 4.6, by decreasing conductivity from 105 to 89 $\mu S\ cm^{-1}\ g^{-1}$. During this treatment, the final average temperature of lentil seeds was 36.2 °C. This is in line with the finding of [Aladjadiyan \(2010\)](#), who reported that microwave treatment of 10 pre-soaked lentil in water at 450 W (22.5 kW m⁻³) for 30 s enhances seed germination and vigour. Seed enhancement effects of microwave treatment on bean seeds were also reported by other researchers ([Jakubowski, 2015](#); [Tylkowska et al., 2010](#)). [Talei et al. \(2013\)](#) exposed fifty seeds of different cultivars of rice to microwave with frequency of 2.45 GHz and power of 1.58 mW and reported that 10 h exposure time increased germination, vigour and germination rate and reduced mean germination time. They also mentioned that temperature of water surrounded the seeds in the container increased to 34°C after this treatment.

The reason for this stimulating effect was explained by [Kontar et al. \(2015\)](#). They mentioned that part of the water in the seeds is used to make a complex of amylose-water-glucose and these water molecules are microwave energy transformer by moving nutrition like glucose from one part of the seed to the other part (embryo), which then is used for the seedling growth. They also indicated that the other part of water content of the seeds causes amylopectin swelling, which causes movement of the amylose-water-glucose complex. These mechanisms lead to more enzyme activities by diluting glucose and so more germination of the seeds. It could be the reason why an enhancement effect of microwave was observed at high moisture content and not in dry seeds. This enhancement effect of microwave and its mechanism can be compared with hydropriming in the case of efficiency, water, and energy consumption in lentil seeds. It should be mentioned that in fitting curves of Figure 4.7, the seed enhancement effect was not considered.

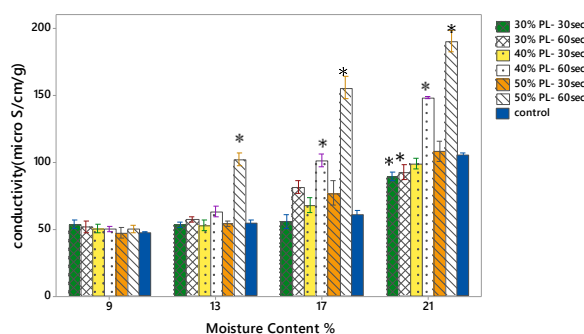


Figure 4.6 Conductivity of lentil seeds treated at different power levels and exposure time, error bars are one standard error from the mean, at each moisture content bars with * are significantly different from their control

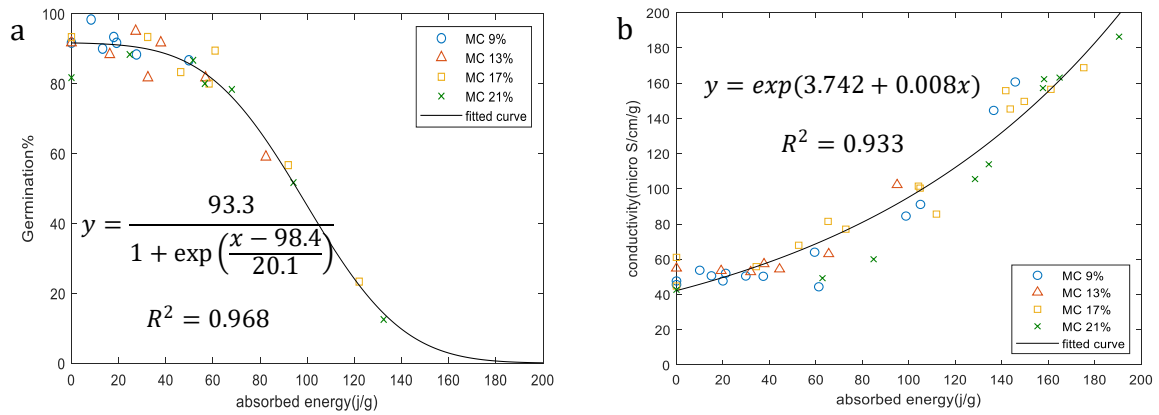


Figure 4.7 (a) Germination and (b) Conductivity of red lentil seeds as a function of absorbed energy from microwave (data are mean)

4.5. Conclusion

The effect of microwave treatment on the heat uniformity, viability, and vigour at different moisture contents of red lentil was investigated in this study. Heat uniformity was the lowest for 13% moisture content (MC) and the highest for 21%, except for power level 5 and 30 s exposure time. It was shown that if we consider viability of the seeds as a function of total absorbed energy (J g^{-1}) from microwave, there could be a single graph independent of the moisture content. Lentil seeds lose their viability and vigour if they absorb more than 70 J g^{-1} of microwave energy at any moisture contents. This was the reason why lentil's vigour and viability were affected at moisture contents of 13, 17 and 21% when exposed to power level of 50% and exposure time of 60 s, which led them to absorb power at rates of 1.53, 2.39 and 2.62 W g^{-1} respectively and moisture contents of 17 and 21% at power-time of 40%-60s, which absorbed 1.74 and 2.02 W g^{-1} of microwave power respectively.

This chapter included a preliminary experiment to study the process parameters and their interactions with lentil seeds in a multimode cavity. Using the results of this chapter, the process parameters were defined for the next experiment to disinfect the lentil seeds contaminated with *Ascochyta blight*. The heat uniformity study was also employed to interpret the results in the next chapter.

Chapter 5. Effect of microwave radiation on internal inoculum of Ascochyta blight in lentil seeds at different seed moisture contents

Taheri, S., Brodie, G. I., Gupta, D., & Dadu, R. H. R. (2019). Effect of Microwave Radiation on Internal Inoculum of Ascochyta Blight in Lentil Seeds at Different Seed Moisture Contents. *Transactions of the ASAE*, 62(1), 33-43. doi: <https://doi.org/10.13031/trans.13088>

5.1. Abstract

Considering the development of pests and pathogens' resistance to chemical treatments and the cost of developing new chemicals, seeking proper physical methods to be considered as a part of integrated pest management seems to be gaining more interest than before. Microwave treatment has been considered as a potential postharvest pest and disease management strategy in legume and grain seeds. The effect of this process, which could be expressed as a very fast and volumetric thermal treatment, has not been fully discovered on pathogens related to legumes. In this study a domestic microwave oven with full output power of 1100 W (company information) or 485 W (calibration with 150 g water) was used to treat red lentil seeds, which were naturally infected with the Ascochyta Blight (AB) pathogen, a problematic seed borne fungi of lentil. Microwave radiations were applied at three moisture contents of red lentil seeds: 9.1, 16, 19% wet base (wb) and two power levels (PL) of 50% and 100%. Three exposure times for each moisture content and power level were selected to achieve the same final average temperatures in low and high moisture contents (approximately 65°C to 85°C). The complementary error function equation was used to model and compare the pathogen and seed death rate, based on the normally distributed susceptibility of the AB pathogen or seed germination to microwave heating. At 9% moisture content with water activity of 0.4, infected seed percentage (IS%) reduced from 17% to 9% without significant loss of seed viability when a power level of 100% and exposure time of 51 s was applied, and final average surface temperature reached to 84°C. However, seed vigour, measured by seed leachate electrical conductivity, decreased at this power level and exposure time. Despite the feasibility of fungi elimination at higher moisture content, at the same final average temperatures, it was very challenging to eliminate the fungi from those seeds without harming their viability and vigour.

5.2. Introduction

Lentil is one of the important pulse crops with a high proportion of protein (21-31% dry base). This is one of the most important legumes in Australia with an annual production of 0.181 Mt ([FAOSTAT, 2016](#)). Most of the produced lentil is exported to the Indian subcontinent and Mediterranean regions with an export value of AUD \$201 million ([PulseAustralia, 2015](#)). However, there has been a sudden decrease in lentil productivity (21%) during 2015-2016 season owing to biotic and abiotic stress factors ([FAOSTAT, 2016](#)). Among the various biotic factors that affect lentil, *Ascochyta* blight (AB) is one of the most expensive diseases of lentil crop in Australia ([Murray, 2012](#)).

AB management includes a combination of cultural and chemical methods. The lentil industry in Australia promotes a three-year rotation for two consecutive lentil crops, 250 m buffer zone between any two lentil paddocks, deployment of resistant cultivars and application of fungicides to contain AB in lentil ([Hawthorne et al., 2015](#)). Amongst all these options, the use of AB resistant cultivars is mostly advocated as a sustainable strategy to manage AB. Accordingly, the development of AB resistance has been an important objective within the Australian lentil breeding program and several cultivars with improved AB resistance have been introduced in Australia ([Rodda et al., 2017](#)). Lately, a change in disease reaction has been identified in prominent resistant cultivars such as Northfield and Nipper because of an apparent increase in the aggressiveness within the *Ascochyta lentis* population in Australia ([Davidson et al., 2016](#)). The usage of fungicides has reportedly been increasing to reduce the losses due to AB though they are environmentally unviable. However, the ability of fungal pathogens to develop resistance to such chemicals is well documented and is a major concern to the agriculture industry ([Owati et al., 2017](#)). Preliminary evidence of fungicidal (Carbendazim) resistance was recently reported in the Australian *Ascochyta lentis* population ([Lopez & Kay, 2017](#)). This then underpins the need to identify some alternative measures to contain *Ascochyta lentis* infection at both primary infection and secondary spread levels.

Supported by favourable conditions, AB can transmit from seed to seedling and decrease the yield dramatically ([Kaiser & Hannan, 1987](#)). [MacLeod \(2002\)](#) reported that AB (caused by *Ascochyta lentis*) of lentil is mainly seed borne and stubble is not a major problem, while in chickpea both seeds and stubbles harbour this fungal pathogen. The fungi are present in both symptomatic and asymptomatic seeds and applying fungicides on seeds improves emergence of the crops ([Wise et al., 2009](#)). Elimination of fungi from seeds could not only increase yield, but it could also help prevent the spread of the disease between countries

when the seeds are imported and exported. Seed testing, according to International Seed Testing Association, is a way of detection of infected seed percentage up to 0.25% ([Davidson & Kimber, 2007](#)). However, very low levels of seed infection are able to cause an epidemic, which makes it necessary to use polymerase chain reaction (PCR) tests to identify the disease in the seeds more accurately and without any distraction from other present fungi ([Phan et al., 2002](#)).

To conclude from the literatures, seedborne *Ascochyta lentis* is one of the primary sources of spread of AB, which is currently eliminated by chemicals and there is no physical means for their eradication. There have been many attempts to discover physical methods to control pests and seed borne pathogens in legumes, cereals, and vegetable seeds. These methods used to be focused on conventional thermal treatments in the past ([Grondeau & Samson, 1994](#)), while new technologies like microwave and radio frequency ([Friesen, 2014](#); [Knox et al., 2013](#)), cold plasma ([Filatova et al., 2012](#)), infrared heating, as well as combinations of these treatments, have been recently gaining attentions.

There was an investigation on the effect of heat treatment for control of *Ascochyta pisi* of pea seeds, which showed that: neither hot water treatments at 50°C for 25 min; hot carbon tetra chloride at 70°C for 1 h; nor a steam/air mixture were effective at controlling this seed borne disease ([Maude & Kyle, 1970](#)). [Kaiser&Hannan \(1987\)](#) reported that *Ascochyta lentis* of lentil seeds could not be controlled by aerated steam or hot water at 45-75°C for 30 min. [Ahmed&Beniwal \(1991\)](#) investigated the control of seed borne AB of lentil and suggested seed germination was not affected by hot water treatment at 50-55°C after 10 min, while after 25 min, recovery of the fungi was 2.5 %, compared to 20 % in the control samples. They also used dry heat at 70°C for 24 h and concluded that dry heat and hot water can only partially reduce the pathogen. In another study, control of *Ascochyta spp.* on bean and pea seeds was reported to be very difficult and among all biological methods and essential oils used in this research, the only ways to partially eradicate it, were using Thyme oil and a strain of *Clonostachys rosea* ([Tinivella et al., 2009](#)). Thyme oil was used at 40°C and it was concluded that the effect might be due to the active components of oil entering the seed coat at higher temperature.

Microwave treatment, which could be considered as a fast thermotherapy method, is beneficial in reducing level of fungal and bacterial diseases ([Friesen et al., 2014a](#); [Knox et al., 2013](#); [Tylkowska et al., 2010](#)) as well as controlling insect pests ([Pande et al., 2012](#); [Purohit et al., 2013b](#); [Vadivambal et al., 2007](#)) and viruses in legumes and cereals. [von](#)

[Hoersten&Luecke \(2001\)](#) carried out different thermal treatments to eradicate *Fusarium culmorum* from wheat seeds and concluded that microwave treatment has a good potential to completely destroy the pathogen without harming the seed germination. [Cwiklinski&von Hörsten \(2001\)](#) used radio frequency to eliminate *Fusarium graminearum* and indicated that a complete eradication was achieved while preserving germination of the seeds. Both studies were carried out on artificially inoculated seeds.

Therefore, considering the potential of microwave treatment in pulse industry and the importance of investigation of non-chemical methods for control of AB pathogen, the objective of this study was to evaluate the feasibility of the control of AB pathogen in naturally infected lentil seeds using microwave treatment, and to determine the effect of seed moisture content on the pathogen's susceptibility to microwave radiation.

5.3. Materials and methods

5.3.1. Materials

5.3.1.1. Fungal material & spore solution

Single spore cultures of the aggressive *Ascochyta lentis* isolate (FT13038) were supplied by Dr. Jenny Davidson, South Australian Research and Development Institute (SARDI). The isolate was sub cultured using potato dextrose agar media (PDA) prepared according to manufacturer's instructions and incubated for 14 days at 22°C, 12h/12h dark/light cycle under florescent (OSRAM TLD/18 W) and near Ultra Violet (UV) lights (PHILIPS BLB/18W). Spore suspensions were prepared according to the method described by ([Dadu et al., 2017](#)). Plates of cultures were flooded with sterile water and spores were harvested by gently disturbing the surface with the help of a sterile glass rod. Later, the spore suspension was filtered through a 250 μ m sieve to separate the spores from mycelia. Spore concentration was adjusted using a haemocytometer to 10⁶ spores ml⁻¹.

5.3.1.2. Lentil Seed preparation

Naturally, infected red lentil (with brown coat) were supplied from a local market. The 1000-seed weight was 33.06 $\pm$ 0.01 g and average seed diameter and thickness was measured as 4.3 and 2.2 mm, respectively. Seeds with severe infection had lesions or discoloration on them. The initial Infected Seed percentage (IS%) was measured by subculturing 200 seeds on Potato Dextrose Agar (PDA) in 9 cm plates (10 seeds per plate) and after 10 days of incubation at 22°C, AB was identified based on fungi's appearance on PDA as well as conidia morphology, which was observed under a microscope (Figure 5.1).

Seed infection was also confirmed by SARDI (South Australian Research and Development Institute).

Lentil seed samples were prepared at three moisture contents of 9.1 (initial), 16 and 19% (wb) by gravimetrically estimating existing seed moisture content and then adding a known amount of sterile distilled water to the seeds and sealing them in polyethylene bags before storing for at least one week in a refrigerator. Samples were mixed several times during storage to make sure the moisture was distributed evenly through all the seeds and all the water was absorbed by the seeds.

5.3.2. Methods

5.3.2.1. Moisture sorption isotherm

Samples were prepared by adding a known amount of water to dry seeds to reach different moisture contents between the initial moisture content and 20% (wb). Moisture contents were determined by drying the samples at 130 °C for 20 h ([Tang & Sokhansanj, 1991](#)). Water activities were measured using a LabMaster-aw (Novasina) water activity meter with accuracy of ± 0.003 at 25 °C and resolution of $0.001a_w/0.1$ °C. The device was calibrated using standard salt solutions with known water activities. Measurements were conducted in triplicate. Then the moisture sorption isotherm was plotted using average values of the moisture contents and water activities.

5.3.2.2. Microwave treatment of spore solutions

A magnetron, working at frequency of 2450 MHz, with power adjustable from 200 to 3000 W and a rectangular waveguide inlet was used for the experiment (refer to Chapter 6, Figure 6.12). The power was adjusted using a custom interface in LabView 2015 (National instruments) by AMT Co. (V1.1.vi). One hundred millilitre spore solutions, with concentration of 10^6 spores ml^{-1} in 250-ml conical flasks, were placed in a cylindrical metal applicator (D: 97 mm, L: 170 mm) with TE_{11} cylindrical mode dictated by the cylindrical geometry. One hundred grams of water absorbed about 0.84 W of microwave energy in this cavity, when the output power is 1 W. Several treatments at output powers of 200, 300 and 400 W and different exposure time (30 to 180 s) were done on the spore solutions and their temperatures were measured immediately after turning off the magnetron (in 1 min), after which the solution were allowed to cool to room temperature.

5.3.2.3. Assessment of spore viability

Post microwave treatment, viability of fungal spores was assessed using the Trypan blue staining method under light microscope. Trypan blue is the common stain used to

distinguish the viable spores from non – viable ones. Spores that absorb the blue coloured dye and appear blue were considered dead and non-viable. Conversely, spores that remain colour-less without absorbing the dye were considered as viable and healthy ([Louis & Siegel, 2011](#)). The percentage of the viable spores was calculated using equation (5.1).

$$\text{Spore Viability\%} = \frac{\text{Number of viable spores}}{\text{Total number of spores observed}} \times 100 \quad (5.1)$$

Simultaneously, the viability of spores was confirmed using the water agar method. Four – five loops full of spores were cultured on water agar and incubated for 24 h as described in section 5.3.1.1. Later, the culture plates were observed under a light microscope to assess the viability of spores. Spores were considered as viable when a germ tube was present regardless of length and number of germ tubes per spore. Conversely, spores lacking germ tubes were considered as dead and non – viable.

5.3.2.4. *Microwave treatment of naturally infected lentil seeds*

A domestic microwave oven (SANYO, EM-S8588V, 1100 W) with frequency of 2450 MHz and 10 power levels (PL: 10% to 100%) and working volume of $33 \times 23 \times 33.5 \text{ cm}^3$ was used in treatment of naturally infected lentil seeds. To improve heat uniformity, a turn table was used, and the samples were mixed immediately after each treatment for 10 s. Based on calibration described by [Brodie et al. \(2009\)](#), 150 g water absorbed 485 W and 200 W at full power and 50% power level, respectively. If the glass container and turn table could be considered transparent to microwave energy, and the reflected power to the magnetron and the absorbed power by the walls could be neglected, the mentioned powers could be considered as output powers of the oven.

Fifty grams of lentil seeds were weighed accurately using a 4-digit scale (resolution of 0.0001 g and accuracy of $\pm 1 \text{ mg}$) before being placed in a glass petri dish with an inner diameter of 138 mm and thickness of 2 mm. Plates were placed on the centre of the turn table in the microwave oven. Two power levels of 100% and 50% were applied to treat the samples. Exposure times were selected based on a regression model developed from a preliminary experiment (data not shown), to reach three final average temperatures of approximately 65, 75 and 85°C for all moisture contents. The applied exposure times for 9.1% moisture content were 77, 102, 127 seconds (s) at power level (PL) of 50% and 38, 51, 64 s at PL of 100%. For seeds at moisture contents of 16 and 19% exposure times of 38, 47, 56 s and 19, 24, 28 s were used at PL of 50% and 100%, respectively.

5.3.2.5. Temperature distribution and absorbed energy

Immediately after each treatment of naturally infected lentil seeds, thermal photos were taken using an infrared thermal camera (FLIR C2 Education kit). For accurate measurement of samples' temperatures, emissivity of the samples, distance of camera, air temperature and relative humidity should be defined in the camera settings. Parameters of emissivity, distance and relative humidity were set as 0.95, 0.3m and 50% for all photos. Thermal photos were processed using ThermoCAM Researcher Pro 2.10. Using the area tool, the area with seeds was selected and then the results of maximum, minimum and average temperature, as well as standard deviation, were extracted.

It was assumed that red lentil was very small, and the temperature was uniform inside the seeds, which was equal to the surface temperature. It was also assumed that total absorbed power of microwave was consumed to firstly raise the initial temperature to the final average temperature and then evaporation would take place at the final average temperature. With this assumption, average absorbed power in the microwave oven was calculated as below:

$$Q = \frac{m_g C_p (T_{av} - T_i) + m_w H_{fg}}{m_g \times t} (W g^{-1}) \quad (5.2)$$

Where

- m_g = lentil's initial mass (g),
- m_w = water loss (g),
- T_{av} = average temperature (°C),
- T_i = initial temperature (°C),
- t = exposure time (s)
- H_{fg} = latent heat of evaporation (J g⁻¹)
- C_p = heat capacity of lentil (J g⁻¹ K⁻¹)

H_{fg} and C_p were calculated according to equation (5.3) ([Ranjbaran & Zare, 2013](#)) and (5.4) ([Tang et al., 1991b](#)) respectively. Both values were calculated based on average temperature (T) and initial moisture content (MC) of the lentil seeds.

$$H_{fg} = 2503 - 2.386T (J g^{-1}); T(^{\circ}C) \quad (5.3)$$

$$C_p = 0.5773 + 0.00709T + \frac{MC}{1000} (62.2 - 91.4(MC/100)) (J g^{-1} K^{-1}); T(^{\circ}C), MC(\%) \quad (5.4)$$

5.3.2.6. Evaluation of AB control in treated lentil seeds

Seeds were surface sterilized by Sodium Hypochlorite 1% for one minute followed by three times rinsing with sterile distilled water prior to culturing. After each microwave treatment, 60 seeds for each replicate (total of 180 seeds for each treatment) were immediately sub-cultured on Potato Dextrose Agar (PDA) in 9 cm petri dishes. After 10 days of incubation at 22 °C, seeds with mycelial growth of *Ascochyta spp.* were counted and percentage of Infected Seeds (IS%) was calculated as the number of seeds with AB pathogen regrowth divided by the total number of sub cultured seeds. AB was identified by its appearance on PDA and its conidia under microscope (Figure 5.1).

To have an estimation of the level of infection or amount of inoculum present in the treated seeds, the diameter of the spread of the AB inoculum on and around each seed on the PDA plate was measured. The infected seeds were first categorised into three levels of infection ($s = 1$ (less than 8 mm around the seed), 2 (less than 20 mm diameter around the seed), 3 (more than 20 mm around the seed)). Further, assessment of the infection severity in the seeds was calculated as below ([Filatova et al., 2012](#)).

$$ID\% = \frac{\sum_i n_i * s}{N * S} * 100 \quad (5.5)$$

Where

- ID% = percentage of infection degree,
- n_i = number of seeds with infection level of s ,
- s = infection level (1, 2, 3)
- N = total number of seeds per replicate,
- S = maximum level of infection (3).

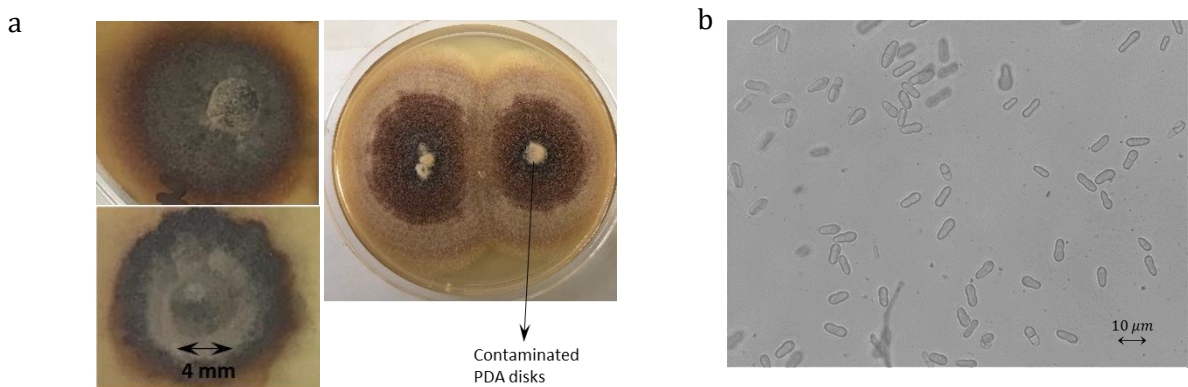


Figure 5.1 (a) *Ascochyta Blight* pathogen growth on the seeds (no treatment) and on PDA (plate with 9 cm diameter), (b) Conidia under microscope 40×

5.3.2.7. Evaluation of lentil seed viability

Lentil seeds, which were sub-cultured on PDA (from the previous part), were observed after five days and those with a 1 mm radicle growth were considered as germinated. Seed germination percentage (SG%) was calculated as the number of germinated lentil seeds divided by the total number of sub cultured seeds (60 per replicate). To evaluate any change in seed vigour after treatments, a conductivity test was done according to International Rules for Seed Testing (ISTA). Measuring electrical conductivity of the water in which seeds are soaked reveals how much of seeds' mineral content leaches into the water through seed cells' membrane, which is an indicator of seed cell's damage. The more the leachate, the less viable and vigorous the seeds will be. [Makkawi \(2008\)](#) showed that measuring electrical conductivity of lentil seed leachates could be a good indicator of seed quality and predict the potential of the seeds in the field.

After each microwave treatment, 100 lentil seeds were counted and, after accurately weighing, placed in the 250-ml Erlenmeyer flasks. Distilled water and seeds were separately left at room temperature ($20 \pm 2^\circ\text{C}$) overnight to reach equilibrium. After this time, 100 ml of distilled water was poured in each container to cover the seeds and provide enough depth to soak the seeds. The containers were covered with aluminium foil to avoid any contaminations, and after $24 \text{ h} \pm 15 \text{ minutes}$ conductivity of the soaking water was measured using an EC probe (HANNA, HI98304) with resolution of 0.01 mS cm^{-1} and accuracy of $\pm 2\%$ F.S. at 25°C . The result was calculated according to equation (5.6). Distilled water was used as blank for background reading of conductivity with the same procedure of the conductivity reading of the soaking water. The test was carried out in triplicate.

$$\begin{aligned} & \text{Conductivity } (\mu\text{S cm}^{-1} \text{ g}^{-1}) \\ &= \frac{\text{conductivity reading } \left(\frac{\mu\text{S}}{\text{cm}}\right) - \text{background reading}}{\text{weight of seeds}} \end{aligned} \quad (5.6)$$

5.3.3. Statistical analysis

The significant difference between the results (responses) was analysed using analysis of variance (ANOVA) in Minitab 18. Comparison between mean values of data groups was done using Fisher pairwise comparison with least significant difference (LSD) method at 95% confidence level. Data are the means of three replicates. Models of complementary error functions (erfc) were developed in MATLAB and goodness of fit was calculated using the fit function.

5.4. Results and Discussion

5.4.1. Water activity

Water activities of the seeds are correlated with expression of fungal growth at different temperatures ([Ayerst, 1969](#)). Water activities are related to seed moisture content and their relationship is crop species specific. Figure 5.2 shows the relation between water activity (a_w) and moisture content (MC) of lentil seeds at 25°C. Most field fungi, such as the AB pathogen, grow at water activity of more than 0.9 ([Mohapatra et al., 2017](#)) and their growth and spore formation could be delayed by drying the host seeds. However, in such condition the fungi assume a dormant form, which could become active again when the conditions are conducive to their growth. Water activity also affects thermal sensitivity of the microorganisms. [Syamaladevi et al. \(2016\)](#) mentioned that low water activity put microorganisms under stress and helps them express specific genes as a response to unfavourable conditions, which may help them survive at higher temperatures. As a result, when a thermal treatment is being considered for controlling fungi, their host's water activity and moisture content should also be taken into account to see if there are any feasibility in eliminating them at higher water activities.

Moisture sorption isotherm of red lentil in Figure 5.2 was expressed by a Smith model according to equation (5.7):

$$MC = 6.33 - 6.13 \cdot \ln(1 - a_w); \quad (a_w: 0.4 - 0.91; R^2 = 0.98; RMSE = 0.694) \quad (5.7)$$

From the result of moisture sorption isotherm of red lentil (equation (5.7) and Figure 5.2), water activity of 0.9 is equivalent to moisture content of 19-20% (wb). Therefore, two moisture contents of 19% and 16%, as well as the initial moisture content of dried seeds (9%) with corresponding initial water activities of 0.87, 0.8 and 0.4, were selected to study the possibility of reducing AB pathogen from the lentil seeds with microwave treatment as well as comparing the efficiency of the treatments at different water activities.

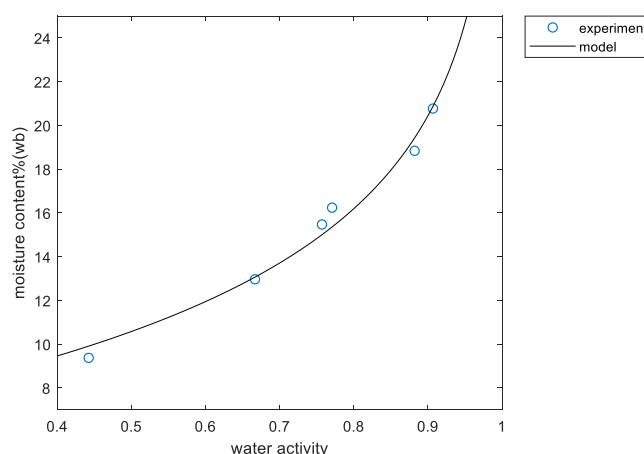


Figure 5.2 Moisture sorption isotherm of red lentil seeds at 25°C

5.4.2. Temperature distribution

The power absorbed by the lentil seeds, which was calculated using equations (5.2), (5.3) and (5.4), as well as final average temperatures are shown in Table 5.1. The reason for applying different exposure times was that lentil seeds with higher moisture content absorb more microwave power and reach a higher temperature faster, due to their higher loss factor and dielectric constant ([Taheri et al., 2018](#)). Therefore, it would be difficult to compare the effect of water activity on elimination of the pathogen. However, by applying lower exposure time for higher moisture contents, this effect could be investigated. As can be seen in Table 5.1, the rate of absorbed power increased with increasing output power, from 0.94 to 1.87 W g⁻¹ at 9% moisture content, from 2.92 to 5.92 at 16% moisture content and from 3.29 to 6.69 at 19% moisture content. As it was expected, at the same output powers, higher moisture contents absorbed more power from the microwave due to their higher dielectric properties. For example, at a power level of 100% with 19 s of exposure time, seeds with 19% moisture content absorb 6.69 W g⁻¹ while seeds at 16% MC absorb 5.92 W g⁻¹.

From the data of Table 5.1, final average temperature increased with increasing power level and exposure time at each moisture content. The same trend was reported by [Vadivambal et al. \(2007\)](#) for microwave treatment of different moisture contents of wheat seeds. Non-uniformity was observed in all the treatments. Maximum temperatures varied between 76-129°C at 9.1% moisture content, 88-115°C at 16% moisture content and 85-107°C at 19% moisture content. To overcome this non-uniformity, samples were thoroughly mixed immediately after each treatment. Maximum and average temperatures at 19% moisture content were the same or lower than those of 16% moisture content, in spite of the same exposure time and more power absorption at 19% moisture content. [Manickavasagan et al.](#)

(2007) also reported that wheat seeds at higher moisture contents reached lower temperatures than lower moisture contents at the same microwave power and exposure times. The reason is that more absorbed power was consumed for moisture evaporation (moisture loss%) than increasing the temperature at 19% moisture content.

5.4.3. *Microwave treatment effect on Ascochyta blight pathogen and lentil seeds' viability*

5.4.3.1. *Effect of microwave treatment on AB infected lentil seeds at different moisture contents*

Table 5.1 shows the results of IS%, ID%, viability and vigour of the seeds (germination% and conductivity) and moisture loss after microwave treatment of naturally AB infected lentil seeds at different moisture contents. Seeds at each moisture content were exposed to two power levels of 50% and 100% and three exposure times. Results of ANOVA showed that moisture content, power level and time significantly affected IS% and ID% ($P < 0.05$). At moisture content of 9%, by applying power level-time combinations of 50%-102s and 100%-51s with final average temperature of 84°C, IS% was significantly reduced from 17.03% (in control) to 9.4 and 8.9% respectively, without any significant loss of lentil seeds' germination ($P < 0.05$). Similarly, ID% decreased from 12.3% (control) to 7.04% and 6.67% respectively. At these treatments, lentil seeds absorbed 0.94 (50% PL) and 1.87 W g⁻¹ (100% PL) of microwave power. However, electrical conductivity of the seeds' soaking water (conductivity) was increased from 44 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (control) to 84.5 (50%-102s) and 91.1 (100%-51s), which means some cell membrane damage occurred in the lentil seeds.

From the results in Table 5.1, at power level of 50%, final average temperatures of the seeds with 9% and 19% moisture content after corresponding exposure times of 77 and 38 s, are not significantly different (63.5 and 66.5 °C for 9 and 19% moisture content respectively), while IS% and ID% of 19% moisture content (10% and 7.04% respectively) were significantly lower than those for 9% moisture content (18.3% and 13.3% respectively). Analysis of variance showed that IS% and ID% were significantly lower at 16% and 19% moisture content than 9.1% moisture content. However, there was no significant difference between 16% and 19% moisture content, which could be the result of their close water activity (0.8 and 0.87 respectively). Hence, it might be concluded that at the same power level and final average temperature, eradication of AB pathogen from the lentil seeds with high moisture content (or water activity) is more feasible than from seeds with low moisture content. This feasibility could either be the result of more energy absorption at higher moisture contents (due to higher dielectric properties) or more susceptibility of the

AB pathogen at higher water activities. This is in agreement with the findings of [Jiao et al. \(2016\)](#) who showed that eradication of *Aspergillus flavus* increased by one log-scale by increasing moisture content of wheat and corn seeds from 12 to 15% during hot air-assisted radio frequency treatment. They concluded that increasing moisture content and correspondingly water activity would be beneficial in fungi inhibition from the grain seeds in radio frequency heating. [Reddy et al. \(1998\)](#) investigated the effect of microwave treatment at powers of 0.3-0.6 W g⁻¹ on wheat seeds with moisture contents of 8, 14 and 20% which were infected with *Fusarium graminearum*. They noted that the percentage of infected seeds could be reduced from 36% to below 7% in wheat seeds without seed germination reduction to below 85%. However, there were not any conclusion about difference in *Fusarium graminearum* elimination from the seeds at different moisture contents.

Germination and conductivity of the lentil seeds were negatively affected by increasing moisture content from 9.1% to 16% and to a lesser extent from 16% to 19% at the same output power and final average temperature. As evident, at power level of 100%, seed germination at 9% moisture content was 76.7% after 38 s exposure time and final average temperature of 62.6°C, while it reduced to 31.1% and 18.9% at 16 and 19% moisture contents respectively. Exposure time of the latter treatments was 19 s with no significant difference between their final average temperatures compared to 9% moisture content. More susceptibility of seed viability to microwave heating at higher moisture contents could be related to higher rate of absorbed energy at the same output power (6.69 W g⁻¹ and 5.92 for 19% and 16% moisture contents compared to 1.87 W g⁻¹ for 9% moisture content). At high moisture contents high microwave field intensity causes water boiling in organic materials and materials' cells could be damaged as a result of micro-steam explosions inside the cells ([Vinden et al., 2004](#)). [Vadivambal et al. \(2007\)](#) also affirmed that germination of wheat seeds was higher at 14% moisture content than 16% and 18% after microwave treatments at powers 250, 300, 400 and 500 W and exposure times of 28 and 56 s. In our study, the factors were the same final temperature with the same output power and different exposure times, whereas they applied the same power and exposure times for different moisture contents and conclusively different final temperatures were obtained. [Campana \(1993\)](#) studied the effect of microwave drying on wheat seeds at moisture contents of 15.6% to 22.7% and found that seed germination was affected by both final temperature and initial moisture content of the seeds. They confirmed that germination of the wheat seeds was lower at higher moisture contents than the lower moisture content at

the same dose of microwave and final average temperature. At higher moisture contents, due to presence of more free water in the seeds, more of the absorbed power is consumed for water evaporation than increasing the temperature. It can be seen from the results of final average temperatures and moisture loss in Table 5.1. For example, at power level of 100% and exposure time of 24 s, final average temperature of seeds with 16% and 19% moisture content are 81.6 and 75.4°C respectively, with corresponding moisture losses of 1.6% and 2.32%. From the results of moisture loss in Table 5.1, moisture loss increased significantly by increasing moisture content from 9% to 16% and 19%. Maximum moisture loss for these moisture contents were 0.55%, 1.99% and 2.86% respectively. More moisture loss at higher moisture contents could be another reason for more viability loss.

Table 5.1 Absorbed power, final average temperature, percentage of seeds with AB growth (IS%), infection degree, germination%, vigour (conductivity) and moisture loss of microwave treated samples, data are mean $\pm$ SE with n=3, data which do not share a letter in the same column are significantly different (based on Fisher pairwise, Least Significant Difference method, $P < 0.05$)

MC %	Power level (output power, W g ⁻¹)	Time (s)	P _{abs} (W g ⁻¹)	T _{av} final	Infected seeds (IS%)	Infection degree (ID%)	Germination %	Conductivity ($\mu S\ cm^{-1}g^{-1}$)	Moisture loss %
	Control			22 ⁱ	17.03 $\pm$ 0.7 ^a	12.3 $\pm$ 1.38 ^a	66.2 $\pm$ 2.4 ^{abc}	44.4 $\pm$ 0.7 ^g	
9.1	50%	77	0.94 $\pm$ 0.05	63.5 $\pm$ 1.2 ^{gh}	18.3 $\pm$ 1.7 ^a	13.33 $\pm$ 0.64 ^a	72.8 $\pm$ 1.5 ^{ab}	44.4 $\pm$ 0.6 ^g	0.05 $\pm$ 0.01 ^f
		102		84.7 $\pm$ 1.3 ^{bc}	9.4 $\pm$ 1.5 ^{bcde}	7.04 $\pm$ 1.30 ^{bcd}	58.9 $\pm$ 5.9 ^{bc}	84.5 $\pm$ 17.8 ^f	0.17 $\pm$ 0.04 ^f
		127		96.8 $\pm$ 0.9 ^a	15.0 $\pm$ 3.3 ^{ab}	10.19 $\pm$ 2.87 ^{ab}	25.0 $\pm$ 1.7 ^{def}	144.5 $\pm$ 6.8 ^{bc}	0.38 $\pm$ 0.05 ^f
	100%	38	1.87 $\pm$ 0.18	62.6 $\pm$ 0.4 ^h	13.3 $\pm$ 1.9 ^{abc}	10.37 $\pm$ 3.16 ^{ab}	76.7 $\pm$ 5.85 ^a	44.9 $\pm$ 0.2 ^g	0.05 $\pm$ 0.01 ^f
		51		84.2 $\pm$ 3.2 ^{bc}	8.9 $\pm$ 1.5 ^{bcde}	6.67 $\pm$ 1.16 ^{bcd}	55.0 $\pm$ 13.5 ^c	91.1 $\pm$ 14.3 ^{ef}	0.20 $\pm$ 0.05 ^f
		64		99.1 $\pm$ 3.9 ^a	6.7 $\pm$ 3.5 ^{cdef}	5.37 $\pm$ 3.05 ^{bcd}	22.2 $\pm$ 8.9 ^{defg}	160.7 $\pm$ 27.7 ^{ab}	0.55 $\pm$ 0.16 ^f
16	50%	38	2.92 $\pm$ 0.44	68.2 $\pm$ 2.1 ^g	11.7 $\pm$ 1.7 ^{abcd}	9.26 $\pm$ 1.30 ^{abc}	26.7 $\pm$ 5 ^{de}	85.6 $\pm$ 9.5 ^{ef}	1.27 $\pm$ 0.10 ^e
		47		84.4 $\pm$ 3.2 ^{bc}	5.0 $\pm$ 0.9 ^{def}	3.52 $\pm$ 0.18 ^{de}	16.7 $\pm$ 0.9 ^{efghi}	156.5 $\pm$ 15.7 ^b	1.82 $\pm$ 0.15 ^{cd}
		56		78.6 $\pm$ 0.2 ^{de}	5.5 $\pm$ 2.0 ^{def}	4.26 $\pm$ 1.58 ^{cde}	12.8 $\pm$ 3.4 ^{efghi}	145.3 $\pm$ 7.1 ^{bc}	1.64 $\pm$ 0.08 ^{de}
	100%	19	5.92 $\pm$ 0.41	66.3 $\pm$ 1.0 ^{gh}	8.3 $\pm$ 1.9 ^{bcde}	5.37 $\pm$ 1.52 ^{bcd}	31.1 $\pm$ 0.5 ^d	100.3 $\pm$ 2.0 ^{def}	1.14 $\pm$ 0.03 ^e
		24		81.6 $\pm$ 0.2 ^{cd}	4.4 $\pm$ 1.5 ^{ef}	3.52 $\pm$ 1.30 ^{de}	14.4 $\pm$ 3.9 ^{efghi}	149.6 $\pm$ 3.6 ^b	1.60 $\pm$ 0.11 ^{de}
		28		88.7 $\pm$ 1.8 ^b	3.3 $\pm$ 0.9 ^{ef}	2.22 $\pm$ 0.96 ^{de}	9.40 $\pm$ 2.9 ^{ghi}	168.7 $\pm$ 8.0 ^{ab}	1.99 $\pm$ 0.02 ^{cd}
19	50%	38	3.29 $\pm$ 0.50	66.5 $\pm$ 3.2 ^{gh}	10.0 $\pm$ 5.7 ^{bcde}	7.04 $\pm$ 4.7 ^{bcd}	15.6 $\pm$ 6.4 ^{efghi}	120.2 $\pm$ 15.1 ^{cd}	2.17 $\pm$ 0.24 ^{cd}
		47		75.7 $\pm$ 1.8 ^{ef}	5.0 $\pm$ 0.9 ^{def}	3.70 $\pm$ 0.74 ^{cde}	12.8 $\pm$ 1.1 ^{efghi}	163.2 $\pm$ 3.7 ^{ab}	2.60 $\pm$ 0.04 ^{ab}
		56		73.6 $\pm$ 0.5 ^f	3.3 $\pm$ 0.9 ^{ef}	1.85 $\pm$ 0.37 ^{de}	12.2 $\pm$ 3.4 ^{fghi}	162.3 $\pm$ 5.3 ^{ab}	2.52 $\pm$ 0.18 ^{ab}
	100%	19	6.69 $\pm$ 0.28	67.5 $\pm$ 1.2 ^{gh}	7.8 $\pm$ 2.4 ^{cde}	6.67 $\pm$ 0.85 ^{bcd}	18.9 $\pm$ 4 ^{defgh}	113.9 $\pm$ 3.2 ^{de}	2.09 $\pm$ 0.08 ^{bcd}
		24		75.4 $\pm$ 0.3 ^{ef}	5.0 $\pm$ 0.9 ^{def}	4.07 $\pm$ 0.80 ^{cde}	7.80 $\pm$ 2.2 ^{hi}	157.2 $\pm$ 7.2 ^b	2.32 $\pm$ 0.14 ^{bc}
		28		83.9 $\pm$ 1.4 ^c	0.5 $\pm$ 0.5 ^f	0.37 $\pm$ 0.37 ^e	4.40 $\pm$ 2.9 ⁱ	186.4 $\pm$ 4.8 ^a	2.86 $\pm$ 0.12 ^a

5.4.3.2. *Effect of microwave heating at low power and exposure time on AB infected lentil seeds with high moisture content*

AB pathogen eradication in the seeds with high moisture content at lower average temperatures was investigated in a separate experiment with two treatments: power level of 40% and 50% with exposure time of 30 s for seeds at moisture content of 19%. As illustrated in Figure 5.3, treating the lentil seeds at power level 40% for 30 s, which increased the final average surface temperature to 44.5°C, did not affect AB pathogen survival. There was an increase in germination of the lentil seeds from 56.7 to 62.8% at power level of 40% and 30 s exposure time, which could be due to the stimulating effect of microwave fields at low power. [Aladjadjiyan \(2010\)](#) reported a 4.3% increase in lentil seed germination because of microwave irradiation at an output power of 450 W and an exposure time of 30 s. Maximum output power and working volume of their microwave oven were 900 W and 0.19×0.33×0.32 m³ respectively. However, in the present study there was a small increase in conductivity of soaking water at the mentioned condition, which means more leakage of minerals from seed cells' membranes occurred. The second treatment at power level of 50% with 30 s exposure time had final average surface temperature of 53.2 °C. At this treatment, IS% and ID% decreased by almost 40%. However, seed germination was also declined by 20%, which could make it unacceptable to process lentil for seed purposes.

In our previous work on artificially inoculated lentil seeds with AB inoculum at 22% moisture content, infected seeds were reduced by more than 80% without significantly harming lentil seed germination at final average temperature of 48°C ([Taheri & Brodie, 2018](#)). This difference between naturally and artificially inoculated seeds could be because of either deeper penetration of AB inoculum into the seeds in naturally infected seeds or more water activity of the lentil seeds (0.92). Here, naturally infected dried seeds were maintained at high moisture contents followed by microwave treatments, while for artificial inoculation, seeds with high moisture content were inoculated with fresh conidia of AB pathogen.

