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Technological Properties of Model System Beef Emulsions with Encapsulated Pumpkin Seed Oil and Shell Powder

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The aim of this research was to examine the technological properties of beef emulsions in which fatty tissue was partially substituted with pumpkin seed oil (PSO) encapsulated in alginate or pectin matrix, and where phosphates (F treatments) were simultaneously substituted with shell powder (C treatments).

Fat replacement (in the amount of 25%) mostly had no significant influence on pH, cooking loss, purge loss, fluid release under pressure, residual nitrite level, and texture properties. On the other hand, higher yellowness and hue angle were observed when backfat was replaced with encapsulated PSO, but only in treatments with phosphates. The use of shell powder as a phosphate replacer led to significantly higher pH values and thus to significantly higher residual nitrite level: 70.87–74.64 mg/kg (C treatments) *vs*. 56.79–62.16 mg/kg (F treatments). The nitrite depletion rate during the seven-week storage was lower in C treatments. Moreover, higher lightness, yellowness and hue angle could be expected, as well as lower hardness, springiness, cohesiveness and chewiness.

For the most part, seven-week storage had no influence on the observed technological properties, except on colour properties in which an opposite trend was observed in terms of yellowness – increase in treatments with phosphates and decrease in treatments with shell powder.

Further research, which would include sensory analysis, should be conducted to determine how these altered colour and textural properties will be perceived by consumers.

INTRODUCTION

For centuries the purpose of meat products was to extend the viability of meat, which is why they were an important source of proteins and energy. Over the past fifty years, the availability of fresh meat has increased, therefore the specific sensory characteristics of meat products have become more important.

Emulsion-type meat products (frankfurters, wieners, bologna, mortadella) are worldwide popular comminuted meat products. These ready-to-serve meat products are made by comminuting and mixing chopped meat, fatty tissue and water/ice. Therefore, their specific technological properties (*e.g.* emulsion formulation and stability, water binding properties, instrumental colour and texture...) which correlate to sensory characteristics, are attributable to the protein/fat/moisture ratio. Furthermore, ingredients such as salt, phosphates, nitrites *etc*. are of significant importance for the formation of these technological (and in turn sensory) properties and their stability.

Salt and phosphates promote the extraction of myofibrillar proteins and contribute to the emulsifying process and emulsion stability. Phosphates exhibit a synergistic effect with salt, improve the water holding capacity of emulsion-type meat products and thus increase the processing yield, reduce storage loss and improve product texture, tenderness, and juiciness [Sebranek, 2009]. However, research indicates that acutely high phosphorus intake affects bone metabolism by decreasing bone formation and increasing bone resorption [Kemi *et al.,* 2007]. Furthermore, nowadays people are showing a greater interest in foods that contain bioactive or functional components [Hygreeva *et al.,* 2014], and the demand for natural, organic and/or clean label meat products has also increased. In that sense, sporadic research studies were conducted with natural calcium powders as phosphate substitutes [Cho *et al.,* 2017].

Fats play an important role in the formation and stabilization of meat emulsions (therefore technological properties)

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and sensory properties of emulsion-type meat products – flavour, juiciness, hardness, and mouthfeel [Choi *et al.,* 2009; Hygreeva *et al.,* 2014]. Pork backfat is the most interesting of fatty tissues due to its technical properties that have a beneficial influence on technological properties of meat products (emulsion formulation and stability, instrumental colour and texture...), which correlates to some sensory characteristics [Ospina-E *et al.,* 2010]. Also, due to its technological properties, beef is slightly preferable to pork [Mittal, 2005]. However, high SFA content and low PUFA content (especially *n*-3 PUFA) are a characteristic of fatty tissue and meat lipids that has been associated with a higher risk of some chronic disorders (*e.g.* cardiovascular diseases).

In general, technological strategies aimed at improving the fatty acid profile entail the replacement of animal fat with a different lipid which would be more in line with health recommendations [Jimenez-Colmenero *et al.,* 2015]. In that sense, a partial replacement of fatty tissue with oils (olive, flaxseed, grape seed, canola, cotton seed, hazelnut, soy, rapeseed, fish) was used to improve the FA profile of different types of meat products [Hygreeva *et al.,* 2014; Jimenez-Colmenero *et al.,* 2015]. Pumpkin seed oil is not used so often as a fatty tissue replacer in meat products as other oils, though it has good nutritional and technical properties. It is rich in oleic and linoleic acids, Δ5- and Δ7-sterols, tocopherols and other bioactive compounds [Montesano *et al.,* 2018; Rezig *et al.,* 2012]. The most beneficial health effect of pumpkin seed oil is that it prevents the growth and reduces the size of the prostate, and it was also associated with reducing cancer and heart disease risk [Montesano *et al.,* 2018]. Due to its chemical composition, it is oxidative-stable at temperatures used in the processing of emulsified-type meat products.

The application of the above-mentioned oils in the modern food preservation industry is quite limited due to difficulties caused by their unstable nature, volatility, rapid evaporation, and degradation under regular conditions. Hence, in order to obtain more stable products, oils were added as an emulsion system, gelled emulsion system or encapsulated. Encapsulation technology could be used as an efficient way of entrapping oils and preventing their degradation and undesirable interactions with the food matrix [Nedović *et al.,* 2013]. Therefore, high PUFA oils can be protected from oxidation in different food systems by different encapsulation techniques, and alginate and pectin are acceptable encapsulating/coating materials commonly used for this purpose [Nedović *et al.,* 2013].

Since the amount of fatty tissue and the presence of phosphates are of significant importance for the formation of meat emulsions and their (storage) stability, and thus for the sensory properties of emulsion-type meat products, the substitution of fatty tissue with high PUFA containing oils in meat emulsions without phosphates poses quite a challenge.

