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Reduced-fat biscuits: interplay among structure, nutritional properties and sensory acceptability

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Abbreviation list*:* ANOVA, one-way analysis of variance; AS, available starch; db, dry basis; G*, complex modulus; n, number of replicates; PCA, principal component analysis; PGPR, polyglycerol polyricinoleate; r, correlation coefficient; RDS, rapidly digestible starch; RS, resistant starch; SEM, scanning electron microscopy; SDS, slowly digestible starch; tanδ, damping factor; TS, total starch; W_1, primary aqueous phase of the double emulsion; W_2, external water phase of the double emulsion; W_1/O, primary emulsion of the double emulsion; $W_1/O/W_2$, water-in oil-in water double emulsion.

Abstract

This work aimed at investigating relationships among structure, nutritional properties, and sensory acceptability of reduced-fat biscuits in comparison with a full-fat biscuit (STD). Four reduced-fat formulations were tested: OPT (46.3% fat reduction), an optimized formulation containing polydextrose and resistant starch; RAW and EXTR, obtained by substituting resistant starch with raw and extruded bean powders, respectively; WOW, in which a double emulsion was used instead of shortening. Fracture strength resulted comparable in STD and OPT, but increased for the other samples due to the structuring role of bean protein or the very low amount of fat. Scanning Electron Microscopy images revealed a well-developed protein structure in all samples, except for WOW that presented a more continuous and closed network. EXTR showed the lowest level of rapidly digestible starch and lipid digestibility. RAW showed the highest protein digestibility, significantly different from STD (12.1 ± 2.7 vs 3.7 ± 1.6 g/100 g proteins). Thanks to the holistic approach used, the influence of fat content on dough and biscuit structure as well as on colour was assessed, demonstrating also an effect on nutritional properties and consumers' acceptance.

Keywords*:* Bean powder; extrusion-cooking; double emulsion; in-vitro digestion; microstructure.

1. Introduction

Fat-replaced biscuits show higher hardness and brittleness and lower crumbliness than the full-fat counterparts, due to a higher development of gluten network (Laguna, Primo-Martín, Varela, Salvador, & Sanz, 2014). The addition of resistant starch (RS) counterbalances texture defects by giving crumblier and less hard biscuits (Laguna, Salvador, Sanz, & Fiszman, 2011; Moriano, Cappa, & Alamprese, 2018). Common bean (*Phaseolus vulgaris* L.) powders may be exploited as RS-rich ingredients with improved nutritional functionality. The presence of poorly digestible starch confers legumes a low glycaemic index compared to cereal grains, providing benefits to consumers suffering for diabetes or cardiovascular diseases (Hoover & Zhou, 2003). Bean powder is generally produced via soaking, blanching and steam-cooking, thus strongly reducing flatulence-causing oligosaccharides and anti-nutritional factors. Extrusion-cooking has been proposed as an alternative process, being versatile, energy-efficient and time-saving (El-Hady & Habiba, 2003). Moreover, extruded bean powders show higher stability to oxidation, leading to food products more acceptable by consumers (Szczygiel, Harte, Strasburg, & Cho, 2017). However, very few studies investigated the use in biscuits of bean powder, both raw (Sparvoli et al., 2016) and extruded (Ai, Jin, Kelly, & Ng, 2017; Siddiq, Kelkar, Harte, Dolan, & Nyombaire, 2013).

An interesting approach for reduced-fat food development is the application of double emulsions water-in-oil-in-water ($W_1/O/W_2$). They consist of an inner water phase (W_1) entrapped as small droplets in oil droplets (O) that are, in their turn, dispersed in another aqueous phase (W_2). Their main advantage lies in the combination of a structure typical of oil-in-water emulsions, but with a reduced fat content. The main issue is obtaining

$W_1/O/W_2$ emulsions able to mimic fat behaviour and with a prolonged stability. Gelling of W_1 may be a useful strategy (Perez-Moral, Watt, & Wilde, 2014), together with the use of a strong lipophilic emulsifier as polyglycerol polyricinoleate (PGPR) (Muschiolik & Dickinson, 2017). To the best of our knowledge, the use of double emulsions as fat replacers in bakery products has not been studied so far.

The aim of this work was to investigate the relationships among structure, nutritional properties, and sensory acceptability of full- and reduced-fat biscuits, making use of carbohydrate fat mimetics, raw and extruded bean flours, or double emulsion in order to modify biscuits properties.

2. Materials and methods

2.1. Biscuit ingredients

Deposited soft-dough biscuits (Table 1) were produced using soft wheat flour ‘00’ (protein content, 10 g/100 g; Molino Dallagiovanna s.r.l., Gragnano Trebbiense, PC, Italy), resistant starch Hi-Maize^{TM} 260 (Ingredion UK Ltd., Manchester, UK; dietary fibre, 56 g/100 g), all-vegetable shortening (Crisco, The J.M. Smucker Co., Orrville, OH, USA), sucrose (Eridania Italia S.p.A, Bologna, Italy), polydextrose (Comprital S.p.A., Settala, MI, Italy), baking powder (Paneangeli, Desenzano del Garda, BS, Italy), salt (Italkali SpA, Palermo, Italy), and deionized water.

Double emulsion contained egg white powder (Lactosan-Sanovo Ingredients Group, Zeven/Aspe, Germany), NaCl (Sigma-Aldrich, Saint Louis, MO, USA), corn oil (Carrefour, Boulogne-Billancourt, France), and PGPR (kindly provided by Lasenor, Barcelona, Spain).

2.2. Double emulsion preparation

A gelled double emulsion was prepared following procedure by Perez-Moral et al. (2014) modified as follows. The inner aqueous phase (W_1) was prepared by rehydrating egg white powder (10 g/100 mL) in NaCl solution (0.4 g/100 mL) and stirring at room temperature for 1 h. The primary emulsion (W_1/O) was obtained by adding 29 mL W_1 to 71 mL corn oil containing 4 g/100 mL PGPR. The phases were mixed using a heavy-duty blender (Waring Laboratory, Torrington, CT, USA) at 18000 rpm for 30 s and at 20000 rpm for further 30 s. W_1/O was then heated at 80 °C for 20 min in a water bath (MR Hei-Standard, Heidolph Intruments GmbH, Schwabach, Germany) in order to induce gelation, and cooled down in iced water for 15 min. The external water phase (W_2) was prepared by dissolving 0.4 g/100 mL NaCl and 5 g/100 mL egg white powder in distilled water, and stirring for 1 h at room temperature. The double emulsion was finally prepared by adding 60 mL W_1/O to 40 mL W_2 and mixing as previously described for W_1/O, thus resulting in 42.6 mL oil in 100 mL emulsion.

