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Use of European pulses to produce functional beverages – From chickpea and lupin as dairy alternatives

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ABSTRACT

Consumption of plant based products as dairy alternatives is increasing steeply. This diet transition can only be achieved if these products keep the nutritional value and meet consumer's sensory acceptance. This work aimed to evaluate the decrease of the "beany" flavour and of raffinose, stachyose and verbascose contents in EU pulse beverages production, and also the best lactic fermentation conditions of the beverages, towards chickpea- and lupin-based yoghurts, with rheology properties similar to the commercial soy yoghurts. The reduction of "beany" volatile compounds of chickpea and lupin beverages during processing was confirmed through GC–MS analysis. Soaking and cooking processes were effective in removing flatulence sugars with almost 48% loss from the initial content in lupin beverage. The fermentation conditions at 40 °C, 12 h and 2% (w/v) of starter concentration evidenced the best viscoelastic structure and flow properties. The lupin yoghurt-type showed a similar gel structure to commercial soy yoghurt.

1. Introduction

In recent years, the interest of vegetarian and vegan diets has been rising. The annual report of 2009 on global health risks of the World Health Organization (WHO), mentioned that more than half of the factors linked to death were related to nutrition-based diseases (Bernat, Cháfer, Chiralt, & Gonzalez-Martínez, 2014). This knowledge led to a growing number of consumers that have come to prefer vegetable-based foods to promote good health or to take it as a lifestyle choice. Adding to that, the dairy alternative global market is projected to grow at a compound annual growth rate of 11.4%, from 2018 to 2023, with the Asia-Pacific region representing the biggest market share (Mintel, 2018; Market and Markets, 2019). There is a high demand for vegetable beverage milk replacers, namely from pulses, for their lactose-, animal protein- and cholesterol-free, but also for their higher sustainable production, ethical issues and environmental concerns (Gerber et al., 2013; Makinen, Wanhalinna, Zannini, & Arendt, 2016).

The major issue that is hampering the production of legume-based beverages is the "beany" flavour, so negatively famous in soymilk. The off-flavour soymilk is primarily due to endogenous enzymes, lipoxygenases (Lv, Song, Li, Wu, & Guo, 2011), which catalyses the formation of non-volatile hydroperoxides from unsaturated fatty acids, which in turn, decompose into medium-chain aldehydes and alcohols such as *n*-hexanal and *n*-hexanol, associated to "beany" flavour. In the case of other legume such as lupin and chickpea, which have much lower oil content (1 to 5%) when compared to soy (>20%), the lipoxygenase action, therefore beanyness, should have lower impact. The volatile beany and herbal flavour are composed of the aldehydes, alcohols, ketones, and furans, whereas the non-volatile bitterness and astringency consist of phenolic acids, isoflavones, saponin, tetrol, and other substances (Murat, Bard, Dhalleine, & Cayot, 2013; Ma et al., 2015; Yang, Smyth, Chaliha, & James, 2016). The technics usually used to remove the "beany" flavour in legume-based beverages are heat inactivation by cooking (Carrão-Panizzi, Beléia, Prudêncio-Ferreira, Oliveira, & Kitamura, 1999), although high temperatures may cause undesirable changes in proteins (e.g. denaturation, aggregation, lower solubility) and some nutrient losses (e.g. vitamins and minerals). On the other hand, fermentation processes of pulses can decrease or mask the properties of undesirable compounds which contribute to this "beany" flavour. Indeed, studies showed that lactic acid fermentation can reduce "beany" flavours and anti-nutritional factors, such as phytic acid, in soymilk based yoghurt (Favaro-Trindade, Terzi, Trugo, Modesta, &

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Couri, 2001). Adding to that, Reddy and co-workers sensory tests evidenced that only 1 out of the 14 tasters detected a "beany" flavour and odour in soy yoghurt, while half of the panel detected it in the soymilk (Reddy, Pierson, Sathe, Salunkhe, & Beuchat, 1983).

Among the anti-nutritional factors present in pulse seeds, the oligosaccharides are also implicated. The most common oligosaccharides in the plant kingdom are α-galactosides and the most ubiquitous group within these are the raffinose family of oligosaccharides which includes raffinose (a trisaccharide), stachyose (a tetrasaccharide), verbascose (a pentasaccharide) and ajugose (a hexasaccharide) (Kadlec et al., 2000). These are commonly linked to an undesirable physiological reaction in the human intestine such as flatulence, due to its non-digestibility in the gut because of the absence α-galactosidase in the human intestinal mucosa (Ribeiro, Teixeira, & Ferreira, 2004; Pedrosa et al., 2012) and further fermentation by gut microbiota, which *per se* is a positive effect. Conventional processing methods are used to reduce the oligosaccharide content in legumes such as soaking, cooking, germination and fermentation (Onyenekwe, Njoku, & Ameh, 2000; El-Adawy, 2002; Han, & Baik, 2006). Since oligosaccharides are relatively heat stable, their reduction is mainly attributed to their difusion into the water during soaking and cooking (Han, & Baik, 2006; Hefnawy, 2011). During fermentation, raffinose, stachyose and verbascose can function as prebiotics stimulating the growth and viability of lactic acid bacteria (LAB) (Chen, Singh, Bhargava, & Ramanathan, 2018) and are hydrolysed during the fermentation (Waters, Mauch, Coffey, Arendt, & Zannini, 2014). Donkor and co-workers showed that the fermentation of soymilk, reduced the amount of flatulence-inducing oligosaccharides depending on the α-galactosidase activity of the strain, and increased the angiotensin-converting enzyme (ACE) inhibitory activity, which results in an anti-hypertensive effect (Donkor, Henriksson, Vasiljevic, & Shah, 2007).

Pulses seeds such as lupin and chickpea, are also known for their nutritional and health-promoting properties, as they are a good source of protein, starch, minerals and fiber (Jukanti, Gaur, Gowda, & Chibbar, 2012; Bernat, Cháfer, Chiralt, & Gonzalez-Martínez, 2014; Lima, Mota, Monteiro, & Ferreira, 2016; Barman, Marak, Barman, & Sangma, 2018; Mota et al., 2021). So, chickpea and lupin yoghurt-like product could present a balanced and interesting nutritional alternative to dairy and soy yoghurts, especially for its similar high content in protein, which can be coagulated during pulse beverage fermentation and contribute to the yoghurt-like texture. Also, resistant starch and amylose usually present in chickpea seeds (Mesquita, Leandro, Alencar, & Botelho, 2020), or non-starch polysaccharides from galactose, xylose and rhamnose polymers present in lupin seeds (Gdala, 1998) can also be hydrolysed and used by LAB, such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, for their growth during pulse beverages fermentation (Marco et al., 2021). Also, the fermentation of vegetable beverages can render the raw material into a more palatable form with good fermentation flavours (Waters, Mauch, Coffey, Arendt, & Zannini, 2014) and also exert health benefits for consumers (probiotics).

Yoghurts are an example of a food gel. Similar yoghurt gel structures, may be differently perceived regarding texture and sensory properties. Thus, the macroscopic behaviour of different gel systems and molecular rearrangements within the network may be compared through rheological measurements (Raymundo, Batista, & Sousa, 2017), which in this case, helped the optimization of the non-dairy pulse-based yoghurts.

In a previous study, Duarte and co-workers evidenced the high digestibility, anti-inflammatory and anti-carcinogenic potential of lupin and chickpea beverages (Duarte et al., 2022). The aims of this study were to evaluate the decrease of i) the "beany" flavour, and ii) of the raffinose, stachyose and verbascose content through pulse beverages production steps, and iii) fermentation of pulse beverages as a way to improve its taste and flavour and the goal was to find the best fermentation conditions comparing its rheological properties with a reference commercial soy yoghurt.

2. Material and methods

2.1. Pulse beverage's production and nutritional composition

The experimental work was previously described (Lopes et al., 2020) and two different pulse seeds (*Lupinus albus L.* and *Cicer arietinum L.*) were used to produce beverages with 10% (w/v) of total dry seeds. Seeds were soaked in warm tap water (around 30 °C) in a proportion of 1:3 (w/v) for 16 h and cooked in boiling water for 30 min in a pressure pan as previously described (Duarte et al., 2022). Liquids were discarded. The cooked seeds were drained, and the corresponding volume of fresh tap water was added to achieve 10% (*w/v*) of dry seeds in water. The mixture was milled in a food processor (Bimby-Worwerk, Wuppertal, Germany) at 20,500 rpm for 4 min with 250 mL of fresh tap water, followed by colloidal milling (Pulverisette 2, Fritsch GmbH, Idar-Oberstein, Germany) at a lab scale, at 70 rpm, for 15 min using the remaining volume of water. The resulting beverage was sieved with a strainer before being bottled in sterilized flasks. In the pasteurization step, the capsulated filled flasks were submitted to a thermal shock, inside the pressure cooker, for 1 min, in boiling water (Fig. 1). The pulse beverages were kept at 8 °C during 24 h before use. Each pulse beverage nutritional composition was previously reported by Lopes et al. (2020).

