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Emulsion filled gels based on inulin and dry-fractionated pulse proteins to produce low-fat baked goods

Graziana Difonzo, Mirella Noviello, Davide De Angelis, Carlo Porfido, Roberto Terzano, Francesco Caponio *

Department of Soil, Plant and Food Science DiSSPA, University of Bari Aldo Moro, Via Amendola, 165/a, I-70126 Bari, Italy

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ABSTRACT

The study evaluated the rheological properties and volatile profile of emulsion filled gels (EFG) composed of inulin, dry-fractionated proteins of red lentil (RL) and black chickpea (BC), and containing three percentages of sunflower seed oil (5%, 10% and 15%). The EFG were then used as fat replacers in the formulation of low-fat brioches. The EFG showed shear thinning behavior, and the EFG containing 15% oil was found to have the highest consistency index. EFG viscosity and viscoelasticity were significantly affected by RL and BC, oil concentration and storage (4 °C for 24 h). EFG made using BC was selected for vegan brioche production due to its lower concentration of undesirable volatile compounds linked to pulse flavor. All the vegan brioches obtained met the criteria for labeling as "low-fat" (Regulation (EC) No 1924/2006). The use of EFG reduced total porosity and pore connectivity but increased hardness and chewiness. The brioche made with EFG containing 15% oil had the lowest levels of hardness and chewiness, and was most similar to the control in its volatile profile and typical brioche odor. These results indicate that EFG containing 15% oil has the potential to replace fat in the production of vegan brioches.

1. Introduction

Emulsion filled gels (EFG) are structured biphasic systems composed of oil droplets dispersed and physically entrapped in an aqueous phase and gelled using hydrocolloids (Patel & Dewettinck, 2016). A solid-like emulsion gel may be obtained from a stable liquid-like emulsion using a continuous phase gelation process and/or aggregating the emulsion droplets (Paciulli et al., 2020). Emulsion gels can be stabilized by polymer molecules such as polysaccharides and protein (Gutiérrez-Luna, Astiasarán, & Ansorena, 2022). Inulin is a polydisperse fructan with β(2–1) bond that is responsible for its reduced energetic value and dietary fiber behavior, and prevents it from being digested like a typical carbohydrate (Niness, 1999). When thoroughly mixed with water or another aqueous solution, inulin forms a particle gel network that produces a white creamy structure, which is spreadable and can easily be incorporated into foods to replace fat (Paradiso et al., 2015). Inulin has been used to cause sol-gel transition of EFG prepared with olive oil, rice bran oil and rosemary essential oil subjected to ultrasonic irradiation (Farjami & Madadlou, 2019; Nourbeheshst, Shekarchizadeh, & Soltanizadeh, 2018; Paradiso et al., 2015). In addition, plant proteins can be used as structuring agents in foods because they can act as emulsifiers to stabilize oil-water mixtures: the oil droplets are dispersed in a continuous protein-gel matrix to form an EFG (Kornet et al., 2022). Various plant-derived proteins have been investigated for use as emulsifiers, including amaranth and quinoa (Gürbüz, Kauntola, Ramos Diaz, Jouppila, & Heinonen, 2018; Lingiardi, Galante, de Sanctis, & Spelzini, 2022), or legume proteins such as soybean and chickpea (Burger & Zhang, 2019; Sharif et al., 2018; Sridharan, Meinders, Sagis, Bitter, & Nikiforidis, 2022). In fact, the increasing global demand for protein requires a transition from animal protein sources to more economical and sustainable plant or microbial protein sources (Aiking & de Boer, 2020). Protein enrichment by dry fractionation processes such as air classification has been proven to be a sustainable approach (van der Goot et al., 2016). Air classified fractions are complex mixtures containing a variety of functional components, such as proteins starch, lipids, and fibers (De Angelis, Latrofa, Caponio, Pasqualone, & Summo, 2024). The functionality of complex ingredients is determined not only by the properties of the individual components but also by their interactions, and synergistic effects have been shown to contribute to improved techno-functionalities (Li & de Vries, 2018). To date, the

* Corresponding author. Università degli Studi di Bari Aldo Moro, Via Amendola 165/a, I-70126 Bari, Italy.
E-mail address: francesco.caponio@uniba.it (F. Caponio).

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techno-functional properties of air classified fractions of legumes have not been widely investigated, and knowledge regarding their behavior as emulsifiers in food remains limited (De Angelis, Latrofa, Caponio, et al., 2024; Funke, Boom, & Weiss, 2022; Gumus, Decker, & McClements, 2017; Saldanha do Carmo et al., 2020). Several authors (De Angelis, Latrofa, Caponio, et al., 2024; Goldstein & Reifen, 2022; Mefleh et al., 2022) have investigated the use of dry-fractioned pulse proteins as ingredients in spreadable plant-based cheese analogues, in bread and bakery products as flour substitutes, or to replace animal proteins (i.e., egg). Nevertheless, a detailed investigation of the sensory and structural impacts of dry-fractionated pulse proteins in EFG could be useful in order to improve understanding of their possible application as ingredient in bakery products.

It has been demonstrated that EFG can be used as fat replacers in food formulations (Giarnetti, Paradiso, Caponio, Summo, & Pasqualone, 2015; Glisic et al., 2019; Paciulli et al., 2020). For example, Giarnetti et al. (2015) have demonstrated that the replacement of 50% of the butter in a shortbread cookie recipe with an EFG based on inulin and extra virgin olive oil gave a final product with healthier properties. A fat replacer is defined by the American Dietetic Association as "an ingredient that can be used to provide some or all of the functions of fat, yielding fewer calories than fat" (Syan et al., 2022). A wide range of food industry products use fat replacers, including meat, dairy and baked products (Colla, Costanzo, & Gamlath, 2018; Guo, Cui, & Meng, 2023; Mefleh et al., 2022).

Fats including butter, margarine, shortening and vegetable oils are typically used in baked goods (Rios, Pessanha, Almeida, Viana, & Lannes, 2014; Wassell, 2014) to lubricate and coat the flour granules, to control water absorption and to ensure the consequent development of the gluten network (Yazar & Rosell, 2023). Fats also retard starch gelatinization, aiding the expansion of gas cells and the development of a porous structure (Yazar & Rosell, 2023). Moreover, they contribute to other important functions of cakes, biscuits, and crackers, such as flavor delivery and shelf-life, by delaying both starch retrogradation and moisture migration (Colla et al., 2018; Lucca & Tepper, 1994; Wang, Li, Copeland, Niu, & Wang, 2015). However, several baked breakfast foods contain substantial quantities of butter and eggs, and many vegan baked goods contain low quality fats such as trans fatty acids (Bojková, Winklewski, & Wszedybyl-Winklewska, 2020). The physical and chemical properties of fats and their fatty acid composition affect their functionality on human health and on the product (Devi & Khatkar, 2018; Omeroglu & Ozdal, 2020).

According to Regulation (EC) No 1924/2006, a food product labeled as "low-fat" must contain no more than 3 g of fat per 100 g (European Commission, 2006). However, the production of low-fat bakery products involves numerous disadvantages for the baking industry, since it has a negative effect on consumer acceptability parameters in baked products, involving reductions in loaf volume, crumb softness, taste (buttery, rich flavor), and shelf-life (Yazar & Rosell, 2023). For these reasons, it is important for product developers and food technologists to understand how fat replacers influence the sensory and physical qualities of baked goods in order to contribute to the development of healthier alternative products.

Nowadays, the bakery industry seeks to enhance the healthy properties of its products to satisfy the needs of specific consumer categories by using ingredients that are both technologically and nutritionally functional.

Within this framework, this study combined different analytical techniques to investigate the effects of inulin and dry-fractionated pulse proteins on the rheological properties of EFG, and then investigated their effects on the structural, microstructural, nutritional, and sensory features of vegan brioches. The outcome of this investigation could lead to the definition of a new vegan brioche recipe with enhanced nutritional characteristics that could attract both vegan and health-conscious consumers.

2. Materials and methods

2.1. Preparation of emulsion filled gels

EFG preparation used commercial inulin with a high degree of polymerization (DP ≥ 20) and purity ≥98.5% (FIBRULINE™ XL chicory root fibre, COSUCRA Groupe Warcoing S.A., Warcoing, Belgium), sunflower seed oil (Bunge, Ravenna, Italy), and dry-fractionated proteins of black chickpea (BC) and red lentil (RL). The BC and RL, purchased from InnovaProt s. r.l. (Gravina in Puglia, Italy), had a protein content of 56.8 and 55.7 g/100 g dry matter (DM), respectively, and a lipid content of 7.3 and 2.5 g/100 g DM, respectively (De Angelis, Latrofa, Squeo, Pasqualone, & Summo, 2024).

Three formulations of EFG were prepared for the dry-fractionated proteins of BC and RL by modifying the liquid fraction ratios with respect to weight (sunflower seed oil:water). In particular, the ingredient ratios by weight (inulin:sunflower seed oil:dry-fractionated protein:water) in BC and RL were as follows: 30:5:2:63 for the EFG containing 5% oil (EFG-BC5 and EFG-RL5); 30:10:2:58 for the EFG containing 10% oil (EFG-BC10 and EFG-RL10); 30:15:2:53 for the EFG containing 15% oil (EFG-BC15 and EFG-RL15).