2.3. Biscuit preparation

A full-fat soft-dough biscuit was used as reference (STD, Table 1). A previously optimized recipe (OPT; 46.3% fat reduction in the dough) (Moriano et al., 2018) containing polydextrose and RS was used as reduced-fat reference. In order to create different structures, OPT formulation was modified as follows. In RAW and EXTR recipes RS was substituted with raw and extruded bean powder (cv. Fuji Otebo), respectively. Bean powders were analysed as reported by Ai, Cichy, Harte, Kelly, and Ng (2016), obtaining the following results: proteins 22.06 ± 0.02 g/100 g dry basis (db); RS 27.8 ± 0.4 and 13.8 ± 0.7 g/100 g db, respectively for the raw and extruded sample.

WOW was formulated by replacing shortening with a double emulsion prepared as described in § 2.2. All biscuit samples were produced as reported by Moriano et al. (2018). Briefly, fat and sugar were creamed in a kneading machine (N-50G, Hobart GmbH, Offenburg, Germany) before the addition of the other powder ingredients and, at last, of water. Immediately after mixing, a fixed weight (15 ± 0.1 g) of dough was dropped in aluminium cups (bottom diameter, 50 mm) and baked for 25 min in a static oven (G255L MF8 16A, Whirlpool S.r.l., Comerio, VA, Italy) preheated to 190 °C. After cooling, the biscuits were stored in hermetic plastic boxes and analysed 24 h after production.

2.4. Biscuit structural characterization

Biscuit doughs were analysed for density and rheology behaviour as detailed in Moriano et al. (2018). Briefly, dough density was measured weighting a known volume (45 mL) of sample (n=3). Strain sweep (strain range, 0.001-10%; frequency, 1 Hz) and frequency sweep (frequency range, 1-0.01 Hz; strain, 0.01%) tests were carried out at 25 °C (n≥2), by using a Physica MCR 300 rheometer (Anton Paar, Graz, Austria) equipped with serrated parallel plates (PP25/P; 2.5 cm diameter).

Biscuits were characterized for moisture, ash, protein, fat, and fibre content, according to official standard methods (AACC 44-15A, 2000; AACC 08-01.01, 1995; AOAC 920.87, 1995; ICC 136, 1984; AOAC 991.43, 1995). Sugars (mono and disaccharides) were evaluated by high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as detailed by Englyst et al. (1999, 2000). Results (g/100 g) represent the average of two replicates. Total starch (TS) was calculated as the 100’s complement of the sum of all the measured components and the theoretical amount of polydextrose. Quality characteristics were assessed as reported in Moriano et al. (2018). Top surface colour was measured (n=3) using a colorimeter Chroma Meter II (Konica-Minolta, Tokyo, Japan), with standard illuminant C. Milk absorption was evaluated (n=5) dipping one biscuit at a time for 10 s in 100.0 g UHT whole milk (Sterilgarda Alimenti, Castiglione delle Stiviere, MN, Italy); after 2 s drainage, the absorbed amount (g/100 g biscuits) was calculated as the difference from the initial and the final weight of milk. Texture was determined by a three-point bending test (n≥10), performed with a TA-HD Plus texture analyser (Stable Micro System, Surrey, UK) equipped with a 500 N load cell and the appropriate device (HDP/3PBeThree Point Bend) at a 30 mm span length; 10 mm/s speed was applied; results were normalized on biscuit dimensions. Thickness, bottom porosity, and surface heterogeneity were determined through image analysis techniques: for each sample, top, bottom, and section images were taken (n=2) at 600 dpi and 24 bit with a flatbed scanner (HP Scanjet 8300, HP Inc., Palo Alto, CA, USA) controlled by the software VueScan (v. 9.5.51, Hamrick Software, Miami, FL, USA). The images were processed using the software Image Pro–Plus (v. 4.5.1.29/XP, Media Cybernetics Inc., Rockville, MD, USA) in order to measure diameter, thickness, heterogeneity (defined as the fraction of pixels whose intensity value deviates more than 10% compared to the average intensity of the entire image), and bottom porosity. The latter was calculated on an area of interest (diameter = 48.3 mm) selected in the central part of each sample image and converted to grey scale (8 bit); pores were automatically identified by adjusting brightness and contrast, and applying a high-pass filter; bottom porosity was expressed as the total area of pores with respect to the area of interest (%).

2.5. Microstructural analysis

Samples were dried at the critical point and coated with gold particles in an automated critical point drier (model SCD 050, Leica, Vienna, Austria). Microstructure was examined by means of scanning electron microscopy (SEM), using a LEO EVO 40 microscope (Zeiss, Oberkochen, Germany) with 20 kV acceleration voltage, and a level of magnification: 1000X, secondary electron mode (Romano, Masi, Aversano, Carucci, Palomba, & Carputo, 2018).

2.6. In vitro digestibility

To assess *in vitro* carbohydrate digestibility, rapidly (RDS) and slowly (SDS) digestible starch fractions were measured as reported by Marti et al. (2017) and expressed as relative percentage (%). Procedure by the same Authors were used to quantify RS, expressed as a percentage of total starch (g/100 g TS). For each sample, 6 analytical replicates were performed.

For *in vitro* protein and lipid digestibility, pepsin from porcine gastric mucosa (P6887, Sigma-Aldrich, Saint Louis, MO, USA) and pancreatin from porcine pancreas cell (P3292, Sigma-Aldrich, Saint Louis, MO, USA) were used. *In vitro* protein digestibility was measured in quadruplicate, following the two-step procedure by Zhou et al. (2017). Undigested peptides (molecular weight > 3000 Da) were evaluated after precipitation with trichloroacetic acid (Sigma-Aldrich, Saint Louis, MO, USA; 10 g/100 mL final concentration), resuspension in NaOH (Sigma-Aldrich, Saint Louis, MO, USA; 2 mol/L) and quantification by Lowry assay (Lowry, Rosebrough, Farr, & Randall, 1951). Protein digestibility was expressed as the amount of digested proteins on the amount of total protein present in the sample at the beginning of each step (g/100 g proteins).

Bovine milk casein (C5890, Sigma-Aldrich, Saint Louis, MO, USA) was digested following the described procedure as a reference protein.