2.2. Evaluation of beverage processing impact on "beany" flavour

To evaluate the decrease of the "beany" flavour through beverages production steps, the volatile compounds extracted from chickpea and lupin milled seeds were analyzed in different stages: dried, soaked and cooked. The volatile compounds were extracted by a combination of headspace solid-phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS), with a GC equipment (Model 6850, Agilent Technologies, USA) coupled to a mass spectrometer (5975C VL MSD). Samples of soaked and cooked seeds were kept inside a desiccator for drying during 5 days before milling. For each sample, 5–6 g was weighted into 10 mL glass vials. For extraction, PDMS-DVB (65 μm) fiber (Sigma-Aldrich® Supelco SPME Fiber Assembly Polydimethylsiloxane/Divinylbenzene), previously conditioned according to the manufacturer's instructions, was exposed to the vial headspace with an extraction time of 25 min, at 70 ± 2 °C. Six replicates of each test were

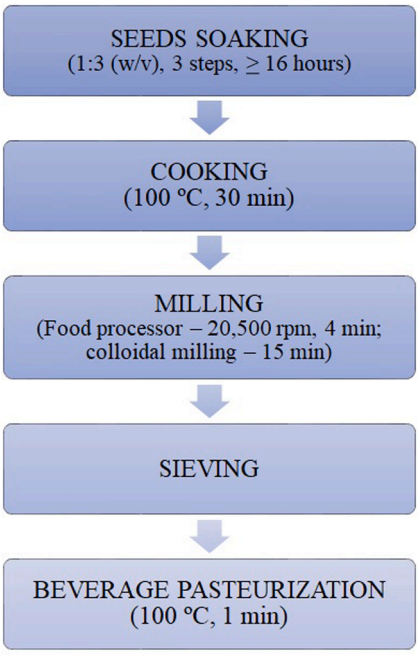

Fig. 1. Pulse beverages manufacturing procedure.

performed.

The identified compounds were assigned according to their mass spectrum and retention indices (RI). Their mass spectra were compared to those present in the reference database (NIST Mass Spectral Data 14), being considered in the present work only the ones with good adjustment (Match > 850). RI were obtained from the NIST software. To determine the key volatile compounds, the percentage ratio of the peak area of the compound to the total area of the chromatogram and its Odour Threshold Value (OTV) was taken into consideration (Brevard et al., 2011; van Gemert, 2011).

The relative percentage of the relevant compounds with olfactory contribution was calculated for each sample profile (Eq. (1)):

$$Relative\% = \frac{[peak\ area\ \%\ of\ compound\ with\ olfactory\ contribuition]}{[Sum\ of\ peak\ areas\ \%\ of\ all\ compounds\ with\ olfactory\ contribuitions]} x100 \quad (1)$$

2.3. Evaluation of beverage processing impact on flatulence sugars

To assess the flatulence sugars decrease through beverages production steps, the oligosaccharides compounds extracted from chickpea and lupin were analyzed in different stages: dried seeds and pasteurized beverages (Lopes et al., 2020). The solid samples of dried seeds were suspended and homogenized in deionized water to a final concentration of 1 $g.L^{-1}$, filtered by a 0.22 μm nylon filter, and the supernatant collected. Pasteurized beverages were treated as above. The oligosaccharides quantification was accomplished by high performance anion exchange chromatography (HPAEC) using a Dionex ICS-3000 system equipped with an electrochemical detector (Pulsed Amperometric Detector, PAD). The separation was carried with both Thermo CarboPac PA10 (4 × 250 mm) and Thermo AminoTrap (4x50 mm) columns, and eluted with a 90 mM NaOH solution at 1 $mL.min^{-1}$ at 25 °C.

All oligosaccharide extractions and analyses were carried out in triplicate.

2.4. Development of a pulse yoghurt-type

Taking into account the soy yoghurt starter (Alpro natural 125 g, Danone, Portugal) a percentage (2%, 5%, 10%, 15% and 18% w/v) with starter cultures (*S. thermophilus* and *L. bulgaricus*) was added to the corresponding volume of pasteurized chickpea beverage to further lactic fermentation. The mixture was warmed in a water bath until reaching the targeted temperature (23 °C, 30 °C, 40 °C) and kept inside an oven during the required time (4 h, 7 h, 12 h) for incubation. After the incubation time, the fermented pulse beverages were dipped in ice to stop the fermentation and to achieve the reference temperature of 20 °C and 8 °C (temperature at which yoghurts are usually consumed).

2.4.1. Evaluation of the fermentation process

pH, titrable acidity and lactic acid.

Non-fermented and fermented beverages were evaluated concerning pH, titrable acidity and lactic acid concentration.

pH was determined using a digital pH meter consisting of an 50 10 T electrode (Hach, France) and a potenciometer (pH meter Basic 20, CRISON Instruments, Spain), standardized at pH 4 and 7.

The titrable acidity of the beverages were analyzed in triplicates in accordance with adapted OIV-MA-AS313-01 international method (2015), with some modifications. Briefly, each sample (20 mL) was diluted with 25 mL of boiled water. The titration was performed with 0.1 N NaOH (aq.) until pH 8.15 was reached. Titrable Acidity was expressed in milliequivalents (mEq) of lactic acid/L.

Lactic acid concentration of fermented and non-fermented beverages was determined by High Performance Liquid Chromatography (HPLC). Samples (1 g) were centrifuged at 16,190 *xg* for 15 min and the supernatant was then diluted (1:20) in sulfuric acid 50 mM, centrifuged for 5 min and filtered using a cellulose acetate membrane of 0.2 μm of pore diameter. Lactic acid standard samples at different concentrations were used to construct the calibration curve. The standard and samples were analyzed at 210 nm in a Hitachi Chromaster UV–vis Detector 5420, connected to a Hitachi Chromaster HPLC system equipped with an Ionic Exclusion Column (Rezex™ ROA Organic Acid H+ (8%) column, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA), at 65 °C, and sulfuric acid (5 mM) was used as a mobile phase at a flow of 0.5 mL/min.

Total lactic acid bacteria viability was determined by counting colony-forming units (CFU). Sequential 10x dilutions of each fermented beverage were prepared and inoculated in MRSA medium with 100 mg/L of chloramphenicol, and incubated at 37 °C. The colonies observed were counted and expressed in terms of CFU per 1 g of fresh matter. Yogurt starter viability was also assessed.

2.4.2. Rheology measurements

All pulse yoghurt-type and soy yoghurt (Alpro natural 125 g, Danone, Portugal) were analysed in a controlled-stress rheometer (Haake MARS III, Germany), equipped with a TMP60 and DC60/2° Ti cone-plate geometry system. Small-amplitude oscillatory shear (SAOS), stress and frequency sweeps, and steady-state flow measurements were conducted.

Viscoelastic parameters, i.e., G' as the storage and G'' as the lost moduli, were determined in oscillatory measurements at low shear stress: shear stress sweeps were previously performed at a constant frequency of 1 Hz (6.28 rad/s) to determine the linear viscoelastic region (LVR). The LVR is the range where G' and G" moduli are independent of the oscillation stress, and structure of the material is preserved. The abrupt decrease of G' indicates the rupture of the gel structure (Paseephol, Small, & Sherkat, 2008). After that, the frequency sweeps from 0.01 to 100 Hz were performed at constant stress values within the LVR for each yoghurt, at 20 °C and 8 °C.

Steady shear viscosity measurements were obtained using a logarithmic ramp of shear rates increasing from 10^{-5} to 10^{3} s^{-1}. The Carreau model (Eq. (2)) was fitted to the flow curves, since the fermented pulse beverages are non-Newtonian fluids and evidenced shear-thinning behaviour, that is, the apparent viscosity decreases as the shear rate increases (Barnes, 2009):

$$\eta = \eta_{\infty} \frac{\eta_0 - \eta_{\infty}}{(1 + (k\dot{\gamma})^2)^{m/2}} \quad (2)$$

where "η_0" is the first limiting ("zero" shear rate) Newtonian viscosity (Pa.s); η_{∞} is the second limiting ("infinite" shear rate) Newtonian viscosity (Pa.s); "$\dot{\gamma}$" is the shear rate (s^{-1}); "k" is the relaxation time (s) and the reciprocal, 1/k ($\dot{\gamma}$ c), is related to the critical shear rate (i.e., onset shear rate for shear-thinning); "m" is the dimensionless constant related to Power Law and accounting for the deviation from the Newtonian behaviour. The TRIOS Software (TA Instruments, v4.1.1.33073) was used for the data analysis.

All the rheology tests were repeated at least three times. Samples were allowed to rest for 300 s after placing it in the measuring device and covered with a layer of paraffin oil to prevent moisture loss.

2.5. Sensory analysis focused on "beany" flavour

Sensory analysis was performed by an untrained sensory panel composed of 35 students and academic staff with 16 men and 19 women, aged 18–39 years. The Institute test room complies with the requirements of ISO 8589 /2007 (R2017). To evaluate the consumer's perception of "beany" flavour in the produced non fermented beverages, two Triangle tests were performed: a) two beverages with cooked chickpea and one with soaked chickpea (uncooked), and 2) two beverages with cooked lupin and one with soaked lupin (uncooked).

