# Effect of whey protein isolate addition on physical, structural and sensory properties of sponge cake

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# Abstract

Whey protein isolate is commonly used in food products due to its functional properties, such as foaming, water binding capacity, emulsifying and gel formation ability. Besides, sponge cake is one of the most consumed cakes by the bakery industry due to its porous structure. The aim of the present study was to determine changes on batter density and viscosity, heating rates, crumb structure, volume, texture and crystallinity in order to evaluate the partial replacement of egg white protein by whey protein isolate. A substitution of 12.5%e25% showed no significant differences (P < 0.05) on the parameters evaluated when compared to control sample and the exploratory consumer sensory evaluation was conducted finding no statistical differences (P < 0.05) between samples. The total replacement of egg white protein by whey protein isolate promoted a more crystalline system and a poorer cake quality. During the second stage of the sponge baking, the air presence could change its heating rate.

# Keywords:

Sponge cake; Whey protein isolate; Crystallinity; Heating rates; Microstructure

## Introduction

Whey, obtained mainly from cheese production, historically had been considered a highly polluting low added value byproduct (Garcia-Garibay, Revah, & Gomez-Ruiz, 1993; Marwaha & Kennedy, 1988). However, its proteins have excellent functional properties, such as high solubility (even at low pH), foaming capability, water binding capacity, emulsifying properties and gel formation ability (Mulvihill & Fox, 1989), being those properties highly appreciated during food product manufacturing, particularly when used as whey protein isolate (WPI) (García-Garibay, Jime'nez- Guzma'n & Herna'ndez -S'anchez); this is commonly used in bakery products, such as cakes, and several authors have described its functionality in Angel food cakes (Arunepanlop, Morr, Karleskind, & Laye, 1996; Berry, Yang, & Foegeding, 2009; Pernell, Luck, Foegeding, & Daubert, 2002). More recently Paraskevopoulou, Donsouzi, Nikiforidis, and Kiosseoglou (2015) evaluated the par- tial and total replacement of whole egg with WPI combined with an emulsifier in order to reduce the egg content in pound cake. Arunepanlop et al. (1996) evaluated appearance, structure, texture and sensory properties of Angel food cakes prepared with a partial substitution of egg white protein (EWP) with WPI using conven- tional and microwave ovens, being WPI cakes not as good as control samples. Those results showed statistically significant differences and the author's analysis concluded that WPI proteins are more heat stable than EWP showing less ability to stabilise air cells when expanded during baking. Pernell et al. (2002) found that cakes with WPI showed low ability to prevent cake structure collapse when starch gelatinisation occurs during baking, and its behaviour is not related to protein denaturation. Additionally, the same authors observed that although proteins added with xanthan gum and heated at 80 °C during 1 min increased the cake volume, they did not reach the cake volume obtained with EWP. The viscosity increased the cake stability but did not avoid the volume decrease. Moreover, the addition of polyvalent cations before foam formation did not increase the volume of the cake when EWP was substituted with 25% and 50% of WPI (Arunepanlop et al., 1996). Yang, Berry, and Foegeding (2009) pointed out that WPI proteins predominate in the cells air/water interface when the foam is formed with WPI and EWP, and that initial smaller cells contribute to higher foam stability. Berry et al. (2009) indicated that sucrose has demon-strated to increase interfacial elasticity in EWP foams, while the inverse effect was observed with WPI and WPI/EWP foams. Interfacial elasticity increase could explain EWP foam stability. The same authors pointed out that EWP foams and batters generate stable structures induced by temperatures lower than those of starch gelatinisation. Besides, EWP early formed structure seems to sta- bilise the system during baking, effect that was not seen in WPI systems. Moreover, the addition of sugar and flour during batter making caused the destabilisation of WPI foams, showing that the interaction with other batter ingredients could be responsible of functional differences between EWP and WPI in Angel food cakes. Yang and Foegeding (2010) found that the increase of sucrose concentration (from 0 to 63.6 g/100 mL) showed a gradual increase in the dissolution viscosity and a decrease in the foaming effect. The incorporation of sucrose modifies the Angel food cakes volume when elaborated with WPI but does not improve its structure; then, sucrose improves the stability of wet foams, but the limited stability of whey proteins during conversion to the dry form (Angel food cake as a dry foam) does not change due to the formation of big cells during heating. Yang and Foegeding (2011) observed the microscopic effect of sucrose addition on the microstructure of EWP and WPI foams. Their findings suggest that small initial cells in EWP systems, produced by the addition of sucrose, improve foam stability due to the permeability of the liquid phase and a slower drainage rate. As it can be seen, the studies that evaluate the addition of WPI in cakes are focused in evaluating physical and structural properties of the foams, although their results indicate that foam properties do not predict the baking yield of Angel food cake, and also demonstrate that the interaction between WPI and other ingredients modifies the final quality characteristics of the cake; therefore, more studies are required to investigate the effect of baked cakes with WPI on their heating behaviour, structure and quality.