In vitro lipid digestibility was assessed in triplicate as reported by Capuano, Pellegrini, Ntone, and Nikiforidis (2018), but using a pH of 7.7 and a reaction time of 20 min. Lipid digestibility was calculated as the quantity of released fatty acids (expressed in oleic acid equivalents) over the total lipids (g/100 g lipids), taking into account the corrections calculated by analysing samples both without enzyme addition and defatted. Extra virgin olive oil (Gaia, S. Rocco al Porto, LO, Italy) was digested following the described procedure as a reference fat.

2.7. Sensory consumers' test

Eighty-six subjects (57% males, 43% females, 18-69 years, mean age 24) participated in the tests conducted in individual booths under white light, avoiding social interaction. Participants evaluated a set of five biscuits presented in blind condition and in random order across subjects. For each sample, consumers rated their liking for appearance, aroma, taste, flavour, texture, and overall liking on a nine-point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like) (Torri, Piochi, Marchiani, Zeppa, Dinnella, & Monteleone, 2016). Consumers were required to rinse their mouth with still water during a 60 s rest interval between samples.

2.8. Data analysis

One-way analysis of variance (ANOVA) was applied to experimental data in order to compare the samples. Sensory data were independently subjected to a two-way mixed ANOVA model (fixed factor: sample; random factor: subject). The Tukey's HSD posthoc test was used to evaluate significant differences ($p < 0.05$) among averages (Statgraphics Centurion 18, Statgraphics Technologies Inc., Warrenton, VA, USA). Principal Component Analysis (PCA) was applied to the results obtained for all the measured variables and a Pearson correlation matrix of all the responses was calculated (Matlab R2018a, MathWorks Inc., Natick, MA, USA).

3. Results and discussion

3.1. Dough rheological properties

Strain sweep curves (Fig. 1A) showed that fat reduction in biscuit doughs prolonged the linear viscoelastic range, in which rheological properties are not deformation dependent (Steffe, 1996), and markedly decreased dough stiffness. This is related to a lower air incorporation rate during creaming. Actually, STD dough density resulted significantly ($p < 0.05$) lower (0.90 ± 0.01 g/mL) than in the other samples (1.1 ± 0.1 g/mL on average), indicating a higher amount of entrapped air. Similarly, dough viscoelasticity measured by frequency sweep tests was strongly affected by formulation changes since all the reduced-fat doughs exhibited lower complex modulus (G*) values with respect to STD (Fig. 1B). The total substitution of shortening with the double emulsion produced the lowest values of G*, while EXTR showed the highest values, suggesting a dough stiffening effect of extruded bean flour similar to that of RS added in OPT. The damping factor (tanδ; ratio between viscous and elastic modulus) resulted lower than 1 (0.31-0.67) throughout the entire frequency range for all the samples (Fig. 1C), according to a solid-like behaviour. The damping factor of WOW showed a different trend with respect to the other samples, being at the lowest values for low frequencies, but tending to increase more with frequency. This behaviour reveals the presence of a tight matrix in a quiescent status, which breaks at higher frequencies due to a weak structuration.

3.2. Biscuit composition

Despite the balance of dough formulations in order to ensure a constant moisture, reduced-fat biscuits showed significantly higher moisture levels (Table 2) due to the presence of ingredients with high water retention capacity, such as polydextrose, resistant starch, and bean powders (Laguna et al., 2011; Zoulias, Oreoupoulou, & Tzia, 2002). Consistently with the recipes, fat content was the highest for STD and reduced of about 44% in OPT, RAW and EXTR; WOW showed the lowest fat content (about 53% reduction), since the double emulsion contained only 42.6 mL/100 mL oil. Protein content was significantly higher for RAW, EXTR, and WOW, containing bean powders and dried egg albumen, and at the lowest level for OPT, according to the partial flour substitution with RS. Samples showed similar values of TS while the highest RS content was obtained for OPT and WOW, consistent with the RS-rich ingredient addition in formulations. Bean flour promoted only a limited increase in RS content, as expected by their composition. Fibre content also was consistent with the ingredients used: STD showed the lowest level, a limited but significant increase was observed in RAW and EXTR due to the bean flour contribution, while OPT and WOW had the significantly highest values, partly related to the presence of the RS-rich ingredient.

3.3. Biscuit quality properties

Biscuit colour (Table 3) was strongly affected by the presence of polydextrose and protein-rich ingredients accounting for a higher development of Maillard reactions (Mieszkowska & Marzec, 2016) and causing a decrease in brightness (L*) and an increase in redness (a*).

Fracture strength resulted comparable in STD and OPT, but increased for the other samples. The differences observed for RAW and EXTR with respect to OPT may be connected to the bean protein structuring role (McWatters, Ouedraogo, Resurreccion, Hung, & Dixon Phillips, 2003). In particular, the significantly higher fracture strength of RAW suggested that bean powder extrusion had an improving effect on the performance of this ingredient in biscuit formulation, yielding a less hard final product. WOW showed a fracture strength nearly six times higher than that of STD, probably ascribable to the very low amount of fat and to the creation of a very compact structure due to the low air incorporation during dough creaming. In fact, WOW dough showed the significantly ($p < 0.05$) highest value of density (1.23 ± 0.01 g/mL). Maybe emulsion viscosity and solid fat content were not appropriate to incorporate and retain enough air during creaming.

Due to the tight matrix, WOW showed the lowest milk absorption ability and porosity. On the other hand, the presence of hygroscopic ingredients, such as polydextrose, resulted in a significantly higher milk absorption for OPT, RAW, and EXTR with respect to STD. The presence of polydextrose in OPT allowed to increase air incorporation during creaming (Kocer, Hicsasmaz, Bayindirli, & Katnas, 2007), thus obtaining a porosity comparable to STD and a dough density of 0.98 ± 0.03 g/mL. Extruded bean powder led to a structure more similar to OPT than the use of raw powder.

3.4. Biscuit microstructure

Representative SEM images are shown in Fig. 2, revealing differences in biscuit internal structure and the gummy aspect of starch granules that were completely covered with an amorphous matrix. Starch is often considered as "inert filler" in biscuits, with the shortening or sugar playing the structuring role (Mamat & Hill, 2014). In particular, microstructural observation of STD and RAW revealed a developed protein-sugar structure. In WOW biscuits, a more closed network than in STD and RAW was evident. On the contrary, starch granules remained quite intact and embedded in a smooth and dense structure in OPT and EXTR, which showed similar microstructure in accordance with porosity results (Table 3).

