

Original article

The effect of popping, soaking, boiling and roasting processes on antinutritional factors in chickpeas and red kidney beans

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Summary This study was conducted to determine the effect that a popping head (like a rice cake machine), a low-shear and low-water processing technology, has on the concentration of antinutritional factors in chickpea and red kidney beans. Seeds were popped under several parameters (popping time, sample format and equipment type) and analysed against soaking (1: 5 w/v in reverse osmosis water for 24 h), roasting (100 g at 180 °C for 20 min) and boiling (1:5 w/v in reverse osmosis water at 100 ± 1 °C for 1 h) processes. Popping and roasting significantly reduced phytic acid content in chickpeas (6%–22%) and red kidney beans (16%–39%). In contrast, phytic acid content after soaking and boiling was not significantly different to raw seeds. Condensed tannins were significantly reduced in red kidney beans after soaking (74%), boiling (100%) and 4 s popping (28%–42%) treatments and increased in both pulses after roasting (137%) and 8 s popping (21%–47%). Further analysis showed that the soluble phenolic content increased with popping, but total and bound phenolic content was reduced. These results demonstrate that the high temperatures and pressures applied during the popping process effectively reduce antinutritional factors in pulses, compared to conventional processing methods.

Keywords Antinutritional factors, condensed tannins, phenolic compounds, phytic acid, popping, pulses, sustainable processing.

Introduction

The demand for protein is estimated to double by 2050, as the global population is set to rise to 9 billion (Flachowsky *et al.*, 2017; Henchion *et al.*, 2017). Animal-derived protein production and consumption is associated with significant land and water usage as well as generating high levels of greenhouse gases; negatively impacting food security, biodiversity and climate change (Henchion *et al.*, 2017). The utilisation of alternative protein sources must therefore be increased to ensure a sustainable food supply chain, especially in developing countries where access to high-quality sources of protein is required to prevent deficiency and malnourishment (Schönfeldt & Gibson Hall, 2012).

Pulses, or grain legumes, are defined as dry edible seeds of major leguminous grain crops having a low-fat content and potentially the capacity to aid future protein demand. Pulse seeds, such as cowpea (*Vigna unguiculata* L. Walp), pigeon pea (*Cajanus cajan* (L.) Millsp.), chickpea (*Cicer arietinum* L.), kidney bean

(*Phaseolus vulgaris* L.), lima bean (*Phaseolus lunatus* L.), lentils (*Lens culinaris* Medik.) and faba beans (*Vicia faba* L.), currently provide 33% of dietary protein globally, as well as being rich in carbohydrates, dietary fibre, vitamins and minerals (Yasmin *et al.*, 2008; Khoury *et al.*, 2015; Bessada *et al.*, 2019; Venkidasamy *et al.*, 2019). In addition, pulse cultivation produces lower greenhouse gas emissions, requires less land and water, is less economically demanding and is more accessible than rearing livestock (Stagnari *et al.*, 2017; Shepon *et al.*, 2018; Acciani *et al.*, 2021). However, the potential of pulses is limited due to the presence of antinutritional factors, which are viewed as a barrier for pulses to meet the ever-growing demand for plant proteins (Kalpanadevi & Mohan, 2013).

Antinutritional factors (ANFs) are commonly defined as natural or synthetic compounds found in food that reduce the bioavailability and utilisation of nutrients, leading to impaired performance of gastrointestinal and metabolic processes (Astley & Finglas, 2016; Nagraj *et al.*, 2020; Samtiya *et al.*, 2020). Examples include phytic acid and tannins, both of which are found in several species of pulses (Singh *et al.*, 2017; Shi *et al.*, 2018). When assessing pulses as

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a future protein demand, the types of ANFs they possess must be considered, in addition to their content, as both will determine nutritional quality.

Phytic acid (myo-inositol hexaphosphate) is present in the cotyledons of pulse seeds and has the ability to chelate mineral ions like Ca^{2+} , Mg^{2+} , Zn^{2+} , Cu^{2+} and Fe^{2+} and K^+ (Shi *et al.*, 2018; Francis *et al.*, 2001; Brouns, 2022), therefore reducing their bioavailability in the gastrointestinal tract (Sarwar Gilani *et al.*, 2012). Raw pulses have varying phytic acid content, for example several chickpea genotypes have been shown to have contents between 424 and 990 mg/100 g (Kaur *et al.*, 2019), with variation being influenced by differing climate, location grown, soil and irrigation conditions, and level of seed maturity (Foster & Samman, 2017; Shi *et al.*, 2018). Importantly, the anionic form of phytic acid (phytate) can also bind to positively charged protein functional groups or indirectly to negatively charged protein groups *via* multivalent cation bridging. These reactions form insoluble protein complexes that can lower the digestibility of protein (Humer *et al.*, 2015). Processing methods are recommended to reduce phytic acid levels in foods because humans have low activity of phytase in the small intestine (Nielsen *et al.*, 2013). Similarly, tannins, located in the hull of pulses (Pal *et al.*, 2016) and commonly divided into two groups (condensed and hydrolysable), are polyphenolic compounds that can inhibit digestive enzymes or can complex with proteins and minerals, reducing their digestibility and bioavailability (Francis *et al.*, 2001; Delimont *et al.*, 2017; Krzyzowska *et al.*, 2017; Ram *et al.*, 2020). Although no standardised safe or acceptable limit currently exists for tannins in pulses (Ojo, 2022), lowering the tannin content reportedly produces food with better palatability, protein digestibility and mineral bioavailability (Gabaza *et al.*, 2016; Pal *et al.*, 2017). Tannin quantity and composition can vary with plant age and species, environmental conditions (soil pH, moisture and nutrient availability) and location (Schweitzer *et al.*, 2008; Top *et al.*, 2017; Gabaza *et al.*, 2018). Indeed, the tannin quantity of lentil samples collected from six cultivars in India ranged in concentration between 218.5 and 421.3 mg/100 g (Khandelwal *et al.*, 2010). Reducing both phytic acid and tannins in pulses, commonly achieved through processing, is essential to maximise the nutritional value that consumption of pulses could contribute to the diet.

