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Impact of ingredients and processing technologies on structural and nutritional properties of reduced-fat foods

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SUMMARY

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ABSTRACT

In the last decades, the demand for nutritionally-improved food has raised, drawing the attention of researchers on new solutions for product development. The design of foods able to satisfy specific sensory and nutritional functionalities requires a better understanding of the relationships between the composition of food materials, the effects of processing on their quality characteristics and structure, and their behavior during digestion. This PhD thesis aimed at evaluating how ingredients and technology affect structural properties of different reduced-fat matrices. Biscuits and whipping cream were used as case studies with different specific aims concerning the effects of ingredients and/or production technology. In particular, resistant starch, raw and extruded bean flour were evaluated as structuring ingredients in reduced fat biscuits, in combination with polydextrose or double emulsion as fat replacers. The effect on structural, nutritional and sensory properties of final biscuits were investigated. For whipping cream, gelatin addition and homogenization condition effects on structure and stability were evaluated, both immediately after production and after three weeks of storage. Design of Experiments techniques coupled with Response Surface Methodology were used for the multivariate investigation of both formulation and processing effect.

Results from the biscuit case study demonstrated that combination of polydextrose and resistant starch allowed to obtain a reduced-fat biscuit (- 44 % fat) with structural characteristics similar to those of a standard full-fat biscuit prepared with shortening or butter. The use of extruded bean flour allowed to obtain biscuits nearly comparable to a traditional reduced-fat product, but with improved nutritional profile. The presented data suggest a hypoglycaemic potential of bean-enriched biscuits, to be confirmed by a dedicated *in vivo* study. Desirable nutritional value of bean powders, including high protein content and slow starch digestibility, may be successfully exploited in biscuits. On the other hand, the study of double emulsions by D-optimal design allowed to improve the knowledge about the effect of internal water gelling and the proportion of water and oil in the emulsion on yield, rheological behaviour and stability. However, the use of double emulsion in reduced-fat biscuits requires further investigations, in order to understand how to improve emulsion structuring and its effect on dough properties.

Results from the whipping cream case-study demonstrated that the combination of gelatin addition and high homogenization pressure may be successfully exploited for the development of reduced-fat whipping cream (25 g/100g fat), with good quality characteristics and stability. D-optimal design and Response Surface Methodology resulted to be effective tools for the study of cream processing at a pilot level, allowing the collection of high quality information on the effect of the studied factors and their interactions, with a limited experimental effort. In particular, gelatin addition at a concentration of 0.25 g/100g, in combination with k-carrageenan, increased initial consistency of cream samples, leading to final overrun values even improved with respect to those obtained for commercial samples, without affecting texture properties and stability. Depending on gelatin

concentration, homogenization pressure revealed a great potential to modulate whipping properties. The multidisciplinary approach adopted, comprehensive of all the functionality aspects related to a food product, may represent a starting point for the design of foods with targeted quality features and behavior during consumption. In such a complex investigation field, Experimental Design techniques coupled with multivariate analyses of the experimental data have confirmed to be effective tools for the characterization and optimization of both food formulation and processing. The developed mathematical models can be applied for reverse engineering and quality-by-design approaches, thus benefit both researchers and companies.

1. INTRODUCTION

Over the past decades, several research efforts have been directed towards new strategies for the production of nutritionally-balanced food. The epidemic dimension of food-related pathologies (World Health Organization 2015) and the increasing consumers' awareness of the role of diet in wellbeing and health maintenance has defined different nutritional targets, i.e. low energy density, low glycemic index, high content of fiber and proteins, addition of micronutrients, etc. However, formulation and process changes required to meet these needs may strongly influence food matrix structure and stability. For instance, fat reduction implies a strong impact on food rheology, flavor, mouthfeel, and texture (Sudha et al. 2007a). The design of products able to satisfy specific sensory and nutritional functionalities requires a better understanding of the relationships between the compositions of food materials, the effects of processing on their structures and interactions, and their behavior during digestion (Lovegrove et al. 2017).

When considering food structure, a fundamental key descriptor is texture, which can give a quantitative information about the structure-deformation properties of a food matrix. Increasing understanding of the effects that reformulation and processing have on food texture may contribute to the creation of a basic background for the design of targeted food structures (Chen and Rosenthal 2015). While it is generally accepted that food structure is a determining factor for food pleasantness and consumers' acceptability (Wilkinson et al. 2001), the impact of micro- and macro-asset of food on its nutritional properties is seldom considered. Food structure may affect nutrition by modifying bioaccessibility and bioavailability of macronutrients and the mechanisms of food intake and satiety (Aguilera 2005). Indeed, focusing on bioaccessibility of macronutrients, a dense food matrix organization has been reported to retard the digestion of starch (Turgeon and Rioux 2011), as well as the presence of fibrous matrices (in particular β-glucans), which would increase the viscosity of gastric content, to slow down enzymatic activity and glycemic response (Jenkins et al. 1978). The presence of carbohydrates and fiber may modulate protein hydrolysis and digestion, as a result of the increased viscosity and the interaction with peptides that limits enzymatic action (Peyron et al. 2006; Villemejane et al. 2016a, 2016b).

Several researches have focused on the link between food processing and nutritional properties: homogenization, heating and extrusion are among the most studied operations. Homogenization process and emulsifier type may affect lipid digestibility by influencing droplet size (McClements 2007; Michalski 2007). Heat treatment applied for pasta drying affects wheat protein digestibility (De Zorzi et al. 2007) and UHT treatment of milk accelerates the kinetics of appearance of plasmatic aminoacids in comparison with pasteurization (Lacroix et al. 2008). Capuano et al. (2018) found that *in vitro* lipid digestibility in oil from roasted hazelnuts was higher than in oil extracted from the raw material. Singh, Gamlath and Wakeling (2007) reviewed the effects of extrusion-cooking on the nutritional quality of different food products (mainly cereals and legumes), concluding that high moisture contents combined with low time-temperature conditions allow to obtain a better

nutritional functionality in terms of starch and protein digestibility. On the other hand, the high temperature and shearing effects reached during extrusion processes are associated to a high degree of starch gelatinization or dextrinization, thus increasing starch digestibility (Singh et al. 2007), and influence the ability of β-glucans to lower serum cholesterol (Wolever et al. 2012). Karkle et al. (2012) reported that hydration regimen during extrusion was a significant factor in determining final starch digestibility of fiber-added snacks.

However, the complexity of a comprehensive investigation of the simultaneous effects of formulation and/or processing on structural, sensory, and nutritional properties may explain why few studies report a comprehensive investigation. In particular, the effects of formulation and/or technological process on these aspects of reduced-fat foods seem understudied. In this perspective, biscuits and whipping cream, being traditionally rich in fat, may represent interesting case studies.

In this context, the multivariate design of experiments (DoE) is an important tool because allows the gathering of large amount of information about complex systems with reduced efforts in terms of time and resources in comparison with the univariate approach (Leardi 2009). Response Surface Methodology (RSM) is one of the most used multivariate techniques; it is a collection of statistical and mathematical methods based on the fit of a polynomial model that reflects the behavior of a data set with the purpose of describing a phenomenon or predicting it. DoE and RSM have been proved to be useful in the food field for the study of processes where responses are affected by several variables and their interactions (Yolmeh and Jafari 2017). Moreover, models developed thank to DoE and RSM can be efficaciously exploited as reverse engineering tools for the optimization of process conditions and food formulations following a Quality by Design approach. When a number of responses should be optimized, the desirability function may be applied setting the proper criteria (Vera Candioti et al. 2014).

1.1. Reduced-fat biscuits

Biscuits are very popular bakery products since they are ready-to-eat, cheap and available in a wide variety. However, biscuits are traditionally rich in fat and its reduction or substitution is a main challenge for researchers. Indeed, lipids are main responsible for flavor, texture and overall quality of the final product. In particular, fat coats flour particles during mixing, limiting protein hydration and gluten development. Moreover, in soft-dough biscuits, fat allows the correct incorporation of air during the creaming phase of dough formation and makes the dough resistant to baking temperatures (Davidson 2016a; Jacob and Leelavathi 2007). Reducing fat results in several undesirable technological effects, such as toughening of the dough, increasing in cohesive, adhesive and elastic dough properties, shrinkage of the final product, loss of color and eating quality (Boobier et al. 2006).

Carbohydrate-based mimetics can be effectively exploited for reduced-fat biscuit production. For instance, polydextrose, a randomly linked polymer of glucose, sorbitol and citric acid, allows to obtain low-fat formulation with proper mouthfeel and creaminess (Sudha et al. 2007b). It can be used as a low-calorie (1 kcal/g) bulking agent, since the random bonds confer a structural compactness and complexity that prevents hydrolysis by mammalian digestive enzymes (Mitchell 1996).

Several Authors analyzed the effects of partially replacing fat in biscuits with carbohydrate-based mimetics such as polydextrose (Aggarwal et al. 2016; Zoulias et al. 2002), maltodextrin (Forker et al. 2012), guar gum and inulin (Błońska et al., 2014; Krystyjan et al., 2015; Laguna et al., 2014; Rodríguez-García et al., 2013). However, to the best of our knowledge, the effect of combining polydextrose and resistant starch in a reduced-fat biscuit formulation has not yet been investigated with a systematic approach.

Generally, fat-replaced biscuits show higher hardness and brittleness and lower crumbliness than the full-fat counterparts, due to a higher development of the gluten network (Chugh et al., 2013; Laguna et al., 2014). The addition of resistant starch was found to counterbalance the texture defects by giving crumblier and less hard textures of baked biscuits (Laguna et al., 2011; Laguna et al., 2012). Moreover, biscuits containing resistant starch rich ingredients may represent an effective way to increase the daily fiber intake of population, recognized as a protective factor towards several food-related diseases (Asp et al. 1996). In this context, legume powders may be exploited as resistant starch rich ingredients, with improved nutritional functionality. In fact, the presence of poorly digestible starch confers legumes a low glycemic index compared to cereal grains, providing benefits to consumers suffering for diabetes or cardiovascular diseases (Hoover and Zhou 2003). In addition, the presence of prebiotic oligosaccharides and fiber shows a regulating effect towards gastrointestinal functions (Tharanathan and Mahadevamma 2003).

Dry common beans (*Phaseolus vulgaris L.*) may represent a very interesting ingredient for their relatively low cost and their high nutritional quality. Bean powder is generally produced via soaking, blanching and steam-cooking followed by drying and particle size reduction, thus strongly reducing flatulence-causing oligosaccharides and anti-nutritional factors. Cooking-extrusion has been proposed as an alternative process for bean powder treatment, being versatile, with low operating costs, energy efficient and time-saving (El-Hady and Habiba 2003). From a product quality point of view, extruded bean powders generally show higher oxidation stability, leading to food products more acceptable by consumers (Szczygiel et al. 2017). Ai et al. (2016) found that extrusion-cooking results in complete starch gelatinization and protein denaturation of bean flour, implying modifications in pasting properties and solvent retention capacities. However, starch digestibility after cooking in boiling water was similar for both extruded and non-extruded powders. Very few studies investigated the use in biscuits of bean powder, both raw (Sparvoli et al. 2016) and extruded (Ai et al. 2017; Siddiq et al. 2013). To the best of our knowledge, no research deals with applying this ingredient to low-fat biscuit formulations.

An interesting approach for the development of reduced-fat foods 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 O/W emulsions, but with a reduced fat content. The main issue connected to real food application is obtaining $W_1/O/W_2$ emulsions able to mimic fat behavior and with a prolonged stability: gelling of the internal aqueous phase may be a useful strategy (Oppermann et al. 2015; Perez-Moral et al. 2014), together with the use of a strong lipophilic emulsifier as polyglycerol polyricinoleate (PGPR) (Muschiolik and Dickinson 2017). The effects of using double emulsions as fat replacers in food products have been studied so far for dairy products (Leong et al. 2017; Lobato-Calleros et al. 2006, 2008, 2009; Márquez and Wagner 2010), meat products (Cofrades et al. 2013; Freire et al. 2016) mayonnaise and dressings (Matsumoto and Kohda 1980; Takahashi et al. 1986). However, to the best of our knowledge, no results have been reported for the application in the bakery field.

1.2.Reduced-fat whipping cream

Whipping cream is one of the most used products in pastry and dessert production; from a technological point of view, it is a foamed dairy emulsion stabilized by the partial coalescence of semi-solid fat particles. Foam structure and persistence basically depend on three factors: development of the air interface, development of a partially coalesced fat network able to stabilize air bubbles, and viscosity of the serum phase (Smith et al. 2000). Thus, fat content (35-40% in commercial whipping creams) is a key factor for good product performance and stability (Van Aken 2001; van Lent et al. 2008) and its reduction is challenging.

Among the common approaches used to improve whipping cream stability and performances, the addition of thickening agents has been widely investigated, thanks to the ability of these ingredients to increase the viscosity of the serum phase and interact with protein components of cream (Camacho et al. 1998; Stanley et al. 1996). Zhao et al. (2009a; 2009b) demonstrated that xanthan gum and hydroxypropyl methylcellulose, used in the right amount, could improve whipping performance, textural properties, and coalescence of fat in whipped cream. On the other hand, Chamacho et al. (1998) claimed an induced foaming resistance in whipping cream due to the addition of locust-bean gum (LBG) and k-carrageenan mixes, although they showed an improvement in cream stability when a low ratio of LBG was present (Camacho et al. 2005). Farahmandfar et al. (2017) demonstrated that basil seed gum, cress seed gum and quince seed gum have a potential as thickening agents and fat replacers in 30% fat whipping cream. However, to our knowledge, no study explored the effect of adding thickening agents in cream containing less than 30% fat.

From a technological point of view, homogenization may be exploited for its stabilization effect on emulsions. Generally, low-pressure homogenization (lower than 5 MPa) is applied in cream manufacture to reduce droplet size and prevent creaming and serum separation during shelf life. High pressures (10-50 MPa) lead to creams with decreased droplet size, a more rigid interfacial layer (Long et al. 2012), and a higher apparent viscosity (Kováčová et al. 2010; Long et al. 2012), resulting in an improved stability of emulsion during storage. Based on fat content, different homogenization pressures may be used to increase cream viscosity. Hub and Hinrichs (1997) reported that for 25% fat cream a pressure between 15 MPa and 20 MPa may produce a viscosity comparable to a full-fat product.

2. RATIONALE and AIM

This PhD thesis aimed at filling the scientific literature gaps about the effects of formulation and/or processing on structural, sensory, and nutritional properties of reduced-fat products. Actually, the complexity of such investigation has limited publications with comprehensive approaches. The results of this research may contribute to better understanding the complex relationships among formulation, processing, structure, and nutritional properties of food thus giving additional information for the design of food products with targeted nutritional properties that may benefit scientific community, industry and consumers.

Biscuits and whipping cream were used as case studies with different specific aims concerning the effects of ingredients and/or production technology on quality and stability of final products.

Case study 1: Reduced-fat biscuits. The general aim of this part of the work was to investigate the effects of adding raw and extruded bean flour or double emulsion on the structural, nutritional and sensory properties of deposited reduced-fat biscuits. This aim was reached with the following subsequent steps:

1. Study of the combined effect of polydextrose and resistant starch on the quality characteristics of reduced-fat biscuits and development of an optimized formulation through Central Composite Experimental design and desirability function.
2. Optimization of a WOW double emulsion to be used as fat substitute, through D-optimal experimental design and desirability function.
3. Application of raw and extruded bean powders and double emulsion to the optimized formulation of reduced-fat biscuits and study of the relationships among structural, nutritional, and sensory properties.

Case study 2 – Reduced-fat whipping cream. In this case, the combined effect of gelatin addition and homogenization conditions on structural properties and stability of cream were evaluated. The case study included two experimental parts:

1. Investigation of the combined effect of gelatin addition and homogenization conditions on the structural properties of reduced fat cream through D-optimal experimental design.
2. Evaluation of storage stability and performance of experimental whipping creams after 3 weeks of storage.

This experimental part was developed in collaboration with the Belgian Institute for Agriculture, Fisheries and Food Research (ILVO) and carried out at ILVO Food Pilot laboratory (Melle, Belgium).

3. RESULTS and DISCUSSION

3.1 Case study 1: Reduced-fat biscuits

Experimental part 1: Reduced-fat soft-dough biscuits: Multivariate effects of polydextrose and resistant starch on dough rheology and biscuit quality.

The aim of this work was to study polydextrose and resistant starch multivariate effects on reduced-fat soft-dough biscuits, focusing on dough rheology and biscuit quality. Moreover, a reduced-fat formulation optimized for biscuit structural properties was designed by using a multiple response optimization.

***Abbreviation list**:* Adj. R^2, adjusted determination coefficient; ANOVA, analysis of variance; b_0, constant value in the polynomial model; b_1 linear coefficient for shortening reduction in the polynomial model; b_{11}, quadratic coefficient for shortening reduction in the polynomial model; b_{12}, interaction coefficient in the polynomial model; b_2, linear coefficient for flour substitution in the polynomial model; b_{22}, quadratic coefficient for flour substitution in the polynomial model; DoE, Design of Experiment; FR, flour replacement; FS, frequency sweep; G*, complex modulus; G', elastic modulus; G'', viscous modulus; LOF, lack of fit (p-value); n, number of replicates; Pred. R^2, predicted determination coefficient; R^2, determination coefficient; SR, shortening reduction; SS, strain sweep; X_1, value of shortening reduction in the polynomial model; X_2, value of flour replacement in the polynomial model; y, response variable value in the polynomial model; ε random error in the polynomial model; ε_f, fracture strain; σ_f, fracture strength.

Biscuit composition

Table 3.1.1 shows the analytical composition of the experimental biscuits developed in accordance with the Design of Experiments reported in section 5, table 5.1.1. Sample name indicates the level of shortening and flour replacement (SR and FR, respectively), i.e. 50-0 is the sample with 50% shortening substitution and 0% flour substitution. Although recipes were balanced to ensure a constant dough moisture, and standardized baking conditions were applied, a small moisture variability within samples can be noticed as a function of the presence of ingredients with high water retention capacity, such as polydextrose and resistant starch (Laguna et al., 2011; Stanyon and Costello, 1990; Zoulias et al., 2002). Protein and lipid contents were consistent with SR and FR levels. As expected, no flour substitution (0-0, 50-0, 25-0) corresponded to the highest protein content (5.46 ± 0.16 g/100g d.b. on average), very similar to that of biscuits made with butter (REF). A progressive protein content decrease was then observed with increasing FR to an intermediate (0-40, 25-40, 50-40) and maximum (0-80, 25-80, 50-80) level (3.44 ± 0.04 g/100g d.b. and 1.43 ± 0.09 g/100g d.b. on average, respectively). In consistency with the rationale of formulation development, samples 0-0 and REF had the same amount of fat, accounting for 60% of flour weight

in the dough, which is quite common for soft-dough biscuits (Davidson 2016b; Manley 2001). In low-fat samples, the shortening relative percentage with respect to flour was lowered to 44% and 30%, thus resulting in an actual fat content decrease of 22% and 51% in samples 25-0 and 50-0, respectively.

Substitution of shortening and flour with polydextrose and resistant starch resulted in a considerable higher content of total dietary fibre in biscuits. Based on a theoretical calculation, the dietary fibre content of 0-0 and REF sample was 1.8 g/100g and 1.7 g/100g , respectively. In all the other samples, fibre content ranged from 9.3 g/100g (sample 25-0) to 35.6 g/100g (sample 25-80), with a maximum value of 46.2 g/100g in sample 50-80.

Table 3.1.1: Sample chemical composition (mean ± standard deviation of two replicates). Results about the reference formulation made with butter (REF) are also reported. See Table 5.1.1, section 5 for sample identification and formulation.

Sample	Moisture	Proteins	Lipids
	g/100g	g/100g d.b.	g/100g d.b.
0-0	3.05±0.20	5.75±0.04	32.10±0.29
50-0	7.67±0.02	5.72±0.14	16.74±0.18
0-80	5.07±0.03	1.61±0.10	31.65±0.93
50-80	5.75±0.06	1.49±0.05	16.77±0.33
0-40	3.32±0.01	3.64±0.08	27.47±0.55
50-40	6.59±0.06	3.70±0.03	17.28±0.30
25-0	6.56±0.01	5.92±0.11	25.65±0.26
25-80	5.22±0.01	1.43±0.02	25.10±0.13
25-40a	5.53±0.02	3.43±0.08	24.1±0.1
25-40b	4.38±0.15	3.47±0.01	24.4±0.1
25-40c	4.78±0.06	3.44±0.02	23.9±0.1
25-40d	4.17±0.01	3.45±0.01	23.8±0.1
25-40e	4.51±0.02	3.45±0.01	23.7±0.2
25-40f	5.47±0.01	3.42±0.03	23.7±0.3
25-40g	6.33±0.03	3.38±0.03	23.5±0.3
REF	5.34±0.18	5.83±0.06	31.4±0.3

Note: Adapted from Moriano et al. (2018)

Dough density and rheological properties

Biscuit dough characteristics are reported in Table 3.1.2. The observed differences in density among samples were most probably ascribable to the fat amount. In fact, the full-fat standard dough (sample 0-0) resulted in the lowest density value, whereas the highest value was found in the dough

with the highest SR (sample 50-0). In addition, sample 0-0 showed a density value lower than REF sample: it can be hypothesized that shortening may exert a better air-entrapping role than butter.

Biscuit dough rheological properties are important for both dough machinability and biscuit quality (Laguna et al., 2011; Laguna et al., 2013; Manley, 2001). A SS test was first carried out in order to find the linear viscoelastic range, i.e. the strain interval in which rheological properties of dough are not deformation dependent (Steffe, 1996). Figure 3.1.1A shows the average SS curves obtained for different biscuit doughs in terms of complex modulus (G*). The linear viscoelastic limit was quite similar for all samples (ranging from 0.04 to 0.07% strain) suggesting that the combination of polydextrose and resistant starch as fat mimetic did not affect dough stability against deformations. However, dough consistency was significantly affected by SR and FR, as shown by G* variations among samples. In particular, decreasing G* values were obtained with SR increase, thus demonstrating the structuring role of fat.

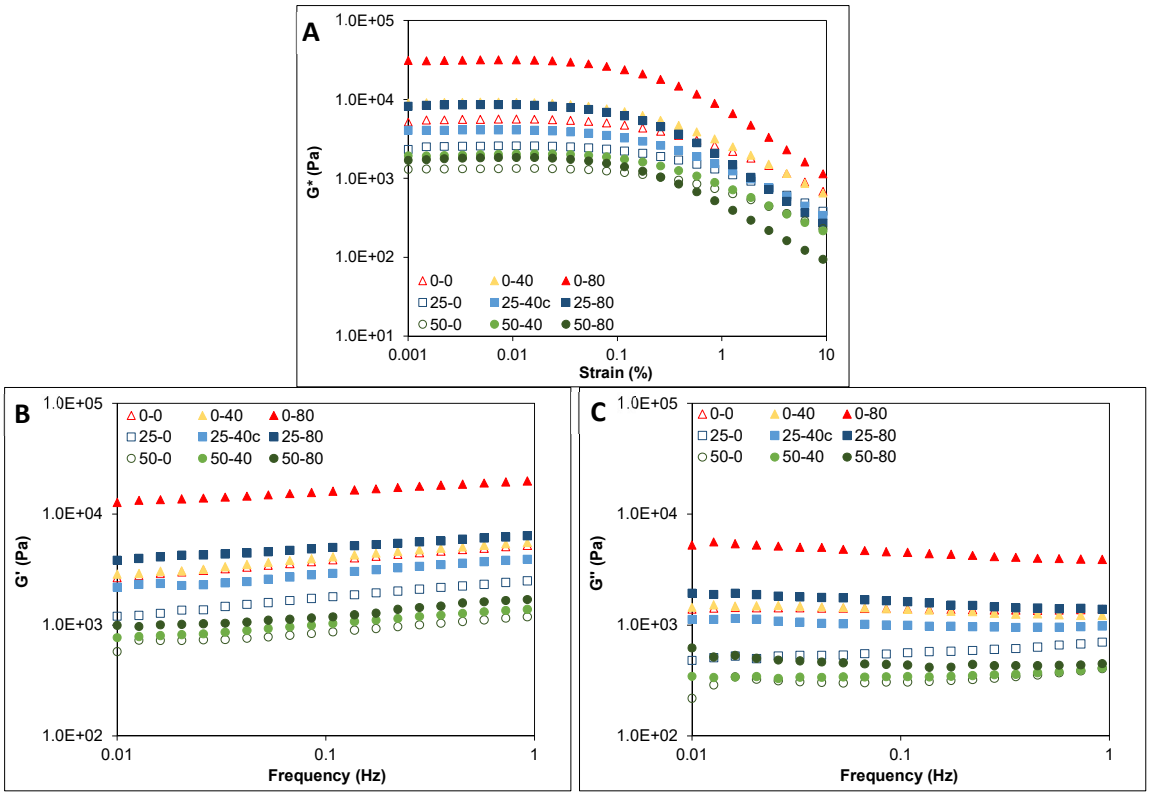

Figure 3.1.1: Average strain sweep (A) and frequency sweep (B, C) curves of some biscuit doughs developed according to the Design of Experiments reported in chapter 5, Table 5.1.1 for the study of a reduced-fat biscuit formulation using polydextrose and resistant starch. Analytical relative standard deviations ranged from 1 to 15% (n = 2). G*, complex modulus; G', elastic modulus; G'', viscous modulus.

Note: Adapted from Moriano et al. (2018).

Similarly, in FS test (Figures 3.1.1B-3.1.1C) both elastic (G') and viscous (G'') modulus decreased with the increase of SR. These results are consistent with Tarancón et al. (2015), who observed significantly lower G' and G'' values in short doughs prepared using different oil-water-cellulose ether emulsions as shortening replacers. The full-fat recipe with the highest amount of resistant

starch (0-80) showed the highest G' and G'' values, revealing a stiffening effect of resistant starch possibly due to its ability to interact and retain water (Laguna et al., 2011). According to a weak gel behaviour, the damping factor (i.e. ratio between G'' and G') was lower than 1 (0.20-0.51) throughout the entire frequency range. Most samples showed a 30-60% decrease of damping factor with the frequency increase, according to the typical stronger solid-like behaviour of materials at high frequencies (Steffe, 1996). The lowest frequency dependence was observed for sample 50-0, probably due to a higher structural stabilization related to a high protein content and the higher water retention capacity provided by polydextrose. Similar results were obtained by Taráncon et al. (2015) in short doughs produced with oil-water-cellulose ether emulsions, due to the presence of glycerol that, increasing the number of hydrogen bonds with water and cellulose, provided structural stabilization. Table 3.1.2 shows G' and G'' modulus values extrapolated from SS and FS curves at a strain of 0.01% and at a frequency of 0.1 Hz, respectively. These data were used for regression modelling of response surfaces. Results obtained for dough made with butter (REF) were very similar to those of the standard formulation (sample 0-0).

Biscuit characteristics

Thickness, colour, milk absorption, texture parameters, bottom porosity and surface heterogeneity were evaluated as quality characteristics of biscuits (Table 3.1.3). The low relative standard deviation values of the central point replicates highlighted a good reproducibility of biscuit production, which is very important in order to obtain reliable models. In particular, all the relative standard deviation values were lower than 10%, with the exception of colour parameter a^* (13%) and fracture strain (14%), for which sample 25-40g showed a higher deviation from the average values with respect to the other central points.

The quality characteristics most influenced by shortening and flour substitution resulted to be fracture strength and a^*, followed by heterogeneity, fracture strain and milk absorption. As expected, redness (a^*) was mainly influenced by the addition of polydextrose, which might provide additional reducing sugars and be responsible for a higher formation of Maillard reaction products during biscuit baking (Mieszkowska and Marzec, 2016; Stanyon and Costello, 1990). On the contrary, fracture strength was significantly decreased mainly by FR, indicating a lower biscuit resistance to snapping. This result is in agreement with the lower protein content of biscuits containing higher amounts of resistant starch, which makes the structure more fragile due to the weaker gluten network. Actually, a significant direct correlation ($r = 0.702$; $p < 0.01$) was calculated between fracture strength and biscuit protein content. A similar discussion can be done also for fracture strain that resulted directly correlated with strength ($r = 0.701$; $p < 0.01$). As already reported by Laguna et al. (2011) for short-dough biscuits, resistant starch has a tenderising effect, not negative for consumers' acceptability. Heterogeneity is a surface texture feature indicating the

smoothness or roughness of a surface, and it is measured by image analysis technique. Biscuit surfaces were mostly smooth, being the heterogeneity values lower than 0.5. Heterogeneity was significantly correlated ($r = 0.738$; $p < 0.01$) with dough rheological parameters: lower values of heterogeneity were obtained with the decrease of G' and G'', both measured by SS and FS tests; thus, lower dough viscoelasticity resulted in a smoother biscuit surface. Milk absorption was higher in samples 50-80 and 50-40, suggesting a relation with both SR and FR. A significant increase of milk absorption was noticed with the reduction of fat ($r = -0.674$; $p < 0.01$), due to the high affinity of polydextrose with water.