To evaluate the impact of fermentation on soaked and cooked pulse beverages, paired tests were conducted: a) between non-fermented and fermented lupin beverage; b) non-fermented chickpea beverage and this beverage fermented at 40 °C, with 10% starter, 12 h; c) non-fermented chickpea beverage and this beverage fermented at 50 °C, with 15% starter, 7 h.

2.6. Statistical analysis

Analysis of variance (one-way ANOVA) was used to assess significant differences between samples at a significance level of 95% ($p < 0.05$). Multiple comparisons were performed by Tukey HSD test. All statistical treatments were performed using SPSS Statistics (v.20, IBM SPSS Statistics, New York, USA).

3. Results and discussion

3.1. "Beany" flavour dissipation through processing steps of pulse beverages

To evaluate the impact of soaking and cooking steps on "beany" flavour reduction in the final beverage, the volatile compounds present in dry, soaked and cooked chickpea and lupin seeds were analyzed through GC–MS.

The chickpea dry seeds (Fig. 2A) evidenced four peaks with olfactory contribute corresponding to 1) 4-ethylbenzoic acid/benzoic acid (ester) with"minty" odour and"medicinal" flavour, and a relevant percentage of 53.9%; 2) *n*-hexadecanoic acid (palmitic acid) with"waxy" odour; 3) pentadecanal with"fresh waxy" odour; and 4) tetradecane with"mild waxy" odour. After soaking (Fig. 2B), all previous volatile compounds

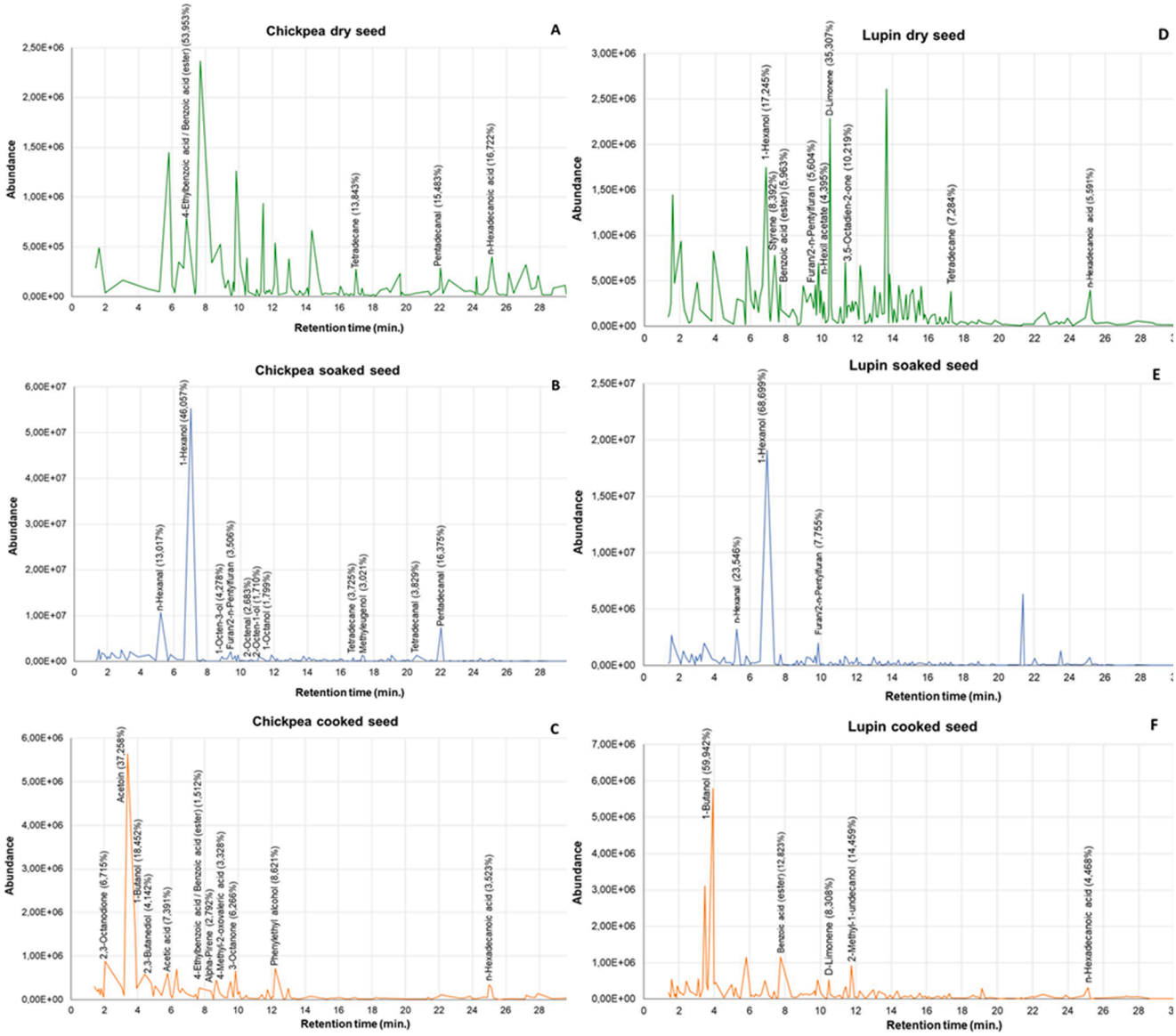

Fig. 2. Chromatograms with the volatile compound profile of chickpea dry seeds (A), soaked seeds (B), cooked seeds (C), lupin dry seeds (D), soaked seeds (E) and cooked seeds (F). The compounds with olfactory contribution are identified and the respective relative percentages are indicated.

are absent except tetradecane which is reduced 73% (from 13.8% in dry seed to 3,7% in soaked seed). Comparing the volatile compounds evidenced after soaking with the ones obtained after cooking (Fig. 2C), only two remained with low percentages: the 4-ethylbenzoic acid/ benzoic acid (ester) which was reduced from 53.9% to 1,5% and the *n*-hexadecanoic acid (palmitic acid) from 16.7% to 3,5%. Adding to this, new compounds with"buttery","herbal" and"floral" odours were shown after cooking, standing out with 37,3% the acetoin with"buttery" odour and"creamy" flavour.

The soaking step of chickpea seeds enhanced the "green" flavour through a rise of new compounds such as *n*-hexanal, 1-hexanol, furan/2-*n*-pentylfuran and 2-octen-1-ol, but also the appearing of"earthy" odour and"mushroom" flavour corresponding to the alcohol 1-octen-3-ol (Fig. 2B).

The cooking step stand out the chickpea richness in"butter-y","fermented","floral" and"herbal" odours (Fig. 2C) and deducts from the initial aromatic matrix (dry and soaked) the unpleasant volatile compounds.

The chromatographic profile of the lupin dry seed (Fig. 2D) presented a wide variety of volatile compounds from "herbal" and "fruity" odours (both with"green" flavour), to"citrus" and"waxy". The"citrus" odour assigned to D-limonene, corresponded to the highest peak area (35.3%). After soaking (Fig. 2E), the lupin seeds evidenced two new volatile compounds, the *n*-hexanal with "green" odour and flavour, and the 1-hexanol with"herbal" odour and"green" flavour which presented the most relevant area percentage of 68,7%. Also, the previously found furan/ 2-*n*-pentylfuran ("fruity" odour and "green" flavour) in dry seeds, evidenced an increase of 38.4% after the soaking step.

After cooking, the lupin seeds (Fig. 2F) showed three new volatile compounds: the 1-butanol with "fermented" odour and "fruity" flavour which presented the most relevant area (59.9%), the D-limonene with "citrus" odour and flavour, and the 2-methyl-1-undecanol with "balsam" odour. There were two volatile compounds in common when comparing the chromatographic profiles of dry and cooked lupin seeds: the Benzoic acid (ester) ("minty" odour and "medicinal" flavour) which increase 115% after cooking, and the *n*-hexadecanoic acid (palmitic acid) ("waxy" odour and flavour) reduced by 20% after cooking.

The extinction of the "green"/"beany" flavour of the pulse seeds by the cooking step was validated successfully when comparing the chromatographic analysis of volatile compounds in dry or soaked pulses seeds. The hot water treatment efficiency was also evidenced in a previous study to reduce the "beany" flavour of soymilk (Lv, Song, Li, Wu, & Guo, 2011), where some of the volatile compounds also found in soaked chickpea and lupin seeds, such as *n*-hexanal, 1-octen-3-ol and *n*-hexanol, diminished after the hot water blanching and grinding processes.

3.2. *"Beany" flavour identification through sensory analysis of pulse beverages*

The majority of the panelists (>22 in 35) identified the presence of "beany" flavour in lupin beverage (91%) and in chickpea beverage (80%), selecting the cooked beverage as the only differences, as expected. This fact allowed to conclude that the cooked and uncooked samples were significantly different from each other ($p < 0.001$).

Table 1
Total oligosaccharide contents in chickpea and lupin seeds, in mg/100 g of dry matter.

