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Original Scientific Paper

Utilization of pumpkin seed protein isolate as a phosphate replacer in model meat emulsions: effects on chemical and techno-functional properties

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A B S T R A C T

This study aimed to evaluate the potential of pumpkin seed protein isolate (PSPI) as a natural alternative to phosphates in model meat emulsions. PSPI was extracted from cold-pressed pumpkin seed oilcake using an alkaline solubilization and acid precipitation method. Model meat emulsions were formulated by replacing sodium tripolyphosphate (STPP) with PSPI at three levels (1%, 1.5%, and 2%) and compared with a phosphate-containing control group. Chemical composition (moisture, fat, protein, ash) and techno-functional properties, i.e., pH, water-holding capacity (WHC), total expressible fluid, expressible fat (EFAT), jelly-fat separation, and cooking yield were evaluated. Results showed that the addition of PSPI significantly reduced fat content and increased protein content in comparison to the control. The control sample exhibited the highest WHC and the lowest fat and fluid separation. However, increasing levels of PSPI improved WHC and reduced EFAT values, with the 2% PSPI group exhibiting emulsion stability comparable to that of the control group. In line with the stability analyses, the highest cooking yield was observed in the control group, while the lowest value belonged to the P1 group. Although the functional performance of PSPI was slightly lower than that of STPP in some parameters, PSPI demonstrated notable potential in maintaining key quality attributes, such as moisture retention and emulsion stability. In conclusion, PSPI can serve as a functional and natural alternative to phosphates in meat emulsions, contributing to the development of healthier and clean-label meat products.

1. Introduction

Phosphates, commonly used in meat products as sodium and potassium salts of phosphoric acid, serve to enhance water-holding capacity, stabilize pH, control lipid oxidation, and improve texture and flavor in processed meats (*Thangavelu et al.*, 2022). Nevertheless, in recent years, changing dietary habits and the increasing awareness of consumers have led to a growing recognition of the relationship between food and health, resulting in an increased demand for "clean label" products. Consequently, phosphates used in meat products are being replaced with various natural alternatives, such as potato starch and rice starch (*Resconi et al.*, 2016), winter mushroom (*Choe et al.*, 2018), *Jerusalem artichoke* powder and sodium carbonate (Öztürk & *Serdaroğlu*, 2018), eggshell calcium powder and low methoxyl pectin (*Kavuşan et al.*, 2021), and seaweed powder dietary

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fiber (*Yuan et al*., 2023), in different kinds of meat products.

Plant-based proteins offer considerable health benefits due to their high nutritional value, low fat absorption, and rich content of branched-chain amino acids (*Xiao et al.*, 2023). They are widely employed as gelling agents to improve the texture of meat products, while their surface-active properties facilitate emulsification and stabilization of fats in processed meats, such as sausages and meatballs (*Ma et al*., 2022). The incorporation of plant-derived proteins, such as pea, flaxseed, pumpkin seed, and sunflower seed proteins, into food products has steadily increased (*Kumar et al.*, 2022; *Langyan et al*., 2022).

Pumpkin seed meal, obtained as a by-product from the extraction of pumpkin seed oil, has attracted attention due to its high protein content of approximately 66.54% *(Gao et al*., 2022a). Pumpkin seed protein is effective as natural functional ingredient in emulsified meat products to improve emulsification, texture, cooking yield, and oxidative stability (*Gao et al*., 2022b; *Tomczyńska-Mleko et al*., 2023; *Rong et al*., 2025). Despite these benefits, studies on the use of pumpkin seed protein isolate (PSPI) in meat products remain limited (*Gao et al*., 2022b; *Baig et al*., 2023). The use of plant protein isolates, such as soybean in chicken sausages (*Muguruma et al*., 2003), pea in beef sausages (*Gomezulu et al*., 2022) and in hybrid meat products (*Wang et al*., 2025), and chickpea in pork meat batters (*Broucke et al*., 2025), as phosphate replacers has been investigated. There is no research available on the use of PSPI for this purpose. Accordingly, this study aims to address the existing gap in the literature by investigating the potential of PSPI as a phosphate replacer. For this purpose, the effects of incorporating PSPI at different levels (1%, 1.5%, and 2%) are based on the equivalent amount of sodium tripolyphosphate (STPP) on the chemical and techno-functional properties of model system meat emulsions.