The aim of this research was to examine the technological properties of beef emulsions in which fatty tissue was partially substituted with encapsulated pumpkin seed oil and where phosphates were simultaneously substituted with shell powder. The stability of these emulsions during 7-week cold storage and the extent of changes were also observed.

MATERIAL AND METHODS

Emulsions preparation

Six different meat emulsions were prepared in order to examine the influence of phosphate substitution with shell powder and fat reduction by pumpkin seed oil (PSO) encapsulated in the calcium alginate and pectin matrices. Two groups of meat emulsions were prepared, each comprising three different meat emulsions. The first was emulsified using a commercial polyphosphate mixture (F emulsions) and the second using shell powder (C emulsions). The pH value of 0.1% (w/w) shell powder water solution (t=20.5°C) was 12.04; whereas the pH value of 0.5% (*w/w*) phosphates mixture water solution (t=20.5°C) was 7.60. Since amounts higher than 1 g/kg of shell powder increased the pH of raw emulsions to around 7 and higher, which will almost suspend colour formation and increase the possibility of spoilage [Feiner, 2006; Shahidi *et al.,* 2014], these emulsions were excluded from measurements. In both groups, ¼ of fatty tissue in two meat emulsions was substituted by PSO encapsulated in the calcium alginate (ALG emulsions) and pectin matrices (PEC emulsions). The full formulations of all meat emulsions are presented in Table 1.

Model system meat emulsions were prepared as follows: fresh beef (round muscles) and pork backfat were bought at a local store, the visible fat and connective tissue were trimmed off the meat and cut into small pieces kept frozen at -20°C until use. Before use, meat and backfat were tempered for 16 h in a cooling chamber (at 0–2°C) to a temperature between -2 and 0°C. Then, they were separately grounded through a 5 mm plate (82H, Laska, Traun, Austria) and weighed. Meat emulsions were prepared according to the following procedure. Ground and weighed meat and backfat were put in a Thermomix TM31 (Vorwerk Elektrowerke GmbH & Co. KG, Wuppertal, Germany). Ice cooled water (0–3°C) with previously dissolved ingredients was added and mixed with meat and backfat using a spoon. Then, the mixture was emulsified for 15 s at a low blender speed, followed by 45 s at the highest speed (10,200 rpm). After that, the encapsulated pumpkin seed oil was added (ALG or PEC treatments) and stirred for 15 s. Only batters with the temperature below 12°C were taken for further processing.

Emulsions were then stuffed in pre-weighted plastic tubes (50 mL, d=27 mm; approximately 50 g each), sealed and centrifuged at 3,000×*g*, 4°C, 90 s (Eppendorf, Centrifuge 5430R, Eppendorf AG, Hamburg, Germany) to eliminate air bubbles. The tubes were heated in a water bath at 80°C until 70°C in the centre was reached (15 min), then cooled and stored in the dark at 3±1°C overnight. Afterwards, all tubes were tempered at room temperature for 1 h, then the content was taken out and wiped with paper towels.

One third from each treatment was examined (day 0) while the remaining ⅔ were vacuum-packed (two in each package) in coextrusive, barrier bags (PA/PE/PE; 85 lm thick, dimensions 200 mm × 350 mm) using a tabletop vacuum machine (MVS 35x; Minipack-Torre SpA, Dalmine, Italy), and stored at 3±1°C for 42 days. During storage, analyses were conducted on day 21 and day 42. Two replications of the experiment were conducted on different days.

TABLE 1. Formulation of model system beef emulsions.

	Phosphate			Shell powder		
	CONF	ALGF	PECF	CONC	ALGC	PECC
Beef meat	500	500	500	500	500	500
Backfat	200	150	150	200	150	150
Water	300	300	300	300	300	300
Encapsulated pumpkin seed oil calcium alginate matrix	/	50		/	50	/
Encapsulated pumpkin seed oil pectin matrix	/	/	50	/	/	50
Nitric salt*	18	18	18	18	18	18
Phosphate mixture**	5	5	5	/	/	/
Shell powder***	/	/	/	1	1	1
Na-isoascorbate	0.5	0.5	0.5	0.5	0.5	0.5

*with 0.6% of $NaNO_2$; ** polyphosphate commercial mixture (sodium tripolyphosphate and disodium pyrophosphate, P_2O_5 content *ca*. 60%); *** Purifact-XTEND 15, Purifact B.V., Roosendaal, NL (CaO content >91%).
CON – treatments with all pork backfat; ALG – treatments with 25% of encapsulated pumpkin seed oil in alginate matrix; PEC – treatments with 25% of encapsulated pumpkin seed oil in pectin matrix; F – treatments with phosphates; and C – treatments with shell powder.

The encapsulation of PSO in the calcium alginate and pectin matrices using electrostatic extrusion was realized according to the procedure described by Stajić *et al.* [2014], with some modifications. The first step in the encapsulation process was the preparation of a shell solution by dissolving sodium alginate (Sigma-Aldrich, St. Louis, MO, USA) or pectin powder (Cargill, Wayzata, MI, USA) in distilled water (0.02 g/mL). Alginate (Pectin)/Oil emulsion (20%, *w/w*) was prepared using Ultra-Turrax T25 (T25 digital ULTRA-TURRAX®, IKA, Germany) at a speed of 10,000 rpm for 5 min. The emulsion was extruded through blunt stainless still needle (22 gauge) using a syringe pump under a constant flow rate of 50 mL/h. Electrostatic potential (5.0 kV) was formed by a high voltage dc unit (Model 30R, Bertan Associates, Inc., New York). The collecting solution was calcium chloride (0.02 g/mL, Analytika, Czech Republic). The distance between the needle tip and the collecting solution was 2.5 cm. After extrusion, the beads were left in the collecting solution for 30 min. After the gelling period, microbeads (Figure 1) were rinsed with distilled water.

