Research Article

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Dry-fractionated protein concentrate as egg replacer in sponge cake: how the rheological properties of the batters affect the physical and structural quality of the products

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Abstract

BACKGROUND: Egg replacement is a notable food trend for academics and industry. Dry-fractionated protein concentrates (DFp) are minimally processed and sustainable ingredients. DFp from chickpea, red lentil and mung bean, prepared as aqueous dispersions at 20–40% (w/w), were used to replace egg in sponge cakes. To understand the effect of DFp on the physicochemical features of sponge cakes, the batter rheological properties (i.e., flow behavior, frequency-dependent and temperature-dependent behaviors) were investigated.

RESULTS: Frequency sweep revealed a higher storage modulus (G') than loss modulus (G''), indicating predominantly elastic-like behavior, dependent on the frequency. Increasing DFp content, especially at 40%, resulted in firmer batters, indicated by elevated apparent viscosity. During temperature sweep, G' increased starting from 80 °C in all DFp-based batters, indicating protein and starch conformational changes. Higher DFp content better simulated the egg behavior, affecting specific volume and thickness variation after baking but resulting in harder cakes. Crumb structure was similar to the control, highlighting that DFp can emulate the egg behavior in cake preparation. Protein content in cakes containing 30% DFp was similar to the control. However, the addition of DFp caused an increase in phytic acid. Sensory analysis of sponge cakes revealed differences in crust color, sweetness and legume flavor, with minimal effect on astringency. Chickpea and lentil DFp are suggested as preferred alternatives because of their to milder sensory impact.

CONCLUSION: Overall, eggs in cake formulation can be substituted by plant-based protein produced by dry fractionation. However, further research is essential to evaluate the nutritional characteristics.

Supporting information may be found in the online version of this article.

Keywords: egg replacers; dry-fractionated protein; rheological properties; sponge cake batter; crumb structure; plant-based protein

INTRODUCTION

The popularity of plant-based proteins is steadily growing, due to increased awareness of the health and sustainability issues associated with the production and consumption of animal-based proteins.[1] In most cases, plant proteins available on the market, used for food preparation, are obtained through a ‘wet fractionation’ procedure, involving water together with alkalis, acids and/or other chemical substances to extract the protein.[2] The conditions used in this process – for example, the acidic environment and/or the temperatures used in the drying operations – often lead to protein denaturation, causing the loss of some functional properties, such as the solubility, foaming ability and gelling capacity.[3] By contrast, the ‘dry fractionation’ process is more resource efficient, as it concentrates proteins by relying exclusively on physical methods.[4] Moreover, the dry fractionation process preserves the protein structure, resulting in an improved protein functionality, especially in terms of foaming ability and foam stability.[3]

Such functional properties of the dry-fractionated protein are particularly suitable to formulate sponge cakes or similar bakery products that require effective air incorporation and the retention of gas cells in the cake matrix.[5] Traditionally, this structuring and

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stabilizing role is played by the egg proteins, which then have a major effect on the quality of the cake. However, consumer concerns related to the presence of cholesterol, allergenicity of egg proteins, as well as the expanding demand for plant-based food,[1] triggered an increasing interest in finding egg replacer alternatives.[1] In fact, the egg replacer market is expected to grow at a compounded average growth rate of 5.61% from 2022 to 2028.[6]

According to a recent review by Yazici and Ozer,[1] legume protein ingredients, especially soy protein, are frequently used as egg replacers. In addition, hydrophobically modified pea protein isolate,[7] aquafaba[8] and lentil protein isolate[9] have been previously proposed as egg substitutes in cake formulations.

Chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* (L.) Medik.) are typical legumes of the Mediterranean area and they were previously studied for their performance during the dry fractionation process.[4,10] Mung bean (*Vigna radiata* (L.) R. Wilczek) is widely produced and consumed in Asian countries, and is gaining interest worldwide due to the nutritional[11] and functional properties of its dry-fractionated protein concentrates.[12] However, to the best of the authors' knowledge, the utilization of dry-fractionated proteins as egg replacers in cake formulation has not been previously reported.

Therefore, in this paper, dry-fractionated proteins extracted from chickpea, lentil and mung bean were used as egg replacer in sponge cake preparation with the aim of investigating their effect on the rheological properties of batters and to assess their influence on the textural properties and the physicochemical quality of sponge cakes.

MATERIALS AND METHODS

Materials

Dry-fractionated chickpea, mung bean and lentil protein concentrates were purchased from InnovaProt s.r.l. (Gravina in Puglia, Italy), and they had 568, 569 and 557 g kg^{-1} protein content (dry matter basis), respectively, and 73, 26 and 25 g kg^{-1} lipid content (dry matter basis), respectively. The seeds were finely milled using a pin mill (CW 250 II, Hosokawa Alpine AG, Augsburg, Germany) and then fractionated using a Turboplex ATP 315 air classifier (Hosokawa Alpine AG, Augsburg, Germany). The protein and lipid contents were determined as described under 'Chemical determinations', below.

Plain wheat flour (type '00' Molino Casillo S.p.A., Corato, Italy), granulated sugar (Eridania, Bologna, Italy), corn starch (Maizena, Rome, Italy), whole chicken eggs (Aia S.p.A., Caselle, Italy), almond drink (Abafoods s.r.l., Badia Polesine, Italy), sunflower oil (Olearia De Santis, Bitonto, Italy) and baking powder (Cameo S.p.A., Desenzano del Garda, Italy) were purchased from the local market.

Preparation of sponge cakes

Sponge cakes were prepared according to Majzoobi *et al*.,[13] with some modifications. The formulation of the sponge cakes is reported in Table 1. To prepare the egg replacer, water and dry-fractionated protein concentrates (DFp) of lentil (L), chickpea (c) and mung bean (M) at ratios of 80:20, 70:30 and 60:40 (w/w) were whipped for 12 min with an electric mixer at 2300 rpm (5KHM5110EWH, Kitchen Aid, Antwerp, Belgium). Sugar was then added and the mixture was whipped for 3 min. For preparation of the control sponge cake (CTRL), eggs and sugar were whipped following the same process. Then, the liquid ingredients – that is, the almond drink and sunflower oil – were added, and mixed for 7 min at 100 rpm. Finally, wheat flour, corn starch and baking powder were added and mixed for 7 min at 100 rpm. The batter was poured into springform cake pans having a diameter of 10 cm and baked in an electric oven (Smeg, Guastalla, Italy) for 35 min at 180 °C, following a Latin square design pattern to minimize any effect of location during baking. The sponge cakes were allowed to cool at room temperature for 2 h, and then analyzed.