Sample 0-0 and REF were similar, with the exception of a harder texture of biscuits containing butter, possibly related to the lower degree of entrapped air during creaming, as discussed about dough density data.

Table 3.1.2: Dough characteristics (mean ± standard deviation values[a]) of biscuits formulated according to the Design of Experiments described in section 5.1. Results about the reference formulation made with butter (REF) are also reported.

Sample	Density (g/mL)	Strain sweep test (0.01% strain)		Frequency sweep test (0.1 Hz frequency)	
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)
0-0	0.74±0.08	5460±1141	1433±356	3870±1138	1393±511
50-0	1.01±0.01	1260±170	439±57	866±139	308±67
0-80	0.96±0.01	31250±778	5965±530	16095±115	4510±475
50-80	0.92±0.01	1785±78	467±52	1335±7	476±9
0-40	0.81±0.03	8995±460	1930±85	4125±445	1380±226
50-40	0.88±0.04	1950±170	596±52	1029±44	342±11
25-0	0.86±0.02	1960±622	549±197	2220±594	792±323
25-80	0.92±0.01	8370±127	1800±14	5010±71	1625±7
25-40a	0.84±0.02	3940±42	982±1	2885±92	976±77
25-40b	0.82±0.02	3910±99	1004±8	2715±163	921±39
25-40c	0.81±0.03	3995±49	1005±21	2925±106	993±25
25-40d	0.81±0.02	3640±156	904±39	2835±7	928±11
25-40e	0.82±0.01	3445±403	847±112	3035±191	1032±139
25-40f	0.82±0.01	4605±247	1165±63	2585±21	847±10
25-40g	0.84±0.04	3935±163	984±52	2560±71	832±32
REF	0.91±0.01	6895±247	1763±74	4785±586	1477±187

[a] Number of analytical replicates (n): density, n = 3; rheological parameters, n = 2. In the case of REF sample, mean and standard deviation values referred to two production replicates. G', elastic modulus; G'', viscous modulus.

Note: Adapted from Moriano et al. (2018).

Table 3.1.3: Quality characteristics (mean ± standard deviation values[a]) of reduced-fat biscuits formulated according to the Design of Experiment described in section 5.1. Results about the reference formulation made with butter (REF) are also reported.

Sample	Thickness (cm)	*L**	*a**	*b**	Milk absorption (g/100g)	Fracture strength (kPa)	Fracture strain (%)	Porosity (%)	Heterogeneity
0-0	1.00±0.03	70.5±2.2	1.6±0.1	31.7±4.2	41±2	102±10	7±1	14±1	0.27±0.04
50-0	1.20±0.02	60.3±1.3	7.5±0.9	36.9±0.4	41±3	258±36	10±3	23±3	0.15±0.04
0-80	1.23±0.08	83.0±1.2	-2.1±0.1	20.4±1.0	34±1	38±7	9±2	12±1	0.42±0.05
50-80	1.20±0.05	73.6±0.8	1.5±0.4	36.1±0.7	74±4	41±7	7±1	19±2	0.24±0.01
0-40	0.97±0.07	77.8±0.3	-0.8±0.1	29.1±0.1	41±2	41±7	5±1	18±2	0.16±0.01
50-40	1.28±0.07	67.7±1.2	5.1±0.6	37.8±0.4	67±2	70±7	8±1	19±2	0.25±0.09
25-0	1.13±0.01	60.9±0.3	6.1±0.8	36.0±0.5	39±1	144±20	10±2	19±1	0.09±0.01
25-80	1.24±0.08	74.7±1.0	0.7±0.2	33.9±0.5	57±3	36±6	6±1	17±2	0.30±0.07
25-40a	1.13±0.01	70.1±0.3	3.4±0.1	36.2±0.2	40±1	53±9	7±2	21±3	0.16±0.01
25-40b	1.06±0.04	68.7±1.1	4.5±0.4	37.0±1.4	49±2	50±10	5±1	19±3	0.30±0.01
25-40c	1.06±0.02	67.9±0.1	4.4±0.1	37.5±0.2	46±1	55±10	5±1	20±4	0.27±0.01
25-40d	1.09±0.05	69.1±0.9	4.1±0.6	37.4±0.3	44±1	49±8	5±1	19±7	0.14±0.01
25-40e	1.07±0.02	67.2±0.6	4.8±0.3	37.7±0.1	44±2	56±8	6±2	18±1	0.12±0.01
25-40f	1.10±0.03	67.6±1.0	4.0±0.1	37.1±0.8	45±2	56±7	6±1	23±2	0.16±0.01
25-40g	1.12±0.01	67.5±1.9	5.1±0.4	37.4±0.7	48±1	53±8	7±1	21±2	0.16±0.01
REF	0.96±0.01	70.6±0.1	1.4±0.2	35.4±0.4	43±1	154±10	9±1	15±2	0.16±0.04

[a] Number of analytical replicates (n): thickness and porosity, n = 2; *L**, *a**, *b**, n = 3; milk absorption, n = 5; fracture strength and strain, n = 15. In the case of REF sample, mean and standard deviation values referred to two production replicates.

Note: Adapted from Moriano et al. (2018).

Multiregression models

Multiregression models were computed for dough and biscuit characteristics, with the exception of biscuit thickness, for which no significant models were found. When non-significant terms were identified by ANOVA, a model simplification was applied, taking into account model hierarchy. Before modelling, some response variables required a mathematical transformation in order to fulfil normal distribution and skewness, as shown in Table 3.1.4, where also the modelling results are reported in terms of coded coefficients and figures of merit (determination coefficient R^2, adjusted R^2, predicted R^2, and p-value of lack of fit). All the calculated models resulted highly significant ($p<0.001$), with the exception of those for biscuit moisture, fracture strain, and porosity, which had R^2 values lower than 0.86 ($p < 0.05$). However, the lack of fit of these models was always not significant, meaning that the calculated response surfaces fitted adequately the design space.

Comparing coefficient values for coded factors, a similar influence of both SR and FR can be inferred. However, in the case of dough characteristics, a slight prevalence of SR effects can be observed. With higher SR, an increase in density and a decrease in rheological parameters were registered. The effect on density is easily explainable by the higher density of polydextrose with respect to fat. As far as rheological parameters, in order to make clearer the effect of the experimental factors, response surfaces are reported in Figures 3.1.2A-3.1.2D in terms of the original response variables (not transformed). The obtained results are due to the structuring effect of fat: decreasing the amount of shortening in the biscuit dough, a less compact material was obtained. In the case of G' and G'' extrapolated from FS curves, also the structuring effect of resistant starch is well visible: response surfaces showed a deep curvature, due to the significance of both main (b_2; $p<0.001$) and quadratic (b_{22}; $p < 0.01$) coefficients. The structuring effect of resistant starch is particularly evident in absence of polydextrose, probably due to the interaction of resistant starch with water, which increases both G' and G''. A similar effect was observed also by Laguna et al. (2014), who studied flour replacement by resistant starch in short-dough biscuits. On the contrary, when both shortening and flour are replaced at the highest levels, the biscuit dough is less firm and compact due to the lack of fat and proteins, which are main structural elements in a biscuit dough.

Table 3.1.4: Second-order polynomial model coefficient values (in terms of coded factors) and results of one-way analysis of variance for the Face-Centered Central Composite Design aimed at studying reduced-fat biscuits containing polydextrose and resistant starch.

	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2	Adj. R^2	Pred. R^2	LOF
Dough properties										
Density (g/mL)	0.832	0.049^{***}	0.033^{**}	-0.076^{***}		0.068^{***}	0.946^{***}	0.924	0.849	$0.486^{n.s.}$
1/√G' SS (1/√kPa)	0.51	0.19^{***}	-0.17^{***}	-0.04^{*}			0.986^{***}	0.982	0.963	$0.373^{n.s.}$
1/√G" SS (1/√kPa)	1.00	0.32^{***}	-0.24^{***}				0.960^{***}	0.952	0.913	$0.201^{n.s.}$
1/√G' FS (1/√kPa)	0.60	0.28^{***}	-0.12^{***}		0.13^{***}	-0.05^{**}	0.989^{***}	0.985	0.966	$0.119^{n.s.}$
1/√G" FS (1/√kPa)	1.04	0.47^{***}	-0.18^{***}		0.22^{***}	-0.11^{**}	0.990^{***}	0.986	0.976	$0.511^{n.s.}$
Biscuit properties										
Moisture	5.2	1.4^{**}	$-0.2^{n.s.}$	-1.0^{*}			0.738^{**}	0.667	0.548	$0.632^{n.s.}$
L*	68.2	-5.0^{***}	6.6^{***}		4.0^{***}		0.982^{***}	0.977	0.972	$0.873^{n.s.}$
√a*+2.343	2.57	0.69^{***}	-0.65^{***}		-0.53^{***}	-0.18^{*}	0.980^{***}	0.972	0.944	$0.305^{n.s.}$
b*^3	51101	14396^{***}	-5703^{***}	5099^{**}	-10390^{***}	-6973^{**}	0.981^{***}	0.971	0.914	$0.262^{n.s.}$
Milk absorption (%)	47	9^{***}	7^{***}	10^{***}			0.919^{***}	0.895	0.807	$0.373^{n.s.}$
Log Fracture strength (Log kPa)	1.73	0.11^{***}	-0.30^{***}	-0.09^{***}		0.16^{***}	0.992^{***}	0.989	0.970	$0.338^{n.s.}$
Fracture strain (%)	5.8	1.6^{**}	-1.8^{**}	-2.7^{***}	1.1^{*}	2.4^{***}	0.859^{**}	0.771	0.678	$0.804^{n.s.}$
Porosity^2.65 (%)	2489	588^{*}	-683^{*}				0.658^{**}	0.589	0.400	$0.299^{n.s.}$
Heterogeneity	0.15	-0.03^{*}	0.11^{***}	0.03^{*}	0.13^{***}	0.05^{**}	0.987^{***}	0.978	0.964	$0.742^{n.s.}$

SR, shortening reduction; FR, flour replacement; R^2, coefficient of determination; Adj. R^2, adjusted R^2; Pred. R^2, predicted R^2; LOF, lack of fit (p-value); G', elastic modulus; G", viscous modulus; SS, strain sweep test; FS, frequency sweep test.

Significance levels: $^{n.s.}$ not significant; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

Note: Adapted from Moriano et al. (2018).

Biscuit moisture was mainly affected by SR. In particular, as evidenced by the highly significant linear term b_1 ($p<0.001$), increasing the amount of polydextrose increased the moisture level, confirming the high water retention capacity of this ingredient.

The significance ($p<0.001$) of both linear terms (b_1 and b_2) for lightness (L^*) indicated a strong influence of the studied factors on this colour parameter: SR produced a decrease in L^*, while FR implied an increase. The high significance of the b_{11} quadratic term ($p<0.001$) suggests that the decrease due to fat substitution is not linear. An opposite tendency was evidenced for the other colour parameters: SR significantly increased redness (a^*) and yellowness (b^*) ($p<0.001$), while FR tended to decrease these parameters ($p<0.001$). Similarly, also for a^* and b^* the effect is not only linear, being the quadratic terms b_{11} and b_{22} significant, as well as the interaction coefficient b_{12} for b^*. Results about colour are related to the effect of polydextrose and resistant starch on Maillard reaction products, as already commented. On the contrary, the partial substitution of flour with resistant starch causes a protein dilution effect, contributing to a reduction in Maillard reaction (Laguna et al., 2011).

Milk absorption was significantly ($p<0.001$) increased by both SR and FR, with a further highly significant increasing effect of the simultaneous presence of the two fat mimetics (significance of b_{12}, $p<0.001$). Actually, the highest milk absorption values can be obtained with the highest SR and FR values, as shown in Figure 3.1.2E. As already highlighted, this result is related to the high hydrophilicity of the carbohydrate-based fat mimetics.

Models obtained for fracture strength and strain revealed that fat reduction gave harder biscuits, less brittle. This is due to the lack of the shortening effect of fat on the gluten network, as already reported by other authors (Chugh et al., 2013; Laguna et al., 2014; Mieszkowska and Marzec, 2016; Sudha et al., 2007; Zoulias et al., 2002). In fact, as shown in Figure 3.1.2F, the hardest biscuits were obtained with the lowest fat content and the standard flour content (sample 50-0). In this sample, gluten network is probably as strength as possible since there is little amount of fat interfering with its formation. Interestingly, the response surface shows also as the presence of resistant starch had a structuring effect. Actually, for FR higher than 40%, a similar fracture strength can be obtained notwithstanding the SR percentage, meaning that resistant starch is able to mimic the texture effect of fat. The hardness-reducing effect of resistant starch in low-fat biscuits has been reported also by Laguna et al. (2011, 2012). The model calculated for porosity suggests that polydextrose favours a higher incorporation of air during dough preparation, as reported also by Kocer et al. (2007) for cake batters.

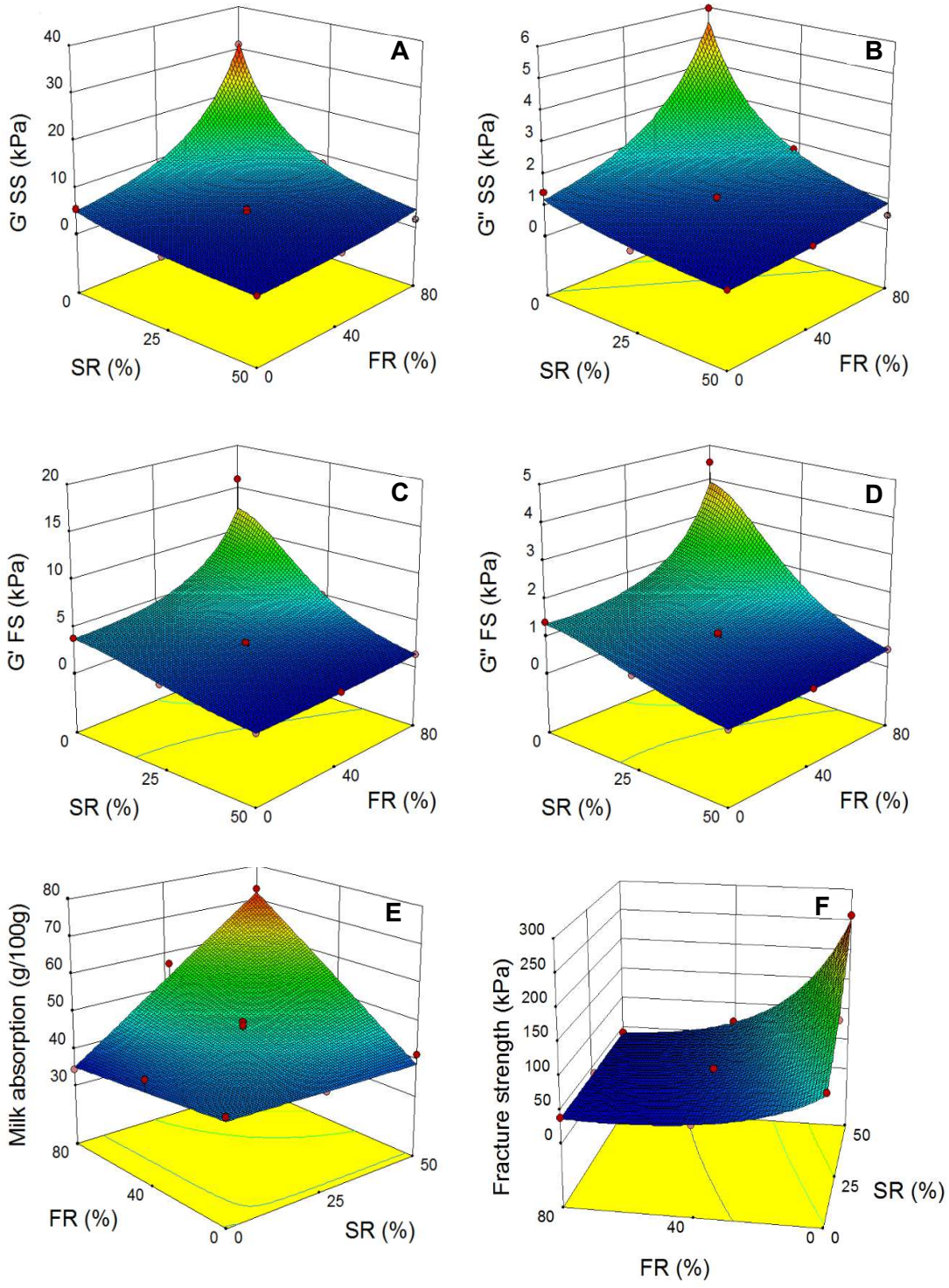

Figure 3.1.2: Response surfaces for reduced-fat dough and biscuit characteristics: dough elastic and viscous moduli extrapolated from strain sweep (A-B) and frequency sweep (C-D) curves; biscuit milk absorption (E); biscuit fracture strength (F). SR, shortening reduction; FR, flour replacement; G', elastic modulus; G'', viscous modulus; SS, strain sweep test; FS, frequency sweep test.

Note: Adapted from Moriano et al. (2018).

Both the fat mimetics had a significant ($p < 0.05$) effect on biscuit bottom porosity, which increased with the addition of polydextrose and decreased with the increase of resistant starch.

The surface aspect of biscuits was mainly influenced by the level of FR even if also SR had an effect, being the interaction and quadratic coefficients significant. The response surface presented a curvature, with a saddle area for SR of 25-40% and FR of 0-16%. In this area, the biscuit surface appears smoother, due to a stronger liquid-like behaviour of dough. In fact, in this experimental range also the viscoelastic moduli resulted to be at their lowest.

Optimization of the reduced-fat biscuit formulation

Models developed for biscuit quality characteristics were used to optimize the reduced-fat formulation by means of a desirability function with the following constraints: 25-50% SR; 41-44 g/100g milk absorption; 100-160 kPa fracture strength. The optimization criteria were chosen in order to obtain a reduced-fat biscuit with good structural properties, similar to those of the full-fat biscuits produced with shortening or butter (sample 0-0 and REF). Thus, SR was imposed at the higher levels, while milk absorption and fracture strength were considered as important quality features. Their ranges were set based on the results obtained for samples 0-0 and REF. The same level of importance was given to all the constraints and a linear desirability function was used (weight = 1). Among the five different solutions having the maximum desirability value (i.e. 1), the formulation with the highest SR was chosen and produced in duplicate to confirm the optimization calculation. The optimized formulation had 46.3% SR and 12.5% FR, thus reaching a fat content of 17.54 ± 0.05 g/100g on the final product. The complete recipe was as follows: 12.38 g/100g shortening, 10.69 g/100g polydextrose, 33.64 g/100g wheat flour, 4.81 g/100g resistant starch, 11.54 g/100g sucrose, 1.23 g/100g leavening agent, 0.08 g/100g salt, and 25.63 g/100g water. Experimental results about the most important characteristics were in good agreement with the predicted values: L^*, 62.5 ± 0.1 vs. 62.2 ± 0.9; a^*, 6.3 ± 0.1 vs. 7.5 ± 0.7; b^*, 37.0 ± 0.1 vs. 37.7 ± 0.9; milk absorption, 43 ± 1 g/100g vs. 43 ± 0.3 g/100g; fracture strain, 8 ± 1% vs. 9 ± 1%. The only exception was represented by fracture strength (108 ± 7 kPa vs. 145 ± 8 kPa), which resulted slightly outside the 95% confidence interval of the predicted value (129-162 kPa), even if in range with the constraints set for the numerical optimization.

Conclusions

In conclusion, the work demonstrated the suitability of Design of Experiments techniques, Response Surface Methodology and desirability function for the multivariate study of the effects of carbohydrate-based fat mimetics on reduced-fat biscuits. In particular, polydextrose and resistant starch were used as shortening replacer and structuring ingredient, respectively. The application of a multiple response optimization enabled the development of a reduced-fat biscuit (fat = 17.54 ±

0.05 g/100g) with structural characteristics similar to those of a standard full-fat biscuit prepared with shortening (fat = 31.1 ± 0.4 g/100g) or butter (fat = 31.4 ± 0.3 g/100g).

According to the Reg. (EC) No. 1924/2006, the product can be claimed as "light" or "with reduced fat content", being the fat reduction higher than 30% (about 44%). Moreover, the use of polydextrose and resistant starch in the biscuit recipe, besides the structuring effect, provided also a high dietary fiber content (about 19.7 g/100g based on theoretical calculation), allowing the use of the "high fiber" claim, reserved to products with at least 6 g/100g fiber. These nutritional claims represent a further benefit for the developed low-fat biscuit.

Experimental part 2: Double emulsions for food applications: An optimization approach using D-optimal design.

The aim of this work was to develop and characterize double emulsion ($W_1/O/W_2$) systems through D-optimal Design of Experiments. A double emulsion to be used in biscuits as shortening replacer was then optimized based on viscosity and yield.

***Abbreviation list**:* Adj. R^2, adjusted determination coefficient; ANOVA, analysis of variance; b_0, constant value in the polynomial model; b_1 linear coefficient for the concentration of proteins in W_1 in the polynomial model; b_2, linear coefficient for the volume of W_1 in W_1/O in the polynomial model; b_3, linear coefficient for the volume of W_1/O in $W_1/O/W_2$ in the polynomial model; b_4, linear coefficient for the W_1 protein type in the polynomial model; b_{12}, b_{23}, b_{34} interaction coefficient in the polynomial model; DoE, Design of Experiment; EW, egg-white; FT-IR, Fourier-transform infrared spectroscopy; K, consistency coefficient; LOF, lack of fit (p-value); MIR, mid-infrared spectroscopy; n, flow behaviour index; O, oil; PCA, principal component analysis; PC, principal component; Pred. R^2, predicted determination coefficient; R^2, determination coefficient; W_1, internal water phase; W_2, external water phase; W_1/O, primary emulsion; $W_1/O/W_2$, double emulsion; WP, whey proteins; X_1, value of concentration of proteins in W_1 in the polynomial model; X_2, value of in the volume of W_1 in W_1/O in the polynomial model; X_3, value of the volume of W_1/O in $W_1/O/W_2$ in the polynomial model; X_4, value of the W_1 protein type in the polynomial model; y, response variable value in the polynomial model; ε random error in the polynomial model.

Theoretical composition of double emulsions

Preliminary trials were performed to define the proper formulation and procedures for the double emulsion preparation and the variation ranges of the experimental factors. Optical microscopy showed the compartmented structure of double emulsions (Figure 3.1.3) consisting of relatively large oil droplets (of different sizes), with finely dispersed smaller water droplets inside, giving a visual impression of homogeneity. Table 3.1.5 reports the experimental matrix and the theoretical composition of the experimental emulsions in terms of water, protein and oil content. Sample denomination was based on the experimental factors: type of protein used to gel the inner water phase W_1 (EW or WP), concentration of protein (0, 5, 10 g/100g), percentage of W_1 in the primary emulsion W_1/O (20, 30, 40 mL/100mL) and percentage of W_1/O in the final double emulsion $W_1/O/W_2$ (40, 50, 60 mL/100mL). As expected, the lowest percentage of W_1 in W_1/O (20%) and the highest W_1/O in $W_1/O/W_2$ corresponded to the maximum oil content (48 g/100g), while increasing levels of W_1 and decreasing levels of W_1/O implied a progressive decrease in fat content, until reaching the minimum (24 g/100g) with the combination W_1 40% - W_1/O 40%. The minimum protein content (1.9 g/100g) was obtained for samples with no protein addition in W_1: in this case

the only protein contribution came from the egg white (EW) or whey proteins (WP) dispersed in the external water phase W_2.

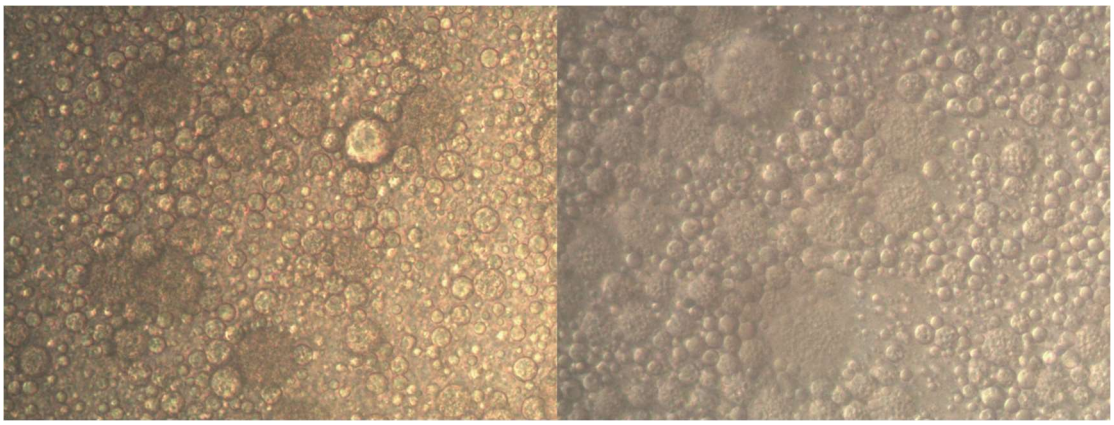

Figure 3.1.3: Optical micrographs (40x magnification) of double emulsions produced as preliminary trials

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Table 3.1.5: D-optimal Design matrix developed to investigate the effect of protein type, protein concentration in the internal water phase W_1, volume of W_1 in W_1/O and volume of W_1/O in $W_1/O/W_2$ on the properties of double emulsion systems: sample identification, factor levels and theoretical composition of experimental emulsions.

	Experimental factors				Theoretical composition		
Sample	**Protein type**	**Protein concentration in W_1 (g/100g)**	**W_1 in W_1/O (mL/100mL)**	**W_1/O in $W_1/O/W_2$ (mL/100mL)**	**Water (g/100g)**	**Proteins (g/100g)**	**Oil (g/100g)**
EW_00_40_40	EW	0	40	40	73.1	2.9	24
EW_10_30_40	EW	10	30	40	68.1	3.9	28
WP_05_40_40	WP	5	40	40	72.4	3.6	24
WP_10_20_60	WP	10	20	60	49.0	3.0	48
WP_00_20_60	WP	0	20	60	50.1	1.9	48
WP_00_40_60	WP	0	40	60	62.1	1.9	36
EW_05_40_50	EW	5	40	50	66.7	3.3	30
WP_05_30_50	WP	5	30	50	61.9	3.1	35
WP_05_30_50	WP	5	30	50	61.9	3.1	35
WP_10_20_40	WP	10	20	40	64.4	3.6	32
EW_00_20_40	EW	0	20	40	65.1	2.9	32
WP_10_30_50	WP	10	30	50	61.3	3.7	35
EW_10_30_60	EW	10	30	60	54.5	3.5	42
EW_00_40_60	EW	0	40	60	62.1	1.9	36
EW_10_20_50	EW	10	20	50	56.7	3.3	40
WP_05_30_50	WP	5	30	50	61.9	3.1	35
WP_05_40_60	WP	5	40	60	61.0	3.0	36
EW_05_30_40	EW	5	30	40	68.6	3.4	28
WP_00_20_40	WP	0	20	40	65.1	2.9	32
WP_00_40_40	WP	0	40	40	73.1	2.9	24
EW_00_20_60	EW	0	20	60	50.1	1.9	48

Characteristics of double emulsions

Yield, creaming stability and rheological properties were measured to characterize double emulsions (Table 3.1.6). To investigate the stability of the inner water phase remaining inside the oil droplets of multiple emulsion samples, expressed as yield, spectroscopic measurements were performed. All the samples showed high yields (> 95%): no differences were observed between emulsions containing EW or WP. Sample EW_00_40_60 showed the same yield as WP_00_40_60 (99.45±0.02% vs 99.45±0.06%): the two emulsions were formulated with the same amounts of proteins in the W_2 phase, water and oil, but with a different protein type. Perez-Moral et al (Perez-Moral et al., 2014) claimed that the lipophilic emulsifier and the gelling of the internal water phase play a major role in ensuring high water encapsulation efficiency In the present study, all the samples were formulated with PGPR that, in the right concentration (above 1%), is able to induce a steric stabilization of the interfacial layer (Márquez et al., 2010). In addition, the structuring effect of proteins on the internal water phase may strongly reduce migration and instability phenomena impacting encapsulation efficiency (Dickinson, 2011; Surh et al., 2007). However, in this experiment, the presence of proteins into W_1 did not produce yield improvements.

