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New functional vegetable ingredients for new consumer needs

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Summary

The research presented in this industrial PhD thesis was developed jointly by HI-FOOD S.p.A. and the Department of Food and Drug of the University of Parma.

HI-FOOD is a company specialised in the Research and Development and commercialisation of plant-based functional ingredients with a technological scope. The core of the project was the study of the technological functionality of two new ingredients developed by HI-FOOD and their application in foods in which a reformulation was necessary to increase their nutritional profile and at the same time to satisfy the consumer request of clean label products.

In particular, the two ingredients studied were HI-MODI FLOUR M and MELTEC® and the results obtained are here briefly summarised:

HI-MODI FLOUR M

A physically modified corn flour obtained through a heating-extrusion process.

Through a multilevel and multi-analytical approach, the flour-water interactions of the new designed ingredient were studied, comparing the latter to a heated and a native corn flours ascertaining its higher water affinity. To prove its feasibility at industrial level its technological functionality was positively tested as a clean label thickening agent in three industrial applications: a carrot soup, a tomato sauce and a meat patty.

Moreover, the thickening properties of the physically modified corn flour were stress-tested in the production of reduced fat mayonnaises. The physicochemical, rheological and sensorial test, corroborated the ability of the flour to act in substitution of fat as clean label thickening agent, stabilizing the continuous phase of the studied reduced-fat mayonnaise, without affecting its organoleptic properties.

MELTEC®

A semi-solid fibre syrup based on maize and chickpea.

The technological functionality as bulking agent in sugar-reduced products was ascertained in three different food matrices. In short bread cookies was pointed out that its presence permitted to partially preserve the structure of the cookies without jeopardize their processability. And more importantly, it was able to enhance both the nutritional profile and the consumer acceptability of cookies when compared to the ones in which sugar was simply removed.

The bulking effect of the semi-solid fibre syrup was also studied in two high-sweet products: a fruit filling and a ripple sauce. In both cases its valuable effect on a technological standpoint to reduce the 30% of sugar was established. Moreover, the obtained products which sugar was reduced by 30% were physiochemically and with rheological properties similar to the full sugar counterpart also during storage, and above all positively accepted by consumers.

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Chapter 1

General Introduction

1 The story behind the food labelling

Food has not always been packed and consequently labelled as we can see nowadays on the shelf of food shops. In the first local food shops consumers purchased foods in bulk, free to choose both the quantity and quality of the items. In this situation, sellers and consumers had a direct relationship. It was indeed the seller the owner of the information about the products sold and the connecting point between the consumers and the producers (Alan, 1995). Then, the massive production of food concomitantly with the development of new packaging materials contributed to pre-packed food diffusion. These novelties in the food products needed to be regulated by the legislators in different countries also to avoid the spreading of food adulteration. The early food labelling legislations were rudimental and were not accurate as the one that we can find nowadays in the EU market (Alan, 1995). One of the first European country which set out a food labelling legislation was the UK. A document dated 1907 which promulgated specific rules on the mandatory indication of certain food ingredients on labels, like additives (Alan, 1995). Subsequently, in 1946 again in the UK, it was established the first formalised legislation on food labelling, the “Labelling of Food Order, 1946” (SR&O 1946, No 2169) in which were imposed detailed label requirements as the name of the packer, the common and appropriate food designation, the list of ingredients and the net weight. Over the years, the attention on consumers rights and the importance of food safety growth in all Europe. During 1978 Europe commission promulgated the first council directive of the European Commission on the approximation of the law of the Member States regarding to the labelling, presentation and advertising of food stuff for sale to consumers (Council Directive 79/112 EEC). In this directive, the focus was addressed to increase the information transparency and to decrease product misleading, obliging the company to identify the manufacturer and the ingredients used for its production. Nowadays in Europe food labelling is regulated by the European regulation on food labelling (Reg. EU 1169/2011) which considerably increased the mandatory information that a food label shall report in order to achieve a higher level of protection for the consumers and to guarantee the highest as possible right on information. In the frame of food labelling and the information to consumer, a cornerstone of the European food legislation is also the EU regulation on nutritional and health claims (Regulation (EC) No 1924/2006) in which are clearly fixed on a scientific base the term and condition for which is possible add on the front of packaging nutritional health claim as “reduced sugar”, “rich in fibre”, “low fat” etc.. Therefore, the European food labelling legislation framework moved hand-in-hand with the growing request of information by consumer which claim to have the highest knowledge as possible for a correct decision before the purchase.

2 The consumer fear for food additives and the rising of “clean label” trend

The request of information needed by the consumers led in the 1960s to the creation in Europe of the today's widely known E-numbers system. This classification was created to reassure the consumers that the ingredients used in the food products for technological reasons were safe for consumption (Faustino et al., 2019; Saltmarsh, 2014). Unfortunately, the presence of codes and numbers on food labels useful for the product description, raised questions and suspects from the public opinion which were emphasised by some publications which suggested to be suspicious on the safety of the food additives (Saltmarsh, 2014). The most famous publications were the book titled “E for additives” (Peters, 1986) and the so called “Villejuif list”, a fake document on the letterhead of a France hospital in which were listed toxic and suspicious, as stated by the author, food additives (Saltmarsh, 2014). The suspect on food additives spread in all Europe countries and consumers were pushed to check for E-number additives in their foods. They began to avoid products in which additives were present looking for more natural ingredients. This interest of the consumer to choose products with “healthy”, “familiar” and “natural” ideological meanings growth year by year and this trend started to be categorised by the term “clean label” (Asioli et al., 2017). The trend is still very popular as evidenced by the Ingredion report in 2019 (Ingredion, 2019) for which “clean label” is stated as a top market trend in food.

Anyhow, a clear and official definition of the term clean label does not exist, leading to different and misleading interpretation of such term. During the “Global Food Forum Clean label Conference”, researchers Hutt and Sloan (Hutt & Sloan, 2015) identified three key characteristics of a clean label food product: (i) a short list of ingredients, (ii) the absence of artificial ingredients, preservatives and additives, (iii) a minimal processing. Interestingly, Ingredion company published a clean label guide (Ingredion, 2014) in which identified as easily accepted ingredients which consumer can easily find in its own kitchen cupboard without any chemical sound name. On the basis of the different interpretation surely the “naturalness” of the ingredients in substitution of the E-numbers is a crucial step which industry need to consider to increase the market value of their products. In Italy for example, during the 2019 the sales volumes of products containing the claim “free from additives” had an increase of 2.7% (Osservatorio Immagino GS1 Nielsen, 2020).

3 Consumer health concerns and food reformulation strategies

Nowadays consumers are no longer driven by the only mere nutritious aspect when buying food. In fact, factors such as price, flavour, taste and culture concur at the time of purchase. Besides the abovementioned clean label, one of the major trends is the attention on health concerns related to food consumption and the increase in knowledge on the strong connection between dietary habits and health (Annunziata & Vecchio, 2011; Kearney, 2010; Roberfroid, 2000). Official institutions and governments are indeed undertaking communication campaigns to sensitise consumers to food-related diseases. In particular, in order to contrast overweight, obesity and the correlated long-term health ailments, type-2 diabetes, cardiovascular disease, hypertension and cancer (Lin et al., 2015; Manson & Bassuk, 2003). A pillar of the international nutritional guideline to contrast overweight and obesity is the reduction of ingestion of food with high caloric density and in particular the reduction of fat (European Food Safety Authority, 2010) and sugar (World Health Organization, 2015). In particular, EFSA in its opinion on the dietary reference for fat (European Food Safety Authority, 2010) suggests a fat intake ranging between 20%-35% of the total daily energy requirement. Unfortunately, in eastern countries the mean fat intake ranges between 28%-45% (Elmadfa & Kornsteiner, 2009). Similarly, the WHO (World Health Organization, 2015) in its guideline suggests that the sugar intake shall be less than 10% of the total energy intake while at the moment in Europe the $\approx 15\%$ - 21% and $\approx 16\%$ - 26% of the energy intake is represented by sugar in adults and children, respectively (Azaïs-Braesco et al., 2017).

Understanding consumer requests for healthier foods, is an important strategy for the food industries to differentiate their products, capturing the interest of consumers (Bigliardi & Galati, 2013). Concomitantly, the smart and innovative product recipe reformulation approach is capable to improve the nutritional profile of food products. However, following this methodology is highly challenging, considering the immense efforts concurring for the development of new products without affecting the overall quality and the consequent consumer acceptance (Judith L. Buttriss, 2013).

In particular, when the reformulation is based on sugar or fat reduction or substitution it is necessary to find solutions able to counterbalance their absence as both have a multifunctional role in foods. In detail, at technological level the presence of macronutrients strongly impacts in the texture, mouthfeel and flavour of food, hence their substitution can be highly challenging ("Handbook of Fat Replacers" 1997; Hutchings et al., 2019).

Fat replacement strategies are diversified and based on different approaches changed over the years on the basis of the different market trends. Initially, the first fat substitutes produced were synthetically produced and, in this range, the most famous was "Olestra", a non-metabolizable sucrose polyester patented by Procter and Gamble in 1971 with physicochemical characteristics similar to a triglyceride. Olestra was created substituting the glycerol molecule of the triglyceride with a sucrose to which were esterified 6 to 8 fatty acids (Prince & Welschenbach, 1998). In the wake of the synthetically based ingredient, in the early 90's, low-calorie fats were created to substitute standard fat. The idea was to modify the long-chain fatty acid esterified with the glycerol of the triglyceride molecule with medium-chain

fatty acid with a lower caloric index and with a lower tendency to be incorporated into tissues as fat deposits (Babayan, 1987; Marten et al., 2006). A different approach able to replace only a part of the total fat of the recipe but without the use of synthetically produced ingredients was the development of maltodextrins (hydrolysed starches with a low dextrose equivalent value) which use in water solution over 20% permitted to create thermo-reversible plastic and spreadable gel similar to fat (Setser & Racette, 1992). In the 90's a new technology was introduced on the market based on whey microparticulated protein; the underlying principle was the surrogation of the fat droplets with the microparticulated proteins ("Handbook of Fat Replacers" 1997). In the years the growing interest through the use of ingredients, also considered healthier, led to the development of fibre bases fat replacers and in particular, gel of fat replacer based on β -glucan or obtained from inulin started to be frequently used and studied and they still are commonly used ("Handbook of Fat Replacers" 1997; Shoib et al., 2016). Another common approach used by the food industry is the stabilisation of the water phase using thickening agent. The most common thickening agents used are additives as hydrocolloids (e.g.: guar gum, xanthan gum) or chemically modified starches (Z. Ma & Boye, 2013; Scrinis & Monteiro, 2018).

Strategies to reduce sugar intake were also highly studied during the years and can be categorized in four categories (i) multisensory integration, (ii) gradual sugar reduction, (iii) food structure innovation (iv) use of sugar substitutes (Hutchings et al., 2019).

In the multisensory integration approach some sensory sensations are enhanced to increase the perception of sweetness in a food product. For example, enhancing odours in a food with the use of aromas which are commonly associated to a sweet sensation can lead in the consumer to an increase of the perceived sweet (Frank & Byram, 1988; Hutchings et al., 2019). A similar approach is the use of specific colour in the design of a food product to influence the sweetness sensation of the consumer (Hutchings et al., 2019). Both multisensory strategies at the moment can lead only to a small sugar reduction and a scientific debate on their utility is still on course. A different approach that the food industry is using, also pushed by local government, is the gradual reduction of sugar in the food products over time trying to wean consumer to high sugar food products (Wise et al., 2016). This strategy cannot be used in all food products, in particular the one containing an high sugar content, and intrinsically needs a long time period to be realized (Hutchings et al., 2019). A completely different approach is to modify the food structure to modify the sensation of sweetness in the mouth. A method is the inhomogeneous distribution of sugar in food, in this way some area of the food contains a high amount of sugar while others contain a lower or absent amount. This technique creates a discontinue perception at the level of taste receptors which leads to an increase in the perception of sweetness (Burseg et al., 2010). Another methodology used under study is the modification of the food fracture mechanism; indeed, it has been observed that more brittle foods which size reduction can be obtained quickly during chewing leads to a rapid release of sugar and therefore to an enhance stimulus of sweetness sensation on the taste receptors (Mosca et al., 2010). For liquid as beverage an approach is the reduction of their viscosity to speed up the transfer of the sugar from the food to the mouth and thus enhancing the sweetness sensation (Hollowood, 2002). All the above mentioned methodologies based on the modification of the

food structures are however limited in the food industry because require an high use of resources in the redesign of food product and can be limited by a negative approach of the consumer to a new food structure different from the one is used to (Hutchings et al., 2019). Overall the most common strategy used by the food industry remains the use of sugar substitutes and in particular non-nutritive sweeteners (eg.: sucralose, aspartame, acesulfame K, saccharin, stevia etc..) to counterbalance the reduction lack of sweetness in a food product or the use of bulking agent as sugar alcohol (polyols), inulin or polydextrose as ingredients able to counterbalance the technological properties of sugar (Di Monaco et al., 2018; Riedel et al., 2015).

Therefore, in general the use of ingredients and in particular food additives to substitute or reduce sugar and/or fat remain at the moment in the most common key strategy applied by the food technologists. However, food additives cannot be used when the scope of the reformulation includes obtaining a clean label status, thus failing in giving the consumer need of “naturalness” (Asioli et al., 2017; Battacchi et al., 2020). Consequently, it is therefore important to work on the research and development of new functional ingredients able to perform at technological level the functionality of E-numbers.

4. HI-FOOD: a R/D company specialised in clean label food ingredients

HI-FOOD is a fast-growing company specialized in the research, development and production of innovative plant-based-clean label functional ingredients for conventional and disruptive food industry. Grown inside the University of Parma, HI-FOOD has been implementing a strong R&D approach to create natural functional ingredients with outstanding functionalities and the best possible ration dosage/cost in use/benefits. The mission of Hi-Food is to develop, produce and supply plant-based-clean label and high technological ingredients for the food industry.

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Research purpose

This industrial PhD thesis focuses on the study of two new vegetable-based ingredients and their application in complex food matrices, especially when a reformulation approach is necessary to enhance the nutritional profile of the product in which they are included.

In particular, two new functional vegetable ingredients and their technological functionality for fat and sugar reduction strategies was exploited.

The first ingredient to be studied was a physically-modified corn flour (HI-MODI FLOUR M) and the results are illustrated in two chapters:

Chapter 2: Probing the Functionality of Physically Modified Corn Flour as Clean Label Thickening Agent with a Multiscale Characterization

A physically modified corn flour was characterised through the use of a multilevel and multi-analytical approach for its physicochemical properties and subsequently tested as thickening agent in a tomato sauce, a vegetable soup and a meat patty.

Chapter 3: Can a physically modified corn flour be used as fat replacer in a mayonnaise?

The functionality of the physical modified corn flour was stress tested as fat replacer to produce clean label reduced-fat mayonnaises which was subsequently analysed for their physicochemical, rheological, stability and sensorial properties.

The second ingredient studied was a semi-solid fibre syrup based on chickpea and maize (MELTEC®) and the results are illustrated in three chapters:

Chapter 4: Use of a semi-solid fibre syrup for the sugar reduction in shortbread cookies

The semi-solid fibre syrup was used as clean label ingredient for sugar reduction in short bread cookies which were subsequently analysed for their physicochemical, rheological and consumer acceptability in comparison to cookies in which sugar was simply eliminated.

Chapter 5: A semi-solid fibre syrup for the sugar reduction in fruit filling for bakery application

Chapter 6: The reduction of sugar with a semi-solid fibre syrup: the ripple sauce case study

The feasibility as bulking agent ingredient in sugar reduction application was proved in two high sweet products: a fruit filling and a ripple sauce. Both matrices were studied during storage at two different temperatures for their physicochemical, rheological, stability and sensorial characteristics.



Chapter 2

Probing the Functionality of Physically Modified Corn Flour as Clean Label Thickening Agent with a Multiscale Characterisation

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Abstract

A multilevel and multi-analytical approach, combining both traditional and unconventional analytical tools, was used to characterise two physically modified (heated and heated-extruded) corn flours to be used as a “clean label” food ingredient. Physical treatments decreased the resistant starch content and increased the water holding capacity and water binding capacity, more extensively in the product subjected to heating-extrusion, as compared to an untreated control. Heated-extruded flour had the highest ability to form homogeneous systems in cold water while all modified flours produced homogeneous systems when mixed with hot water. Systems made with heated-extruded flour were “more rigid” than other samples at all levels of investigation as they were harder (macroscopic) and had higher storage modulus (mesoscopic), as well as lower proton ^1H mobility (molecular). Overall, the results highlighted the ability of the multiscale method to give a thorough overview of the flour–water interactions and showed highest water affinity of heated-extruded flour. Heated-extruded flour was then tested in three real-food industrial applications (carrot soup, tomato sauce and a meat patty), where it was successfully implemented as a clean label thickening agent.

Keywords

corn flour; clean label; physical modification; multilevel method; molecular mobilities



1 Introduction

Currently, consumers are very interested in the quality of food they eat and are turning in favour of products considered more “natural”, “healthy” and “familiar”. These choices are leading the food industry to develop “clean label” food products with a limited number of ingredients, free from E-numbers additives and/or from substances consumers are not acquainted with (Asioli et al., 2017). The food industry is therefore pushed to search for new natural ingredients with comparable technological functionality to E-number additives. Starches and flours are commonly used by the food industry as thickening and gelling agents. These ingredients show a limited functionality in their native form; for this reason, chemical and physical modifications are usually performed to improve and modulate their functionality. Ingredients that have undergone chemical (crosslinking, hydroxypropylation, phosphorylation, etc.) modifications need to be labelled as E-numbers, on the basis of EU Regulation 1333/2008 on food additives. On the contrary, flours subjected to physical modification must not be labelled with E-numbers and represent a way to fulfil the demand for a clean functionalization of ingredients. Heat treatment and extrusion processes are two types of the physical modifications used by the industry to obtain pregelatinized starch and flours. Starch gelatinization occurs during heat treatment and extrusion processes that alter the paracrystalline structure of starch granule, increase the exposure of hydroxyl groups of amylose and amylopectin and the portion of starch in the amorphous state, facilitating and ameliorating the interaction with water and, therefore, inducing the starch thickening effect (Hoover, 2001; Mason, 2009; Ye et al., 2018; Zavareze & Dias, 2011). Anyway, the use of flour should be preferred over the use of starch because of its lower cost of production for the absence of the starch extraction phase. Pregelatinized flour has been tested as clean label thickening agents in bread (M. Martínez et al., 2013), in reduced-fat mayonnaise (Román et al., 2015) and in sauces (Román et al., 2018). A multiscale approach to highlight and an in-depth understanding of the effect of physical modification on functional properties of flours at the macroscopic, mesoscopic and molecular level may be of high interest. Traditional techniques used for the characterisation of flours/starch usually focus on their macroscopic and mesoscopic properties, in particular studying their hydration (e.g., moisture content, water binding capacity and water holding capacity), pasting (viscosity during heating cycle), thermal (differential scanning calorimetry) and viscoelastic (rheometer) properties (Cornejo & Rosell, 2015; Lai, 2001; M. M. Martínez et al., 2014; Sandhu et al., 2007; Sarangapani et al., 2016; Singh et al., 2003; Zhang et al., 2016). Unconventional techniques could be useful to better elucidate, clarify and understand changes occurred at the molecular level determining the macroscopic and mesoscopic functionalities, which are detected by the traditional techniques. The effectiveness of ^1H low resolution NMR spectroscopy to study the protons mobility and dynamics at the molecular level in food systems has been well reported. In fact, this technique is able to detect different protons populations related to different environments characterized by different molecular mobilities. Polymers in flour interact with water altering its mobility and dynamics and ultimately affecting rheological and physicochemical functionality of products. Different studies have already been performed



on starch and flour model systems to assign the different protons domains to different components of flour biopolymers (Bosmans et al., 2012, 2016; Luyts et al., 2013; Tananuwong & Reid, 2004; Tang et al., 2000). In this perspective, the aim of this study was the evaluation of the technological functionality of physically modified corn flours using a multiscale investigation at the macroscopic, mesoscopic and molecular level, as compared to a native corn flour. Additionally, the thickening properties of the most performing flour were tested as a clean label ingredient in real food applications.

2. Materials and Methods

2.1. Materials

A native corn flour used as the control sample (CWF14) and two corn flours obtained with two different physical treatments (CWPF14 and HI-MODI FLOUR M) were obtained from local producers (CWF14 and CWPF14 by MartinoRossi (Cremona, Italy) and HI-MODI FLOUR M by HI-FOOD (Parma, Italy). All flours had the following granulometry ($>355 \mu\text{m}$ 0–2%, $>250 \mu\text{m}$ 0–6%, $>150 \mu\text{m}$ 55–65%, $<150 \mu\text{m}$ 20–30%) and a similar nutritional profile (carbohydrate 80%, proteins 6%, lipids 1% and fibre 1.5%), as reported in their technical data sheets. All flours were obtained starting from white corn horny grits (*Zea mays* L.). Corn grits were milled to obtain native flour (C) or pre-treated, dried and then milled, to obtain physically modified samples, as described in the following. Grits pre-treatments consisted of heating or a combination of heating and extrusion processes and were carried out by the MartinoRossi company (Cremona, Italy). The heat treatment was carried out using a conventional steam cooker (CVB, Ocrim, Italy) operating at temperature = 100 °C, pressure = 1.03 bar and time = 120 min on grits at 30% moisture content (g water/100 g of sample); this sample was named M_1 . The combined heating and extrusion treatment were carried out using a twin screw extruder with a length to diameter ratio of 20:1 (HMR, Ocrim, Italy) operating at temperature = 90 °C, pressure = 5 bar and time = 30 min at 30% moisture content of the grits; this sample was named M_2 . M_1 and M_2 were subsequently dried to $\approx 13\%$ moisture content and milled in a roller mill (MPI, Ocrim, Italy) at 25 °C.

2.2. Flours Physicochemical Characterization

2.2.1. Amylose Content

The amylose content of the flour samples was determined using the Megazyme Amylose/Amylopectin test kit K-AMYL 12/16 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland) according to the assay procedure, which is based on a modification of the Con A method (Yun & Matheson, 1990). Three replicates were performed for each sample.



2.2.2. Resistant Starch Content

Resistant starch (RS) content was measured in accordance to the AOAC method 2002.02 (McCleary et al., 2002) using the Megazyme resistant starch test kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland) according to the assay procedure. Three replicates were performed for each sample.

