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ORIGINAL ARTICLE

The effect of different pre-treatments on unformulated pulse-based milk analogs: physicochemical properties and consumer acceptance

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Abstract This is the first part of a study on developing pulse-based milk analogs using chickpea, faba bean, and cowpea as raw materials. The objectives of the present study were to determine the processing conditions for pulse-based milk analog production at laboratory-scale and to investigate the effects of some pre-treatments such as dry milling (control), soaking and wet milling, blanching, blanching and dehulling, vacuum, and germination on lipoxygenase (LOX) activity of the raw material and some physicochemical and sensory properties of the final products. Dry milling provided the lowest LOX activity and the highest yield while soaking and wet milling resulted in a substantial increase in LOX activity, lower product yield, and a final product with lower whiteness value, regardless of the pulse type. Germination caused a significant decrease in LOX activity in all pulse types, while milk analogs produced from germinated pulses received the lowest acceptability scores from consumers.

Keywords Milk analogs · Plant-based milk alternatives · Pulses · Legumes · Germination · Lipoxygenase activity · Consumer acceptance

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Introduction

The interest in plant-based products has remarkably increased in recent years with the clear recognition that animal products play a crucial role in the release of greenhouse gases that cause global warming. It is estimated that animal products are responsible for 56–58% of food-related emissions while disproportionally providing only 18% of the calories and 37% of the protein from food (Poore and Nemecek 2018; Tamburino et. al. 2020). Therefore, in recent years, food industry has produced plant-based analogs of animal products and re-presented the familiar products to consumer in a more sustainable context. One of these novel animal-analog food products is plant-based milk analogs which are colloidal suspensions of plant material.

Considerable number of people prefer plant-based milk analogs over dairy milk for a wide variety of reasons such as cow's milk allergy, lactose intolerance, hypercholesterolemia, hormones and antibiotic residues, vegan/vegetarian diet, animal welfare, and environmental concerns namely extensive land use, GHG emissions, and water footprint (Mäkinen et. al, 2016). On the other side, animal milk is known as nature's most complete food (Park 2009). Milk and dairy products are nutrient-dense foods

which supply high-quality protein and a range of essential micronutrients, especially vitamin D and calcium. Although plant-based milk analogs resemble cow's milk in terms of appearance and consistency, they have completely different dynamics in terms of nutritive and sensory properties. Besides, the production process is very challenging and unique to the plant material.

In general, milk analogs are nutritionally poor, technologically unstable and sensorially unpleasant (Chalupa-Krebzdak et. al. 2018; Jeske et. al. 2017). Pulses come forward as raw materials for the production of plant-based milk analogs since they have high protein content and low environmental impact. However, off-flavors characterized as beany-like flavor limit the use of pulses in food applications.

The main objectives of this study were to develop pulse-based milk analogs using 3 non-soy pulses namely chickpea, faba bean, and cowpea as raw materials, to optimize the production steps at laboratory-scale, and to investigate the effects of various pre-treatments such as dry milling (control), soaking and wet milling, blanching, blanching and dehulling, vacuum, and germination on some physicochemical properties and consumer acceptance of the final products.

Materials and methods

Material

Chickpea (*Cicer arietinum* L.), cowpea (black-eyed pea) (*Vigna unguiculata* L.), faba bean (broad bean) (*Vicia faba* L.) seeds were purchased from different retail stores and same types of the samples were combined to better represent the population.

Pre-treatments

Dry milling (Control)

Pulse seeds (~ 10% of moisture content) were ground in dry form with a laboratory type mill (Karaerler Makina, Ankara, Turkey) and sieved through 300 µm sieve.

Soaking

The dry seeds were soaked in distilled water (1:3 w/v) at 25 °C for 24 h. Soaking water was discarded and hydrated seeds were wet milled firstly with a Waring blender for 2 min and then with a knife mill (Retsch/GM 200, Haan, Germany) for 3 more min with fresh water.

Blanching

The dry seeds were directly soaked in boiling water for 30 – 300 s and immediately after in an ice-water for 2 min. Optimum blanching time was determined according to the lipoxygenase (LOX) activity inhibition for each pulse type.

Blanching and dehulling

The dry seeds were soaked in boiling water for the pre-determined optimum time and cooled in ice-water. Then, the seeds were soaked in alkaline water (arranged to pH 9 with sodium carbonate) (Gao et. al. 2020) for 1 h to loosen the seed coat and dehulled manually.

Vacuum

The dry milled seed flours hydrated by mixing with fresh distilled water at room temperature for 30 min on a magnetic stirrer with constant mixing. Then the suspension was treated with constant vacuum (0.08 MPa) using rotary evaporator at 50 °C for 30 min (with 50 rpm rotation).

Germination

The seeds were steeped in tap water containing 1% sodium hypochlorite (NaOCl, v/v) for 30 min. Then, the seeds were washed under tap water for about 10 min to remove the NaOCl and soaked in distilled water 1:5 (w/v) for 24 h for hydration. Hydrated seeds were transferred to clean trays lined with wet cheesecloth and germinated in a climate cabinet (Nüve, TK 120, Ankara, Turkey) at 25 °C and 95% relative humidity. In order to keep the samples moist during germination, distilled water was given at every 12–18 h. The Cereals and Grains Association stated that germinated or sprouted grains can be considered as whole grains as long as sprout growth does not exceed the kernel length and nutrient values have not diminished (Anonymous 2020). Therefore, germination process was carried out at 2 levels and terminated based on sprout length. Chickpea and cowpea samples germinated rapidly and the sprout lengths were longer than the seed length at end of the 48 h. Therefore, germination period for chickpea and cowpea was determined as 24 h for germination level 1 and 48 h for germination level 2. On the other hand, germination of the faba beans at the noted conditions took longer, and the germination time was 48 h for germination level 1 and 72 h for germination level 2. Germination process was terminated by drying the seeds at 45 °C in a laboratory dryer (Tre Spade Atacama, F77000, Torino, Italy) until the moisture content of the seeds fall below 10%. Dried germinated seeds were pulverized to

a fine powder using a laboratory mill (Karaerler Makina, Ankara, Turkey) and sieved through 300 µm sieve to obtain uniform particle size.