Cakes are very appreciated in the bakery industry (Paraskevopoulou et al., 2015) and these can range from light Angel food cakes to dense, rich cheesecakes, and numerous other types between these two extremes (Zelch, 2001); the cake type and their quality characteristics depend on its elaboration process and on its formulation. Sponge cake is a baked product which porous structure determines its commercial functionality due to its ability to retain liquids such as milk syrup (Díaz-Ramírez et al., 2013). As Angel food cake, sponge cake is classified as a foam type cake, but sponge cake uses whole eggs and sometimes is enriched with the addition of egg yolks, so its aeration properties and structure are based on its whole egg content and on its foam and emulsion stability. Otherwise in batter type cakes, as Pound cake, those pa- rameters are based on shortening/oil included in the formula. For that reason, during cake mixing different colloidal systems could be formed depending on the cake type formula, on the process and on the interaction between their ingredients, hence unique final cake quality characteristics. During baking, gas cells expand and/or coalesce; water and temperature increment promote starch gelatinisation and the development of the structure around gas cells giving strength to the resulting cake. The interaction among sponge cake ingredients with WPI could produce quality and structural changes in the final product. Therefore the aim of the present study was to determine changes on batter density and viscosity, heating rates, crumb structure, volume, texture, crystallinity and sensory properties in order to evaluate the partial replacement of egg white protein (EWP) by whey protein isolate (WPI).

# Materials and methods

## Materials

"Saratoga" flour commercialised by Harinera de Me´xico, S.A. de C.V. was used in all treatments, its characteristics were supplied by the producer: moisture 13.97 g/100 g, protein 10.30 g/100 g, ash 0.570 g/100 g and particle size measured in a #120 sieve 94.08 g/ 100 g. Fresh eggs were purchased from a local supplier (San Juan, S.A. de C.V., Me´xico); egg yolks and whites were manually separated. Baking powder was supplied by Royal, Kraft Foods De Me´xico, S. De R.L. De C.V, A´lvaro Obrego´n, DF, Me´xico. The rest of the ingredients (milk, vanilla and sugar) were obtained from local suppliers. Whey protein isolate (WPI) with 89% protein content was from Isopure Company, LLC (195 Engineers Road Hauppauge, NY 11788, United States).

## Protein substitution

The protein solution was prepared according to Berry et al. (2009). WPI was hydrated at room temperature  $(22 \pm 2 \circ C)$  with constant agitation in deionised water until a final concentration of 11% (w/v), in order to equate and standardise the protein content to egg white protein (previously analised and standardised to 11% for all treatments). Egg white was substituted by 0%, 12.5%, 25%, 50% and 100% of WPI solution.

## Cake elaboration and heating temperature tracking

Sponge cake was prepared using an American sponge cake formulation according to Levy (1982) adding 30.1% of sugar, 20.1% of wheat flour, 27.2% of fresh egg white, 16.9% of fresh egg yolk, 4.5% of liquid milk, 0.6% of baking powder and 0.5% of vanilla. The batter was prepared in three steps; first, fresh egg yolks 95% sugar, milk, vanilla, wheat flour and baking powder were mixed in a N50, 5 Quart Hobart Mixer (Troy, OH, USA) during two minutes at high speed (580 rpm). In another bowl, 5% sugar and fresh whites or WPI solution, were mixed during three minutes at high speed (580 rpm). Finally, the two batters were mixed together during two more minutes at low speed (136 rpm). The final batter (125 mL) was poured into 500 mL aluminium pans (0.12 m diameter, 0.05 m height) and baked at 170  $\circ$ C for 40 min in a Simon Rotary Test Baking Oven (Henry Simon Limited, Stockport, Cheshire, UK). After baking, the cakes were removed from the oven, taken out from the pan and cooled in a Mini AK Kiln (AFOS, Hull, East Yorkshire, UK) at 20  $\circ$ C for 140 min and were immediately evaluated. A temperature kinetics analysis was carried out for each cake using a K-type