Methods

For each treatment, six samples were weighed after stuffing and after wiping the cooked and cooled emulsions, and the weight loss was calculated by the difference between these measurements and expressed as a percentage.

During storage (days 21 and 42), six vacuum packages were used for determining purge loss. The package content (two cooked tube contents) was taken out, wiped with paper towels, and weighed. Purge loss was reported as a percentage of the initial weight (day 0). The samples were then used for further analysis.

On days 0, 21, and 42, pH values, fluid release under pressure (FRP), nitrite content [ISO2918:1975], instrumental colour on cross section, and texture profile were determined. Samples were held for equilibration to room temperature for 1 h before measurements were taken.

Eight samples (one per tube content) from each treatment were used for pH value measurements with an HI 83141 pH-meter (Hanna Instruments, Sarmeola di Rubano, Italy) equipped with a penetration probe, which was calibrated with standard buffer solutions (pH=4 and pH=7) before each measurement.

Regarding FRP, on days 0, 21, and 42, one sample height 10±0.5 mm (original diameter) was taken from six cooked tube contents of each treatment, weighed, and compressed between two filter papers (dried at 103°C, 30 min and cooled at room temperature in exicator), using the weight of 200±2 g for 5 min. After that, the sample was removed and both papers were measured. The amount of the released fluid relative to initial sample weight represents fluid release under pressure (expressed as %).

Sixteen samples (eight cooked tube contents with two cross-sections samples) were used for instrumental colour measurement using the Computer vision system (CVS) as described by Tomasevic *et al.* [2019]. Three readings were taken from each cross-section from RAW photographs and 5 × 5 pixels measuring area, using a Photoshop Average Colour Sampler Tool. Average values of these measurements were calculated and used as one iteration for statistical analysis. C* (chroma) and h (hue angle) were calculated using the following equations:

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \text{ and } h = \arctan b^*/a^*$$

Total colour difference (TCD), relative to CONF, was determined on days 0, 21, and 42 using the standard equation:

$$TCD = \sqrt{(L^*_X - L^*_{CONF})^2 + (a^*_X - a^*_{CONF})^2 + (b^*_X - b^*_{CONF})^2}$$

where: X – treatments (ALGF, PECF, CONC, ALGC, PECC).

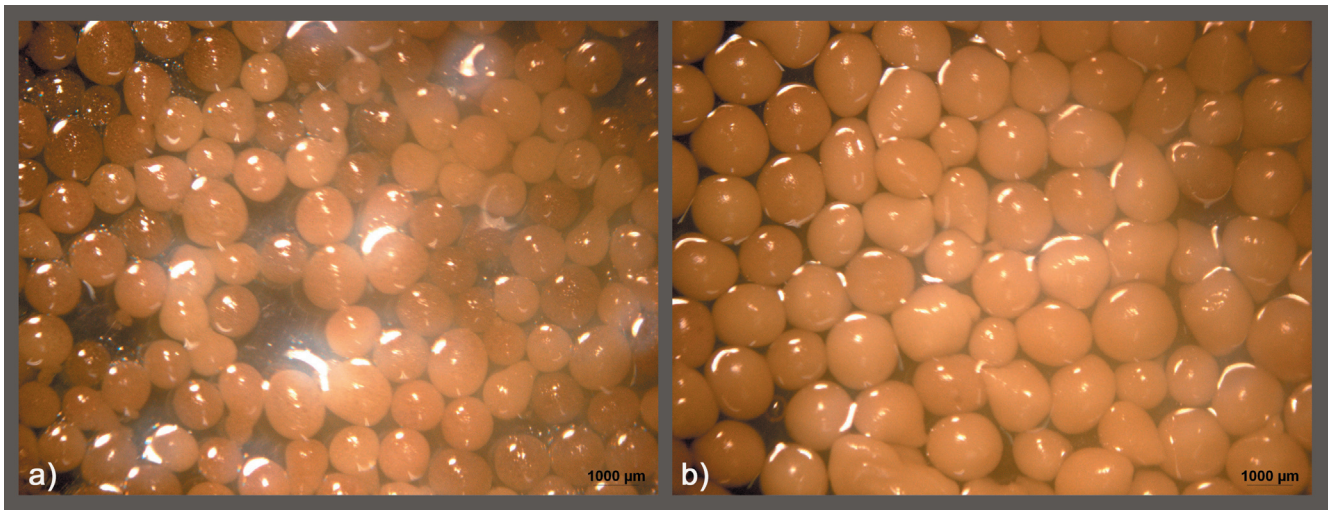

FIGURE 1. Visual appearance of encapsulated pumpkin seed oil: a) in alginate matrix; b) in pectin matrix.

Texture profile analysis was performed in the same manner as described by Stajić *et al.* [2018]. Using the available computer software, the fallowing texture parameters were obtained [Bourne, 2002]: hardness (g): the height of the force peak on the first compression cycle; adhesiveness (g × s): the work necessary to pull the compressing probe away from the sample (the negative force area of the first bite); cohesiveness (dimensionless): the ratio of the positive force areas under the first and second compressions; springiness: the ratio that the sample recovered its height after first and before second compression cycle; and chewiness (g): calculate as hardness × cohesiveness × springiness and represents work required to masticate a sample before swallowing. Twelve samples (six cooked tube contents with two sample cores, d=16 mm, h=12 mm) were used.

Statistical analysis

Two-way ANOVA was used to evaluate the effect of additive replacement, fat replacement, and its interaction. Statistica 12.5 (StatSoft, Inc., Tulsa, OK, USA) software was used to perform statistical analysis. Differences between means were determined using Tukey's HSD test at the significance level of $p<0.05$, while during the storage period they were determined using Repeated measures ANOVA.