Proximate analysis of the seed flours

The moisture, crude fat, crude protein, ash, and dietary fiber contents of the seed flours were determined by using standard methods (AACC 2000; AOAC 2000). Moisture content of the flours was measured with a moisture analyzer (Ohaus MB27, New Jersey, USA). Crude fat content was determined by the Soxhlet extraction method using hexane. Crude protein content was determined according to the macro-Kjeldahl method using 6.25 as the N conversion factor. Ash content was measured gravimetrically by burning the samples in a muffle furnace at gradually increasing temperatures up to 650 °C. Soluble, insoluble and total dietary fiber analyses were carried out according to the official enzymatic–gravimetric AACC method (Method No: 32–07) (AACC 2000) using a commercially available enzyme kit (Megazyme, Wicklow, Ireland). All results were expressed as dry weight (d.w.).

Lipoxygenase activity

LOX activity was performed according to Lampi et. al. (2020) with some modifications. Briefly, 10 g seed was weighed and wet milled with 100 mL of water using Waring blender for 2 min. Then, the suspension was centrifuged at 9000 rpm speed at 4 °C for 15 min and the supernatant was filtered through a filter paper. The substrate solution was 10 mM linoleic acid (Product number: L1376, Sigma) solution in 1% Tween 20 in water which was clarified with 1 M NaOH. Filtered sample solution was diluted with M/15 buffer. To 2.6 mL M/15 buffer, 0.2 mL substrate solution and 0.2 mL diluted sample solution was added and started to record the absorbance immediately against blind (2.8 mL M/15 buffer and 0.2 mL substrate solution) at 234 nm. Results were expressed as enzyme activity (U/g sample) and calculations were done according to the formula reported by Baltierra-Trejo, et.al. (2015) as follows:

$$U/L = \frac{(\Delta A)(Vt)(Df)\left(10^6\right)}{(t)(\varepsilon)(d)(Vs)}$$

where; U is the enzyme activity (µmol min^{-1} L^{-1} or unite L^{-1}), (ΔA) is the difference between final absorbance and initial absorbance, (Vt) is total volume of the reaction (mL), (Df) is the dilution factor, (10^6) is the correction factor (µmol mol^{-1}), (t) is the reaction time (min), (ε) is the molar extinction coefficient (M^{-1} cm^{-1} or L mol^{-1} cm^{-1}), (d) is optical path (1 cm), and (Vs) is the sample volume (mL).

Production of pulse-based milk analogs

All milk analogs were produced at fixed concentration of 1:10 solid to water ratio (w/v) for comparison purposes. Basically, the production steps of the pulse-based milk analogs were; (a) pre-treatment (explained above), (b) wet-milling or dry milling followed by hydration (30 min mixing on a magnetic stirrer at room temperature), (c) filtration < 100 µm, (d) gelatinization of starch (heating the suspensions above 80 °C), (e) starch hydrolysis with commercial α-amylase (1 µL/ g solid material, Spezyme LT-300, DuPont, Delaware, USA) according to instructions, (e) homogenization using Ultraturrax (IKA T25, Staufan, Germany) at 15,000 rpm for 5 min, (f) sterilization using autoclave at the reference temperature of 121.1 °C for 5 min. The milk analogs were produced with a batch size of 1 L and all products were made in triplicates of the same batch size. Totally, 21 L of unformulated milk analogs (7 pre-treatment × 3 replicates) were produced for each pulse type.

Yield

The total yield was estimated as the proportion of the weight of the final product (milk analog obtained after the heat treatment) to the sum of weight of the solid material and water used at the beginning and calculated as % (Moscoso Ospina et. al. 2022).

pH and titratable acidity

The pH of the milk analogs (50 mL) was measured directly using a digital pH-meter (Mettler Toledo / S20, Ohio, USA). For the measurement of titratable acidity, 25 mL of sample was titrated with 0.1 N NaOH in the presence of phenolphthalein indicator. The results of titratable acidity were presented as lactic acid equivalents.

Viscosity

Viscosity measurement was performed with a viscometer (Brookfield, LVDV-II + Pro, Toronto, Canada) using the SC4-18 spindle at 200 rpm rotational speed. The temperature of the suspension kept constant at 20 °C.

Color

Color characteristics of the crude milk analogs were measured using a Minolta CR-400 model colorimeter (Minolta Co., Osaka, Japan) by putting the samples in a cylindrical cuvette (CR-A505 & CR-A504 35 × 34 m∅). Lightness (L^*), greenness (-a^*) or redness (+a^*), blueness (- b^*) or yellowness (+b^*) were measured and other color attributes were calculated as follows:

$$\text{Chroma} = \left[\left(a^{*2} + b^{*2}\right)\right]^{1/2}$$

$$\Delta E = \left[(\Delta L)^{*2} + (\Delta a)^{*2} + (\Delta b)^{*2}\right]^{1/2}$$

$$\text{Whiteness} = 100 - [(100 - L)^{*2} + a^{*2} + b^{*2}]^{1/2}$$

Sensory analysis

The effect of the pre-treatments on sensory properties of the unformulated pulse-based milk analogs was assessed with consumer acceptance test (Meilgaard et. al. 1999). The panelists (aged between 19 and 53, ~ 70% female and 30% male) were recruited among staff and students from Food Engineering and Food Technology Departments. Each milk analog type was evaluated individually in different sessions. The samples were randomly coded with a three-digit number and served to panelists in 20 mL plastic cups. The panelists were asked to rate the samples on a 9-point hedonic scale (1 = "Dislike extremely", 2 = "Dislike", 3 = "Dislike moderately", 4 = "Dislike slightly", 5 = "neither like nor dislike", 6 = "Like slightly", 7 = "Like moderately", 8 = "Like", and 9 = "Like extremely"). To eliminate the carry-over impact, tap water was provided as neutralizer between samples.

