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ORIGINAL ARTICLE

Effect of sunflower protein meal and electrostatic complexes of sunflower meal-pectin on the cake crumb structure and color

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Abstract Sunflower protein meal could be an alternative and sustainable plant-based protein source, with the potential to add value and replace egg (E) in cakes due to its surface-active properties, however, contains chlorogenic acid and its oxidation leads to greening. Additionally, the electrostatic complexes prepared from the mixture of proteins and polysaccharides can be an important tool to improve the technological properties compared to them are used individually. The aim of this study was the replacement of E in cakes by sunflower protein meal dispersion (SPMD) or electrostatic complexes of sunflower protein meal-pectin (ECSPM-P), evaluating their effects on the cake batter and fresh and aged baked product. Results of the Rapid Visco Analyser (RVA) analysis indicated higher initial viscosity of the cake batters with SPMD and ECSPM-P and lower peak viscosity and less swelling of the starch granules of the cake batter with ECSPM-P. The formulation with SPMD showed the greatest aeration of the cake batter, however resulted in a cake with collapsed structure. The formulation with ECSPM-P showed lighter color (ΔE = 9.95) and improvement in the cake structure, where the cake crumb was similar to the cake with E. During aging, cake formulated with SPMD (ΔE = 36.25) showed intensification in the greening of the cake crumb. The positive effects on the structure and color of the cake crumb showed the potential uses of the ECSPM-P as E replacer in cakes, besides promising ingredient in terms of the trend towards plant-based foods and healthiness.

Keywords Plant-based protein · Egg substitute · Viscosity profile · Chlorogenic acid-greening · Sunflower byproduct

Abbreviations

ΔE	Color difference
DM	Degree of methylation
E	Egg
ECSPM-P	Electrostatic complexes of sunflower protein meal-pectin
GDL	Glucono delta-lactone
IP	Isoelectric point
P	Pectin
RVA	Rapid Visco Analyser
SPM	Sunflower protein meal
SPMD	Sunflower protein meal dispersion

Introduction

Cake is a highly acceptable, frequently consumed bakery product. Cake batter can be defined as an oil in water emulsion system containing air bubbles in a fatty phase (discontinuous phase), the remaining ingredients being dissolved or dispersed in the aqueous phase (continuous phase) (Bennion and Bamford 1997).

Egg (E) is one of the main ingredients of cake formulations, with special foaming and emulsifying properties during the cake batter preparation, and structure formation during baking, providing the proper volume and soft texture characteristic of the product (Wilderjans et al. 2013). Factors such as E proteins allergy, tendencies for foods with vegan or vegetarian compositions or special diets,

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have motivated the substitution of E proteins by other protein sources that can offer similar functional properties (Arozarena et al. 2001; Lin et al. 2017). In addition, such substitutions result in significant cost reductions since animal proteins represent high production and market costs, and also the possibility of more sustainable products with a reduction in environmental impacts due to the use of plant source proteins (Paraskevopoulou et al. 2015).

Sunflower protein meal (SPM), a byproduct of sunflower oil extraction, has been shown to be fairly competitive as an alternative protein source with respect to productivity, economic and environmental issues. It contains large amounts of proteins (40–50%) with the functional properties required for food production (Carrão-Panizzi and Mandarino 1994; González-Pérez et al. 2002), highlighting mainly the emulsifying properties, with an interesting potential to enhance the use of sunflower proteins (González-Pérez and Vereijken 2007) as E replacer. However, according to González-Pérez and Vereijken (2007), the oil extraction conditions could result in protein denaturation and influence its functional properties. Other problem related to SPM is the presence of large amounts of phenolic compounds such as chlorogenic acid, hinders its application in food products with alkaline pH values, due to oxidation and undesirable darkening and greening, being mainly used for animal feed (Wildermuth et al. 2016).

Electrostatic complexes can be prepared from the mixture of proteins and polysaccharides to improve the performance of proteins in the formation and stability of foams, emulsions, and gels, since they combine the functional properties of both ingredients (Schmitt and Turgeon 2011). The obtaining of these complexes depends on the electrostatic interactions caused by the attraction between components, generally between positively charged proteins (pH < isoelectric point–IP) or near to the IP and anionic polysaccharides such as pectin (Dickinson 2008; Patino and Pilosof 2011). Pectin (P) presents a high molecular weight linear chain constituted of α-1,4-d-galacturonic acid units and it classified by the degree of methylation (DM). The high methoxy P (DM > 50%), form gels in high solute concentration, like sugar, and low pH whereas the low methoxy P (DM < 50%), form gels in presence of calcium (Chan et al. 2017).