3.5 Starch, lipid, and protein in vitro digestibility

RDS and SDS represent starch fractions becoming available for rapid or slow small intestine absorption, respectively, thus modulating glycaemic response (EFSA, 2011b) that appears to be directly related to the amount of RDS, while the insulin demand is inversely correlated to SDS fraction (Garsetti, Vinoy, Lang, Holt, Loyer, & Brand-Miller, 2005). Bean powder significantly increased SDS fraction in RAW and EXTR (Fig. 3A), maybe due to both the soluble dietary fibre content of beans (Los, Zielinski, Wojeicchowski, Nogueira, & Demiate, 2018) and their starch properties, characterized by high amylose content and large granule size (Sandhu & Lim, 2008). RAW revealed a significant but slightly lower SDS level with respect to EXTR that differed from all the other samples for the highest SDS value, probably due to the tighter protein network as well as to protein and starch modifications occurring during extrusion. EXTR showed the lowest RDS level, significantly different from that of STD, OPT, and WOW, but comparable to the RAW one. OPT and WOW showed SDS values similar to STD, because the higher amount of RS is not able to modulate starch digestion by increasing viscosity, but its effect is mainly due to the replacement of available carbohydrates

(EFSA, 2011a).

Lipid digestibility kinetic of WOW differed from that of the other samples (Fig. 3B), although final lipid digestibility (19.9 ± 1.3 g/100 g lipids) resulted comparable to OPT (17.4 ± 0.3 g/100 g lipids) and STD (17.6 ± 0.5 g/100 g lipids). This peculiar behaviour may be ascribed to the different type of fat used and to the double emulsion preparation process that reduces the oil droplet size and increases the surface exposed to lipase attack. RAW and EXTR had comparable lipid digestibility (14.6 ± 0.7 and 14.3 ± 0.1 g/100 g lipids, respectively), significantly lower than that of STD, probably due to the tight protein network, as already discussed for starch. Considering the extra-virgin olive oil matrix, a low digestibility (14.8 ± 0.1 g/100 g lipids) was obtained probably ascribable to the absence of added emulsifiers that, in biscuit samples, can enhance lipid-enzyme interaction.

The gastric attack produced a small protein digestion rate (ranging from 2.4 ± 0.3 to 5.9 ± 0.6 g/100 g proteins, for OPT and casein respectively), thus only results about the pancreatic phase are shown (Fig. 3C). The highest amount of digested proteins (25.5 ± 1.3 g/100 g proteins) were assessed for casein due to the absence of interferences. Among biscuit samples, RAW showed the highest protein digestibility, significantly different from STD (12.1 ± 2.7 vs 3.7 ± 1.6 g/100 g proteins). The other samples did not differ significantly from STD and RAW. Reaction kinetic reflects these observations: while casein digestion follows a first order kinetic, the other samples show a zero order kinetic that reveals a digestion rate strongly slower than that of casein. RAW resulted more readily digestible than EXTR: this appears in contrast with previous studies reporting an increase in digestibility of extruded and extruded-cooked legumes (Linsberger-Martin, Weiglhofer, Thi Phuong, & Berghofer, 2013; El-Hady & Habiba, 2003). However, it is possible that the baking process added to extrusion-cooking was responsible for the creation of indigestible aggregates of proteins and other macromolecules (Carbonaro, Maselli, & Nucara, 2015).

3.6. Consumers' acceptability of biscuits

Biscuit sensory attributes were affected by both the addition of bean flours and double emulsion (Table 4). OPT and STD showed the highest and comparable scores, in agreement with the structural data and the efficacy of the optimization strategy. RAW and EXTR did not show significant differences between them and obtained scores lower than STD for all the considered parameters. Texture perception by consumers confirmed the instrumental results, being WOW the sample with the lowest scores. These results affected overall liking, although appearance and odour of WOW resulted comparable to OPT.

3.7. Interplay among structure, nutritional properties and sensory acceptability

The interplay among structure, nutritional properties, and sensory acceptability of biscuits was explored by a first PCA performed with all the analytical data. Then, the less important variables (according to loading values) and some of the highly correlated responses (identified by the degree of overlapping on the PC1 vs PC2 plan and by the calculation of a Pearson correlation matrix) were eliminated. Comparing the final PC1 vs PC2 score and loading plots (Fig. 4), it is possible to infer that STD had high values of all the sensory attributes, brightness (L*), and fat content; bean-containing samples (RAW and EXTR) were mainly characterized by high absolute values of protein digestibility, moisture, and SDS; WOW was located at low values of PC1 and high values of PC2, far from all the other samples, and it was characterized by high values of dough density, biscuit fracture strength, and lipid digestibility, as well as by low values of texture- and overall-liking.

The relationships among structure, nutritional properties, and sensory acceptability of biscuits are highlighted by the loading correlation plot (Fig. 4B) and were confirmed by the Pearson correlation matrix. G* values, determined both by strain and frequency sweep tests, resulted directly correlated with fat level ($r = 0.947$ at $p < 0.05$, and $r = 0.961$ at $p < 0.01$, respectively). Similarly, dough density resulted inversely correlated with G* measured by strain sweep tests ($r = -0.892$, $p < 0.05$). Fat content lies in the same loading plot region of the liking attributes, confirming a higher consumers' acceptability for full-fat biscuits. A significant and inverse correlation ($r = -0.984$, $p < 0.01$) was found between perceived texture and fracture strength, thus confirming that an instrumental method gives results well correlated with consumers' responses.

Brightness (L*) was directly correlated to both appearance ($r = 0.878$, $p < 0.05$) and odour of biscuits ($r = 0.982$, $p < 0.01$), while redness (a*) showed an inverse correlation with the same responses ($r = -0.887$, $p < 0.05$; $r = -0.947$, $p < 0.05$), demonstrating the high influence of colour parameters on food sensory perception. SDS resulted directly correlated with damping factor ($r = -0.597$, $p < 0.01$), being also located in the same region of the loading plot. Similarly, fracture strain and lipid digestibility are in the same area of the loading plot, thus suggesting a relationship between biscuit structure and *in vitro* digestibility of starch and fat, worth of further investigations.