Rheological properties of batters

The rheological properties of batters were measured with a rheometer (HAAKE MARS iQ Air, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a parallel plate geometry (P35/Ti-02180932). All the determinations were carried out at 1 mm gap between the plates.

The shear rate ramp test was performed by increasing the shear rate from 1 to 100 s^{-1} at 25 °C. The data were fitted to the Ostwald–de Waele model, according to the following equation[14]:

$$\tau = K \times \dot{\gamma}^{n}$$

where τ = shear stress (Pa), K = consistency index (Pa s^n); $\dot{\gamma}$ = shear rate (s^{-1}) and n = flow behavior index.

The oscillatory frequency sweep and temperature sweep were carried out following the procedures described in Li *et al*.,[15] with some modifications. For the oscillatory frequency sweep the frequency varied from 0.1 to 10 Hz, at a strain value of 0.5%, within the linear viscoelastic region of the batters. The storage modulus (G') and loss modulus (G'') as a function of the frequency were recorded. The loss factor (tan δ) was calculated as the ratio between G'' and G'.[12]

The temperature sweep test was performed at 0.5% strain and 1 Hz frequency. The batters were heated from 25 to 95 °C at a heating rate of 5 °C min^{-1}. They were kept at 95 °C for 5 min. The geometry containing the sample was covered with a sample hood during the analysis to prevent water evaporation. Th' G' an" G'' moduli were recorded as a function of the temperature. Two technical replicates for each batter were performed.

Physical and textural properties of the sponge cake

The specific volume of the sponge cakes (mL g^{-1}) was determined following the rapeseed displacement method, as described in the method AACC 10-05.01.[16] Thickness variation after baking was evaluated with a caliper as described by Pasqualone *et al*.[17] and calculated as follows:

$$\%\text{Variation of thickness} = \frac{\text{Thickness after baking} - \text{Thickness before baking}}{\text{Thickness before baking}} \times 100$$

Texture profile analysis was performed using a Z1.0 TN texture analyzer (Zwick Roell, Ulm, Germany) equipped with a cylindrical probe (36 mm diameter) and a 50 N load cell, according to the method described in Gómez *et al*.,[18] with some modifications. The samples were cut into cubes (25 mm side) and compressed twice at 1 mm s^{-1} speed until 50% deformation was reached, with 5 s pause between the two compressions. The following parameters were evaluated, according to Summo *et al*.[19]: firmness (N), indicating the maximum force recorded during the first compression; springiness, measured by the height detected during the second compression divided by the original height of the sample; cohesiveness, measured as the area of work during the second compression divided by the area of work during the

Table 1. Formulation of the sponge cake control and sponge cake with egg replacer

Ingredient (g)	C20	C30	C40	L20	L30	L40	M20	M30	M40	CTRL
Eggs	—	—	—	—	—	—	—	—	—	100
Chickpea protein concentrate	20	30	40	—	—	—	—	—	—	—
Lentil protein concentrate	—	—	—	20	30	40	—	—	—	—
Mung bean protein concentrate	—	—	—	—	—	—	20	30	40	—
Water	80	70	60	80	70	60	80	70	60	—
Other ingredients for each trial[a]	430									

[a] Other ingredients for each trial: sugar (80 g), corn starch (40 g), plain wheat flour (160 g), baking powder (10 g), sunflower oil (40 g), almond milk (100 g).

first compression; and chewiness (N), calculated as firmness × cohesiveness × springiness.

Image analysis of the crumb structure was carried out using the method described in De Angelis *et al.*[20] using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Briefly, sponge cakes were cut in half, and an RGB image was collected using a Canon EOS 600D camera (Canon, Tokyo, Japan) equipped with a Canon EF-S 18–200 mm f/3.5–5.6 IS lens. The image was first converted into 8-bit grayscale, and an area of 40 × 40 mm was considered for the analysis. The threshold function was used to process the image and carry out the cell count, selecting cells having an area ≥ 0.05 mm^2. Cell density was calculated as the number of cells divided by the area used for the analysis.

Chemical determinations

Protein content (N × 6.25) of the dry-fractionated proteins and sponge cakes were determined according to the method AOAC 979.09.[21] The lipid content was determined using a Soxhlet apparatus (SER 148 extraction system, Velp Scientifica s.r.l., Usmate Velate, Italy) following the method AOAC 945.38F,[21] with diethyl ether as extraction solvent. Phytic acid was determined using the phytic acid assay kit K-PHYT 05/07 (Megazyme, Bray, Ireland), in accordance with the instructions of the manufacturer.

Sensory analysis

Quantitative descriptive analysis of sponge cakes was performed by a panel of nine trained members composed of four men and five women (aged between 26 and 57 years). Panelists were informed of the objectives of the study, and each participant provided written informed consent via the statement 'I am aware that my responses are confidential, and I agree to participate in this survey', where an affirmative reply was required to enter the survey. They indicated that they did not suffer from any food intolerances or allergies. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption. A training session to acquire confidence with the lexicon was carried out, to fix the scale anchors, define the list and the intensity of the descriptors, as described by Pasqualone *et al.*[22] The panelists evaluated the sponge cakes using an unstructured 10 cm scale in terms of crust color (i.e., 0: yellow–9: brown) and crumb color (i.e., 0: white–9: dark yellow), sweetness, legume flavor and astringency (i.e., 0: not perceived–9: very intense).

Statistical analysis

Two different batters for each formulation were prepared, and for each batter two different baking trials have been performed. Accordingly, the determinations on the batters were repeated twice ($n = 2$), whereas the analysis on the cakes were repeated four times ($n = 4$). All the experimental data of the trials containing the egg replacer were subjected to two-way analysis of variance (ANOVA) followed by the Tukey's HSD test for multiple comparisons at $\alpha = 0.05$, considering the amount of DFp and the type of legume as independent variables, together with their interaction. The differences between the samples and the control were evaluated using Dunnett's multiple comparisons test at $\alpha = 0.05$. The data of the physical and textural properties of the sponge cake were also subjected to a Pearson correlation at 95% confidence interval. All data were processed by Minitab Statistical Software (Minitab Inc., State College, PA, USA).

