Impact of thermal processing on levels of acrylamide in a wheat-lentil flour matrix

Drew Portman1,2 | Pankaj Maharjan2 | Chris Blanchard1 | Mani Naiker3 | Joe F. Panozzo2,1

1School of Biomedical Science, Charles Sturt University, Wagga Wagga, New South Wales, Australia
2Agriculture Victoria Research, Grain Innovation Park, Horsham, Victoria, Australia
3School of Health, Medical and Applied Science, Central Queensland University, Rockhampton, Queensland, Australia

Correspondence
Drew Portman, School of Biomedical Science, Charles Sturt University, Wagga Wagga, New South Wales 2650, Australia.
Email: drew.portman@agriculture.vic.gov.au

Abstract
A growing demand for plant-based protein has seen a resurgence in the utilisation of pulses such as lentil and chickpea as a protein-rich substrate to produce food products traditionally based on animal or cereal protein. Additionally, pulses offer metabolic benefits due to the bioactivity of compounds including phenolic acids, simple and complex carbohydrates and a more complete amino-acid profile which is not found in cereal grains. However, there is an increasing concern in the formation of acrylamide in food which occurs when asparagine, an abundant amino acid found in pulses, forms a complex with the reducing sugars; glucose, fructose, and maltose. Recent studies in animal models have shown that acrylamide is carcinogenic and therefore is a concern for humans. High levels of acrylamide have been reported particularly in fried products, primarily due to the Maillard reaction which amplifies the formation of acrylamide. This study investigated the levels of acrylamide in bread, cookies, and extruded products prepared with wheat-lentil composite flour. Our research found that extrusion resulted in a significantly lower concentration of acrylamide compared to traditional baking. Additionally, increasing the amount of lentil within the composite significantly increased the net concentration of acrylamide for all bread, cookies and extrudate products.

KEYWORDS
acrylamide, baking, extrusion, free asparagine, reducing sugar

1 | INTRODUCTION

Lentil is a highly nutritious pulse, that is protein rich, high in complex sugars and has a dietary fibre (Bessada et al., 2019). Traditionally, lentils are consumed whole or as dahl and are a staple throughout India, Southeast Asia and the Subcontinent (Venkidasamy et al., 2019). The use of pulses flour including lentil is becoming popular with food manufacturers for the production of snack foods such as crisps, cookies, pasta, and puffed breakfast cereals (Morales et al., 2015). A benefit of utilising pulse flours for food production is the ability to simultaneously improve digestibility and nutritional quality (Pasqualone et al., 2020). However, processes that rely on heating, has the potential to initiate the formation of carcinogenic compounds including aldehydes, heterocyclic amines, polycyclic aromatic hydrocarbons and acrylamide (acrylamide) (Tanguler & Kabak, 2019).

Acrylamide is a by-product formed through the Maillard reaction during the thermal processing of foods (Kocadağlı et al., 2012). During the Maillard reaction, amino acid groups of free asparagine react with the carbonyl groups of reducing sugars forming a Schiff-base and through decarboxylation, form 3-aminopropionamide, which is further...
degraded to form acrylamide (Mesias et al., 2019). Acrylamide has been identified as a potential carcinogen for humans. Studies using animal models concluded that 1–2 mg of acrylamide per kg of body weight per day is sufficient to induce cancer in rats (Keramat et al., 2011). A European Union funded-study in 2003, investigated heat generated food toxicants (HEATOX) including acrylamide in foods and recommended that the maximum intake of acrylamide should not exceed 0.05 mg per kg of body weight per day (Keramat et al., 2011). The project concluded that in addition to acrylamide, more than 50 other genotoxic compounds can be formed through the thermal processing of food (Zeheer & Akhtar, 2016). It is generally agreed that acrylamide in food is a result of the reaction of free asparagine and reducing sugars mediated by heat, moisture, temperature, and time (Crawford et al., 2019). Farming practices and grain storage conditions can alter the concentrations of water-soluble carbohydrates such as sucrose and glucose (Maharjan et al., 2019) which can during normal heating could indirectly influence the formation of acrylamide. (Galani et al., 2017; Stojanovska & Tomovska, 2015). Pulses including lentil also retain a more complex carbohydrate profile compared to wheat, and can be altered through processing conditions which include fermentation (Portman, Blanchard, et al., 2019).