RESULTS AND DISCUSSION

Changes of pH values

The use of shell powder as a phosphate replacer had a significant influence on pH values of meat emulsions (Table 2). Before cooking, all treatments with phosphates (F treatments) had significantly lower pH values relative to equivalent treatments with shell powder (C treatments). After cooking, pH values increased in all F treatments, while the opposite effect was observed in C treatments. When meat is subjected to heat treatments at 70–80°C, pH increases because of the reduction of free acidic groups of proteins and because of liberation cations (Ca^{2+} and Mg^{2+}) [Medyński *et al.*, 2000]. In sausage batters with phosphates, heating can increase pH value by 0.0 to 0.4 pH units [Puolanne *et al.*, 2001]. Medyński *et al.* [2000] stated that due to limitations in the reactivity of meat proteins with increased amount of lactic acid, pH value after cooking can decrease when lactic acid was added in meat. It is possible that a similar limitation can occur when a higher amount of calcium ions was added into the meat batter, especially assuming that the lower hardness of C treatments can contribute to poorer solubilisation (activation) of myofibrillar proteins when shell powder was added. The texture section covers this in more detail.

The results indicated a significant impact of additive replacement on pH values on day 0 and throughout storage, while fat replacement (and encapsulating agents as well) and interaction of factors had a sporadic no significant impact. During storage, a slight increment of pH values was observed (up to 0.09), significantly only in CONF and ALGF, but without an influence on the relations within treatments. Though significant differences were found, the pH values of all model system emulsions, on day 0 and throughout storage, were within the range for the emulsified-type of sausages made of beef [Vural & Javidipour, 2002], pork/beef [Yotsuyanagi *et al.,* 2016], and goat/beef [Stajić *et al.,* 2020].

Lee *et al.* [2011] reported that different levels (0.15–0.5%) of oyster shell powder as phosphate substituent, increased pH values in emulsified pork sausages up to the values slightly above 7. Similarly, Cho *et al.* [2017] reported significantly higher pH values in ground pork meat products when replacing 0.3% of phosphates with 0.5% of oyster shell powder. Choi *et al.* [2014] also reported a significant increase of pH values (0.5–0.7) when replacing 0.3% of phosphates with different levels (0.15–0.5%) of oyster shell powder and 0.5% of whey protein in restructured pork hams. In contrast to them, Bae *et al.* [2017] found no effect of phosphate (0.3%) replacement with 0.5% oyster shell powder on the pH of ground pork meat products before and after cooking, which differs from the results presented in this research study.

Research data indicate that partial (or even total) fat replacement with different preparations of plant oils could not lead to pH changes in emulsified-type sausages [Pintado *et al.,* 2016a; Salcedo-Sandoval *et al.,* 2015].

TABLE 2. Technological properties of uncooked* and cooked model system beef emulsions.

Technological properties	Storage time	Additive 1			Additive 2			Significance (p)		
		Fat 1	Fat 2	Fat 3	Fat 1	Fat 2	Fat 3			
		CONF	ALGF	PECF	CONC	ALGC	PECC	Additive	Fat	Additive* Fat
pH	Day 0*	5.93±0.02^{b}	5.92±0.02^{b}	5.91±0.04^{b}	6.69±0.06^{ab}	6.64±0.03^{a}	6.74±0.04^{a}	0.0000	0.0028	0.0007
	Day 0	6.06±0.02^{bB}	6.06±0.02^{bB}	6.06±0.02^{b}	6.49±0.03^{a}	6.47±0.05^{a}	6.51±0.06^{a}	0.0000	NS	NS
	Day 21	6.09±0.04^{bB}	6.09±0.02^{bB}	6.09±0.04^{b}	6.51±0.02^{a}	6.47±0.05^{a}	6.52±0.05^{a}	0.0000	NS	0.0476
	Day 42	6.13±0.01^{bA}	6.15±0.02^{bA}	6.12±0.07^{b}	6.52±0.07^{a}	6.51±0.05^{a}	6.52±0.06^{a}	0.0000	NS	NS
Cooking loss (%)	Day 0	3.77±0.30^{ab}	4.12±0.45^{ab}	3.26±0.40^{b}	4.14±0.80^{ab}	4.31±0.57^{a}	4.39±0.53^{a}	0.0034	NS	NS
Purge loss (%)	Day 21	5.04±0.50^{B}	5.48±0.55^{B}	5.54±0.57^{B}	4.67±0.51^{B}	5.15±0.37^{B}	5.13±0.47^{B}	0.0349	0.0385	NS
	Day 42	6.61±1.03^{A}	7.13±0.96^{A}	7.20±0.77^{A}	5.97±0.88^{A}	6.49±0.86^{A}	6.36±0.78^{A}	0.0235	NS	NS
Fluid release under pressure (%)	Day 0	4.14±0.46^{abA}	4.25±0.61^{abA}	3.64±0.38^{b}	4.14±0.69^{ab}	4.89±0.54^{a}	5.09±0.61^{aA}	0.0007	NS	0.0128
	Day 21	3.29±0.39^{bB}	3.40±0.54^{bB}	3.42±0.67^{b}	4.63±0.46^{a}	4.75±0.20^{a}	4.48±0.35^{aAB}	0.0000	NS	NS
	Day 42	3.05±0.31^{bB}	3.13±0.23^{bB}	3.03±0.37^{b}	4.03±0.33^{a}	4.05±0.60^{a}	3.70±0.43^{abB}	0.0000	NS	NS
Nitrite (mg/kg)	Day 0	56.79±2.95^{b}	62.16±2.16^{ab}	61.27±7.09^{ab}	72.98±7.52^{a}	70.87±6.09^{a}	74.64±7.99^{a}	0.0000	NS	NS
	Day 21	44.39±1.69^{b}	50.15±2.74^{b}	50.23±3.85^{b}	66.02±6.20^{a}	67.25±8.47^{a}	70.73±8.30^{a}	0.0000	NS	NS
	Day 42	28.39±1.42^{bc}	27.07±2.09^{c}	30.09±6.77^{abc}	42.76±9.59^{a}	42.55±7.33^{a}	41.12±3.68^{ab}	0.0000	NS	NS

Additive 1 – phosphates (F treatments); Additive 2 – shell powder (C treatments); Fat 1 – all pork backfat (CON treatments); Fat 2 – with 25% of encapsulated pumpkin seed oil in alginate matrix (ALG treatments); Fat 3 – with 25% of encapsulated pumpkin seed oil in pectin matrix (PEC treatments); NS – not significant. $^{a–c}$ Values (mean±SD) in the same row with different superscripts are significantly different ($p<0.05$). $^{A, B}$ Uppercase letters are used for comparing the samples considering the effect of storage. Values in the same column for the same property, with different superscripts are significantly different ($p<0.05$).