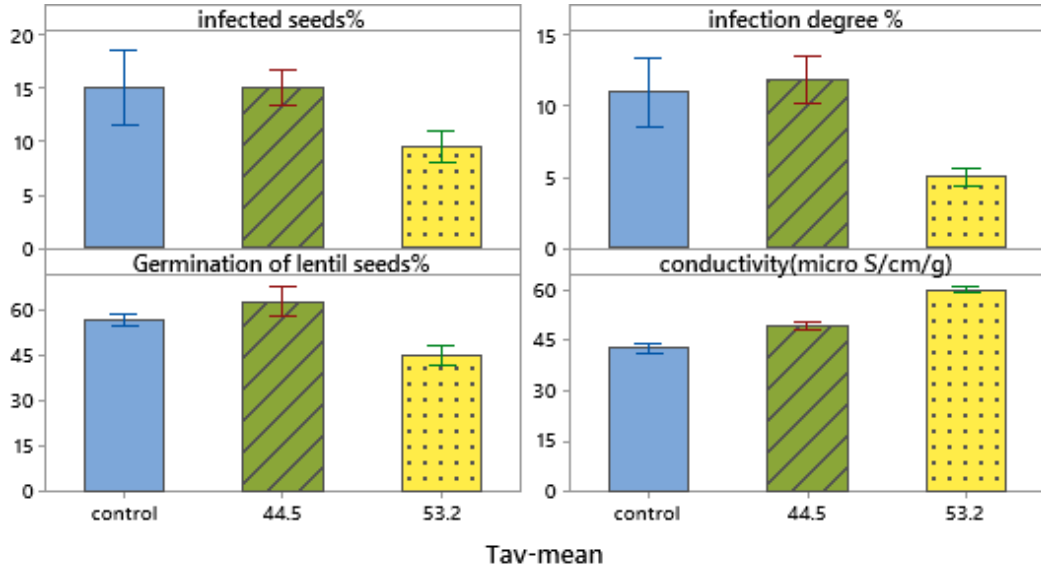


Figure 5.3 Results of lentil seed treatment with moisture content of 19% at power levels of 40% (44.5°C) and 50% (53.2°C) and 30 s exposure time, bars are standard errors

5.4.3.3. Complementary error function model for seeds and fungi viability

Microwave disinfection is not an isothermal process and so it may not be easy to use a first order kinetic reaction model to express the results. Therefore, to have a comparison between different responses like seed germination and IS%, the term of time was eliminated. Instead, they were plotted against final average temperature, which itself is a function of power level and time, at different moisture content (Figure 5.4). These results were fitted using a complementary error function to allow comparison between them. Using this model is another way of visualizing the results and drawing conclusions to see if the thermal process is reasonable to be considered to eliminate the microorganism in a host. The complementary error function (erfc) and its derivatives are expressed as below:

$$\text{erfc}(x) = 1 - \frac{2}{\sqrt{\pi}} \int_0^x e^{-t^2} dt$$

$$\frac{d(\text{erfc}(x))}{dx} = \frac{2}{\sqrt{\pi}} e^{-x^2} ; \frac{d^2(\text{erfc}(x))}{dx^2} = -\frac{4x}{\sqrt{\pi}} e^{-x^2}$$

If the second derivative equals zero, the obtained x value, which will be zero, is the point at which a 50% reduction in the response will occur. By fitting the responses of spores survival to this solution (survived cell%), seed germination% (SG%) and infected seeds% (IS%), according to Figure 5.5 (a) and Figure 5.6 (a) and (b) respectively, in a model as indicated in equation (5.8), and considering zero value for x, or here ($T_{av}-C$), C is the average temperature at which germination and infected seeds will be reduced by 50%, and A is percentage of survivors at $T_{av} = C$.

$$survivors\% = A \cdot \text{erfc}[B(T_{av} - C)] \quad (A, B, C \text{ are constant}) \quad (5.8)$$

Equation (5.8) is plotted in Figure 5.4 to show constant A and C and the effect of increasing constant B. As can be seen, a lower value of the C constant shows more susceptibility of the spores/ seeds/ pathogen inoculum to the thermal process and a higher value of the B constant shows less gradual death of pathogen inoculum or alternatively, they are more prone to a sudden death at C.

The equation parameters are shown in Table 5.2. By comparing constant C in equations (5.9), (5.11) and (5.13), it can be concluded that seed germination will reduce to 50% at final temperatures of 61 and 58.2°C (after microwave treatment), at moisture contents of 16 and 19% respectively. From equations (5.10), (5.12) and (5.14), final average temperature required for 50% reduction in IS% could be predicted as 62 and 64.4°C at 16 and 19% moisture content respectively. These latter temperatures are higher than those for seed germination reduction, which indicates that for 50% reduction in infected seeds more than 50% reduction in seed germination is expected at moisture contents of 16% and 19%. Furthermore, this model was not a good fit for IS% at 9% moisture content as R^2 for this fit was 38%. Therefore, there is no conclusion about reduction of infected lentil seeds at 9% moisture content based on erfc model.

Considering water content, these results, together with required temperature of 56°C (equation (5.15)) for 50% reduction of spores in spore solution, indicate that by increasing moisture content and the corresponding water activity, the average temperature for eradication of the AB pathogen can be reduced or in other words its thermal sensitivity will increase. However, a significant difference between these temperatures was not observed for 16% and 19% MC, which could be due to their close water activity values (0.8 and 0.87 respectively).

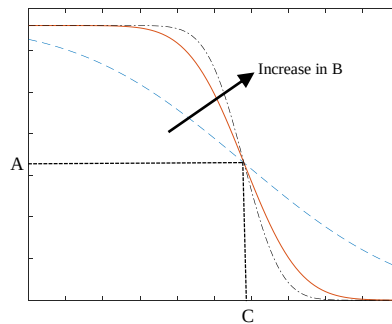


Figure 5.4 Complementary error function, effect of increasing constant B with the same constant C

Table 5.2 Fitting model of seed germination percentage (SG%), infected seeds (IS%), and spore survival in spore solution (survived spores%) as a function of average temperature (T_{av} , °C) according to Figure 5.5 and Figure 5.6

MC	Complementary error function	R ²	RMSE (root mean square error)	Equation no.
9	$SG\% = 35 \operatorname{erfc}(0.06(T_{av} - 94.3))$	0.98	3.25	(5.9)
	$IS\% = 9 \operatorname{erfc}(0.02(T_{av} - 97.9))$	0.38	4.32	(5.10)
16	$SG\% = 35.4 \operatorname{erfc}(0.03(T_{av} - 61))$	0.98	3.37	(5.11)
	$IS\% = 9.4 \operatorname{erfc}(0.02(T_{av} - 62))$	0.96	1.18	(5.12)
19	$SG\% = 33.7 \operatorname{erfc}(0.04(T_{av} - 58.2))$	0.99	2.28	(5.13)
	$IS\% = 8.5 \operatorname{erfc}(0.04(T_{av} - 64.4))$	0.74	3.48	(5.14)
Spore solution	$\text{survived spores}\% = 45.8 \operatorname{erfc}(0.4(T_{av} - 56))$	0.96	7.88	(5.15)

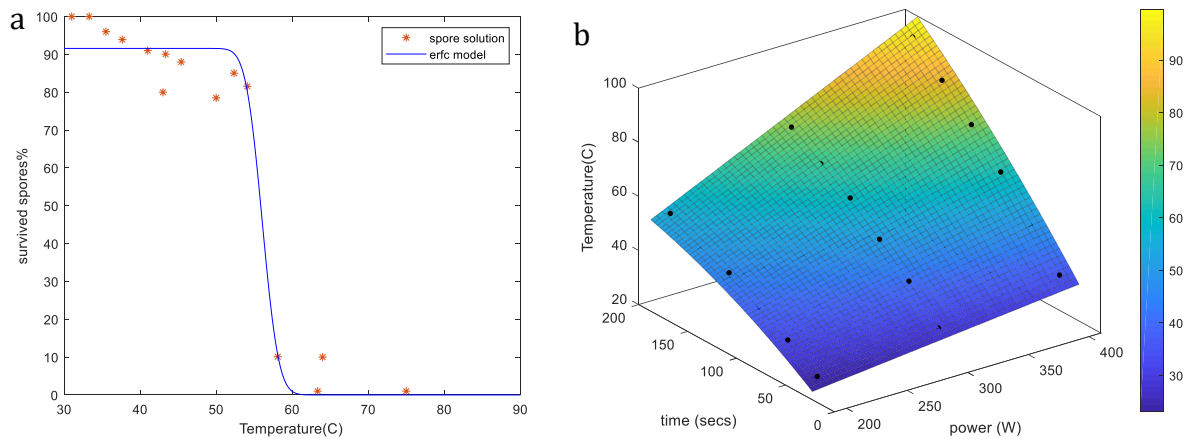


Figure 5.5 (a) Change in spore population (survived spores%) in water at different final temperatures reached after microwave treatments and (b) Combination of output power-time in a single mode cavity to reach the final temperatures(T)

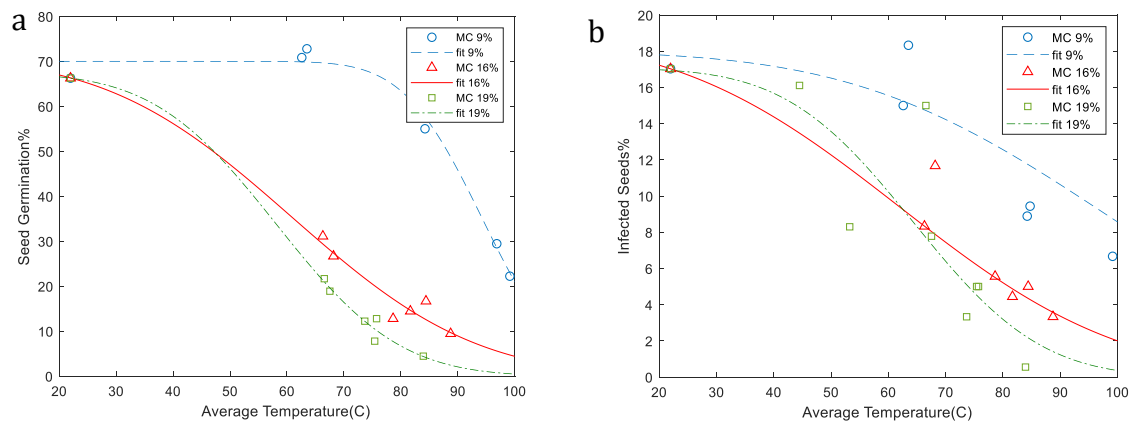


Figure 5.6 (a) Seed germination% and (b) IS% of different moisture contents of lentil seeds at different final average temperatures reached after microwave treatments

5.5. Conclusion

In this study the effect of microwave treatment on the *Ascochyta* blight pathogen in red lentil seeds, at moisture contents of 9.1, 16 and 19% (wb), was evaluated. Increasing moisture content (MC) increased AB pathogen susceptibility to microwave treatment at the same output powers and final average temperatures. However, a decrease in seed

germination percentage upon increasing moisture content negated the value of microwave irradiation control the AB pathogen at high moisture content. In dry lentil (9% MC), reduction of infected seeds from 17% to 9.4% and 8.9% (at output power levels of 50% and 100% respectively) could be obtained at a final average surface temperature of 84°C without harming the seeds' germination. This final average temperature may seem too high because it could affect protein structure of the seeds and so studying the effect of microwave output power level of 50% or 100% to reach this final average temperature on protein structure of dry lentil is suggested. In a separate experiment on the infected seeds with 19% moisture content, it was shown that IS% could be reduced by 40% with an output power level of 50% and 30 s exposure time (final average temperature of 53.2°C), while 20% reduction in seed germination occurred. A complementary error function was used to model and visualize the results of the reduction in seed infection as well as the reduction in lentil seed germination. From the fitted model, it was predicted that the required final average temperature, because of microwave treatment, for 50% reduction of infected seeds was more than that for 50% reduction in seed germination at moisture contents of 16% and 19%. It is also recommended to find the water activity level for optimum growth of the pathogen, which may be more than 0.9, and then treat the infected seeds at the corresponding moisture content (more than 20%) to see if a reduction in IS% could be achieved at a lower power, exposure time and final average temperature.

The results in the two previous chapters indicated that it was very challenging to disinfect lentil seeds in a multimode cavity with a turntable without seed vigour loss. This challenge was attributed to the high non-uniformity of heating. Therefore, a microwave fluidised bed was considered for disinfection of lentil seeds with the aim of improving heat uniformity. The next chapter will discuss the simulation and heat uniformity study in a microwave fluidised bed.

Chapter 6. Preliminary studies for the design of microwave fluidised bed in a single-mode cavity

6.1. Abstract

In the two previous chapters, treatment of the lentil seeds in a multimode cavity with a turntable was considered. However, the disinfection affected seed vigour, which could be attributed to the non-uniformity of heating and a large difference between the hot and cold spots. Therefore, a microwave fluidised bed will be designed and evaluated for its heat uniformity and ability to disinfect lentil seeds. Due to the high costs of designing systems by experimental increments, it is beneficial to predict the potential results of candidate systems by doing computer simulation. The aim of this chapter is the prediction of heat uniformity in lentil and Kabuli chickpea seeds while treating in a single-mode cavity before and after adding hot air across the seeds. Due to the limitations in the software, the movements of the seeds were not considered. Any improvement in the heat uniformity was predicted by writing code in MATLAB to solve the system of partial differential equations (PDEs). XFDTD® was used for electromagnetic simulation and finding the power dissipation in the cavity and the seeds followed by extracting the results in .mat file and finding the temperature distribution in MATLAB using the PDE solver. The results showed that forced hot air flow through the seed bed improved heat uniformity by 80%, and therefore a hybrid microwave/hot air system was considered for the treatment of legume seeds.

6.2. Introduction

Simulation was described as one of the pillars of industry 4.0 (fourth industrial revolution) in the food industry ([Hasnan & Yusoff, 2018](#)). It facilitates testing, analysing, and optimising of a process before performing any actual changes. This saves time and money and helps companies remain competitive and robust in today's fast-growing world of technologies.

Microwave energy is increasingly being utilised to facilitate thermal processing and reduce process time in industries. However, one of the major problems related to this technology is the non-uniform distribution of the electric field. Computer simulation could assist the proper design of the microwave applicator, predicting the heat uniformity and determination of the best process parameters for the host material with specific dielectric properties.

Microwave thermal processing can be mathematically modelled using different numerical methods. The most common methods are Finite-Difference-Time-Domain (FDTD) and

Finite Element Method (FEM) ([Birla & Pitchai, 2017](#)). These numerical techniques have been implemented in a number software tools to visualise and facilitate the procedure of simulation. Some of them like Xfdtd® has been developed for solving the electromagnetic equations alone and is useful for finding the electric field distribution and design of an applicator. Quickwave™ is another software which uses the FDTD method, which provides the option of solving the coupled electromagnetic and thermal equations together. Another example is COMSOL™, a complete Multiphysics software package, which could solve the electromagnetic and heat and mass transfer problems, besides providing the opportunity to consider the material's properties as a function of temperature. These methods and related software, as well as their pros and cons, are reviewed by [Birla&Pitchai \(2017\)](#).

In the present study, the Xfdtd® was used to solve the electromagnetic equations followed by exporting the result to MATLAB and solving the heat transfer equation using Partial differential equation (PDE) method. Therefore, we did not solve coupled electromagnetic and thermal equation, which means considering the microwave power distribution as a constant value over time but being spatially variable regarding the location within the 3D object. This made solving the equations much easier and faster, though the accuracy of prediction is much lower. However, simulation in this study aimed to compare heat uniformity for various scenarios and not the prediction of the final temperature. Thus, the assumptions and the method of simulation seem to be reasonable.

The objective of this chapter were: to design the sample holder of microwave fluidised bed which could hold the seeds in the hot spot of the microwave cavity; to compare the heat uniformity of a bed of lentil and Kabuli chickpea seeds in the single-mode cavity before and after adding forced hot air.

6.3. Materials and methods

6.3.1. *Sequence of study*

1. 3-D design of a sample holder using Autodesk Inventor Professional 2017 (Autodesk Inc., USA) to hold the lentil seeds in the hot spot of the cavity
2. Simulation of the power dissipation in the seeds with XFtdt® 7.5.0 (Remcom, Inc., State College, PA), using the power dissipation from the outcome of XFtdt® and solving the heat balance equation in MATLAB and comparing the temperature results of simulation and experiment.

3. Performing the simulation in MATLAB with different boundary conditions to investigate the difference in heating uniformity before and after applying hot air across the seedbed.

6.3.2. Temperature uniformity index

To study heat uniformity of the process, the temperature uniformity index was calculated, for both experiment and simulation, according to equations 6.1 and 6.2 ([Uyar et al., 2016](#)).

$$TUI = \frac{\sqrt{\sum_1^N (T_i - T_{av})^2 / N}}{T_{av} - T_{initial}} \quad (6.1)$$

$$T_{av} = \frac{\sum_1^N T_i}{N} \quad (6.2)$$

Where N is the number of the points in which temperatures (T_i) are calculated. $T_{initial}$ is the initial temperature of the samples. For the power uniformity index, the same equations can be applied by replacing temperature with power.

6.3.3. Governing equations for simulation

6.3.3.1. Assumptions and limitations in simulation

Assumptions used in the simulation are as below:

- The dielectric properties of the materials are assumed to be constant with any change in temperature, due to the limitation of entering the values in the software
- Kabuli chickpea is assumed to be an ellipse with length of 9.97 mm and width of 8.15 mm and lentil is a lens shape with dimensions 6.5 mm and 2.4 mm.
- Due to a very low change in moisture content at low initial seed moisture content, the mass transfer equation was not solved. However, the effect of moisture loss cooling on the temperature was considered. For this reason, the average rate of moisture loss was considered in the heat balance equation.
- Seeds are isotropic materials and their properties do not change with location.
- Radiation from seeds is assumed to be negligible.
- The geometry considered for these simulations is based on 100 g of each legume seeds.
- Power dissipation was considered as constant with time
- Any movement of the seeds was not considered, but the boundary condition from each seed was assumed to be just convection from and to the air flow.

6.3.3.2. Thermo-physical properties used in the simulation

Properties of Kabuli chickpea and lentil seeds, which were used in the simulation, are represented in Table 6.1 and Table 6.2. Change in moisture content, which causes moisture cooling, could be solved in MATLAB using a system of PDE. Nevertheless, at this stage, due to very small evaporation, final moisture contents, as functions of time, were extracted from literature and their derivatives are used here as good estimations for moisture loss rates.

Table 6.1 Properties of Kabuli chickpea used in the simulation, $T(^{\circ}\text{C})$, $MC(\%)$, $\rho(\text{g cm}^{-3})$, $t(\text{s})$

Properties	Value	Reference
Thermal conductivity ($\text{W m}^{-1} ^{\circ}\text{C}^{-1}$)	$k = 0.1027356 + 0.000829257 T + 0.00568746 MC$	Sabapathy&Tabil (2004)
Specific heat ($\text{J kg}^{-1} ^{\circ}\text{C}^{-1}$)	$C_p = 1.060073 + 0.017232672 \left(\frac{MC}{100}\right) + 0.004910467T$	Sabapathy&Tabil (2004)
Density (kg m^{-3})	$\rho = (1090/MC) + 1239$	Taheri et al. (2018)
Change of moisture content ($\text{kg m}^{-3} \text{s}^{-1}$)	$\partial X/\partial t = 0.0074\rho(MC/100) 0.8762\exp(-0.0074t)$	Cil&Topuz (2010)
Dielectric constant (at room temperature)	$\varepsilon' = 4.98$	(Taheri et al. 2018)
Loss factor (at room temperature)	$\varepsilon'' = 1.11$	(Taheri et al. 2018)

Table 6.2 Properties of lentil used in simulation, $T(^{\circ}\text{C})$, $MC(\%)$, $\rho(\text{g cm}^{-3})$, $t(\text{s})$

properties	Value	Reference
Thermal conductivity ($\text{W m}^{-1} ^{\circ}\text{C}^{-1}$)	$k = 0.0004MC^2 - 0.0066MC + 0.2224$	Gharibzahedi et al. (2014)
Specific heat ($\text{J kg}^{-1} ^{\circ}\text{C}^{-1}$)	$C_p = 577.3 + 7.09T + MC (62.2 - 91.4(MC/100))$	Tang et al. (1991b)
Density (kg m^{-3})	$\rho = 1755.5/MC^{0.0099}$	(Taheri et al. 2018)
Change of moisture content ($\text{kg m}^{-3} \text{s}^{-1}$) at microwave power of 300 W	$\frac{\partial X}{\partial t} = 2.7942\rho \left(\frac{MC}{100}\right) * 0.00201^{2.7942} * t^{1.7942} \exp((-0.00201t)^{2.7942})$	lik et al. (2011)
Dielectric constant (at room temperature)	$\varepsilon' = 3.29$	(Taheri et al. 2018)
Loss factor (at room temperature)	$\varepsilon'' = 0.489$	(Taheri et al. 2018)

6.3.3.3. Power dissipation

Power dissipation was found using XFDTD® software by solving Maxwell's equations (equations (6.3) to (6.6)) using the Finite-Difference Time-Domain Method and the boundary conditions which was explained by [Yee \(1966\)](#). Geometries of lentil and chickpea were designed in Autodesk inventor based on having a 100-g of each which resulted to have a 10-layer lentil and a 3-layer chickpea geometry in a 90-mm diameter. Calculations were performed based on the whole geometry and not the individual seeds.

$$\nabla \times E = -j\omega\mu H \quad (6.3)$$

$$\nabla \times H = -j\omega\varepsilon_0\varepsilon E \quad (6.4)$$

$$\nabla \times E = 0 \quad (6.5)$$

$$\nabla \times H = 0 \quad (6.6)$$

where E and H are electric and magnetic fields. ω is the angular frequency ($2\pi f$), μ is the material's permeability, ε_0 is the permittivity of free space and ε is the complex permittivity of the material. All boundaries are considered perfect conductors and quartz is a perfect insulator. To run the simulation in XFDTD®, the physical and dielectric properties of lentil or Kabuli chickpea were applied, based on Table 6.1 and Table 6.2. The convergence point was considered -30 dB. All the materials of the 3-D object (cavity, sample holder) were selected from the software's library and the physical and electromagnetic properties of lentil seeds were also added to the library (Taheri et al., 2018) and used in the simulation. The result of power dissipation was then extracted into MATLAB (.mat file) to be applied in the heat balance equation.

The first simulation was conducted in the chamber with the geometry of the seeds to define the power density in the seed bed and hot spots of the cavity as well as to determine the effect of blowing hot air on the samples. The second simulation was performed in the cavity with the sample holder without any samples to show that the sample holder for the fluidised bed reactor hold the seeds in the hot spot of the cavity. In both cases, the excitation was considered at power of 1 W at the rectangular entrance of the waveguide.

Having considered a mesh size of less than 1 mm, the power dissipation in the whole geometry of the seeds were obtained from XFDTD. Maximum and average power dissipations were then calculated in the horizontal and vertical directions separately by having the values at each point. Number of points were $92 \times 20 \times 96$ (axis of x, y, z respectively) for lentil and $80 \times 12 \times 92$ (axis of x, y, z respectively) for chickpea. Axis are shown in Figure 6.2 (a) and are the same for both geometries.

6.3.3.4. Conservation of energy

Heat balance for the whole geometry is based on Fourier's law as indicated below:

$$\rho C_p \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) + P_{MW} - \frac{\partial X}{\partial t} H_{fg} \quad (6.7)$$

Where ρ , C_p and k are density, specific heat capacity and thermal conductivity of the seeds. P_{MW} is microwave power dissipation per unit volume of the geometry and H_{fg} is the enthalpy of evaporation of seeds moisture. $\frac{\partial x}{\partial t}$ is the rate of moisture loss per unit volume. According to [Law et al. \(2016\)](#) the latent heat ($J\ kg^{-1}$) can be written as equation (6.8).

$$H_{fg}(J\ kg^{-1}) = -0.0469(T + 273)^2 - 13.493(T + 273) + 52202 ; T(^{\circ}C) \quad (6.8)$$

Microwave power dissipation term, which can be found from electric field distribution according to equation (6.9), were extracted from XFDTD® software and then its distribution in the geometry was obtained by interpolation in MATLAB. This power density is, in fact, variable with temperature and time, but here we did not consider this change.

$$P_{MW} = 2\pi f \epsilon_0 \epsilon'' |E|^2 \quad (6.9)$$

6.3.3.5. Initial and boundary conditions of the heat balance equation

The initial temperature of the seeds was considered the same as the ambient temperature. At the surface of the seeds, heat is lost by natural or forced convection according to equation (6.10), where k and h are thermal conductivity and heat transfer coefficient. T_s is surface temperature and T_{air} is surrounding air temperature.

$$-n \cdot (k \nabla T) = h(T_s - T_{air}) \quad (6.10)$$

Heat transfer coefficient (h) depends on the air velocity, material shape and surface area, air temperature and thermal properties. According to the literature, the heat transfer coefficient for natural convection was assumed to be $5\ Wm^{-2}K^{-1}$ ([Zhang et al., 2001](#)). Heat fluxes at the contact points of seeds are equal, $n_1 \cdot (\nabla T_1) = n_2 \cdot (\nabla T_2)$. The geometry of the whole bed of the seeds was extracted from XFDTD as one .stl file. Therefore, it was not possible to apply all boundary conditions by using PDE solving function of MATLAB. As a result, the only boundary condition, which could be reasonably applied to the geometry, was natural or forced convection from all the seeds.

6.4. Results and discussion

6.4.1. Temperature and power distribution from simulation and experiment

Temperature distribution in bulk samples of lentil and Kabuli chickpeas are shown in Figure 6.1 and Figure 6.2. According to these results, heat is concentrated in the centre of the top layer. The thickness of Kabuli chickpeas along the y-axis is 2.85 cm, which is almost the same as half of the wavelength, when the seeds are at 11.8% moisture content and room

temperature (2.72 cm). As the samples are aligned with the electric field in the cavity, those near the maximum electric field have a higher temperature and by getting close to the minimum electric field, samples have lower temperature as they receive less power from the microwave. It can be more obviously observed in Figure 6.3. This figure illustrates the maximum and average power dissipated in Kabuli chickpeas and lentil along the thickness of geometries (vertical axis). The maximum points in the graph are inside the seeds. [Law et al. \(2016\)](#) reported the same maximum points inside the seeds of soybean when loosely packed seeds (with no connection between them) were simulated. However, they observed the maximum power densities at the contact points of the soybean when they were in contact with one another. This result from our simulation could be because the geometries are considered as separate seeds with no contacts in the XFDTD simulation.

Figure 6.4 indicates that Kabuli chickpea seed samples absorbed less microwave power in the centres than on the surfaces along the x-axis, while lentil seed samples have more power density at their centres than their surfaces like their maximum power distribution along the thickness of the geometry. This result for Kabuli chickpea could stem from the ellipsoid seed shape, which has a longer diameter along the x-axis. This finding is similar to the results described by [Brodie \(2008\)](#). He showed that the hot spot of a sphere moved from centre to the surface as the diameter or the loss factor increased. From this finding, it can be concluded that Kabuli chickpea seed with a diameter of 9.97 mm and the mentioned dielectric properties should be considered as a large object in microwave processing.

The maximum and minimum temperatures of Kabuli chickpea and lentil seeds after treatment with forwarded power of 300 W and 60 s exposure time are represented in Figure 6.5 and Figure 6.6 respectively. The difference between them could be the result of simplifications and assumptions made for calculations. In reality, particles have connection points and so heat is transferred from one to another by conduction too. The other point is that the maximum temperature of the seeds' surfaces was considered for experimental data, whereas in simulation, the maximum temperature is obtained for the whole body of the geometry. From these graphs, maximum temperatures increase by increasing power and exposure time. In a steady state (data are not shown), this maximum temperature will level off after 30 min, at which time the temperature will go up to 450°C in Kabuli chickpea seeds.

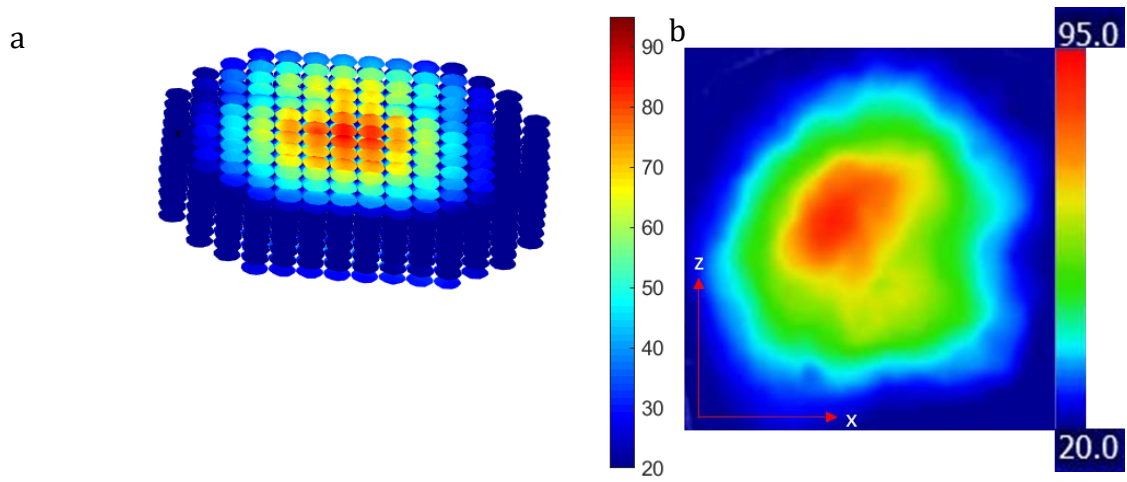


Figure 6.1 Temperature distribution of lentil in a single-mode cavity at the forwarded power of 300 W after 60 s, (a) Simulation and (b) Experiment (photo from the top layer after the treatment)

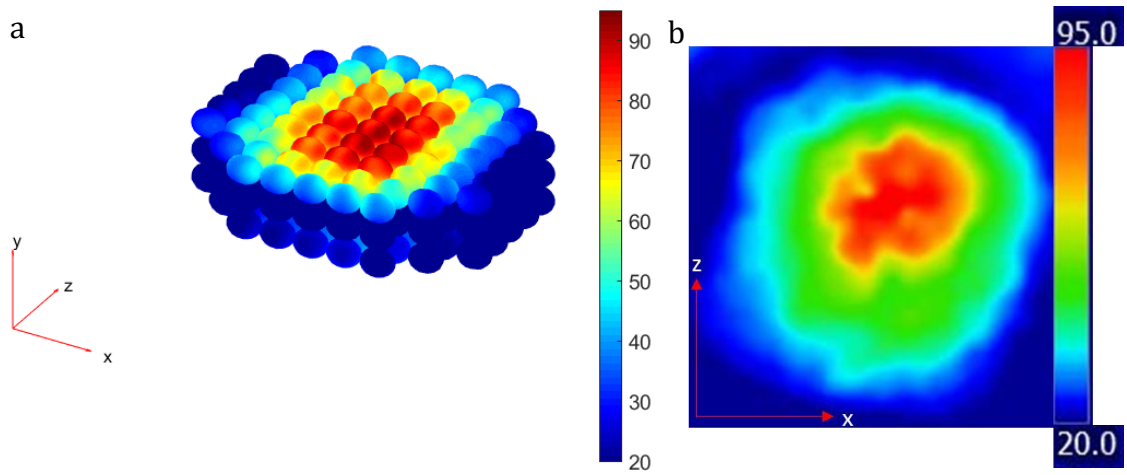


Figure 6.2 Temperature distribution of Kabuli chickpea in a single-mode cavity at the forwarded power of 300 W after 60 s, (a) Simulation and (b) Experiment (photo from the top layer after the treatment)

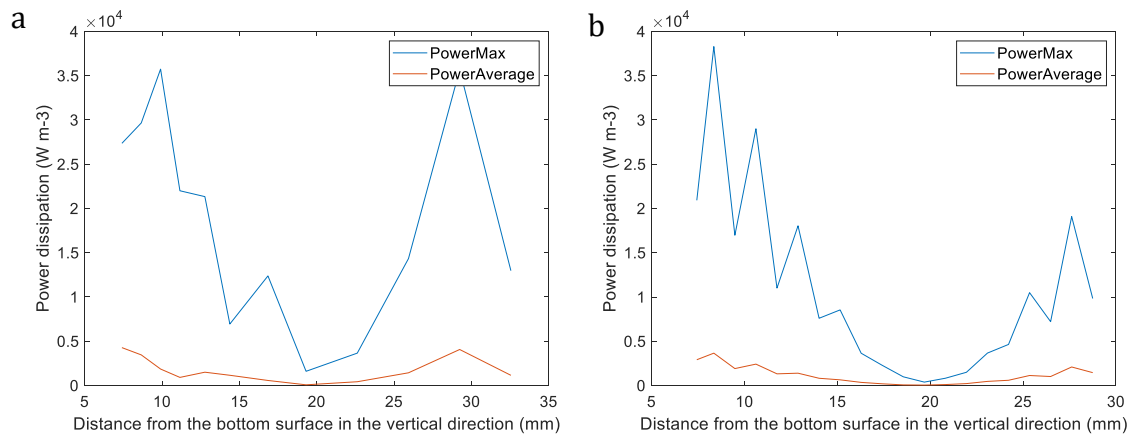


Figure 6.3 Maximum and average power dissipated in (a) Kabuli chickpea and (b) Lentil in each cross-section in vertical position (y-axis), at the forwarded power of 300W after 60 s, results obtained from simulation

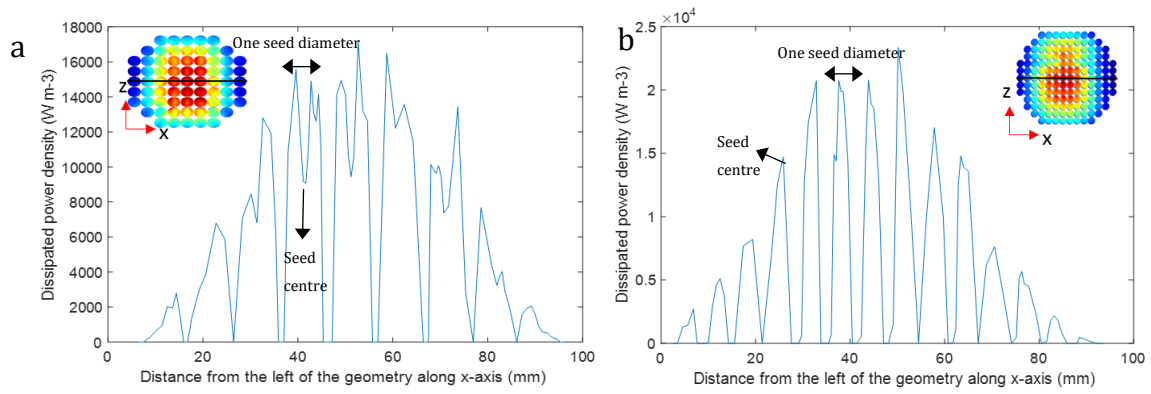


Figure 6.4 Power dissipation from simulation in (a) Kabuli chickpea and (b) Lentil along the x-axis in the centre of the geometry on the top layer (the black line in the geometry), at the forwarded power of 300 W after 60 s.

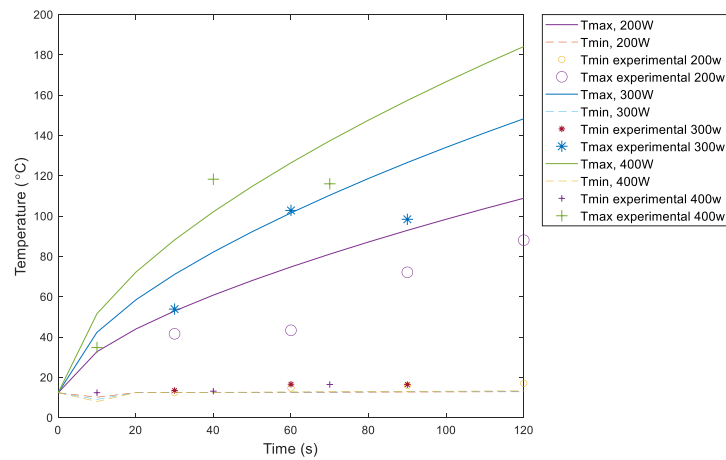


Figure 6.5 Maximum and minimum temperatures of Kabuli chickpeas after treatment with forwarded power of 300 W and 60 s exposure time, simulation (continuous lines), and experiment (discrete symbols), powers are forwarded powers

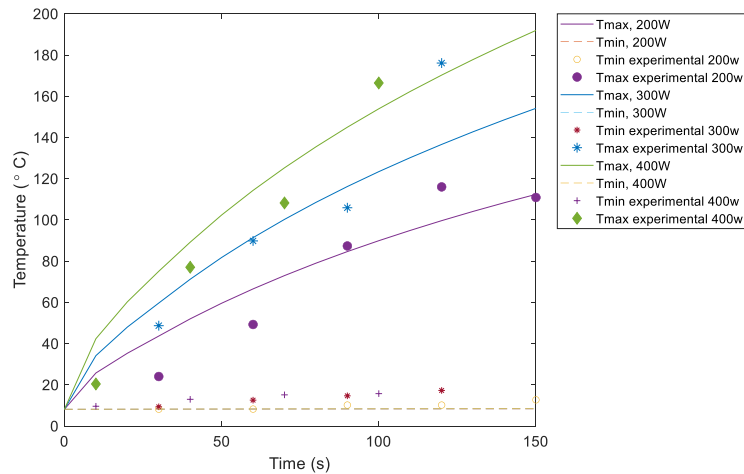


Figure 6.6 Maximum and minimum temperatures of lentil after treatment with forwarded power of 300 W and 60 s exposure time, simulation (continuous lines), and experiment (discrete symbols)- powers are forwarded powers

6.4.2. Heat uniformity study before and after adding hot air by simulation

Average temperatures and temperature uniformity indexes, which were calculated from the results of the simulation, are shown in Figure 6.7 to Figure 6.10. Average temperatures increased with increasing power level and exposure time. Temperature uniformity indexes decreased with increasing power level when heat transfer is natural convection with a coefficient of $5 \text{ W m}^{-2} \text{ K}^{-1}$.

It is hypothesised that by blowing hot air on the samples, heat uniformity will improve. In the simulation, hot air at 55°C with a speed of 2.5 m s^{-1} was used to study the effect on the heat uniformity in a single-mode microwave. For this purpose, forced convective heat transfer coefficient (h) was calculated by assuming that the particles are spheres and using Nusselt (Nu), Reynolds (Re) and Prandtl (Pr) numbers as below ([Ramaswamy & Zareifard, 2003](#)) :

$$Nu = 2 + [0.4\sqrt{Re}] + 0.06\sqrt[3]{Re^2} Pr^{0.4} \left(\frac{\mu_{air}}{\mu_{surface}} \right)^{\frac{1}{4}} = \frac{hD}{k} \quad (6.11)$$

$$Re = \frac{vD}{\vartheta} \quad (6.12)$$

where v is the speed of air, μ_{air} is the air dynamic viscosity at the air bulk temperature, $\mu_{surface}$ is the air dynamic viscosity at the boundary surface temperature (here the seed surface temperature), $\frac{\mu_{air}}{\mu_{surface}}$ is assumed to be 1, and D is the equivalent diameter of the shape. Thermal properties of air, used in the calculation, are represented in table Table 6.3.

Table 6.3 Thermal properties of air at 55°C

Thermal properties of air at 55°C	Value
Dynamic viscosity, μ (kg s^{-1})	1.962×10^{-5}
Kinematic viscosity, ϑ ($\text{m}^2 \text{s}^{-1}$)	1.807×10^{-5}
Pr	0.701
Thermal conductivity, k ($\text{W m}^{-1} \text{K}^{-1}$)	0.02816

According to these data, the convective heat transfer coefficient (h) will be $65 \text{ W m}^{-2} \text{ K}^{-1}$. It is assumed that moisture loss is the same as the process without the hot air. The effect of blowing air on the average temperatures and heat uniformity indexes are plotted in Figure 6.8 and Figure 6.10 for Kabuli chickpeas and lentil respectively. By applying this change to the process, heat uniformity improves by 80% for Kabuli chickpeas (from 1 to 0.2 after 50 s with a microwave power of 200 W) and 90% for lentil (from 1 to 0.1 after 50 s with a microwave power of 200 W). Furthermore, the temperature reached a steady state after a very short time (50 s) in lentil seeds.