The plot of the rheological data and of the sensory properties (i.e., a heatmap) were made using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Rheological properties of batters

Frequency sweep analysis and flow behavior of batters

The storage modulus (G') and loss modulus (G'') of the batters as a function of frequency (Hz) are depicted in Fig. 1. They represent the total resistance of the batter to flow as a function of the linear frequency.

All the batters containing DFp exhibited a storage modulus (G') higher than loss modulus (G''), indicating the predominance of an elastic-like behavior,[23] with a frequency-dependent relationship. Notably, both moduli exhibited an increase with rising frequency, a phenomenon previously documented in cake batters supplemented with both vegetable and animal proteins.[23,24] The CTRL batter showed a frequency-dependent behavior similar to the batters containing the DFp. However, the absolute values of the moduli were comparatively lower than those in the other samples, indicating a batter with a more fluid-like behavior. Moreover, the moduli nearly overlapped, especially at higher frequency, reaching the crossover point at 10 Hz. This observation confirms that the CTRL batter demonstrated a more liquid-like rather than elastic behavior in comparison to the DFp-based batters.[24,25] This is also evidenced by the tan δ calculated at 1 Hz for all the batters and reported in Table 2. In particular, the CTRL batter showed the highest tan δ, which indicates a more liquid-like behavior, without significant similarities to any of the batters containing DFp. Consequently, the inclusion of DFp conferred a more elastic solid-like behavior to the batters. This effect becomes particularly evident when examining the flow curves, as depicted in Fig. 2, where apparent viscosity is plotted against shear rate. The batters

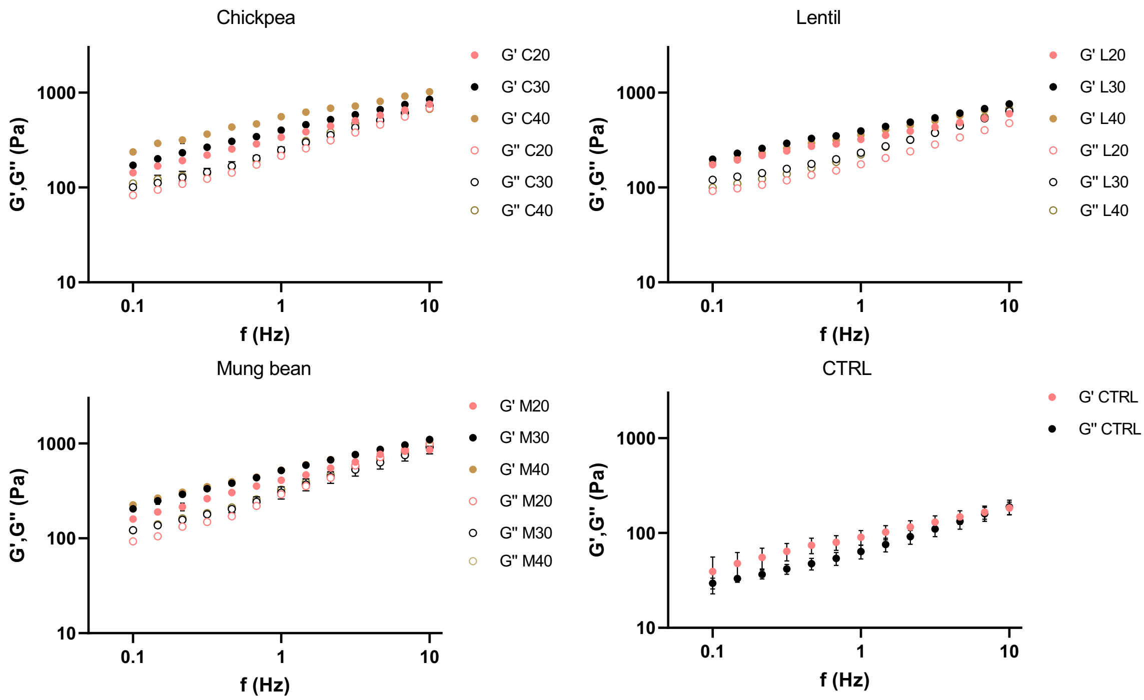

Figure 1. Mean storage modulus (G', Pa) and loss modulus (G'', Pa) of sponge cake batters prepared with an aqueous dispersion of chickpea (C), lentil (L) and mung bean (M) proteins at 20–40% as a function of the linear frequency (f, Hz). Bars indicate standard deviation ($n = 2$). CTRL, control sponge cake batter.

Table 2. Rheological properties of cake batters prepared with an aqueous dispersion of chickpea (C), lentil (L) and mung bean (M) proteins at 20–40%

	Flow behavior (shear rate ramp)		Frequency sweep at 1 Hz			Temperature sweep at 95 °C
Sample	K (Pa s^n)	n	G' (Pa)	G'' (Pa)	tan δ	G' (Pa)
C20	40.62 ± 10.63b	0.54 ± 0.06a	338 ± 2c	215 ± 2c	0.64 ± 0.00b	16 673 ± 794c
C30	57.89 ± 6.31b	0.52 ± 0.03a	400 ± 8b	247 ± 11b	0.62 ± 0.02b	21 714 ± 735b
C40	128.65 ± 10.55a	0.36 ± 0.02b	667 ± 22a	447 ± 18a	0.67 ± 0.00a	29 192 ± 712a
L20	69.58 ± 7.93b	0.49 ± 0.04a	320 ± 16c	176 ± 9b	0.55 ± 0.00a	20 415 ± 1285c*
L30	79.85 ± 8.38b	0.51 ± 0.03a	393 ± 5a	232 ± 7a	0.59 ± 0.01a	32 510 ± 100b
L40	260.00 ± 33.70a	0.28 ± 0.04b	356 ± 6b	223 ± 26a	0.63 ± 0.08a	41 035 ± 1485a
M20	45.85 ± 1.59c	0.53 ± 0.00a	408 ± 7b	290 ± 2a	0.58 ± 0.01a	20 730 ± 470b*
M30	124.55 ± 1.45b	0.46 ± 0.00b	517 ± 23a	325 ± 10a	0.54 ± 0.01b	27 920 ± 670a
M40	242.25 ± 9.05a	0.46 ± 0.01b	520 ± 20a	303 ± 32a	0.55 ± 0.00b	25 718 ± 1338a
CTRL	18.06 ± 0.72	0.54 ± 0.01	90 ± 11	64 ± 7	0.71 ± 0.00	19 480 ± 283