Emulsions stability

As mentioned before, phosphates are very important in the production of emulsified meat products. Alkaline phosphates, mostly used in meat processing [Mills, 2014], increase the pH value and facilitate the extraction of myofibrillar proteins, which enhances water binding properties and emulsification process (thus improves processing yield), gel formation during thermal processing (textural properties), and product stability during retail storage. Water binding properties are of great importance for the quality of meat systems, hence cooking loss, purge loss, and fluid release under pressure (FRP) were useful parameters to evaluate this. In general, pH values higher than the isoelectric point of meat proteins increase the water binding properties of meat systems [Mills, 2014]. Moreover, Puolanne *et al.* [2001] reported a maximum water-holding capacity for beef cooked sausages with phosphates (0.25% P_2O_5) for 2% of NaCl added and pH 6.5.

Regarding cooking loss, phosphate replacement had a significant impact. However, despite significantly lower pH values, all F treatments had lower cooking loss relative to the equivalent C treatments, significantly so only between PECF and PECC (and ALGC). Somewhat similarly to these results are the results of Cho *et al.* [2017] who reported significantly higher cooking loss in ground pork products when replacing 0.3% of phosphates with 0.5% of oyster shell powder. Furthermore, Choi *et al.* [2014] reported significantly higher cooking loss in restructured pork hams (despite higher pH values) when replacing 0.3% of phosphates with different levels (0.15–0.50%) of oyster shell powder and 0.5% of whey protein. However, Lee *et al.* [2011] found no significant differences in cooking loss of emulsified meat products when replacing 0.3% of phosphates with different levels (0.15–0.50%) of oyster shell powder and 0.5% of whey protein. Similar relations to those of cooking loss were found for FRP on day 0 – higher values in C treatments relative to equivalent F treatments, but significantly so only between PECC (and ALGC) and PECF.

After 21 days of storage, the amount of purged liquid was higher in F treatments relative to the equivalent C treatments, however no significant differences were found between any of the treatments. The purge loss increased at the end of storage (day 42), significantly in all treatments, however without an influence on the relations between treatments. On the other hand, FRP decreased during storage, significantly so in CONF, ALGF and PECF, which can be correlated with the increase of purge loss – the higher purge loss, the lower (free) water content which can be released by sample compression. Decreases of FRP during storage influenced the relations between treatments – on days 21 and 42 all F treatments (except PEC on day 42) had significantly lower FRP relative to the equivalent C treatments.

Residual nitrite

As shown in Table 2, the residual nitrite level was significantly influenced by phosphate replacement, while fat replacement and interaction of factors had no significant influ-

TABLE 3. Instrumental colour of cooked model system beef emulsions.

Instrumental colour properties	Storage time	Additive 1			Additive 2			Significance (p)		
		Fat 1	Fat 2	Fat 3	Fat 1	Fat 2	Fat 3			
		CONF	ALGF	PECF	CONC	ALGC	PECC	Additive	Fat	Additive* Fat
L*	Day 0	67.65±2.32^{ab}	66.81±1.60^{b}	67.88±1.02^{abA}	69.00±1.36^{aA}	68.69±1.34^{aA}	68.60±1.85^{aA}	0.0002	NS	NS
	Day 21	67.33±2.09	66.21±0.61	65.54±1.56^{B}	67.00±1.94^{B}	65.60±2.57^{B}	66.33±1.31^{B}	NS	0.0076	NS
	Day 42	67.69±0.98^{a}	66.60±1.72^{ab}	67.38±1.26^{abAB}	67.38±1.0^{abB}	66.06±1.66^{bB}	66.75±0.88^{abB}	NS	0.0017	NS
a*	Day 0	10.27±0.68^{C}	9.52±0.38^{B}	9.35±0.99^{B}	10.17±1.01^{B}	9.67±1.26^{C}	9.77±0.94^{C}	NS	0.0076	NS
	Day 21	12.92±0.76^{aB}	11.56±0.48^{bcdA}	12.52±0.60^{bcAB}	10.94±1.18^{dB}	12.63±1.65^{abB}	11.52±1.26^{cdB}	0.0043	NS	0.0000
	Day 42	13.83±1.37^{abA}	11.81±1.53^{cA}	12.85±1.56^{bcA}	12.85±1.77^{bcA}	15.29±1.92^{aA}	14.31±2.59^{abA}	0.0007	NS	0.0000
b*	Day 0	5.90±1.03^{bA}	8.17±1.15^{aC}	8.04±1.17^{aB}	8.85±1.05^{aA}	7.83±1.00^{aA}	8.56±0.66^{aA}	0.0000	0.0017	0.0000
	Day 21	5.02±0.51^{cB}	9.81±1.25^{aB}	10.23±1.78^{aA}	7.79±0.94^{bB}	7.21±1.23^{bA}	7.81±1.25^{bAB}	0.0034	0.0000	0.0000
	Day 42	6.00±1.03^{cA}	11.33±1.19^{aA}	10.48±1.72^{aA}	7.92±0.77^{bB}	7.77±0.70^{bA}	7.35±0.63^{bB}	0.0000	0.0000	0.0000
C*	Day 0	11.88±0.80^{cC}	12.58±0.75^{abC}	12.37±1.17^{abB}	13.49±1.37^{aB}	12.45±1.51^{abC}	13.00±0.98^{abB}	0.0029	NS	0.0112
	Day 21	13.87±0.79^{bcB}	15.19±0.96^{abB}	16.21±1.43^{aA}	13.45±1.27^{cB}	14.55±2.00^{bcB}	13.96±1.43^{bcB}	0.0002	0.0001	0.0167
	Day 42	15.10±1.50^{cA}	16.46±0.68^{abA}	16.69±1.33^{abA}	15.13±1.64^{cA}	17.17±1.86^{aA}	16.12±2.44^{abA}	NS	0.0002	NS
h	Day 0	29.75±4.41^{bA}	40.43±4.39^{aAB}	40.60±4.52^{aA}	41.01±2.06^{aA}	39.03±2.62^{aA}	41.28±2.66^{aA}	0.0000	0.0000	0.0000
	Day 21	21.24±1.92^{dB}	40.17±3.49^{aB}	38.95±4.25^{aA}	35.48±3.48^{bB}	29.62±1.93^{cB}	34.08±4.30^{bB}	NS	0.0000	0.0000
	Day 42	23.41±3.19^{cB}	43.91±6.38^{aA}	39.14±6.55^{aA}	31.84±3.92^{bC}	27.11±2.78^{bcC}	27.64±3.80^{bcC}	0.0000	0.0000	0.0000