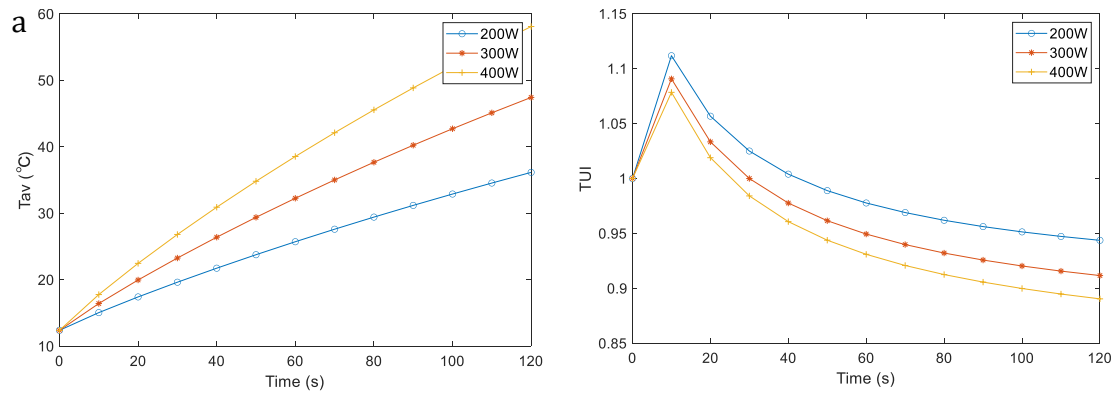


Figure 6.7 (a) Average temperature and (b) Temperature uniformity index for the whole body of Kabuli chickpeas, obtained by simulation with no air flow across the seeds

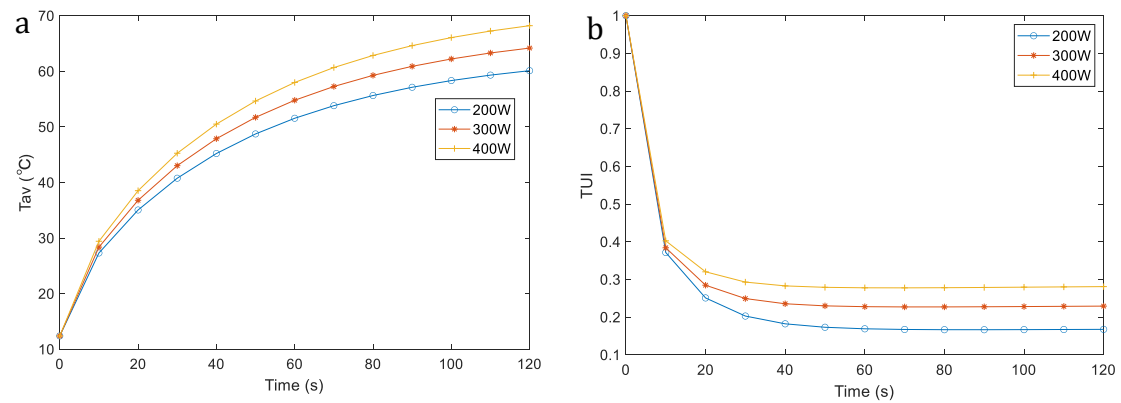


Figure 6.8 (a) Average temperature and (b) Temperature uniformity index of Kabuli chickpeas after blowing hot air at 55°C and velocity of 2.5 m s⁻¹ across the seeds, obtained by simulation

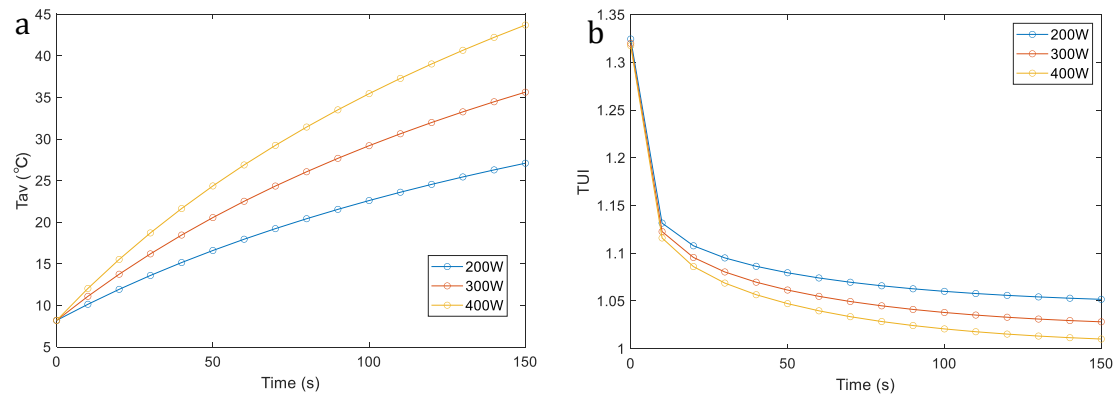


Figure 6.9 (a) Average temperature and (b) Temperature uniformity index for the whole body of lentil, obtained by simulation with no air flow across the seeds

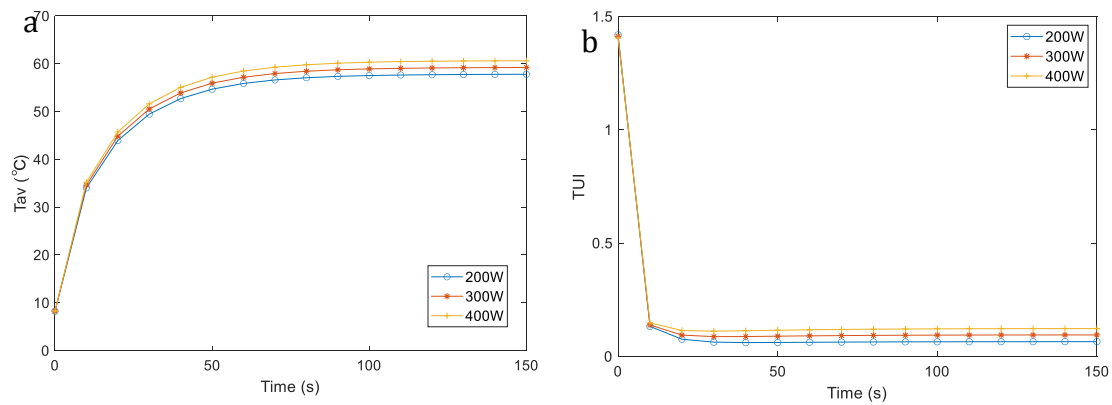


Figure 6.10 (a) Average temperature and (b) Temperature uniformity index of lentil after blowing hot air at 55°C and velocity of 2.5 m s⁻¹ across the seeds, obtained by simulation

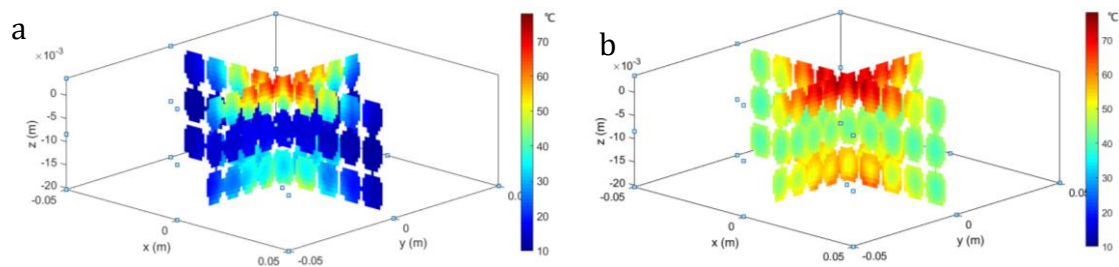


Figure 6.11 Two cross-sections of Kabuli chickpea seeds (a) Before and (b) After blowing hot air, power 200 W after 60 s, obtained by simulation

Figure 6.11 illustrates two cross-sections in the middle of Kabuli chickpea geometry for a power of 200 W after 60 s of exposure time. On the left, there is no air on the samples and the minimum and maximum temperatures are 10°C and 70°C. On the right, the temperature distribution is shown after blowing air on the samples, which raises the minimum temperature to above 40°C. As a result, blowing hot air can improve the heat uniformity after a short time, and it can be reassured that all parts of the material reach a minimum temperature, which is a necessity in the disinfection. However, the temperature and the speed of air need to be optimized if moisture loss is not desired. Moreover, the seeds receive a higher amount of microwave power in their upper layers, which justifies blowing hot air from their bottom layers to compensate for the lower temperature.

6.4.3. Electric field distribution in the cavity and the sample holder for fluidised bed dryer

As the result of simulation indicated, blowing hot air across the samples could be justified by improving the heat uniformity by 80%. Therefore, a sample holder and a new lid were designed to be implemented in the single mode cavity to turn it to a microwave fluidised bed reactor. The drawings of the sample holder can be found in Figure 6.12(b). The sample holder is made of Teflon (polytetrafluoroethylene or PTFE), which is almost transparent to microwave radiation. The perforated plate that holds the sample is also made of Teflon, but

the perforated plate at the bottom of the cone is made of Stainless steel. To minimize the leakage of microwave radiation from the bottom part of the sample holder, the parts which are outside of the cavity were covered and sealed in all directions with aluminium tape. However, a very small amount of leakage from the small holes of the vents was unavoidable. This leakage was experimentally measured and was less than 0.5 W cm^{-2} . The angle of the cone was selected based on two criteria: firstly, to locate its upper part in the hot spot of the cavity, and secondly, to have a diameter at the bottom surface with an equivalent surface of the two smaller holes (vents of air) on the lid of the cavity. The latter was to make sure the inlet and outlet of the forced air had the same area and there is not a change in the speed of air inside the cavity. The angle of the cone part (the diameter of the spout or orifice) is an important parameter when the reactor is to be used as a spouted bed for small particles as it defines how particles float back and how the spout would be shaped. This also depends on the physical properties of the particles ([Sutkar et al., 2013](#)). However, this was not of concern here, because we did not consider using a spouted bed sample holder. The angle is also important in terms of developing the air flow, but the considerations regarding the fluid dynamics were also neglected due to the importance of keeping the samples in the hot spot of the cavity and the limitations regarding the size of the cavity. The result of the simulation using the sample holder inside the cavity is represented in Figure 6.12(a). The sample holder could hold the seeds on its perforated part in the hot spot of the cavity, and therefore the seeds could absorb the most forwarded microwave energy.

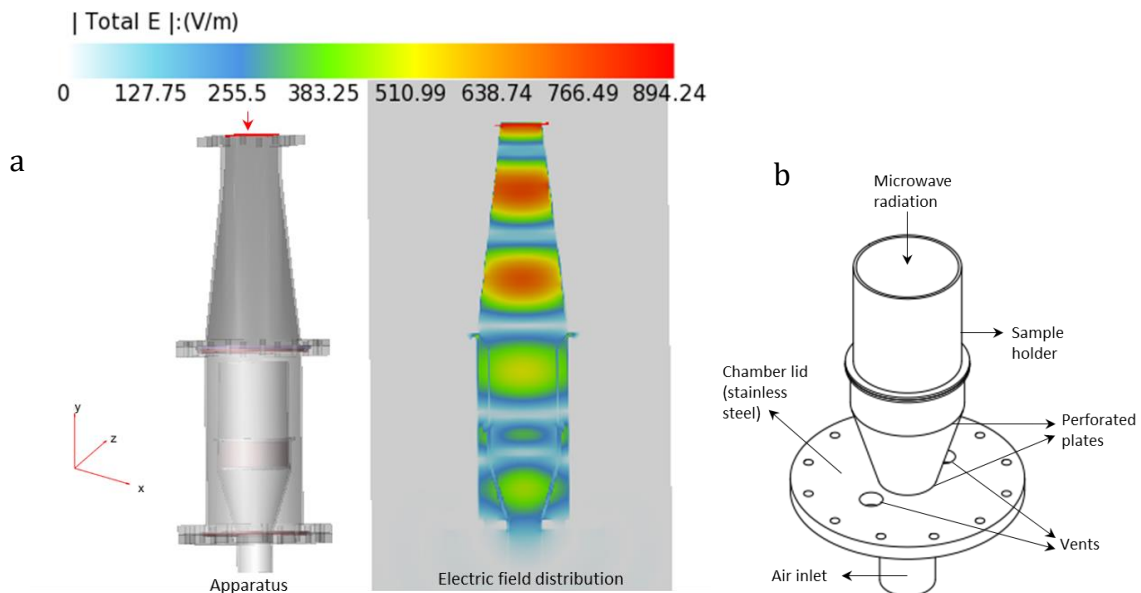


Figure 6.12 (a) Apparatus (red arrow shows the excitation point) and The result for the simulation of electric field distribution in the microwave fluidised bed reactor without samples in the sample holder, (b) Different parts of the sample holder.

6.5. Conclusion

In this chapter, the heat uniformity of lentil and Kabuli chickpea seeds, treated in a microwave single-mode cavity, was studied by simulation and validation by an experiment. The power distribution was found in the cavity and bulk of lentil and Kabuli chickpea seeds in XFDTD®. The power dissipation was then exported to MATLAB where the heat transfer equation was solved to find temperature distribution. The effect of adding hot air at a specific speed on the heat uniformity was also evaluated by considering heat transfer coefficient (h) for forced air at 55°C and speed of 2.5 m s⁻¹. The result of the simulation showed that the heat uniformity could be improved by 80% after adding air across the seedbed. This result indicated that the microwave hybrid system is promising for the treatment of the seeds with regard to the heat uniformity. The microwave fluidised bed will be evaluated for its effectiveness in disinfection of lentil seeds in the following chapter.

The result of this chapter indicated that the heat uniformity could be improved by fluidising the seeds using a forced hot air flow. Therefore, this system was manufactured and utilised to study the disinfection of lentil seeds. The effect of process parameters and the effectiveness of this system will be evaluated in the next chapter.

Chapter 7. Effectiveness of a microwave fluidised bed dryer in eradication of seed-borne *Botrytis grey* mould of lentil

Taheri, S., Brodie, G., & Gupta, D. (2019). Effectiveness of a microwave fluidised bed dryer in eradication of seed-borne *Botrytis grey* mould of lentil. *Proceeding of the 17th International Conference on Microwave and High frequency heating, Valencia, Spain*. ISBN: 978-84-9048-719-8; DOI: <http://dx.doi.org/10.4995/Ampere2019.2019.9635>

7.1. Abstract

A single mode microwave cavity, with a 2.45 GHz microwave source, was modified to have a microwave fluidised bed and its potential to eliminate *Botrytis grey* mould (BGM) pathogen of lentil seeds was evaluated. Air speed was maintained at a constant value and was just enough to fluidise 100 g of red lentil seeds in the sample holder. Two wet-based (w.b.) seed moisture contents (MC) of 10.5% and 18.5% were prepared and the process parameters were selected as air temperature at 50 and 60°C; microwave power at 0, 300, 400 W for 18.5% MC and 0, 400, 500 W for 10.5% MC; and exposure times of 5 and 10 min. The effect of the process parameters on seed moisture loss, seed germination, the electrical conductivity of seed soaking water and the percentage of infected seeds (IS%) were analysed. The most effective factors on moisture loss, after seed moisture content, was exposure time, followed by microwave power and air temperature. The final bed temperature was mostly affected by air temperature, and then by microwave power. Furthermore, based on general full factorial regression and Pareto chart of standardized effects, moisture content had a major influence on the reduction of IS%. Seed pathogen inoculum reduction, without significant seed viability loss, was obtained by applying microwave power of 300 W and set air temperature of 60°C (actual inlet air temperature of $57 \pm 1^\circ\text{C}$) on seeds with MC of 18.5% for 10 min. This gave a 27% reduction in IS%, from 82% to 55%. Thus, these results showed that applying microwave fluidised bed dryer was able to reduce BGM pathogen in lentil seeds and it could be considered as part of integrated disease management.

7.2. Introduction

Botrytis cinerea (BC) is one of the important seed-borne pathogens causing *Botrytis grey* mould (BGM) disease of chickpea and lentil crops, one of the economically important seed-borne diseases in Australia. It can survive in the form of micro and macroconidia, mycelium,

sclerotia and chlamydospores ([Yigal Elad, 2007](#)). [Lindbeck et al. \(2009\)](#) showed that *BC* is mostly located externally on lentil and chickpea seeds. They mentioned that seeds, which are infected during early stages of growth, may have an internal infection, and will be discoloured and shrivelled, so they could be recognized by their appearance. However, those infected later, close to the harvest time, do not have any symptoms and might be used for sowing in the next season. The later seeds are mostly externally infected.

BGM control in lentil crop consists of integrated management including the application of resistant cultivars, healthy seeds, biological agents, seed treatment, foliar treatment with fungicides and cultural practices ([Beniwal & Trapero-Casas, 1994](#); [Yigal Elad, 2007](#)). Lentil varieties grown in Australia do not have complete resistance against BGM and need fungicide application to control the disease ([Lindbeck et al., 2008](#)). Therefore, using chemicals for seed and foliar treatments has remained the easiest and the most popular way of BGM control in this crop. However, there are still concerns about BGM pathogen's ability to develop resistance as well as environmental and health impacts due to over usage of fungicides ([Fillinger & Elad, 2016](#)). There have been some attempts to find a physical treatment for control or eradication of BGM pathogen. [Burgess \(1997\)](#) showed that *BC* is mostly present externally in chickpea seeds and can be controlled by moist heat at 45°C for 10 min or 50°C for 5 min. These treatments decreased seed infection to 2.5 and 0% respectively with no adverse effect on the seed germination and seedling emergence. However, the same treatment on the freshly harvested seeds reduced the infection to 18%, which could be the result of initial inoculum or changes in heat tolerance of the pathogen during treatment time. They also indicated that infected seeds did not necessarily have any visual symptoms to distinguish them from healthy seeds. This highlighted the need for seed testing. [Marquenie et al. \(2002\)](#) applied UV-C, thermal treatment, and their combination to inactivate spores of *BC*. They used first-order kinetic models to describe the inactivation of the spore, with temperature and UV dose as the independent factors for thermal and UV-C treatment, respectively. They observed that there was no survival of conidia at 45°C after 15 min and at 48°C after 5 min. It was concluded that the combination of UV and thermal treatment allowed inactivation at lower temperature and lower UV doses. Nevertheless, there is still a lack of information on a proper physical method for elimination of *BC* from important legume crop seeds, such as lentil.

The objective of this experiment was to determine the feasibility of control or eradication of seed-borne *BC* from lentil seeds in a microwave fluidised-bed drier. For this purpose, the

effect of different process parameters on moisture loss, temperature, seed viability and infected seed percentage (IS%) were investigated.

7.3. Materials and Methods

7.3.1. Lentil Seeds preparation

Red lentil seeds (Type Bolt) were purchased from PBseeds Co, Horsham, Victoria, Australia. The 100-seeds weight was 4.27 ± 0.09 g and their average diameter and thickness, which were measured using a digital calliper, were 5 ± 0.19 and 2.4 ± 0.14 mm (average of twenty seeds). The initial moisture content was measured by drying 10 g of unground seeds at 130 °C for 20h and measuring the weight difference before and after drying ([Tang & Sokhansanj, 1991](#)). To reach the desired moisture content, a calculated volume of sterilised distilled water was added to a known amount of dried seeds in a resealable polyethylene bag and after thorough shaking, they were kept in a refrigerator for at least one week to let all the water absorbed by the seeds, and used in the experiment within one month, to make sure the seed viability did not change in storage, especially for moisture contents of above 17%. During the first week of storage, before the start of the experiment, seeds were mixed two times per day to make sure moisture was uniformly distributed among the seeds.

7.3.2. Inoculation of lentil seeds

Botrytis cinerea (174/02) spores, isolated from lentil seeds in Australia in 2002, were provided by Dr. Jenny Davidson, from the South Australian Research and Development Institute (SARDI). The isolate was subcultured on potato dextrose agar (PDA) and incubated at 22°C, 12h/12h dark/light cycle under fluorescent (OSRAM TLD/18W) and near ultraviolet (UV) lights (PHILIPS BLB/18W). After two weeks, when the cultures were full of conidia, the spore solution was prepared by flooding with sterilized distilled water (SDW) (containing 0.01% Tween 20) on plates of BC on PDA and harvesting the spores by gently rubbing the surface of the culture using a glass rod. About 100 ml of spore solution, with 10^5 - 10^6 spore ml⁻¹, was obtained from two plates.

Inoculation of the lentil seeds was done according to the method described by [Burgess et al. \(1997\)](#). Briefly, lentil seeds were surface sterilized with Sodium Hypochlorite (1%) for one minute, followed by rinsing three times with sterile distilled water. Sterilized seeds were placed on a sterilized cheesecloth in a safety biological cabinet to let the excess water dry before putting them inside an autoclaved Scott bottle. The spore solution was diluted with sterilized distilled water to reach a concentration of 10^4 - 10^5 spore ml⁻¹. This solution was poured onto the lentil seeds in the Scott bottle to reach 10^3 spores seed⁻¹. The spore

solution's volume was enough to wet all the seeds (for red lentil 25 ml per 100 g). After pouring the spore solution, the bottle was tightly closed and mixed thoroughly and left overnight in the dark to make sure the spores are attached to the seeds. The bottle was shaken two times to make sure the spores are attached to all the seeds. On the next day, the inoculated seeds were spread on sterilized aluminium foil in a biological safety cabinet and weighed regularly to reach the required moisture content of 10.5 and 18.5% (wb). The moisture contents were calculated from the weight loss of the seeds (by evaporation in the laminar hood) by knowing the initial moisture content (10.5%), initial weight of the seeds and weight of the added water. To reach the initial moisture content, they were left for three nights. After reaching the desired moisture contents, seeds were transferred in the Scott bottles, kept at 5°C and used in the experiment within one month. Before each treatment, bottles of the seeds were taken out of the refrigerator and left overnight in the room to reach room temperature.

7.3.3. Microwave fluidised bed system and treatment of the lentil seeds

Schematic of the system is illustrated in Figure 7.1. For details of the equipment and critical parameters refer to section 8.3.3 (chapter 8).

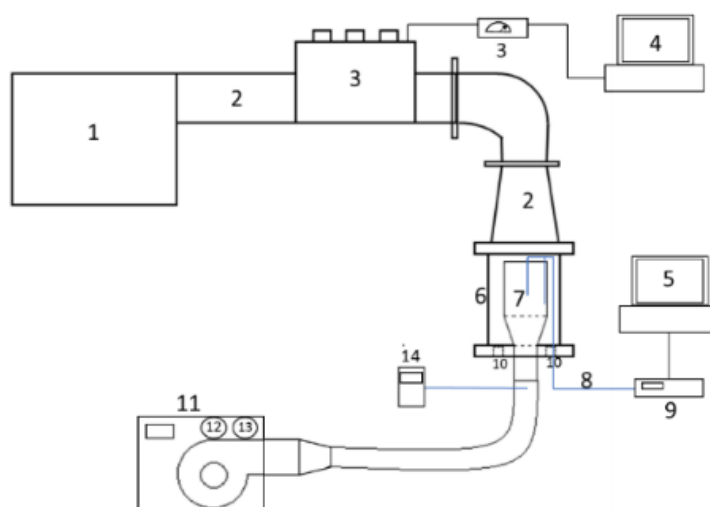


Figure 7.1 Microwave fluidised-bed system for drying and disinfection of particles, 1-Magnetron, 2-Waveguide, 3- Three-stub tuner, 4- Absorbed power monitoring, 5- Data acquisition system and microwave power control, 6- Microwave cavity, 7- Sample holder (fluidised bed), 8- Fibre optic probes, 9- Temperature monitor, 10- Vents, 11- Air blower and heater, 12- Air speed potentiometer, 13- Air temperature potentiometer, 14- Inlet air temperature monitor; bigger dashed line is the Teflon perforated plate for holding the samples and the smaller dashed line in the metal perforated plate for inlet air

For each treatment, 90 g of healthy seeds were mixed with 10 g of inoculated seeds, which their surfaces were marked with a small point using a red marker. The effect of marking on the growth of fungi compared to the unmarked seeds was evaluated by subculturing both, and it was proved that the marking did not have any effect on the reduction of the fungal

growth. The total of 100 g of seeds was transferred into the sample holder with a perforated bottom. Three fibre optic sensors were placed in the hot spot (top centre of the bed), the cold spot (corner of the cylindrical sample holder) and at the bottom of the sample holder, where the air enters. This was considered as inlet air temperature. Hot and cold spots were obtained from the results of simulation of microwave power distribution in XFDTD® 7.5.0 (Remcom, Inc., State College, PA) using dielectric properties ([Taheri et al., 2018](#)) and physical properties ([Gharibzahedi et al., 2014](#); [Tang et al., 1991b](#)) of red lentil seeds. Autodesk Inventor Professional 2017 (Autodesk Inc., USA) was used to design the 3-D geometry of lentil seeds with length of 6.8 and width of 2.4 mm and 36 mm bed thickness (15 layers of lentil seeds), which was then placed in the sample holder inside the microwave cavity to perform the simulation (for the information about the simulation refer to section 6.3.3.1 and section 6.3.3.3).

Here, the real dimensions of the lentil seeds were not considered, because the real dimensions were too small, and the computer's space was not enough to solve the finite-difference-time-domain equations. However, the thickness of 36 mm was the same as 100 g of red lentil at 18.5% MC, which was used in the experiment. The amount of sample was selected, firstly, for having a proper power density, which did not increase the hot spot temperature more than 75-80°C and harm the seeds and secondly, for having a thickness less than the penetration depth of the microwave at 2450 MHz in lentil seeds ([Taheri et al., 2018](#)). As the seeds are fluidised by hot air, fibre optic probes did not show the real seed surface temperatures and it is assumed that the measured temperatures are an average of air and seeds surface temperature, which is henceforth called bed temperature. Air speed was adjusted to fluidise the bed. Turning the potentiometer of the flow rate, a fracture below 560 l min⁻¹ turned the fluid bed into a static bed.

7.3.4. Evaluation of lentil seed viability and vigour after treatments

Seed viability was examined by the standard germination test. Twenty seeds per replicate and a total of 60 seeds per treatments were placed on Whatman filter paper no.1 in 9-cm petri dishes. Each plate contained 10 seeds which were wetted by 4 ml of sterilized distilled water. Germination of the seeds was examined after two and five days. Vigour of the seeds was evaluated by measuring electrical conductivity (EC) of the seed soaking water according to [ISTA \(2016\)](#). Following each treatment, 50 lentil seeds were counted and, accurately weighed and placed in 250-ml Erlenmeyer flasks. One hundred ml distilled water was poured in each container to cover the seeds and provide enough depth to soak the seeds. The containers' heads were covered with aluminium foil and after 24 h ± 15 min

electrical conductivity of the soaking water was measured using an EC probe (model HI98304, HANNA Instruments Inc., Melbourne, Australia) with a resolution of 0.01 mS cm⁻¹ and accuracy of ±2% F.S. at 25°C. The result was calculated according to equation (7.1). Distilled water was used as a blank for background reading of conductivity and the test was carried out in triplicate.

$$\begin{aligned} &\text{Conductivity } (\mu\text{S cm}^{-1} \text{ g}^{-1}) \\ &= \frac{\text{Conductivity reading } \left(\frac{\mu\text{S}}{\text{cm}}\right) - \text{Background reading}}{\text{Weight of seeds}} \end{aligned} \quad (7.1)$$

7.3.5. Evaluation of BGM pathogen viability after treatments

Botrytis selective media (BSM) was prepared according to [Burgess \(1997\)](#) and [Edwards&Seddon \(2001\)](#). The media contained (g l⁻¹): glucose 2; PDA 5; Agar 10; tannic acid 5; Ridomil 0.02; Pentachloronitrobenzene (PCNB) solution 0.5% in ethanol 4; Zineb 0.00091; and streptomycin sulphate 0.1. a mixture of glucose. The PDA and Agar were autoclaved before adding to the rest of the ingredients.

After each treatment, the contaminated seeds, which had been marked, were separated and 30 seeds per replicate with a total of 90 seeds per treatment were subcultured on BSM. The plates were incubated at 23°C and after 4 and 10 days were examined to see the pathogen's growth. BC was recognized, based on the appearance of the mycelium and their conidia under microscope (after 2 weeks) as well as the colour change of the selective media to brown, which was the result of tannic acid oxidation.

7.3.6. Evaluation of thermal resistance of the BGM pathogen in a water bath

To see the susceptibility of the pathogen to thermal treatment at different seed moisture contents, five grams of inoculated lentil seeds at moisture contents of 10.5% (initial), 12.5%, 14.5%, 16.5% and 18.5% (wb) were placed inside a resealable polyethylene bag and immersed in a water bath at temperatures 60, 65, 67, 70, 75 °C for 10 min. The seeds were sub-cultured on BSM and examined for the percentage of infected seeds after 4 and 10 days. Five-Minute exposure time was also examined for those temperatures, which were effective in 100% elimination of the pathogens. The results of these treatments helped the selection of the microwave power to treat the seeds with selected moisture content. The powers were selected to raise the seed surface temperatures to those which were lethal to the pathogen so that the bed temperature was at least 5°C above these temperatures. It must be noted that, here, the seeds were not directly exposed to water, which creates 100% water activity and lowers the lethal temperature to its minimum. The seeds were sealed in the resealable

polyethylene bag and the moisture was not allowed to scape, and water activities could be considered as the seeds water activity at the corresponding moisture content at each temperature. If the exposure time is enough to reach the equilibrium in the closed bags, relative humidity (RH) might be considered close to the water activity.

7.3.7. Experimental design and statistical analysis

To investigate the effect of different process parameters on moisture loss, final temperature, germination, and frequency of pathogen isolation from the seeds or percentage of infected seeds (IS%), an experiment was designed with four factors, each with 2 or 3 levels and with 3 replications. Factors and levels were selected as follow: moisture content (MC) at 10% (dry form) and 18% wet base (wb); air temperature set at 50 and 60 °C; microwave forwarded power at 0, 200, 300 W for 18% MC and 0, 400, 500 W for 10% MC; and exposure times of 5 and 10 min. The set air temperature of 50 and 60°C was measured as 50±1 and 57±1 °C at the inlet of the sample holder. Forwarded powers were adjusted as it was explained in section 5.3.2.2 (chapter 5).

This experiment was set out as a randomized full factorial design in Minitab version 18.1 (Minitab Inc., Pennsylvania, USA). The effect of microwave power, air temperature, exposure time and initial moisture content on final sample temperature, moisture loss, seed viability and percentage of infected seeds were investigated using a general factorial regression and analysis of variance. The most effective factors were found from a Pareto chart of the standardized effects with $\alpha = 0.05$. Significant differences from the control were analyzed by one-way analysis of variance. All the statistical analysis was performed in MINITAB Statistical Software version 18.1 (Minitab, Inc., Pennsylvania, USA) and presented data are mean of three replicates.

7.4. Results and discussion

7.4.1. Simulation of power density inside the single mode cavity

Simulated microwave power dissipation inside 100 g of lentil seeds are shown in Figure 7.2. As can be seen in the power distribution in the bottom layer of the lentil seeds (Figure 7.2 (a)), the hot spot of the microwave radiation is predicted to be in the centre of the sample and the cold spot is along the circumference of the cylindrical sample holder (for the axis x, y and z refer to Figure 6.2(a), Figure 6.4(b), Figure 6.12(a)). The same distribution was obtained for the top layer of the samples (data not shown). In Figure 7.2 (b), the maximum and average predicted microwave power density are represented (for the definition of the maximum and minimum powers refer to section 6.3.3.3). Here, the maximum microwave

power is predicted to be on the top layer of lentil samples and the minimum power is absorbed in the middle layers, 20-25 mm distance from the top. Therefore, the hot spot was in the centre of the top layer and the cold spot in the corner of the middle layer. Fibre optic probes were placed in these two locations to obtain hot and cold spot temperatures during the treatments. Additionally, hot air was assumed to be evenly distributed in the bed after entering through the perforated plate.

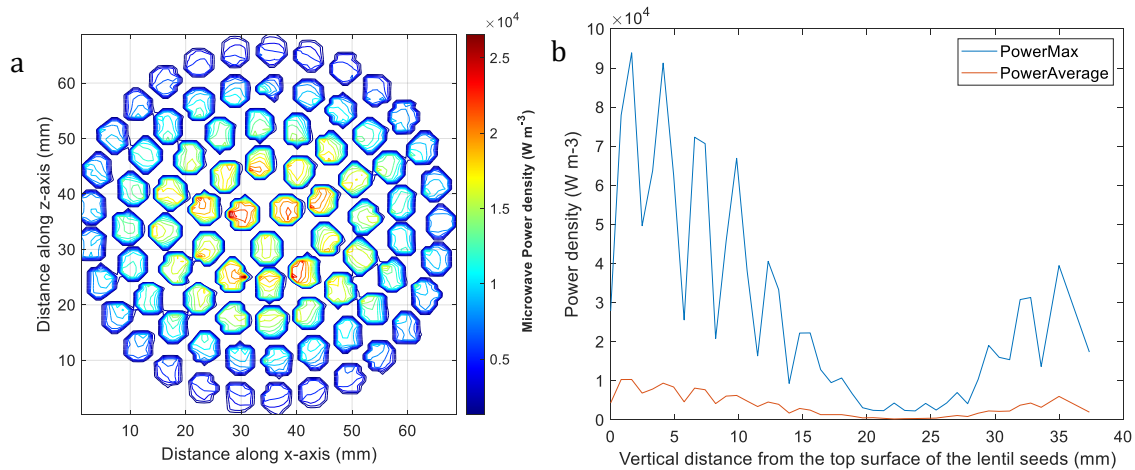


Figure 7.2 Power dissipation in lentil seeds, the result of simulation; (a) Absorbed power density in a cross section of the bottom layer of lentil seeds (x-z); (b) Vertical (y-axis) maximum and average power density in 100 g of lentil seeds in a cylindrical sample holder (W m^{-3})

7.4.2. Thermal sensitivity of the BGM pathogen in a water bath

Results of thermal sensitivity of the BGM pathogen on lentil seeds, with different seed MCs, are shown in Figure 7.3. A 10-min exposure time led to complete eradication of the pathogens from the lentil seeds at 65°C for seed MC of 18.5%, 16.5%, and 14.5%, at 70°C for 12.5% seed MC and at 75°C for 10.5% MC (Figure 7.3(a)). A temperature of 65°C was also lethal to seed pathogen after 5 min exposure of the contaminated seeds at 18.5% and 16.5% MC. However, seeds with MC of 14.5%, 12.5%, and 10.5% required temperatures of 70°C, 75°C and 80°C, respectively to remove the pathogen completely, after 5 min. [Taheri et al. \(2019d\)](#) also found that Ascochyta blight pathogen of lentil seeds could be reduced more at higher seed moisture content and the same final temperature, by applying microwave treatment.

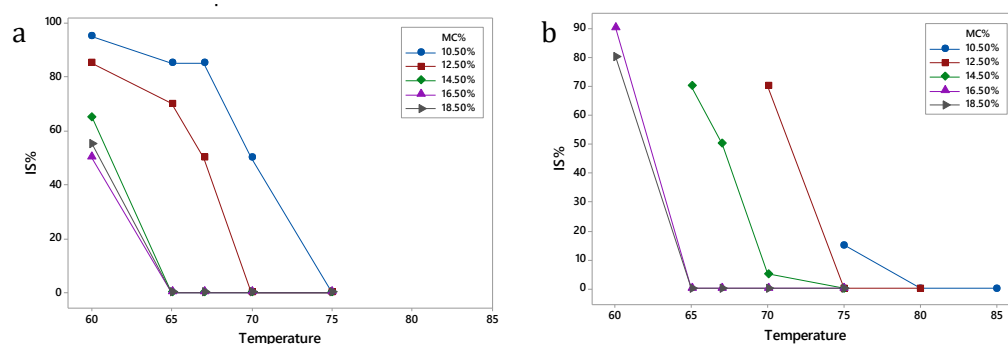


Figure 7.3 Percentage of *Botrytis grey* mould infected lentil seeds after treatments in a water bath at different temperatures (°C) after (a) 10 min and (b) 5 min. IS%- infected seeds percentage; MC%-seed moisture content(w.b.)

7.4.3. Effect of microwave power, air temperature and exposure time on moisture loss and final temperatures of lentil seeds

Results of the Pareto chart of the standardized effects from general factorial regressions ($\alpha = 0.05$) for moisture loss and bed temperature are represented in Figure 7.4, (a) and (b) respectively. The x-axis of these charts shows the coefficient of each factor in the regression model divided by its standard error. The factors, with the standardized effect of more than 2.04 (red vertical line), have significant effects on the response and the more the standardized effect is, the more effective the factor will be. Pareto chart of moisture loss (Figure 7.4(a)) suggested that all the factors including seed moisture content, microwave power, air temperature and exposure time significantly affected the seed moisture loss, whereas, seed moisture content being most effective factor followed by exposure time. Additionally, microwave power had more contribution to moisture removal than the air temperature. According to Figure 7.4, (b) lentil seeds bed temperature was mostly affected by air temperature followed by microwave power, while other factors did not have any significant effect. Final lentil bed temperature is shown in Figure 7.5 The lentil seeds' bed temperature corresponded with increasing air temperature and microwave power. Its maximum value was 70°C at the power of 400 W for 18.5% MC and 500 W for 10.5% MC and air temperature of 60°C. Lentil seeds' surface temperatures were different from the bed temperature as the bed temperature was an average of seed surface and air temperature.

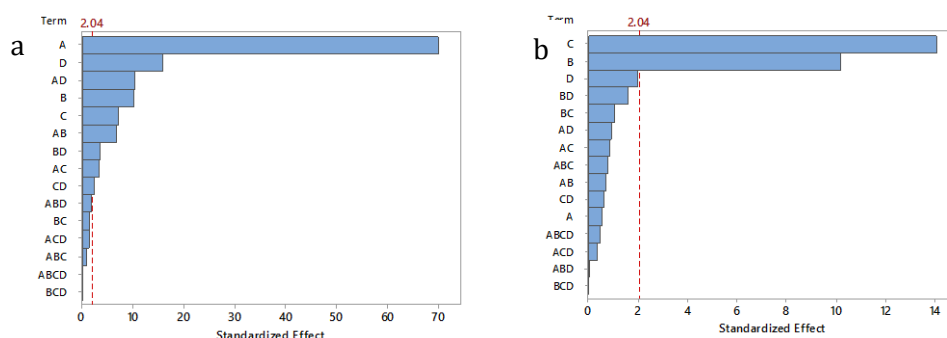


Figure 7.4 Pareto Chart of the standardized effects for (a) Moisture loss and (b) Bed temperature with $\alpha = 0.05$; A = moisture content (10.5%, 18.5%), B = microwave power (0, 300, 400 W for 18.5% MC and 0, 400, 500 W for 10.5% MC), C = air temperature (50, 60°C), D = exposure time (5, 10 min)

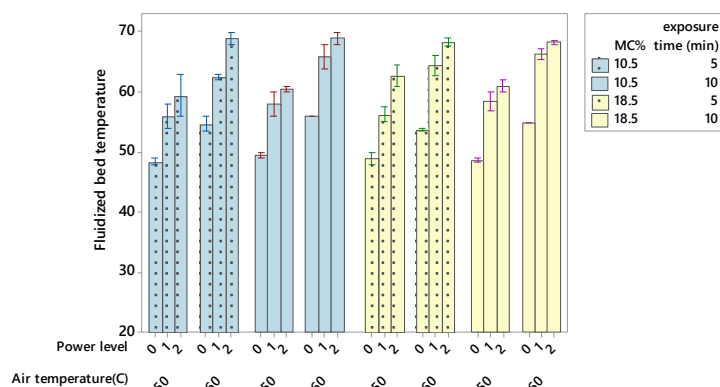


Figure 7.5 Final bed temperature at two set air temperatures of 50 and 60°C, two exposure times of 5 and 10 min, microwave powers of 0, 300, 400 W for 18.5% MC (power level of 0, 1, 2 respectively) and 0, 400, 500 W for 10.5% MC (power level of 0, 1, 2 respectively); bars are SE from the mean; MC-moisture content

7.4.4. Effect of microwave power, air temperature and exposure time on seed viability and percentage of infected lentil seeds at each seed moisture content as a categorical factor

Generally, initial seed moisture content had by far the most influence on IS% using general full factorial regression with $R^2 = 79\%$, F-Value = 42.62 and P-Value = 0.000. Therefore, statistical analysis for seed viability and IS% were carried out separately for each of moisture contents of 10.5% and 18.5%. The Pareto chart of standardized effects for germination percentage and infected seed percentage counted after 4 days (IS%-4d) for treated lentil seeds at 18.5% MC is shown in Figure 7.6, (a) and (b) respectively. Both microwave power and air temperature had a significant effect on seed germination and IS%-4d. However, the effect of microwave power on IS%-4d was more than the effect of air temperature, while they affect seed germination almost equally. According to the results of main effect plots for the seeds at 18.5% MC, IS% decreased with increasing microwave power, air temperature and exposure time. Similarly, seed germination was negatively affected by increasing air temperature and microwave power from 300 to 400 W, while

increasing microwave power from 0 to 300 W had a positive effect on seed germination. For 10.5% MC, the general factorial regression did not provide a good fit and hence, no conclusion was derived about the effect of process parameters on IS% and seed viability.

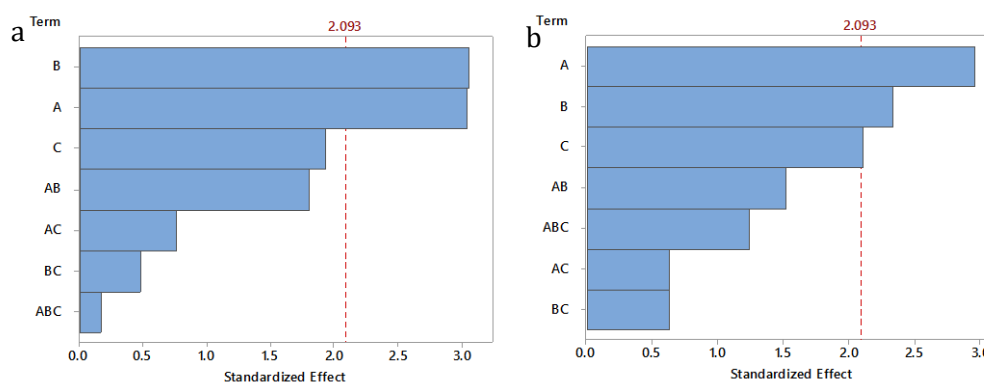


Figure 7.6 Pareto Chart of the standardized effects for (a) Germination percentage and (b) Infected seed percentage counted after 4 d of lentil seeds at 18.5% MC; A = microwave power (0, 300, 400 W), B = air set temperature (50, 60 °C), C = exposure time (5, 10 min); $\alpha = 0.05$

Table 7.1 represents the results of germination percentage (G%), infected seed percentage counted after 4 days (IS% 4d) and 10 days (IS% 10d), and electrical conductivity of soaking water (EC) after microwave fluidised bed treatments of lentil seeds at two moisture contents of 10.5% and 18.5% with exposure times of 5 and 10 min. For seeds at 10.5% MC, none of the treatments significantly ($p > 0.05$) affected germination, electrical conductivity or IS%, even at bed temperature of close to 70°C (seed surface temperature of 75-80°C), which was obtained by microwave power of 500 W and air temperature of 60°C and exposure time of 10 min, while in the experiment with the water bath, the pathogen was eliminated at 75°C. The difference between these results could stem from the difference between relative humidity (RH) around the seeds at the time of treatments. Equilibrium RH around the seeds (water activity of the seeds) at 10.5% MC at 60°C is between 60 to 75% (Menkov, 2000) and as the water activity (and corresponding equilibrium RH) of the carbohydrate/protein rich foods increases by increasing temperature (Syamaladevi et al., 2016), RH of the seeds sealed in the bag in the water bath at 10.5% MC and temperature of 75°C will be more than 60%. However, in microwave fluidised bed treatments, RH of air at the mentioned speed and temperature of 60 °C was measured as 11%, when ambient RH was about 50%, and this amount can be even less when the microwave is also used in combination with hot air. Therefore, the very low RH in the microwave fluidised bed could be the reason for increasing the lethal temperature of the seed pathogen. The other reason, which was mentioned by Syamaladevi et al. (2016), might be very fast desiccation of the

pathogen in the microwave fluidised bed which could increase the pathogen's resistance to irreversible damage due to high temperatures.

Results for seeds with 18.5 % MC showed that by applying microwave power of 300 W and air temperature of 60°C for 10 min, IS% counted after 10 d (IS%-10d) reduced from 82% to 55% without any significant loss of viability. IS% -10d was reduced from 82% to 51.6% by applying microwave power of 400 W and air temperature of 60°C for 10 min; however, germination was also decreased from 95% to 60%. No significant change in electrical conductivity was observed as a result of these treatments. Here again, the difficulty of eliminating the seed pathogen could be related to the lower RH around the seeds. Nevertheless, it was more feasible to reduce the pathogen's load at higher MC of 18.5% than the dry seeds.

BGM pathogen growth on the seeds in BSM was evaluated on day 4 and 10, as after 4 days the pathogen mycelium, as well as the separated brown areas on BSM (tannin oxidation) were clearly discriminated and were comparable with the non-treated seeds. After 10 days, the mycelial growth was more and IS% in almost all the plates, including the control. The difference between the treated and control samples was less than that on day 4. After 2 weeks of incubation, conidia were observed on the mycelium, however, the mycelium grew beyond the initially contaminated seeds and infected healthy lentil seeds. Conclusively, reduction of IS% was considered after 4 (start of the growth) and 10 days (when healthy seeds could still be separated).

Table 7.1 Germination percentage (G%), infected seed percentage counted after 4 days (IS% 4d) and 10 days (IS% 10d), and electrical conductivity of soaking water (EC) after microwave treatments at different powers and air temperatures for 5 and 10 min; values with * are significantly different from their control based on Dunnett multiple comparison with $\alpha = 0.05$ ¹

Moisture content% (wb)	MW power (W)	Set temperature (°C)	air	Exposure time															
				5 min								10 min							
				G%	SD	IS% 4d	SD	IS% 10d	SD	EC (μ S cm ⁻¹ g ⁻¹)	SD	G%	SD	IS% 4d	SD	IS% 10d	SD	EC (μ S cm ⁻¹ g ⁻¹)	SD
18.5	0	50		95.0	7.1	67.5	3.5	80.0	7.1	96.0	9.3	86.7	14.4	48.3	18.9	68.3	14.4	95.3	1.6
		60		85.0	0.0	55.0	0.0	70.0	7.1	92.0	9.1	85.0	10.0	66.7	5.8	82.0	2.9	96.7	5.0
	300	50		96.7	2.9	53.3	16.0	75.0	18.0	85.4	5.4	92.5	3.5	47.5	3.5	72.5	3.5	93.0	0.4
		60		91.7	7.6	40.0	10.0	68.3	5.8	102.7	11.4	90.0	10.0	26.7*	7.6	55.0*	0.0	100.0	6.9
	400	50		95.0	0.0	57.5	3.5	77.5	3.5	96.9	2.3	82.5	3.5	42.5	3.5	60.0	0.0	91.5	7.4
		60		73.3*	12.6	40.0	22.9	71.6	12.6	103.8	0.8	60.0*	13.2	20*	18.0	51.6*	11.5	97.6	5.1
	control			95.0	5.0	58.3	10.4	82.0	12.6	98.7	6.7	95.0	5.0	58.3	10.4	82.0	12.6	98.7	6.7
10.5	0	50		80.0	14.1	50.0	7.1	85.0	0.0	82.6	2.3	92.5	3.5	65.0	21.2	95.0	7.1	84.6	3.9
		60		100.0	0.0	57.5	3.5	90.0	14.1	90.9	8.1	92.5	3.5	50.0	14.1	90.0	0.0	87.4	5.6
	400	50		90.0	0.0	42.5	10.6	92.5	3.5	84.3	6.0	85.0	7.1	45.0	28.3	90.0	0.0	80.7	9.9
		60		95.0	7.1	62.5	17.7	97.5	3.5	85.1	10.5	85.0	0.0	50.0	0.0	90.0	0.0	87.3	14.7
	500	50		87.5	3.5	67.5	10.6	90.0	0.0	86.1	8.9	95.0	0.0	67.5	10.6	92.5	10.6	87.2	5.4
		60		95.0	7.1	55.0	7.1	87.5	10.6	91.7	1.8	95.0	0.0	52.5	17.7	85.0	7.1	81.8	4.9
	control			97.5	3.5	80.0	14.1	100.0	0.0	87.5	15.5	97.5	3.5	80.0	14.1	100.0	0.0	87.5	15.5

¹ MW-microwave, G%-germination percentage, IS%- infected seed percentage, d - day, EC- electrical conductivity, SD- standard deviation

7.5. Conclusion

Microwave fluidised bed processing was developed in this study to evaluate its potential for eradication of BGM pathogen from lentil seeds. The effect of process parameters on moisture loss, bed temperature, seed germination and infected seed percentage (IS%) were investigated. Most effective factors on moisture loss and bed temperature were seed moisture content and air temperature, respectively and microwave power was the most effective factor in IS% reduction. Air temperature and microwave power equally affected seed germination. Additionally, it was observed that eradication of BGM pathogen from lentil seeds in a microwave fluidised bed was more difficult than doing this in a sealed polyethylene bag in a water bath at the same temperature, which was concluded to be due to the difference in RH of the air around the seeds at the time of treatment. It is therefore recommended to investigate the effect of increasing RH of inlet air on seed pathogen reduction in future studies. BGM pathogen reduction, without significant seed viability loss in a microwave fluidised bed, was obtained by applying microwave power of 300 W and set air temperature of 60°C on the seeds with a moisture content of 18.5% for 10 min. This gave a 27% reduction in IS% from 82% to 55%. Therefore, microwave fluidised bed dryer can be considered as part of integrated disease management of BGM of lentil if the seed moisture content and process parameters are standardized for effective irradiation of the pathogen inoculum from the lentil seeds.