The EFG were prepared by mechanical homogenization for 4 min at 24,000 rpm (Ultraturrax T25, IKA, Staufen, Germany). This was followed by ultrasonic homogenization by Sonopuls HD 3200 (Bandelin Electronic, Berlin, Germany) with a tapered tip KE 76 (6 mm diameter) at 108 W for 4 min. The duration of homogenization corresponded to the time required to obtain a homogenous mixture.

2.2. Characterization of emulsion filled gels

2.2.1. Rheological parameters

EGG rheological properties were measured using a HAAKE MARS iQ Air rheometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a parallel plate geometry (P35/Ti-02180932). All measurements were carried out at 25 °C, using a 0.8 mm gap between the plates. These measurements were then repeated after storage of EFG at 5 °C for 24 h.

EFG flow behavior was evaluated via a shear rate ramp test, increasing the shear rate from 1 to 100 1/s at 25 °C (De Angelis, Latrofa, Squeo, et al., 2024). The viscosity data (η, expressed in Pa s) were fitted to the Ostwald–de Waele model (Power law), according to the following equation:

$$\eta = K\,\dot{\gamma}^{n-1}$$

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

The frequency-dependent behavior of the EFG was determined by an oscillatory frequency sweep, conducted as described in literature (Krystyjan, Ciesielski, Khachatryan, Sikora, & Tomasik, 2015), with some modifications. In particular, the frequency varied from 0.1 to 10 Hz at 1% strain (which is within the linear viscoelastic regime). The storage modulus (G') and loss modulus (G'') as a function of the frequency were recorded. Moreover, the moduli data were also fitted to the Power law model (Nilsson et al., 2023). These measurements were conducted in triplicate.

2.2.2. Volatile profile

Volatile Organic Compounds (VOC) in the different samples were determined through headspace solid phase micro-extraction (HS-SPME), coupled with gas chromatography/mass spectrometry as previously reported by Difonzo et al. (2018), slightly modified. EFG (0.5 g) were placed into 12 mL vials with 150 μL of internal standard solution (1-propanol, 160 ppm) and 4 mL of NaCl (20% w/v) aqueous solution. Aluminum crimp caps with a butyl rubber septum were used to close the vials. Before VOC extraction, vials were shaken for 2 min using a laboratory vortex in order to improve sample homogenization. Volatile compounds were separated using an Agilent 6850 gas-chromatographer fitted with an Agilent 5975 mass-spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) and polar capillary column (60 m length × 0.25 mm i. d. × 0.25 μm film thickness, HP–Innowax, Agilent Technologies Inc., Santa Clara, CA, USA). Volatiles were extracted by exposing a 75 μm SPME carboxen/polydimethylsiloxane (CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) in the headspace of the vials at 50 °C for 40 min; this was then desorbed in the injection port of the gas-chromatographer, operating in splitless mode at 250 °C for 2 min. The volatile compound separation was carried out as follows: helium was used as a carrier gas with a flow of 2 mL/min; the injector temperature was 250 °C; the oven temperature was held at 35 °C for 5 min, and then increased by 5 °C/min until 50 °C. This temperature was held constant for 5 min, then raised to 230 °C at 5.5 °C/min, and then held constant at 230 °C for 5 min. The mass detector was set as follows: the source temperature was 250 °C, the scan range was 33–270 amu and ionization energy was 70 eV. The volatile compounds were quantified via the standardization of the peak area of the internal standard with the peak areas of the compounds of interests. Each sample was analyzed in triplicate.

2.3. Brioche preparation

In the light of EFG characterization, only the three EFG containing dry-fractionated of black chickpea (EFG-BC5, EFG-BC10, EFG-BC15) were chosen as fat replacers for the preparation of the three experimental brioches (B_5, B_{10}; B_{15}) compared to the control (B_C). Two different batches were prepared for each thesis.

The traditional brioche recipe, as reported below, was used for B_C: 1000 g of soft wheat flour 00 with moisture, ash and protein contents of 15.5%, 0.55% and of 13.5%, respectively (Agugiaro & Figna Molini S. p. a., Collecchio, Italy), 300 g milk (Latte Rugiada S. r.l, Matera, Italy), 264 g egg (Avicola Debernardis, Altamura, Italy), 175 g sunflower seed oil (Bunge, Ravenna, Italy), 100 g sugar (Südzucker, Mannheim, Germany), 50 g egg yolk (Avicola Debernardis, Altamura, Italy), 15 g brewer's yeast (2% lipids, 2% carbohydrates, 10% fibers, 16% proteins; Lievitalia, Barra S. r.l, Cuneo, Italy), and 12.5 g salt (ITALKALI S. p.a., Palermo, Italy). The three experimental brioches (B_5, B_{10}, and B_{15}) were produced as described for B_C but with the following modifications: sunflower seed oil was totally replaced with the three different EFG (EFG-BC5, EFG-BC10, EFG-BC15), milk was replaced with 450 g oat-based beverage (Bioavena, Stammibene, Abafoods S. r.l., Badia Polesine, Italy), and eggs were not added to the dough.

Brioche preparation was carried out by mixing the ingredients for 25 min using a mechanical mixer (Dito Sama, Z.I. du Mont 23200 Aubusson, France). The doughs were left to rise at 30 °C and 78% humidity in retarder prover cabinets of the FERMENTUM series (ACF S. r.l., Galliera, Bologna, Italy) for 2 h. B_C dough had the lowest moisture level (33.3%) compared to B_5, B_{10} and B_{15} (37.6%, 37.1% and 36.6%, respectively). After production, a part of each dough was used for rheological analysis. The remainder was formed by hand into braids that were then left to rise for 2 h, as described above. Finally, the doughs were baked in an electric oven (Aroma® by Naboo®, LAINOX®, Vittorio Veneto, Treviso, Italy) at 170 °C for 7 min.

2.3.1. Viscoelastic properties of brioche doughs

The temperature sweep analysis was conducted according to Hesso et al. (2015), with some modifications, using a HAAKE MARS iQ Air rheometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a parallel plate geometry (P35/Ti-02180932). The dough was analyzed at 1 Hz frequency, 0.05% strain (within the linear viscoelastic regime), and with a 2 mm gap between plates. The temperature varied from 25 to 98 °C at a heating rate of 3 °C/min. The storage modulus G′, and the loss modulus G'' (Pa) were measured as a function of the temperature. A sample holder was used to cover the dough during the analysis in order to prevent sample drying (De Angelis, Latrofa, Squeo, et al., 2024). The measurements were performed in duplicate.

2.4. Brioche characterization

2.4.1. Nutritional composition

The moisture content of each sample was measured with a Thermobalance (Ladwag Mac 110/NP, Radwag, Poland). The protein content (total nitrogen × 6.25) and ash content were estimated by the standard AOAC procedure, following methods 979.09 for protein and 923.03 for ash (AOAC, 2006). The lipid content was determined by a Soxhlet apparatus, using diethyl ether as an extracting solvent, according to AOAC method 945.38F (AOAC, 2006). The enzymatic-gravimetric procedure was used to determine total dietary fiber, as reported in AOAC method 985.29 (AOAC, 2006). The carbohydrate content was determined by subtracting the protein, ash, moisture, total dietary fibers, and lipid content from 100. The results were expressed as g/100 g of product. Given that 1 g of fiber, carbohydrates, protein and fat correspond, respectively, to 2 kcal, 4 kcal, 4 kcal, and 9 kcal (Karp, Wyrwisz, Kurek, & Wierzbicka, 2017; Menezes et al., 2016), the energy value (kcal/100 g) was calculated by summing the % fiber (×2 kcal/g), carbohydrate (× 4 kcal/g), protein (× 4 kcal/g) and fat (× 9 kcal/g) available (Brito et al., 2022; European Commission, 2011). Each sample was analyzed in triplicate.

2.4.2. Textural profile analysis (TPA)

TPA was performed on cubes (20 × 20 × 20 mm) cut from the inside of each brioche sample (crumb) (Onacik-Gür, Żbikowska, Kapler, & Kowalska, 2016). TPA used a Z1.0 TN texture analyzer (Zwick Roell, Ulm, Germany) with a stainless-steel cylindrical probe (36 mm diameter) and a 50 N load cell(Troilo, Difonzo, Paradiso, Pasqualone, & Caponio, 2022). Two compressive cycles (50% sample deformation in both compressions) were carried out at a probe compression rate of 1 mm/s, with 5s pauses before the second compression. Data were acquired using TestXPertII version 3.41 software (Zwick Roell, Ulm, Germany). Hardness, springiness, chewiness, and cohesiveness were evaluated in duplicate.

2.4.3. Specific volume determination

Brioche volume was calculated by rapeseed displacement following AACC method 10–05.01 (AACC, 2000). Specific volume consists of the ratio between the volume and weight of the sample. Each sample was analyzed in duplicate.

2.4.4. Micro-CT 3D analysis

A SkyScan 1272 μ-CT (Bruker Nano GmbH, Berlin, Germany) was used to investigate the internal structure of one brioche sample for each thesis (B_C, B_5, B_{10} and B_{15}). Specimens were cut into a cylinder approximately 3 cm high with a diameter of 1 cm; the same specimens were wrapped with plastic kitchen film to minimize the moisture loss and related artifacts during μ-CT scanning. The SkyScan was equipped with a W micro-focus source (60 kV, 160 μA, <5 μm spot size) and a 16 Mp CCD detector. The acquisition settings were pixel size 5.4 μm, rotation step 0.3° (from 0 to 180°), and exposure time of 2300 ms. To improve data quality, flat field correction, averaging 3 frames and random movement of 10 pixels was used during acquisition. The angular projections were reconstructed using NRecon software version 1.6.10.4, while CTAnalyser software (version 1.15.4.0 + 4, Bruker μ-CT) and CTvox (version 3.1.1 r1191, Bruker μ-CT) were used for measuring quantitative parameters and for the 3D rendering, respectively.