Statistical analyses

Analysis of variance (ANOVA) was used to assess the effect of the pre-treatments and pulse type on the noted physicochemical properties of the milk analogs at a significance level of 95% ($p < 0.05$). Multiple comparisons were performed by Tukey. All results were presented as mean (at least three replicates for all analyses) ± standard error. The results of the consumer acceptance test were evaluated with Kruskal–Wallis test and multiple comparisons were performed by Dunn's test ($p < 0.05$).

Results and discussion

Proximate composition

Proximate composition of the pulse flours was presented in Table 1. It was observed that chickpea was superior in terms of crude fat and faba bean was superior in terms of protein among the seeds used in the present study in accordance with the literature (Langyan et. al. 2022). Some of our results slightly differed from the reported literature since the analyses were performed directly on the raw material which was sieved through 300 µm. Crude protein content of the pulse flours ranged between 22.50 and 30.56% (d.w.). Considering that the average protein content of cow's milk is 3.5%, a milk analog including the noted pulses, especially faba bean, at a 10% level may have protein content close to cow's milk. However, it should be noted that the qualifications of the protein such as amino acid profile may not be comparable to the same extent. Nevertheless, pulses are good sources for milk analogs in terms of protein content compared to many other plant materials such as cereals and some seeds. On the other side, high fat content of the chickpea may increase the sensory appeal of the product especially in terms of mouthfeel. The total dietary fiber content of the pulse flours ranged between 8.04 and 12.38% (d.w.). Although consumption of dietary fiber is associated with variety of health benefits, high amount of dietary fiber may favor sedimentation during the storage of plant-based milks which is an important problem in terms of colloid stability.

Lipoxygenase activity

LOX enzymes have been found responsible from the off-flavors in milk analogs in many studies (Gao et. al. 2020; Li et. al. 2008; Mäkinen et. al. 2016). The highest LOX activity was found in cowpea among the pulses analyzed in this study. The amount of LOX activity in cowpea was almost 2.5- and 5-fold higher than chickpea and faba bean, respectively (Table 2).

Milling or grinding is the first main process in production line of pulse-based milk analogs. Although wet milling is

Table 1 Proximate composition of the pulse flours (< 300 µm)*

Pulse type	Moisture (%)	Crude fat (%, d.w.)	Ash (%, d.w.)	Crude protein(%, d.w.) (Nx6.25)	Insoluble dietary fiber (%, d.w.)	Soluble dietary fiber (%, d.w.)	Total dietary fiber (%, d.w.)
Chickpea	8.31 ± 0.06^{B}	9.35 ± 0.78^{A}	2.99 ± 0.01^{B}	22.50 ± 0.57^{C}	7.80 ± 0.20^{C}	0.24 ± 0.02^{B}	8.04 ± 0.18^{C}
Faba bean	8.73 ± 0.23^{B}	5.10 ± 0.21^{B}	3.60 ± 0.01^{A}	30.56 ± 0.02^{A}	12.00 ± 0.03^{A}	0.38 ± 0.01^{A}	12.38 ± 0.02^{A}
Cowpea	10.56 ± 0.03^{A}	3.48 ± 0.48^{B}	3.71 ± 0.07^{A}	24.05 ± 0.03^{B}	10.91 ± 0.04^{B}	0.37 ± 0.01^{A}	11.28 ± 0.04^{B}

*Means followed by different capital letters are significantly different by Tukey ($p < 0.05$)

Table 2 The effect of the pre-treatments on lipoxygenase activity*

Lipoxygenase activity (unit/g sample)			
Pulse type	Chickpea	Faba bean	Cowpea
Dry milling-no hydration	0.64 ± 0.08^{F}	1.31 ± 0.09^{D}	7.04 ± 0.16^{D}
Dry milling-15 min hydration	2.78 ± 0.09^{EF}	1.34 ± 0.06^{D}	7.72 ± 0.28^{D}
Dry milling-30 min hydration	2.79 ± 0.12^{EF}	3.15 ± 0.34^{D}	7.58 ± 0.06^{D}
Dry milling-60 min hydration	4.27 ± 0.35^{E}	4.33 ± 0.52^{CD}	6.96 ± 0.50^{D}
Direct wet milling	13.34 ± 0.05^{BC}	7.16 ± 0.60^{C}	33.76 ± 0.80^{B}
Soaking (24 h)	16.16 ± 0.46^{AB}	15.65 ± 1.17^{B}	46.32 ± 0.73^{A}
Alkali soaking (1 h) and dehulling	18.94 ± 0.74^{A}	25.68 ± 1.34^{A}	44.30 ± 2.49^{A}
Germination (1st level)	12.87 ± 1.63^{C}	3.44 ± 0.27^{D}	35.37 ± 0.40^{B}
Germination (2nd level)	7.84 ± 0.82^{D}	1.47 ± 0.07^{D}	25.77 ± 1.05^{C}

*Means followed by different capital letters for the same pulse type are significantly different by Tukey ($p < 0.05$)

more prevalent, the process can be done in two ways: Dry milling and wet milling. In general, dry milled pulses are expected to have higher LOX activity due to more aeration or oxygenation during the procedure. Although there are studies comparing cold and hot grinding of pulses with regard to LOX activity (Zhang et. al. 2012), we could not reach any study comparing the effects of dry and wet milling. It was found that wet milling resulted in higher LOX activity for all samples (Table 2). To exclude the effect of the time for pulse and water interaction in wet milling, dry milled pulse flours were also hydrated with water up to 1 h on a magnetic stirrer with continuous mixing before the LOX analyses. As a result, despite there was a slight increase in LOX activity with increasing hydration time in some samples, it was found that LOX activity was still notably higher in wet milled samples compared to dry milled samples, especially for cowpea (Table 2).