The result of this chapter showed that the exposure time was an effective factor in the reduction of infected seeds at high seed moisture content. Having considered this result, an experiment with prolonged exposure time with higher seed moisture content, which is indeed drying the seeds to their safe storage moisture content, was designed in the next chapter to study the simultaneous drying and disinfection of the lentil seeds.

Chapter 8. Microwave fluidised bed drying of red lentil seeds: drying kinetics and reduction of Botrytis grey mould pathogen

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8.1. Abstract

Botrytis grey mould (BGM) is a problematic seed-borne disease of lentil crop, with little known about its possible physical control. Microwave fluidised bed dryers have been widely studied for grain, fruit and vegetable drying. In the present study, simultaneous drying and disinfection of red lentil seeds, were investigated in a microwave fluidised bed at 4 microwave powers of 0, 300, 400 and 500 W using fluidising hot air at 50 and 60 °C. Moisture diffusivities of lentil seeds were defined by considering the Fick's second law of diffusion in one direction in a sphere with convective boundary conditions as well as negligible external resistance to mass transfer. They varied from 0.44×10^{-10} for hot air alone at 50 °C to $3.06 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ for microwave power and air temperature combinations of 500 W and 60°C by considering convective boundary condition for the seeds. Drying times obtained from well-fitted Page's model were applied for drying of the seeds contaminated with BGM. The results indicated that infected seed percentage (IS%) could be reduced by 30% by applying combinations of microwave power and hot air at 300 W and 60°C or 400 W and 50°C, without a significant loss of seed viability. Thus, microwave fluidised bed drying has a good potential to reduce seed born BGM in lentil crop.

8.2. Introduction

Lentil is one of the important pulse crops, which is a valuable source of protein, carbohydrate, and fiber as well as essential vitamins and minerals. However, there is a wide range of fungal diseases which affect the plant during different stages of growth. Some of these diseases have proven to be seed-borne, which necessitate seed treatment before sowing ([Taylor et al., 2007](#)). Botrytis grey mould (BGM), caused by *Botrytis cinerea* (BC), is one of the serious diseases of lentil, especially in wet and humid climates. This is a seed borne pathogen and there is no relationship between the external appearance of the seeds and the frequency of isolation of the pathogen from the seeds ([Burgess, 1997](#); [Lindbeck et al., 2009](#)). There has also been some evidence that grey mould disease is seed-borne in other

plants, such as *Primula* and lettuce, and could transmit from symptomless seeds ([Barnes & Shaw, 2003](#); [Sowley et al., 2010](#)). However, the level of seed infection or initial inoculum in each seed is an important factor contributing to the transmission of the disease from seed to seedling. Therefore, the reduction of seed inoculum could be helpful in preventing the transmission of the pathogen.

Lentil seeds should be harvested at a moisture content of at least 15% to avoid mechanical damage and be further dried to below 12% for their safe storage. If the seeds are to be stored at a moisture content of more than 12%, they need to be air cooled during the storage to prevent seed viability loss and growth of storage fungi ([GRDC, 2018c](#)).

Microwave processing has shown great potential in drying, disinfection, and disinfestation of grain seeds ([Reddy et al., 1998](#); [Taheri et al., 2019d](#); [Wray & Ramaswamy, 2015](#); [Yadav et al., 2014](#)). However, there remain some concerns over the non-uniformity of heat, which is an intrinsic characteristic of microwave heating. Applying hot air to create a fluid or spouted bed can provide agitation in the particles to obtain better microwave energy distribution in the material. Although, utilizing air to fluidise particles in a microwave cavity can be a good solution for making the temperature distribution uniform ([Feng & Tang, 1998](#); [Nawirska-Olszańska et al., 2017](#); [Wang & Chen, 2000](#)), it is crucial that the best “microwave power-air temperature” combination be used in the drying process to have both temperature uniformity and energy efficiency. If the microwave power is too high, the particles’ surface temperature will be much higher than the air temperature and air will have a cooling effect rather than supplying evaporation energy ([Kudra, 1989](#); [Wang & Chen, 2000](#)). In this scenario, microwave energy will be spent on warming up the air.

The energy efficiency and time-saving of a combined process, including microwave heating, along with quality changes in the food, should be compared with conventional methods to assure the advantage of the new method. [Reyes et al. \(2007\)](#) compared a tunnel dryer, a fluidised bed and a microwave fluidised bed dryer for drying of potato slices. They observed that drying was 70% faster in the fluidised bed dryer than the tunnel dryer and by applying microwave energy in the fluidised bed system, the time was further reduced by 85%. [Goksu et al. \(2005\)](#) also reported that adding 2.1 and 3.5 W g⁻¹ of microwave energy to a fluidised bed dryer, for drying macaroni beads, reduced drying time by 50%. They indicated improved diffusivity by increasing air temperature and microwave power. [Zare&Ranjbaran \(2012\)](#) showed that 4.3 and 5.3 W g⁻¹ of microwave power and an air temperature of 50 and 60°C for drying soybeans, reduced energy usage by more than 90%.

Although grain drying in microwave and microwave assisted technologies has been studied, there is not enough information on the effect of such drying processes on post-harvest pest and pathogen control. In a study, [Pande et al. \(2012\)](#) evaluated disinfection and disinfestation of green gram seeds as well as their nutritional quality after microwave drying to safe storage moisture contents. They showed that by optimizing microwave power to 808 W and exposure time to 80 s, green gram seeds could be dried from moisture contents of 13.4% to 8.9% with 99.5% reduction in infesting insects and no fungal growth on Potato Dextrose Agar. There has also been a recent investigation of the effectiveness of microwave drying of thick layers of rice on its microbial counts using an industrial 915-MHz microwave ([Smith & Atungulu, 2018](#)). An optimization technique was applied in this study to minimize microbial counts using specific microwave energy for drying, which itself was a function of microwave output power, exposure time and rice mass or thickness. They concluded that applying a microwave specific energy of 692.79 kJ kg⁻¹, significantly reduced *Aspergillus flavus* mould from the rice seeds while drying the seeds from moisture content of 24% to 12.5%. Conclusively, the simultaneous study of drying and disinfection could be a novel tool to evaluate microwave potential in the grain industry. However, there is still a lack of information about the effect of this process on important seed borne pathogens of grains, such as BGM, in lentil seeds during drying. In addition, drying kinetics and effective diffusivity of lentil seeds had been examined before in conventional thin layer dryer ([Tang, 1994](#)), microwave alone ([Iik et al., 2011](#)) and microwave-infrared combination drying ([Chayjan & Radmard, 2016](#)), but there is no information about their moisture diffusivity in microwave assisted fluidised bed dryer.

Therefore, the objectives of this study were, Modelling the drying curves of lentil seeds in a microwave fluidised bed; Defining required exposure times to reach a specific moisture content, using an empirical model; Determining effective moisture diffusivities of lentil seeds by considering convective boundary conditions from the seed surface or constant seed surface moisture content; and treatment of lentil seeds, contaminated with BGM, at defined exposure time to specify how much of the seed borne BC could be eliminated from lentil as part of its drying process without significant seed viability loss.

8.3. Materials and methods

8.3.1. Lentil seed preparation

Red lentil seeds (Type Bolt) were purchased from a local grower located in Horsham, Victoria, Australia. The initial moisture content was measured by drying seeds at 130°C for

20h ([Tang & Sokhansanj, 1991](#)). The 100-seed weight was 4.27 ± 0.09 g and the average diameter and thickness, measured by a digital caliper, were 5 ± 0.19 and 2.4 ± 0.14 mm (average of twenty seeds). To reach 16.5% moisture content (wb) from an initial amount of 10.5%, 7.2 g of sterilised distilled water was added to each 100 g of dry seeds in a ziplock bag and after thorough shaking, they were kept in a refrigerator for at least one week and were used in the experiment within one month, to make sure the seed viability did not change during storage. During the first week of storage, seeds were mixed two times per day to make sure moisture was uniformly distributed among the seeds ([Rajagopal, 2009](#)).

8.3.2. Lentil seed inoculation with the BGM pathogen

Botrytis cinerea (isolate: 174/02) (BC), which was isolated from lentil seeds in Australia in 2002, were provided by Dr. Jenny Davidson, South Australian Research and Development Institute (SARDI). The isolate was subcultured on potato dextrose agar (PDA) and incubated at 22°C, 12h/12h dark/light cycle under fluorescent (OSRAM TLD/18W) and near Ultraviolet (UV) lights (PHILIPS BLB/18W). After two weeks of incubation, the spore solution was prepared by flooding sterile distilled water (SDW) plus 0.01% tween 20 on the plates. The spores were harvested by gently rubbing the surface of the culture using a glass rod following by passage through two layers of sterilized cheesecloth to obtain the spore solution.

Lentil seeds were inoculated with the BGM pathogen according to the method suggested by [Burgess et al. \(1997\)](#) with minor modification. Lentil seeds were surface sterilized with sodium hypochlorite 1% for one minute, followed by three times rinsing with SDW. Sterilized seeds were placed on a sterilized cheesecloth in a safety biological cabinet to let the excess water dry before placing them inside an autoclaved Scott bottle. The spore solution was diluted with SDW to reach the concentration of 10^4 - 10^5 spore ml⁻¹. This solution was poured on the lentil seeds in the Scott bottle to reach 10^2 - 10^3 spore seed⁻¹. The spore solution's volume was enough to wet all the seeds (for red lentil 25 ml per 100 g). After pouring the spore solution, the bottle was tightly closed, mixed thoroughly, and left overnight in the dark to make sure the spores were attached to the seeds. The bottle was shaken gently 3 times during the first 2 h and then two more times during the next 2 hours, if any water was observed at the bottom of the bottle, to make sure water dispersed evenly in the seedbed and the spores were attached to all the seeds. Next day, the inoculated seeds were spread on sterilized aluminum foil in a biological safety cabinet and weighed regularly to reach the required moisture content. The moisture content of the seeds was estimated from their weight loss and confirmed by the oven method, which was previously explained.

Inoculated seeds were then transferred in the Scott bottles, kept at 5°C and used in the experiment within one month. Before each treatment, seeds were taken out of the refrigerator and left overnight in the room to reach room temperature.

8.3.3. Microwave fluidised bed dryer

A single mode cylindrical microwave cavity (D: 97 mm, L: 170 mm), designed to operate at a frequency of 2.45 GHz, was modified to have a microwave fluidised bed system. A sample holder was designed in Autodesk Inventor Professional 2017 (Autodesk Inc., USA) and fabricated in the workshop of the Physics Department of the University of Melbourne. The sample holder was a cylinder, made of Teflon, with an inner diameter of 66 mm and height of 111 mm with a perforated bottom to let air pass through the seeds. Perforation was performed in a squared pattern with the whole diameter of 1 mm. The cylinder was located on a conical part with an angle of 17°. The cone's height was 62 mm, to hold the bottom surface of the sample holder in the hot spot of the microwave cavity. The bottom outer diameter of the cone was 38 mm and was attached to the inlet air tube with the same inner diameter. The cavity's lid had four additional vents, with diameters of 16 mm each, to provide enough ventilation for outlet air. Microwave leakage was monitored during the treatments using a microwave leakage detector (Tenmars Electronics Co., LTD., Taipei, Taiwan) and it did not exceed 0.5 mW cm⁻². The bottom part of the cone was attached to an air tube which itself was attached to the air blower and heater. A 3700-W hot air source (Hotwind System, Leister, Kaegiswil, Switzerland) with two potentiometers, one for air flow control and one for air temperature control, as well as a monitor to show the real and set air temperatures, was utilized to provide airflow at desired speeds and temperatures. The air temperature was adjustable from room temperature to 650°C with 5°C resolution and air flow could be adjusted between 200-900 l min⁻¹ (at 20°C). The whole system is illustrated in Figure 8.1.

Microwave absorbed and reflected power were monitored using an Autotuner (S-Team – HOMER Automatic Impedance Analyzer and Matching System, Bratislava, Slovak Republic) using HomSoft software (Version 5.0.0.2). Results of absorbed powers were saved every 5min and an average of the power during the drying time was calculated for each microwave power. The temperature of the inlet air, as well as the cold and hot spots of the lentil bed, were measured using three fiber optic temperature sensors (FISO Technology Inc., Quebec, Canada) with an accuracy of ±0.1°C and a Nortech temperature monitor (NoEMI-TS series, Fibronic Inc.). The probes were fixed using pieces of tapes in the sample holder in two positions, in the centre of seeds top layer (hot spot) and close to the sample holder wall in

the middle layer of the seeds (cold spot). As the seeds were fluidising, the probes might have not measured the exact seed surface temperature. Therefore, the hot and cold spot temperatures are assumed to be close to these temperatures. Temperature data were recorded every 10 seconds using custom software, implemented using Net/C#. The hot and cold spots of the bed were obtained by simulation and validation in our previous study (refer to Figure 6.1 and Figure 6.3 (b), chapter 6).

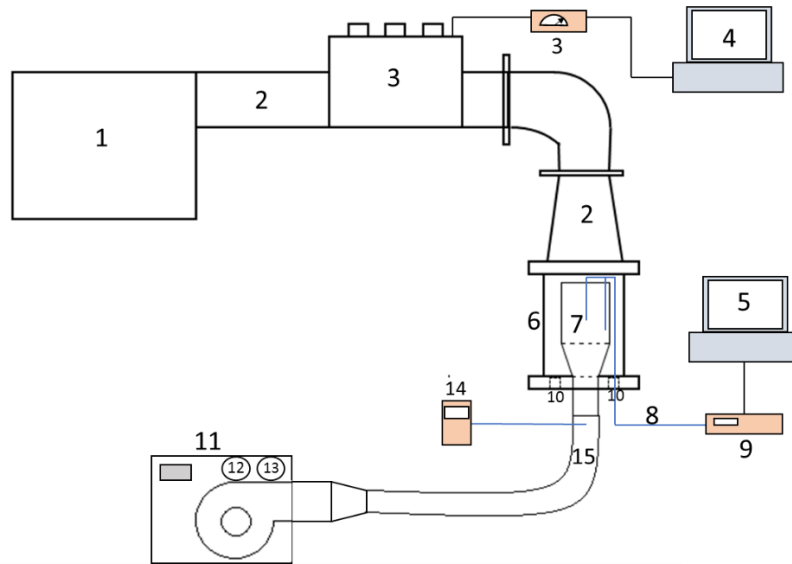


Figure 8.1 Microwave fluidised-bed system for drying and disinfection of particles, 1-Magnetron, 2-Waveguide, 3- Three-stub tuner, 4- Absorbed power monitoring, 5- Data acquisition system and microwave power control, 6- Microwave cavity, 7- Sample holder (fluidised bed), 8- Fibre optic probes, 9- Temperature monitor, 10- Vents, 11- Air blower and heater, 12- Air speed potentiometer, 13- Air temperature potentiometer, 14- Inlet air temperature monitor, 15- Air tube; bigger dashed line is the Teflon perforated plate for holding the samples and the smaller dashed line in the metal perforated plate for inlet air

8.3.4. Microwave fluidised bed drying of the seeds

Air speed was adjusted to fluidise the lentil seedbed. Turning the potentiometer, a fraction below 560 l min^{-1} (corresponding speed of 3.2 m s^{-1}), turned the fluid bed to a static bed. For each drying process, 100 g of lentil seeds with 16.5% moisture content (MC) at room temperature was weighed on a 2-kg digital scale with a precision of 0.01 g. The weighed seeds were placed inside the Teflon sample holder followed by placing the sample holder inside the microwave cavity. The sample holder was taken out every 5 min in microwave-hot air drying and every 10 min when no microwave was applied (hot air alone) and weighed on a scale with 0.01 g precision. Weighing the samples took 60 to 90 s, during which the temperature of the lentil seeds, measured by fibre optics, did not change. The moisture content of the lentil seeds at each time interval was calculated from the weight loss of the seeds and recorded to plot the drying curve and fit the drying models. Ambient and forced air temperature and relative humidity (RH) were recorded using a temperature and

humidity data logger with a temperature range of -40 to 70°C with an accuracy of $\pm 2^\circ\text{C}$ and RH range of 0-100% with an accuracy of 0.3%.

8.3.5. The empirical model applied for drying of lentil seeds

There are several empirical models which have been suggested for grain drying curves. They are summarised by [Erbay&Icier \(2010\)](#) and [Bala \(2016\)](#). One of these empirical models which has been reported to be a good fit for drying curves of grains ([Khoshtaghaza et al., 2015](#); [Tang, 1994](#)) is Page's model. It is described in equation (8.1). This model was applied in this work to fit the drying curve, relative moisture content (MR) against time, and finding the constants of k and n, and finally prediction of the required time to reach a specific moisture content of lentil seeds.

$$MR = \frac{MC - MC_e}{MC_i - MC_e} = \exp(-kt^n) \quad (8.1)$$

- MC: moisture content of the seeds at time t (dry based, db);
- MC_e : equilibrium moisture content at each air temperature and RH (db);
- MC_i : initial moisture content of the seeds (db);
- k (1 min^{-1}) and n: constants of page's model

The equilibrium moisture content at specific air temperature (T_{air} , °C) and relative humidity (RH) of the forced air was calculated according to equation (8.2) ([Tang, 1994](#)).

$$MC_e = \left[\frac{-\ln\left(\frac{RH}{100}\right)}{\exp(10.5 - 0.018T_{\text{air}})} \right]^{-0.47} \quad (8.2)$$

To find the dry based (db) MC from wet based (wb), equation (8.3) was applied:

$$MC = MC_{db} = \frac{MC_{wb}}{100 - MC_{wb}} \times 100 \quad (8.3)$$

8.3.6. Determination of effective moisture diffusivity of lentil seeds

Liquid diffusion is widely accepted to be the main mechanism in the drying of agricultural commodities in the falling rate period ([Bala, 2016](#); [Srikiatden & Roberts, 2007](#)). [Jumah&Raghavan \(2001\)](#) observed that in microwave spouted bed drying of wheat kernels during the falling rate period, the drying process was dominated by moisture diffusion inside the kernels and external mass transfer resistance could be neglected.

Lentil seed has the shape of a lens and by considering it as an oblate spheroid with dimensions of $5 \times 5 \times 2.4 \text{ mm}$ (length (a), width (a) and thickness (b) respectively), the

spherical equivalent diameter ($2r_e$) and sphericity (ϕ) were calculated using equations (8.4) and (8.5) ([Jennings, 1988](#); [Maneesh Kumar et al., 2018](#)):

$$r_e = \frac{(a^2 \times b)^{1/3}}{2} \quad (8.4)$$

$$\phi = \frac{\text{surface area of the sphere with the same volume of the particle}}{\text{surface area of the particle}} = \frac{2r_e}{a} \quad (8.5)$$

By considering $a=5$ and $b=2.4$ for lentil seeds, the equivalent radius and sphericity will be 1.95 mm and 0.8 respectively, which shows it can be considered as a sphere with a good approximation. Based on Fick's second law of diffusion for constant moisture diffusivity and negligible shrinkage, transfer of water mass in a sphere, with one-dimensional mass transfer, can be described by equation (8.6).

$$\frac{\partial MC}{\partial t} = \frac{D_{eff}}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial MC}{\partial r} \right) \quad (8.6)$$

- MC: mass of water diffusing in the solid or here dry based moisture content of the seeds
- t: time
- r: length of the direction in which diffusion occurs (radius direction of the sphere)
- D_{eff} : effective moisture diffusivity of lentil seeds

To find effective moisture diffusivity (D_{eff}) of lentil seeds from equation (8.6), moisture transfer was assumed to occur from the seed's centre to its surface through its radius. The initial and boundary conditions for solving this equation were considered as:

$$\text{at } t = 0 \text{ and } 0 < r < r_e ; MC = MC_i \quad (8.7)$$

$$\text{at } r = 0 \text{ and } t > 0 ; \frac{\partial MC}{\partial r} = 0 \quad (8.8)$$

$$\text{at } r = r_e \text{ and } t > 0 ; MC = MC_e \quad (a)$$

$$\text{at } r = r_e \text{ and } t > 0 ; -D_{eff} \frac{\partial MC}{\partial r} = \alpha (MC - MC_e) \quad (b)$$

where α is the mass transfer coefficient of the gas phase (m s^{-1}) and r_e is the equivalent spherical radius of lentil seeds. Here, MC of the seeds' surface at any time during the drying process can be considered equal to equilibrium MC (equation ((8.9)(a)) and in fact negligible external resistance is assumed ([Tang, 1994](#)), or the rate of moisture transferring from a seed to its surface can be considered equal to the rate of moisture leaving the seed

surface into the surrounding air, which is, in fact, the convective boundary condition (equation (8.9)(b)) ([Carmo & Lima, 2005](#); [Yuniarti et al., 2018](#)). Both these boundary conditions were considered to find D_{eff} for lentil seeds.

Analytical solutions to equation (8.6), for a sphere, by considering initial and boundary conditions of (8.7), (8.8) and (8.9)(a) is represented in equation (8.10) and when considering initial and boundary conditions of (8.7), (8.8) and (8.9)(b), the solution will be equation (8.11) ([Crank, 1979](#)). These solutions give the total moisture which transferred from the whole seed at any time.

$$MR = \sum_{n=1}^{\infty} \frac{6}{\pi^2 n^2} \exp\left(\frac{-D_{eff} n^2 \pi^2 t}{r_e^2}\right) \quad (8.10)$$

$$MR = \sum_{n=1}^{\infty} \frac{6L^2}{\beta_n^2 (\beta_n^2 + L^2 - L)} \exp\left(\frac{-\beta_n^2 D_{eff} t}{r_e^2}\right) \quad (8.11)$$

Where $L = \frac{r_e \alpha}{D_{eff}}$, α is the mass transfer coefficient in the gas phase ($m s^{-1}$) and β_n are positive roots of $\beta \cot \beta = 1 - L$. The summative series in equations (8.10) and (8.11) converge quickly and it has been stated that just the first element of the series could be considered with very small error under the condition that the time of drying is sufficiently long and MR is less than 0.3 ([Feng, 2000](#)) or $\frac{D_{eff} t}{r_e^2}$ is more than 0.1 ([McCabe et al., 1993](#)). By considering the first elements, equations (8.10) and (8.11) can be rewritten to become equations (8.12) and (8.13) respectively, or they can be generally written as $MR = A \exp(-Bt)$ with different A and B constants.

$$MR = \frac{6}{\pi^2} \exp\left(\frac{-D_{eff} \pi^2 t}{r_e^2}\right) \quad (8.12)$$

$$MR = \frac{6L^2}{\beta_1^2 (\beta_1^2 + L^2 - L)} \exp\left(\frac{-\beta_1^2 D_{eff} t}{r_e^2}\right) \quad (8.13)$$

To find D_{eff} from equation (8.12), data of $\ln(MR)$ were simply plotted against time to find the slope of linear plot and goodness of fit, followed by calculation of D_{eff} from the slope by knowing the equivalent radius of the lentil seeds. For equation (8.13), the whole model was fitted into the drying data using custom code in MATLAB R2018a (MathWorks Inc., Natick, MA, USA). To calculate D_{eff} from equation (8.13), firstly β_1 for different values of L were considered as represented in Table 8.1 ([Crank, 1979](#)) and coefficient A ($\frac{6L^2}{\beta_1^2 (\beta_1^2 + L^2 - L)}$) was calculated using corresponding values for L and β_1 . By having values of A from the fitted

equations, the corresponding value for β_1 was found from Table 8.1 by a linear interpolation strategy. Finally, D_{eff} was calculated from the coefficient B ($\frac{-\beta_1^2 D_{eff}}{r_e^2}$) by knowing β_1 and r_e . As can be seen from the data of Table 8.1, equations (8.12) and (8.13) are the same when L value becomes very large (infinite), which can be obtained by high values of mass transfer coefficients (α) and negligible external resistance to mass transfer.

Table 8.1 Values of L, β_1 , and calculated first coefficient of equation (8.13)

L	β_1	$A = \frac{6L^2}{\beta_1^2(\beta_1^2 + L^2 - L)}$
0.01	0.1730	1.0009
0.1	0.5423	0.9996
0.2	0.7593	0.9994
0.5	1.1656	0.9959
1	1.5708	0.9855
2	2.0288	0.9534
5	2.5704	0.8533
10	2.8363	0.7607
100	3.1102	0.6259
∞	3.1416	0.6079

8.3.7. Microwave fluidised-bed disinfection of lentil seeds

For the disinfection experiment, 10 g of infected seeds were weighed, and each seed was marked by a red marker to be discriminated from the healthy seeds. Infected seeds were mixed thoroughly with 90 g of healthy seeds at the same moisture content, followed by placement into the sample holder, inside the microwave cavity. Exposure time for each microwave power and the air temperature was found from the empirical model to reach 10.5% MC (initial moisture content of the seeds). After each treatment, the seeds were weighed and transferred to polyethylene bags. The infected seeds (with red marks) were sub-cultured and healthy seeds were examined for their germination and the electrical conductivity of the soaking water on the same day.

As stated before, lentil seedbed temperature was monitored using three fixed fibre optic probes in the inlet air stream as well as in the hot and cold spot of the microwave field in the bed. The data was a good indicator for studying heat tolerance of the BGM pathogen in the lentil seed. Furthermore, heat uniformity in the drying and disinfection in the microwave fluidised bed was studied by the strategy explained in references for microwave and radio frequency ([Chen et al., 2017](#); [Taheri et al., 2019c](#); [Wang et al., 2005](#)). Here, temperature data were considered during the whole processing time (every 10 s) for three positions inside the bed and thus initial average temperature was not considered in the formula. Briefly, average temperature (over space in the selected area) and standard deviation were calculated from all the data obtained for temperatures during the whole

drying time, before cooling down the seed, and from all three fibre optic probes. Maximum temperatures were also extracted from the same data. Heat uniformity index, which is negatively correlated with heat uniformity, was defined as the coefficient of variation (COV) of all data, according to equation (8.14). This is an indicator which shows the degree of variation between different drying processes.

$$TUI = COV = \frac{STD}{T_{av}} \quad (8.14)$$

where TUI = temperature uniformity index; STD = standard deviation; T_{av} = average temperature in the selected area.

8.3.8. Evaluation of BGM pathogen viability on lentil seeds

Botrytis selective media (BSM) was prepared based on the recipes suggested by [Burgess \(1997\)](#) and [Edwards&Seddon \(2001\)](#). It contained (g l⁻¹): glucose 2; PDA 5; Agar 10; tannic acid 5; Ridomil 0.02; Pentachloronitrobenzene (PCNB) solution with concentration of 0.5% in ethanol 4; Zineb 0.00091; streptomycin sulphate 0.1. A mixture of glucose, PDA and agar were autoclaved and cooled to around 50°C before adding other ingredients.

After each treatment, the contaminated seeds, which had been marked by a red marker, were separated, and subcultured on BSM. Thirty seeds per replicate and a total of 90 seeds per treatment were subcultured. The plates were incubated at 23°C and after 4 and 14 days were examined to see the pathogen's growth. BC was recognized based on the appearance of the mycelium and their conidia under the microscope (after sufficient time of incubation) as well as a change of color of the selective media to brown, which was the result of tannic acid oxidation.

8.3.9. Evaluation of lentil seed viability and vigour

Healthy seed viability was examined by the standard germination test. Twenty seeds per replicate and a total of 60 seeds per treatment were placed on Whatman filter paper no.1 in 9-cm petri dishes. Each plate contained 10 seeds and 4 ml of sterilized distilled water. Germination of the seeds was examined after 5 days. Seeds with radicle emergence were considered as germinated and the percentage was considered as Germination% ([ISTA, 2016](#)).

Vigour of the seeds was evaluated by electrical conductivity measurement of the seed soaking water according to [ISTA \(2016\)](#). After each microwave treatment, 50 lentil seeds were counted, accurately weighed and placed in Erlenmeyer flasks with a volume of 250 ml.

Distilled water and seeds were left at room temperature ($20 \pm 2^\circ\text{C}$) overnight to reach equilibrium. After this time, 100 ml of distilled water was poured in each container to cover the seeds and provide enough depth to soak the seeds. The containers were covered with aluminum foil and after $24 \text{ h} \pm 15 \text{ min}$ electrical conductivity of the soaking water was measured using an EC probe (model HI98304, HANNA Instruments Inc.; Melbourne, Australia) with a resolution of 0.01 mS cm^{-1} and accuracy of $\pm 2\%$ F.S. at 25°C . The result was calculated according to equation (8.15). Distilled water was used as blank for background reading of conductivity and the test was carried out in triplicate.

$$\begin{aligned} \text{Conductivity } (\mu\text{S cm}^{-1} \text{ g}^{-1}) \\ = \frac{\text{Conductivity reading } \left(\frac{\mu\text{S}}{\text{cm}}\right) - \text{Background reading}}{\text{Weight of seeds}} \end{aligned} \quad (8.15)$$

8.3.10. Experimental design and statistical analysis

The experiment was designed as a multilevel factorial design with two factors of microwave power and air temperature at levels of 0, 300, 400 and 500 W for microwave power and 50 and 60°C for air temperature. Two separate experiments were carried out; the first one with interval weighing of the lentil seeds to find the drying curves and required time of drying. Fitting models and calculations of this stage were done in MATLAB R2018a (MathWorks Inc., Natick, MA, USA). In the second experiment, which aimed at disinfection of the contaminated seeds at specified exposure times, the effect of the factors on germination%, IS% and electrical conductivity of the soaking water was evaluated using general factorial regression and effects Pareto chart. Differences between responses and control value were examined by one-way Analysis of Variance (ANOVA) and Dunnett multiple comparisons with a control with a confidence level of 95% in Minitab version 18.1 (Minitab Inc., Pennsylvania, USA). All the treatments and post-tests were carried out in triplicate.

8.4. Results and discussion

8.4.1. Microwave forward power regime and air temperature

Table 8.2 shows, average microwave power at set points of 300, 400 and 500 W, which were measured as 216, 324 and 422 W, respectively. Measured air temperatures were also as 49 and 56°C for set temperatures of 50 and 60°C respectively. The difference between set and measured air temperatures at 60°C could stem from either the inaccuracy of the device or the waste of heat between the air blower and the air inlet to the sample holder. Figure 8.2 provides two typical microwave power regimes in a short time (around 30 s) during one treatment for microwave set power of 300 W (a) and 500 W (b). The difference between the

microwave power set point and the measured microwave forward power is due to the proprietary software that was used to manage the microwave generator, via the CanBus Interface of the microwave generator. There may have been some discrepancy in the calibration functions used in the software coding. There was also fluctuation in the power output due to the resonance of the chamber, which may have caused the autotuner to begin “hunting” behaviour, as is sometimes observed in other automatic governor behaviour on devices such as engines. This caused the powers to fluctuate between two higher and lower powers around the set points to reach an average value. The data of these power regimes, during the whole process of drying, were used to calculate the average microwave power for each set power (Table 8.2).

Table 8.2 Setpoint and average measured microwave powers and air temperatures

Set power(W)	Average measured forwarded power (W)	Set air temperature (°C)	Measured air temperature (°C)
300	216±13.1	50	49±1
400	324±21	60	56±1
500	422±13.8		

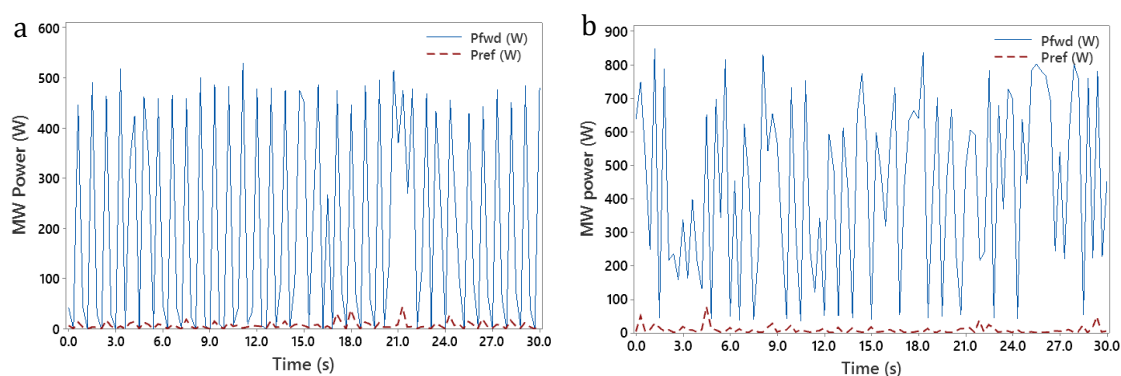


Figure 8.2 Typical regime of microwave forwarded (P_{fwd}) and reflected (P_{ref}) power at set microwave powers of (a) 300 W and (b) 500 W during about 30 s

8.4.2. Seeds moisture history and drying kinetics

8.4.2.1. Seed moisture history

Ambient air temperature and RH during the experiment varied between 23 to 25°C and 32% to 45% respectively. RH of forced air at 50°C was measured as 11-14% and at 57°C was recorded as 8-10%. The deviation in RH of forced air could be the result of a difference in ambient air RH on different days during the experiment. Changes in seed moisture content (wb) during drying at different microwave powers are plotted in Figure 8.3 at air temperatures of 50°C (a) and 60°C (b). As can be seen in these figures, drying time to reach a specific seed moisture content decreased with increasing microwave power and air

temperature. Additionally, the slope of these graphs, which has a direct relation with drying rate, increased with increasing microwave power at each air temperature and time point. It means adding microwave at higher power facilitated the drying process and for the same exposure time more seed moisture reduction was achieved at higher microwave power. [Kahyaoglu et al. \(2012\)](#) also observed that the drying rate of parboiled wheat in a microwave fluidised bed increased with increasing microwave power and air temperature. [Jumah&Raghavan \(2001\)](#) described that in microwave spouted bed drying of wheat, higher microwave power led to higher particle temperatures and thus higher moisture diffusivity inside the wheat kernels, which finally resulted in a higher drying rate. No constant drying rate has been observed from the curves in Figure 8.3, which indicates the whole drying occurred in the falling rate period. Based on the dielectric properties of red lentil seeds, [Taheri et al. \(2018\)](#) indicated that 16.8% could be the critical moisture content as they observed a sudden increase in loss factor from 16.8% to 19% MC. The critical moisture content had been explained by [Erbay&Icier \(2010\)](#), to be the point at which constant rate drying rate period would finish and falling rate would begin.

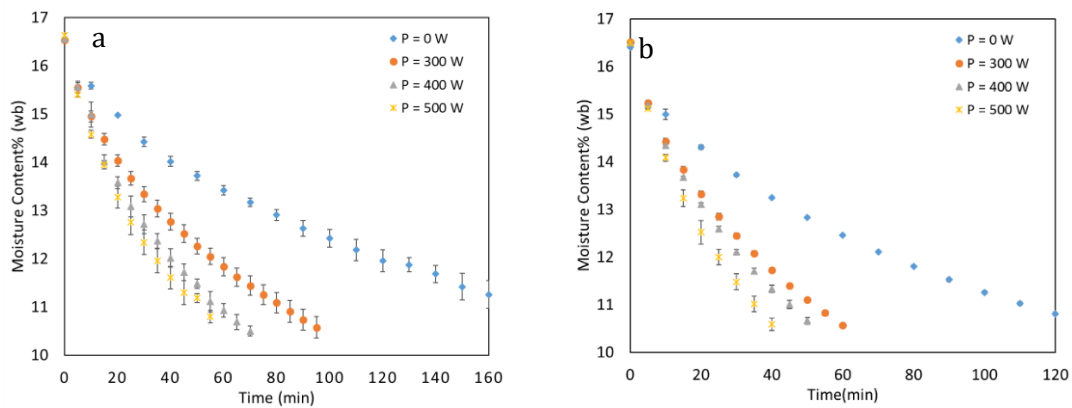


Figure 8.3 Moisture content% of lentil seeds vs drying time after drying of the seeds at different microwave power (P) and an air temperature of (a) 50°C and (b) 60°C; bars are standard deviation (SD) from the mean with $n=3$.

8.4.2.2. Page's model

Page's model (equation (8.1)) was fitted into the experimental data according to Figure 8.4 for an air temperature of 50 (a) and 60°C (b). The results for the goodness of fit, evaluated by the root mean square error (RMSE), R^2 and p-value as well as model's coefficients (k and n), predicted time of drying to reach 10.5% MC (wb) and time-saving percentages, as a result of adding microwave heating, are represented in Table 8.3. The high values of R^2 and low values of RMSE and p-values indicate that the Page's model is a good fit for the drying data and prediction of drying time from this model could be reliable. Values of k and n were higher when microwave power was added to the fluidised bed compare with the hot air

drying alone. However, there was no obvious trend by increasing microwave power and air temperature. A closer inspection of the k values reveals that at both air temperatures, k values (min^{-1}) decreased with increasing microwave power from 300 to 400 W, with corresponding values of 0.034 to 0.033 for air temperature of 50°C and from 0.042 to 0.041 for air temperature of 60°C , but increased with increasing power from 400 to 500 W, with corresponding values of 0.033 to 0.038 for air temperature of 50°C and 0.041 to 0.043 for air temperature of 60°C . The same trend was observed in the data reported by [Khoshtaghaza et al. \(2015\)](#), when they applied an air speed of 3.1 m s^{-1} and air temperatures between 80 to 140°C for drying of soybean kernels. Their k value ranged from 0.009 to 0.039. This slight drop in k value by increasing microwave power from 300 to 400 W might seem unusual as we expect it to increase by increasing microwave power.

The required times of drying, in Table 8.3, were calculated from the obtained values for k and n at each microwave power and air temperature and using equation (8.1) for the final MC of 10.5% (wb). These times were later applied to investigate the effect of the process on disinfection of lentil seeds from BGM pathogen. As can be seen in Table 8.3, predicted times of drying varied between 41 min at a microwave power of 500 W and air temperature of 60°C to 216 min for hot air alone at 50°C . Drying time reduction varied between 54% to 72% after applying microwave to fluidising hot air. It means adding microwave to the fluidised bed dryer had a significant contribution to increasing the drying rate and seed moisture removal.

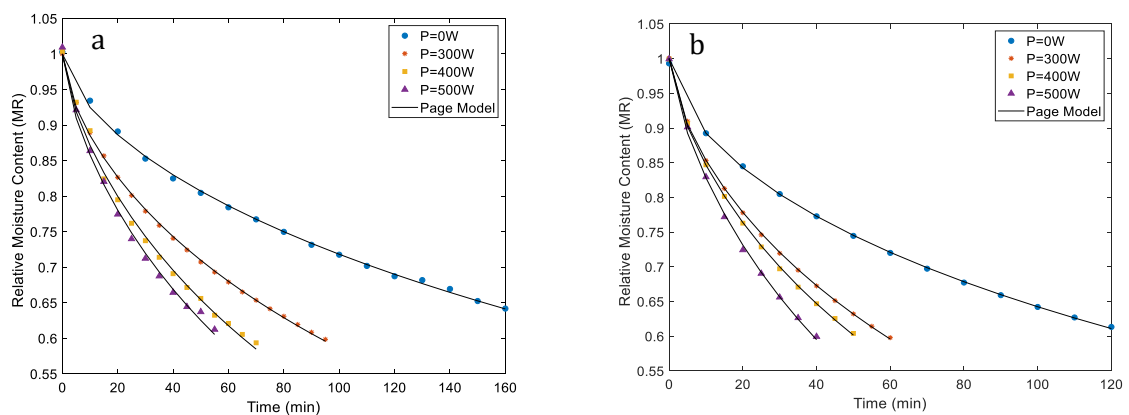


Figure 8.4 Mean experimental Relative Moisture Content (MR) for lentil seeds dried in microwave fluidised bed at different microwave powers (P) and fitted page's model (black line) for an air temperature of (a) 50°C and (b) 60°C

Table 8.3 Drying model parameters based on Page's model (k (min^{-1}) and n), the goodness of fit of the model at each microwave power and air temperature and predicted time for drying; RMSE: root mean square error

Microwave power (W)	Air temperature($^{\circ}\text{C}$)	RMSE	R^2	p-value	Coefficients		Predicted time to reach 10.5% MC (min)	Time saving %
					k (min^{-1})	n		
0	50	0.00468	0.9991	1.43e-33	0.023195	0.68099	216	0
300	50	0.00162	0.9999	6.76e-48	0.034528	0.71104	98	54
400	50	0.0102	0.9971	1.41e-24	0.03379	0.77757	68	68
500	50	0.00943	0.9975	1.26e-19	0.038954	0.76909	60	72
0	60	0.00323	0.9996	1.35e-26	0.035735	0.63809	137	0
300	60	0.00132	0.9999	7.00e-31	0.042531	0.71741	62	54
400	60	0.00106	1.0000	2.18e-26	0.041761	0.75007	53	61
500	60	0.00451	0.9995	2.20e-16	0.043147	0.79345	41	70

8.4.2.3. Effective moisture diffusivity

Data of goodness of fits for equation (8.12) and (8.13) as well as calculated D_{eff} are represented in Table 8.4. As it was expected from the obtained time of drying, effective moisture diffusivity, D_{eff} , increased with increasing microwave power and air temperature. The increase in D_{eff} with increasing microwave power at each air temperature could stem from either increasing seed temperature, or more production of water vapor and thus more vapor pressure inside the seeds due to absorbing more microwave power. It led to a greater moisture movement inside the seeds, as also explained by [Kahyaoglu et al. \(2012\)](#). These two phenomena could also occur at the same time and be coupled to increase the D_{eff} by increasing microwave power ([Brodie, 2007](#)). At an air temperature of 50°C , D_{eff} increased from 0.27×10^{-10} to $0.95 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ by increasing microwave power from 300 to 500W and correspondingly rose from 0.37×10^{-10} to $1.25 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ at an air temperature of 60°C by using equation (8.12). The results of D_{eff} obtained from equation (8.12) were smaller than the corresponding values calculated from equation (8.13) (corresponding values of 0.44-2.15 and $0.55\text{-}3.06 \text{ m}^2 \text{ s}^{-1}$), which could have its root in the assumptions for boundary conditions, the goodness of fit into the models or consideration of the first terms of the series of equations (8.10) and (8.11) for fitting the data. However, both values are within the range of 10^{-10} - 10^{-11} . [Tang \(1994\)](#) calculated lentil seeds' diffusivity as $0.22 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ in cotyledon, $69.16 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ in Hilum and $0.03 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ in seed coat by considering lentil as a slab in a conventional thin layer drying with air at 40°C . They

assumed a boundary condition where the surface was at a constant moisture content equal to an equilibrium moisture content of the seeds. [Carmo&Lima \(2005\)](#) also investigated the drying kinetics of lentil seeds using numerical simulation by considering shrinkage and the shape of lentil seed as an oblate spheroidal body. They estimated lentil seeds' diffusivity to be in the range of $0.05 - 0.26 \times 10^{-10} m^2 s^{-1}$ during conventional drying with air at 23, 40 and 60 °C with different relative humidity and speed of $0.3 m s^{-1}$, and concluded that diffusion coefficients increased dramatically by increasing air temperature. The difference among the reported results in the literature could stem from using different lentil varieties, different processes, and process parameters as well as different equations used for fitting the data.