This study aimed to determine the extent of acrylamide formation in products made from wheat-lentil flour composites under the conditions of fermentation, baking and extrusion.

2 | MATERIALS AND METHODS

2.1 | Materials

Lentil (L. culinaris. Medik.) cv. Northfield used in this study is a commercially grown variety characterized by a grey seed coat and orange-red cotyledon. The lentil flour was prepared as previously described and incorporated with a commercial baker’s flour in varying proportions (Portman et al., 2018). Wheat-lentil composite flours were prepared in ratios of 0%, 20%, 40% and 100% lentil, each blend was homogenized in a screw mixer (Chopin Technologies, France) for 30 min.

2.2 | Bread

All bread samples were baked using the Straight Dough procedure as described by Portman et al. (2018) (AACC Approved Methods 10-09.01. AACC, 2000).

2.3 | Cookies

Cookies were prepared and baked using the (AACC Approved Methods Procedure 10-50.05 AACC, 2000).

2.4 | Extrusion

A co-rotating, interlocking, twin-screw extruder (Brabender KETSE 20/40; Brabender® GmbH & Co. KG Duisburg, Germany) with a flighted length ratio 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 were 8 kg/h. Water dosing was supplied by a peristaltic pump (Watson Marlow 120 U/DV) with a constant melt moisture content of 25%. There were six programed Heating Zones (HZ) and were as follows, HZ1: 50 °C, HZ2: 95 °C, HZ3: 110 °C, HZ4: 125 °C, HZ5: 125 °C, HZ6: 140 °C. The die-head melt temperature was 137 °C.

2.5 | Water-soluble carbohydrates UPLC/ELSD

The analysis of Water-soluble carbohydrates in composite wheat-lentil flours was performed using Ultra-high Performance Chromatography and a Evaporative Light Scattering Detector (UPLC/ELSD) described in (Portman, Blanchard, et al., 2019).

2.6 | Acrylamide sample extraction

The extraction of acrylamide in each product was performed using the method described by Mastovska and Lehotay (2006) with modifications. Each sample, (1.5 g) was weighed into Teflon tubes containing HPLC grade hexane (5 mL) and vortexed for 1 min. Subsequently reverse osmosis (RO) water (10 mL), acetonitrile (ACN) (10 mL), MgSO4 (4 g), and NaCl (0.5 g) were added. The sample was then shaken vigorously for 1 min and centrifuged at 2655 rcf. After centrifugation, the hexane layer was decanted and an aliquot (1 mL) of the upper ACN layer transferred into a (2 mL) Eppendorf tube pre-packed with (50 mg) of PSA and (150 mg) of magnesium sulphate. The sample was vortexed for 30 sec and centrifuged for 1 min at 2655 rcf, before being filtered through a 0.2 μm PTF syringe filter (FILTER-BIO® into UPLC vials for analysis. The samples were analysed for acrylamide content by ultra-performance liquid chromatography–mass spectrometry (UPLC-MS).

2.7 | Acrylamide analysis UPLC-MS

The quantification of acrylamide was performed on a Waters UPLC-ACQUITY system (Waters Corporation, Milford, MA, USA), equipped with a Photodiode Array Detector (PDA), ACQUITY Mass Detector (QDa) and Binary Solvent Manager (BSM). The compound separation was achieved using a UPLC-BEH C18 Column (2.1 x 50 mm, 1.8 μm). An isocratic mobile phase was used over a run time of 10 min. The mobile phase consisted of methanol (2.5%) and MilliQ (97.5%), with formic acid (0.1%). Run conditions were as follows; Flow rate (0.2 mL/min), column temp (30 °C), PDA wavelength (scan mode) λ (210–400 nm), MS detector mass range (0–1250 Da), Cone Voltage (20 v), and Selective Ion Recording (SIR) in positive mode of 72 Da, respectively. The acrylamide peak was identified by its molecular
weight (71 g/mol) and the retention time of an acrylamide standard (Sigma-Aldrich Pty Ltd, Castle Hill, Australia). The analysed acrylamide concentration was determined by the area under the curve (ACU) and quantified using an acrylamide concentration curve with a liner modelling characteristic of ($R^2 > 0.999$). An internal standard was used to verify the detection of acrylamide with a limit detection of 0.003 μg/mL. Acrylamide recovery from bread, cookies and extrudate were 100%, 99.6% and 98.6%, respectively. All samples were analysed in triplicate and the data was processed using Empower 3 software (Waters Corporation, Milford, MA, USA).

