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Effect of dry-fractionated pea protein on the physicochemical properties and the nutritional features of gluten-free *focaccia* flat bread

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ABSTRACT

The aim of this work was to formulate a gluten-free *focaccia* flat bread based on rice and corn flour fortified with dry-fractionated pea protein concentrate (55 g/100 g protein content). A simplex-centroid mixture design with ten formulations helped to study how the flour ratios influenced the physical and sensory properties of dough and breads. The special cubic model significantly described all the responses determined in the dough and flour mixes, and most of those determined in the *focaccia*. The pea protein concentrate influenced the pasting properties of the flour mixes resulting in a decrease of viscosity. The midpoint of the experimental domain (*focaccia* containing 5 g/100 g of pea protein concentrate and 20 g/100 g of rice flour and corn flour each) was optimal, being not affected by the discolorations typical of pea ($a^* = 11.97$, $b^* = 31.86$, corresponding to an orange hue), having crumb hardness and chewiness of 9.11 N and 4.83 N, respectively, and moderate legume odor and flavor (5.6 and 5.3 c.u. in a 0–9 scale, respectively). The selected formulation could be labelled as "source of protein" (energy value provided by proteins >12%), "source of fiber" (fiber >3 g/100 g), and "low-fat" (fat <3 g/100 g).

1. Introduction

Flat breads are among the most ancient processed foods (Pasqualone, 2018), but are still very popular, being easily adaptable to different consumer needs. Depending on their thickness and on their mono- or bi-layered structure, flat breads indeed can be either rolled around, or stuffed in their "pocket", or topped with a variety of ingredients to give palatable street foods, which fit well the modern pace of life (Boukid, 2022). A recent survey showed the existence of a multitude of traditional flat breads throughout the Mediterranean basin, with Italy specialized in the production of the mono-layer topped ones, garnished with several ingredients which vary according to local availability and taste (Pasqualone et al., 2022). Among these Italian garnished flat breads, *focaccia* (related to the French *fougasse* and to the Spanish *coca de recapte*) is particularly popular, after pizza, and is consumed throughout the entire country under various regional names reflecting the seasoning type or the geographical origin. With a simple recipe typically based on wheat flour, vegetable oil, yeast and salt, *focaccia* has been the object of previous investigations substantially aimed at improving its nutritional features by raising the content of proteins and fibers and reducing lipids or improving their quality (Pasqualone et al., 2019; Vurro, Summo, Squeo, Caponio, & Pasqualone, 2022). However, such a widely appreciated bakery product has not been formulated in a gluten-free version yet, despite the increased request of gluten-free foods.

Gluten-free breads are often characterized by a poorer nutritional composition compared to the conventional counterparts, due to higher lipids, lower proteins, and unbalanced amino acid composition (Skendi, Papageorgiou, & Varzakas, 2021). The list of ingredients is usually long due to the additives used to mimic the gluten behavior (Roman, Belorio, & Gomez, 2019). Consumers, instead, prefer low-processed products with few ingredients and are attracted by clean labels (Noguerol, Pagán, García-Segovia, & Varela, 2021). This is a challenging aspect since consumers also require products with sensory and nutritional properties similar to the gluten-containing reference foods (Šmídová & Rysová, 2022).

Among the available gluten-free flours, corn and rice flours are the

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most used (Gobbetti et al., 2018). Nevertheless, incorporating pulse flours can improve the nutritional value in terms of protein, dietary fiber and mineral content (Boukid, Zannini, Carini, & Vittadini, 2019; García-Segovia, Igual, & Martínez-Monzó, 2020; Skendi et al., 2021). However, the addition of pulse flours to bread, especially at the doses required for reaching a high protein content, negatively impacts on the textural and sensory features (Boukid et al., 2019).

To overcome these issues, protein concentrates or isolates from pulses can be used, generally in a relatively low amount, comprised between 2% and 10% (Skendi et al., 2021). However, most of the pulse proteins proposed so far were obtained through a resource-intensive process, i.e., wet fractionation, in terms of consumption of water and chemicals (De Angelis et al., 2021), while more sustainable and less processed ingredients should be used (Van der Goot et al., 2016). From this perspective, the pulse protein concentrates obtained by dry-fractionation are a promising alternative, being obtained by solely physical methods, usually an air fractionation, and reaching protein contents of about 55 g/100 g (De Angelis et al., 2021). Compared to wet-extracted protein, dry-fractionated protein concentrates have a different functionality in terms of water absorption and solubility, which are important properties for the breadmaking process. However, the application of pulse protein concentrates obtained by dry-fractionation is still poorly investigated. In particular, there are some studies concerning the use of chickpea (Xing, Kyriakopoulou, Zhang, Boom, & Schutyser, 2021) and faba-bean proteins (Hoehnel et al., 2020) for preparing wheat-based bread, and pea proteins for wheat-based cakes (Gómez, Doyagüe, & de la Hera, 2012), pointing out the need to better study the effect of dry-fractionated protein in gluten-free breadmaking.

The formulation of a food product is a complex and challenging task that should be supported by rational tools, such as the design of experiments, to reach the best compromise between the efforts employed during product development and the result acquired (Squeo, De Angelis, Leardi, Summo, & Caponio, 2021).

Therefore, the aim of this study was to formulate a gluten-free *focaccia* enriched of dry-fractionated pea protein concentrate. The optimal formulation was selected through a simplex-centroid mixture design, to point out the effect of the three main ingredients, namely dry-fractionated pea protein concentrate, rice and corn flours, on the physicochemical and sensory properties of the final product.

2. Materials and methods

2.1. Raw materials

Rice flour (Lo Conte, Rome, Italy) (fat 0.5 g/100 g; carbohydrates 82 g/100 g; fiber 0.5 g/100 g; protein 7 g/100 g), corn flour (Mulino Rossetto, Portelongo, Italy) (fat 1.0 g/100 g; carbohydrates 75 g/100 g; fiber 2.9 g/100 g; protein 7.5 g/100 g), psyllium husk powder (*Plantago ovata* Forsk, Biotiva, Straßlach-Dingharting, Germany), yeast (*Saccharomyces cerevisiae*, Mulino Caputo, Naples, Italy), sea salt (Com-Sal Srl, Pesaro, Italy) were purchased from local retailers. Dry-fractionated pea protein concentrate (55 g/100g of protein) was kindly provided by Innovaprot srl (Gravina, in Puglia, Italy).

2.2. Experimental design and focaccia preparation

A simplex-centroid mixture design was planned to study the effects of three components, namely the main ingredients of *focaccia* (rice and corn flours, pea protein concentrate), with the following constrains (g/100 g): rice flour ($15 \leq x1 \leq 30$); corn flour ($15 \leq x2 \leq 30$); pea protein concentrate ($0 \leq x3 \leq 15$). The sum of the components was 45 g/100 g, whereas the other 55 g/100 g were constituted by the other ingredients and kept constant (Table 1). The experimental points were chosen according to the D-optimality criterion and a special cubic model, which require seven experiments for three variables (Squeo et al., 2021). Moreover, three replicate points were also included in the model to consider the variability related to the preparation process. In fact, three replicates, for a total of ten experiments are often used in research activities based on three-components mixture design (Squeo et al., 2021).