The partial or total replacement of E by other protein sources (plant or animal origin), hydrocolloids and emulsifiers has already been evaluated, and the authors highlighted the difficulties involved in obtaining adequate cake batters and baked products, from the technological and sensorial points of view (Arozarena et al. 2001; Lin et al. 2017; Paraskevopoulou et al. 2015; Rahmati and Tehrani 2015).

The aim of this study was to evaluate the effect of E replacement on the cake formulations by SPM or by electrostatic complexes of sunflower protein meal-pectin (ECSPM-P) on cake crumb structure and greening of fresh and aged cake. This application could contribute to plant-based product development and add value to sunflower byproduct.

Materials and methods

Materials

SPM obtained from a commercial mixture of seeds by grinding the cake after extraction of the oil, was donated by the Fraunhofer Institute–IVV (Freising, Germany) as part of the bilateral cooperation project Brazil-Germany SUNPRO. The proximate composition of SPM determined by AOAC (1997), presented moisture (w.b.), protein and ash content of 6.51, 53.01 and 8.32%, respectively. The crude fat determined by Bligh and Dyer (1959) and total carbohydrates by difference, showed content of 1.50 and 30.67%, respectively. Low-methoxy pectin (GENU pectin type LM CG-22, degree of esterification 47.2%, molecular mass 90 kDa) was donated by CPKelco (Grossenbrode, Germany). The other ingredients were kindly donated as follows: palm fat by Agropalma (Limeira, Brazil); dry whole egg (Ovopan®) by Shinoda Alimentos Ltda. (Indaiatuba, Brazil); glucono delta-lactone (Dairy Mix GDL®) by Ashland/Germinal (Cabreúva, Brazil); sodium propionate by Metachem Industrial Comercial Ltda. (São Paulo, Brazil); and liquid sorbic acid preservative (Shelf Life®) by PMAN (Guarulhos, Brazil). Wheat flour, sugar, baking powder and salt were purchased on the local market.

Methods

Cake ingredients and preparation

The cakes formulations and preparation were according to Bennion and Bamford (1997) with some modifications. The formulation of the cakes was 26.9% of wheat flour, 22.5% of sugar, 9.6% of palm fat, 2.8% of baking powder, 0.3% of sodium propionate, 0.2% of salt, 33.7% of water and 4% dry whole E (assay 1–formulation with E) or 4% SPM (assay 2–formulation with SPMD) or 3.9% SPM and 0.1% P (assay 3–formulation with ECSPM-P). To prepare the egg (ingredient E), dry whole E was weighed and reconstituted with 15.3% of the total water from the formulation, according to the supplier's recommendations, and homogenized in a blender (Britânia Eletrodomésticos S.A., Joinville, Brazil). To prepare the sunflower protein meal dispersion (ingredient SPMD) SPM was weighed and mixed with 30.6% of the total water from the formulation. To prepare the electrostatic complexes of sunflower protein meal-pectin (ingredient ECSPM-P) was mixing SPM, water (30.6% of the total water from the formulation), previously hydrated P in the mass ratio of 21:1 (w/w) with respect to protein of the SPM and P, and glucono delta-lactone (GDL), in the proportion 0.6 g GDL/g protein of the SPM. The pH was monitored with an UltraBasic pHmeter (Denver Instrument, Bohemia, USA) and when the dispersion reached approximately pH 3.7, it was heated to 98 °C and then cooled to room temperature.

Whipping of the cake batter and baking were carried out as described in the sequence. The sugar and palm fat were mixed for 10 min in an industrial mixer at speed 3 (Irmãos Amadio Ltda., São Paulo, Brazil). The E or SPMD or ECSPM-P ingredient was added into the bowl and mixed for 4 min at speed 2. The powdered ingredients were then added and mixed for a further 5 min (speed 2), followed by the water which was added and mixed for another 5 min (speed 2). The cake batter was poured into muffin pans (base diameter 55 mm, upper diameter 75 mm and internal height 30 mm) and baked for 47 min at 160 °C using an electric convection oven (Vipinho 0448, Perfecta, Curitiba, Brazil). After baking, the cakes were removed from the pans and cooled for approximately 50 min until reaching room temperature. Liquid preservative was then applied to the cakes, which were individually wrapped in metalized BOPP/BOPP (biaxially oriented polypropylene) packaging and stored in a chamber at 25 °C with 75% relative humidity.