Thermal inactivation of LOX is the most prevalent approach (Yalcin and Basman 2015; Stephany et al. 2015). Blanching was the designed pre-treatment for LOX inactivation since it is an easy, inexpensive and a practical procedure. LOX activity gradually decreased with prolonged blanching time for chickpea and cowpea and after 2 min of blanching, LOX was completely inactivated. However, LOX inactivation in faba bean followed a fluctuation trend of decrease-increase–decrease despite numerous replications (Fig. 1). This result was attributed to thicker husk and bigger size of faba bean seeds compared to other pulses, which probably prevented the homogenous heat transfer into the seed. Nonetheless, faba bean LOX was also inactivated after 5 min. Optimum blanching time was defined as the mean LOX activity < 0.1 U/g and determined as 5, 2, and 2 min for faba bean, cowpea, and chickpea, respectively. Similar approaches were reported in previous studies. Zhang et al. (2012) reported that although hot grinding of soy gave lower solid and protein recoveries, either LOX activity or off-flavor is significantly reduced by hot grinding when compared to cold and ambient grinding procedures. Soaking the seeds in distilled water at 25 °C for 24 h lead to a significant increase in LOX activity in all type of pulses when compared to direct wet milling of the dry seeds ($p < 0.05$). The highest increase was observed in faba bean (Table 2). This was an expected result since LOX requires water to be activated which is facilitated by soaking.

Alkali soaking and dehulling resulted in further increase in LOX activity. Although dehulling pre-treatment involves blanching and alkaline soaking steps together, blanching step was skipped solely for the LOX analysis because otherwise there will be no parameter to be measured. Therefore, this activity increase was directly related with alkaline soaking (pH 9) for 1 h and manual dehulling. Soaking in alkaline environment may have favored LOX activity since alkaline region is reported to be the optimum environment for LOX isozymes which are commonly classified as type I (Kumar et. al. 2006). Besides, increase in LOX activity may also be related to dehulling itself due to the distribution of LOX in the seed. Stephany et.al. (2015) reported a specific LOX activity of 1004 and 994 U/mg protein for whole and dehulled lupine seeds, respectively, which shows that LOX is mainly located in the cotyledon of lupine.

Germination or sprouting is one of the main food processes mostly performed to increase the nutritive value of grains, legumes and seeds. It is a complex phenomenon of a variety of biochemical changes leading to an increase in the activity of many enzymes (Mäkinen and Arendt 2015). Therefore, an increase in LOX activity would be expected as a result of germination. However, in our study, germination lead to a significant decrease in LOX activity regardless of the pulse type ($p < 0.05$). Moreover, the activity of LOX tended to decrease with prolonging the germination time in all pulse types. The results of the LOX analyses either performed directly on the germinated pulse seeds (Table 2) or germinated, dried and ground pulse flours (data not shown) showed that germinated pulses have significantly

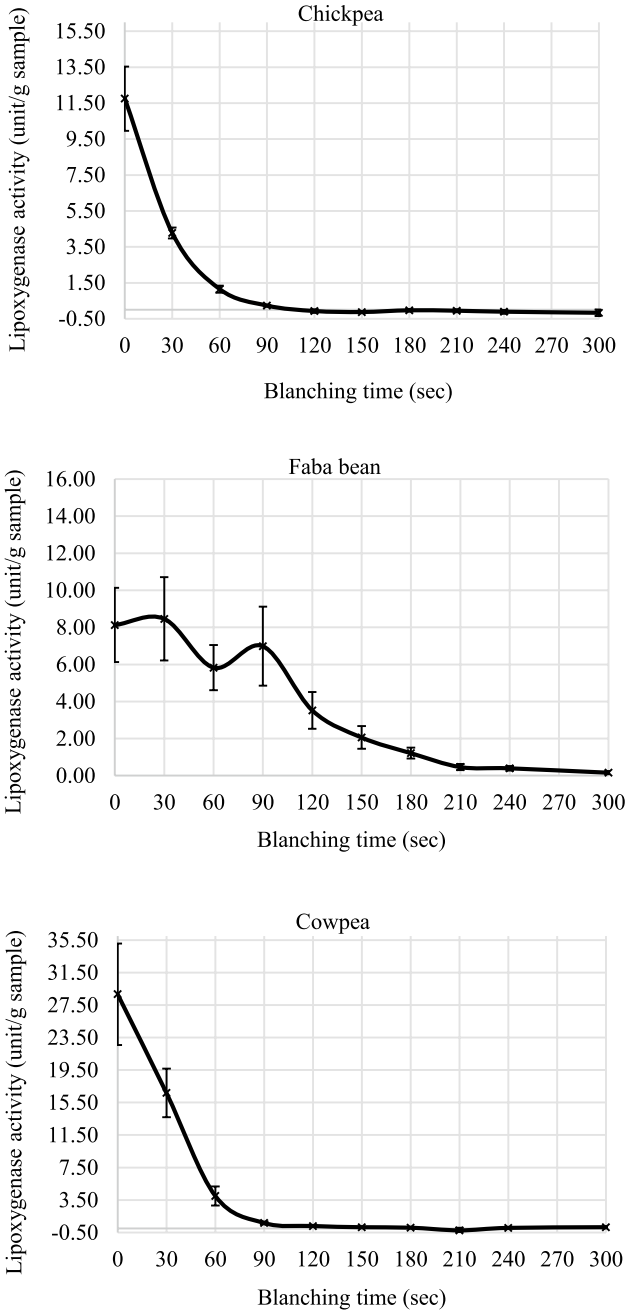

Fig. 1 Lipoxygenase activity of the pulses as a function of blanching time (unit/g sample) Numer acceptability

lower LOX activity compared to control seeds for any pulse type ($p < 0.05$).

There are conflicting results related to the subject in the literature. Hu et. al. (2022) reported that LOX activity of soybeans increased first (0–12 h) and then decreased (12–36 h) with increasing germination period. The authors found no significant difference in LOX activity between different soybean varieties. Xu et al. (2020) found a significant increase in LOX activity of chickpea, lentil and yellow pea flours after 5 days of germination ($p < 0.05$). On the other hand, Akkad et al. (2021) found a notable (50%) decrease in LOX activity of a low-tannin faba bean cultivar (Snowbird) after 72 h germination period. Kumar et al. (2006) investigated the changes in the activity of LOX isozymes in soybean during germination at two different temperatures for 144 h. The researchers found that either LOX type I or LOX type II + III were degraded continuously during germination process for any soybean genotype and the degradation rate was higher at germination temperature of 35 °C when compared to 25 °C. In this context, one of the hypotheses explaining the decrease in LOX activity due to germination is the degradation of LOX during the process, while another is that LOX enzymes do not involve the lipid mobilization but act as a storage protein during germination (Wang et. al. 1999).

