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Article type : Research

Frost-affected lentil (*Lens culinaris* M.) compositional changes through extrusion: Potential application for the food industry

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ABSTRACT

Background and objectives: Lentil (*Lens culinaris* M.) is a high value, highly nutritional grain which originated in Middle-East. More recently lentil has gained favour in western countries due to the high value in production and the benefits they provide agronomically, however growing lentil in countries such as Australia, Canada, and the United States is not without its challenges. One is the high probability of damage due to radiant frost either before flowering or during pod-filling. The effects of which is most noticeable in the appearance of the seed reducing the value and usability of crops. On the other hand, a generation of well informed, health-conscious, and environmentally concerned consumers has driven the demand for affordable and healthy food alternatives. Such demand has resulted in a growing industry for protein extraction and novel food production. If used for protein extraction or novel food production the visual appearance of seeds is no longer an important quality trait. Lentil seeds damaged through frost that still retain a high nutritional composition may be the perfect candidate for a low-cost substrate in novel food production whilst improving the outcomes for growers and industry alike.

Findings: This study used as a model extrusion technology to investigate the use of flour derived from Grade 1 (premium quality), and downgraded frost-damaged lentil and to monitor compositional changes during extrusion. The study concentrated on how total protein, individual carbohydrates, and phenolic acids changed through high-temperature, high-pressure extrusion. Overall flours made from composite lentil-wheat flour had significantly higher concentrations of protein and carbohydrates than the base wheat flour.

No significant differences were observed for total protein or carbohydrates between Grade 1 and frost-damaged flours however extrusion significantly reduced total protein concentration as well as maltose and glucose concentration but did not alter the concentration of fructose, sucrose or the raffinose family oligosaccharides (RFOs).

As expected, phenolic acids, procyanidin, kaempferol glycoside, and kaempferol trihexose were detected in lentil-wheat composites but not in wheat. All phenolic acids significantly increased with increasing concentrations of lentil flour in composite, however their concentration decreased as a result of the extrusion process. Differences in concentrations of procyanidin and kaempferol glycoside were detected between Grade 1 and frost-affected lentil in both the composite flour and in the extrudate.

Conclusion: The extrusion process has the effect of altering the composition of the raw material. This was evident by a decrease in protein percentage phenolic compounds and to a lesser effect the water-soluble carbohydrates. No changes in RFOs was observed. The complete loss of glucose and a significant reduction of maltose provides a healthier carbohydrate profile as the carbohydrates are low fermentable sugars. The reduction of phenolic acids a result of extrusion may help to reduce the antinutritional activity particularly in grains where the concentration of phenolic acids is high. This study found that functionality of down-graded lentil is similar to the premium grade, and more expensive raw material. This knowledge may assist in reducing the increasing issue of food waste where often down-graded products are not used in the production of food.

Significants and novelty: In order to meet world food security needs it is predicted that global food production will need to increase by at least 70% by 2050. Therefore, the utilization of all possible protein sources including down-graded pulses, such as lentil, will become increasingly more important. Furthermore, due to climate change increasingly more variable weather conditions will result in the production of below optimum grains. Understanding how to best utilise both premium and downgraded grains is a desirable outcome to minimize food waste.

KEYWORDS

extrusion, frost, lentil, wheat, phenolic acids, protein, carbohydrate.

1 | INTRODUCTION

The quality of lentil (*Lens culinaris* M.) is generally determined by its physical traits such as seed size, shape, and colour (Shahin & Symons, 2003) however, these traits can be negatively impacted by abiotic stress including frost during pod-filling. Radiant frost occurs when plants and soil absorb sunlight during the day and then absorb radiant heat throughout the night when the air is still, and the sky is clear (Maqbool, Shafiq, & Lake, 2010). Dense chilled air settles into the lower areas of the canopy of the crop and in severe cases the cold air causes the intracellular fluid in the plant to crystallize, (Nucleation). Crystallisation results in the rupturing of the intracellular plasma membrane. Pulses including lentil, field pea, and faba bean are all highly susceptible to radiant frost damage (White, 2000). Such events during flowering and podding are phenotypically expressed as flower abortion, poor pod set, a reduction in pod filling all contributing to lowering grain yield and quality (Delahunty, Perry, Wallace, Brand, & Nuttall, 2019; Maqbool et al., 2010; White, 2000). Visually seeds from plants exposed to radiant frost have severe darkening and wrinkling of the seed coat. The seed shape is often distorted and are much smaller than healthy seeds. Frost events are a common occurrence in Australia, Canada and the United Kingdom (Crimp et al., 2016; Maqbool et al., 2010; White, 2000). Damaged lentil that would likely be downgraded as rejects and discarded or used as stock feed still retain a high level of protein, carbohydrates, and other nutritional compounds (Portman, Blanchard, Maharjan, Naiker, & Panozzo, 2019). Frost-damaged lentil seeds that retaining a sufficiently high nutritional composition could be milled to flour for use as an additive in novel food production. This may provide an economical substrate to the food industry whilst improving the outcomes for growers when crops are affected by frost.