2.4.5. Volatile profile

VOC in the different samples were determined through headspace solid phase micro-extraction (HS-SPME), coupled with gas chromatography/mass spectrometry, as reported above in 2.2.2. In this case, ground brioches (0.5 g) were placed in 12 mL vials containing 150 μL of internal standard solution (1-propanol, 160 ppm) and 4 mL of NaCl

(20% w/v) aqueous solution. The volatile compounds were quantified by the standardization of the peak area of the internal standard with the peak areas of the compounds of interest. Each sample was analyzed in triplicate.

2.4.6. Sensory analysis

Quantitative descriptive analysis (QDA) of the brioches was performed at the University of Bari Aldo Moro (Bari, Italy), by a trained panel of nine judges (5 males and 4 females, with ages ranging between 25 and 60). Ethical guidelines for the laboratory and standard procedures described in Pasqualone et al. (2019) were followed. Preliminary sessions were held to define the list of descriptors and their intensity range (Juárez-Barrientos et al., 2019; Pasqualone et al., 2019). The four brioche samples (B_C, B_5, B_{10} and B_{15}) were presented to each panelist in a completely randomized order. Brioches were evaluated using a 9-point anchored scale from 0 to 9, indicating the intensity of the selected attributes. Each brioche was assessed by judges using a tasting evaluation sheet that included the following descriptors: (1) visual and tactile descriptors indicating crust color (0 = white, 9 = brown), crumb color (0 = white, 9 = beige), elasticity (indicating return to the original shape after moderate finger pressure) (0 = not elastic, 9 = very elastic), crust and crumb homogeneity (0 = unhomogeneous, 9 = homogeneous); (2) olfactometric descriptors indicating odor intensity (0 = not intense, 9 = very intense), odor of brioche (0 = different from the typical odor of commercial brioche, 9 = typical commercial brioche odor), off-odor intensity (0 = not perceived, 9 = very intense); (3) taste descriptors indicating sweetness intensity (0 = unperceived, 9 = very intense) and off-taste intensity (0 = not perceived, 9 = very intense); (4) textural attributes indicating hardness (0 = not hard, 9 = very hard) and dryness (0 = moist; 9 = dry); (5) finally, an overall rating was assigned to each sample.

2.5. Statistical analysis

The graphics of the rheological data for EFG and doughs were created using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA). EFG rheological parameters were analyzed by three-way analysis of variance (ANOVA) with third order interaction considering the type of protein, EFG oil content, and storage as variables. This was followed by Tukey's HSD post-hoc test at $P < 0.05$. A clustering analysis with the construction of a polar heatmap was used to determine the volatile composition of the EFG. For this purpose, hierarchical cluster analysis was performed after data standardization for VOC. Then, VOC and EFG were clustered applying the Ward method and the Euclidean distance type. In addition, two-way analysis of variance (ANOVA) with interactions was performed on these analytical data, considering the independent factors of protein type and oil percentage and their interaction. Differences between the samples and the control brioche were evaluated using Dunnett's multiple comparisons test at $P < 0.05$. Moreover, all results of experimental brioche characterization were analyzed by one-way analysis of variance (ANOVA), and means comparisons were carried out by Tukey's HSD post-hoc test at $P < 0.05$. OriginPro 2021 (OriginLab Corporation, Northampton, MA, USA) was used for the statistical analysis.

3. Results and discussion

3.1. Characterization of the fat replacers

3.1.1. Flow behavior and frequency sweep analysis

EFG viscosity was measured using a shear rate ramp, while viscoelastic properties were studied using an oscillatory frequency sweep performed immediately after preparation of the emulsion and following refrigeration at 4 °C for 24 h.

Fig. 1 (Panel A) shows the apparent viscosity of the EFG, and it can be observed that all the samples had a shear thinning behavior, since viscosity decreased as the shear rate increased. This behavior is typical of non-Newtonian pseudoplastic fluids like emulsions and gels (Hu, Li, Tan, McClements, & Wang, 2022), and indicates that the EFG microstructure is subjected to rearrangement and elongation with the flow (Hu et al., 2022). Table 1 reports the results of fitting the viscosity data with the Ostwald-de Waele power law model (Tsatsaragkou, Methven, Chatzifragkou, & Rodriguez-Garcia, 2021), together with the viscosity values at 1, 10, and 100 1/s. The results of three-way ANOVA with interactions are also reported. Considering the main effects, oil concentration was highly significant for all parameters. In fact, the EFG containing 15% oil presented the highest consistency index and viscosity, and the lowest flow behavior index, thus corroborating the findings of previous studies (Mu, Ren, Shen, Zhou, & Luo, 2022; Xu et al., 2020). The increased viscosity at higher oil content could be explained by the Krieger-Dougherty model, which suggests that higher oil content corresponds to a higher packing volume fraction (Fuhrmann, Sala, Stieger, & Scholten, 2019; Xu et al., 2020).

The variable 'type' (dry-fractionated BC and RL) was only significant for the consistency index and viscosity at 10 and 100 1/s. A significant interaction was found between type of protein and oil concentration, highlighting that the magnitude of the differences was affected by the combination of both factors. Overall, the flow behavior index ranged between 0.106 and 0.289, which is typical of shear thinning materials (Mu et al., 2022). Interestingly, at 100 1/s, the second order interactions 'type × storage' and '% oil × storage', and the third order interaction were no longer significant, and were affected only by 'type × % oil', and by the single factors. From a practical point of view, it is estimated that mixing and stirring involves a range of shear rates comprising between 10 and 100 1/s (Steffe, 1996). It has been reported that dough formation may begin to occur at shear rates above 50 1/s (Tietze, Jekle & Becker, 2017). Therefore, EFGs tend to behave in a similar way at these shear rates. Refrigerated storage influenced EFG viscosity, whereas 'concentration × storage' also affected the consistency index and the viscosity at 1 and 10 1/s. Further comments on the effect of storage on rheological properties of the EFG are given with regard to viscoelastic behavior.

The frequency sweep analysis was carried out to evaluate the viscoelastic properties of the EFG in the non-destructive deformation range, simulating the behavior on short (high frequency) and long (low frequency) time scales (Ramli, Zainal, Hess, & Chan, 2022). Fig. 1 (Panel B) shows the results of the frequency sweep evaluation of the EFG. The storage modulus (G') was always higher than the loss modulus (G'') in all samples over the 0.1–10 Hz frequency range, and both moduli were slightly dependent on the frequency, as they tended to increase moderately at higher frequencies. The dependence of G' and G'' on frequency was evaluated by calculating the relaxation exponent (n) derived from fitting the curves using the power law model (Nilsson et al., 2023).

As reported in Table 1, the relaxation exponents were very low, indicating a small slope and consequently a slight dependence on frequency, which is typical of emulsion gel (Hu et al., 2022; Lorenzo, Zaritzky, & Califano, 2013). Overall, G' was more dependent on the frequency than G'', which suggests the existence of a tridimensional network mainly formed via non-covalent bonds (Tanger, Müller, Andlinger, & Kulozik, 2022). The small slopes of G' and G'' did not result in a clear tendency to crossover within the frequency range of 0.1–10 Hz, indicating the solid-like behavior of the EFG (Steffe, 1996). This means that a dynamic equilibrium may exist between the formation and disruption of intermolecular interactions, giving the structure stability during long observation times (Lorenzo et al., 2013). These observations indicate that EFG present solid-like behavior characterized by elastic deformations (Nilsson et al., 2023). Although some significant differences were found among the three oil concentrations, the frequency sweep data suggest that the EFG behaved in a similar way in the 0.1–0 Hz frequency range. Refrigerated storage affected the viscoelastic properties of the fat replacers, since a moderate increase in G' and G'' was detected in the stored samples. Moreover, the effect of storage and its interaction with oil concentration were significant for n (G''), whereas

Table 1
Rheological parameters of emulsion filled gels (EFG) derived from the shear rate ramp and frequency sweep analysis and results of the three-way ANOVA with third order interaction. K: consistency index; n: flow behavior index.

