https://doi.org/10.17113/ftb.62.02.24.8356 original scientific paper

Characterization of Pea Milk Analogues Using Different Production Techniques

Running title: Characterization of Pea Milk Analogues

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Received: 9 September 2023
Accepted: 29 May 2024

SUMMARY

Research background. Peas stand out among legumes for their high protein content, low glycemic index, and exceptional versatility. However, its potential as a food is often hindered by its undesired off-flavor and taste. Hence, this study focused on minimizing off-flavors through simple pre-treatments, with the goal of improving its compatibility for pea milk analog (PMA) production. PMAs are a burgeoning type of plant-based milk alternative in the growing plant-based market.

Experimental approach. Pea seeds were exposed to different pre-treatments, dry milling, blanching-alkaline soaking-dehulling and vacuum. Typical physicochemical properties such as pH, viscosity, color, titratable acidity and yield were measured to provide a brief outlook on the products. Consumer acceptance test, descriptive sensory analysis, gas chromatography-mass spectrophotometry and gas chromatography-olfactometry techniques were used to present the complete sensory profile and appeal of the pea milk substitutes.

Results and conclusions. *L** values of PMA were quite lower than that of cow's milk, while *a**, *b**, viscosity and pH values were similar. In the descriptive sensory analysis, sweet, astringent, pea-

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like, cooked, hay-like, boiled corn, and green notes received relatively higher scores. Vacuum-treated PMA got higher flavor and overall acceptability scores in consumer acceptance test. Pre-treatments caused significant alterations in the volatile profiles of the PMA. Some volatiles, which are typically associated with off-flavor, such as hexanal, were found in higher concentrations in blanched PMA. Among the pre-treatments applied, vacuum emerged as the most effective method for reducing the level of volatile off-flavor compounds.

Novelty and scientific contribution. This study stands out as a rare investigation into characterizing pea milk analogs and assessing the impact of simple pre-treatments on improving its sensory properties. The findings from this study could aid in the advancement of milk alternatives that offer both nutritional value and strong appeal to consumers.

Keywords: plant-based milk analogues; plant-based milk substitutes; off-flavour; lipoxygenase; vacuum; gas chromatography-olfactometry

INTRODUCTION

In recent years, consumers have reduced their consumption of animal products due to growing awareness of sustainability, environmental impact of foods, and the concern of diseases associated with animal-based diets (*1*). In response to these trends, food manufacturers and researchers are developing plant-based alternatives such as meat and dairy analogs. The market for plant-based foods, poised for further expansion and innovation, has experienced rapid growth in recent years and is expected to reach USD 161.9 billion by 2030 (*2*). Plant-based milk analogs (PBMA) constitute the largest product category of plant-based market (*3*). PBMA are water-soluble extracts of plant materials and they resemble cow's milk in appearance and consistency.

Pulses are recognized as the key raw materials for PBMA, owing to their protein-rich and nutrient-dense properties. Commercially, the most popular and accessible pulse-based milk analog is soy milk (*4*). Soybean is one of the richest sources of protein among pulses. However, soy allergy restricts the consumption of soy products (5). In addition, antinutrients such as enzyme inhibitors and tannins decrease the bioavailability of soy protein (*6*).

Pea, soybean, wheat and rice are the major sources to produce plant-based alternatives (*7*). Peas are emerging as a promising alternative to soy in the production of PBMA due to their low allergenicity, widespread availability, and high nutritional value, thus gaining increased attention (*8*). Pea (*Pisum sativum* L.) is one of the oldest cultivated crops globally, grown in 84 countries, including Australia, Canada, China, and the United States (*9*). Moreover, pea constitutes the largest share (36 %) of total pulse production over the world (*10*). Therefore, it is recognized as an outstanding

nutritional source, especially for its high-quality protein. Pea protein (~ 20-25 % of the pea seed) is rich in essential amino acids such as tryptophan and lysine, and characterized for its high digestibility and notably less allergenic responses compared to soybean or other plant proteins (*10*). Pea is also high in soluble and insoluble fiber, low in fat and sodium, and is a notable source of complex carbohydrates, B group vitamins, folate, and minerals especially iron, calcium, and potassium (*9*). Furthermore, consuming peas has been associated with various health benefits such as anti-cancer, anti-obesity, anti-diabetic, and cardio-protective effects (*11*). However, the utilization of peas in food products is limited, partly due to their undesirable sensory attributes, known as "beany off-flavor" (*12*).

The off-flavor of pea can either be inherent or can be developed during processing and storage (*13*). The primary off-flavors in peas have been described as green, beany, earthy, hay-like, bitter and astringent. These are associated with volatile compounds such as aldehydes, ketones, and alcohols, as well as non-volatile compounds such as isoflavones and saponins (*13,14*). The presence of off-flavor related volatiles is mostly attributed to oxidation of unsaturated fatty acids favored by enzymatic reactions (*15*). In this context, lipoxygenase (LOX), hydroperoxide lyase enzymes, and indirectly lipase have been reported to play significant roles in the formation of volatile off-flavor compounds (*16,17*).

There are limited studies on enhancing the sensory properties of products made from green pea seeds. Azarnia *et al*. (*18*) evaluated the volatiles of yellow, green, and dun cotyledon field pea cultivars grown under uniform conditions to assess the effect of cultivar, crop year, and processing methods (dry milling, cooking, and dehulling) on volatile flavor compounds. The authors indicated that the volatile flavor compounds in peas were affected by the cultivar, crop year, and processing conditions. Moreover, cooking significantly reduced the total area counts of these volatile compounds.

Bi *et al*. (*19*) conducted roasting (160 °C for 30 min), high hydrostatic pressure (HHP) (200-550 MPa for 10 min), and inhibitor (ascorbic acid, quercetin, epigallocatechin-3-gallate [EGCG], and reduced glutathione) treatment to improve sensory properties of pea milk. The authors found that high hydrostatic pressure (HHP) combined with quercetin had the best inhibitory effect on LOX-2 enzyme activity, which significantly correlated with hexanal content.

