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Nutritional and functional properties of cookies made using down-graded lentil – A candidate for novel food production and crop utilisation

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ABSTRACT

Background and objectives: Lentil (*Lens culinaris* M.) are a high-value grain used traditionally as a minimally processed product. Lentil crops are well-suited to Mediterranean-type climates with mild winters and hot dry summers which results in the production of high-value grain. However, extreme weather conditions, such as frost, can impact on the lentil seed e.g. a darkened seed colour and distorted seed shape resulting in a downgrade of its market value. The quality parameters of lentil when milled as wholemeal flour is not reliant on visual or physical seed traits such as seed size and color which impact on the market value in traditional market specifications. Instead, quality parameters such as those applied in the assessment of wheat, these include flour yield and colour would be appropriate parameters to determine the value of lentil flour. It is proposed that flour, even from down-graded lentil could be supplemented with wheat flour and used to enhance the quality profile of baked products including breads, pastas and cookies.

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Findings: This study investigated the use of premium and frost damaged lentil flour in cookie making. Overall cookies made from wheat-lentil composite blends resulted in flatter and wider cookies that were darker than the cookies made from 100% wheat flour. Cookies made by incorporating lentil and wheat flour resulted in a significant increase in total protein, insoluble fiber and oligosaccharides ($p < 0.05$). In addition, the phenolic acids kaempferol and procyanidin were detected in cookies made using wheat-lentil contributing to an increase in antioxidant activity ($p < 0.05$). These phenolic acids were not detected in cookies made from 100% wheat flour. Our results show that using concentrations of up to 25% lentil flour resulted in cookies that had expectable hardness and colour characteristics in comparison with a 100% wheat biscuit.

Conclusion: This research showed that the nutritional value and functional properties of cookies can be significantly enhanced by using either premium or down-graded lentil and the quality of the cookies was not impacted by the market-grade of the raw material.

Significance and novelty

Investigations into the use of pulse flours such as lentil in novel food products including pasta and snack foods is gaining popularity particularly by the health-conscious consumer. The visual appearance of lentil is not critical when utilised in such products. Lentil that have been visually or physically affected by adverse weather conditions, but its nutritional and functional value has not been compromised may prove a viable inclusion in the creation of highly nutritious food alternatives. The use of down-graded lentil as flour in novel food production could ultimately add value to pulse grain affected by adverse environmental conditions.

KEY WORDS

Lentil, Frost, Protein, Fiber, Oligosaccharides, Kaempferol, Procyanidin

INTRODUCTION

Premium quality lentil seeds (Figure 1A) are classified based on physical seed quality traits relating to size, shape, colour and absence of defective seeds (McDonald, Panozzo, Salisbury, & Ford, 2016). Seed characteristics and grain yield are genetically determined but strongly influenced by environmental conditions. Adverse conditions such as frost, impact on the appearance of the harvested seed can create a market challenge for growers (Nuttall et al.,

2018). Frost events are a particular concern for lentil growers, as the economic loss to growers through frost-affected lentil crops in Australia is estimated to be US \$250 million per annum (Delahunty, Perry, Wallace, Brand, & Nuttall, 2019).

(Insert FIGURE 1)

The investigation into the alternate use of pulse flour such as wheat-based products including pasta, breakfast cereals and snack foods is gaining popularity in western countries (Dziki, Różyło, Gawlik-Dziki, & Świeca, 2014; Sparvoli et al., 2016; Tosh & Yada, 2010). Research has shown that pulses including lentil are highly nutritious and have functional properties that may play a protective role in human health (Rochfort & Panozzo, 2007; Takruri & Issa, 2013). An opportunity exists to exploit the compositional components of down-graded pulses such as frost-damaged lentil seeds to produce wheat-based food products. Classifying damaged seed based on its proximal composition for use as a flour additive could provide a substantial nutritional and dietary supplement whilst concurrently improving returns for growers and lowering the cost of production.