Physicochemical properties

The total yield of the pulse-based milk analogs was between 68 and 86% (Table 3). It is worth to mention that yield may widely vary depending on many factors such as the raw material used, extraction process (pH arrangement, enzyme use), filtration system, etc. Therefore, higher yields can be obtained in scale-up conditions.

Among the pre-treatments applied, blanching gave the lowest yield results for any type of pulse material ($p < 0.05$). This result was attributed to the gelatinization of starch which prevents water leakage and therefore decreases the yield. Obtaining the lowest yield result (68%) from the milk analog produced from faba beans which were blanched for the longest time supports this inference. Soaking-wet milling also resulted in a relatively lower yield among the pre-treatments. During the soaking procedure, the soaking water was discarded and the hydrated pulses were wet-milled with fresh water. Therefore, probably leaching losses lead to lower yield results. The highest yield was obtained from control samples which were produced from dry milled pulses without any pre-treatment.

The pH of the pulse-based milk analogs was close to neutral and ranged between 5.9 and 6.9 for all samples. Lopes et. al. (2020) also reported similar pH values for lupin-based (~ pH 6), and chickpea-based (~ pH 7) milk analogs. In general, it is accepted that pulse proteins have the lowest solubility in a pH range between 4 and 6, however the solubility exhibits a sharp rise when the pH is arranged to more acidic (< pH 4) or to neutral and alkaline (> pH 6) environments (Kiosseoglou and Paraskevopoulou 2011). Therefore, the pH values of the final products were suitable for good protein solubility which favors physical stability.

Titratable acidity of the pulse-based milk analogs ranged between 0.05 and 0.13%. Three different brands of full-fat and semi-fat commercial cow's milk samples were also

Table 3 Some physicochemical properties of the pulse-based milk analogs*

Pulse type	Pretreatment	Yield (%)	pH	Titratable acidity (%)	Viscosity (cP)
Chickpea	Control (Dry milling)	83.21 ± 1.75 ^{A}	6.76 ± 0.01 ^{A}	0.07 ± 0.01 ^{B}	3.69 ± 0.01 ^{A}
	Soaking and wet milling	75.70 ± 2.31 ^{AB}	6.65 ± 0.01 ^{B}	0.05 ± 0.01 ^{C}	3.19 ± 0.02 ^{BC}
	Blanching	71.40 ± 0.69 ^{B}	5.96 ± 0.01 ^{F}	0.09 ± 0.01 ^{A}	2.57 ± 0.04 ^{D}
	Blanching and dehulling	75.87 ± 1.76 ^{AB}	6.30 ± 0.01 ^{E}	0.07 ± 0.01 ^{B}	2.61 ± 0.01 ^{D}
	Vacuum	82.62 ± 2.31^{A}	6.57 ± 0.01 ^{D}	0.08 ± 0.01 ^{B}	3.28 ± 0.01 ^{B}
	Germination (1st level)	82.20 ± 1.22 ^{A}	6.62 ± 0.01 ^{C}	0.07 ± 0.01 ^{B}	3.29 ± 0.02 ^{B}
	Germination (2nd level)	81.16 ± 1.82 ^{A}	6.55 ± 0.01 ^{D}	0.08 ± 0.01 ^{B}	3.09 ± 0.03 ^{C}
Faba bean	Control (Dry milling)	84.70 ± 1.15 ^{A}	6.79 ± 0.01 ^{F}	0.10 ± 0.01 ^{B}	2.48 ± 0.01 ^{A}
	Soaking and wet milling	70.02 ± 2.89 ^{C}	6.87 ± 0.01 ^{C}	0.09 ± 0.01 ^{C}	2.02 ± 0.01 ^{CD}
	Blanching	68.87 ± 1.13 ^{C}	6.83 ± 0.01 ^{E}	0.09 ± 0.01 ^{C}	2.03 ± 0.02 ^{CD}
	Blanching and dehulling	71.73 ± 1.88 ^{BC}	6.90 ± 0.01 ^{B}	0.09 ± 0.01 ^{C}	1.92 ± 0.04 ^{D}
	Vacuum	83.53 ± 1.93 ^{A}	6.96 ± 0.01 ^{A}	0.09 ± 0.01 ^{C}	2.47 ± 0.01 ^{A}
	Germination (1st level)	78.90 ± 1.21 ^{AB}	6.85 ± 0.01 ^{D}	0.09 ± 0.01 ^{C}	2.12 ± 0.02 ^{C}
	Germination (2nd level)	80.55 ± 1.16 ^{A}	6.86 ± 0.01 ^{CD}	0.11 ± 0.01 ^{A}	2.27 ± 0.02 ^{B}
Cowpea	Control (Dry milling)	86.16 ± 1.70 ^{A}	6.76 ± 0.01 ^{B}	0.10 ± 0.01 ^{B}	2.33 ± 0.04 ^{B}
	Soaking and wet milling	76.59 ± 1.74 ^{B}	6.75 ± 0.01 ^{B}	0.09 ± 0.01 ^{B}	1.78 ± 0.02 ^{D}
	Blanching	76.09 ± 0.63 ^{B}	6.76 ± 0.01 ^{B}	0.09 ± 0.01 ^{B}	2.03 ± 0.01 ^{C}
	Blanching and dehulling	79.60 ± 1.71 ^{AB}	6.80 ± 0.01 ^{A}	0.06 ± 0.01 ^{C}	1.75 ± 0.01 ^{D}
	Vacuum	85.83 ± 1.16 ^{A}	6.79 ± 0.01 ^{A}	0.09 ± 0.01 ^{B}	2.05 ± 0.04 ^{C}
	Germination (1st level)	84.22 ± 1.76 ^{A}	6.71 ± 0.01 ^{C}	0.10 ± 0.01 ^{B}	2.97 ± 0.03 ^{A}
	Germination (2nd level)	83.99 ± 1.15 ^{A}	6.65 ± 0.01 ^{D}	0.13 ± 0.01 ^{A}	± 0.03 ^{A}

*Means followed by different capital letters for the same pulse type are significantly different by Tukey ($p < 0.05$)

measured with the same analytical procedures and the mean pH and titratable acidity values were 6.67 and 0.18, respectively. Therefore, it can be said that unformulated pulse-based milk analogs had similar acidity to commercial cow's milk samples.