4. Conclusions

This work, thanks to the holistic approach used, allowed to investigate the complex relationships among structure, nutritional features, and sensory acceptability of reduced-fat biscuits. The role of fat content and type on dough and biscuit structure was assessed, demonstrating also an effect on nutritional properties. Consumers' acceptance was in its turn affected by fat content and biscuit structure and colour.

As regards the technologically-advanced ingredients used in fat-reduced biscuits, the extruded bean flour resulted in biscuits nearly comparable to a traditional reduced-fat product but with improved nutritional profile, while the use of double emulsion deeply modified the final product structure. Both ingredients are worth of further investigations, having interesting potential in the reduction of biscuit glycaemic impact or in low-fat product development.

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Legends to figures

Fig. 1. Average strain sweep (A) and frequency sweep curves (B, C) of biscuit doughs. G*, complex modulus; tanδ, damping factor. Analytical relative standard deviations ranged from 1 to 15% ($n \geq 2$). ■, STD; ◇, OPT; △, RAW; ○, EXTR; ∗, WOW. See Table 1 for sample identification.

Fig. 2. Scanning Electron Microscopy images of biscuits (see Table 1 for sample identification).

Fig. 3. *In vitro* digestibility of biscuits (see Table 1 for sample identification). A, starch digestibility (average ± standard error, n=6); RDS, rapidly digestible starch (dotted bars); SDS, slowly digestible starch (striped bars). For each parameter, different superscript letters indicate a significant difference ($p < 0.05$). B, lipid digestibility (average ± standard error, n=3); ■, STD; ◇, OPT; △, RAW; ○, EXTR; ∗, WOW; ●, OIL, extra-virgin olive oil reference. C, protein digestibility (average ± standard error, n=3); ■, STD; ◇, OPT; △, RAW; ○, EXTR; ∗, WOW; ●,CAS, milk casein reference.

Fig. 4. PC1 vs PC2 score plot (A) and loading correlation plot (B) obtained from Principal Component Analysis applied to the measured characteristics of biscuit samples (see Table 1 for sample identification).

Legends to coloured figures

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Table 1

Formulation (g/100 g) of biscuit doughs: STD, standard formulation; OPT, optimized formulation; RAW, formulation with raw bean powder; EXTR, formulation with extruded bean powder; WOW, formulation with double emulsion ($W_1/O/W_2$).

Sample	Flour	Shortening	Sugar	Salt	Water	Yeast	Polydextrose	Resistant starch	Bean powder	$W_1/O/W_2$
STD	38.45	23.07	11.54	0.08	25.63	1.23	-	-	-	-
OPT	33.64	12.38	11.54	0.08	25.63	1.23	10.69	4.81	-	-
RAW	33.64	12.38	11.54	0.08	25.63	1.23	10.69	-	4.81	-
EXTR	33.64	12.38	11.54	0.08	25.63	1.23	10.69	-	4.81	-
WOW	33.64	-	11.54	0.08	11.16	1.23	10.69	4.81	-	26.85

Table 2

Composition (g/100 g) of biscuits (mean ± standard error[1]). See Table 1 for sample identification.

Sample	Moisture	Ash	Fat	Proteins	Sugars	Total starch[2]	of which resistant starch	Fibre
STD	2.84±0.08[a]	1.22±0.01[b]	32.1±0.1[c]	5.7±0.1[b]	16.8±0.6[a]	40.0	1.9±0.2[a]	1.2±0.1[a]
OPT	3.86±0.07[c]	1.16±0.01[a]	17.5±0.1[b]	4.7±0.1[a]	18.0±0.6[a]	37.4	7.7±0.2[c]	6.7±0.1[c]
RAW	4.05±0.04[c]	1.40±0.01[d]	17.8±0.1[b]	6.2±0.1[c]	17.3±0.6[a]	41.5	2.8±0.2[a]	2.4±0.1[b]
EXTR	3.93±0.01[c]	1.41±0.01[d]	18.0±0.2[b]	6.0±0.1[c]	16.1±0.6[a]	39.3	2.1±0.2[a]	2.6±0.1[b]
WOW	3.42±0.01[b]	1.31±0.02[c]	14.9±0.1[a]	6.2±0.1[c]	18.2±0.6[a]	39.7	6.5±0.2[b]	6.6±0.1[c]

[1] Number of analytical replicates (n): moisture, ash, fat, proteins, sugars, fibre, n=2; resistant starch, n=6.
[2] Total starch was calculated as the 100's complement of the sum of all the measured components and the theoretical amount of polydextrose.
nd: not detectable
[a-d] For each parameter, different superscript letters indicate a significant difference ($p < 0.05$) among biscuit samples.

Table 3

Quality characteristics of biscuits (mean ± standard error[1]). See Table 1 for sample identification.

Sample	Thickness (cm)	L*	a*	b*	Milk absorption (g/100g)	Fracture strength (kPa)	Fracture strain (kPa)	Porosity (%)
STD	0.87±0.02[a]	70.2±0.4[c]	0.7±0.1[a]	31.4±0.5[a]	47±2[b]	93±4[a]	6±1[a]	8.2±0.6[b]
OPT	1.02±0.03[b]	60.4±0.9[b]	6.6±0.2[b]	35.7±0.5[b]	62±2[c]	100±4[a]	5±1[a]	9.4±0.2[b]
RAW	1.01±0.02[b]	53.2±0.4[a]	9.1±0.2[d]	32.1±0.6[a]	58±2[c]	229±1[b]	4±1[a]	4.9±0.2[a]
EXTR	1.02±0.01[b]	59.1±0.4[b]	7.5±0.4[bc]	34.8±0.3[b]	59±2[c]	168±5[ab]	5±1[a]	9.9±0.8[b]
WOW	0.84±0.02[a]	59.7±0.4[b]	8.3±0.4[cd]	33.8±0.6[ab]	25±0.7[a]	599±44[c]	6±1[a]	nd

[1]Number of analytical replicates (n): thickness, porosity n=2; L*, a*, b* n=3; Milk absorption, n=5; fracture strength, fracture strain, n≥10.

nd: not detectable.

[a-d] For each parameter, different superscript letters indicate a significant difference ($p < 0.05$) among biscuit samples.