Table 8.4 Effective diffusion coefficients of lentil seeds ($D_{eff}(m^2 s^{-1})$) at different microwave powers and air temperatures and goodness of fits (R^2 and RMSE) for fitting equation (8.10) and (8.11) into experimental drying data

Equation number	Microwave power (W)	Air temperature(°C)					
		50			60		
		$D_{eff} \times 10^{10}$	R^2	RMSE	$D_{eff} \times 10^{10}$	R^2	RMSE
(8.10)	0	0.27	0.975	0.035	0.37	0.971	0.040
	300	0.55	0.983	0.034	0.81	0.982	0.034
	400	0.80	0.983	0.038	0.96	0.986	0.031
	500	0.95	0.979	0.041	1.25	0.989	0.031
(8.11)	0	0.44	0.969	0.027	0.55	0.964	0.031
	300	0.87	0.977	0.026	1.38	0.977	0.026
	400	1.61	0.981	0.026	1.86	0.983	0.024
	500	2.15	0.978	0.028	3.06	0.987	0.022

From the data of Table 8.4, by fitting equation (8.12) and (8.13) into experimental drying data (MR-t), R^2 was in the range of 0.964-0.987 and RMSE was between 0.022-0.031. Values of the first coefficient in equation (8.13) ($A = 2L^2 / [\beta_1^2 (\beta_1^2 + L^2 + L)]$) varied between 0.91-0.96. Therefore, values of L and β_1 were in the range of 2-5 and 2.02-2.57 respectively, based on the data of Table 8.1. The values of L could be a reason why the external resistance to mass transfer cannot be neglected when microwave is added to forced air for drying. It is stated that external resistance to drying is negligible when using conventional hot air drying with air speed of more than $1 m s^{-1}$ and external resistance will be important for Biot number ($Bi_m = \frac{kL}{D\rho}$) of less than 10 ([Srikiatden & Roberts, 2007](#)). Here, both conditions are met, and external resistance should be negligible. However, microwave penetrates inside the seeds and warms up the inside, up to a penetration depth, in addition to the heat transfer from the

outer hot air. This volumetric heating generates water vapor inside the seeds and mass transfer could occur as a result of pressure gradient (Feng, 2001). To prove if the error of considering only the first term of the series (equations (8.10) and (8.11)) is sufficiently low, values of MR were calculated from the first term of the equations (8.10) and (8.11) and also from adding the first five terms of these series. Calculated D_{eff} for each treatment from Table 8.4 as well as corresponding values of β_1 to β_5 from Crank (1979) and the drying times to reach 10.5% MC were applied to calculate MR, and then percentage of errors were found. Data of error% are represented in Table 8.5. All the errors are below 0.5% and they decreased by increasing microwave power from 300 to 500 W, which could be the result of bigger values of $\frac{D_{eff}t}{r_e^2}$.

Table 8.5 Percentage of error (%) caused by comparing first and sum of the first five sentences of the equation (8.10) and (8.11) by using D_{eff} s from Table 8.4 and drying times from Table 8.3

Equation number	Microwave power (W)	Air Temperature (°C)	
		50	60
(8.10)	0	0.29	0.74
	300	0.46	0.46
	400	0.15	0.25
	500	0.06	0.07
(8.11)	0	0.19	0.37
	300	0.27	0.27
	400	0.12	0.18
	500	0.06	0.07

8.4.3. Disinfection of lentil seeds with the obtained time from the drying experiment

8.4.3.1. Lentil bed temperature

The average and maximum bed temperature (bars) as well as COV (lines) during lentil seeds drying processes are plotted in Figure 8.5 (a) and (b) for air temperature of 50 and 60°C respectively. Both average and maximum bed temperatures increased with increasing microwave power and air temperature and it varied between 48 to 68°C and 52 to 81°C for average and maximum values, respectively. As can be seen from the line chart, COV increased with increasing microwave power at each air temperature, which means temperature uniformity decreased and temperature deviations from the average values were more in the three points of the bed as well as over the full range of the heating time. However, COV did not necessarily increase by raising air temperature at each microwave

power. The highest heat uniformity was achieved by applying hot air alone at 50 and 60°C, with COV equal to about 0.03 and the least heat uniformity occurred in the bed when applying microwave power of 500 W and air temperature of 50 °C, with COV of 0.13.

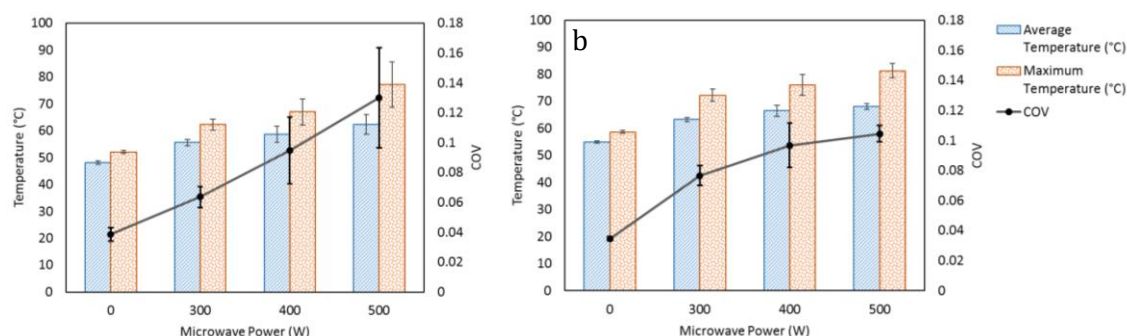


Figure 8.5 Coefficient of Variation (COV), Average and maximum lentil bed temperatures during the whole drying process at different microwave power (W) and air temperatures of (a) 50°C and (b) 60°C; bars are two standard errors from the mean with n=3.

8.4.3.2. Seed moisture contents after the disinfection process

Results of final moisture contents of lentil seeds after three replicates of drying at predicted times from Page's model (equation (8.1)) are graphed in Figure 8.6 in comparison with the expected moisture content (dashed line). Data of three replicates of drying with hot air alone at 50 or 60°C appeared to be clustered close to the expected MC of 10.5%, while those from combining microwave at powers of 300, 400 and 500 W spread farther from each other as well as from the horizontal dashed line. This inaccuracy could stem from a lack of proper control of microwave power during the drying which is an intrinsic characteristic of a magnetron.

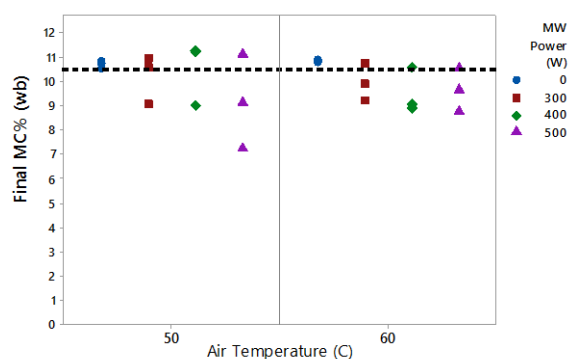


Figure 8.6 Actual seed moisture contents (dots) after three replicates of drying at different microwave powers (MW Power, W) and two air temperatures of 50 and 60 °C; dashed line shows the predicted moisture content by the model

8.4.3.3. Results of disinfection and lentil seeds viability

According to the result of general factorial regression, both microwave power and air temperature had significant effects on seed germination ($R^2 = 0.78, p = 0.002$) and IS%

($R^2 = 0.69, p = 0.017$) with a confidence level of 95%. Both factors were negatively correlated with IS% and germination, while being positively correlated with electrical conductivity of the seed soaking water. From the effects' Pareto charts, microwave power had far more effect on both IS% reduction ($p=0.002$) and viability loss ($p=0.000$) than the air temperature (with corresponding p-value of 0.046 and 0.022).

Results of infected seed percentage counted after 4 and 14 days (IS%-4d and IS%-14d respectively) for different microwave power (P) and air temperature (T) combinations are plotted in Figure 8.7 (a) and (b). There was no significant change in IS%-4d by applying hot air alone at 50 or 60 °C while there was a significant drop when the microwave was combined with the hot air at all microwave powers. According to Figure 8.7(a), IS%-4d declined from 50% to about 20% by applying microwave power of 300 and 400 W and an air temperature of 50°C (P300-T50 and P400-T50) and it further decreased to just below 10% at a microwave power (W) and air temperature (°C) combinations of P300-T60, P400-T60, P500-T60, and P500-T50. As can be seen in Figure 8.7(b), IS%-14d significantly reduced from 70% to 40% by applying microwave power of 300W and an air temperature of 60°C (P300-T60) and to around 30% by applying a microwave power of 400 W and an air temperature of 60 °C (P400-T60) and a microwave power of 500 W with both air temperatures of 50 and 60 °C (P500T50 and P500T60). Taken together, these results suggest that by comparing the pathogen's growth with the control sample, there was more reduction in IS% counted after 4d than after 14 d. For example, by applying the process of P300-T60, there was a 40% reduction of IS% counted after 4d (IS%-4d), while it was 30% after 14 d of growth (IS%-14d). This result might stem from the fact that the microwave-assisted process was able to reduce the initial inoculum of each seed, which delayed the appearance of the BGM mycelium in the seeds on BSM. This result agrees with the unpublished finding from Dr. Brodie's lab, which showed that either inhibition of fungi growth or a reduction in the inoculum load of soil fungi could occur after microwave soil treatment.

Figure 8.8 provides the results obtained for lentil seed viability as measured by germination% after 2 days (a) and electrical conductivity of seed soaking water (seed vigour indicator) (b). As can be seen from the Figure 8.8 (a), lentil seeds' germination was negatively affected at a microwave power of 400 W and air temperature of 60°C (P400-T60) as well as microwave power of 500 W and air temperatures of 50 and 60°C (P500-T50 and P500-T60) with a reduction from 80% (control) to below 20%. Electrical conductivity for the mentioned process parameters also significantly increased from 100 to above 150 $\frac{\mu S}{cm.g}$.

A comparison of Figure 8.7 and Figure 8.8 reveals that by applying microwave power of 300 W and air temperature of 60°C for drying lentil seeds from 16.5% MC, 30% of the infected seeds were free from BGM pathogen without significant seed viability and vigour loss. However, process parameters of P300-T50 and P400-T50 were also able to reduce IS%-4d by 30% without seed viability and vigour loss, which could reduce disease development later in crop plants as explained earlier.

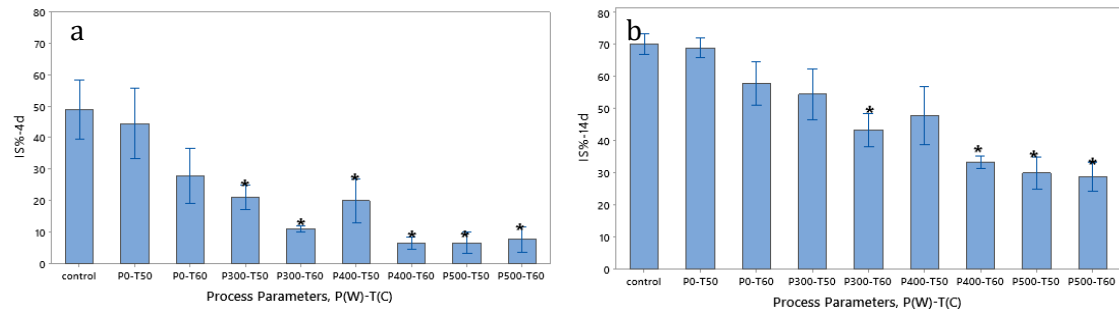


Figure 8.7 Infected seed percentage (IS%), counted after (a) 4 days and (b) 14 days for treated seeds at different power(P) and air temperatures (T) and untreated seeds (control); values with * are significantly different from control by Dunnett multiple comparison ($\alpha = 0.05$); bars are standard errors from the mean ($n=3$)

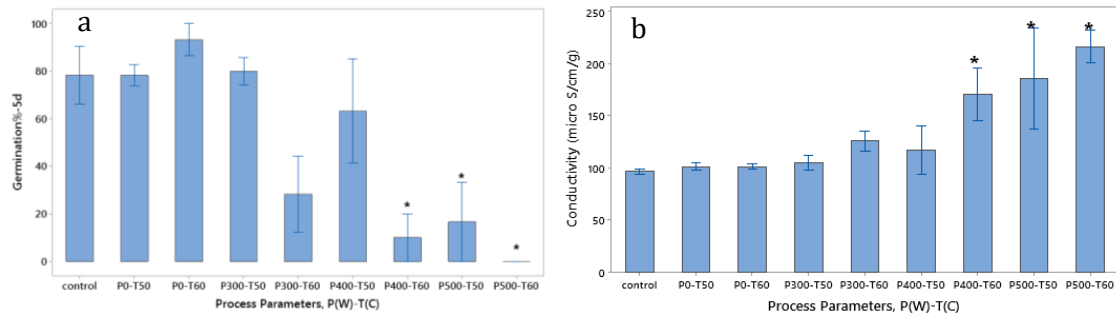


Figure 8.8 (a) Lentil seed germination and (b) Electrical conductivity of seed soaking water after 24h for treated seeds at different power (P) and air temperatures (T) and untreated seeds (control); values with * are significantly different from control by Dunnett multiple comparisons ($\alpha = 0.05$); bars are standard errors from the mean ($n=3$)

8.5. Conclusion

The aim of the present work was to study the drying kinetics of red lentil seeds in a microwave-assisted fluidised bed dryer and evaluate the possibility of eradication of seed-borne BGM pathogen from the seeds during the drying process. Drying curves of red lentil seeds at microwave powers of 0, 300, 400 and 500 W and air temperatures of 50 and 60°C were well fitted by empirical Page's model. There was no obvious trend for the 'k' value with microwave power, but it increased with increasing air temperature at each microwave power. Additionally, the investigation of lentil's moisture diffusivity showed that they increased with increasing microwave power and air temperature. They varied between

$0.27 \times 10^{-10} - 1.25 \times 10^{-10} \text{ (m}^2 \text{ s}^{-1}\text{)}$ by considering negligible external resistance and between $0.44 \times 10^{-10} - 3.06 \times 10^{-10} \text{ (m}^2 \text{ s}^{-1}\text{)}$ by considering convective boundary conditions. In the second part of the experiment, lentil seeds that were contaminated with BGM pathogen were dried from 16.5% (wb) to 10.5% by the duration time calculated from the first experiment, using Page's model and the reduction of the pathogen load was evaluated at each microwave power and air temperature combinations. The finding of this experiment suggested that by applying microwave power of 300 W and air temperature of 60°C or microwave power of 400 W and air temperature of 50°C for drying 100 g of lentil seeds from 16.5% to 10.5% (wb), infected seed percentage (IS%) could be reduced by 30% without a significant loss of seed viability. This is the first study on drying kinetics of red lentil seeds in a microwave fluidised bed and the first study to evaluate the possibility of a seed-borne disease reduction (BGM pathogen) as part of this drying process. The question raised by this research was the possibility of reducing spore load on each seed by applying the drying treatments, which could possibly reduce the transmission of the disease from seed to seedling. To address this question, the spore load of each seed is recommended to be defined before and after the drying process to measure the average spore reduction per seed. One of the obstacles in this study was the lack of data on the number of spores per seed which can cause seed to seedling transmission of the BGM pathogen. Thus, it is recommended to investigate this limit to pave the way for further studies on physical control of this seed-borne pathogen.

In this chapter the simultaneous drying and disinfection of lentil seeds in a microwave fluidised bed was studied. The effect of these treatments on the seed quality will be explored in the next chapter.

Chapter 9. Fluidisation of lentil seeds during microwave drying and disinfection could prevent detrimental impacts on their chemical and biochemical characteristics

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9.1. Abstract

A microwave fluidised bed has proven to be substantially more efficient than a conventional fluidised bed dryer in food and agriculture. However, its effect on the quality attributes of the grains, have not yet been fully discovered. In the present study, the effect of microwave fluidised bed drying with the aim of pest and pathogen control, utilising microwave powers of 0, 300, 400, 500 W (0, 3.6, 4.8 and 6 W g⁻¹ dry seeds, respectively) and fluidising air temperature of 50 and 60 °C, on the quality traits of lentil seeds were examined by measuring antioxidant enzyme activities, cooking quality and macronutrients analysis using Fourier transform infrared micro spectroscopy. A reduction in Peroxidase activity was observed at powers of 3.6 and 4.8 W g⁻¹ and fluidising air at 60°C. The hardness of soaked and cooked seeds increased after drying at 6 W g⁻¹ with both air temperatures and at 4.8 W g⁻¹ with an air temperature of 60°C. Based on the results of FTIR, no conformational changes in the protein secondary structure were observed at microwave powers of 0, 3.6, and 4.8 W g⁻¹. Therefore, controlling microwave radiation utilising hot air for agitation could prevent any negative effect on some of the grain quality attributes.

Keywords: Microwave fluidised bed drying, lentil, quality, FTIR, antioxidant enzymes

9.2. Introduction

Lentil is one of the important pulse crops with a high protein content of between 20.6-31.4% (Jarpa-Parra, 2018). However, there are many pests (GRDC, 2018a) and pathogens (Taylor et al., 2007) of lentil that threaten the yield pre and post farmgate. The current methods of controlling pests are heavily dependent on pesticides, which raise the concerns of developing resistant species as well as health and environmental impacts.

There have been many attempts to find a proper physical method for grain disinfection/disinfestation. One of these methods which has recently gained more attention

is applying electromagnetic radiation with frequencies of mainly 27 MHz (Radio frequency) and 2.45 GHz (Microwave). Radio frequency (RF) has proven to be a great tool for insect ([Jiao, 2012](#)) and fungal ([Jiao et al., 2016](#)) control in the grain industry. Microwave has also shown great potential for removing grain insects ([Vadivambal et al., 2007](#)), storage fungi ([Knox et al., 2013](#)) and field fungi ([Taheri et al., 2019d](#)). There have been several reports indicating that grain quality, including germination, was negatively affected by microwave treatments ([Dalmoro et al., 2018](#); [Singh et al., 2012](#)). Quality loss is attributed to non-uniformity of heating in microwave radiation and a very big difference between hot and cold spot temperatures, which can be as high as 50°C in lentil seeds ([Taheri et al., 2019a](#)) and 80°C in canola, barley and wheat seeds ([Manickavasagan et al., 2006](#)). Therefore, the loss of seed quality could be overcome by controlling the hot spot temperature and increasing the uniformity of radiation.

There are several recommended strategies for having uniform heating in microwave treatments or drying of particles such as grains, which could be beneficial in preventing negative effects on the host quality. They include surface temperature and microwave power control ([Cheng et al., 2006](#); [Koné et al., 2013](#); [Li et al., 2010](#)); applying mode stirrers and different materials for the turntable ([Ye et al., 2017](#)) and agitating the particles using hot air ([Kudra, 1989](#)). Agitation of the grain in a microwave fluidised bed has been proven to have a very good heat uniformity ([Feng & Tang, 1998](#)) and therefore can be used for drying, disinfection, and disinfestation of grain, but its potential should still be investigated more deeply by exploring the grain quality after the treatments.

Lentil seeds are recommended to be harvested at the seed moisture content of not less than 15% (wb) to prevent physical damage during harvesting. However, the recommended safe storage moisture content for lentil seeds to prevent fungal infestation and maintain seed viability is below 12%. Therefore, lentil seeds need to be dried before storage. In our previous work ([Taheri et al., 2020](#); [Taheri et al., 2019b](#)), the possibility of eliminating *Botrytis* grey mould of lentil seeds as part of drying with a microwave fluidised bed was evaluated. It was proven that by using a combination of microwave and hot air, infected seeds could be reduced by 30% without significant seed germination loss. The applied microwave power and air temperature in this research also meet the minimum requirements for insect control in lentil seeds, which has been reported as 60°C and 10min of holding time ([Wang et al., 2010](#)). Therefore, the aim of the present study was to have an elaborate evaluation of the seed quality as a result of treatments by microwave fluidised bed with the aim of drying, disinfection and disinfestation.

9.3. Materials and methods

9.3.1. Microwave drying of lentil seeds

The drying experiment and the microwave fluidised bed equipment were explained in our previous study ([Taheri et al., 2020](#)) (refer to Chapter 8). The microwave fluidised bed consisted of a single mode cylindrical cavity with a diameter of 97 mm and a height of 170 mm, and a cylindrical sample holder (D: 66 mm, L: 111 mm) with a perforated bottom which was located in the hot spot of the cavity. It was operated by a magnetron working at a frequency of 2.45 GHz and variable powers up to 3 kW. Air flow was fixed at 560 l min⁻¹ for fluidisation of 100 g of lentil seeds. Red lentil seeds (Type Bolt) were purchased from a field located in Horsham, Victoria, Australia. The initial moisture content was measured as 10.5% (wb) by drying unground seeds at 130°C for 20 h ([Tang & Sokhansanj, 1991](#)). The 100-seeds weight was 4.27±0.09 g and their average diameter and thickness, measured by a digital caliper, were 5 ± 0.19 and 2.4 ± 0.14 mm (average of twenty seeds). To reach 16.5% moisture content (MC) (wb), 7.5 g of sterilized distilled water was added to each 100 g of dry seeds in a ziplock bag and after thoroughly shaking, they were kept in the refrigerator for at least one week to reach equilibrium. The seeds were shaken at least once each day during this week to make sure that the moisture spread homogenously among them. For each drying process, 100 g of lentil seeds with 16.5% MC at room temperature was placed inside the Teflon sample holder of the microwave fluidised bed. The samples were dried to reach 10.5% MC (initial MC of dry seeds) and times of drying were: 216, 98, 68 and 60 min for microwave powers of 0, 300, 400 and 500 W (corresponding power densities of 3.6, 4.8 and 6.0 W g⁻¹ dry seeds) respectively at fluidising air temperature of 50°C; 137, 62, 53 and 41 min for corresponding microwave powers of 0, 300, 400 and 500 W at fluidising air temperature of 60°C. Lentil seed temperatures were measured and monitored using two fibre optics probes (FISO Technology Inc., Quebec, Canada). After drying the seeds with the mentioned microwave powers and air temperatures, lentil seeds were evaluated for quality attributes. Seed germination and electrical conductivity measurement methods were explained before ([Taheri et al., 2020](#)). Evaluation methods for the other parameters are outlined in the remaining parts of this section.

9.3.2. Antioxidant enzymes activity

9.3.2.1. Enzyme extraction

Ten lentil seeds were weighed accurately before adding 5 ml of water and incubating for 17 h at room temperature. After this time the seeds were ground with a pre-chilled pestle and mortar in 50 mM potassium phosphate buffer with a pH of 6.8 for Peroxidase (POX) and

Catalase (CAT) activities and pH of 7.2 for Ascorbate peroxidase (APX) ([Biju et al., 2017](#)). The mixtures of the seeds and buffer were centrifuged at 11000 g at 4°C for 10 min and the supernatant was collected and kept in a water and ice mixture to be analysed for their enzymatic activities. Enzyme extracts were analysed within 12 h. Protein contents of the enzyme extracts were measured according to [Kruger \(1994\)](#) and [Bradford \(1976\)](#) using Bradford reagent for 1-1400 $\mu\text{g ml}^{-1}$ protein (Sigma-Aldrich, NSW, Australia). Briefly, 20 μl of the enzyme extract was mixed with 200 μl of the reagent in microplate wells and after resting for 5 min at room temperature (to let the reaction complete), their absorbance were measured against a blank containing phosphate buffer and the reagent at 595 nm. A standard curve was prepared using BSA (Bovine Serum Albumin) at concentrations of 0.1-1mg l⁻¹ and the ratio of standard solution/reagent was the same as the ratio of enzyme extract/reagent.

9.3.2.2. Peroxidase (POX)

To evaluate POX activity of the lentil seeds, fresh enzyme extract (500 μl) was mixed with 10 mM H₂O₂ (250 μl), 20 mM guaiacol (250 μl), and 50mM phosphate buffer with pH 6.8 (2ml). A blank was prepared in which the enzyme extract was replaced with a phosphate buffer. The absorbance of the mixtures, at 470 nm using a UV/visible spectrophotometer, was recorded every 30 s for 5 min. This recording helped to monitor the oxidation of guaiacol in the presence of H₂O₂, which is an indicator of POX activity. Total activity was calculated for the initial weight of the seeds and the volume of the enzyme extract. Specific POX activity was calculated as mM guaiacol. mg⁻¹ protein. s⁻¹ for each gram of lentil seeds (M indicates moles) by considering the extinction coefficient of guaiacol as 26.6 mM⁻¹cm⁻¹ ([Maehly, 1954](#)).

9.3.2.3. Catalase (CAT)

The CAT activity was measured based on the breakdown of H₂O₂ in the presence of the enzyme at 240 nm using a UV/visible spectrophotometer ([Maehly, 1954](#)). Fresh enzyme extract (500 μl) was mixed with 30% H₂O₂ (500 μl) and 50 mM phosphate buffer with pH 6.8 (2 ml) and a blank was prepared with the enzyme extract being replaced by phosphate buffer. CAT activity, for each gram of lentil seeds, was defined as mmol H₂O₂. mg⁻¹ protein. s⁻¹ by considering 0.04 mM⁻¹.cm⁻¹ for the extinction coefficient of H₂O₂.

9.3.2.4. Ascorbate peroxidase (APX)

APX activity was determined by monitoring the decrease in absorbance of a reaction mixture in one minute as a result of ascorbate oxidation at 290 nm with a UV/visible

spectrophotometer. The reaction mixture consisted of enzyme extract, 50 mM phosphate buffer pH 7.2 (500 μ l), 0.5mM Ascorbic acid (500 μ l), 30% H₂O₂ (500 μ l), and 50 mM phosphate buffer with pH of 7.2 (1.5 ml). APX activity was expressed as μ mol ascorbate.mg⁻¹ protein. s⁻¹ by considering 2.8 mM⁻¹cm⁻¹ for the extinction coefficient of ascorbate at 290 nm ([Biju et al., 2017](#)). Measurements were performed against a blank with phosphate buffer replacing the enzyme extract.

9.3.2.5. Hydrogen peroxide content (H₂O₂)

H₂O₂ concentration in the seeds was measured based on the peroxide mediated oxidation of Fe⁺² and then the reaction of Fe⁺³ with xylene orange with an extinction coefficient of 220 mM⁻¹cm⁻¹ ([Daniela Bellincampi, 2000](#)). H₂O₂ of the seeds was extracted using 10 mM potassium phosphate buffer in a prechilled mortar and pestle in an ice bath. The mixtures were centrifuged at 11000 g at 4°C for 10min and the supernatant was collected and kept in an ice bath. An 80 μ l extract was mixed with 220 μ l of the reagent consisting of equal amounts of 0.5 mM ammonium ferrous sulphate, 50 mM H₂SO₄, 200 mM sorbitol, and 0.2 mM xylene orange in the wells of a microplate. The absorbance of the Fe⁺³-xylene orange complex was read at 560 nm after 45 min of incubation using a microplate reader (Multiskan Go, Thermo scientific). Accumulation of H₂O₂ in the seeds was obtained using the standard curve of H₂O₂ and expressed as μ mol g⁻¹ of the seeds. The standard curve was prepared by reading the absorbance of the mixture of 80 μ l of different concentrations of H₂O₂ and 220 μ l of the reagent at 560 nm.

9.3.3. Cooking quality

Cooking quality was defined by measuring the hardness of the soaked and cooked seeds ([Ioshi et al., 2010](#); [Tang et al., 1991a](#)). One gram of treated and non-treated lentil seeds was soaked in 10ml of distilled water in 15-ml centrifuge tubes for 20 h. To test the hardness of cooked seeds, the closed tubes, containing water and soaked seeds, were placed in the water bath at 98.5°C for 10 min and then the excess water was immediately poured out. The hardness of the soaked and cooked seeds was determined using Lloyd texture analyser LS5 (Lloyd Instruments Ltd., Largo, FL, USA) with a load cell capacity of 500 N. Double Bite compression test with 0.5 s interval and up to 50% compression was selected and the parameters were set as: preload stress = 0.5 N; preload speed = 25 mm min⁻¹ (0.41mm s⁻¹); datum speed = 50 mm min⁻¹; datum load = 0.5 N; test speed = 0.8 mm s⁻¹. A minimum of 15 seeds for each treatment was examined for their texture profile. The probe used for compressing the seeds was an aluminium cylinder with a diameter of 13 mm and the height of the probe's bottom surface was adjusted to one centimetre above the parallel table plate

before performing each test. Data were recorded in the software Nexygen plus 3 (Ametech) and exported to Excel for further analysis.

9.3.4. Fourier Transform Infrared (FTIR) micro spectroscopy

9.3.4.1. Sample preparation for FTIR test

One millilitre of distilled water was added to ten lentil seeds from each treatment and left for 17 h at 5°C to allow the seeds to absorb the water. After this time, the seeds were frozen in liquid nitrogen and kept at -80°C for further analysis. The frozen samples were mounted on the sample holder with a very small amount of OCT (optimal cutting temperature) compound and cross-sectioned to 6 μm thickness in a Cryostat at -21°C ([Yu, 2005](#)). As water might exist in the protein, the pathlength of less than 10 μm is required to avoid the overlapping of water absorbance (at near 1640 cm^{-1}) with the protein ([Fabian & Măntele, 2006](#)). A minimum of three seeds for each treatment was cross-sectioned and mounted on BaF_2 windows (13 $\times$ 1 mm) for FTIR micro spectroscopy analysis. The sectioned samples on the windows were kept in a desiccator overnight at room temperature to make sure they were dry and water's IR absorption did not affect the spectra acquisition.

9.3.4.2. Spectra acquisitions

Infrared spectra of lentil seeds' sections were recorded using Nicolet™ Continuum™ Infrared (IR) Microscope (IS 50, Thermo Scientific) coupled with spectroscopy and equipped with a KBr beam splitter. The microscope housed a 15 $\times$ IR objective and a 10 $\times$ light objective for visible light viewing. Data were collected in absorbance mode with a fixed aperture size of 50 $\times$ 50 μm , resolution of 32 (3.875 cm^{-1}) and a minimum of 30 scans per spectrum in the wavenumber range of 740-4000 cm^{-1} (the range specified for the BaF_2 window). The background spectra were collected from the sample free area of each window. These were subtracted from the sample spectra for correction of CO_2 and H_2O in the air. Data were acquired using Omnic software. Data from the parts full of starch granules (S in Figure 9.1) were considered as starch-dominant spectra and the parts with mostly the protein bodies (P in Figure 9.1) were considered as protein-dominant spectra. Three spectra were collected for each part of the protein and starch dominant areas of the cotyledons of each lentil seeds and three lentil seeds were evaluated for each treatment. Therefore, the spectrum obtained for each drying condition was an average of 27 spectra.

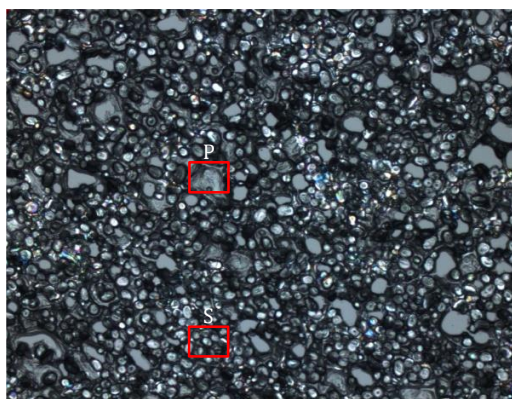


Figure 9.1 Light microscopic image ($\times 10$) of the cross-section of lentil seeds (cotyledon), P: protein-dominant area, S: starch-dominant area

9.3.4.3. Data analysis and spectra treatments

Spectra were exported into excel files for further analysis. The raw spectra were pre-processed using an Extended multiplicative signal correction (EMSC) procedure to reduce the effect of baseline scattering and biases and then second derivatives of these spectra were obtained by 9 Savitzky-Golay smoothing points and a third polynomial degree, in order to increase the resolution and discrimination of the peaks. All the pre-processing procedures were performed in the Unscrambler software 2013 (v. X 10.3, Camo Software, Oslo, Norway). Principal component analysis (PCA) was carried out on all the pre-processed spectra from the starch and protein dominant area to study their difference and outliers. Afterward, the PCA was performed on each group in wavenumbers of amid I ($1600\text{--}1700\text{ cm}^{-1}$) to determine the important wavenumbers (i) from their loading plot. Any conformational change in the protein secondary structure after each treatment was then further studied by calculating the weight of the peak in the amide I region according to equation 9.1 ([Carbonaro et al., 2008](#)).

$$W_i = \frac{SD_i}{\sum_{1600}^{1700}(SD_i)} \quad (9.1)$$

i: Wavenumber at the peak position

SD_i : the intensity of the second derivative at wavenumber of i

W_i : the weight of the peak at wavenumber of i

Lipid/protein and phosphate/protein indexes were obtained using the integration of their second derivative within their band area. For protein, phosphate, and lipid, band areas of $1500\text{--}1700$, $1075\text{--}1080$ and $2800\text{--}3000\text{ cm}^{-1}$ were applied, respectively. Here the ratios are not the actual ratios in the seeds because molecular absorption coefficients were not considered in the calculation. The aim was just to compare the different treatments with the

control samples. The calculations, averaging and plotting were carried out using MATLAB R2018a (MathWorks Inc., Natick, MA, USA) or MINITAB® 18.1 statistical software (Minitab Inc., Pennsylvania, USA).

9.3.5. Statistical analysis

Microwave fluidised bed drying was performed three times and all the other tests were done with at least two replicates for each drying replicate. Means were compared using Dunnett multiple comparison method with control or least square difference with a 95% confidence level. Analysis of the means and multivariate analysis were carried out in MINITAB® 18.1 statistical software (Minitab Inc., Pennsylvania, USA).

9.4. Results and discussion

9.4.1. Lentil seeds' temperature

Lentil seeds' average and maximum temperatures, as a result of drying in the microwave fluidised bed at different microwave powers and air temperatures, are represented in Figure 9.2 (a) and (b). The average temperature varied between 48°C to 68°C for microwave power-air temperature combinations of 0W-50°C and 500W-60°C respectively for which the corresponding maximum temperatures were 51 and 81°C. As it was expected, both the average and maximum temperatures increased with increasing microwave power and air temperature.

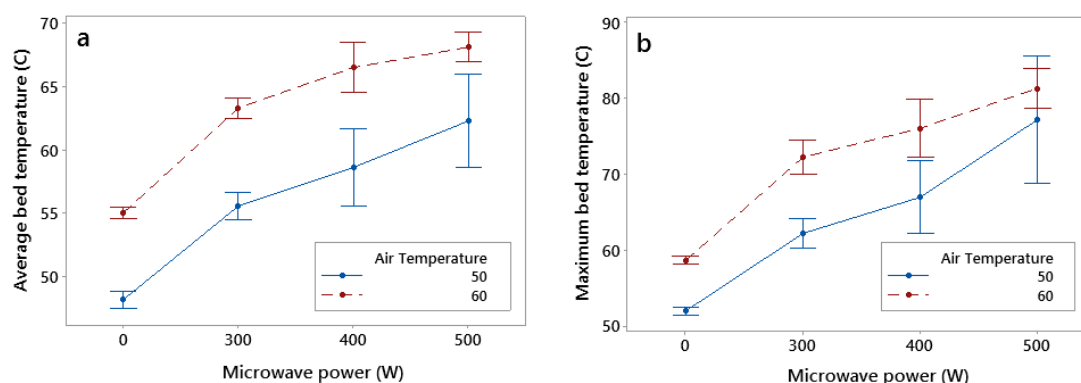


Figure 9.2 (a) Average and (b) Maximum lentil bed temperatures during the whole drying process at different microwave power (MW Power, W) and air temperatures (°C), bars are one standard error from the mean with $n=3$.

9.4.2. Seed viability, vigour, and antioxidant enzymes activity

The results of seed germination and electrical conductivity are shown in Figure 9.3 (a) and (b). These results explain that the seeds' viability (measured by germination%) and vigour (measured by electrical conductivity) significantly decreased after drying the seeds with

microwave power-air temperature combinations of 500W-50°C, 500W-60°C, and 400W-60°C. These reductions in viability and vigour could be the result of high seed temperatures at higher microwave powers or much faster absorption of microwave power. These results are adopted from [Taheri et al. \(2020\)](#) to relate them to other quality changes, which will be presented here.

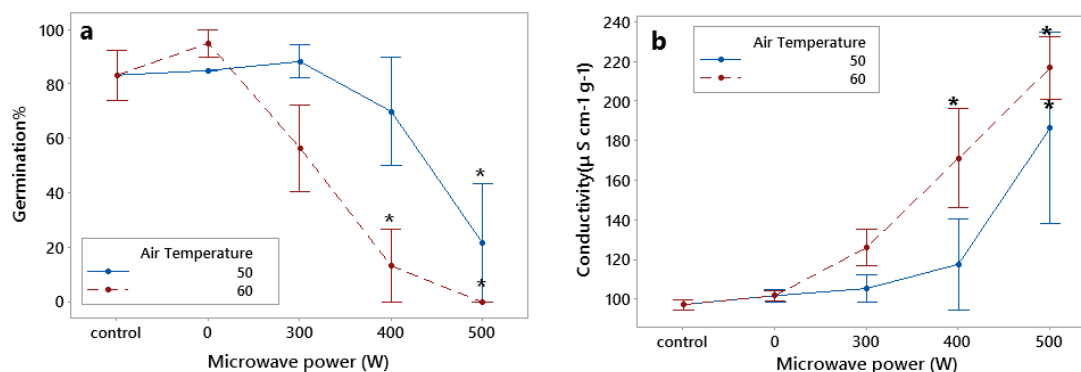


Figure 9.3 (a) Number of germinated lentil seeds after 2 days and (b) Electrical conductivity of seed soaking water after 24 h for treated seeds at different power (P) and air temperatures (T) and untreated seeds (control); values with * are significantly different from control by Dunnett multiple comparisons ($\alpha = 0.05$); bars are standard errors from the mean ($n=3$)

Deterioration of aged seeds is believed to be related to oxidative damage caused by reactive oxygen species (ROS) accumulation, which is negatively correlated with seed vigour. H_2O_2 , which is produced under oxidative environments, is mainly scavenged by CAT and APX. Therefore, any change in the activity of these enzymes or H_2O_2 could be the result of seed response to oxidative damage. The results of lentil seeds' antioxidant enzyme activities are represented in Figure 9.4 and Figure 9.5. Due to the dramatic reduction of germination rate at the microwave power of 500 W, no enzymatic activity tests were performed on the related treatments. There were no significant changes in APX and CAT activities and in H_2O_2 content after hot air or microwave fluidised bed drying of lentil seeds. However, there was a decrease in POX activities from 0.0003 to 0.00017 $mM\ guaiacol.mg^{-1}.s^{-1}$ after drying the seeds with a microwave power of 300 and 400 W with fluidising hot air at 60°C (Figure 9.4(a)), which increased maximum bed temperatures to more than 70°C, based on the data of Figure 9.2. This is in agreement with the findings of other researchers who stated that peroxidase activity decreased in highly aged seeds ([Cakmak et al., 2010](#); [Scialabba et al., 2002](#)). [Scialabba et al. \(2002\)](#) indicated that POX activity was high in the aged seeds with high viability and a decline was recorded with increasing aging time. This change in POX activity mostly occurred in the seeds' coat and cotyledon rather than their embryonic axis. In the experiment done by [Yin et al. \(2014\)](#) on aging rice seeds, CAT and APX activities decreased and correspondingly accumulation of H_2O_2 and O_2^- increased in aged seeds,

while in the dead seeds with very low germination (2%), these ROS were lower compared with the control. They did not find a significant reduction of superoxide dismutase and glutathione reductase in the aged seeds. As there was not a significant change in accumulation of H_2O_2 in the seeds after drying (Figure 9.5 (b)), decreasing POX activity could be due to the formation of other peroxides such as lipid peroxides, which will be explained more in the FTIR section of these results. Drying of the lentil seeds with forced air at 50°C in combination with either 300 or 400 W did not have any significant negative effect on the antioxidant defence system of the seeds.

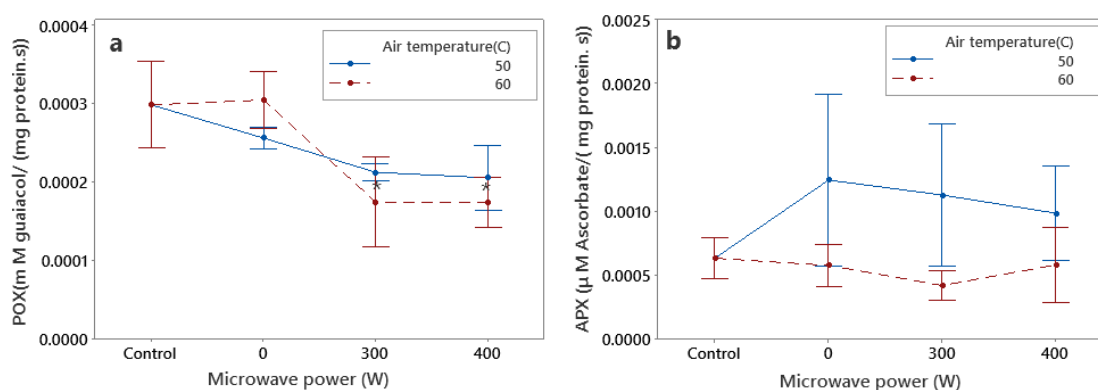


Figure 9.4 (a) Peroxidase (POX) and (b) Ascorbate peroxidase (APX) activity of lentil seeds after drying under microwave (MW) powers of 0, 300 and 400 W and air temperatures of 50 and 60°C; bars are standard errors from the mean with $n=3$.

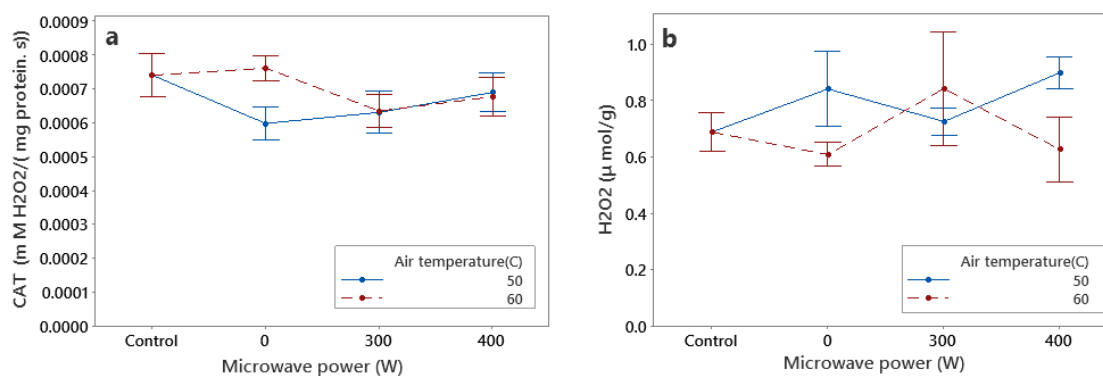


Figure 9.5 (a) Catalase (CAT) activity and (b) Concentration of H_2O_2 in lentil seeds after drying under microwave (MW) powers of 0, 300 and 400 W and air temperatures of 50 and 60°C; bars are standard errors from the mean with $n=3$.

9.4.3. Cooking quality

Hardness in the first bite for soaked and cooked lentil seeds dried at different microwave powers and air temperatures is represented in Figure 9.6 (a) and (b) respectively. The hardness of soaked lentil seeds dried at microwave power and air temperature combinations of 500W-50°C, 500W- 60°C and 400W- 60°C increased from 23 N (control) to 26, 28 and 27 N, respectively. The hardness of cooked seeds also slightly increased at the

150

same process parameters from 16.5 N (control) to corresponding values of 20.5, 20.5 and 19.9 N. There was no significant change in the hardness of the soaked and cooked seeds dried with the other microwave power-air temperature combinations compared with the control samples. The more the hardness of the seeds is after a specific time of cooking, the poorer would be the cooking quality.

As the water uptake of the lentil seeds after 24 h of soaking did not change (data not shown), the decrease in cooking quality of the lentil seeds (bars with asterisks in Figure 9.6 a and b) might be attributed to the reduction of phytic acid content, which could be the topic of further study. Phytic acid is one of the non-proteins antinutritional factors in legume seeds, which alters their protein and minerals digestibility and absorption. Cooking quality of lentil seeds was previously found to be positively correlated with their phytic acid content ([Bhatty & Slinkard, 1989](#); [Yadav et al., 2007](#)). There have been several reports that phytic acid content of legumes decreased as a result of microwave treatment and the reduction was intensified by increasing microwave power ([Ertas, 2013](#); [Pande et al., 2012](#)). On the other hand, phytic acid, as a source of phosphorus and a natural antioxidant, has a crucial role in the metabolism of germinating seeds and its deficiency could lead to lower plant vigour, producing less vigorous plants ([Zaghdani, 2002](#)). Therefore, its reduction after treatment by power-air temperature combinations of 500W-60°C, 500W-50°C, and 400W-60°C could also be one of the reasons for seed viability and vigour loss (Figure 9.3 a and b). [Hentges et al. \(1991\)](#) observed that the development of hard-to-cook defect in bean seeds was directly related to the reduction of phytate and water-soluble pectin and increase in EDTA-soluble pectin and electrical conductivity of seed soaking water. They explained that this could be due to the interactions of phytate-mineral and mineral-pectin. Reduction of starch gelatinization and protein solubility, as well as not-enough cell separations, could also be involved in this phenomenon ([Liu & Bourne, 1995](#)).