Note: Different letters for the same parameter indicate significant differences within the same species, according to Tuckey's HSD test ($P \leq 0.05$).
Abbreviations: K, consistency index; n, flow behavior index; CTRL, control sponge cake batter.
*The value is similar to the control (CTRL) according to Dunnett's test ($P > 0.05$). $n = 2$.

containing 40% DFp showed the highest viscosity, and they were consequently characterized by the highest consistency index (K), calculated by the Ostwald–de Waele model (Table 2). The hardening effect of the vegetable protein was previously observed[23,26] and can be related to the water absorption of the protein and to a reduction in free water in the batter cake.[9] Moreover, Bustillos *et al.*[24] reported that the viscosity and K in cake batter increased at higher concentrations of pea protein because protein particles tended to connect to each other in the continuous phase of the batter. Furthermore, the batters showed a shear thinning behavior, having a flow behavior index (n) lower than 1 (Table 2). This suggests that as the shear rate increases the microstructure of the batter aligns in the direction of flow, leading to a reduction in apparent viscosity.[9]

The viscoelastic properties of cake batter affect the quality features of the final product, such as the volume, crumb porosity and texture.[1] In particular, during the baking process, a lower batter viscosity can easily raise the bubbles to the surface and cause them to be lost in the atmosphere, resulting in cakes collapsing in the oven.[26] Conversely, if the consistency of the batter is excessively high, this might hinder the expansion of air bubbles during baking,[1] thereby compromising the overall quality of the product.

Viscoelastic properties of the batters during heating

The temperature-dependent behavior of the batters is depicted in Fig. 3. The temperature sweep was used to simulate and understand the main rheological phenomena occurring during the baking process. The trend observed was similar in the batters containing egg replacer prepared with the three legumes under investigation.

In the early stage of the analysis, G' exhibited relative stability with minor fluctuations. The latter were probably given by the temperature increase and by the heat transfer in the batter, resulting in changes in the viscosity of the product. A sharp increase of G' was then observed at approximately 80 °C in all the formulations containing DFp. This increase persisted at a consistent rate until reaching 95 °C. During the isotherm step at 95 °C, G' slightly increased in all the formulations. This behavior explains the conformational changes due to protein denaturation, coagulation and subsequent gelation, together with starch gelatinization.[27] Similar heating patterns were observed in previous studies on batters formulated with legume proteins.[23,27] In particular, it was previously reported that the globulin contained in legume proteins denatures at a temperature range of 78–83 °C, causing an increase in the G' modulus.[12,28] Therefore, it is reasonable to assume that the structure of the product started to develop at around 80 °C. This transformation shifted the product from a fluid, aerated emulsion, to an elastic and porous structure.[14] In this respect, temperature sweep tests can be considered an excellent tool to understand the structural modifications in batters during the heating process, providing information on viscoelastic changes during the cooking process.[27] A closer examination reveals a significant effect on G' modulus recorded at the end of the analysis (Table 2), related particularly to both protein concentration and protein type. It is evident that increasing the protein concentration led to a significant increase in G' in all the three proteins under investigation, indicating that the proteins were responsible for strengthening the elastic behavior of the batter. Moreover, considering absolute values, lentil-based batters showed the highest G'. This might be related to the protein fractions that are mainly composed of legumin-like and vicilin-like

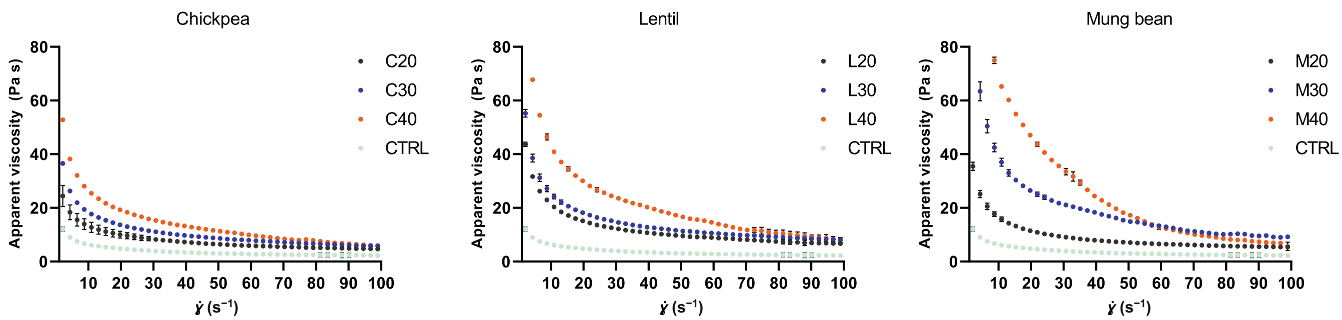

Figure 2. Apparent viscosity (Pa s) of sponge cake batters prepared with an aqueous dispersion of chickpea (C), lentil (L) and mung bean (M) proteins at 20–40% as a function of the shear rate ($\dot{\gamma}$, s^{-1}). Bars indicate standard deviation ($n = 2$). CTRL, control sponge cake batter.