Table 1
Formulation of the *focaccia* samples according to the simplex-centroid mixture design. The baking time was chosen to guarantee optimal cooking in all the trials.

Trial	Rice flour (x1)(g/100 g)	Corn flour (x2)(g/100 g)	Pea protein concentrate (x3) (g/100 g)	Other ingredients** (g/100 g)	Baking time (min)
1	30	15	0	55	20
2	20	20	5	55	15
3	15	15	15	55	11
4	22.5	15	7.5	55	13
5*	15	15	15	55	11
6*	30	15	0	55	20
7	22.5	22.5	0	55	20
8	15	30	0	55	20
9	15	22.5	7.5	55	13
10*	15	30	0	55	20

*Replication. ** Other ingredients: water (50 g), yeast (1 g), salt (1.5 g), and psyllium husk powder (2.5 g).

The *focaccia* samples were prepared according to the following procedure. Firstly, the flours (rice, corn, dry-fractionated pea protein concentrate), psyllium husk powder (2.5 g) and yeast (1 g), were mixed with 25 mL of water at low speed for 1 min with a spiral kneader (G3 Ferrari, Rimini, Italy). Secondly, salt (1.5 g) was added, dissolved in additional 25 mL of water, and kneading continued for 5 min. The dough was flattened manually, using pastry rings having a diameter of 10.8 cm (Tescoma, Cazzago San Martino, Italy), then left to rise for 1 h and 30 min at 35 °C, RH = 33.5% (Memmert proofer, EN.CO. Srl, Spinea, Italy), and baked in an electric oven (Oem Ali Group Srl, Bozzolo, Italy) at 220 °C for the min reported in Table 1. Owing to the different physicochemical properties of the doughs it was not possible to keep the baking time constant. By contrast, after preliminary trials it was decided to keep constant the hydration level, by varying the baking time, and guaranteeing the optimal cooking of all the 10 formulations. Further explanations about the baking time are discussed in paragraph 3.3.

2.3. Analyses of the flour mixes and dough

The water absorption index and the water solubility index were determined on the ten flour mixes, according to the procedures reported in Du, Jiang, Yu, and Jane (2014). The analysis was carried out in triplicate.

The pasting behavior of flour mixes was analyzed by Rapid Visco Analyzer (RVA 4500; Perten Instruments, Hägersten, Sweden). The flours were blended in a roller mixer (Fisher Scientific, Massachusetts, USA) for 24 h. Flour blends (3.5 g, 14% moisture basis) were suspended into 25 mL of distilled water. Slurries were stirred at 960 rpm for 10 s to complete dispersion and then kept at 160 rpm during the assay. Heating started at 50 °C for 1 min, followed by a temperature increase up to 95 °C in 3 min 42 s, held for 2 min 30 s at 95 °C, and then cooling down to 50 °C in 3 min 48 s, which was kept for 2 min. Pasting parameters included: onset (time at which viscosity starts to increase), viscosity at 95 °C, setback rate (slope during cooling phase), viscosity at 50 °C and final viscosity (Santamaria, Garzon, Moreira, & Rosell, 2021). The analysis was carried out in triplicate.

The color coordinates (L^*, a^*, b^*) of the doughs were determined by means of the CM-600d spectrophotometer (Konica Minolta, Tokyo, Japan). Five replicates were carried out.

2.4. Analyses of focaccia samples

The textural properties were evaluated by a texture profile analysis

(TPA), according to Pasqualone et al. (2019) with few modifications, using a ZI.0 TN texture analyzer (ZwickRoell GmbH & Co. KG, Ulm, Germany), equipped with 50 N load-cell and a compression probe having a 36 mm diameter. The sample was cut in uniform pieces of 30 mm side and compressed twice at 1 mm/s with 5 s of pause within the two compressions, reaching 40% sample deformation in both the compressions. Four replicates were carried out.

The image analysis was carried out as in De Angelis et al. (2020). Briefly, the images of the crumb were acquired with a Sony α-6100 mirrorless camera, equipped with a Sony 16–50 mm f/3.5-5-6 lens (Sony Corporation, Tokyo, Japan), and processed by the ImageJ software (National Institutes of Health, Bethesda, USA), after being converted into 8-bit grayscale. An image section of 65 × 12 mm was cropped from the center, filtered by thresholding function to obtain the best cell resolution. The number of cells and the percentage of cells with an area higher than 5 mm^2 were determined (Zorzi, Garske, Flôres, & Thys, 2020). Four replicates were carried out.

The variations of weight, thickness and diameter induced by baking were determined as percentage with a technical balance (Mettler Toledo, Columbus, Ohio, USA) and a caliper, respectively. Four replicates were carried out.

Color (L^*, a^*, b^*) of crust and crumb was determined by means of the CM-600d spectrophotometer (Konica Minolta, Tokyo, Japan). Five replicates were carried out.

2.5. Quantitative descriptive analysis of focaccia samples

The sensory evaluation was carried out according to the Quantitative Descriptive Analysis (QDA) methodology by a trained panel of eleven people (5 male, 6 female, age 23–55 y), following the ethical guidelines of the laboratory of Food Science and Technology of the Department of Soil, Plant and Food Science of the University of Bari, Italy, and the standard procedures described in Vurro et al. (2022). The panelists were regular consumers of bakery products and legumes, and did not suffer any food intolerances or allergies. They were informed about the study aims and signed an individual written informed consent. The typical odor associated with legume/pea and the typical odor of corn were scored for their intensity on an anchored 9-points scale, using the following contractual units: 0 contractual units (not perceived), 3 (mildly perceived), 6 (distinctively perceived) to 9 (highly perceived). The evaluation was carried out in triplicate.

2.6. Selection of the optimal formulation and nutritional evaluation

The optimal formulation of the gluten-free *focaccia* was selected by the overall observation of the contour plots representing the variation of the analytical characteristics of the flour mixes, doughs and *focaccia* samples in the experimental domain, as well as considering the calculated nutritional values, with particular attention to the content of proteins and fibers. The selected *focaccia* (the one with 5 g/100 g pea protein concentrate) was subjected to the analysis of proximate composition as follows. Protein content (total nitrogen × 6.25) was determined with the AACC method 46–11.02 (AACC International, 2009); the lipid content was determined by a Soxhlet apparatus (Velp Scientifica srl, Usmate, Italy), using diethyl ether as solvent, according to the AOAC method 945.38 F (AOAC, 2006). The total dietary fiber was determined by the enzymatic-gravimetric AOAC method 991.43 (AOAC, 2006). The moisture content was determined at 105 °C by an automatic moisture analyzer (Radwag Wagi Elektroniczne, Radom, Poland). The carbohydrate content was determined as the difference by subtracting the protein, moisture, and lipid contents from 100. The energy value (kcal) was calculated considering the contribution of 4 kcal/g from proteins and carbohydrates, 9 kcal/g from lipids and 2 kcal/g from total dietary fibers, according to the Annex XIV of Regulation (EC) No. 1169/2011 (European Parliament and Council, 2011). Three replicates were carried out.