In general, viscosity of an unformulated pulse-based milk analog depends on solid to water ratio or concentration, enzyme including temperature and types and amounts of the enzymes used in the hydrolysis procedure (Yılmaz Tuncel et. al. 2022). Although the most important factor affecting the viscosity of an unformulated milk analog is starch properties, the effect of the pre-treatments was also found significant on viscosity ($p < 0.05$). Among the applied pre-treatments, blanching, blanching-dehulling, and soaking resulted in lower viscosity in all milk analogs likewise the yield results ($p < 0.05$) (Table 3). Gelatinization occurred during blanching prevented starch from being extracted with water and lead to a decrease in viscosity of the final product. Similarly, soaking of pulses also decreased the final viscosity because any leaching compound contributing viscosity was also discarded with discarding soaking water. Same results may not be found in milk analogs produced with soaking water instead of fresh water. Besides, chickpea milk had higher viscosity results compared to faba bean and cowpea milk analogs although it was subjected to the hydrolysis treatment with the same amount of enzyme. This result was attributed to higher starch content of chickpea compared to faba bean and cowpea (Table 1).

Color

Color characteristics of the milk analogs were presented in Table 4. Lightness of the final product was the highest in samples pre-treated with blanching-dehulling procedure regardless of the pulse type ($p < 0.05$). This is an expected result since the hulls of the pulses contain the pigments which give the characteristic color to the seed and dehulling removes the most colored part of the seed. Interestingly, soaking-wet milling pre-treatment resulted in significantly lower L^* values in the final products for any pulse type. Whiteness may be a quality criteria for a milk analog since consumers are very accustomed to the opaque white color of cow's milk. Whiteness value exactly followed the same trend with L^* value (Table 4).

Almost all milk analogs showed a very slight greenness ($-a^*$). The highest variation in b^* value was observed in chickpea milk analogs. Chickpea milk analog produced from soaked and wet milled chickpeas showed the lowest yellowness ($+b^*$) explaining the intense yesllow color of the discarded soaking water. Blanching significantly increased the yellowness ($+b^*$) of the samples regardless of the pulse type ($p < 0.05$). Blanching also had an increasing effect on

Table 4 Color characteristics of the milk analogs*

Pulse type	Pretreatment	L^*	a^*	b^*	Chroma	Whiteness	ΔE
Chickpea	Control (Dry milling)	59.30 ± 0.01^{D}	-3.45 ± 0.01^{F}	7.48 ± 0.01^{B}	8.56 ± 0.01^{A}	58.41 ± 0.01^{D}	–
	Soaking and wet milling	53.79 ± 0.01^{G}	-2.92 ± 0.01^{D}	2.57 ± 0.01^{F}	3.89 ± 0.01^{D}	53.62 ± 0.01^{F}	33.28 ± 0.06^{A}
	Blanching	61.59 ± 0.02^{B}	-2.67 ± 0.01^{B}	8.29 ± 0.01^{A}	8.71 ± 0.01^{A}	60.62 ± 0.02^{B}	2.63 ± 0.02^{E}
	Blanching and dehulling	62.88 ± 0.02^{A}	-2.79 ± 0.01^{C}	6.93 ± 0.01^{C}	7.47 ± 0.01^{B}	62.14 ± 0.02^{A}	4.47 ± 0.01^{D}
	Vacuum	59.09 ± 0.05^{E}	-3.18 ± 0.01^{E}	6.82 ± 0.09^{C}	7.53 ± 0.09^{B}	58.40 ± 0.04^{D}	1.41 ± 0.21^{F}
	Germination (1st level)	59.92 ± 0.01^{C}	-2.78 ± 0.01^{C}	4.22 ± 0.01^{E}	5.06 ± 0.01^{C}	59.60 ± 0.01^{C}	13.95 ± 0.01^{B}
	Germination (2nd level)	58.29 ± 0.01^{F}	-2.40 ± 0.01^{A}	4.45 ± 0.01^{D}	5.05 ± 0.01^{C}	57.98 ± 0.01^{E}	12.94 ± 0.05^{C}
Faba bean	Control (Dry milling)	33.50 ± 0.01^{D}	-0.05 ± 0.01^{D}	0.46 ± 0.01^{F}	0.46 ± 0.01^{E}	33.49 ± 0.01^{D}	–
	Soaking and wet milling	30.01 ± 0.01^{F}	0.61 ± 0.01^{B}	1.32 ± 0.01^{C}	1.46 ± 0.01^{C}	30.00 ± 0.01^{F}	4.30 ± 0.01^{D}
	Blanching	32.04 ± 0.02^{E}	1.36 ± 0.01^{A}	2.58 ± 0.01^{A}	2.92 ± 0.01^{A}	31.97 ± 0.02^{E}	6.55 ± 0.04^{B}
	Blanching and dehulling	41.46 ± 0.01^{A}	-1.41 ± 0.01^{F}	1.03 ± 0.01^{E}	1.74 ± 0.01^{B}	41.43 ± 0.01^{A}	8.40 ± 0.01^{A}
	Vacuum	29.21 ± 0.05^{G}	0.52 ± 0.01^{C}	1.13 ± 0.01^{D}	1.25 ± 0.01^{D}	29.20 ± 0.05^{G}	4.77 ± 0.06^{C}
	Germination (1st level)	35.33 ± 0.01^{B}	-0.14 ± 0.01^{E}	0.36 ± 0.01^{G}	0.39 ± 0.01^{F}	35.33 ± 0.01^{B}	1.84 ± 0.01^{F}
	Germination (2nd level)	35.21 ± 0.01^{C}	-0.06 ± 0.01^{D}	1.42 ± 0.01^{B}	1.42 ± 0.01^{C}	35.19 ± 0.01^{C}	2.63 ± 0.01^{E}
Cowpea	Control (Dry milling)	36.32 ± 0.17^{C}	-0.69 ± 0.08^{CD}	0.31 ± 0.06^{E}	0.78 ± 0.03^{E}	36.31 ± 0.17^{C}	–
	Soaking and wet milling	34.92 ± 0.18^{E}	-0.72 ± 0.06^{CD}	1.47 ± 0.03^{B}	1.64 ± 0.05^{C}	34.90 ± 0.18^{E}	0.30 ± 0.10^{F}
	Blanching	35.81 ± 0.03^{D}	0.28 ± 0.02^{A}	2.26 ± 0.03^{A}	2.28 ± 0.04^{B}	35.77 ± 0.03^{D}	1.84 ± 0.11^{D}
	Blanching and dehulling	42.09 ± 0.03^{A}	-1.68 ± 0.01^{E}	1.43 ± 0.01^{B}	2.21 ± 0.01^{B}	42.04 ± 0.03^{A}	2.24 ± 0.04^{C}
	Vacuum	35.10 ± 0.01^{E}	-0.36 ± 0.01^{B}	0.62 ± 0.01^{D}	0.72 ± 0.01^{E}	35.10 ± 0.01^{E}	5.96 ± 0.03^{A}
	Germination (1st level)	41.37 ± 0.05^{B}	-0.55 ± 0.01^{BC}	1.11 ± 0.01^{C}	1.24 ± 0.01^{D}	41.36 ± 0.05^{B}	1.29 ± 0.01^{E}
	Germination (2nd level)	42.23 ± 0.02^{A}	0.86 ± 0.01^{D}	2.40 ± 0.01^{A}	2.55 ± 0.01^{A}	42.17 ± 0.02^{A}	5.12 ± 0.05^{B}