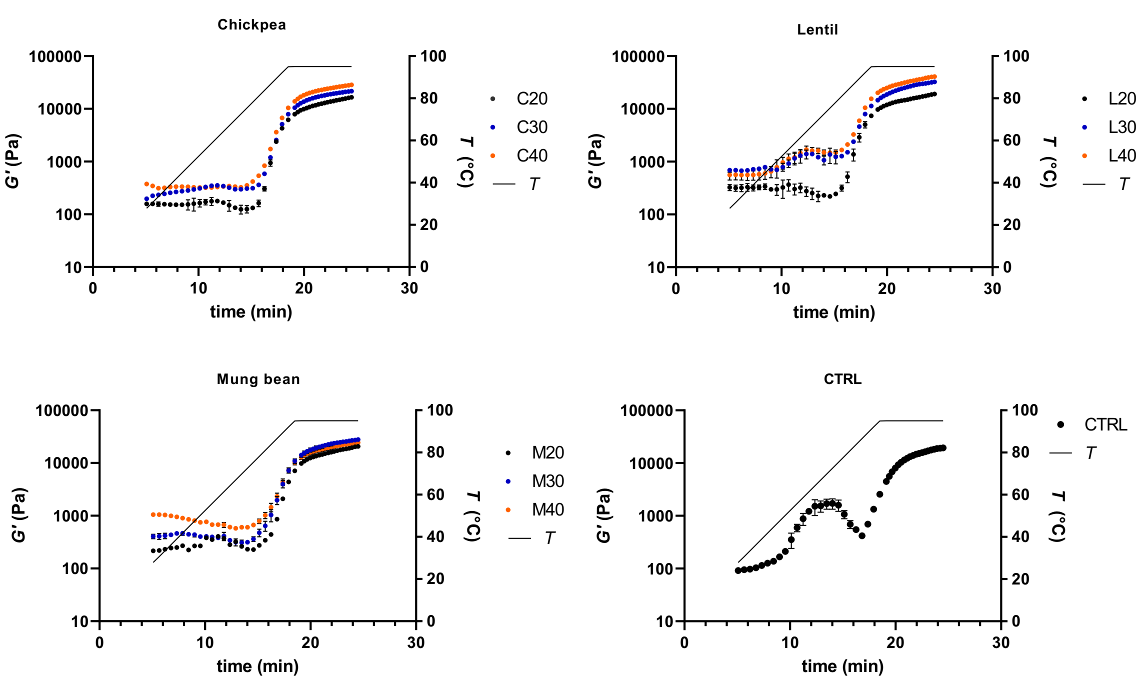

Figure 3. Storage modulus (G', Pa) of sponge cake batters prepared with an aqueous dispersion of chickpea (C), lentil (L) and mung bean (M) proteins at 20–40% as a function of temperature (T, °C). Bars indicate standard deviation ($n = 2$). CTRL, control sponge cake batter.

globulins and albumin (approximately 15%). Lentils contain a slightly higher quantity of globulins (approximately 70%) compared to mung bean (approximately 62%)[29] and chickpea (approximately 53–60%).[30] Therefore, such differences may explain the G' values, being the globulin fraction predominantly involved in the gelation reactions.[30] Moreover, G' values of L20 and M20 were significantly similar to the CTRL batter. During heating, the CTRL batter showed two inflection points, at 45–50 °C and at about 85 °C, with a short drop in the G' modulus observed between 80 and 85 °C. This trend was previously observed in control batters prepared with wheat flour and whole eggs,[14] and it was explained by a drop in viscosity due to sugar dissolution in the batter.[14] The higher consistency of the batters containing the egg replacer might have hidden this drop, mitigating the effect of sugar dissolution. The mechanism of the structure formation of egg-based cakes during baking has been reported in a recent review,[31] and it majorly involves the denaturation of ovalbumin contained in the egg white and coagulation of the egg yolk proteins (livetin, phosvitin and lipoproteins), which contribute to network development through the formation of disulfide bonds and other intermolecular interactions.[31]

Overall, despite the differences in the type of protein involved, the development of consistency during thermal treatment showed similarities between the batter containing the egg replacer and the CTRL, indicating that DFp are able to imitate the egg viscoelastic behavior when used in cake batters.

Physical and textural properties of the sponge cake

The physical and textural properties of the sponge cakes are reported in Table 3. The 'type of legume' variable significantly influenced all the parameters under investigation, with the exception of cell density, whereas the variable 'DFp amount' was not significant only for the cell class $0.5 < x < 1$ mm^2. Moreover, a significant interaction between species and concentration was found for all the physical properties of the cakes, except for the cell class $0.5 < x < 1$ mm^2 ($P = 0.056$), meaning that the magnitude of the changes observed in the parameters was not solely dependent on the species or concentrations (main effects) but also on their combination. In particular, considering the specific volume, it appeared to be positively affected by the quantity of DFp used in the sponge cake formulation, with the highest values found for the sponge cakes containing 40% DFp. Species also affected the specific volume, and the sponge cakes produced with lentil DFp showed the highest specific volume, followed by those that were chickpea based and mung bean based. The CTRL sponge cake showed a specific volume of 2.4 mL g^{-1}, which was only comparable to the L40 sponge cake. The other trials showed a lower specific volume compared to the CTRL, indicating that a lower protein concentration resulted in a less expanded cake structure. It was recently reviewed that the specific volume is highly variable in cake formulation, ranging from 1.38 to 4.08 mL g^{-1};[31] therefore, the results obtained with the formulations proposed in this study fall in this range. The specific volume, being dependent on the ability of the cake batter to retain gases during the baking process, is a direct indicator of the quality of the sponge cakes.[1,32] In particular, the specific volume could be related to both batter viscosity and to the gel-forming ability of batter during baking.[1] In fact, the observed results are corroborated by the rheological properties of the batters, which highlighted the highest apparent viscosity (Fig. 2) and the highest G' after heat treatment (Fig. 3) in the batters containing 40% DFp. Therefore, a higher quantity of DFp might have improved the viscoelastic properties of the batters, also enhancing retention of the gases developed during baking,[9,26] without hampering the expansion of air bubbles. Consequently, a similar trend was observed in the thickness variation recorded after baking, as also demonstrated by the significant and positive correlation with the specific volume ($r = 0.753$, $P = 0.011$). Therefore, it may be inferred that high DFp concentrations are needed to replicate the expansion phenomena given by egg in cake preparation.

The results of the texture profile analysis of the sponge cakes are also reported in Table 3. Both species and concentration significantly influenced the textural properties of the cake. In fact, the variable 'type of legume' was highly significant for all the textural properties ($P \leq 0.001$). In particular, the lentil protein conferred a stronger structure to the products compared to the other proteins. Overall, the lentil-based cakes showed the highest firmness and chewiness. These results might be explained by the rheological properties observed during the temperature sweep analysis (Fig. 3). In fact, the batters containing DF lentil protein showed the development of the highest G' modulus during heating, meaning the formation of the strongest viscoelastic network (Table 2). Moreover, it should be considered that lentil protein is characterized by a higher content of globular protein compared to mung bean[29] and chickpea,[30] which may be responsible for the strong gelling ability of the protein.