2.5. Statistical analysis

The responses of the experimental design were modeled according to the postulated special cubic model and the regression coefficients (R^2), the adjusted coefficients of determination (R^2 adj), as well as their significance ($p \leq 0.05$) were calculated by the software Design-Expert 11 (StatEase Inc., Minneapolis, USA). Data were subjected to one-way analysis of variance ANOVA followed by Tukey's HSD (Honestly Significant Differences) test for multiple comparisons at a significance level $\alpha = 0.05$ by using the Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Model evaluation

The regression models calculated for the responses of the flour mixes, dough, and *focaccia* samples together with their significance, are shown in Table 2 *focaccia*. The responses determined on flour mixes and dough were all highly significant (R^2 ≥ 0.98 and R^{2}adj ≥0.93, respectively), meaning that the selected models adequately describe the relationship between the experimental factors and the response variables. Instead, not all the responses calculated on *focaccia* were significant, but the values of R^2 and R^{2}adj of the significant ones were very high (≥0.95 and ≥ 0.86, respectively). The processing steps of *focaccia*-making, i.e., kneading, leavening, and baking, probably resulted in a higher variability of the responses, reducing their significance. However, it should be considered that the interpretation of the model coefficients of mixture designs is not so clear as it happens for the designs for independent variables, because the coefficients are not directly related to the effects (Squeo et al., 2021). Therefore, perusal of the contour plots depicting the variations of each parameter is fundamental to easily have an immediate and comprehensive overview of the phenomena occurring in the experimental domain.

3.2. Characteristics of flours and dough

The water absorption index (WAI) and water solubility index (WSI) of flour mixes were significantly affected by the ingredient ratios (Table 3). The addition of pea protein concentrate led to a decrease of WAI and an increase of WSI (Fig. 1a and b). Rice flour, instead, was responsible of the highest WAI value, and corn flour had an intermediate effect. WAI is mostly due to hydrated and swollen starch after the hydrothermal treatment of flour, while WSI measures the amount of soluble solids after the same treatment. Both WAI and WSI are related to the chemical composition of flours, in terms of content and properties of starch and proteins therein (Tas, Ertugrul, Grunin, & Oztop, 2022) Generally, the dry-fractionated pulse proteins are characterized by low WAI and high WSI (De Angelis et al., 2021) because of their low starch content and the presence of soluble proteins (Vogelsang-O'Dwyer et al., 2020).

Also the pasting behaviors of flour mixes varied according to the different ingredient ratios (Fig. 1c–g). The onset was lower in mixes with high amount of rice flour, indicating an earlier swelling of the rice starch, which was delayed in the presence of the other flours (Fig. 1c). Indeed, the well-shaped peak of viscosity during heating was only visible when high amount of rice flour, and low amount of pea protein concentrate, were present (Fig. 2), probably due to the starch dilution when the level of pea protein increased. These findings confirmed the observed values of WAI. Particularly, the flour mixes with the highest amount of rice had the highest viscosities at 95 °C (2274, 2303 mPa s), at 50 °C (3256, 3171 mPa s), and final viscosity (3608, 3521 mPa s) (Table 3), evidencing the formation of a firmer gel able to resist thermal and mechanical stress. Furthermore, these blends also displayed higher slope during cooling, which indicates a faster retrogradation rate of amylose. The addition of pea flour modified these parameters. Again, by

Table 2
Regression coefficients of the model and their significance for all the responses determined on the flour mixes and on the gluten-free *focaccia* flat bread produced by the simplex-centroid mixture design. (A: rice flour; B: corn flour; C: pea protein concentrate).

	A	B	C	AB	AC	BC	ABC	R^2	*Adjusted* R^2	*p-value*
Flour mixes										
WAI	**4.55**	**3.90**	**3.46**	−0.35	−0.31	−0.64	0.20	**0.99**	**0.98**	**0.002**
WSI	**0.03**	**0.03**	**0.13**	0.01	−0.01	0.01	−0.06	**1.00**	**0.99**	**0.001**
Onset (min)	**3.35**	**4.15**	**4.13**	0.00	0.05	0.05	1.73	**0.98**	**0.93**	**0.014**
Viscosity at 95 °C (mPa s)	**2288**	**1072**	**296**	**602**	**−1249**	**−933**	**−4409**	**1.00**	**1.00**	**< 0.001**
Viscosity at 50 °C (mPa s)	**3213**	**2728**	**1089**	67	**−753**	**−850**	−1949	**1.00**	**1.00**	**< 0.001**
Final viscosity (mPa s)	**3564**	**3127**	**1262**	89	**−722**	**−973**	−1698	**1.00**	**1.00**	**< 0.001**
Setback rate	**754**	**648**	**380**	**−87**	**110**	**202**	309	**1.00**	**1.00**	**< 0.001**
*L**	**72.12**	**69.27**	**56.62**	−2.49	−1.57	−4.98	0.44	**0.99**	**0.98**	**0.002**
*a**	**2.94**	**5.21**	**−1.26**	−0.92	−4.25	−4.43	**37.98**	**0.99**	**0.98**	**0.002**
*b**	**19.56**	**30.13**	**33.12**	−8.15	−1.63	−6.33	**145.28**	**0.99**	**0.98**	**0.003**
Gluten-free Focaccia flat bread										
N. cells	**108.00**	**137.00**	**145.00**	**−98.00**	**63.33**	**−34.67**	703.00	**0.99**	**0.97**	**0.005**
Cells >5 mm^2 (%)	**13.65**	**10.01**	**9.26**	5.82	−7.86	0.03	−38.48	**0.95**	**0.86**	**0.040**
Firmness (N)	**4.37**	**11.71**	**18.27**	**−19.03**	**14.27**	−5.02	−33.72	**1.00**	**0.99**	**0.000**
Springiness	0.82	0.89	0.87	−0.15	−0.27	−0.06	1.09	0.92	0.75	0.096
Chewiness (N)	**2.49**	**7.03**	**8.94**	**−11.67**	6.05	−6.36	0.26	**0.98**	**0.93**	**0.016**
Cohesion	0.70	0.67	0.56	0.04	−0.03	−0.31	0.49	0.87	0.62	0.166
Weight loss (%)	23.79	22.92	14.47	−2.98	−16.08	−18.11	−68.07	0.93	0.80	0.068
Variation in diameter (%)	−14.64	−9.00	−4.84	−4.26	2.07	−6.64	33.10	0.94	0.82	0.060
Variation in thickness (%)	68.26	55.73	80.31	**312.02**	45.57	**118.58**	−319.74	**0.99**	**0.97**	**0.004**
*L** crumb	72.12	70.43	67.54	0.10	−16.85	−25.02	−58.37	0.84	0.51	0.237
*a** crumb	**5.24**	**6.99**	**3.01**	**−5.23**	−0.42	0.81	**39.95**	**1.00**	**0.99**	**0.001**
*b** crumb	**30.03**	**39.67**	**34.52**	**−29.80**	**−8.69**	**−12.66**	**75.52**	**0.99**	**0.98**	**0.002**
*L** crust	68.88	61.33	58.46	14.45	−14.68	−2.78	−79.02	0.86	0.58	0.194
*a** crust	18.13	26.53	168.20	−54.52	−489.64	−523.75	1541.59	0.76	0.27	0.382
*b** crust	**26.71**	**38.36**	**39.13**	−3.01	**18.19**	4.57	**−136.65**	**1.00**	**0.99**	**0.001**
Corn odor	**5.72**	**5.78**	**1.85**	1.99	−8.48	−6.87	0.87	**0.97**	**0.90**	**0.026**
Legume odor	**0.10**	**0.22**	**6.89**	−0.24	**11.36**	**12.18**	16.43	**0.99**	**0.97**	**0.004**

WAI = water absorption index; WSI = water solubility index; bold font indicates significant terms ($p \leq 0.05$).