Ma *et al*. (*8*) applied different pre-treatments to dried yellow peas, such as dehulling, blanching, acid soaking, alkaline soaking, and their combinations. The authors produced pea milk yogurt and found that a combination of blanching and acid soaking resulted in the highest sensory scores, as evaluated by a panel of ten trained members. It was concluded that this pre-treatment improved the sensory appeal compared to the control sample. Yen *et al*. (*15*) reported that vacuum microwave dehydration treatment significantly reduced the total volatile compounds in pea protein and has great potential for reducing off-flavor intensity. Lan *et al*. (*20*) evaluated the effects of solid dispersion-based

spray-drying on the sensory properties of pea protein isolate and found that dispersions with gum arabic and maltodextrin reduced the beany aroma. Tanger *et al*. (*21*) reported that both spray-drying and freeze-drying reduced the beany off-flavor and improved the sensory characteristics of pea protein.

The main objective of this study was to assess the effectiveness of simple pre-treatments (dry milling-control, blanching-alkaline soaking-dehulling, vacuum) that can be easily scaled up to large-scale production, in mitigating the characteristic off-flavor in PMA and to investigate the correlation between LOX activity and sensory acceptance. Additionally, it was aimed to examine the impacts of the pre-treatments on the physicochemical properties and sensory characteristics of PMA.

MATERIALS AND METHODS

Materials

Pea (*Pisum sativum* L.) seeds were obtained from local markets (Migros). Pea seeds from three distinct brands were combined to enhance the representation of the sample. The pooled material exhibited average moisture, crude protein, ash, crude fat, insoluble, soluble, and total dietary fiber contents of 9.25, 24.05, 2.83, 2.32, 7.97, 0.56 and 8.53 %, respectively. The moisture content was measured at 130 °C (*22*). Crude protein content was measured according to macro Kjeldahl method, with a nitrogen (N) conversion factor of 6.25 applied to calculate protein content (*23*). The ash content of the samples was measured by linear heating up to 650 °C (*24*). The crude fat content was determined using the Soxhlet method, with hexane as the solvent (*25*). Soluble, insoluble, and total dietary fiber contents was analyzed using a commercial enzyme kit (Megazyme, Wicklow, Ireland) through an enzymatic-gravimetric mechanism (*26*).

Additionally, three different brands of whole and two different brands of semi-skimmed commercial cow's milk samples were purchased to compare some physicochemical properties.

Pre-treatments

Three different pre-treatments were employed to pea seeds, (*i*) dry milling (control): pea seeds were grounded with a laboratory-type grinder (Yuhong Industry, IC-02A, Jiangsu, China) and sieved through 300 μm sieve; (*ii*) blanching-alkaline soaking-dehulling: pea seeds were blanched by immersing them in boiling water (~100 ºC) for 3 min to inactivate the LOX enzyme. They were then soaked in alkaline water (pH=9) for 1 h, manually dehulled, and wet milled using Waring blender (8011S, Connecticut, USA) and Retch GM200 (Haan, Germany); and (*iii*) vacuum: the pea seeds were dry milled and then hydrated for 30 min on a magnetic stirrer at room temperature. Subsequently, the suspension (1:10 solid-to-water ratio (*m*/*V*)) was transferred to a rotary evaporator

(IKA, RV 8, Staufen, Germany) and subjected to constant vacuum (0.08 MPa) at 50 °C for 30 min with a rotational speed of 50 rpm. The PMAs produced from peas subjected to the mentioned pre-treatments were labeled as DPMA, BPMA, and VPMA.

Determination of LOX activity

LOX activity of pea seeds was determined according to Lampi *et al*. (*27*) with some modifications. To extract LOX, 10 g of pea seeds were weighed and milled for 2 min with distilled water (1:10) using a blender (Waring Commercial, 8011S, Connecticut, USA). The mixture was centrifuged (Nüve, NF 800R, Ankara, Turkey) at 9000 rpm for 15 min at 4 °C and the supernatant was used as the enzyme extract after diluting with M/15 buffer. The substrate was 10 mM linoleic acid (Product number: L1376, Sigma) solution in 1 % Tween 20 in water which was clarified with 1 M NaOH. The change in absorbance at 234 nm was promptly recorded (Shimadzu, UV-160A, Kyoto, Japan) after adding 0.2 mL of enzyme extract to a mixture consisting of 2.6 mL of M/15 buffer and 0.2 mL of substrate solution, for a duration of 270 s. LOX activity results were calculated using the equation below which was suggested by Baltierra-Trejo *et al.* (*28*):

$$U \cdot L^{-1} = (\Delta A \cdot V_t \cdot D_f \cdot 10^6)/(t \cdot \varepsilon \cdot d \cdot V_s) \qquad /1/$$

where U is the enzyme activity (μmol/min·L), Δ*A* is the difference between final and initial absorbance, (*Vt*) is the total reaction volume (mL), *Df* is the dilution factor, 10^6 is the correction factor (μmol/mol), *t* is the reaction time (min), *ε* is the molar absorption coefficient (26 000 $M^{-1} \cdot cm^{-1}$), *d* is the optical path (1 cm) and *V*s is the final sample volume (mL).

Production of PMA

All PMA samples were prepared at 1:10 solid-to-water ratio (*m*/*V*) for comparison purposes. The suspension, which was exposed to the noted pre-treatments above, was filtered through <100 μm and heated at about 80 °C for starch gelatinization. Starch hydrolysis step was performed with commercial α-amylase enzyme (Spezyme, LT-300, Dupont, Delaware, USA) according to instructions (1 μL enzyme solution per g sample). Then, the mixture was homogenized (IKA, T25 Digital, Staufan, Germany) at 15000 rpm for 5 min and was sterilized in screw-capped glass bottle (1 L) at 121.1 °C for 5 min using autoclave (Hirayama, HV-110L, Tokyo, Japan).