	Flow Curves					Frequency Sweep	
	K (Pas^{n-1})	*n*	η (Pas)	η (Pas)	η (Pas)	n G′	n G″
			1 1/s	10 1/s	100 1/s		
EFG-BC5 pre	193±9d	0.257 ± 0.018abc	205 ± 15d	28±0d	2.17 ± 0.03cd	0.152 ± 0.011 abcd	0.102 ± 0.01BCE
EFG-BC10 pre	100±0f	0.194 ± 0.010abcde	104±2f	17±1e	1.27 ± 0.08ef	0.139 ± 0.002bcd	0.132 ± 0.001 ab
EFG-BC15 pre	411±1b	0.106 ± 0.000e	396±2BCE	26±2d	2.76 ± 0.30BCE	0.184 ± 0.008a	0.153 ± 0.015a
EFG-BC5 post	312 ± 11c	0.267 ± 0.013 ab	330±2c	46±2b	3.29 ± 0.11 ab	0.142 ± 0.00bcd	0.105 ± 0.005abc
EFG-BC10 post	151±4def	0.258 ± 0.040abc	163 ± 10def	26±1d	2.06 ± 0.05cd	0.150 ± 0.009 abcd	0.108 ± 0.027abc
EFG-BC15 post	467 ± 71 ab	0.16 ± 0.003de	462 ± 76b	53±3a	3.26 ± 0.52 ab	0.141 ± 0.038bcd	0.101 ± 0.047BCE
EFG-RL5 pre	147 ± 11def	0.168 ± 0.035cde	167 ± 20def	17±0e	1.20 ± 0.01ef	0.146 ± 0.006bcd	0.086 ± 0.006BCE
EFG-RL10 pre	111±0ef	0.261 ± 0.036abc	110±4ef	13±1e	1.10 ± 0.05f	0.123 ± 0.003cd	0.064 ± 0.001c
EFG-RL15 pre	323±0c	0.174 ± 0.005bcde	347±7c	40±0c	3.48 ± 0.08 ab	0.164 ± 0.002 ab	0.111 ± 0.002abc
EFG-RL5 post	186 ± 22d	0.211 ± 0.067abcd	206 ± 25d	29±2d	2.01 ± 0.35d	0.117 ± 0.009d	0.096 ± 0.003BCE
EFG-RL10 post	170±4de	0.289 ± 0.020a	189±3de	26±0d	1.92 ± 0.02de	0.158 ± 0.006abc	0.069 ± 0.001c
EFG-RL15 post	494 ± 23a	0.199 ± 0.055abcde	555 ± 49a	45±0b	3.89 ± 0.50a	0.164 ± 0.008 ab	0.089 ± 0.003BCE
Type	***	ns	ns	***	*	ns	***
% oil	***	***	***	***	***	***	*
Storage	***	**	***	***	***	ns	*
Type × % oil	***	***	***	***	***	ns	*
Type × Storage	ns	ns	ns	***	ns	ns	ns
Conc. × Storage	*	ns	*	***	ns	***	*
Type × % oil × Storage	***	ns	***	***	ns	*	ns

Different letters for the same parameter mean significant differences according to the post-hoc Tuckey's HSD test at $P < 0.05$ for the third order interaction 'Type × % oil × Storage'. Significance: ns, *, **, and ***, not significant or significant at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively. Data are represented as means ± standard deviation.

the relaxation exponent of G' was only affected by the oil content, the concentration × storage and the third order interaction. Klost and Drusch (2019) hypothesized the formation of weak non-covalent links in the gel upon resting and cooling for 24 h at 4 °C. Moreover, the authors reported a slight increase in the complex modulus caused by a modest change in the viscous component. In view of the hypothetical applications of EFG in discontinuous production cycles and the significant effect of storage on their viscosity and viscoelastic properties, further studies may focus on validation of these findings in an industrial environment.

3.1.2. Volatile profile

Fig. 2 shows the results of VOC analysis presented as a polar heatmap with a circular dendrogram, allowing characterization of the volatile patterns and their distribution on the samples. In addition, mean concentrations and standard deviations with two-way ANOVA results are reported in Table S1 of Supplementary Materials.

As shown in Fig. 2, EFG clustered in two homogeneous groups, indicating that dry-fractionated proteins of BC and RL conferred specific features on the volatile profile. The samples were further classified into four categories: (i) EFG-BC5; (ii) EFG-BC10, and EFG-BC15; (iii) EFG-RL5; (iv) EFG-RL10, and EFG-RL15. It could be indicated that the content of volatile compounds was changed by modifying the percentage of oil in EFG of both BC and RL.

Many of the VOC identified and quantified in the EFG samples were previously found in EFG using similar ingredients (Paradiso et al., 2015). Analysis of the circular heatmap revealed six clusters. The main cluster included hexanal, 2,4-heptadienal, 1-pentanol, *trans*-2-hexenal and benzaldehyde characterized the EFG obtained with dry-fractionated RL, in which these compounds were more concentrated (data not shown). Hexanal, *trans*-2-hexenal and 2,4-heptadienal were the major aldehydes produced from the oxidation of unsaturated free fatty acids (linoleic and linolenic acid) (Shahidi & Hossain, 2022), which occurs during legume seed development, storage, or stress conditions (i.e., milling, dehulling, and starch or protein production) (Karolkowski, Guichard, Briand, & Salles, 2021). A similar trend was observed for hexanoic acid linked to pungent and rancid notes (Xu, Jin, Lan, Rao, & Chen, 2019), and for 2, 4-hexadienal. A different cluster composed of 2-pentylfuran was found in EFG of both BC and RL with 5 and 10% oil. Moreover, 2-ethylhexan-1-ol, 6-methyl-5-hepten-2-one and 3-octen-2-one were particularly present in EFG-RL samples containing 5% oil. Finally, 2-heptanal, 2-octenal and nonanal characterized EFG-RL containing 15% oil. Some compounds found in the highest concentrations in EFG-RL are contributors of flavor linked to pulses, and are described as green, grassy, fatty, or beany (Karolkowski et al., 2021; Xu et al., 2019). It is well known that these off-flavors have impeded and discouraged the use and consumption of pulses in foods (Roland, Pouvreau, Curran, van de Velde, & de Kok, 2017). In order to minimize the content of these notes in vegan brioches, EFG-BC were selected for use in brioche preparation.

3.2. Dough characterization

3.2.1. Temperature sweep

Fig. 3 shows the temperature-dependent behavior of the dough, with the G′ modulus as a function of the temperature. Temperature sweep analysis was carried out to simulate the baking process and then to investigate the main rheological phenomena and structural modifications in the dough, providing information on viscoelastic changes during cooking (De Angelis, Latrofa, Squeo, et al., 2024; Hesso et al., 2015). G′ was always higher than G″ for both control and experimental doughs, indicating the predominance of elastic-like behavior from the early stage of measurement. The greatest differences were observed in the absolute values of the moduli. In fact, G′ and G″ of B_C dough were approximately one magnitude lower than those of the experimental doughs, indicating a lower viscoelastic response. This might be related to the role of fat in limiting gluten formation by coating flour particles and inhibiting their interaction with water, giving a less elastic dough (Krystyjan, Gumul, Ziobro, & Sikora, 2015). In fact, lipids have a recognized role in optimizing ~~the~~ dough density and viscosity, aiding the stabilization of gas cell dimensions and their distribution (Pycarelle & Delcour, 2021). The experimental doughs obtained using the EFG had a fat content of 1.3%, 1.8% and 2.3%, respectively, which was much lower when compared to the 12.1% of the control dough.

Moreover, the polysaccharide chains of inulin may form a gel-like structure during mixing and hydration by self-association (Peressini,

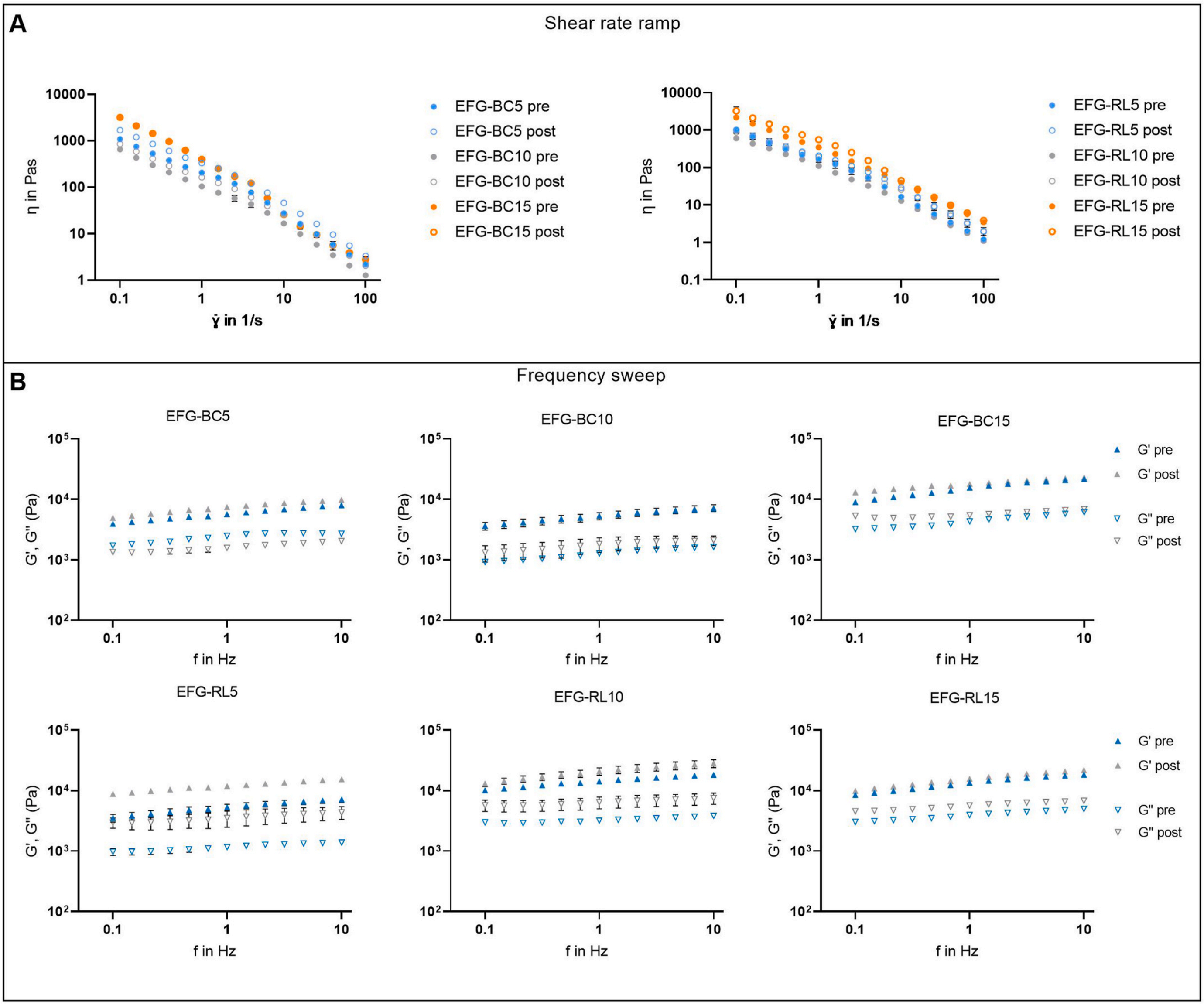

Fig. 1. Apparent viscosity as a function of the shear rate (**A**) and storage (G′) and loss (G″) modulus as a function of the linear frequency (Hz) (**B**) of emulsion filled gels (EFG) containing 5, 10, and 15% of oil, prepared using dry-fractionated proteins of black chickpea (BC) and red lentil (RL). Filled symbols indicate measurement before refrigeration, while empty symbols indicate values recorded after EFG refrigeration.