The variable 'amount of protein' also showed a highly significant effect on the textural properties and, accordingly, higher DFp concentrations led to a significant increase in firmness and other textural indices. Therefore, the products containing 40% of DFp showed the highest firmness, springiness, chewiness and cohesiveness. However, this behavior is in contrast to the increase in specific volume recorded when higher concentrations of DFp were used. In particular, it is generally reported that the firmness of cakes is inversely correlated with specific volume.[33] In fact, higher protein content in the batter can increase the overall firmness of the product owing to a combination of factors including: (i) the gelling ability and formation of a stronger protein network[1]; (ii) the reduction in free water due to the water absorption capacity of the vegetable protein.[13]

The textural indices of all the trials showed similarities to the CTRL, as determined by Dunnett's test, except for C20. In fact, the CTRL showed a firmness of 7.14 N, which was similar to that of M30 and C30. Overall, these results suggest that varying the protein source and the protein quantity can yield different textural properties, allowing the production of products with the desired texture in contrast to traditional egg-based cakes.

Image analysis was carried out to evaluate the crumb structure of the sponge cakes, and the results are reported in Table 3. Slight but significant differences were detected in the three porous classes under investigation and in the cell density. In particular, the crumb structure was characterized by the presence of small cells, with the majority of the observations – that is, 52–59% – having an area ≤ 0.5 mm^2. C20 showed the highest porosity in this dimensional range, whereas the other trials showed similar values compared to the CTRL. C20 also showed the lowest percentage of pores with an area ≥ 1 mm^2, and therefore this trial showed the major differences compared to the crumb structure of the CTRL. The other trials showed slight but significant differences among them, without following a clear pattern considering the species and the concentrations. The cell density was always similar to the CTRL. The variable 'type of legume' was significant for the porous classes and not significant for the cell density. It can be observed that the chickpea-based cakes were characterized by

Table 3. Mean value, standard deviation and results of statistical analysis two-way ANOVA of the physical characteristics of sponge cakes prepared with an aqueous dispersion of chickpea (C), lentil (L) and mung bean (M) proteins at 20–40%

Sample	Specific volume (mL g^{-1})	Thickness variation (%)	Firmness (N)	Springiness	Chewiness (N)	Cohesiveness	≤0.5 mm^2	0.5 < x < 1 mm^2	≥1 mm^2	n. cells cm^{-2}
C20	1.84 ± 0.03d	122 ± 2d	4.67 ± 0.27f	0.73 ± 0.01abc	1.01 ± 0.13d	0.30 ± 0.04d	76.61 ± 8.74a	12.31 ± 2.62b*	13.69 ± 3.50d	16.72 ± 2.91ab*
C30	2.12 ± 0.06abc	126 ± 6d	6.58 ± 0.25de*	0.71 ± 0.02abc	1.58 ± 0.03 cd	0.34 ± 0.01 cd*	58.72 ± 4.26b*	13.31 ± 1.17ab*	25.78 ± 2.19bc*	17.31 ± 0.44ab*
C40	2.21 ± 0.17ab	152 ± 4b*	9.33 ± 0.33c	0.74 ± 0.03abc	2.11 ± 0.15bc*	0.31 ± 0.01d	54.94 ± 1.03b*	13.48 ± 0.10ab*	31.58 ± 1.13ab*	16.00 ± 0.81b*
L20	2.13 ± 0.02abc	138 ± 2c*	6.30 ± 0.10e	0.70 ± 0.02bcd	1.51 ± 0.05 cd	0.34 ± 0.02cd*	59.09 ± 4.83b*	17.13 ± 3.06a*	23.79 ± 1.77c*	20.13 ± 0.31a
L30	2.09 ± 0.08abc	154 ± 6b	11.40 ± 0.40b	0.80 ± 0.01ab*	2.93 ± 0.07b*	0.32 ± 0.01 cd	51.69 ± 1.69b*	14.77 ± 0.37ab*	33.53 ± 2.06a*	15.22 ± 0.47b*
L40	2.29 ± 0.05a*	170 ± 2a	15.20 ± 0.17a	0.69 ± 0.01 cd	5.53 ± 0.02a	0.53 ± 0.01ab	59.34 ± 0.07b*	13.65 ± 0.74ab*	27.00 ± 0.81abc*	17.91 ± 0.97ab*
M20	1.86 ± 0.05d	106 ± 6e	3.45 ± 0.05 g	0.61 ± 0.04d	1.03 ± 0.08d	0.42 ± 0.01c*	59.32 ± 4.16b*	12.97 ± 0.20ab*	27.72 ± 3.96abc*	17.59 ± 0.03ab*
M30	1.95 ± 0.05 cd	110 ± 2e	7.46 ± 0.08d*	0.48 ± 0.08e	1.54 ± 0.01 cd	0.43 ± 0.01bc*	56.24 ± 3.76b*	12.40 ± 2.06ab*	31.36 ± 1.70ab*	16.94 ± 1.81ab*
M40	2.02 ± 0.03bcd	122 ± 2d	9.64 ± 0.02c	0.81 ± 0.02a*	4.73 ± 0.06a	0.63 ± 0.06a	52.84 ± 0.57b*	15.05 ± 1.41ab*	32.11 ± 1.99ab*	14.66 ± 0.91b*
P-value type of legume × amount	0.012	0.005	<0.001	<0.001	<0.001	<0.001	0.002	0.056	<0.001	0.006
F-value	4.34	5.50	35.79	30.14	29.47	11.08	6.64	2.81	11.41	5.07
CTRL	2.4 ± 0.09	142 ± 6	7.14 ± 0.36	0.85 ± 0.01	2.48 ± 0.31	0.42 ± 0.01	57.45 ± 2.39	14.14 ± 1.69	28.41 ± 0.70	17.66 ± 2.09
C	2.06 ± 0.20b	133 ± 15b*	6.86 ± 2.04b*	0.73 ± 0.02a*	1.57 ± 0.49c*	0.31 ± 0.03c*	63.42 ± 11.15a*	13.03 ± 1.53b*	23.69 ± 8.19b*	16.68 ± 1.63a*
L	2.17 ± 0.10a	154 ± 14a*	10.97 ± 3.87a*	0.73 ± 0.05a*	3.32 ± 1.77a*	0.40 ± 0.10b*	56.71 ± 4.55b*	15.18 ± 2.21a*	28.11 ± 4.53a*	17.75 ± 2.20a*
M	1.94 ± 0.07c	113 ± 8c	6.85 ± 2.74b*	0.64 ± 0.15b	2.43 ± 1.78b*	0.49 ± 0.11a*	56.13 ± 3.98b*	13.47 ± 1.74ab*	30.39 ± 3.12a*	16.40 ± 1.68a*
P-value type of legume	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.002	0.031	<0.001	0.086
F-value	22.18	240.25	459.46	21.70	85.78	52.10	8.67	4.26	19.29	2.82
20	1.94 ± 0.14c	122 ± 14c*	4.81 ± 1.28c	0.68 ± 0.05b	1.18 ± 0.40c*	0.35 ± 0.04b*	65.01 ± 10.25a*	14.14 ± 3.03a*	21.73 ± 6.86b*	18.15 ± 2.12a*
30	2.05 ± 0.14b	130 ± 20b*	8.48 ± 2.25b*	0.66 ± 0.15b	2.02 ± 0.71b*	0.36 ± 0.05b*	55.55 ± 4.28b*	13.49 ± 1.59a*	30.23 ± 3.87a*	16.49 ± 1.36b*
40	2.17 ± 0.18a	148 ± 21a*	11.39 ± 2.87a*	0.74 ± 0.06a*	4.12 ± 1.59a	0.49 ± 0.15a	55.71 ± 2.93b*	14.06 ± 1.09a*	30.23 ± 2.71a*	16.19 ± 1.61b*
P-value amount	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	0.671	<0.001	0.009
F-value	23.42	99.75	887.90	12.66	604.46	37.05	15.47	0.41	39.93	6.14