2. Materials and methods

2.1. Materials

Pumpkin seed (*Cucurbita pepo*) oilcake obtained by cold pressing was kindly donated by Mecitefendi Herbal Products Food Industry and Trade Limited Company (Izmir, Turkey). Beef (72.21% moisture, 22.1% protein, 4.5% fat, 1.19% ash, pH: 5.6) and beef fat were purchased from Dincer Meat & Steakhouse (Tekirdag, Turkey). All the chemicals were of analytical grade (Sigma-Aldrich Chemie GmbH, Germany) and used without purification.

2.2. Production of pumpkin seed protein isolate

PSPI was prepared with modifications based on the method of *Boye et al*. (2010). Pumpkin seed oilcake was milled (60 mesh) and defatted with hexane (1:5, w/v). The defatted flour was dispersed in water (1:20, w/v), homogenized (UltraTurrax, 12000 rpm, 5 min), and the pH was adjusted to 8.5 using 1 M NaOH. After stirring (25 °C, 200 rpm, 65 min), the suspension was centrifuged (4000 rpm, 15 min, 25 °C). The supernatant was acidified to pH 4.5 (1 M HCl) and centrifuged again. The resulting protein precipitate was washed twice (distilled water, 1:2, w/v), freeze-dried (-55 °C, 0.250 mBar), and stored at -20 °C until use. The chemical composition of PSPI was 7.47% moisture, 85.45% protein, 5.72% fat, and 1.36% ash.

2.3. Production of model system meat emulsion and experimental design

For the model meat emulsions, the control (C) sample was prepared using 500 ppm sodium tripolyphosphate (STTP), while the other treatment groups were formulated with 1% (P1), 1.5% (P2), and 2% (P3) PSPI as a replacement for STTP (Table 1). Model meat emulsions were produced according to *Yüncü-Boyacı et al*. (2024). Firstly, beef and beef fat were minced separately using a 3 mm plate grinder (Arnica, Turkey). Minced meat was then homogenized in a Thermomix (TM5, Vorwerk, Germany) at 500 rpm for 1 min. Curing agents (salt, sodium nitrite) were added and mixed at 500 rpm for 2 min. Fat, PSPI, and/or STPP, and half of the ice were added and emulsified at 1100 rpm for 3 min, followed by the addition of the remaining ice and further emulsification for another 3 min. The final batter was homogenized at 2000 rpm for 1 min, maintaining the temperature below 10 °C throughout. Portions (30 g) were filled into 50 mL polypropylene tubes and centrifuged (Nüve NF 400, Turkey) at 2500 g for 15 min to eliminate air bubbles. Model emulsions were cooked in a water bath at 70 °C for 30 min, then immediately cooled. All analyses were conducted within 72 h of production.

Table 1. Formulation of model meat emulsions

Ingredients (%)	Treatments*			
	C	P1	P2	P3
Beef	68	68	68	68
Beef fat	20	20	20	20
Ice	10	10	10	10
Salt	1.5	1.5	1.5	1.5
Sodium ripolyphosphate (STPP)	0.5	-	-	-
Pumpkin seed protein isolate (PSPI)	-	1	1.5	2
Sodium nitrite	0.015	0.015	0.015	0.015

*C: Model meat emulsions containing sodium tripolyphosphate (500 ppm). P1: Model meat emulsions produced using 1% PSPI. P2: Model meat emulsions produced using 1.5% PSPI. P3: Model meat emulsions produced using 2% PSPI.