Soaking, roasting and boiling are traditional methods that are reported to reduce ANFs in plant-based foods. Water-soluble tannins are typically targeted by soaking and boiling processes, as they can be discarded in the treatment medium (Smeriglio *et al.*, 2017). Mubarak (2005) observed a 39% and 46% reduction in tannins when mung beans were soaked for 12 h and boiled for 90 min respectively. On

the other hand, phytic acid is heat-labile and previous studies show that roasting is effective at reducing this ANF (Khattab & Arntfield, 2009) with Mesfin *et al.* (2021) reporting that Kabuli-type chickpeas were 40% lower in phytate when roasted at 150 °C for 30 min in a hot air oven. Alternative methods of thermal treatment, microwave cooking and infra-red micronization have also demonstrated the potential to reduce ANFs, lowering phytic acid in horse gram by 40–54% and 33–39% respectively (Vashishth *et al.*, 2021). Unfortunately, current ANF reduction methods (soaking, thermal treatment and a combination) have high cooking and processing times for pulses (Divekar *et al.*, 2017) and can require large quantities of water and energy. Furthermore, soaking has been shown to significantly lower total protein by as much as 22% in chickpea. Boiling pre-soaked white- and pink-mottled cream beans also reduced total protein by 14% and 23% respectively (Aguilera *et al.*, 2009), meaning alternative treatments should be explored to preserve pulse nutritional value while reducing ANF content.

One such alternative technology considered in this study was compression popping, a food processing technique that forms ready-to-eat snacks with desirable nutritional and sensory properties. Described as heating pulses or grains until internal moisture causes expansion, popping is commonly practiced in Asia, Africa and Latin America to process commodities such as rice, wheat, corn, amaranth and chickpea. Snack preparation through popping involves high-temperature short-time (HTST) treatment and is viewed in the food industry as more sustainable as the process is fast, easy to operate, cheap and uses low water (Sreerama *et al.*, 2008; Kaur, 2009; Reyes-Moreno *et al.*, 2019). However, existing literature on the effect of popping on ANFs is limited. In principle, the high temperatures and pressures generated during the popping process should be sufficient to thermally degrade ANFs, or cause changes to their structure and extractability, even after a short processing time. Popping techniques have been reported to be effective at reducing heat-labile ANFs in horse gram and amaranth (Sreerama *et al.*, 2008; Amare *et al.*, 2016). Therefore, the aim of the current research was to assess popping methodologies of two key pulses, chickpea and red kidney bean, and to determine impacts on the phytic acid, condensed tannins and phenolic content of these pulse varieties.

Materials and methods

Materials

Dried, whole beige Kabuli chickpeas and red kidney beans were purchased from Buy Whole Foods Online Limited (Kent, UK) and stored at room temperature.

Processing

Quantities of raw chickpeas and red kidney beans were each processed once and separately using the following methods.

Soaking

Whole, raw chickpeas and red kidney beans (200 g) were each soaked in 1000 mL reverse osmosis (RO) water (1:5 w/v) for 24 h at room temperature (~25 °C). Samples were then sieved and rinsed three times with RO water, dried with paper roll to remove excess water and freeze-dried (Frozen in Time Ltd, York, UK) until consistent mass (at least 72 h).

Boiling

Whole, raw chickpeas and red kidney beans (300 g) were boiled in 1500 mL RO water (1:5 w/v) at 100 ± 1 °C for 1 h. Samples were sieved and rinsed three times with RO water, dried with paper roll and oven dried (Tom Chandley Ltd, Manchester, UK) at 50 ± 2 °C for 24 h.

Roasting

Whole, raw chickpeas and red kidney beans (100 g) were roasted in aluminium foil containers (197 × 105 × 50 mm) in a fan assisted electric oven (Whirlpool Corporation, Michigan, USA) at 180 °C for 20 min. Halfway through the roasting process, the containers were shaken to redistribute the seeds.