thermocouple (20 diameter T/C wire) which was placed in each cake pan at 3 cm from the bottom and coupled to a digital scanning thermometer (Digi-Sens 69202e30, Eutech Instruments, Ayer Rajah Crescent, Singapore). Temperatures were recorded in a personal computer with the Scanlink software v 1.2.1 (Barnant Co. Barrington, IL, USA) and registered every 1 min. Each treatment batch yielded four cakes, and three independent experiments were run for each treatment to obtain twelve samples per treatment.

## Batter characteristics

# Batter density

Batter density was determined as the ratio of the weight of a standard container (10 g/cm3) filled with batter (Fox, Smith, & Sahi, 2004) to that of the same container filled with water ( $21 \circ C$ ) and determined as the average of three independent measurements per treatment.

# Viscosity

The viscosity, after mixing and prior to baking, was evaluated with a 0.015e2000 Pa s-1 Brook-LVT, Brookfield viscometer (Brookfield Engineering, Middleboro, MA, USA) using the 4-spindle. Samples were tempered at 22  $\circ$ C and speed was set up to 0.3 rpm.

Three independent measurements per treatment were done. Ki- nematic viscosity has been calculated as the ratio between viscosity and relative density of the cake batter.

#### Sponge cake quality

All samples were weighed on an Ohaus Adventurer Pro AV4101 precision balance (Ohaus Corp, Pine Brook, NJ, USA). The baking loss (%) was determined by weighing each batter before baking (W) and each cake after baking and cooling (W) and using the and flavour. The panellists rinsed their mouth with water between samples.

#### Texture analysis

Texture was measured on 60 mm-width60 mm-length 40 mm-height samples using a texture analyser (TA-XT plus, Stable Micro Systems, Godalming, Surrey, U.K.), equipped with a 50 mm diameter aluminium cylinder probe and applying a double compression test to penetrate 50% (20 mm) of the sample's height at a test speed of 2 mm s—1. Upper dome and crust sides were removed from all samples as recommended by Go'mez, Ruiz, and Oliete (2011) and Karaouglu, Kotancilar, and Gercekaslan (2008) and determinations were carried out by triplicate. Firmness, cohesiveness, springiness, resilience and chewiness were obtained from the texture profile analysis (TPA). Firmness was defined as the peak of the first compression; the cohesiveness was defined as the ratio of the force area during the second compression and the first compression; the springiness is the ratio between the time of the compression in the second compression cycle and the time of the compression in the first compression cycle; the resilience is the ratio between the area of decompression and the area of compression during the first compression cycle; and the chewiness 0 f is the result of multiplying firmness by cohesiveness by springiness. equation (1); where W0 is the weight (in grams) of batter before baking and Wf is the weight (in grams) of cake after baking. The volume was determined by the seed displacement method and the specific volume was calculated by dividing the product volume by its weight (Lee, Hoseney, & Varriano-Marston, 1982) with a volume accuracy of  $\pm 10$  cm3. Weight, baking loss and specific volume were measured on six independent cake samples per treatment. After the crust was removed, the crumb from the bottom, centre and top was milled for 30 s in a Braun Aromatic KSM2 kitchen blender (Procter & Gamble, Cincinnati, OH, USA). A 4 g sample was placed in an Ohaus MB45 moisture analyser balance (Ohaus Corp, Pine Brook, NJ, USA) and moisture was calculated following the manufacturer's procedure (Pastry method: step drying program at a drying tem- perature of  $170 \circ C/130 \circ C$ ). The average of the moisture content was calculated using six independent samples per treatment.