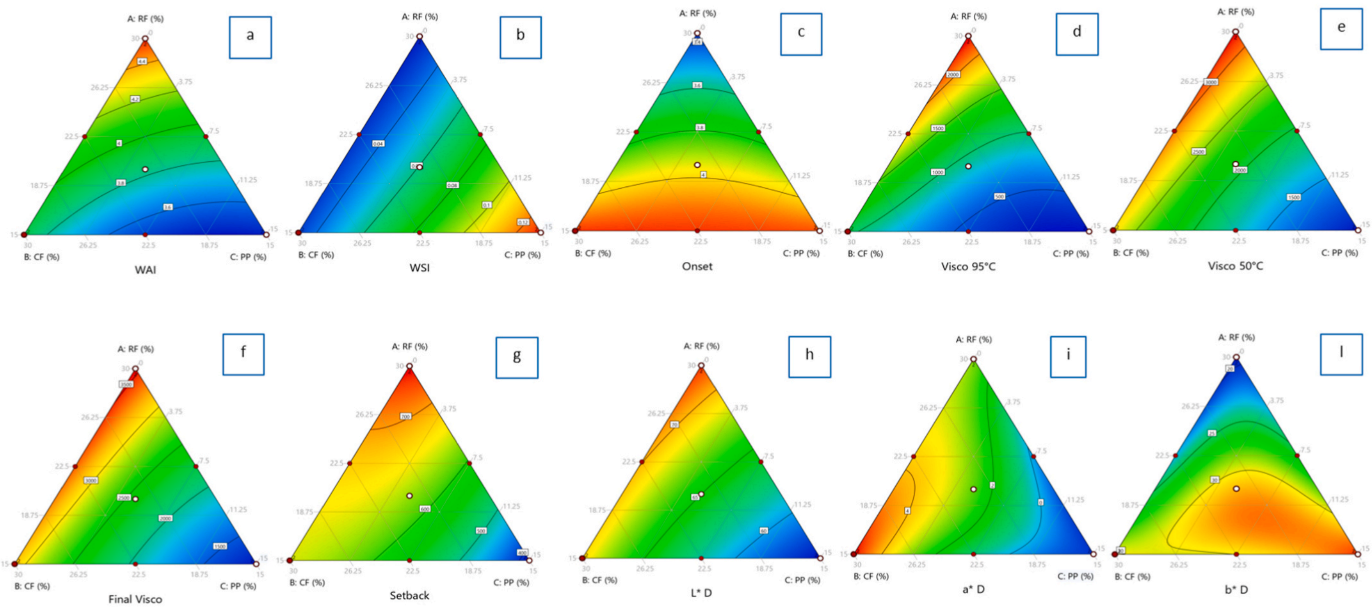

Fig. 1. Contour plots depicting the variations of the physic-chemical parameters and pasting properties of flour mixes and dough at different ratios of corn flour (CF, from 15 to 30 g/100g), rice flour (RF, from 15 to 30 g/100g), and pea protein concentrate (PP, from 0 to 15 g/100 g). For flour mixes: WAI = water absorption index (a); WSI = water solubility index (b); onset of gelatinization (c); viscosity at 95 °C (d); viscosity at 50 °C (e); final viscosity (f); setback (g). For dough: *L** index (h); *a** index (i); *b** index (l). Color variation from blue to red indicates an increase of the considered parameter. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

diluting starch as pea flour increased, there was limited starch swelling and amylose leaching which, in turn, corresponded to weaker gel. The mixture with 15 g/100 g pea flour, indeed, presented lower viscosities at 95 °C (309, 284 mPa s), 50 °C (1117, 1062 mPa s) and final viscosity (1291, 1233 mPa s), as well as lower setback rate during cooling. The latter could be a positive feature of the fortified *focaccia*, indicating that high protein pea flour limits starch retrogradation affecting the realignment of the amylose chains.

The color of dough was significantly influenced by varying the ratio of the three ingredients in the flour mixes (Fig. 1h–l). The highest lightness (*L**) was observed when rice flour was more abundant, while the addition of pea protein concentrate led to a decrease of *L** and of the

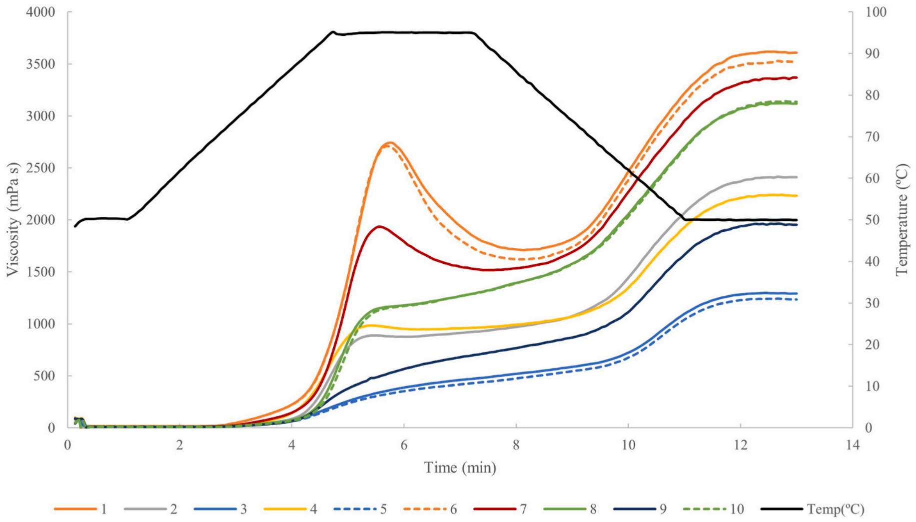

Fig. 2. Viscoamylogram, based on temperature changes due to heating and cooling phases, of flour mixes at different ratios of corn flour, rice flour, and pea protein concentrate. Flour mix compositions, coded from 1 to 10, are reported in Table 1.

green/red coordinate (*a**). The latter reached negative values, corresponding to green, when the pea protein concentration was ≥7.5 g/100g, unless relevant quantities of corn flour (22.5 g/100 g) were also present, as in trial 9 ($a^* = 0.87$) (Table 3). A green hue is particularly critical, being unusual in bakery products and capable of negatively influencing consumer perception (Maina, 2018). The midpoint of the experimental domain, corresponding to 5 g/100 g of pea protein concentrate, was not affected by this unwanted discoloration ($a^* = 2.64$). The highest value of the blue/yellow coordinate (*b**) was observed in the lowest part of the contour plot, where rice flour was in the lowest concentration, substituted by pea protein and corn flour (Fig. 1l).