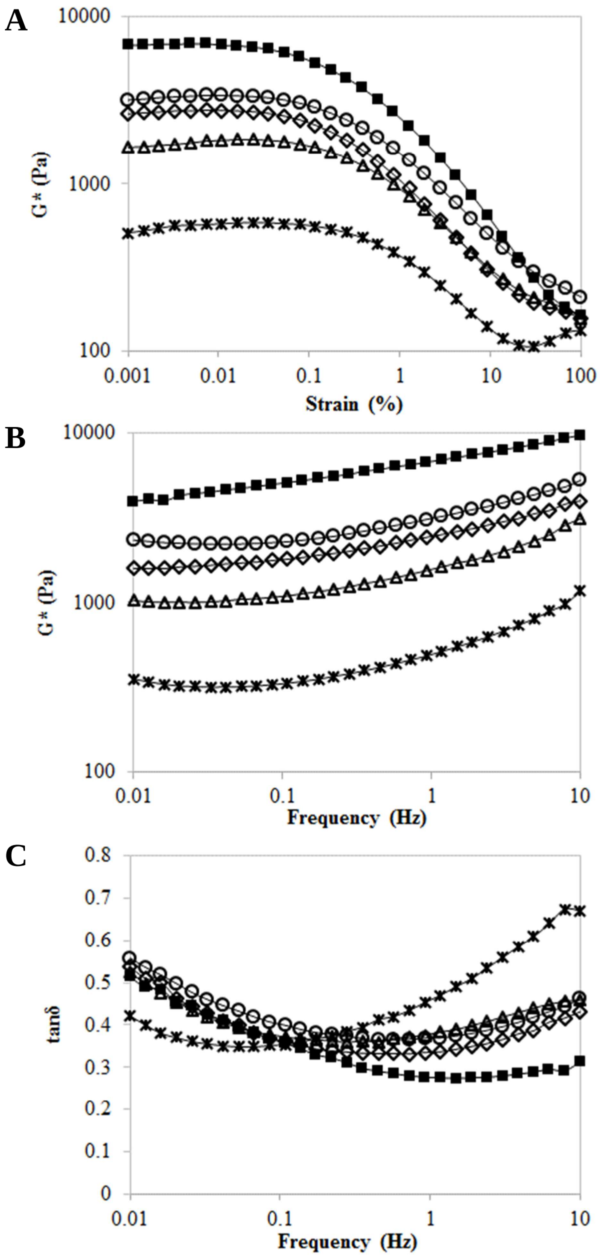
Table 4

Sensory liking attributes (mean ± standard error; n = 86) of biscuits. See Table 1 for sample identification.

Sample	Appearance	Odour	Taste	Flavour	Texture	Overall
STD	5.3±0.2[a]	4.9±0.2[a]	4.6±0.2[a]	4.5±0.2[a]	5.2±0.2[a]	4.7±0.2[a]
OPT	4.8±0.2[ab]	4.5±0.2[ab]	4.3±0.2[a]	4.2±0.2[a]	4.9±0.2[ab]	4.5±0.2[a]
RAW	4.2±0.2[bc]	4.0±0.2[b]	3.3±0.2[b]	3.1±0.2[b]	3.7±0.2[c]	3.3±0.2[bc]
EXTR	4.1±0.2[c]	4.3±0.2[b]	3.5±0.2[b]	3.4±0.2[b]	4.3±0.2[bc]	3.7±0.2[b]
WOW	4.5±0.2[bc]	4.3±0.2[b]	3.4±0.2[b]	3.5±0.2[b]	1.8±0.2[d]	2.8±0.2[c]

[a-d] For each parameter, different superscript letters indicate a significant difference ($p < 0.05$) among biscuit samples.

Fig. 1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100
G* (Pa)
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0.01
0.1
1
10
100
Strain (%)
B
10000
1000
100
G* (Pa)
0.01
0.1
1
10
Frequency (Hz)
C
0.8
0.7
0.6
0.5
0.4
0.3
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0.1
1
10
Frequency (Hz)