2.2.3. Differential Scanning Calorimetry (DSC) Measurement

Flours thermal properties were studied using a differential scanning calorimeter (DSC-Q100 TA instruments Waters, New Castel, DE, USA). The instrument was calibrated with indium (melting temperature: 156.6 °C, melting enthalpy: 28.71 J/g) and mercury (melting temperature: -38.83 °C, melting enthalpy: 11.44 J/g). Briefly, 30 g of corn flour were mixed with 90 mL of distilled water and equilibrate overnight at room temperature. Wet flour samples were then weighted (5–10 mg range) and placed into hermetic stainless-steel pans (PerkinElmer, Waltham, MA, USA) and heated from 10 to 100 °C at a rate of 5 °C/min. Thermal properties T_o (onset transition temperature), T_p (peak transition temperature), T_e (end transition temperature) and ΔH (transition enthalpy, J/g sample) were obtained from the thermograms using the Universal Analysis Program (Version 1.9 D; TA Instruments, New Castle, DE, USA). At least three measurements were performed for each sample.

2.2.4. Flour Hydration Properties

Water holding capacity (WHC, defined as the amount of water retained by the sample without being subjected to any stress) and the water binding capacity (WBC, defined as the amount of water retained by the sample under low-speed centrifugation) of corn flours were measured using the method described by Sarangapani and co-workers with slight modifications (Sarangapani et al., 2016). To measure WHC, corn flour samples (2.00 ± 0.05 g) were mixed with distilled water (20 mL) and kept at room temperature for 24 h in vials covered with Parafilm® to avoid water evaporation. The supernatant was decanted with vacuum pump and filter paper to assure that all water was collected. WHC was expressed as grams of water retained per gram of solids. Measurements were taken in triplicates. To measure WBC, corn flour samples (2.00 ± 0.05 g) were mixed with distilled water (20 mL) and centrifuged at 2000 x g for 10 min with centrifuge ALC PK121R (A.L.C. International S.r.l., Milano, Italy). WBC was expressed as grams of water retained per gram of solids. Measurements were obtained as the average of three replicates.

2.3. Multiscale Characterisation of Flour–Water Systems

2.3.1. Flour–Water Systems Preparation



Flour–water systems (C, M₁ and M₂) were prepared at different flour:water ratios (1:3, 1:4, 1:5, 1:6, 1:7, 1:8 and 1:9) in cold (CC, water temperature 25 ± 2 °C) and in hot conditions (HC, water temperature 100 ± 2 °C) to evaluate the range of flours functionality in a water-based system. Water and flour were placed in a mixer equipped with a whisk (Artisan, Kitchen Aid, Benton Harbor, MI, USA) and mixed at 135 rpm for 2 min. The obtained systems were then placed into aluminium molds and stored overnight at room temperature prior to being analysed. Two batches of each system were produced in two different days.

2.3.2. Macroscopic Characterisation

Water Activity and Moisture Content

Water activity (a_w) was measured at 25 °C with an Aqualab 4 TE (Decagon Devices, Pullman, WA, USA). Moisture content (MC, g of water/100 g of sample) was measured by weight loss by drying in a forced-air oven (ISCO NSV 9035, ISCO, Milan, Italy) at 105°C to constant weight. At least three measurements were taken for each system for a total of six determinations for both parameters.

Bostwick Running Distance and Texture Analysis

Bostwick running distance was tested with a Bostwick consistometer (LS100, Laboscintifica, Parma, Italy). The Bostwick consistometer chamber was filled with samples and the distance (cm) travelled after 30 s from the release of the gate of the chamber, was recorded. Three measurements were performed for each system for a total of six determinations. Hardness (maximum compression height of the peak, N) was performed using a TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) using a spherical probe P/1SP. Samples were placed in an aluminium mold (85 mm wide and 40 mm height) and analysed at room temperature after overnight rest. The probe penetrated into the sample to 10% strain at a rate of 2 mm/s. At least five measurements were performed for each sample for a total of at least ten determinations.

2.3.3. Mesoscopic Characterization

Rheological Properties

Rheological properties of the different systems were determined using a controlled stress rheometer (Anton Paar, MCR 702 twin drive) at 25 °C with a 50 mm diameter plate–plate geometry and a gap of 1 mm. The linear viscosity range (LVR) was determined with a preliminary strain sweep test at 10 Hz. Viscoelastic properties were studied using a frequency sweep test from 0.1 to 10 Hz at 25 °C applying a constant strain of 0.2%. Once the gap was taken to test the gap, the sample was trimmed and Vaseline oil was applied to the edges of the samples, which are not in contact with the plate surfaces. After sample loading, sample went through a resting time until axial force reached about 0 N prior to the start of the experiment to allow for temperature equilibration and system relaxation. The storage modulus (G'), loss modulus (G'') and $\tan \delta$ ($\delta = G''/G'$) were recorded. Three measurements were performed for each sample for a total of six determinations.



2.3.4. Molecular Characterization

¹H Molecular Mobility NMR

¹H molecular mobility was studied with a Low Resolution Nuclear Magnetic Resonance (NMR) spectrometer (20 MHz, the MiniSpec, Bruker Biospin, Milano, Italy) working at 25.0 ± 0.1 °C. Approximately 4 g of sample were placed into an NMR tube (10 mm diameter) and sealed with Parafilm® to avoid water loss during the test. ¹H free induction decay (¹H FID) and proton transverse relaxation time (¹H T₂) experiments were performed to investigate the mobility of the more and the less rigid protons, respectively. ¹H FIDs were acquired using a single 90° pulse, followed by a dwell time of 7 μs, a recycle delay from 2 to 7 s, depending on sample relaxation time, a 0.5 ms acquisition window and 32 scans. ¹H FID relaxation curves were fitted with a two-component model (exponential and Gaussian; (Le Grand et al., 2007)) to obtain quantitative information about the relaxation time and percentage of protons belonging to the more rigid and more mobile proton populations detectable within the FID experimental time frame (ranging from 7 to 500 μs). The fitting was performed with the SigmaPlot v.6 software (Systat Software Inc., San Jose, CA, USA) according to the following equation:

$$f = y_0 + A * e^{\left(-\frac{t}{TA}\right)} + B * e^{\left(-\frac{t}{TB}\right)^2}$$

where y_0 is the FID decay offset, A and B are the intensities of each relaxation component, TA and TB are the apparent relaxation times. ¹H T₂ relaxation time was measured with a Carr–Purcel–Meiboom–Gill (CPMG) pulse sequence with a recycle delay of 5 s, interpulse spacing of 0.04 ms, 32 scans and 15.000 data points. ¹H T₂ curves were analysed as quasi-continuous distributions of relaxation times with the UPENwin software (Alma Mater Studiorum, Bologna, Italy). Default values for all software UPEN parameters were used with the exception of one parameter (LoXtrap) that was set to 1 to avoid extrapolation of relaxation times shorter than the first experimental point. Experimental curves were also fitted with a discrete multiexponential model (SigmaPlot, v.6, Systat Software Inc., San Jose, CA, USA).

2.4. Industrial Application

Based on the results of flours and water–flour systems characterisation, the most performing flour (M₂) was selected, and the effectiveness of its technological functionality was assessed in real food. For this purpose, three food products were selected: a carrot soup, a tomato sauce and a meat patty.

2.4.1. Industrial Food Recipes Preparation



(i) Carrot soup: a commercial fresh carrot soup (Zerbinati, Alessandria, Italy) based on water, carrots, potatoes, celery, EVO and salt was used as the standard (STD). Increasing the level of M₂ flour was added to the standard (1–3%, g of flour/100 g sample; S1–S3 samples) and mixed and heated up to 70 °C for 4 min in a cooking mixer (Thermomix®, Vorwerk, Germany). All samples were prepared in duplicate. (ii) Tomato sauce: an industrial recipe of tomato sauce was used as the standard (STD). Mix tomato puree (Metro Chef, Milano, Italy; 56.1 wt %), water (30.6 wt %), EVO oil (Farchioni, Perugia, Italy; 6.1 wt %), mirepoix (5.1 wt %), basil (1.2 wt %) and salt (0.8 wt %; ESCO, Hannover, Germany) were mixed at 300 rpm and heated up to 90 °C, cooked under vacuum for 15 min at 115 °C in a bowl chopper (Polyfunctional Qbo 8-3, Roboqbo, Italy). On the basis of the standard recipe, three samples with increasing level of M₂ (1–3%, g of flour/100 g sample; S1–S3 samples) were also prepared in duplicate. (iii) Meat patty: an industrial recipe of meat patty was used as the standard (STD). Beef minced meat (93.7 wt %), water (5 wt %), salt (1.2 wt %; ESCO, Hannover, Germany) and ascorbic acid (0.1 wt %; Faravelli, Milano, Italy) were mixed in a kitchen machine equipped with a whisk at 120 rpm for 5 min (Major KMM77XX, Kenwood, Treviso, Italy) and subsequently 70 g formed in a burger patty mold. On the basis of the standard recipe, three samples with an increasing level of M₂ flour (1–3%, g of flour/100 g sample; S1–S3 samples) were also prepared in duplicate.

2.4.2. Food Characterisation

Rheological properties of carrot soups and tomato sauces were tested with a Bostwick consistometer (LS100, Laboscintifica, Parma, Italy). The Bostwick consistometer chamber was filled with sample and the distance (cm) travelled by the sample after 30 s from the release of the gate of the chamber, was recorded. Three measurements were performed for each sample.

Cooking yield (CY) of the meat patties was measured on the basis of the method of Madane and colleague (Madane et al., 2019) by weighting the sample before and after cooking on a pan. CY was obtained in a percentage on the basis of the following equation:

$$\text{Cooking yield} = \frac{\text{weight of cooked meat patty}}{\text{weight of raw meat patty}} \times 100$$

2.5. Statistical Analysis

Significant differences ($p \leq 0.05$) among different samples were assessed by a one-way-analysis of variance (ANOVA) with a Duncan post-hoc test and a Student's t-test using IBM SPSS statistical software (Version 24.0, SPSS Inc., Armonk, NY, USA).



3. Results and Discussions

3.1. Flours' Physicochemical Characterisation

Flours were analysed for their amylose and resistance starch (RS) content and relative results are reported in Table 1.

Table 1. Physicochemical properties of three different corn flours (C, M₁ and M₂).

	C	M₁	M₂
Amylose (%)	27.41 ± 4.06 ^a	25.16 ± 5.27 ^a	28.32 ± 2.48 ^a
Resistant Starch (%)	5.45 ± 0.01 ^a	1.74 ± 0.28 ^b	1.94 ± 0.05 ^b
To (°C)	62.927 ± 2.21 ^a	57.28 ± 0.42 ^b	57.55 ± 0.04 ^b
Tp (°C)	4.04 ± 0.57 ^a	68.49 ± 0.16 ^b	68.18 ± 0.32 ^b
Te (°C)	84.02 ± 2.37 ^a	84.54 ± 2.50 ^a	81.10 ± 0.59 ^a
ΔH (J/g)	2.32 ± 1.09 ^a	0.84 ± 0.26 ^b	0.44 ± 0.11 ^c
WHC (g/g)	2.36 ± 0.30 ^c	4.67 ± 0.20 ^b	6.44 ± 0.09 ^a
WBC (g/g)	1.65 ± 0.04 ^c	3.51 ± 0.11 ^b	4.34 ± 0.13 ^a

All data are expressed as mean ± standard deviations. Means with different superscript letters for each parameter considered differ significantly ($p < 0.05$).

Amylose content of C, M₁ and M₂ was found to be respectively ≈27.4%, ≈25.2% and ≈28.3%. These values were in line with common corn starch, which amylose content was reported in the range 25–27% (Cauvain, 2009; Zhang et al., 2016). Physical treatments did not affect the amylose content of the three flours, as previously reported in the literature (Sagum & Arcot, 2000; Zhang et al., 2016). An amylose increase would have been possible only under severe shear degradation processes during extrusion, which would lead to amylopectin fragmentation (Roman et al., 2019). A significant effect of the physical treatment was instead found on RS that decreased in M₁ (≈1.7%) and M₂ (≈1.9%) if compared with C (≈5.5%). Pre-gelatinisation on M₁ and M₂ due to the effect of temperature and pressure during physical treatment favour the loss of starch granule integrity and an opening of their crystalline structures making easier the access of hydrolytic enzymes (M. M. Martínez et al., 2014; Roman et al., 2019; Sagum & Arcot, 2000; Sopade, 2017; Ye et al., 2018). Gelatinisation process occurred on the grits during the physical treatments were highlighted studying the thermal properties of the different flours by DSC (representative thermograms are shown in Figure 1, while To, Tc, Tp and ΔH are reported in Table 1). One endothermic peak was identified in all samples in the range 60–85 °C and it was attributed to starch gelatinisation.

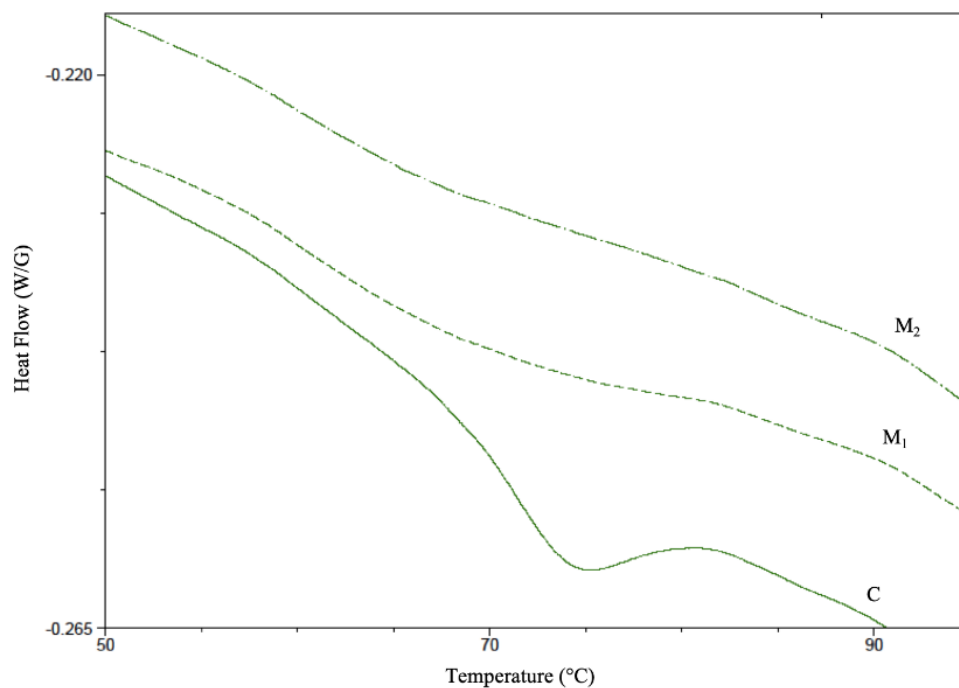


Figure 1. Differential scanning calorimetry (DSC) representative thermograms in the 50–95 °C temperature range of the three different corn flours C, M₁ and M₂.

To and T_p of M₁ and M₂ samples shifted to significantly lower values if compared to C indicating a structure alteration of native starch that favoured the occurrence of gelatinisation at lower temperature. ΔH of the C flour was ≈ 2.3 J/g, and it was found significantly decreased in M₁ (≈ 0.8 J/g) and M₂ (≈ 0.4 J/g), with significant differences due to the physical treatment applied on grits. The lower gelatinisation enthalpy, T_o and T_p in modified flours as compared to native ones is associated to the physical treatments these samples were subjected to that induced partial starch gelatinisation during processing. The combined heating and extrusion physical treatment was found the most effective on the starch gelatinisation. Physical treatments and the consequently pre-gelatinisation enhanced the hydration properties (WHC and WBC) of the flours with M₂ the flour with the highest water holding and binding capacity. The WHC and WBC increase is associated to the breakage of intra and inter hydrogen bonding, the presence of a less ordered molecular structure and consequently an increase of hydroxyl groups able to bind more water molecules (Cornejo & Rosell, 2015; Ye et al., 2018; Zhang et al., 2016).

3.2. Flour–Water Systems

To study the functionality of physically modified flours, flour–water systems were produced considering a wide range of flour:water ratios (1:3, 1:4, 1:5, 1:6, 1:7, 1:8 and 1:9) in the cold condition (CC) and hot condition (HC; Table 2).



Table 2. Physical appearance of corn flour:water systems subjected to mixing for 2 min at 135 rpm under the cold condition (CC) or hot condition (HC). H means that flour:water systems obtained were homogeneous while S means that flour:water systems showed water syneresis.

Preparation Condition	Flour	Flour:Water Ratio						
		1:3	1:4	1:5	1:6	1:7	1:8	1:9
CC	C	S	S	S	S	S	S	S
	M1	H	H	S	S	S	S	S
	M2	H	H	H	S	S	S	S
HC	C	H	H	H	S	S	S	S
	M1	H	H	H	H	H	H	H
	M2	H	H	H	H	H	H	H

An empirical evaluation on systems has been performed to check the absence of water syneresis and the formation of lumps at room temperature for 30 min. Different treatments on flours affected their ability to form homogenous systems avoiding syneresis. In CC, C was not able to form homogeneous systems, as expected. M₁ was able to avoid water syneresis up to 1:4 while M₂ up to a 1:5 flour:water ratio. In HC, C was able to avoid water syneresis up to 1:5 while M₁ and M₂ up to a 1:9 flour:water ratio. Only the homogenous systems were subsequently analysed. The higher ability to avoid water syneresis for M₂ in CC were related to the higher pre-gelatinisation level occurred on this sample by physical treatment (as confirmed by thermal properties) and to the consequent higher WHC and WBC. Increasing ability of all flours to interact with water in HC can be attributed to the increased starch gelatinisation occurred during samples preparation in the presence of a high amount of water and temperature over $\approx 60^{\circ}\text{C}$.



3.3. Macroscopic Characterization

Moisture contents (MC) and Bostwick running distances are reported in Table 3.

Table 3. Moisture content (MC) and Bostwick running distance of corn flour-water systems prepared with three corn flours (C, M₁ and M₂) at different flour:water ratio (1:3, 1:4, 1:5, 1:6, 1:7, 1:8 and 1:9) in cold (CC) and hot conditions (HC).

		Moisture Content (MC) % (g H ₂ O/100 g)	Bostwick Running Distance (cm)
Cold Condition (CC)			
M ₁	1:3	75.3 ± 0.6 aB	1.25 ± 0.20 B
	1:4	80.2 ± 0.6 bA	8.42 ± 0.12 aA
M ₂	1:3	75.2 ± 0.1 aC	0
	1:4	80.9 ± 0.5 bB	0.50 ± 0.16 bB
	1:5	83.8 ± 0.2 A	4.29 ± 0.29 A
Hot Condition (HC)			
C	1:3	75.9 ± 0.5 aC	0
	1:4	80.7 ± 0.9 aB	0
	1:5	83.9 ± 0.4 aA	1.58 ± 0.14
M ₁	1:3	74.8 ± 0.5 aF	0
	1:4	80.2 ± 0.2 aE	0
	1:5	83.0 ± 0.6 aD	0
	1:6	85.1 ± 0.8 aC	0
	1:7	87.6 ± 0.3 aB	0.58 ± 0.20 C
	1:8	88.4 ± 0.4 aA	3.54 ± 0.19 aB
M ₂	1:9	90.6 ± 0.3 aA	4.21 ± 0.12 aA
	1:3	75.2 ± 0.4 aE	0
	1:4	80.6 ± 0.9 aD	0
	1:5	83.1 ± 0.7 aC	0
	1:6	84.9 ± 0.3 aC	0
	1:7	87.2 ± 0.3 aB	0
	1:8	88.4 ± 0.4 aA	0.46 ± 0.19 bB
	1:9	90.3 ± 0.4 aA	2.37 ± 0.14 bA

MC of systems increased with the increase of the water level in all flours, as expected, with no significant differences due to the flour treatment applied to grits. For clarity purposes, the subsequent characterisation results on systems will be described on the basis of the approximate moisture content of the samples rather than the flour:water ratio. a_w values of systems at all moisture content were higher than 0.99 (data not shown) with no significant differences among systems obtained by different flours, indicating that physical treatment on grits did not affect the water–solids interaction at the macroscopic level. The Bostwick Running Distance is inversely related to the systems consistency. Bostwick Running Distance increased with increasing moisture content in all samples, due to the plasticising effect of water that made systems more liquid-like. In both CC and HC, significantly lower Bostwick running distance was observed for M₂, followed by M₁ and lastly C. A comparison of systems consistency formed with all flours could be possible only at HC and 83% moisture content; running distance for C, M₁ and M₂ was ≈1.6, 0 and 0 cm, respectively. Systems textural properties were also analysed using a texture analyser by the measure of hardness (Figure 2).

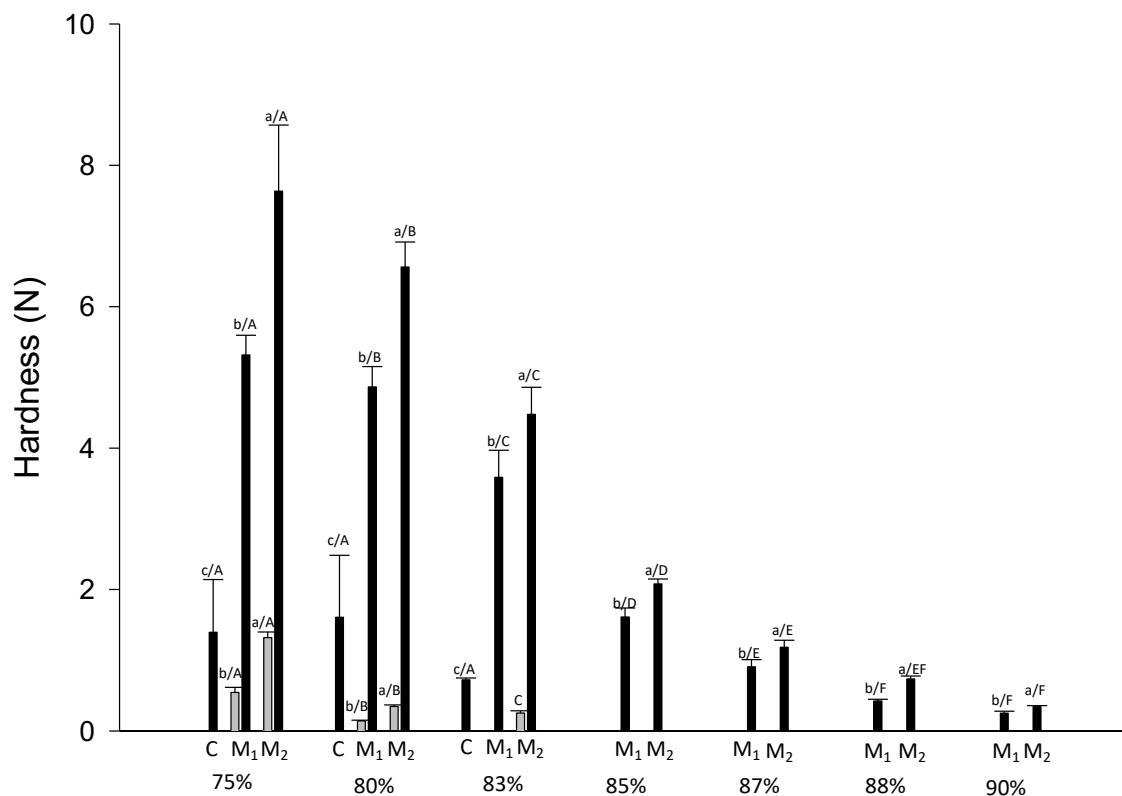


Figure 2. Hardness value of systems formed using three different flours C, M₁ and M₂ at different conditions (black HC and grey CC) and different moisture content (75%, 80%, 83%, 85%, 87%, 88% and 90%). Different letters indicate significant differences among samples ($p \leq 0.05$) where the small letters refer to the differences due to the flours and capital letters refer to the moisture content at the same preparation condition.