Popping

Whole, raw chickpeas and red kidney beans were ground to a flour using a Thermomix TM31 (Vorwerk, Wuppertal, Germany), to fit a 425 micron screen. The sieved samples were popped using two heat presses of different scales to create final products similar to rice cakes. Chickpea and red kidney bean flour (100 g) was volumetrically fed into a modified SYP9001 automatic rice popper (10 cm diameter, Shinyoung Mechanics Ltd, Bucheon-si, South Korea) at 230 ± 4 °C for 4 and 8 s, referred to in this study as the large head. The same flours were popped using a manual smaller heat press (4.5 cm diameter, custom build, University of Nottingham, UK) referred to in this study as the small head. For the small head, 1.8 g of sample was weighed into an aluminium foil tray which had been moulded to fit the dimensions of the heat press base platen recess (4.5 cm diameter, 1 cm depth). The heat press head and base platens were both set to 230 ± 2 °C, and all samples were popped at this temperature for 4 and 8 s under a gauge pressure of 4 bar. Separate whole chickpeas and red kidney beans (50 g) were also popped in aluminium foil trays (1.8 ± 0.05 g per tray) using the small head.

Preparation of flour for analysis

Raw and processed seeds (soaked, boiled, roasted and popped) were ground using a Thermomix TM31 (Vorwerk, Wuppertal, Germany) for 30 s (in 10 s bursts to prevent temperature increase). Samples were then passed through a 425 micron screen and stored in sealed plastic screw-cap bottles at room temperature prior to analysis.

Phytic acid analysis

Phytic acid content was quantified using a Megazyme K-PHYT phytic acid (phytate)/total phosphorus assay kit (Megazyme International, County Wicklow, Ireland) and the method of McKie & McCleary (2019). In short, samples were agitated overnight in 0.66 M HCl, centrifuged and neutralised with 0.75 M NaOH before being dephosphorylated with phytase and alkaline phosphatase to liberate inorganic phosphate from all forms of myo-inositol phosphate (InsP₁₋₆) in the sample. A modified colorimetric molybdenum blue assay was then used to convert phosphate into total phosphorus and then phytic acid, using a standard curve of known phosphorus concentrations (0–7.5 µg/mL). Samples were analysed in three independent replicates.

Extraction and determination of phenolics

Soluble and bound phenolic extractions were completed in line with the method of Gonzales *et al.* (2014). Briefly, soluble phenolics were extracted with pure methanol and reextracted with 80% methanol. Using the leftover pellet, bound phenolics were subsequently hydrolysed under sonication using 2 M NaOH before extraction with 0.1% formic acid in methanol. Phenolics were then quantified according to Singleton *et al.* (1999), using three technical replicates per sample. A standard curve (0–0.05 mg/mL) was produced using gallic acid monohydrate (Sigma-Aldrich, Missouri, USA) and phenolic concentration was expressed in mg gallic acid equivalents/100 g.

Condensed tannin analysis

Condensed tannin content was determined according to the vanillin-HCl method (Price *et al.*, 1978), using the soluble phenolic extract and three technical replicates for each sample. Catechin hydrate (Sigma-Aldrich, Missouri, USA) was used to prepare a standard curve (0–0.3 mg/mL) and condensed tannin concentration was expressed in mg catechin equivalents/100 g. Samples with an average absorbance ≤ 0 at 500 nm were classified as 'not measurable'.

Statistical analysis

Statistical analyses were carried out using Genstat 21st edition software. One-way ANOVAs followed by Tukey multiple comparison tests were used to identify significant effects. Significance was defined as $P < 0.05$. Samples with 'not measurable' values were not included in statistical analysis.

Results and discussion

Effect of processing conditions on the phytic acid (PA) content of chickpea and red kidney beans

The phytic acid (PA) content of raw chickpeas and red kidney beans used in this study is shown in Table 1. Raw chickpeas and red kidney beans contained 945.21 mg/100 g and 1201.08 mg/100 g of PA respectively. The PA contents are comparable with previous studies, for which 943–990 mg/100 g was reported for chickpeas (Kaur *et al.*, 2019) and 1158–1188 mg/100 g for raw kidney bean (Khattab & Arntfield, 2009).

Soaking chickpeas and red kidney beans failed to cause any significant ($P > 0.05$) changes in PA levels, as shown in Table 1. Similar findings have previously been reported in chickpeas, red kidney beans, faba beans, peas and lupins (Vidal-Valverde *et al.*, 1998; Wang *et al.*, 2008; Yasmin *et al.*, 2008; Embaby, 2010; Shi *et al.*, 2018). However, significant reductions have

Table 1 Effect of different processes on phytic acid (PA) content in chickpea and red kidney bean (Data are representative of means \pm SD, $n = 3$; superscript letters indicate significance ($P < 0.05$ as determined using Tukey's test))