Cavarape, Brennan, Gao, & Brennan, 2020). Additionally, the inulin gel is strengthened during heating (Sirbu & Arghire, 2017). Furthermore, inulin gel easily interacts with water, binding it and conferring a highly elastic-like behavioron the dough (Yovchev & Le-Bail, 2021).

It is evident that while the overall behavior was similar among the trials, minor differences were observed in the temperatures at which the slope of the reaction changed. After an initial slight decrease in the modulus owing to changes in viscoelastic behavior caused by the increase in temperature and the melting of the fat (De Angelis, Latrofa, Squeo, et al., 2024; Hesso et al., 2015), a marked increase in G’ was observed at approximately 65 °C in B_C, whereas this increase occurred at approximately 5 °C lower in the doughs containing EFG. The increase in the modulus is generally related to starch gelatinization and to protein unfolding and denaturation (Yovchev & Le-Bail, 2021). The greater fat content of B_C might also explain the slightly delayed increase in the moduli when compared to EFG doughs, because fat may inhibit water absorption, consequently delaying starch gelatinization (Devi, Sindhu, & Khatkar, 2020).

At the same time, a slight increase was also observed in the G″ modulus, although at a lower rate than G′ due to the predominance of elastic-like behavior. The rate of increase in G′ diminished at approximately 90–92 °C but continued to increase until the end of the analysis (98 °C). The final G′ and G″ values were similar for the doughs containing the fat replacer, indicating that the different levels of oil contained in the EFG resulted in only minor modifications to the viscoelastic properties and structural development.

3.3. Brioche characterization

3.3.1. Nutritional composition

Table 2 reports the nutritional composition of the brioches obtained. In general, their chemical composition was in accordance with those reported in other studies and in similar products (Altamirano-Fortoul & Rosell, 2011; Caggia et al., 2020). As expected, the lipid content of B_C (15.8 g/100 g) was significantly higher than that of the other samples. The lipid value dropped to 0.9 g/100 g in samples containing EFG prepared with 5% oil and increased as the percentage of oil in the EFG increased. Overall, “low-fat” brioches were obtained when an EFG was

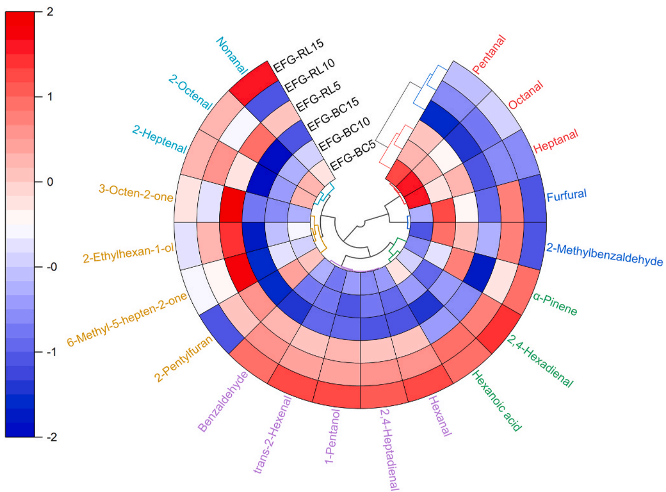

Fig. 2. Polar heatmap with a circular dendrogram derived from a hierarchical cluster analysis of the volatile profiles of the emulsion filled gels (EFG) containing 5, 10, and 15% oil, prepared with black chickpea (BC) and red lentil (RL) dry-fractionated proteins.

used in the formulation; according to Regulation (EC) 1924/2006, a product may be labeled as "low-fat" when it contains no more than 3 g of lipids per 100 g (European Commission, 2006).

All the experimental brioches differed from the control in their protein content: B_C had the highest protein content, probably due to the eggs used in the recipe. In fact, eggs consist of 27% protein, of which 11% is in the white and 16% in the yolk (Hedayati, Jafari, Babajafari, Niakousari, & Mazloomi, 2022). Total dietary fiber remained statistically unchanged among the experimental brioche samples but was higher than in the control. However, only B_{15} differed significantly from B_C (3.20 *vs* 2.65 g/100 g, respectively). These differences could be associated with the use of inulin, a non-digestible dietary fiber used in fat replacer formulation (Colla et al., 2018). According to Krystyjan, Gumul, et al. (2015), an increase in gelled inulin content, used as a fat replacer in biscuits, determined an increase in total dietary fiber (60–87%). Moreover, oat milk, which contains fiber (Yu et al., 2023), was used as a replacement for cow's milk and may have determined this difference.

Finally, the amount of energy derived from the consumption of 100 g of brioche was evaluated by considering the fat, protein, carbohydrate, and fiber contents. The use of EFG to replace fat, which is the component with the highest energy value, was responsible for reducing the total calorie content of the brioches. In fact, all the experimental brioches differed significantly from B_C, which had the highest energy content (365.25 Kcal/100 g); in fact, B_5 had the lowest energy content (280.57 Kcal/100 g), while B_{10} and B_{15} had higher contents (288.96 and 296.12 Kcal/100 g, respectively).

3.3.2. Micro-CT 3-D analysis and textural profile analysis (TPA)

Table 3 reports the results of 3-D analysis obtained after micro-CT data processing. The object volume (OV) indicates the volume occupied by the solid fraction of the brioche within the total volume of interest volume (TVV) investigated, which comprises the pore spaces; it can be observed that B_C has the smallest OV value, while B_5 has the highest. In fact, total porosity ($P_{(tot)}$), which is conversely greatest for B_C

Table 2
Nutritional composition of brioches using emulsion filled gels containing 5%, 10% and 15% oil (B_5, B_{10}, and B_{15}, respectively) and of the control brioche (B_C).

Parameters	B_5	B_{10}	B_{15}	B_C
Energy Value (Kcal/100 g)	280.57 ± 0.56b*	288.96 ± 5.56 ab*	296.12 ± 6.23a*	365.25 ± 5.24
Lipids (g/100 g)	0.96 ± 0.02c*	1.51 ± 0.03b*	2.13 ± 0.18a*	15.82 ± 0.22
Proteins (g/100 g)	7.82 ± 0.02a*	8.25 ± 0.41a*	8.16 ± 0.06a*	9.45 ± 0.05
Ash (g/100 g)	0.89 ± 0.02a	0.64 ± 0.02b*	0.80 ± 0.16 ab	0.97 ± 0.01
Carbohydrates (g/100 g)	58.68 ± 0.11a*	59.14 ± 1.09a*	59.48 ± 1.54a*	45.80 ± 1.13
Total dietary fibre (g/100 g)	3.00 ± 0.20a	2.90 ± 0.10a	3.20 ± 0.20a*	2.65 ± 0.15

In row, different letters indicate statistically significant differences between experimental brioches at $P < 0.05$ according to one-way ANOVA followed by Tukey's test, while * shows statistically significant differences to the control according to Dunnett's multiple comparison test at $P < 0.05$. Data are represented as means ± standard deviation.

Table 3
Micro-CT 3D analysis of brioches using emulsion filled gels containing 5%, 10% and 15% oil (B_5, B_{10}, and B_{15}, respectively) and of the control brioche (B_C).

	B_5	B_{10}	B_{15}	B_C
TVV (mm^3)	912.4	941.2	862.4	949.3
OV (mm^3)	431.5	355.3	324.2	283.8
OV (%)	47.3	37.8	37.6	29.9
TVS (mm^2)	656.0	624.6	606.3	633.0
OS (mm^2)	7999.7	7595.4	6756.2	5619.0
OS/OV (mm^{-1})	18.5	21.4	20.8	19.8
StTh (mm)	0.20 ± 0.09	0.16 ± 0.07	0.17 ± 0.08	0.19 ± 0.09
StSp (mm)	0.33 ± 0.23	0.43 ± 0.25	0.41 ± 0.22	0.64 ± 0.36
OSD (mm^{-1})	8.77	8.07	7.83	5.92
$PN_{(cl)}$	5390	1701	1374	772
$PV_{(cl)}$ (mm^3)	9.50	2.84	2.48	1.62
$PS_{(cl)}$ (mm^2)	400.66	123.07	102.58	63.90
$P_{(cl)}$ (%)	2.15	0.79	0.76	0.57
$PV_{(op)}$ (mm^3)	471.41	583.03	535.69	663.88
$P_{(op)}$ (%)	51.66	61.95	62.12	69.93
$P_{(tot)}$ (%)	52.71	62.25	62.40	70.10

Abbreviations: TVV: Total Volume of interest; OV: Object Volume; TVS: Total Volume of interest Surface; OS: Object Surface; OS/OV: Object Surface/Volume Ratio; StTh: Structure Thickness; StSp: Structure Separation; OSD: Object Surface Density; $PN_{(cl)}$: Number of Closed Pores; $PV_{(cl)}$: Volume of Closed Pore Space; $PS_{(cl)}$: Surface of Closed Pores; $P_{(cl)}$: Closed Porosity (percent); $PV_{(op)}$: Volume of Open Pore Space; $P_{(op)}$: Open Porosity (percent); $PV_{(tot)}$: total Volume of Pore Space; P(tot): total Porosity (percent).