Lentil is a popular widely grown crop throughout India, the Mediterranean and, Southeast Asia, where it has been highly prized as a food staple (Yadav, McNeil, & Stevenson, 2007). Traditional uses include dahl and soups as well as being consumed whole after soaking and boiling. Along with other pulses such as chickpeas, faba beans, field peas, and dry beans, lentil plays a key role in assisting populations obtain sufficient levels of protein and other important function compounds in their diet (Bresciani & Marti, 2019). These compounds include dietary fiber, carbohydrates, starch and minerals (Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014; Li et al., 2014; Portman et al., 2019; Tosh & Yada, 2010; Wang & Toews, 2011). In western countries, a growing trend is the use of pulse-flours in novel food products. These products are well suited to vegetarian and vegan diets but may also generate products that increase the mainstream consumption of pulses including lentil (Bresciani & Marti, 2019). There are challenges to overcome in order to increase the acceptance of pulse-based products in western society. These include an unpleasant beany-flavours that can be produced and also the presence of antinutritional compounds such as non-digestible oligosaccharides (Bresciani & Marti, 2019) and nutrient-limiting compounds such as phenolic acids (Champ, 2002; Gilani, Xiao, & Cockell, 2012; Thavarajah, Thavarajah, & Vandenberg, 2009). Conversely, oligosaccharides and phenolic acids are reported to have properties that may have a positive health benefit. (Ganesan & Xu, 2017; Johnson, Combs, & Thavarajah, 2013; Rao, Chinkwo, Santhakumar, & Blanchard, 2018; Vaher, Matso, Levandi, Helmja, & Kaljurand, 2010). In understanding the benefits and limitations of lentil as an additive wheat based flour, it is study the effect that processing has on the above-mentioned compounds as different types of processing including fermentation, baking and, extrusion may alter the composition (Bartolomé, Estrella, & Hernandez, 1997; Han & Baik, 2008; Hansen et al., 2002). Extrusion is a cooking process where a substrate such as flour when mixed with water undergoes a variety of conditions including, kneading and melting (Offiah, Kontogiorgos, & Falade, 2018). Post mixing and hydration the doughs are subjected to sheer forces and heating in a die-head were different levels of gelatinization are achieved depending on the desired outcome (Offiah et al., 2018). The resulting extrudate can be manipulated to form shapes through the selection of different die-heads and by controlling the exit speed and temperature. Extrusion cooking has many advantages, including the ability to produce a diverse range of products using inexpensive raw materials with minimal processing times (Offiah et al., 2018). For example, operating an extruder under a low-shear profile can be used to create pasta-style products, whereas medium-shear profiles can be used to create meat analogs from plant-based material and, high-shear extrusion profiles allow for the creation of expanded snack foods (Dogan, Gueven, & Hicsasmaz, 2013). Furthermore, extrusion can alter the final composition, through protein denaturation, enzyme inactivation (Dogan et al., 2013), and a reduction of antinutritional compounds (Nikmaram et al., 2017; Rathod & Annapure, 2016) including phenolic acids (Brennan, Brennan, Derbyshire, & Tiwari, 2011; Dogan et al., 2013). Several studies using extrusion has been completed using chickpeas beans, peas, sorghum, and legume blends, there have been few studies have been undertaken using red lentil. (Dogan et al., 2013). To our knowledge this is the first study where down-graded lentil damaged by frost have been used in extrusion. Grade 1 and frost-damaged lentil seeds were milled to flour and used as an additive with wheat flour in high-temperature and high-pressure extrusion. This study investigated the compositional changes in total protein, water soluble carbohydrates and phenolic acids, during extrusion.