MATERIALS AND METHODS

Two grades of commercially available red lentil (*Lens culinaris* M.) were used in this study. The samples consisted of premium-grade lentil with a Grade 1 classification and down-graded frost-affected lentils classified as rejects and destined for stock feed. Lentil seeds were cleaned using a vacuum separator (KimSeed, WA, Australia) to remove any field debris. Lentil seeds were milled to flour using a cyclone mill fitted with a 0.5 mm screen (Laboratory Mill 120; Perten Instruments, Huddinge, Sweden). The cotyledon was not separated from the seed coat and seeds were milled whole. Composite wheat-lentil flours were prepared by blending high-grade soft baking flour (II Molino Chiavazza, Casalgrasso, Italy) with either premium-grade or frost affected lentil flour (Table 1).

(Insert Table 1)

Preparation of Cookies

Cookies were made from doughs prepared with wheat flour supplemented with either premium or frost affected lentil-flour (Insert TABLE 1) concentrations and from dough containing only wheat flour as a control. The cookies were baked according to International Method No. 10-50.05 AACC (AACC, 2000). Cookie flours along with additional ingredients (Table 2) were mixed using a Dough-LAB 3100 mixer (Perten Instruments, Huddinge, Sweden). The dough was manually sheeted to 1mm thickness and cookies were cut using a round cutter

with an internal diameter of 49 mm. Cookies were baked for 10 minutes in a pilot scale electric baking oven (Rotel, APV Inc., QLD, Australia) at 210 °C.

Physical Analysis

The physical characteristics were determined 24 hours after baking. The thickness of individual cookies was measured using a digital height gauge and their weights recorded. Cookie hardness was measured using a TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) in accordance with the American Institute of Baking (AIB) standard procedure for hardness of cookies (AIB, 2012). The average force required to snap each cookie was expressed as a measure of firmness (N). Images of each cookie were taken using a digital camera and analysed with MATLAB R2016b software (MathWorks, MA, USA) for diameter and area. The lightness of each cookie was measured using the Commission International del'eclairage tristimulus color parameters (CIE) L* with a Chroma Meter CR-410 colorimeter (Minolta Co., Osaka, Japan).

Total Protein Analysis

Total protein content of cookies was measured using the Dumas combustion method AACC 46-30.01 (AACC International, 2002) and was performed using a Leco TruMac analyzer (Leco Corp, St Joseph, MI, USA). Moisture content was determined using a thermogravimetric analyser (TGA) (Leco Corp, St Joseph, MI, USA). Total protein was reported on a % dry basis and all sample evaluations were completed in triplicate.

Neutral Detergent Fiber Analysis (NDF)

Insoluble fiber (lignin, hemicellulose and cellulose) contained in cookies was measured using the ANKOM Neutral Detergent Fiber (NDF) assay as described by (Van Soest & Robertson, 1981).

Analysis of Raffinose Family Oligosaccharides (RFOs)

The raffinose family oligosaccharides (RFOs) was measured by Ultra Performance Liquid Chromatography/ Evaporative Light Scattering Detection (UPLC/ELSD) using the method as described in Portman et al., (2019).

Phenolic Acid Extraction

Ground cookies (0.25 g) was weighed into 15 mL polyethylene tubes. One mL of 70% acetone was added and the sample was vortexed and placed on a thermo shaker (Thermo Scientific, Australia) set at 5000 rpm at room temperature for 1 hour. Samples were then centrifuged at 4000 rcf (Eppendorf Centrifuge 5810, Hamburg, Germany) for 5 minutes and the supernatant transferred to 15 mL tubes. This extraction step was repeated, and the

supernatant collected. An aliquot (1 mL) of pooled supernatant was dried at 60°C under a nitrogen gas stream in a heated block (Ratek Instruments, Victoria, Australia). After drying, samples were resuspended in 1 mL 10% methanol and filtered through 0.22 µm PTF syringe filter (FILTER-BIO®) into UPLC vials for analysis.