%Bakingloss Wf — W0 x100 (1)

W0

Sensory evaluation

A preliminary affective sensory test was conducted by 125 volunteer students as untrained panellists (20e22 years of age). Only for sensory test, the samples were hermetically sealed with a plastic film (Cryovac®D-955 Multipurpose Shrink Film,

Gauge 125, Sealed Air, Duncan, SC, U.S.A.) after cooling and kept in controlled environmental conditions at 20 oC during 24 h, then were evaluated on the basis of acceptability of their appearance, colour, odour and

flavour in a hedonic 7-point scale, where 7 was written as the most liked, 4 as neither like nor dislike and 1 as the most disliked. The control cake was presented simultaneously with the rest of the samples (Pedrero & Pangborn, 1989). The sensory analysis was performed under white light and at room temperature; the cakes were presented on plastic plates coded with three-digit random numbers. This evaluation were done in two stages. Firstly, the

Texture analysis was done on six samples per treatment.

# Cake structure

Crumb images were taken from the bottom, centre and top of cylindrical samples (10.6 cm diameter) with a scanner (Scanjet 3010, Hewlett-Packard Development Company LP, Houston, Texas, USA) at 600 dpi. Cell area, cell density, maximum length and shape factor were calculated and analysed according to Díaz-Ramírez et al. (2013), removing all objects smaller than or equal to

0.02 mm2 and larger than or equal to 3.19 mm2. This was the result of previous analysis (results not showed), since they determined this size range in the majority of the porous (histograms). Image analysis evaluations were carried out applying the ImageJ freeware (National Institutes Health, Bethesda, MD, U.S.A.). Six independent cakes were analysed for each treatment.

$$\frac{W_f - W_0}{V_4} \times 100$$

# Cake crystallinity by X-ray diffraction analysis

Sugar and freeze-dried ( $50 \circ C$  during 24 h) crumb samples (Labconco 75034 Bench Top Freeze Dryer from Labconco, Kansas City, MO, USA), taken from the cake were milled for 30 s in a kitchen blender (Braun Aromatic KSM2, Procter & Gamble, Cincinnati, OH, USA). Samples were placed into the sample holder of an X pert-pro X-ray diffractometer (PANalytical B.V., Almelo, Netherlands) adjusted to 45 kV and 40 mA, producing CuKa radiation of 10.154 nm, scanning 2q from  $3\circ$  to  $50\circ$ . The step size was  $0.01\circ$  with a measurement time of 50 s per step. Three independent samples of every treatment were analysed. The X-ray patterns were obtained and the crystallinity was calculated (equation (2)) by integrating the area of the peaks obtained (IT) taking into account the amorphous phase (IA) using the Peak-fit V.4.12 software (Sea-Solve Software Inc, Framingham, MA, U.S.A.) according to Ribotta, Cuffini, Leo'n, and An~o'n (2004). whole cakes were sliced at the middle and without crust were presented to panelists for determining appearance and colour; secondly, 20 g cube samples cut from the centre of the cakes without crust were given to panelists for analysing the colour odour and flavour. The panellists rinsed their mouth with water between samples.

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An ANOVA was done to evaluate differences between samples and the Tukey test was performed if significant differences were found (P < 0.05) using SigmaPlot V.11.0 (Systat Software Inc, San Jose, CA, USA).