2 | MATERIALS AND METHODS

2.1 | Materials

A commercially available red lentil (*Lens culinaris* M.), *cv.* PBA Jumbo was used in this study and consisted of two samples; Grade 1 (premium quality) and, a frost-damaged (down-graded lentil) which had a high proportion of small and dark seeds. Both samples were cleaned with a vacuum separator (KimSeed, WA, Australia) to remove any plant residue matter. Whole lentil seeds including the seed coat were milled to a flour with a cyclone mill that was fitted with a 0.5 mm screen (Laboratory Mill 120; Perten Instruments, Huddinge, Sweden). Composite wheat-lentil flours were prepared by blending a commercial baker's flour (Perfection Bakers Flour, Allied Mills, Australia) with either Grade 1 or frost-affected lentil flour using ratios of lentil at 0, 10, 20, 40, and 100%.

2.2 | Extrusion cooking

A co-rotating, interlocking, twin-screw extruder (Brabender KETSE 20/40; Brabender® GmbH & Co. KG Duisburg, Germany) with a flighted length ration of 20/40 was used in this study. The screw configuration was comprised of a feed zone, compression zone with kneading block, and a melting zone. A round strand die head with a nozzle diameter of 2.5 mm was also used. The screw speed was 250 rpm and the material feed rate was 8 kg/h. Water dosing was supplied by a peristaltic pump (Watson Marlow 120U/DV) with a constant melt moisture content of 25%. Heating zones (HZ) were as follows, HZ1: 50°C, HZ2: 95°C, HZ3: 110°C, HZ4: 125°C, HZ5: 125°C, HZ6: 140°C. The melt temperature in the die head was 137° C.

2.3 | Sample preparation

Extruded products were ground to a fine powder with a mortar and pestle and stored at 4° C until further analysis.

2.4 | Total protein

The total protein of extrudates was measured using the Dumas combustion method AACC 46-30.01 (AACC, 2000) and was performed using a Leco TruMac analyzer (Leco Corp, St Joseph, MI, USA). The moisture content of samples was determined through a thermogravimetric analyzer (TGA) (Leco Corp, St Joseph, MI, USA). All sample evaluations were reported in triplicate and total protein was reported on a % dry basis.

2.5 | Water-soluble carbohydrate extraction

Carbohydrate extraction was performed as described by Maharjan, Jacobs, Deighton, and Panozzo (2018) with modification. Ground extrudate, (0.4g) was weighed into polyethylene tubes and suspended in 5 mL of reverse osmosis (RO) water. Each sample was sonicated for a total of 30 min at a frequency of 50 hertz (FXP 12; Unisonic Pty Ltd, Australia), and then vortexed (Thermolyne Maxi Mix II; Thermoline Scientific, IA, USA), at 10-minute intervals throughout the sonification process. The above process was repeated twice more. The pooled extract was then centrifuged at 10,000 g for 10 minutes (Eppendorf Centrifuge 5810, Hamburg, Germany). An aliquot, 0.75 mL of the resulting supernatant, was transferred to 2 mL Eppendorf tubes and 0.75 mL of 100% acetonitrile added. Each sample was then centrifuged at 3,000g for 10 minutes (Eppendorf Centrifuge 5430R, Hamburg, Germany) before being filtered through a 0.2 µm PTF syringe filter (FILTER-BIO®) into UPLC Vials for analysis. The samples were then analyzed for water-soluble carbohydrates (WSC) using the UPLC/ELSD method outlined by Maharjan et al. (2018).

2.6 | Water-soluble carbohydrate (WSC) UPLC-ELSD analysis

Water-soluble carbohydrates (WSCs) was measured by Ultra Performance Liquid Chromatography/ Evaporative Light Scattering Detection (UPLC/ELSD) using the method described by Maharjan et al. (2018).

2.7 | Phenolic acid extraction

Ground extrudate (0.25 g) was weighed into 15 mL polyethylene tubes. One mL of 70% acetone was added to the sample which was then 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 2000 rcf (Eppendorf Centrifuge 5810, Hamburg, Germany) for 5 minutes and the supernatant transferred to 15 mL Teflon tubes. This extraction step was repeated, and the supernatant collected. An aliquot (1 mL) of pooled supernatant was dried at 60°C under a stream of nitrogen gas in a heated block (Ratek Instruments, Victoria, Australia). Following drying, the samples were resuspended in of 10% methanol (1 mL) and filtered through a PTF syringe filter (0.22 µm) (FILTER-BIO®) into UPLC Vials for analysis.