Cake batter analysis

Specific gravity The specific gravity of each cake batter was determined using cylindrical glass containers and calculating the relationship between the weight (g) and volume (cm^3). The measurements were made in triplicate.

Viscosity properties The viscosity properties of the cake batters were studied using a Rapid Visco Analyser–RVA (Rapid Visco Analyser RVA 4500, Perten Instruments, Hägersten, Sweden), simulating the oven-baking with the conditions defined by Wilderjans et al. (2008). Cake batter samples (20 g) were submitted to the following temperature profile: 5 min at 25 °C, linear temperature increase up to 95 °C with a heating rate of 3.5 °C/min and holding step of 10 min at 95 °C, totaling 30 min of analysis. Four replicates were prepared.

Optical microscopy Samples of fresh cake batters were placed on microscope slides, covered with thin coverslips and analyzed by optical microscopy (Microscope BX4, Olympus, Tokyo, Japan). The sample images were captured in duplicate with a magnification of × 10.

Cake analysis

The cakes were evaluated one day after processing (specific volume, moisture, color and texture) and again after 8, 15, 22 and 29 days, with the exception of the specific volume. The crumb grain was analyzed 2–7 days after processing.

Specific volume The cake specific volume was measured by rapeseed displacement using the AACC (2010) method n° 10–05.01, calculating the relationship between the volume and the weight of the cakes (cm^3/g).

Moisture The cake moisture content was determined in two phases. The samples were first prepared using AACC (2010) method n° 62–05.01, and the moisture content determined using AACC (2010) method n° 44–15.02. The analyses were carried out in triplicate.

Color The cake crumb color was determined using a portable colorimeter (CR450, Konica Minolta, Tokyo, Japan), with the CIEL*a*b* coordinate system. The measurements were made of three different cakes from each formulation. The color difference (ΔE) of cakes with 0 and 29 days of the aging was calculated according to Eq. 1.

$$\Delta E = \left[\left(L_t^* - L_0^* \right)^2 + \left(a_t^* - a_0^* \right)^2 + \left(b_t^* - b_0^* \right)^2 \right]^{1/2} \quad (1)$$

where, L^*_0, a^*_0 and b^*_0 values are the reference values obtained from formulation with E; L^*_t, a^*_t and b^*_t values are the teste values obtained from formulation with SPMD and ECSPM-P.

Texture The top of the cakes was cut horizontally, removed, and the cake crumb firmness measured in the compression mode using a TAXT2i texture analyzer (Stable Micro Systems Ltd., Surrey, UK) with a HDP/90 platform and SMS P/36R aluminum probe. The test conditions were: pre-test speed = 1.0 mm/s; test speed = 1.7 mm/s; post-test speed = 10.0 mm/s; and distance = 10.0 mm, according to method n° 74–09.01 (AACC, 2010). At least ten cakes were analyzed.

Analysis of the cake crumb structure The cake crumb structure was analyzed based on the method described by Sánchez-Pardo et al. (2008). Central slices were cut from three cakes and scanned using a HP Scanjet G2710 scanner (California, USA). Sub images of each picture were selected with dimensions of 240 × 165 pixels (32.43 × 22.30 mm) and processed using the ImageJ software (National Institutes of Health, USA). Parameters such as the total number of pores, total pore area (mm^2), mean pore area (mm^2) and pores/cm^2 were evaluated and calculated.

Statistical analysis

The cakes were prepared in triplicate. The means and standard deviations of the results were calculated and evaluated using the analysis of variance and Tukey's multiple comparison test ($p < 0.05$).

Results and Discussion

Effect of sunflower protein meal dispersion and electrostatic complexes of sunflower protein meal-pectin on the cake batters

The formation of the electrostatic complexes was evaluated at another stage of the research (data not shown), when the zeta potential of SPM, P and mixtures of SPM:P prepared at the ratio of 25:1 and 15:1 (w/w) were evaluated at pH range 2.0–8.0 (Giarola 2018). The zeta potential profile of the studied ratios presented negative and intermediate charge values to that of SPM and P, indicating the occurrence of electrostatic interactions between SPM and P contained in mixtures. In addition, the viscosity in function of shear rate of the mixtures mentioned above, of SPM and P was evaluated at pH 3.7, room temperature and heated to 98 °C (Giarola 2018). SPM and P showed newtonian behavior, in which the viscosity does not vary with shear rate. Whilst, mixtures SPM:P showed pseudoplastic behavior, with viscosity behavior dependent on the shear rate where the interactions and structure between biopolymers are disrupted by shear. The rheological characteristics of dispersions are due to the electrostatic interactions and structure formation between protein and P (Lutz et al. 2009). This change in the behavior of the samples indicates the formation of electrostatic complexes. The proportion of SPM and P was adjusted for the cake formulation based on preliminary tests and fixed in 21:1 (w/w) with respect to protein of the SPM and P. Thus, the formation of electrostatic complexes in the ECSPM-P ingredient was considered based on the results presented in SPM:P mass ratio 25:1 and 15:1 (w/w), in view of the use of intermediate proportion in the cake formulation.