2.8 | Statistical analysis

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

3 | RESULTS

3.1 | Acrylamide analysis UPLC-PDA-MS

The acrylamide for all samples eluted between 1.5 and 1.6 min. Figure 1 shows the peak areas and retention time comparing bread-crust, cookie and extrudate made from a 60%–40% wheat-lentil composite flour. Based on SIR data acquisition at 72 Da, significant differences in peak height were recorded for bread crust (Peak 1), cookie (Peak 2), and extrudate (Peak 3). Similarly, significant differences were observed for bread crust, cookie and extrudate prepared from 100% wheat, 80%–20% wheat-lentil and for cookies and extrudate prepared with 60%–40% and 100%.

3.2 | Carbohydrate composition UPLC-ELSD

The carbohydrate profile of simple sugars for the 80%–20% and 60%–40% wheat-lentil composite blends is presented in Figure 2. The addition of lentil flour resulted in a significant increase in all sugars ($p < 0.05$).

3.3 | Effect of baking and extrusion on the synthesis of acrylamide

The acrylamide content (μg/kg) of bread, cookies, and extrudate after thermal processing (baking and extrusion) are presented in Figure 3. Acrylamide concentration differed significantly between bread crust and crumb and between cookies and extrudate ($p < 0.05$). The highest concentration of acrylamide was detected in bread crust (2.83–5.92 μg/kg), whilst a significantly lower concentration of acrylamide ($p < 0.001$) was detected in bread crumb.

![FIGURE 1](a) Example of the PDA (280 nm) of acrylamide detected for 60%–40% wheat-lentil composite (bread crust: (P1), cookie: (P2), extrudate (P3))
Acrylamide concentration in cookies ranged between (1.96–2.43 μg/kg) and was significantly lower than the acrylamide concentration detected in bread crust (p < 0.05). The lowest concentration of acrylamide between the three products was in the extrudate (<2.24 μg/kg).

3.4 | Effect of baking time on formation of acrylamide in cookies

A separate experiment was conducted to assess the relationship between baking time and formation of acrylamide in cookies.
The temperature within the baking oven was held constant (210° C) and cookies were removed at time intervals of 4, 6, 8, 10 and 12 min. Colour measurements were taken and L* was used as a measurement of darkening.

Darkening of cookies was negatively correlated \( r = -0.061 \) with increasing concentration of acrylamide measured in the baked product (Figure 5). For instance, a two-fold increase in acrylamide was observed at 8 minutes for both the 80%–20% and 60%–40% wheat-lentil cookies. Furthermore, an increase was observed at 6 min in cookies made using 100% lentil, both increased were highly significantly \( p < 0.001 \). Visually, cookies became darker with increasing concentration of lentil flour in the composite (Figure 6) which are

**FIGURE 4** Visual appearance of cookies made using varying concentrations of wheat-lentil flour after 10 min of baking

**FIGURE 5** Measured L* colour values of cookies for varying concentrations of wheat-lentil flour and different baking times

**FIGURE 6** Time course of fold change in acrylamide concentration wheat-lentil flour (W-L)
most-likely due to increases in Maillard reaction (Kocadağh et al., 2012).

The acrylamide concentration for cookies baked at each time interval are presented in Figures 6 and 7. Overall, the highest concentrations of acrylamide (3.13–6.96 μg/kg) were measured in cookies after 12 min. Significant differences (p < 0.001) were observed in acrylamide concentration between 6 and 10 min for each composite and ranged from 1.85 to 3.13 μg/kg for 100% wheat-cookies and from 1.95 to 6.96 μg/kg in 100% lentil cookies. Overall, the concentration of acrylamide in the cookies increased significantly (p < 0.05) as the concentration of lentil flour in the composite wheat-lentil cookies was increased Figure 6.