Physicochemical analysis

Viscosity measurements of the final PMA (after sterilization) were performed at 20 °C using a viscometer (Brookfield, LVDV-II+Pro, Toronto, Canada) equipped with spindle SC4-18 rotating at a shear rate of 264 per s. Color of the final PMA was measured according to CIE *L*a*b** system using

a cylindrical cuvette (Minolta, Cell Holder CR-A503, Tube Cell CR-A504, Osaka, Japan) and a colorimeter (Minolta, CR-400, Osaka, Japan). Whiteness was calculated according to Milovanovic *et al.* (*29*). A digital pH meter (Mettler Toledo, S20, Ohio, USA) was used for pH measurements. Titratable acidity was determined according to Nielsen (*30*) and results were expressed as lactic acid equivalents (*w* lactic acid/%). The yield was determined according to Moscoso Ospina *et al*. (*31*) and calculated as a proportion of the mass of sterilized PMA to the initial mass (total solids + water) of PMA.

Consumer acceptance test

The effect of the pre-treatments on the sensory appeal of the PMA was evaluated using a consumer acceptance test according to Meilgaard *et al*. (*32*). The participants (around 60 % female and 40 % male) were mostly university staff and students (*N*=58) with ages ranging from 21 to 53 years. 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, 9=like extremely) was used for the evaluation. PMA samples were coded with random three-digit numbers and served to panelists in plastic cups (~20 mL) at room temperature and under daylight. Drinking water was provided in between samples to cleanse the palate.

Descriptive sensory analysis

The sensory attributes of the PMA were assessed using a descriptive sensory analysis according to Meilgaard *et al*. (*32*). Seven trained panelists (5 females, 2 males) aged between 27 and 54 developed potential sensory terms by tasting different types of commercial PBMA at several rounds. The definitions and references of the developed descriptive terms are presented in Table 1. Each type of milk analog was assessed in duplicate for the sensory attributes using a 15-point scale (0 represents no attribute and 15 indicates a strong presence of the attribute). PMA samples were coded with random three-digit numbers and served to panelists in plastic cups (~30 mL) at room temperature. Unsalted crackers and drinking water were provided in between samples to cleanse the palate.

Gas chromatography-mass spectrometry (GC-MS) analysis

Volatile compounds of PMA were extracted with the headspace solid-phase microextraction (HS-SPME) technique and identified with GC-MS. Briefly, 5 mL of sample, 1 g of NaCl and 10 µL of internal standard (10 µL of 2-methyl-3-heptanone in 5 mL methanol) were mixed in a 40-mL amber vial which was capped with a PTFE/silicone septa (Supelco, Bellefonte, PA, USA). The content was

incubated in 50 °C water bath for 30 min. Then, SPME fiber (Carboxen/DVB/PDMS 50/30 µm 2 cm, Supelco, 57348-U, Bellefonte, USA) was inserted into the vial and incubated at the same conditions for another 30 min to absorb volatile compounds. At the end of the period, SPME fiber was injected into GC-MS (GC 6890, MS 6890N, Agilent, Delaware, USA) in splitless mode. HP-INNOwax column (60 m, 0.25 mm i.d., 0.25 µm film thickness, J and W Scientific, 19091N-136, California, USA) was used for the separation of volatile compounds. Helium with a flow rate of 1 $mL.min^{-1}$ was used as carrier gas. The GC oven temperature was initially set at 40 °C for 1 min, then ramped up to 250 °C at a rate of 4 °C per min, with a final hold time of 10 min. The MS was operated at 70 eV ionization energy, 280 °C interface temperature, 35 to 350 *m*/*z* mass range, and 4.45 scan/s scan rate. National Institute of Standards and Technology (NIST) and Wiley Registry of Mass Spectral Data libraries were used for the identification of volatile compounds (based on >70 match score). Retention indices were calculated according to Van den Dool and Kratz (*33*) by using *n*-alkane series (C_7-C_{23}) (Sigma-Aldrich, Missouri, USA) as external references.

Gas chromatography-olfactometry (GC-O) analysis

Aroma active compounds of PMA were extracted with the HS-SPME technique as mentioned above with the exception of internal standard addition. Subsequently, SPME fiber was injected into GC system (GC 6890, Agilent, Delaware, USA) which was equipped with an olfactory detection port. DB-5 column (30 m, 0,32 mm i.d., 0,25 µm film thickness, J and W Scientific, 122-5032, California, USA) was used for the identification of aroma active compounds. Helium with a flow rate of 1.7 mL/min was used as carrier gas. The GC oven temperature was initially set at 40 °C for 3 min, then ramped up to 200 °C at a rate of 10 °C per min, with a final hold time of 10 min. Intensities of aroma active compounds were determined with a 10-point scale (left side: 0=no intensity, right side; 10=strong intensity). Odor descriptions were compared with a) *n*-alkane series (C_7-C_{23}) (Sigma-Aldrich, Missouri, USA) which were injected at the same chromatographic conditions and the retention indices of each compound were matched to the NIST database and literature, b) data obtained with GC-MS, c) authentic standard compounds which were analyzed at the same chromatographic conditions.

Statistical analysis

The data was evaluated using Minitab v. 21.4.2 (*34*), SPSS v. 27.0.1.0 (*35*) and NCSS v. 11 (*36*) statistical software. Parametric data was assessed with analysis of variance (One-Way ANOVA) and multiple comparisons were made with Tukey's test ($p<0.05$). Non-parametric data were assessed with the Kruskal-Wallis test and multiple comparisons were made with Dunn's test ($p<0.05$). All data

was expressed as mean ± standard error. The means consist of three replicates except for the GC-O analyses which were conducted twice.

RESULTS AND DISCUSSION

LOX activity

It is widely acknowledged that the volatile compounds responsible for inducing off-flavors primarily result from LOX enzyme activity, which catalyzes the oxidation of unsaturated fatty acids in the presence of oxygen (*17*). Additionally, the LOX enzyme is associated with quality loss as it leads to discoloration, pigment degradation, and loss of essential fatty acids (*16*). In this regard, the inactivation of the LOX enzyme appears to be crucial for pea processing. The effect of blanching on LOX activity as a function of process time is shown in Fig. 1. It was determined that LOX was completely inactivated after 3 min of blanching treatment. In addition, it was observed that LOX activity increased in the early stages (0 - 60 s) of blanching and thereafter, it showed a decreasing trend (Fig. 1). This is most likely due to inhomogeneous heat transfer. In other words, different regions of the grain reached the temperature at which the enzyme is inactivated at different times. Similar results were found by Gökmen *et al*. (*37*) who reported complete inactivation after blanching at 80 °C for 2 min.