The evaluation of the specific gravity and the viscosity properties of the cake batter may provide indications of desirable characteristics in the final product, such as good volume and soft texture. The effects of E replacement on the cake batters are presented in Table 1.

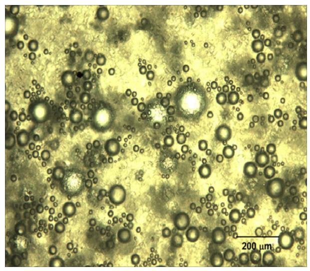
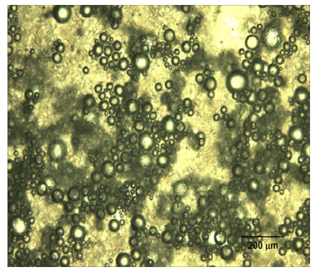
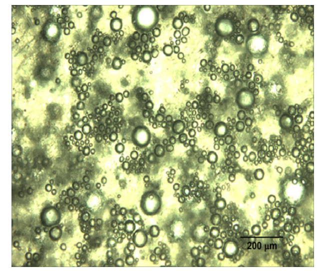
Specific gravity is related to air bubble incorporation in the cake batter during the mixing step, hence lower values are desirable, indicating greater aeration of the cake batter and better cake structure. The substitution of E by SPM resulted in a decrease in the specific gravity of the cake batter (0.944 ± 0.025 to 0.886 ± 0.019 g/cm^3, respectively), suggesting better aeration in the formulation with SPMD during the mixing step. Lin et al. (1974) evaluated the excellent aeration capacity of the sunflower meal in an aqueous system with a basic pH as comparable to that of E and suggested that other constituents besides the proteins might improve foam formation. Formulations with E and ECSPM-P showed no statistical differences ($p > 0.05$) in this analysis (Table 1). The optical microscopy of the cake batters can be seen in Table 1 and indicated large amount of air bubbles in the formulation with SPMD, which could be correlated with the better cake batter aeration. The appearance of the cake batter with E and ECSPM-P were similar in quantity and size of the air bubbles (Table 1).

The incorporation of SPM (formulation with SPMD) contributed to an increase in the initial viscosity of the cake batter (4960 ± 102 cP) as compared to formulation with E (2447 ± 149 cP), due to its composition including proteins, polysaccharides and fibers (Table 1). Rahmati and Tehrani (2015) used a dispersion of water and full-fat soy flour in different proportions in the partial and total replacement of E in cake formulations and verified a gradual increase in the viscosity of the cake batter, which reached its highest value with total replacement of the E. They also highlighted the high water absorption of the soy flour due to its composition of proteins and carbohydrates, a condition similar to that of the present study.

The increase in initial viscosity was even more significant for the formulation with ECSPM-P (6970 ± 525 cP) (Table 1), with the incorporation of electrostatic complexes containing P, and this probably helped reduce aeration of the cake batter during the mixing step, when compared to formulation with SPMD. Polysaccharides such as P presents various functionalities such as gelling agents and stabilizers and are frequently added to different formulations due to their ability to bind with water and change the properties of other ingredients (Li and Nie 2016). Cake batter viscosity is important for the retention of air bubbles and stability of cake batter, however, when it is very high, such as in the formulation with ECSPM-P, it hamper the beating and air incorporation processes, leading to high batter specific gravity, which would hinder the expansion of the air bubbles in the oven, resulting in cakes with a low specific volume (Lin et al. 2017; Sahagún et al. 2018; Wilderjans et al. 2008).