3.3. Crumb structure, texture and baking-induced variations of focaccia

The results of the image analysis performed on the crumb of *focaccia*, reported in Table 4, were highly significantly modeled (Table 2). Overall, the samples showed a crumb characterized by a dense network of fine cells. Indeed, the percentage of cells with an area greater than 5 mm^2 was less than 14% of the total cells detected. The contour plots (Fig. 3a and 2b) highlight that the addition of pea protein concentrate increased the number of cells and decreased their size. By contrast, when rice flour prevailed, less pores with larger dimensions were observed. The lower gelling properties observed in the flour mixes added of pea protein concentrate (Table 3) probably had an impact on the features of cell crumb network, reducing the viscosity of the dough matrix and, in turn, its ability to retain fermentation gas. However, the differences detected were significant for the number of cells observed but not for the percentage of cells >5 mm^2 (Table 4).

The textural properties of the *focaccia* samples were influenced by the ratio of the mix ingredients, and significantly varied among the ten trials (Table 4). In particular, *focaccia* firmness varied between 3.28 and 18.52 N, covering a wide range of values typical of both gluten-free (Matos & Rosell, 2012) and wheat-based breads (García-Segovia et al., 2020; Pasqualone et al., 2019). The special cubic model significantly fitted the responses of firmness and chewiness, whereas it was not

Table 3
Physicochemical properties of the flour mixes and doughs prepared according to the mixture design.

Trial	WAI	WSI	Pasting properties					Dough color		
			Onset (min)	Viscosity at 95 °C (mPa s)	Viscosity at 50 °C (mPa s)	Final viscosity (mPa s)	Setback rate	*L**	*a**	*b**
1	4.50 ± 0.02^{a}	2.76 ± 0.29^{a}	3.4 ± 0.0^{c}	2274 ± 136^{a}	3256 ± 30^{a}	3608 ± 33^{a}	758 ± 46^{a}	73.01 ± 0.20^{a}	2.94 ± 0.23^{c}	19.86 ± 0.80^{f}
2	3.83 ± 0.04^{d}	6.06 ± 0.07^{d}	4 ± 0.1^{ab}	880 ± 34^{c}	2101 ± 46^{d}	2410 ± 35^{d}	$630{\pm}3^{bc}$	65.02 ± 0.16^{d}	2.64 ± 0.21^{c}	31.19 ± 0.71^{bc}
3	3.43 ± 0.01^{e}	13.34 ± 0.11^{e}	4.1 ± 0.1^{a}	309 ± 40^{d}	1117 ± 83^{f}	1291 ± 96^{f}	$381{\pm}0^{d}$	56.61 ± 0.22^{g}	-1.63 ± 0.23^{g}	32.31 ± 0.31^{ab}
4	3.93 ± 0.01^{cd}	7.48 ± 0.24^{cd}	3.8 ± 0.1^{b}	980 ± 10^{c}	1963 ± 49^{d}	2233 ± 37^{d}	595 ± 13^{bc}	63.98 ± 0.14^{e}	-0.22 ± 0.08^{e}	25.93 ± 0.43^{d}
5	3.49 ± 0.01^{e}	12.48 ± 0.15^{e}	4.2 ± 0.0^{a}	284 ± 11^{d}	1062 ± 18^{f}	1233 ± 23^{f}	379 ± 16^{d}	56.63 ± 0.11^{g}	-0.88 ± 0.10^{f}	33.92 ± 0.54^{a}
6	4.60 ± 0.00^{a}	2.41 ± 0.06^{a}	3.3 ± 0.1^{a}	2303 ± 250^{a}	$3171{\pm}5^{a}$	3521 ± 60^{ab}	750 ± 66^{a}	71.23 ± 1.12^{b}	2.93 ± 0.39^{c}	19.26 ± 1.07^{f}
7	4.14 ± 0.07^{b}	3.05 ± 0.06^{b}	3.8 ± 0.1^{b}	1831 ± 30^{b}	2987 ± 28^{b}	3368 ± 29^{b}	$679{\pm}5^{ab}$	70.07 ± 0.41^{c}	3.84 ± 0.21^{b}	22.81 ± 1.38^{e}
8	3.86 ± 0.05^{cd}	3.19 ± 0.06^{cd}	4.1 ± 0.0^{a}	1090 ± 53^{c}	2733 ± 20^{c}	3117 ± 21^{c}	$642{\pm}2^{bc}$	69.11 ± 0.58^{c}	5.09 ± 0.30^{a}	29.67 ± 1.32^{c}
9	3.52 ± 0.00^{e}	8.26 ± 0.09^{e}	4.2 ± 0.1^{a}	451 ± 16^{d}	1696 ± 44^{e}	1951 ± 44^{e}	$565{\pm}3^{c}$	61.70 ± 0.53^{f}	0.87 ± 0.15^{d}	30.04 ± 0.26^{c}
10	3.94 ± 0.05^{c}	3.25 ± 0.00^{c}	4.2 ± 0.00^{a}	1055 ± 45^{c}	2722 ± 31^{c}	3137 ± 39^{c}	653 ± 10^{abc}	69.43 ± 0.19^{c}	5.34 ± 0.3^{a}	30.59 ± 0.62^{bc}

WAI = water absorption index; WSI = water solubility index; Data are presented as means ± SD of the replicates; Different letters in the same column indicate significant differences at $p < 0.05$.

Table 4
Crumb structure, texture baking-induced variations, color and odor of the *focaccia* samples prepared according to the mixture design.