2.8 | Analysis of phenolic acids UPLC-PDA-MS

The identification of phenolic acids of extrudates was performed using the method outlined by Maharjan, Penny, Partington, and Panozzo (2019). All analyses were performed on a Waters UPLC ACQUITY system (Waters Corporation, Milford, MA, USA) equipped with a Photodiode array detector (PDA) and ACQUITY mass detector (QDa). Compound separation was achieved using a UPLC-BEH C18 Column (2.1 x 50 mm, 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. Changes in compound concentration was determined by an assessment of area under the curve (ACU). All samples were run in triplicate and the data was processed using Empower 3 software (Waters Corporation, Milford, MA, USA).

2.9 | Statistical analysis

All data were subjected to analysis of variance (ANOVA) and Fisher's protected with GenStat statistical software 17th edition (VSN International, Hemel Hempstead, UK). Means were analyzed for the least significant difference at a probability level of $p < 0.05$. Results are expressed as a mean value ± *SD*. All analyses were conducted in triplicate.

3 | RESULTS AND DISCUSSION

3.1 | Extrusion

All extrusion runs were performed using the same round die-head and temperatures, whilst only altering the water and flour dosing rate to maintain a 14 % moisture ration for each composite flour. Visually, the extrudate had a tubular-like shape with air pockets resulting from expansion and giving the extrudate a "pretzel-like" appearance. As the lentil concentration in the wheat flour increased, the colour of extrudates changed from light golden brown to dark brown (Figure. 1)

Insert Figure. 1

3.2 | Protein

As expected, increasing the concentration of lentil flour in composite blends resulted in a significant increase of total protein percentage ($p < 0.05$) (Table. 1).

Extrusion did result in a significant reduction of total protein content compared to their respective flours (Table. 1), ($p < 0.05$). These findings confirm those of Dogan et al. (2013) who showed that proteins become denatured through extrusion and Camire and King (1991) proposed that such a reduction occurs during extrusion when the hydrophilic amino acid regions of protein become exposed, changing the solubility of proteins in water also affecting nitrogen solubility. Furthermore, the effect that extrusion has on protein solubility has implications for the final product. Hydrophobicity may lead to a rapid reduction of water at the die-head which could enhance product expansion (Camire & King, 1991).

No significant differences were observed for protein percentage in the extrudates from the Grade 1 composite flours compared with the corresponding flour of frost-affected lentil (Figure. 2).

Insert Table. 1

3.3 | Water-soluble carbohydrates

A comparison of water-soluble carbohydrates for the 60 - 40% wheat-lentil composite flour and the corresponding extrudate is demonstrated in Figure 3. The water soluble carbohydrates present in the highest concentrations were fructose, glucose, sucrose, maltose as well as the raffinose family of oligosaccharides (RFOs). The similar chromatographic profiles were observed for all of the wheat-lentil flour composites..

Insert Figure. 3

This study found no significant differences for percentage total WSCs concentration between each composite flour from either the Grade 1, or downgraded lentil (Table. 2).

With increasing concentrations of lentil flour in the composite, there was an incremental increase in water soluble carbohydrates ($p<0.05$), however the concentration of individual water soluble carbohydrates were altered differentially during extrusion as is shown in Figure 3 for the 60-40% composite. The largest change in concentration was observed for glucose and maltose whereas frucose and sucrose were not altered as a result of the extrusion process.

Raffinose, ciceritol, stachyose, and verbascose are not detected in wheat but as expected were present in the composite flours due to the contribution of the lentil flour and were not altered in the extrusion process. This is in contrast to the changes in RFOs which occurred in a bread-study where fermentation significantly ($p < 0.05$) reduced the level of RFOs (Portman et al., 2019). The RFOs are adjuncts of sucrose with the addition of a α-1, 6- galactosyl extension forming complex FODMAPs (fermentable oligo-, di-, mono and poly- saccharides) (Sengupta, Mukherjee, Basak, & Majumder, 2015). In plants RFOs serve as; transport-sugar, sugar-storage units which protect seeds by desiccation during instances of high abiotic stress (Fàbregas & Fernie, 2019). However, RFOs are considered predominantly antinutritional when consumed by mono-gastric animals and humans, because of insufficient α-galactosidase enzymes (α-GAL) (Sengupta et al., 2015). Instead, α-GAL hydrolyses the non-reducing α-D-galactose residues liberating simple sugars and larger complex carbohydrate molecules remain undigested entering the small intestine where they are partially fermented by microbial flora producing carbon dioxide, hydrogen, and small quantities of methane (Raman, Saiprasad, & Madhavakrishna, 2019). RFOs are of particular concern when developing foods based on pulse flours as RFOs and their by-products are the primary cause of irritable bowel syndrome (IBS) (Berrios, Morales, Cámara, & Sánchez-Mata, 2010; Johnson et al., 2013; Siva & Thavarajah, 2018). Conversely, there is evidence showing the beneficial effects of RFOs which undergo partial fermentation in the large intestine acting as a prebiotics and enhancing the growth of lactobacilli and other bifidobacterial ultimately contributing to improved gut health (Berrios et al., 2010; Siva & Thavarajah, 2018). Furthermore, RFOs are reported to quench reactive oxygen species (ROS) by a reduction of N-nitroso compounds which can be formed in the gut (Devi, Lakhera, & Kumar, 2019; Van Loo et al., 1999). RFOs may also interact with Tol-like receptors stimulating the innate immune system (Crittenden & Playne, 1996; Rochfort & Panozzo, 2007).