*Means followed by different capital letters for the same pulse type are significantly different by Tukey ($p < 0.05$)

saturation (chroma) of the milk analogs. Among all samples, the highest total color change (ΔE) was observed in chickpea milk analog produced from soaked and wet milled chickpeas. This result was mainly associated with the decrease in b^* value due to soaking. Blanching-dehulling caused the highest total color change in faba bean milk samples, however, only a slight variation was observed in chickpea and cowpea milk analogs due to this pre-treatment. This may be an expected result since faba bean hull has an intense dark color, while chickpea and cowpea do not. Surprisingly, the highest ΔE was observed in vacuum treated samples for cowpea milk analogs. Vacuum treated samples were subjected to dry milling as the control sample, however, the pulse flour–water suspension was exposed to 50 °C for 30 min together with the vacuum with the aim of volatilizing the components that may cause off-flavor. Therefore, the variation in ΔE is most probably associated with discoloration of cowpea pigments, mainly anthocyanins, even with this mild heat treatment rather than the vacuum treatment.

Furthermore, the color characteristics of the milk analogs produced from control and dehulled pulses were also measured before and after sterilization treatment (at 121 °C for 5 min) to evaluate the effect of heat (data not shown). Average ΔE values were 1.16, 3.14, and 4.18 for milk analogs produced from control and 1.24, 2.00, and 1.83 for milk analogs produced from blanched and dehulled pulses namely chickpea, faba bean, and cowpea, respectively. Lopes et. al. (2020) stated that ΔE values higher than 3 are detectable by human eye. In this regard, it can be speculated that no visually noticeable color change occurred in milk analogs produced from dehulled pulses due to sterilization treatment applied at the noted conditions. However, sterilization caused noticeable color changes in milk analogs produced from faba bean and cowpea flours without a dehulling process.

Sensory analysis

The effect of the pre-treatments was insignificant ($p > 0.05$) on appearance scores of chickpea milk analogs, while the panelist gave significantly higher appearance scores for faba bean and cowpea milk analogs which were subjected to blanching and dehulling pre-treatments ($p < 0.05$). On the other hand, mean appearance scores of chickpea milk was significantly higher than that of faba bean and cowpea milk analogs independent of the effect of pre-treatments ($p < 0.05$). These results were in accordance with the instrumental color characteristics (L^* and whiteness values) of the samples and indirectly show that consumers tend to prefer a whiter product.

Although blanching and dehulling pre-treatment resulted in lower viscosity values (Table 3), the panelists gave

generally higher scores for the consistency attribute of the milk analogs produced from blanched and dehulled pulses. However, the effect of the pre-treatments on consistency scores of faba bean milk analog was insignificant ($p > 0.05$).

In general, flavor scores and overall acceptability scores followed the same trend with regard to the pre-treatments for all pulse types. Therefore, it can be claimed that the most influential factor on overall consumer acceptance of milk analogs is flavor. For both chickpea and cowpea milk analogs, the samples that were pre-treated with blanching and dehulling were the samples that received the highest flavor and overall acceptability ratings ($p < 0.05$) (Table 5). In faba bean milk analogs, although the control sample, which was not subjected to any treatment, received the highest flavor and overall acceptability score, samples produced from blanched and dehulled faba beans got the second highest score and shared the first place. Besides, vacuum also stands out as a pre-treatment which resulted in final products with high sensorial ratings for all pulse types. Vacuum process was employed at laboratory-scale conditions in the present study, and it may be more effective in removal of undesired off-flavor notes when applied on an industrial scale and with stronger suction power. Vacuum-treated milk analogs were not subjected to any thermal pre-treatment, which is the most prevalent way of LOX inactivation, and produced from dry-milled pulses such as the control sample. Therefore, besides from blanching and dehulling, vacuum may also be employed as a simple treatment for removal of off-flavors.