Note: Different letters for the same parameter mean significant differences according to the Tuckey's HSD test ($P \leq 0.05$).
Abbreviation: CTRL, control sponge cake.
*The value is similar to the control (CTRL) according to Dunnett's test ($P > 0.05$). $n = 4$.

the highest presence of pores with an area ≤ 0.5 mm^2, and the lowest quantity of pores with an area ≥ 1 mm^2.[34] The protein amount showed a significant effect on the crumb porosity, and the cakes produced with 30% and 40% DFp showed the highest presence of large pores (i.e., with an area ≥ 1 mm^2). This confirms that high DFp content is necessary to imitate the egg behavior in sponge cake preparation. Overall, all these trials showed similarities to the CTRL, highlighting that the DFp allowed imitation of the egg behavior in cake preparation. This is particularly important, considering that cakes produced with egg replacers are reported to have inferior crumb structures compared to the controls[1] and that, to overcome this drawback, emulsifiers are often used.[1] Moreover, it was previously reported that the increased viscosity of batters containing pea protein hampered the air incorporation during mixing, causing a reduction in the cell dimension.[24,31,35] Therefore, it has been suggested that a vigorous mixing is then necessary to overcome the drawbacks given by the protein addition.[24] The crumb cell dimensions are generally used as a quality parameter for bakery goods[1] and they contribute to the textural properties of the products.[1,36] However, it should be remembered that alongside the cell dimension the surrounding matrix – that is, the cell wall – also contributes to the mechanical strength of the product.[36] In fact, no significant correlations were found between the results of the image analysis and the textural evaluation of the sponge cakes (data not shown). Therefore, it is reasonable to assume that the viscoelastic properties of the batters may better explain the phenomena occurring during baking, which are responsible for the quality of the products.

Chemical determinations

The chemical characteristics of the sponge cakes are presented in Fig. 4. Protein and lipid contents proportionally increased as the concentrations of DFp increased in the formulation. The protein content of the formulations containing 30% DFp was similar to the CTRL, and it was significantly higher in the trials containing 40% DFp. However, egg is considered to be a balanced source of protein in terms of amino acid composition,[37] whereas legumes and cereals themselves are not able to provide a balanced amino acid intake.[32] By contrast, their combination is a good strategy to balance the amino acid composition.[9] Further investigation of the nutritional value and protein digestibility could be the object of future studies.

The lipid content of the CTRL was higher than that of all the formulations containing the egg replacer, the only exception being C40. In fact, the whole egg usually has a lipid content of around 95 g kg^{-1},[37] which is higher than the lipid content of the legume proteins used in this study.

Phytic acid was also determined since it is naturally found in legume flours and has been observed to follow the protein fraction during the dry fractionation process.[4] Indeed, its levels increased proportionally with the higher concentrations of DFp used in the cake formulations. The CTRL showed a phytic acid content of 2.0 g kg^{-1}, which was similar only to the C20 formulation. Wheat flour also contains phytic acid in a range of 3.4–7.6 g kg^{-1}.[38] Phytic acid is considered an antinutritional factor because it reduces the bioavailability of minerals and micronutrients.[39] However, some antioxidant and beneficial health roles have been also associated with phytic acid,[39] suggesting that further studies are needed to comprehensively evaluate the nutritional value of the food formulated with these kinds of plant-based ingredients.

Sensory analysis

The heatmap shown in Fig. 5 describes the results of quantitative descriptive sensory analysis carried out to evaluate the sponge cakes in terms of crust and crumb color, sweetness, legume flavor and astringency, whereas the mean data, standard deviation and results of statistical analysis are reported as Supplementary Material (S1). The differences were particularly evident for the crust color, which appeared darker in the cakes produced with the egg replacer compared to the CTRL, regardless of the species and of the concentration. The darkening effect of the protein incorporation in bakery goods has been previously reported,