Trial	1	2	3	4	5	6	7	8	9	10
Number of cells	108.33 ± 6.24^{bc}	143.75 ± 9.54^{a}	143.00 ± 25.2^{a}	135.75 ± 19.19^{ab}	147.00 ± 10.23^{a}	107.67 ± 10.66^{bc}	94.00 ± 11.22^{c}	133.50 ± 10.47^{ab}	129.50 ± 9.04^{ab}	140.50 ± 7.42^{a}
Cells > 5 mm^2 (%)	13.64 ± 5.13^{a}	9.32 ± 2.20^{a}	10.04 ± 3.24^{a}	9.49 ± 4.47^{a}	8.47 ± 2.06^{a}	13.66 ± 4.47^{a}	13.28 ± 3.52^{a}	10.44 ± 2.10^{a}	9.64 ± 1.89^{a}	9.57 ± 1.53^{a}
Hardness (N)	4.80 ± 0.72^{d}	9.11 ± 2.45^{c}	18.52 ± 2.14^{a}	14.89 ± 0.70^{ab}	18.03 ± 1.27^{a}	3.93 ± 0.38^{d}	3.28 ± 0.62^{d}	11.51 ± 2.60^{bc}	13.73 ± 1.78^{b}	11.90 ± 1.03^{bc}
Springiness	0.81 ± 0.03^{de}	0.85 ± 0.02^{bcd}	0.85 ± 0.02^{abcd}	0.77 ± 0.01^{e}	0.89 ± 0.02^{ab}	0.83 ± 0.02^{cde}	0.82 ± 0.04^{cde}	0.88 ± 0.01^{ab}	0.87 ± 0.01^{abc}	0.90 ± 0.03^{a}
Chewiness (N)	2.69 ± 0.42^{de}	4.83 ± 1.28^{cd}	8.07 ± 0.91^{ab}	7.23 ± 1.51^{abc}	9.81 ± 1.88^{a}	2.29 ± 0.25^{de}	1.84 ± 0.19^{e}	6.95 ± 1.71^{bc}	6.39 ± 0.57^{bc}	7.11 ± 0.89^{bc}
Cohesion	0.70 ± 0.02^{a}	0.63 ± 0.05^{abc}	0.51 ± 0.02^{c}	0.62 ± 0.10^{abc}	0.61 ± 0.10^{abc}	0.70 ± 0.02^{a}	0.70 ± 0.06^{a}	0.68 ± 0.04^{a}	0.54 ± 0.02^{bc}	0.66 ± 0.07^{ab}
Weight loss (%)	25.79 ± 2.41^{a}	13.74 ± 3.49^{b}	16.06 ± 1.49^{b}	15.11 ± 2.46^{b}	12.88 ± 1.68^{b}	21.79 ± 2.68^{a}	22.61 ± 2.32^{a}	22.32 ± 2.17^{a}	14.17 ± 1.37^{b}	23.51 ± 2.27^{a}
Variation in diameter (%)	−13.92 ± 3.56^{de}	−9.25 ± 0.29^{bcd}	−3.13 ± 1.49^{a}	−9.23 ± 1.27^{bcd}	−6.56 ± 1.61^{ab}	−15.36 ± 2.24^{e}	−12.89 ± 2.85^{cde}	−8.84 ± 1.25^{bcd}	−8.58 ± 2.91^{bc}	−9.16 ± 0.87^{bcd}
Variation in thickness (%)	65.57 ± 9.88^{bc}	109.20 ± 25.00^{ab}	85.00 ± 23.80^{bc}	85.68 ± 11.89^{bc}	75.60 ± 25.00^{bc}	70.96 ± 5.37^{bc}	140.00 ± 11.55^{a}	56.94 ± 6.99^{c}	97.70 ± 24.50^{b}	54.51 ± 7.47^{c}
L[a] crumb	75.18 ± 0.89^{a}	63.23 ± 0.66^{ef}	68.12 ± 1.60^{cd}	65.62 ± 0.72^{de}	66.96 ± 1.66^{cd}	69.05 ± 1.33^{bc}	71.30 ± 1.28^{b}	69.29 ± 1.68^{bc}	62.73 ± 1.17^{f}	71.57 ± 1.01^{b}
a[a] crumb	5.03 ± 0.25^{c}	6.02 ± 0.34^{b}	3.04 ± 0.43^{e}	4.02 ± 0.31^{d}	2.98 ± 0.35^{e}	5.44 ± 0.55^{bc}	4.80 ± 0.43^{cd}	6.99 ± 0.39^{a}	5.20 ± 0.32^{c}	6.98 ± 0.36^{a}
b[a] crumb	29.68 ± 1.62^{de}	31.85 ± 1.53^{cd}	35.13 ± 1.08^{b}	30.10 ± 1.39^{de}	33.90 ± 1.25^{bc}	30.37 ± 1.43^{d}	27.40 ± 1.13^{e}	39.52 ± 1.50^{a}	33.93 ± 1.24^{bc}	39.82 ± 0.91^{a}
L[a] crust	72.34 ± 0.92^{a}	59.63 ± 2.31^{de}	60.13 ± 1.30^{d}	60.00 ± 0.43^{d}	56.79 ± 1.34^{e}	65.41 ± 2.03^{c}	68.72 ± 1.64^{b}	61.39 ± 1.38^{d}	59.20 ± 0.78d^{e}	61.28 ± 0.90^{d}
a[a] crust	7.79 ± 0.78^{f}	11.97 ± 1.00^{ab}	8.52 ± 0.56^{def}	9.78 ± 1.00^{cde}	10.27 ± 1.03^{bcd}	9.84 ± 0.67^{cde}	8.38 ± 0.91^{ef}	11.11 ± 0.92^{abc}	10.23 ± 0.88b^{cd}	12.12 ± 0.48^{a}
b[a] crust	26.17 ± 1.34^{c}	31.86 ± 1.30^{b}	38.74 ± 0.75^{a}	37.47 ± 0.71^{a}	39.51 ± 0.57^{a}	27.24 ± 1.80^{c}	31.78 ± 1.24^{b}	38.73 ± 1.48^{a}	39.88 ± 1.05^{a}	37.98 ± 0.92^{a}
Corn odor (c.u.)[a]	6.00 ± 2.18^{a}[a]	3.00 ± 1.51^{bc}	2.20 ± 1.99^{c}	1.67 ± 1.66^{c}	1.50 ± 1.08^{c}	5.44 ± 2.19^{ab}	6.25 ± 1.98^{a}	5.13 ± 1.73^{ab}	2.10 ± 2.38^{c}	6.44 ± 1.33^{a}
Legume odor (c.u.)	0.20 ± 0.42^{b}	5.60 ± 1.96^{a}	6.22 ± 1.48^{a}	6.33 ± 1.73^{a}	7.56 ± 1.24^{a}	0.00 ± 0.00^{b}	0.10 ± 0.32^{b}	0.44 ± 1.01^{b}	6.60 ± 2.59^{a}	0.00 ± 0.00^{b}

[a] C.u. = contractual units. Data are presented as means ± SD of the replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$.

significant for springiness (p = 0.096) and cohesion (p = 0.166) (Table 2). The contour plots of the textural properties of *focaccia* samples are reported in Fig. 3c–f. The addition of pea protein concentrate led to an increase of firmness, as shown by the red areas in the right angle of the contour plot (Fig. 3c). By contrast, higher concentrations of rice flour led to softer texture, as displayed by the blue area in the top left. A similar behavior was shown by chewiness (Fig. 3f), highlighting that the firmness was directly related to the effort needed to masticate the sample.

The cohesion and springiness of *focaccia* samples were significantly affected by the ratio of ingredients, but with little variations (Table 4). The addition of high doses (15 g/100 g) of pea protein concentrate led to a less cohesive product, meaning that a lower effort was required to compress twice the samples, compared to the products prepared without the pea protein (Fig. 3e and f). Generally, baked goods with a high cohesion remain compact during the mastication and this is a preferred quality feature (Matos & Rosell, 2012). The springiness showed the highest value when the maximum concentration of corn flour was used (trial 10), indicating that this flour gave a better ability to withstand a second deformation compared to the others.

The increase of hardness and other textural parameters after the addition of pea protein was previously recorded (Ziobro, Juszczak, Witczak, & Korus, 2016). This behavior agreed with the results of the image analysis on the crumb structure, which indicates the presence of a more compact network in the focaccia obtained by the addition of pea protein.

The baking-induced variations in weight and size of *focaccia* samples are reported in Table 4. Despite not being significantly described by the special cubic model (p = 0.069), weight loss was significantly affected by the addition of pea protein concentrate, regardless of its amount. In particular, all the samples containing pea protein showed a weight loss below 16%, which is significantly lower compared to the products containing only rice and corn flour, having a weight loss >21%. This trend is evident by observing the contour plot shown in Fig. 3g. These results, usually due to higher water binding capacity of pulse proteins, in our case could be explained by the lower baking time necessary for the products containing the pea protein, which was 5–9 min lower compared to the other flat breads (Table 1). The cooking time, in turn, can be explained by the different physicochemical properties of the doughs, being positively correlated ($p < 0.05$, R from 0.82 to 0.97) with all viscosity parameters during cooking and cooling. Indeed, the doughs prepared with the pea protein concentrate had a lower ability to bind water (Table 3) compared to the other formulations, which means that during the baking process, the water easily evaporated from the dough, leading to a higher heat transfer and temperature rice (Zhang & Datta, 2006). Although several commercial pea protein isolates have high water binding capacity, lower values for this parameter have been reported for dry-fractionated pulse protein (De Angelis et al., 2021). This can be related to the presence of protein in the native state which tends to absorb less water compared to the isolates, in which protein denaturation may have occurred during the extraction procedures (Vogelsang-O'Dwyer et al., 2020).

