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Desirability-based optimization of bakery products containing pea, hemp and insect flours using mixture design methodology

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ABSTRACT

Simplex lattice design was used to design 15 sponge cakes formulations combining pea (PP), hemp (HP) and insect (IP) flours representing 15% of dough composition. Moisture, protein content, baking loss, specific volume, texture and cost of the 15 samples plus the control (0% added protein) were analysed. Results showed that the effect of PP, HP and IP on cake properties could be modelled with linear regressions (96.80% < R^2 < 99.96%). Ternary diagrams showed the effect of the combination of the three proteins in each response. The desirability function was used to obtain a multi-response optimization of the samples with maximum protein, maximum specific volume and minimum incremental cost. Sensory results of the 5 optimised samples showed that by combining 3.75% pea, 3.75% hemp and 7.5% insect it was possible to obtain a dairy- and egg-free sponge cake without significant differences from the control with animal-derived proteins.

1. Introduction

The growing food industry is driving forward the protein market and the need for alternative protein food sources and their derived ingredients as a response to climate change, increasing population, and changing diets. Furthermore, consumers are seeking sustainability and transparency in their food supply (Wognum, Bremmers, Trienekens, Van der Vorst, & Bloemhof-Ruwaard, 2011). In accordance, food industries are interested in commercializing products formulated with ingredients derived from environmentally sustainable sources, that deliver optimal nutrition, flavour, and functionality. The global protein ingredients market was valued at USD 38.5 billion in 2020 and is expected to expand at a rate (CAGR) of 10.5% from 2021 to 2028 (Research, 2021, pp. 2021–2028). Demonstrating how these alternative sources can partially or wholly replace functionalities of traditional animal protein ingredients in sectors like meat, dairy and bakery, is essential for protein market success.

Particularly, in the bakery sector, the animal proteins historically used for formulating are from dairy and egg origin. For instance, sponge cakes are a sweet baked treat that is enjoyed by people from all walks of life. This is primarily owing to its ready-to-eat aspect, as well as its availability in a variety of flavours and an affordable price. Sponge cakes are a type of cereal product that is made primarily of wheat flour, eggs, milk protein, sugar and fat, and has a flexible and elastic alveolar crumb (Paraskevopoulou, Donsouzi, Nikiforidis, & Kiosseoglou, 2015). On the one hand, milk functional properties such as casein, which is responsible for binding fat and water, and lactose, which together with the protein, promote the Maillard browning reaction adding to the characteristic colour of baked products, are both of relevant importance when it comes to developing baked products (Mironeasa, 2022).

On the other hand, egg protein properties are also related to binding as well as emulsifying. Furthermore, egg protein produces foams when whipped, essential for volume building, lightness, and smooth texture. They also provide a desirable yellow colour and participate in the above mentioned Maillard reactions responsible for desirable flavour and crust browning.

Therefore, when dairy and egg proteins are removed from the equation due to their animal origin, important losses in texture, flavour and colour need to be replaced. With that, it is necessary to optimize new formula to improve the qualitative characteristics of dairy and egg-free bakery products.

Subsequently, there have been many alternative protein sources found fit to possibly substitute these ingredients from animal origin, such as proteins that come from plant-based products. Plant-based

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proteins are key ingredients within certain food formulations due to their many desired attributes like thickening, gelling, foaming, water-holding, emulsifying and fat absorption abilities. These functionalities are quite convenient in these sorts of products, although most of these ingredients' properties are difficult to control because of their elevated sensitivity to pH alterations, their meagre water-solubility, and their ionic strength and temperature, thus limiting their range of use (Nikbakht Nasrabadi, Sedaghat Doost, & Mezzenga, 2021).

Alternative proteins come in different formats (flours, concentrates and isolates) and they differ in protein content and functional characteristics. Furthermore, most plant-based proteins have certain off-tastes, such as beany aftertaste (soy) or earthy aroma (legumes). In addition, protein isolates or concentrates may taste bitter or astringent depending on the peptide breakdown or the presence of other compounds (Nadathur & Carolan, 2017). Other sources might add pleasant flavour notes such as nutty (hemp, rice, insects) and cocoa (larvae from lesser mealworm) (Mishyna, Chen, & Benjamin, 2020; Roncolini et al., 2020). The unattractive factor in insect proteins and flours may be greater than any strong off-tastes.

Formulators have a lot of options to consider when working with alternative proteins, especially when combining them. Apart from the techno-functional properties, the nutritional value of the alternative protein ingredients used should be equivalent or superior to the animal-origin ones. In fact, García-Segovia, Igual, Noguerol, and Martínez-Monzó (2020) and Igual, García-Segovia, and Martínez-Monzó (2020) used combinations of pea and insect protein from *Alphitobius diaperinus*, *Tenebrio molitor*, and *Acheta domesticus* to improve the nutritional profile of extruded snacks. That study showed that high AD *(Acheta domesticus)* concentration in mixtures cause low expansion and crunchy extrudate. They concluded recommending the use of 7.5% AD percentage in mixtures for extruded snacks.

Another consideration when choosing protein ingredients is legislation. Novel protein ingredients are considered viable alternatives for existing animal/plant-based protein ingredients (Lähteenmäki-Uutela, Rahikainen, Lonkila, & Yang, 2021). Some examples of novel food dossiers received by EFSA (as per October 2021) include proteins from alternative sources such as insects in larvae form (*Tenebrio molitor*, *Alphitobius diaperinus*, *Hermetia illucens*, *Apis mellifera*). For bakery applications, insect-derived proteins have shown potential due to their nutty flavour and brownish colour (Gravel & Doyen, 2020; Hawkey, Lopez-Viso, Brameld, Parr, & Salter, 2021). Roncolini et al. (2020) studied *Alphitobius diaperinus* as a novel baking ingredient for high-protein snacks.

However, consumer perception of a particular protein source can influence its market uptake. It is obvious why insect protein can cause neophobia, but this is not limited only to the appearance of arthropods, also ingredients coming from hemp seeds can cause confusion as many consumers are not aware of the differences between cannabinoids (CBD) and hemp (Metcalf, Wiener, & Saliba, 2021). Although protein from hemp seeds (*Cannabis sativa* L., <0.2% tetrahydrocannabinol content) is not considered Novel Food in EU, it is currently a relatively niche product. Nevertheless, it's making impact in the plant-based protein industry due to its sustainable production and health benefits (Aluko, 2017; Shen, Gao, Fang, Rao, & Chen, 2021).

In many cases, the potential benefits of novel ingredients take time to reach consumers via new food products in the market. Research can accelerate market uptake by investigating how new applications of these protein ingredients in different food matrices can build upon the nutritional profile, sensory properties, safety and consumer trust of foods containing alternative proteins.

In this study, three protein ingredients from different alternative sources (legumes, oilseeds and insects) were chosen based on their Novel Food status (insects), low consumer acceptance (hemp) and well-known source (pea). Also, nutritional quality reported by other authors was considered. Jensen, Miklos, Dalsgaard, Heckmann, and Norgaard (2019) reported an *in vitro* Protein Digestibility (IVPD) for *Alphitobius diaperinus* of 91–94%, House, Neufeld, and Leson (2010) reported up to 84.1–86.2% for whole hemp seed and Boye, Zare, and Pletch (2010) reported 60.4%–74.4% for pea protein. The aim of the present work was to replace dairy and egg ingredients with alternative protein-rich flours, namely pea, hemp and insect flour, to investigate the effects of these flour mixtures on some physicochemical and sensory properties of sponge cakes using simplex lattice mixture design approach. This type of mixture design is used to determine the relative proportion of ingredients that optimizes a formulation with respect to a specified variable(s) or outcome. This technique has been already employed for the design and optimization of flour mixes in bakery (Cherie, Ziegler, Fekadu Gemede, & Zewdu Woldegiorgis, 2018; Kayacier, Yüksel, & Karaman, 2014; Yuksel, Yavuz, & Baltacı, 2022). In order to increase the nutritional, functional, and acceptability qualities of alternative protein sponge cakes, an egg- and dairy-free sponge cake formulation was optimised using the desirability function.

2. Methods

2.1. Raw materials

Wheat flour (75/100 g carbohydrates, 10/100 g of proteins, and 2/100 g of fat), sugar, refined crystal salt, baking powder, and oat milk (7,7/100 g carbohydrates, 1,3/100 g of proteins, and 0,8/100 g of fat) were purchased at Makro store (Erandio, Spain). Commercially available yellow pea protein concentrate containing 55% protein (<13.5% moisture, 50–60% protein, < 16% starch, < 6% fat, < 5% crude fibre, and <8% ash) provided by Herba Ingredients B.V. (Wormer, Netherlands), organic hemp protein powder (<8% moisture, > 80% protein, < 10% carbohydrates) from Healthy Proteins B.V. (Schoorl, Netherlands), and whole buffalo powder obtained from the mealworm, *Alphitobius Diaperinus*, (<5% moisture, 59.6/100 g of protein, 28.7/100 g of fat, 2.7/100 g of carbohydrates, 3.3/100 g of fibre, 0.9/100 g of salt) from Protifarm NV (Ermelo, Netherlands).

2.2. Physicochemical analysis of flours

The particle size distribution of flours was measured by Static Light Scattering Instrument Master-Sizer 3000 (Malvern instrument Ltd, US) using bidistilled water as dispersion agent (refractive index = 1.33) following other studies by David-Birman, Raften, and Lesmes (2018); Luo et al. (2021). The d_{50} (μm) is the maximum particle diameter below which 50% of the sample volume exists - also known as the median particle size by volume. The most common percentiles reported are the d_{10} (μm), d_{50} (μm), d_{90} (μm). The volume moment mean or De Brouckere Mean Diameter (D[4, 3]) is also shown as it reflects the size of those particles which constitute the bulk of the sample volume. It is most sensitive to the presence of large particulates in the size distribution.

Swelling capacity of flours was determined according to Robertson et al. (2000). For water solubility index (WSI) of flours, a sample of 2 g was dispersed in 100 mL of distilled water in a water bath at 80 °C during 30 min. The dispersion was then centrifuged at 1100×*g* during 10 min at room temperature and the supernatant was collected carefully and dried at 103 ± 2 °C to determine its solid content. WSI was expressed as the percentage of the total of the original sample that was present in the soluble fraction (Eq. (1)):

$$\mathrm{WSI}(\%)\mathrm{WSI} = \frac{\mathrm{Weight\ \ of\ \ dissolved\ \ solid\ \ in\ \ supernatant}}{\mathrm{Weight\ \ of\ \ dy\ \ solids}} \times 100 \tag{1}$$

Water holding capacity (WHC) was measured using Eq. (2):

$$WHC = \frac{(Wetsampleweight - dysampleweight) - weightofwaterretainedbydisc}{sampleweight} \times 100 \tag{2}$$

Table 1
Physico-chemical characteristics of protein ingredients.