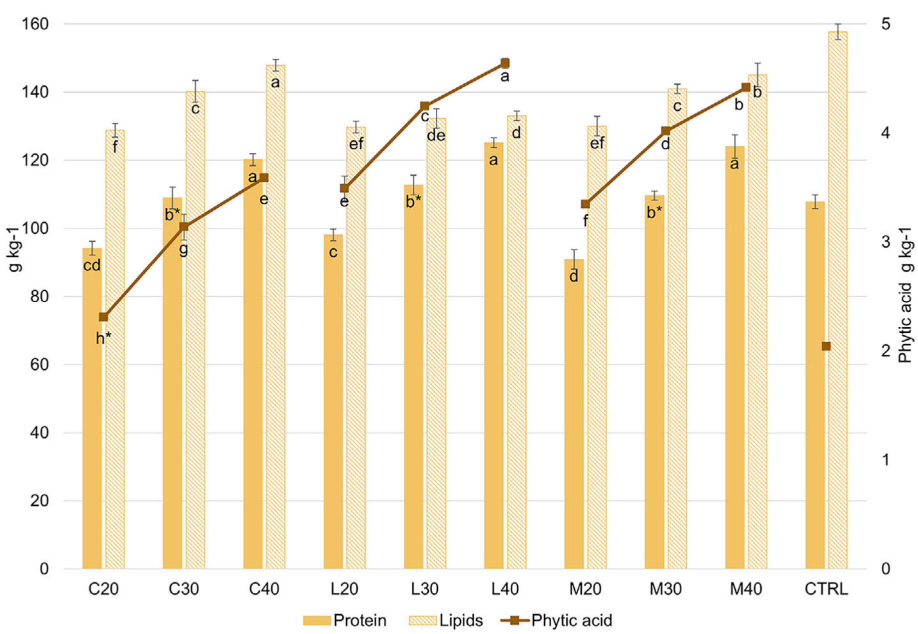

Figure 4. Protein, lipid and phytic acid contents of sponge cakes prepared with an aqueous dispersion of chickpea (C), lentil (L) and mung bean (M) proteins at 20–40%. Different letters for the same parameter mean significant differences according to the Tuckey's HSD test ($P \leq 0.05$). Abbreviation: CTRL, control sponge cake. *The value is similar to the control (CTRL) according to Dunnett's test ($P > 0.05$). $n = 4$.

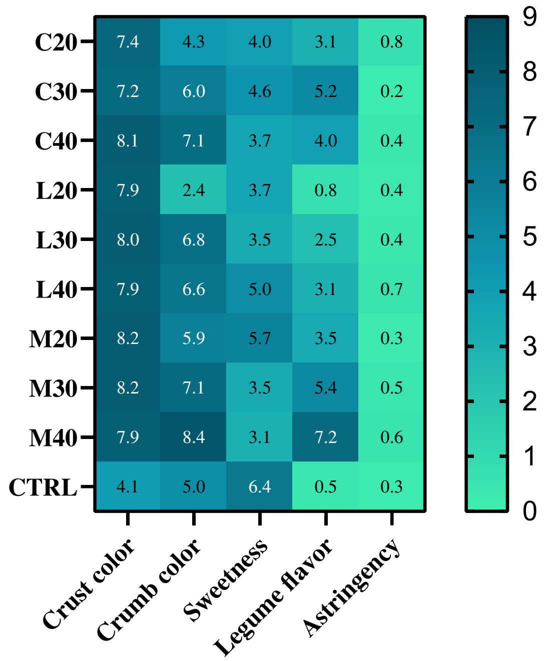

Figure 5. Heat map describing the sensory attributes of sponge cakes prepared with the aqueous dispersion of chickpea (C), lentil (L) and mung bean (M) proteins at 20–40% evaluated by quantitative descriptive analysis. CTRL, control sponge cake. $n = 4$.

and can be related to an increase in Maillard reaction precursors.[13,40] More heterogeneous values were obtained for the crumb color. In fact, it is mainly affected by the color of the protein ingredient and not by the Maillard reactions or sugar caramelization.[13] No significant similarities with the CTRL were recorded.

The CTRL cake showed the highest score for sweetness, whereas the addition of egg replacer lowered the sweetness perception, which was always evaluated with a score below 4.5 out of 9. Moreover, the cakes produced with 30% and 40% DFp showed the lowest sweetness. These results were probably due to the bitter taste reported in other studies for DFp,[41] which might have lowered the sweetness perception.[42]

The intensity of the legume flavor was most pronounced in the cakes formulated with mung bean protein as egg replacer, and it was affected by the concentration used. By contrast, in the other trials the legume flavor was only slightly perceptible, with scores below 4 out of 9. Overall, the legume flavor is a critical aspect to consider in new food development, because it is generally not well accepted by consumers.[41] Therefore, the use of chickpea and/or lentil DFp should be preferred for this kind of formulation, having a milder impact on the sensory properties compared to mung bean. However, further consumer tests could be carried out in order to establish the consumer preference and acceptance for such products.

Finally, astringency, which is commonly associated with legumes,[41] was little perceived by panelists, indicating a low impact of the egg replacers on this sensory descriptor.

CONCLUSIONS

Egg replacement is gaining interest worldwide, and the results of this study suggest that dry-fractionated proteins are a sustainable alternative that could be used in sponge cake preparation as egg replacer. However, their introduction in food must be evaluated in order to understand their effect on the physicochemical quality of the products. DFp addition resulted in a more pronounced elastic-like behavior of the batters, as seen by the higher G' modulus and the higher apparent viscosity compared to the CTRL formulation. Moreover, DFp concentration significantly affected rheological behavior during baking, and lentil-based batters showed the highest G' after heat treatment. Through temperature sweep analysis, it was possible to explain the phenomena occurring during baking that are responsible for the quality features of the products. In fact, higher specific volume, thickness variation and firmness were found in the products containing the highest concentration of DFp.

All the cakes prepared with DFp showed similarities in the crumb structure compared to the CTRL, regardless of the protein type and concentration. The protein content of the cakes containing at least 30% DFp was similar to the CTRL. However, the addition of DFp was directly related to an increase in phytic acid content. Sensory analysis highlighted a lower impact of the chickpea and lentil protein on legume flavor, compared to mung bean.

The overall results of this study confirm that eggs in cake formulations can be substituted by legume protein produced by dry fractionation, which was able to imitate egg behavior and the physical structure of the products. Furthermore, different textural properties can be obtained by changing the protein source and amount of protein, in order to obtain products with the desired texture compared to the egg-based cake.

This is particularly important considering that, although the dry fractionation process was comprehensively studied, limited food applications are reported in the scientific literature. However, further studies would be needed to evaluate the nutritional characteristics – for example, amino acid composition and digestibility – of the DFp-based cakes in comparison with the traditional egg-based products, as well as to assess the overall acceptability of the products by carrying out consumer acceptability tests.

AUTHOR CONTRIBUTIONS

Davide De Angelis: conceptualization, investigation, formal analysis, writing – original draft preparation, writing – review and editing; Vittoria Latrofa: conceptualization, investigation, formal analysis writing – original draft preparation; Giacomo Squeo: conceptualization, investigation, formal analysis, writing—review and editing; Antonella Pasqualone: project administration, resources, writing—review and editing; Carmine Summo: conceptualization, resources, project administration, writing—review and editing.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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