In all studied conditions, hardness decreased with increasing moisture content, as expected, due to the plasticising effect of water on macroscopic consistency. In the CC and HC systems obtained by M₂ flour had higher hardness than those obtained by M₁; where a comparison was possible, systems formed with C had always the lowest hardness value. In general, flours with higher WBC value led to the formation of systems with lower Bostwick running distance and higher hardness. At HC, the reduced Bostwick running distance and increased hardness can be associated to the increased gelatinisation process occurred using hot water and the associated increase of systems viscosity after cooling.

3.4. Mesoscopic Characterisation

At a mesoscopic level, homogeneous systems were investigated for their viscoelastic properties. G' and G'' curves versus frequency for samples at selected moisture content (75%, 85% and 90%) at HC are reported in Figure 3, while the effect of water temperature on rheological attributes of the systems is shown in Figure 4 for 75% moisture content samples. At all moisture contents and temperature conditions the storage modulus (G') was higher than the loss modulus (G'') in the selected experimental frequency range (0.1–10 Hz) and $\tan \delta < 1$ indicating solid-like properties of all systems. G' and G'' were also frequency dependent increasing with the increase of frequency at low moisture contents. With the



increase of moisture content, the frequency dependence of G' and G'' was found lower (Figure 3). G' higher than G'' for corn flour systems obtained by heating was also observed by Singh and colleagues (Singh et al., 2003) and by Rosalina and Bhattacharya, which have analysed corn starch–water systems (Rosalina & Bhattacharya, 2002). To compare G' values of different samples, a single frequency 0.858 Hz was selected (Figure 5).

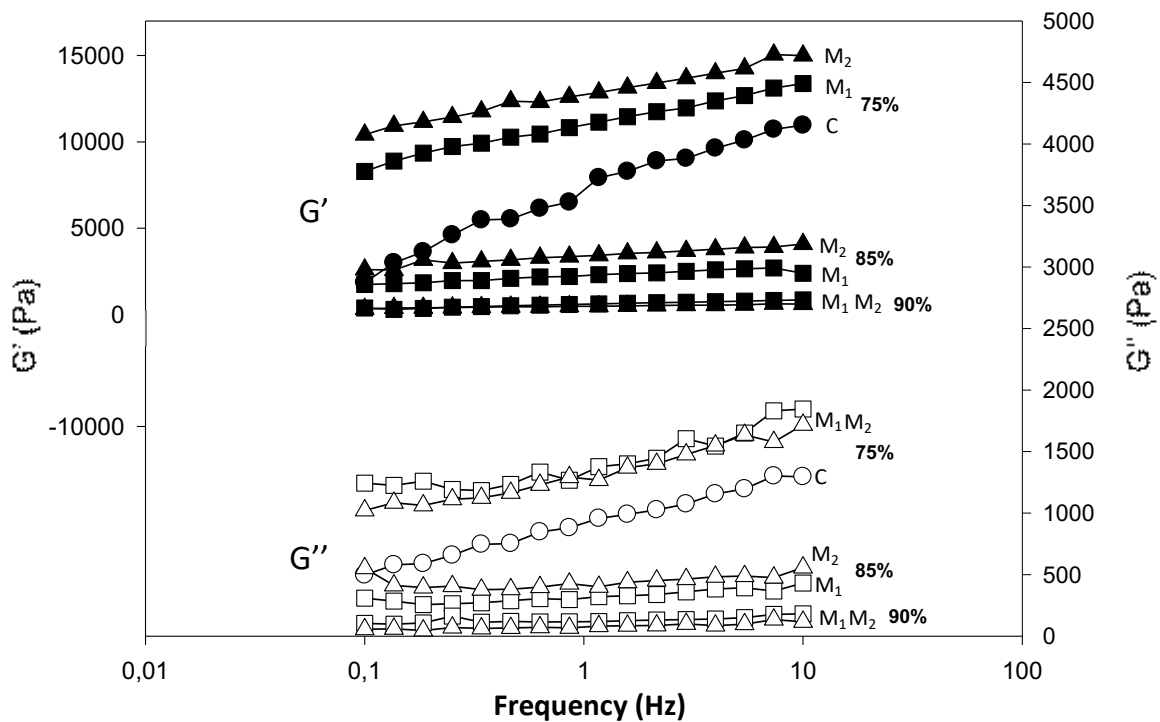


Figure 3. Frequency sweep curves of the systems formed with the three flours (circle: C, square: M₁ and triangle: M₂) at three different moisture contents (75%, 85% and 90%) at the hot condition (HC).

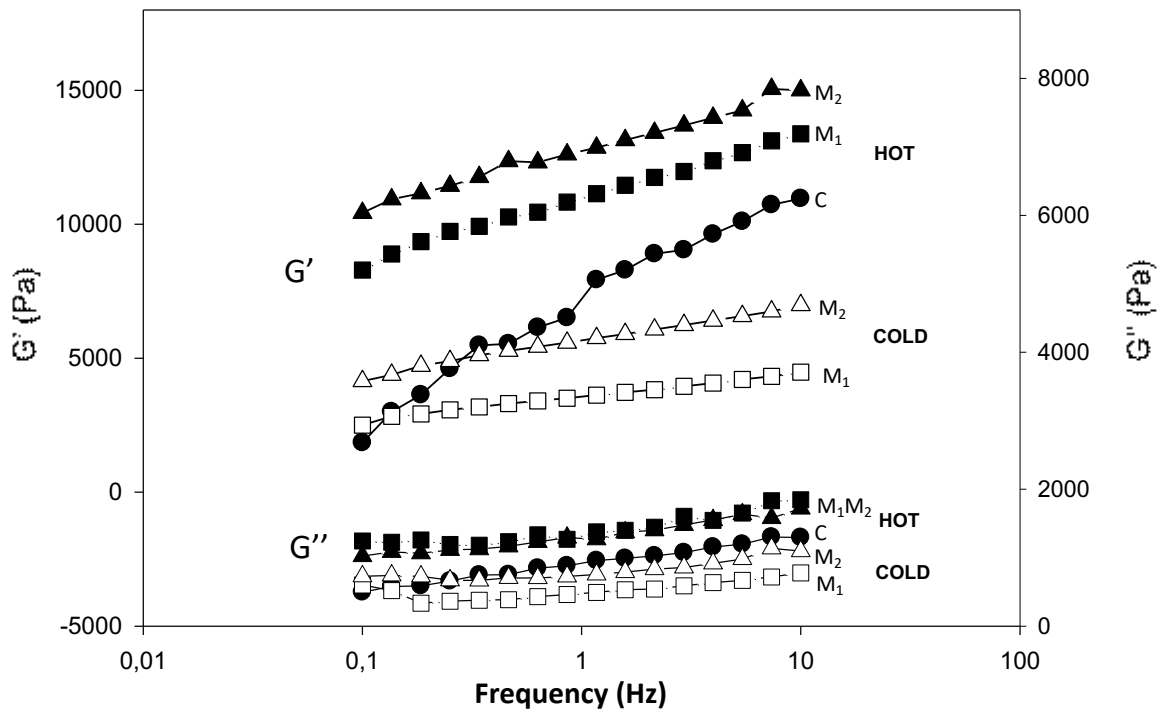


Figure 4. Frequency sweep curves of the systems formed with the three flours (circle: C, square: M₁ and triangle: M₂) at the 75% moisture content in the cold condition (cold, white) and hot condition (hot, black).

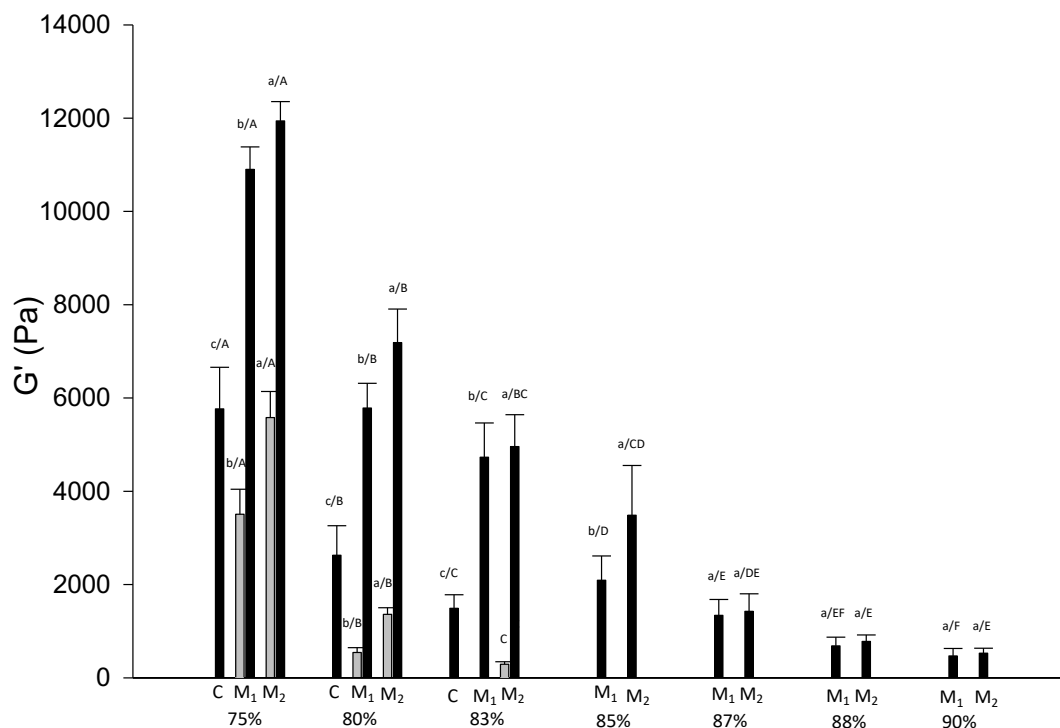


Figure 5. Storage modulus (G') of systems formed using three different flours, in the CC (grey) and HC (black) and different moisture content (75%, 80%, 83%, 85%, 87%, 88% and 90%). Different letters indicate significant differences among samples ($p < 0.05$) where the small letters refer to the differences due to the flours and capital letters refer to the moisture content at the same preparation condition.



The increase of the moisture content always reduced G' of the systems formed with the different flours. Furthermore, where a comparison was possible, both CC and HC systems formed with M_2 had always the highest G' followed by M_1 and lastly C. G'' followed the same trend of G' while $\tan \delta$ had an inverse trend (data not shown). Higher G' of M_2 , keeping in mind that G' represents the recoverable energy of systems after deformation, suggested a more rigid structure when the flour used was obtained with the combination of heating and extrusion process. The rheological properties were in agreement with the Bostwick running distances and hardness values obtained at the macroscopic level.

3.5. Molecular Characterization

Relaxation times and relative abundances of ^1H FID populations A (popA) and B (popB) are reported in Table 4.



Table 4. Relaxation times (T_A , T_B , T_C , T_D and T_E) and relative abundances (pop A (%), pop B (%), pop C (%), pop D (%) and pop E (%)) of ^1H populations (A and B from ^1H FID, and C, D and E from ^1H T_2 experiment) of systems formed with three corn flours (C, M_1 and M_2) at different moisture contents (75%, 80%, 83%, 85%, 87%, 88% and 90%) and in the cold condition (CC) and hot condition (HC).

Moisture Content		Pop A (%)	T_A (ms)	Pop B (%)	T_B (ms)	Pop C (%)	T_C (ms)	Pop D (%)	T_D (ms)	Pop E (%)	T_E (ms)
Cold Condition (CC)											
M_1	75%	35.12 ± 0.94 aA	0.022 ± 0.000 bA	64.89 ± 0.94 bA	0.49 ± 0.01 aA	7.03 ± 0.54 aB	6.11 ± 0.58 bB	26.86 ± 1.43 bB	33.99 ± 1.38 bB	66.79 ± 0.95 aA	145.04 ± 2.55 aB
	80%	34.49 ± 1.19 aA	0.022 ± 0.000 bA	64.50 ± 1.19 bA	0.51 ± 0.03 aA	10.51 ± 1.75 aA	12.85 ± 1.99 aA	36.76 ± 2.68 aA	76.64 ± 3.64 aA	53.33 ± 0.60 bB	166.71 ± 3.93 aA
M_2	75%	32.10 ± 0.30 bA	0.025 ± 0.001 aA	67.90 ± 0.30 aB	0.46 ± 0.00 bC	6.11 ± 0.30 bB	6.85 ± 0.42 aC	31.81 ± 1.63 aA	38,42 ± 2,20 aC	62.08 ± 1.78 bB	78.49 ± 0.70 bC
	80%	31.42 ± 0.41 bB	0.024 ± 0.001 aB	68.58 ± 0.41 aB	0.49 ± 0.01 aB	6.22 ± 0.78 bB	8.16 ± 1.20 bB	25.94 ± 0.87 bB	43,91 ± 2,78 bB	67.84 ± 1.60 aA	106.06 ± 2.61 bB
	83%	23.28 ± 0.7 C	0.024 ± 0.00 B	76.72 ± 0.7 A	0.56 ± 0.02 A	8.09 ± 0.88 A	14.05 ± 0.6 A	32.80 ± 2.2 A	72.32 ± 3.2 A	59.35 ± 2.5 C	147.14 ± 3.9 A
Hot Condition (HC)											
C	75%	22.81 ± 0.55 cA	0.018 ± 0.000 bA	77.19 ± 0.55 aC	0.69 ± 0.01 aB	4.99 ± 0.38 aA	10.58 ± 0.75 aB	17.25 ± 1.12 b	52.80 ± 2.48 aB	77.76 ± 1.50 aA	122.95 ± 0.87 aB
	80%	20.98 ± 0.59 cB	0.017 ± 0.000 cAB	79.02 ± 0.59 aB	0.70 ± 0.02 aB	4.40 ± 0.86 aA	11.04 ± 0.69 aAB	17.20 ± 0.29 aA	58.50 ± 3.41 aA	78.63 ± 0.93 bA	135.33 ± 3.92 aA
	83%	15.60 ± 1.56 cC	0.017 ± 0.000 bB	84.40 ± 1.56 aA	0.77 ± 0.03 aA	4.08 ± 0.12 aA	12.06 ± 0.46 aA	17.74 ± 0.46 aA	61.17 ± 1.00 aA	78.17 ± 0.42 cA	140.41 ± 3.40 aA
M_1	75%	27.96 ± 1.02 bA	0.019 ± 0.000 bA	72.04 ± 1.02 bE	0.58 ± 0.00 bF	4.50 ± 0.18 aA	5.70 ± 0.34 bF	20.47 ± 1.45 abA	33.18 ± 1.49 bG	75.03 ± 1.62 abE	66.38 ± 0.84 bG
	80%	23.05 ± 0.65 bB	0.018 ± 0.001 bA	77.17 ± 0.79 bD	0.65 ± 0.01 bE	3.62 ± 0.23 abB	7.07 ± 0.98 bE	12.78 ± 1.64 bC	43.35 ± 2.39 bF	83.60 ± 1.87 aC	100.03 ± 2.01 bF
	83%	18.12 ± 1.04 bC	0.019 ± 0.000 aA	81.88 ± 1.04 bC	0.69 ± 0.02 bD	2.85 ± 0.17 bD	8.53 ± 0.19 bD	11.02 ± 0.27 cDE	52.03 ± 1.48 bE	86.13 ± 0.37 aAB	134.41 ± 0.99 bE
	85%	10.38 ± 0.11 bD	0.019 ± 0.000 bA	89.62 ± 0.11 aAB	0.79 ± 0.04 aBC	3.45 ± 0.23 aB	11.85 ± 0.48 aC	15.27 ± 1.81 aB	73.54 ± 1.07 aD	81.28 ± 1.99 bD	150.81 ± 3.08 aD
	87%	10.50 ± 0.20 bD	0.019 ± 0.001 aA	89.49 ± 0.12 aAB	0.78 ± 0.05 aC	3.13 ± 0.08 aC	13.34 ± 0.51 aB	11.96 ± 0.50 aCD	80.15 ± 2.10 aC	84.91 ± 0.51 aBC	197.10 ± 3.69 aC
	88%	10.21 ± 0.70 bD	0.019 ± 0.001 aA	89.79 ± 0.70 aA	0.83 ± 0.03 aAB	2.80 ± 0.10 aD	15.13 ± 0.57 aA	10.17 ± 0.50 bE	86.73 ± 1.49 aB	87.02 ± 0.55 aA	224.75 ± 2.92 aB
	90%	11.54 ± 0.85 bD	0.017 ± 0.002 aB	88.51 ± 0.95 aB	0.88 ± 0.06 aA	2.75 ± 0.05 aD	15.72 ± 0.90 aA	11.54 ± 0.21 bCDE	104.55 ± 1.68 aA	85.71 ± 0.25 aAB	291.32 ± 4.85 aA
M_2	75%	29.07 ± 1.15 aA	0.024 ± 0.001 aB	70.92 ± 1.15 bE	0.48 ± 0.01 cF	3.86 ± 0.28 bA	3.29 ± 0.37 cE	23.89 ± 2.92 aA	24.90 ± 1,87 cG	72.98 ± 2.25 bB	50.18 ± 1.79 cG
	80%	29.24 ± 0.5 aA	0.021 ± 0.001 aC	70.75 ± 0.51 cE	0.50 ± 0.02 cE	2.88 ± 0.31 bBC	3.53 ± 1.29 cE	13.19 ± 0.30 bB	27.79 ± 1,16 cF	83.94 ± 0.17 aA	76.36 ± 1.65 cF
	83%	21.37 ± 1.29 aB	0.025 ± 0.002 aA	78.63 ± 1.29 cD	0.58 ± 0.01 cD	3.07 ± 0.04 bB	7.04 ± 1.44 cD	12.82 ± 1.2 bB	39.23 ± 0,45 cE	84.11 ± 1.16 bA	98.58 ± 3.43 cE
	85%	17.50 ± 0.42 aC	0.023 ± 0.001 aB	82.51 ± 0.42 bC	0.60 ± 0.02 bC	2.85 ± 0.17 bBC	8.53 ± 0.19 bC	11.02 ± 0.27 bB	52.03 ± 1.48 bD	86.13 ± 0.37 aA	134.41 ± 0.9 bD
	87%	14.15 ± 0.96 aD	0.020 ± 0.001 aC	85.85 ± 0.96 bB	0.69 ± 0.02 bB	2.63 ± 0.31 bC	11.75 ± 1.01 bB	14.06 ± 3.99 aB	66.46 ± 1.56 bC	83.97 ± 4.84 aA	149.29 ± 3.15 bC
	88%	11.80 ± 0.22 aE	0.021 ± 0.001 aC	88.20 ± 0.22 bA	0.72 ± 0.01 bA	2.77 ± 0.24 aBC	12.03 ± 0.34 bB	13.01 ± 1.24 aB	72.88 ± 2.44 bB	84.22 ± 1.47 bA	163.93 ± 0.01 bB
90%	11.73 ± 1.33 aE	0.020 ± 0.001 aC	88.54 ± 0.15 aA	0.74 ± 0.02 bA	2.84 ± 0.13 aBC	14.99 ± 0.35 aA	13.65 ± 1.13 aB	86.33 ± 1.09 bA	83.51 ± 1.26 bA	190.94 ± 5.06 bA	

All the data are expressed as mean ± standard deviations; different letters close to the number indicate significative differences among samples ($p \leq 0.05$) where the small letters refer to the differences due to the flours and capital letters refer to the moisture content at the same preparation condition.