Analysis of Phenolic Acids

Identification of phenolic acids in cookies was undertaken as described by (Maharjan, Penny, Partington, & Panozzo, 2019). All analyses were performed on a Waters UPLC ACQUITY system (Waters Corporation, Milford, MA, USA) with Photodiode array detector (PDA) and ACQUITY QDa mass detector. Separation was achieved using a UPLC-BEH C18 column (2.1 X 50mm, 1.8 µm). The mobile phase consisted of (solvent A) acetonitrile with 0.1% acetic acid and (solvent B) MilliQ water with 0.1% acetic acid. Peaks were identified based on their molecular weight and UV profiles. All data was processed using Empower 3 software.

Ferric Reducing Antioxidant Power Assay (FRAP)

The antioxidant activity of wheat-lentil cookies was determined using the FRAP method described by Benzie and Strain (1996) with some modifications. The FRAP reagent was prepared by mixing 300mM acetate buffer at pH 3.65, 20 mM ferric chloride and 10 mM of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, Sydney, Australia) in the ratio of 10:1;1. Ferrous sulphate solution was used as a standard at concentrations of (0.1, 0.2, 0.4, 0.6, 0.8, and 1 mM). A 0.1 mL aliquot of sample and 3 mL of FRAP reagent were combined in a test tube and incubated at 37 °C for 4 minutes. One mL aliquot of each sample was transferred to a 96-well microplate and the absorbance was measured at 593 nm using a micro-plate reader (Multiscan GO, Thermo Scientific, USA). The antioxidant power of samples was calculated using ferrous sulphate standard calibration curves and expressed as Fe²⁺ equivalent µmol/100g.

Statistics

All data was analysed by analysis of variance (ANOVA) with GenStat statistical software 17th edition (VSN International, Hempstead, UK). Means were analyzed for the least significant difference at a probability level of $p < 0.05$. Results are expressed as mean values $\pm$ SD. All analyses were completed in triplicate unless otherwise stated.

RESULTS AND DISCUSSION

Cookie Quality

For all cookie traits, the addition of lentil flour resulted in a significant reduction in cookie height ($p < 0.05$), and a significant reduction in cookie weight ($p < 0.05$) compared to the cookies baked with 100% wheat flour. The addition of lentil also caused a significant increase in hardness ($p < 0.05$), and a significant increase in cookie diameter and surface area ($p < 0.05$) (Table 3). Cookies containing lentil flour became significantly darker as the proportion of lentil flour increased ($p < 0.05$). Overall this study showed that the addition of lentil flour resulted in cookies that were harder, thinner, and darker than a cookie made from 100% wheat flour (Figure 2).

The main aim of the study was to show the potential applications for both premium and down-graded lentil flour in producing cookies. This study was a proof of concept and sensory evaluation was not performed. Although wheat-lentil composite cookies are in commercial production, further work using sensory evaluation would be necessary to assess the palatability of cookies using a wheat flour composite made with down-graded lentils. The observed changes in the physical attributes of cookies made using lentil flour is likely due to a combination of interactive factors. Competition for water-binding between lentil and wheat proteins (Portman et al., 2018; Turfani, Narducci, Durazzo, Galli, & Carcea, 2017), the dilution of gluten reduced the hydrophilic regions available for water binding (Portman et al.; Tuhumury, 2014) and an increase in Maillard reaction due to an increase in protein and carbohydrate content (Martins, Jongen, & Van Boekel, 2000; Portman et al., 2018) all contribute to these changes.

(Insert Table 3)

Protein and Fiber Content of Cookies

The proximal analyses of protein and neutral detergent fibre (NDF) of cookies are presented in (Figure 3). As expected, the addition of lentil flour regardless of lentil-grade resulted in an increase in both total protein and NDF of cookies compared to a 100% wheat cookie ($p < 0.05$). However, no significant difference in the concentration of protein or NDF fiber was observed when comparing cookies made from flour of either premium or frost damaged lentil.