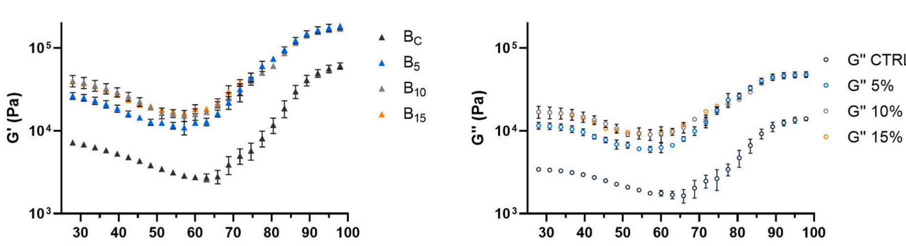

Fig. 3. Storage modulus (G′) and loss modulus (G″) development of the control brioche dough (B_C) and of the brioche doughs using emulsion filled gels (EFG) containing 5%, 10% and 15% oil (B_5, B_{10}, B_{15}, respectively) as a function of the temperature (°C).

(70.1 %), appears to be reduced when using the fat replacer at the lowest EFG oil concentration (52.7 %), and rises to higher values (62 %) as the oil concentration in EFG increases to 10 and 15%. In a similar way, Oh and Lee (2018) reported the lowest total porosity of a muffin when shortening was completely replaced with hydroxypropyl methylcellulose oleogels, while porosity was similar to the control at replacement levels of up to 50 %. In addition, Lim et al. (2017) reported that the total porosity of muffins in which shortening was replaced with oleogels (grape seed oil and candelilla wax) decreased at the highest level of shortening substitution. In fact, the fat improves the incorporation of air bubbles and the development of a porous structure with an increase in volume (Garvey, O'Sullivan, Kerry, & Kilcawley, 2020a). It cannot be excluded that the reduced total porosity found in samples B_5, B_{10} and B_{15} might also be due to the absence of egg, and not only to the replacement of sunflower oil with EFG. During heating, the network of air bubbles created by the egg white proteins coagulates and forms a porous aerated structure that is appreciated in baked products like brioches (Arunepanlop, Morr, Karleskind, & Laye, 1996; Garvey et al., 2020a; Hedayati et al., 2022).

Moreover, besides the $P_{(tot)}$ values, further insights can be obtained from 3-D analysis of pore spaces. The number of closed pores ($PN_{(cl)}$) in B_5 is considerably higher than in the other samples, suggesting the formation of a great quantity of small air bubbles, which in turn results in a higher percentage of closed porosity ($P_{(cl)}$). In B_{10} and B_{15}, however, $PN_{(cl)}$ and $P_{(cl)}$ are much lower and more similar to B_C, suggesting that the increase in oil content to 10 and 15% in the fat replacer favored the formation of a more connected and aerated pore network inside the brioches (Fig. 4 and Supplementary Video 1). This trend was in accordance with Lim et al. (2017), where increased rates of fat substitution with oleogel increased the number of closed pores. Moreover, the OV/OS (object volume to object surface ratio) values do not differ significantly because the increase in OS corresponds to an increase in OV.

With regard to the structure separation parameter (StSp), which describes the average distance separating object structures (i.e., the solid boundaries of a bubble in the brioches), the minimum value for B_5 testifies the abundant presence of closed micro-pores, as described above. In this regard, a lower StSp value would suggest a more compact structure, while higher values indicate larger and connected air bubbles. On the contrary, despite the increase in OV or the reduction in total porosity, structure thickness (StTh) remains unchanged. This is justified by the increase in the number of closed pores in the experimental brioches.

In summary, the micro-CT results indicate that the use of EFG to replace fat affected the structure of the brioches by reducing total porosity (thus aeration) and pore connectivity. However, while such parameters changed almost drastically in B_5 compared with B_C, these differences are much more limited in B_{10} and B_{15}, for which 3-D analysis gave very similar structural parameters. Despite this, visual comparison of B_C, B_5, B_{10} and B_{15} by both 2-D (Fig. 4) and 3-D renderings (Supplementary Video 1) shows that B_{15} is the most similar to B_C in terms of structure. Table 4 reports the specific volume results, and although there is not statistically significant difference between the samples, a trend can be detected: B_5 had the lowest specific volume when compared to the other samples and resulted in less aerated products. This trend is supported by the decrease in $P_{(tot)}$ as previously described.

Texture parameter evaluation highlighted that all the experimental brioches were significantly different from the control brioche in terms of hardness, chewiness, and cohesiveness. B_5, B_{10} and B_{15} had higher hardness values than B_C, in accordance with the total porosity results. Similarly, other researchers have shown that the use of different inulin preparations as fat replacers increased the hardness of shortbread cookies, biscuits, sponge cakes, cakes, or muffins (Majzoobi et al., 2018; Paciulli et al., 2020; Rodríguez-García, Puig, Salvador, & Hernando, 2012; Rodríguez-García, Laguna, Puig, Salvador, & Hernando, 2013; Zahn et al., 2010). B_{15} was significantly less hard than B_5 and B_{10}, which

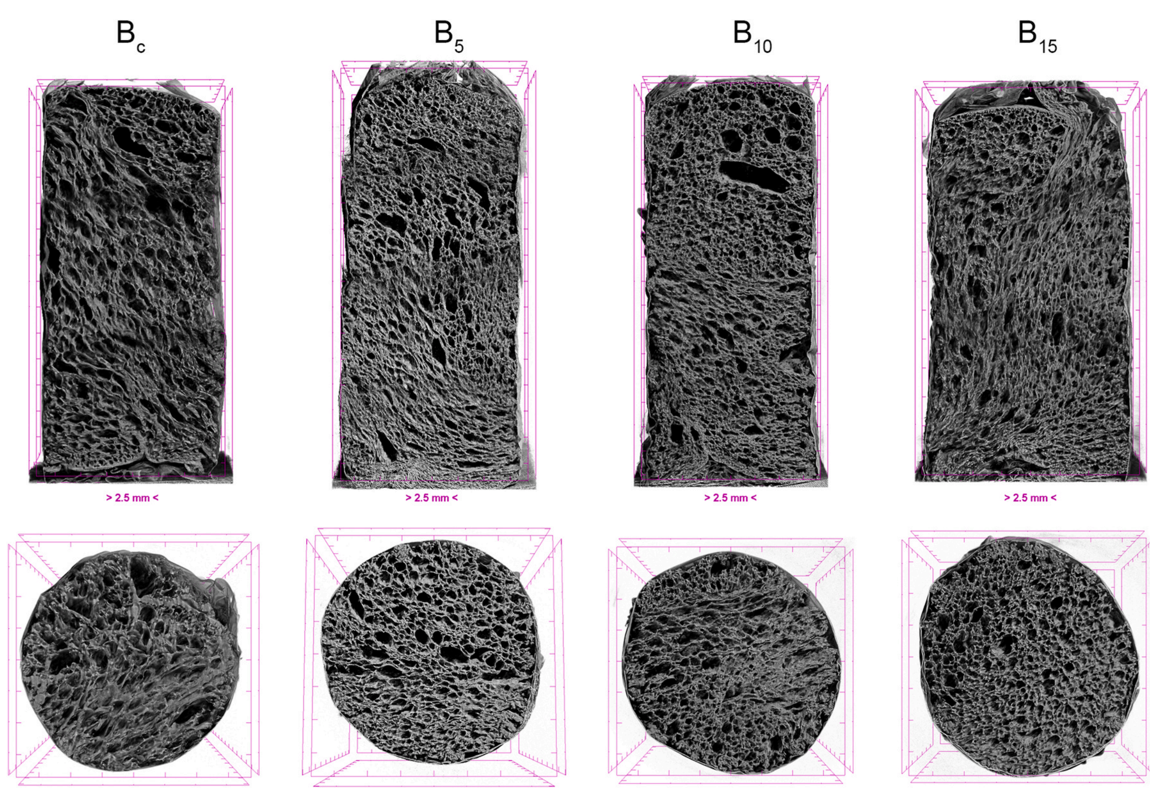

Fig. 4. For the control brioche (B_C) and brioches using emulsion filled gels containing 5%, 10% and 15% oil (B_5, B_{10} and B_{15}, respectively), a coronal and a transversal section after the 3-D rendering of micro-CT analysis is shown. The >2.5 mm < indication refer to the scale marks of the clipping box of the 3-D renderings.

Table 4
Specific volume and texture analysis of brioches using emulsion filled gels containing 5%, 10% and 15% oil (B_5, B_{10}, and B_{15}, respectively) and brioche control (B_C).