The occurrence of starch gelatinization during baking is an important parameter for cake quality, because it is responsible for the porous structure of the cake (Sakiyan et al. 2011), and should occur at the correct time, rate and extension to obtain an adequate texture (Howard et al. 1968 as cited by Gómez et al. 2010). There were no significant

Table 1 The specific gravity, viscosity properties and optical microscopy (10 × magnification) of cake batters formulated with egg (E), sunflower protein meal dispersion (SPMD) and electrostatic complexes sunflower meal-pectin (ECSPM-P)

	Cake batters		
	E	SPMD	ECSPM-P
Specific gravity (g/cm^3)	0.944 ± 0.025^a	0.886 ± 0.019^b	0.944 ± 0.004^a
Initial viscosity (cP)	2447 ± 149^c	4960 ± 102^b	6970 ± 525^a
Pasting temperature (°C)	84.64 ± 0.01^a	84.70 ± 0.06^a	84.65 ± 0.01^a
Peak viscosity (cP)	44,296 ± 1046^b	53,797 ± 3695^a	33,404 ± 1565^c
Optical microscopy			

Means and deviations, in the same line, followed by the same letters do not differ statistically by Tukey test ($p < 0.05$)

differences ($p > 0.05$) in the pasting temperatures among formulations with E, SPMD and ECSPM-P (Table 1).

During baking, the increase in viscosity is attributed to a combination of starch gelatinization and E protein denaturation and coagulation (Wilderjans et al. 2013). The peak viscosity varied from 33,404 ± 1565 to 53,797 ± 3695 cP for the formulations with ECSPM-P and SPMD, respectively, while the formulation with E presented intermediary values (44,296 ± 1046 cP), all showing significant differences from the others ($p < 0.05$) (Table 1). These results showed that the formulation with ECSPM-P presented a lower peak viscosity and less swelling of the starch granules. The reduction in this parameter compared to the other formulations may be due to interactions with the other ingredients, considering that it contains P, and possibly the reduced availability of water for starch granule hydration which would make swelling difficult (BeMiller 2011), a fact that may be related to the high initial cake batter viscosity (Table 1).

Effect of sunflower protein meal dispersion and electrostatic complexes of sunflower protein meal-pectin on the cakes after baking

The cakes formulated with E presented the highest specific volume, 2.24 ± 0.11 cm^3/g, whereas cakes formulated with SPMD and ECSPM-P showed significantly ($p < 0.05$) lower results, 1.40 ± 0.19 and 1.55 ± 0.17 cm^3/g, respectively (Fig. 1). The low cake specific volume in the formulations containing SPM (SPMD and ECSPM-P) as compared to cakes formulated with E, may be related to the high initial viscosity of the cake batter (Table 1),

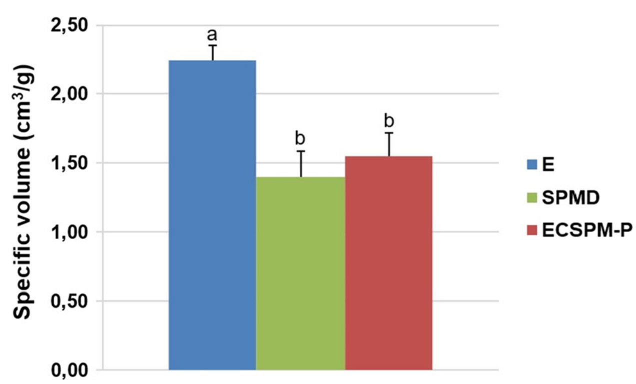
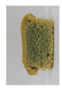


Fig. 1 Specific volume of the cakes formulated with egg (E), sunflower protein meal dispersion (SPMD) and electrostatic complexes sunflower meal-pectin (ECSPM-P) Values followed by the same letter are not different by Tukey test ($p < 0.05$)

hampering expansion of the air bubbles during the first stage of baking (Sahagún et al. 2018).

The images presented in Table 2 shows the differences in cakes structure formulated with E, SPMD and ECSPM-P the day after processing. A thicker crust and collapsed structure were observed for the cake formulation with SPMD. Arozarena et al. (2001) also reported collapse of the cake structure after cooling. The authors used white lupine protein as E substitute and suggested that the foam in the batter was not stable and denaturation of the white lupine proteins was not sufficient to maintain the structure as the coagulation of E proteins. Sosulski (1979) verified that sunflower flour presents a low capacity to form firm gels. Thus, the formulation with SPMD probably presented instabilities in the air bubble in the cake batter and denaturation of the SPM was not sufficient to keep the

Table 2 Color parameters (L*, a*, b*), color difference (ΔE) and images of the cakes formulated with egg (E), sunflower protein meal dispersion (SPMD) and electrostatic complexes sunflower meal-pectin (ECSPM-P)