Additive 1 – phosphates (F treatments); Additive 2 – shell powder (C treatments); Fat 1 – all pork backfat (CON treatments); Fat 2 – with 25% of encapsulated pumpkin seed oil in alginate matrix (ALG treatments); Fat 3 – with 25% of encapsulated pumpkin seed oil in pectin matrix (PEC treatments); NS – not significant. $^{a–d}$ Values (mean±SD) in the same row with different superscripts are significantly different ($p<0.05$). $^{A–C}$ Uppercase letters are used for comparing the samples considering the effect of storage. Values in the same column for the same property, with different superscripts are significantly different ($p<0.05$).

ence. Regarding partial fat replacement with different plant oil preparations, literature data varied from the reduced residual nitrite content to no effect [Salcedo-Sandoval *et al.,* 2015] as was the case in this research. Since the decrease of pH values increases the reactivity of nitrite [Skibsted, 2011], the significant influence of phosphate replacement on residual nitrite level can be attributed to the higher pH values of treatments with shell powder. The higher pH values could be the reason why nitrite depletion rate (at the end of storage) was lower [Honikel, 2008a] in C treatments [39.96% (ALGC)–44.91% (PECC)] compared to F treatments [50.01% (CONF)–56.45% (ALGF)].

Colour properties

Colour parameters were significantly influenced by phosphate and fat replacement, as well as their interaction (Table 3).

On day 0, C treatments were lighter than F treatments, however significantly only to ALGF. Regarding redness, no significant differences were found between treatments. The pH values are of significant importance for colour formation in meat systems with nitrite. The nitric oxide (NO) formulation (from added sodium nitrite), which reacts with myoglobin and forms nitrosylmyoglobin (NOMb, bright red colour), is pH dependable – lower pH values accelerate NO formation [Sebranek, 2009; Skibsted, 2011]. In meat batters with the usual pH values of 5.5–6, the nitric oxide production is low and is even lower in meat systems with higher pH values [Honikel, 2014]. At pH values above 6.5, NO formulation is almost suppressed [Feiner, 2006]. However, the addition of ascorbic acid / ascorbate accelerates nitric oxide formation and in turn colour formation as well. Furthermore, the reactivity of ascorbic acid / ascorbate increases with increasing pH [Skibsted, 2011] which could be sufficient for the NOMb formulation in meat systems with pH values slightly higher than 6.5, as was the case in this research. This is in line with the research of Glorieux *et al.* [2017] who used different types of phosphates and did not find significant differences in a* values (also in L* and b*) in emulsified-type pork sausages (with nitrite added) with pH values within the interval of 5.70–6.53. Furthermore, Bae *et al.* [2017] found no effect of phosphate (0.3%) replacement with 0.5% oyster shell powder on redness of ground pork meat products.

Phosphate and partial fat replacement resulted in higher b* and h values in all treatments compared to CONF, while significantly higher chroma values were found only in CONC relative to CONF. Bae *et al.* [2017] found no effect of phosphate (0.3%) replacement with 0.5% oyster shell powder on b* values while Cho *et al.* [2017] reported significantly lower yellowness. Though research data indicate that partial replacement of backfat in frankfurters with different oils (stabilised

in different systems) can increase b* and reduce a* values [Jiménez-Colmenero *et al.,* 2010; Pintado *et al.,* 2016b], the results of this research show no significant differences within C treatments with in any of the observed colour parameters on day 0. The significantly higher b* and h values in CONC relative to CONF could be attributed to the Maillard browning reactions which are promoted by higher pH values in cooking temperatures. Regarding F treatments, colour parameters indicating yellow tones (b* and h values) were significantly higher in treatments with PSO compared to CONF. The significantly higher b* and h values in ALGF and PECF treatments relative to CONF could be attributed to the better stability of PSO microbeads in the presence of Ca^{2+} ions [LeRoux *et al.,* 1999] from shell powder which could be also the reason for the increase of these values during storage.