α-GOS (mg/100 g d.m.)	Chickpea	Lupin
Raffinose	397	436
Stachyose	1470	5346
Verbascose	0	580
Total	1867	6362

3.3. *Flatulence sugars reduction through beverage processing*

To evaluate the impact of beverage processing steps on the amount α-oligosaccharides (α-GOS) in the final beverage, raffinose, stachyose and verbascose were identified and quantified first in the dry chickpea and lupin seeds, and then in the corresponding beverages by HPAEC-PAD.

Pulses raw seeds contained considerable amounts of total α-oligosaccharides (Table 1), ranging from 1867 mg/100 g of dry matter (d.m.) in chickpea to more than three time fold in lupin (6362 mg/100 g d.m.). The identified α-GOS were raffinose, verbascose and stachyose, where the last was undoubtedly predominant in chickpea and lupin seeds (1470 and 5346 mg/100 g d.m., respectively). Raffinose was present in moderate amounts in both seeds, as reported by a previous study (Reddy, Pierson, Sathe, & Salunkhe, 1984), and the highest quantities were detected in lupin (436 mg/100 g d.m.). As for verbascose, it was identified only in lupin seeds at moderate concentration (580 mg/100 g d.m.).

During soaking and cooking processes, oligosaccharides are diffused to water (Han, & Baik, 2006; Hefnawy, 2011), which is in agreement with the results obtained for chickpea and lupin beverages (Table 2).

The total α-GOS loss (expressed as percentage of the initial content) ranged from 4.40% in chickpea to 47.61% in lupin. According to Njoumi and co-workers, the α-GOS loss in chickpeas was expected to be higher (around 40%), which was probably due to the fact that soaking process, by itself, being enough to remove the α-GOS from chickpea seeds (Njoumi, Amiot, Rochette, Bellagha, & Mouquet-Rivier, 2019). During soaking, seeds absorb water, and raffinose, stachyose and verbascose, which are all water-soluble, leach out of the seeds into the soaking water (Han, & Baik, 2006; Coffigniez et al., 2018). In this case, we expect the majority α-GOS to be in the discharged water. However, α-GOS can also undergo through an enzymatic degradation into lower molecular weight sugars (Vidal-Valverde et al., 2002; Martinez-Villaluenga, Chicholoska, Kliber, & Gulewicz, 2008; Berrios, Morales, Cámara, & Sánchez-Mata, 2010). In lupin, for instance, soaking process was not probably enough to remove the α-GOS, since the major loss occurred only after cooking. Usually, cooking after soaking leads to the development of better conditions for the expression of endogenous α-galactosidase activity, responsible for the enzymatic degradation of seeds after leaching (Coffigniez et al., 2018), therefore, in lupin seeds, this enzymatic process could have more expression than the leaching.

The different behavior during the processing of beverages can be due to several natural aspects such as α-GOS spatial distribution in seeds, the germination conditions, maturation state of the seed, which influences not only the above mentioned α-GOS spatial distribution, but also the concentration of the α-GOS on the different cotyledons, embryonic axe and coat fractions of seeds (Sreerama, Neelam, Sashikala, & Pratape, 2010).

3.4. *Evaluation of the impact of beverages' fermentation conditions*

Pulse beverages can be directly consumed as alternatives to milk with nutritional composition advantages (Duarte et al., 2022), to diversify their use, the preparation of fermented products was tested. The benefits of human nutrition fermented products are well known,

Table 2
Total oligosaccharide contents in chickpea and lupin beverages, in mg/mL, and loss % after processing.

	α-GOS (mg/mL)		α-GOS loss (%)	
	Chickpea	Lupin	Chickpea	Lupin
Raffinose	0.025	0.158	7.06	41.57
Stachyose	0.049	2.180	3.70	46.78
Verbascose	0.000	0.303	0.00	59.83
Total	0.074	2.641	4.40	47.61

Table 3
pH, titrable acidity (mEq lactic acid/L) and lactic acid concentration (g/L) of fermented and non-fermented chickpea and lupin beverages.

Beverages	Lactic Acid (g/L)	pH	Titrable acidity (mEq lactic acid/L)
Non-fermented			
lupin	0	5.91 ± 0.000	0.03 ± 0.009
chickpea	0.31 ± 0.001	7.02 ± 0.000	0.02 ± 0.000
Fermented			
lupin: 40 °C, 10% S, 4 h	0.57 ± 0.047	5.07 ± 0.014	0.09 ± 0.000
chickpea: 40 °C, 10% S, 4 h	0.72 ± 0.022	5.34 ± 0.007	0.08 ± 0.000
chickpea: 40 °C, 10% S, 12 h	1.30 ± 0.020	4.82 ± 0.021	0.12 ± 0.000
chickpea: 23 °C, 10% S, 12 h	0.67 ± 0.012	5.34 ± 0.000	0.07 ± 0.009
chickpea: 30 °C, 5% S, 7 h	0.65 ± 0.018	5.38 ± 0.021	0.07 ± 0.009
chickpea: 50 °C, 5% S, 7 h	0.52 ± 0.013	5.76 ± 0.014	0.04 ± 0.009
chickpea: 50 °C, 15% S, 7 h	0.87 ± 0.058	5.17 ± 0.014	0.10 ± 0.091

including the enhanced sensory characteristics and benefits for the gut microbiome (Simões et al., 2022).

The yoghurt like development by the fermentation pulse beverages was based on optimal LAB fermentation conditions, testing several incubation temperatures, times and starter concentrations to assess if fermentation changed their structure and investigate further modifications on composition.

3.4.1. *Evaluation of fermentation process*

pH, titrable acidity and lactic acid concentration were evaluated before and after the fermentation process of lupin and chickpea beverages (Table 3).

There was a markedly increase in the lactic acid concentration when comparing non-fermented and fermented beverages, also resulting in an increase of titrable acidity and a concomitant decrease in pH values. This occur as a consequence of the lactic acid fermentation performed by viable LAB from the inoculated starter culture (1.14 x10^5 CFU/g). At the end of fermentation, all chickpea beverages presented viable LAB cells (from 1.95x10^3 to 3.09x10^5 CFU/g, depending on the incubation conditions). In lupin fermented beverage, despite the increase in lactic acid concentration revealing that fermentation occurred, no viable cells were detected in the final product. Chickpea fermented beverages showed higher lactic acid concentration than lupin (0.72 g/L vs 0.57 g/L, at 40 °C, 10% starter, 4 h), which is related to the higher starch content (1.39 g/100 mL vs 0.01 g/100 mL) and fermentable sugars of the non-fermented chickpea beverages (Duarte et al., 2022).

Incubation time had a marked effect in lactic acid content, varying between 0.72 g/L and 1.30 g/L when time increased from 4 h to 12 h at 40 °C and with 10% starter concentration, reaching a final pH value of 4.82 after 12 h. As expected, when incubation temperature decreased to suboptimal temperatures (from usual 40 °C to 23 °C), a considerable decrease of lactic acid was observed (1.30 g/L to 0.67 g/L) since LAB are termophilic bacteria. In a similar way, when increasing temperature from 30 °C to 50 °C, LAB loose viability (from 2.12x10^5 CFUs/g at 30 °C, 5% starter, 7 h, to 2.24x10^4 CFUs/g at 50 °C, 5% starter, 7 h), the fermentation rate decreases and the lactic acid concentration also decreases (0.65 g/L vs 0.52 g/L).

The overall results showed that fermentation of these pulse beverages allowed the development of a fermented functional food with expected positive health benefits. However, pH values higher than 4.5 can represent a drawback in terms of food safety. Therefore, a pasteurization step could be necessary, obtaining a final product enriched in *para*-probiotic and post-biotics (Cuevas-González et al., 2020).

3.4.2. *"Beany" flavour identification through sensory analysis in fermented beverages*

Sensory analysis was conducted to determine if fermentation promoted the reduction of the "beany" flavor in the lupin and chickpea beverages, in comparison to the non-fermented ones. 81% of the panelists detected a decrease of "beany" flavor with fermentation of the lupin beverage at 40 °C, 10% starter and 4 h. Chickpea beverages fermented at 40 °C, 10% starter, 12 h and at 50 °C, 15% starter, 7 h were compared with the non-fermented beverage. A decrease in "beany" flavor was pointed by 88% of the panelists when using 50 °C, 15% starter, 7 h, but at 40 °C, 10% starter, 12 h only 31% detected this reduction. It is probably due to a markedly decrease in the pH (4.82) and lactic acid production when a longer incubation time was applied, interfering in the detection of the "beany" flavors.

3.4.3. *Rheology of fermented beverages*

The incubation temperature effect was detected through gelification of chickpea beverage resulting from the acid coagulation of globular proteins naturally present (Peng, Ren & Guo, 2016), as a consequence of LAB activity. Temperature provide enhanced rheology as a higher degree of gel structure of the fermented chickpea beverage was observed when the temperature of incubation was 50 °C and the less structured gel was obtained at 23 °C (Fig. 3).