	B_5	B_{10}	B_{15}	B_C
Specific Volume (ml/g)	1.78 ± 0.2a	1.92 ± 0.1a	2.18 ± 0.2a	2.08 ± 0.3
Hardness (N)	18.73 ± 0.60a*	19.00 ± 0.14a*	16.24 ± 0.44b*	3.92 ± 0.54
Springiness (−)	0.72 ± 0.02a	0.70 ± 0.04a	0.70 ± 0.03a	0.77 ± 0.06
Chewiness (N)	5.01 ± 0.11a*	4.82 ± 0.26 ab*	4.29 ± 0.03b*	1.53 ± 0.43
Cohesiveness (−)	0.39 ± 0.01a*	0.36 ± 0.04a*	0.39 ± 0.03a*	0.51 ± 0.04

In row, different letters indicate statistically significant differences between experimental brioches at P < 0.05 according to one-way ANOVA followed by Tukey's test, while * shows a statistically significant difference to the control according to Dunnett's multiple comparison test at P < 0.05. Data are represented as means ± standard deviation.

might be related to the slightly higher oil content of the sample. Rodríguez-García et al. (2013) highlighted the greater accessibility of water to flour components when fat was replaced with inulin in biscuits; this leads to a harder structure since the flour particles are less surrounded by fats. In fact, B_C had the lowest percentage of moisture (25.31 ± 0.88), followed by B_{15} (26.23 ± 1.70), B_{10} (27.56 ± 1.35) and B_5 (28.66 ± 0.28).

In our study, the use of EFG as a fat replacer led not only to a significant increase in hardness but also in chewiness, which is in accordance with the closed porosity results. The increase in chewiness was like that reported by other authors (Rodríguez-García et al., 2012), in which the chewiness of a sponge cake increased significantly with full fat replacement by inulin. The chewiness values of the experimental brioches decreased as higher levels of oil were used in the EFG.

No differences in terms of cohesiveness were found among B_5, B_{10} and B_{15}, which had lower values than B_C. Therefore, brioches produced with the fat replacers were more consistent and more resistant to deformation than the control and required more energy to make them ready to swallow (Marcet, Collado, Paredes, & Díaz, 2016). This trend also corroborates the results of temperature sweep analysis of the doughs (Fig. 3), confirming that EFG addition resulted in brioches with higher level of elastic-like behavior. No significant differences were found regarding springiness.

3.3.3. *Volatile profile*

Table 5 reports the mean concentration of VOC as measured in B_C, B_5, B_{10} and B_{15}, and their odor description. A total of twenty-one VOC were identified and quantified, including alcohols, aldehydes, furans, ketones, alkane, ester, carboxylic acid, which are widely identified in baked products (Garvey et al., 2020a, 2020b; Garvey et al., 2023; Garvey et al., 2021; Krause et al., 2022). In order to distinguish differences in the volatile profiles of brioche samples, a PCA was undertaken with the strongest associations shown in Fig. 5. The first two components of the PCA explained ~76% of the total variance among the samples. The first principal component (PC1) accounted for 54.64% and clearly separated B_C from B_5 and B_{10}, which were in the positive area of this axis, particularly characterized by aldehydes and furans. On the contrary, B_C was in the negative PC1 axis due to the higher concentration of alcohols. However, it should be noted that B_{15} was almost at the center of the biplot, due to its greater similarity to the control. Moreover, B_C, B_{15} and B_{10} were located on the negative side of PC2 (21.25% of variance), primarily due to aldehydes, unlike B_5, which was located in the positive axis.

Six alcohols were identified, and ethanol and isoamyl alcohol were the most abundant VOC found in all samples (Table 5). The presence of alcohols (e.g., ethanol, 2-phenylethanol or isoamyl alcohol) mainly

Table 5
Mean concentrations of volatile compounds (μg/g) and their odor descriptions for brioches using emulsion filled gels containing 5%, 10% and 15% oil (B_5, B_{10}, and B_{15}, respectively) and for the control brioche (B_C).

Compounds (μg/g)	Chemical class	Odor description	B_5	B_{10}	B_{15}	B_C
2-Methylbutyraldehyde	Aldehyde	Musty, cocoa, coffee, nutty, malty[1]	1.0 ± 0.1c	2.8 ± 0.3a*	1.6 ± 0.1b	1.3 ± 0.1
3-Methylbutyraldehyde	Aldehyde	Chocolate, ethereal, aldehydic, peach, fatty, malty[1]	3.1 ± 0.2b*	4.3 ± 0.3a	3.3 ± 0.03b*	4.2 ± 0.3
Ethanol	Alcohol	Alcoholic, sweet[3]	129.2 ± 6.2 ab*	120.7 ± 5.9b*	143.5 ± 4.8a	147.8 ± 3.3
Hexanal	Aldehyde	Floral, fruity, herbal, cut grass, green[1]	32.4 ± 0.3c	42.1 ± 0.3a*	36.0 ± 1.1b	35.4 ± 2.1
Isobutanol	Alcohol	–	20.8 ± 0.3a	18.1 ± 1.7a*	17.9 ± 1.5a*	21.5 ± 0.6
Dodecane	Alkane	Musty, damp, earthy[2]	2.4 ± 0.3a	2.4 ± 0.3a	2.1 ± 0.1a	2.2 ± 0.2
Heptanal	Aldehyde	Fresh, green, sweet, herbal[1]	0.27 ± 0.04a*	0.26 ± 0.03a*	0.24 ± 0.02a*	–
***trans*-2-Hexenal**	Aldehyde	–	2.3 ± 0.1b*	3.2 ± 0.2a*	2.2 ± 0.2b*	1.5 ± 0.1
2-Pentylfuran	Furan	Butter, floral, fruit, green bean, mushroom, raw nuts[5]	1.1 ± 0.1b*	1.3 ± 0.02a*	1.3 ± 0.05 ab*	0.4 ± 0.01
Isoamyl alcohol	Alcohol	–	141.8 ± 7.2a*	93.1 ± 0.3b*	89.0 ± 4.2b*	201.7 ± 2.6
Acetoin	Ketone	Sweet, butter, creamy, dairy product, caramel, fatty[4]	20.7 ± 1.6 ab	17.9 ± 1.5b*	24.1 ± 1.1a	21.3 ± 1.7
6-Methyl-5-hepten-2-one	Ketone	–	0.9 ± 0.1a*	0.9 ± 0.1a*	0.8 ± 0.1a	0.6 ± 0.1
Nonanal	Aldehyde	Aldehydic, waxy, citrus, orange, green, peel[1]	3.7 ± 0.4a*	3.8 ± 0.3a*	2.9 ± 0.3b*	1.3 ± 0.1
Ethyl octanoate	Esters	Fruity, wine, waxy, sweet, apricot, banana, brandy[1]	1.7 ± 0.1b*	2.8 ± 0.3a*	2.0 ± 0.04b*	1.2 ± 0.1
1-Hexanol	Alcohol	Ethereal, oil, alcohol, green, grass, fruity sweet, woody, floral[4]	16.2 ± 1.0a*	17.1 ± 1.7a*	16.2 ± 0.1a*	11.7 ± 1.0
Furfural	Furan	Sweet, woody, almond, fragrant, bready[1]	1.5 ± 0.1a	1.4 ± 0.1a	1.2 ± 0.1a	1.4 ± 0.1
Benzaldehyde	Aldehyde	Sweet, bitter, almond, cherry[1]	11.2 ± 0.6a*	11.9 ± 0.8a*	9.3 ± 0.5b*	7.0 ± 0.4
2-Ethylhexan-1-ol	Alcohol	Citrus, fresh, floral, oily[1]	9.1 ± 0.7a*	4.9 ± 0.4b*	3.4 ± 0.3c	2.7 ± 0.3
4-Ethylbenzaldehyde	Aldehyde	–	0.8 ± 0.05c	1.5 ± 0.02a*	0.9 ± 0.01b*	0.8 ± 0.05
Hexanoic acid	Carboxylic acid	Sour, fatty, sweat, cheesy, goat-like[4]	5.5 ± 0.3a*	3.1 ± 0.2b*	2.2 ± 0.1c	2.3 ± 0.1
2-Phenylethanol	Alcohol	Bready, flowery, sweet[3]	6.3 ± 0.2a*	6.0 ± 0.7a*	6.2 ± 0.4a*	9.6 ± 0.3

In row, different letters indicate statistically significant differences between experimental brioches at P < 0.05 according to one-way ANOVA followed by Tukey's test, while * shows a statistically significant difference to the control according to one-way ANOVA with Dunnett's test at P < 0.05. Data are represented as means ± standard deviation. Odor description references: [1]Garvey et al. (2020a); [2]Garvey et al. (2021);[3]Heitmann et al. (2017); [4]Galoburda, Straumite, Sabovics, & Kruma (2020); [5]Cepeda-Vázquez et al. (2018).