Treatment	Phytic acid (PA) (mg/100 g DM)			
	Chickpea	Compared to raw (%)	Red kidney bean	Compared to raw (%)
Raw	945.21 \pm 8.19 ^{de}	–	1201.08 \pm 15.82 ^d	–
Soaked	979.72 \pm 8.06 ^e	+3.65	1226.55 \pm 29.52 ^d	+2.12
Boiled	897.67 \pm 33.41 ^{cd}	–5.03	1197.66 \pm 41.61 ^d	–0.28
Roasted	887.97 \pm 5.90 ^c	–6.06	999.34 \pm 10.75 ^c	–16.80
Popped W	811.27 \pm 23.60 ^b	–14.17	1011.56 \pm 28.71 ^c	–15.78
SH 4 s				
Popped W	799.94 \pm 7.56 ^b	–15.37	779.59 \pm 57.86 ^{ab}	–35.09
SH 8 s				
Popped G	795.60 \pm 9.16 ^b	–15.83	929.53 \pm 16.71 ^c	–22.61
SH 4 s				
Popped G	786.48 \pm 5.09 ^{ab}	–16.79	728.52 \pm 71.67 ^a	–39.34
SH 8 s				
Popped G	793.76 \pm 21.34 ^{ab}	–16.02	886.59 \pm 29.46 ^{bc}	–26.18
LH 4 s				
Popped G	738.68 \pm 5.47 ^a	–21.85	745.21 \pm 20.12 ^a	–37.96
LH 8 s				

Abbreviations: G, ground; LH, large head; SH, small head; W, whole.

been observed for cowpea and mung beans (Mubarak, 2005; Kalpanadevi & Mohan, 2013) as well as dehulled Canadian and Egyptian kidney beans (Khattab & Arntfield, 2009) using comparable conditions to this study. A reduction in PA content after soaking is thought to be due to the activation and subsequent hydrolysis of PA by endogenous phytase, resulting in the products leaching into the soaking medium (Alonso *et al.*, 2000; Lestienne *et al.*, 2005; Embaby, 2010). The contrasting results may be due to the presence of the hull for samples investigated in this study. It has been shown for faba beans that the reduction in PA content of dehulled beans (20%) was greater than soaking whole seeds (9%), with the presence of the hull restricting the diffusion of hydrolysis products into the soaking medium (Luo *et al.*, 2009).

Boiling also had no significant ($P > 0.05$) effect on the PA content of chickpeas or red kidney beans, as well as parallel work by Shi *et al.* (2018) following the assessment of red kidney beans, pinto beans, navy beans and black beans. The enzyme phytase, responsible for the degradation of PA, is activated during soaking, and subsequently deactivated when heated above 80 °C (Liu *et al.*, 2019). This observation explains the lack of PA reduction reported in the current work.

Both forms of dry thermal treatment (roasting and popping) significantly ($P < 0.05$) decreased PA content. Roasting reduced PA by 6% in chickpeas and 17% in red kidney beans, as displayed in Table 1. Popping significantly reduced PA levels compared to roasting for chickpea samples across all parameters (time, processing equipment, sample format) and for kidney bean samples when 8 s of popping was conducted. For chickpeas, ground samples popped for 8 s (large head) had the biggest reduction in PA (22%), with the same popping duration and sample format in the small head the most effective at reducing PA in red kidney beans (39%).

A reduction in PA content after popping or roasting is not unexpected as PA is heat-labile and other authors have reported significant decreases in roasted chickpeas, pigeon peas, mung beans, urid bean, cowpea, kidney bean and peas (Chitra *et al.*, 1996; Khattab & Arntfield, 2009). Moreover, Sreerama *et al.* (2008) reported a 46% reduction of PA in horse gram that had been popped at 230–250 °C for 20–30 s using hot sand. Reduction in PA levels can be initiated by different mechanisms during thermal treatment. Heat applied during roasting and HTST popping can cause hydrolysis of myo-inositol hexaphosphate (PA) into penta-, tetra- and triphosphates or form insoluble complexes between phytate and proteins and minerals (Alonso *et al.*, 2000; Kalpanadevi & Mohan, 2013; Nikmaram *et al.*, 2017; Kataria *et al.*, 2021; Sarkhel & Roy, 2022). This study therefore suggests that at

temperatures of ~100 °C (boiling), PA is stable to heat, which has been reported by other authors (Martín-Cabrejas *et al.*, 2009; Embaby, 2010), but at higher temperatures used in roasting and popping, reduction mechanisms are initiated.

The extent of PA reduction for popped pulses varied between popping parameters and samples. For chickpeas, all popping significantly ($P < 0.05$) reduced PA content compared to the unprocessed chickpea sample. However, the processing conditions (4 or 8 s, popping ground or whole samples and popping head used) did not significantly affect the PA content.

All popping significantly ($P < 0.05$) reduced PA in red kidney beans. No significant difference in red kidney bean PA content was observed between each 4 s popping treatment, with the same trend observed at 8 s, indicating that the popping head and sample format did not influence the degree of PA reduction for red kidney beans. However, increasing the popping time from 4 s to 8 s resulted in a significant ($P < 0.05$) reduction in PA, suggesting that the longer the pulse was under the popping head, the more PA was degraded.

Unlike in chickpeas, the PA content in red kidney beans decreased with an increase in popping time, likely due to differences in the phytate–mineral complexes in both seeds. In mature pulse seeds, phytate is stored in globoids in protein storage vacuoles of the cotyledon, either as a complexed salt with Ca, Mg and K or bound to protein and starch (Angel *et al.*, 2002; Madsen & Brinch-Pedersen, 2020). Sun *et al.* (2021) suggested that although there are negligible differences in the thermal stability of metal phytates, Ca phytate is said to decompose significantly more (28%) than Al, Fe, Cu, Zn, Mn, Cd, Na and Mg phytates (0.05%–1.6%) under the same thermal treatments. Clearly, further analysis is required to determine the types of phytate mineral complexes and their thermal stability in our tissue samples.