The trial at 40 °C had viscoelastic values ($G'_{0.1Hz}$ 52.1 Pa (20 °C) and 51.5 Pa (8 °C)) between the ones fermented at 23 °C and 50 °C (23 °C: 28.6 Pa (20 °C) and 31.9 Pa (8 °C); 50 °C: $G'_{0.1Hz}$ 130.2 Pa (20 °C) and 80.4 Pa (8 °C)) (Fig. 3), which may be explained by the fact that lactic acid bacteria such as *S. thermophilus* and *L. bulgaricus* have an optimal growth temperature between 39 and 44 °C, and between 40 and 46 °C, respectively (Béal, & Helinck, 2019). The incubation temperature affects the rates of chemical and biochemical reactions, and bacterial membrane permeability (Béal, & Helinck, 2019) during the fermentation, allowing a more extensive protein acid coagulation for fermented beverages incubated at 50 °C.

Besides the role of the protein gelled network, the structural reinforcement at 50 °C of incubation may be related to the impact of temperature on the starch granules, since starch properties are largely affected by hydrothermal treatments (Liu et al., 2022). The pulse beverages developed by Lopes and co-workers were submitted to cooking and pasteurization steps as explained previously (Fig. 1) (Lopes et al., 2020), which lead to starch gelatinization and subsequent retrogradation during cooling and storage at 8 °C, before the fermentation. When preparing the fermented beverages, further incubation at high temperatures (50 °C) allowed the reorganization of the starch molecules leading to a firmer structure (Liu et al., 2022). Wang and co-workers also developed fresh and fermented plant-based beverages made with chickpeas and evaluated its composition, fermentability and acceptability. They reported a starch retrogradation upon cooling, leading to syneresis (expulsion of water due to contraction of the gel matrix) (Wang, Chelikani, & Serventi, 2018).

The enhanced structure with temperature of incubation in the fermented chickpea beverages, was also reflected on its higher zero-shear viscosity (η_0) values as shown in Fig. 3C-D. The flow curves of all fermented pulse beverages represent a non-Newtonian shear-thinning behavior as apparent viscosity decreases with increasing shear rates (Fig. 3C and Fig. 4C). This may be explained by the conformational changes of the protein clusters due to the shear stress between the flowing layers, reducing the binding interactions and resulting in shear thinning (Lee & Lucey, 2006).

The incubation time effect on fermented chickpea beverages, at the same temperature and starter concentration, of 4 h, evidenced a less structured gel ($G'_{0.1Hz}$ 29.0 Pa (8 °C)) when compared to 12 h of fermentation at 40 °C ($G'_{0.1Hz}$ 51.5 Pa (8 °C)) (Fig. 4A-B). The same expected trend was found for incubations at 50 °C (with the same starter

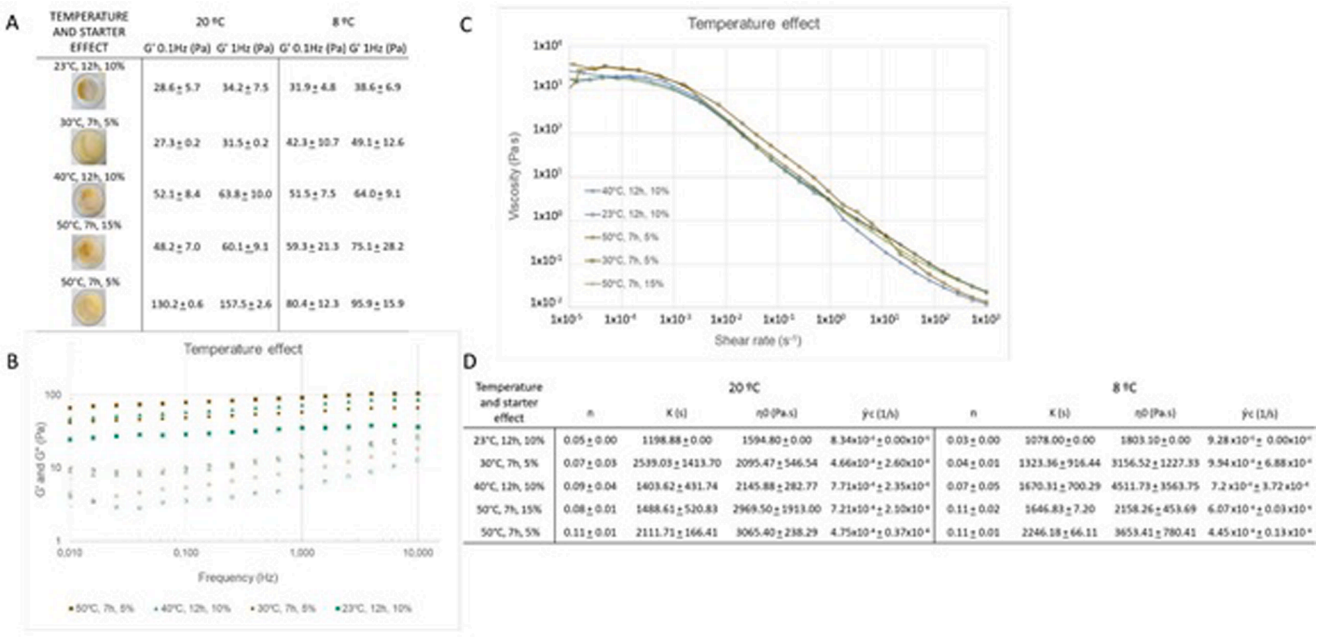

Fig. 3. The effect of temperature on (A) storage modulus Ǵ' at 0.1 and 1 Hz measured with the Haake Mars III Thermo scientific rheometer at 20 °C and 8 °C, and respective fermented chickpea beverages illustrations (B) mechanical spectra (G' (full signs) and G'' (lost modulus - empty signs)) at 8 °C. (C) Flow curves of the fermented chickpea beverages with the same incubation time and starter concentration measured at 8 °C with the Haake Mars III Thermo scientific rheometer. (D) Parameters obtained after fitting the flow curves to the Carreau model (n – rate index; K - consistency; η_0 - zero-shear viscosity; and $\dot{\gamma}$ c - critical shear rate), at 20 °C and 8 °C. The results are presented as mean ± SD (n = 3).

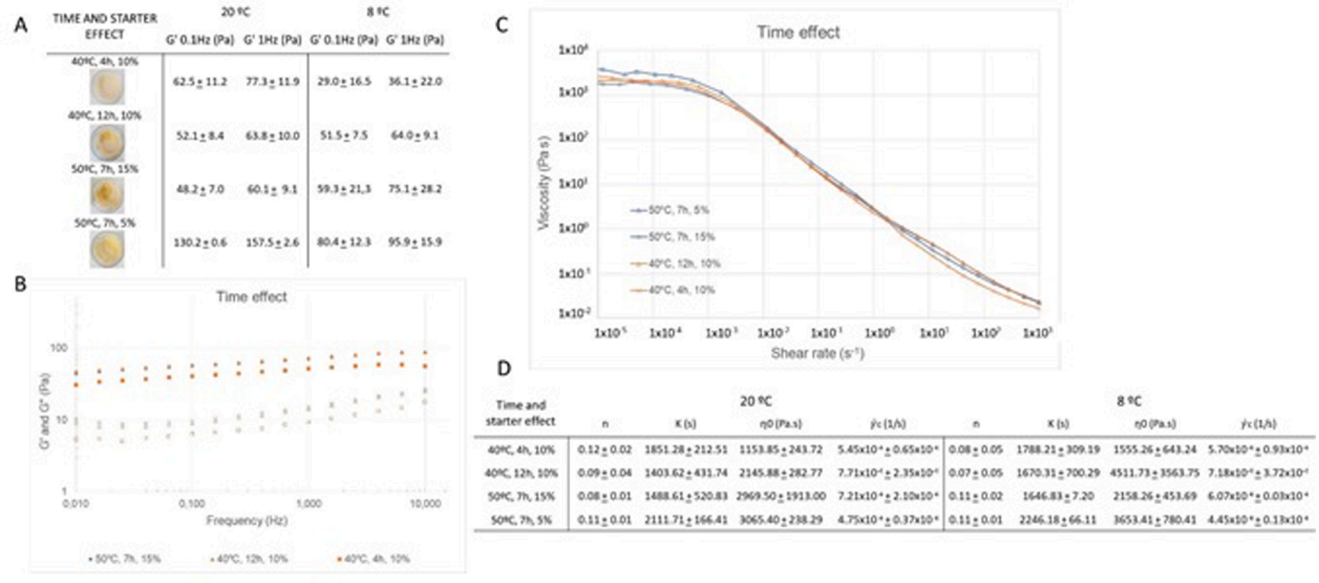

Fig. 4. The effect of incubation time on (A) storage modulus G' at 0.1 and 1 Hz measured with the Haake Mars III Thermo scientific rheometer at 20 °C and 8 °C, and respective fermented chickpea beverages illustrations (B) mechanical spectra (G' (full signs) and G'' (lost modulus - empty signs)) at 8 °C. (C) Flow curves of the fermented chickpea beverages with the same incubation temperature and starter concentration measured at 8 °C with the Haake Mars III Thermo scientific rheometer. (D) Parameters obtained after fitting the flow curves to the Carreau model (n – rate index; K - consistency; η_0 - zero-shear viscosity; and $\dot{\gamma}$ c - critical shear rate), at 20 °C and 8 °C. The results are presented as mean ± SD (n = 3).

concentration of 5% or 15% and over 12 h of incubation - data not shown). The longer incubation times allowed to achieve more structured gels with higher $G'_{0.1Hz}$ values. Also, the zero-shear viscosity (η_0) values were higher when the incubation time was extended, confirming the gel structuring with time as shown in Fig. 4C-D. In line with increasing of the storage modulus (Ǵ') with incubation time, the flow measurements revealed an increase of zero-shear viscosity when time increase from 4 h to 12 h of incubation at 40 °C (η_0 2145.88 Pa.s (20 °C) and 4511.73 Pa.s (8 °C) at 12 h).