	Pea protein	Hemp protein	Insect protein
Functional properties			
Moisture (%)	$6.40^{b} \pm 0.25$	$11.99^{a} \pm 1.82$	$6.28^{b} \pm 0.44$
Swelling capacity (mL/g)	$4.56^{b} \pm 0.39$	$7.30^{a} \pm 0.38$	$7.21^{a} \pm 0.89$
Water solubility index (g/100 g)	$41.90^{a} \pm 1.03$	$35.60^{b} \pm 1.74$	$37.17^{b} \pm 0.42$
Water holding capacity (g/g)	$1.48^{b} \pm 0.06$	$2.44^{a} \pm 0.16$	$2.39^{a} \pm 0.04$
Oil holding capacity (g/g)	$1.75^{a} \pm 0.24$	$1.97^{a} \pm 0.27$	$2.00^{a} \pm 0.00$
Color parameters			
L*	$75.45^{a} \pm 7.02$	$76.84^{a} \pm 4.73$	$60.72^{b} \pm 2.52$
a*	$-1.03^{c} \pm 0.20$	$-0.70^{b} \pm 0.06$	$1.12^{a} \pm 0.04$
b*	$15.48^{b} \pm 1.05$	$7.19^{c} \pm 0.43$	$18.81^{a} \pm 0.61$
Particle size characteristics			
d_{10} (μm)	$4.58^{b} \pm 0.14$	$5.71^{a} \pm 0.71$	$4.84^{b} \pm 0.38$
d_{50} (μm)	$14.67^{c} \pm 0.60$	$57.09^{a} \pm 16.82$	$35.0^{b} \pm 3.16$
d_{90} (μm)	$36.96^{c} \pm 2.58$	$178.27^{b} \pm 17.25$	$628.17^{a} \pm 40.27$
$D[3,2]$ (μm)	$9.36^{b} \pm 9.36$	$11.25^{a} \pm 1.95$	$11.23^{ab} \pm 0.67$
$D[4,3]$ (μm)	$29.6^{c} \pm 8.8$	$77.7^{b} \pm 11.7$	$164.8^{a} \pm 19.5$

Means with different superscript letters in the same column are significantly different according to Tukey's test ($p < 0.05$).

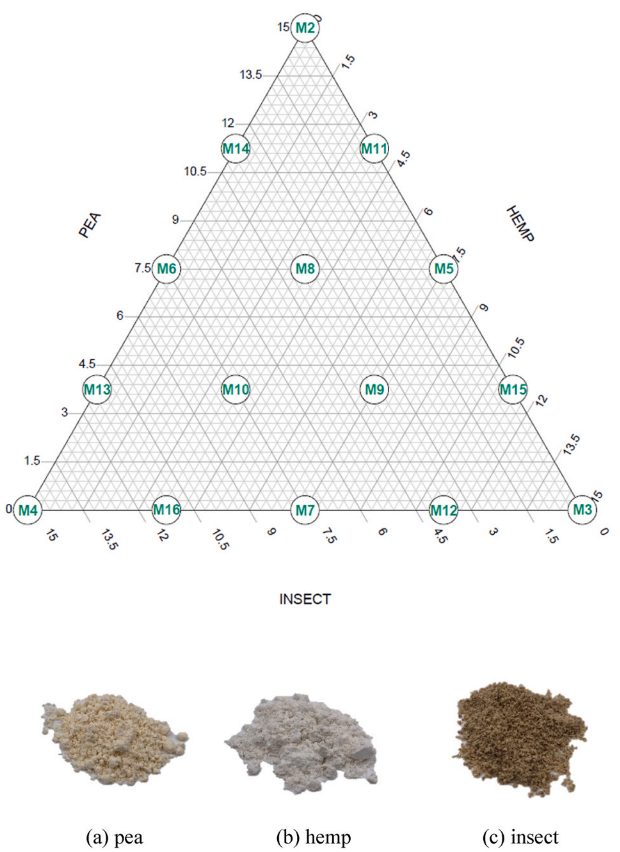

Fig. 1. Simplex lattice design plot representing 15% of sponge cake formula using combination of (a) pea protein, (b) hemp protein and (c) insect protein ingredients.

Oil holding capacity (OHC) was measured using Eq. (3).

$$OHC = \frac{(Wet sample weight - dy sample weight) - weight of water retained by disc}{sample weight} \times 100 \tag{3}$$

The colour of the samples was measured using a colorimeter (Konica Minolta, Inc, Japan) in the CIE L*a*b* system. The colorimetric parameters L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) were determined.

2.3. Sponge cake

2.3.1. Experimental design and sponge cake preparation

The procedure used in this study included design, modelling, optimization, and validation steps.

A three-factor simplex lattice design methodology was used in order to obtain all possible mixtures (15 essays): pea protein, hemp protein and insect protein (the sum of the component proportions was equal to 1). The control sample did not include any type of protein, so the effect of the different alternative proteins and their combination could be studied. The control batter included 250 g wheat flour, 250 g of oat milk, 100 g of sugar, 7 g of baking powder and 1 g of salt. All 15 mixes were made so that 15% of wheat flour was replaced with alternative protein ingredients. The experimental design was carried out in triplicate. Table 1 and Fig. 1 show the mixture design with the corresponding compositions.

For the 15 combinations, the constant ingredients were 212.5 g of wheat flour, 250 g of oat milk, 100 g of sugar, 7 g of baking powder and 1 g of salt, representing 85% of the mixture. The other 15% was 37.5 g of alternative protein (pea, hemp and/or insect powder). All ingredients were weighed with a high precision scale (Mettler Toledo, AB304-S, ± 0.0001 g) and mixed during 30 s at a low speed followed by 3 min at maximum speed using a planetary mixer Sammic BM-5 (Sammic S.L., Azkoitia, Spain). After mixing, 35 g of batter were placed in each one of the 12 rectangular-shaped cells in a rubber mould and baked in an electric oven (De Dietrich, Basingstoke, UK) with combined heat (fan, top and bottom heat) for 20 min at 180 °C. Subsequently, the baked cakes were removed from the mould, cooled at room temperature for approximately 20 min, packaged in vacuum plastic bags, labelled, and stored at – 20 °C in the freezer until further characterisation. By the selection of desired ranges of multiple responses from an industrial scalability perspective, the optimum sponge cake formulae that maximized protein content and specific volume as well as minimized the cost was determined by Derringer and Suich (1980).

2.3.2. Physicochemical characterisation of sponge cake

Moisture was analysed as in ISO 1442:1997 and protein content was analysed by using the Digestion Unit K-435 and a distillation unit B-324 (Buchi Labortechnik AG, Flawil, Switzerland). A correction factor of 6.25 was used as recommended by ISO 937:1978. The specific volume was calculated using the ratio between the cake volume and its weight. Volume was calculated by the displacement method using sesame seeds (ρ sesame = 580 kg/m^3). The colour measurements of the cake samples were made using the colorimeter described above. The baking loss (%) was determined on three independent samples using equation (2), (24) h after baking using Eq. 7, where W_f is the weight of the sample after baking and W_0 is the weight before baking:

$$\% \, Baking \; loss = \left(\frac{W_f - W_0}{W_0}\right) x \; 100 \tag{4}$$

2.3.3. Textural properties

Textural properties were determined with TA-HD plus texture analysis device (Stable Micro Systems, Godalming, Surrey, UK (AACC, 2010). For this purpose, Texture Profile Analysis (TPA) was applied on the samples with a P/75 compression platen probe, typically used for texture analysis in bakery products. The obtained force-time curve, hardness, adhesiveness, springiness, cohesiveness, chewiness and gumminess values were calculated. The mechanical conditions (test parameters) for the test were adjusted as follows: Pre-test speed: 3 mm/s. Test speed: 1 mm/s. Post-test speed: 5 mm/s. Deformation: 50%. Activation force: 5.0 g. Loading cell: 2 kg. The sponge-cake was cut into small 2 × 2 cm units and was analysed.

2.3.4. Sensory analyses

Due to Novel Food legislation regarding insect protein, this study was not able to be conducted with consumers for sensory evaluation. Only some EU countries (e.g., Belgium, Netherlands, Denmark, Finland and Germany) have adopted their own internal regulations for the trading of insect-based food. Instead, 8 panellists were trained following the guideline for the measurement of the performance of a quantitative descriptive sensory panel following ISO1112:2021,2021. .

A sensory evaluation of five egg- and dairy-free sponge cakes was conducted. The selection of the sponge cakes assessed was based on the optimization results of the mixture design using the desirability function. This function optimizes/selects the optimal ingredient-mixing ratio. For the optimization, each response must have a low and high value assigned to each goal. In this study, maximum protein content, maximum specific volume and minimum cost were selected as the responses to be optimised following a cost-effective approach.

A quantitative descriptive analysis (QDA) was performed for assessing the sensory characteristics of the dairy- and egg-free sponge cakes. A preliminary recall training was preceded by four sessions at which a trained panel of 8 assessors consisting of 7 women and 1 man was re-trained to identify and evaluate sensory characteristics of a sponge cake being the same recipe of samples but with animal-derived protein: 250 g wheat flour, 250 g of cow milk, 8 eggs, 100 g of sugar, 7 g of baking powder and 1 g of salt.

They were also trained in the use of 0–10 intensity scale. In these sessions they generated descriptors and their definitions. They were asked to mention the attributes they considered important for descriptive evaluation of sponge cake and their definitions through open discussion. Finally, a QDA scale (see supplementary material, Table 8) was stablished by the panellists based on an animal-derived protein sponge cake in order to get a sample as closed as possible to the values obtained for the QDA in the following sessions. 9 attributes related to appearance, flavour, aroma and texture were generated by trained assessors.

After training, a statistical analysis to identify the discrimination capacity, repeatability and reproducibility for each panellist as well as the robustness of the panel was conducted. Each panellist evaluated the 5 samples presented in random order. Having a robust trained panel, the sensory evaluation of the dairy-and egg-free sponge cakes was carried out.

Each assessor was provided with 5 samples on the plate which were coded with a 3-digit random number for the evaluation. Sample evaluations were performed at room temperature under normal lightning conditions. The attributes were evaluated on a 0–10 intensity scale. All samples were assessed in random order in three replicates. The panellists defined each sample in intensities based on the generated attributes and the results were analysed in order to see if there were significant differences between the products and what attributes make it different. As well as, how those samples could resemble to the animal protein sponge cake compared to the QDA.

2.4. Statistical analysis and modelling of experimental data

The experimental design, modelling and optimization was carried out using R-project software (v 4.1.2). The packages used were "readxl", "tidyverse", "mixexp", "rsm", "ggtern", "Ternary", "ggplot2", "plotly", "rjson", "agricolae" and "desirability".

The polynomial equation used was:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \tag{5}$$

Where Y is the estimated response; β_1, β_2, β_3, β_{12}, β_{13}, β_{23} and β_{123} are constant coefficients for each linear and nonlinear (interaction) term produced for the prediction models of processing components. The computational work, including ternary contour plots was performed using R-project software (v 4.1.2), ternary package. The model for each response was calculated based on the fitting quality, the coefficient of determination (R^2), the adjusted coefficient of determination ($R^2_{\ adj}$) and the significant level of regression ($p < 0.05$). A one-way analysis of variance (ANOVA) and a Tukey HDS test was used to determine pairwise differences between groups ($p < 0.05$). The desirability package was used for the optimization of the experimental design based on maximum protein content, maximum specific volume and minimum cost.