Pop A relaxed in the range ≈ 0.017 – 0.025 ms and represented ≈ 10 – 35% of total protons detected in the ^1H FID frame. Pop A significantly changed as a function of the water content, physical treatment and condition of preparing systems. Pop A was previously attributed to the CH protons of the rigid crystal phase of starch in a corn starch–water system (Bosmans et al., 2012). In this study, the relaxation time and relative abundance of this less mobile Pop A increased and decreased, respectively, with moisture content increase (in both CC and HC) indicating an increase of mobility of these protons due to the plasticizing effect of water on biopolymers. Similar results were observed by Bosmans and colleagues (Bosmans et al., 2016) who have observed a decrease of the area of Pop A with the increase of water concentration in samples prepared with potato and rice starch. Comparing samples obtained by different physical treatments at the same moisture content, it could be observed a different trend based on systems prepared in CC or in HC. In fact, Pop A abundance decreased with the increase of strength of physical treatment ($M_1 > M_2$) when systems were prepared in CC, or increased ($C < M_1 < M_2$) when systems were prepared in HC. In CC, the higher mobility of the system (lower abundance of Pop A) in M_2 than M_1 was related to the decrease of rigidity of the starch granule due to a higher gelatinization level reached because of stronger physical treatment (as also confirmed by thermal analysis) used on flour. When HC was applied in preparing systems, an additional effect of temperature could have further altered the starch granules integrity. In fact, it can be noticed a higher Pop A% in samples formed in CC than those formed at HC. Same results were noticed by Bosmans and co-workers (Bosmans et al., 2012) studying a corn starch–water model system after the heating process at 110 °C for 10 min. If the starch granules integrity was further altered when HC was applied in preparing systems, a higher amount of amylose release from the starch region could be hypothesised. During systems cooling, the released amylose underwent fast recrystallisation possibly resulting in a decrease of mobility of these protons (Pop A abundance $C < M_1 < M_2$). Indeed, as also hypothesised by Bosmans and colleagues (Bosmans et al., 2012), Pop A% of a starch–water system after the heating process can represent CH protons of amorphous starch immobile after heating and/or newly formed amylose crystals formed during cooling of the sample. As mentioned before, relaxation time of the more rigid population T_A of the systems formed in the CC and HC at all moisture content ranged between ≈ 0.017 and 0.025 ms with significant but slight differences depending on different samples and therefore no additional discussion was deserved. Pop B relaxed in the range ≈ 0.5 – 0.9 ms increasing its mobility (relaxation time) with moisture content increase while becoming less mobile when flour was subjected to physical treatment (T_B significant different as $C < M_1 < M_2$). Pop B % changes were linked to the relative changes of Pop A abundance. Bosmans and colleagues assumed that Pop B represents non-exchanging CH protons in the amorphous part of the granule (Bosmans et al., 2012). Three not well resolved ^1H populations named population C (Pop C), population D (Pop D) and population E (Pop E) were observed by the ^1H T_2 distribution of relaxation times relaxing in the range ≈ 2 – 10 ms, ≈ 33 – 105 ms and ≈ 50 – 290 ms, respectively. ^1H T_2 experimental curves were also fitted with a three exponential model and ^1H relaxation times and relative abundances of the three populations were therefore obtained and are reported in Table 4. Three ^1H T_2 populations were also observed in studies conducted by Bosmans and colleagues (Bosmans et al., 2012) on the corn starches–water model system



relaxing at lower relaxation times than relaxation times we found. This misalignment is probably due to the much higher moisture content of samples studied than those studied by Bosmans and colleagues. Anyway, the assignment of protons to different domains performed by those authors can be used to explain our changes due to experimental variables. In all samples, Pop E was the predominant population as it encompassed $\approx 53\text{--}86\%$ of the total protons observed in the ^1H T_2 experimental window. The mobility and abundance of this population significantly changed as a function of moisture content, preparation condition and physical treatment used on flour. These protons were previously assigned to water protons that exchange with starch hydroxyl protons in the extragranular space in an unheated corn starch–water sample and to hydroxyl protons of starch and water in the gel network containing the granule remnants in a heated corn starch–water sample (Bosmans et al., 2012). Moreover, Pop C was related to the CH protons of amorphous starch in little contact with water and Pop D to contained hydroxyl protons of intragranular water and starch (Bosmans et al., 2012). Relaxation times of the three ^1H T_2 populations C, D and E shifted towards higher values with a moisture content increase in all samples, indicating an increase of mobility due to the plasticisation effect of water at the molecular level. Considering the effect of the condition used to prepare systems, it could be noticed that samples prepared in CC, had Pop C and D more abundant than systems prepared in HC, that, on the contrary, were characterised by high abundance of Pop E. Taking in mind that protons of Pop E, as reported before, represent the gel network domain, this different behaviour could be related to the achievement of a higher gelatinisation degree during the preparation of systems in HC, due to the high temperature of water used.

Physical treatment on flours significantly affected the ^1H T_2 mobility of systems, both in the CC and HC. In particular, major changes can be related to the decrease of T_E . In fact, the lowest mobility of Pop E was detected in M_2 samples, followed by M_1 and lastly by C samples. A decrease of the T_2 mobility of the Pop E in a wheat flour–water model system after heating has been observed by Luyts and colleagues, which have suggested that the decreased mobility is predominately attributed to the formation of a structured network and consists predominately of mobile exchanging protons of water, starch and gluten (Luyts et al., 2013). The stronger condition of the physical treatment used to obtain M_2 flour allowed us to increase starch gelatinisation, as also probed by DSC, and explain the overall higher rigidity of these systems. Overall, lower molecular mobility in M_2 samples was found in agreement to their higher hardness and G' values. The molecular results obtained were related with the macroscopic (Bostwick Running Distance, hardness) and mesoscopic (G' , G'') properties of the systems, confirming the usefulness of ^1H low resolution NMR spectroscopy as a tool to deeper investigate the physicochemical functionality of ingredients as the physically modified flours analysed in this study.

3.6. Industrial Applications

Physicochemical characterisation of flour–water systems showed M_2 as the flour with the highest thickening ability among samples considered, due to its major ability to interact with water. To prove its feasibility in real complex foods, the flour has been used as a clean label food ingredient in the formulation



of (i) carrot soup, (ii) tomato sauce and a (iii) meat patty. These foods were selected as they represent examples of systems to which thickening ingredients are frequently added by the food technologists for different purposes. In fact, in carrot soups no water syneresis and a proper pulpiness is required; the tomato sauce has to not present water–oil syneresis and has to be creamy, while the meat patty has to retain water during cooking showing a proper juiciness. To assess these features industrial familiar analytical tools have been used: a Bostwick consistometer for the carrot soup and tomato sauce systems, and cooking yield for the meat patty. Both analytical tools are used by the food industry for the quick evaluation of food quality parameters (Pietrasik, 2003; Swainson & McWatt, 2010). The Bostwick Running Distance (RD) of carrot soup (Table 5) significantly decreased with the increase of M_2 in the formulation. The overall appearance of the soup also improved with the elimination of the undesired water syneresis effect present in the commercial carrot soup, as it can be observed in Figure 6.

Table 5. Bostwick running distance of carrot soup and tomato sauce, and cooking yield of a meat patty at increasing M_2 inclusion level, 1%, 2% and 3%, g of flour/100 g sample (S1, S2, S3).

	STD	M_2 Inclusion Level		
		S1	S2	S3
Bostwick Running Distance (cm)				
Carrot Soup	9.2 ± 0.3 a	7.8 ± 0.3 b	5.8 ± 0.8 c	4.5 ± 0.5 d
Tomato Sauce	15.3 ± 0.6 a	13.2 ± 0.3 b	12.2 ± 0.8 b	8.8 ± 0.3 c
Cooking Yield (%)				
Meat Patty	78.6 ± 0.2 b	78.4 ± 0.5 b	82.8 ± 0.4 a	82.7 ± 0.2 a

All the data are expressed as mean ± standard deviations; different letters close to the number indicate a significative difference among the sample ($p < 0.05$) due to the flour inclusion level amount.

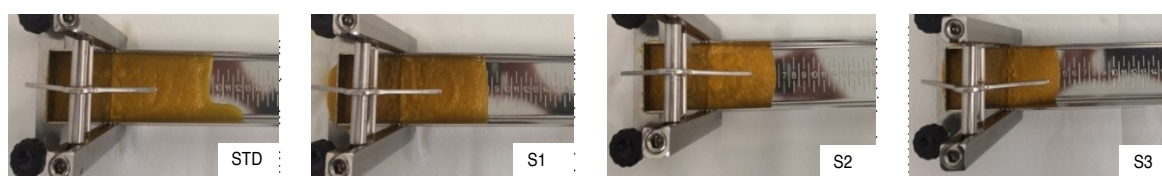


Figure 6. Appearance of cooked commercial carrot soup (STD) and carrot soup samples at increasing M_2 level (S1–S3).



The Bostwick Running Distance of the different cooked tomato sauce samples are reported in Table 5. As reported for the carrot soup the RD significantly decreased with the increase of M_2 . As it can be observed in Figure 7, the overall appearance of the tomato sauce improved as a creamier texture without the presence of undesired water and oil syneresis up to a 2% M_2 inclusion level. Instead, the S3 sample had a jelly and steady texture, which is undesired in sauces.

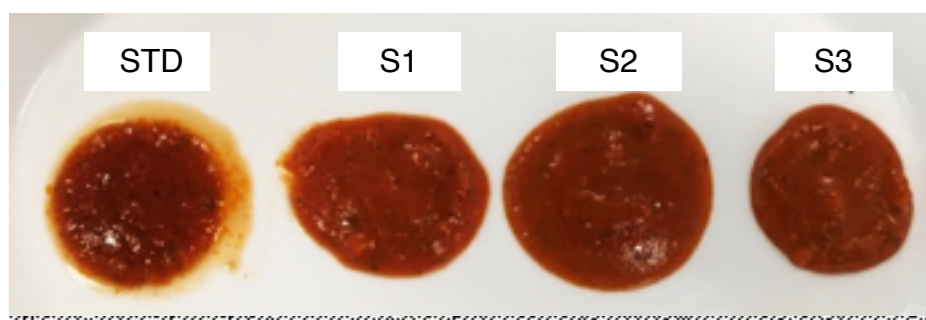


Figure 7. Appearance of tomato sauce (STD) and tomato sauce samples at increasing M_2 level (S1–S3).

For the meat patty, a significant increase of the cooking yield (CY) was observed for S2 and S3 recipe (≈ 82) compared to STD and S1 ($\approx 78\%$; Table 5). The CY increase led to an increase of water retention in the patty after cooking and a possible improvement in its juiciness. Overall, the technological functionality of M_2 as a thickening agent and water/oil binder was confirmed by the industrial application in real foods. Additional sensorial analysis would be necessary to ascertain the consumer acceptability.

4. Conclusions

A multilevel and multi-analytical approach was used to study the technological functionality of two physically modified corn flours subjected to different production processes (heating and heating-extrusion, respectively) and a native corn flour. Overall, the results have highlighted the ability of the multiscale method to provide a thorough and global understanding of the occurred flour–water interactions. In particular, differences due to the moisture content, the preparing condition of systems and the physical treatment used on the flour were highlighted at the molecular level and were in agreement with changes showed at the macroscopic (Bostwick running distance, hardness) and mesoscopic (G' and G'') level. The strengthening of the physical treatment on grits allowed it to further alter the starch native structure towards a more gelatinised structure that was therefore more able to interact with water when flour was used to prepare systems at different moisture content. This modified flour could be therefore used in a wide range of food applications, both in the cold or hot condition. The use in the hot condition allows one to increase the gelatinisation degree and therefore to further modulate macroscopic properties, which were driven by molecular changes, as probed before. Particularly important could be



the application of this ingredient when a clean label is searched for, as probed by its effectiveness as a clean label thickening agent in real foods.

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Chapter 3

Can a physically modified corn flour be used as fat replacer in a mayonnaise?

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Abstract

Physically modified flours have high potential as fat-replacer in low-fat mayonnaise formulations as they are cheaper than modified starches and successfully respond to the consumers' request for clean label products. Starting from a standard full-fat ("FF", 80% oil) sample formulation, three mayonnaises with reduced-fat amount were produced (60%, 40%, 25% oil) substituting oil with a physically modified flour, used as fat-replacer. Mayonnaises (both untreated and pasteurised) were investigated for their physicochemical properties and sensorial attributes. Emulsions were well formed for all mayonnaise formulations and presented acceptable consistency, with the only exception of the full-fat sample which was unstable after heating. High and comparable emulsion stability was noticed among reduced-fat mayonnaise samples. Mayonnaise with 25% oil content showed hardness and G'' comparable to FF, while mayonnaises with 40% and 60% oil content were harder and showed higher G' than other samples. Overall, rheological results corroborate the ability of the fat-replacer to counterbalance the absence of fat gelling/thickening the continuous phase of the emulsion. Regarding the colour, a fading (L^* increase and a^* and b^* decrease) of the product with increasing fat reduction was found. Sensory acceptability was positive for all the samples, with 40% oil mayonnaise being the most preferred. The colour of the reduced-fat mayonnaises was perceived as paler and the flavour less persistent than the FF sample. The physically modified corn flour was found to be a valuable fat replacer in reduced-fat mayonnaise.

Keywords

Mayonnaise; Fat reduction; Clean label; Physically modified flour



1. Introduction

Mayonnaise is one of the most popular and commercialized sauces worldwide. It is a semi-solid oil-in-water emulsion and is traditionally produced mixing oil, egg yolk, salt, vinegar and spices (especially mustard). Traditional mayonnaise is a very caloric and high fat food as it contains approximately 70–80% lipids (Depree & Savage, 2001; Honold et al., 2016). Fat reduction is a pillar of international guidelines to reduce the risk of developing type 2 diabetes mellitus and the risk of cardiovascular diseases (European Food Safety Authority, 2010). From a technological point of view, fat has a pivotal role in the physicochemical and sensory properties making its reduction in food formulations highly challenging. In food emulsions fat impacts on the texture, colour, lubricity and flavour properties (Ma & Boye, 2013). Reduced-fat mayonnaises available in the market are obtained by formulations in which different non-fat ingredients are used to mimic fat quality attributes. Guar gum and/or xanthan gum are commonly used in industrial low-fat mayonnaise formulations (Ma & Boye, 2013) but they are additives and have to be labelled with “E-numbers”. Such additives are not appreciated by the consumer who is searching for “clean” labels and, for this reason, their replacement with “natural”, “healthy” and “familiar” ingredients has become a priority for the food industry (Asioli et al., 2017). Different works have already been performed to study the use of innovative ingredients in low-fat mayonnaise applications. Mun and co-workers (Mun et al., 2009) developed a 50% reduced-fat mayonnaise with rheological properties and emulsion stability comparable to a full fat mayonnaise, using an enzymatically (4 α Gase) modified rice starch (5.6%) in combination with xanthan gum (0.1%). Shen and colleagues (Shen et al., 2011) optimised the recipe of a 30% reduced-fat mayonnaise using oat dextrin (dextrose equivalent of 8.1). The obtained low-fat mayonnaise showed higher viscosity, similar acceptability, colour, odour and lower caloric value than the full-fat counterpart. Worrasinchai and co-workers (Worrasinchai et al., 2006) used β -glucans from spent brewer’s yeast as fat replacer in a 50% reduced-fat mayonnaise that was sensorial acceptable, with similar firmness and adhesiveness and higher storage stability than the full-fat counterpart. Overall, the best strategy to adopt is to use ingredients able to enhance the viscosity of the aqueous phase or to impart the proper gel-like structure, partly lost due to the fat reduction (D. Julian McClements & Demetriades, 1998). Pre-gelatinised starches obtained by physical treatments (e.g., heating and/or extrusion) are ingredients widely used by food industry for fat reduction applications in food emulsions. Anyway, scarce information is present in the literature on physically modified flours; their use should be promoted, because their production is more sustainable (more economic and with a lower environmental impact) than the starch extraction process (Eckhoff & Watson, 2009; Román et al., 2015). Roman and colleagues successfully used an extruded corn flour (pre-gelatinised) for the production of a reduced-fat mayonnaise even though its consumer acceptability was not ascertained (Román et al., 2015). In this paper a commercially available physically modified corn flour (HI-MODI FLOUR M, HI-FOOD, Parma, Italy) was used in reduced-fat mayonnaise formulations that were characterised for their physicochemical and sensory properties. HI-MODI FLOUR M is a clean ingredient and is designed to



give texture stability in various products. It works as natural thickening agent and water binder in cold and hot processes (Carcelli et al., 2020). It can also partially absorb oils avoiding separation of water–oil phases in final applications.

2. Materials and Methods

2.1. Materials

A physically modified commercial corn flour (PMCF), HI-MODI FLOUR M, was obtained from a local producer (HI-FOOD, Parma, Italy). It was derived from white corn horny grits (*Zea mays* L., 30% moisture content) which were subjected to a cooking-extrusion process (90 °C and 5 bar for 30 min, twin screw extruder) and subsequently dried to 11% (g water/100 g product) and milled in a drum mill. Other mayonnaise ingredients were: potassium sorbate (Dr Paul Lohmann, Emmerthal, Germany), citric acid (Brenntag, Milan, Italy), salt (ESCO, Hannover, Germany), sugar (British Sugar, Peterborough, UK), pasteurised egg yolk (AIA, Verona, Italy), vinegar (Ponti, Novara, Italy) and mustard (Columbus, Parma, Italy).

2.2 Fat replacer preparation

Fat replacer gel was prepared mixing PMCF and water (1:9 ratio) in a cooking mixer (Thermomix, Vorwerk, Wuppertal, Germany). The mixture was heated to 90 °C and held for 25 min while mixing at 500 rpm to obtain a gel that was then stored at 4 °C overnight before use.

2.3. Mayonnaise preparation

Full-fat (FF) and reduced-fat (RF) mayonnaises were prepared on the basis of an industrial recipe shown in Table 1.

Table 1. Mayonnaise recipes (% wt).

	Full fat FF	Reduced-fat to 60% (RF60%)	Reduced-fat to 40% (RF40%)	Reduced-fat to 25% (RF25%)
Sunflower oil	80	60	40	25
Water	5.92	-	-	-
Fat replacer gel	-	25.92	45.92	60.92
Egg yolk	6	6	6	6
Mustard	2	2	2	2
Sugar	1.9	1.9	1.9	1.9
Salt	0.9	0.9	0.9	0.9
Vinegar	3	3	3	3
Citric acid	0.25	0.25	0.25	0.25
Potassium sorbate	0.03	0.03	0.03	0.03



RF mayonnaises were prepared replacing sunflower oil and water with the fat replacer gel to have a fat content of 60% (RF 60%), 40% (RF 40%), and 25% (RF 25%) (g fat/100 g product). All mayonnaises (FF and RF) were prepared using a bowl chopper (Polyfunctional Qbo 8-3, Roboqbo, Bologna, Italy) by stirring (3000 rpm for 60 s) egg yolk, water (FF recipe) or fat replacer (RF recipe), mustard, sugar and salt to obtain a homogeneous matter. Oil was then slowly added while stirring at 3000 rpm for 200 s under vacuum ($p = 1$ bar). As a last step, vinegar, citric acid and potassium sorbate (previously mixed together using a hand blender [Minipimer MQ5035, Braun, Germany]) were added to the mixture stirring at 3000 rpm for 30 s. Mayonnaise aliquots (160 g) were then transferred into glass jars. Half production was pasteurised in a vertical autoclave (Tecno-Gaz, Italy, 65 °C, 5 min) to replicate industrial processing, while the other half was stored at 25 °C. Untreated and pasteurised samples were named U and P, respectively. Two batches of mayonnaise were produced for each formulation in two different days.

2.4. Mayonnaise characterisation

2.4.1. Mayonnaise microstructure

Mayonnaise microstructure was observed by placing a drop of mayonnaise on a glass microscope slide covered with a cover slip and analysed using a H550 L Eclipse microscope (Nikon, Japan) at a 40X magnification. Pictures were taken by internal digital camera; representative images were selected among 5 replicates.

2.4.2. Water activity and pH

Water activity (a_w) was measured at 25 °C with an Aqualab 4 TE (Decagon Devices, Inc. WA, USA). pH was measured with a potentiometer pH7 Food (XS Instruments, Modena, Italy). At least three measurements were taken for each formulation for a total of six determinations.

2.4.3. Bostwick consistency

Bostwick consistency was tested with a Bostwick consistometer (LS100, Laboscientifica, Parma, Italy). The Bostwick consistometer chamber was filled with 100 ml mayonnaise and the distance (cm) travelled by the samples after the release of the chamber gate was recorded after 30 s (running distance, RD). Three measurements were performed for each formulation for a total of six determinations.

2.4.4. Texture

Texture properties were determined using TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) with a P/45C conical probe and analysed using Texture Expert software (Stable Micro Systems, Godalming, UK). Mayonnaises were kindly transferred to complete fill a cylindrical probe (85 X 40 mm) and analysed after overnight rest. Each mayonnaise sample was subjected to a penetration test to a depth of 10 mm at a speed of 1 mm/s and then returned at 1 mm/s. Peak maximum force (N) and



adhesiveness (area of the negative peak, N mm) were taken as hardness and adhesiveness, respectively. Four measurements were performed for each formulation for a total of eight determinations

2.4.5. Rheological properties

Rheological properties of mayonnaise were measured with a controlled stress rheometer (Advanced Rheometric Expansion System, Rheometric Scientific, Inc. Piscataway, NY, USA) at 25 °C with a 50 mm diameter plate–plate geometry and 1 mm gap. Linear viscosity range (LVR) was determined using a strain sweep test in the range 0.25–10% at a fixed frequency of 5 Hz. Viscoelastic properties were studied using a frequency sweep from 0.1 to 13.34 HZ at 25 °C applying a constant strain of 0.5% which was found within LVR. Storage modulus (G'), loss modulus (G'') and $\tan \delta$ (G''/G') were recorded. Prior to each experiment, sample was loaded and covered with paraffin oil on the exposed surface and allowed to rest for 3 min for sample relaxation and temperature equilibration. Three measurements were performed for each formulation for a total of six determinations.

2.4.6 Colour

Colour was determined using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65 and a 10° position of the standard observer. The results were expressed in accordance with the CIE Lab system. The parameters measured were: L^* [0 (black) – 100 (white)], a^* ($-a^*$ = greenness and $+a^*$ = redness) and b^* ($-b^*$ = blueness and $+b^*$ = yellowness), and the overall colour difference ΔE was calculated using FF as Ref. (Limbo & Piergiovanni, 2006). At least ten determinations were performed for each formulation for a total of at least twenty determinations.

2.4.7 Mayonnaise stability

Emulsion stability of mayonnaise was measured using the method described by Mun and co-workers (Mun et al., 2009). 15 g of sample (F_0) were transferred into a 50 ml falcon tube, sealed with plastic cap and stored at 50 °C for 48 h. After storage, samples were centrifugated at 3000 rpm for 10 min (centrifuge, Eppendorf, Milan, Italy). The weight of the precipitated fraction (F_1) was measured and emulsion stability was calculated as percentage (%) = (F_1/F_0) . Three tests were performed for each formulation for a total of six determinations. Mayonnaise stability was also observed after 12 months of storage at room temperature.