(Insert FIGURE 3)

The increase in NDF content caused by the addition of lentil flour is predominantly due to the inclusion of the seed coat material from lentil, which contains cellulose, hemicellulose and lignin fibers (Dalgetty & Baik, 2003). The total dietary fibre (TDF) found in lentils is reported to be around 15% (Williams, Mikkelsen, Flanagan, & Gidley, 2019). In humans, it is

commonly stated that the majority of insoluble dietary fiber (IDF) undergoes microbial fermentation in the large intestine (Tosh & Yada, 2010), which is largely due to the number of microbes and their activity. However, Matthews, Howarth, and Butler (2012) describe a multiple pathway of IDF along with other dietary macronutrients with fermentation occurring at a lower rate in the stomach and small intestine. The ability to ferment IDF is not only based on solubility but its structure, not all forms of IDF are fermented in the human digestive system (Matthews et al., 2012). Microbial fermentation of IDF can provide many health benefits (Rochfort, Ezernieks, Neumann, & Panozzo, 2011). Such benefits may be found in the production of short-chain fatty acids (SCFAs), which acts as an anti-inflammatory (Li et al., 2018), suppresses blood cholesterol absorption (Hara, Haga, Aoyama, & Kiriya, 1999) and may reduce tumour growth (Matthews et al., 2012). In this study Insoluble fibre in wheat-lentil composite cookies was measured by the Neutral Detergent Fibre method (NDF). This method of fibre analysis measures most structural components found in plant cell walls, predominantly lignin, hemicellulose and cellulose. Thus, NDF was used as a rapid screen closely related to dietary fibre but does not measure the content of other constituents such as pectin (Van Soest & Robertson, 1981). Therefore, product development arising from this research would require a more mainstream analyses to measure total dietary fibre, such as the AACC total dietary fibre (TDF) method, 32-05.01 (McCleary, 2010; Menkovska et al., 2017) or with the Megazyme K-TDFR total dietary fibre assay (Hollmann, Themeier, Neese, & Lindhauer, 2013).

Raffinose Family Oligosaccharides (RFOs)

The addition of lentil flour introduced the Raffinose Family Oligosaccharides (RFOs) to the wheat-lentil composite flour. Wheat predominantly contains the reducing sugars fructose, glucose, sucrose and maltose but no RFOs. The α -galacto-oligosaccharides from lentil detected include raffinose, stachyose verbascose, and the pinitol digalactoside ciceritol (Figure 4).

(Insert FIGURE 4)

Increasing the concentration of lentil flour significantly increased RFO concentration ($p < 0.05$). However, no significant difference in RFO concentration was observed at any concentration level between cookies made from either premium or frost damaged lentil flour (Table 4).

(Insert TABLE 4)

A previous bread study using wheat-lentil composite flours demonstrated that RFOs with the exception of ciceritol, were significantly reduced through the fermentation process (Portman, Blanchard, Maharjan, Naiker, & Panozzo, 2019). However, cookies prepared according to AACC method 10-50.05 (AACC, 2000) do not undergo fermentation and therefore the concentration of RFOs was not significantly reduced in wheat-lentil cookies.

The presence of RFOs in flour and baked products can be both problematic and beneficial. A high concentration of RFOs can contribute to Irritable Bowel Syndrome (IBS). Symptoms include abdominal bloating and diarrhea (Fleming, 1981; Gilani, Xiao, & Cockell, 2012). IBS is caused by the production of hydrogen, carbon dioxide and methane gasses when RFOs are fermented in the colon (Gilani et al., 2012). Conversely, fermentation of RFOs in the colon have been associated with several health benefits in humans when fermented in the large intestine (Siva et al., 2018; Siva & Thavarajah, 2018). These include improving gut health by increasing levels of lactobacilli and fidobacteria (Berrios, Morales, Cámara, & Sánchez-Mata, 2010), a reduction of opportunistic pathogens including enterobacteria (Berrios et al., 2010), protection against reactive oxygen species (ROS) through the reduction of N-nitroso (NOC) compounds formed in the gut (Van Loo et al., 1999), and aiding in stool softening (Berrios et al., 2010). Additionally there is evidence that RFO can stimulate the innate immune system by their interactions with Tol-like receptors (TLRs) (Rochfort & Panozzo, 2007). The addition of fermentable carbohydrates such as RFOs may also block the fermentation of protein which can lead to the generation of cancer-promoting metabolites (Matthews et al., 2012).