Fig. 1 Colour version for online only

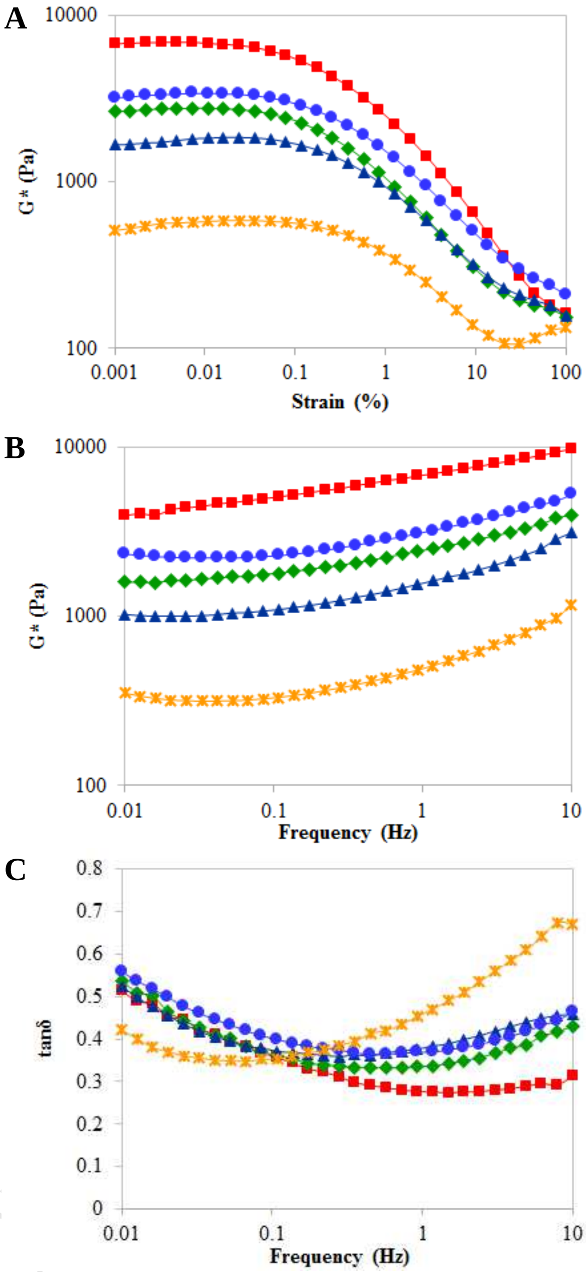
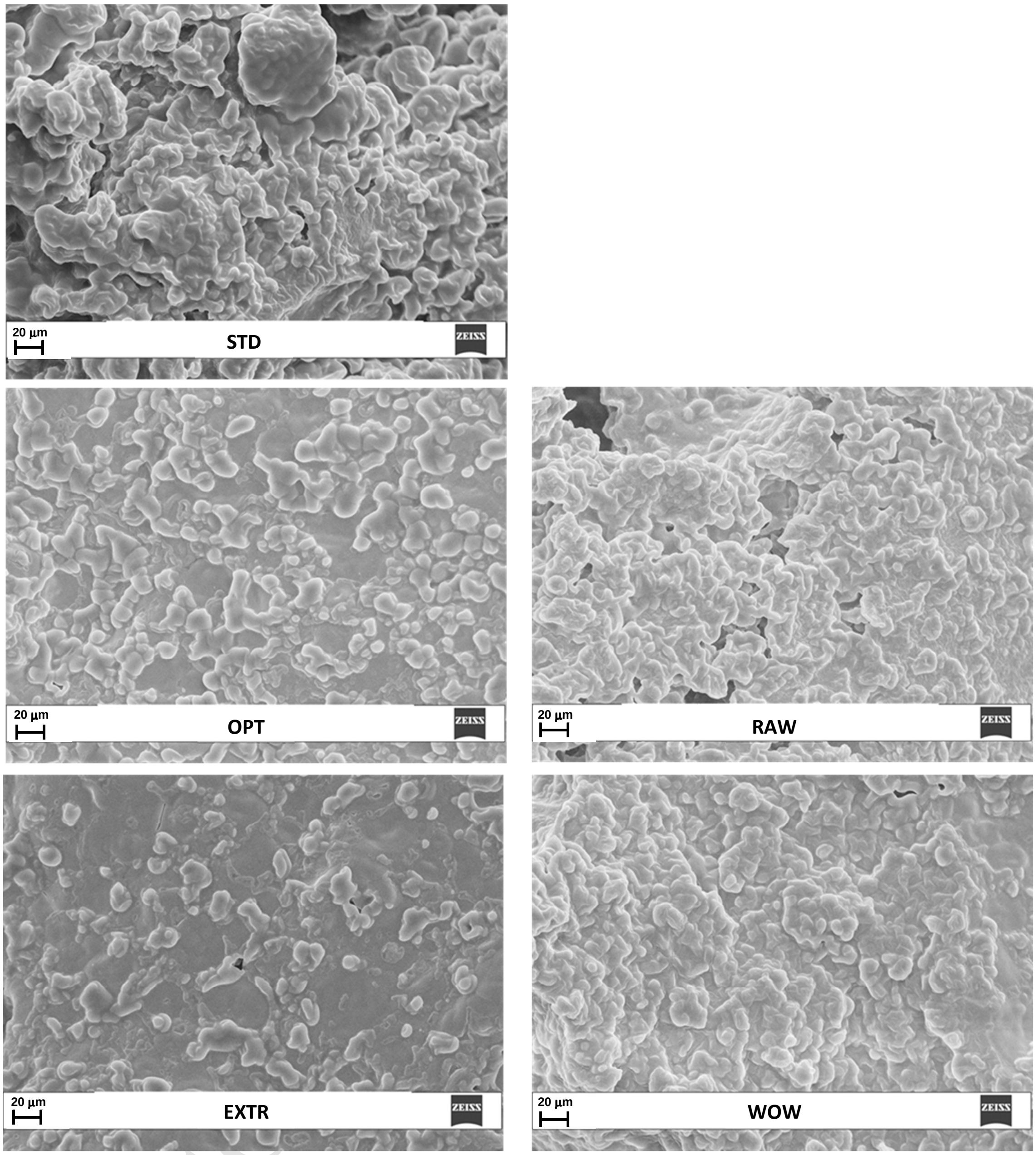


Fig. 2.


20 µm
STD
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RAW
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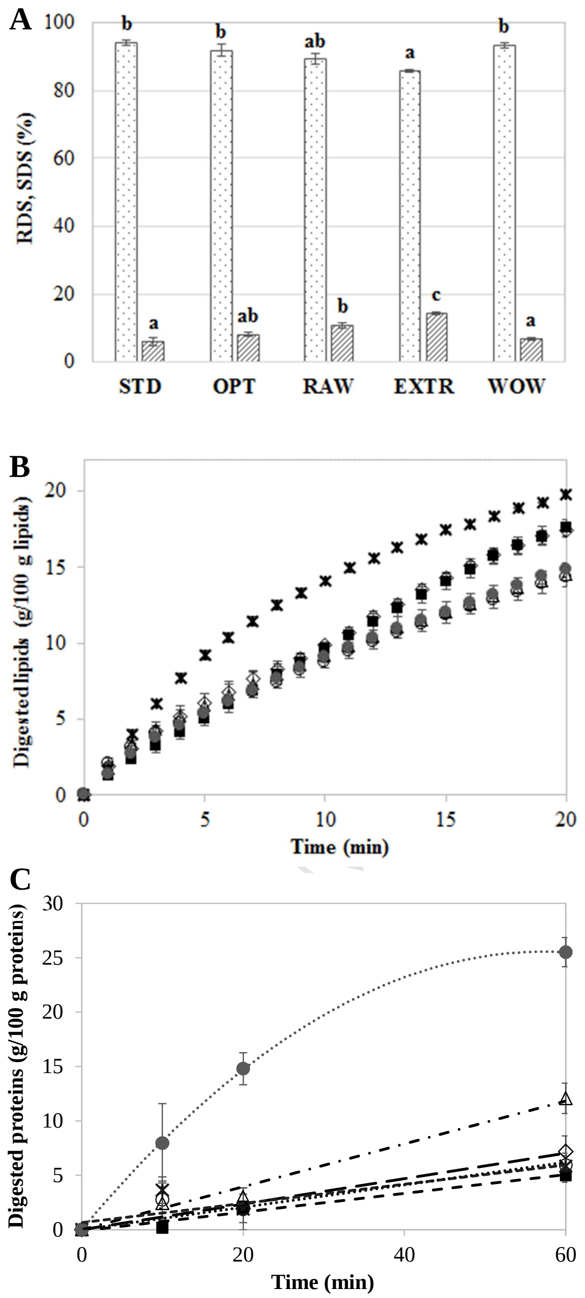
Fig. 3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RDS, SDS (%)
100
80
60
40
20
0
b
a
b
ab
ab
b
a
c
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a
STD
OPT
RAW
EXTR
WOW
B
Digested lipids (g/100 g lipids)
20
15
10
5
0
Time (min)
C
Digested proteins (g/100 g proteins)
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25
20
15
10
5
0
0
20
40
60
Time (min)