Assay	Color L*		Color a*		Color b*		ΔE		Image	
	0 day	29 days	0 day	29 days	0 day	29 days	0 day	29 days	0 day	29 days
E	73.75 ± 0.58^{aA}	72.74 ± 0.71^{aA}	2.07 ± 0.20^{aA}	3.25 ± 0.09^{bA}	26.76 ± 0.51^{aA}	27.38 ± 1.28^{aA}	*	*		
SPMD	55.70 ± 1.40^{aC}	45.01 ± 0.67^{bC}	1.54 ± 0.55^{aA}	− 2.20 ± 0.24^{bC}	16.40 ± 0.87^{aB}	4.68 ± 0.53^{bC}	20.82	36.25		
ECSPM-P	70.18 ± 0.91^{aB}	69.36 ± 0.85^{aB}	1.85 ± 0.03^{aA}	2.28 ± 0.09^{bB}	17.48 ± 0.36^{aB}	18.29 ± 0.56^{aB}	9.95	9.75		

*Values of the formulation with E was used as reference values to calculate the color difference (ΔE)

Averages followed by the same lower case letter, in the same line and assay are not different by Tukey test ($p < 0.05$)

Averages followed by the same capital letter, in the same column, are not different by Tukey test ($p < 0.05$)

structure of the cakes. During baking process, the proteins must be able to prevent instabilities involving the air bubbles (coalescence and disproportion) and their escape from the cake batter (Bennion and Bamford 1997; Paraskevopoulou et al. 2015; Kiosseoglou and Paraskevopoulou 2006). In addition, protein coagulation and aggregation properties together with starch gelatinization and gluten development are responsible for the final structure of the cake (Kiosseoglou and Paraskevopoulou 2006; Wilderjans et al. 2013).

In comparison, the cakes of the formulation with ECSPM-P showed a finer crust, uniform structure and not collapsed (Table 2). Electrostatic complexes, prepared with different types of proteins and polysaccharides, are extensively studied for their ability to improve the interfacial properties in relation to pure proteins, producing films with less gas permeability in foams, improving the stability of emulsions, as well as contributing to the gelling properties (Schmitt and Turgeon 2011). Thus, the formulation with ECSPM-P probably had greater stability of the air bubbles in the batter and in expansion during baking, in addition to the contribution to better coagulation of the proteins due to the presence of electrostatic complexes with P (Table 2).

In relation to the color parameters (Table 2), on the 0 day, the cake crumb with E was the lightest (L* = 73.75 ± 0.58) and most yellow (b* = 26.76 ± 0.51). The yellow tones are characteristic of the yolk egg. The color parameter a* (positive values = red color) showed no significant difference between any of the cake samples. Between the cakes made from formulations with SPMD and ECSPM-P were significantly different ($p < 0.05$) for color parameter L*, where the cake crumb of formulation with SPMD were darker (L* = 55.70 ± 1.40) than those of formulation with ECSPM-P (L* = 70.18 ± 0.91), as seen in the image (Table 2). This difference in cake crumb lightness was due to formulation with SPMD having been prepared with SPM with its natural pH (6.2—6.5), showing a slightly green and brown color due to the presence of chlorogenic acid. Under alkaline pH conditions, like cake batters, this phenolic compound undergoes oxidation, being able to polymerize with amino groups of the proteins through strong bonds, like covalent bonds, resulting a green coloration (Rawel and Rohn 2010; Wildermuth et al. 2016; Yabuta et al. 2001). The formulation with ECSPM-P contained electrostatic complexes, which were produced by reduction of the pH value to 3.7 and heated to 98 °C, thus fixing the lighter coloration. Rawel and Rohn (2010) and Sastry and Rao (1990) explained that the change in pH alters the number of binding sites between chlorogenic acid and sunflower protein and the temperature also affects the binding affinity between them (Sastry and Rao 1990). The color difference was calculated to the formulation with SPMD and ECSPM-