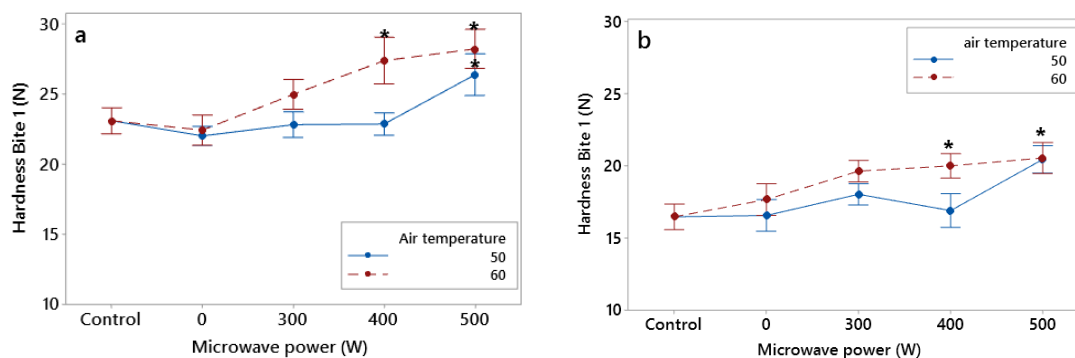


Figure 9.6 Hardness of the lentil seeds (a) Soaked for 17 h and (b) Cooked for 10 min for treated seeds at different power(P) and air temperatures (T) and untreated seeds (control); values with * are

significantly different from control by Dunnett multiple comparisons ($\alpha = 0.05$); bars are standard errors from the mean ($n=15$)

9.4.4. FTIR Micro spectroscopy

9.4.4.1. Raw spectra comparisons

Assignment of the peaks to their related chemical groups and the interpretation of the spectra were carried out based on the procedure explained by [Coates \(2006\)](#) and [Movasaghi et al. \(2008\)](#). Some of these band assignments and the results obtained in the present study (shown in Figure 9.7 and Figure 9.8) are represented in Table 9.1. FTIR spectra of starch-dominant and protein-dominant areas of dried seeds at different microwave powers and air temperatures, as well as the control sample, are shown in Figure 9.7 and Figure 9.8 respectively. The overall absorbance did not change significantly after drying treatments with any of the microwave powers and air temperature combinations. [Qiu et al. \(2018\)](#) reported that the overall absorbance of heat damaged and aged corn seeds, measured by FT-NIR, reduced in comparison with viable seeds. Here, no change in the overall absorbance was observed even for the seeds treated with a microwave power of 500W and air temperature of 60°C, which dramatically reduced seed viability.

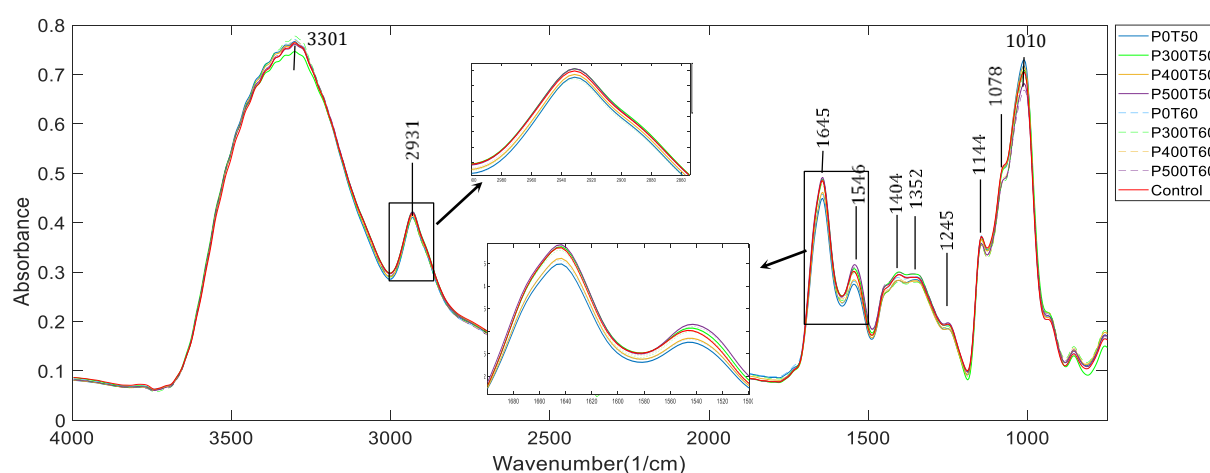


Figure 9.7 FTIR spectra of the starch-dominant area of lentil seeds' cotyledon dried at different microwave powers and air temperatures; each spectrum is average of 9 replicates

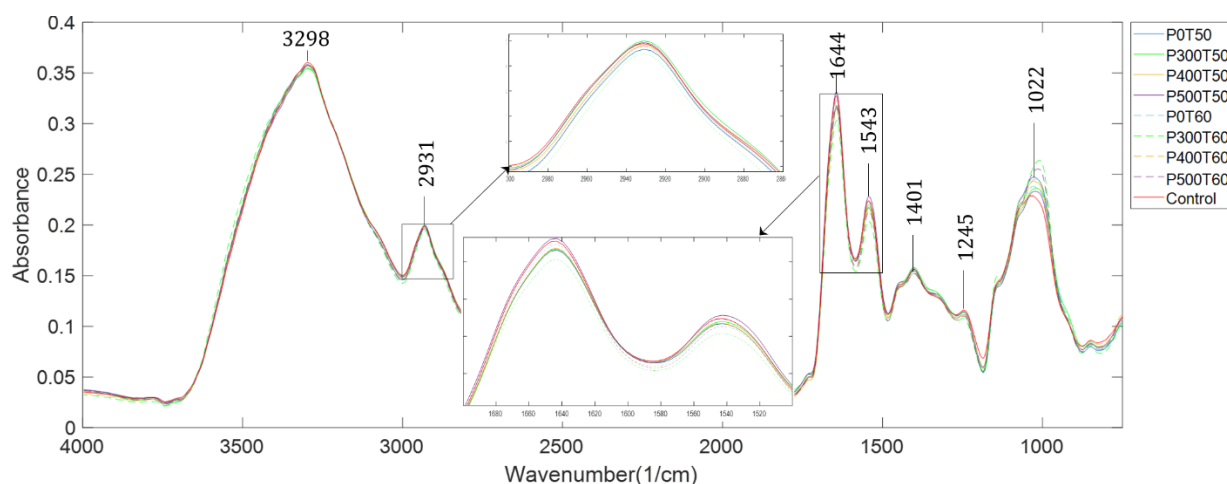


Figure 9.8 FTIR spectra of the protein-dominant area of lentil seeds' cotyledon dried at different microwave powers and air temperatures; each spectrum is average of 9 replicates

Table 9.1 Peak positions in FTIR spectra and band assignments to them (from OMNIC library) with the result of the present study

Peak position (cm ⁻¹)	Assignment			Peak found in the present study
		Chemical formula	Band name	
Near 3350-3180 3320-3070	Primary Secondary	NH stretching	Acetamide	3301- 3298
Near 1650 1680-1630 1670-1630	Primary Secondary Tertiary	C=O stretching	Amide I	1644 - 1645 1666, 1639
1650-1620 1570-1515	Primary Secondary	NH bending & CN stretching (out-of-phase)	Amide II	1543 - 1546
Near 1270	Secondary	CN stretching & NH bending (in-phase)	Amide III	
1078		Symmetric phosphate, stretching and C-O-H deformation motions		1078
1145		Phosphate & Oligosaccharides		1144
1238		Asymmetric stretching PO ₂		1238
2975-2950 Near 2930		CH ₃ asymmetric stretching CH ₂ asymmetric stretching	Aliphatic group	2930
2885-2865 2870-2840 1470-1440		CH ₃ symmetric vibration CH ₂ symmetric vibration CH ₃ asymmetric deformation		2883 1450
1150-1050		C-O stretch	Aliphatic Ether	1144

9.4.4.2. Second derivatives and multivariate analysis

The second-derivative analysis helps to resolve the overlapping bands ([Yang et al., 2015](#)) and therefore by emphasizing small spectral variations provides a better understanding of any changes. Principal component analysis (PCA) of the second derivatives in the lipid and fingerprint regions of protein and starch dominant areas are represented in Figure 9.9, a and b respectively. In both lipid and fingerprint regions, protein-dominant spectra (blue circles) and starch-dominant spectra (red squares) cluster together and separately from the other group, which can also be seen in Figure 9.10 as two separate clusters (blue and red). The difference between these two groups is mostly in PC1, which is almost the whole range of the wavenumbers and all the important peaks. This difference indicates that, for studying the seed macromolecules using FTIR micro spectroscopy, it is of crucial importance to select the same area across the seed sections in all the samples. The reason is that the seed structure is heterogenous, which could cause an error in the interpretation when comparing two different seeds. [Yu \(2005\)](#) also showed that FTIR spectra of each pericarp, Aleurone and endosperm regions of wheat seeds cluster together and separate from each other when applying PCA analysis. The reason could be different concentrations of macromolecules like protein, starch, and lipid in different parts of the seeds, which causes different absorbance of the IR radiation. The dendrogram of cluster analysis of starch-dominant (red) and protein-dominant (blue) areas is represented in Figure 9.10. The analysis was performed on the second derivatives of the spectra at the wavenumbers related to fingerprint and lipid parts. Here, it is also obvious that there is little similarity between the two clusters of starch and protein dominant areas and additional differences among the treatments in each group were revealed from PC2. In both PCA and cluster analysis, it can be seen that the similarities among the treatments in the protein-dominant area are more than those in the starch-dominant area (sum of squares 1.57×10^{-5} for the protein-dominant compare to 6.53×10^{-5} for the starch-dominant area), which could be the result of more susceptibility of the starch-dominant area to the treatments.

In Figure 9.11, the second derivatives of Amide I and II of protein macromolecules are plotted for starch-dominant (a) and protein-dominant (b) areas of the cotyledon. In both figures, the peak for the β – *sheet* is much more noticeable. It could be the result of protein components of lentil seeds, which is mostly globulin (56%, legumin and vicilin) rather than albumin (12%) ([Liu & Bourne, 1995](#)). [Saguer et al. \(2012\)](#) observed that globulin is less sensitive to protein aggregation due to thermal treatments than albumin and showed that globulin is mostly β – *sheet* with its peak near 1640cm^{-1} and albumin is mostly α – *helix*

with the peak near 1660 cm^{-1} . There was not a noticeable peak of α – *helix* at around 1655 cm^{-1} in the second derivatives of the spectra, and the reason could be short α – *helix* chain of peptides in the lentil seeds. [Barth \(2007\)](#) stated that the α – *helix* with less than 6 peptides did not always give the typical α – *helix* peak absorbance.

According to Figure 9.11, no change in peak positions of Amid I was observed. [Divekar et al. \(2017\)](#) noticed a shift in the β – *sheet* peak from 1639 cm^{-1} to 1630 cm^{-1} when they treated lentil seeds with moisture contents of 10% and 18% with a microwave power of 600 W for 56 s. They mentioned that this shift was the result of a change in protein secondary structures or break down of H-bonds. The difference in the obtained results could stem from either the controlling of the seed temperature in our study with fluidising air, which did not let the hot spot increase beyond the destroying temperature of protein, or the lower applied microwave power.

The second derivative of the spectra of lentil seeds, before and after drying at different microwave power and air temperatures, in the lipid band area ($2800\text{-}3000\text{ cm}^{-1}$) are shown in Figure 9.12. In both starch (a) and protein (b) dominant parts of the cotyledon, there is a principal peak near 2930 cm^{-1} , which is the C-H stretching of CH_2 in lipid and a weaker peak near 2960 cm^{-1} , which is C-H stretching of CH_3 . The more pronounced peak of CH_2 than CH_3 could be the result of a long chain of fatty acids presents in lentil seeds. [Caprioli et al. \(2016\)](#) indicated that lentil fat mostly consisted of Polyunsaturated fatty acid (PUFAs) (42-57%) with C18:2 (n-6 class) four times more than C18:3 (n-3 class) rather than monounsaturated fatty acid (MUFA), C18:1 (23-28%). They also confirmed that lentil's fat composed of 17.7-19% saturated fatty acids (SFA) of C16:0 (14-16%) and C18:0(2-3%). The peak near 2960 cm^{-1} is more obvious in the protein-dominant area (Figure 9.12, b) than in the starch-dominant area (Figure 9.12, a). The C-H peak at this wavenumber could be due to the asymmetric stretch mode of the methyl groups from cellular protein or lipids ([Movasaghi et al., 2008](#)). Therefore, the peak for the protein-dominant area is more obvious because it could stem from both protein and lipid molecules, while in the starch-dominant area, it is mostly from lipids. The other possible reason could be the presence of different lipid structures in the protein and starch of the seeds and the presence of more PUFA (with more CH_3 compared to CH_2 groups) in the protein-dominant area than MUFA and SFA. This difference can also be seen from the more obvious peak of the protein-dominant area at 1730 cm^{-1} (Figure 9.8 compared to Figure 9.7), which is related to the absorbance of fatty acid esters ([Movasaghi et al., 2008](#)).

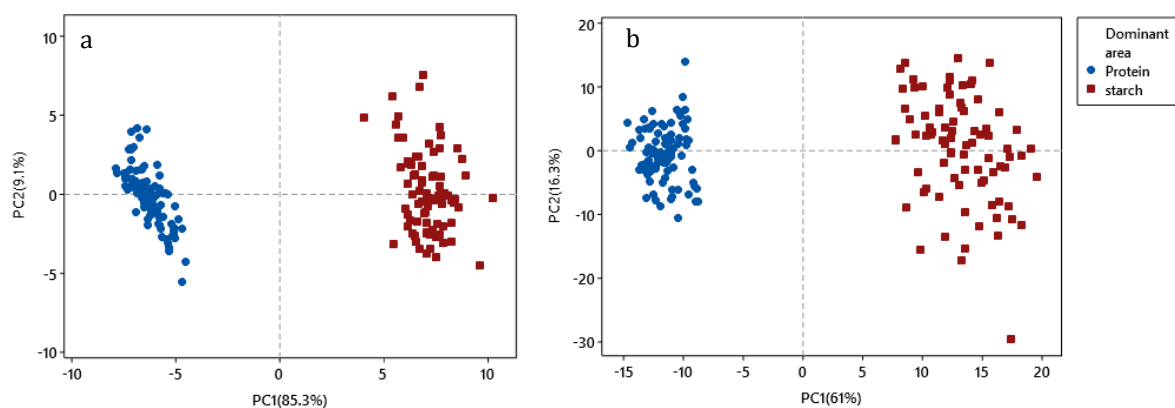


Figure 9.9 The PCA analysis of the second derivatives of FTIR spectra of lentil seeds in the (a) Lipid ($3100\text{--}2700\text{ cm}^{-1}$) and (b) Fingerprint ($1800\text{--}740\text{ cm}^{-1}$) regions for protein reach area (circle) and starch reach area (square) of cotyledon

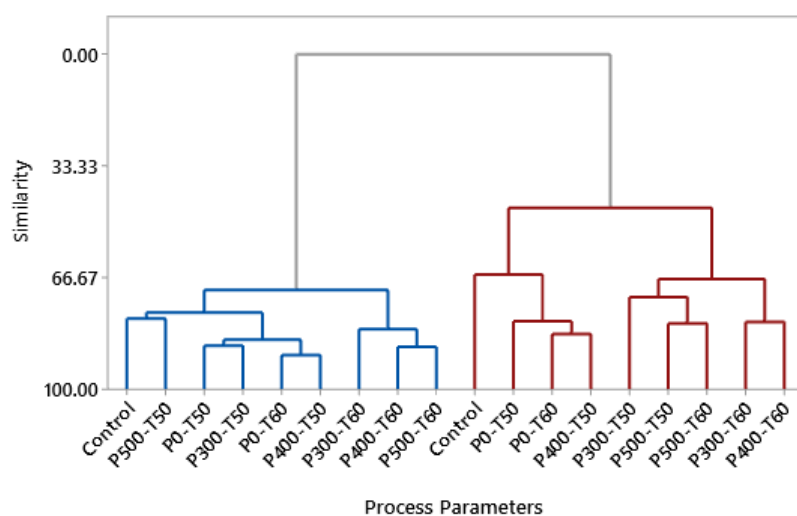


Figure 9.10 Cluster analysis of the second derivatives of the spectra in starch-dominant (red) and protein-dominant (blue) areas at fingerprint ($1800\text{--}740\text{ cm}^{-1}$) and lipid ($3100\text{--}2700\text{ cm}^{-1}$) regions with Pearson distance and complete linkage method

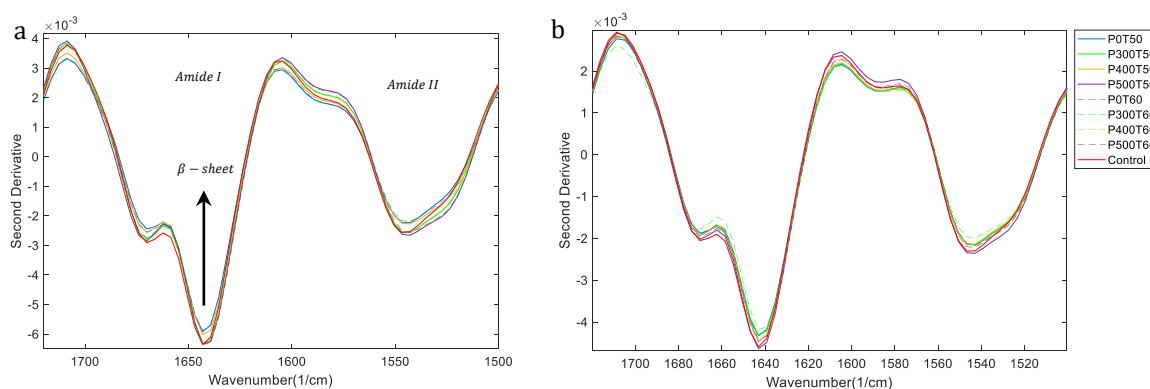


Figure 9.11 Second derivative of Amide I and II regions for (a) Starch-dominant and (b) Protein-dominant areas of the lentil seeds

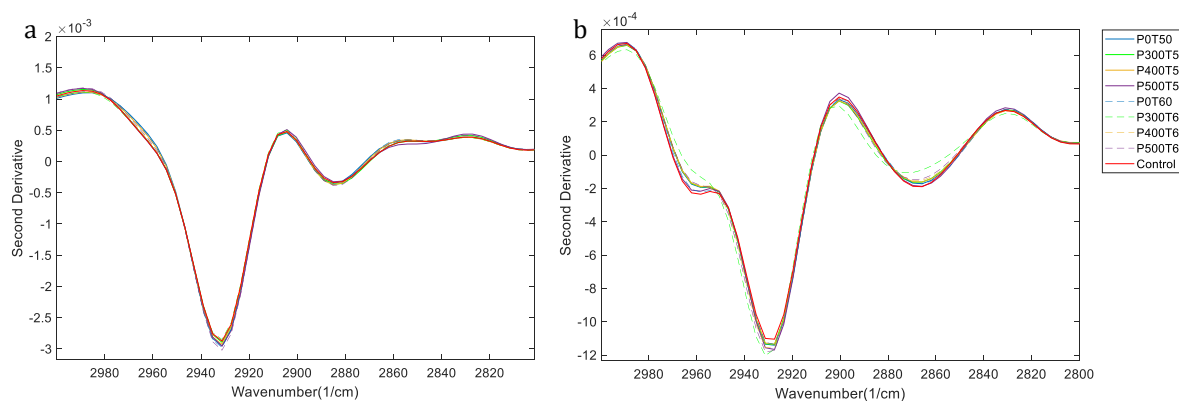


Figure 9.12 Second derivative of lipid region for (a) Starch-dominant and (b) Protein-dominant areas of the lentil seeds

Lipid & phosphate study

The lipid/protein index for the spectra of starch-dominant areas are compared in Figure 9.13. The ratio increased for the treatments by hot air at both air temperatures of 50 and 60°C and without applying microwave but decreased after applying microwave power of 500 W at both air temperatures as well as microwave powers of 300 and 400 W at the air temperature of 60°C. A reduction in lipid content and lipid/protein ratio, after microwave treatments, was also observed by [Echereobia et al. \(2014\)](#) for cowpea seeds and by [Zhong et al. \(2015\)](#) for black soybean seeds. In contrast, the fat content of lentil's starch isolate and lipid/protein ratio of lentil seeds were reported to increase after microwave radiation ([Divekar et al., 2017](#); [González & Pérez, 2002](#)). [Christensen \(1957\)](#) also indicated that fatty acid content of grains increased as a result of seed deterioration due to heat, storage and fungi invasion. A decrease in lipid/protein ratio could be the result of lipid peroxidation, which led to the formation of peroxides. The accumulated peroxides are hypothesized to break up by peroxidase and subsequently result in the reduction of peroxidase activity as explained in section 9.4.2. Conclusively, the reduction of lentil seeds viability could be the result of lipid peroxidation. However, no statistical difference was found between the treatments and the control samples by Dunnett multiple comparisons with 95% confidence level.

The most important components in the legumes' protein molecules/complexes are salts of phytic acid (~10%) and storage protein (~80%). Red lentil seeds contain approximately 0.3-1.5% phytic acid, which is mostly located in the protein body of cotyledon (90% of the total amount) ([Schlemmer et al., 2009](#)) and its ratio to the protein content of lentil seeds account for 2.7% ([Bennetau-Pelissero, 2018](#)). Phytic acid or its salt, phytate, have peaks in the IR region due to the presence of organic phosphate, P=O band (1250-1350 cm⁻¹);

aliphatic phosphate, P-O-C stretching ($990\text{-}1050\text{ cm}^{-1}$) and aromatic phosphate, P-O-C stretching ($850\text{-}995/1190\text{-}1240\text{ cm}^{-1}$) (Coates, 2006). However, the peak near 1000 cm^{-1} could overlap with the peaks resulted from the bands of starch content of the seeds which is more abundant in lentil seeds. Therefore, to study phosphate part just the region of $1075\text{-}1080\text{ cm}^{-1}$ was considered as the peak for symmetric P-O stretch. Figure 9.14 shows the relative change of the phytic acid compound as phosphate/protein index. As can be seen from the bars, the trend of changes is like that for lipid/protein index and a decrease in the index at microwave power of 500 W and both air temperatures (50 and 60 °C) can be observed. As previously explained (section 4.3 cooking quality), this reduction in the phytic acid/protein ratio could be the reason for increasing lentil seeds' hardness and decreasing of cooking quality as it was explained in section 9.4.3.

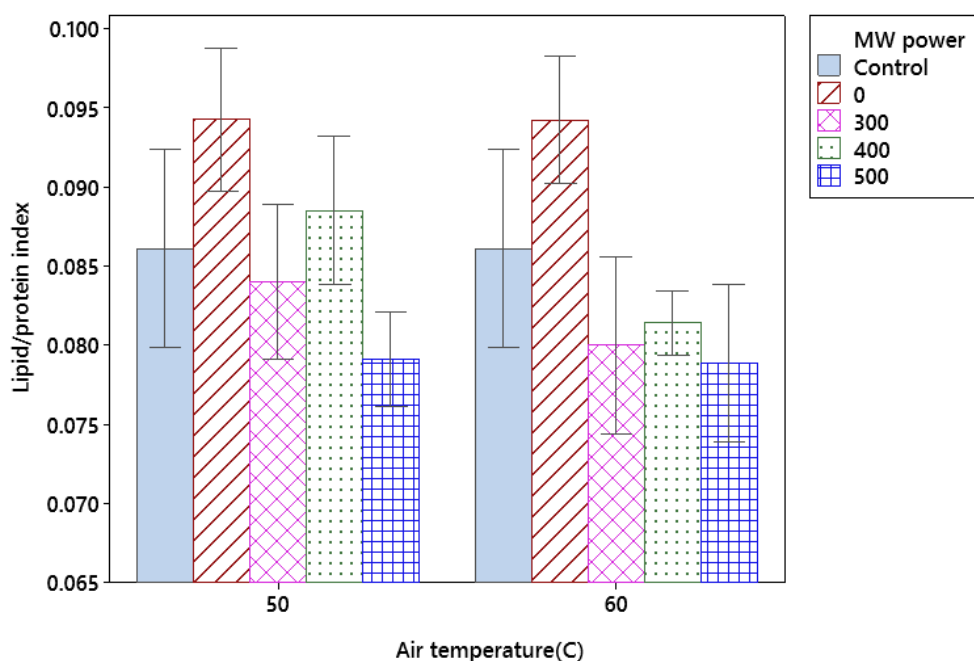


Figure 9.13 Lipid/protein index of the seeds treated at different microwave powers and air temperatures for the spectra obtained from the starch-dominant area of the lentil seeds; bars are standard errors of the mean with $n=9$.

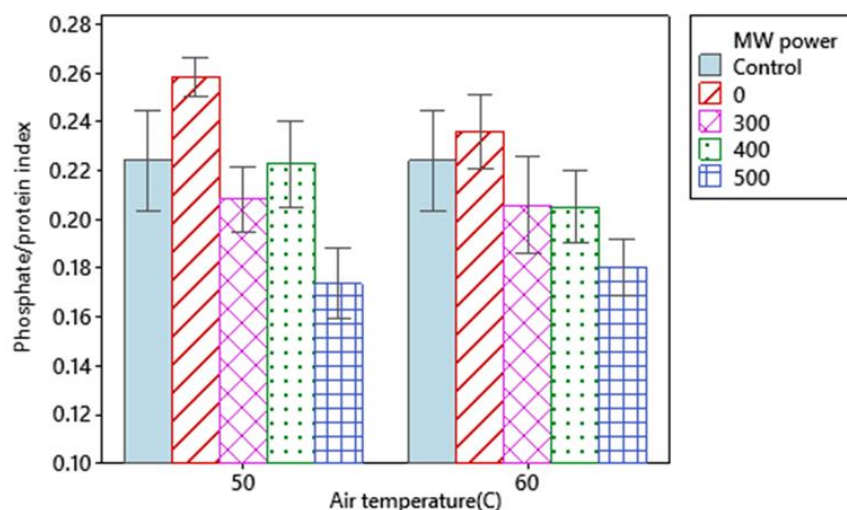


Figure 9.14 Phosphate/protein index for wavenumber range of $1075\text{--}1080\text{ cm}^{-1}$ of the seeds treated at different microwave powers and air temperatures for the spectra obtained from the starch-dominant area of the lentil seeds; bars are standard errors of the mean with $n=9$.

Protein secondary structure in amid I region

The secondary structure of the protein is usually studied by employing 80% C=O stretch near 1650 cm^{-1} for the amide I band, 60% N-H bend and 40% C-N stretch near 1550 cm^{-1} for the amide II, and 40% C-N stretch and 30% N-H bend near 1300 cm^{-1} for amide III (Pelton & McLean, 2000). However, in the FTIR spectra, amide II could arise from complex vibrations that involve multiple functional groups and makes them less useful for studying protein structure prediction. The amide I region ($1700\text{--}1600\text{ cm}^{-1}$) is very useful for studying the secondary structure of the protein and any conformational changes. As this band is sensitive to small molecular vibrations, different secondary structures can give rise to a different C=O stretching frequency. The susceptibility of the amide I band to conformational changes makes it more useful in identifying protein folding, unfolding or aggregation (Carbonaro, 2012), by focusing on the secondary structures including α – helix, β – sheet and β – turn.

A PCA analysis was carried out for each protein and starch-dominant area separately and the important wavenumbers to study the peaks in the amide I band ($1600\text{--}1700\text{ cm}^{-1}$) found from their loading plots. Weights of secondary structures at these wavenumbers (W_i) are represented in Table 9.2. Band assignment was carried out based on literature (Bonwell et al., 2008; Carbonaro, 2010, 2012; Pelton & McLean, 2000). No significant changes in the low and high-frequency β – sheet (at 1639 and 1682 cm^{-1}) were observed. Carbonaro et al. (2008) showed that there was no unfolding or structural reorganization in the protein secondary structure of lentil seeds after dry heat treatment at 120°C for 30 min. They attributed it to the high content of β – sheet in the protein structure of lentil seeds.

However, in the present study $W_{1658}(\alpha - helix)$ decreased (from 0.094 to 0.076 and 0.077) and W_{1624} (aggregated β - sheet) increased (from 0.016 to 0.027 and 0.031) significantly as a result of lentil seed drying at microwave power of 500W and both air temperature of 50 and 60°C. There was also a reduction in turns (W_{1666}). The decrease in $\alpha - helix$ weight and increase in aggregated β - sheet could be explained by denaturation of the protein secondary structure after microwave fluidised bed drying at high power (500 W). [Saguer et al. \(2012\)](#) treated protein from porcine plasma and its fractions at temperature between 30-95°C and reported an increase with increasing temperature for the absorbance intensities of the peaks near 1620 and 1680 cm^{-1} and a decrease for those near 1640 and 1650 cm^{-1} . However, protein mixture significantly affected the aggregation pattern. They concluded that $\alpha - helix$ was more thermally sensitive than native $\beta - sheet$. [Fabian&Mäntele \(2006\)](#) explained that there was a two-part transition in protein structure change as a result of thermal denaturation. In the first part (temperature below 60°C), there was not a notable change in $\beta - sheet$, but a significant decrease in $\alpha - helix$, which could lead to an increase of antiparallel $\beta - sheet$ bonds. In the second part (temperature more than 60 and up to 95°C), the $\alpha - helix$ would remain constant while the $\beta - sheets$ start to unfold and shape irregular protein structures. From the data of Table 9.2, it can be concluded that protein denaturation of the seeds at microwave power of 500 W (both air temperatures) occurred in the first part of the proteins' secondary structure change and it could be reassured that the rest of the microwave power-air temperature combinations did not cause any significant ($P<0.05$) harm to lentil's storage protein.

Table 9.2 Weight of the band assignments in the amide I region of the protein in lentil seeds before and after treatments in the microwave fluidised bed¹

Air temperature (°C)	Microwave power (W)	W_{1624} (Aggregates)	W_{1639} ($\beta - sheet$)	W_{1658} ($\alpha - helix$)	W_{1666} (Turn)	W_{1682} ($\beta - antiparallel$)
Control		0.016±0.013	0.208±0.009	0.094±0.017	0.096±0.010	0.025±0.005
50	0	0.019±0.007	0.217±0.005	0.092±0.011	0.090±0.006	0.018±0.004
	300	0.027±0.010	0.205±0.006	0.080±0.011	0.085±0.007*	0.027±0.006
	400	0.024±0.008	0.210±0.008	0.083±0.007	0.086±0.007*	0.023±0.008
	500	0.027±0.020	0.199±0.013	0.076±0.011*	0.083±0.006*	0.033±0.009
60	0	0.020±0.008	0.210±0.007	0.090±0.008	0.091±0.007	0.022±0.005
	300	0.024±0.011	0.207±0.008	0.082±0.015	0.089±0.008	0.028±0.008
	400	0.023±0.008	0.209±0.005	0.085±0.010	0.090±0.005	0.026±0.008
	500	0.031±0.015*	0.203±0.013	0.077±0.015*	0.082±0.009*	0.027±0.008

¹ data with * are significantly different from their control in the same column based on Dunnett multiple comparisons with a 95% confidence level; data are mean ± SD with n=9

9.5. Conclusion

This study investigated the effect of microwave fluidised bed drying and disinfection on the quality attributes of red lentil seeds including antioxidant defence system, cooking quality and macronutrients using FTIR micro spectroscopy. Four microwave powers of 0, 300, 400 and 500 W (corresponding power densities of 0, 3.6, 4.8 and 6.0 W g⁻¹ dry seeds) and two air temperatures of 50 and 60°C were applied to dry the seeds from 16.5% to 10.5% MC. Among antioxidant enzymes, there was a slight reduction in POX activity of the seeds treated at microwave powers of 300 and 400 W with air temperature of 60°C (no measurement was done for 500 W), but no change was observed in APX and CAT activities. There was no change in cooking quality of the seeds treated at power-air temperature combinations of 300W-50°C, 300W-60°C, 400W-50°C as well as with no microwave power and both air temperatures. Analysis of FTIR spectra recorded from protein-dominant and starch-dominant areas of the cotyledon showed no difference between their overall absorbances. There was no change in the peak positions of amid I and lipid as a result of any of the treatments, but a slight reduction in lipid/protein and phosphate/protein indexes was observed after treating the seeds with a power of 500 W and air temperature of 60°C, which could be the result of lipid peroxidation and reduction of phytic acid content. The second derivative analysis of amid I of the protein showed a reduction in α – *helix* and an increase in the aggregated β - sheet in the seeds treated with a microwave power of 500 W, which was concluded to be the result of the first stage of protein denaturation at this microwave power. However, no significant conformational changes were observed in the protein secondary structure of the seeds treated at microwave powers of 300 and 400 W. It may be concluded that by controlling the hot spot of the product while treating with microwave radiation, negative quality effects can be prevented. The fluidising hot air used in this study avoided crossing the temperature limitation and agitated the seeds so that they received the same amount of microwave radiation during the treatment.

The effect of thermal treatment using microwave energy was investigated so far. However, some of the seed types or even different lots of one type of legume seed can react differently toward the thermal treatment. Therefore, non-thermal treatment using microwave plasma, will be evaluated in the next chapter.

Chapter 10. Afterglow of atmospheric non-thermal plasma for disinfection of *Botrytis grey* mould from infected lentil seeds

Under revision following peer review for "Innovative food science and emerging technologies"

10.1. Abstract

Seed-borne fungal diseases of grains are a serious threat to the crops' yield in the agriculture industry due to lack of either resistant crop varieties or too much dependence on fungicides. Use of fungicides still raises concerns about the environmental and human health impacts. Therefore, there is a growing demand to develop more sustainable and environmentally friendly technologies of crop protection for integrated pest management. In the present study, the effectiveness of atmospheric downstream microwave plasma, which is one of the emerging technologies with great potential in eliminating surface microorganisms, was investigated for the eradication of lentil seed-borne *Botrytis grey* mould (BGM). Working gases of argon, air, and a combination of the two gases (30% air/70% argon) were applied to create the plasma. The artificially inoculated seeds were then treated at different treatment times and holding times. There was a 41% reduction in the percentage of infected seeds (IS%) after 100 s of treatment with the afterglow of air plasma followed by 24 h holding in a sealed bottle. A 32.3% reduction occurred when a 30% air/70% Argon was applied for 10 s and 60 min of holding time. Except for the holding time of 24 h, which triggered catalase activity, no change in the antioxidant enzyme activity was observed in other treatments. This treatment changed lentil seed colour causing a red/yellow ting. Conclusively, the afterglow of atmospheric microwave plasma/plasma processed air (PPA) has a good potential in the eradication of the seed-borne BGM and could be considered as a part of integrated disease management strategies in lentil crops.

10.2. Introduction

Botrytis grey mould (BGM) is one of the important seed-borne diseases of pulse crops and vegetables. It can be transmitted from seed to seedling, without any visible symptoms, in many agricultural products such as lentil ([Lindbeck et al., 2009](#)), primula ([Barnes & Shaw, 2003](#)) and lettuce ([Sowley et al., 2010](#)). BGM is caused by *Botrytis cinerea* (BC) in lentil and it can reduce the crop yield dramatically, especially in cool and humid seasons.

Lentil is one of the important pulse crops and a valuable source of protein, carbohydrate, and fibre. This is known as a cool-season crop in Australia and its yield is threatened by

fungal diseases, including BGM. BGM is a seed-borne pathogen and is primarily controlled by fungicides ([Lindbeck et al., 2009](#)). There are still concerns about the emergence of resistance in pathogen populations of crops as well as the environmental and health impacts due to the overuse of these chemicals. There have been attempts to find a proper non-chemical method to be considered as part of an integrated disease management strategy in pulse crops, including conventional ([Burgess, 1997](#)) and microwave ([Taheri et al., 2020](#); [Taheri et al., 2019b](#)) thermal treatment. However, limitations exist with these thermal treatments including the concern of seed quality damage, long exposure times and high energy demands. These limitations have been the motivation for developing a fast and non-thermal physical method for seed treatment.

Among the physical methods of seed treatment, non-thermal plasma has gained an increasing interest in recent years. A considerable amount of literature has been published on the potential of cold plasma for plant growth enhancement ([Randeniya, 2015](#)) and removing pathogenic bacteria and storage fungi from the seeds of various crops, including rice ([Khamsen et al., 2016](#)), wheat ([Thomas-Popo et al., 2019](#)), maize ([Zahoranová et al., 2018](#)) and other grains ([Selcuk et al., 2008](#)). Although the fungicidal effect of cold plasma has been confirmed for some of the pathogens related to grains ([Filatova et al., 2012](#); [Jo et al., 2014](#); [Pérez Pizá et al., 2018](#); [Zahoranová et al., 2016](#)), there is still a lack of information about its effect on a wide range of seed-borne pathogens and the quality of legume seeds.

There are different sources and apparatus for creating a cold plasma for seed treatment. Electromagnetic waves, at a frequency of 2.45 GHz (microwave), is one of the sources, which can be utilised to create the plasma without the need for an electrode. This source has been used to produce plasma processed air (PPA) that has been explored for its antimicrobial activity on fresh produce and seeds ([Hertwig et al., 2015](#); [Schnabel et al., 2018](#)) and also to process water, usually called plasma-activated water (PAW), that has been proven to have decontamination ([Schnabel et al., 2019](#)) and plant growth enhancement ([Kang et al., 2019](#)) effects.

Therefore, the objective of this study was to explore the potential of microwave downstream plasma to decontaminate lentil seeds from the seed-borne BGM pathogen. For this purpose, contaminated seeds were exposed directly to plasma with argon gas or remotely utilising air or a mixture of air and argon as the process gas. Any positive or negative effect of plasma treatments on the seed quality was also investigated.

10.3. Materials and Methods

10.3.1. Lentil seeds preparation

Lentil seeds (type Bolt) were collected from a local farm in Horsham, Victoria, Australia. The 100-seed weight was 4.27 ± 0.09 g, and average diameter and thickness of twenty seeds, measured using a digital calliper, were 5 ± 0.19 and 2.4 ± 0.14 mm respectively. The initial moisture content of the seeds was determined as 10% (wb) by drying the seeds at 130°C for 20 h. Preparation of BGM culture and the procedure of inoculation of the seeds with the spores was followed from previously published work from our lab ([Taheri et al., 2020](#); [Taheri et al., 2019b](#)) (refer to section 8.3.2).

10.3.2. Plasma system

The plasma system consists of a 3-kW magnetron and three-stub auto-tuner to minimize reflection of the microwave energy. Plasma is created in the downstream plasma source with TE_{10} mode developed by Sairem Co. (WR340 3 XX, Neyron, France). The plasma reactor is a rectangular stainless-steel chamber with a length of 220 mm and height of 190 mm and the quartz tube with a length of 500 mm passed through the centre of the reactor where the gas interacted with microwave and created plasma. The system works at atmospheric pressure and can be fed by gases of Argon and air. The plasma in the quartz tube is cooled down by the water and airflow. Each stainless-steel tube in the top and bottom which were used for circulation of water around the quartz tube was 150 mm in length. A mass flow controller (EL-Flow Prestige, Bronkhorst®, Netherlands) was used to control the gas flow rate and mix the gas flows at an accurate ratio where needed. Forwarded and reflected microwave powers were monitored using the Auto-tuner (S-Team – HOMER Automatic Impedance Analyzer and Matching System, Bratislava, Slovak Republic) with HomSoft software (Version 5.0.0.2). The whole schematic of the system is shown in Figure 10.1.

10.3.2.1. Simulation of electric field and temperature of the treated seeds

A simulation of the system was performed using the commercial software XFDTD® 7.5.0 (Remcom, Inc., State College, PA) with argon gas at atmospheric pressure as the solid sensor inside the quartz tube and microwave frequency of 2.45 GHz and power of 800 W. Here, the solid sensor means the material that absorb microwave radiation and the simulation was performed by considering argon at atmospheric pressure just before plasma ignition to show the electric field distribution inside the plasma reactor. The temperature of the lentil seeds after treatments was measured using an infrared thermal camera (FLIR C2 Education

kit) by considering lentil's emissivity as 0.95 (Taheri et al., 2019c), and a vertical distance of 20 cm of the camera from the seeds.

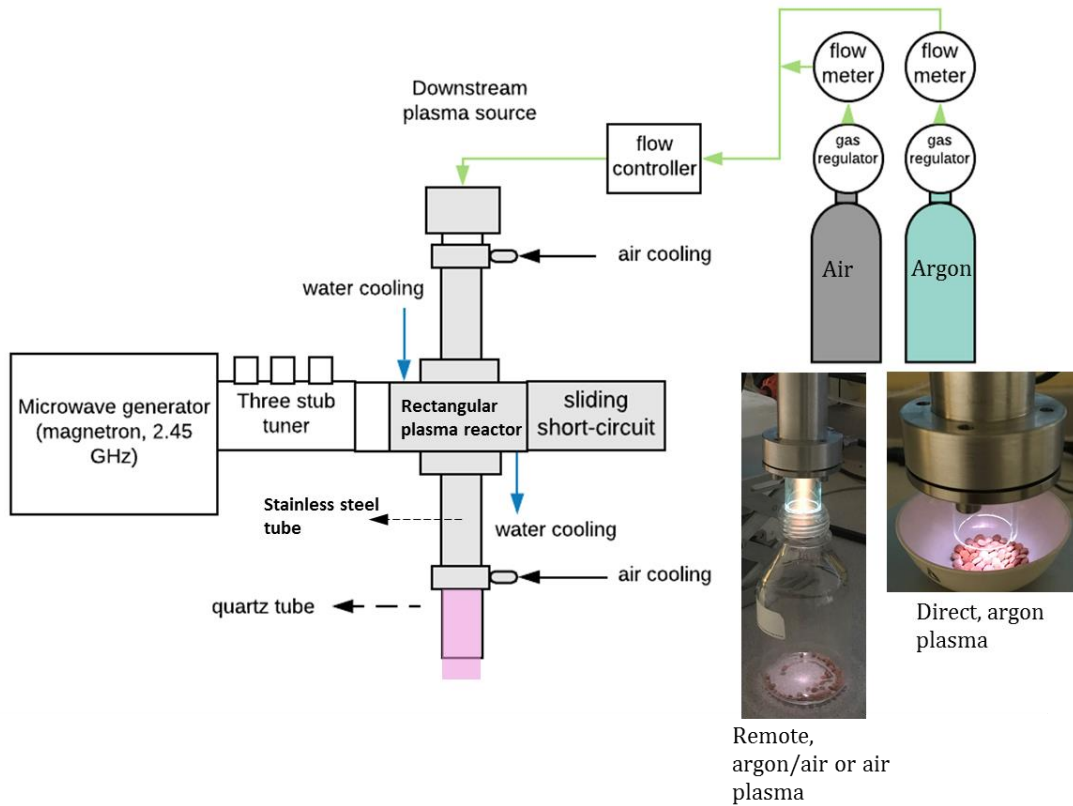


Figure 10.1 Downstream cold plasma system

10.3.3. Design of the experiment

10.3.3.1. Direct plasma

In a preliminary experiment, the effect of process parameters including microwave power (300 and 500 W), gas flow (15 and 40 nl min⁻¹ (n indicates normal)), exposure time (15 and 45 s) and seed moisture content (10% and 16%) on IS% reduction and seed viability were investigated using direct exposure to argon plasma (2 cm below the end of quartz tube). The plasma treatments were carried out in triplicates and all the quality tests were done at least twice for each treatment replicate. The process parameters were chosen in order not to exceed the temperature of 50°C in the lentil seeds. For direct argon plasma treatments, 2.5 g of contaminated lentil seeds were placed inside a ceramic dish and the seeds were exposed to plasma at a distance of 2 cm below the quartz tube, which is about 20 cm below the entrance of microwave into the plasma source. The container was shaken continuously to make sure all sides of the seeds were treated equally. The ceramic dish diameter was 100 mm with a height of 20 mm.