Physicochemical properties of PMA

The physicochemical properties of the PMA are presented in Table 2. Viscosity is a critical physical parameter utilized in quality control related to mouthfeel. During the preliminary assessments, it was noted that the viscosity of the PMA was primarily correlated with the concentration of solids and the hydrolysis of starch. It was not possible to obtain a final product with a drinkable viscosity after sterilization if starch hydrolysis was not performed. The viscosity of the PMAs, which were prepared at the same solids concentration (10 %), ranged between 2.53-3.25 mPa·s. The viscosity of both whole and semi-skimmed commercial cow's milk samples from various brands, measured using the same method, ranged between 1.9 and 2.1 mPa·s. Similar viscosities for semi-skimmed (1.56 mPa·s) and whole cow’s milk (2.00 mPa·s) were reported by Nikmaram and Keener (*38*). Jeske *et al.* (*39*) evaluated the physicochemical properties of 17 commercial PBMA and found that the viscosity of PBMA varied widely between 2.21–47.80 mPa·s. It is worth mentioning that the viscosity of the final product can be significantly modified by the hydrolysis step for raw materials high in starch content.

The pH and titratable acidity values of unformulated PMA ranged between 6.84–6.86 and 0.06–0.08 (*w* lactic acid/%), respectively (Table 2). Similar pH and titratable acidity values were

reported in other studies related to PBMA (*40*). On average, the pH and titratable acidity of commercial cow's milk samples were measured as 6.5 and 0.16 (*w* lactic acid/%), respectively.

The yield of the PMA ranged between 72.21 and 87.20 %, with dry milling resulting in a significantly higher yield compared to wet milling ($p<0.05$) (Table 2). Previous studies have reported much lower yield values, such as 50–60 % (*41*). The variability in yield values may be attributed to different process stages, particularly the filtration and milling of the raw material, as well as variations in calculation methods.

Color is a sensory attribute that significantly influences consumer preference. *L** and whiteness values of PMA were quite low compared to cow's milk. The *L** value of PMA ranged between 43.46 and 47.89 (Table 2), whereas the *L** value of commercial cow's milk samples was measured between 76 and 79 (data not shown). The darker color of the PMA was attributed to the chlorophyll degradation and non-enzymatic browning reactions which may occur during sterilization. Likewise, studies have reported that the color of soy milk subjected to heat treatment at elevated temperatures is adversely affected by Maillard reactions. Additionally, the browning index of soy milk has been observed to increase with longer holding times at high temperatures (*42*). The lower *L** value in BPMA, which involves a dehulling step, suggests that the pigments are not concentrated in the hulls of peas, unlike other pulses such as lentils, faba beans and mung beans (*43*). It is also important to note that ingredients added during the formulation step of PBMA can have a significant impact on the color of the final product. For instance, incorporating oil and homogenizing the mixture can result in a significant rise in the *L** value (data not shown). The calculated whiteness value followed exactly the same trend as *L** value (Table 2). Negative *a** values indicating greenness and positive *b** values indicating yellowness were observed in this study (Table 2), and the results were similar to those of the commercial cow's milk samples. On the other hand, Oliveira *et al.* (*44*) reported a decrease in *L** and an increase in *a** and *b** values when pea protein isolate was added to skimmed cow's milk with increasing concentrations.

Consumer acceptance of PMA

The results of the consumer acceptance test of PMA are presented in Table 3. During consumer acceptance tests, food products are typically presented in their final form, as they would be consumed. However, PMA were produced and presented in unformulated form to eliminate the masking effect of ingredients such as sugar and flavor. Therefore, it's crucial to emphasize that these results apply to unformulated samples. Additionally, the addition of ingredients, particularly sugar during the formulation stage, significantly enhances consumer acceptability. Despite being unformulated, all samples received scores higher than 5 (indicating neither like nor dislike) on a 9-

point hedonic scale (Table 3). The participants could not identify any significant difference between samples exposed to different pre-treatments with regard to appearance and consistency ($p>0.05$). However, VPMA received the highest aroma/flavor and overall acceptability scores, which can be attributed to the volatilization of undesired off-flavors at 50°C in the water bath and their subsequent elimination under vacuum. The vacuum treatment was performed on a laboratory scale, indicating that more efficient outcomes may be attained with vacuum systems on an industrial scale. Vacuum treatment has also been reported as an effective strategy for removing beany flavor from soymilk (*45*). While no statistically significant difference was observed between the consumer scores, DPMA got the lowest overall acceptability score on average, which was very close to that of BPMA (Table 3). Therefore, it can be hypothesized that blanching and dehulling pre-treatments did not have a positive effect on general sensory perception of the PMA. In other words, the inactivation of LOX did not provide an additional benefit in terms of enhancing consumer appeal. Similarly, Murat *et al.* (*46*) reported that off-flavors can still occur even if LOX is inactivated. On the other hand, it is also worth to mention that consumer acceptance testing is highly subjective in nature and may not be reproducible when applied to another consumer community or significantly larger one.

Descriptive sensory analysis of PMA

The results of the descriptive sensory analysis of PMA are depicted in Fig. 2. The panelists developed fifteen flavor descriptors, including astringent, pea-like, cooked, sulphureous, nutty, earth, hay-like, boiled corn, polish, dirty wet towel, metallic, green, fermented dough, medicinal, and wet cartoon. Among these, sweet, astringent, pea-like, cooked, hay-like, boiled corn, and green received relatively higher scores compared to other descriptive terms (Fig. 2). Statistically, significant differences were found in the scores of “astringent”, “boiled corn”, and “green” with respect to the pre-treatments. Similar descriptive terms have been reported in previous studies regarding pea milk (*47,48*). Zhang *et al*. (*47*) found that the “earthy” notes received the highest score in pea milk, followed by “grassy/green”, “mushroom” and “sweet” notes. Bi *et al.* (*19*) conducted a sensory evaluation of pea milk, where trained panelists were instructed to list as many attributes as possible to describe the sensory profile. The researchers noted that the five terms with the highest frequency among all defined attributes were “raw beans,” “grassy,” “milk-like,” “earthy,” and “fatty”. Moreover, Trikusuma *et al*. (*48*) reported that “beany”, “potato”, “pasta” and “cooked green bean” notes was the highest in pea protein beverage.