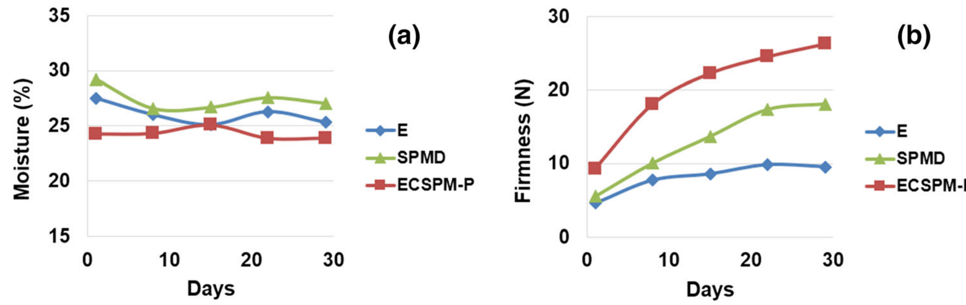
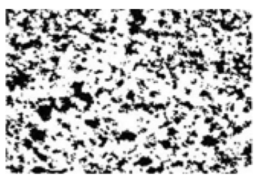
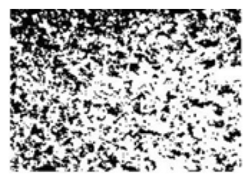
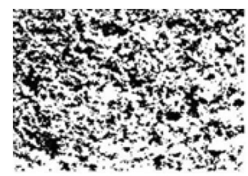


Fig. 2 Moisture content (**a**) and firmness (**b**) of the cakes formulated with egg (E), sunflower protein meal dispersion (SPMD) and electrostatic complexes of sunflower meal-pectin (ECSPM-P) during aging

Table 3 Crumb grain structure parameters by image analysis of cakes formulated with egg (E), sunflower protein meal dispersion (SPMD) and electrostatic complexes of sunflower protein meal-pectin (ECSPM-P)

	Cakes		
	E	SPMD	ECSPM-P
Total number of pores	1123 ± 91^{a}	859 ± 32^{b}	1083 ± 93^{a}
Total pores area (mm^2)	69.296 ± 6.042^{a}	46.526 ± 2.497^{c}	54.830 ± 5.155^{b}
Mean pores area (mm^2)	0.062 ± 0.003^{a}	0.054 ± 0.001^{b}	0.050 ± 0.001^{c}
Pores/cm^2	51.75 ± 4.20^{a}	39.58 ± 1.46^{b}	49.89 ± 4.29^{a}
Images			

Means and deviations, in the same line, followed by the same letters do not differ statistically by Tukey test ($p < 0.05$)

P compare to formulation with E on the 0 day and showed value 20.82 and 9.95, respectively, indicating that cake of the formulation with ECSPM-P presented color closer to the cake with E.

Cake formulations with E and SPMD presented higher moisture contents, 27.51 ± 0.74 and 29.20 ± 0.21%, respectively, whereas cake formulation with ECSPM-P showed lower values, 24.26 ± 1.24% (Fig. 2a). Starch contributes to the retention of moisture in the cakes (Bennion and Bamford 1997), and probably the less swelling of the starch granules, observed by the lower peak viscosity in the RVA analysis from the cake batter with ECSPM-P (Table 1), resulted in the lower moisture content this cake. Thus, an adjustment in the amount of water in the formulation with ECSPM-P would be necessary to reduce competition with the other ingredients for water absorption and to promote adequate swelling of the starch granules (higher peak viscosity).

The structure of the cake crumb was evaluated by images and the parameters were calculated from them (Table 3). The total number of pores in the crumb of cake formulations with E and ECSPM-P were statistically equal (1123 ± 91 and 1083 ± 93, respectively), however the mean pore area formed was lower in formulation ECSPM-P (0.050 ± 0.001 mm^2), also resulting in a lower total pore area (54.830 ± 5.155 mm^2). The formulation with SPMD produced cakes with a lower total number of pores (859 ± 32) and lower pores per total area (39.58 ± 1.46 pores/cm^2), even though it presented an intermediary mean pore area (0.054 ± 0.001 mm^2). Paraskevopoulou et al. (2015) observed that the application of whey isolate protein with the complete replacement of the E resulted in changes in the cake crumb, producing a reduced number of pores and lower area covered by pores. In the same study, the effects on the cake crumb were associated with low air retention capacity. The lower number of pores in the formulation with SPMD (Table 3) may be related to the more fragile structure of the cake observed in Table 2, with the loss of air bubbles, as reported in the study above and discussed earlier in this study. In the case of formulation with ECSPM-P, the appearance of the cake batter was like formulation with E (Table 1) and resulted in statistically equal total number of pores (Table 3), however, the reduced pore size can be justified by the reduced swelling of the starch granules, indicated by lower peak viscosity (Table 1), which promotes shrinkage of the air cells during cooling of the cakes (Keppler et al. 2018).