Insert Table. 2

3.4 | Phenolic acids

The addition of lentil flour to the composite resulted in an increase in the relative absorbance of phenolic acids measured at 280nm. Three main phenolic acids were detected; procyanidin and two kaempferol derivatives which are signature compounds associated with lentil not detected in wheat (Figure. 4). The phenolic acid profile of frost-damaged lentil was similar to the Grade 1 lentil except for kaempferol derivatives which were detected in lower concentrations.

Insert Figure. 4

A possible explanation for changes in phenolic compounds that occurred in frost-affected lentil may be their role in plant functions including growth and reproduction (Yedidia, Schultz, Golan, Gottlieb, & Kerem, 2019). Phenolic compounds also respond in a protective mechanism during times of abiotic stress such as ambient-frost (Yedidia et al., 2019). Furthermore, increased expression of kaempferol derivatives has been reported in plants exposed to short-term frost prior to harvest (Lee & Oh, 2015).

The effect of extrusion on the phenolic compounds is shown in Figure 5 . Extrusion reduced the concentration of all three compounds. These observations align with other studies that have shown that extrusion improves nutrient bioavailability, by significantly reducing the measurable phenolic compounds in food products (Singh, Kaur, Singh, Singh, & Singh, 2019).

Insert Figure. 5

A possible explanation for a reduction of phenolic compounds may be due to high barrel temperatures in the extruder resulting in decarboxylation and polymerisation of phenolic compounds.

The reduction of antinutritional factors which includes phenolic acids, phytic acid, trypsin inhibitors and tannins is an important outcome resulting of thermal processing including extrusion (Patterson, Curran, & Der, 2017). All pulses have a relatively high concentration of antinutritional factors compared with cereals and can affect the bioavailability of minerals and inhibit protein digestibility (Rathod & Annapure, 2016). A substantial reduction or complete removal of anti-nutritional factors is recommended for safe food production (Nikmaram et al., 2017). The amount of reduction for anti-nutritional compounds during extrusion is reliant on optimal processing conditions such as temperature, extrusion-time, and moisture content. Achieving the optimal extrusion conditions can enhance the bioavailability of foods and digestibility of protein and starch (Rathod & Annapure, 2016). For example, Rathod and Annapure (2016) demonstrated that increasing the temperature from 140°C to 180°C during extrusion, reduced the phenolic content by 65%, and reduced tannin and phytic acid content by 90For example, there is a high correlation between the intake of kaempferol and its derivatives and a reduced risk of disease including lung, gastric, ovarian cancer and cardiovascular disease (Calderon-Montano, Burgos-Morón, Pérez-Guerrero, & López-Lázaro, 2011).

4 | CONCLUSION

Extrusion has the effect of altering the physical structure and composition of the final product which facilitates the development of a wide range of food products. This study has demonstrated that the composition of the raw material is differentially affected as a result of extrusion depending on the processing conditions. Extrusion can advantageously be used to decrease the bioactivity of anti-nutritional compounds particularly those found in pulses. This study has shown there are no functional differences between Grade 1 and frost-damaged lentil which would impede its use in extrusion technology . Extrusion can be used to create many types of products beyond the basic extrudate created in this research. Further research using extrusion technology and its application with substrates including downgraded grain-crops affected by a range of abiotic conditions may provide a cost-effective way in addressing the growing global demand for additional dietary protein.

ACKNOWLEDGMENTS

The authors would like to acknowledge Mr. Jurgen M. Cyrulla from CI Scientific Australia for his technical advice with the extruder. The authors acknowledge the ongoing contribution to this research made by the technical staff of the Seed Phenomics and Quality Traits Laboratory Agriculture Victoria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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TABLE 1 Difference in total protein % dry basis before and after extrusion of Grade 1 Lentil.