In the present study, it was found that vacuum pre-treatment resulted in significantly lower intensities of “astringent”, “boiled corn”, and “green” notes ($p<0.05$). In addition, the intensities of the sensory attributes “pea-like”, “earth”, “polish”, “dirty wet towel”, “metallic”, “fermented dough”, and “wet

cartoon" were lower in VPMA (Fig. 2). The sensory descriptors mentioned above are primarily perceived as undesirable and are commonly associated with off-flavors. In this regard, it can be concluded that the results of the descriptive sensory analysis align with those of the consumer acceptance test. On the other hand, the intensities of "pea-like" and "green" notes were the highest in BPMA, which were exposed to blanching pre-treatment to inactivate LOX (Fig. 2). This finding implies that the off-flavor of pea cannot solely be attributed to LOX enzyme activity, as highlighted by other researchers as well (*13*).

GC-MS analysis of PMA

The volatile compounds of PMA, which were identified by GC-MS, are presented in Table 4. Among the total of 21 compounds detected in PMA, 9 of them—specifically, 2-ethyl-furan, 1-pentanal, hexanal, butanoic acid/2-methylpropyl ester, 2-heptanone, (Z)-2-heptenal, thujone, benzaldehyde, and 2-furanmethanol—were present in all samples. Identified volatiles belong to various groups such as aldehydes, alcohols, ketones, esters, furans, and phenols. Most of these identified volatiles emerge as a result of oxidation, enzymatic activity and/or Maillard reactions in materials such as pea flour, pea protein isolates, and pea milk (*12,46,49*).

In this study, the main volatiles found in relatively higher amounts (>10 μg/L) were hexanal and 2-heptanone for DPMA, 2-ethyl-furan, 1-pentanal, hexanal, 2-heptanone, 2-pentyl-furan and 1-pentanol for BPMA and 2-ethyl-furan, hexanal, 2-heptanone, 2-pentyl-furan and thujone for VPMA (Table 4). Similarly, Ma *et al*. (*8*) reported that pre-treatments such as blanching and dehulling can significantly alter the contents and types of volatile compounds. The majority of these compounds primarily stem from linoleic acid, the most prevalent fatty acid in peas. The concentration and interaction of these compounds in the system significantly influence sensory properties (*12,50*).

Multiple studies suggest that hexanal is a pivotal compound linked to off-flavors, and removing this compound from the material may improve its flavor (*48,51*). The hexanal content of BPMA, which was heat treated to inactivate LOX, was higher than that of the PMA exposed to other pre-treatments (Table 4). This result implies that the formation of hexanal in PMA is not solely attributed to LOX activity but may also arise from other reaction pathways (*46*). Even the heat treatment itself, utilized to deactivate LOX, could potentially contribute to increased hexanal formation. Lin and Blank (*52*) found that hexanal is the major odor-active volatile degradation product of heated phospholipids. Similarly, Trikusuma *et al*. (*48*) reported an increase in amounts of hexanal, 1-pentanol, 1-octen-3-ol, 2-heptanone, and 2-pentyl-furan in pea protein beverage after UHT treatment. Moreover, Bi *et al*. (*19*) reported that although they found a significant correlation between the hexanal content and LOX

activity in pea milk, only a 55 % reduction in hexanal content was observed versus 90 % inhibition in LOX activity.

Volatile compounds that cause off-flavor in pea can be either present naturally in the seed or emerge during processing and storage. Several molecules such as hexanal, 2-pentyl-furan, 1-hexanol, nonanal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decadienal are reported to have important impacts on flavor of pea milks (*8,17,47,48*). Furthermore, certain molecules such as 3-methyl-1-butanol, 1-octen-3-ol, 1-pentanol, 3-isopropyl-2-methoxypyrazine, and (*E,E*)-2,4-heptadienal are held responsible for beany off-flavor of pea. However, it has become evident that the flavor cannot be solely attributed to the presence of specific volatiles (*17*).

GC-O analysis of PMA

The aroma-active compounds of PMA are presented in Table 5. A total of 29 compounds were identified in PMA as a result of GC-O analysis, with 11 of them being present in all samples namely, 2,3-butandione (butter), hexanal (green, grass), 2-methyl-3-furanthiol (medicinal), styrene (gasoline), methional (boiled potato), 2-acetyl-1-pyrroline (popcorn, rice), 1-octen-3-one (mushroom), (Z)-1,5-octadien-3-one (geranium, metal), benzyl alcohol (fresh, flower) and (E)-2-nonenal (hay) (Table 5). Most of the identified aroma-active compounds have been previously reported in studies on pea materials, belonging to various groups such as aldehydes, alcohols and ketones (*47*).

Hexanal (green, grass), 2-acetyl-1-pyrroline (popcorn, rice), 1-octen-3-one (mushroom), (Z)-1,5-octadien-3-one (geranium, metal), benzyl alcohol (fresh, flower) and durene (dirty, oxide) were identified as the main aroma active compounds for DPMA with intensities greater than 3 (Table 5). The intensities of hexanal (green, grass), styrene (gasoline) and 2-acetyl-1-pyrroline (popcorn, rice) increased, while the intensities of (*Z*)-1,5-octadien-3-one (geranium, metal) and benzyl alcohol (fresh, flower) decreased in either BPMA or VPMA when compared to DPMA (control) (Table 5). Zhang *et al*. (*47*) reported that the aroma-active compounds which showed higher intensities in olfactometric analysis of pea milk were hexanal, 1-octen-3-ol and (*E,E*)-2,4-nonadienal. Liu *et al*. (49) identified aroma-active compounds of hexanal, methyl hexanoate, methional and benzyl alcohol in pea protein powders (concentrates and isolates). Ebert *et al*. (*53*) found hexanal, 2-nonanol, (*E*)-2-nonenal, and 2-pentyl-pyridine in pea protein isolate.