Concerning the texture (Fig. 2b), cake formulations with E and SPMD showed no significant differences ($p > 0.05$) between them for firmness, 4.62 ± 0.15 and 5.52 ± 0.42 N, respectively, and formulation with ECSPM-P presented the highest value, 9.32 ± 0.91 N. The soft nature may be related to the higher moisture content and collapsed structure in the cakes with SPMD, whereas the greater firmness of formulation with ECSPM-P could be due to lower peak viscosity of the cake batter (Table 1) and less swelling of the starch granules, which made the cake crumb denser (Table 2 and 3), and lower moisture content (Fig. 2a).

Therefore, the overall analysis of the results indicated that an adjustment in the amount of water in the formulation with ECSPM-P would be necessary to decrease the initial viscosity of the cake batter and facilitate the incorporation of air bubbles, as well as promoting the swelling of the starch granules (higher peak viscosity), leading to the higher specific volume, the higher moisture content, and lower firmness on the cakes.

Effect of sunflower protein meal dispersion and electrostatic complexes of sunflower protein meal-pectin on the cakes during aging

During aging, the color parameter L* remained stable in the samples of cake made from formulations with E and ECSPM-P, but the samples of cake made from formulation with SPMD showed a reduction in values (55.70 ± 1.40 on the 0 day, to 45.01 ± 0.67 on the 29 day of evaluation), indicating darkening of the cake crumb (Table 2). According to color parameter a*, samples made from formulations with E and ECSPM-P presented an increase in reddish tones (positive values), whereas cakes made with SPMD showed negative values, corresponding to a green color (Table 2). The color parameter b*, which represents the variation from blue (-b) to yellow (+ b), was stable in cakes made from formulations with E and ECSPM-P (Table 2) and the cake crumb of the formulation with SPMD showed a great reduction in this parameter, from 16.40 ± 0.87 to 4.68 ± 0.53. In the cakes made from the formulation with SPMD the greening and darkening, already observed on the 0 day, were intensified considering that the presence of oxygen, moisture and the storage time are factors that contribute the oxidation reaction of the chlorogenic acid and development of the green color (Wildermuth et al. 2016). Liang and Were (2018) also observed greater greening in sunflower butter cookies as a function of different storage conditions (like pH, moisture and storage time). In the case of formulation with ECSPM-P no darkening of the cake crumb occurred during aging (Table 2), due to the acidic pH and temperature to prepare electrostatic complexes prevented color change. The changes in color become even clearer when the color difference is observed, where the cake with SPMD and ECSPM-P showed 36.25 and 9.75 values, respectively (Table 2). Cakes with SPMD were more distant from cakes with E, comparing day 0 and 29, while cakes with ECSPM-P showed a minimal difference.

In relation to the texture, there was an increase in firmness for all the cake samples (Fig. 2b). This fact usually correlates with the aging process during storage due to the loss of moisture content and the phenomenon of starch retrogradation in bakery products. The cakes made from the formulations with SPM (SPMD and ECSPM-P) showed a greater increase in firmness comparing to those formulated with E. The cake with ECSPM-P showed lower moisture content (Fig. 2a) and less swelling of starch granules (Table 1), that may have contributed to the greater firmness observed during aging.

Conclusion

The substitution of E by SPM or ECSPM-P produced effects on the cake batter, and consequently on the cakes. The cake batter with SPMD and ECSPM-P showed greater initial viscosity in comparison to cake batter with E. The cake batter with SPMD also showed better aeration, however the structure of the cake collapsed and occurred greening of the cake crumb due to the presence and oxidation of the chlorogenic acid. The moisture and firmness of the cakes with SPMD were similar to cakes formulated with E. The cake batter with ECSPM-P showed less swelling of the starch granules, but the cakes presented improvement in structure, preventing collapse and better retention of air bubbles, with cake crumb similar to cakes formulated with E. In addition, the reduction of pH during formation of the complexes maintained the light color of the product. During aging, the cakes formulated with SPMD showed greater greening and both formulations with SPMD and ECSPM-P presented greater firmness compared to cakes formulated with E. Therefore, additional studies in relation to adjustment of the cake batter viscosity, P concentration decrease for electrostatic complexes formation, and use of improver ingredient are necessary to produce softer cakes with a larger volume.

The positive effects on the structure and color of the cake crumb showed the potential uses of the ECSPM-P as E replacement and promising ingredient for vegan and healthy food product.

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Availability of data and material The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare that they have no conflicts of interest.

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