Apparent viscosity and consistency coefficient (K) showed a comparable trend, with samples containing 60% W_1/O resulting in the highest values. Emulsion containing EW were characterized by flow behavior indexes (n) ranging from 0.357±0.061 to 0.905±0.025, indicating a non-Newtonian pseudoplastic behavior ($n < 1$) of different intensity. On the contrary, WP samples showed a range between 0.935±0.001 and 1.105±0.003, revealing a more similar Newtonian behavior. Shear thinning behavior of EW emulsions may be connected to the presence of aggregated droplets (Panagopoulou et al., 2017), i.e. flocculated droplets, which are deformed and disrupted as the shear rate increased (Demetriades et al., 1997). The results obtained in this experiment may suggest that the differences observed between the protein types is linked to their ability to interact with other components and create a homogenously dispersed phase.

Creaming stability is of paramount importance for double emulsions intended as alternative fats in food formulation, since they have to be prepared beforehand and stored until use. In this experiment, a chilled storage of 24 h was performed as the most suitable for such an ingredient. All the experimental samples showed no creaming 1 h after production (data not shown), while, after 24 h, samples with the lowest W_1/O in W_1/O/W_2 (40%) resulted in the lowest stability (<80%), independently of the protein type, except for sample WP_00_20_40 (88±1%). In particular, the highest water content (about 70g/100g) due to the combination of the highest level of W_1 in W_1/O and the lowest level of W_1/O in W_1/O/W_2, produced a stability lower than 50% for samples EW_00_40_40, WP_05_40_40, WP_00_40_40, suggesting the unsuitability of these emulsions to be used as ingredients after storage.

Table 3.1.6: Characteristics of double emulsions (mean ± standard deviation values[a]) formulated according to the D-Optimal design described in section 5.1. See Table 5.1.2 for sample identification and composition.

[a] Number of replicates: yield: n=8; apparent viscosity, K, n and stability: n=2.

Sample	Yield (%)	Apparent viscosity (mPa*s^n)	K (mPa*s^n)	n	Stability (%)
EW_00_40_40	99.23 ± 0.03	6.76 ± 0.12	16.38 ± 1.06	0.847 ± 0.008	50 ± 1
EW_10_30_40	98.84 ± 0.06	13.70 ± 0.42	43.77 ± 5.14	0.809 ± 0.016	73 ± 1
WP_05_40_40	98.39 ± 0.13	4.29 ± 0.02	2.36 ± 0.05	1.105 ± 0.003	40 ± 1
WP_10_20_60	98.53 ± 0.05	32.70 ± 0.28	47.16 ± 0.42	0.935 ± 0.001	80 ± 1
WP_00_20_60	99.31 ± 0.06	28.65 ± 0.49	39.71 ± 1.78	0.943 ± 0.005	100 ± 1
WP_00_40_60	99.45 ± 0.06	12.98 ± 0.30	13.11 ± 0.45	0.999 ± 0.002	80 ± 1
EW_05_40_50	98.69 ± 0.05	9.89 ± 0.11	17.46 ± 0.34	0.900 ± 0.001	80 ± 1
WP_05_30_50	98.88 ± 0.05	9.63 ± 0.32	7.45 ± 0.18	1.045 ± 0.001	100 ± 1
WP_05_30_50	99.16 ± 0.18	11.05 ± 0.21	9.17 ± 0.21	1.032 ± 0.001	89 ± 1
WP_10_20_40	96.60 ± 0.11	9.05 ± 0.13	6.95 ± 0.17	1.046 ± 0.001	75 ± 1
EW_00_20_40	97.23 ± 0.04	8.59 ± 0.27	19.60 ± 2.68	0.857 ± 0.018	60 ± 1
WP_10_30_50	98.74 ± 0.10	10.97 ± 0.35	8.26 ± 0.37	1.050 ± 0.002	89± 1
EW_10_30_60	99.30 ± 0.02	63.06 ± 3.69	3089 ± 440	0.357 ± 0.061	100 ± 1
EW_00_40_60	99.45 ± 0.02	18.06 ± 0.27	85.35 ± 0.77	0.731 ± 0.001	90 ± 1
EW_10_20_50	95.47 ± 0.08	22.17 ± 0.94	142.42 ± 12.84	0.677 ± 0.008	90 ± 1
WP_05_30_50	98.79 ± 0.03	11.43 ± 1.17	8.56 ± 0.55	1.039 ± 0.005	90 ± 1
WP_05_40_60	99.21 ± 0.02	9.73 ± 0.24	7.35 ± 0.23	1.049 ± 0.001	100 ± 1
EW_05_30_40	97.62 ± 0.02	7.46 ± 0.35	12.97 ± 2.46	0.905 ± 0.025	63 ± 4
WP_00_20_40	95.83 ± 0.23	5.23 ± 0.26	3.28 ± 0.19	1.082 ± 0.002	88 ± 1
WP_00_40_40	99.01 ± 0.06	3.76 ± 0.01	2.54 ± 0.01	1.103 ± 0.001	47 ± 1
EW_00_20_60	98.61 ± 0.05	43.12 ± 0.18	545.21 ± 1.70	0.559 ± 0.001	100 ± 1

FT-IR spectroscopy analysis

Figure 3.1.4 shows FT-IR spectra of double emulsion samples after spectral interval reduction, performed to eliminate the less informative regions and the absorption band of CO_2. The considered spectral ranges were between 3728 and 2754 cm^{-1} and between 2272 and 719 cm^{-1}. The figure also shows spectra of corn oil and solutions of EW and WP (5 and 10%). A principal component analysis (PCA) was applied to the reduced raw spectra, in order to identify possible sample patterns and to observe the contribution of each variable. Figure 3.1.5 shows the PC1 vs PC2 score plot and the related loading plots. Emulsion samples tend to differentiate along PC1 depending on the type of protein used. Loadings reveal that the most influent variables in the differentiation of samples along PC1 corresponded to the absorption peaks of water OH bond (3500-3000 cm^{-1}) and vegetable oils, as reported in table 3.1.7. It can be therefore assumed that the differentiation of emulsion samples based on the types of proteins is linked to a different interaction of the proteins themselves with oil and water phases.

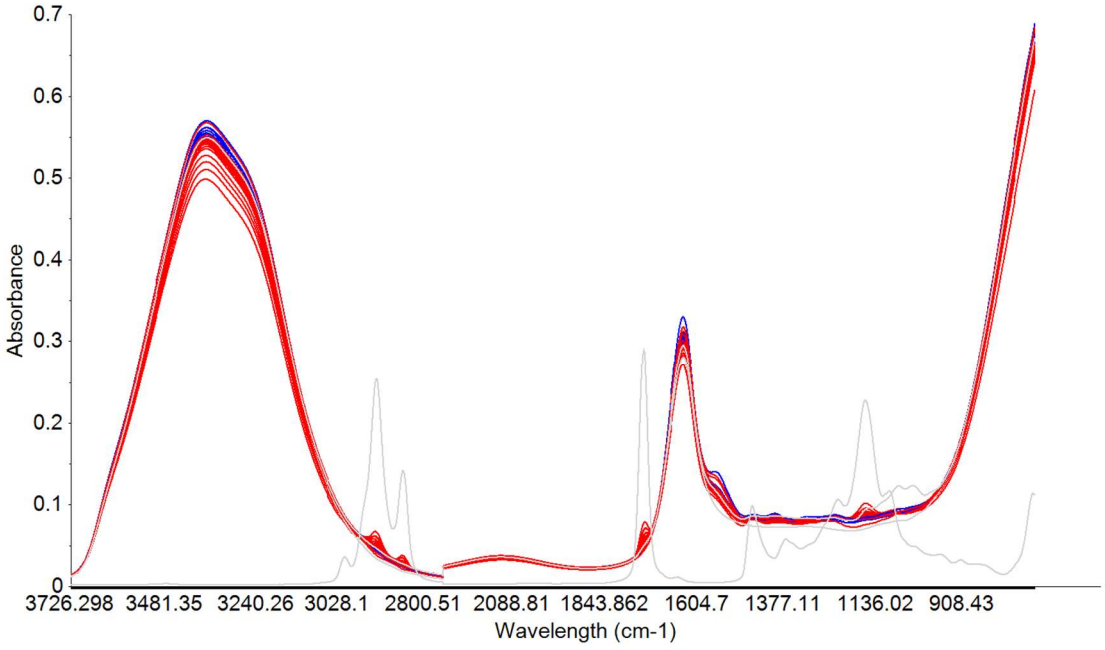

Figure 3.1.4: reduced FT-IR spectra of double emulsion samples (red); spectra of corn oil (gray) and solutions (5-10%) of egg white and whey protein powders (blue) are also reported.

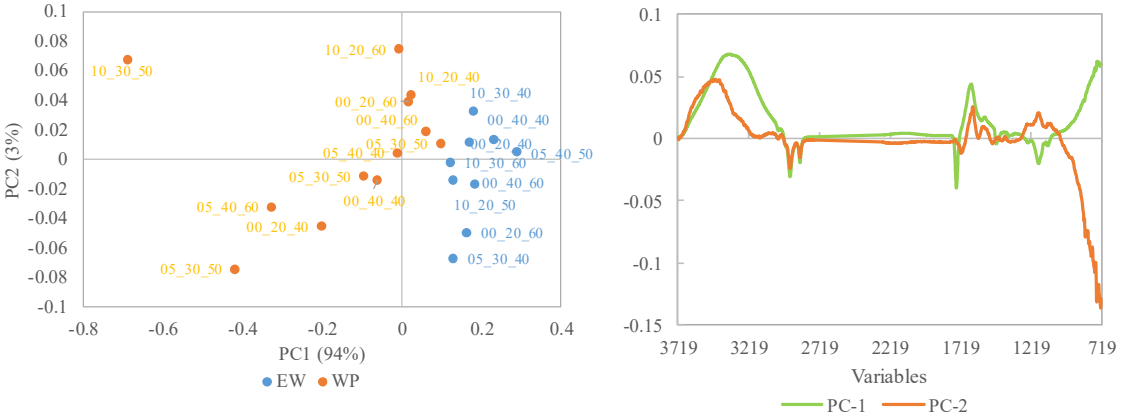

Figure 3.1.5: PC1 vs PC2 score plot and related loading plots obtained from the PCA analysis of the FT-IR spectra of the double emulsions samples.

Table 3.1.7: wavenumber (cm^{-1}), functional groups, vibration mode and peak intensity for vegetable oils analyzed by MIR spectroscopy.

Wavenumber (cm^{-1})	Functional group	Vibration mode	Intensity	Reference
2925	Acyl group CH_2	Stretching asym	vst	Guillén and Cabo (1997)
2856	Acyl group CH_2	Stretching sym	vst	Guillén and Cabo (1997)
1745	Carbonilic ester C=O	Stretching	vst	Jolayemi et al. (2017)
1643	-C=C- (cis-)	Stretching	vw	Guillén and Cabo (1997)
1163	$-CO_2$, $-CH_2$,	Stretching, bending	st	Guillén and Cabo (1997)

vst, *very strong*; st, *strong*; vw, *very weak*

In a following step, a smoothing pre-treatment was applied to the spectral data using the Savitzky-Golay algorithm (11 smoothing points), in order to reduce the noise influencing data, and two separate PCA were applied to spectral data of samples containing EW or WP (Figure 3.1.6 and 3.1.7). In the PC1 vs PC4 score plot (Figure 3.1.6A) a differentiation between samples with a protein:oil concentration ratio in the final emulsion greater or less than 0.1 can be observed. In addition, Figure 3.1.6 B shows that on the PC3 vs PC6 score plot, despite the small percentage of explained variance, a pattern of the double emulsion samples as a function of protein concentration in the internal aqueous phase can be recognized. In the PC4 vs PC6 score plot (Figure 3.1.6C) samples are arranged according to the primary emulsion content (% W_1/O in $W_1/O/W_2$). The same principal components also reveal a distinction of samples based on fat content (Figure 3.1.6D): samples containing less than 30% fat are placed towards high values of PC4; samples containing between 30% and 40% fat have low PC4 values and high PC6 values; finally, samples containing more than 40% fat are placed at low PC6 values.

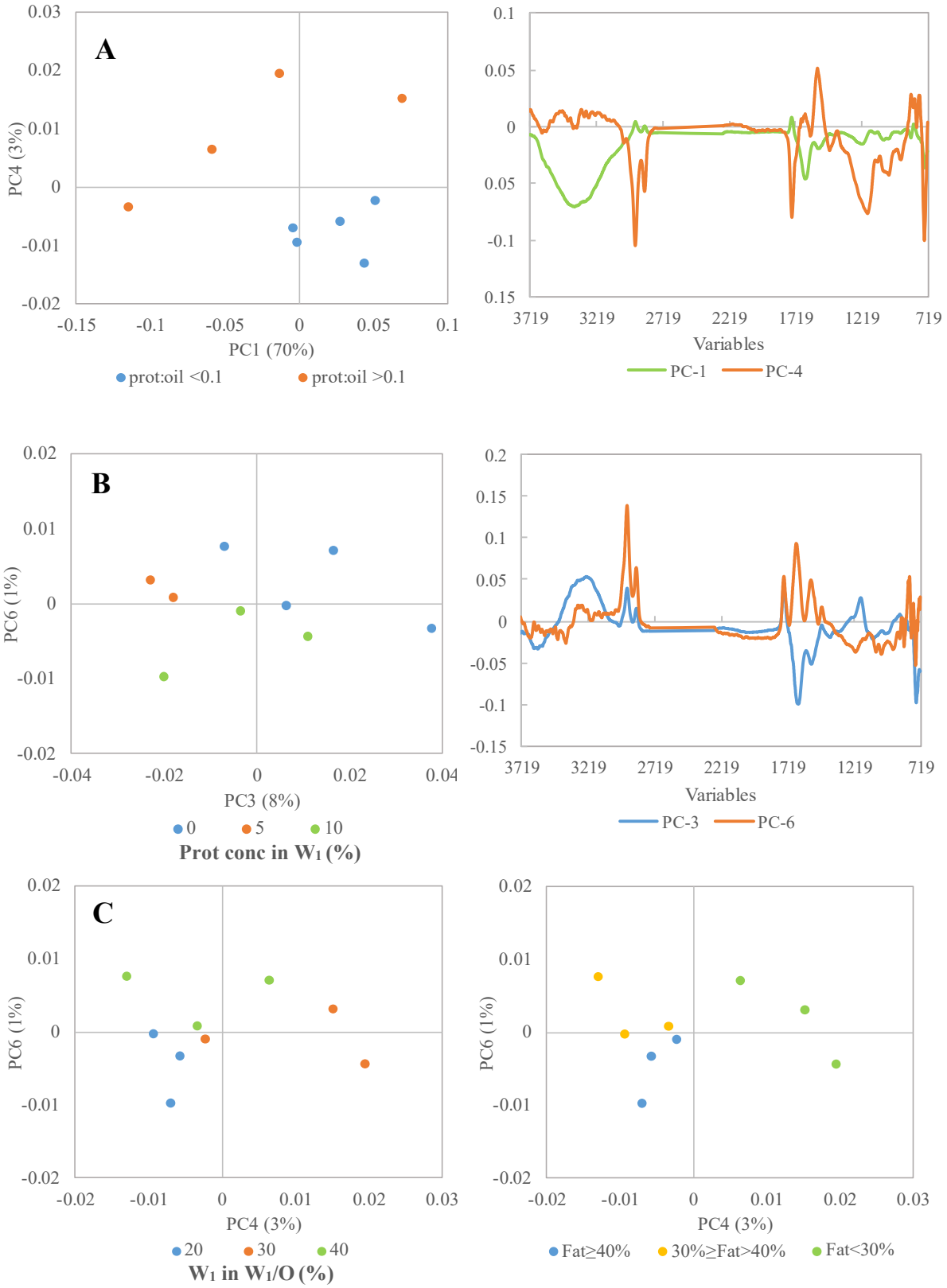

Figure 3.1.6: score plots and related loading plots obtained from PCA applied to FT-IR spectra of double emulsions containing egg white powder (EW).

As far as samples containing WP are concerned (Figure 3.1.7), samples were distinguished only for the protein concentration in the internal aqueous phase W_1. In this case the PCs involved (PC2 and PC3) contribute to explaining a small amount of variance in the system (6%).

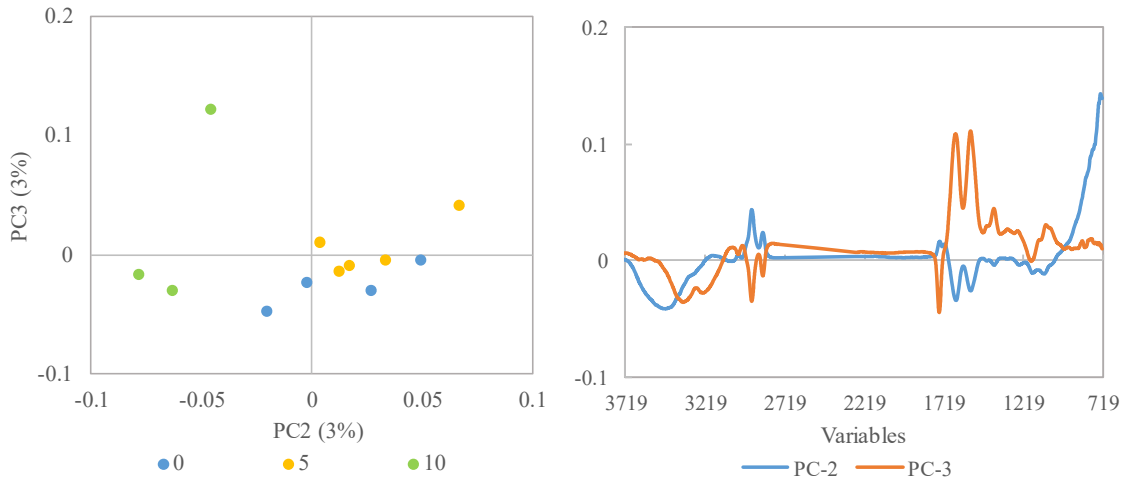

Figure 3.1.7: PC2 vs PC3 score plot and related loading plots obtained from PCA applied to FT-IR spectra of double emulsions containing whey protein powder (WP). Colors in the score plot identify different protein concentrations (g/100g).

Since the PCA applied to the FT-IR spectra of the WP-containing double emulsions did not give useful information for the characterization of the samples, the investigation was focused on more limited spectral ranges, characterized by a greater variation for the samples under investigation. In particular, the range between 1800-1660 cm^{-1} was chosen. Furthermore, it was decided to exclude from the analysis a replica of the sample WP_05_30_50 identified as an outlier. Figure 3.1.8 shows the result of the PCA in the range 1800-1660 cm^{-1}. In the PC2 - PC3 score plot, the samples are grouped based on the protein: water concentration in the final emulsion.

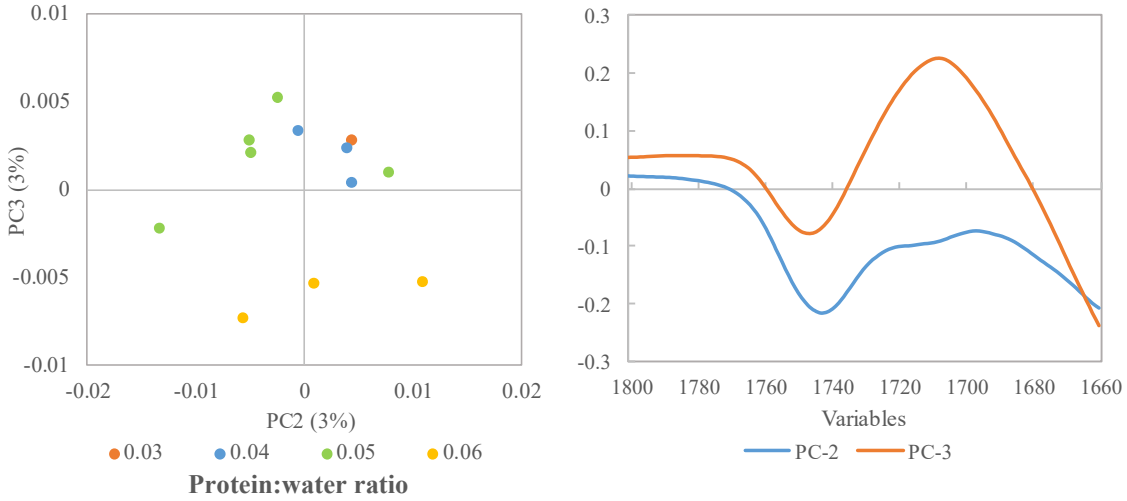

Figure 3.1.8: PC2 vs PC3 score plot and related loading plots obtained from PCA applied in the 1800 - 1660 cm^{-1} range of FT-IR spectra of double emulsions containing whey protein powder (WP).

In conclusion, FT-IR analysis resulted a powerful tool to highlight differences among double emulsions samples. Data showed that whey proteins have a greater interaction with water and oil phases, not found for egg white proteins.

Multiregression models

Multiregression models were computed for yield, apparent viscosity at 310 s^{-1}, K, n, and creaming stability. In order to simplify models, when non-significant effects were identified by ANOVA, they were excluded, respecting model hierarchy. Before modelling, some response variables required a mathematical transformation in order to fulfil normal distribution and skewness criteria; in particular, apparent viscosity was transformed with inverse square root, while K was $\log_{10}$ transformed. Table 3.1.8 reports the modelling results in terms of coded coefficients and figures of merit (determination coefficient R^2, adjusted R^2, predicted R^2, adequate precision, and p-value of lack of fit). The calculated models resulted highly significant ($p<0.001$); rheology variables resulted in models with determination coefficients higher than 0.90, while yield and stability had R^2 around 0.80. The lack of fit of the models was always not significant, except for the model for n ($p<0.01$), meaning that the response surface did not fit adequately the experimental domain. Samples EW_10_30_60 and EW_00_20_60 were excluded from K and n model calculations because they resulted outliers.

Observing coefficient values, W_1 and W_1/O appeared to be the most influent factors. In particular, for yield model, coefficients related to these terms were positive and highly significant ($p<0.001$), revealing a positive effect of increased W_1 and W_1/O on the retaining ability of internal water by the double emulsion. This effect can be observed in Figure 3.1.9A: response surface is characterized by a slight curvature, due to the significance ($p<0.05$) of the interaction term between W_1 and W_1/O (b_{23}). This means that the effect of W_1/O on yield increase is more accentuated working at low level of W_1 (20%) than at the high level. In this case, nor protein concentration or type showed to have significant effect, contrarily to what reported by Opperman et al. (2015) and Perez – Moral et al. (2014), that found an increase in double emulsion yield with the gelation of the inner water phase W_1. The observed difference may be due to the fact, the cited studies considered a standard double emulsion formulation (Opperman et al., 2015: 30% W_1, 30%W_1/O; Perez-Moral et al., 2014: 40%W_1, 27%W_1/O) without investigating the variability associated to the variation of relative amounts of oil and water as we did in this study. Thus, in our case the effect of this variation on yield is more marked than the effect of gelling W_1. In addition, we used proteins (5 g/100g) also to the external water phase, in order to further reduce water mobility in the system: this may have leveled the effect of internal water gelation.

Viscosity results may be very interesting for fat reduction strategies since linked both to air incorporation ability of the system (Márquez and Wagner, 2010) and to sensory perception of

emulsion creaminess (Akhtar et al., 2005; van Aken et al., 2011). Apparent viscosity was significantly influenced by all the factors, with a tendency to increase at higher concentrations of protein in W_1 ($p<0.001$) and of W_1/O ($p<0.001$), and at lower percentages of W_1 (Figure 3.1.9B). The effect of protein concentration appears in agreement with the findings of Opperman et al. (2016), that demonstrated that gelation of W_1 allowed to obtain viscosity levels higher than those of single oil in water emulsions, despite the lower oil content. In addition, they found that all the fat-related mouth-feel and after-feel attributes increased in gelled samples, balancing the effects of fat reduction, and revealing an interesting potential of internal water gelation for sensory optimization of $W_1/O/W_2$ systems. In this case, also the protein type resulted significant ($p<0.01$), demonstrating EW to be a better structuring agent for the final emulsion. Similarly, protein type and percentage of W_1/O had a strong positive effect ($p<0.001$) on K (Figure 3.1.9 C-D), with EW-containing samples showing the highest values. On the other hand, W_1 showed an opposite effect ($p<0.001$), leading to a decrease of K at increasing levels. Protein concentration in W_1 was involved in significant interactions both with W_1 (b_{12}, $p<0.05$) and W_1/O (b_{13}, $p<0.05$). In fact, at high protein concentration (10%), K tended to a reduction with the decrease of W_1 more marked than at low protein concentration (0-5%). Similarly, at high (10%) and intermediate (5%) levels of protein concentration, the increase of K while increasing W_1/O was less marked than at 0% proteins. The effect reversed when considering the low level of W_1, because of the significant interaction b_{23} ($p<0.05$). More focused investigation may be carried out on air incorporation ability and sensory perception by consumers, in order to exploit the possibility to use viscosity multiregression models obtained in this study for the prediction of structuring and sensory properties of double emulsion systems. Flow behaviour index was strongly influenced by the protein type ($p<0.001$) (Figure 3.1.9E): WP-containing emulsions revealed a quite Newtonian behaviour ($n\approx1$), while EW-containing emulsions resulted to be mostly shear thinning ($n<1$). W_1 showed a direct effect ($p<0.05$) on n, while W_1/O resulted in an opposite effect ($p<0.01$): the less the W_1/O percentage, the higher the n values. Creaming stability was significantly ($p<0.001$) improved as the percentage of W_1/O increased, while an inverse significant effect ($p<0.01$) was found for the percentage of W_1 (Figure 3.1.9F). These findings were in agreement with previous studies, demonstrating that 20–30% of dispersed phase (W_1) to continuous phase (O), in primary emulsions was the optimum phase volume ratio in terms of stability (Su et al. 2006). As reported above for yield, also for stability we did not observe a significant effect of the internal water phase gelling, confirming that the other factors had a stronger effect on the system.

To our knowledge, no study in literature deals with a systematic and comprehensive approach that include all the factors considered in this research. Thus, the obtained multiregression models may represent a useful starting point for the development of double emulsion systems designed for targeted purposes.

Table 3.1.8: Model coefficient values (in terms of coded factors) and significance levels for the D-optimal design developed to study double emulsions.

R^2, coefficient of determination; Adj. R^2, adjusted R^2; Pred. R^2, predicted R^2; LOF, lack of fit (p-value).