Usually, the initial concentration of starter strongly influences beverage fermentation, so the higher it is, the faster the fermentation (Béal, & Helinck, 2019). During the fermentation, the pH decreases, and acidification occurs, so the isoelectric point of chickpea proteins (pH 4.0–5.0) is reached and proteins lack a net charge, thus the repulsions

are null between them and they can associate to form a network (Kaur & Singh, 2007). The cooling step helped the gel to set with a higher intermolecular bridge forming. On the other hand, the acidification may affect starch reorganization during the incubation, and subsequent cooling, since higher inoculum level causes higher activity during fermentation. This was shown in our results at 40 °C and 12 h of incubation with $G'_{0.1Hz}$ (8 °C) values of 71 Pa, 51 Pa and 42 Pa, and with zero-shear viscosity (η_0) values (8 °C) of 6265 Pa.s, 4512 Pa.s and 1870 Pa.s, for 2%, 12% and 18% of starter concentration respectively (results not shown). From Figs. 3 and 4 it is possible to confirm a reduction in Ǵ' and η_0 values with more starter concentration when comparing the trials 50 °C, 7 h, 5% vs 50 °C, 7 h, 15%, with a higher lactic acid concentration for 15% starter (Table 3, 0.87 g/L vs 0.52 g/L).

Chickpea beverage is a mixed protein-polysaccharide system, where interactions between starches and proteins may be more important than the physicochemical properties of the individual components. Such interactions are affected by the intrinsic nature of the polymers, protein/starch ratio, temperature, pH, and ionic strength (Nunes, Raymundo, & Sousa, 2006; Liu et al., 2022). Therefore, some of the existing differences between gel properties are due to the changed kinetics of gel formation depending on all these factors. From our results, one can see that pH decrease has an important effect on starch-protein interactions. As reported by Bravo-Núñez and co-workers, acidic pH decreased the apparent peak viscosity of starch–protein (pea, rice and whey) mixtures during heating and cooling (Bravo-Núñez, Garzón, Rosell, & Gómez, 2019). According to these authors, the influence of pH on starch pasting properties is higher than its influence on proteins.

The optimal conditions for pulse beverage fermentation can be obtained using commercially available vegetable-based yoghurt-like characteristics as targets, such as soy yoghurt (Fig. 5). This will help to set the adequate processing conditions to produce a fermented pulse beverage with physical properties close to the chosen target. For this comparison, the lupin beverage fermented at 40 °C (4 h, 10% starter) is presented. Different from chickpea, the lupin beverage did not show a structural reduction for long incubation times, neither an increase in the gel structure with temperature increase (from 40 °C to 50 °C – data not shown) since lupin beverage has an extremely low starch content (Lopes et al., 2020).

The structure of fermented chickpea beverages produced at incubation temperatures of 40 °C can be approximated to soy yoghurt with adjustments of the incubation times and starter concentration, becoming more appealing to the consumer's (Fig. 5). For equal fermentation conditions (40 °C, 4 h, 10%), lupin gel (Fig. 5, bars in brown) presented a higher structure degree, due to its higher protein content of 4.05 g/100 mL when compared to chickpea gels with 3.24 g/100 mL (Lopes et al., 2020), as less protein can be associated with lower gel development and viscosity (Fig. 5). Wang and co-workers also showed that fermented chickpea beverage evidences a weaker protein network compared to fermented soymilk (Wang, Chelikani, & Serventi, 2018), which is consistent with the lower protein content of chickpea seed compared to soybean. Since lupin beverage is poor in starch, it is probable that LAB synthesized more exopolysaccharides (EPS) with lupin proteins, which can increase the viscosity of the final product (Khurana & Kanawjia, 2007) through higher elasticity and more resistance to stirring

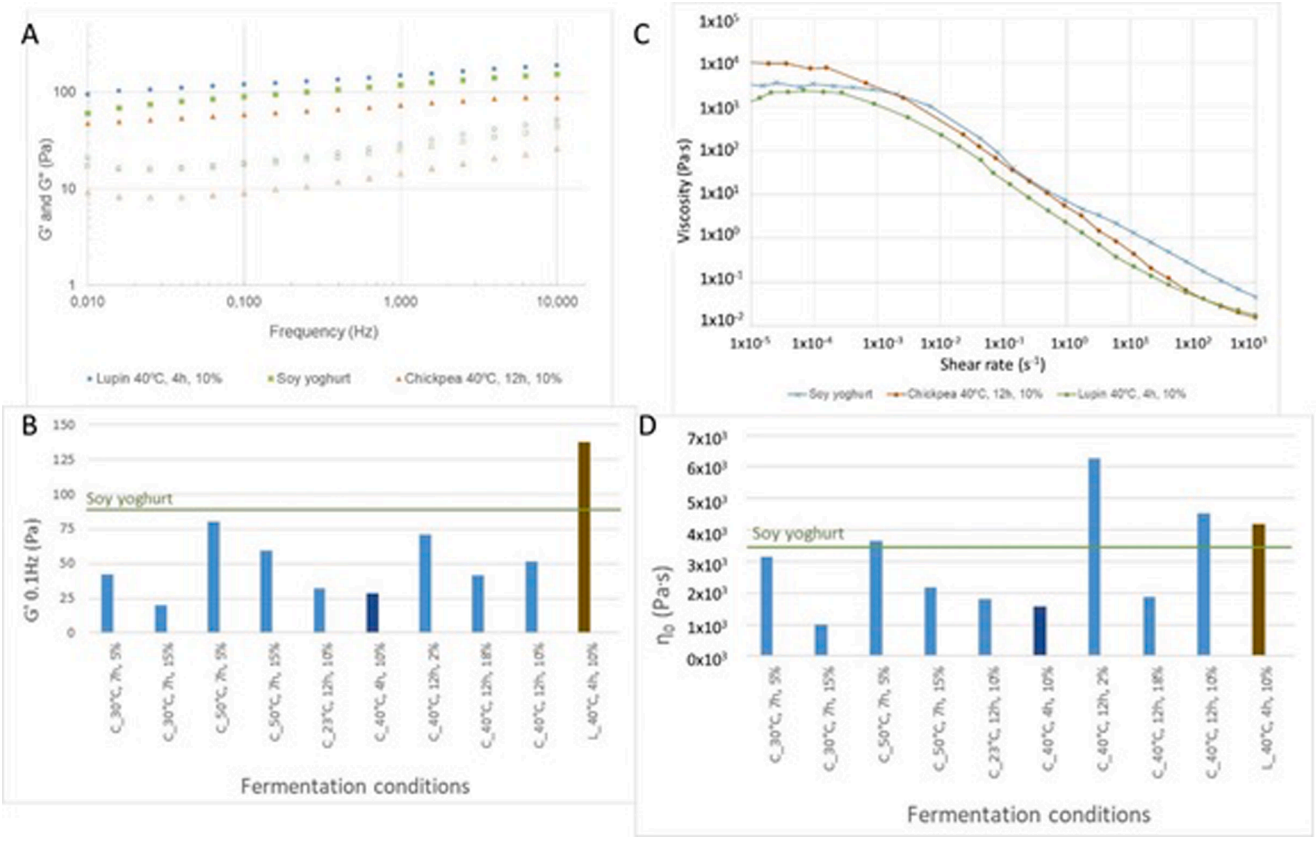

Fig. 5. (A) Mechanical spectra at 8 °C for commercial soy yoghurt, fermented chickpea beverage incubated at 40 °C, 12 h, 10% starter, and the fermented lupin beverage. (B) Comparison of respective average values obtained for $G'_{0.1Hz}$ between all fermented chickpea beverages and the fermented lupin beverage with soy yoghurt (89 Pa) (green line). (C – chickpea; L – lupin; dark blue and brown bars are the same fermentation conditions for chickpea and lupin, respectively). (C) Flow curves at 8 °C showing the shear viscosity evolution for a commercial soy yoghurt, fermented chickpea beverage incubated at 40 °C, 12 h, 10% (w/v) starter, and the fermented lupin beverage. (D) Comparison of respective average values obtained from zero-shear viscosity between all fermented chickpea beverages and the fermented lupin beverage, with soy yoghurt (3381 Pa.s) (green line). (C – chickpea; L – lupin; dark blue and brown bars are the same fermentation conditions for chickpea and lupin, respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Folkenberg, Dejmek, Skriver, & Ipsen, 2005), or the EPS-lupin protein interactions were stronger, when compared to the chickpea proteins.