The variations in thickness and diameter were not significantly described by the model. Slight but significant differences indeed were recorded (Table 4). The decrease in diameter varied from −3.13%, at the highest level of pea protein, to −15.36%, when pea protein was absent. Also in this case, it is reasonable to hypothesize that the different heat transfer during baking influenced the size changes occurring in *focaccia*. Indeed, it was previously reported that a more drastic heating, i.e. happening in the *focaccia* with pea proteins, leads to an early protein denaturation and/or starch gelatinization, hardening the dough matrix and making the expansion phenomena difficult (Zhang & Datta, 2006). This also corroborates with the results of the image analysis which demonstrated the presence of a crumb network constituted of small and

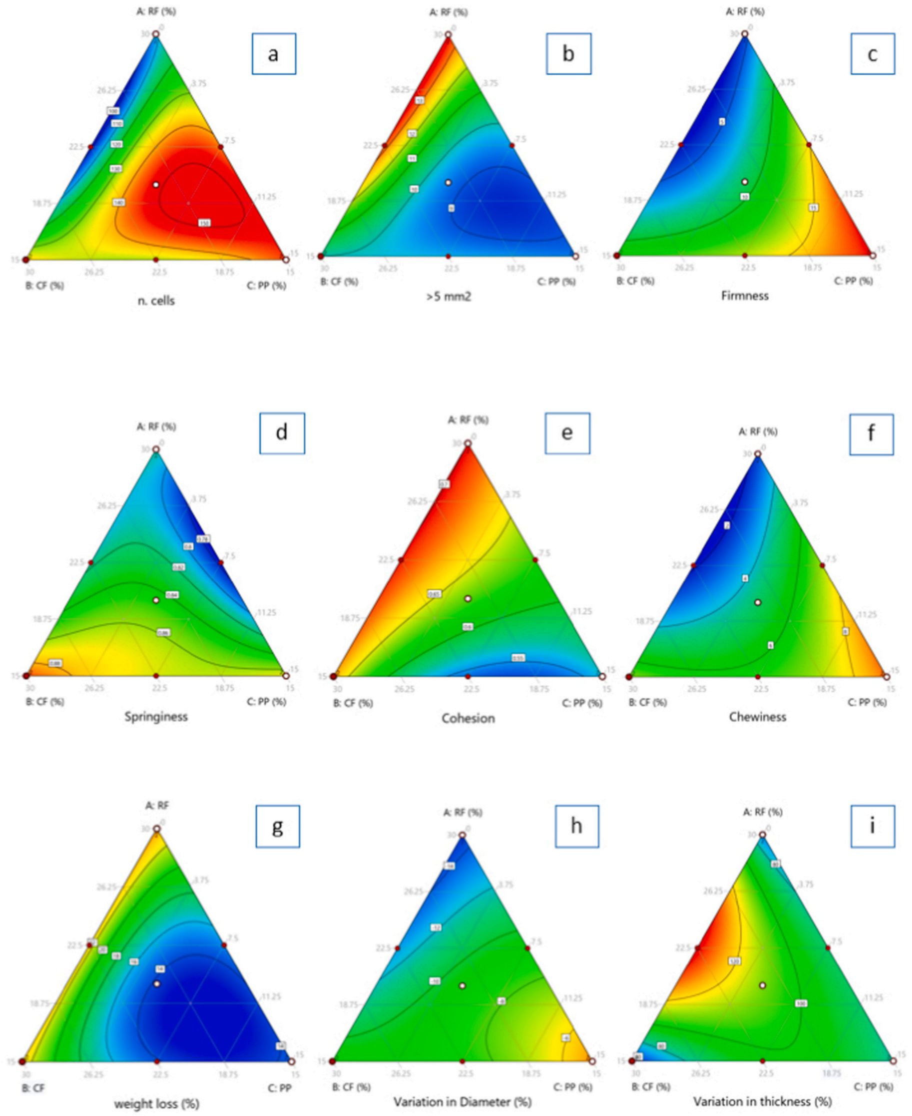

Fig. 3. Contour plots depicting the variations of the textural properties and crumb cell characteristics of *focaccia* samples prepared with different ratios of corn flour (CF, from 15 to 30 g/100g), rice flour (RF, from 15 to 30 g/100g), and pea protein concentrate (PP, from 0 to 15 g/100 g). Number of cells (a); percentage of cells >5 mm^2 (b); firmness (c); springiness (d); cohesion (e); chewiness (f). Color variation from blue to red indicates an increase of the considered parameter. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

numerous pores.

3.4. Color and odor of focaccia

The ratio of the ingredients significantly influenced the color of crust and crumb. Similar trends were observed for the color indices of crumb and crust, except for b^*, which in the crumb was positively related only to the level of corn flour, while in the crust was positively influenced also by the addition of pea protein concentrate (Table 4, Fig. 4a–f). L^* decreased with the addition of the dull, greenish, pea protein concentrate because of its intrinsic color contribution, as already observed in the dough (Table 3), and due to the higher concentration of substrates of the Maillard reaction which, with baking, resulted in a darker crust (Pico, Reguilón, Bernal, & Gómez, 2019). Elevated additions of pea protein concentrate determined the lowest values of a^*, especially in *focaccia* crumb (Fig. 4b), while corn flour increased the same parameter, in agreement with the results observed in the dough. However, a^* values were always positive, indicating that the Maillard reaction and sugar caramelization induced by baking were sufficient to turn the color towards an orange hue, eliminating the greenish one which affected the appearance of unbaked dough. Similar results were observed by Pico et al. (2019) and Ziobro et al. (2016), both in the crumb and in the crust of gluten-free bread fortified with pea protein, as well as by Matos, Sanz, and Rosell (2014) in gluten-free muffins added of pea protein isolate, and by Mancebo, Rodriguez, P., & Gomez (2016) in gluten-free cookies enriched of pea protein.

The typical sensory notes of the main odorous ingredients were perceived by the panelists. The odor of legumes was perceivable in all the samples containing pea protein concentrate, with an intensity scored 5.60 c.u. out of a 9 point scale when 5 g/100 g of pea protein were added, up to 7.56 c.u. at the level of 15 g/100 g (Table 4). The contour plot (Fig. 4g) shows that the perception of this sensory descriptor becomes negligible at a level of about 2 g/100 g of pea protein concentrate. The odor of corn was perceivable but partly masked by the legume odor contributed by the pea protein concentrate. In absence of the latter, corn odor was scored >6 (Fig. 4h). It was previously reported that the presence of chickpea flour in bakery products led to a distinct perception of the typical legume odor (Pasqualone et al., 2019). However, this is one of the critical aspects to consider when developing formulations containing legumes, because the overall acceptability of the products is negatively influenced by the legume odor and flavor (Boukid et al., 2019). In our case, the use of a protein concentrate implies that to reach certain nutritional goals, such as a relevant protein content, the addition of a relatively low quantity of legume would be sufficient, mitigating the impact on the sensory properties.