3. Results

3.1. Physicochemical characterisation of protein ingredients

The proximate composition, functional properties and colour parameters of pea, hemp and insect protein is summarised in Table 1.

Moisture content of pea, hemp and insect protein ingredients were 6.28, 11.99 and 6.40%, respectively, with no significant differences ($p > 0.05$) between pea and insect protein ingredients. This is in accordance with the supplier specifications for pea (<13.5%), about 30% higher than the moisture specified for hemp protein (<8%), and a slightly higher for insect protein (<5%). However, this range of moisture content (6–12%) is particular to powder products obtained from alternative sources such as pea (Nascimento, Rosa, Andreola, & Taranto, 2020; Yen & Pratap-Singh, 2021), hemp (Aluko, 2017; Zahari et al., 2020) and insects (Cho & Ryu, 2021; Sun et al., 2021).

Swelling capacity was significantly lower ($p < 0.05$) for pea protein (4.56 mL/g) when compared with hemp and insect protein, that had the highest values without significant differences ($p > 0.05$) among them (7.30 and 7.21 mL/g, respectively). WHC was significantly higher ($p < 0.05$) for hemp and insect protein (2.44 and 2.39 g/g) than for pea protein (1.48 g/g). However, WSI was significantly higher ($p < 0.05$) for pea protein (41.90 g/100 g) but not among hemp or insect protein (35.60 and 37.17 g/100 g, respectively). These differences on hydration properties between pea, hemp and insect protein might be due to the particle size differences (Table 1). Indeed, the three type of powder ingredients studied consisted mainly of average particle diameter (d_{50}) from 14.67 μm for pea flour, 35.0 μm for insect flour to 57.09 μm for hemp flour. It is important to note that for insect protein d_{90} was 628.17 μm, meaning that there were coarse particles that reach such size. For hemp protein, d_{90} was 178.27 μm and for pea 36.96 μm. This ease in solubility of pea protein might be due to smaller particle-size (Recharla, Riaz, Ko, & Park, 2017; Talens, Arboleya, Castro-Giraldez, & Fito, 2017). On average, the sizes of fine particles ($D[3,2]$) were similar among pea and insect (9.36 μm and 11.23 μm, respectively) and significantly different ($p < 0.05$) among pea and hemp (11.25 μm). The size of coarse particles ($D[4,3]$) was significantly higher for insect flour (164.8 μm), followed by hemp flour (77.7 μm) and pea flour (29.6 μm). These might be caused to differences during the milling step, were the size of the mesh (unknown to the authors) will impact the particle size of the flours, and therefore, their technological properties. Larger particles size can increase the swelling (SC) and water holding capacity (WHC) due to an increased porosity (Talens et al., 2017), but also to the presence of other

Table 2
Responses to different formulations of sponge cakes containing pea. hemp and insect protein.

Mixture	Pea	Hemp	Insect	MC (%)	Protein Content (%)	Baking los (%) (%)	Specific volume (cm^3/g)	Hardness (N)	Resilience (%)	Cohesiveness	Springiness (%)	Chewiness	ΔCost (Δ€)
M1	0	0	0	$30.8^{a} \pm 2.4$	$4.29^{e} \pm 0.13$	$13.1^{b} \pm 4.7$	$0.40^{g} \pm 0.03$	$5.99^{ab} \pm 1.33$	$16.22^{ij} \pm 2.62$	$0.48^{h} \pm 0.06$	$69.33^{g} \pm 5.74$	$204.96^{cdef} \pm 64.71$	0.0
M2	1	0	0	$27.3^{a} \pm 1.9$	$7.69^{bcd} \pm 0.28$	$19.8^{a} \pm 2.2$	$0.57^{def} \pm 0.04$	$6.22^{ab} \pm 1.89$	$27.86^{ab} \pm 3.19$	$0.67^{ab} \pm 0.03$	$91.20^{a} \pm 2.46$	$384.74^{a} \pm 106.60$	0.2
M3	0	1	0	$29.5^{a} \pm 2.0$	$9.50^{a} \pm 0.30$	$18.4^{ab} \pm 5.9$	$0.65^{abcdef} \pm 0.02$	$6.19^{ab} \pm 0.50$	$28.41^{a} \pm 3.85$	$0.68^{a} \pm 0.03$	$89.82^{ab} \pm 2.59$	$384.27^{a} \pm 98.43$	1.6
M4	0	0	1	$29.3^{a} \pm 6.6$	$8.10^{bcd} \pm 0.11$	$17.4^{ab} \pm 2.9$	$0.78^{a} \pm 0.05$	$2.54^{e} \pm 0.84$	$14.60^{j} \pm 2.34$	$0.48^{h} \pm 0.06$	$70.60^{fg} \pm 10.05$	$88.81^{g} \pm 36.16$	1.6
M5	0.5	0.5	0	$29.3^{a} \pm 2.4$	$7.80^{bcd} \pm 0.08$	$22.3^{a} \pm 2.6$	$0.66^{abcde} \pm 0.05$	$6.66^{a} \pm 1.79$	$28.13^{a} \pm 3.14$	$0.68^{ab} \pm 0.03$	$90.35^{ab} \pm 3.62$	$410.70^{a} \pm 93.41$	0.9
M6	0.5	0	0.5	$28.6^{a} \pm 1.3$	$7.33^{d} \pm 0.23$	$19.0^{ab} \pm 2.1$	$0.71^{abcd} \pm 0.05$	$3.41^{cde} \pm 1.34$	$17.03^{hij} \pm 1.56$	$0.53^{gh} \pm 0.04$	$74.75^{efg} \pm 4.59$	$142.33^{efg} \pm 67.45$	0.9
M7	0	0.5	0.5	$29.9^{a} \pm 1.9$	$8.01^{bcd} \pm 0.00$	$19.0^{ab} \pm 2.6$	$0.66^{abcde} \pm 0.05$	$3.06^{cde} \pm 0.49$	$19.69^{efghi} \pm 2.87$	$0.59^{cdefg} \pm 0.05$	$79.00^{cde} \pm 2.87$	$146.50^{degf} \pm 29.46$	1.6
M8	0.5	0.25	0.25	$29.2^{a} \pm 3.3$	$7.47^{cd} \pm 0.16$	$18.4^{ab} \pm 5.3$	$0.72^{abc} \pm 0.05$	$4.70^{bcd} \pm 0.37$	$22.74^{cdefg} \pm 2.93$	$0.61^{bcdef} \pm 0.03$	$85.95^{abcd} \pm 4.44$	$251.49^{bcd} \pm 32.27$	0.9
M9	0.25	0.5	0.25	$27.7^{a} \pm 1.3$	$7.83^{bcd} \pm 0.13$	$19.0^{ab} \pm 2.7$	$0.73^{ab} \pm 0.04$	$3.87^{cde} \pm 0.49$	$24.83^{abcd} \pm 3.15$	$0.65^{abcd} \pm 0.04$	$86.80^{abc} \pm 3.76$	$221.03^{cdef} \pm 28.36$	1.3
M10	0.25	0.25	0.5	$28.1^{a} \pm 1.8$	$7.51^{cd} \pm 0.14$	$19.6^{a} \pm 1.6$	$0.68^{abcde} \pm 0.04$	$3.40^{cde} \pm 0.56$	$18.59^{ghij} \pm 2.02$	$0.56^{fg} \pm 0.03$	$79.10^{cde} \pm 5.58$	$156.56^{defg} \pm 41.46$	1.3
M11	0.75	0.25	0	$27.8^{a} \pm 2.1$	$8.31^{b} \pm 0.25$	$18.7^{b} \pm 3.3$	$0.51^{bg} \pm 0.04$	$4.65^{bcd} \pm 0.59$	$25.93^{abc} \pm 3.45$	$0.66^{abc} \pm 0.04$	$88.74^{ab} \pm 4.00$	$277.60^{bc} \pm 45.31$	0.6
M12	0	0.75	0.25	$29.4^{a} \pm 2.2$	$8.38^{b} \pm 0.16$	$17.5^{ab} \pm 7.0$	$0.73^{ab} \pm 0.05$	$3.88^{cde} \pm 0.91$	$23.28^{bcdef} \pm 2.68$	$0.63^{abcdef} \pm 0.04$	$85.41^{abcd} \pm 4.22$	$216.30^{cdef} \pm 67.84$	1.6
M13	0.25	0	0.75	$29.8^{a} \pm 2.4$	$7.77^{bcd} \pm 0.21$	$17.3^{ab} \pm 3.0$	$0.57^{ef} \pm 0.04$	$3.08^{cde} \pm 1.51$	$19.35^{fghi} \pm 2.25$	$0.57^{fg} \pm 0.04$	$78.91^{cde} \pm 6.14$	$145.03^{efg} \pm 80.64$	1.3
M14	0.75	0	0.25	$30.3^{a} \pm 3.2$	$7.70^{bcd} \pm 0.23$	$19.1^{ab} \pm 1.7$	$0.70^{abcde} \pm 0.05$	$4.79^{abc} \pm 1.36$	$20.86^{defghi} \pm 2.06$	$0.58^{defg} \pm 0.04$	$85.36^{abcd} \pm 3.50$	$240.52^{bcde} \pm 68.64$	0.6
M15	0.25	0.75	0	$31.1^{a} \pm 1.4$	$8.13^{bc} \pm 0.25$	$19.6^{a} \pm 2.8$	$0.63^{bcdef} \pm 0.04$	$5.78^{ab} \pm 0.87$	$24.04^{abcde} \pm 2.77$	$0.64^{abcde} \pm 0.03$	$86.73^{abc} \pm 5.87$	$329.53^{ab} \pm 65.90$	1.3
M16	0	0.25	0.75	$28.6^{a} \pm 1.2$	$7.85^{bcd} \pm 0.21$	$16.8^{ab} \pm 4.2$	$0.67^{abcde} \pm 0.04$	$2.89^{de} \pm 0.36$	$19.37^{fghi} \pm 3.07$	$0.57^{efg} \pm 0.05$	$77.93^{def} \pm 5.72$	$131.79^{fg} \pm 25.01$	1.6

Means with different superscript letters in the same column are significantly different according to Tukey's test ($p < 0.05$).

components, such as starches and fibres, that can trap water molecules, can affect these properties. Ahmed, Thomas, and Arfat (2019) showed how, as the particle size of quinoa flours decreased, their rheological behaviour also decreased (less viscous, less rigid). This was associated to a reduced starch content as particle size decreased (therefore, less water retention potential). The flours used in this study also contained carbohydrates and other molecules contributing to such properties. Regarding OHC, there were no significant differences ($p > 0.05$) among samples.