Phytic acid content of chickpea and kidney beans were quantified and compared following soaking, boiling, roasting and popping of the samples. It is noteworthy that the assay used measures all forms of myo-inositol phosphate (InsP₁₋₆) in the sample, including PA (InsP₆), which is nutritionally relevant as it can negatively affect mineral bioavailability, while lower forms of myo-inositol phosphate are said to have a poorer ability to inhibit mineral absorption (Lönnerdal, 2002; Nielsen *et al.*, 2013). Therefore, it is possible that the true PA content of samples may be lower than the assay suggests, underestimating the effect of processing.

Effect of processing conditions on the condensed tannin (CT) content of chickpeas and red kidney beans

Condensed tannins (CT) are commonly quantified in plants using the colorimetric vanillin-HCl assay (Price

et al., 1978). The method is sensitive and specific for flavan-3-ols, their oligomers and polymers (CT) and dihydrochalcones (Muchuweti *et al.*, 2005), which when reacted with vanillin to form red-coloured complexes (Schofield *et al.*, 2001). It is noteworthy that catechin, a monomer of CT, and its oligomers will produce a positive result (Gabaza *et al.*, 2018), which can lead to a misvaluation of antinutritional risk, as it is higher molecular weight tannins that are associated with an increased degree of protein precipitation and trypsin inhibition, with lower molecular weight tannins/catechins said to have less of an effect (Naumann *et al.*, 2014; Cirkovic Velickovic & Stanic-Vucinic, 2018). Furthermore, Yoneda & Nakatsubo (1998) reported that a higher degree of CT polymerisation is directly linked to an increase in the relative stability of chelates between CT and aluminium. Processing could also lead to a misvaluation due to the breakdown of tannins to lower molecular weight tannins, which needs to be considered in the analysis presented. Although the vanillin-HCl assay does not allow differentiation between CT structures in the sample, processing will likely alter the ratio of CT compounds and their molecular weights, meaning changes to measurable CT levels will help with assessing the antinutritional risk.

The CT content of chickpea and red kidney bean (mg catechin equivalents/100 g DM) is reported in Table 2. Raw red kidney beans contained CT levels of 81.02 mg CE/100 g, which are comparable to the study by Marzo *et al.* (2002) who reported levels in the range of 112 mg/100 g in raw kidney beans. In contrast, CT levels were not measurable in unprocessed chickpeas, as they contained an average absorbance ≤ 0 in the vanillin-HCl assay, as described in Section 2.6. Wang *et al.* (2010) found low levels of CT, 4 mg/100 g in Kabuli chickpeas, indicating that values obtained in the current research were comparable to published studies. Seeds that are darker in colour have been associated with having a higher CT content (Díaz *et al.*, 2010; Varma Penmetsa *et al.*, 2016). Higher levels of CT have been reported for raw dark red kidney beans and Desi chickpeas, containing 1370 mg/100 g and 21 mg/100 g respectively (Wang *et al.*, 2010).

A reduction in tannins after soaking and boiling can be attributed to their water-soluble nature (Smeriglio *et al.*, 2017). When pulses are soaked or boiled, tannins will leach out of the seed and into the treatment medium, which is then discarded (Kalpanadevi & Mohan, 2013; Kataria *et al.*, 2021). In this study, soaking was found to significantly ($P < 0.05$) reduce the CT content by 74% in red kidney beans. This is comparable to the study by Khattab & Arntfield (2009), which observed that tannins in soaked Canadian and Egyptian kidney beans were reduced by

59% and 86% respectively. Similarly, Yasmin *et al.* (2008) recorded an 82% decrease in tannin levels after boiling red kidney bean seeds. As shown in Table 2, CT were not measurable in boiled red kidney beans, indicating that they had leached into the treatment medium.

Soaking and boiling had the opposite effect on CT in chickpeas, causing an increase from 'not measurable' levels in raw seeds. It is hypothesised that both processes slightly increased the extractability and solubility of monomeric CT and flavan-3-ol compounds associated with the bound phenolic fraction, possibly due to loosening or thermal degradation of the cell wall matrix (Wan *et al.*, 2022; Zhao *et al.*, 2022).

Roasting increased the CT content in chickpeas and red kidney beans, a trend which has also been reported in thermally treated finger millet and teff (Gabaza *et al.*, 2016; Kataria *et al.*, 2021).

Popping had a differing effect on CT in both pulses. For chickpeas, all popped cakes were significantly ($P < 0.05$) higher in CT than the unprocessed samples, other than one sample which was popped whole for 4 s in the small popping head, which also contained not measurable levels. Chickpeas that were 4 s-popped (regardless of popping head and sample format) were found to have a lower content of CT than samples

popped at 8 s (Popped W SH 8 s, Popped G SH 8 s and Popped G LH 8 s). These results highlight that increases in quantified CT for chickpeas compared to the raw seed correlate with popping duration rather than sample format or popping head utilised. In red kidney beans, ground samples popped for 4 s in the small and large popping heads were significantly ($P < 0.05$) lower in CT compared to raw seeds. Increasing the popping time to 8 s increased the CT content for both whole and ground red kidney beans, similarly to chickpeas. The sample format, whether whole or ground, impacted the CT in kidney beans after 8 s popping (small head), with samples popped whole having significantly higher CT contents than ground samples.