Results and discussion

Batter properties and cake quality

Batter density can be related to the amount of air incorporated in the batter during the mixing process (Fox et al., 2004) while viscosity plays an important role in gas cell stability (Sahi & Alava, 2003). Kinematic viscosity denotes the behaviour of the cake batter as a fluid, right before baking, discarding the forces that generate the batter's motion; it represents better the viscosity of the static fluid subjected to heating. All these parameters determine the final quality of the baked cake. According to the results shown in Table 1, the addition of 25% of WPI reduces significantly (P < 0.05) the batter density due to a higher quantity of air entrapped in this batter during mixing while at 50% and 100% of EWP substitution these values were comparable with the control. Regarding viscos-ity, a decreasing tendency was observed between control and 25% of WPI substitution. Sahi and Alava (2003), mentioned that air incorporation depends on beater speed, viscosity and surface ten- sion, among others and included that "the efficiency of air retention depends on the speed at which air bubbles rise out of the batter". It seems that at 25% of WPI substitution the batter viscosity allows the expansion of the bubbles before they rise out from the batter as a decrease in cell density (Table 5) and a higher specific volume (Table 2) were observed. However when viscosity continues decreasing (50% and 100%) an air rising out of the batter takes place and lower specific volume is shown (Table 2). Otherwise 12.5% and 25% of WPI do not show significant differences (P < 0.05) in the kinematic viscosity of the cake batter if compared with the control, but the addition of 100% showed significant differences (P < 0.05) being those values lower than the control. These results are consistent with the specific volume, where 50% and 100% substitution samples presented significantly (P < 0.05) lower values (Table 2) which could be related to air loss during baking. In cakes such as sponge cake, egg proteins (egg yolk and egg white) assist in entrapping large air quantities due to their high capacity to foam air into water based batters. Besides, egg yolk proteins coagulation and gellifying capacity helps to improve crumb cell stability, even without EWP in 100% WPI samples. Some attempts were done to substitute EWP by WPI (Berry et al., 2009; Pernell et al., 2002; Yang & Foegeding, 2010) but more recently Paraskevopoulou et al. (2015) substituted liquid whole egg with WPI and an emulsifier with no significant improvement of cake texture and volume. During the present study, the addition of 12.5% and 25% of WPI did not affect significantly (P  $\leq$ 0.05) the batter viscosity and the addition of 25% of WPI reduce density significantly showing that EWP could be replaced by WPI without decreasing batter air entrapment and cell stability prior to heating.

According to Lostie, Peczalski, Andrieu, and Laurent (2002), two heating rates are observed during sponge cake baking, these au- thors identified the first stage as the "heating up" rate with water migration from the core to the surface by diffusion and heat transfer from the surface to the core of the batter by conduction; and the second stage, named "crust and crumb" rate, the drying velocity continuously increases until reaching a constant moisture content of the crust and the crumb. In the present study and as it can be seen in Table 1 and Fig. 1, during the first stage (up to the first twenty minutes, reaching temperatures close to 90  $\circ$ C), the heating rate was not affected significantly (P < 0.05) by the addition of WPI, showing that the physical changes of the batter constituents did not affect the heat transference during this stage.

The heating rate at the second stage increased significantly (P < 0.05) at 12.5% and 25% substitution (0.281 oC/min and 0.236 oC/min respectively). Additionally, in these cases the specific volume was higher than at the other levels of substitution (Table 2), meaning that the cake network was able to retain more air. In this regard, literature points out that thermal properties (thermal conductivity and heat capacity) (Coimbra et al., 2006) and dena- turation temperature (Donovan, Mapes, Davis, & Garibaldi, 1975; McMahon, Yousif, & Kal'ab, 1993) of WPI and EWP proteins are similar, hence the difference among these systems is trapped air.

Air is a non-conducting component, which exposed to a higher internal pressure in a porous system could promote a heating rate increase.

At 50% and 100% WPI substitution, an opposite effect was observed during the second stage, where the heating rates values decreased significantly from those observed in the control sample ( $0.013 \circ C/min$  and  $0.66 \circ C/min$  respectively). Although negative data were obtained, it means that the temperature was kept con- stant at its maximum value after the first heating stage. Decreasing EWP content (50% substitution) while increasing WPI (50% and 100%) probably affects the interaction between proteins showing a behaviour more related to a WPI gelling system due to the whey proteins has the ability to form heat-induced geles (Frydenberg, Hammershøj, Andersen, Greve, & Wiking, 2016), but also the lower air presence could explain this behaviour.