Fig. 3 Colour version for online only

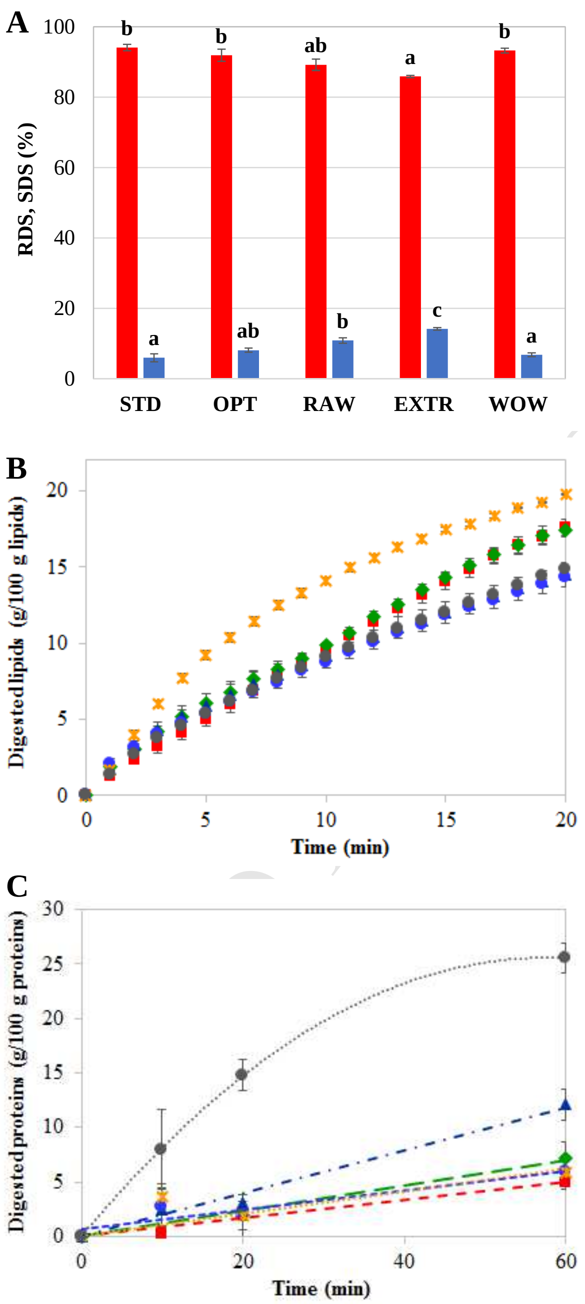
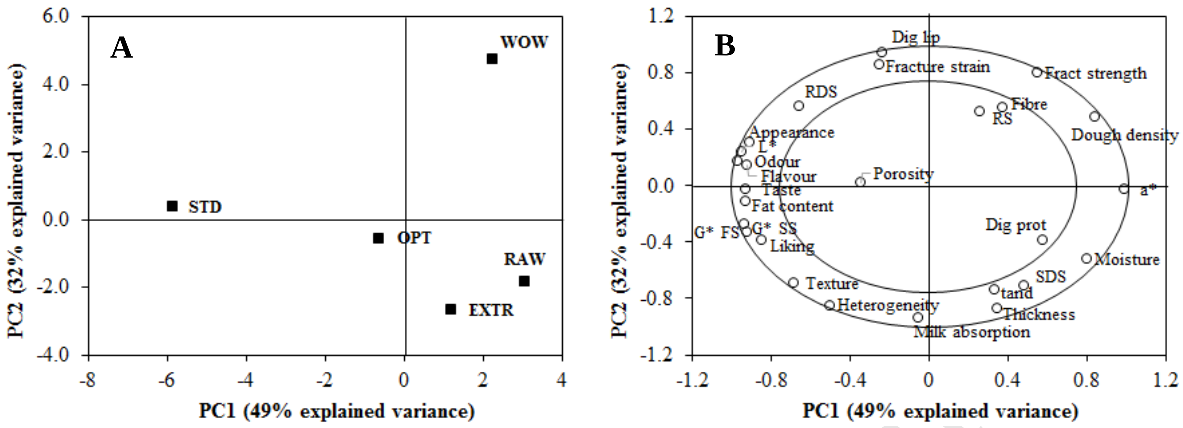


Fig. 4



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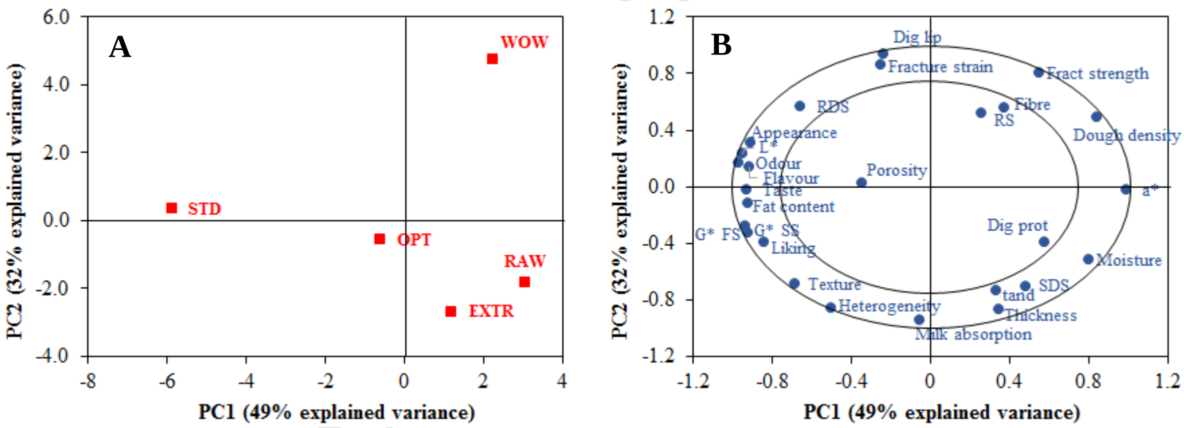

Highlights

- Use of raw and extruded bean flours or double emulsion affect biscuit properties.
- Bean powders have a structuring role in reduced-fat biscuits.
- Double emulsion gives biscuits with closed structure and high fracture strength.
- Extruded bean powder gives biscuits with low level of rapidly digestible starch.
- The role of fat on dough rheology and biscuit liking was assessed.