2.4. Analyses

Moisture and ash content were measured according to the *AOAC* (2012). Fat content was determined by the chloroform-methanol extraction according to *Flynn and Bramblett* (1975). Crude protein was determined using the Kjeldahl method by multiplying 6.25 (nitrogen-to-protein conversion factor) (*Morr*, 1981). Triplicate measurements of pH values were conducted using a WTW pH 3110 set 2 pH meter from Germany. Water holding capacity (WHC), total expressible fluid (TEF), and expressible fat (EFAT) were determined in triplicate according to *Hughes et al.* (1997) with some modifications. The amounts of separated gel and fat in the meat emulsions were determined using the method of *Bloukas and Honikel* (1992). The cooking yield (CY) was calculated based on the methodology established by *Murphy et al.* (1975). The impact of PSPI on chemical composition and techno-functional properties of model meat emulsions was assessed using analysis of variance (ANOVA) followed by Duncan's post-hoc tests in the SPSS software.

3. Results and discussion

3.1. Chemical composition

Table 2 presents the moisture, fat, protein, and ash contents of the meat emulsion systems. Moisture values indicated that the C, P1, and P3 groups had the highest moisture content, while the P2 group showed a comparatively lower moisture content (62.28%) ($P<0.05$). This situation is explained by the high protein content in this group. The fat content of treatments decreased with the addition of PSPI regardless of the utilization ratio ($P<0.05$). This finding is consistent with the results reported by *Wang et al.* (2025), who studied the reduction of phosphate levels using PSPI. Besides, no statistical differences were observed between P2 and P3 ($P>0.05$). In contrast to the fat contents, the addition of PSPI to the formulation led to an increase in protein content ($P<0.05$); however, no statistical difference was observed between the P2 and P3 ($P>0.05$). This finding can be attributed to the higher protein content of the PSPI (ort protein içeriği). The protein content reported by *Gao et al.* (2022a), in a study utilizing PSPI, was 15.22%, which is lower than the values observed in the current study. Similar to our results, it was determined that the fat content of low-fat duck meat sausages with added soy protein isolate decreased compared to the control group (*Moirangthem et al*., 2022). The C group exhibited the highest ash content (2.25%), while the P3 group (1.81% ash) showed the second highest value among all treatments ($P<0.05$). On the contrary, some researchers have reported that the addition of pea protein isolate or rice protein to beef patties, and of soy protein isolate to low-fat duck meat sausages, did not result in a significant difference in ash content compared to the control groups (*Baugreet et al.,* 2016; *Moirangthem et al*., 2022). The pH values, which have a significant impact on the technological properties of meat products, are presented in Table 2. The pH value of the treatments decreased with the addition of PSPI to the meat emulsions, and the highest pH value was observed in group C containing STPP ($P<0.05$). On the other hand, no statistical differences were observed between the P2 and P3 groups ($P>0.05$). In a study by *Öztürk and Turhan* (2020), pumpkin seed kernel flour was used as a fat replacer in beef meatballs, and the pH values ranged from 5.98 to 6.22. These values are consistent with those obtained in the present study.

Table 2. Chemical composition and pH value of model meat emulsion batters

Treatments*	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	pH
C	64.14±0.72[a]	20.39±0.99[a]	12.56±1.31[c]	2.25±0.22[a]	6.20±0.01[a]
P1	65.61±0.95[a]	16.22±0.20[b]	16.86±0.66[b]	1.31±0.48[b]	5.90±0.01[b]
P2	62.28±0.14[b]	14.85±0.37[c]	21.42±0.35[a]	1.45±0.11[b]	5.88±0.01[c]
P3	64.11±1.08[a]	14.18±0.70[c]	19.90±0.96[a]	1.81±0.01[ab]	5.88±0.01[c]

*C: Model system meat emulsion containing sodium tripolyphosphate (500 ppm), P1: Model system meat emulsion formulated with 1% pumpkin seed protein isolate, P2: Model system meat emulsion formulated with 1.5% pumpkin seed protein isolate, P3: Model system meat emulsion formulated with 2% pumpkin seed protein isolate. [a-d] Different letters in the same row indicate a significant difference (P < 0.05).