Note: Wheat-Lentil blend (W-L). Data are means ± SD. Values with different alphabetical letters differ significantly ($p < 0.05$). Data based on the mean result of six replicates for each sample, sampled from different sections of extrude during a 30-minute extrusion run.

	100% Wheat	W-L 80% - 20%	W - L 60% -40%	100% Lentil
Flour	13.3 ± 0.1^{a}	15.9 ± 0.1^{a}	18.3 ± 0.1^{a}	26.6 ± 0.1^{a}
Extrudate	12.7 ± 0.1^{b}	15.5 ± 0.1^{b}	16.9 ± 0.1^{b}	25.4 ± 0.1^{b}

TABLE 2 Water soluble carbohydrate (WSC) % in extrudate prepared from Grade 1 and Frost affected lentil.

	100% Wheat	W-L 90%-10%	W-L 80%-20%	W-L 60%-40%	100% Lentil
Lentil (Grade1)	-	3.3 ± 0.3^{b}	4.8 ± 0.6^{c}	5.6 ± 0.6^{d}	11.0 ± 0.6^{e}
Lentil (Frost)	-	3.9 ± 0.9^{b}	4.4 ± 0.4^{c}	5.7 ± 0.2^{d}	10.0 ± 0.3^{e}

Note: Data are means ± SD. Values with different letters differ significantly as ($p < 0.05$).

Figures

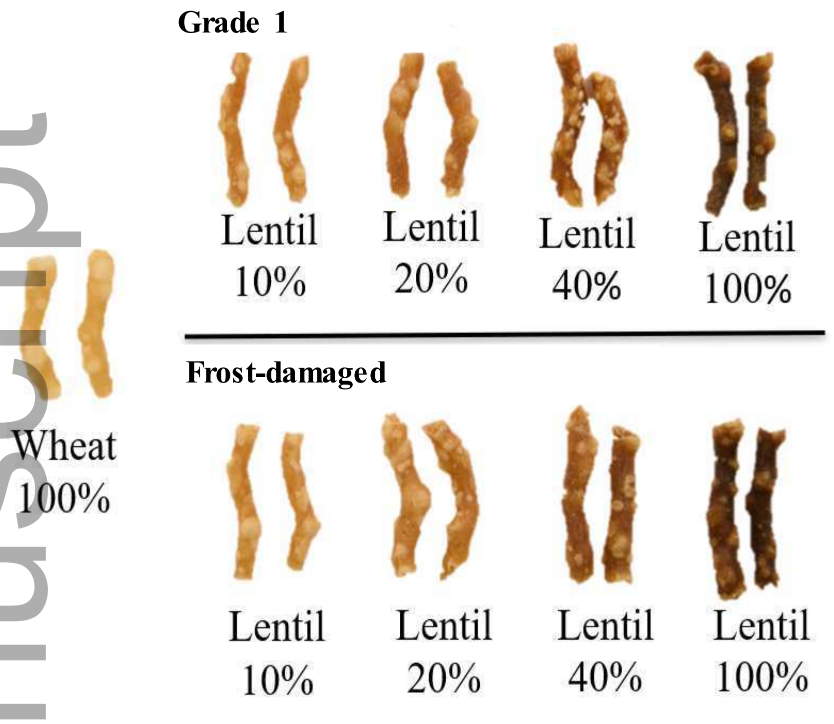

FIGURE 1 Extrudate made from composite Grade 1 and frost-damaged wheat-lentil flour.