4 | DISCUSSION

4.1 | Thermal processing

Acrylamide has been reported in a wide range of wheat products, including white bread up to 364 μg/kg and cookies up to 1796 μg/kg (Abt et al., 2019). In this research the higher concentration of acrylamide detected in bread crust compared to cookies is likely due to a higher exposure to temperature on the surface of the bread and longer baking time, compared with cookies. In this study temperatures during extrusion are less than baking (140°C) and as the extrudate passes through the extruder at a rate of 480 g/min the exposure to even moderate temperatures is minimal. In a separate study, (Farouk et al., 2000) reported that extrusion induced both gelatinization and caramelization of the extrudate however the lower temperatures and short processing times, resulted in moderate Maillard reactions and limited the formation of acrylamide.

Our study concluded that the increased percentage of lentil intensified the Maillard reaction and concomitantly an increased concentration of acrylamide was also measured. Research conducted by Liu et al., (2018) demonstrated that acrylamide in bread can be reduced by coating the wheat-dough with a starch film prior to baking. The application of the starch film was reported to retain the moisture content within the bread crust. However, our research indicates that the formation of acrylamide is more dependent on the cooking-time at high temperature.

The rate of increase in acrylamide was more rapid when the concentration of lentil flour increased above 20%. Above this concentration both an increased coloration in the cookies and reduced optimal baking time was also noted. Cookies containing more than 20% lentil flour and baked for 6 min were observed to snap cleanly indicating they were completely cooked at this time period (AACC Approved Methods Procedure 10-50.05 AACC, 2000). It was concluded that reducing the baking time for wheat-lentil composites greater than 20% lentil may achieve the desired coloration and cookie-snap and limit the Maillard reaction and formation of acrylamide.

**FIGURE 7**  Baking time trial of cookies made using different concentrations of wheat-lentil flour (W-L). Letters that are not the same differ significantly (p < 0.05)
4.2 | Disaccharides and their influence on acrylamide formation

The precursors to acrylamide in food have been reported to be free-asparagine and reducing sugars. However, other sugars in addition to fructose and glucose can contribute to the formation of acrylamide. As an example, Figure 4 shows the carbohydrate profile of an 80%–20% wheat-lentil composite compared with 60%–40% composite resulted in increases in both sucrose and maltose. During dough fermentation the hydrolysis of sucrose produces additional glucose and fructose, furthermore two glucose molecules are produced through the hydrolysis of maltose (Stojanovska & Tomovska, 2015). The hydrolysis of both sucrose and maltose could potentially contribute to an increase the acrylamide formation in food products.

5 | CONCLUSION

The research outlined here-in has focused on processing factors including variables such as time and temperature investigating the formation of acrylamide. Previous research has shown that the formation of acrylamide can also be controlled by the addition of asparaginase (Vinci et al., 2012). In addition, studies have shown the polyphenol procyanidin as well as its derivatives catechin and epicatechin, found in abundance in lentil (Bresciani & Marti, 2019; Portman, McDonald, et al., 2019) may inhibit the production of acrylamide as shown within a cellular model (Zhao et al., 2019).

Acrylamide has been reported in both extruded and baked foods (Kocadağı et al., 2012) derived from legume flours in the range of 401–750 μg/kg (Galani et al., 2017). It is well known that processing conditions such as temperature and cooking time, impact on the formation of acrylamide. Extrusion processing proved to be the most effective in minimising the formation of acrylamide by limiting the Maillard reaction due to the rapid cooking time. The sequential addition of lentil flour to the composite resulted in Maillard reactions occurring at an earlier stage of the baking process. Overall products derived from extruded lentil flour had a significantly lower concentration of acrylamide than has been reported for fried or other cereal-based foods (Vinci et al., 2012).

A combined approach to overcome this issue is to use optimised conditions or food-processing additives. Alternatively, targeted plant-breeding to reduce ‘acrylamide precursors’ in new cultivars may offer a solution to achieve increasingly more stringent food safety standards and reduce levels of acrylamide in food products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.


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