3.2. Techno-functional properties

Water-holding capacity (WHC) is a vital quality parameter in the meat industry, as it directly influences tenderness and juiciness—two essential attributes that determine consumer acceptance of meat products (*Yüncü-Boyacı et al.*, 2024). The highest WHC was observed in the C group (81.55%), which contains STPP (P<0.05). In the other groups, a significant increase in the proportion of PSPI incorporated into the formulation resulted in a progressive enhancement of the WHC values of the meat emulsions (P<0.05). In a study conducted by *Helikh and Yongfeng* (2025), the WHC was reported as 74.2%, which closely aligns with the findings of the present study that utilized PSPI. TEF values ranged between 15.40% (C) and 29.92% (P1). These results indicate that the TEF value of treatments increased with the addition of PSPI (P<0.05). Similarly, in a study by *Wang et al.* (2025), where phosphate was replaced with modified chickpea protein in pork emulsions, the total released fluid ranged from 13.29% to 21.79%, and consistent with the present findings, the control group containing phosphate showed the lowest fluid release. Similar to the TEF value, the highest EFAT value (6.24%) among the experimental meat models was observed in our P1 group, which contained 1% PSPI (P<0.05). On the other hand, the lowest values (ranging from 2.73% to 3.23%) were recorded in the C and P3 groups (P<0.05). Therefore, it can be concluded that incorporating PSPI at levels above 2% improves emulsion stability, exhibiting an effect comparable to that of STPP. Jelly and fat separation of meat emulsions were significantly affected by the replacement of STPP with PSPI (Table 3). While the lowest value was observed in the C group, reformulated samples had higher values (P<0.05). Besides that, no statistical differences were observed between the reformulated samples (P>0.05). This result was consistent with the WHC, TEF, and EFAT values. Compared to the study conducted by *Helikh and Yongfeng* (2025), which involved the addition of PSPI, the fat holding capacity (73.8%) reported in their work was higher than that observed in the present study. In terms of cooking yield, the highest value was observed in our control group with 85.28% (P<0.05). In the groups formulated with PSPI, cooking yields decreased, with the lowest cooking yield detected in the P1 group at 63.09% (Table 3). This indicates that increasing PSPI levels led to a partial improvement in cooking yield.

Table 3. Techno-functional properties of model system meat emulsions

Treatments*	WHC (%)	TEF (%)	EFAT (%)	Jelly and Fat Separation (%)	Cooking Yield (%)
C	81.55±0.92[a]	15.40±0.63[c]	2.73±0.14[c]	9.50±1.00[b]	85.28±1.48[a]
P1	72.45±1.01[d]	29.92±1.47[a]	6.24±0.57[a]	21.25±0.25[a]	63.09±0.87[c]
P2	74.34±0.92[c]	26.23±0.29[b]	4.88±0.48[b]	20.83±1.26[a]	68.50±0.90[b]
P3	75.99±0.38[b]	25.01±0.56[b]	3.23±1.11[c]	22.04±0.71[a]	68.10±0.69[b]

Legend: *C: Model system meat emulsion containing sodium tripolyphosphate (500 ppm), P1: Model system meat emulsion formulated with 1% pumpkin seed protein isolate, P2: Model system meat emulsion formulated with 1.5% pumpkin seed protein isolate, P3: Model system meat emulsion formulated with 2% pumpkin seed protein isolate. WHC: Water holding capacity; TEF: Total expressible fat, EFAT: Expressible fat. [a-d] Different letters in the same row indicate a significant difference (P < 0.05).

4. Conclusion

The findings of this study demonstrate the potential of PSPI as a promising phosphate replacer in meat emulsions. Incorporating PSPI at increasing levels led to significant reductions in fat content and increases in protein content. However, replacing sodium tripolyphosphate (STPP) with PSPI resulted in decreased WHC, TEF, cooking yield, and jelly and fat separation values in the model meat emulsions. Although the reformulated samples showed slightly lower performance than the phosphate-containing control group in some functional parameters, the use of 2% PSPI exhibited comparable effects to STPP, particularly in terms of TEF. These results highlight the potential of PSPI to serve as a clean-label alternative to phosphates in meat products. Further studies may focus on optimizing PSPI functionality through protein modification techniques and evaluating its effects on sensory attributes and shelf-life characteristics in real product formulations.

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