10.3.3.2. *Remote plasma treatment (exposure to afterglow) or plasma processed air (PPA)*

To investigate the effect of exposure to the plasma afterglow on the infected seeds, the gas flow of 20 nl min⁻¹ (n indicates normal condition) containing air/argon (30/70 v/v) and 100% air were utilised to create plasma. Plasma was ignited with 1.5 kW of microwave power being injected into the plasma source and then the power was reduced to 800 W and the treatments were carried out using the plasma operated with 800 W. The auto-tuner was on during the ignition of the plasma and also after the establishment of the plasma to match the changes in the impedance. For each treatment, 2.5 g of contaminated lentil seeds were placed inside a 500-ml Schott Duran bottle and the bottleneck (inner diameter of 30 mm) was adjusted just below the quartz tube with no more than 1 mm distance. Seed temperature did not exceed room temperature during the exposure times in any of the treatments. After each treatment with specified treatment time (10 and 100 s, as well as 150, 200, 300 and 400 s), the bottle's lid was immediately closed and sealed using aluminium tape to trap the processed gas. This was followed by holding the seeds inside the closed bottle for a specified holding time (0, 5, 15, 30, 60 min and 24 h). The seeds were shaken two or three times during the holding times to expose both sides of the seeds to the reactive gas species. After the holding times, the bottle was opened to let the PPA out and then after shaking the seeds into a beaker to make sure there was no trapped process gas in them, they were transferred into the polyethylene Ziplock bags until further examinations. Evaluation of the fungicidal effect of plasma

Botrytis grey mould selective media (BSM) was prepared with the following ingredients (g l⁻¹): glucose 2; PDA 5; Agar 10; tannic acid 5; Ridomil 0.02; Pentachloronitrobenzene (PCNB) solution with concentration of 0.5% in ethanol 4; Zineb 0.00091; streptomycin sulphate 0.1 ([Taheri et al., 2020](#)). A mixture of glucose, PDA and agar was autoclaved and cooled to below 50 °C before adding other ingredients. Direct and remote treatments of the seeds were performed three times and after each treatment by the downstream plasma, 24 lentil seeds per replicate (total of 72 seeds per treatment) were sub-cultured on BSM and the plates were incubated at 23°C with 12 h/12 h dark/light cycle under fluorescent (OSRAM TLD/18W) and near-ultraviolet (UV) lights (PHILIPS BLB/18W). After four days mycelium growth and the colour change of the BSM to golden brown was examined. Seeds with fungal growth were considered infected and the percentage of infected seeds (IS%-4d) was calculated by dividing the number of infected seeds by the total number of sub-cultured seeds. IS% of the treated seeds was compared with that of the untreated control samples.

The same evaluation was carried out after 7 d of incubation (IS%-7d). Infection degree after 4 d of incubation (ID%-4d) was also calculated based on the degree of fungi growth on the seeds. The degree of growth was considered as 1 (low growth of mycelium on the seeds) and 2 (high growth of the mycelium on and around the seeds on the media) and then ID% was calculated as:

$$\frac{(\text{no. of infected seeds with degree of } 1 \times 1 + \text{no. of infected seeds with degree of } 2 \times 2)}{\text{total no. of seeds} \times 2} \times 100 \quad (10.1)$$

10.3.4. Lentil's quality assessment

10.3.4.1. Seed viability

The seeds were examined for their viability using the standard germination test by placing 10 healthy-looking (symptomless, without discolouration) seeds per replicate (three replicates) in a 90-cm plate containing No.1 Whatman filter paper and 4 ml of sterilized distilled water. Germinated seeds were counted after 4 d of incubation at room temperature. To evaluate seed vigour, seedling fresh weight was determined after 4 d using a four-digit scale and vigour index was calculated as below:

$$\text{Vigour Index} = \frac{\text{Seedling fresh weight} \times \text{Germination}\%}{\text{Number of seeds}} \quad (10.2)$$

10.3.4.2. Antioxidant enzymes activity of the seedling (POX, CAT, SOD, H₂O₂)

Ten of the four-day-old lentil seedlings were accurately weighed using a 4-digit scale. The weighed seedlings were mashed using a pre-chilled pestle and mortar in an ice bath and the enzymes were extracted in 50 mM potassium phosphate buffer with a pH of 6.8 for peroxidase (POX) and catalase (CAT) activities and 100 mM potassium phosphate buffer (pH 7.6) for superoxide dismutase (SOD) followed by centrifugation of the homogenised mixture with 11000g at 4°C for 10 min. The supernatants were then collected in the centrifuged tubes and kept in an ice bath for a maximum of 12 h. The CAT activity of the enzyme extract was measured based on the breakdown of H₂O₂ in the presence of the enzyme at 240 nm using a UV/visible spectrophotometer ([Maehly, 1954](#)). POX activity of the extract was assayed according to the method described by [Maehly \(1954\)](#). H₂O₂ content was measured based on the peroxide mediated oxidation of Fe⁺² and then the reaction of Fe⁺³ with xylenol orange with an extinction coefficient of 220 mM⁻¹cm⁻¹ ([Daniela Bellincampi, 2000](#)). SOD activity was defined according to the method described by [Dhindsa et al. \(1981\)](#), which is based on the ability of the enzyme for inhibition of the photochemical reduction of Nitro Blue Tetrazolium (NBT) by 50%. Protein contents of the enzyme extracts

were determined according to [Bradford \(1976\)](#) using Bradford reagent for 1-1400 $\mu\text{g ml}^{-1}$ protein (Sigma-Aldrich, NSW, Australia) by mixing 20 μl of the enzyme extract and 200 μl of the reagent in microplate wells followed by measuring their absorbance at 595 nm after resting for 5 min at room temperature against a blank containing phosphate buffer and the reagent. The protein concentrations were decided using the standard curve of BSA (Bovine Serum Albumin) at concentrations of 0.1-1 mg l⁻¹.

10.3.4.3. *Lentil seeds' colour assessment*

Lentil seed's colour was assessed by a Nix™ Pro colour sensor. Seven seeds were arranged in a circle on a black surface, and their colours were scanned, followed by extraction of the CIE.L.a.b colour space data (L*: lightness, a*: red/green value, b*: blue/yellow value), using the Nix™ Pro mobile application (v 2.6.4). The overall change in the colours, compared to control samples, was determined by calculation of ΔE^* as below ([Mokrzycki & Tatol, 2011](#)):

$$\Delta E^* = \sqrt{(L^* - L_{control}^*)^2 + (a^* - a_{control}^*)^2 + (b^* - b_{control}^*)^2} \quad (10.3)$$

According to [Mokrzycki & Tatol \(2011\)](#), clear differences in the colours could be observed by human eyes for $\Delta E^* > 3.5$.

10.3.4.4. *Contact angle measurement*

The effect of plasma treatments on the wettability of the lentil seeds' coat was evaluated by measuring water droplet contact angle using Attension Theta Optical Tensiometer (Biolin Scientific, Frolunda, Sweden) with an accuracy of ± 0.1 degrees. RO (Reverse osmosis) water with a volume of 2.5 μl was dropped on each seed and simultaneously the video was captured and contact angles of right and left sides of the droplet on the seeds were automatically measured and recorded with the OneAttension software every 0.03 s up to 10 s. The final picture of the water droplet on the seeds as well as the mean value of the right and left contact angle at the time 0 and 10 s were extracted from the software.

10.3.5. *Statistical analysis*

General factorial regression or general linear model (GLM) was used to fit the results followed by the evaluation of the significant effects of the factors and their interactions by ANOVA (analysis of variance). The responses were compared and grouped using Fisher LSD (least significant differences) method with 95% confidence level. All the statistical analysis was performed in Minitab version 18.1 (Minitab Inc., Pennsylvania, USA).

10.4. Results

10.4.1. Electric field distribution and microwave power

Electric field distribution in the downstream plasma reactor, for microwave power of 800 W, is shown in Figure 10.2. By applying this input power, the maximum electric field was estimated as $56,816 \text{ V m}^{-1}$. Forward power, for the set power of 800 W, fluctuated between 400 W and 1200 W due to the sinusoidal excitation at frequency of 2.45 GHz. Average forwarded and reflected microwave power, after the plasma ignition, was calculated to be 660 W and 10.68 W respectively for the 800 W set power, which shows that there is less than 2% of the microwave reflection in the downstream plasma system.

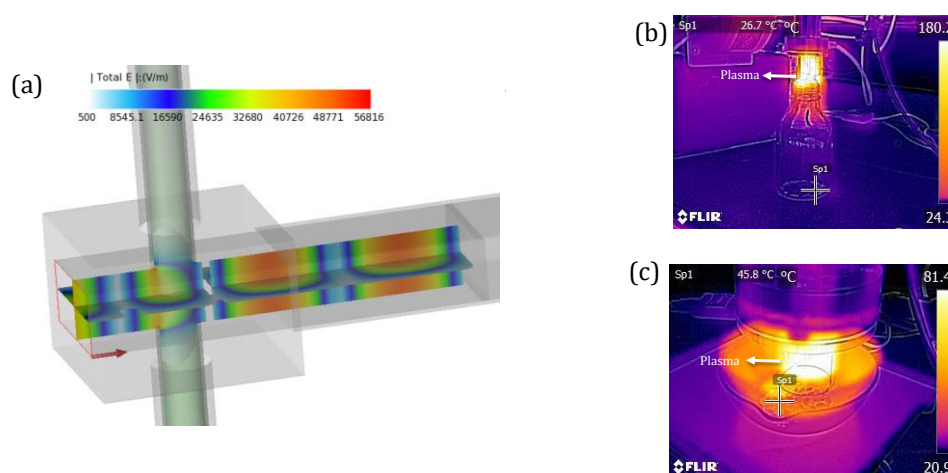


Figure 10.2 (a) Electric field distribution in the downstream plasma reactor with sliding short distance of 12 cm and microwave power of 800 W, the red arrow shows the direction of microwave entry; Images of the thermal camera for (b) Indirect, air and (c) Direct, argon plasma treatments of lentil seeds.

10.4.2. Direct exposure to argon plasma, fungicidal effect, and seed viability

According to the result of the general factorial regression on the results from the preliminary experiment, gas flow rate (p-value=0.01 and F-value=7.27) was the most effective factor in reducing the IS%. Based on these results (results in supplementary data), microwave power (300-500 W) did not affect the results, and since it was challenging to reach a stable plasma with a power of 300 W, microwave power of 400 W was applied in the next step with three levels of argon gas flow (20, 30 and 40 nl min^{-1}) and 25-125 s for exposure times. The results are illustrated in Figure 10.3 (a-d).

As can be seen in Figure 10.3(c), IS% (after 4 d of incubation) decreased from 70% to 66%, 56% and 51% after 100 s of exposure and 45%, 37% and 12% after 125 s exposure for gas flow rates of 20, 30 and 40 nl min^{-1} respectively. Germination and vigour index (Figure 10.3 (a & b)) were not significantly affected up to 100 s, while there was a dramatic reduction in

germination from 87.5% to 66.6%, 15% and 0%, at 125 s for gas flow rates of 20, 30 and 40 nl min⁻¹ respectively. The seeds' temperature increased to more than 80°C after 125 s (Figure 10.3(d)). To avoid the contribution of the heat, the seeds were treated in a pulse mode to keep the temperature below 60°C. In pulse mode treatment, a time of 150 s was provided using 2 cycles of 75 s, 3 cycles of 50 s or 6 cycles of 25 s. Similarly, a time of 250 s was provided using 10 cycles of 25 s with interval cooling to below 45°C (the results in the supplementary data). There was no significant difference among the treatments according to one-way ANOVA with 95% confidence level, but there was a significant reduction in IS% (4d of incubation) from 70% in the control to 52% in the seeds treated with 10 or 6 cycles of 25 s.

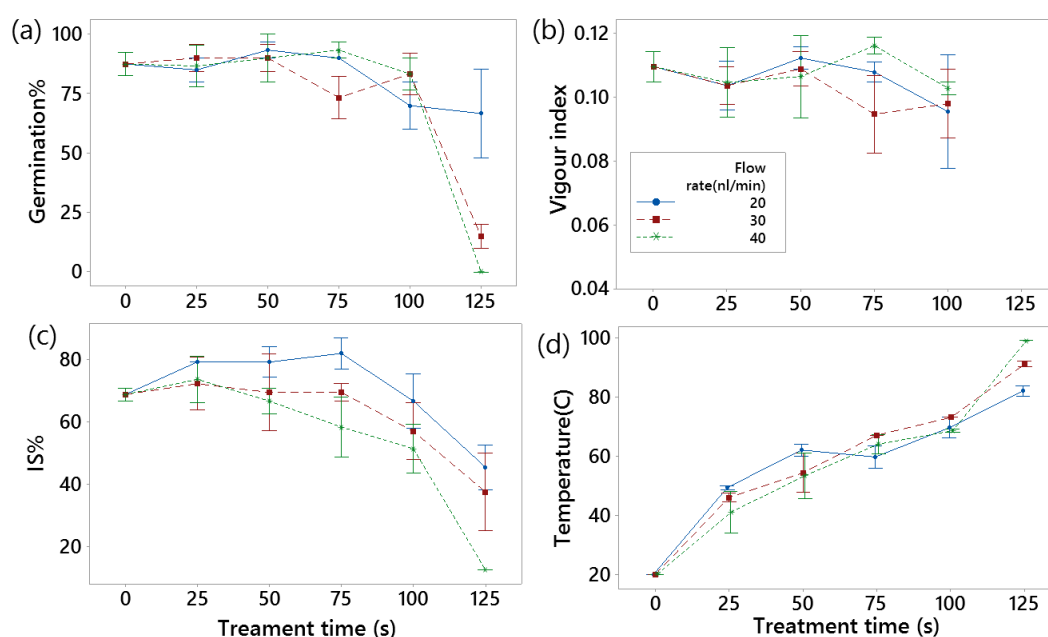


Figure 10.3 Results of the treatments with 100% argon plasma at a microwave power of 400 W, seed moisture content of 10%, different gas flow rates and exposure times; (a) Germination rate after 5 d, (b) Vigour index, (c) Infected seed percentage (IS%) after 4 d of incubation, (d) Seed temperature after treatments; bars are standard errors of means for n=3.

10.4.3. Remote treatment of the seeds with afterglow flow of plasma, fungicidal effect, and seed viability

Analysis of IS% using general factorial regression with three factors of treatment time (A), holding time (B) and air% (C) revealed that the three-way interaction (A.B.C) was significant (p-value = 0.041). Therefore, the two-way ANOVA was applied to compare the obtained results for each air% (30% and 100%) separately. Figure 10.4 (a-f) represents the results of lentil seeds treated remotely, which can be called the afterglow of the plasma, using a mixture of 30% air/ 70% argon plasma. Although there were slight increases in the germination and vigour index of healthy seeds (symptomless seeds) after some of the

treatments, such as treatment time of 100 s and holding time of 15 and 60 min (an increase from 92.5% to 100% in germination and from 0.1 to 0.12 in vigour index) (Figure 10.4 (a and b)), no statistically significant differences were found among them. IS% after 4 d of incubation (c), ID% after 4 d of incubation (d), and IS% after 7 d of incubation (f) were significantly affected by treatment (p-value of 0.000) and holding times (corresponding p-values of 0.026, 0.015, 0.004). However, increasing the treatment time from 10 s to 100 s was not beneficial in decreasing the IS% or ID%. The best process parameters at this stage were treatment time (t) – holding time (H) of t10s-H60min and t100s-H15min, which reduced the IS%-4d from 88.5% to 40.3% and 36.1% respectively and IS%-7d from 94.4% to 63.9% and 73.6% respectively. Germination of infected seeds on the selective media (Figure 10.4 (e)) increased significantly at t10s-H15min (from 38.9% to 68%) and decreased at t100s-H24h (from 38.9% to 19.4%). Although holding time significantly affected IS% reduction, no significant change was observed by increasing it from 60 min to 24 h.

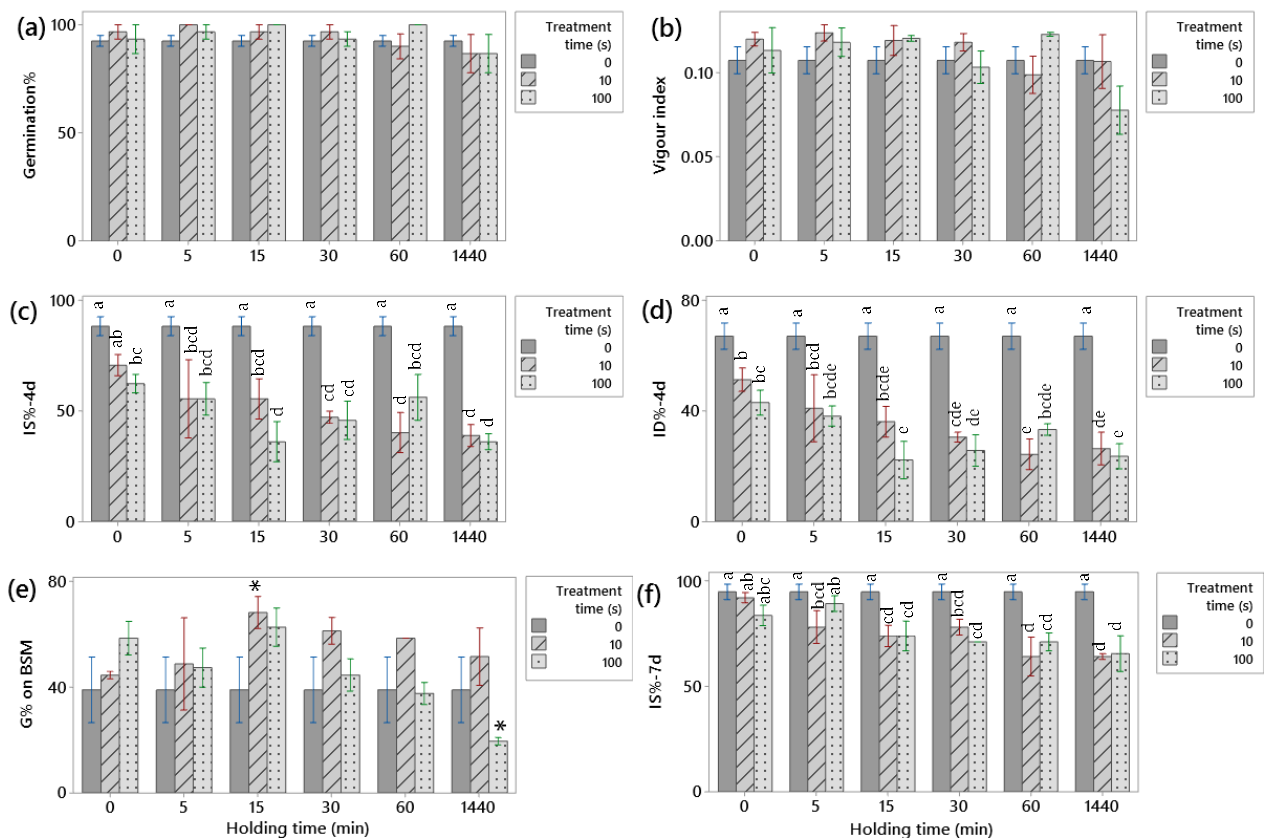


Figure 10.4 (a) Germination% of the healthy seeds, (b) Vigour index, (c) Infected seeds% after 4 d of incubation, (d) Infected degree% after 4 d of incubation, (e) Germination% of the contaminated seeds on BSM and (f) Infected seed% after 7 d of incubation for the lentil seeds exposed to 30% air plasma remotely at different treatment and holding times; error bars are $\pm$ SE with $n=3$; bars which do not share a letter are significantly different according to Fisher's LSD method with 95% confidence level.

The results of lentil seed treatments using 100% air plasma are shown in Figure 10.5 (a-f). Here again, no significant changes were observed in germination and vigour index of the healthy-looking seeds, but treatment time and holding time significantly affected IS%-4d, IS%-7d and ID%-4d. A treatment time of 100 s with the holding time of 5 min reduced IS%-4d from 88.5% to 33.3%, ID%-4d from 67.2% to 19.4% and IS%-7d from 95.8% to 72%. Although no more reduction in IS%-4d and ID%-4d occurred for 100 s treatment by increasing holding time from 5 min to 24 h, a further reduction in IS%-7d to 56.9% was achieved after 24 h of holding time. The results for 10 s treatment time were quite different from those for 30% air plasma. With 10 s treatment time, the results for IS%-4d and ID%-4d were significantly affected by a holding time after 60 min and 24 h (44.8% and 26.4% respectively), while IS%-7d was just significantly reduced after 24 h of holding time (from 95.8% to 73.6%), compared with the control samples. Germination of infected seeds on the selective media (Figure 10.5 (e)) increased significantly with a treatment time of 100 s and holding time of 15 min.

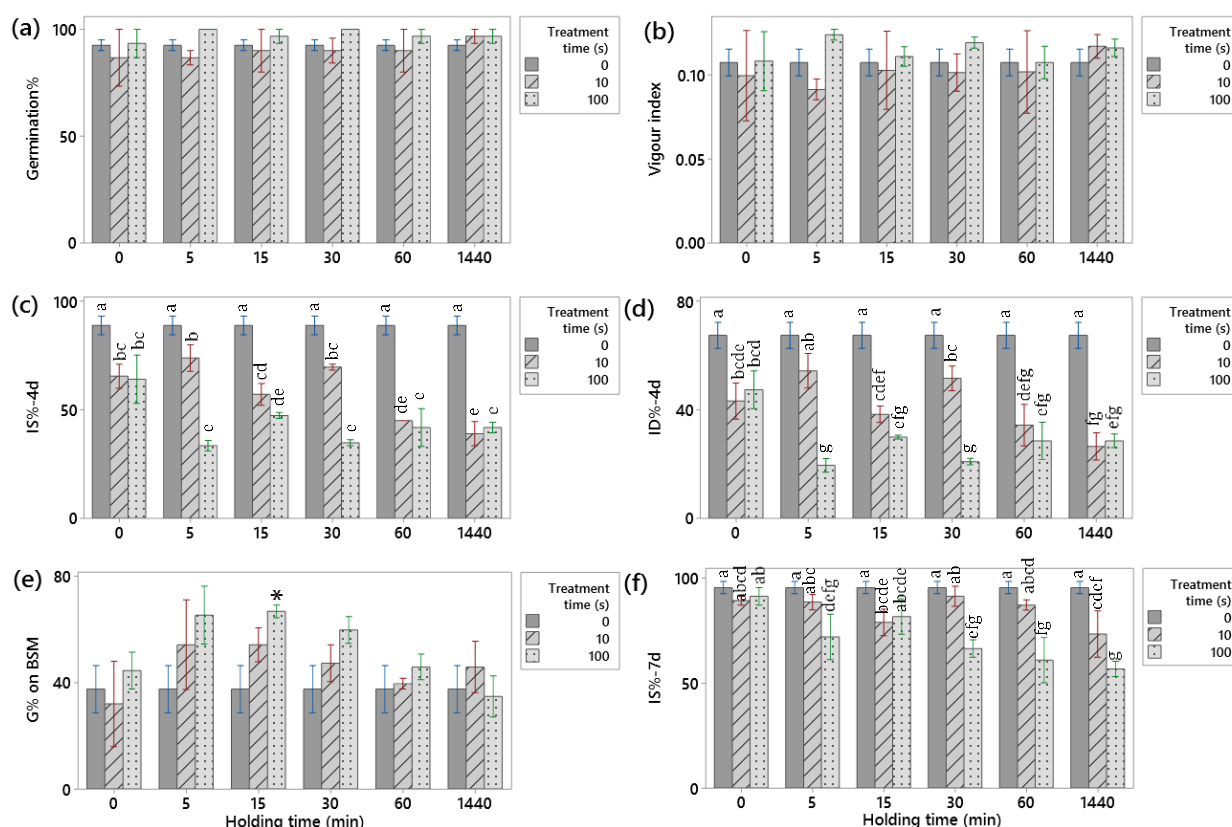


Figure 10.5 (a) Germination% of the healthy seeds, (b) Vigour index, (c) Infected seeds% after 4 d of incubation, (d) Infected degree% after 4 d of incubation, (e) Germination% of the contaminated seeds on BSM and (f) Infected seed% after 7 d of incubation for the lentil seeds exposed to 100% air plasma remotely at different treatment and holding times; error bars are $\pm$ SE with $n=3$; bars which do not share a letter are significantly different according to Fisher's LSD method with 95% confidence level.

Considering that the treatment time was the most effective factor in the reduction of IS%, treatment time was increased up to 400 s to treat the seeds with 100% air plasma following by a holding time of 5 min. The results (Figure 10.6 (a-d)) indicated that increasing treatment time from 100 s to 400 s did not reduce IS%-4d to lower than 33%. However, IS%-7d decreased to 58% after 200 s treatment compared to 72% for 100 s treatment time. There was also no more change in the germination of infected seeds on BSM and ID%-4d by increasing the treatment time from 100 s to 400 s (data not shown).

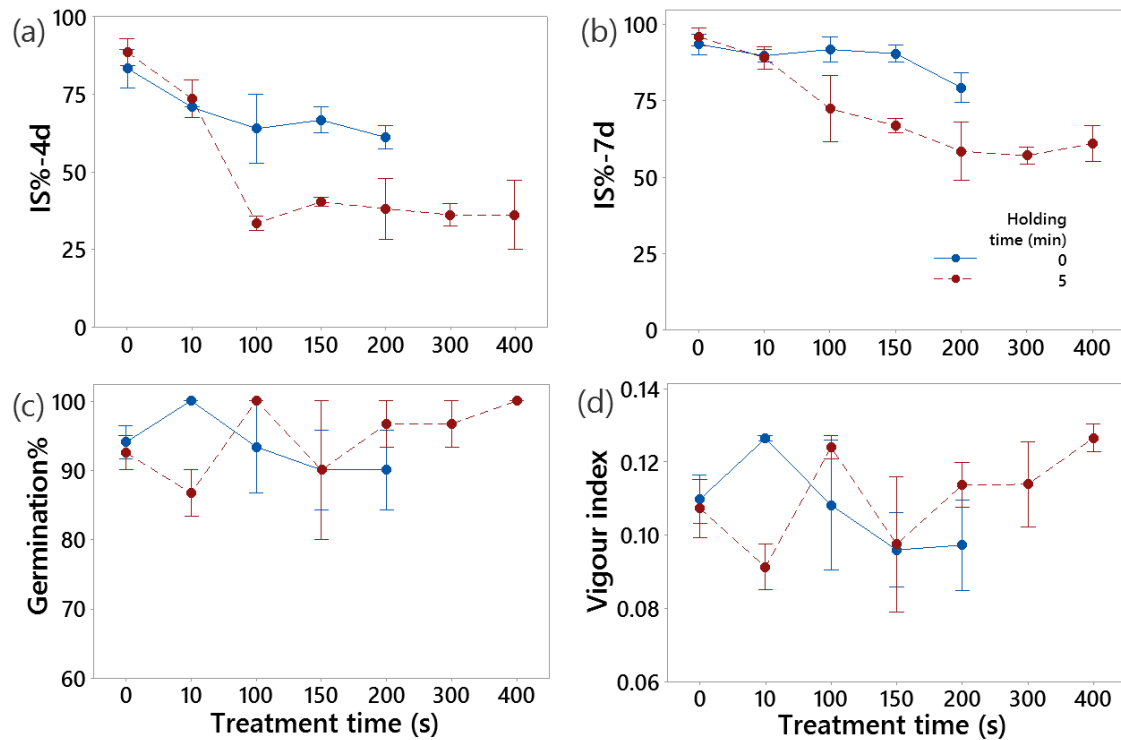


Figure 10.6 (a) Infected seeds% after 4 d of incubation, (b) Infected seed% after 7 d of incubation, (c) Germination% of the healthy seeds and (d) Vigour index of the lentil seeds exposed to remote plasma with 100% air as working gas and holding time of 5 min at different treatment times. error bars are $\pm$ SE with $n=3$.

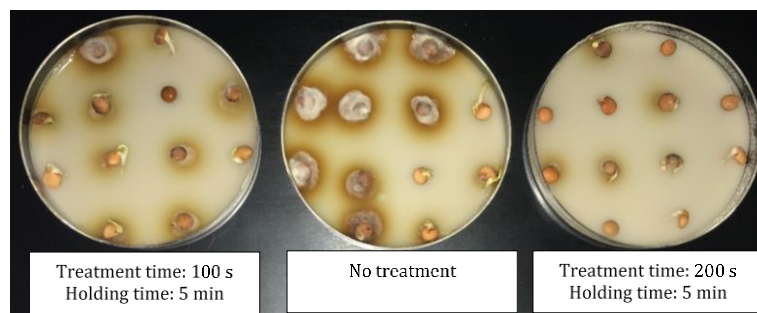


Figure 10.7 Growth of BGM of lentil seeds on Botrytis selective media (BSM), non-treated and treated with remote air plasma for 100 and 200 s and holding time of 5 min.

10.4.4. Effect of the treatments on lentil's antioxidant enzyme activity

CAT, POX, SOD activities, as well as H₂O₂ content of lentil seedlings for effective treatments from the direct and remote mode of exposures, are presented in Table 10.1. There were no significant changes in POX and SOD activities after any of the treatments. However, CAT activity increased slightly through all the treatments and this increase was statistically significant in the seeds treated with 30% air for 10 s and a holding time of 24 h, which increased CAT from 0.8 to 1.1 mM H₂O₂ mg⁻¹ min⁻¹. Correspondingly, H₂O₂ content significantly decreased at the treatment time of 10 s and holding time of 24 h for both remote treatments with 30% (from 0.88 to 0.62 μ mole g⁻¹) and 100% air (from 0.88 to 0.67 μ mole g⁻¹) plasma.

Table 10.1 Antioxidant enzymes activities of lentil seedlings before and after the treatments with direct and remote plasma¹

Mode of treatment	Air /Argon%	Treatment time (s)	Holding time	CAT (mM H ₂ O ₂ mg ⁻¹ min ⁻¹)		POX (mM guaiacol mg ⁻¹ min ⁻¹)		SOD (Unit mg ⁻¹ min ⁻¹)		H ₂ O ₂ (μ mole g ⁻¹)	
Remote	100/0	100	5 min	1.02 $\pm$ 0.10	ab	7.88 $\pm$ 0.62	a	57.82 $\pm$ 3.26	a	0.78 $\pm$ 0.02	ab
		400	5 min	0.94 $\pm$ 0.08	abc	9.145 $\pm$ 0.67	a	55.35 $\pm$ 6.29	a	0.79 $\pm$ 0.02	a
		10	24 h	0.99 $\pm$ 0.06	abc	8.09 $\pm$ 0.56	a	58.32 $\pm$ 3.72	a	0.67 $\pm$ 0.07	bc
	30/70	100	15 min	0.94 $\pm$ 0.07	abc	8.96 $\pm$ 0.69	a	54.50 $\pm$ 10.4	a	0.90 $\pm$ 0.02	a
		10	24 h	1.10 $\pm$ 0.06	a	7.876 $\pm$ 0.76	a	51.15 $\pm$ 2.10	a	0.63 $\pm$ 0.02	c
Direct	0/100	100	-	0.99 $\pm$ 0.06	abc	9.35 $\pm$ 0.61	a	65.69 $\pm$ 7.00	a	0.83 $\pm$ 0.05	a
Control	-	-	-	0.81 $\pm$ 0.04	bc	8.46 $\pm$ 0.49	a	56.69 $\pm$ 7.01	a	0.88 $\pm$ 0.04	a

¹ data are $\pm$ SE with n=6; numbers in each column which do not share a letter are significantly different according to Fisher's LSD method with 95% confidence level.

10.4.5. Effect of the treatments on the lentil seed colour

The results of the CIE Lab and the overall colour change compared to the untreated control samples (ΔE^*) are represented in Table 10.2. Remote treatment of the seeds with 30% air plasma for 10 and 100 s and holding time of 24 h reduced lightness from 51.4 to 48.02 and 43.4 but increased a* from 13.87 to 16.23 and 16.7 and b* from 21.43 to 24.27 and 26.15, respectively. Correspondingly, for 100% air plasma, a holding time of 24 h and treatment times of 10 and 100 s caused a reduction in the L* from 51.4 to 48.02 and 43.42, an increase in a* from 13.87 to 15.08 and 15.67 and an increase in b* from 21.43 to 23.45 and 24.87. Likewise, ΔE^* dramatically increased to over 4 for 24 h holding time at both treatment times and air percentages. Figure 10.8 illustrates the results of CIE Lab for longer treatment times and 5 min holding time for 100% air plasma. This shows that the same change in colour as 24 h holding time could happen at 400 s treatment time with a reduction in lightness and an increase in redness (a*) and yellowness (b*). Colour change of the lentil seeds treated

with 30% air can be observed in Figure 10.9 for the treatment time of 100 s and holding times of 0, 30 min and 24 h.

Table 10.2 CIE L^* a^* b^* data for untreated and treated lentil seeds with direct and remote plasma at different holding times¹

Mode of treatment	Air /Argon%	Treatment time (s)	Holding time	L^*	a^*	b^*	ΔE^*
Remote	100/0	100	0 min	51.1±0.6 ab	13.3±0.6 e	21.3±0.5 efgh	0.59
		100	5 min	50.3±0.9 ab	14.6±0.4 bcde	22.1±0.43 defg	1.48
		100	24 h	43.4±2.1 *d	15.6±0.6 abc	24.9±1.66 *abc	8.87
		10	24 h	48.0±0.9 bc	15.0±0.7 abcd	23.4±1.00 bcde	4.12
	30/70	100	0 min	50.9±0.8 ab	12.9±0.4 e	19.8±0.48 gh	1.89
		100	15 min	48.6±0.5 b	14.1±0.6 cde	22.9±0.48 cdef	3.17
		100	24 h	44.5±1.4 *d	16.7±0.6 *a	26.1±0.99 *a	8.80
		10	24 h	45.0±0.7 *cd	16.2±0.6 *ab	24.3±0.43 *abcd	7.37
Direct	0/100	100	-	51.3±1.2 ab	13.0±0.6 e	19.3±0.90 h	2.26
Control	-	-	-	51.4±0.6 a	13.9±0.2 de	21.4±0.40 fg	

¹ Data are $\pm$ SE with $n=6$; numbers in each column which do not share a letter are significantly different according to Fisher's LSD method with 95% confidence level; numbers with * are significantly different from control according to Dunnett method with 95% confidence.

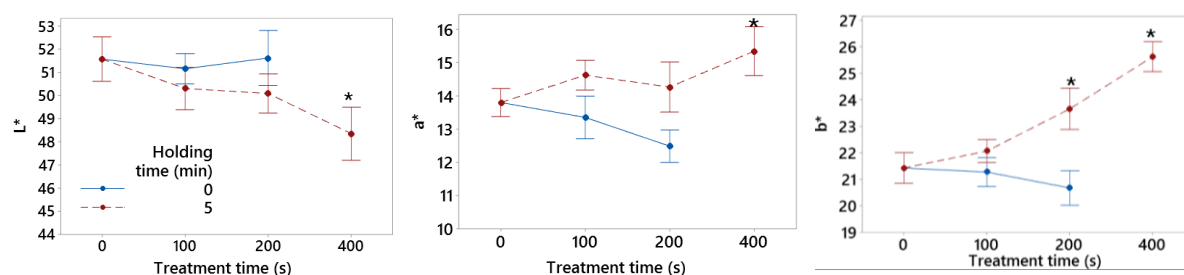


Figure 10.8 L^* a^* b^* data of the lentil seeds exposed to remote plasma with 100% air as working gas at different treatment and holding times; ΔE^* for 200 s and 400 s are 2.6 and 5.4 respectively; error bars are $\pm$ SE with $n=6$; data with * are significantly different from control according to Fisher's LSD method with 95% confidence level



Figure 10.9 Colour change of lentil seeds treated by remote plasma with 30% air for 100 s at different holding times

10.4.6. Contact angle

Figure 10.10 shows the water droplet contact angles on the control and treated lentil seeds. The mean contact angle at the beginning was 117° in control samples and 95° in the samples treated with 30% air with a treatment time of 10 s and holding time of 24 h (30%Air-t10s-H24h). This was 101° and 96.6° for the seeds treated with 100% air for 100 s and holding

times of 5 min and 24 h respectively. After 10 s of capturing all these values reduced and it was lowest for 30%Air-t10s-H24h with 73.3° compared to 101.6° for the control samples.

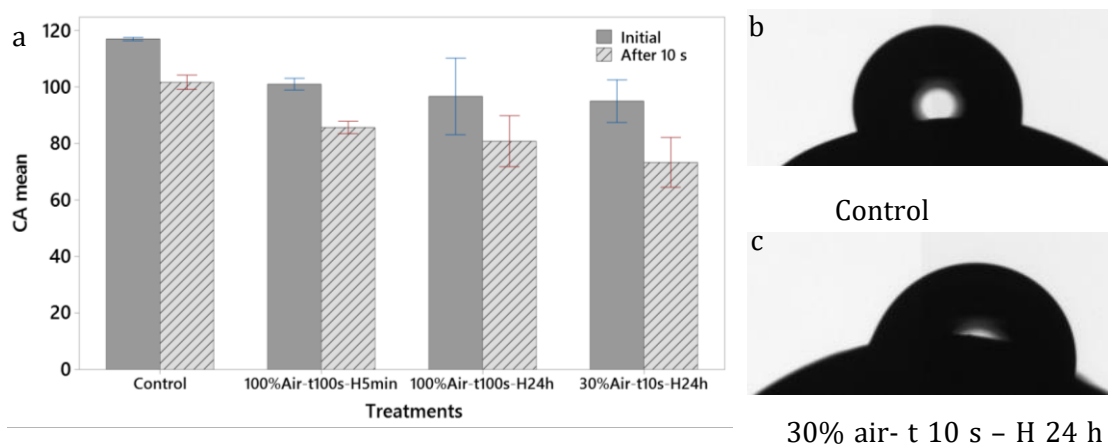


Figure 10.10 (a) Mean Contact Angle (CA) of the water droplet on the control and treated seeds with 100% air, treatment time of 100 s and holding time of 5 min (100% Air-t100s-H5min) and holding time of 24 h (100% Air-t100s-H24h), and with 30% air, treatment time of 10 s and holding time of 24 h (30% Air-t10s-H24h); the image of contact angle measurement of (b) Control and (c) The seed treated with 30% air, treatment time of 10 s and holding time of 24 h

10.5. Discussion

In the direct argon plasma treatments, less IS% was observed by increasing gas flow and exposure time. However, the temperature of the seeds increased to more than 60°C after 75 s of exposure time. Therefore, any reduction of the pathogen from the seeds after this point could be the result of the synergistic effect of the heat and plasma. Interestingly, the IS%-4d slightly increased (although with no statistical significance) after treating the seeds with an argon gas flow of 20 nl min⁻¹ and treatment times of 25, 50 and 75 s. It shows that the effect of plasma on growth enhancement of fungi and seeds should be the same, although the dosage and the treatment time might be different. Šimončicová et al. (2018) showed that plasma treatment could induce antioxidant enzyme activities of *Aspergillus flavus* as a result of the hyphae reacting to the stress. Similar effects might have been evident in the BGM pathogen, which led to their recovery and even more growth later when enough food was provided through media. However, longer treatment times or higher gas flow rates caused enough damage to them to decrease their growth after 4 d of incubation.

The best result of this stage (minimum IS% and maximum germination rate) was achieved by treating the lentil seeds utilising a gas flow of 40 nl min⁻¹ for 100 s. This raised the temperature to 64°C (Figure 10.3, (d)) and reduced IS% (4 d of incubation) from 70% to 50% (Figure 10.3 (c)) without any deteriorative effect on seed viability (Figure 10.3, (a) and (b)). This reduction in IS% might be due to the contribution of heat, UV and reactive species (mostly excited argon atoms and their derivatives) of the plasma. The results of pulse mode

treatment indicated that an increasing number of cycles from 6 to 10, did not have a beneficial effect on the reduction of IS%, though it adversely affected seed viability. Treatment of the seeds with 6 cycles of 25 s slightly increased their germination. In conclusion, pulse mode treatment in direct argon plasma did not have an added value to the continuous treatment with 100 s exposure time regarding the reduction of IS%. However, it was beneficial in keeping the seeds at a lower temperature and protected them from the deteriorative effect of high temperature on the seed viability and possibly other quality attributes. Therefore, a reduction of 20% in IS%-4d was achieved, but there was no difference between IS% of the treated and non-treated seeds after 7 d of incubation, which suggests that pathogen recovered after a longer time of incubation.

In the treatment of the seeds with the afterglow of the plasma (remote treatment), the reactive species from the air (ROS and RNS) are involved in the reduction of the BGM pathogen, while in the direct plasma treatments, the reactive species are mainly excited argon atoms. The reactive species in the afterglow of air plasma are mainly atomic oxygen (O), atomic nitrogen (N), singlet oxygen ($O_2(a^1\Delta_g)$), excited metastable species of nitrogen ($N_2(A)$ and $N_2(a')$) ([Moisan et al., 2014](#)) and the species produced from their reactions such as NO as well as the energy released in the form of UV. In the present study, the air was used as the working plasma gas, which is a combination of about 21% oxygen and 78% of nitrogen. However, [Moisan et al. \(2013\)](#) showed that a combination of N_2 - O_2 with 0.5% of oxygen provided the most UV intensity, which was the result of having the most NO γ molecular systems in the afterglow of the microwave plasma. [Takamatsu et al. \(2014\)](#) also indicated that the most NO radical production occurred when a 1:1 ratio of N_2 : O_2 was used. Therefore, the proportions of the reactive species could vary when 100% or 30% with argon was used as the plasma working gas. This could be the reason for the difference in their efficiency in the reduction of IS% at the same treatment or holding times (for example, ID% of 43% for 100% air compared to 51% for 30% air after 10 s treatment and without any holding time).

Any reduction in IS% during treatment of the seeds with the afterglow of plasma (remote treatments) and without any retention time could be the results of bombardment with the afore-mentioned reactive species from argon and air. However, the pathogen elimination during the holding times (from 5 min to 24 h) could stem from exposure to the long-lived species (such as singlet oxygen ($a^1\Delta_g$)) as well as the result of their reactions. Here, the damage to the pathogen might be due to the exposure to singlet oxygen which led to structural or permanent damage to the spores due to oxidation. The other explanation of

pathogen damage during the holding time could be penetration of atomic oxygen and nitrogen into the stack of surface spores, molecular collisions and production of UV radiation and finally damage of the pathogen ([Moisan et al., 2014](#)).

As suggested before, the damage to the spores of the pathogen, caused by the reactive species and their interactions, could be structural or to DNA material. An *in-vitro* (on water agar) investigation of the effect of surface dielectric barrier discharge on the conidia of BC by [Ambrico et al. \(2020\)](#) showed that the plasma caused the erosion of the conidia's cell wall at first and after three minutes of exposure the damage was irreversible. By increasing the exposure time, they proved that the conidia were destroyed completely after 5 minutes. If the damage is structural due to erosion of the spores' cell wall, they might be able to recover, which could be the reason for the difference between the reduction of IS% after 4 and 7 d of incubation. For example, treatment of the seeds by the afterglow of 30% air for 100 s with 15 min holding time caused a 52.4% reduction in IS%-4d (88.5% to 36.1%), while this reduction after 7 d of incubation was about 21% (94.4% to 73.6%). In our previous study, the results of the microwave thermal treatment of the lentil seeds, which were contaminated with BGM ([Taheri et al., 2020](#)), indicated that the proportion of the IS% reduction remained almost the same after 4 and 7 d of incubation, suggesting permanent damage to the pathogen. However, plasma afterglow treatments caused non-permanent damage to some of the seed pathogen spores, which led to their recovery and regrowth later in their incubation time.

No significant reduction in the IS% was observed by increasing treatment time to longer than 200 s or for a holding time over one hour. This could be due to the deep penetration of the BGM pathogen in the seed coats. The reactive species from the plasma, as well as UV, cannot penetrate deeply into the seed, which made them a very good surface sterilizer. Therefore, apart from the melanin present in the BC's cell wall ([Fillinger & Elad, 2016](#)), which makes them resistant to UV radiation, pathogen location in the seeds could also be a limitation to achieve complete eradication.

The increase in the germination of the seeds on the media (Germination% on BSM), in Figure 10.4 (e) and Figure 10.5 (e), stems from cleaning them from the pathogen and might not necessarily be the result of the stimulating effect of plasma, which has been mentioned in the literature, as an attractive application of the cold plasma. The reason for this conclusion is that we did not find the same growth enhancement in the symptomless seeds (Figure 10.4 (a and b) and Figure 10.5 (a and b)).

As the results of antioxidant enzyme activities showed, the remote plasma treatment and holding time could trigger CAT activity for scavenging hydrogen peroxide which could lead to fortifying the defence system of lentil plants and make them more immune towards contaminations later in the plants' developmental stages. It has been reported that direct or indirect treatment with plasma could decrease antioxidant enzymes activities such as CAT and SOD in wheat ([Kučerová et al., 2019](#)) and soybean (DBD, atmospheric pressure) ([Pérez Pizá et al., 2018](#)), while an increase in these enzyme activities was observed in sweet basil seeds (RF, vacuum) ([Singh et al., 2019](#)), wheat (RF, vacuum air) ([Sabeti et al., 2018](#)), artichoke (RF, vacuum N₂) ([Hosseini et al., 2018](#)), soybean (DBD, atmospheric pressure argon) ([Zhang et al., 2017a](#)) and mung bean (micro plasma, atmospheric air) ([Zhou et al., 2016](#)). The difference among the results could stem from different plasma devices with different gases and working pressure or different stages of plant growth used to measure the enzyme activity. [Surowsky et al. \(2016\)](#) explained that carbonylation as a result of an oxidative attack in plasma treatments could be the reason for enzyme reduction such as catalase. Carbonylation is an irreversible reaction of producing free carbonyl groups in some amino acids such as arginine, histidine, lysine, proline, threonine, and tryptophan. They also mentioned that Ozone or UV alone could result in enzyme inactivation. However, no enzyme inactivation was observed in the present study, which might be because the seeds temperature did not increase, or the seeds did not go under an oxidative stress due to overdosage of plasma species.