CONCLUSIONS

The findings indicated that the physicochemical properties of the PMAs subjected to various pre-treatments were generally similar, except for the yield, which was greater in DPMA. Vacuum treatment reduced the green and pea-like notes in the descriptive sensory analysis. Additionally,

vacuum-treated PMA received higher scores for aroma, flavor, and overall acceptability in the consumer acceptance test. The concentration of certain volatile compounds, believed to contribute to off-flavors, such as hexanal, 1-octen-3-ol, and 1-pentanol, was elevated in BPMA, which was exposed to blanching-alkaline soaking-dehulling pre-treatment. Although LOX is recognized for its role in promoting off-flavor production, the findings suggest the existence of varying mechanisms, as evidenced by the highest level of off-flavor markers observed in PMAs derived from blanched (LOX inactivated) peas. Overall, olfactometric intensities exhibited minimal variation across the various pre-treatments.

The study results demonstrated that the off-flavor in pea milk analogs cannot be explained solely by LOX activity. However, vacuum pre-treatment proved to be an effective method for removing the off-flavor. Nevertheless, additional research is required to fully explore the effectiveness of vacuum treatment in a more efficient and large-scale system.

CONFLICT OF INTEREST

None of the authors have any conflict of interest.

AUTHORS' CONTRIBUTION

Ali Emre Andaç contributed to the research by conducting formal analysis, curating data, interpreting data, and reviewing the relevant literature. Necati Barış Tuncel provided vital contributions to the research, including conceptualization, supervision, and the design of the analysis. Neşe Yılmaz Tuncel contributed significantly to the research by conceptualizing the study, designing the analysis methods, supervision, and actively participating in writing and editing the manuscript.

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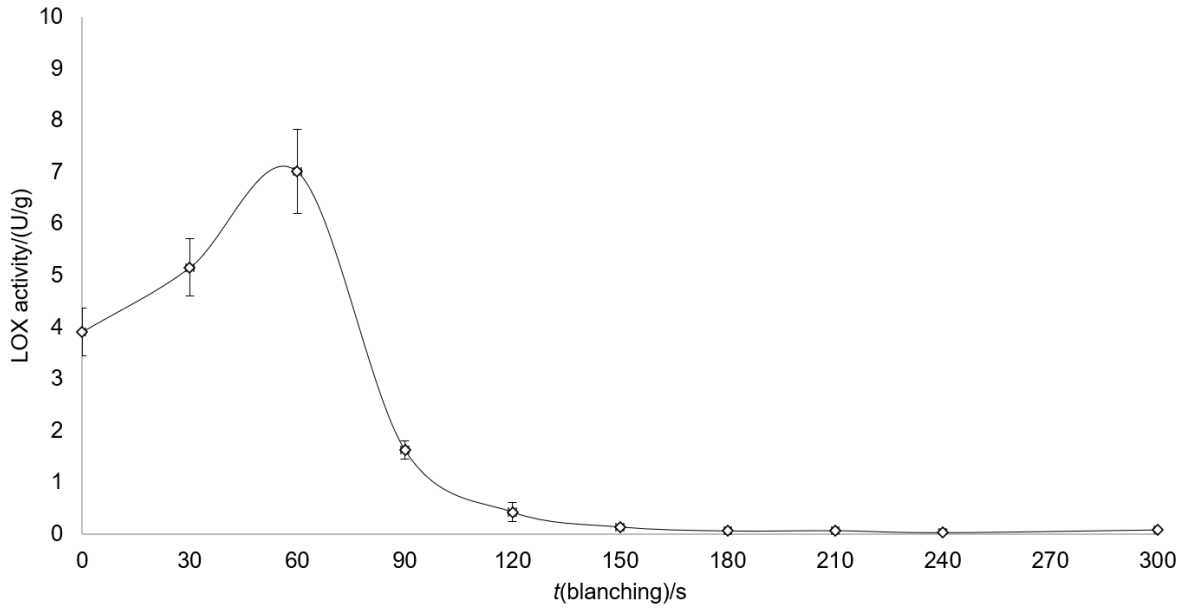