High temperatures generated by dry thermal treatment (roasting and popping) are likely to depolymerise bound CT into lower molecular weight forms that are more soluble and assayable (Duodu, 2014; Gabaza *et al.*, 2016), and therefore the measurable CT content increases after processing. Awika *et al.* (2003) observed that extrusion of sorghum significantly reduced the quantity of mixed CT polymers with a degree of polymerisation >10 , while CT monomers, dimers, trimers and tetramers were significantly increased, indicating that heat treatment caused the higher molecular weight tannins to depolymerise. Therefore, it could be assumed that a proportion of CT that is quantified is likely to be lower molecular weight forms such as catechin and its oligomers, resulting from the breakdown of higher molecular weight polymers during processing. Furthermore, structural variations in CT can affect the colour yield in the assay, with monomers and subunits of CT polymers having variable reactivity with vanillin (Schofield *et al.*, 2001), meaning the distribution of monomers (catechin), oligomers and polymers of CT, altered by thermal treatment, can influence the degree of red colour formation in the assay and contribute to changes in reported CT levels (Wu *et al.*, 2013).

A decrease in measurable CT, as seen for the 4 s-popped red kidney bean (ground), could relate to a reduction in extractability or a change in structure (Alonso *et al.*, 2000; Duodu, 2014).

It was also found that the increase in CT after 8 s popping (small head) was greater for whole-popped red kidney beans, compared to ground. When popping whole, the first point of contact between the hot platen would be the seed coat, where CT and phenolics are concentrated (Pal *et al.*, 2016; Elessawy *et al.*, 2021), meaning the aforementioned changes to CT should occur at an increased rate, compared to popping as a homogenous powder where the hull is evenly distributed in the sample.

Other authors have also reported a rise in phenolics, flavonoids and CT after thermal treatment, due to the

Table 2 Effect of different processes on the condensed tannin content in chickpea and red kidney bean (Data are representative of means \pm SE, $n = 3$; superscript letters indicate significances ($P < 0.05$ as determined using Tukey's test))

Treatment	Condensed tannins (CT) (mg CE/100 g DM)			
	Chickpea	Compared to raw (%)	Red Kidney Bean	Compared to raw (%)
Raw	NM	–	81.02 \pm 1.74 ^{de}	–
Soaked	5.15 \pm 3.05 ^a	–	20.83 \pm 4.96 ^a	–74.29
Boiled	5090 \pm 1.43 ^{ab}	–	NM	–100
Roasted	9.12 \pm 2.02 ^{ab}	–	192.12 \pm 1.76 ^b	+137.13
Popped W SH 4 s	NM	–	73.15 \pm 9.03 ^{cd}	–9.71
Popped W SH 8 s	39.79 \pm 6.83 ^{cd}	–	118.82 \pm 3.00 ^g	+46.66
Popped G SH 4 s	12.92 \pm 2.58 ^{ab}	–	58.10 \pm 1.83 ^{bc}	–28.29
Popped G SH 8 s	41.84 \pm 6.58 ^{cd}	–	98.20 \pm 1.25 ^{ef}	+21.20
Popped G LH 4 s	25.92 \pm 4.12 ^{bc}	–	47.23 \pm 0.74 ^b	–41.71
Popped G LH 8 s	51.34 \pm 3.28 ^d	–	103.21 \pm 3.02 ^{fg}	+27.39

Note: No measurable values are classified as samples with an average absorbance ≤ 0 at 500 nm.

Abbreviations: G, ground; LH, large head; NM, not measurable; SH, small head; W, whole.

release of bound phenolics and a subsequent increase in extractability (Wu *et al.*, 2013; Duodu, 2014). Catechin has been quantified as an insoluble-bound flavonoid in red kidney beans (Shahidi & Yeo, 2016; Wang *et al.*, 2016), which may be released at the high temperatures and will produce a positive result for CT, increasing the absorbance of the red-coloured adduct, a plausible reason for the increase in chickpea and red kidney bean CT content after roasting and popping. Giusti *et al.* (2019) also reported that catechin in the soluble phenolic fraction of unprocessed chickpea was not detectable, however, 2.65 mg/100 g catechin was quantified in the bound phenolic fraction.

Therefore, it is likely that processing caused depolymerisation and/or a decrease in bound phenolics, resulting in an increase in soluble phenolics and explaining the increase in measurable contents of CT seen in chickpea and red kidney bean samples after roasting and popping (Table 2). The Folin–Ciocalteu total phenolic assay was therefore conducted to quantify the phenolic content of soluble and bound extracts, as discussed in Section 3.3.