2.4.8 Sensorial analysis

Sensory analysis of mayonnaise samples was performed using an acceptability and a rapid profiling method check all-that-apply (CATA) tests. All mayonnaise samples were named with a three-digit random number and presented to 50 untrained judges. Judges were allowed to drink sparkling water and eat salt free crackers between samples to cleanse the palate. In the acceptability test, judges were asked to grade the samples for consistency, taste, flavour and overall acceptability using a 9-points



hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike, 4 = dislike slightly, 5 = neither like nor dislike, 6 = likes slightly, 7 = like, 8 = like very much and 9 = like extremely). Overall scores obtained were subjected to an ANOVA test to verify significant differences among samples. Further insight on consumers' perception of mayonnaises was captured using a CATA test, by asking consumers to select appropriate attributes for the sample from a given list (Ares et al., 2014; Dooley et al., 2010). In particular, judges were asked to identify from a list of attributes all those that applied to the samples and to their ideal concept of mayonnaise. The attributes listed (presented to the judges in a balanced and randomized manner) were: opaque, pale, shiny, yellow, bad flavour, good flavour, delicate flavour, lemon taste, low persistent taste, vegetable taste, anomalous taste, acid, vinegar taste, persistent taste, egg taste, oily taste, bitter, sweet, salty, thick, good consistency, creamy, sandy, good, mediocre, unpleasant, good aftertaste, bad aftertaste. A correspondence test was performed on the results counting the times each attribute was selected for each sample.

2.5. Statistical analysis

Significant differences ($p \leq 0.05$) among different samples were assessed by one-way-analysis of variance (ANOVA) with a Duncan post hoc test using an IBM SPSS statistical software (Version 24.0, SPSS Inc., Armonk, New York, USA). The contingency table of CATA dataset was created on the basis of samples and attributes. A correspondence analysis was performed to summarize the relationship between samples and attributes using as software Statistica (Version.13.3, TIBCO Software Inc.).

3. Results and discussion

3.1 Fat replacer and reduced-fat mayonnaise recipe optimisation

Commercial RF mayonnaise recipes include a range of additives that are added with the aim of bind the high number of water molecules present and to thicken the product. In an attempt to use clean label ingredients, a commercial PMCF was used as thickener in RF mayonnaise recipe. Preliminary trials revealed that PMCF, when inserted in product's formulation in powder form, did not provide a stable mayonnaise. In other matrices fat reduction was obtained using structured gel prepared with the use of technological functional ingredients (Câmara et al., 2020; Curti et al., 2018). PMCF was, therefore, tested for the production of a fat replacer gel that was then used in reduced-fat mayonnaise samples. Several preliminary and empirical tests have been performed to identify the best methodology for fat replacer production to identify optimal PMCF: water ratio and, cooking conditions (temperature, time, shear). The best fat replacer gel was obtained subjecting a mix of PMCF and water (1:9 flour:water ratio) to cooking at 90 °C and mixing speed of 500 rpm for 25 min. The gel thus obtained showed a shiny surface, off-white colour, a self-standing cuttable gel consistency and water syneresis did not occur when it was held at refrigerated temperature for 12 h. After overnight rest, the fat replacer gel was diced (2 X 2 X 2 cm³)



and added to RF mayonnaise applications in partial substitution of oil as compared to the FF mayonnaise, and the overall quality (appearance, taste and stability) of the mayonnaises formed was tested by preliminary acceptability assessment by the researchers of the development team. It was found that good quality mayonnaise could be obtained up to an oil content of 25% in the formulation, lower fat content could not be obtained without sacrificing the taste of the mayonnaise. Thus, three RF mayonnaises in which fat (oil) was reduced to 60%, 40% and 25% (RF60%, RF40%, RF25%) were produced to reduce the fat content of the 25%, 50% and 70%, respectively. A FF mayonnaise was also produced and taken as control. Additionally, half of the production of each mayonnaise was also thermally treated to replicate the pasteurisation processes usually performed in industrial settings.

3.2 Mayonnaise microstructure

Optical microscopy has been used to evaluate the microstructure of the different mayonnaises, which consists of oil droplets dispersed in a water phase (Langton et al., 1999). Representative microstructure images of the mayonnaise are reported in Figure 1.

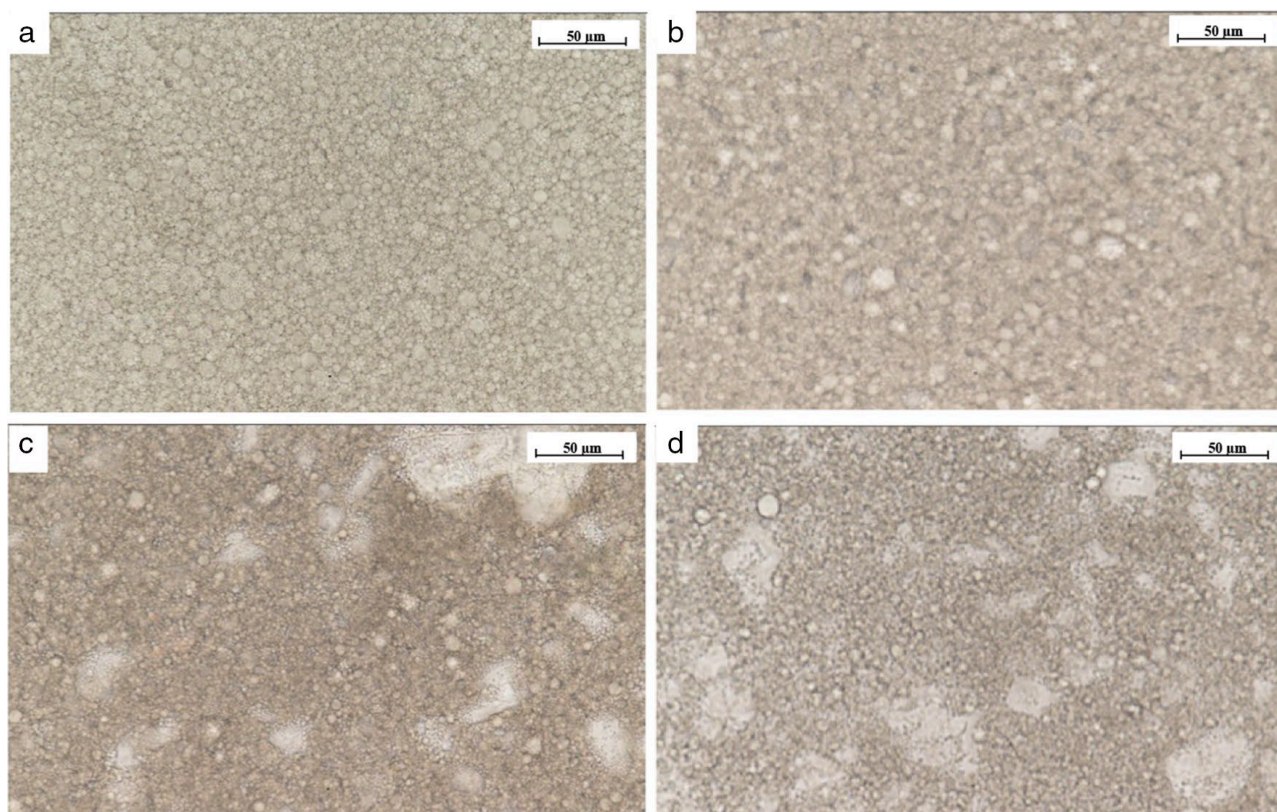


Figure 1. Light microscopy images of mayonnaises: a full fat FF, b RF60%, c RF40%, d RF25%.

In FF mayonnaise the oil content is above 75%, and therefore, the classical emulsion microstructure formed by spherical droplets packed within the continuous phase change was not observed: oil droplets lost sphericity and assume a distorted shape favouring close packing and interaction of fat globules



providing the characteristic consistency to mayonnaise (Depree & Savage, 2001; Langton et al., 1999). FF mayonnaise microstructure reflected what expected, with small fat droplets closely packed among larger droplets, interacting strongly among each another (Fig. 1a). RF mayonnaises were characterized by aggregation of droplets and interspaced void spaces, that increased with decreasing fat amount (Fig. 1b–d). Microstructural images suggested the gelling of the continuous phase of the emulsion thanks to the thickening properties of the PFMC.

3.3 Mayonnaise characterisation

All mayonnaises prepared were well formed emulsions (both U and P) and presented an acceptable consistency, with the exception of the standard pasteurised mayonnaise (FF) that, when subjected to the heating treatment, underwent emulsion disruption and could not be analysed. The instability of the FF mayonnaise after the heat treatment was associated to oil droplets coalescence, since the only stabilizer present in its recipe was egg yolk, as previously reported by Mun and co-workers (Mun et al., 2009). Mayonnaise samples were initially characterized on their water activity (a_w), pH, running distance (RD), colour, and the results are reported in Table 2.



Table 2. Water activity (a_w), pH, running distance (RD), colour (L^* , a^* , b^* , ΔE), emulsion stability (ES) of mayonnaises at variable fat content (FF = full fat, RF60% = Reduced-fat to 60%; RF40% = Reduced-fat to 40%; RF25% = Reduced-fat to 25%).

	FF	RF60%		RF40%		RF25%	
	U	U	P	U	P	U	P
a_w	0.947 ± 0.002 c	0.969 ± 0.00 1b	0.973 ± 0.008 b	0.979 ± 0.009 a	0.977 ± 0.008 ab	0.982 ± 0.009 a	0.984 ± 0.001 a
pH	3.45 ± 0.02 c	3.72 ± 0.03 b	3.70 ± 0.05 b	3.74 ± 0.03 b	3.79 ± 0.01 a	3.86 ± 0.02 a	3.77 ± 0.05 a
RD (cm)	0.2 ± 0.1 b	0	0	0.4 ± 0.1 b	0.4 ± 0.2 b	0.8 ± 0.2 a	0.6 ± 0.1 a
L^*	89.4 ± 0.8 c	91.6 ± 1.2 b	91.7 ± 0.6 b	92.7 ± 0.7 a	92.2 ± 0.5 a	91.5 ± 0.9 b	91.5 ± 0.7 b
a^*	3.1 ± 0.2 a	2.4 ± 0.2 b	2.3 ± 0.2 a	2.0 ± 0.1 c	2.0 ± 0.1 b	2.3 ± 0.4 b	2.3 ± 0.2 a
b^*	42.1 ± 0.4 a	34.1 ± 2.0 b	34.1 ± 1.0 b	31.4 ± 0.5 c	31.7 ± 0.4b	34.6 ± 1.5 b	34.0 ± 1.1 a
ΔE	/	7.69 ± 1.29 b	8.07 ± 0.74 b	11.09 ± 0.41aA	10.72 ± 0.34 aB	7.97 ± 1.05 bB	8.49 ± 1.08 bA
ES (%)	99.9 ± 0.2 a	99.9 ± 0.1 a	99.9 ± 0.1 a	100.0 ± 0.1 a	99.9 ± 0.1 a	99.7 ± 0.5 a	99.9 ± 0.1 a

All the data are expressed as mean ± standard deviations; different letters close to number indicate significative difference among sample ($p \leq 0.05$) where the small letters to the difference due to the fat amount and capital letter due to the thermal treatment.



The a_w of all mayonnaises studied were high, between 0.95 and 0.98, in line with the average value that is usually between 0.93 and 0.95 (Chirife et al., 1989). Due to the high a_w values of the products, pH and thermal treatment are crucial to guarantee the microbiological safety of the product. Mayonnaise pH for microbiological safety reasons should be below 4.1, that is mostly achieved with the acetic acid present in the vinegar used for the production of mayonnaise and in minor part from other acidifying ingredients used as citric acid or lemon juice (Jay, 1992). All the mayonnaises studied presented a pH value between 3.5 and 3.9 (Table 2). Comparable values were noticed between RF60% and RF40%, while significant differences were noticed among all the other samples. No significant differences could be noticed between U and P samples with the same oil concentration. Overall, an increase of the pH with decreasing fat content was observed, likely due to the increase of the amount of hydrophilic phase and the resulting dilution of the acetic acid in the recipe (Hathcox et al., 1995). Colour, in particular lightness (L^*), is an important aspect of the appearance and, therefore, of the acceptability of mayonnaise; indeed, it has been appointed that the reduction of fat reduces droplets concentration leading to a lowering of the L^* -value and consequently of product acceptability (McClements & Demetriades, 1998; Mun et al., 2009). FF mayonnaise was characterized by the significantly lowest L^* -value (≈ 89.4) compared to RF counterpart (between ≈ 91.5 and ≈ 92.7). Previous works reported that mayonnaise lightness increased with the increase of starch and gum in the recipe (Mun et al., 2009) and that emulsion colour changed from grey to bright white due to an increase in light scattering (Chantrapornchai et al., 1999; McClements & Demetriades, 1998). Therefore, it can be supposed that the incorporation of the fat replacer gel has modified the scattering ability of the mayonnaise, leading to the observed paler appearance. A significant lowering of a^* value (redness) and b^* (yellowness) for RF mayonnaises compared to the FF counterpart was also noticed: this phenomenon can be associated to the increase of the lightness and the decrease of oil content which cause a fading of the colours. Worrasinchai and colleagues observed that FF mayonnaise showed a shiny bright yellow colour, whereas the colours of the RF mayonnaise obtained with β -glucan were too pale (less coloured) due to the addition of fat replacer and due to the decrease of oil content that has a yellow colour (Worrasinchai et al., 2006). In the mayonnaises studied, the removal of fat led to a paler and less shiny colour with strong colour difference (ΔE) between all RF mayonnaise samples and FF counterpart. In particular, ΔE of RF40% was the highest (≈ 11.09), significantly different from RF25% (≈ 7.97) and RF60% (≈ 7.69), among which no significant difference was measured. The highest ΔE observed for RF40% is mainly related to its b^* value (yellow) which value was significantly different than in RF60% and RF25%. It can be hypothesized that in RF40% the decrease of yellow colour was more intense, because the decrease of oil content was higher than RF60% and was not adequately compensate by the fat replacer as for RF25%. Pasteurisation led only to a slight significantly differences of ΔE between URF40% and PRF40% samples, respectively, ≈ 11.09 and ≈ 10.72 and URF 25% and PRF 25% samples, respectively, ≈ 7.97 and ≈ 8.49 . If RF mayonnaises studied were found paler, their high yellow colour and the absence of grey led to acceptable products as identified in the sensorial analysis, data described subsequently. Furthermore, colour of RF products could be adjusted during the industrial phase using natural colouring agent. Mayonnaise consistency was analysed using a Bostwick



consistometer, an easy and fast analytical tool often used by food manufacturers to assess product's quality and conformity with standards. A low running distance indicates high mayonnaise consistency, characteristic of FF mayonnaise with 80% fat with thick and heavy body structure (Rasor & Duncan, 2014). All samples presented a short running distance (Table 2) indicating good product consistency. A slightly but significantly higher running distance compared to the other samples was found only in RF25%, while no significant differences were ascribable to pasteurisation treatment. The lowest Bostwick consistency of RF25% can be attributed to the decreased fat and increased water contents in the product. Textural and rheological properties of the different mayonnaises were further investigated to better describe the effect of fat reduction on mayonnaise rheology.

3.4 Mayonnaise texture and rheological properties

A combined empirical and fundamental rheological investigation (Chang et al., 2017; Worrasinchai et al., 2006) was carried out in an attempt to describe rheological and quality properties of mayonnaises. In particular, mayonnaises hardness and adhesiveness throughout the texture analysis were measured as they are important textural attributes influencing the mouthfeel (Chang et al., 2017) and workability of the product, and related to the viscosity and flow behaviour of the system. Moreover, a combined investigation can be useful in an attempt to find agreement between the two approaches (empirical and fundamental). This is particularly important for the food industry realities that cannot sustain costs and advanced skills related to the implementation of fundamental methods. All RF mayonnaises showed hardness higher or comparable to the FF counterpart (Figure 2).

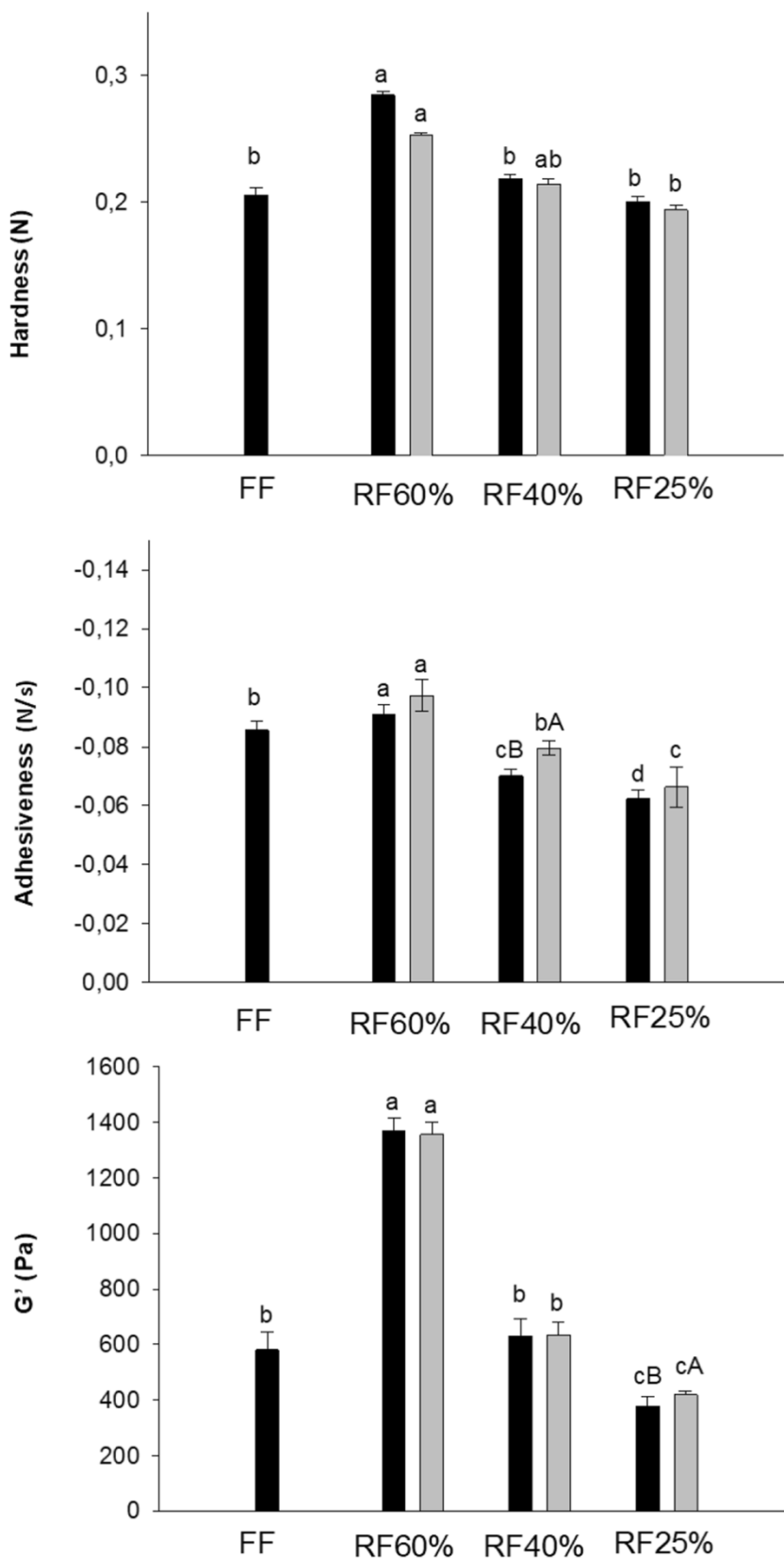


Figure 2. Hardness, adhesiveness and storage modulus (G' at selected frequency of 1 Hz) of FF and RF mayonnaises, untreated samples U (black) and pasteurized samples P (gray). Different letters close to number indicate significant difference among sample ($p \leq 0.05$) where the small letters to the difference due to the fat amount and capital letter due to the thermal treatment.



In particular RF60% was significantly harder (0.24 ± 0.00 N) than FF (0.19 ± 0.01 N), RF40% (0.20 ± 0.00 N), and RF25% (0.18 ± 0.01 N), which were comparable among themselves. Pasteurisation did not significantly affect textural properties of each product. It can be concluded that fat replacer used in the formulation of RF mayonnaises was able to provide structural strength to the product even with the significant fat reduction achieved. RF60% was also found to be significantly more adhesive than all other products, while RF40% and RF25% were less adhesive than the FF counterpart. A slightly significant difference due to pasteurisation was noticed only in the adhesiveness of RF40% and RF25% mayonnaises. It can be hypothesised that the highest hardness and adhesiveness of the RF 60% mayonnaise compared to all other samples (including the FF counterpart) were associated to the increase of the viscosity of the continuous phase due to the gelling/thickening effect of PMFC (despite microscopic images revealed the aggregation of droplets and the presence of interspaced void spaces) and the formation of a strong three-dimensional structured network among oil droplets and the fat replacer, as suggested by Worrasinchai and co-workers (Worrasinchai et al., 2006) who obtained 50% and 75% fat reduced mayonnaise with firmness and adhesiveness comparable to the full fat counterpart using β -glucans as texturizing agent. The structured network in RF40% and RF25% was weaker than in RF60% probably because of a lower amount of oil in the dispersed phase. Viscoelastic properties of the mayonnaise were also analysed using a dynamic oscillatory test. The dependence of moduli G' and G'' upon frequency for all samples are reported in Figure 3.

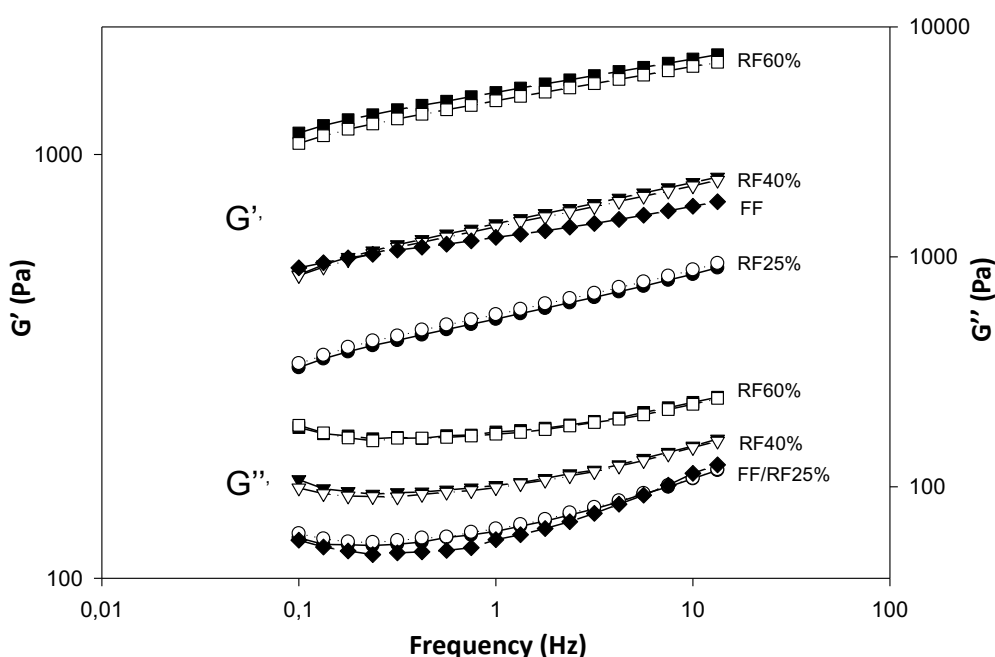


Figure 3. Storage Modulus (G') and Loss Modulus (G'') as a function of frequency of mayonnaises (\blacklozenge FF, \blacksquare RF60%, \blacktriangledown RF40%, \bullet RF25%), untreated samples U (black symbols) and pasteurized samples P (white symbols).