During storage, all C treatments became less light, significantly so on day 42 compared to the beginning of storage, while lightness of F treatments was not changed. Redness increased significantly during storage in all treatments, and on day 21 it was significantly higher in CONF relative to all modified treatments except ALGC, and PECC at the end of storage. At the end of storage, yellowness values in C treatments were lower (significantly in CONC and PECC) compared to day 0, while in F treatments with pumpkin seed oil they were significantly higher, and unchanged in CONF. This changed the relations between treatments so that all C treatments had significantly lower b* values compared to F treatments with pumpkin seed oil, but still significantly higher than CONF. Calcium alginate lipid containing microbeads are stable under acid and neutral conditions [Zeeb *et al.,* 2015], hence lower pH values in F treatments should not influence the PSO release during storage and increase of b* values. However, research data [LeRoux *et al.,* 1999] indicate that the presence of Na^{+} can reduce the strength of alginate gels due to ion exchange (Ca^{2+} with Na^{+}) in the gel network. The increase of b* values could be a result of the release of the PSO into the meat system (lower stability of PSO microbeads) due to the higher sodium content (due to presence of sodium phosphate) in F treatments, while PSO microbeads were more stable in systems with lower sodium and with the presence of Ca^{2+} ions from shell powder. The PSO was characterised as vegetable oil with very high b* values [Rezig *et al.,* 2012]. A significant decrease of hue angle values in C treatments during storage was also observed, which indicates less yellow product.

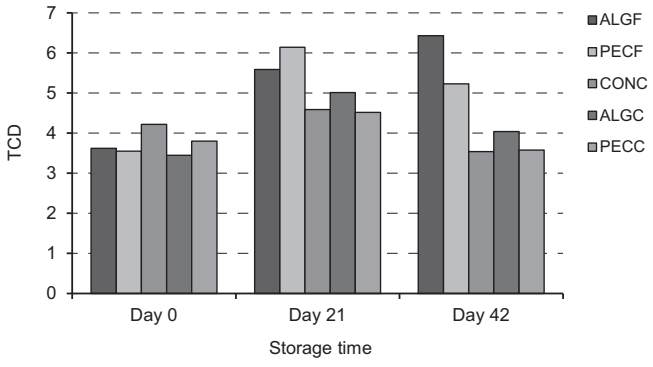

FIGURE 2. Total colour difference (TCD) compared to CONF and during the storage period; F treatments – with phosphates added; C treatments – with shell powder added; CON – all pork backfat; ALG treatments – with 25% of encapsulated pumpkin seed oil in alginate matrix; PEC treatments – with 25% of encapsulated pumpkin seed oil in pectin matrix.

TCD was calculated by a comparison to CONFs' L*a*b* values because CONF represents the model system which is usually prepared (with phosphates added and all fatty tissue). The results (Figure 2) on day 0 were very similar between modified treatments 3.5 (ALGC) – 4.2 (CONC). These values indicate that colour differences were probably perceptible by consumers [Brainard, 2003; Ramírez-Navas & Rodríguez De Stouvenel, 2012]. During storage, TCD values were higher in F treatments compared to all C treatments, reaching values higher than 6, which [Ramírez-Navas & Rodríguez De Stouvenel, 2012] marked as significant values.

Texture properties

The replacement of phosphates with shell powder had a significant influence on the observed textural properties, while fat substitution and interaction of factors had no influence or sporadic influence (Table 4). In meat systems, phosphates exhibit several actions which affect the stability of meat emulsions [Feiner, 2006; Honikel, 2008b]: alkaline phosphates (mostly used in emulsified-type meat products) increase pH, influence actomyosin complex dissociation, enhance protein solubilisation (activation) and increase the ionic strength, hence their substitution poses quite a challenge.

All F treatments had significantly higher hardness, springiness, cohesiveness and chewiness relative to equivalent C treatments on day 0 and throughout storage. On day 0, phosphate replacement with shell powder increased adhesiveness, significantly in all C treatments relative to CONF (a commonly used emulsion system – with phosphates added and all fatty tissue). Backfat replacement with encapsulated PSO led to increased adhesiveness in F and C treatments without any observed effect of encapsulating agents (alginate *vs*. pectin). During storage, the changes of the observed textural properties within the same treatment were mostly not significant except cohesiveness, where a significant increase was observed after 21 days of storage, without further significant changes until the end of storage. However, this did not lead to different relations between treatments. An increase in cohesiveness during storage was observed in emulsified-type meat products with different formulations [Pintado *et al.,* 2016b; Salcedo-Sandoval *et al.,* 2013; Stajić *et al.,* 2020].

Lee *et al.* [2011] replaced 0.3% of phosphates with 0.15, 0.30, and 0.50% of oyster shell powder (+0.50% whey protein in each) in emulsified-type pork sausages and reported a significant increase in hardness, cohesiveness, springiness and chewiness. However, Cho *et al.* [2017] reported significantly lower hardness, cohesiveness, springiness and chewiness when 0.3% of phosphates are replaced with 0.5% of oyster shell calcium powder in ground pork meat products. Similar results were also reported by Bae *et al.* [2017].

There were no differences in the values of cooking loss and purge loss between treatments, which implies similar moisture contents. Thus, differences in textural properties were probably not the result of different moisture/protein

TABLE 4. Texture profile analysis of cooked model system beef emulsions.