The colour of the ingredients used for baking is important because it directly affects the colour of the cake crumb (Köten, 2021). In fact, pea, hemp and insect protein ingredients significantly varied in colour ($p < 0.05$) (Table 1). Pea and hemp protein had the highest L* value (75.45 and 76.84, respectively), without significant differences ($p > 0.05$) among them, which means that insect protein had the darkest L* value (60.72). Moreover, a* and b* were statistically different ($p < 0.05$) among the three samples, meaning that insect protein had the higher a* values (1.12), followed by hemp protein (−0.7) and pea protein (−1.03). Positive values for a* indicate closeness to red, and negative values indicate colours that are closer to green. Regarding b* value, increasing positive values for *b indicates closeness to yellow. Also, insect protein had the highest value (18.81), followed by pea (15.48) and hemp (7.19). Previous studies have also reported a more yellow colour of *Alphitobious diaperinus* flour (Gravel et al., 2020; Hawkey et al., 2021). Another study reported that only the samples with animal proteins and the highest percentage of pea protein presented significant differences with respect to control ($p < 0.05$) in the color attribute (Sahagún, Bravo-Núñez, Báscones, & Gómez, 2018).

3.2. Physicochemical characterisation of sponge cakes

The physicochemical characteristics and textural parameters of the different sponge cake formulations and a control sample without protein (M1) are shown in Table 2. No significant differences ($p > 0.05$) were found in the moisture content of the sponge cake (ranging from 27.7% to 30.8%) mainly because the proportion of ingredients that differed among samples represented only 15% of total composition. Therefore, the reformulation approach of this study did not affect moisture content of the samples.

The protein content in the samples ranged from 7.33% of sample M6 to 9.50% of sample M3. M6 contained pea and insect protein in the same proportion, but it did not contain hemp. Instead M3 contained 100% hemp protein, meaning that hemp protein ingredient contributed with the highest amount of protein to the sponge cake. In addition, the higher percentage of hemp protein was used, the higher the protein content of the sponge cake. This is in accordance with the specification from the supplier that claimed a protein concentration for these ingredients of >80%. Sample M2 (only pea protein) and sample M4 (only insect protein) did not differ significantly among them ($p > 0.05$), although the protein content for M4 was slightly higher (8.10%) than for M2 (7.80%). All samples differed significantly ($p < 0.05$) from control sample, with 4.29% protein content. Protein content was in the range of egg-containing sponge cakes, from 7 to 11%, as reported by recent studies and reviews (Miller, 2016; Pycarelle, Bosmans, Nys, Brijs, & Delcour, 2020). In sponge cakes, protein has a very important role in trapping the air bubbles for structure creation.

Table 2 also shows baking loss percentage and specific volume variation between the different formulations of sponge cakes. Baking loss was minimum for the control sample (13.1%), being significantly lower ($p < 0.05$) than M7, M9, M11 and M4 (19.6–22.3%). These samples contained insect protein in different percentages, being M4 (100% insect protein), the one with the highest baking loss. This is a crucial parameter for the creation of the sponge cake structure (Ammar et al., 2021). All samples had significantly higher ($p < 0.05$) specific

Table 3
Analysis of the predicted model equation (linear) for the studied responses as function of three components (alternative protein ingredients).

	Response	Coefficients			Statistics		
		A (Pea)	H (Hemp)	I (Insect)	p-value	R^2(%)	R^{adj} (%)
Textural characteristics	Baking loss (%)	0.18***	0.18***	0.20***	<0.0001	99.67	99.59
	Hardness (N)	582.92***	556.83***	189.14***	<0.0001	98.32	97.9
	Resilience	25.53***	27.03***	14.38***	<0.0001	99.43	99.28
	Cohesiveness	0.64***	0.68***	0.50***	<0.0001	99.86	99.83
	Springiness	89.48***	89.26***	71.56***	<0.0001	99.93	99.91
	Chewiness	265.69***	347.19***	56.60	<0.0001	96.80	96.0
Protein content	Protein content (%DM)	7.51***	8.73***	7.63***	<0.0001	99.81	99.76
Volume	Specific volumen (g/cm^3)	0.60***	0.67***	0.72***	<0.0001	99.20	99.0
Cost	Cost (€)	0.23***	1.61***	1.61***	<0.0001	99.96	99.95

p-value (dimensionless); R^2: coefficient of determination (%); R^2_{Adj}: Adjusted coefficient of determination (%).
*: significant effect ($p < 0.05$); **: very significant effect ($p < 0.01$); ***: very highly significant effect ($p < 0.001$).

volume than the control (0.4 cm^3/g). The samples with the highest specific volume (0.73–0.65 cm^3/g) compared to control were also M4, M7, M9. This relationship meant that the samples with higher baking loss had the highest volume (cm^3). Water evaporation during baking determines how the structure of the sponge cake is created in terms of volume. As water evaporates during baking, starch gelatinizes, granules

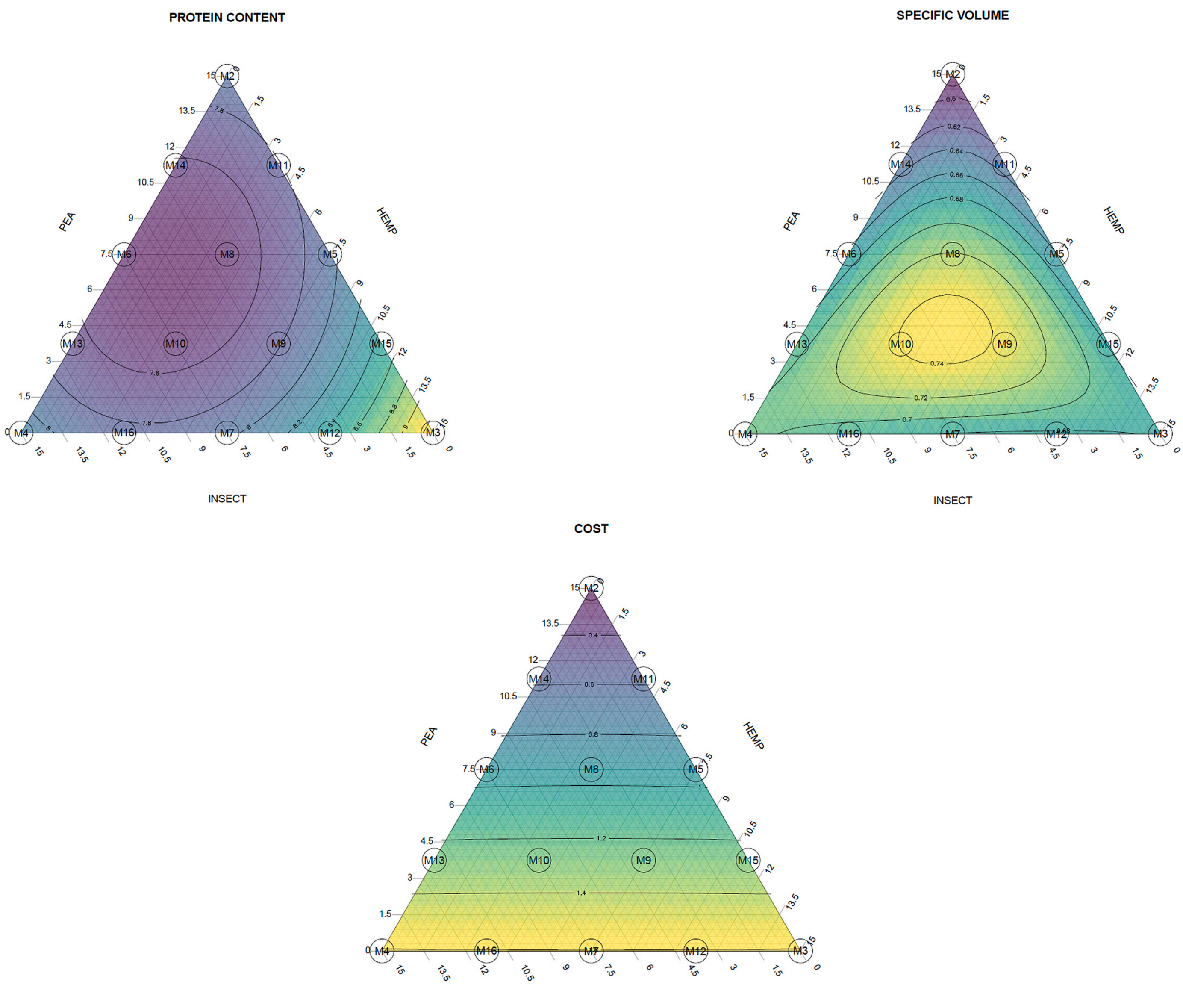

Fig. 2. Ternary contour plots as a function of three variables (protein ingredients) of the 3 optimised responses: protein content, specific volume and cost. Sponge cakes samples (M1-M15) are represented in each ternary plot (the other 6 responses are shown in Supplementart Material).

Table 4
Colour analyses of the different formulations of sponge cakes containing pea. hemp and insect.

	CRUST			CRUMB		
	L*	a*	b*	L*	a*	b*
M1	$44.13^{a} \pm 4.04$	$12.83^{a} \pm 1.15$	$31.50^{a} \pm 3.71$	$56.95^{a} \pm 5.30$	$4.36^{bc} \pm 1.34$	$31.99^{ab} \pm 1.00$
M2	$41.99^{ab} \pm 2.32$	$12.90^{a} \pm 0.85$	$27.09^{abc} \pm 3.54$	$53.16^{abc} \pm 2.72$	$6.88^{a} \pm 0.83$	$32.53^{ab} \pm 0.85$
M3	$44.08^{a} \pm 5.31$	$12.47^{a} \pm 2.05$	$30.55^{ab} \pm 4.60$	$55.51^{ab} \pm 3.23$	$4.656^{abc} \pm 1.20$	$31.22^{ab} \pm 0.94$
M4	$33.05^{c} \pm 3.01$	$13.05^{a} \pm 0.68$	$21.63c \pm 3.89$	$47.67^{c} \pm 1.85$	$6.59^{ab} \pm 1.06$	$30.59^{ab} \pm 1.08$
M5	$40.60^{ab} \pm 2.18$	$12.36^{a} \pm 0.68$	$25.27^{abc} \pm 3.77$	$52.13^{abc} \pm 1.93$	$6.16^{abc} \pm 0.95$	$31.24^{ab} \pm 0.86$
M6	$39.37^{abc} \pm 4.71$	$12.95^{a} \pm 0.81$	$27.44^{abc} \pm 5.47$	$52.58^{abc} \pm 2.76$	$5.29^{abc} \pm 0.78$	$31.13^{ab} \pm 1.26$
M7	$40.59^{ab} \pm 4.28$	$12.72^{a} \pm 1.58$	$28.12^{abc} \pm 4.36$	$52.78^{abc} \pm 3.04$	$4.24^{a} \pm 1.14$	$30.01^{b} \pm 1.77$
M8	$42.08^{ab} \pm 3.24$	$12.63^{a} \pm 0.84$	$28.33^{abc} \pm 3.70$	$52.09^{abc} \pm 3.53$	$5.40^{abc} \pm 0.97$	$30.34^{ab} \pm 0.73$
M9	$41.41^{ab} \pm 1.30$	$13.26^{a} \pm 0.79$	$30.33^{ab} \pm 2.20$	$54.44^{ab} \pm 2.53$	$4.42^{bc} \pm 0.60$	$31.07^{ab} \pm 0.96$
M10	$40.71^{ab} \pm 4.56$	$12.78^{a} \pm 1.35$	$27.85^{abc} \pm 4.51$	$54.19^{ab} \pm 2.50$	$4.56^{bc} \pm 0.56$	$30.55^{ab} \pm 0.76$
M11	$39.78^{ab} \pm 4.15$	$13.26^{a} \pm 0.79$	$26.59^{abc} \pm 5.47$	$52.61^{abc} \pm 3.03$	$5.63^{abc} \pm 1.39$	$31.29^{ab} \pm 0.92$
M12	$40.31^{ab} \pm 4.87$	$13.56^{a} \pm 1.18$	$27.67^{abc} \pm 5.02$	$51.95^{abc} \pm 4.12$	$5.19^{abc} \pm 1.14$	$30.41^{ab} \pm 0.98$
M13	$39.11^{abc} \pm 3.70$	$13.09^{a} \pm 0.77$	$27.48^{abc} \pm 5.52$	$50.55^{bc} \pm 6.90$	$5.76^{abc} \pm 3.00$	$29.85^{b} \pm 4.30$
M14	$44.18^{a} \pm 3.98$	$12.10^{a} \pm 1.53$	$32.31^{a} \pm 2.76$	$55.85^{ab} \pm 1.68$	$4.57^{bc} \pm 0.56$	$31.33^{ab} \pm 1.19$
M15	$43.64^{ab} \pm 4.46$	$12.66^{a} \pm 1.54$	$28.72^{abc} \pm 4.43$	$53.22^{abc} \pm 3.88$	$5.72^{abc} \pm 2.91$	$30.73^{ab} \pm 1.33$
M16	$37.15^{bc} \pm 4.9$	$12.82^{a} \pm 1.38$	$24.27^{bc} \pm 5.26$	$50.35^{bc} \pm 2.73$	$5.67^{abc} \pm 0.75$	$30.54^{ab} \pm 0.72$