Previous work on FF mayonnaise (Ma & Barbosa-Cánovas, 1995) and RF mayonnaise (Liu et al., 2007; Mun et al., 2009) concluded that mayonnaise have a gel-like characteristics (typical of emulsions). The gel-like characteristic of mayonnaise confers desirable textural properties (Corradini & Peleg, 2005) and



reducing the fat content may cause a change from gel-like to low viscosity fluid with important changes in mouthfeel and, therefore, in the acceptability of the product (McClements & Demetriades, 1998). In the case of the mayonnaises object of this study, the fat replacer was able to counterbalance the fat decrease gelling the continuous phase of the emulsion and resulting in a solid-like behaviour in all samples categorizing them as gels. Indeed, the storage modulus (G') was higher than the loss modulus (G'') for all samples in the selected frequency range (0.1–13.34 Hz), with $\tan\delta > 0.1$. Comparison of G' at the selected frequency of 1 Hz for the different mayonnaises studied are reported in Figure 2. RF60% showed the significantly highest G' compared to all other samples, while G' of RF40% and FF were comparable and higher than RF25%. Pasteurisation treatment led to a slightly significant difference in G' only for RF 25%, in which pasteurized sample presented higher value than untreated. G' has been associated to the fat content of the mayonnaise, as magnitude of the elastic modulus usually increases with increasing oil content (Chang et al., 2017). The presence of the PMCF based fat replacer gel led to the development of the elastic component (G') for RF60%, RF40%, RF25%, respectively, higher, similar and lower to the FF counterpart. The results obtained confirm the ability of the fat replacer gel to strengthen the RF mayonnaise structure, probably by an increase of viscosity of the continuous phase. Similar results were previously found when pectin weak gel, microparticulated pectin gel and, β -glucan from spent brewer's yeast were used as fat replacer in a mayonnaise matrix (Liu et al., 2007; Worrasinchai et al., 2006). A positive correlation has been observed between hardness and G' values ($r^2 = 0.916$) confirming the agreement between empirical and fundamental rheological techniques. Stability of the FF and RF mayonnaises was checked on the basis of a method previously reported by Mun and co-workers to assess emulsion stability of RF mayonnaises (Mun et al., 2009).

3.5. Emulsion stability

All mayonnaises developed in this study showed high stability (> 99%) with no water and oil release (Table 2). In addition, there was no evidence of creaming or emulsion instability for all the mayonnaises observed after 12 months of storage at room temperature. Emulsion instability is usually associated to droplet coalescence, flocculation and creaming. In high fat products, as FF mayonnaise, oil droplets are packed together and their movement is impeded. In RF products the oil droplets movement is usually reduced using thickening agents as gum or starches in the aqueous phase (McClements, 2007). In the RF mayonnaise of this study, the increased rigidity structure of the fat replacer gel can have replicated the technological functionality of the above cited additives creating strong gel-like interactions.



3.6. Sensory analysis

Results of sensory evaluations of the mayonnaise are reported in Figure. 4.

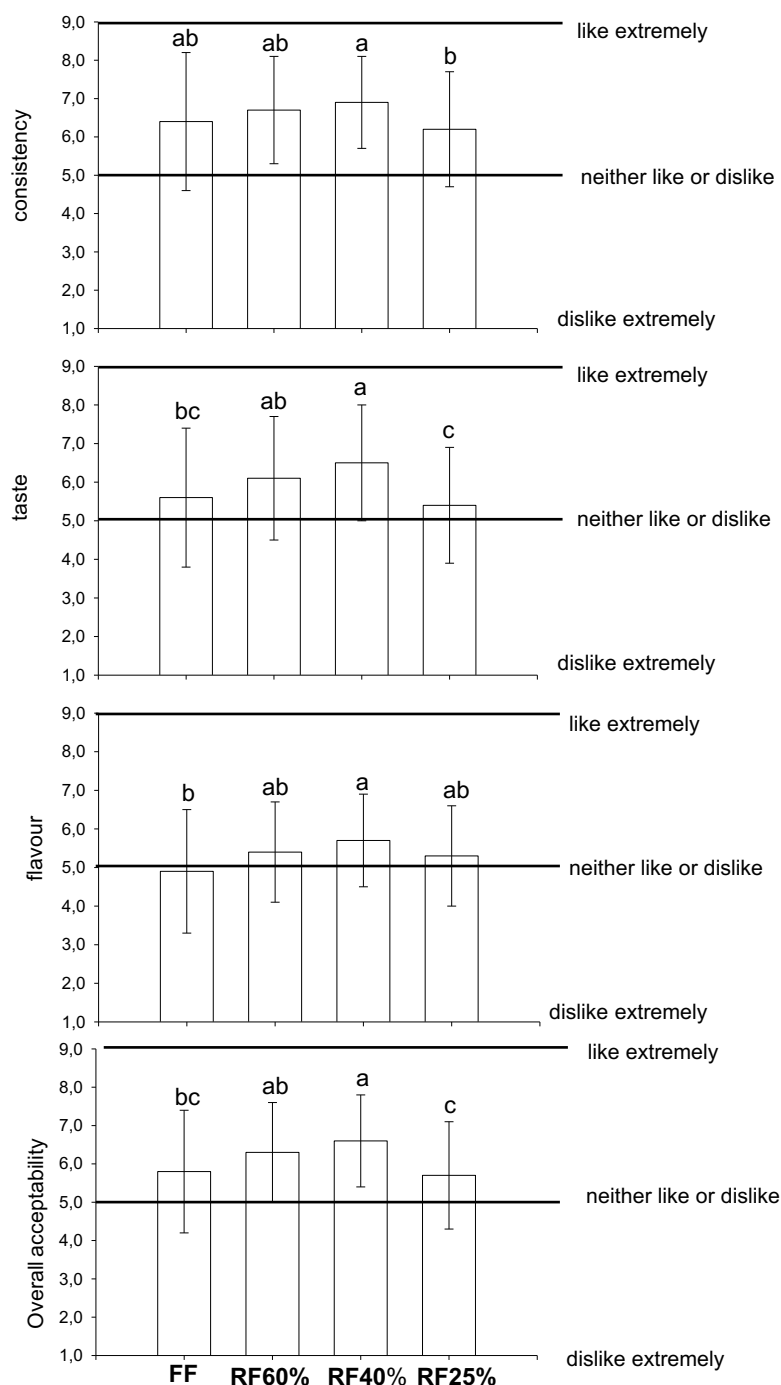


Figure 4. Sensory scores for consistency, taste, flavor and overall acceptability of mayonnaises prepared with different fat amount. Different letters indicate significative differences among the samples ($p \leq 0.05$).

Overall sensory acceptability was positive for all samples, with RF40% and RF60% mayonnaise being significantly preferred by a consumer panel followed by FF and RF25%. RF40% presented the highest score in all attributes with score close to 7 (like) except for flavour. FF and RF25% showed the lowest score for all attributes but value above 6 (likes slightly) for consistency. Flavour of all samples was graded



below 6 (likes slightly) indicating a low acceptability of this parameter for all the recipes tested. The good results obtained and in particular the highest scores on RF40% is encouraging, also because no information on the benefit of the health claim of the product was revealed to judges during the test. CATA data were analysed using a correspondence analysis, in Figure 5 the projection of the “IDEAL” product and the different mayonnaises on the factor plane are graphically represented.

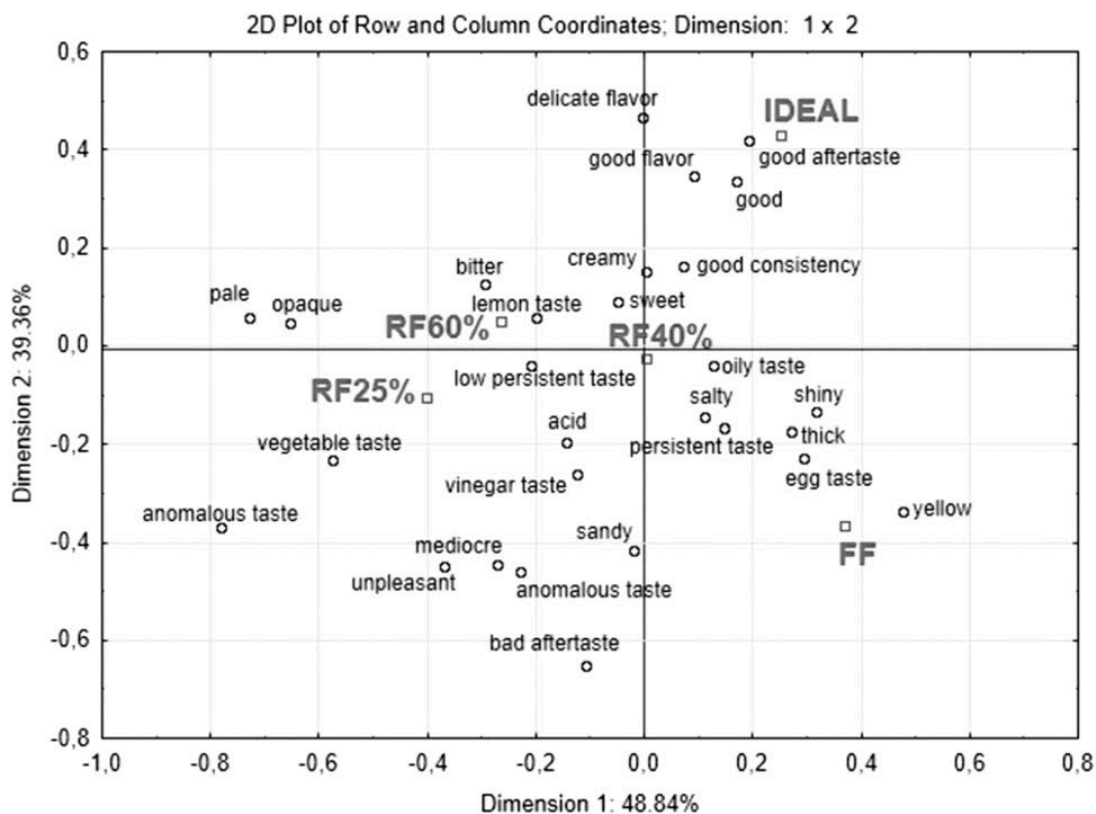


Figure 5. Correspondence analysis of the CATA test data of mayonnaises prepared with different fat amounts.

The two dimensions explained $\approx 88\%$ of the variance with dimension 1 explaining $\approx 49\%$ and dimension 2 explaining $\approx 39\%$. The ideal product was identified by generic descriptors as good, good aftertaste, good flavour, far from mayonnaise samples. FF was described by the attribute yellow, egg taste, thick, shiny, persistent taste, opposite to RF 25% attributes which were pale, opaque, low persistent taste, vegetable taste, and to RF 60% that was described with the lemon taste and bitter attributes. RF 40% could not be discriminated by any attribute. On the flavour part the CATA results were consistent with the acceptability test results: indeed, all the ideal descriptors as good flavour and good aftertaste were not associated to any mayonnaise formulation including FF. This negative satisfactory perception of the flavour of the mayonnaises can be associated with the initial basic formulation used in this study developed to focus on structural and rheological aspects of the product. Flavour attributes can be easily corrected and brand customized in an industrialisation step with the addition of natural flavouring agents into the recipe. No negative descriptors are linked to consistency parameters and all scores obtained are over 6; therefore, the structure created using the fat replacer has not negatively influenced the



mayonnaise prepared in this study. Overall, RF 40% mayonnaise was appreciated by the panel and the recipe proposed can be a good starting point for industrialisation.

4. Conclusions

A physically modified corn flour-based gel has been successfully used as fat replacer in clean label mayonnaise. The reduced-fat mayonnaise formed presented a Bostwick consistency, texture, rheological properties and stability similar or higher to the full-fat counterpart corroborating the ability of the flour to counterbalance the absence of fat in clean label products. Differences in colour and sensorial attributes were noticed but can be mitigated with slight recipe modification during the industrialisation steps.

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Chapter 4

Use of a semi-solid fibre syrup for sugar reduction in shortbread cookies

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Abstract

Sugar reduction is a pillar of international nutritional guideline and the food industry is constantly looking for new ingredients able to replace sugar technological functionality and in meantime satisfy the consumer request of clean label ingredients. A new semi-solid fibre syrup, with consistency similar to honey was tested as “bulking agent” in shortbread cookies. From a standard product recipe four reformulation strategies were tested without/with addition of the syrup to obtain a 30 and 50% sugar reduction. Cookies were subsequently characterised for their physicochemical, rheological and as well sensorial attributes. Syrup addition did not hinder dough workability and did not require changes in shortbread production procedure. Sugar reduction cause a hardness reduction of short breads in a manner proportional to the level of sugar reduction. The bulking effect of the syrup was found to partially preserve structural strength. Sugar reduced products presented all a colour distinguishable from the control with the syrup containing products having a more marked red component. Syrup application resulted in cookies with a better sensory and nutritional profile confirming the positive outcome of the use of the syrup as a bulking agent.

Keywords

Shortbread cookies, sugar reduction, fibre, clean label

1. Introduction

Overweight and obesity (especially in children and teenagers) are considered an epidemic concern with serious long-term health effects associated to premature mortality, type 2 diabetes, cardiovascular disease, hypertension and cancer (Lin et al., 2015; Manson & Bassuk, 2003). A strategy to diminish obesity risk factor is the reduction of sugar in diet because its overconsumption has been associated to the increase of body weight and therefore to the correlated health problems (Te Morenga et al., 2012). At the present time in Europe, sugar represents $\approx 15\%$ - 21% and $\approx 16\%$ - 26% of the energy intake in adult and children, respectively (Azaïs-Braesco et al., 2017), and the WHO (World Health Organization, 2015) recommends to reduce its intake to less than 10% of the total energy intake. Cookies are a very popular food and, due to their high sugar content (30-40% of the recipe), represent a high source of added sugar. In USA around the 15% of the children overall sugar intake derive from sweet bakery products, in particular cookies (Bailey et al., 2018), while in Europe sweet products (including cookies) contribute for around 50% to the total sugar intake of children (Azaïs-Braesco et al., 2017). In Italy, cookies represent for children and teenager around 4-5% of their total energy intake (Sette et al., 2011). Sugar has a pivotal



role in product quality because it contributes to the colour, structure and sensorial properties (Biguzzi et al., 2014; Chevallier et al., 2000; Sai Manohar & Haridas Rao, 1997; van der Sman & Renzetti, 2019). Therefore, the reduction of high levels of sugar is a difficult task for food technologists when product quality must not fail. In fact, if sweetness can be adjusted by the use of intensive sweetener (e.g. sucralose, aspartame, saccharin, stevia) (van der Sman & Renzetti, 2019), other quality features linked to sugar are difficult to preserve. Indeed, sucrose thanks to its hygroscopicity and crystallisation properties contributes to different aspects to the final cookies quality: (i) its melting (in combination with shortening) during cooking lead to a lowering of dough viscosity and consequently allowing its spreading (Sahin et al., 2019; Sumnu & Sahin, 2008), (ii) its tendency to recrystallise during cooling increases final surface hardness (Belcourt & Labuza, 2007; Gallagher et al., 2003; Pareyt et al., 2009) (ii) its involvement in the Maillard reaction contributes to the formation of cookies colour and flavour (Davis, 1995).

Sugar reduction can be reached using different approaches as multisensorial integration, food structure innovation, gradual sugar reduction, the use of sugar substitutes like bulking agents (Hutchings et al., 2019). This latter strategy is the most covered, (Hutchings et al., 2019) and different studies have been conducted to reduce sugar in cookies using bulking agent, including oligosaccharides, polyols and dextrans (Di Monaco et al., 2018; Struck et al., 2014; van der Sman & Renzetti, 2019). Sugar reduced cookies obtained substituting 30% of the sucrose with a chicory based fructo-oligosaccharide (Raftilose®) were found to have a lower hardness and a darker colour at the surface compared to standard (Gallagher et al., 2003). The use of inulin allowed to obtain consumer acceptable cookies substituting up to 25% of sucrose, higher substitution level led to a reduction in cookies crispiness and a detrimental consumer acceptability (Laguna et al., 2013). Instead, tagatose (a minimally absorbed ketohexose) was able to replace half of sucrose with a positive sensory score despite the reduced sugar cookie was found harder and darker than control (Taylor et al., 2008). However, in the study of different bulking agents usable for sugar reduction, it has also to be considered that a new consumer trend (clean label) is establishing. This “new” consumer is particularly careful to products containing ingredients easily “recognisable”, “familiar”, “natural” and “healthier” (Asioli et al., 2017). These “new” consumers need to be taken in consideration when a reduced sugar formulation has to be designed with the use of bulking agents.

In this perspective, a win-win approach can be the use of ingredients easily recognisable by the consumer like the dietary fibres as bulking agents. In particular, dietary fibres could be used with a double effect: technological ingredient for sugar reduction with its bulking and humectant properties and nutritional improver as source of dietary fibres. In a previous work of Pareyt and colleagues (Pareyt et al., 2011), arabinoxylans obtained from wheat bran have been successfully used to replace 30% of sugar and increase the fibre content in cookies while Handa and co-workers (Handa et al., 2012) positively used fructooligosaccharide to reduce sugar up to 60% increasing the total dietary fibre content.

In this frame, the objective of this work was to study the use of a new syrup-like syrup based on chickpea and corn fibre (MELTEC®, HI-FOOD, Parma, Italy) as bulking agent in shortbread cookies which were further analysed on their psychochemical attributes and sensorial acceptability.



2. Materials and Methods

2.1. Materials

MELTEC® was obtained from HI-FOOD S.p.A. (Parma, Italy). MELTEC® is a clean label ingredient with a consistency similar to honey, a gold brownish colour, sugar content lower than 1.0% and ≈25% of moisture content. Wheat flour 00 (W:120), fresh egg, butter, leavening agent, sucrose, used for the shortbread cookies formulations were obtained from a local supermarket.

2.2. Semi-solid fibre syrup characterisation

The semi-solid fibre syrup was firstly basically characterised for its physicochemical properties. °Brix of semi-solid fibre syrup was measured using a portable refractometer HB 95 (Lega Italy, Ravenna, Italy). Water activity was measured at 25 °C with an Aqualab 4 TE (Decagon Devices Inc., Pullman, WA, USA). Moisture content (MC, g of water/100 g of sample) was obtained by weight loss by drying in a forced-air oven (ISCO NSV 9035, ISCO, Milan, Italy) at 70 °C to constant weight. pH was analysed with a potenziometer pH7+ DHS Food (XS Instruments, Modena, Italy). Colour analysis was performed using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65 and a 10° position of the standard observer. The results were obtained according to CIE Lab system. The parameters obtained were: L^* [0 (black) - 100 (white)], a^* ($-a^*$ = greenness and $+a^*$ = redness) and b^* ($-b^*$ = blueness and $+b^*$ = yellowness). At least three measurements were taken for each analysis

2.3. Shortbread cookies preparation

From the standard recipe, sucrose content was reduced to 30% and 50% with and without the addition of MELTEC® (Table 1).

Table 1. Short bread cookies recipes (g).

Ingredients	Sucrose	Sucrose	Sucrose 70%	Sucrose	Sucrose 50%
	100%	70%	Meltec 30%	50%	Meltec50%
	S100	S70	S70M30	S50	S50M50
Wheat flour	100	100	100	100	100
Egg	28	28	28	28	28
Butter	30	30	30	30	30
Yeast	3	3	3	3	3
Sucrose	40	28	28	20	20
Meltec®	/	/	12	/	20

In particular, starting from the standard recipe (S100, containing 100% of sucrose), four different sugar reduced cookies were prepared: S70, containing 70% of sucrose, S70M30 containing 70% of



sucrose and 30% of MELTEC[®], S50, containing 50% of sucrose, S50M50 containing 50% of sucrose and 50% of MELTEC[®].

Shortbread cookies doughs were prepared mixing all the ingredients in a mixer (Kitchen Aid, St Joseph, USA) at 60 rpm for 5.5 min. Shortbread cookies dough was manually sheeted until reaching a thickness of 4 mm and cut in pieces of shape 0.4 cm x 2 cm x 5 cm, and then cooked at 180°C for 20 min in a forced convection oven (Electrolux EOB8747AOX, Stockholm, Sweden). Cookies were placed on rack and cooled at room temperature and, subsequently, stored in a plastic bag for 24 h before the analysis. Two batches of cookies of each formulation were produced in two different days.

2.4. Shortbread cookies characterisation

2.4.1. Nutritional label

Macronutrients content of the different recipes of shortbread cookies was obtained using European Institute of Oncology database (IEO-DBA, 2020) for standard ingredients and using the nutritional information reported in the technical data sheet for MELTEC[®]. Energy values (kJ and kcal) were calculated using the energy factors reported in the EU Regulation on labelling of food products (Regulation (EU) No 1169/2001) [in details: carbohydrate = 17 kJ (4 kcal); protein = 17 kJ (4 kcal); fat = 37 kJ (9 kcal); fibre = 8 kJ (2 kcal)].

2.4.2. Water activity and moisture content

Water activity was measured at 25 °C with an Aqualab 4 TE (Decagon Devices Inc., Pullman, WA, USA). Moisture content (MC, g of water/100 g of sample) was obtained by weight loss by drying in a forced-air oven (ISCO NSV 9035, ISCO, Milan, Italy) at 70 °C to constant weight.

In both analysis, at least three measurements were taken for each formulation for a total of six determinations.

2.4.3. Texture property

Food Texture Analyzer (TA1 Texture Analyzer, AMETEK, USA) equipped with a 100 N load cell. Hardness (N) was evaluated by means of cutting test (at 2 mm/s, trigger force = 0.1 N) using a flat blade (FG/WBJ) and was measured as the maximum force at brake (N).