The comparison between fermented chickpea and lupin gels also evidenced that whatever the incubation time (4 h or over 12 h – data not shown), the lupin gel presented a stronger structure (G') than chickpea's (Fig. 5). Indeed, according to a study about the least gelation concentration (LGC) (Kaur & Singh, 2007), a lower LGC corresponds to a better gelling ability of the proteins. So, the LGC of soy protein found ranged between 16 and 17%, for chickpea protein was between 14 and 18%, which allowed to form a firm and resistant gel, but the lupin protein LGC of 14% were even better than soy (Kaur & Singh, 2007), as evidenced in the fermented lupin structure.

4. Conclusions

The extinction of the "green"/"beany" flavour of pulse seeds by cooking has been clearly confirmed by the chromatographic analysis of volatile compounds in uncooked pulses. In addition, other pleasant odours and the unpleasant volatile compounds in the original aromatic matrix have been eliminated by cooking, as evidenced by chromatographic methods.

In general, soaking and cooking processes were effective in reducing α-oligosaccharides, the so-called flatulence sugars, in chickpea and lupin, however the α-GOS loss is clearly higher in lupin processed beverages.

This study also showed that lactic acid fermentation of chickpea and lupin beverages is an interesting alternative to obtain added-value products with a gelled structure, which are promising alternatives to soy "yoghurt". Chickpea beverage has a high starch content and a structured gel could be obtained, although protein-polysaccharide interactions in this mixed system are highly dependent on the pH. Lupin fermented beverages are richer in protein presenting an enhanced structure. Rheology of fermented pulses is greatly affected by fermentation conditions and the selection of the fermentation parameters is required to achieve gel structure and apparent viscosity values similar to the commercial targets. It was shown that different temperature–time-starter combinations are possible, its selection will also depend on industrial costs. Further work will be necessary to evaluate these gels on the basis of sensory characterization and consumer acceptance.

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None.

6. Ethics statement**a

Not applied.

CRediT authorship contribution statement

C.M. Duarte: Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **M.C. Nunes:** Validation, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **P. Gojard:** Investigation, Writing - original draft. **C. Dias:** Investigation, Writing - original draft. **J. Ferreira:** Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **C. Prista:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **P. Noronha:** Methodology, Writing – review & editing, Supervision. **I. Sousa:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Barman, A., Marak, C. M., Barman, R., & Sangma, C. S. (2018). Chapter 1: Legume seed nutraceutical research. In J.C. Jimenez-Lopez & A. Clemente (Eds), *Nutraceutical properties of legume seeds and their impact on human health* (pp. 1-22). IntechOpen. 10.5772/intechopen.78799.

Barnes, H. A. (2009) Chapter 9: Shear-thinning liquids. In Institute of Non-Newtonian Fluid Mechanics, Department of Mathematics, University of Wales: Aberystwyth, UK (Eds), *Handbook of Elementary Rheology* (pp. 55–61) (1st ed.).

Béal, C., & Helinck, S. (2019). Fabrication des yaourts et des laits fermentés. *Techiques de l' Ingénieur.* Bioprocédés. F6315 v2. 1-23. https://www.techniques-ingenieur.fr/base-documentaire/procedes-chimie-bio-agro-th2/filiere-de-production-produits-d-origine-animale-42432210/fabrication-des-yaourts-et-des-laits-fermentes-f6315/definitions-et-reglementation-f6315v2niv10001.html.

Bernat, N., Cháfer, M., Chiralt, A., & Gonzalez-Martínez, C. (2014). Vegetable milks and their fermented derivative products. *International Journal of Food Studies., 3*, 93–124. https://doi.org/10.7455/ijfs/3.1.2014.a9

Berrios, J., Morales, P., Cámara, M., & Sánchez-Mata, M. C. (2010). Carbohydrate composition of raw and extruded pulse flours. *Food Research International, 43*, 531–536. https://doi.org/10.1016/j.foodres.2009.09.035

Bravo-Núñez, A., Garzón, R., Rosell, C. M., & Gómez, M. (2019). Evaluation of starch–protein interactions as a function of pH. *Foods, 8*, 155–165. https://doi.org/10.3390/foods8050155

Brevard, H., Cantergiani, E., Cachet, T., Chaintreau, A., Demyttenaere, J., French, L., … Sherlock, A. (2011). Guidelines for the quantitative gas chromatography of volatile flavouring substances, from the working group on methods of analysis of the international organization of the flavour industry (IOFI). *Flavour and Fragrance Journal, 26*, 297–299.

Carrão-Panizzi, M. C., Beléia, A. P., Prudêncio-Ferreira, S. H., Oliveira, M. C. N., & Kitamura, K. (1999). Effects of isoflavones on beany flavour and astringency of soymilk and cooked whole soybean grains. *Pesquisa Agropecuária Brasileira, 34*, 1045–1052. https://doi.org/10.1590/S0100-204X1999000600017

Chen, X., Singh, M., Bhargava, K., & Ramanathan, R. (2018). Yogurt Fortification with Chickpea (*Cicer arietinum*) Flour: Physicochemical and Sensory Effects. *Journal of the American Oil Chemists' Society, 95*, 1041–1048. https://doi.org/10.1002/aocs.12102

Coffigniez, F., Briffaz, A., Mestres, C., Alter, P., Durand, N., & Bohuon, P. (2018). Multi-response modeling of reaction-diffusion to explain alpha-galactoside behavior during the soaking-cooking process in cowpea. *Food Chemistry, 242*, 279–287. https://doi.org/10.1016/j.foodchem.2017.09.057

Cuevas-González, P. F., Liceaga, A. M., & Aguilar-Toalá, J. E. (2020). Postbiotics and paraprobiotics: From concepts to applications. *Food Research International, 136*, Article 109502. https://doi.org/10.1016/j.foodres.2020.109502

Donkor, O., Henriksson, A., Vasiljevic, T., & Shah, N. P. (2007). α-Galactosidase and proteolytic activities of selected probiotic and dairy cultures in fermented soymilk. *Food Chemistry, 104*, 10–20. https://doi.org/10.1016/j.foodchem.2006.10.065

Duarte, C. M., Mota, J., Assunção, R., Martins, C., Ribeiro, A. C., Lima, A. I., … Sousa, I. (2022). New alternatives to milk from pulses: Chickpea and lupin beverages with improved digestibility and potential bioactivities for human health. Frontiers. *Nutrition.* Accepted for publication on June 8th, 2022 - in press.

El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum L.*) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition, 57*, 83–97. https://doi.org/10.1023/a:1013189620528

Favaro-Trindade, C., Terzi, S., Trugo, L., Modesta, R., & Couri, S. (2001). Development and sensory evaluation of soymilk based yogurt. *Archivos Latinoamericanos de Nutrición, 51*, 100–104.

Folkenberg, D. M., Dejmek, P., Skriver, A., & Ipsen, R. (2005). Relation between sensory, texture properties and exopolysaccharide distribution in set and in stirred yoghurts produced with different starter cultures. *Journal of Texture Studies, 36*, 174–189. https://doi.org/10.1111/j.1745-4603.2005.00010.x

Gdala, J. (1998). Composition, properties, and nutritive value of dietary fibre of legume seeds. A review. *Journal of Animal and Feed Sciences, 7*, 131–149. 10.22358/jafs/69204/1998.

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. *Food and Agriculture Organization of the United Nations (FAO)*, Rome. Retrieved from http://www.fao.org/3/a-i3437e.pdf. Accessed March 22, 2019.

Han, H., & Baik, B.-K. (2006). Oligosaccharide Content and Composition of Legumes and Their Reduction by Soaking, Cooking, Ultrasound, and High Hydrostatic Pressure. *Cereal Chemitry, 83*, 428–433. https://doi.org/10.1094/CC-83-0428

Hefnawy, T. (2011). Effect of Processing Methods on Nutritional Composition and Anti-Nutritional Factors in Lentils (*Lens Culinaris*). *Annals of Agricultural Sciences, 56*, 57–61. https://doi.org/10.1016/j.aoas.2011.07.001

International Organization of Vine and Wine. Type I methods: Total Acidity (OIV-MA-AS313-01: R2015). In Compendium of International Methods of Wine and Must Analysis; IOVW: Paris, France, 2019; Volume I.

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): A review. *British Journal of Nutrition, 108*, S11–S26. https://doi.org/10.1017/S0007114512000797

Kadlec, P., Bjergegaard, C., Gulewicz, K., Horbowicz, M., Jones, A., Kintia, P., … Urban, J. (2000). Carbohydrate chemistry. In C. L. Hedley (Ed.), *Carbohydrates in grain legume seeds: improving nutritional quality and agronomic characteristics* (pp. 15–59). Wallingford: CAB International.