Response variables	b_0	b_1 Protein concentration in W_1 (g/100mL)	b_2 W_1 in W_1/O (mL/100mL)	b_3 W_1/O in $W_1/O/W_2$ (mL/100mL)	b_4 Protein type	b_{12}	b_{13}	b_{23}	R^2	Adj. R^2	Pred. R^2	Adequate precision	LOF (p-value)
Yield (%)	98.57	0.079	0.68^{***}	0.65^{***}	0.069			-0.44^{*}	0.791^{***}	0.723	0.561	9.873	0.114^{ns}
1/√Apparent viscosity (1/√mPa*s)	0.29	-0.026^{**}	0.041^{***}	-0.09^{***}	-0.034^{***}				0.934^{***}	0.920	0.882	24.97	0.201^{ns}
Log K (Log (mPa s^n))	1.3	5.14E-004	-0.29^{***}	0.36^{***}	0.39^{***}	-0.13^{*}	-0.11^{*}	-0.11^{*}	0.967^{***}	0.946	0.871	23.55	0.127^{ns}
n	0.91	-7.20E-003	0.034*	-0.059^{**}	-0.12^{***}				0.915^{***}	0.891	0.836	18.24	0.010*
Stability (%)	83.07	4.92	-9.29^{**}	18.09^{***}	-2.04				0.806^{***}	0.751	0.652	12.46	0.298^{ns}

Significance levels: $^{n.s.}$ not significant; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

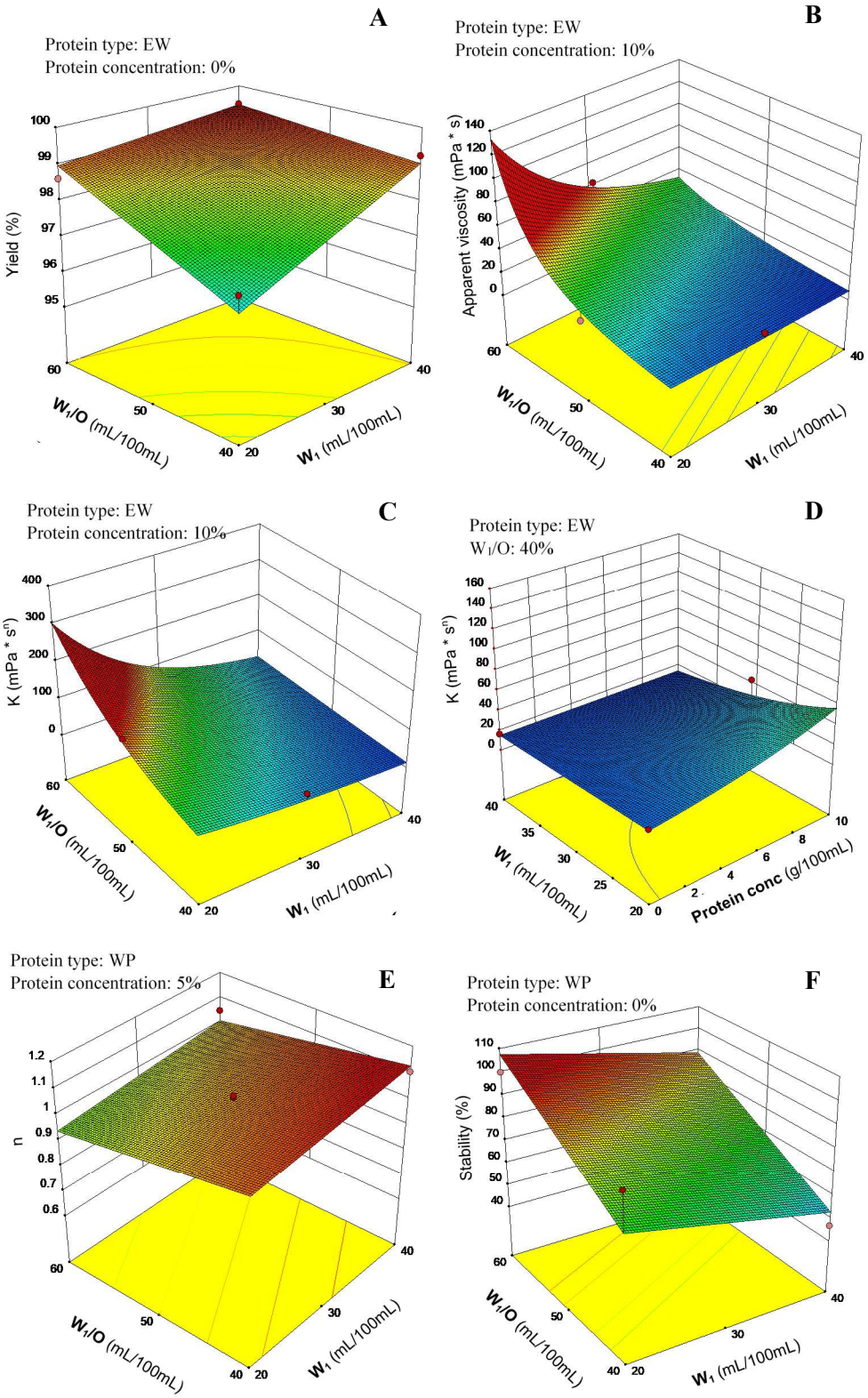

Figure 3.1.9: Response surfaces for double emulsion characteristics: emulsion yield (A); apparent viscosity at 310 s^{-1} (B); consistency coefficient, K (C-D) and flow behaviour index, n (E); stability (F).

Optimization of a double emulsion as shortening replacer

Models developed for double emulsion characteristics were used in order to optimize a double emulsion intended for shortening replacement in biscuit formulations. Optimization was performed by means of the desirability function, maximizing apparent viscosity, yield and stability. The optimization criteria were chosen in order to obtain an emulsion with the maximum structuration and stability as possible. The same level of importance was given to all the constraints and a linear desirability function was used (weight = 1). The calculated formulation had the highest desirability value (d=0.990) and it was composed of 10% EW in W_1, 29% W_1 in W_1/O, and 60% W_1/O in $W_1/O/W_2$, thus reaching a fat content of 42.6 g/100g and 3.5 g/100g proteins. The optimized emulsion was produced and characterized in duplicate, giving results in good agreement with the predicted values: yield, 98.71±0.05% vs. 99.35± 0.30%; apparent viscosity, 70.4±6.6 mPa*s vs. 63.1± 0.1 mPa*s; stability, 100±1% vs. 100±1%. The only exceptions were represented by K, (3763±597 mPa*s^n vs 100.78±0.10 mPa*s^n) and n (0.31±0.02 vs 0.72±0.1). As for K, the explanation may lay in the fact that the data related to two experimental samples containing EW and with a similar formulation to the optimized emulsion (EW_10_30_60 and EW_00_20_60) were excluded by model calculation due to their outlier nature. On the other hand, n model showed a significant lack of fit, revealing the inadequacy to fit the experimental domain. For these reasons, the two responses were not considered for optimization.

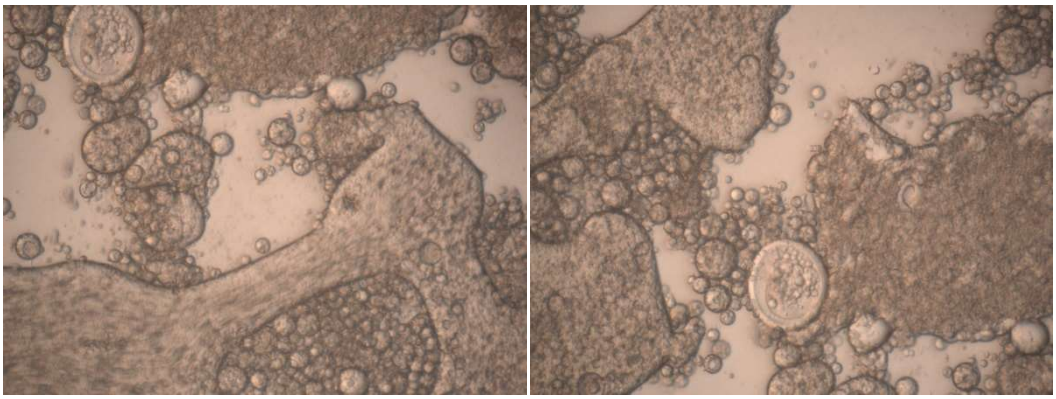

Figure 3.1.10: Optical micrographs (40x magnification) of the optimized double emulsion.

Conclusions

The present work aimed at (1) characterizing double emulsion systems for potential application as fat replacers and (2) optimizing a high viscosity double emulsion for shortening replacement in biscuit dough. Response surface methodology and desirability function demonstrated their effective applicability for the study of complex multivariate systems and allowed to reach the goals, leading to the development of a double emulsion containing 42.6 g/100g of fat and providing a structured shortening-alternative to be tested in reduced-fat biscuits.

Experimental part 3: Reduced-fat biscuits with bean powders and double emulsions: interplay between food structure, nutritional and sensory properties

The aim of this work was to investigate the effects of the addition of raw and extruded bean flours, as structuring ingredients, or double emulsion, as fat replacer, on the structural, nutritional, and sensory properties of reduced-fat biscuits.

***Abbreviation list**:* ANOVA, analysis of variance; AS, available starch; db, dry basis; EXTR, reduced-fat sample containing extruded bean powder; FS, frequency sweep; G*, complex modulus; n, number of replicates; OPT, optimized reduced-fat sample; PCA, principal component analysis; PC, principal component; PGPR, polyglycerol polyricinoleate; r, correlation coefficient; RAW, reduced-fat sample containing raw bean powder; RDS, ready digestible starch; RS, resistant starch; SDS, slowly digestible starch; SEM, scanning electron microscopy; SS, strain sweep; STD, reference sample; tan δ, damping factor; TS, total starch; W_1, internal water phase; W_2, external water phase; W_1/O, primary emulsion; $W_1/O/W_2$, double emulsion; WOW, reduced-fat sample containing double emulsion.

Dough rheological properties

Dough formulations are reported in section 5, Table 5.1.3. SS curves (Figure 3.1.11A) showed that the reduction of fat in biscuit doughs tended to prolong the linear viscoelastic range, in which rheological properties are not deformation dependent (Steffe 1996), and to markedly decrease dough stiffness. In addition, dough viscoelasticity measured by FS tests was strongly affected by formulation changes, since all the reduced-fat doughs exhibited lower and less linear complex modulus (G*) values than STD (Figure 3.1.11B). The total substitution of shortening with the

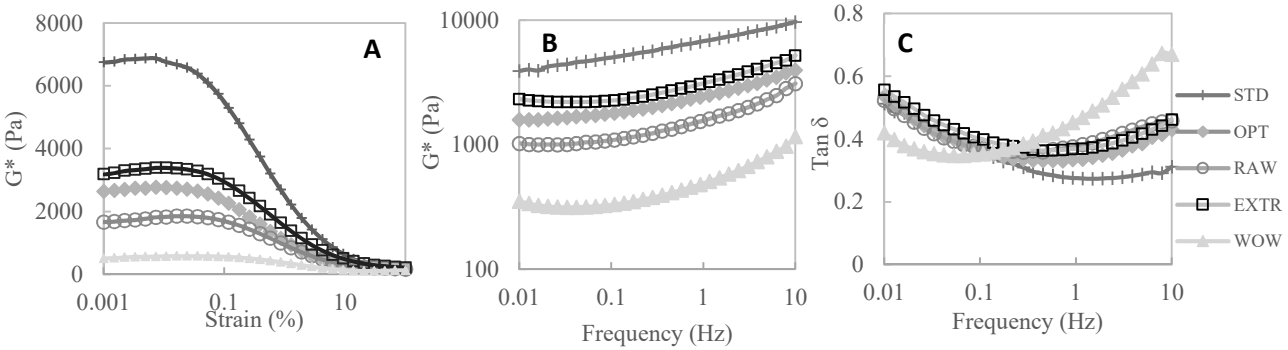

Figure 3.1.11*:* Average strain sweep (A) and frequency sweep curves (B, C) of biscuit doughs. G*, complex modulus; Tanδ, damping factor. Analytical relative standard deviations ranged from 1 to 15% (n≥2).

double emulsion produced the lowest values of G*, while EXTR showed the highest values, suggesting a dough stiffening effect of the extruded bean flour similar to that of resistant starch added in OPT formulation. The damping factor (ratio between viscous and elastic modulus) resulted

lower than 1 (0.31-0.67) throughout the entire frequency range for all the samples (Figure 3.1.11C), according to a solid-like behavior of all samples. For WOW dough, the damping factor 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 behavior may reveal the presence of a tight matrix in a quiescent status, which breaks at higher frequencies due to a weak structuration.

Biscuit composition

Table 3.1.9 reports the analytical composition of the experimental biscuits. Despite the fact that dough formulations had been balanced to ensure a constant moisture, final samples showed significant differences for this parameter. In fact, the presence of ingredients with high water retention capacity, such as polydextrose, resistant starch and bean powders (Laguna et al., 2011; Stanyon and Costello, 1990; Zoulias et al., 2002) may explain the higher moisture level measured for all the reduced-fat biscuit samples. WOW biscuits showed a lower water content with respect to OPT, EXTR, and RAW, probably due to the inclusion of a part of water in the oil droplets of the double emulsion. Consistently with the recipes, fat content was the highest for STD sample and reduced of about 44% in OPT, RAW and EXTR; WOW showed the lowest fat content (about 53% reduction), since the double emulsion used to substitute the remaining shortening contained only 42.6% oil. Protein content was significantly higher for samples RAW, EXTR and WOW that contained, respectively, bean powders and dried egg albumen. OPT showed the lowest protein content, according to the substitution of a fraction of flour with resistant starch. Total starch values were calculated as the 100's complement of the sum of all the measured components included the theoretical amount of polydextrose. In fact, specific analyses on the polidextrose used as ingredient revealed that only 27.9% of it was detected as fiber. Thus, subtracting this amount from the one added in the dough, the theoretical amount of polydextrose in the final biscuit was calculated and reported in table 3.1.9. Samples showed similar values of total starch, while significant differences were observed for resistant starch. EXTR revealed a not detectable resistant starch content, probably due to the combined effect of bean powder cooking-extrusion (Ai et al., 2017) and dough cooking, causing a marked decrease of resistant starch in final biscuits. This seems to be confirmed by the fact that sample containing raw bean powder (RAW) showed a higher resistant starch level, comparable to STD. The highest resistant starch content was obtained for OPT, according to the addition of the resistant-starch-rich ingredient in the formulation. On the contrary, WOW showed a lower content of resistant starch with respect to OPT, probably ascribable to a different interaction of the ingredients during biscuit processing. In particular, the low amount of available water in the dough, due to the encapsulation in the double emulsion, could have limited starch gelatinization during dough cooking, with a consequent lower starch retrogradation level, accounting for the lower amount of resistant starch (Wang et al., 2015). Regarding fiber, as expected, STD showed the lowest level, while OPT and WOW had the significantly highest fiber content, probably ascribable to the

contribution of the resistant-starch-rich ingredient added in the formulation.

Biscuit properties

Quality characteristics of experimental biscuits are reported in Table 3.1.10. Color was strongly affected by the presence of polydextrose, which provides reducing sugars, as well as protein-rich ingredients (i.e. bean powder in RAW and EXTR and dried egg albumen used in the double emulsion of WOW formulation), accounting for a higher development of Maillard reactions (Mieszkowska and 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 structuring role of bean protein (McWatters et al., 2003). In particular, the significantly higher value measured in RAW suggested that bean powder extrusion had an improving effect on the powder performance in biscuit formulation, yielding a less hard final product. Sample WOW showed a fracture strength nearly six times higher than STD, probably ascribable to the very low amount of fat (14.86±0.03 g/100g) and to the creation of a very compact structure due to the low air incorporation during dough creaming. It can be hypothesized that the viscosity and solid fat content of the emulsion was not enough to incorporate and retain the right amount of air during the creaming phase. 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 absorption for OPT, RAW and EXTR, with respect to STD. The presence of polydextrose in OPT allowed to increase air incorporation during dough preparation (Kocer et al. 2007), thus obtaining a porosity comparable to STD. The presence of extruded bean powder led to a structure more similar to OPT than the use of raw powder.

Table 3.1.9: Composition of biscuits (mean ± standard error [a]). See Table 5.1.3, section 5, for sample identification and composition.

Sample	Moisture (g/100g)	Ash (g/100g)	Fat (g/100g)	Proteins (g/100g)	Sugars (g/100g)	Total starch* (g/100g)	of which resistant starch (g/100g)	Fiber (g/100g)	Polydextrose ** (g/100g)
STD	2.84±0.08^{a}	1.22±0.01^{b}	32.1±0.1^{c}	5.65±0.07^{b}	16.8±0.3^{a}	40.0	4.7±0.5^{a}	1.2±0.1^{a}	-
OPT	3.86±0.07^{c}	1.16±0.01^{a}	17.5±0.1^{b}	4.75±0.03^{a}	18.0±0.4^{a}	37.4	11.6±0.9^{c}	6.7±0.1^{c}	10.6
RAW	4.05±0.04^{c}	1.40±0.01^{d}	17.8±0.1^{b}	6.19±0.01^{c}	17.3±0.7^{a}	41.5	7.0±0.4^{ab}	2.4±0.1^{b}	10.6
EXTR	3.93±0.01^{c}	1.41±0.01^{d}	18.0±0.2^{b}	6.02±0.07^{c}	16.1±0.6^{a}	39.3	nd	2.6±0.1^{b}	10.6
WOW	3.42±0.01^{b}	1.31±0.02^{c}	14.9±0.1^{a}	6.16±0.04^{c}	18.2±0.9^{a}	39.7	8.7±0.4^{b}	6.6±0.1^{c}	10.6

*Total starch was obtained by calculation.

**Theoretical polydextrose amount not detected by the fiber assessment method used.

[a] Number of analytical replicates (n): moisture, fat, proteins, n=2; resistant starch, n=6. For each parameter, different superscript letters indicate a significant difference ($p < 0.05$). nd: not detectable.

Table 3.1.10: Quality characteristics of biscuits (mean ± standard error [a]). See Table 5.1.3, section 5, for sample identification and composition.

Sample	Thickness (cm)	L*	a*	b*	Milk absorption (g/100g)	Fracture strength (kPa)	Fracture strain (%)	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±^{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

[a] Number of analytical replicates (n): thickness, porosity n=2; L*, a*, b* n=3; Milk absorption, n=5; fracture strength, fracture strain, n≥10. For each parameter, different superscript letters indicate a significant difference ($p < 0.05$). nd: not detectable.

Microstructural characterization

To study differences among biscuit samples at microstructural level, SEM analyses were performed. Representative SEM images of samples are shown in Figure 3.1.12. SEM images revealed differences in internal structure of the biscuits and the gummy aspect of starch granules, which were completely covered with an amorphous matrix due to the gelatinization process (Ovando-Martínez et al., 2013). In particular, microstructural observation of STD and RAW cookies revealed a well-developed protein structure due to dough mixing and thermal treatment during cooking, as well as to sufficient amount of added water in formulations. In OPT and EXTR a similar microstructure with starch granules embedded in a very dense protein matrix was also observed. WOW presented a more continuous and closed network structure, as compared to the STD and RAW samples.

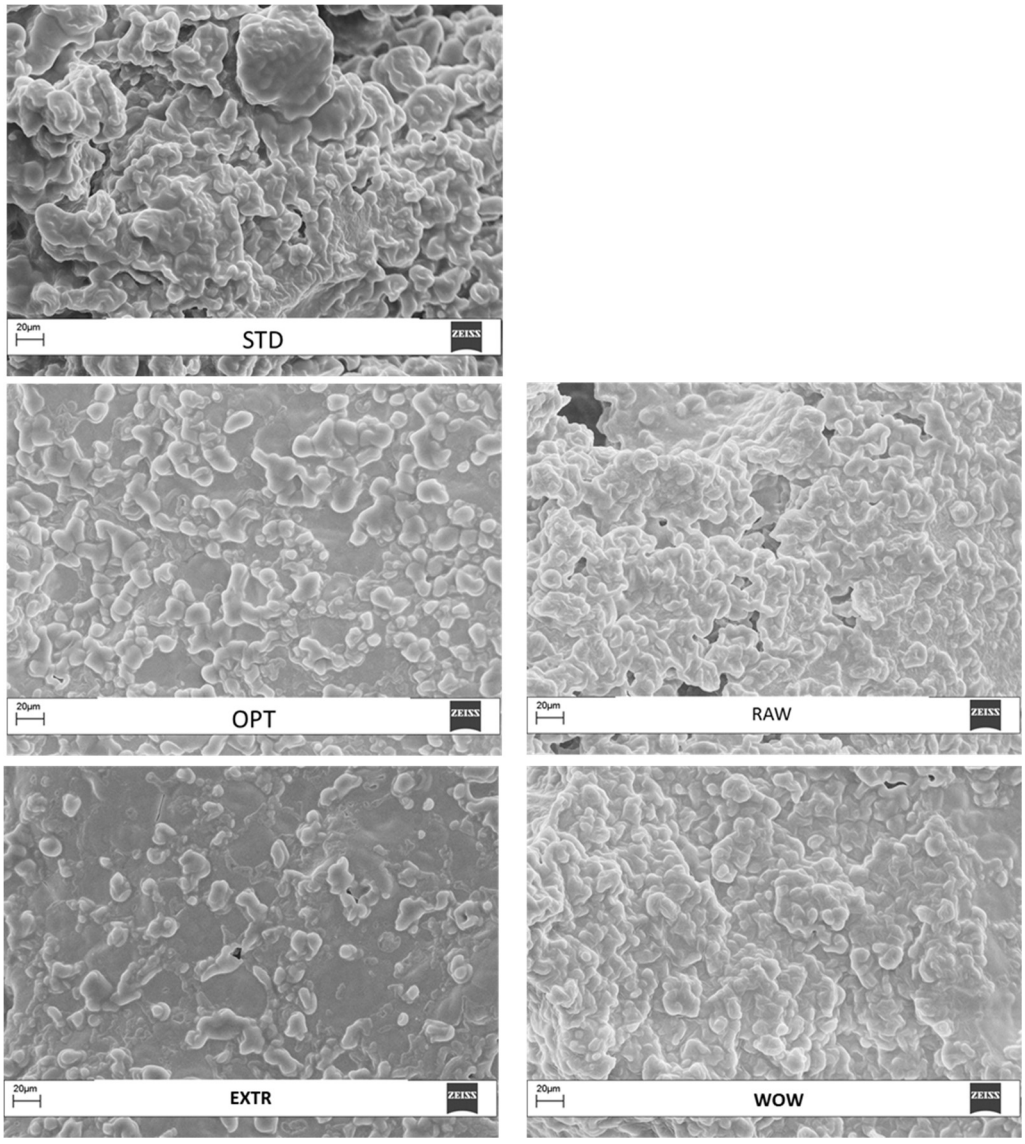

Figure 3.1.12: SEM images of samples: STD, OPT, RAW, EXTR and WOW.

In vitro digestibility

All the digestibility analyses were carried out on samples treated only with a process of grinding and dispersion, in order to mimic what occurs in the digestive system, even if this led to a lower efficiency in the hydrolytic action of the enzymes. *In vitro* starch digestibility was expressed in terms of ready digestible starch (RDS) and slowly digestible starch (SDS) as a percentage referred to 100g available starch (AV) in biscuits, considered as the difference between total starch and resistant starch. The assay used in this study to measure starch susceptibility to digestive enzymes, is a commonly used method to estimate the potential glycemic response of foods (EFSA, 2011). Indeed, the glycemic response appears to be directly related to the amount of RDS and the insulin demand is inversely correlated to SDS fraction (Garsetti et al., 2005). Samples containing extruded bean powder significantly differed from the others for the highest content of slowly digestible starch

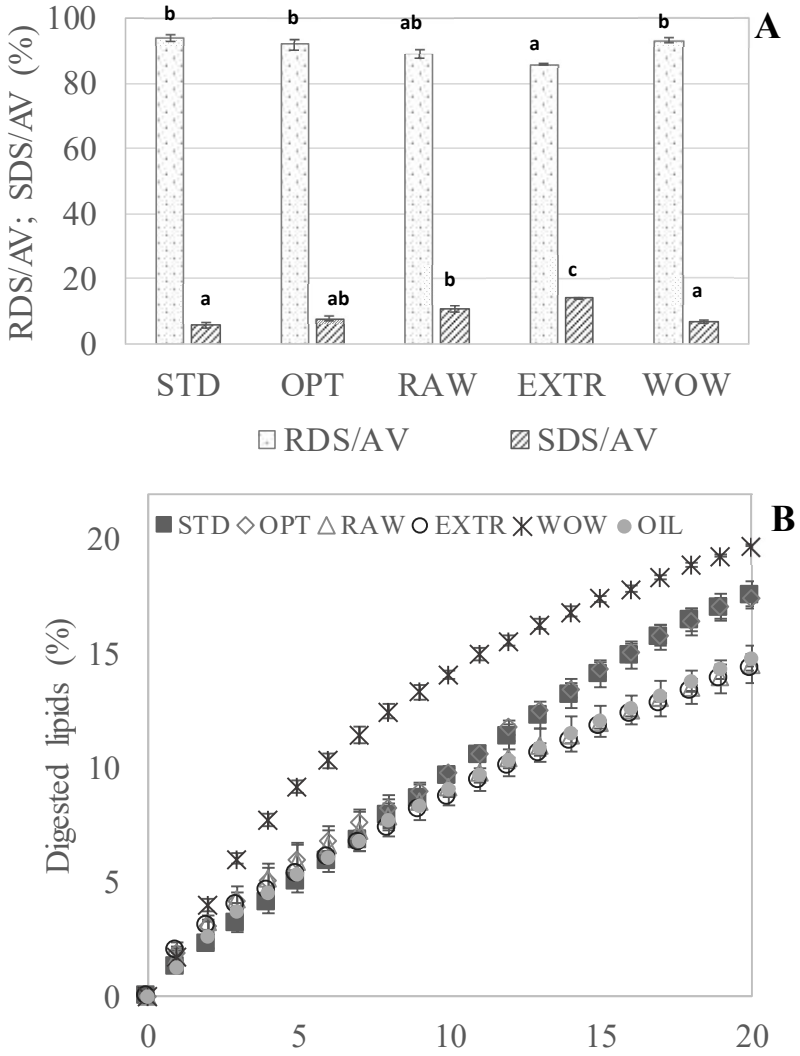

Figure 3.1.13: (A) In vitro starch digestibility of biscuit samples: rapidly digestible (RDS) and slowly digestible (SDS) starch fractions are expressed as a percentage of available starch (AV) (average ± standard error, n=6). For each parameter, different superscript letters indicate a significant difference ($p < 0.05$). (B) In vitro lipid digestibility expressed as the percentage of digested lipids referred to the initial lipid content (average ± standard error, n=3). OIL represents an extra-virgin olive oil sample analyzed as reference.

(Figure 3.1.13A): considering that this formulation was characterized by the lowest content of resistant starch (see Table 3.1.9), it can be argued that, during dough preparation and cooking, the portion of RS contained in the original powder (12.6% vs 24.7% of the raw powder) was gelatinized and converted into the slowly digestible fraction. Moreover, a role of the tighter protein network observed by texture analysis and SEM can be hypothesized. RAW sample revealed a significant but slightly lower SDS content with respect to EXTR, comparable to the level of OPT. This result means that protein and starch modification occurring during bean powder extrusion actually change *in vitro* starch digestibility of biscuits, with a minor improvement of nutritional performances. Regarding ready digestible starch, which is the fraction that account for the rapid glucose release in blood, EXTR showed the lowest level, differing significantly from STD, OPT, WOW but comparable to RAW. These results might be due to the legume starch properties, such as high amylose content and large size granules, reducing starch degradability (Sandhu and Lim 2008). Moreover, a contribution of the tight protein network created by bean powder can't be excluded.