Phenolic Compounds

Three prominent phenolic compounds were detected in cookies made from either premium or frost damaged lentil that were not detected in a 100% wheat cookie (Figure 5). The three compounds were tentatively identified as the dietary flavanols kaempferol trihexose and kaempferol glycoside as well as the flavonoid procyanidin. These compounds have previously been reported in lentils (Duenas, Hernandez, & Estrella, 2006; Mirali, Ambrose, Wood, Vandenberg, & Purves, 2014; Żuchowski, Pecio, Reszczyńska, & Stochmal, 2016), and are summarised below (Table 5). Epidemiological studies have shown a high association between the consumption of fruits and pulses that contain kaempferol derivatives and reduce risk of disease including lung, gastric, ovarian

(Insert FIGURE

(Insert TABLE 5)

cancer and cardiovascular disease (Calderon-Montano, Burgos-Morón, Pérez-Guerrero, & López-Lázaro, 2011). For example, a case study of 558 lung cancer sufferers, found that dietary intake of approximately 2 mg of kaempferol daily was inversely associated with lung cancer risk (Cui et al., 2008). Kaempferol and procyanidin derivatives have also been identified as potent superoxide scavengers in low concentration (Calderon-Montano et al., 2011; Rao, Santhakumar, Chinkwo, & Blanchard, 2018). Superoxides are highly reactive and can damage DNA, protein and lipids (Aniya et al., 2005). Research has also identified the potential protective mechanisms of procyanidin to include anti-diabetic, anti-platelet, anti-cholesterol, anti-microbial, and anti-aging properties (Ma & Zhang, 2017).

Ferric Reducing Antioxidant Power (FRAP)

The antioxidant activity of wheat-lentil cookies was measured by their ability to reduce Iron 3 oxide (Fe III) to Iron 2 oxide (Fe II) forming a ferrous- tripyridyltriazine complex (FeII - TPTZ). The results (Figure 6) demonstrated a significant increase in antioxidant activity with increased proportions of lentil flour ($P < 0.05$). Importantly, no significant difference in antioxidant activity was observed between cookies made from either premium or frost damaged lentil.

(Insert FIGURE 6)

Conclusion

Cookies made using either premium and frost damaged lentil had higher concentrations of protein and fiber compared to cookies made from 100% wheat. No significant difference was observed in in percent protein or total fiber when comparing cookies made from either premium or frost damaged lentil flour. Furthermore, combining wheat and lentil flour in cookie making resulted in the addition of RFOs, which are considered soluble dietary fiber and help promote regulatory function in humans. Wheat-lentil composite cookies introduced the phenolic compounds kaempferol trihexose, kaempferol glycoside and procyanidin which were not detected in cookies made from 100% wheat. The potential beneficial roles of these phenolic compounds in human health has been widely published. Down-graded pulses such as frost-damaged lentil can be milled to high-grade flour comparable to using premium grade lentil. Thereby enhancing the nutritional and functional value of wheat-based products while increasing the potential value of damaged pulse crops.

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TABLE 1 Blending ratio of wheat and lentil flour

Sample	Wheat Flour (g)	Lentil Flour (g)
Wheat 100%	225.0	0
Lentil 25%	180.0	45.0
Lentil 50%	112.5	112.5
Lentil 100%	0	225.0

TABLE 2 Cookie baking formulation

Ingredients	Weight (g)
Shortening	64
Sugar	130
Sodium chloride	2.1
Sodium bicarbonate	2.5
Dextrose solution*	33
Water	16
Flour	225

Notes* Dextrose solution consisted of 5.9 g dextrose in 100 ml water.