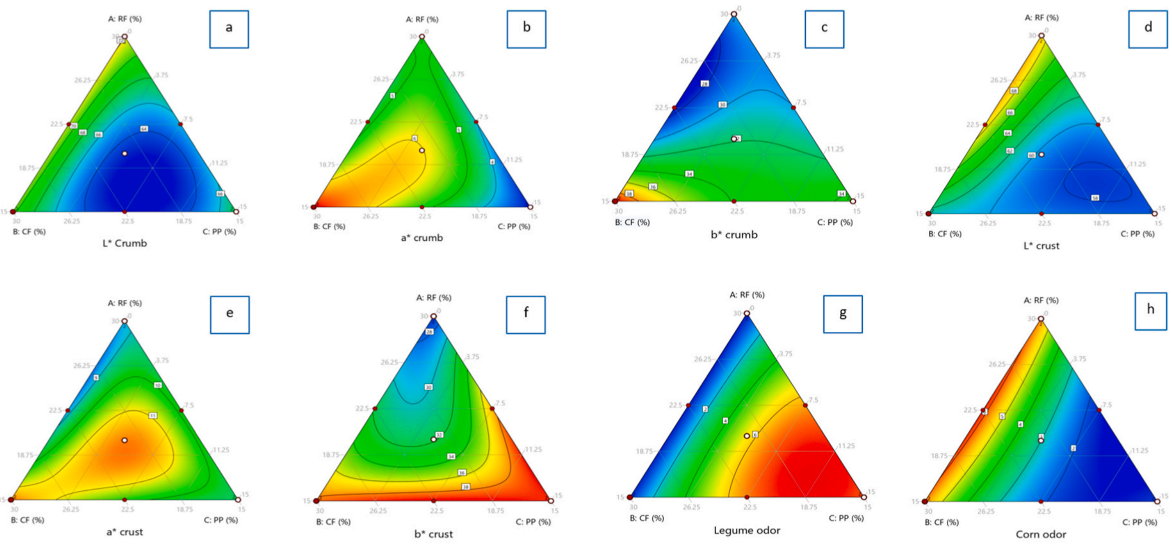

Fig. 4. Contour plots depicting the color and odor of *focaccia* samples prepared with different ratios of corn flour (CF, from 15 to 30 g/100g), rice flour (RF, from 15 to 30 g/100g), and pea protein concentrate (PP, from 0 to 15 g/100 g). L^* index of crumb (a); a^* index of crumb (b); b^* index of crumb (c); L^* index of crust (d); a^* index of crust (e); b^* index of crust (f); legume odor (g); corn odor (h). Color variation from blue to red indicates an increase of the considered parameter. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.5. Selection of the optimal formulation and definition of the nutritional properties of the fortified focaccia

Based on the observed impact of pea protein concentrate on the main physic-chemical properties of dough and final product (gelling ability, texture, color and odor) it is appropriate to keep relatively low this ingredient in a fortified gluten-free *focaccia*. The formulation containing 5 g/100 g of pea protein concentrate and 20 g/100 of corn flour and rice flour each (trial 2, Table 1), which represents the midpoint of the experimental domain, was selected because it best balanced the nutritional aims of the fortification study with the textural and sensory features. This level of addition was esteemed to allow an increase of protein content of about 75–78% compared to the formulations without pea flour.

The nutritional composition of the selected *focaccia* was then experimentally determined (Supplementary Table 1). As intended, the protein content of the selected *focaccia* was high enough to reach the conditions required to label it with the “source of protein” nutritional claim (European Parliament and Council, 2006), i.e., more than 12% of the energy value of the final product was supplied by proteins (30 kcal out of a total energy value of 234 kcal). This result is interesting compared to previous trials which required a double concentration of legume flour to achieve a similar or even lower protein content (Cunha et al., 2019), resulting in a higher alteration of the sensory characteristics (Boukid et al., 2019).

Moreover, to further improve its nutritional features, the formulation of the experimental gluten-free *focaccia* did not include oils (which, instead, are abundant in the conventional *focaccia*). The lipid content, therefore, derived exclusively from the flours used and was therefore below the maximum limit of 3 g/100 g imposed by the Regulation (EC) No. 1924/06 (European Parliament and Council, 2006) to label “low-fat” the final product. The fortified focaccia fulfilled the EC Reg. 1924/06 also for another nutritional claim. Fibers, indeed, important to reduce the risk of chronic and metabolic diseases (Santos, Aguiar, Centeno, Rosell, & Capriles, 2020), reached the amount (>3 g/100 g) needed for the “source of fiber” claim (European Parliament and Council, 2006).

The moisture content of fortified *focaccia* was slightly higher than that reported by other authors in gluten-free flat breads fortified with legumes or other cereals different from rice and corn (Omran & Mahgoub, 2022). This result was probably due to the higher thickness of *focaccia* (approximately 2 cm, compared to the few millimeters of the other flat breads considered in these studies) or to the effect of psyllium husk powder, known to limit the evaporation of water during baking (Franco & Gómez, 2022).

4. Conclusion

The sector of gluten-free baked goods is increasingly attracting the interest of food companies and researchers, particularly concerned by the need to formulate clean label and sustainable foods, with good nutritional and sensory characteristics. The dry-fractionated protein concentrates are low-processed ingredients with a low environmental impact, which could fulfill the consumer expectations for simple, genuine, and transparent food products.

This study explored the effect of different ratios of rice, corn and a dry-fractionated pea protein concentrate used in the formulation of a gluten-free *focaccia* flat bread, to evaluate how they influenced the properties of dough and final product. The simplex-centroid mixture design was effective in helping the evaluation. The results have shown that the addition of pea protein concentrate influenced the pasting properties of the flour mixes, also leading to differences in crumb porosity, color, texture and sensory characteristics. Balancing the physical and sensory properties with the nutritional value, the trial containing 5 g/100 g of pea protein, 20 g/100 g of corn flour and 20 g/100 g of rice flour was chosen as the optimal. Despite this low addition level, the fortified *focaccia* could be labelled as a “source of protein”, “source of fiber” and “low-fat” according to the EC Regulation No. 1924/06, showing 8.27, 0.73 and 3.92 g/100 g of protein, lipids and fiber, respectively.

Overall, considering the need to propose new gluten-free flat breads, having high nutritional value and sensorially acceptable, the use of dry-fractionated pea protein concentrate has proved to be an effective and sustainable strategy. Moreover, starting from our findings, further studies could investigate the use of other pulse species with different protein composition, and consequently, with different functional properties, in order to expand the knowledge about the effect of dry-fractionated protein on the physicochemical and sensory quality of the products.

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CRediT authorship contribution statement

Davide De Angelis: Data curation, Formal analysis, Writing – original draft. **Francesca Vurro:** Investigation, Writing – original draft. **Maria Santamaria:** Investigation, Writing – original draft. **Raquel Garzon:** Formal analysis, Investigation. **Cristina M. Rosell:** Formal analysis, Writing – review & editing, Project management. **Carmine Summo:** Formal analysis, Writing – review & editing. **Antonella Pasqualone:** Conceptualization, Formal analysis, Writing – review & editing, Project management.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114873.

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