Effect of processing conditions on the phenolic content of chickpea and red kidney beans as measured by the Folin–Ciocalteu assay

As discussed in Section 3.2, it is hypothesised that the increase in CT levels seen after roasting and popping is likely due to depolymerisation and/or release of bound phenolics, causing an overall increase in soluble phenolics and measurable CT, including monomers of CT such as catechin.

The soluble, bound and total phenolic (soluble and bound combined) content of chickpea and red kidney bean (mg gallic acid equivalents/100 g DM) are presented in Table 3. Processing of chickpea and red kidney bean significantly ($P < 0.05$) decreased the total phenolic content in all samples aside from roasted, which was not significantly different for raw chickpea and increased for red kidney bean. Furthermore, 8 s-popped chickpeas (ground) in the small head resulted in the greatest reduction in total phenolic content for chickpea and red kidney bean, suggesting that this process was the most intense form of heat treatment as more phenolics were thermally degraded.

Soaking and boiling enabled soluble phenolics present in the raw seed to leach into the solution (Tajoddin *et al.*, 2014; Liu *et al.*, 2021) and therefore decrease the overall phenolic content as shown in Table 3. In addition, both processes would be expected to soften the pulse by loosening or rupturing the matrix of the cell wall (Zhao *et al.*, 2022) and therefore bound phenolics would be solubilised, as confirmed by a significant ($P < 0.05$) decrease in bound phenolics for soaked and boiled chickpea and red kidney bean.

In chickpeas, soluble phenolics increased after soaking, in line with the slight increase in CT shown in Table 2, similar results have also been reported for soaked rice bran (Zhao *et al.*, 2022). Interestingly, the percentage reduction in total phenolics after soaking red kidney beans was 23%, greater than the reduction after boiling (10%), we hypothesise that these results are linked to the effect of the different processes on the cell microstructure, which needs further investigation.

Total phenolic content after roasting was unchanged for chickpeas but significantly ($P < 0.05$) increased for red kidney beans. Soluble phenolics were significantly increased in roasted chickpeas and red kidney beans, which were also found in the CT assay presented in Table 2. It is expected that a portion of the bound phenolic content was solubilised after roasting, as this process can also rupture the cell wall matrix (Shahidi & Yeo, 2016), thus increasing the soluble content. However, the formation of Maillard reaction products (MRP) and melanoidins during roasting, which has been reported to give a positive result with the Folin–Ciocalteu reagent (Górnaś *et al.*, 2016), explains why bound and total phenolics did not decrease in roasted samples (Chandrasekara & Shahidi, 2011).

All popping parameters significantly ($P < 0.05$) reduced total and bound phenolic content in chickpea and red kidney bean samples. Furthermore, the soluble phenolic content of all popped samples was significantly ($P < 0.05$) increased by popping, except for ground red kidney beans popped for 4 s in the small and large heads, which had no significant difference and were significantly lower respectively. Phenolics can be covalently bonded to cell wall components including structural proteins, pectin, cellulose and arabinoxylan. The high temperatures and pressures applied during popping can result in the disruption of the cell wall matrix and depolymerisation of high molecular weight phenolics, leading to the solubilisation of bound phenolics, as well as thermal degradation of phenolic compounds causing an overall reduction in their total content (Duodu, 2014; Shahidi & Yeo, 2016; Liu *et al.*, 2020; Kataria *et al.*, 2021).

Samples popped with the small head (ground) showed a greater reduction in total and bound phenolics to samples popped in the larger popping head (also ground), the smaller sample size to heated plateau surface could be expected to be more effective at heat transfer and therefore degrading and extracting phenolics.

The longer the popping duration, the greater the reduction in bound and total phenolic content for red kidney bean samples, however, there was no significant ($P > 0.05$) difference between chickpea samples popped for 4 or 8 s. Anthocyanins, which are heat-labile flavonoids, are natural pigments found in high concentrations in red and black coloured seed coats, with pale

Table 3 Effect of different processes on soluble, bound and total phenolic content in chickpea and red kidney bean (Data are representative of means \pm SE, $n = 3$; superscript letters indicate significances ($P < 0.05$ as determined using Tukey's test))