Lipid digestibility was measured as oleic acid equivalent of free fatty acids released during 20 min of pancreatic attack and it was expressed as a percentage on the total initial lipid amount. Figure 3.1.13B reports the reaction kinetics. Final lipid digestibility data were compared with ANOVA and post-hoc LSD test. WOW kinetic differed from that of the other samples, although final lipid digestibility (19.9±1.3%) resulted comparable to OPT (17.4±0.3%) and STD (17.6±0.5%). On the other hand, RAW and EXTR (14.6±0.7% and 14.3±0.1%) were comparable among each other and significantly lower than STD. For the sample containing double emulsion, the peculiar behavior may be explained by the type of fat (oil) that differed from the one used for the other samples (shortening) and by the treatment of the emulsion itself, that reduces the size of oil droplets, increasing the surface exposed to lipase attack. On the other hand, the presence of legume powders in RAW and EXTR may reduce digestibility due to the presence of a tight protein network, as discussed above for starch. In Figure 3.1.13B the digestibility of extra-virgin olive oil is also reported as a reference. Considering the simple matrix and in particular the absence of interfering compounds in the oil, this sample showed a low digestibility, probably ascribable to the presence, in the other samples, of emulsifiers (derived from shortenings and double emulsion) enhancing lipid-enzyme interaction. Figure 3.1.14 reports the results for protein digestibility during the pancreatic phase, which produced the major digestion. Protein digestibility was calculated considering digested the peptides with molecular weight lower than 3000 Da, that at the beginning of the reaction were about 60% (ranging from 60.4% to 73.0%) of the total protein content for all the samples. Protein digestibility values were normalized with respect to the initial value of each digestion phase. Bovine casein digestibility was also analyzed, as a pure protein reference. The gastric attack (pH 2, pepsin 0.115 µg/mL, 37°C, 1 h), indeed, produced a digestion ranging from

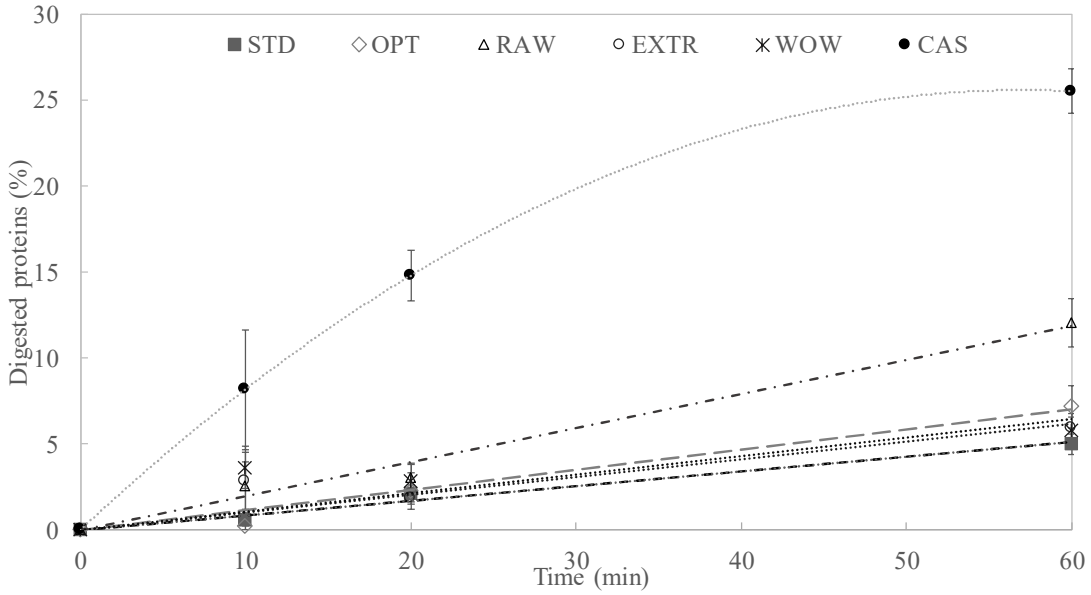

Figure 3.1.14: In vitro protein digestibility expressed as the percentage of digested proteins with respect to the initial protein content (average ± standard error, n=3). CAS represents a milk bovine casein sample analyzed as reference.

2.4±0.3% of OPT to 5.5±0.5% of EXTR (data not shown). WOW resulted not digested, while STD and RAW showed intermediate values (3.6±0.9% and 2.5±0.5%) CAS showed a digestion by pepsin of 5.9±0.6%. After the pancreatic attack (pH 6.8, pancreatin 0.75 mg/mL, 37°C, 1 h), casein resulted, as expected, in the highest amount of digested proteins (25.5±1.3%), due to the complete absence of interference by other macro-molecules. Among biscuit samples, RAW showed the highest protein digestibility and differed significantly from STD (12.08±2.74% vs 3.74±1.62%). The other sample did not differ significantly from STD and RAW. Reaction kinetic (Figure 3.1.14) reflects these observation: while casein digestion follows a first order kinetic, the other samples show a linear tendency that reveals a digestion rate strongly slower than that of casein. RAW resulted more readily digestible than EXTR: this appears in contrast to what observed in previous studies, reporting an increase in digestibility of legumes subjected to extrusion and extrusion-cooking as a protein denaturation and degradation of protein complexes (El-Hady and Habiba 2003; Linsberger-Martin et al. 2013). In our study, the extruded powder was used as ingredient, thus passing through a second processing: the severe thermo-mechanical process may be responsible for the creation of indigestible aggregates between proteins and other macromolecules (Carbonaro et al. 2015). Further investigations are needed to clarify the macromolecular modification involved in digestibility differences.

Consumers' acceptability

Cookie sensory liking attributes (appearance, odor taste, flavor and texture) were affected by both the addition of bean flours and double emulsion (Table 3.1.11). OPT and STD showed the highest and comparable scores, in agreement with the previously observed structural data and the efficacy

of the optimization strategy. Samples containing bean powders did not show significant differences among each other and showed scores lower than STD for all the considered parameters, mainly due to the off-flavours related to bean presence. Very few studies report results for consumers' acceptability of baked foods enriched with bean powders and they are focused on gluten-free products (Szczygiel et al. 2017; Sparvoli et al., 2016), thus a direct comparison is not possible. Texture perception by consumers confirmed the analytical results: sample WOW showed very low scores for texture and overall liking, although appearance and odor resulted comparable to OPT.

Table 3.1.11: Appearance, aroma, taste, flavor, texture and overall liking data (mean ±standard error) of biscuits from consumers' acceptability tests. See Table 5.1.3, section 5, for sample identification and composition.

Product	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}

For each parameter, different superscript letters indicate a significant difference ($p < 0.05$).

Interplay among structure, nutritional and sensory properties

A Principal Component Analysis was performed to explore both the location of experimental sample with respect to the measured variables and possible correlation among variables, then verified by a correlation matrix (data reported only in the text). From the bi-plot PC1 vs PC2 (Figure 3.1.15) it can be observed that the biscuit samples tend to locate in the four different quarters of the PC's plan. In particular, STD showed high absolute values for all the sensory attributes, lightness

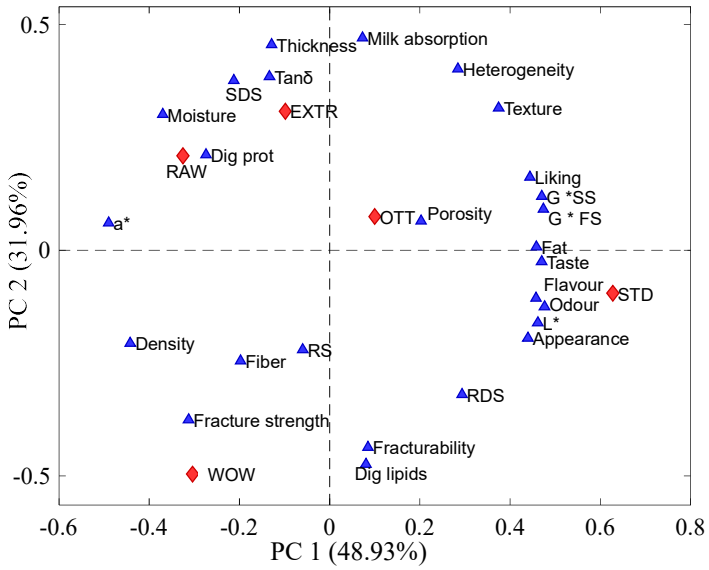

Figure 3.1.15: Bi-plot PC1 vs PC2 obtained from PCA applied to all the measured variables for biscuit samples.

and fat. On the other hand, bean-containing samples were mainly characterized by high absolute values for protein digestion, moisture and slowly digestible starch content. WOW located at low values of both PC1 and PC2, far from all the other samples and was characterized by high absolute values for dough density, fracture strength and lipid digestibility. Regarding variable correlation, it can be observed that dough density resulted inversely correlated with complex modulus from SS ($r=-0.892$, $p<0.05$); G*from SS showed a direct correlation with fat content ($r=0.947$, $p<0.05$) similarly to G* from FS (0.961, $p<0.01$). Perceived texture and fracture strength resulted inversely correlated ($r=-0.984$, $p<0.01$) and color parameter L* was directly correlated to both appearance ($r=0.878$, $p<0.05$) and odor of samples ($r=0.982$, $p<0.01$), while redness (a*) showed an inverse correlation with the same responses ($r= -0.887$, $p<0.05$; $r=-0.947$, $p<0.05$). Slowly digestible starch fraction (SDS) resulted directly correlated with damping factor ($r=-0.597$, $p<0.01$).

Conclusions

The present research allowed to investigate the effect of applying technological-modified ingredients, i.e. extruded bean flour and double emulsions, to a reduced-fat food matrix. The use of extruded bean flour allowed to obtain biscuits nearly comparable to a traditional reduced-fat product, but with improved nutritional profile. As expected, the presented data suggest a hypoglycemic potential of bean-enriched biscuits, but this have to be confirmed by a dedicated *in vivo* study. In fact, desirable nutritional value of bean powders, including high protein content and low starch digestibility, may be successfully exploited in biscuits, although sensory and structural results suggest that a focused improvement is still needed. On the other hand, the use of double emulsion requires further investigations, in order to understand how to improve emulsion structuring and its effect on dough properties.

3.2 Case study 2: Reduced-fat whipping cream

Experimental part 1: Reduction of fat in whipping cream: effect of gelatin addition and homogenization conditions.

The aim of this work was to investigate the combined effect of gelatin type and concentration and homogenization conditions on the properties of reduced-fat whipping cream (25% fat) by means of D-optimal design of experiments.

***Abbreviation list**:* Adj. R^2, adjusted determination coefficient; ANOVA, analysis of variance; b_0, constant value in the polynomial model; b_1 linear coefficient for the gelatin concentration in the polynomial model; b_{11}, quadratic coefficient for the gelatin concentration in the polynomial model; b_2, linear coefficient for the homogenization pressure in the polynomial model; b_{22}, quadratic coefficient for the homogenization pressure in the polynomial model; b_3, linear coefficient for the gelatin type in the polynomial model; b_4, linear coefficient for the number of homogenization steps; b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34}, interaction coefficients in the polynomial model; $d_{[4,3]}$, particle mean volume; $d_{(0.1)}$, diameter at which 10% of the sample's mass is comprised of particles with a diameter less than this value; $d_{(0.5)}$, diameter at which 50% of the sample's mass is comprised of particles with a diameter less than this value, e.g. volume weighted median diameter; $d_{(0.9)}$, diameter at which 90% of the sample's mass is comprised of particles with a diameter less than this value; DoE, Design of Experiment; K, consistency coefficient; LOF, lack of fit (p-value); n, flow behavior index; PCA, principal component analysis; PC, principal component; r, correlation coefficient; Pred. R^2, predicted determination coefficient; R^2, determination coefficient; TPA, texture profile analysis; X_1, value of gelatin concentration in the polynomial model; X_2, value of homogenization pressure in the polynomial model; X_3, value of the gelatin type in the polynomial model; X_4, value of the number of homogenization steps in the polynomial model; y, response variable value in the polynomial model; ε random error in the polynomial model.

Whipping cream characteristics

Table 3.2.1 reports the experimental factors considered in DoE with the corresponding levels (see section 5, Table 5.2.1) and the quality characteristics of whipping cream samples used to calculate multi-regression models by Response Surface Methodology. Samples were identified by codes indicating the levels of the experimental factors tested (i.e.: sample B0.25_15_0 contained gelatin type B at 0.25g/100g concentration and was treated at 15 MPa with a single homogenization step). As reported in table 5.2.2 of chapter 5, the two tested gelatins differed for gel strength and particle size. All the experimental samples contained k-carrageenan at a concentration of 0.25 g/100g.

Rheological behaviour of whipping creams was described by apparent viscosity at 2 s^{-1}, consistency factor (K) and flow behaviour index (n) calculated from the flow curves. Since K and apparent viscosity gave the same information, only K was reported and commented. Gelatin concentration may be considered the most influent factor on rheological parameters. Contrarily to the expectations, samples without gelatin showed the highest K values (ranging from 20.27 mPa*s^n to 55.49 mPa*s^n), suggesting the ability of k-carrageenan to retain high amounts of water and create a very compact network that increases system viscosity (Kováčová et al., 2010). Ji et al. (2008) demonstrated that concentration of k-carrageenan >0.025% in reconstituted skim milk powder/k-carrageenan systems formed gels during cooling, when no shear was applied. In fact, during heating, the reactive sites of carrageenan molecules associate with each other and form an effective matrix, which traps the kappa casein micelles, thus forming a thixotropic strong gel (Dickinson, 1998) able to slow down the coalescence and separation of the fat globules (Lal et al., 2006). This may have occurred in the experimental samples prepared in this study, which underwent a UHT treatment and a following storage at 4°C. In presence of gelatin, the competition for water absorption tends to reduce the strength of carrageenan network, giving a less viscous matrix. This hypothesis seems confirmed by the fact that higher gelatin concentrations resulted in K decreases, reaching the minimum for samples with 0.25 g/100g added gelatin (range between 2.94 mPa*s^n and 10.88 mPa*s^n). Experimental samples differed significantly from commercial references, which showed a K range between 0.21 and 0.76 mPa*s^n.

Table 3.2.1. D-optimal Design matrix developed to investigate the effect of gelatin type and concentration, as well as homogenization pressure and pressure ratio between the second (p2) and the first (p1) homogenization step on the properties of reduced fat whipping cream: sample identification, actual factor levels and whipping cream properties. Results are reported as average[a]. The property ranges of commercial reference samples (REF) are also reported.

	Experimental factors				**Whipping cream properties**			
Sample name	Gelatin concentration (g/100g)	Homogenization pressure (MPa)	Gelatin type	p_2/p_1	K (mPa*s^n)	n	$d_{[4,3]}$ (μm)	$d_{(0.5)}$ (μm)
B0.25_15_0	0.25	15	B	0	10.88	0.42	12.22	8.41
A0_15_0	0	15	A	0	55.49	0.18	23.72	8.04
B0_15_0	0	15	B	0	28.66	0.35	21.30	8.35
A0.125_17.5_0.2	0.125	17.5	A	0.2	9.16	0.38	17.50	8.57
A0.125_20_0.2	0.125	20	A	0.2	16.44	0.32	21.90	10.59
A0.125_17.5_0	0.125	17.5	A	0	13.39	0.48	18.00	10.80
A0_15_0.2	0	15	A	0.2	25.53	0.29	5.94	4.07
B0_15_0.2	0	15	B	0.2	29.27	0.30	7.05	4.43
A0.125_17.5_0.2	0.125	17.5	A	0.2	13.90	0.36	9.44	4.80
A0.25_17.5_0.2	0.25	17.5	A	0.2	2.94	0.67	9.92	5.07
A0_20_0	0	20	A	0	26.79	0.31	10.05	6.55
B0.125_20_0	0.125	20	B	0	18.76	0.35	11.37	6.86
A0.25_15_0	0.25	15	A	0	7.58	0.50	11.02	7.31
B0.25_15_0.2	0.25	15	B	0.2	3.59	0.58	7.93	4.22
A0.125_17.5_0.2	0.125	17.5	A	0.2	7.27	0.46	10.63	5.11
B0.25_17.5_0	0.25	17.5	B	0	8.69	0.47	12.81	7.98
B0_20_0.2	0	20	B	0.2	20.27	0.26	22.39	8.223
B0.25_20_0.2	0.25	20	B	0.2	6.77	0.48	24.08	8.01
A0.25_20_0	0.25	20	A	0	9.90	0.44	14.56	8.89
REF	-	-	-	-	0.21-0.76	0.60-0.67	3.05-3.25	3.02-3.17

[a] Number of replicates: K, n: n=2; $d_{[4,3]}$, $d_{(0.5)}$: n=6. Analytical relative standard deviations were lower than 20%.

Table 3.2.2: Pearson correlation matrix of cream quality characteristics.

Variables	K	n	$d_{(3,4)}$	d (0.5)	Span	WT	OV	Hard	Cohes	Adhes	Spring	ST	WR
K	1.000	-0.829***	0.275	0.039	0.247	-0.798***	-0.468*	0.781***	-0.427	0.385	0.023	-0.535*	0.263
n		1.000	-0.299	-0.141	-0.225	0.609**	0.412	-0.695***	0.250	-0.390	-0.226	0.482*	-0.225
$d_{(3,4)}$			1.000	0.764***	0.826***	0.035	-0.334	0.203	-0.115	0.085	0.267	-0.363	-0.265
d (0.1)				0.776***	0.034	-0.136	-0.266	0.053	0.240	-0.185	0.319	-0.617**	-0.189
d (0.5)				1.000	0.294	0.279	-0.162	-0.156	0.232	-0.236	0.431	-0.399	-0.417
d (0.9)					0.887***	0.064	-0.283	0.207	-0.151	0.120	0.227	-0.270	-0.279
Span					1.000	-0.019	-0.226	0.312	-0.311	0.264	-0.015	-0.068	-0.184
WT						1.000	0.649**	-0.751***	0.399	-0.338	0.060	0.588**	-0.698***
OV							1.000	-0.595**	0.559*	-0.032	-0.037	0.812***	-0.628*
Hard								1.000	-0.666**	0.661**	-0.293	-0.406	0.535*
Cohes									1.000	-0.291	0.551*	0.132	-0.406
Adhes										1.000	-0.178	0.112	0.248
Spring											1.000	-0.436	-0.167
ST												1.000	-0.364
WR													1.000

Significance levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. WT: Whipping time, OV: overrun, Hard: hardness; Cohes: cohesiveness; Adhes: adhesiveness; Spring: springiness; ST: stability; WR: whipping rate.

The marked difference observed may be ascribable to the fact that commercial sample did not contain gelatin and did contain k-carrageenan stabilizer, but probably of a different type and/or in a different amount. The stabilizer used in this study was added to cream samples in the concentration suggested by the producer, e.g. 0.25%. It can be argued that this concentration was too high for the samples under study, containing a high amount of reconstituted skim milk powder. Among the samples without gelatin, A0_15_0 and B0_15_0 are replicates of the same operative conditions, as well as A0_15_0.2 and B0_15_0.2. They were included in the experimental plan since they could be useful, together with the replicates of the central points (A0.125_17.5_0.2) for the estimation of the experimental variance. The variability between samples demonstrated a good reproducibility and a low experimental variance for nearly all the measured variables. Only the viscosity parameters (K and n) resulted different between A0_15_0 and B0_15_0, probably connected to the creation of a non-homogeneous gelled structure due to the presence of k-carrageenan. Further specific investigations may clarify the effect of this ingredient on viscosity properties.

All samples exhibited pseudoplastic behavior ($n<1$), with increasing n values observed for increasing levels of gelatin concentration. This trend was opposite in comparison with K. Actually, a negative highly significant correlation was found between K and n ($r=-0.829$, $p<0.001$) (Table 3.2.2) as already observed by Padiernos et al (2009). Commercial references resulted in flow behavior index values (0.60-0.67) higher than those of all the experimental samples, except for A0.25_175_0.2 ($n=0.67$), while K was definitely lower than in experimental samples. In the context of a reduced-fat cream matrix, viscosity ensures the structuring effect essential for air incorporation and retaining during whipping, but it may represent a limiting factor for moving and pumping operations during further processing. Thus, for an industrial use of reduced-fat cream, viscosity will have to be considered in the design and control of the equipment.

Particle size distribution of cream samples was measured by laser diffraction and in Figure 3.2.1A some examples are reported; the average particle size distribution of commercial reference is also reported in Figure 3.2.1B. Complete data, expressed as volume weighted mean diameter $d_{[4, 3]}$ and median diameter $d_{(0.5)}$ are reported in table 3.2.1. The $d_{[4, 3]}$ is a measure of the average globule size (5.94 to 24.08 μm), while $d_{(0.5)}$ is the volume weighted median diameter, measuring the diameter at which 50% of the sample's mass is comprised of particles with a diameter less than this value. All the samples showed a bimodal distribution of particle size, more clearly defined for commercial ones, with one population of particles from about 0.04 to 0.5 μm and the other from 2 to about 100 μm. The peak corresponding to the lowest size may be due to the presence of casein micelles that are removed from the exterior membrane of the fat globule (Cropper et al., 2013). Some samples showed minor peaks in the range 10-100 μm, probably ascribable to the appearance of casein–carrageenan aggregates (Kováčová et al., 2010). In general, fat globules in cream show dimensions ranging from 0.8 to 20 μm (Daw and Hartel, 2015; Walstra et al., 1999): in this experiments, most of the samples resulted included in this range (Table 3.2.1), except for A0_15_0, B0_15_0,

B0_20_0.2, A0.125_20_0.2, B0.25_20_0.2. For the first three samples (no gelatin addition) these results are consistent with the viscosity data: higher droplet size may be associated with higher emulsion viscosity as reported by Tippets and Martini (2012) for milk protein stabilized emulsion containing carrageenan, pectin and gelatin. For the latter two samples, characterized by the highest homogenization pressure level (20 MPa) applied in two steps, it can be argued that the combined effect of UHT treatment, homogenization pressure and presence of
k-carrageenan and/or gelatin caused agglomeration effects that increased mean volume of particles (Kováčová et al., 2010). Multiregression models developed in the following part of this experiments may give additional information on these phenomena. Commercial reference samples showed markedly lower $d_{[4, 3]}$ and $d_{(0.5)}$ values, indicating smaller particle size.

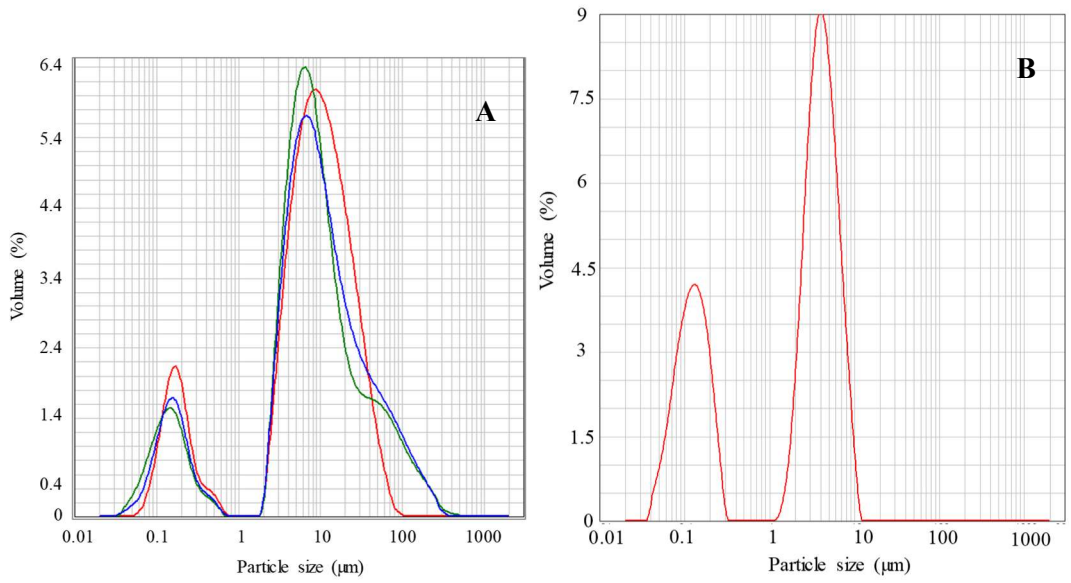

Figure 3.2.1: Examples of particle size distribution of cream samples: experimental samples (A) and commercial reference (B).

Whipped cream characteristics

Whipped cream quality characteristics are reported in Table 3.2.3 in terms of whipping time, whipping rate, overrun, hardness, cohesiveness, adhesiveness, springiness, and stability. Whipping time was defined by optimal relationship overrun-hardness. Samples containing 0.25 g/100g gelatin showed a tendency to take time for whipping (from 6.75 to 7.50 min) longer than samples without gelatin addition (4.50-7.00 min). In this last group, an effect of pressure may be evidenced, since samples treated at 15 MPa showed whipping times (average 4.75 min) shorter than samples treated at 20 MPa (average 6.63 min). Kovacova et al. (2010) reported an increasing effect of UHT treatment and homogenization on whipping time, while Stanley et al. (1996) found that cream without additives tended to reach higher overrun in short whipping times than cream containing stabilizers: this is due to the fact that stabilizers increase viscosity, while depressing overrun at first. However, all the samples resulted in whipping times shorter than those reported in literature, more

similar to commercial reference ones (2.00-3.00 min). This effect may be explained by the reduced presence of fat and the viscosity increase due to hydrocolloids: in fact, whipping time showed an inverse correlation with K ($r=-0.798$, $p<0.001$).

Air incorporation rate was calculated by fitting whipping curves by an exponential equation. As expected, whipping rate resulted highly and inversely correlated with whipping time ($r = -0.698$; $p<0.001$). The highest rate values were obtained for samples B0_15_0, B0_15_0.2 and A0.125_175_0.2, that probably represented an outlier data. Whipping rate was also inversely correlated with overrun ($r = -0.628$; $p<0.05$), which showed values ranging from 126% to 214%. Experimental samples reached overrun values higher than those obtained for commercial samples (93.7-113.6%). Similar findings were obtained by Zhao et al. (2009; 2009), who underlined a connection between long whipping times and increasing overrun. Overrun is an indicator of the air holdup in the whipped cream (Jakubczyk and Niranjan, 2006): the thickening property of gums makes this air incorporate into the bubbles and it becomes hard to elapse. Once most of the air is incorporated into the emulsion, the emulsion shows strong combination and reinforces the stabilization of the already incorporated air cells (Allen et al., 2006). It is easy to understand that extension of whipping time can facilitate more air being incorporated into the emulsion system, resulting in a higher overrun percentage. Notwithstanding viscosity of cream is a key factor to ensure air incorporation (Farahmandfar et al., 2017), it can be observed that in our experimental samples overrun was inversely correlated with K ($r = -0.468$; $p<0.05$). This was probably due to the fact that, as described above, in samples without gelatin the formed gelled structure, due to k-carrageenan, was too strong and inhibited air incorporation over a certain level.Texture properties were assessed by means of TPA and reported in terms of hardness, adhesiveness, cohesiveness and springiness (Table 3.2.3). Hardness represents the force opposed by sample to a given deformation (N). Samples containing only stabilizer (0 g/100g gelatin) showed hardness values (4.5–5.4 N) very similar to those of commercial reference (4.70-4.77 N). In fact, a highly significant direct correlation ($r = 0.781$; $p<0.001$) was found with K, while whipping time and overrun resulted inversely correlated ($r = -0.751$; $p<0.001$ and $r = -0.595$; $p < 0.01$) to hardness. Cohesiveness represents the strength of internal bonds making up the body of the product and it is calculated as the ratio of the positive force areas under first and second compression peaks (Zhao et al. 2009).

Table 3.2.3: Quality characteristics (average of three replicates[a]) of whipped cream samples. Result ranges of commercial reference samples (REF) are also reported.

Sample	**Whipped cream properties**							
	Whipping time (min)	Whipping rate (%/min)	Overrun (%)	Hardness (N)	Cohesiveness	Adhesiveness (mJ)	Springiness	Stability (%)
B0.25_15_0	6.75	0.011	198	2.7	0.90	27	1.18	98.3
A0_15_0	4.50	0.024	148	5.4	0.64	34	1.14	97.1
B0_15_0	5.00	0.102	154	5.4	0.67	38	1.10	98.5
A0.125_17.5_0.2	6.25	0.101	126	4.4	0.68	29	1.13	97.2
A0.125_20_0.2	6.75	0.021	171	3.9	0.75	33	1.17	98.0
A0.125_17.5_0	6.50	0.010	181	3.6	0.87	32	1.14	98.1
A0_15_0.2	4.75	0.087	175	4.5	0.75	35	1.13	99.1
B0_15_0.2	4.75	0.145	163	4.9	0.69	36	1.12	98.5
A0.125_17.5_0.2	6.00	0.040	181	3.9	0.75	34	1.11	99.3
A0.25_17.5_0.2	6.75	0.034	189	3.3	0.69	30	1.08	100.0
A0_20_0	6.25	0.028	184	5.0	0.66	32	1.08	99.3
B0.125_20_0	6.75	0.020	214	3.7	0.82	32	1.12	100.0
A0.25_15_0	7.50	0.008	193	3.5	0.72	33	1.14	99.6
B0.25_15_0.2	6.75	0.064	194	3.6	0.77	33	1.13	99.7
A0.125_17.5_0.2	7.00	0.015	196	4.1	0.76	36	1.11	100.0
B0.25_17.5_0	7.50	0.010	204	3.6	0.74	34	1.10	100.0
B0_20_0.2	7.00	0.011	194	4.6	0.75	38	1.13	99.9
B0.25_20_0.2	7.25	0.013	190	3.5	0.75	31	1.13	100.0
A0.25_20_0	7.25	0.010	186	2.9	0.74	28	1.12	99.9
REF	2.00-3.00	0.695-0.928	93.7-113.6	4.7-4.8	0.72-0.77	38-39	1.13	100

[a] Analytical relative standard deviations were lower than 10%, except for adhesiveness (< 25%).