Fig. 1. LOX activity as a function of the blanching time

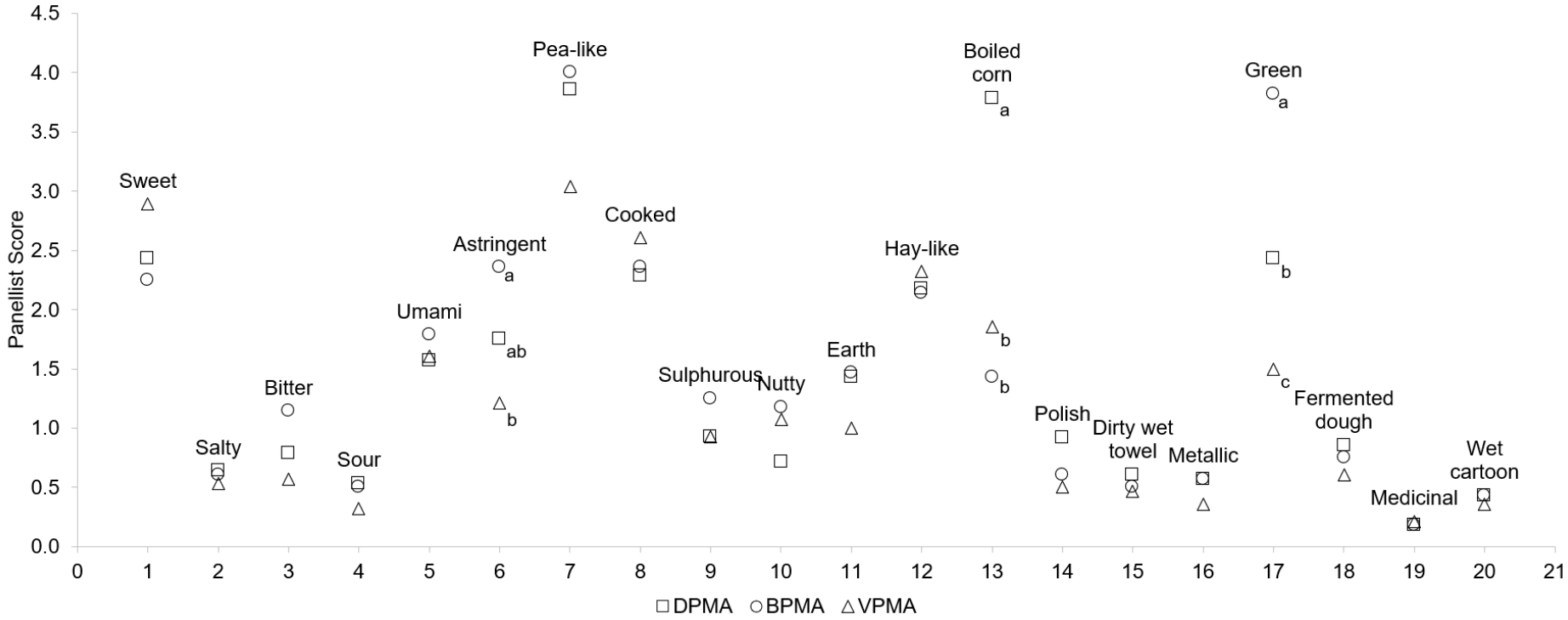

Fig. 2. Descriptive sensory analysis results of the PMAs. Means followed by different letters are significantly different ($p<0.05$). (15-point hedonic scale was used, where 0 represents no attribute and 15 indicates a strong presence of the attribute)

Table 1. Definitions and references for the developed descriptive terms developed in descriptive sensory analysis

Sensory term	Description	Reference
Sweet	Taste sensation elicited by sugars	2 % sucrose, solution in water=2.0* 5 % sucrose, solution in water=5.0
Salty	Taste sensation elicited by salts	0.2 % sodium chloride, solution in water=2.5 0.35 % sodium chloride, solution in water=5.0
Bitter	Taste sensation elicited by caffeine	0.05 % caffeine, solution in water=2.0 0.08 % caffeine, solution in water=5.0
Sour	Taste sensation elicited by citric acid	0.05 % citric acid, solution in water=2.0
Umami	Taste sensation elicited by certain amino acids (glutamate and aspartate) and nucleotides	0.5 % monosodium glutamate, solution in water=3.0 0.75 % monosodium glutamate, solution in water=4.5
Astringent	The shrinking or puckering of the tongue surface caused by substances such as tannins or alum	Tea (brewed)
Pea-like	Aromatics associated with pea	Pea (boiled)
Cooked	Aromatics associated with cooked cereals and pulses	Bulgur (boiled)
Sulphurous	Aromatics associated with sulphurous compounds	Egg (boiled)
Nutty	Aromatics associated with hazelnut/peanut	Hazelnut/ peanut (crushed)
Earth	Aromatic notes associated with damp soil, wet foliage or slightly undercooked potatoes	Green potato skin

Hay-like	Aromatics associated with neutral notes	Oats (soaked)
Boiled corn	Aromatics associated with boiled sweet corn	Canned sweet corn
Polish	Aromatics associated with polish	Flaxseed (oxidized)
Dirty wet towel	Aromatics associated with dirty and wet towel	Reference not used/ Assignment by panelist
Metallic	Aroma of minerals and metals commonly associated with metal spoon	Reference not used/ Assignment by panelist
Green/flower	Aromatics associated with freshly cut leaves, grass and unripe fruits	Freshly cut green grass
Fermented Dough	Aromatics associated with fermented dough	Dough (fermented)
Medicinal	Aromatics associated with medicine	Crushed vitamin B complex
Wet cartoon	Aromatics associated with wet cartoon	Wet cardboard (soaked)

*Reference numbers for the basic tastes indicate their position on the 15-point hedonic scale. For instance, a 2 % sucrose solution in water corresponds to 2.0 points on the scale

Table 2. Physicochemical properties of the PMA

Property	DPMA	BPMA	VPMA
η/(mPa·s)	$(2.97\pm0.07)^{b}$	$(2.53\pm0.03)^{c}$	$(3.25\pm0.03)^{a}$
pH	$(6.86\pm0.01)^{a}$	$(6.85\pm0.01)^{ab}$	$(6.84\pm0.01)^{b}$
TA as *w*(lactic acid)/%	$(0.08\pm0.01)^{a}$	$(0.06\pm0.01)^{b}$	$(0.08\pm0.01)^{a}$
Y/%	$(87.20\pm1.75)^{a}$	$(72.21\pm1.60)^{c}$	$(83.22\pm1.90)^{b}$
*L**	$(44.46\pm0.03)^{b}$	$(43.46\pm0.01)^{c}$	$(47.89\pm0.02)^{a}$
*a**	$(-4.04\pm0.01)^{c}$	$(-2.98\pm0.01)^{a}$	$(-3.93\pm0.01)^{b}$
*b**	$(5.05\pm0.01)^{b}$	$(4.77\pm0.01)^{c}$	$(6.47\pm0.01)^{a}$
Whiteness	$(44.08\pm0.03)^{b}$	$(43.18\pm0.01)^{c}$	$(47.35\pm0.02)^{a}$

Results are expressed as mean±standard error. Means followed by different letters within the same row are significantly different ($p<0.05$)

Table 3. Consumer acceptance test results of the PMA

PMA type	Appearance*	Consistency*	Aroma/Flavor*	Overall acceptability*
DPMA	(5.64±0.33)	(6.16±0.28)	(5.20±0.40)	(5.74±0.33)
BPMA	(5.68±0.35)	(6.20±0.31)	(5.60±0.32)	(5.80±0.26)
VPMA	(5.48±0.35)	(6.20±0.27)	(6.08±0.32)	(6.20±0.29)