Treatment	Phenolic content (mg GAE/100 g DM)					
	Chickpea			Red Kidney Bean		
	Soluble	Bound	Total	Soluble	Bound	Total
Raw	15.89 \pm 0.19 ^b	455.03 \pm 2.90 ^g	470.92 \pm 2.83 ^e	49.62 \pm 0.22 ^c	861.81 \pm 9.22 ^g	911.43 \pm 9.12 ^g
Soaked	30.56 \pm 0.14 ^f	370.80 \pm 10.43 ^{de}	401.36 \pm 10.56 ^{cd}	39.39 \pm 0.17 ^b	663.07 \pm 10.54 ^{cd}	702.46 \pm 10.50 ^{cd}
Boiled	12.01 \pm 0.22 ^a	405.76 \pm 15.02 ^{ef}	417.76 \pm 15.16 ^d	25.27 \pm 0.17 ^a	792.20 \pm 3.58 ^f	817.47 \pm 3.74 ^f
Roasted	19.99 \pm 0.20 ^c	442.82 \pm 14.79 ^{fg}	462.81 \pm 14.94 ^e	95.31 \pm 0.68 ^g	883.92 \pm 9.00 ^g	979.24 \pm 8.39 ^h
Popped W SH 4 s	24.59 \pm 0.27 ^e	348.47 \pm 3.29 ^{bcd}	373.06 \pm 3.03 ^{abc}	52.57 \pm 0.26 ^d	671.92 \pm 2.13 ^d	724.49 \pm 2.19 ^{cd}
Popped W SH 8 s	37.81 \pm 0.18 ^h	329.75 \pm 5.48 ^{abc}	367.56 \pm 5.66 ^{abc}	88.75 \pm 0.26 ^f	521.25 \pm 5.30 ^b	610.00 \pm 5.17 ^b
Popped G SH 4 s	22.67 \pm 0.22 ^d	320.28 \pm 4.12 ^{ab}	342.95 \pm 4.10 ^{ab}	49.30 \pm 0.15 ^c	677.78 \pm 1.42 ^d	727.09 \pm 1.37 ^d
Popped G SH 8 s	33.90 \pm 0.52 ^g	302.54 \pm 3.98 ^a	336.44 \pm 4.42 ^a	60.53 \pm 0.36 ^e	461.84 \pm 4.77 ^a	522.37 \pm 4.75 ^a
Popped G LH 4 s	24.41 \pm 0.10 ^e	365.31 \pm 1.67 ^{cde}	389.72 \pm 1.76 ^{cd}	41.00 \pm 0.63 ^b	740.22 \pm 4.36 ^e	781.23 \pm 4.59 ^e
Popped G LH 8 s	34.16 \pm 0.12 ^g	348.60 \pm 4.93 ^{bcd}	382.75 \pm 4.86 ^{bcd}	62.10 \pm 0.07 ^e	634.92 \pm 1.92 ^c	697.02 \pm 1.90 ^c

Abbreviations: G, ground; LH, large head; SH, small head; W, whole.

or white coloured seed coats having low or negligible levels (Díaz *et al.*, 2010; Zhou *et al.*, 2017; Kan *et al.*, 2018). The presence of anthocyanins in red kidney beans could explain the significant decrease in bound and total phenolics between 4 and 8 s of popping, as the process would degrade more of these phenolics. The soluble phenolic content of popped chickpea and red kidney bean samples was significantly increased after 8 s of popping, in line with CT results presented in Table 2.

Sample format significantly ($P < 0.05$) affected soluble phenolics in small head-popped chickpeas and red kidney beans, with ground samples having significantly lower soluble phenolics than the whole. Ground red kidney beans were also significantly lower in total and bound phenolics than whole samples when small head-popped for 8 s, indicating that the structure of the ground sample made it easier for heat to degrade phenolics.

Conclusion

This research has validated the use of popping to reduce the levels of PA and rearrange phenolic compounds, specifically releasing bound phenolics which are likely to entrap nutrients such as proteins and minerals in chickpeas and red kidney beans.

Popping for 8 s reduced PA to lower levels than all traditional processes (soaking, boiling and roasting) used in this study, with the high temperatures and pressure generated likely to accelerate PA degradation. Expectedly, soaking and boiling were the most effective treatment for removing water-soluble CT and phenolics as they are discarded in the treatment medium. Popping and roasting increased the content of measurable condensed tannins. It was hypothesised that the

high temperatures the samples are exposed to during popping and roasting, are enough to cause changes to structure and extractability of phenolic compounds in chickpeas and red kidney beans, including depolymerisation of high molecular weight tannins, and release of bound phenolics, enhancing the solubility and therefore measurability of CT compounds. The Folin–Ciocalteu assay confirmed that whilst total phenolics decreased after popping due to thermal degradation, bound phenolic content decreased in all popped samples and soluble content increased in most.

Although lower molecular weight PA and CT compounds may still be present in popped chickpeas and red kidney beans, which could impact mineral bioavailability and protein digestibility when consumed, it is suggested that structural changes caused by popping conditions could lower the antinutritional effect of these compounds on essential nutrients, as well as releasing nutrients from PA, CT and phenolic complexes, making them more accessible. Further investigation is needed to confirm this.

Incorporation of pulses into popped foods should be considered, not only due to their high nutritional value, but as the antinutritional risk is reduced in popped snacks compared to raw seeds. Further investigation into the sensory and functional properties of popped pulses, as well as the digestibility, bioaccessibility and bioavailability of nutrients pre- and post-popping, is required in order for humans to maximise the benefits from the production and consumption of popped pulse snacks.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

Ethics approval was not required for this research.

Author contributions

Joseph Godrich: Conceptualization (equal); data curation (lead); investigation (lead); methodology (equal); visualization (equal); writing – original draft (lead); writing – review and editing (equal). **Peter Rose:** Conceptualization (equal); supervision (equal); writing – review and editing (equal). **Molly Muleya:** Conceptualization (equal); methodology (equal); writing – review and editing (equal). **Joanne Gould:** Conceptualization (equal); funding acquisition (lead); methodology (equal); project administration (lead); resources (lead); supervision (equal); visualization (equal); writing – review and editing (equal).

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Data availability statement

All data supporting this study are openly available from the University of Nottingham data repository at <http://doi.org/10.17639/nott.7229>.

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