Higher cohesiveness values were obtained for samples with 0.25 g/100g gelatin or treated at high homogenization pressures, suggesting an effect of these factors on maintaining an intact structure of whipping cream under deformation. Cohesiveness was inversely correlated with hardness ($r=-0.666$, $p<0.01$) and directly correlated with overrun ($r=0.559$, $p<0.05$), meaning that a higher amount of air makes the cream softer and more malleable. Adhesiveness is defined as the work necessary to overcome the attractive forces between the surface of the food product and the surface of other materials that come in contact with it. As reported also by Farahmandfar et al. (2017), adhesiveness showed a trend similar to hardness, being the two parameters directly correlated ($r=0.661$, $p<0.01$). Moreover, adhesiveness increased with gelatin concentration decrease. Finally, springiness can be considered a measure of sample elasticity, meaning the ability to recover the original height after deformation. All the samples showed values comparable to the commercial reference (1.13), suggesting a very limited effect of fat reduction, gelatin concentration and homogenization treatment on this parameter.

Foam stability resulted very high for all the combinations of tested factors, ranging from 98.0% to 100%. This was probably due to the presence of ingredients with a very strong water binding capacity, preventing water migration and structure collapse. As expected, significant positive correlations were found with whipping time ($r=0.588$, $p<0.01$) and overrun ($r=0.812$, $p<0.001$).

Explorative PCA

PCA allowed to identify the contributions of the different experimental factors to the properties of cream, both before and after whipping. Bi-plot of PC1 vs PC2 reported in Figure 3.2.2 reflects the

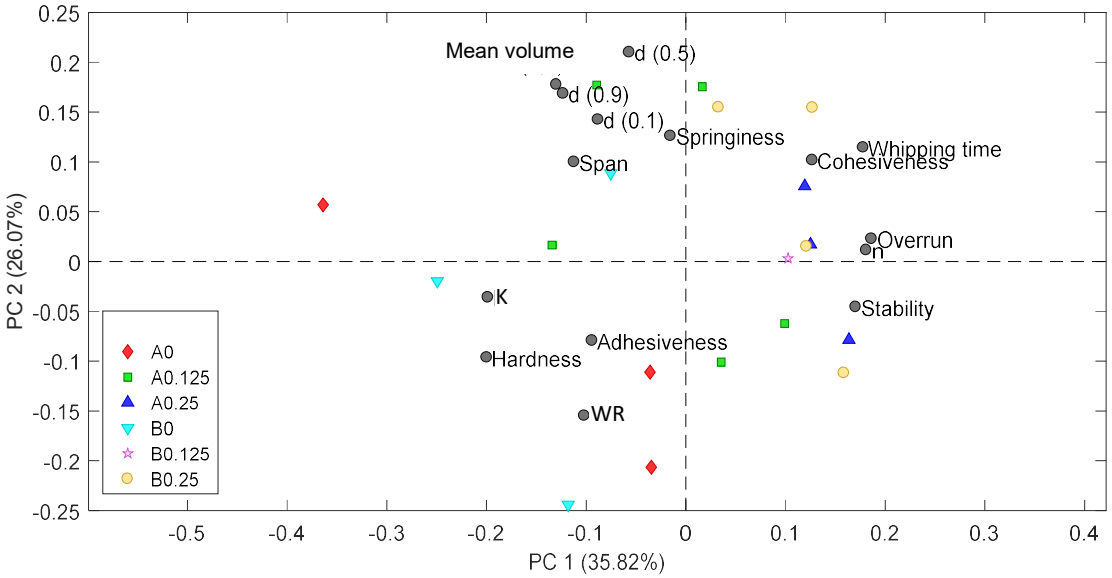

Figure 3.2.2: Bi-plot PC1 vs PC2 obtained from PCA applied to the variables measured for reduced-fat cream samples. Symbol's shape and color refers to the gelatin type and concentration used in each sample.

location of cream samples colored as a function of gelatin type and concentration. In particular, irregarding of the gelatin type, it can be observed that samples with higher gelatin concentration

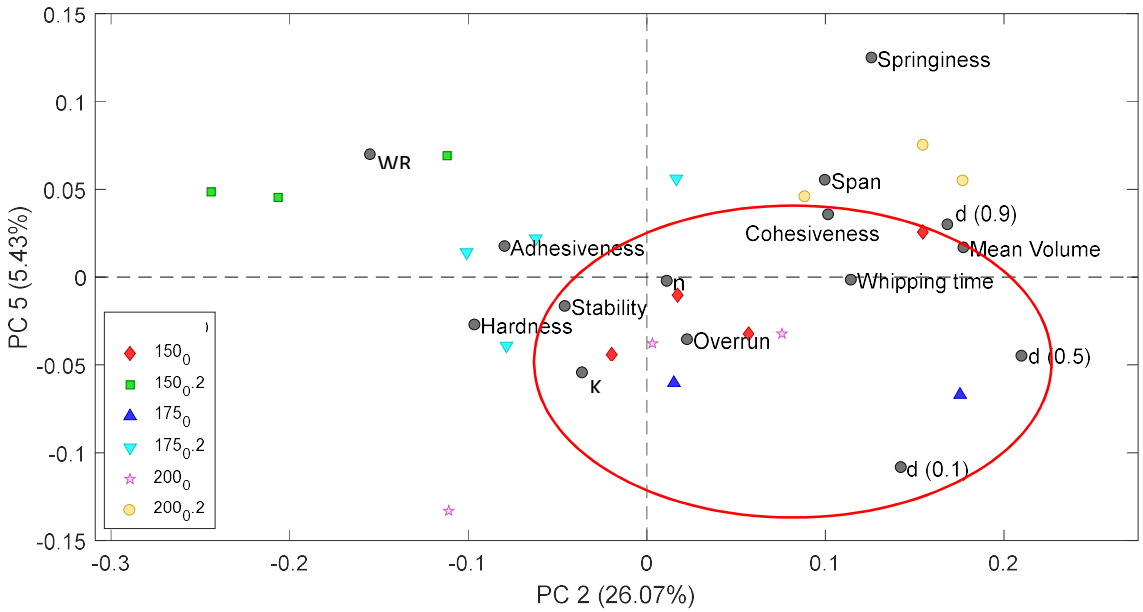

Figure 3.2.3: Bi-plot of PC2 vs PC5 obtained from PCA applied to measured variables for reduced-fat cream samples.

tend to position to progressively higher values of of PC1. This distribution is mainly affected by sample viscosity (K, n), texture (hardness, adhesiveness and cohesiveness), whipping time, overrun and stability. Flow behavior index (n), whipping time, overrun, cohesiveness and stability showed positive loadings on PC1; on the contrary, consistency index (K), hardness, adhesiveness and whipping velocity showed negative loadings. Indeed, samples without gelatin showed higher hardness, viscosity and whipping velocity. The presence of gelatin tended to reduce the hardness of the final whipped cream, but to increase overrun and stability. Samples with 0.25% gelatin showed also higher whipping times. Figure 3.2.3 shows the bi-plot of PC2 vs PC5, where a distribution of samples along PC5 (5%) based on the number of homogenization steps (1 or 2) can be identified: samples treated with one step (_0) tend to locate at negative values of PC5, while those treated with two steps (_0.2) have mainly positive values. Springiness and whipping rate appear to be the most influent variables on PC5; $d_{(0.1)}$ and $d_{(0.5)}$ are the other variables with heavy negative loadings on PC5. Actually, most of the samples treated in one step showed lower springiness and whipping velocity but higher d(0.1) than samples treated in two steps. PC2 allows to identify a pattern for samples treated at 15 MPa: samples treated in two steps tend to group at high values of PC2, while samples treated in one step tend to assume lower values of PC2. In this case, particle size parameters as mean volume, $d_{(0.5)}$ and $d_{(0.9)}$ are the heaviest variables and assume positive loadings. Indeed, samples treated with one step showed higher values for this parameters.

Multiregression models

Table 3.2.5 reports the multiple regression models in terms of coded coefficients. Variables to be modeled were selected according to the relevance for final cream performance and quality. Adequacy of the models was verified through the determination coefficient R^2, the adjusted R^2, the predicted R^2, the adequate precision and the lack of fit test (LOF). When non-significant terms were identified by ANOVA, a model simplification was applied, respecting model hierarchy. The calculated models resulted highly significant ($p<0.001$) for K, whipping time, overrun, hardness, and adhesiveness, while the other models were significant for $p<0.01$. LOF of the models was always not significant, and determination coefficients very good considering the pilot nature of the experimentation.

Consistency coefficient (K) showed an indirect strong dependence on gelatin concentration ($p<0.001$) (Figure 3.2.4A), probably due to the fact that the only presence of the k-carrageenan stabilizer led to a strong gel structure, reduced by the presence of gelatin for a mechanism of water competition. In addition, two-step homogenization caused a decrease in consistency ($p<0.01$), suggesting a fluidizing effect (Figure 3.2.4B). Flow behavior index was directly affected by the increase in gelatin concentration ($p<0.001$), describing a tendency towards Newtonian behavior of the matrix as discussed above (Figure 3.2.4C). Mean diameter volume of particles increased as pressure increased ($p<0.01$) (Figure 3.2.4D), as previously reported by Marshall and Arbuckle (1996). A possible explanation may be that shear forces and the high fluid outlet temperature (70°C) may have denatured whey proteins and impaired their emulsifying properties (Thiebaud et al., 2003). This effect was more accentuated at high levels of gelatin concentration, due to the significance ($p<0.05$) of the interaction between the two factors. The interaction between homogenization pressure and number of steps (b_{24}) resulted positive and highly significant ($p<0.001$), suggesting that the effect of high pressure on particle size increase was more evident working with 2 steps. Overrun was positively affected by gel concentration ($p<0.001$), pressure ($p<0.01$) (Figure 3.2.4E) and gelatin type ($p<0.05$), suggesting higher overrun reached with gelatin B, characterized by lower gel strength and higher particle size than gelatin A. Interaction term between gelatin concentration and homogenization pressure (b_{12}) resulted highly significant ($p<0.001$) and negative, indicating that the effect of pressure was more accentuated at low gelatin concentration levels. In addition, the negative significant interaction b_{24} ($p<0.01$) suggests that one step homogenization implied a less evident effect of homogenization pressure (Figure 3.2.4F). Gelatin type resulted relevant in significant interactions b_{23} and b_{34} (respectively for $p<0.01$ and $p<0.05$), indicating that overrun increasing effect of homogenization pressure was more evident for samples containing gelatin B and that overrun of samples with gelatin A was more affected by the number of homogenization steps. Gelatin concentration and homogenization pressure affected directly whipping time confirming the tendency described above (Figure 3.2.5A). Consistently, the

terms for the two factors resulted significant ($p<0.05$) and positive for the whipping rate model, indicating a decreasing effect: due to the inverse square root transformation applied to the variable, the mathematical signs represent the opposite of the real effect. Interestingly, the number of homogenization steps showed a positive effect on whipping rate ($p<0.05$), suggesting that samples treated in two steps had a faster air incorporation (Figures 3.2.5C-D). However, the significance of the interaction terms b_{14} and b_{24} ($p<0.05$) revealed that the effect of the number of homogenization steps was dependent on gelatin concentration and pressure levels, with a stronger effect at low amounts of gelatin and at higher pressures. Moreover, the significant interaction b_{34} ($p<0.05$) indicates that, working with gelatin B, the steps effect was less evident. Consistently with the data discussed above, both hardness and adhesiveness were indirectly affected only by gelatin concentration ($p<0.001$) (Figure 3.2.5B). For springiness, interestingly the quadratic terms b_{11} and b_{22} resulted significant ($p<0.01$), indicating a maximizing effect on springiness at the lowest values of gelatin concentration and high values of homogenization pressure.

Table 3.2.5. Coded second-order polynomial equation coefficients and results of one-way analysis of variance for the D-Optimal Design aimed at studying the effect of gelatin type, gelatin concentration, homogenization pressure and ratio between pressure applied in the second (p2) and in the first step (p1) on the properties of a reduce-fat (25 g/100g) whipping cream.

	b_0	b_1 Gel conc	b_2 Pressure	b_3 Gel type	b_4 Steps	b_{12}	b_{13}	b_{14}	b_{23}	b_{24}	b_{34}	b_{11}	b_{22}	R^2	Adj. R^2	Pred. R^2	Adequate precision	LOF
Whipping cream																		
Log K (Log kPa)	1.14	-0.34 ***	0.01	0.01	-0.11 **									0.849 ***	0.807	0.722	12.5	0.604 n.s.
Log n	0.39	0.12 ***	-0.01	-0.01	0.02									0.705 **	0.621	0.474	7.7	0.371 n.s.
d [4,3] (μm)	14.24	-1.15	3.04 **	0.50	-0.69	2.28 *				5.44 ***				0.801 **	0.701	0.456	10.8	0.477 n.s.
Whipped cream																		
Whipping time (min)	6.38	0.81 ***	0.53 **	0.06	-0.04									0.802 ***	0.745	0.596	11.3	0.664 n.s.
1/√WR (1/√(%/min))	6.80	1.26 *	1.19 *	-0.26	-0.99 *	-0.36	0.26	-1.29 *	0.50	1.17 *	0.67 *			0.888 **	0.748	0.741	8.5	0.994 n.s.
Overrun (%)	186	9 ***	6 **	4 *	-0.9	-10 ***	-0.01	-3	7 **	-7 **	-4 *			0.956 ***	0.893	0.818	14.8	0.952 n.s.
Hardness (N)	4.1	-0.8 ***	-0.1	0.1	0.1									0.980 ***	0.972	0.944	10.5	0.305 n.s.
Adhesiveness	33	-2 **	-0.4	0.7	0.5									0.981 ***	0.971	0.914	6.1	0.262 n.s.
Springiness	1.12	0.01	-0.01	-0.01	0.01		0.01 *	-0.02 **		0.01 **		-0.03 **	0.04 **	0.864 **	0.728	0.433	10.1	0.364 n.s.

R^2, coefficient of determination; Adj R^2, Adjusted R^2; Pred R^2, predicted R^2; LOF, Lack of fit (p-value).

Significance levels: n.s. not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

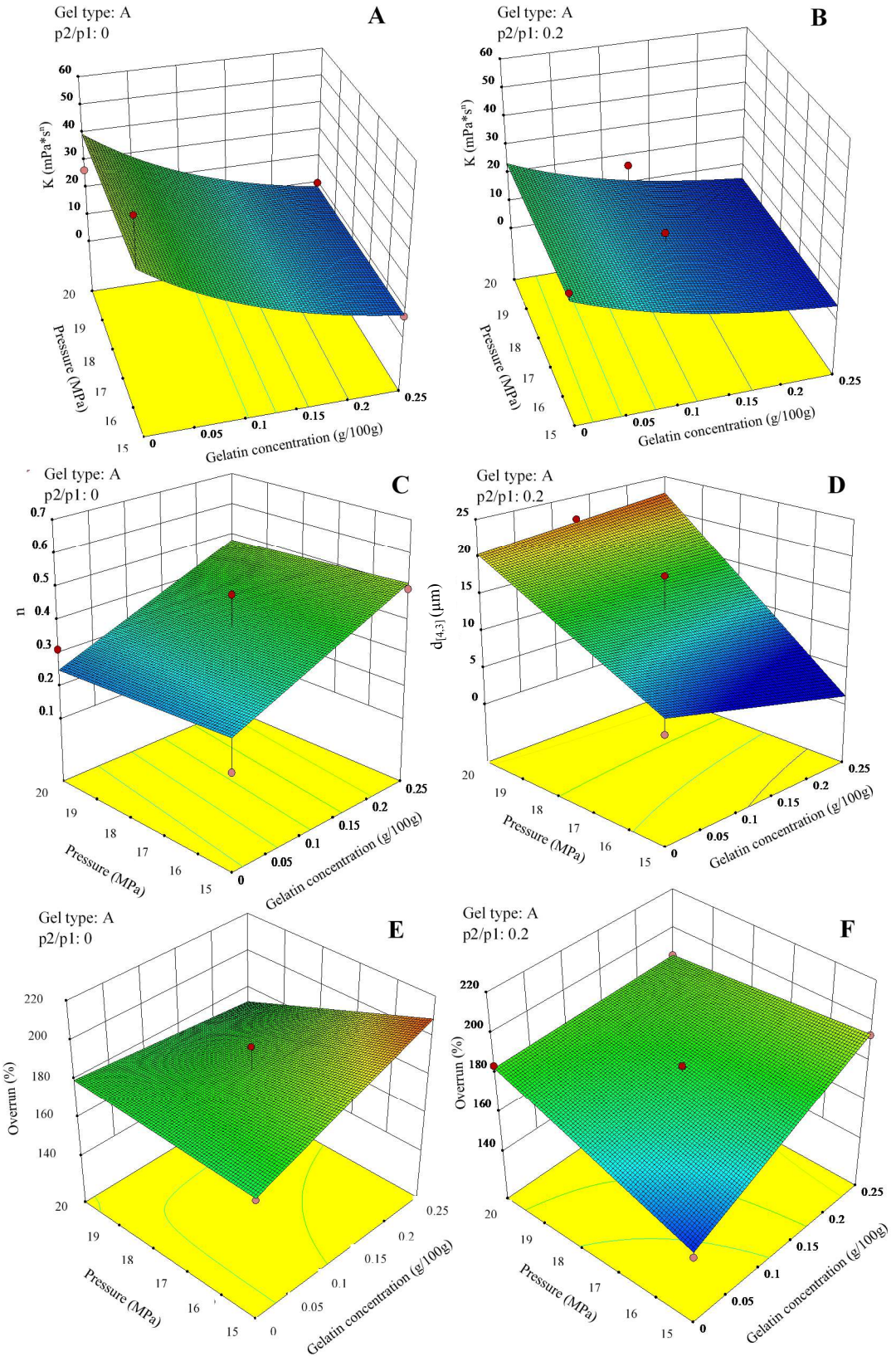

Figure 3.2.4: Response surfaces for reduced-fat whipping and whipped cream characteristics: consistency coefficient, K (A-B) and flow behaviour index, n (C); particle mean volume d $_{[4,3]}$ (D); overrun (E-F).

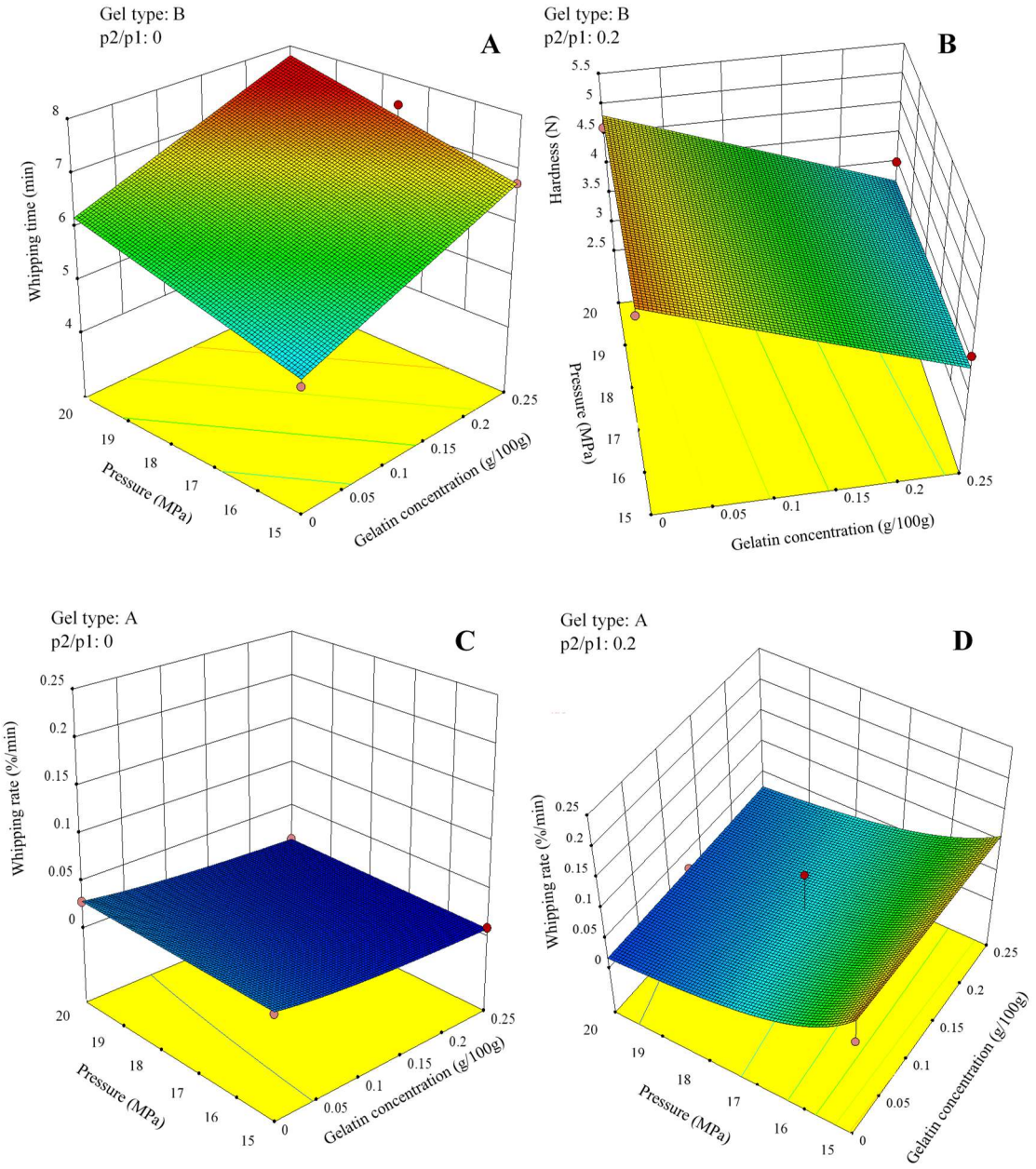

Figure 3.2.5: Response surfaces for reduced-fat whipped cream characteristics: whipping time (A); hardness (B); whipping rate (C-D).

Conclusions

Results from this study demonstrated that the combination of gelatin addition and high homogenization pressure may be successfully exploited for the development of reduced-fat whipping cream. D-optimal design and Response Surface Methodology resulted to be effective tools to study food processing at a pilot level, allowing the collection of high quality information

on the effect of the studied factors and their interaction, with a limited experimental effort. In particular, gelatin addition at a concentration of 0.25 g/100g, in combination with k-carrageenan, allowed to increase initial consistency of cream samples, leading to final overrun even improved than those obtained for commercial samples, without affecting texture properties. On the basis of gelatin concentration, homogenization pressure revealed a great potential to modulate whipping properties. In a future work development, calculated multiregression models may be exploited to optimize the formulation and processing conditions to obtain the most suitable reduced-fat product in terms of whipping performance and stability, directing optimization criteria towards commercial reference characteristics. Then, microstructural investigation, sensory consumers' test and nutritional *in vitro* digestibility analyses may be carried out to obtain a comprehensive evaluation of the relationship among the different matrix functionalities.

Experimental part 2: Reduced – fat whipping cream storage stability

The aim of this work was to evaluate the effects of cold storage on the stability and performance of reduced–fat whipping creams containing gelatin (developed in Experimental part 1).

***Abbreviation list**:* ANOVA, analysis of variance; $d_{[4,3]}$, volume weighted mean; $d_{(0.5)}$, volume weighted median diameter; K, consistency coefficient; LSD, least significant difference; n, flow behaviour index; PCA, principal component analysis; PC, principal component; TSI, Turbiscan stability index.

Turbiscan stability index

Figure 3.2.6 reports TSI (Turbiscan Stability Index) data during 7 days of storage at 4°C: samples are shown in three graphs according to the gelatin concentration, e.g. 0 g/100g, 0.125 g/100g and 0.25 g/100g. The TSI is a relative number that evaluates the stability of dispersions, and it is calculated from the variations in the rate of backscattering/transmission intensity of the stored sample with respect to the product at the time of production. A high value of TSI indicates instability and high probability of phase separation, whereas a low TSI value indicates the opposite. Samples produced without gelatin (Figure 3.2.6A) revealed that the two-step homogenization (_0.2) tended to increase stability in the first days of storage (lower initial TSI values) for both samples treated at 15 MPa and 20 MPa. However, after 3 days only the sample B0_20_0.2 maintained TSI values lower than those of samples treated in a single step. In addition, samples treated in two steps or at higher pressure (20 MPa) showed a variability in TSI higher than that of the other samples, which had a rather constant stability. The addition of 0.125 g/100g gelatin (Figure 3.2.6B) implied different trends: the sample treated at 17.5 MPa with double step homogenization showed lower stability, while at 20 MPa no differences were evidenced according to the number of steps. At a 0.25 g/100g gelatin level (Figure 3.2.6C), samples treated at 15 MPa showed comparable behaviours, with a slight increase in stability when the double step homogenization was applied. Samples treated at 20 MPa showed the lowest TSI values, independently of the number of steps, while samples treated at 17.5 MPa showed a behaviour similar to the 15 MPa samples containing the same amount of gelatin. Gelatin B apparently conferred a more constant stability throughout the studied period of storage (3 weeks), although the higher pressures seemed to level the observed difference. As observed in previous studies (Kováčová et al., 2010; Long et al., 2012), the use of higher homogenization pressure tended to increase cream stability towards creaming, and the presence of gelatin did not alter markedly this effect. Moreover, the effect of using a double step was evidenced only at the lowest pressure used (15 MPa). Since instability may be due to sedimentation, creaming, and clarification kinetics, further development of this work may include

the identification, through backscattering profiles, of the prevalent phenomena in experimental samples.

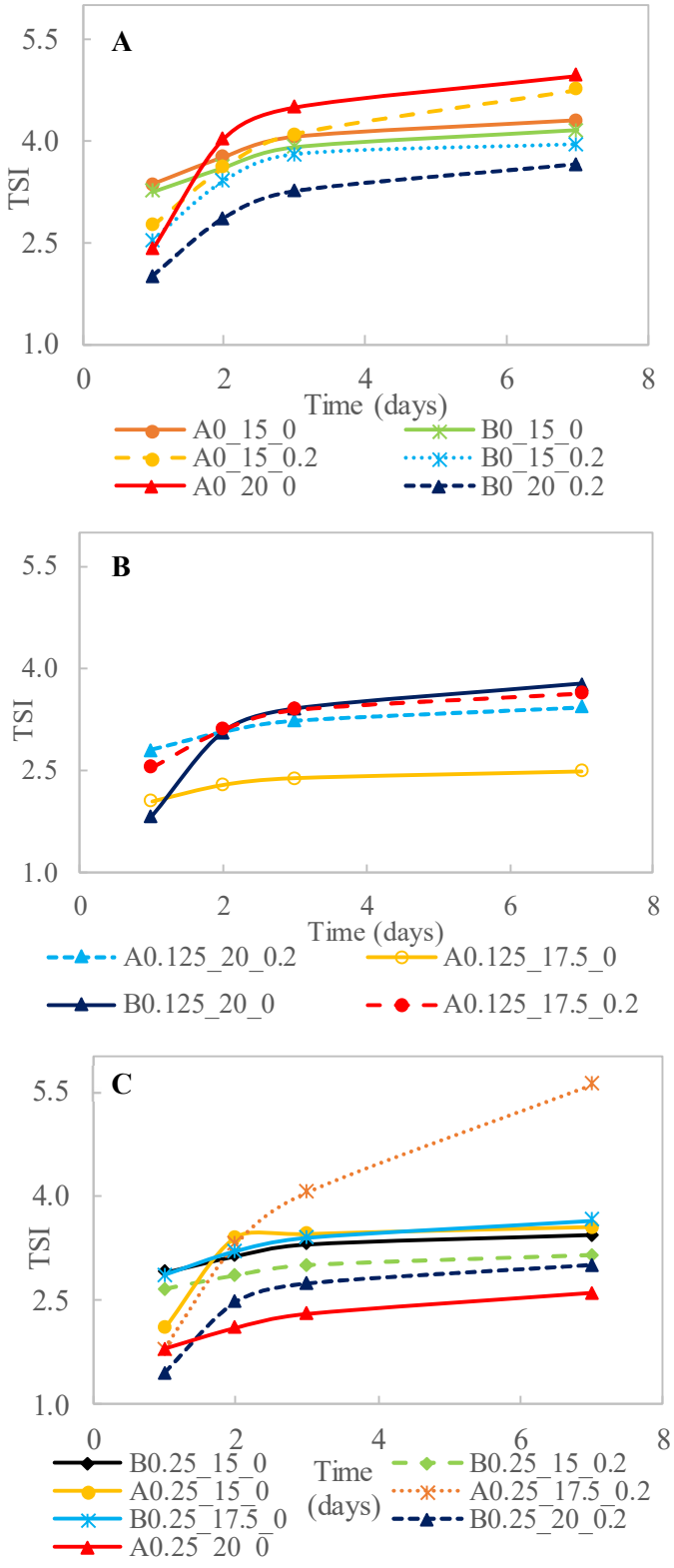

Figure 3.2.6. Physical stability (TSI) of reduced-fat cream samples during 7 days of storage. (A): samples without gelatin; (B): samples with 0.125 g/100g gelatin; (C) samples with 0.25 g/100g gelatin. Analytical relative standard deviation ranged from 0 to 22% (n=3).