The results of lentil seeds colour measurement proved that prolonged exposure of the seeds to the afterglow of the plasma or a longer holding time could cause a reduction of the lightness (L*) and a shift toward redness (a*) and yellowness (b*). However, treatment of the seeds for 200 s did not cause any change in L* and a*, while it led to an increase in b*. Values of the overall change in colours, ΔE^* , also indicated large changes (more than 4) as a result of prolonged holding time (24 h). This colour change, which mostly occurred during the holding time, could be the result of photodegradation of the lentil seed coat (reaction with polyphenols, fibres or lipids) by the UV produced as a result of the collision of atomic oxygen and nitrogen, as stated before. It could also be the result of oxidation of the seed coat with singlet oxygen ([Wypych, 2018](#)) and could be mostly lipid oxidation, which led the seed coat to be more hydrophile as confirmed by a reduction in the contact angle. An increase in the seed wettability after cold plasma treatments were also reported by other researchers for wheat ([Velichko et al., 2019](#)), rice ([Khamsen et al., 2016](#)) and soybean ([Pérez Pizá et al., 2018](#)). [Li et al. \(2018\)](#) applied FTIR spectroscopy on the spinach seed coats and showed that

lipid molecules of the coat were altered after plasma treatment leading to more surface hydrophilicity. This phenomenon could lead to the more water uptake and increasing germination of the seeds because of plasma treatment. Overall, plasma processed air has a good potential to permanently remove the seed-borne pathogen of lentil seeds as far as the seed colour is not of concern. As the seeds in this study are aimed to be used for sowing purpose, the colour change might not be considered as a detrimental effect on their marketability.

10.6. Conclusion

Treatment of lentil seeds by a combination of the afterglow of atmospheric microwave plasma and holding them in the trapped processed gas could significantly reduce the BGM infected seeds and consequently increase their germination. A holding time after each treatment times was necessary for the plasma treatments to effectively reduce the infected seeds. The prolonged retention time of 24 h changed lentil seeds' colour dramatically but triggered the CAT activity and increased surface wettability. Applying a mixture of 30% air/70% argon or 100% air as the plasma working gas significantly affected the results. At the same holding times, there were more differences between the results of low and high treatment times with 100% air than those for the mixture of air/argon, which suggested a difference between the effective reactive species. The results suggest that afterglow of microwave plasma is a promising tool to be implemented in pulse seeds' integrated disease management as a sustainable and environmentally friendly process.

Chapter 11. General Discussion

The purpose of the present study was to design a microwave process for the eradication of important seed-borne pathogens of lentil seeds. For this purpose:

- 1- The dielectric properties of key legumes were measured in the microwave frequency range over a range of seed moisture contents, and the proper penetration depth of microwave fields into lentil and chickpea seeds was deduced. Dielectric properties showed a dependency on seed moisture content, temperature, and seed bulk density. The penetration depth of microwave fields at 2450 MHz, in red lentil seeds, was calculated to be 3.1 cm at room temperature and it decreased with increasing temperature and seed moisture content. The values obtained here were used in simulations to find the power dissipation in lentil seeds and therefore to study heat uniformity in the working systems. The penetration depth was also helpful to define a proper thickness of lentil seeds to be processed in a multimode microwave cavity. By choosing a thickness that is less than the penetration depth, more heat uniformity could be achieved along the vertical axis.
- 2- Heat uniformity of lentil was determined experimentally in a microwave cavity with a turntable, using thermal imaging, at seed moisture contents of 9%, 13%, 17%, and 21%. It was proven that the temperature difference between hot and cold spots could reach 50 °C and this difference increased with increasing microwave power and seed moisture content up to the moisture content of 17%. This was in agreement with the finding of [Manickavasagan et al. \(2006\)](#), who reported that the difference between the maximum and minimum temperatures increased with increasing microwave power from 100 to 500 W for canola, barley, and wheat. Therefore, applying lower powers for longer times might be more beneficial in reducing the difference between hot and cold spot temperatures. Additionally, the absorbed energy after microwave treatments, was calculated using the thermal and physical properties of lentil seeds and was related to their viability. It was confirmed that lentil seeds could lose their viability if they absorb more than 70 J g⁻¹ of microwave energy at any moisture content. Further, this experiment suggested that microwave power at a rate of 1.1 W g⁻¹ for 30 s (i.e. 30.3 J g⁻¹) could have a stimulating effect on the seeds by increasing their germination and vigour and decreasing the electrical conductivity of the seed's soaking water. This stimulating effect on lentil seeds was also reported by [Aladjadjiyan \(2010\)](#) at 450 W power and 30 s of exposure time. It was also confirmed by [Horikoshi et al. \(2017\)](#) who showed

that microwave energy could activate the plants' growth at a very low power. Therefore, microwave energy could be used for germination enhancement of lentil seeds at very low dose and short treatment time. It was found that heat uniformity was the lowest at 13% seed moisture content, therefore, this level of moisture content was not considered for the next experiment on infected seeds.

- 3- The possibility of removing an important seed-borne pathogen of lentil seeds, namely *Ascochyta blight* (AB), was evaluated in the multimode microwave cavity with the movement of the seeds using a glass turntable. It was first confirmed that for the elimination of the spores of the pathogen from the spore solution, a minimum temperature of 60°C was required. Therefore, the process parameters were selected to reach the same final temperature of at least 60 °C with two power levels of 50% and 100% (of the total input power of 1100 W) at different exposure times. The percentages of infected seeds were lower compared to the control sample for all the treatments, especially at higher seed moisture content. The maximum reduction without a significant loss of seed germination was about 50% (from 17% to 8.9%) at a seed moisture content of 9%. However, seed vigour was negatively affected at this process parameters (100% power level for 51 s). The reason for the dramatic reduction of the seed viability was attributed to the high difference between hot and cold spots, which was mentioned in the previous section. These results showed the necessity of high heat uniformity for seed treatment when the aim is the eradication of a pathogen, as the heat could damage the seed at specific hot spots before the elimination of the pathogen from the cold spots. An important result was that the elimination of AB from lentil seeds at higher water activities was much faster than at lower water activities. For example, at the same final average temperature of 84°C and the same power level(100%), IS% reduced from 18.3% to 8.9% (after 51 s) at 9% seed moisture content, while it reduced to 0.5% (after 28 s) at 19% seed moisture content. Water activities of seed at moisture contents of 9% and 19% were 0.4 and 0.89, respectively. This was in agreement with the findings of [Jiao et al. \(2016\)](#) who treated the grains using hot air assisted RF (65°C, 10 min) and reported that the reduction of *Aspergillus flavus* from wheat and corn increased by one log-scale (CFU/g) when their moisture content increased from 12% to 15%. However, in our study, the seed viability was also very susceptible at higher seed moisture content and a dramatic loss was observed with increasing moisture content. Controlling the hot spots at a special temperature by keeping the power level and

exposure time constant was very challenging, which could be attributed to the lack of controllability of the magnetron.

- 4- To improve heat uniformity, with the aim of controlling the temperature of the hot spot and maintaining the viability of the seed, a microwave fluidised bed was designed and manufactured. The result of the simulation in a single mode cavity reassured that the uniformity could be improved by 80% after adding hot air across the seed bed at a speed of 2.5 m s^{-1} . It should be noted that the simulation was not performed with the actual speed of air which could fluidise the seed. This was because of the limitation in the method and software used for the simulation. Therefore, the simulation was done for a bed with hot air blowing across them. However, the heat uniformity of the fluidised bed would be better than that indicated by the simulations. Temperature uniformity index (TUI) was calculated to be below 0.1 after 10 s, while it was above 0.2 in the multimode cavity even after 30 s, as it was studied in Chapter 4 (section 2 of the discussion). In this experiment, microwave energy was utilised as the heat source and forced hot air was used to fluidise the seeds to make sure that they receive uniform microwave radiation. A microwave fluidised bed was firstly evaluated by [Kudra \(1989\)](#) who implemented a fluidised bed dryer into a multimode microwave cavity and utilised it for drying wheat seeds. From then on, there have been many researchers who worked on this system and its efficiency for drying of agricultural commodities such as apple slices ([Feng & Tang, 1998](#)), wheat ([Jumah & Raghavan, 2001](#)) and peppercorns ([Weerachai & Somchai, 2004](#)). Although a fluidised bed, with conventional heating, has shown to have a great potential in disinfestation of wheat ([Dermott & Evans, 1978](#)), to the best of our knowledge, a microwave fluidised bed has not been evaluated for its efficiency in disinfection/ disinfestation of particulate materials. To investigate this potential, the system was evaluated by treating lentil seeds contaminated with Botrytis grey mould (BGM). The number of infected seeds reduced by 30% at higher seed moisture content (18%) and 10 min exposure time without significantly affecting the seed viability. One of the interesting outcomes of the statistical evaluation of the factors, using pareto chart of standardised effects, was that microwave power had more effect than air temperature in the reduction of IS%, while the effect of microwave power and air temperature were the same on the seed germination. The other effective factor on IS% reduction, but not on seed germination, was the exposure time at higher seed moisture content. This led to the

design of an experiment with prolonged exposure time until drying the seeds to its safe moisture content to evaluate if any more reduction of IS% could be achieved. Here, the exposure time of 10 min at the microwave power of 500 W did not change seed germination at low moisture content, whereas seed germination was reduced dramatically from 72.8% to 25% after 127 s (Chapter 5) at the power level of 50% (400 W). The reason, as stated before, is the control of the hot spot temperature in the microwave fluidised bed.

- 5- Disinfection of the lentil seeds was investigated as part of the grain drying process with the drying times defined from Page's model. Drying kinetics of lentil seeds were evaluated in this experiment, and lentil seeds diffusivities were found at different microwave powers and air temperatures in the microwave fluidised bed system. These data are very useful for the simulation of this process. An empirical model, known as Page's model, was applied to find the final time to reach a specific seed moisture content. Page's model has previously been shown to have a good fit for the drying data of many food materials and agricultural products, such as lentil ([Tang, 1994](#)) and garlic cloves ([Sharma & Prasad, 2001](#)). The result from this experiment confirmed that there was not more than a 30% reduction of the percentage of infected seeds (without the seed viability loss) at prolonged exposure times, which resulted in drying the seeds to its safe storage moisture content (10%). Therefore, increasing the treatment time to longer than 10 min was not beneficial in removing the pathogen from the seeds, but it proved to save drying time by at least 54% compared with the conventional fluidised bed dryer. The reason for this could be attributed to the fast desiccation of the seeds and the pathogen as well. By drying the seeds, water activity reduced and therefore the resistance of the pathogen towards the thermal treatment could increase at lower water activities (as confirmed by conventional thermal treatment in chapter 7), which could be the reason for the difficulty in removing the pathogen using this drying process. The hot spot of the microwave fluidised bed was controlled by the forced hot air and conclusively the seed temperature did not exceed a specific value during the whole process of drying. Therefore, this process could be applied for drying lentil seeds to the safe seed moisture content as well as partially controlling the seed borne BGM and possibly other pests and pathogens, which could be a focus of further studies.
- 6- After the drying and disinfection of lentil seeds, any quality change was investigated by measuring seed viability (germination%), seed vigour (electrical conductivity),

soaked and cooked seeds' hardness, antioxidant enzymes activities and macromolecule changes using Fourier-Transform Infrared (FTIR) micro spectroscopy. It was shown that there was no significant change in any of the mentioned quality attributes and no change in the protein secondary structure of lentil seeds at the effective process parameter (microwave power of 400 W and air temperature of 50°C) with 30% reduction in the infected seeds. However, at the higher microwave power of 500 W and air temperature of 60°C, there was a dramatic loss in the seed viability and vigour as well as an increase in the hardness of the cooked seeds and reduction in the peroxidase (POX) activity. This deteriorative effect was found to be the same as what happens in seed aging during storage ([Cakmak et al., 2010](#); [Scialabba et al., 2002](#)). POX inactivation occurred at the air temperature of 60°C and both microwave powers of 300 W and 400 W (from 0.0003 to 0.00017 $mM\ guaiacol.mg^{-1}.s^{-1}$), which could be due to the susceptibility of the enzyme in the lentil seeds to the temperatures higher than 60°C. Furthermore, a close examination of the second derivatives of the FTIR spectra showed that at microwave powers of 500 W and air temperature of 60°C, the α – *helix* weight in the protein's secondary structure reduced from 0.094 to 0.077 and the weight of aggregates increased from 0.016 to 0.031, which showed that protein unfolding occurred at high microwave power and air temperature. Protein unfolding in legumes was also reported by [Divekar et al. \(2017\)](#) who observed a shift in the β – *sheet* peak position due to microwave heating at high power (600 W), and by [Carbonaro \(2012\)](#) who showed that the weight of aggregates and α – *helix* changed in the thermally treated legume flour.

- 7- To exploit the potential of the microwave as an indirect source for the treatment of contaminated lentil seeds, a non-thermal plasma, driven from a microwave source, was considered. A downstream plasma source was installed to the 2.45-GHz magnetron and two gases (air and argon) were considered for creating the plasma. Argon plasma at a microwave power of 400 W with a gas flow rate of 40 nl min⁻¹ and 100 s exposure time, as a direct treatment, in terms of the distance from the end of the quartz tube, could reduce IS% by 20% after 4 days of incubation, but there was no significant difference in IS% between the treated samples and the control after 7 days of incubation. This means that all the spores could recover eventually, and the damage was only structural and not permanent. Remote treatment of the seeds with the afterglow of air or air/argon plasma (power of 800 W and gas flow of 20 nl min⁻¹

¹); however, was able to permanently destroy the pathogen and reduce the IS% by 32% and 40% using a combination of treatment time-holding time of 10 s – 60 min and 200 s – 5 min with 30% air and 100% air respectively, without any negative effect on the seed viability and antioxidant enzymes activities. However, holding the seeds in the trapped processed gas for 24 h changed their colour, which was assumed to be due to oxidation of the seed coat's polymers with the singlet oxygen or their photodegradation with the UV that was produced as a result of the collision of atomic oxygen and nitrogen. This conclusion was made based on the results of [Moisan et al. \(2014\)](#) who showed that the reactive species present in the afterglow of microwave air plasma were mainly atomic oxygen (O), atomic nitrogen (N), singlet oxygen ($O_2(a^1\Delta_g)$), excited metastable species of nitrogen ($N_2(A)$ and $N_2(a')$), and indicated that NO_x species could produce as a result of collision of O and N with the emission of energy in the form of UV. Overall, plasma processed air has a good potential to permanently remove seed-borne pathogens of lentil seeds, if seed colour is not of concern. However, the impact of this colour change on their resistance to plant pathogens in the soil and the later stages of the growth should be investigated. The reason is that the polyphenols in the lentil seed coat act as protection for the plant and its oxidation would mean losing this protection at an early stage of growth, though, we are not sure if the polyphenols were also oxidised.

Future perspectives

The potential of microwave has been explored for the treatment of lentil seeds, mainly for sowing purposes, and the findings from this work may be utilised to complete the development of a sustainable technology to be implemented as a part of integrated disease management system in grain crops.

- As the seeds and the pathogens have different thermal susceptibility and seeds are different in weight, density, and initial moisture content, an accurate protocol should be developed for microwave-assisted drying and disinfection for seeds of each crop by defining limits of applied microwave power, air temperature, and air speed. These protocols could be defined in the lab-scale equipment and then used for scale-up.
- Where the aim is not drying, treatment of the grain for sowing purposes with the microwave alone is not advisable. The reason is that the large difference between hot and cold spots affects the seed viability. However, the new solutions such as

surface temperature controlling or mechanical agitation with an auger could be investigated and applied. The uniformity could initially be investigated by simulation of the process, which is much cheaper than experimental works. For this purpose, the dielectric properties of the target grain need to be measured and modelled at different seed moisture content and temperatures at the working microwave frequency.

- To increase the efficiency of decontamination with the microwave fluidised bed system, an increase in the relative humidity of the air flow could be recommended. As it was shown in chapter 7, the susceptibility of the pathogen to the thermal treatment increased by increasing water activity (seed moisture content). Therefore, increasing the relative humidity of the air flow could prevent the fast desiccation of the seed and the pathogen, and more infected seeds at the same final temperature could be decontaminated.
- Treatment with the afterglow of a cold plasma in a glass bottle was studied. A compartment for trapping the processed gas could be designed in a future study and the effect of different microwave powers and gas flow rates could also be investigated and be optimized regarding energy efficiency, quality change, and seed pathogen reduction. As this will be a batch process, the feasibility of that on an industrial scale should be considered. One possibility (conceptual) could be filling sealed or gas-tight silos (currently used for fumigation) with this processed gas and after a specific time, if necessary, purging the processed gas with atmospheric air. This idea seems to be practical because aeration, with the aim of seed moisture reduction and pest control, or fumigation in the airtight silos are common practices even in large silos.
- One of the interesting findings of the plasma experiment was that after 4 d of incubation of the treated infected seeds, there was a large difference between IS% of the control and treated samples, which was narrowed after 7 d of incubation. It was concluded that a part of the damage caused by plasma was structural and not permanent. A question, here, is if this damage to the pathogen makes them more susceptible to other conventional treatments. This could be the topic of further study to combine the plasma treatment with other conventional or physical methods of disinfection.
- It was shown that holding the seeds in the trapped processed gas triggered seedlings' catalase activity and reduced hydrogen peroxide content. It was concluded that it could immune the plant toward the diseases in later stages of the

growth. Therefore, employing afterglow of the microwave plasma for vaccination of the plants could be further investigated.

- One of the limitations of the seed treatment with the plasma, directly or indirectly, is the limited penetration depth of the reactive species from the gas phase into the seeds. To overcome this limitation, treatment of the product with the plasma activated water could be recommended. By activating water, the reactive species could travel deeper inside the seeds and increase the efficiency of decontamination. It could have the potential to reduce more infected seeds without causing any harm to the seed quality.

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Appendix A. Chapter 6

A.6. 1 MATLAB code for finding temperature distribution in the sample holder in the microwave cavity after finding power distribution in XFDTD

```
% finding Temperature distribution in a geometry with specified
dissipated
% power distribution from XFDTD
% Dielectric properties from our measurement in Townsville at 2450 MHz :
% at 24C e' = 2.3639 , e'' = 0.2515 ; at 45C e' = 4.721 , e'' = 0.8167
% for "Dissipated Power - 100 g LentilDPsOfSeedCalculatedFromPowdered"
DPs
% are : dielectric constant: 3.2964; loss factor: 0.4892
clear
clc

%% specify constants For Lentil
MC = 19; % moisture content
Tamb = 60; % degC
TambK = Tamb+273; % deg K
Tinitial = 22; % degC
inputPower = 200; % W
hc = 65; % w/m2 K
t = 300; % time of heating s
emisivity = 0.9; % assumption

%% Geometry & pde
FourierEquation = createpde('thermal', 'transient');
importGeometry(FourierEquation, 'cylinder sample for single mode.stl');
figure
pdegplot(FourierEquation, 'FaceLabels', 'on', 'FaceAlpha', 0.5);

%% specify pde coefficients
k = 0.0004*MC^2 - 0.0066*MC + 0.2224 ; % w/m C
Cp = @(~,state) 1000*((0.5773+0.00709.*state.u)+(6.22-
9.14*(MC/100))*(MC/100) ) ; % j/kg C
Rho = 1755.5/(MC^0.099) ; % our previous experiment for M:8-19.8
thermalProperties(FourierEquation, 'thermalConductivity', k,
'MassDensity', Rho, 'SpecificHeat', Cp);

%% Microwave heat source
load('PowerDensitycylinderLentil21%24CinSampleHolderNotube55mmConeH.ma
t');
powerInterpole = griddedInterpolant((Z_Dimension_1, Y_Dimension_2,
X_Dimension_3), DissipatedPowerDensity, 'linear'); % W
% Q actual pattern
Q = @(region,~) inputPower * powerInterpole(region.z.*1000-68.8223,
region.y.*1000+35-35.1992 , region.x.*1000+35-34.7490) ; % because input
power is 1W,

internalHeatSource(FourierEquation, Q);

%% Power uniformity Index
[I,J,K] = size(DissipatedPowerDensity);
SumSD = 0;
SumP = 0;
```

```

Np = I*J*K;
for i = 1:I
    for j = 1:J
        for k = 1:K
            SumP = SumP + DissipatedPowerDensity(i,j,k);
        end
    end
end
Pav = SumP/Np;
for i = 1:I
    for j = 1:J
        for k = 1:K
            SumSD = SumSD + ((DissipatedPowerDensity(i,j,k) - Pav )^2);
        end
    end
end
PUI = sqrt(SumSD/Np)/Pav;

%% generate Mesh
generateMesh(FourierEquation);
figure;
pdeplot3D(FourierEquation);
axis equal

%% wait for user
prompt = 'Proceed to solving PDE? Y/N [N]: ';
str = input(prompt,'s');
if isempty(str)
    str = 'N';
end

if(str=='y' || str=='Y')

    %% specify Boundary Conditions
    n = FourierEquation.Geometry.NumFaces;

    % 1-natural convection from the top surface
    for j = 1 : n
        thermalBC(FourierEquation, 'Face', j , 'ConvectionCoefficient',
hc , ...
        'AmbientTemperature', Tamb);
    end
    % 2- heat flux from the bottom layer which is in contact with
    quartz,
    % % is zero.
    % for m = 1 : n
    %     thermalBC(FourierEquation, 'Face', m , 'HeatFlux', 0);
    % end
    % thermalBC(FourierEquation, 'Face', 14, 'Temperature', Tinitial);
    % thermalBC(FourierEquation, 'Face', 18, 'Temperature', Tinitial);
    % 3- samples are in quartz beaker, so sides & bottom surfaces are
    insulated
    % and heat flux or the temperature gradient is zero.

    % % 3- radiation from the top surface - find the emisivity of the sample
    % FourierEquation.StefanBoltzmannConstant = 5.670373E-8;
    % for i = 1 : n
    %     thermalBC(FourierEquation, 'Face', i , 'Emissivity',emisivity,
    'AmbientTemperature', TambK);

```

```

%     end
%% specify Initial Condition
thermalIC(FourierEquation,Tinitial);
%% solve and plot T distribution
% time
tList = 0:10:t;

thermalresults = solve( FourierEquation, tList );

% steady state result
figure

pdeplot3D(FourierEquation,'ColorMapData',(thermalresults.Temperature(:
,end)));
title(sprintf('Transient Temperature at Final Time (%g seconds)',
tList(numel(tList))));
axis equal

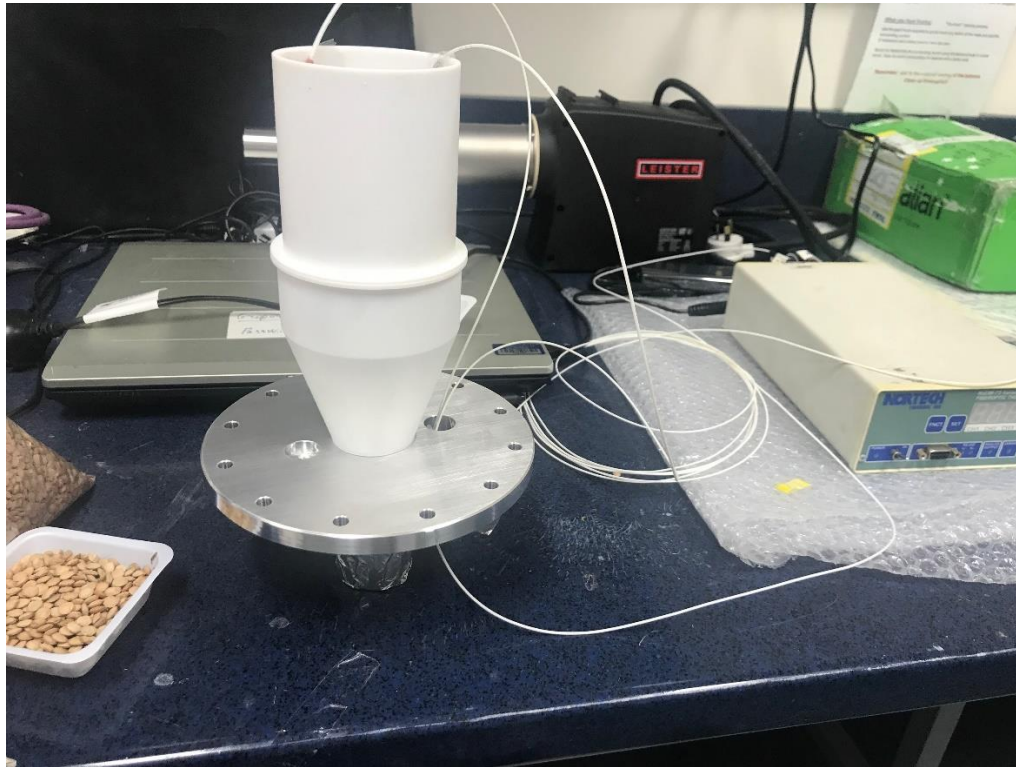
% animate the transient result
h = figure;
for i = 2:numel(tList)
    Tmin(i) = min(thermalresults.Temperature(:,i));
    Tmax(i) = max(thermalresults.Temperature(:,i));
    caxis([Tmin(i),Tmax(i)])

pdeplot3D(FourierEquation,'ColorMapData',(thermalresults.Temperature(:
,i)));
    title(['Temperature at Time ' num2str(tList(i))]);
    M(i-1) = getframe;
end
figure;
plot(tList,Tmax,tList,Tmin,'--');
xlabel('time');
ylabel('T');
legend('Tmax','Tmin');

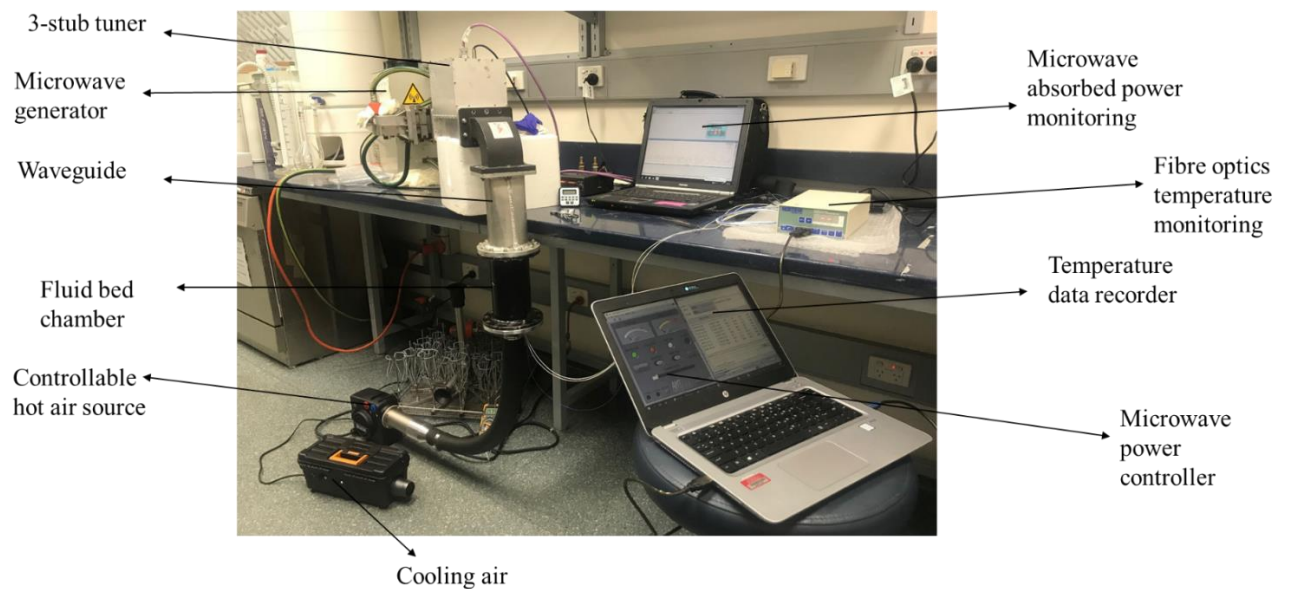
% Heat uniformity Index graph
N = length(thermalresults.Temperature);
Tav = zeros(numel(tList),1); Sum = zeros(numel(tList),1);
SDT = zeros(numel(tList),1); TUI = zeros(numel(tList),1);
for j = 1:numel(tList)
    Tav(j) = mean(thermalresults.Temperature(:,j));
    Sum(j) = 0;
    for k = 1 : N
        Sum(j) = Sum(j) + ((thermalresults.Temperature(k,j) -
Tav(j) )^2);
    end
    SDT(j) = sqrt(Sum(j)/N);
    TUI(j) = SDT(j)/(Tav(j)-Tinitial);
end
figure;
plot(tList,TUI);
xlabel('time');
ylabel('TUI');
title(['TUI for MC =' num2str(MC) ' & hc= ' num2str(hc)...
' & input power=' num2str(inputPower)]);
grid on
grid minor
end

```

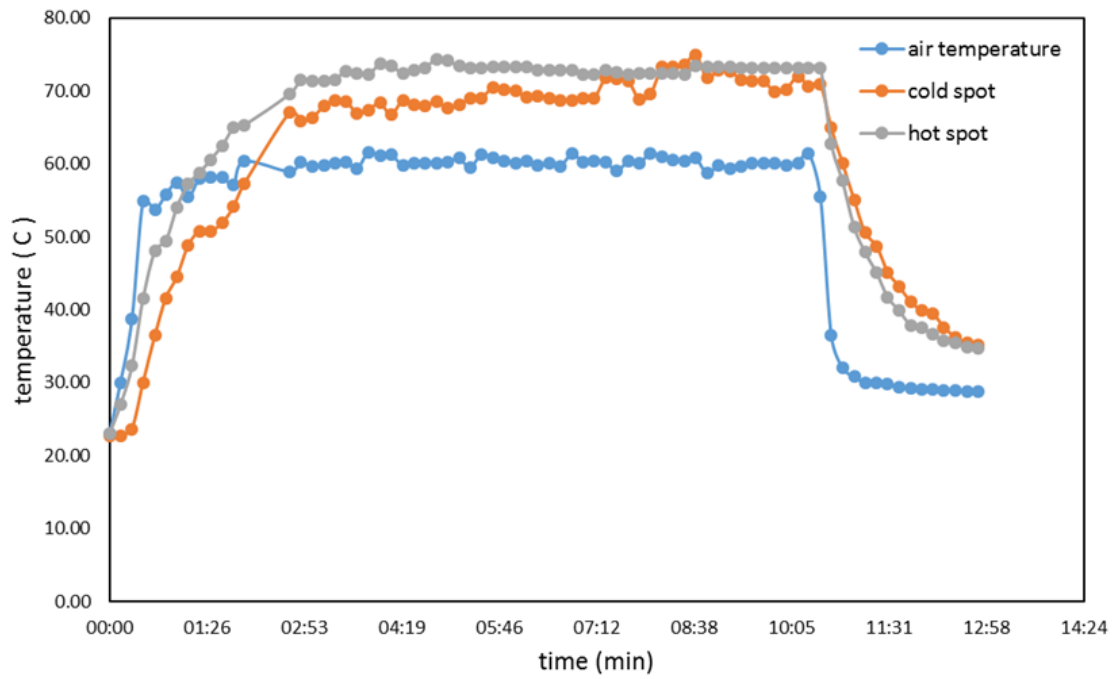

Appendix B. Chapter 7



B.7. 1 Sample holder for fluidised bed dryer after manufacturing with fibre optic probes inside the samples

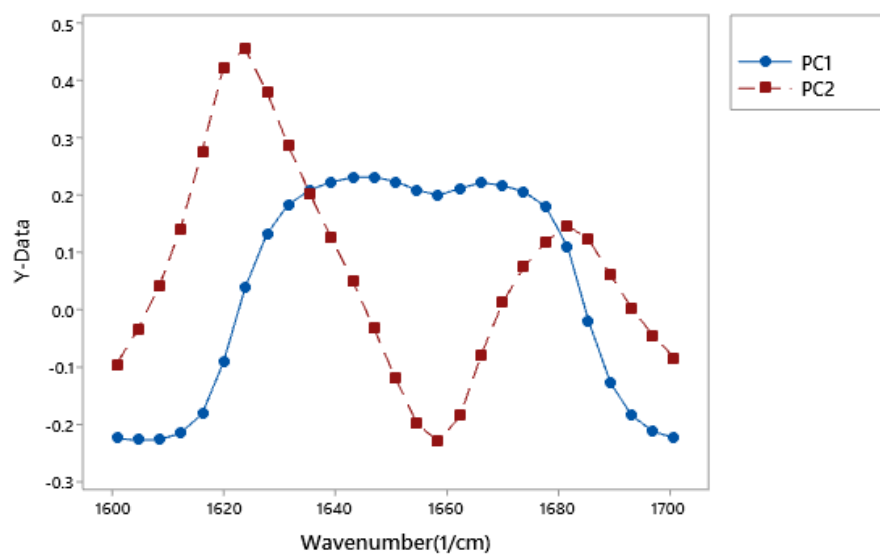


B.7. 2 The whole unit of microwave fluidised bed

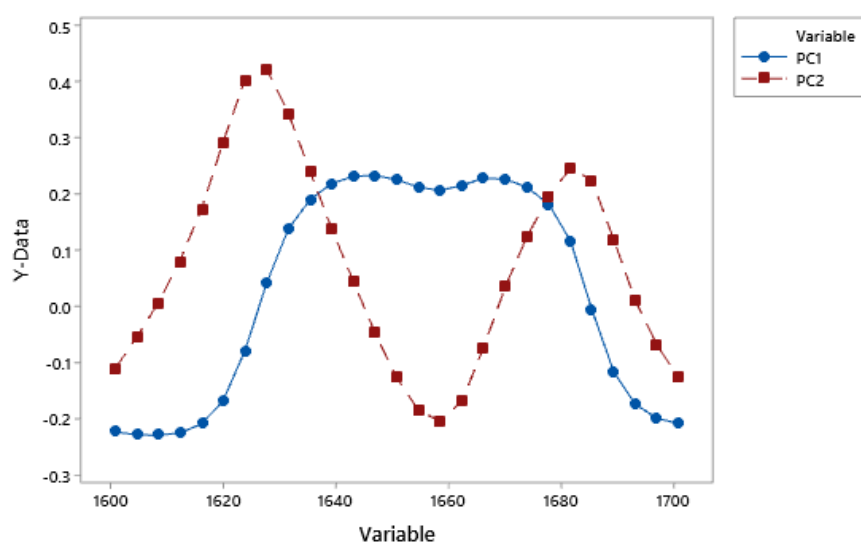


B.7. 3 Time-temperature history of 100g lentil seeds at 18% moisture content in microwave fluidised-bed at power of 400 W and air temperature of 60°C, recorded by fibre optic probes in hot and cold spots of the lentil bed and at the inlet of air flow.

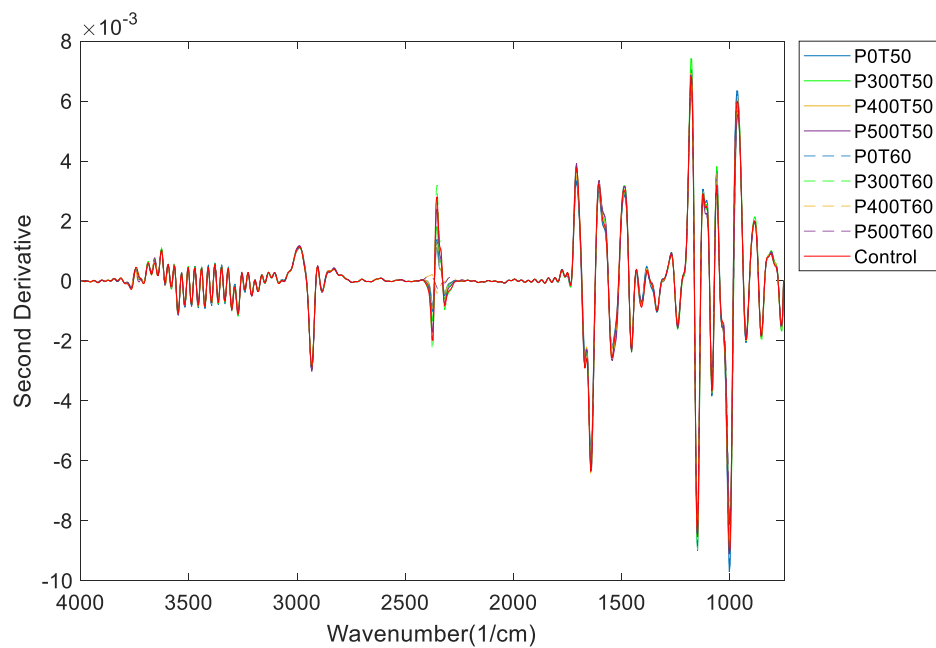
Appendix C. Chapter 9



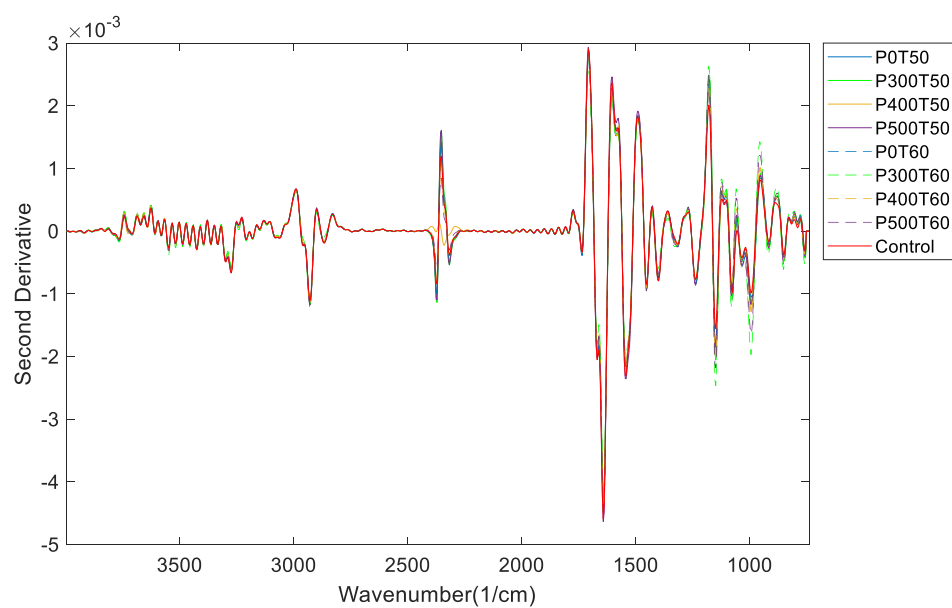
C.9. 1 Loading plot of PCA analysis for starch-dominant area of lentil seeds in the amide I region



C.9. 2 Loading plot of PCA analysis for protein-dominant area of lentil seeds in the amide I region



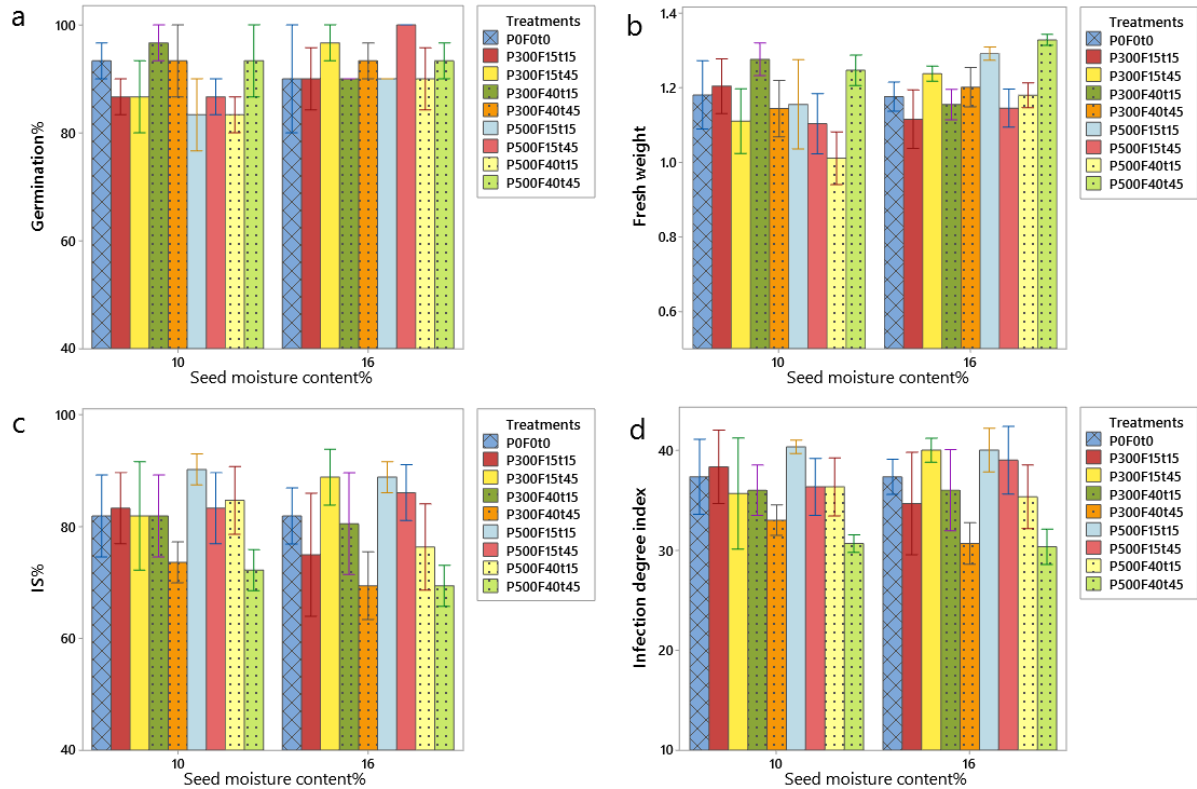
C.9. 3 Second derivative of the spectra in the starch-dominant area in the whole wavenumber region



C.9. 4 Second derivative of the spectra in the protein-dominant area in the whole wavenumber region

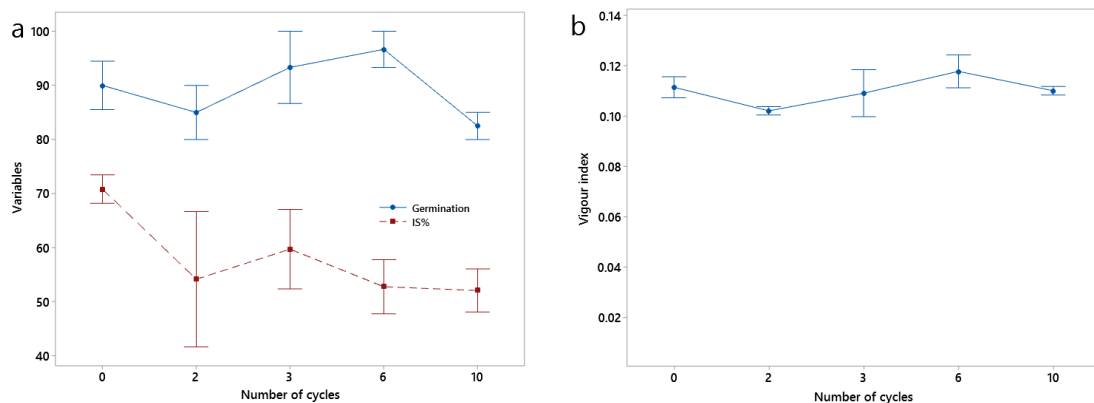
Appendix D.Chapter 10

Step1: 100% Ar & direct exposure, effect of process parameters



D.10. 1 Results of the treatments with 100% argon plasma at different microwave powers, P (W); gas flow rates, F (nl min^{-1}); and exposure times, t (s) and two seed moisture content of 19 and 16%; (a) Germination rate%, (b) Fresh weight of germinated seeds (g), (c) Infected seed percentage (IS%), (d) Infection degree index; bars are standard errors of means for $n=3$.

100% Ar & direct exposure, pulse mode treatments



D.10. 2 (a) Germination% and IS%, and (b) Vigour index of lentil seeds after treatments with argon plasma in a pulse mode; Number of cycles: 0=control, 2= $2 \times 75\text{s}$, 3= 3×50 , 6= $6 \times 25\text{s}$, 10= $10 \times 25\text{s}$; bars are standard errors of means with $n=3$.