Ten measurements were taken for each formulation for a total of twenty determinations.

2.4.4. Colour

Colour analysis was performed using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65 and a 10° position of the standard observer. The results were obtained according to CIE Lab system. The parameters obtained were: L^* [0 (black) - 100 (white)], a^* ($-a^*$ = greenness and $+a^*$ = redness) and b^* ($-b^*$ = blueness and $+b^*$ = yellowness). ΔE was obtained



using as reference the full sugar recipe cookie S100 (Limbo & Piergiovanni, 2006). At least ten determinations were performed for each formulation for a total of at least twenty determinations.

2.4.5. Sensorial analysis

Sensorial analysis of shortbread cookies samples was realized using both an acceptability and a rapid profiling method check-all-that-apply (CATA). All shortbread cookies were named with a three-digit random number and presented to 50 untrained judges. In the acceptability test a 9-points hedonic scale was used, (1=dislike extremely, 2=dislike very much, 3=dislike, 4=dislike slightly, 5=neither like nor dislike, 6=likes slightly, 7=like, 8=like very much and 9=like extremely). Judges were allowed to drink water between the samples to cleanse the palate. Overall, scores undergone to an ANOVA test to verify significant differences among samples.

For CATA test judges were requested to recognize all the attributes that applied to the samples and to their ideal version. The attributes were randomly reported in the questionnaire and were: excellent, good, mediocre, bad taste, crispy, crumbly, hard, soft, gummy, golden, pale, dark, very sweet, optimal sweetness, low sweet, good after taste, bad after taste.

2.5. Statistical analysis

Significant differences ($p \leq 0.05$) among different samples were assessed by one-way-analysis of variance (ANOVA) with a Duncan post-hoc test using an IBM SPSS statistical software (Version 24.0, SPSS Inc., Armonk, New York, USA). The contingency table of CATA dataset was obtained on the basis of samples and attributes. A correspondence analysis was performed to summarize the relationship between samples and attributes using the software Statistica (Version.13.3, TIBCO Software Inc.).



3. Results and discussion

3.1. Semi-solid fibre syrup characterisation

The semi-solid fibre syrup, before its use in cookies, was firstly physicochemical characterised for its °Brix, water activity (a_w), moisture content (MC), colour and pH, results in Table 2.

Table 2. Physicochemical properties of the semi-solid fibre syrup.

Semi-solid fibre syrup	
°Brix	74.55 ± 0.55
a_w	0.88 ± 0.01
MC (g H ₂ O/ 100 g sample)	25.03 ± 0.98
pH	6.37 ± 0.04
L^*	23.51 ± 0.15
a^*	0.34 ± 0.03
b^*	3.34 ± 0.42

All data are expressed as mean ± standard deviations

The results indicated a value of ≈ 75 °Brix due to presence of the fiber in the product. The values of a_w and MC were respectively ≈ 0.9 and ≈ 25 (g H₂O/ 100 g sample) due to the high presence of water in the product and the weaker water interaction ability of the fibre. The results confirm the values of moisture content reported also in the technical data sheet of the product. The pH observed was ≈ 6.4 therefore indicating a value close to neutral of the syrup. The colour properties (L^* , a^* , b^*) and in particular the positive values for a^* and b^* indicated the more marked presence of redness and yellowness values, thus confirming the brownish colour reported in the technical data sheet of the product.

3.2. Nutritional information

The main objective of the study was the achievement of shortbread cookies with a high nutritional profile without jeopardize their structure and consumer acceptability. The nutritional profiles of the different cookies are reported in Table 3.

Table 3. Nutritional label of shortbread cookies at different sucrose (S) /MELTEC® (M) ratio (%).

	S100	S70	S70M30	S50	S50M50
Energy (kJ)	1570	1938	1899	1965	1882
Energy (kcal)	452	463	454	470	450
Fat	16,1	17,5	16,8	18,6	17,1
-of which saturated	15,8	17,4	16,3	18,1	16,6
Carbohydrates	67,7	66,2	63,5	64,8	59,9
-of which sugars	22,9	17,4	16,6	13,1	12,1
Fibre	1,4	1,6	6,2	1,6	9,4
Protein	8,6	9,4	9,1	10	9,3
Salt	0,1	0,1	0,1	0,1	0,1



Energy values of all shortbread cookies were not markedly influenced by the different formulation used and they did not decrease with the decrease of sucrose in the recipe. Indeed, on the basis of EU Regulation on labelling of food products (Regulation (EU) No 1169/2001) the energy conversion factor is not directly associated to the sugar content but to the total carbohydrate content which value did not diminish consistently in the cookies. Instead, the nutritional profile of the shortbread cookies reformulated recipes was improved. In fact, all reformulated shortbread cookies presented lower sugar and increased fibre contents when the semi-solid fibre syrup was used. In particular, sugar content decreased from ≈ 23 g/100 g of the standard recipe (S100) to ≈ 17 g/100 g for S70 and S70M30, to ≈ 13 g/100 g for S50 and to ≈ 12 g/100 g for S50M50. In percentage, it was possible to obtain a reduction of $\approx 30\%$ and $\approx 50\%$ of the sugar content respectively for S30-S30M70 and S50-S50M50. Considering a cookie daily portion of 30 g (SINU, 2014), it can be estimated that in the nutritional label of the standard recipe (S100) can be indicated that it contributes to a 7.6% to the daily reference intake of sugar in adult basing on the reference intake reported in the EU Regulation on labelling of food products (Regulation (EU) No 1169/2001). On the contrary, the reformulated cookies lowered the contribution to $\approx 5.8\%$ in S70, to $\approx 5.3\%$ in S70M30, to $\approx 4.4\%$ in S50 and to $\approx 4.0\%$ in S50M50. Fibre content in the reformulated cookies in which the semi-solid fibre syrup was used increased from ≈ 1.4 g/100 g (S100) to ≈ 6.2 g/100 g and ≈ 9.4 g/100 g for S70M30 and S50M5, respectively. Based on the sugar reduction and increased fibre values in S70M30 and S50M50 shortbread cookies, a dual health claims “reduced in sugar” and “high in fibre” could be used on the label of these products on the basis of the EU regulation on nutritional and health claims (Regulation (EC) No 1924/2006).

3.3. Shortbread cookies characterisation

Sugar reduction in this study was evaluated with and without the use of the semi-solid fibre syrup in substitution of sucrose to evaluate its technological functionality effectiveness. All shortbread cookies doughs were easily workable and it was not necessary any modifications in the cookie production process neither when the semi-solid fibre syrup was included in the recipe.

Shortbread cookies were initially characterised for their water activity (a_w) and moisture content (MC) to evaluate if the reduction of sugar, and/or the presence of the semi-solid fibre syrup could lead to any variation of these parameters, results are reported in Table 4.



Table 4. Water activity (a_w), moisture content (MC), and color (L^* , a^* , b^* , ΔE) of short bread cookies at variable sucrose (S) / MELTEC® (M) ratio (%) (S100, S70, S70M30, S50, S50M50).

	S100	S70	S70M30	S50	S50M50
a_w	0.32 ± 0.02 ab	0.31 ± 0.02 abc	0.29 ± 0.02 bc	0.34 ± 0.02 a	0.26 ± 0.02 c
MC	5.47 ± 0.93 a	4.55 ± 0.87 ab	3.73 ± 0.76 b	4.19 ± 0.55 b	3.53 ± 0.73 b
L^*	79.71 ± 0.83 b	80.71 ± 1.14 a	79.70 ± 0.99 b	80.77 ± 0.98 a	78.08 ± 0.97 c
a^*	-1.43 ± 1.01 c	-1.92 ± 0.46 c	1.12 ± 0.31 a	-2.00 ± 0.55 c	0.48 ± 0.55 b
b^*	44.66 ± 1.62 b	44.95 ± 1.28 b	41.73 ± 1.17 c	47.33 ± 1.84 a	44.89 ± 0.79 b
ΔE	/	2.86 ± 1.26 a	3.87 ± 1.39 a	3.68 ± 1.86 a	3.19 ± 1.20 a

All the data are expressed as mean ± standard deviations; different letters close to number indicate significative difference among sample ($p \leq 0.05$).

Overall, a_w and MC were in the range 0.26-0.34 and 3.5-5.5 g H₂O /100 g sample, values in accordance for the product category (average value are ≈ 0.3 and below 6 g/100 g respectively for a_w and MC, (Curti et al., 2018; Pareyt & Delcour, 2008). Therefore, the use of the semi-solid fibre syrup did not affect the two parameters which are strongly linked to product stability.

Cookies quality is strongly related to texture properties, in particular to hardness (Pareyt & Delcour, 2008) which values for shortbread cookies as a function of different formulation are reported in Figure 1.

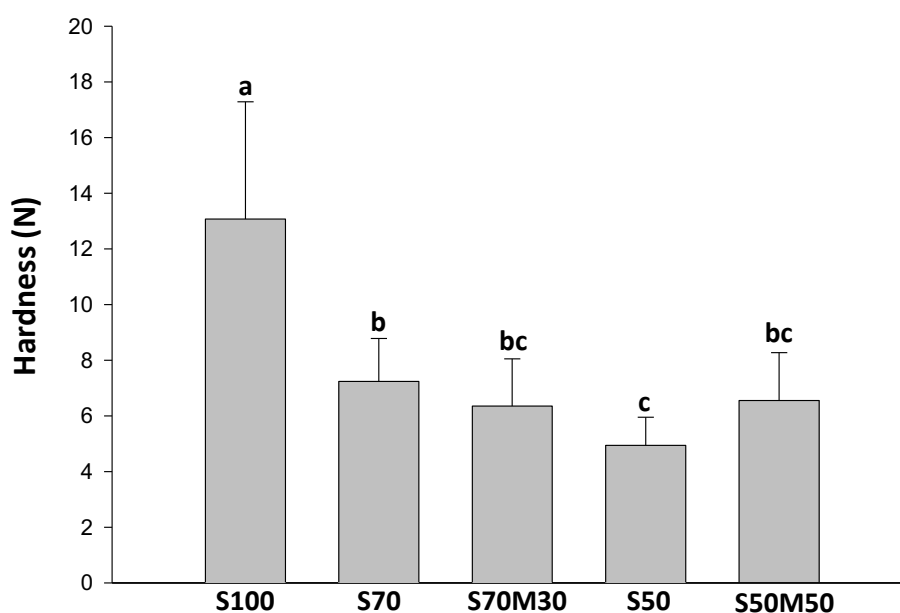


Figure 1. Hardness of short bread cookies at variable sucrose/MELTEC® content. Different letters close to number indicate significative difference among sample ($p \leq 0.05$).

Hardness of the shortbread cookies significantly decreased with the reformulation in a proportional manner with the reduction of sugar when the semi-solid fibre syrup was not included in the formulation. S100 was the hardest sample (13.7 ± 2.8 N) and a decrease of hardness with the sugar reduction was found (7.2 ± 1.5 N for S70 and 4.9 ± 1.0 N for S50). The presence of the semi-solid fibre syrup



significantly lowered hardness which was 6.4 ± 1.7 N for S70M30 and 6.6 ± 1.7 N for S50M50. Interestingly, with the 50% of sugar reduction, the presence of the semi-solid fibre syrup increased hardness indicating a bulking role at certain amount in the formulation. Softening of the sugar reduced cookies is explained by the absence of sugar, which crystallise during cooling, causing a hardening effect on the cookies (Gallagher et al., 2003). Similar softening effects, due to sucrose replacement on cookies, were reported by Gallagher and colleagues (Gallagher et al., 2003) using a chicory-based oligosaccharide (Raftilose®), by Kulthe and co-workers using stevia leaves powder (Kulthe et al., 2014) and by Handa and colleagues using fructooligosacharide (Handa et al., 2012). On the contrary, Taylor and co-workers using tagatose increased cookies hardness because it is less soluble than sucrose and therefore tend to crystallise to a larger extent (Taylor et al., 2008).

Cookies colour parameters (L^* , a^* , b^*) are reported in Table 4. Sugar reduced cookies produced without the use of the bulking agent (S70 and S50) presented comparable surface lightness with S100, as indicated by the L^* value ≈ 80 in all the three samples. A darkening effect associated to a significantly decrease of L^* was observed in the presence of the semi-solid fibre syrup (≈ 75 and ≈ 78 for S70M30 and S50M50, respectively, with the latter significantly darker than the former). The darkening effect on the surface can be attributed to the intrinsic brownish colour of MELTEC®. a^* value indicates redness or greenness respectively for positive or negative values; a^* decreased with the increase of sugar reduction when no bulking agent was present moving from ≈ -1.43 for S100 to ≈ -1.92 for S70 and to ≈ -2.0 for S50. On the contrary, the presence of the semi-solid fibre syrup allowed to increase the a^* values leading to a more pronounced red colour, with $a^* \approx 1.12$ and ≈ 0.48 for S70M30 and S50M50, respectively. b^* value (yellowness) of the different cookies was found comparable with S100 in the case of S70 and S50M50 (≈ 45) while it decreased and increased in the case of S70M30 (≈ 42) and S50 (≈ 45), respectively. Overall, sugar reduced cookies produced presented all a distinguishable colour when compared to the standard ones. A quite distinguishable colour difference (ΔE) was observed for all samples with comparable values between them, in particular S70 (≈ 2.9), S70M30 (≈ 3.7), S50 (≈ 3.7) and S50M50 (≈ 3.2).



3.4. Sensorial analysis

Sensory evaluation test results are reported in Figure 2.

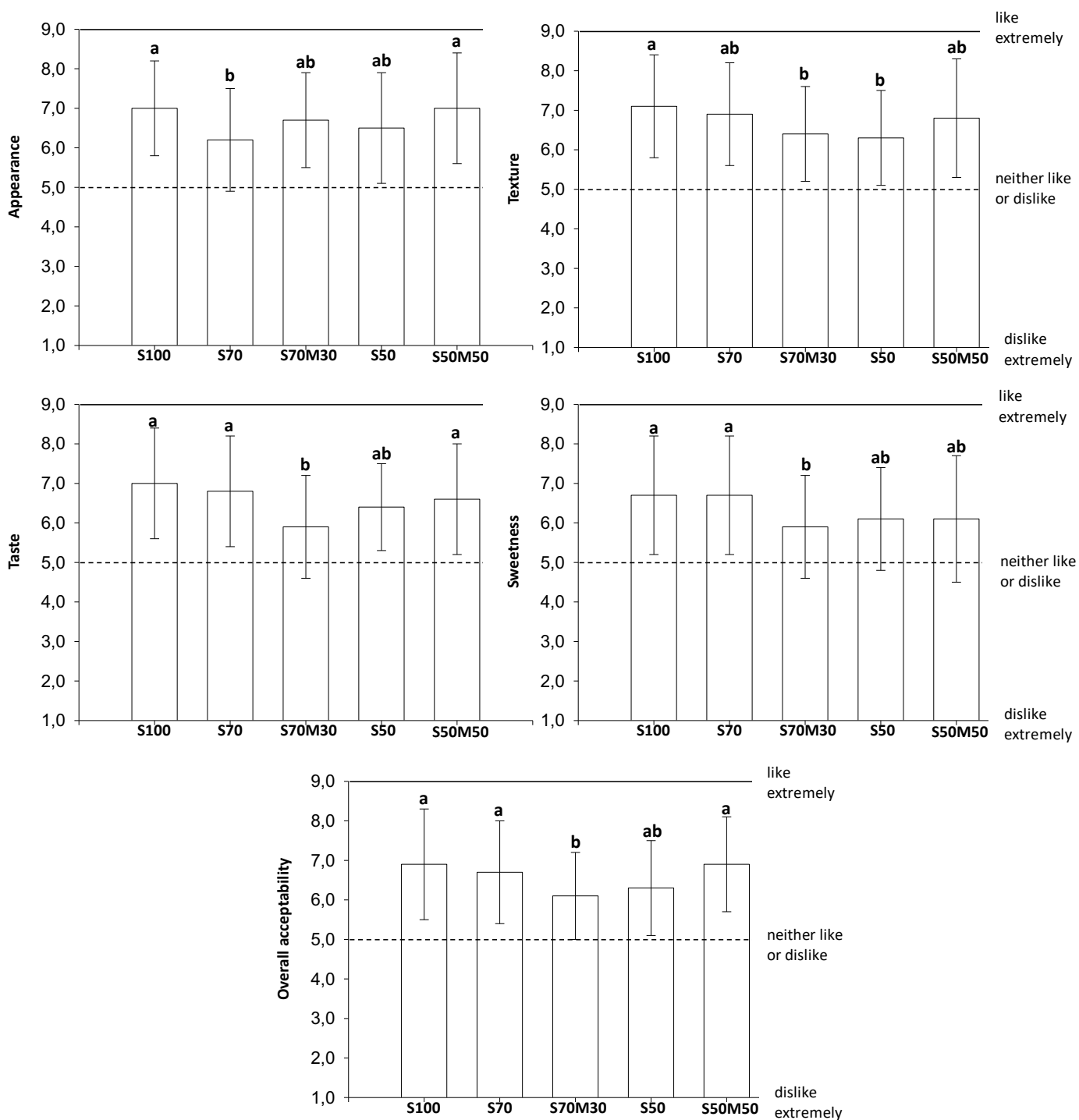


Figure 2. Sensory scores for appearance, texture, taste, sweetness, and overall acceptability of short bread cookies at variable sucrose/MELTEC® content. Different letters close to number indicate significant difference among sample ($P \leq 0.05$).



In terms of overall acceptability, all shortbread cookies tested were positively accepted by the consumer panel, with the most preferred S100, S70 and S50M50 samples in which quite all attributes were close to 7 (like). In particular, in S70 the lowest score was attributed to the appearance while in S50M50 was attributed to sweetness. The low score of the appearance in S70 increased with the use of the semi-solid fibre syrup in S70M30 from ≈ 6 (likes slightly) to ≈ 7 (like) highlighting that the high colour difference previously observed by colour analysis, did not hinder consumer product acceptability. The high scores identified for S50M50, in particular for appearance and texture, are particularly important because they highlight the efficacy of the semi-solid fibre syrup as bulking agent when a high sugar reduction is searched for. Instead, the lower scores reported for sweetness, can be easily corrected with the use of sweeteners.

Detailed information on consumers' perception of shortbread cookies was obtained using a CATA test, requiring to consumers panel the selection of appropriate attributes for the sample from a previous specified list (Ares et al., 2014; Dooley et al., 2010).

CATA results were analysed using a correspondence analysis which the output is shown in Figure 3.

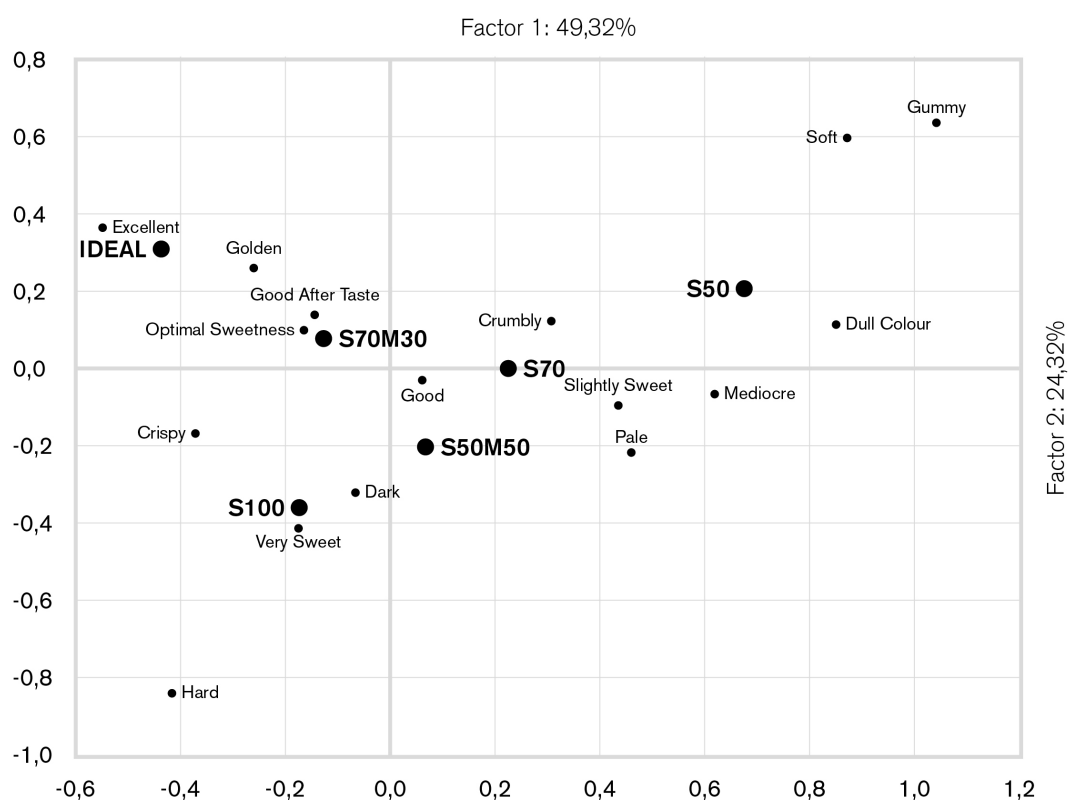


Figure 3. Correspondence analysis of the CATA test data of shortbread cookies prepared with different sucrose/MELTEC® content

In the factor plane are represented the “IDEAL” product and the different shortbread cookies recipes. The two dimensions explained $\approx 74\%$ of the variance with dimension 1 explaining $\approx 49\%$ and dimension 2 explaining $\approx 24\%$. “IDEAL” sample was described as “excellent”, “golden”, “good after taste”, “optimal



sweetness”; similar descriptors were used also for S70M30. S100 was described by the attributes “hard”, “very sweet”, “crispy”, “dark”; S50 was described with negative indicators “crumbly”, “soft”, “gummy”; S70 was not discriminated by any attribute; S50M50 was described by “slightly sweet”, “mediocre”, “bad after taste”. The negative attributes indicated for S50M50 reflect the lower sweetness scores reported in the acceptability test. In the final view to obtain sugar reduced and high fibre cookies encouraging results have been obtained combining acceptability and CATA test. Indeed, cookies in which sugar has been substitute with the semi-solid fibre syrup (S50M50) presented a better consumer outcome than cookies in which sugar has been simply eliminated (S50). The lower scores and attributes related to the taste can be adjusted with the use of sweetener in the recipe, not used in this study in which the main purpose was the study of the technological effect of the semi-solid fibre syrup. These results are even more interesting if it is considered that the consumer panel was not aware of the healthy claims accompanying the product and that fibre-enriched products are usually associated to a general disliking (Biguzzi et al., 2014; Brennan & Samyue, 2004).