Table 3 Effect of lentil flour concentration on cookie quality

Blend	Height (mm)	Weight (g)	Hardness (N)	Diameter Max (cm)	Diameter Min (cm)	Area (cm ²)	Lightness (L*)
Wheat 100%	9.2 ± 0.3 ^a	18.4 ± 1.3 ^a	3406 ± 191 ^a	7.1 ± 0.1 ^a	6.8 ± 0.1 ^a	38.3 ± 0.6 ^a	65.2 ± 0.3 ^a
Lentil 25%	8.3 ± 0.2 ^b	18.4 ± 0.5 ^a	3684 ± 125 ^a	8.6 ± 0.2 ^{bc}	8.3 ± 0.3 ^b	56.5 ± 2.7 ^b	61.8 ± 0.6 ^b
Lentil 50%	8.2 ± 0.1 ^b	17.3 ± 0.5 ^b	3957 ± 86 ^b	8.7 ± 0.3 ^{bc}	8.3 ± 0.4 ^b	57.2 ± 4.2 ^b	52.3 ± 0.9 ^c
Lentil 100%	6.3 ± 0.7 ^c	16.5 ± 0.5 ^c	7015 ± 184 ^c	8.85 ± 0.3 ^c	8.5 ± 0.3 ^{bc}	59.4 ± 4.0 ^c	46.3 ± 0.8 ^d

Note: Data are means $\pm$ SD. Values in the same column with different alphabetical letters differed significantly as determined by ANOVA following a Tukey's HSD test ($P < 0.05$).

TABLE 4 Concentration of raffinose family oligosaccharides (RFOs) in wheat-lentil cookies

Blend	Raffinose	Ciceritol	Stachyose	Verbascose	R+C+S+V
Wheat 100%	ND	ND	ND	ND	ND
Premium Lentil 50%	0.16 $\pm$ 0.1 ^a	2.0 $\pm$ 0.1 ^a	0.7 $\pm$ 0.1 ^a	0.3 $\pm$ 0.1 ^a	3.3 $\pm$ 0.1 ^a
Frosted Lentil 50%	0.15 $\pm$ 0.1 ^a	1.9 $\pm$ 0.1 ^a	0.8 $\pm$ 0.1 ^a	0.3 $\pm$ 0.1 ^a	3.2 $\pm$ 0.1 ^a
Premium Lentil 100%	0.24 $\pm$ 0.1 ^b	3.1 $\pm$ 0.1 ^b	1.2 $\pm$ 0.1 ^b	0.6 $\pm$ 0.1 ^b	5.3 $\pm$ 0.1 ^b
Frosted Lentil 100%	0.27 $\pm$ 0.1 ^b	3.2 $\pm$ 0.1 ^b	1.3 $\pm$ 0.1 ^b	0.6 $\pm$ 0.1 ^b	5.5 $\pm$ 0.1 ^b

Note: ND; not detected. Data are means $\pm$ SD. Values in the same column with different alphabetical letters differed significantly as determined by ANOVA following a Tukey's HSD test ($P < 0.05$).

TABLE 5 Peak identification using UPLC/QDa

Peak	Tentative Identification	Formula	m/z ^[+]	Retention Time	Average Mass	λ Max
P1	Kaempferol trihexose	C ₃₃ H ₄₀ O ₁₅	901.43	1.513	902	265/346
P2	Kaempferol glycoside	C ₁₅ H ₁₀ O ₆	477.25	1.651	478	265/346
P3	Procyanidin	C ₃₃ H ₄₀ O ₁₅	577.07	1.513	578	279/218

FIGURE 1 (A) Premium grade red lentils with a mean seed size of 4.5mm, uniform colour and characteristic lens shape. (B) Down-graded red lentils affected by frost with a majority seed size below 3.5 mm, major discoloration as well as damaged split and malformed seed.

FIGURE 2 Wheat-lentil composite cookies baked using different concentrations of lentil flour with wheat.

FIGURE 3 A: Comparison of % protein and 3 B: % fiber of composite wheat-lentil cookies: (WL). Letters that are the same are not significantly different ($p < 0.05$).

FIGURE 4 Chromatogram of oligosaccharides in a 50% frost damaged wheat-lentil cookie, (peaks: 1, raffinose; 2, ciceritol; 3, stachyose; and 4, verbascose).

FIGURE 5 Chromatogram comparing the phenolic acids detected in 50% wheat-lentil cookies. Premium lentil; solid line, and frost damaged lentil; broken line. Peaks P1; Kaempferol trihexose, P2; Kaempferol glycoside, P3; Procyanidin.

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