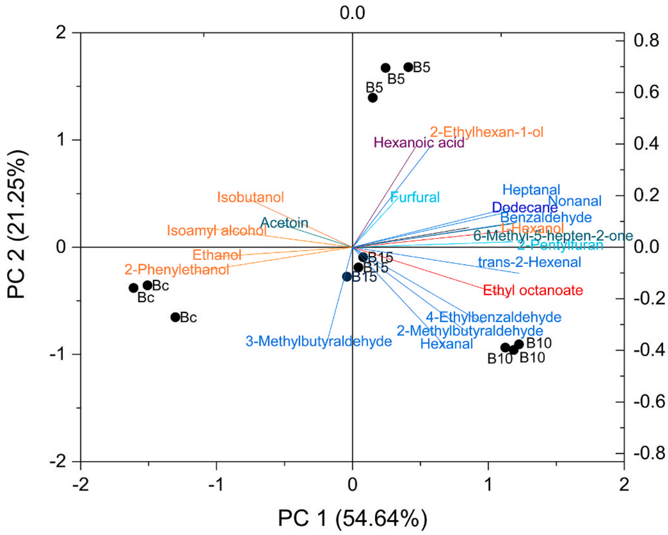

Fig. 5. Biplot resulting from the principal component analysis of the VOC divided into alcohols ●, aldehydes ●, furans ●, ketones ●, alkane ●, ester ●, and carboxylic acid ●, and associated with brioches using emulsion filled gels containing 5%, 10% and 15% oil (B_5, B_{10}, and B_{15}, respectively) and the control brioche (B_C).

derives from the microbiological activities occurring during brioche preparation processes and from the fermentative action of yeast (Birch, Petersen, & Hansen, 2014; Heitmann, Zannini, Axel, & Arendt, 2017; Pasqualone et al., 2014), such as *Saccharomyces cerevisae,* the yeast species most widely used in bakery products (Xi et al., 2021) and was also used in our products. Moreover, other alcohols (such as 1-hexanol or 2-ethylhexan-1-ol) can also derive from lipid oxidation occurring in bakery products during ingredient preparation in the presence of oxygen, or during baking or storage (Garvey et al., 2020a). The slight differences in alcohol concentrations may be attributable to differences in microbial activity in the four brioche doughs. No statistically significant difference in ethanol concentration was found between B_{15} and B_C. However, B_{10} contained the lowest amount of this compound among the experimental brioches.

The concentration of isoamyl alcohol was higher in the control brioche than in the experimental brioches, of which B_5 had the highest concentration. With regard to 2-phenylethanol, a volatile compound characterized by a flowery aroma, B_C had a higher concentration of this compound (9.6 μg/g) than the experimental brioches (6.2 μg/g on average). We identified 1-hexanol (oil, alcohol, green) and 2-ethylhexan-1-ol (citrus, fresh and floral aroma) in all brioche samples. The highest concentration of 1-hexanol was found in the experimental brioches, presenting significant differences from the control. Of the experimental brioches, B_5 contained the highest concentration of 2-ethylhexan-1-ol, followed by B_{10} and B_{15}. The concentration of 2-ethylhexan-1-ol was higher in the experimental brioches than in the control, probably because this compound was contained in the EFG (Table S1). However, this increase was significantly different only for B_5 and B_{10}.

Eight aldehydes were identified in all the brioche samples: 2-methylbutyraldehyde, 3-methylbutyraldehyde hexanal, heptanal, *trans*-2-hexenal, nonanal, benzaldehyde, and 4-ethylbenzaldehyde. The results suggest that the concentration of nonanal, heptanal, *trans*-2-hexenal and benzaldehyde was higher in the experimental brioches than in the control. These aldehydes are commonly contained in bakery products (Garvey et al., 2020a); these compounds may be transferred from the EFG (Table S1) to the experimental brioches and are linked to a green-grassy or sweet odor, as in the case of benzaldehyde (Table 5).

Two furan compounds were identified in all samples: 2-pentylfuran and furfural. The former is associated with a buttery, floral, fruity, green bean odor (Cepeda-Vázquez, Rega, Descharles, & Camel, 2018) and was present in the experimental brioches at levels three times higher than in the control. Slight differences in 2-pentylfuran, although statistically significant, were found among the experimental brioches: B_{10} had the highest concentration, followed by B_{15} and B_5; 2-pentylfuran was identified in all EFG of BC (Table S1). It is considered as one of the beany flavor markers in chickpea, lentil, and yellow pea flour (Xu et al., 2019). The presence of 2-pentylfuran in B_C was correlated to the oxidation of wheat flour lipid (Birch, Petersen, Arneborg, & Hansen, 2013). On the contrary, there was no significant change in the concentration of furfural, which can be generated through caramelization or Maillard reaction during cooking (Cepeda-Vázquez et al., 2018; Srivastava et al., 2018).

Acetoin and 6-Methyl-5-hepten-2-one were the only two ketones identified in all samples. The concentration of acetoin, produced after the addition of baker's yeast (Raimondi et al., 2017), remained almost unchanged. However, the acetoin concentration of B_{10} was lower than for B_5, B_{15} and the control. On the other hand, all experimental brioches had a higher concentration of 6-Methyl-5-hepten-2-one, which was probably released by the EFG. However, no significant difference in concentration was found between B_{15} and B_C.

3.3.4. Sensory analysis

The mean scores for sensorial attributes of brioches were plotted in a spider plot (Fig. 6). Significant differences ($P < 0.05$) between control and experimental brioches were found in nine of the 13 attributes across the samples. The color of both crust and crumb differed among the samples; specifically, B_5, B_{10} and B_{15} had a lighter crust and crumb color (tending towards white) than B_C. Brioche color was probably affected by colored compounds formed during baking and by the color of the main ingredients (such as egg) used (Kaur, Singh, Kaur, & Singh, 2019; Shevkani, Kaur, Kumar, & Singh, 2015).

B_{15} was the most similar to B_C with regard to the typical brioche odor. The results also showed that the brioches made using EFG scored higher for hardness and dryness than the control brioche, in line with the results obtained by TPA. Moreover, the low crumb porosity of the EFG-based brioches (Table 3, Fig. 4) can also explain the results of the sensory evaluation. Of the brioches prepared with EFG, B_{15} received the highest overall rating from panelists.

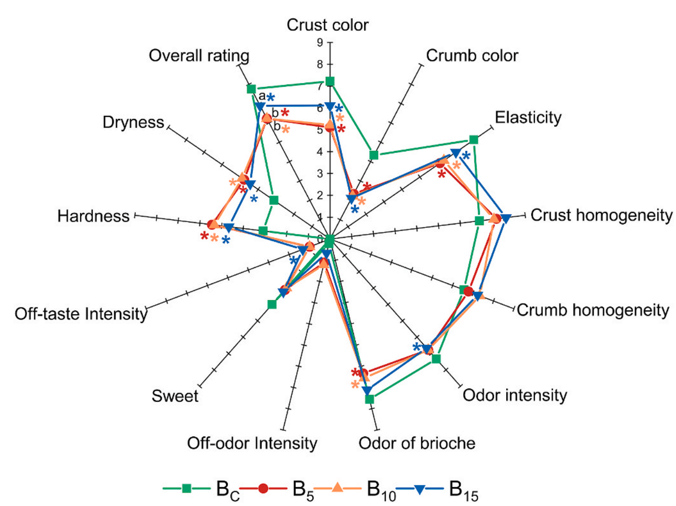

Fig. 6. Sensory profile of the control brioche (B_C) and brioches using emulsion filled gels containing 5%, 10% and 15% oil (B_5, B_{10} and B_{15}, respectively). The different letters indicate statistically significant differences between experimental brioches at $P < 0.05$ according to one-way ANOVA followed by Tukey's test, while * shows a statistically significant difference with the control according to Dunnett's multiple comparison test at $P < 0.05$.

4. Conclusions

The rheological properties and the volatile profile of EFG composed of inulin, dry-fractionated proteins of RL and BC at three different oil percentages (5%, 10% and 15%) were evaluated before their use as fat replacers. The EFG presented shear thinning behavior, and the frequency sweep revealed the predominance of solid-like behavior characterized by elastic deformations. Viscosity and viscoelastic properties were significantly affected by the type of dry-fractionated pulses, oil content and storage, highlighting the presence of significant interactions among the variables. In view of these effects, further studies may consider the validation of these findings in an industrial context, during continuous or discontinuous production cycles. However, the lower concentration of volatile compounds linked to pulse flavor meant that dry-fractionated BC protein was preferred for use in the EFG. When the EFG were used to replace fat in vegan brioches, the lipid content was reduced below 3 g per 100 g of product, allowing the brioches to be labeled as "low-fat" products according to Regulation (EC) No. 1924/2006 (Official Journal of the European Union, 2006). Despite wide structural and sensory differences compared to the control brioche, the effect of EFG in the formulation of vegan brioches depended on the oil content of the EFG. All the vegan brioches were particularly characterized by aldehydes and furans, which were probably released by EFG. From a sensory point of view, B_{15} was the most similar to B_C in terms of crust color, elasticity, brioche odor and overall rating.

Consequently, EFG containing 15% oil has potential for use as a fat replacer in brioche recipes to obtain a low-fat product, although an improvement is required in the formulation, and consumer acceptance of the product will need to be evaluated by a consumer acceptability test. Finally, our study shows an agreement between the results of the temperature sweep of the dough and the structural and micro-CT analysis of the brioches, confirming that a combination of different analytical techniques can be useful in providing a complete evaluation of product properties.

CRediT authorship contribution statement

Graziana Difonzo: Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation, Conceptualization. **Mirella Noviello:** Writing – original draft, Software, Methodology, Investigation. **Davide De Angelis:** Writing – original draft, Software, Methodology, Investigation. **Carlo Porfido:** Writing – original draft, Software, Methodology, Investigation. **Roberto Terzano:** Writing – review & editing, Supervision. **Francesco Caponio:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

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

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.2024.116620.

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