## Cake quality and sensory evaluation

The sponge quality after baking, measured as baking loss and reported in Table 2, shows that there are no differences (P < P0.05) between control cake weight (0%) and samples with 12.5%, 25% and 50% of WPI substitution, showing only statistical differences (P < 0.05) when 100% WPI was used (19.43  $\pm$  1.8%); this effect could 12.5% WPI substitution samples. When evaluating an overall quality, the panellists did not find differences between 0%, 12.5% and 25% WPI substitution samples. As mentioned by Paraskevopoulou, Amvrosiadou, Biliaderis, and Kiosseoglou (2014), besides the development of a mixed gel structure, heating egg yolkeWPI mixtures is also expected to result in gel flavour characteristics widely different from those of the heat treated single constituents, meaning that aroma and flavour could change as a synergistic effect of both ingredients, and in this case, as a result of the baking process as well. However, a more thorough study focused on the sensory effect of egg yolk-WPI mixes is required. be due to the significant lower weight  $(99.90 \pm 2.8 \text{ g})$  resulted from the moisture loss  $(36.60 \pm 1.1 \text{ g}/100 \text{ g})$  of the crumbs at the bottom of the cake. Sensory evaluation analysis (Table 3) showed that when comparing 12.5% and 25% WPI substitutions to the control, panel-lists did not find any significant difference (P < 0.05) in odour, flavour or appearance; however, they did find significant differ- ences (P < 0.05) in the colour of the cake when comparing 0% and 3.4. Texture and crystallinity. According to the results shown in Fig. 2 and Table 4, the addition of WPI at 12.5%, 25% and 50% did not show significant differences (P <0.05) in the cake crystallinity values; however, at higher WPI concentration (100%) a more crystalline structure become evident. The peaks observed in the X-ray patterns correspond to sucrose crystals (8.36°, 11.7°, 13.2°, 16.7°, 19.6°, 24.7°) and  $38.3\circ$ ) (Fig. 2, diffraction pattern f). In the control cake (0%), the available water is used to solubilise the added sucrose, to form the foam structure and a subsequent gelatinisation of the starch, but in substituted sam- ples, WPI competes with the added sucrose for the available water; hence, as WPI increases, sucrose's solubility decreases, causing it to crystalise when exposed to heat. Chevallier, Colonna, Bule'on, and Della Valle (2000) evaluated the X-ray patterns of dough, crumb and crust of biscuits, attributing the presence of crystalline sucrose in crust to the higher drying rate of the surface. Crystallinity could also decrease the quality of the cake by modifying the texture of the crumbs. A crystalline structure promotes more fragile and less resilient cakes such as it is observed in Table 4. Besides, firmness which is the most studied textural parameter associated with hu- man perception of freshness (Carr & Tadini, 2003) showed a significant reduction (P < 0.05) at 25% WPI substitution, while its value did not increase significantly (P < 0.05) at 50% and 100%. On the other side, chewiness which is the energy required to chew a cake crumb for swallowing, reduced significantly at all WPI substitutions except to 100%.

#### Cake microstructure

The microstructure (Table 5) was also affected as pore area significantly increased (P < 0.05) and pore density significantly decreased (P < 0.05) at 12.5% and 25% WPI substitution; these re- sults are associated with smaller cohesiveness and

firmness, and in the case of 25% WPI substitution higher specific volume; however at 50% and 100% WPI substitution these values changed, leading to smaller areas and higher pore densities than the control. The shape factor (a parameter used to measure pore similarity to a circle) only changed at 100% WPI substitution, being significantly (P < 0.05) higher. Material science has established that as the pore shape gets more circular (higher circularity), its thermal resistance to heat transfer is lower (Polaki, Xasapis, Fasseas, Yanniotis, & Mandala, 2010). Whey proteins have been reported to be very heat sensible (Singh & Havea, 2003); based on these findings, the increase of the shape factor in the cake with 100% WPI substitution could be explained by the heat-denaturation of the protein during baking which changes the protein's elasticity and its interactions with the surrounding medium.

# Conclusions

The substitution of EWP with WPI at 12.5% or 25% does not show significant differences (P < 0.05) in the batter behaviour and in the final quality of the sponge cake. The first stage of the heating rate was not affected by the addition of 12.5%, 25%, 50% and 100% WPI, but at the second stage, the heating rate increased significantly (P < 0.05) when using 12.5% and 25% WPI. One possibility that could explain this effect is that the air trapped in the system affects the thermal conductivity of the batter during the solidification of the foam which exposed to a higher internal pressure in a porous system could promote an increase in the heating rate. Another element that influences the behaviour of the batter during baking is the ability of WPI to form heat-induced geles. Nevertheless, more studies are needed to a better understanding of this phenomenon.

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