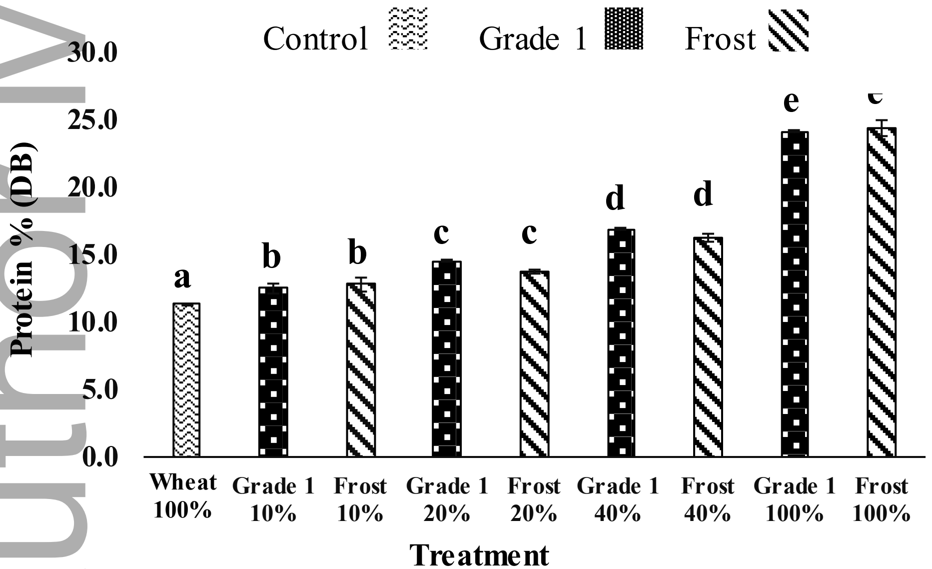

FIGURE 2 Total protein of wheat-lentil extrudate. Letters that are the same indicate no significant difference, ($p < 0.001$) L.S.D = 0.47.

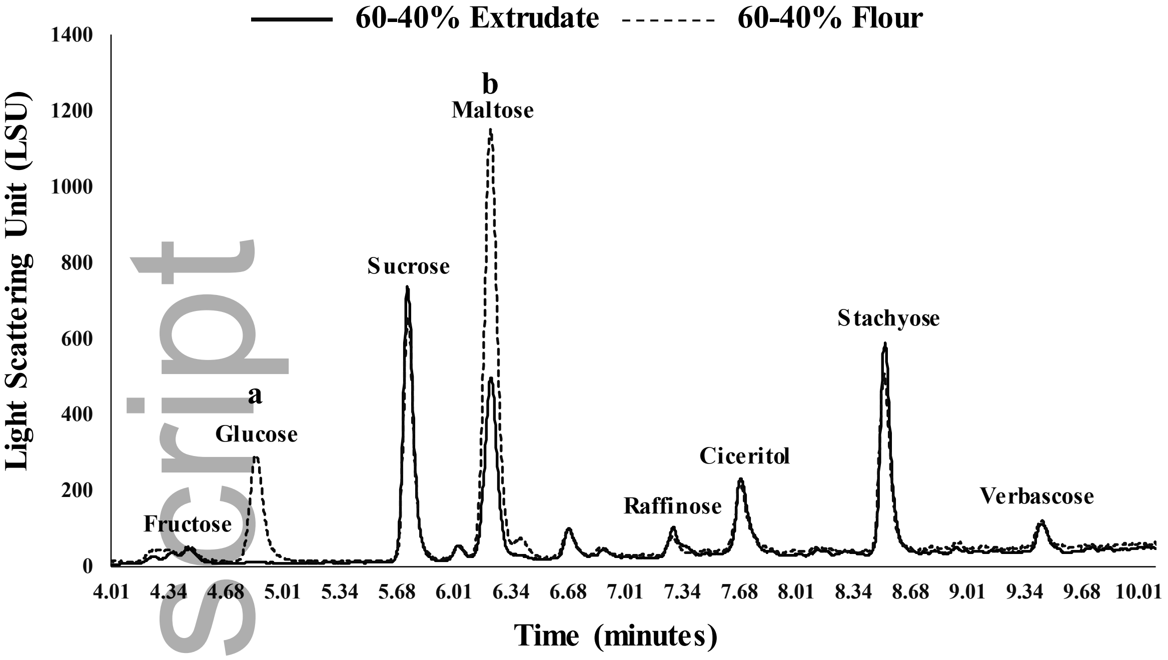

FIGURE 3 Carbohydrate profile of 60 – 40% wheat lentil composite flour (broken line) and extrudate (solid line).