Kaur, M., & Singh, N. (2007). Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. *Food Chemistry, 102*, 366–374. https://doi.org/10.1016/j.foodchem.2006.05.029

Khurana, H., & Kanawjia, S. (2007). Recent trends in development of fermented milks. *Current Nutrition and Food Science, 3*, 91–108. https://doi.org/10.2174/157340131070310091

Lima, A. I. G., Mota, J., Monteiro, S. A. V. S., & Ferreira, R. M. S. B. (2016). Legume seeds and colorectal cancer revisited: Protease inhibitors reduce MMP-9 activity and colon cancer cell migration. *Food Chemistry, 197*, 30–38. https://doi.org/10.1016/j.foodchem.2015.10.063

Liu, X., Huang, S., Chao, C., Yu, J., Copeland, L., & Wang, S. (2022). Changes of starch during thermal processing of foods: Current status and future directions. *Trends in Food Science & Technology, 119*, 320–337. https://doi.org/10.1016/j.tifs.2021.12.011

Lee, W.-J., & Lucey, J. A. (2006). Impact of gelation conditions and structural breakdown on the physical and sensory properties of stirred yogurts. *Journal of Dairy Science, 89*, 2374–2385. https://doi.org/10.3168/jds.S0022-0302(06)72310-4

Lopes, M., Pierrepont, C., Duarte, C. M., Filipe, A., Medronho, B., & Sousa, I. (2020). Legume beverages from chickpea and lupin, as new milk alternatives. *Foods, 9*, 1458–1474. https://doi.org/10.3390/foods9101458

Lv, Y.-C., Song, H.-L., Li, X., Wu, L., & Guo, S.-T. (2011). Influence of blanching and grinding process with hot water on beany and non-beany flavour in soymilk. *Journal of Food Science, 76*. https://doi.org/10.1111/j.1750-3841.2010.01947.x. S20–S25.

Ma, L., Li, B., Han, F., Yan, S., Wang, L., & Sun, J. (2015). Evaluation of the chemical quality traits of soybean seeds, as related to sensory attributes of soymilk. *Food Chemistry, 173*, 694–701. https://doi.org/10.1016/j.foodchem.2014.10.096

Makinen, O. E., Wanhalinna, V., Zannini, E., & Arendt, E. K. (2016). Foods for special dietary needs: Non-dairy plant-based milk substitutes and fermented dairy-type products. *Critical Reviews in Food Science and Nutrition, 56*, 339–349. https://doi.org/10.1080/10408398.2012.761950

Marco, M. L., Sanders, M. E., Gänzle, M., Arrieta, M. C., Cotter, P. D., De Vuyst, L., … Hutkins, R. (2021). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. *Nature Reviews Gastroenterology & Hepatology, 18*, 196–208. https://doi.org/10.1038/s41575-020-00390-5

Market and Markets. (2019). Retrieved from https://www.marketsandmarkets.com/Market-Reports/dairy-alternative-plant-milk-beverages-market-677.html. Accessed February 28, 2019.

Martinez-Villaluenga, C., Chicholoska, J., Kliber, A., & Gulewicz, K. (2008). Raffinose family oligosaccharides of lupin (*Lupinus albus* L. cv multolupa) as a potential prebiotic. *Proceedings of the Nutrition Society, 67*(OCE1), E55. https://doi.org/10.1017/S0029665108006642

Mesquita, M. C., Leandro, E. dos S., de Alencar, E. R., & Botelho, R. B. A. (2020). Fermentation of chickpea (*Cicer arietinum* L.) and coconut (*Coccus nucifera* L.) beverages by *Lactobacillus paracasei* subsp *paracasei* LBC 81: The influence of sugar content on growth and stability during storage. *LWT – Food Science and Technology, 132*, 109834. 10.1016/j.lwt.2020.109834.

Mintel (2018). Food and Drink. Consumer Market Research Report. Retrieved from https://www.mintel.com/press-centre/food-and-drink/us-non-dairy-milk-sales-grow-61-over-the-last-five-years. Accessed March 19, 2019.

Mota, J., Direito, R., Rocha, J., Fernandes, J., Sepodes, B., Figueira, M. E., … Ferreira, R. B. (2021). *Lupinus albus* protein components inhibit MMP-2 and MMP-9 gelatinolytic activity *in vitro* and *in vivo*. *International Journal of Molecular Sciences, 22*, 13286–13302. https://doi.org/10.3390/ijms222413286

Njoumi, S., Amiot, M. J., Rochette, I., Bellagha, S., & Mouquet-Rivier, C. (2019). Soaking and cooking modify the alpha-galacto-oligosaccharide and dietary fibre content in five Mediterranean legumes. *International Journal of Food Sciences and Nutrition, 70*, 1–11. https://doi.org/10.1080/09637486.2018.1544229

Nunes, M. C., Raymundo, A., & Sousa, I. (2006). Gelled vegetable desserts containing pea protein, κ-carrageenan and starch. *European Food Research and Technology, 222*, 622–628. https://doi.org/10.1007/s00217-005-0170-3

Murat, C., Bard, M.-H., Dhalleine, C., & Cayot, N. (2013). Characterisation of odour active compounds along extraction process from pea flour to pea protein extract. *Food Research International, 53*, 31–41. https://doi.org/10.1016/j.foodres.2013.03.049

Onyenekwe, P., Njoku, G., & Ameh, D. (2000). Effect of cowpea (*Vigna unguiculata*) processing methods on flatus causing oligosaccharides. *Nutrition Research, 20*, 349–358. https://doi.org/10.1016/S0271-5317(00)00128-7

Paseephol, T., Small, D., & Sherkat, F. (2008). Rheology and texture of set yogurt as affected by inulin addition. *Journal of Texture Studies, 39*, 617–634. https://doi.org/10.1111/j.1745-4603.2008.00161.x

Pedrosa, M. M., Cuadrado, C., Burbano, C., Allaf, K., Haddad, J., Gelencsér, E., … Muzquiz, M. (2012). Effect of instant controlled pressure drop on the oligosaccharides, inositol phosphates, trypsin inhibitors and lectins contents of different legumes. *Food Chemistry, 131*, 862–868.

Peng, X., Ren, C., & Guo, S. (2016). Particle formation and gelation of soymilk: Effect of heat. *Trends in Food Science and Technology, 54*, 138–147. https://doi.org/10.1016/j.tifs.2016.06.005

Raymundo, A., Batista, A., & Sousa, I. (2017). Rheology applied to food product design. In M. D. T. Pérez (Ed.), *Advances in Rheology Research* (pp. 63–118). Nova Science Publishers Inc.

Reddy, N. R., Pierson, M. D., Sathe, S. K., Salunkhe, D. K., & Beuchat, L. R. (1983). Legume-based fermented foods: Their preparation and nutritional quality. *C R C Critical Reviews in Food Science and Nutrition, 17*, 335–370. https://doi.org/10.1080/10408398209527353

Reddy, N. R., Pierson, M. D., Sathe, S. K., & Salunkhe, D. K. (1984). Chemical, nutritional and physiological aspects of dry bean carbohydrates - A review. *Food Chemistry, 13*, 25–68. https://doi.org/10.1016/0308-8146(84)90026-8

Ribeiro, A. C., Teixeira, A. R., & Ferreira, R. B. (2004). Characterization of globulins from common vetch (*Vicia Sativa* L.). *Journal of Agricultural Food Chemistry, 52*, 4913–4920. https://doi.org/10.1021/jf049833p

Simões, S., Santos, R., Bento-Silva, A., Santos, M. V., Mota, M., Duarte, N., … Prista, C. (2022). Improving nutritional quality of unripe tomato through fermentation by a consortium of yeast and lactic acid bacteria. *Journal of the Science of Food and Agriculture, 102*, 1422–1429. https://doi.org/10.1002/jsfa.11476

Sreerama, Y. N., Neelam, D. A., Sashikala, V. B., & Prata pe, V.M. (2010). Distribution of nutrients and antinutrients in milled fractions of chickpea and horse gram: seed coat phenolics and their distinct modes of enzyme inhibition. *Journal of Agricultural and Food Chemistry, 58*, 4322–4330. 10.1021/jf903101k.

van Gemert, L. J. (2011). *Odour Thresholds: Compilations of Odour Threshold Values in Air, Water and Other Media (second enlarged and* (revised edition). Utrecht: Oliemans Punter & Partners B.V.

Vidal-Valverde, C., Frias, J., Sierra, I., Blazquez, I., Lambein, F., & Kuo, Y.-H. (2002). New functional legume foods by germination: Effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology, 215*, 472–477. https://doi.org/10.1007/s00217-002-0602-2

Wang, S., Chelikani, V., & Serventi, L. (2018). Evaluation of chickpea as alternative to soy in plant-based beverages, fresh and fermented. *LWT –. Food Science and Technology, 97*, 570–572. https://doi.org/10.1016/j.lwt.2018.07.067

Waters, D. M., Mauch, A., Coffey, A., Arendt, E. K., & Zannini, E. (2014). Lactic acid bacteria as a cell factory for the delivery of functional biomolecules and ingredients in cereal based beverages: A review. *Critical Reviews in Food Science and Nutrition, 55*, 503–520. https://doi.org/10.1080/10408398.2012.660251

Yang, A., Smyth, H., Chaliha, M., & James, A. (2016). Sensory quality of soymilk and tofu from soybeans lacking lipoxygenases. *Food Science & Nutrition, 4*, 207–215. https://doi.org/10.1002/fsn3.274