Means with different superscript letters in the same column are significantly different according to Tukey's test ($p < 0.05$).
The colorimetric parameters: L* (lightness), a* (redness/greenness) and b* (yellowness/blueness).
(**M1**: 15% PP, 0% HP, 0% IP; **M2**: 0% PP, 15% HP, 0% IP; **M3**: 0% PP, 0%HP, 15% IP; **M4**: 7.5% PP, 7.5% HP, 0% IP; **M5**: 7.5% PP, 0% HP, 7.5% IP; **M6**: 0% PP, 7.5% HP, 7.5% IP; **M7:** 0% PP, 7.5% HP, 7.5% IP; **M8:** 7.5%PP, 3.75% HP, 3.75% IP; M9: 3.75% PP, 7.5% HP, 3.75% IP; **M10**: 3.75% PP, 3.75% HP, 7.5% IP; **M11**: 11.25% PP, 3.75% HP, 0% IP; **M12**: 0% PP, 11.25% HP, 3.75% IP, **M13**: 3.75% PP, 0% HP, 11.25% IP; **M14**: 11.25% PP, 0% HP, 3.75% IP; **M15**: 3.75% PP, 11.25% HP, 0% IP; **M16**: 0% PP, 3.75% HP, 11.25% IP.).

enlarge, and air cells expand (Assad Bustillos, Jonchère, Garnier, Réguerre, & Della Valle, 2020). The higher swelling capacity of insect protein leads to higher granule particle when hydrated, and consequently, a higher size of air pores is formed after water evaporates.

Table 2 also summarises the textural properties of the sponge cake formulation. Interestingly, the hardness of M2, M3, M5, M11, and M15 was significantly similar ($p > 0.05$) to the control. Those were the samples that contained pea and/or hemp in a proportion of 1 and/or 0.75 in their composition. To a lesser extent, M8 and M14 were also similar, with a 0.25 proportion for insect protein. When insect protein is introduced in the formulation, samples significantly reduce ($p < 0.05$) their hardness from 679.29 g for M5 to 259.63 g for M4. This is in accordance with previous discussion on the higher pore size of sponge cakes containing insect protein. The addition of ingredients that enhanced specific volume and porosity of cakes, result in softer textures (Marchetti, Califano, & Andrés, 2018; Pycarelle et al., 2020).

Finally, Table 2 shows the incremental cost of the sponge cakes compared to the control without added protein. This increase is about 0.2 €/Kg when pea protein is introduced in the formula and 1.6 €/Kg when either hemp or insect protein (less industrialised) are introduced. The combination of proteins allows to reduce costs from 0.9 to 1.3 €/Kg, when the three protein ingredients are combined in different proportions; while improving the textural properties (i.e. reduce hardness), protein content and specific volume.

On the contrary, for cohesion, springiness and resilience, sponge cakes with higher content of insect protein (M4) were not significantly different ($p > 0.05$) from the control. Whereas the samples with pea and hemp as major ingredients were the most significantly different ($p < 0.05$) from the control. For gumminess and chewiness, the same trend was observed, the samples with the lowest values were the six samples (M4, M16, M6, M13, M7, M10) that contain insect protein as major ingredient, in proportions of 1, 0.75 or 0.5. Springiness, cohesiveness, resilience and chewiness seem to determine how fast the degree of structure is reduced to the swallowing plane, meaning that higher values of these parameters increase the number of bites needed before swallowing (Wee, Goh, Stieger, & Forde, 2018).

Table 3 shows the linear regression and coefficients for each variable. Results show how the coefficients of pea, and hemp protein had a very highly significant effect on all the responses. No significant differences were found in the coefficients for the quadratic or special cubic models, meaning that there were no interactions among ingredients (PP * HP; HP * IP, PP * IP; PP*HP*IP)

For baking loss, the effect of each ingredient was small and very similar, which is in line with the absence of significant differences for moisture content (or moisture lost during baking). For hardness, the effect of pea and hemp (5.72 and 5.46 N, respectively) was higher than the effect of insect protein (1.85 N). This meant that, as pea and hemp protein appeared in the formula, hardness increased by almost 3-fold when insect protein was used.

For protein content, the higher effect was for the hemp protein (8.73) as the protein concentration of this ingredient was higher than the rest. For specific volume, the coefficient for insect protein (0.72) was slightly higher than for pea and hemp protein (0.60 and 0.67, respectively). Their impact was also very highly significant for the prediction this response. The same occurred for cost, where very highly significant effects were found in the prediction of the incremental cost. In this case, when hemp and insect protein appeared in the formulation, the incremental cost increased (1.61 for both). This was not the case for pea protein, which was the one with the least impact on incremental cost (0.23). This might be due because hemp and insect protein are less industrialised and production costs are higher than pea protein, which it has been in the market for a while. These effects are also illustrated in Fig. 2 by means of ternary plots (see also Figure 2 cont. in the Supplementary Material).

3.3. Colour of the sponge cakes

Colour analysis of the crust and the crumb of the sponge cakes is shown in Table 4. For crust, there were significant difference ($p < 0.05$) for L* and for b*. L* was significantly darker for M4 (33.05), the sample that contained 100% insect protein. L* increased with decreasing amounts of insect protein and increasing amounts of pea (41.99) and hemp protein, being the latter in the group with the highest L* value (44.08). Regarding b* value, samples with insect protein were significantly less yellow in the crust (21.63) than the control (31.50), being the only samples that were statistically different. For crumb, significant differences were found for L*, a* and b* values. L* followed the same trend than for the crust colour, being the darker samples the one with highest content of insect protein. Regarding a* values, the sample with hemp and insect protein in identical proportions had the lowest value

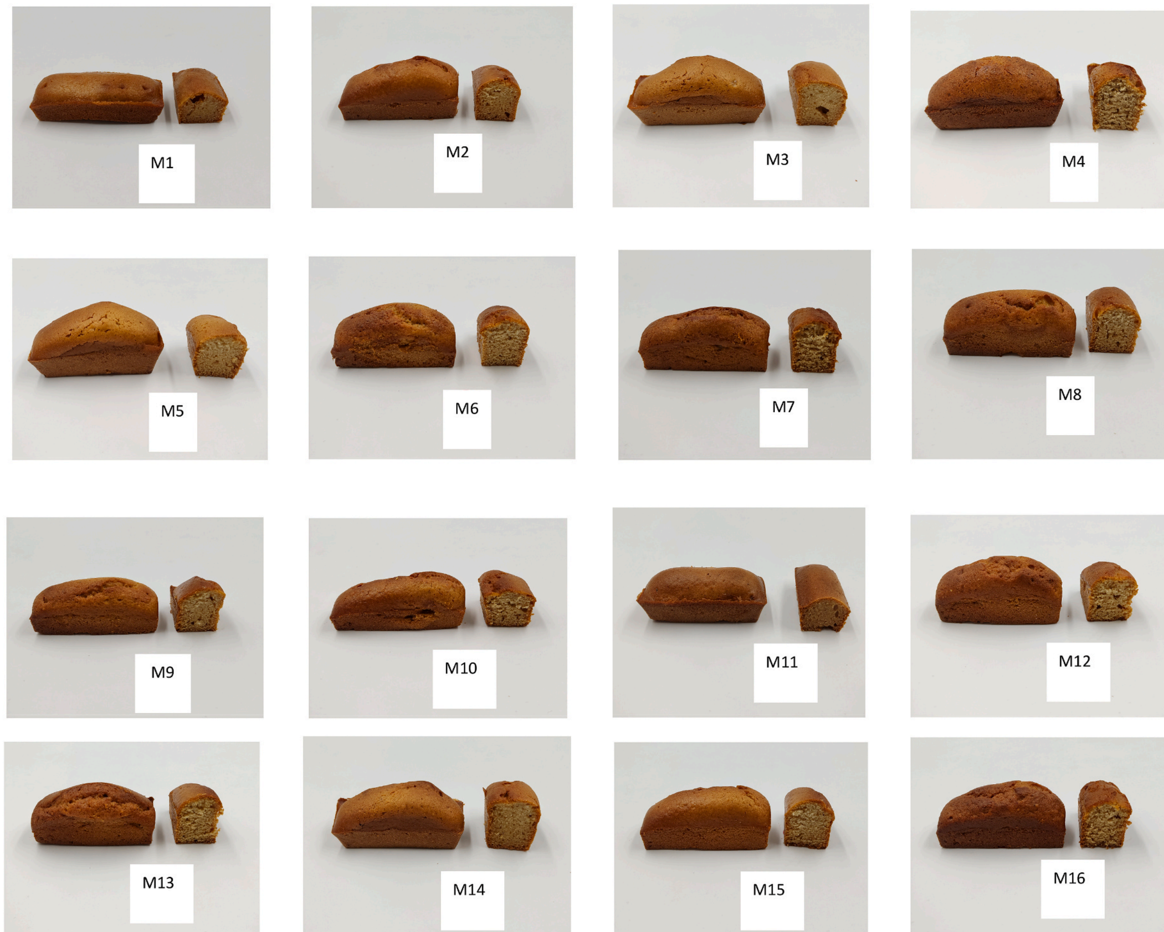

Fig. 3. Images of the 15 samples from the experimental design plus the control without alternative protein ingredient. M1: 15% PP, 0% HP, 0% IP; M2: 0% PP, 15% HP, 0% IP; M3: 0% PP, 0%HP, 15% IP; M4: 7.5% PP, 7.5% HP, 0% IP; **M5**: 7.5% PP, 0% HP, 7.5% IP; **M6**: 0% PP, 7.5% HP, 7.5% IP; **M7:** 0% PP, 7.5% HP, 7.5% IP; **M8:** 7.5%PP, 3.75% HP, 3.75% IP; **M9**: 3.75% PP, 7.5% HP, 3.75% IP; **M10**: 3.75% PP, 3.75% HP, 7.5% IP; **M11**: 11.25% PP, 3.75% HP, 0% IP; **M12**: 0% PP, 11.25% HP, 3.75% IP, **M13**: 3.75% PP, 0% HP, 11.25% IP; **M14**: 11.25% PP, 0% HP, 3.75% IP; **M15**: 3.75% PP, 11.25% HP, 0% IP; **M16**: 0% PP, 3.75% HP, 11.25% IP.