On the other hand, blanched and dehulled samples got higher ratings when compared to the samples pre-treated with solely blanching for all pulse types. This result shows that blanching-dehulling was more effective than blanching alone with regard to the removal of off-flavors. Dehulling may have caused a reduction in the content of some non-volatile compounds such as isoflavones, saponins, and phenolics which are also responsible from the off-notes observed in pulses (Roland et al. 2017).

Surprisingly, milk analogs produced from germinated pulses received the lowest flavor and overall acceptability ratings regardless of the pulse type ($p < 0.05$). In general, germination or sprouting is known as a process which improves sensory properties (Lopes et. al. 2020; Roland et. al. 2017). However, in our study we observed the opposite. Off-flavors in pulses are mostly associated with the activity of LOX enzyme (Roland et. al. 2017). While an increase in the activities of all enzymes, including LOX, was expected during germination process, the lowest LOX activity was found in the germinated pulses as explained above. Therefore, the off-flavors associated with pulses may not be directly related with the LOX activity. Lower sensory ratings of milk analogs produced from germinated pulses attributed

Table 5 Consumer acceptance of the pulse-based milk analogs subjected to different pre-treatments*

Pulse type	Pre-treatment	Appearance	Consistency	Flavor	Overall acceptance
Chickpea ($n=64$)	Control (Dry milling)	5.23 ± 0.22	5.35 ± 0.24^{AB}	4.23 ± 0.28^{B}	4.73 ± 0.25^{CD}
	Soaking and wet milling	5.60 ± 0.23	5.53 ± 0.21^{AB}	4.10 ± 0.25^{B}	4.84 ± 0.22^{BCD}
	Blanching	5.46 ± 0.24	5.43 ± 0.24^{AB}	4.56 ± 0.26^{AB}	5.07 ± 0.22^{ABC}
	Blanching and dehulling	5.89 ± 0.20	5.89 ± 0.22^{A}	5.05 ± 0.24^{A}	5.57 ± 0.22^{A}
	Vacuum	5.70 ± 0.20	5.93 ± 0.19^{A}	4.85 ± 0.26^{AB}	5.42 ± 0.21^{AB}
	Germination (1st level)	5.73 ± 0.26	4.96 ± 0.25^{B}	2.29 ± 0.19^{D}	3.42 ± 0.23^{E}
	Germination (2nd level)	6.04 ± 0.25	5.14 ± 0.23^{B}	3.36 ± 0.26^{C}	4.09 ± 0.26^{D}
Faba bean ($n=61$)	Control (Dry milling)	4.27 ± 0.25^{B}	5.67 ± 0.19	5.50 ± 0.20^{A}	5.41 ± 0.20^{A}
	Soaking and wet milling	3.86 ± 0.28^{B}	5.06 ± 0.21	4.09 ± 0.26^{CD}	4.42 ± 0.24^{BC}
	Blanching	4.19 ± 0.28^{B}	5.19 ± 0.18	4.63 ± 0.24^{BC}	4.73 ± 0.24^{B}
	Blanching and dehulling	5.44 ± 0.27^{A}	5.24 ± 0.19	4.54 ± 0.26^{BC}	5.04 ± 0.22^{AB}
	Vacuum	3.63 ± 0.28^{B}	5.09 ± 0.22	4.85 ± 0.27^{AB}	4.86 ± 0.22^{AB}
	Germination (1st level)	3.86 ± 0.24^{B}	4.96 ± 0.19	3.60 ± 0.23^{DE}	3.90 ± 0.19^{CD}
	Germination (2nd level)	3.57 ± 0.22^{B}	4.72 ± 0.20	3.06 ± 0.24^{E}	3.52 ± 0.19^{D}
Cowpea ($n=46$)	Control (Dry milling)	3.91 ± 0.26^{B}	4.89 ± 0.25^{BC}	4.28 ± 0.27^{B}	4.41 ± 0.24^{B}
	Soaking and wet milling	4.15 ± 0.24^{B}	4.63 ± 0.26^{BC}	4.13 ± 0.23^{B}	4.41 ± 0.21^{B}
	Blanching	4.37 ± 0.25^{B}	5.06 ± 0.23^{AB}	4.82 ± 0.22^{AB}	4.89 ± 0.20^{B}
	Blanching and dehulling	6.41 ± 0.26^{A}	5.71 ± 0.26^{A}	5.31 ± 0.24^{A}	5.87 ± 0.25^{A}
	Vacuum	4.23 ± 0.25^{B}	5.06 ± 0.26^{AB}	4.43 ± 0.27^{B}	4.81 ± 0.22^{B}
	Germination (1st level)	4.04 ± 0.32^{B}	4.26 ± 0.28^{C}	3.10 ± 0.25^{C}	3.47 ± 0.25^{C}
	Germination (2nd level)	3.82 ± 0.31^{B}	4.28 ± 0.30^{BC}	2.69 ± 0.26^{C}	3.06 ± 0.25^{C}

*Means followed by different capital letters for the same pulse type are significantly different ($p < 0.05$)

to unpleasant off-flavor notes which may be generated during the germination process.

Conclusion

To sum up, it was found that dry milling of pulses resulted in the lowest LOX activity and the highest yield. On the other hand, soaking and wet milling caused a significant increase in LOX activity, gave lower milk yields and resulted in a final product with the lowest whiteness value regardless of the pulse type. Blanching was an effective pre-treatment in terms of LOX inactivation, however, decreased the yield and viscosity of the final product. Milk analogs with the highest whiteness value were obtained from pulses which were blanched and dehulled, regardless of the pulse type. The acidity of the unformulated milk analogs was similar to that of commercial cow's milk. It was not possible to produce a homogeneous and drinkable product without a starch hydrolysis procedure. Viscosity of the final product was affected by starch hydrolysis more than any factor. Blanching-dehulling and vacuum treatments were the most promising techniques for removal of off-flavors in the pulse-based milk analogs with regard to the consumer acceptance test. It should also be noted that addition of sugars, flavorings, acids, etc. in the formulation step may further improve the sensory characteristics and acceptance of the pulse-based milk analogs.

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Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

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