Instrumental texture properties	Storage time	Additive 1			Additive 2			Significance (p)		
		Fat 1	Fat 2	Fat 3	Fat 1	Fat 2	Fat 3			
		CONF	ALGF	PECF	CONC	ALGC	PECC	Adv	Fat	Additive* Fat
Hardness (g)	Day 0	1225.28±229.82^{a}	1198.17±160.77^{aAB}	1222.81±245.60^{a}	768.55±219.49^{b}	781.27±120.95^{b}	724.68±70.90^{b}	0.0000	NS	NS
	Day 21	1215.77±130.14^{a}	1074.27±108.32^{aB}	1065.05±225.98^{a}	765.40±101.11^{b}	708.46±88.49^{b}	706.61±95.47^{b}	0.0000	0.0123	NS
	Day 42	1341.55±218.34^{a}	1239.99±123.99^{aA}	1227.07±184.52^{a}	698.07±82.54^{b}	750.17±98.17^{b}	781.96±109.11^{b}	0.0000	NS	NS
Adhesiveness (g × s)	Day 0	-29.09±17.77^{b}	-16.95±4.67^{abAB}	-20.29±16.57^{ab}	-13.07±7.85^{aB}	-11.84±7.86^{a}	-7.29±2.03^{a}	0.0000	0.0478	NS
	Day 21	-20.63±10.62^{c}	-13.41±6.72^{bcA}	-11.17±6.78^{ab}	-5.86±2.86^{aA}	-6.07±2.70^{ab}	-6.11±3.81^{ab}	0.0000	0.0339	0.0236
	Day 42	-26.09±10.51^{c}	-22.20±8.65^{bcB}	-19.68±6.55^{abc}	-10.98±6.52^{aAB}	-12.79±9.28^{ab}	-10.76±7.32^{a}	0.0000	NS	NS
Springiness	Day 0	0.93±0.02^{ab}	0.94±0.02^{aA}	0.92±0.04^{ab}	0.83±0.06^{c}	0.86±0.05^{c}	0.87±0.08^{bc}	0.0000	NS	NS
	Day 21	0.92±0.03^{a}	0.91±0.02^{aB}	0.91±0.02^{a}	0.83±0.05^{b}	0.85±0.04^{b}	0.82±0.05^{b}	0.0000	NS	NS
	Day 42	0.92±0.02^{a}	0.89±0.03^{aB}	0.91±0.04^{a}	0.87±0.05^{b}	0.82±0.06^{b}	0.84±0.04^{b}	0.0000	0.0143	NS
Cohesiveness	Day 0	0.77±0.01^{aB}	0.76±0.02^{aB}	0.75±0.02^{aB}	0.61±0.09^{bB}	0.60±0.08^{bB}	0.63±0.08^{bB}	0.0000	NS	NS
	Day 21	0.81±0.02^{aA}	0.79±0.01^{aA}	0.79±0.02^{aA}	0.71±0.03^{bA}	0.70±0.04^{bA}	0.69±0.04^{bA}	0.0000	NS	NS
	Day 42	0.79±0.01^{aA}	0.79±0.01^{aA}	0.79±0.01^{aA}	0.70±0.06^{bA}	0.68±0.03^{bA}	0.70±0.03^{b}	0.0000	NS	NS
Chewiness (g)	Day 0	871.74±144.95^{a}	851.18±113.27^{a}	844.29±153.05^{a}	403.14±159.96^{b}	407.96±107.80^{b}	402.41±92.54^{b}	0.0000	NS	NS
	Day 21	898.03±111.48^{a}	778.42±80.62^{ab}	768.22±172.31^{b}	451.90±74.22^{c}	422.08±68.59^{c}	401.34±66.31^{c}	0.0000	0.0074	NS
	Day 42	973.74±149.71^{a}	874.07±109.60^{a}	877.54±123.76^{a}	429.35±83.73^{b}	419.46±72.36^{b}	459.53±82.19^{b}	0.0000	NS	NS

Additive 1 – phosphates (F treatments); Additive 2 – shell powder (C treatments); Fat 1 – all pork backfat (CON treatments); Fat 2 – with 25% of encapsulated pumpkin seed oil in alginate matrix (ALG treatments); Fat 3 – with 25% of encapsulated pumpkin seed oil in pectin matrix (PEC treatments); NS – not significant. $^{a–c}$ Values (mean±SD) in the same row with different superscripts are significantly different ($p<0.05$). $^{A–B}$ Uppercase letters are used for comparing the samples considering the effect of storage. Values in the same column for the same property, with different superscripts are significantly different ($p<0.05$).

content. Moreover, the results indicate that fat replacement had no significant influence on the observed textural properties (except adhesiveness). Phosphates promote dissociation of actomyosin complex and myofibrillar proteins extraction, enhance gelation, and in turn increase the hardness of meat products [Glorieux *et al.,* 2017]. The type of phosphates probably had little impact on textural properties [Glorieux *et al.,* 2017]. Moreover, the optimum gelling capacity of myofibrillar proteins at the temperature of 65°C occurs at pH around 6.0, while Ca^{2+} in small quantities enhances gelation [Xiong, 2014]. The possible explanation for lower hardness, springiness, cohesiveness, and chewiness could be better solubilisation (activation)/gelation of myofibrillar proteins in the presence of phosphates.

Partial replacement of backfat with different plant oil preparations does not necessary lead to significantly different textural properties [Pintado *et al.,* 2016a, b], as was observed in this research.

CONCLUSIONS

The substitution of phosphates with shell powder in model system beef emulsions significantly increased pH values (from around 0.8 in raw to about 0.4 in cooked emulsions). However, this did not alter cooking loss, purge loss, and fluid release under pressure. Nitrite content was significantly higher in the treatments with higher pH values, but no significantly lower redness values were measured. Phosphates substitution with shell power will probably lead to higher parameters indicating yellow tones (b* and h values). All the observed textural parameters were significantly altered by the phosphates substitution with shell powder – significantly lower hardness, springiness, cohesiveness, and chewiness were observed, probably due to better solubilisation (activation) of myofibrillar proteins in the presence of phosphates.

Backfat substitution with encapsulated pumpkin seed oil to the level of 25% as well as encapsulating agents altered mostly colour parameters, especially these indicating yellow tones (b* and h values) in the treatments with phosphates. Furthermore, yellowness and hue angle significantly increased during storage in these treatments, while the decrease in the treatments with shell powder was possibly due to the better stability of PSO microbeads in systems with lower sodium and with presence of Ca^{2+} ions from shell powder.

Further research should involve the application of the obtained (positive) results with the aim to develop products and perform examinations which will include microbiological and oxidative stability, and nutritional and sensory quality of meat products obtained by the optimization of a model system into a production system.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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