(less red) than the samples with only pea or only insect protein, indicating closeness to red. For b* value, sample M2 (only pea protein) was significantly higher ($p < 0.05$) than M3 and M7, with only hemp protein (M3), and hemp and insect protein in the same proportion (M7), indicating less yellowness. Fig. 3 shows the pictures of the 16 sponge cake formulations from the mixture design where some colour differences can be observed also by the human eye. These results are in accordance with other studies that report a higher yellowness for sponge cakes with *Alphitobius diaperinus* (Köten, 2021; Roncolini et al., 2020; Sun et al., 2021).

3.4. Optimization of the sponge cake quality

After the response results were obtained, the ingredients proportions were optimised by multi-response analysis. Multi-objective optimization was considered to achieve targeted and optimum output. The desirability function was used to optimize and select the five samples that will undergo the sensory analysis by trained panellists (see Supplementary material).

A highly well-known method that provides the most desirable response values by detecting the pertaining operating conditions, is the desirability function approach. It is widely used to optimize multiple response processes and values can be found from 0 to 1, where 0 indicates that the conditions have led to an unwanted response, and 1 shows optimal responses to the studied conditions (Derringer et al., 1980). These authors proposed a categorization of the desirability functions that allowed the visualization of the different functions to be used depending on if a particular response Y_i has to be minimized, maximized or is a target value. The global desirability function D was determined later on by finding the geometric average of all of the responses within the individual functions $d_i(Y_i)$, where n is the number of responses, and the ideal solutions are found by maximizing D.

$$D = \left(\prod_{i=1}^{n} d_i(Y_i) \right)^{\frac{1}{n}}$$

Based on cost-effective criteria, the desirability was calculated for the maximum protein content, specific volume and minimum cost. The optimization generated five best solutions showed in Table 6 (see supplementary material, M15, M5, M9, M8 and M10, in descending order of desirability) and Table 7 (see supplementary material) that shows the relation between the ingredients and their proportions (4 levels) in each sponge cake.

3.5. Sensory analysis of the sponge cakes

Fig. 4 shows the robustness of the trained panel by indicating the average rating (red diamond) and mean of each panellist (colour spots) per sample and per attribute. The red line indicates the agreed QDA (see Table 8 in supplementary material). The dark grey area represents QDA ± 1 point, meaning that the scores inside this area can be considered similar to QDA. M10 fell inside the dark grey area for all attributes

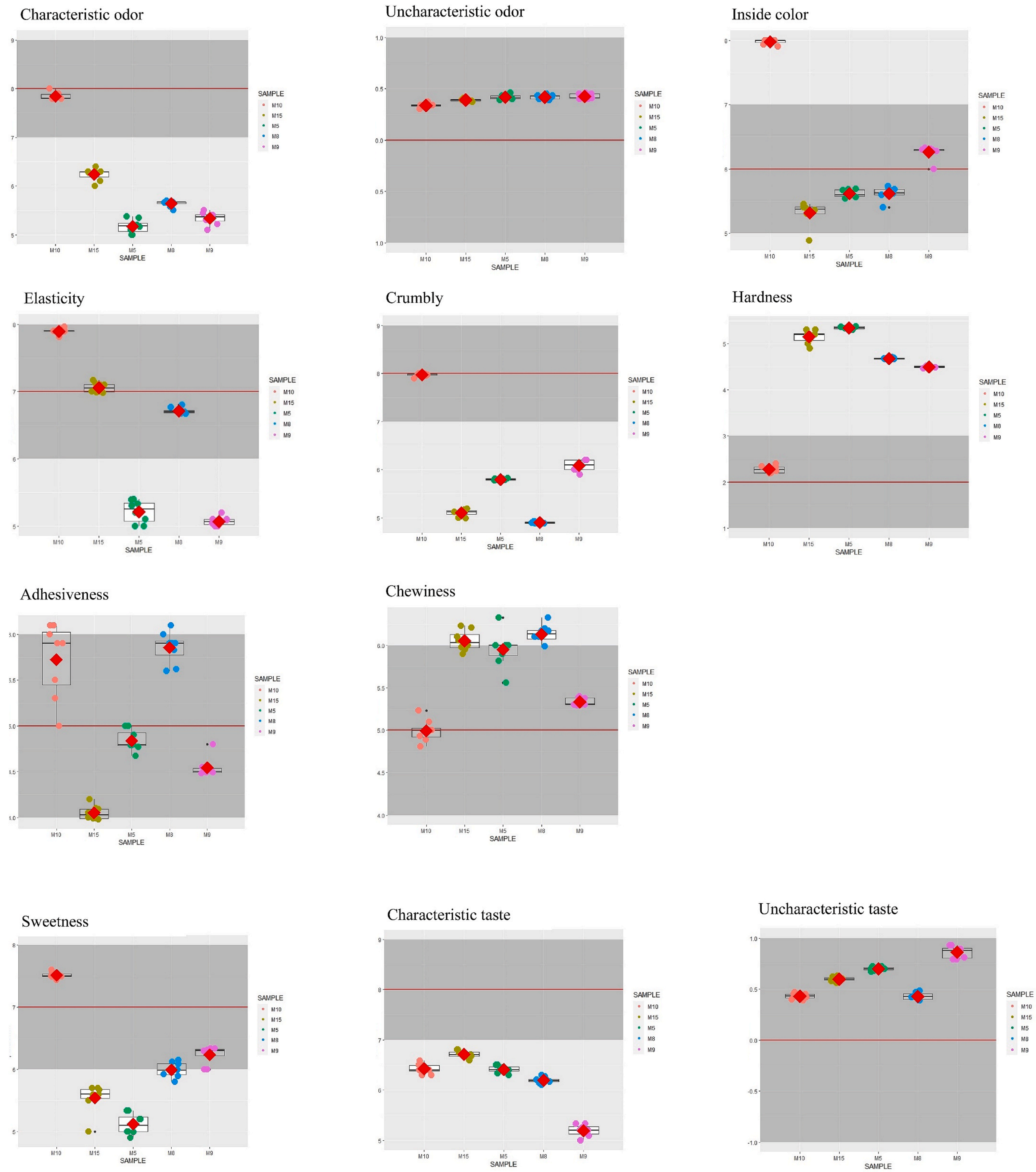

Fig. 4. Sensory analyses of the 5 optimised samples (desirability function) characterised by the trained panel in terms of characteristic odour, uncharacteristic odour, inside color, elasticity, crumby, hardness, adhesiveness, chewiness, sweetness, characteristic taste, and uncharacteristic taste. Red line indicates QDA of control sample with dairy and egg ingredients, dark grey area represents QDA ±1. For each attribute, the box plots include; ♦(mean), different color spots (mean of each panellist for each sample): **M10:** 3.75% PP, 3.75% HP, 7.5% IP **M15:** 3.75% PP, 11.25% HP, 0% IP; , **M5:** 7.5% PP, 0% HP, 7.5% IP, **M8:** 7.5%PP, 3.75% HP, 3.75% IP and **M9:** 3.75% PP, 7.5% HP, 3.75% IP
*PP = pea protein, HP = hemp protein, IP = insect protein.

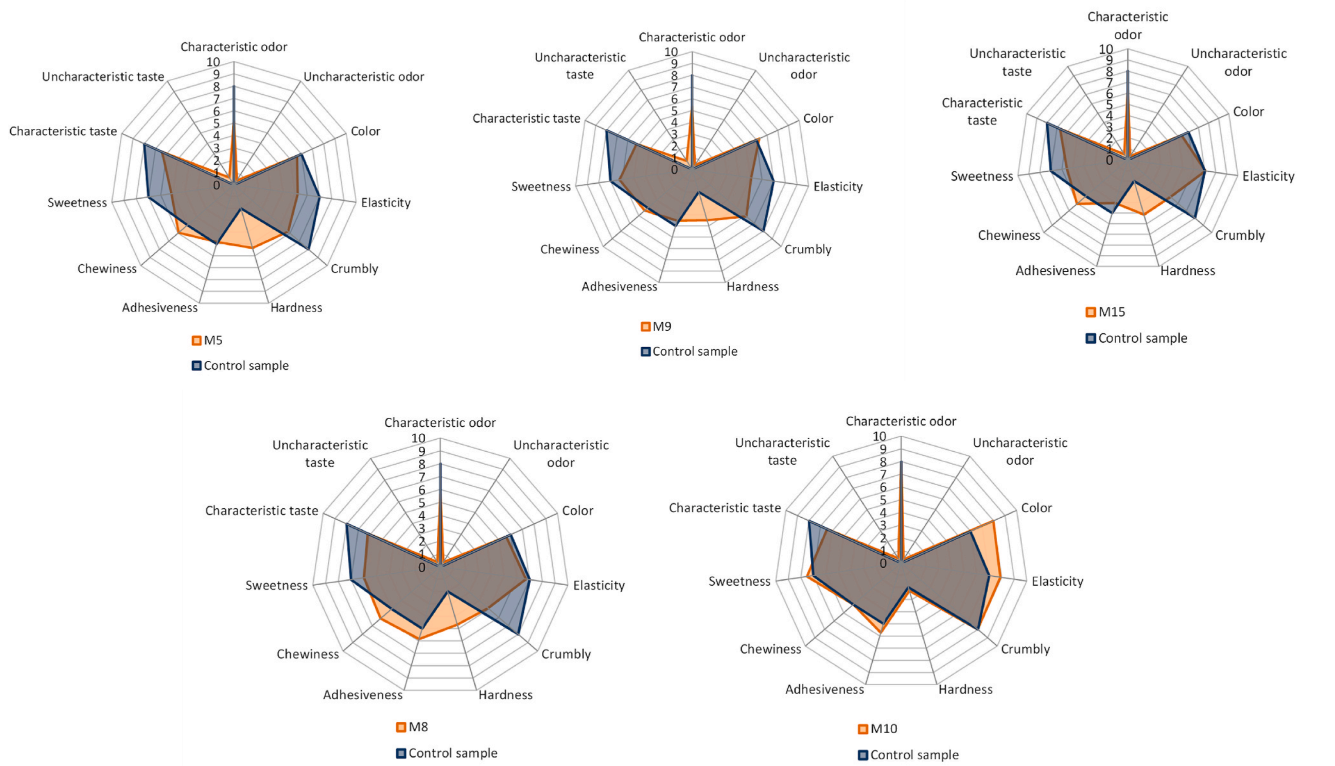

Fig. 5. Spidercharts showing the sensory results obtained from the trained panelists, for each attribute of the 5 sponge cake samples with alternative proteins, and the control sample with dairy and egg ingredients. **M5**: 7.5% PP, 0% HP, 7.5% IP; **M9**: 3.75% PP, 7.5% HP, 3.75% IP, **M15**: 3.75% PP, 11.25% HP, 0% IP, **M8**: 7.5% PP, 3.75% HP, 3.75% IP; and **M10**: 3.75% PP, 3.75% HP, 7.5% IP. M5.

except for inside colour, meaning that M10 was similar to QDA for most of the attributes. Fig. 5 shows the spiderweb charts where each sample is compared with the QDA scale for each attribute. Once more, M10 diagram was the closest to QDA.