Results are expressed as mean±standard error. *The effect of the treatments is not significant ($p>0.05$). 9-point hedonic scale was used, where 1=dislike extremely, 2=dislike, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like, 9=like extremely

Table 4. Volatile profile of the PMA by GC-MS

Compound	RT	RI	Aroma description	γ/(μg/L)		
				DPMA	BPMA	VPMA
2-Ethyl-furan	5.92	944	Sweet, burnt	(4.06±0.81)^{b}	(15.02±3.44)^{ab}	(26.80±5.43)^{a}
1-Pentanal	6.46	980	Almond, malt, pungent	(6.10±0.01)^{b}	(11.37±1.86)^{a}	(8.03±0.17)^{ab}
Acetic acid butyl ester	7.93	1053	Pear	-	(0.83±0.03)^{a}	(0.37±0.01)^{b}
Hexanal	8.08	1060	Green	(47.36±3.32)^{b}	(100.16±0.34)^{a}	(43.05±4.02)^{b}
1-Penten-3-ol	9.83	1131	Pungent	-	(8.46±3.62)	-
Butanoic acid, hexyl ester	9.85	1132	Green	(0.48±0.02)	-	-
Butanoic acid, 2-methylpropyl ester	9.89	1133	Fruity	(2.19±0.06)	(4.53±1.02)	(2.75±0.07)
2-Heptanone	10.96	1171	Soap	(12.35±1.88)	(56.40±17.90)	(19.27±1.95)
2-Pentyl-furan	11.65	1195	Green bean	-	(23.59±0.96)^{ab}	(47.13±9.60)^{a}
1-Pentanol	12.98	1232	Balsamic	-	(84.63±7.03)^{a}	(4.46±1.81)^{b}
1-Hexanol	17.11	1344	Resin, flower, green	(4.66±0.44)	-	(3.85±0.18)
(Z)-2-Heptenal	18.90	1394	Fish	(4.19±1.63)	(6.54±0.94)	(2.02±0.10)
Furfural	21.02	1459	Bread, almond, sweet	-	(2.28±0.28)	(1.91±0.01)
1-Octen-3-ol	21.61	1478	Mushroom	-	(4.48±0.22)	-
Thujone	22.30	1499	Thujonic	(5.91±0.28)	(7.26±0.09)	(11.09±2.68)
Benzaldehyde	23.53	1540	Almond, burnt sugar	(3.79±1.25)^{ab}	(1.58±0.37)^{b}	(6.34±0.66)^{a}
1-Octanol	24.58	1575	Oily, aldehyde	-	-	(2.33±0.15)
2-Furanmethanol	25.08	1592	Burnt	(9.00±0.72)	(8.91±2.28)	(8.62±1.38)

Metoxyphenyl oxime	28.20	1732	Wet towel	-	-	(2.82±0.31)
α-Terpineol	28.84	1755	Oil, aniseed, mint	(5.54±0.20)	(4.99±0.51)	-
2-Metoxy-4-vinylphenol	39.37	2142	Clove, curry	(1.41±0.07)[a]	-	(0.57±0.03)[b]

Results are expressed as mean±standard error. Means followed by different letters within the same row are significantly different ($p<0.05$)

Table 5. Aroma active compounds of the PMA (GC-O)

Aroma description	Calculated RI	Reference RI	Compound	Identification	DPMA	BPMA	VPMA
Butter	632	593	2,3-Butandione	O, RI, MS	0.65	0.75	0.65
Sulfurous	705	711	Methyl thiocyanate	O, RI, MS	0.65	-	-
Green, grass	825	801	Hexanal	O, RI, MS, STD	3.00	3.50	4.00
Sour, pungent	844	847	Isopropyl butyrate	O	0.65	-	-
Medicinal	894	868	2-Methyl-3-furanthiol	O, RI	2.25	0.40	1.00
Flower	922	-	Unknown	O	-	-	0.40
Gasoline	927	893	Styrene	O, RI	1.75	4.50	4.50
Boiled potato	934	909	Methional	O, RI, STD	1.75	1.00	2.00
Popcorn, rice	951	930	2-Acetyl-1-pyrroline	O, RI, STD	4.00	4.50	5.00
Fresh	963	1000	Methyl hexanoate	O	-	0.50	-
Rubber	999	974	2-Octanone	O, MS	-	-	2.00
Mushroom	1004	977	1-Octen-3-one	O, RI	6.50	7.00	4.00
Geranium, metal	1010	983	(Z)-1,5-Octadien-3-one	O, RI	7.00	6.50	5.00
Fresh, flower	1031	1036	Benzyl alcohol	O, RI, MS	3.00	2.00	2.50

Dirty, oxide	1084	1087	Durene	O, RI	5.00	5.50	4.00
Fat	1089	1100	3-Nonenal	O, RI	1.50	-	-
Dirty, burnt	1123	-	Unknown	O	-	5.00	-
Dirty	1147	-	Unknown	O	-	0.75	-
Fresh	1158	-	Unknown	O	-	0.50	-
Fat	1181	1192	2-Pentylpyridine	O, RI	-	0.50	-
Cucumber	1187	1187	2-Nonanol	O	-	1.25	-
Hay	1193	1162	(E)-2-Nonenal	O, RI, MS	2.00	2.50	2.00
Fat	1268	1263	Decanol	O, RI, MS	-	-	0.25
Fish market	1281	-	Unknown	O	-	1.00	-
Dirty, oxide	1350	1373	Decanoic acid	O	-	-	1.25
Fresh	1353	1329	Ethylhydroxyhexanoate	O	0.25	0.50	-
Fat	1367	1333	4-Oxodecanal	O	-	1.75	-
Sweet	1367	1350	2-Undecenal	O, RI	0.50	-	-
Hay	1374	-	Unknown	O	-	1.00	-

The results represent the olfactory intensity on a 10-point scale, where 0=none or not perceptible intensity, and 10=extremely high intensity. O=olfactory identification, RI=retention indices matched to the NIST database and literature, STD=authentic standard compounds which were analyzed at the same chromatographic conditions, MS=mass spectrophotometric identification