Explorative PCA

As an explorative tool, PCA was performed on all the data obtained from cream analyses at T0 (immediately after production) and T3 (after three weeks of storage). Figure 3.2.8 reports the PC3 vs PC4 bi-plot: samples analyzed after cold storage tended to position at high values of PC3 and low values of PC4, suggesting that storage may have influenced the consistency index, located in the same position. The PC2 vs PC3 bi-plot (Figure 3.2.7) allowed to identify a tendency of stored samples to locate at high values of PC3. These samples showed high absolute values of hardness and adhesiveness (variables located at positive values of PC3) and low values of overrun (located at negative values of the same PC). Thus, it can be argued that storage influenced significantly whipped cream textural properties and air incorporation ability. The observed effects of storage on measured variables was verified by multifactor ANOVA, as reported in the following section.

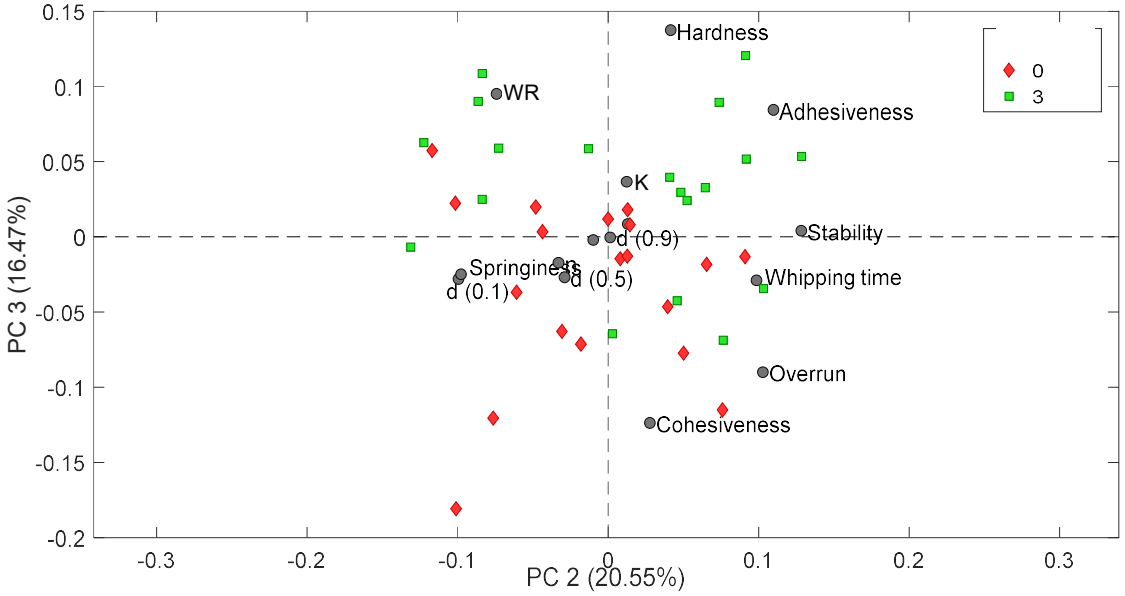

Figure 3.2.7. Bi-plot PC2 vs PC3 obtained from PCA applied to measured variables for reduced-fat cream samples immediately after production (T0) and after 3 weeks of storage (T3).

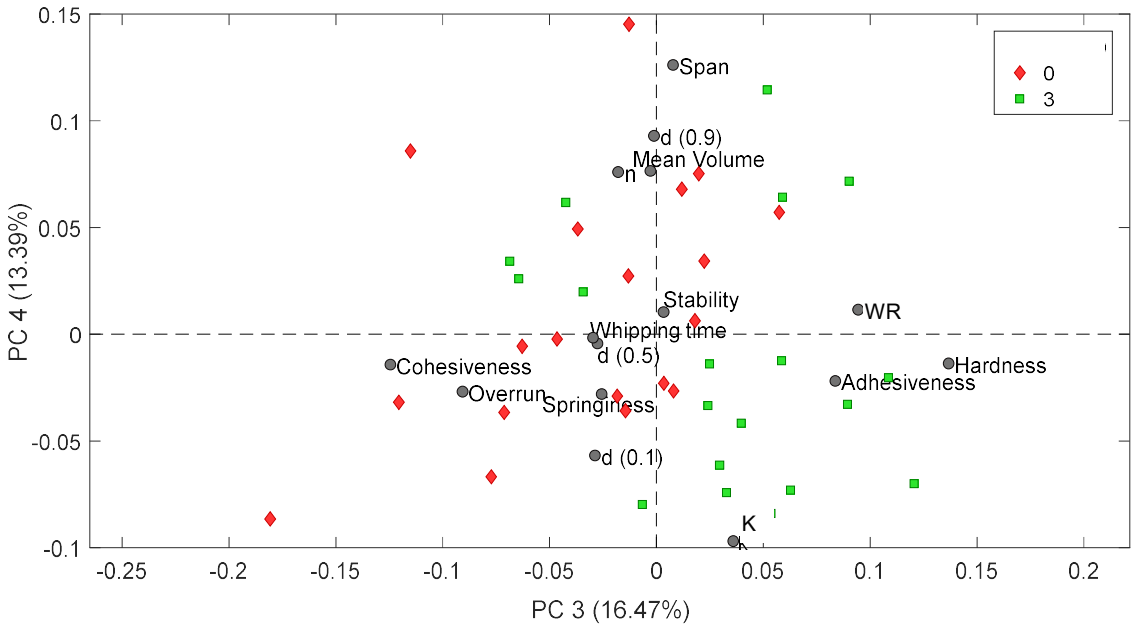

Figure 3.2.8. Bi-plot of PC3 vs PC4 obtained from PCA applied to measured variables for reduced-fat cream samples immediately after production (T0) and after 3 weeks of storage (T3).

Whipping cream properties

Results of whipping cream characteristics and LSD test after multifactor ANOVA are shown in Table 3.2.6. Interactions of storage time with gelatin concentration, homogenization pressure and steps were also considered and commented in the text only when significant ($p<0.01$). Cream viscosity was affected by both storage and experimental factors. In particular, storage significantly increased consistency coefficient while decreasing flow behavior index ($p<0.05$) with respect to fresh samples. It can be argued that the water retention ability of stabilizer and gelatin was increased by storage, due to the stabilization of the gel structure, thus causing a viscosity-enhancing effect on cream. Similar findings were obtained by Long et al. (2012), who observed an increase of K in correspondence with high pressure treatment and storage. Gelatin concentration increase produced a significant decrease of consistency index ($p<0.05$) and revealed an inverse effect on n, probably due to the competition for water between stabilizer and gelatin discussed in experimental part 1. The significant interaction storage time x gelatin concentration ($p<0.001$) revealed that storage affected more markedly samples without gelatin, while the presence of gelatin allowed to obtain viscosity properties more similar to those of the fresh sample. Homogenization pressure above 15 MPa and the use of two steps tended to decrease significantly K ($p<0.05$), while no differences were found for samples treated at 17.5 and 20 MPa. Sample particle size was not affected by storage, while a significant decreasing effect ($p<0.05$) was found for gelatin addition on volume weighted mean diameter $d_{[4,3]}$. Homogenization pressure showed a direct significant effect ($p<0.05$) on $d_{[4,3]}$ and $d_{(0.5)}$, revealing an increase of particle size. This effect may be due to the formation of fat

globules aggregates as a consequence of homogenization at high pressures (Chandrapala et al., 2016). Two-step homogenization produced a significant decrease of the volume median diameter $d_{(0.5)}$: the presence of a second homogenization step helped to partially disaggregate clusters, leading to a decrease in particle size (Thiebaud et al., 2003).

Table 3.2.6: Reduced-fat whipping cream properties (mean ± standard error values) as a function of the storage time and the three experimental factors studied (gelatin concentration, pressure and number of homogenization steps).

	K (mPa*s^n)	n	$d_{[4,3]}$ (μm)	$d_{(0.5)}$ (μm)
Storage time (weeks)				
0	17.28±1.09^a	0.40±0.01^b	14.55±0.79^a	7.28±0.25^a
3	26.52±1.10^b	0.34±0.01^a	16.37±0.77^a	7.95±0.25^a
Gel concentration (g/100g)				
0	40.27±1.65^c	0.26±0.02^a	18.88±1.15^b	7.94±0.36^a
0.125	17.61±1.55^b	0.35±0.02^b	14.23±1.15^a	7.86±0.36^a
0.25	7.83±1.23^a	0.50±0.01^c	13.26±0.93^a	7.04±0.30^a
Pressure (MPa)				
15	26.08±1.51^b	0.34±0.02^a	13.44±1.06^a	7.11±0.34^a
17.5	19.14±1.55^a	0.43±0.02^b	14.42±1.17^a	7.21±0.37^a
20	20.49±1.35^a	0.33±0.02^a	18.53±0.99^b	8.51±0.33^b
Steps				
1	24.65±1.16^b	0.35±0.01^a	15.84±0.82^a	8.50±0.26^b
2	19.16±1.04^a	0.38±0.01^a	15.08±0.77^a	6.73±0.25^a

a-c within the same factor, results with different superscript letters in the same column are significantly different ($p < 0.05$) on the basis of multifactor ANOVA followed by the LSD test.

Whipped cream properties

Table 3.2.7 reports results of multifactor ANOVA for whipped cream characteristics. Whipping rate and time were not significantly influenced by cream storage, while overrun decreased significantly after 3 weeks ($p<0.05$). As discussed in the experimental part 1 (Par n.), whipping time and whipping rate showed an inverse trend. Increasing gelatin concentration caused a significant increase of whipping time, while the lowest rate was observed for samples with the highest gelatin concentration (0.25 g/100g) and the fastest whipping was recorded for samples without gelatin. Samples at 0.125 g/100g gelatin were comparable to the other two groups. Homogenization pressure produced a significant increase in whipping time ($p<0.05$) and an inverse effect on whipping rate ($p<0.05$), although, for this parameter, samples treated at 15 MPa and 17.5 MPa were comparable. The number of homogenization steps did not affect significantly whipping properties, included overrun. The highest gelatin concentration (0.25 g/100g) and the highest homogenization

pressure (20 MPa) caused a significant ($p<0.05$) increase of air incorporation capacity of reduced fat cream samples. Regarding texture properties, hardness and adhesiveness tended to increase significantly ($p<0.05$) after storage, while no significant changes were observed for cohesiveness and springiness. Increasing gelatin concentration implied a significant decrease of hardness ($p<0.05$) and adhesiveness, although for this parameter samples at 0.125 g/100g and 0.25 g/100g resulted comparable. Pressure conditions did not affect significantly hardness and adhesiveness. On the other hand, cohesiveness showed a significant increase ($p<0.05$) with gelatin addition, irrespective the concentration considered. Samples treated at 20 MPa resulted in a significantly higher cohesiveness ($p<0.05$) than samples treated at 17.5 MPa, although the treatment at 15 MPa did not produce significant differences compared to the other samples. Interestingly, the interaction storage time x pressure steps resulted significant ($p<0.01$), revealing a synergistic effect of the two factors: cream samples after three weeks of storage showed a decrease in cohesiveness when treated in one step and an increase when subjected to double-step homogenization. Samples springiness decreased significantly ($p<0.05$) with pressures above 15 MPa, while no effect was observed for the number of steps. The interaction storage time x pressure was significant ($p<0.001$) and indicated that while samples treated at 15 and 20 MPa showed a decrease of springiness after storage, homogenization at 17.5 MPa caused an increase of this parameter. Further investigation should be carried out to clarify the mechanism underlying this observation. Finally, it can be observed that storage did not affect stability of experimental creams, as gelatin concentration and number of homogenization step. The only influent factor was pressure: the treatment at 20 MPa produced whipped creams significantly ($p<0.05$) more stable than those treated at 15 MPa.

Table 3.2.7: Reduced-fat whipped cream properties (mean ± standard error values) as a function of the storage time and the three experimental factors studied (gelatin concentration, pressure and number of homogenization steps)

	Whipping time (min)	Whipping rate (%/min)	Overrun (%)	Hardness (N)	Cohesiveness	Adhesiveness (mJ)	Springiness	Stability (%)
Storage time (weeks)								
0	6.4±0.1^{a}	0.04±0.01^{a}	181±2^{b}	4.08±0.05^{a}	0.74±0.01^{a}	33.1±0.6^{a}	1.122±0.003^{a}	99.1±0.3^{a}
3	6.6±0.1^{a}	0.07±0.01^{a}	171±2^{a}	4.49±0.05^{b}	0.72±0.01^{a}	37.1±0.6^{b}	1.123±0.003^{a}	99.3±0.3^{a}
Gel concentration (g/100g)								
0	5.7±0.1^{a}	0.08±0.02^{b}	166±3^{a}	5.28±0.07^{c}	0.69±0.01^{a}	38.4±0.9^{b}	1.110±0.005^{a}	99.5±0.4^{a}
0.125	6.5±0.1^{b}	0.05±0.02^{ab}	171±3^{a}	4.09±0.07^{b}	0.76±0.01^{b}	34.3±0.9^{a}	1.138±0.005^{b}	98.6±0.4^{a}
0.25	7.2±0.1^{c}	0.03±0.01^{a}	190±3^{b}	3.49±0.06^{a}	0.74±0.01^{b}	32.5±0.7^{a}	1.120±0.004^{a}	99.5±0.4^{a}
Pressure (MPa)								
15	6.0±0.1^{a}	0.07±0.01^{b}	170±3^{a}	4.35±0.07^{a}	0.73±0.01^{ab}	34.9±0.8^{a}	1.139±0.005^{b}	98.4±0.4^{a}
17.5	6.4±0.1^{b}	0.08±0.02^{b}	171±4^{a}	4.34±0.07^{a}	0.71±0.01^{a}	35.0±0.9^{a}	1.113±0.005^{a}	99.6±0.4^{ab}
20	7.0±0.1^{c}	0.01±0.01^{a}	187±3^{b}	4.17±0.06^{a}	0.75±0.01^{b}	35.3±0.8^{a}	1.115±0.004^{a}	99.7±0.4^{b}
Steps								
1	6.6±0.1^{a}	0.05±0.01^{a}	176±2^{a}	4.29± 0.05^{a}	0.73±0.01^{a}	34.6±0.6^{a}	1.125±0.004^{a}	98.9±0.3^{a}
2	6.4±0.1^{a}	0.06±0.01^{a}	176±2^{a}	4.28±0.05^{a}	0.74±0.01^{a}	35.5±0.6^{a}	1.120±0.003^{a}	99.5±0.3^{a}

a-c within the same factor, results with different superscript letters in the same column are significantly different ($p < 0.05$) on the basis of multifactor ANOVA followed by the LSD test.

Conclusions

The presented results demonstrated that storage affected to a limited extent reduced-fat whipping cream properties. The use of high pressures (20 MPa) and the combined presence of k-carrageenan and gelatin conferred the best stability towards creaming phenomena during the first week of storage. Regarding whipping performances, after storage overrun tended to decrease slightly, but an improvement effect was observed on texture properties of whipped samples. Consistency index tended to increase after storage, but this result was influenced by the strong gelling effect of k-carrageenan in absence of gelatin. Thus, also in this case, gelatin concentration resulted a determining factor for the final properties of experimental creams, suggesting the need for clarifying the molecular mechanisms involved in water retention phenomena and competition amongst ingredients. On the other hand, high homogenization pressures allowed to obtain the better overrun and stability of whipped cream, in correspondence with the lowest initial viscosity of non-whipped cream.

4. CONCLUSIONS

Beyond the conclusions reported for the individual experimental sections, results from this PhD thesis may contribute to the creation of a knowledge background, still scarce in literature, about the effects of ingredients and processing technology on the structural, nutritional and sensory properties of reduced-fat matrices. The multidisciplinary approach adopted, comprehensive of all the functionality aspects related to a food product, may represent a starting point for the design of foods with targeted quality features and behavior during consumption. In such a complex investigation field, Experimental Design techniques coupled with multivariate analyses of the experimental data have confirmed to be effective tools for the characterization and optimization of both food formulation and processing. The developed mathematical models can be applied for reverse engineering and quality-by-design approaches, thus benefit both researchers and companies.

5.MATERIALS and METHODS

5.1 Case study 1: Reduced-fat biscuits

Experimental part 1: Reduced-fat soft-dough biscuits: Multivariate effects of polydextrose and resistant starch on dough rheology and biscuit quality.

Preparation of biscuits

Soft wheat flour '00' (protein content = 10 g/100g; Molino Dallagiovanna s.r.l., Gragnano Trebbiense, Italy), resistant starch Hi-Maize™ 260 (Ingredion, Manchester, UK; dietary fibre 56 g/100g), all-vegetable shortening (Crisco, Orrville, OH, USA), sucrose (Eridania Italia S.p.A, Bologna, Italy), polydextrose (Comprital S.p.A., Settala, Italy), leavening agent (Paneangeli, Desenzano del Garda, Italy), salt and deionized water were used to produce soft-dough biscuits (1 kg dough per each formulation). Soft-dough biscuit formulations were developed according to the levels of the experimental factors reported later on, varying the amount of shortening and flour as shown in Table 1 and keeping constant the other ingredients (added water, sucrose, leavening agent, and salt). A theoretical moisture content of about 30 g/100g was considered, since the soft-dough biscuits are produced by depositing rather than by moulding. The formulation 0-0 is a standard full-fat soft-dough recipe produced with shortening. A reference full-fat product (named REF) made with butter (Dalla Torre Dorotea s.r.l., Tassullo, Italy; 82 g/100g milk-fat) was also produced in duplicate, with the same formulation of sample 0-0, with the exception of shortening, substituted by 28.13 g/100g butter in order to assure the same fat amount. Added water was reduced to 20.57 g/100g, taking into account the water brought in by butter.

The production procedure was developed through preliminary trials and standardized assuming constant ambient moisture and temperature. Fat and sugar were creamed at room temperature in a kneading machine (N-50G, Hobart GmbH, Offenburg, Germany) for 4 min at low speed and 4 min at medium speed. Then, the other powder ingredients were added in two steps, with a mixing time of 30 s each, at low speed. Finally, water was added to the dough and mixed (2 min at low speed; 2 min at medium speed), thus obtaining a rather liquid dough. Immediately after preparation, a fixed weight (15 ± 0.1 g) of dough was portioned in 32 aluminium cups (height = 39 mm, top diameter = 85 mm, bottom diameter = 55 mm) by means of a spoon, spreading on the whole cup bottom surface, and baked for 25 min in a static oven preheated to 190 °C. The placement in the oven was kept constant for all the prepared samples. After cooling, the biscuits were stored in hermetic plastic boxes (27 x 20 x 7.5 cm) and analysed 24 h after production.

Experimental design

Experiments were planned according to a two-factor, three-level Face-Centered Central Composite Design. The two considered factors were the shortening reduction (SR) and the level of flour replacement (FR) with resistant starch in biscuit formulation. Based on preliminary trials, experimental factor levels were set as follows: 0%, 25%, and 50% for SR (replaced by the same amount of polydextrose) and 0%, 40%, 80% for FR. The design consisted of 15 experimental trials, including 7 replicates of the central point (Table 5.1.1). The order of experiments was fully randomized in order to avoid possible bias related to systematic effects and uncontrolled variations.

Dough characterization

Dough density was measured in triplicate pouring the soft dough in small containers of known volume (45 mL) and weighting them. Density was calculated as the weight/volume ratio, and expressed as g/mL. Dough rheological characterization was performed at 25 ± 0.1 °C with a Physica MCR 300 rheometer (Anton Paar, Graz, Austria) equipped with serrated parallel plates (PP25/P; 2.5 cm diameter). Strain sweep (SS; strain range, 0.001-10%; frequency, 1 Hz) and frequency sweep (FS; frequency range, 1-0.01 Hz; strain, 0.01%) tests were carried out at least in duplicate for each sample.

Biscuit characterization

Biscuit moisture, protein and fat contents were determined according to official standard methods (AACC 44-15A, 2000; AOAC 920.87, 1995; ICC Standard Method No. 136, 1984). Results represent the average of two replicates and are expressed as g/100g.

Top surface colour was measured using a colorimeter Chroma Meter II (Konica-Minolta, Tokyo, Japan), with standard illuminant C. Results are expressed in CIE $L^*a^*b^*$ scale as the mean of three determinations.

Milk absorption properties of biscuits were evaluated dipping one biscuit at a time for 10 s in 100.0 g UHT whole milk (Sterilgarda Alimenti, Castiglione delle Stiviere, Italy). After 2 s drainage, the absorbed amount was calculated as the difference from the initial and the final weight of milk and expressed as g/100g biscuits. Results are the average of five replicates.

Biscuit fracture properties were determined by a three-point bending test, performed with a TA-HD Plus texture analyser (Stable Micro System, Surrey, UK) equipped with a 500 N load cell. Samples were subjected to fracture at 10 mm/s speed, using the appropriate device (HDP/3PBeThree Point Bend) with a 30 mm span length. Measurements were replicated on 15 biscuits for each formulation. From the force-deformation curves, the maximum force opposed to fracture (N) and the breaking

deformation (mm) were obtained and then normalised on biscuit dimensions, calculating the fracture strength (σ_f, kPa) and strain (ε_f, %) with the following equations (Bruns and Bourne, 1975):

$$\sigma_f = \frac{3 \cdot F \cdot g}{2 \cdot d \cdot t^2} \quad \text{Eq. (5.1.1)}$$

$$\varepsilon_f = \frac{6 \cdot D \cdot t}{g^2} \cdot 100 \quad \text{Eq. (5.1.2)}$$

where F is the breaking force (N), g the span length (mm), d the sample diameter (mm), t the sample thickness (mm), and D the breaking deformation (mm).

Geometric indexes (thickness, diameter, area) and bottom porosity of biscuits were determined through image analysis techniques. The surface, bottom, and cross section images of two biscuits for each sample were taken at 600 dpi resolution and 24 bit colour depth 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 (mm; data not shown), thickness (mm), area (mm^2; data not shown), heterogeneity, and bottom porosity. Heterogeneity is a texture surface parameter defined as the fraction of pixels whose intensity value deviates more than 10% compared to the average intensity of the entire image. Heterogeneity ranges from 0 to 1 for homogeneous (smooth) and heterogeneous (rough) surfaces, respectively (Fongaro and Kvaal, 2013). For bottom porosity, a square area of interest (diameter = 48.3 mm) was selected in the central part of each sample image and converted to grey scale (8 bit). Pores were identified by automatically adjusting brightness and contrast, and applying a high-pass filter. Then, pores were counted and dimensionally measured. Biscuit bottom porosity was expressed as the total area of pores with respect to the area of interest (%), as the mean of two replicates.

Table 5.1.1: Face-Centered Central Composite Design matrix developed for the multivariate study of reduced-fat biscuits containing polydextrose and resistant starch: sample identification and formulation. A reference full-fat formulation (REF) made with butter is also reported.
SR, shortening reduction; FS, flour replacement.

Sample name	Actual factor levels		Dough formulation (g/100g)							
	SR (%)	FR (%)	Shortening	Polydextrose	Wheat flour	Resistant starch	Sucrose	Leavening agent	Salt	Water
0-0	0	0	23.07	-	38.45	-	11.54	1.23	0.08	25.63
50-0	50	0	11.54	11.54	38.45	-	11.54	1.23	0.08	25.63
0-80	0	80	23.07	-	7.69	30.76	11.54	1.23	0.08	25.63
50-80	50	80	11.54	11.54	7.69	30.76	11.54	1.23	0.08	25.63
0-40	0	40	23.07	-	23.07	15.38	11.54	1.23	0.08	25.63
50-40	50	40	11.54	11.54	23.07	15.38	11.54	1.23	0.08	25.63
25-0	25	0	17.30	5.77	38.45	-	11.54	1.23	0.08	25.63
25-80	25	80	17.30	5.77	7.69	30.76	11.54	1.23	0.08	25.63
25-40a	25	40	17.30	5.77	23.07	15.38	11.54	1.23	0.08	25.63
25-40b	25	40	17.30	5.77	23.07	15.38	11.54	1.23	0.08	25.63
25-40c	25	40	17.30	5.77	23.07	15.38	11.54	1.23	0.08	25.63
25-40d	25	40	17.30	5.77	23.07	15.38	11.54	1.23	0.08	25.63
25-40e	25	40	17.30	5.77	23.07	15.38	11.54	1.23	0.08	25.63
25-40f	25	40	17.30	5.77	23.07	15.38	11.54	1.23	0.08	25.63
25-40g	25	40	17.30	5.77	23.07	15.38	11.54	1.23	0.08	25.63
REF	-	-	28.13*	-	38.45	-	11.54	1.23	0.08	20.57

*butter was used instead of shortening

Data analysis

The experimental design allowed to estimate, for each response variable, the coefficients of the following postulated quadratic model (Montgomery, 2001):

$$y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 + \varepsilon \qquad \text{Eq. (5.1.3)}$$

where y is the response for the considered variable, X_1 the SR level, X_2 the FR level, b_0 the constant value, b_1 and b_2 the linear coefficients, b_{12} the interaction coefficient, b_{11} and b_{22} the quadratic coefficients, and ε the random error. The significance of each coefficient was determined by one-way analysis of variance (ANOVA). A multi-objective optimization of the low-fat biscuit formulation was carried out, constructing an overall desirability function (Alamprese et al. 2007; Montgomery 2001). Design Expert v. 10 software (Stat-Ease Inc., Minneapolis, MN, USA) was used for DoE development, regression modelling, and optimization. A Pearson correlation matrix of all the response variables was calculated using Statgraphics Plus 5.1 (Statistical Graphics Corp., Herndon, VA, USA).

Experimental part 2: Double emulsions for food applications: An optimization approach using D-optimal design.

Experimental design

A D-optimal design was used to investigate the effects of 4 factors (protein type and concentration, volume fraction of internal and external aqueous phase) on the characteristics of WOW double emulsions (Table 5.1.2). A total of 19 experiments plus 2 replicates of the central point were performed.

Table 5.1.2: Factors and levels of the experimental design for the study and optimization of double emulsions

	Factor			
Level	**Protein type**	**Protein concentration (g/100mL)**	**W_1 in W_1/O (mL/100mL)**	**W_1/O in $W_1/O/W_2$ (mL/100mL)**
-1	WP	0	20	40
0		5	30	50
1	EW	10	40	60

Double emulsion preparation

Protein solutions (5 g/100mL or 10 g/100mL) were prepared dissolving whey protein concentrate (WP, Milkiland EU, Poland) or dried egg white (EW, Sanovo, Germany) in NaCl solution (0.4 g/100mL) and stirring at ambient temperature for 1 h. These solutions were used as aqueous phase (W_1) in the primary water-in-oil emulsions (W_1/O). When level 0g/100mL of protein concentration in W_1 was set by experimental design, a water solution at 0.4g/100mL without protein addition was used as W_1. W_1/O emulsions were prepared by adding 20, 30 or 40 mL/100mL of aqueous phase to mays oil (Carrefour, France) containing 4 g/100mL polyglycerol polyricinoleate (Lasenor, Barcelona, Spain). The phases were mixed using a heavy duty blender (Waring Laboratory, New Hartford, CT, USA) at 18000 rpm for 30 s and at 20000 rpm for further 30 s. When the obtained W_1/O contained proteins, they were heated at 80°C for 20 min and cooled down in iced water for 15 min in order to induce gelation. External water phase (W_2) was prepared by dissolving 0.4 g/100mL NaCl and 5 g/100mL protein powder in distilled water, and stirring for 1 h at ambient temperature. Double emulsions were prepared by adding 40, 50 or 60 mL/100mL of W_1/O to W_2 and mixing as described previously for the primary emulsions. Double emulsions were stored at 4°C overnight before performing viscosity, yield and stability analyses.