The score points of the sensory analysis are detailed in Table 5. Samples were described as different depending on the combinations of alternative proteins used. Tukey's test showed that parameters such us characteristic odour, inside colour and crumbly were significantly different ($p < 0.05$) from other parameters regarding the ingredient protein used. These results agree with the differences encountered in the instrumental analysis of L*a*b* for crumb (Table 4) and cohesiveness in TPA (Table 2), equivalent to inside colour and crumbliness, respectively, in the sensory analysis.

The uncharacteristic odour was rated exceptionally low for all samples (0.34–0.42), with small significant differences among them. Also, uncharacteristic taste was rated <1 for all samples, with slightly significant differences among all samples. The 15% that represented the combination of ingredients in the total composition of the sponge cake had a small effect on the uncharacteristic odour and taste perception.

For characteristic odour, there were significant differences ($p < 0.05$) among all samples. The highest score was for M10 (7.84) followed by M15 (6.24), M8 (5.64), M9 (5.33) and M5(5.17).

Inside colour was rated closer to yellow in sample M10 (7.97) followed by M9 (6.26). Then, the whiteness increased being M5 and M8 the samples rated with the lowest scored (5.62).

When looking at the composition of the samples, it is possible to conclude that the scores for characteristic odour and colour increased with the percentage of insect protein ingredient, followed by hemp and pea (Table 5).

The sample rated with the highest elasticity (7.89) was again M10 followed by M5, both differing by 1 point from sample M8 and by 2 points from samples M5 and M9. Table 5 also shows how the effect that protein addition had on elasticity was higher for samples that contained insect protein, followed by hemp and pea. The same trend was observed for crumbliness, hardness, adhesiveness and chewiness. For crumbliness, sample M10 reached the highest score (7.97) followed by M9 (6.08), M5 (5.80), M15 (5.10) and M8 (4.90). For hardness, samples M5 and M15 were rated as the hardest, followed by M9 and M8. Sample M10 was considered the softest sample. Regarding adhesiveness, M10 (5.72) and M8 (5.86) did not have any significant differences ($p > 0.05$), and they were 1.5 points more adhesive than M15 (4.05) and M5 (4.84). M15 (4.05) was considered less adherent than the rest. In terms of sweetness, the 5 samples were significantly different ($p < 0.05$) among them. M10 was rated as the sweetest one (7.50) followed by M9 (6.23), M8 (5.99), M15 (5.54), M5 (5.12). A study of bread enriched with alternative proteins, showed that higher amounts of pea protein incorporation increased hardness values (García-Segovia, Igual, & Martínez-Monzó, 2020).

For chewiness, M8 (6.13), M15 (6.05) and M5 (5.95) received the highest scores but did not have significant differences among them ($p > 0.05$). They were followed by M9 (5.33) that was significantly different from M10 (4.99). Therefore, it can be concluded that, for chewiness intensity, the effect of protein addition was higher for pea protein, followed by hemp and insect protein.

Finally, the characteristic taste was rated higher for M15 (6.71) followed by M10 (6.42) and M5 (6.41), which were significantly higher ($p < 0.05$) than M8 (6.19) and M9 (5.19). Looking at the samples' composition in Table 5, it was shown that as hemp and insect protein appeared in the formula, the characteristic taste was considered similar to the control with animal-derived ingredients. Fig. 5 illustrates the spider web diagrams of each of the samples compared with the control. It is possible to observe how the pattern followed by M10 is closer to closest to the control. M5, M9, M15, M8 differ from the control in characteristic taste and crumbliness.

Table 5
Sensory analysis of the optimised sponge cakes containing pea, hemp and insect protein.

SAMPLES	Characteristic odour	Uncharacteristic Odour	Inside color	Elasticity	Crumbliness	Hardness	Adhesiveness	Chewiness	Sweetness	Characteristic taste	Uncharacteristic taste
M10	$7.84^{a} \pm 0.08$	$0.34^{c} \pm 0.02$	$7.97^{a} \pm 0.04$	$7.89^{a} \pm 0.04$	$7.97^{a} \pm 0.03$	$2.27^{e} \pm 0.08$	$5.72^{a} \pm 0.41$	$4.99^{c} \pm 0.13$	$7.50^{a} \pm 0.05$	$6.42^{b} \pm 0.10$	$0.43^{d} \pm 0.03$
M15	$6.24^{b} \pm 0.13$	$0.39^{b} \pm 0.01$	$5.31^{d} \pm 0.18$	$7.05^{b} \pm 0.07$	$5.10^{d} \pm 0.07$	$5.15^{b} \pm 0.14$	$4.05^{c} \pm 0.08$	$6.05^{a} \pm 0.12$	$5.54^{d} \pm 0.23$	$6.71^{a} \pm 0.07$	$0.59^{c} \pm 0.02$
M5	$5.17^{e} \pm 0.14$	$0.42^{ab} \pm 0.02$	$5.62^{c} \pm 0.06$	$5.21^{d} \pm 0.17$	$5.80^{c} \pm 0.01$	$5.34^{a} \pm 0.03$	$4.84^{b} \pm 0.12$	$5.95^{a} \pm 0.22$	$5.12^{e} \pm 0.17$	$6.41^{b} \pm 0.07$	$0.69^{b} \pm 0.02$
M8	$5.64^{c} \pm 0.07$	$0.42^{ab} \pm 0.02$	$5.62^{c} \pm 0.10$	$6.71^{c} \pm 0.05$	$4.90^{e} \pm 0.01$	$4.68^{c} \pm 0.02$	$5.86^{a} \pm 0.17$	$6.13^{a} \pm 0.11$	$5.99^{c} \pm 0.12$	$6.19^{c} \pm 0.07$	$0.43^{d} \pm 0.04$
M9	$5.33^{d} \pm 0.13$	$0.42^{a} \pm 0.03$	$6.26^{b} \pm 0.11$	$5.07^{e} \pm 0.07$	$6.08^{b} \pm 0.11$	$4.50^{d} \pm 0.02$	$4.54^{b} \pm 0.11$	$5.33^{b} \pm 0.05$	$6.23^{b} \pm 0.14$	$5.19^{d} \pm 0.11$	$0.86^{a} \pm 0.06$

Means with different superscript letters in the same column are significantly different according to Tukey's test ($p < 0.05$). **M10:** 3.75% PP, 3.75% HP, 7.5% IP, **M15:** 3.75% PP, 11.25% HP, 0% IP, **M5:** 7.5% PP, 0% HP, 7.5% IP, **M8:** 7.5%PP, 3.75% HP, 3.75% IP and **M9:** 3.75% PP, 7.5% HP, 3.75% IP.

4. Conclusion

This study describes the optimization of an egg- and dairy-free formulation of sponge cakes using various combinations of pea, hemp and insect protein ingredients. The procedure used included design, modelling, optimization, and validation steps. By the selection of desired ranges of multiple responses, the optimum sponge cake formula that maximises protein and specific volume and minimizes the cost was determined by the desirability function. The sensory analyses carried out on the optimum sponge cake samples exhibited good agreement with the regression models and experimental measurements. The results of this study indicated that it is possible to improve the specific volume, texture and cost of substituting egg and dairy ingredients in sponge cakes by a combination of pea, hemp and insect protein. The incorporation of hemp protein contributed to increase the final protein content of the sponge cakes, whereas the incorporation of insect protein contributed to increasing the specific volume and reducing hardness. Pea protein had a positive effect in reducing reformulation costs.

According to the organoleptic evaluation, differences were found in each studied attribute. The combination of all 3 of the alternative proteins ingredients (in particular, 3.75% PP, 3.75% HP, 7.5% IP) generated sponge cakes that were more similar to the control with animal-derived ingredients, than when using just one ingredient on its own. Another observation was that the more insect-protein powder was added into the formula, the less hardness, more crumbliness, sweeter flavour and the more perceivable the yellowish internal colour by expert panellists.

CRediT role

Clara Talens: Conceptualization, Methodology, Investigation, Supervision, Writing-original draft, Funding acquisition **Maider Lago**: Formal analysis, Data curation. **Laura Simó-Boyle:** Formal analysis, Writing -review and editing. **Isabel Odriozola-Serrano**: Supervision, Writing -review and editing. **Mónica Ibargüen**: Conceptualization, Funding acquisition.

Declaration of competing interest

The Authors declare no Competing Financial or Non-Financial Interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113878.