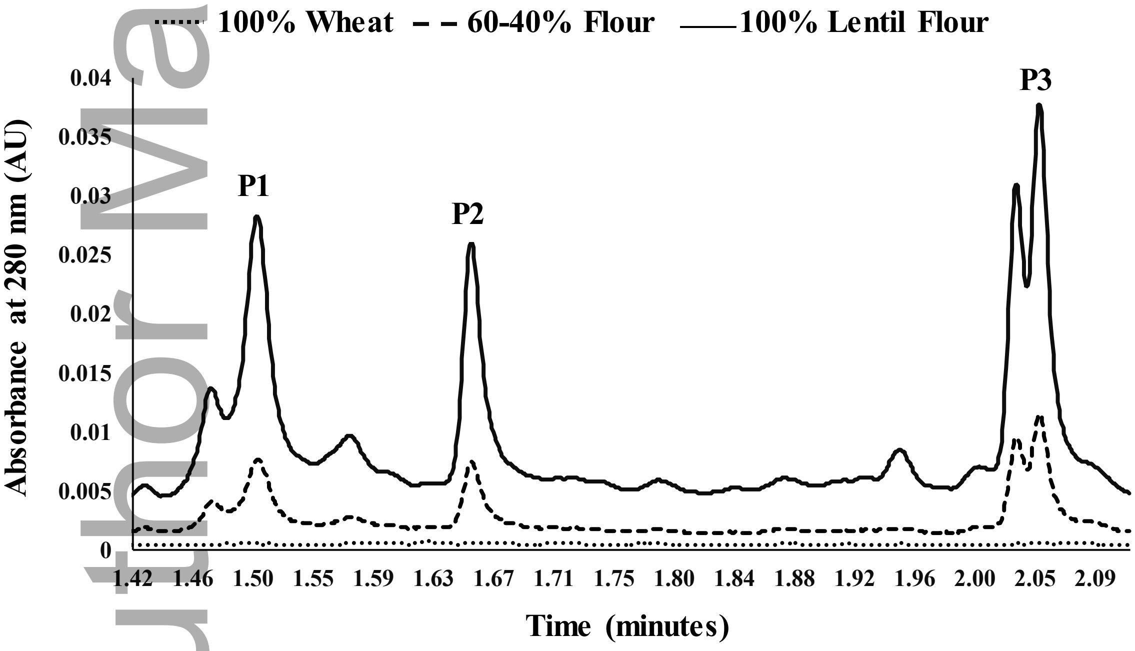

FIGURE 4 Phenolic profile of procyanidin **(P1)**; kaempferol glycoside; **(P2)** and kaempferol trihexose; **(P3)**. Wheat flour 100% (dotted line), wheat-lentil composite 60-40% (broken line) and lentil flour 100% (solid line).

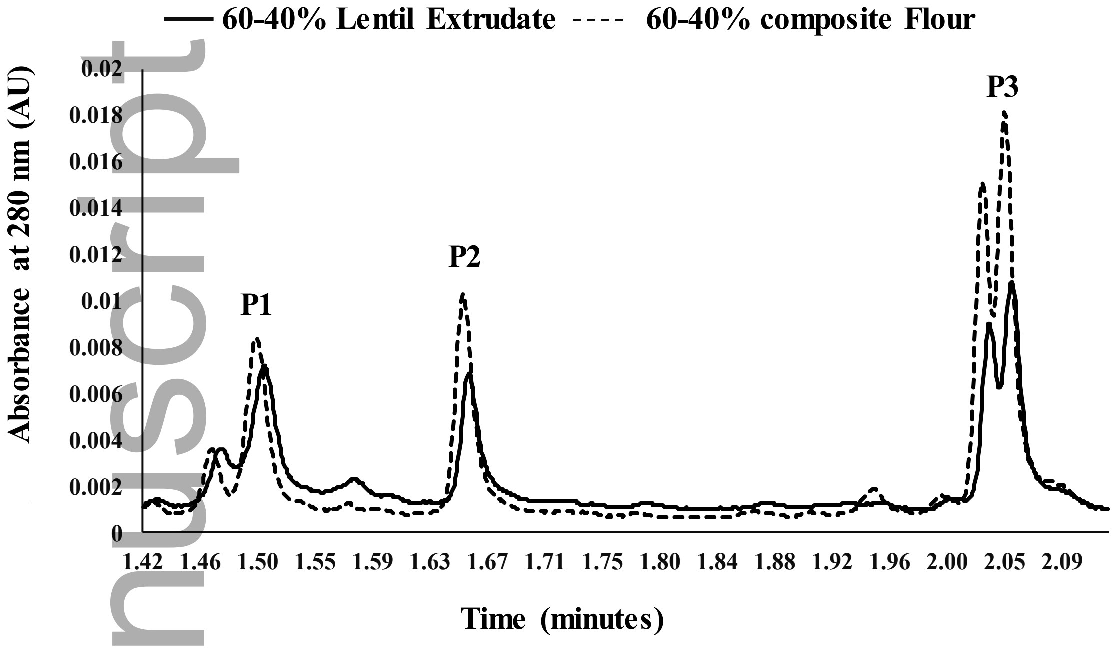

FIGURE 5 Phenolic of procyanidin **(P1)**; kaempferol glycoside **(P2)**, and kaempferol trihexose **(P3)**. lentil flour 60-40% (broken line) and extruded lentil 60-40% (solid line).