Emulsion characterization

Flow curves were measured at 25°C using a Physica MCR 300 rheometer (Anton Paar, Graz, Austria) equipped with coaxial cylinders (CC27), in a 30-500 s^{-1} range of shear rate. From the flow curves of the emulsions, apparent viscosity was taken at 310 s^{-1}, while consistency coefficient K and flow behavior index n were obtained by the power law equation (Eq. 5.2) fitting the curves in the range 152-500 s^{-1}. Results are expressed as the average of at least two replicates.

$$\tau = K \cdot \dot{\gamma}^n \qquad \text{Eq. (5.1.4)}$$

where τ is the shear stress (mPa) and $\dot{\gamma}$ is the shear rate (s^{-1}) (Steffe 1996).

Yield, expressed as percentage of emulsified water droplets remaining inside the secondary oil droplets, was measured spectrophotometrically with a method adapted from Perez-Moral et al. (2014). $W_1/O/W_2$ were prepared following the procedure described above but using buffer sodium phosphate 5 mM with NaCl 100 mM (pH 7) as W_1. PTSA (1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt hydrate, Sigma Aldrich, St. Louis, USA) was added as tracer to W_1, and a "blank" WOW was also prepared without dye addition. The obtained $W_1/O/W_2$ emulsions were centrifuged in a benchtop centrifuge (Centrikon T-42K, Neufahrn, Germany) at 20000 rpm for 20 min at 10°C to separate fat and continuous phase. The concentration of dye appearing in the continuous phase was measured spectrophotometrically at 375 nm against a calibration curve and hence the percentage of dye remaining encapsulated within the $W_1/O/W_2$ emulsion droplets was calculated with the following equation adapted from Surh et al. (2007):

$$Yield\ (\%) = 1 - \left[\left(\frac{Cf}{Ci - C}\right) * \left(\frac{1 - Øwow}{Øwo * Øwow}\right)\right] \qquad \text{Eq. (5.1.5)}$$

where: C_i is the initial PTSA concentration in W_1, expressed as g/100mL; C_f is the final PTSA concentration in W_1, expressed as g/100mL; $Ø_{W/O}$ is the volume phase of W_1 in W_1/O, expressed as fraction; $Ø_{W/O/W}$ is the volume phase of W_1/O in $W_1/O/W_2$, expressed as fraction.

For creaming stability evaluation, double emulsions were prepared as previously described, but in this case the oil phase was colored with the pigment *Oil red O* (0.0015 g/100g). Immediately after preparation, emulsions (10 mL) were transferred into 10 mL graduated cylinders. The stability of the emulsions was evaluated by observing the separation of a cream layer after 1h and 24 h of storage at 4°C. Emulsions separated into a red top layer and a white bottom layer with a similar appearance to the original emulsion. Creaming stability (CS) was expressed as:

$$CS(\%) = 100 - \left[\left(\frac{H_t}{H_e}\right) * 100\right] \qquad \text{Eq. (5.1.6)}$$

where H_t is the height of the lipid layer creamed after 1 h or 24 h and H_e is the total height of the double emulsion. The method was adapted from Karaca et al (2011).

FT-IR spectra of double emulsion samples were acquired by a spectrometer (VERTEX 70, Bruker Optics, Ettlingen, Germany) equipped with horizontal germanium crystal multiple ATR plate (Pike Technologies, Inc., Madison, USA). The spectral data were collected in the range 4000–400 cm^{-1} at 20°C, with a 4cm^{-1} resolution and 32 scans both for background and samples. Opus software (v. 6, Bruker Optics, Ettlingen, Germany) was used for spectral acquisition, instrument control and preliminary file manipulation.

Data analysis

A quadratic model (Eq. 5.5) was postulated for each of the considered response variables. Data elaboration was performed with Matlab R2016b (Mathworks, Natick, MA, USA) and Design Expert 10.0.6 (Stat-Ease Inc., Minneapolis, MN, USA). The significance of each coefficient was determined by one-way analysis of variance (ANOVA). A multi-objective optimization of the double emulsion was carried out, constructing an overall desirability function (Alamprese et al., 2007; Montgomery, 2001).

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + \varepsilon \quad \text{Eq. (5.1.7)}$$

Experimental part 3: Reduced-fat biscuits with bean powders and double emulsions: interplay between food structure, nutritional and sensory acceptability.

Sample formulation and preparation

Deposited biscuit dough formulations are reported in Table 5.1.3. A standard biscuit formulation (STD) containing vegetable shortening was used as reference. As reduced-fat formulation, the optimized recipe (OPT; 46% fat reduction) developed in the Experimental Part 1 was used (Moriano, Cappa and Alamprese, 2018). Based on OPT recipe, RAW and EXTR formulations were obtained by substituting the amount of resistant starch used in OPT with raw and extruded bean powder, respectively. Bean powders were kindly provided by professor Perry K.W. Ng, Department of Food Science and Human Nutrition of the Michigan State University. WOW sample was formulated by substituting the remaining portion of shortening (12.38 g/100g) with the double emulsion optimized in the Experimental Part 2, prepared with 10 g/100g of egg white powder in the internal water phase W_1, 29 mL/100mL W_1 in W_1/O, and 60 mL/100mL W_1/O in $W_1/O/W_2$ (the final corn oil content was 42.6 mL/100mL). The optimization criteria for the double emulsion were the maximization of apparent viscosity, yield and stability, in order to resemble as much as possible a shortening. All biscuit samples were produced as described in Moriano et al. (2018) with a standardized procedure developed assuming constant ambient moisture and temperature.

Table 5.1.3: Formulation (g/100g) 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.

Sample	Flour	Shortening	Sugar	Salt	Water	Yeast	Polydextrose	Resistant starch	WOW	Bean powder
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	-

Doughs and biscuits were characterized as detailed in experimental part 1. Compositional analyses were enriched with the determination of ash, sugar and fiber content. Ash were determined according to official AACC method (AACC, 1995); sugars were assessed by HPLC–anion exchange chromatography with pulsed amperometric detection (Zygmunt et al. 1982). Total, soluble, and insoluble dietary fiber contents were determined by an enzymatic–gravimetric procedure (AOAC method 991.43 [AOAC International 1995], which corresponds to AACCI Approved Method 32-07.01).

Microstructural analysis

Microstructural characterization was performed at the Centre for Food Innovation and Development in the Food Industry of the University of Naples (Portici, Naples, Italy). 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 of samples was examined by means of Scanning Electron Microscopy (LEO EVO 40, Zeiss, Germany) with a 20 kV acceleration voltage and a ×1000 magnification.

In vitro digestibility

Englyst's method was applied as previously reported by Marti et al. (2017) to assess *in vitro* carbohydrate digestibility by estimating rapidly (RDS) and slowly (SDS) digestible starch from glucose release after 20 min and 120 min, respectively. After these digestive phases, resistant starch (RS) was quantified by gelatinizing and hydrolyzing the remaining starch. The amounts of glucose released were quantified by HPLC and starch fractions were calculated as follows:

$$\mathrm{RDS} = \mathrm{glucose}_{20} * 0.9 \qquad \text{Eq. (5.1.8)}$$

$$\mathrm{SDS} = (\mathrm{glucose}_{120} - \mathrm{glucose}_{20}) * 0.9 \qquad \text{Eq. (5.1.9)}$$

$$\mathrm{RS} = \mathrm{total\ starch} - (\mathrm{RDS} + \mathrm{SDS}) \qquad \text{Eq. (5.1.10)}$$

where $\mathrm{glucose}_{20}$ is the glucose released at 20 min and $\mathrm{glucose}_{120}$ is the glucose released at 120 min. RDS and SDS were expressed as percentage of available starch (RDS + SDS) (Englyst et al. 2000), while RS was expressed as percentage of total starch in biscuits. A total of 6 analytical replicates were performed for each sample.

Pepsin from porcine gastric mucosa (P6887, Sigma Aldrich, St. Louis, MO, USA) and pancreatin from porcine pancreas cell (P3292, Sigma Aldrich, St. Louis, MO, USA) were used for *in vitro* protein and lipid digestibility. *In vitro* protein digestibility was measured in two steps, simulating first gastric environment (pH 2, pepsin 0.115 µg/mL, 37°C) and then intestinal digestion (pH 6.8, pancreatin 0.75 mg/mL, 37°C). An amount of sample containing 150 mg proteins was dispersed in 25 mL HCl 0.2 mol/L and pH was adjusted at 2 with NaOH 6 mol/L. Then, 25 µL of pepsin solution was added and the sample was incubated at 37°C in a shaking bath for 1 hour. Afterward, 10 mL of phosphate buffer 2 mol/L and 2 mL of NaOH 0.6 mol/L were added to inactivate pepsin by neutralizing pH that was then adjusted to 6.8 with NaOH 6 mol/L prior to add 1 mL of pancreatin solution and incubate for 1 hour at 37°C in a shaking bath. Aliquots of 2.5 mL were taken immediately before the addition of pepsin, after 1 hour of acid hydrolysis, and after 10, 20 and 60 min of pancreatic attack. Undigested peptides (molecular weight > 3000 Da) contained in extracted

samples were precipitated with tricloroacetic acid (TCA) 10 g/100mL (final concentration) and quantified by Lowry assay (Lowry et al. 1951). Pepsin and pancreatin attacks were considered as separate phases and protein digestibility was expressed as the percentage of digested proteins on the amount of total protein present in the sample at the beginning of each step. Casein from bovine milk (C5890, Sigma Aldrich, St. Louis, MO, USA) was digested following the described procedure to verify the process. Four replicates were performed for each sample.

In vitro lipid digestibility was assessed by free fatty acid titration during pancreatic lipase activity in a thermostatic reaction vessel maintained at 38°C. An amount of sample containing 300 mg lipids and 300 mg of bovine bile extract (B3883, Sigma Aldrich, St. Louis, MO, USA) were weighted and mixed in the reaction vessel with a solution of 1.5 mL NaCl 1.5 M, 1.98 mL $CaCl_2$ 0.075 M and 11.52 mL of distilled water under constant stirring. After 5 minutes, pH was adjusted to 7.7 with NaOH 0.5 M; after 10 minutes from the beginning of the analysis, 100 µL of 50 mg/mL pancreatin solution were added to the suspension. Thus, the final composition of the sample in the reaction vessel was: 300 mg of lipids, 20 mg/mL of bile extract, 0.33 mg/mL pancreatin, and 1.09 mg/mL $CaCl_2$. Acidity was titrated with NaOH 0.01 M and the volume used was recorded every minute, for a total of 20 minutes. Lipid digestibility (%) was calculated in oleic acid equivalents as follows:

$$\text{Lipid digestibility (\%)} = \frac{V_{NaOH} * M_{NaOH} * MW_{oleic\ acid}}{W_{lipids}} * 100 \qquad \text{Eq. (5.1.11)}$$

where V_{NaOH} corresponds to the volume of NaOH required to neutralize the free fatty acids produced (mL), M_{NaOH} is the concentration of the NaOH solution used (M), $MW_{oleic\ acid}$ is the molecular weight of oleic acid (282.47 g/mol) and W_{lipids} is the mass of lipids initially present in the reaction vessel (g). Since the sample per se and released aminoacids can contribute to pH changes during digestion, samples were also analyzed both without enzyme addition and defatted by Soxhlet extraction. V_{NaOH} was calculated by subtracting the volume of NaOH necessary to neutralize the acidity developed by the sample per se and during digestion of defatted samples from the volume of NaOH necessary to neutralize the original samples. Three replicates were performed for each sample.

Consumer's acceptability of biscuits

Eighty-six subjects (57% males,43% females, 18-69 years, mean age 24) participated in the study carried out at the Food and Wine Sensory Laboratory of the University of Gastronomic Sciences (Bra, Italy). They had seen or received an invitation and volunteered based on their interest and availability. All tests were conducted in individual booths under white light, social interaction was not permitted. Participants evaluated a set of five biscuits presented in blind condition in coded, clear disposable plastic cups (237 mL) hermetically sealed with a clear plastic lid and in random order across subjects. For each sample, the consumers rated the appearance, aroma, taste, flavor, texture and overall liking on a nine-point scale ranging from 1 (extremely dislike) to 9 (extremely

like) (Peryam and Pilgrim, 1957). Consumers were required to rinse their mouth with still water during a 60 s rest interval between samples.

Data analysis

One-way analysis of variance (ANOVA) was applied to experimental data in order to compare the samples. Liking data (overall acceptability, liking for appearance, aroma, taste, flavor and texture) from consumers were independently subjected to a two-way mixed ANOVA model (fixed factor: sample; random factor: subject). The Tukey's HSD post-hoc test at $p<0.05$ was used to evaluate significant differences among averages (Statgraphics Plus 5.1, Statistical Graphics Corp., Herndon, VA, USA). Overall acceptability data expressed by all 86 subjects were subjected to the principal component analysis in order to obtain an internal preference map for explorative purposes. PCA was also applied with explorative purposes to the results obtained for all the measured variables (structural, nutritional, sensory) and then a Pearson correlation matrix of all the response variables was calculated using Matlab (R2018a, The MathWorks Inc., Natick, MA, USA).

5.2 Case study 2: Reduced-fat whipping cream

Experimental part 1: Reduction of fat in whipping cream: effect of gelatin addition and homogenization conditions.

Experimental design

Experiments were planned according to a D-optimal design, considering four independent factors and levels as follows: gelatin concentration (0g/100g, 0.125g/100g, and 0.25g/100g); total homogenization pressure (15 MPa, 17,5 MPa, and 20 MPa); gelatin type (A and B); ratio between pressure applied in the second homogenization step and pressure applied in the first homogenization step (0 and 0.2, where 0 means that total pressure was applied in one step only). A total of 17 experiments plus 2 replicates of a central point were carried out. The experimental matrix is reported in Table 5.2.1.

Table 5.2.1: D-optimal Design matrix developed to investigate the effects on properties of a reduce-fat (25 g/100g) whipping cream of gelatin type, gelatin concentration, homogenization pressure, and ratio between pressure applied in the second (p2) and in the first step (p1).

Sample name	Experimental factor levels			
	Gelatin concentration (g/100g)	Homogenization pressure (MPa)	Gelatin type	p_2/p_1
B0.25_15_0	0.25	15	B	0
A0_15_0	0	15	A	0
B0_15_0	0	15	B	0
A0.125_17.5_0.2	0.125	17.5	A	0.2
A0.125_20_0.2	0.125	20	A	0.2
A0.125_17.5_0	0.125	17.5	A	0
A0_15_0.2	0	15	A	0.2
B0_15_0.2	0	15	B	0.2
A0.125_17.5_0.2	0.125	17.5	A	0.2
A0.25_17.5_0.2	0.25	17.5	A	0.2
A0_20_0	0	20	A	0
B0.125_20_0	0.125	20	B	0
A0.25_15_0	0.25	15	A	0
B0.25_15_0.2	0.25	15	B	0.2
A0.125_17.5_0.2	0.125	17.5	A	0.2
B0.25_17.5_0	0.25	17.5	B	0
B0_20_0.2	0	20	B	0.2
B0.25_20_0.2	0.25	20	B	0.2
A0.25_20_0	0.25	20	A	0

The order of experiments was fully randomized, in order to avoid possible bias related to systematic

effects and uncontrolled variations. Samples were identified by codes including the levels of the experimental factors tested (i.e.: sample B0.25_15_0 contained gelatin type B with 0.25g/100g concentration and was treated at 15 MPa with a single homogenization step).

Cream production

Experimental cream samples (45 kg each) were prepared at the pilot plant of ILVO - Food Pilot (Melle, Belgium) by using heavy cream (40g/100g fat) (Lactis Pur Natur, Sint-Pieters-Leeuw, Belgium), skim milk powder (Belgomilk, Kallo, Belgium), gelatin 250 PS 60 (A), gelatin 100 PS 30 (B) (Rousselot BVBA, Ghent, Belgium), k-carrageenan stabilizer XP SGW 901 (Ingrizo, De Pinte, Belgium), and tap water. Gelatins were kindly provided by Rousselot (Table 5.2.2 reports gelatin characteristics), while stabilizer was kindly provided by Ingrizo. Firstly, skim milk powder was dissolved in warm water and both stabilizer and gelatin (when present) were added while stirring (Typhoon, Raamdonksveer, The Netherlands); then, heavy cream was added in order to obtain a standardized fat content of 25g/100g and the mixture was subjected to a second milder stirring. The UHT treatment (120°C for 6 s) was carried in an indirect tubular heat exchanger (SPX Corporation, Charlotte, North Carolina, United States) and it was followed by homogenization (70°C) by a high pressure homogenizer (SPX Corporation, Charlotte, North Carolina, United States) at different pressures (15 MPa, 17.5 MPa, and 20 MPa) and with one or two steps according to the experimental plan. The treated cream was then cooled down to 4°C, packed in 1 L volume bag-in-box and stored at 4°C. Analyses were carried out 24 h after cream production.

Table 5.2.2: Characteristics of gelatins used for reduced-fat cream development.

Gelatin type	**Characteristics**		
	Origin	Gel strength (Bloom)*	Particle size (mm)
A	Pig skin	250	0.25
B	Pig skin	100	0.60

*Bloom indicates the force required to depress a prescribed area of the surface of a 6.67% gelatin gel at 10°C to a distance of 4 mm.

Note: Adapted from www.rousselot.com

Particle size analysis

Particle size analysis was performed by laser diffraction using a Mastersizer 2000 with the sample dispersion unit Hydro 2000 G (Malvern Instruments Ltd., Malvern, UK). The refractive index of milk fat globules was taken to be 1.46, absorption 0.01 and water refractive index 1.33. Volume weighted mean diameter of fat globules $d_{[4,3]}$ (μm), span, d(0.1), d(0.5) and d(0.9) were calculated by the software Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK).

Apparent viscosity

Apparent viscosity of cream samples was analyzed at 10°C by MCR 302 rheometer (Anton Paar, Graz, Austria) equipped with parallel plates PP50 (50 mm diameter; 1 mm gap), in a 1-100 s^{-1} range of shear rate. Each sample was analyzed at least twice. Flow curves were fitted by the power law equation (Eq. 5.1.2), in order to calculate the consistency coefficient (K) and the flow behavior index (n).

Overrun

Creams (1 L) were whipped in a domestic mixer Kenwood Cooking Chef Major (De Longhi Group, Treviso, Italy), at 641 rpm (speed 6). The mixer bowl was previously cooled in ice. Overrun was determined by weighing a fixed volume (150 mL) of raw cream and the same volume of whipped cream and using the following equation:

$$\text{Overrun } (\%) = \frac{M_1 - M_2}{M_2} \text{x} 100 \qquad \text{Eq. (5.2.1)}$$

where M_1 is the weight of a fixed volume of raw cream and M_2 the weight of the same volume of whipped cream. In order to describe the air incorporation as a function of whipping time, the overrun measurement was performed at 15 s intervals. Whipping was stopped when a decrease in overrun values was observed. The test allowed to choose a reference whipping time for each sample, used in the following texture analysis. Whipping data were fitted by the following equation:

$$y = a + b\,(1 - \exp^{-ct}) \qquad \text{Eq. (5.2.2)}$$

where y is the overrun, t is the whipping time, a and b are constant values (a+b corresponds to overrun tending to infinity), and c is the rate of air incorporation.

Texture Profile Analysis

Texture measurements were performed by LF Plus Texture Analyzer (Lloyd Instruments Ltd, Bognor Regis, UK), carrying out a Texture Profile Analysis (TPA) (Bourne, 1978). Each sample was analyzed at five different whipping times: the time corresponding to the maximum overrun value and the ±15 s and ±30 s times. For each time, 1 L of cream was whipped and TPA was performed on sample contained in three different cups of fixed volume (150 mL, the same used for overrun measurement), positioning the sample at the center of the probe area. A cylindrical probe (diameter 50 mm) was used to double compress the samples up to 40% of their original height. Test speed was 60 mm/min and a 2 seconds rest period was set between the two compressions. Cream hardness, cohesiveness, adhesiveness, and springiness were calculated by the Nexigen Plus 3.0

software (Lloyd Instruments Ltd, Bognor Regis, UK).

Whipped cream stability

Whipped cream stability was evaluated by leakage of liquid after 24 h of storage at 4°C. Immediately after whipping, 150 mL of whipped cream was placed on a metal sieve and the amount of leakage liquid after 24 h storage was weighted. Stability was calculated as the percentage of remaining cream, according to the following equation:

$$Stability\ (\%) = \frac{C_{start} - W_{serum}}{C_{start}} * 100 \qquad \text{Eq. (5.2.3)}$$

where C_{start} is the whipped cream weight at the test start and W_{serum} is the serum weight after 24 h of storage.

Data analysis

Design Expert 10 (Stat-Ease Inc., Minneapolis, MN, USA) and Matlab R2017a (The MathWorks Inc., Natick, MA, USA) were used to set the experimental design and to estimate, for each response variable, the coefficients of the following postulated quadratic model:

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22}X_2^2 + \varepsilon \qquad \text{Eq. (5.2.4)}$$

where y is the response for the considered variable, X_1 the gelatin concentration level, X_2 the pressure level, X_3 the gelatin type level, X_4 the ratio between pressure in step 2 and pressure in step 1, b_0 the constant value, b_1, b_2, b_3, b_4 the linear coefficients, b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34} the interaction coefficients, b_{11} and b_{22} the quadratic coefficients, and ε the random error. The significance of each coefficient was determined by ANOVA.

Principal component analysis (PCA) and Pearson correlation matrix were calculated using Matlab R2018a (The MathWorks Inc., Natick, MA, USA), in order to explore data variability. The data set was pre-treated dividing each response variable by its mean and standard deviation.

Experimental part 2: Reduced – fat whipping cream storage stability

Turbiscan stability

Creaming stability of samples was determined through Turbiscan LAB® (Formulaction, Toulouse, France). Cream (20 mL) was poured into glass cylindrical vials to a height of approximately 55 mm. Each sample was analyzed in triplicate at 0, 1, 3 and 7 days from production; samples were stored at 4°C between analyses. Stability was evaluated by monitoring the delta backscattering profile variation (ΔBS, %) and by calculating the Global Turbiscan Stability Index (TSI) at the different storage times.

Structural characterization

Characterization of cream samples was carried out after 3 weeks of storage at 4°C performing the same analyses described in experimental part 1.

Data analysis

Multifactor analysis of variance (ANOVA) was applied to the analytical data in order to study the effects of the considered factors: gelatin concentration (0 g/100g, 0.125 g/100g, 0.25 g/100g), homogenization pressure (15 MPa, 17.5 MPa, 20 MPa), homogenization steps (1, 2) and storage time (0, 3 weeks). The 2-factor interaction terms for storage time (gelatin concentration × time, homogenization pressure × time, homogenization steps × time) were also considered in ANOVA. The Least Significant Difference (LSD) test at $p < 0.05$ was used to evaluate significant differences among the averages (Statgraphics Plus 5.1, Statistical Graphics Corp., Herndon, VA, USA). Principal component analysis (PCA) was calculated using Matlab R2018a (The MathWorks Inc., Natick, MA, USA), in order to explore data variability. The data set was pre-treated dividing each response variable by its mean.

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Honey, trehalose and erythritol as sucrose-alternative sweeteners for artisanal ice cream. A pilot study

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ABSTRACT

The use of sucrose-alternative sweeteners in ice cream production could satisfy requirements of modern consumers focused on natural and nutritionally balanced foods. The aim of this work was to fill the gap in basic knowledge about the effects of honey, trehalose, and erythritol on the properties of artisanal ice cream. A milk-based sucrose-sweetened ice cream was produced as reference sample (REF), using then the alternative sweeteners to partially (50%) or totally (100%) substitute sucrose. With respect to REF, honey-containing ice cream mix revealed a significantly lower value of soluble solids (30.4 °Bx *vs.* 34.5 °Bx) and apparent viscosity (36.5 mPa s *vs.* 47.6 mPa s) and a significantly higher extrusion time (8.18 min *vs.* 7.04 min). The total substitution of sucrose with trehalose and erythritol led to a melting rate (2.07 and 1.56 g/min, respectively) significantly lower than REF (2.75 g/min), a very high firmness (508 and 725 N *vs.* 4 N), and a higher extrusion temperature (−7.1 and −5.3 °C *vs.* −9.3 °C). The results of this study represent a guideline for the successfully utilization of honey, trehalose, and erythritol in peculiar ice cream formulations (e.g. non-sweet or low-calorie products).

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Organogels as novel ingredients for low saturated fat ice creams

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ABSTRACT

The aim of this work was to evaluate the use of sunflower oil organogels made with phytosterols and γ-oryzanol as milk cream substitutes in artisanal ice creams. Fat amount (4 and 8 g/100 g) and type (milk cream, sunflower oil, and organogels containing two levels of gelators) were considered as factors. The higher fat amount significantly decreased density (1.08 ± 0.01 g/mL *vs* 1.10 ± 0.01 g/mL) and soluble solid content (27.2 ± 0.3 °Bx *vs* 30.1 ± 0.3 °Bx) of mixes, as well as ice cream overrun (31.1 ± 0.6% *vs* 37.3 ± 0.6%) and melting rate (2.5 ± 0.1 g/min *vs* 2.9 ± 0.1 g/min). The use of organogels with the highest gelator concentration yielded ice creams with quality characteristics comparable to those of the samples containing milk cream, and even better overrun (42.4 ± 0.8% *vs* 37.1 ± 0.8%) and melting starting time (20 ± 1 min *vs* 16 ± 1 min). Thus, the application of organogels in artisanal ice creams is a successful approach in order to obtain "low saturated fat" products (saturated fat < 0.9 g/100 g) "with added plant sterols and stanols" intended for people who want to lower their blood cholesterol level.

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Effect of physicochemical and empirical rheological wheat flour properties on quality parameters of bread made from pre-fermented frozen dough

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ABSTRACT

The objective of this study was to examine the influence of flour quality on the properties of bread made from pre-fermented frozen dough. The physicochemical parameters of 8 different wheat flours were determined, especially the protein quality was analysed in detail by a RP-HPLC procedure. A standardized baking experiment was performed with frozen storage periods from 1 to 168 days. Baked bread was characterised for specific loaf volume, crumb firmness and crumb elasticity. The results were compared to none frozen control breads. Duration of frozen storage significantly affected specific loaf volume and crumb firmness. The reduction of specific loaf volume was different among the used flours and its behaviour and intensity was highly influenced by flour properties. For control breads wet gluten, flourgraph E7 maximum resistance and RVA peak viscosity were positively correlated with specific loaf volume. However, after 1–28 days of frozen storage, wet gluten content was not significantly influencing specific loaf volume, while other parameters were still significantly correlated with the final bread properties. After 168 days of frozen storage all breads showed low volume and high crumb firmness, thus no significant correlations between flour properties and bread quality were found. Findings suggest that flours with strong gluten networks, which show high resistance to extension, are most suitable for frozen dough production. Furthermore, starch pasting characteristics were also affecting bread quality in pre-fermented frozen dough.

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Reduced-fat soft-dough biscuits: Multivariate effects of polydextrose and resistant starch on dough rheology and biscuit quality

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ABSTRACT

The aim of this work was a multivariate study of polydextrose and resistant starch (high amylose maize starch; Hi-Maize™ 260) effects on reduced-fat soft-dough biscuits. Design of Experiments and Response Surface Methodologies were applied to model the effects on dough rheology and biscuit quality of a partial substitution of fat with polydextrose (0–50%) and of flour with resistant starch (0–80%). The calculated models evidenced highly significant effects ($p < 0.001$) of the experimental factors on dough density and rheological behaviour and on most biscuit characteristics (colour, milk absorption, and fracture strength). With the increase of shortening reduction, higher density and lower complex modulus of dough were observed. The biscuit quality characteristics most influenced by shortening and flour substitution were fracture strength and strain, redness (a^*), heterogeneity, and milk absorption. The obtained models were used in a desirability function for the optimization of the reduced-fat formulation based on the following constraints: 25–50% shortening reduction; 41–44 g/100 g milk absorption; 100–160 kPa fracture strength. The optimized product (17.54 ± 0.05 g/100 g fat), obtained with 46.3% shortening reduction and 12.5% flour replacement, had quality characteristics similar to those of full-fat biscuits (31.1 ± 0.4 g/100 g fat).