References

Ahmed, J., Thomas, L., & Arfat, Y. A. (2019). Functional, rheological, microstructural and antioxidant properties of quinoa flour in dispersions as influenced by particle size. *Food Research International, 116*, 302–311. https://doi.org/10.1016/j.foodres.2018.08.039

Aluko, R. E. (2017). Chapter 7: Hemp seed (Cannabis sativa L.) proteins: Composition, structure, enzymatic modification, and functional or bioactive properties. In S. R. Nadathur, J. P. D. Wanasundara, & L. Scanlin (Eds.), *Sustainable protein sources* (pp. 121–132). San Diego: Academic Press.
Ammar, I., Gharsallah, H., Ben Brahim, A., Attia, H., Ayadi, M. A., Hadrich, B., et al. (2021). Optimization of gluten-free sponge cake fortified with whey protein concentrate using mixture design methodology. *Food Chemistry, 343*, Article 128457. https://doi.org/10.1016/j.foodchem.2020.128457
Assad Bustillos, M., Jonchère, C., Garnier, C., Réguerre, A. L., & Della Valle, G. (2020). Rheological and microstructural characterization of batters and sponge cakes fortified with pea proteins. *Food Hydrocolloids, 101*, Article 105553. https://doi.org/10.1016/j.foodhyd.2019.105553
Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International, 43*(2), 414–431. https://doi.org/10.1016/j.foodres.2009.09.003
Cherie, Z., Ziegler, G. R., Fekadu Gemede, H., & Zewdu Woldegiorgis, A. (2018). Optimization and modeling of teff-maize-rice based formulation by simplex lattice mixture design for the preparation of brighter and acceptable injera. *Cogent Food & Agriculture, 4*(1), Article 1443381. https://doi.org/10.1080/23311932.2018.1443381
Cho, S. Y., & Ryu, G. H. (2021). Effects of mealworm larva composition and selected process parameters on the physicochemical properties of extruded meat analog. *Food Sciences and Nutrition, 9*(8), 4408–4419. https://doi.org/10.1002/fsn3.2414
David-Birman, T., Raften, G., & Lesmes, U. (2018). Effects of thermal treatments on the colloidal properties, antioxidant capacity and in-vitro proteolytic degradation of cricket flour. *Food Hydrocolloids, 79*, 48–54. https://doi.org/10.1016/j.foodhyd.2017.11.044
Derringer, G., & Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology, 12*(4), 214–219. https://doi.org/10.1080/00224065.1980.11980968
García-Segovia, P., Igual, M., & Martínez-Monzó, J. (2020). Physicochemical properties and consumer acceptance of bread enriched with alternative proteins. *Foods, 9*(7), 933. https://www.mdpi.com/2304-8158/9/7/933.
García-Segovia, P., Igual, M., Noguerol, A. T., & Martínez-Monzó, J. (2020). Use of insects and pea powder as alternative protein and mineral sources in extruded snacks. *European Food Research and Technology, 246*(4), 703–712. https://doi.org/10.1007/s00217-020-03441-y
Gravel, A., & Doyen, A. (2020). The use of edible insect proteins in food: Challenges and issues related to their functional properties. *Innovative Food Science & Emerging Technologies, 59*, Article 102272. https://doi.org/10.1016/j.ifset.2019.102272
Hawkey, K. J., Lopez-Viso, C., Brameld, J. M., Parr, T., & Salter, A. M. (2021). Insects: A potential source of protein and other nutrients for feed and food. *Annual Review of Animal Biosciences, 9*(1), 333–354. https://doi.org/10.1146/annurev-animal-021419-083930
House, J. D., Neufeld, J., & Leson, G. (2010). Evaluating the quality of protein from hemp seed (Cannabis sativa L.) products through the use of the protein digestibility-corrected amino acid score method. *Journal of Agricultural and Food Chemistry, 58*(22), 11801–11807. https://doi.org/10.1021/jf102636b
Igual, M., García-Segovia, P., & Martínez-Monzó, J. (2020). Effect of Acheta domesticus (house cricket) addition on protein content, colour, texture, and extrusion parameters of extruded products. *Journal of Food Engineering, 282*, Article 110032. https://doi.org/10.1016/j.jfoodeng.2020.110032
Jensen, L. D., Miklos, R., Dalsgaard, T. K., Heckmann, L. H., & Norgaard, J. V. (2019). Nutritional evaluation of common (Tenebrio molitor) and lesser (Alphitobius diaperinus) mealworms in rats and processing effect on the lesser mealworm. *J Insects Food Feed, 5*(4), 257–266. https://doi.org/10.3920/jiff2018.0048
Kayacier, A., Yüksel, F., & Karaman, S. (2014). Simplex lattice mixture design approach on physicochemical and sensory properties of wheat chips enriched with different legume flours: An optimization study based on sensory properties. *Lebensmittel-Wissenschaft und -Technologie, 58*(2), 639–648. https://doi.org/10.1016/j.lwt.2014.03.032
Köten, M. (2021). Influence of raw/roasted terebinth (Pistacia Terebinthus L.) on the selected quality characteristics of sponge cakes. *International Journal of Gastronomy and Food Science, 24*, Article 100342. https://doi.org/10.1016/j.ijgfs.2021.100342
Lähteenmäki-Uutela, A., Rahikainen, M., Lonkila, A., & Yang, B. (2021). Alternative proteins and EU food law. *Food Control, 130*, Article 108336. https://doi.org/10.1016/j.foodcont.2021.108336
Luo, S., Yan, X., Fu, Y., Pang, M., Chen, R., Liu, Y., … Liu, C. (2021). The quality of gluten-free bread made of brown rice flour prepared by low temperature impact mill. *Food Chemistry, 348*, 129032-129032.
Marchetti, L., Califano, A. N., & Andrés, S. C. (2018). Partial replacement of wheat flour by pecan nut expeller meal on bakery products. Effect on muffins quality. *Lebensmittel-Wissenschaft und -Technologie, 95*, 85–91. https://doi.org/10.1016/j.lwt.2018.04.050
Metcalf, D. A., Wiener, K. K. K., & Saliba, A. (2021). Comparing early hemp food consumers to non-hemp food consumers to determine attributes of early adopters of a novel food using the Food Choice Questionnaire (FCQ) and the Food Neophobia Scale (FNS). *Future Foods, 3*, Article 100031. https://doi.org/10.1016/j.fufo.2021.100031
Miller, R. (2016). Cakes: Types of cakes. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 579–582). Oxford: Academic Press.
Mironeasa, S. (2022). Current approaches in using plant ingredients to diversify range of bakery and pasta products. *Applied Sciences, 12*(6), 2794. https://www.mdpi.com/2076-3417/12/6/2794.
Mishyna, M., Chen, J., & Benjamin, O. (2020). Sensory attributes of edible insects and insect-based foods – future outlooks for enhancing consumer appeal. *Trends Food Science and Technology, 95*, 141–148. https://doi.org/10.1016/j.tifs.2019.11.016
Nadathur, S. R., & Carolan, M. (2017). Chapter 23: Flavors, taste preferences, and the consumer: Taste modulation and influencing change in dietary patterns for a sustainable earth. In S. R. Nadathur, J. P. D. Wanasundara, & L. Scanlin (Eds.), *Sustainable protein sources* (pp. 377–389). San Diego: Academic Press.
Nascimento, R. F., Rosa, J. G., Andreola, K., & Taranto, O. P. (2020). Wettability improvement of pea protein isolate agglomerated in pulsed fluid bed. *Particulate Science & Technology, 38*(4), 511–521. https://doi.org/10.1080/02726351.2019.1574940
Nikbakht Nasrabadi, M., Sedaghat Doost, A., & Mezzenga, R. (2021). Modification approaches of plant-based proteins to improve their techno-functionality and use in food products. *Food Hydrocolloids, 118*, Article 106789. https://doi.org/10.1016/j.foodhyd.2021.106789
Paraskevopoulou, A., Donsouzi, S., Nikiforidis, C., & Kiosseoglou, V. (2015). Quality characteristics of egg-reduced pound cakes following WPI and emulsifier incorporation. *Food Research International, 69*. https://doi.org/10.1016/j.foodres.2014.12.018
Pycarelle, S. C., Bosmans, G. M., Nys, H., Brijs, K., & Delcour, J. A. (2020). Stabilization of the air-liquid interface in sponge cake batter by surface-active proteins and lipids: A foaming protocol based approach. *Food Hydrocolloids, 101*, Article 105548. https://doi.org/10.1016/j.foodhyd.2019.105548
Recharla, N., Riaz, M., Ko, S., & Park, S. (2017). Novel technologies to enhance solubility of food-derived bioactive compounds: A review. *Journal of Functional Foods, 39*, 63–73. https://doi.org/10.1016/j.jff.2017.10.001
Research, G. V. (2021). *Protein ingredients market size, share & trends analysis report by product (plant proteins, animal/dairy proteins, microbe-based proteins, insect proteins), by application, by region, and segment forecasts*. https://www.grandviewresearch.com/industry-analysis/protein-ingredients-market.
Robertson, J. A., de Monredon, F. D., Dysseler, P., Guillon, F., Amado, R., & Thibault, J.-F. (2000). Hydration properties of dietary fibre and resistant starch: A European collaborative study. *Lebensmittel-Wissenschaft und -Technologie, 33*(2), 72–79. https://doi.org/10.1006/fstl.1999.0595
Roncolini, A., Milanović, V., Aquilanti, L., Cardinali, F., Garofalo, C., Sabbatini, R., … Osimani, A. (2020). Lesser mealworm (Alphitobius diaperinus) powder as a novel baking ingredient for manufacturing high-protein, mineral-dense snacks. *Food Research International, 131*, Article 109031. https://doi.org/10.1016/j.foodres.2020.109031
Sahagún, M., Bravo-Núñez, Á., Báscones, G., & Gómez, M. (2018). Influence of protein source on the characteristics of gluten-free layer cakes. *Lebensmittel-Wissenschaft und -Technologie, 94*. https://doi.org/10.1016/j.lwt.2018.04.014
Shen, P., Gao, Z., Fang, B., Rao, J., & Chen, B. (2021). Ferreting out the secrets of industrial hemp protein as emerging functional food ingredients. *Trends Food Science and Technology, 112*, 1–15. https://doi.org/10.1016/j.tifs.2021.03.022
Sun, H., Velazco, O. N., Lakemond, C., Dekker, M., Cadesky, L., & Mishyna, M. (2021). Differences in moisture sorption characteristics and browning of lesser mealworm (Alphitobius diaperinus) ingredients. *Lebensmittel-Wissenschaft und -Technologie, 142*. https://doi.org/10.1016/j.lwt.2021.110989
Talens, C., Arboleya, J. C., Castro-Giraldez, M., & Fito, P. J. (2017). Effect of microwave power coupled with hot air drying on process efficiency and physico-chemical properties of a new dietary fibre ingredient obtained from orange peel. *Lebensmittel-Wissenschaft und -Technologie, 77*, 110–118. https://doi.org/10.1016/j.lwt.2016.11.036
Wee, M. S. M., Goh, A. T., Stieger, M., & Forde, C. G. (2018). Correlation of instrumental texture properties from textural profile analysis (TPA) with eating behaviours and macronutrient composition for a wide range of solid foods. *Food & Function, 9*(10), 5301–5312. https://doi.org/10.1039/c8fo00791h
Wognum, N., Bremmers, H., Trienekens, J., Van der Vorst, J., & Bloemhof-Ruwaard, J. (2011). Systems for sustainability and transparency of food supply chains - current status and challenges. *Advanced Engineering Informatics, 25*, 65–76. https://doi.org/10.1016/j.aei.2010.06.001
Yen, P. P. L., & Pratap-Singh, A. (2021). Vacuum microwave dehydration decreases volatile concentration and soluble protein content of pea (Pisum sativum L.) protein. *Journal of the Science of Food and Agriculture, 101*(1), 167–178. https://doi.org/10.1002/jsfa.10627
Yuksel, F., Yavuz, B., & Baltacı, C. (2022). Some physicochemical, color, bioactive and sensory properties of a pestil enriched with wheat, corn and potato flours: An optimization study based on simplex lattice mixture design. *International Journal of Gastronomy and Food Science, 28*, Article 100513. https://doi.org/10.1016/j.ijgfs.2022.100513
Zahari, I., Ferawati, F., Helstad, A., Ahlström, C., Östbring, K., Rayner, M., et al. (2020). Development of high-moisture meat analogues with hemp and soy protein using extrusion cooking. *Foods, 9*(6), 772. https://www.mdpi.com/2304-8158/9/6/772.