4. Conclusions

A semi-solid fibre syrup based on corn and chickpea fibres was firstly physicochemical characterised and subsequently positively used as clean label bulking agent for sugar reduction in shortbread cookies. The semi-solid fibre syrup did not hinder dough workability and no modifications in cookie preparation were necessary. The syrup was able to partially preserved the structure of the sugar reduced cookie and contributed to enhance their nutritional and consumer acceptability profile. Colour differences noticed did not jeopardize consumer acceptability.



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Chapter 5

A semi-solid fibre syrup for sugar reduction in fruit filling for bakery application

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Abstract

Food industry is looking for new ingredients able to reduce sugar in food and meanwhile satisfy consumer request of clean label products. A new semi-solid fibre syrup has been used to formulate fruit fillings with reduced-sugar and increased fibre content. Three reduced-sugar recipes (by 30, 50 and 70%) were produced starting from the standard recipe. The overall physicochemical stability and sensory attributes of the fruit fillings were assessed during 180 days of storage at two different temperatures (5°C and 25°C). As a result of the syrup increase, water activity and moisture content of the fruit fillings increased, without affecting their shelf-life stability. Filling properties were significantly affected by the increase of syrup with an increment of hardness, adhesiveness, K-consistency, bake stability and differences in colour samples. During storage, in all fillings bake stability and colour were the most affected parameters leading to less bake stable and darker products. Finally, sensory features of the fruit filling with a 30% sugar reduction were the most appreciated by consumers, and its sensory attributes were not distinguishable from those of the FS formulation. Similarities at rheological and sensorial level confirmed the technological functionality of the syrup when used to obtain a 30% sugar reduction.

Keywords

Fruit filling, Sugar reduction, semi-soli fibre syrup, dietary fibre



1. Introduction

In Europe, the estimated energy intake of sugar of the total daily energy intake in adult ranges between 15 and 21% while in children between 16% and 26% (Azaïs-Braesco et al., 2017). These values are not in line with the World Health Organization guidelines (World Health Organization, 2015) which recommend to reduce sugar intake to less than 10% of the total energy intake since higher sugar intake is related to health problems as obesity, diabetes, and dental caries (Schwingshackl et al., 2017; Te Morenga et al., 2012; Touger-Decker & van Loveren, 2003). Therefore, awareness campaigns from institutions and governments are going in different countries to inform consumers (Piernas et al., 2013). As a result, consumers are searching for products on the market with a reduced-sugar content (Piernas et al., 2013). In 2018 food with the claim “low sugar” were the 9% of all newly launched foods (FIE Global, 2020), while during 2018 in the USA, 8% of new products contained a claim associated to sugar reduction (Food Navigator, 2020). Also in Italy similar trends were found as product with the claim “reduced-sugar” increased of the 7.6% during 2019 (Osservatorio Immagino GS1 Nielsen, 2020).

Although the increased market request, it is well known that the development of reduced-sugar products is not an easy challenge for the food technologists. This is due to the important technological role of sugar on the sweetness delivering and structuring food products. Different strategies to reduce sugar in foods have been proposed by the literature: (i) multisensorial integration in which aroma and colour stimuli are enhanced to counterbalance the reduction of sugar, (ii) food structuring technologies act to create an inhomogeneous distribution of sucrose in the matrix and the consequently discontinuous stimulation of taste receptors or to modify the fracture mechanisms of food to modify its sweetness perception, (iii) the gradual reduction of sugar content to lower the desired level of sweetness of the consumers (iv) the use of ingredients to substitute sugar. The latter is considered the most appropriate so far and the most viable in an industrial context (Hutchings et al., 2019). The sweetener role of sugar can be nowadays easily replaced using synthetic or natural non-caloric sweeteners e.g., sucralose, aspartame, saccharin, stevia. On the contrary, the replacement of the bulking effect of sugar with a proper ingredient is still a challenge. This is particularly difficult for high sugar content products as jam, jelly, fruit filling in which sugar can be up 60% of the product (Di Monaco et al., 2018). Polyols as sorbitol, lactitol and xylitol have been largely studied as sugar replacer due to their double effect as sweetener and bulking agents; nevertheless, their laxative effect above 10% concentration forces their use only in confectionary products consumed in low amounts (Riedel et al., 2015).

Moreover, the search of ingredients able to replace sugar should also take into account the request of consumers for clean label products with ingredients easily recognizable, healthy and familiar to which the consumer are acquainted with (Asioli et al., 2017; Osborn, 2015). An ingredient able to simultaneously satisfy the technological requirement and the clean label standard would be of great interest for the food industry. Dietary fibres could potentially satisfy both requirements: they are easily recognisable and positively accepted by the consumers (Dhingra et al., 2012) and they are used either as bulking agents



and as nutritional improvers. Different studies have underlined the use of non-digestible carbohydrates as inulin, fructooligosaccharides (FOS) and/or modified starches as bulking agent ingredients in high sugar products (Buttriss, 2017). In the frame of high sugar foods, bakery products which contain fruit based filling are a growing sale sector (Cropotova et al., 2016). In this product a high percentage of sugar is represented by the fruit filling which are mainly based on sugar, water, fruit puree/jam, flavour and thickening agent (Wei et al., 2001). Despite the high sugar content of this food preparations, very few researches have been carried out to reduce their sugar content. Agudelo and co-workers (Agudelo et al., 2015a; Agudelo et al., 2015b) used polydextrose (E1200) to replicate the bulking effect of sucrose in fruit filling preparations obtaining a similar structure of the full-sugar counterpart without evaluating its stability over storage.

In this paper, a new semi-solid fibre was used as sugar substitute to develop reduced-sugar fruit filling formulations. Fruit fillings with different reduced-sugar levels were developed and characterised for their physicochemical properties, stability and sensory profile at 5°C and 25°C for a storage period of 180 days.

2. Materials and Methods

2.1. Materials

A semi-solid syrup (MELTEC®) was obtained from HI-FOOD S.p.A. (Parma, Italy). MELTEC® is a clean label ingredient based on chickpea fibres, corn fibres and water with an appearance and rheology similar to a syrup or to honey. Its sugar content is below 1% and its moisture content is $\approx 25\%$ (g water/100 g sample). Other ingredients used in the fruit filling recipes were: low-methoxyl (LM) pectin Classic AB 902 (Herbstreith&Fox KG, Werder, Germany), glucose syrup 60 dextrose equivalent DE “Glucoplus 361” (Uniglad Ingredienti, Cuneo, Italy), tricalcium citrate (Giusto Faravelli, Milano, Italy), potassium sorbate (Giusto Faravelli, Milano, Italy), crystalline sucrose (British sugar, Peterborough, UK), apricot jam (Rogelfrut, Cuneo, Italy), citric acid (Brenntag, Milano, Italy), apricot flavour NATLQ10989 (International Taste Solution, Newbury, UK).

2.2. Fruit filling preparation

Full-sugar (FS) and reduced-sugar (RS) fruit filling recipes, based on industrial recipe, are shown in Table 1.



Table 1. Fruit filling recipes (% wt). FS: full sugar; RS30: 30% sugar reduction; RS50: 50% sugar reduction; RS70: 70% sugar reduction.

	FS	RS30	RS50	RS70
Sucrose	32	30.6	19.3	8.3
Glucose syrup	20	-	-	-
MELTEC®	-	21.4	32.7	43.7
Water	26.9	26.8	26.75	26.72
Apricot jam	20	20	20	20
Pectin	0.8	0.8	0.8	0.8
Citric acid	0.14	0.24	0.29	0.32
Tricalcium citrate	0.08	0.08	0.08	0.08
Potassium sorbate	0.05	0.05	0.05	0.05
Apricot flavour	0.03	0.03	0.03	0.03

RS fruit fillings were prepared replacing all the glucose syrup and increasing level of crystalline sucrose with MELTEC® in a 1:1 weight ratio, achieving a sugar reduction of 30% (RS30), 50% (RS50) and 70% (RS70).

All fruit filling samples (FS and RSs) were produced using a bowl chopper (Polyfunctional QB 8-3, Roboqbo, Bologna, Italy). As a first production step, LM pectin was mixed with crystalline sucrose in a 1:5 ratio, the mixture obtained was then dissolved in half of the water present in the recipe and pre-heated at 80°C with a blender (Minipimer MQ5035, Braun, Germany) at 13500 rpm for 5 minutes. In parallel, the other ingredients as crystalline sucrose, tricalcium citrate, glucose syrup (FS recipe) or MELTEC® (for RS recipes), apricot jam and the remaining water in which was previously dissolved the potassium sorbate, were added together in the bowl chopper. The ingredients in the bowl chopper were subsequently mixed at 500 rpm and subjected to a heat treatment at 75°C until reaching 73 °Brix. Subsequently, the pre-mix of LM pectin and crystalline sucrose was added to the bowl chopper continuing the mixing process at 500 rpm and heating at 90°C under vacuum (1 bar) to concentrate the preparation at 72 °Brix. Afterwards, the citric acid dissolved in few drops of water and the liquid apricot flavouring were added checking the pH until reaching a value of 3.6. As a final step, the fruit filling obtained was cooled under vacuum (1 bar) at 800 rpm until reaching the temperature of 65°C.

The produced fruit filling was directly transferred into plastic container to be analysed after cooling to RT (t0) or stored at 25°C and 5°C for analysis after 30, 60 and 180 days (t30, t60, t180). Two batches of fruit filling of each formulation were produced in two different days.

2.3. Fruit filling characterisation

2.3.1. °Brix and pH

°Brix of fruit filling was measured using a portable refractometer HB 95 (Lega Italy, Ravenna, Italy), and pH with a potenziometer pH7+ DHS Food (XS Instruments, Modena, Italy). At least three



measurements were taken at 25°C for each formulation for a total of six determinations. Both parameters were also used to control the production process.

2.3.2. Nutritional profile

Macronutrient profile of FS and RS fruit fillings was calculated using the European Institute of Oncology database (IEO-DBA, 2020) Nutritional information of MELTEC® was provided by its technical data sheet. Energy (kJ and kcal) was obtained multiplying all macronutrients for their energy factors cited in the EU Regulation on food labelling (Regulation (EU) No 1169/2001) [carbohydrate = 17 kJ (4 kcal); protein = 17 kJ, (4 kcal); fat = 37 kJ, (9 kcal); fibre = 8 kJ, (2 kcal)].

2.3.3. Water activity and moisture content

Water activity was measured at 25 °C with an Aqualab 4 TE (Decagon Devices Inc. WA, USA). Moisture content (MC, g of water/100 g of sample) was measured by weight loss by drying in a forced-air oven (M120-TBR, MPM Instruments Srl, Milano, Italy) at 70 °C to constant weight. At least three measurements were taken for each formulation for a total of six determination at each storage time.

2.3.4. Texture

Texture properties were determined using TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK). A cylindrical container (58 X 70 mm) was completely filled with the fruit filling which it was subjected to a penetration test (50% strain at a rate of 0.8 mm/s) with a P/20 probe. Force deformation curves were analysed using Texture Expert software (Stable Micro Systems, Godalming, UK) to determine hardness (peak force, N) and adhesiveness (negative area, N mm). Five measurements were taken for each formulation for a total of ten determinations at each storage time.

2.3.5. Rheological properties

Rheological properties of fruit filling were determined using a controlled stress rheometer (MCR 702 twin drive, Anton Paar, Graz, Austria) at 25°C with a 50 mm diameter plate-plate geometry and a gap of 1 mm. Before each analysis, the exposed surface of samples was protected with paraffin oil, besides the samples were allowed to rest for 3 minutes for until axial force reached about 0 N prior and temperature equilibration. Flow curves were obtained increasing shear rates from 1 to 100 s⁻¹. Flow behaviour was characterized fitting the experimental data with the Power law equation according with:

$$\sigma = K\gamma^n$$

where σ is the Shear stress (Pa), γ is the shear rate (s⁻¹), K is the consistency coefficient (Pa•sⁿ) and n the non-Newtonian index (dimensionless).



Three measurements were performed for each formulation for a total of six determinations.

2.3.6. Colour

Colour analysis was performed for each fruit filling sample using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65 and a 10° position of the standard observer. The results were expressed in accordance with the CIE Lab system. The parameters determined were: L^* [0 (black) - 100 (white)], a^* ($-a^*$ =greenness and $+a^*$ =redness) and b^* ($-b^*$ =blueness and $+b^*$ =yellowness). ΔE was obtained using FS as reference (Limbo & Piergiovanni, 2006). At least ten determinations were performed for each formulation for a total of at least twenty determinations.

2.3.7. Syneresis and bake stability

Syneresis was measured as described by Crobotova and co-workers (Crobotova et al., 2009): 5 g of sample (F_0) were transferred into a 50 ml falcon tube, sealed with plastic cap and centrifugated at 3000 rpm for 20 min (centrifuge 5910R, Eppendorf, Milan, Italy). The weight of the precipitated fraction (F_1) was measured and grade of syneresis was calculated as percentage (%) = (F_1/F_0). Three tests were performed for each formulation for a total of six determinations.

Bake stability has been evaluated following Young and colleagues with slight modifications (Young et al., 2003). 10 g of fruit filling were placed into a circular mould (\varnothing 35 mm) on a 7x7 cm layer of short crust. On the circular sample obtained 4 points have been marked on the external part to measure the diameter pre and post baking. Baking has been performed on a conventional oven (903.008.05, IKEA, Leida, Netherlands) at 200° C for 10 minutes. Bake stability percentage (B.S.%) has been calculated on the basis of the following equation:

$$B.S.\% = 100 - \left[\left(\frac{\varnothing_{pb} - \varnothing_{bb}}{\varnothing_{bb}} \right) * 100 \right]$$

where \varnothing_{pb} is diameter post baking while \varnothing_{bb} is diameter before baking.

Three measurements were performed for each formulation changing the position of the short crust in the oven for a total of six determinations.

2.3.8. Sensorial analysis

Sensory analysis of fruit filling samples was realized using both an acceptability and a rapid profiling check-all-that-apply (CATA) method performed on samples immediately after production and after 60 and 180 days (t_0 , t_{60} , t_{180}). All fruit filling samples were coded with a three-digit random number and presented to 50 untrained judges. In the acceptability test a 9-points hedonic scale was used, (1=dislike extremely, 2=dislike very much, 3=dislike, 4=dislike slightly, 5=neither like nor dislike, 6=likes slightly, 7=like, 8=like very much and 9=like extremely). Judges were allowed to drink water between the samples to cleanse the palate. The ANOVA test was used to verify significant differences of the overall scores



among samples. For CATA test judges were requested to recognize all the attributes that apply in the samples and in the ideal version using the descriptors indicated in a specific list (Ares et al., 2014; Dooley et al., 2010). The attributes random reported in the questionnaire were: pleasant colour, unpleasant colour, opaque, orange, brown, shiny, acid, bitter, sweet, very sweet, slightly sweet, good taste, bad taste, mediocre taste, vegetal taste, apricot taste, good aftertaste, bad aftertaste, pleasant consistency, unpleasant consistency, melty, jelly, sticky, fluid, sandy.

2.4. Statistical analysis

A three-way ANOVA was performed using three fixed factors: recipe (R), storage time (St) and storage temperature (ST). The evaluation of single factor and their interactions in the variability of each parameter was obtained with the partition of total variance of sum square (SS%). Significant differences ($p \leq 0.05$) among different samples were assessed by one-way-analysis of variance (ANOVA) with a Duncan post-hoc test using an IBM SPSS statistical software (Version 24.0, SPSS Inc., Armonk, New York, USA). The contingency table of CATA dataset was obtained on the basis of samples and attributes. A correspondence analysis was performed to summarize the relationship between samples and attributes using Statistica software (Version.13.3, TIBCO Software Inc.).



3. Results and Discussion

Fruit fillings used by food manufacturers of bakery products are mainly formulated using high levels of sucrose and glucose syrup which strongly affect their overall quality. In this study, all the glucose syrup and increasing level of crystalline sucrose have been partially replaced with a commercially semi-solid fibre syrup, in an attempt to develop products with reduced-sugar content. All the recipes (FS and RSs) were prepared keeping constant pH and °Brix to replicate the industrial processes for this products category. In particular, a pH of 3.6 ± 0.1 and a °Brix of 72 ± 1 were reached in all samples increasing the citric acid content and prolonging (where necessary) the cooking time, respectively.

pH and °Brix were also monitored throughout the entire storage time (data not showed) showing significant but slight changes and remaining in the desired range.

3.1. Nutritional label information

Pillar of the study was the development of a fruit filling formulation with an improved nutritional profile in terms of lower sugar and higher dietary fibre contents, if compared with a standard fruit filling formulation. The nutritional labels of the different fruit filling recipes (FS and RSs) are reported in Table 2.

Table 2. Nutritional label based on Reg EU 1169/2011 of fruit fillings at different sucrose content (g/100g). FS: full sugar; RS30: 30% sugar reduction; RS50: 50% sugar reduction; RS70: 70% sugar reduction.

	FS	RS30	RS50	RS70
Energy (kJ)	964	831	724	620
Energy (kcal)	230	198	173	148
Fat	0	0	0	0
-of which saturated	0	0	0	0
Carbohydrates	57.0	41	30.5	20
-of which sugars	55.8	39	27.5	17
Fibre	1	15	22.5	30
Protein	0	0	0.6	0.9
Salt	0	0	0	0

Energy decreased remarkably with the increase of the semi-solid fibre syrup moving from 964 KJ/230 Kcal of standard formulation (FS) to 831 KJ/198 Kcal, 724 KJ/173 Kcal and 620 KJ/148 Kcal of RS30, RS50 and RS70, respectively. The decrease of the energy was associated to the decrease of the carbohydrates in favour of the dietary fibres which have a lower energy conversion factor (Regulation (EU) No 1169/2011). Moreover, RS fillings presented a sugar content reduced by 30% (RS30, from 55.8 g/100 g to 39 g/100 g), 50% (RS50, from 55.8 g/100 g to 27.5 g/100 g) and 70% (RS70, from 55.8 g/100 g to 17 g/100) if compared with FS. The increase of the semi-solid fibre syrup in the recipe led to an increase in the fibre content from 1 g/100 g of FS to 15 g/100 g, 22.5 g/100 g and 30 g/100 g for RS30,



RS50 and RS70, respectively. The decrease of sugar and the increase of fibre contents would allow to label all the RS fruit fillings with the double nutritional claims “reduced in sugar” and “high in fibre” on the basis of EU regulation on nutritional and health claims (Regulation (EC) No 1924/2006).

3.2. Moisture content and water activity

Product stability at rheological, chemical and microbiological level is strongly related to water activity (a_w) and moisture content (MC) values. Variations for both parameters during storage for all samples are reported in Table 3.

Table 3. Moisture content (MC), water activity (a_w), of fruit filling at different sugar ratio during storage at different temperature.

		Water activity (a_w)			
		t0	t30	t60	t 180
5 °C	FS	0.800 ±0.010 dA	0.801 ±0.014 dA	0.805 ±0.007 dA	0.794 ±0.004 dA
	RS30	0.836 ±0.007 cAB	0.836 ±0.009 cAB	0.842 ±0.004 cA	0.832 ±0.003 cB
	RS50	0.861 ±0.005 bA	0.858 ±0.005 bA	0.864 ±0.005 bA	0.857 ±0.008 bA
	RS70	0.874 ±0.003 aA	0.874 ±0.002 aA	0.876 ±0.003 aA	0.866 ±0.003 aB
25° C	FS	0.800 ±0.010 dA	0.798 ±0.009 dA	0.804 ±0.010 dA	0.784 ±0.004 dB
	RS30	0.836 ±0.00 cA	0.833 ±0.009 cA	0.839 ±0.003 cA	0.824 ±0.006 cB
	RS50	0.861 ±0.005 bA	0.857 ±0.004 bA	0.858 ±0.010 bA	0.846 ±0.010 bB
	RS70	0.874 ±0.003 aA	0.871 ±0.004 aA	0.875 ±0.003 aA	0.865 ±0.002 aB
		Moisture content (MC) g H ₂ O/ 100 g of sample			
		t0	t30	t60	t180
5 °C	FS	20.50 ±0.02 bB	22.50 ±0.01 bA	23.50 ±0.01 bA*	22.83 ±0.01 bA
	RS30	22.67 ±0.01 aB	23.83 ±0.01 abA	24.17±0.01 abA*	23.83 ±0.01 abA
	RS50	22.50±0.01 aB	24.50±0.02 aA	25.00±0.02 aA	24.67±0.02 aA
	RS70	23.00±0.0 aB	23.67±0.01 abB	25.00±0.01 aA	23.67±0.01 abB
25° C	FS	20.50 ±0.02 bB	22.00 ±0.01 bA	22.83 ±0.01 bA*	22.33 ±0.01 bA
	RS30	22.67 ±0.01 aB	23.33 ±0.01 aAB	23.67 ±0.01 bA*	23.83 ±0.01 aA
	RS50	22.50±0.01 aB	24.33 ±0.01 aA	24.67 ±0.01 aA	24.00 ±0.01 aA
	RS70	23.00±0.01 aB	23.67±0.01 aB	24.67±0.01 aA	23.83±0.01 aAB

All the data are expressed as mean ± standard deviations; different letters close to number indicate significative difference among sample ($p \leq 0.05$), where the small letters due to the sugar content, capital letter due to the time of storage and * due to temperature of storage.



Additionally, the evaluation of the effect of all single factor and their interactions in the variability of each parameter is reported in Table 4.

Table 4. Partition of the total variance of sum square (SS%) of every single factor (R: recipe, St: storage time; ST: storage temperature) and their interactions for every parameter studied.

	a_w	MC	Hardness	Adhesiveness	n	K	L^*	a^*	b^*	Bake stability
R	96.6	45.6	48.5	63.4	17.8	78.7	25.9	29.5	30.7	2.9
St	2.5	45.7	39.4	21.5	37.8	9.7	64.6	57.5	55.6	85.0
ST	0.3	1.3	2.4	5.0	11.1	4.9	1.6	0.2	4.4	2.2
R*St	0.1	5.8	5.0	4.7	15.5	1.0	5.7	2.5	5.3	5.1
R*ST	0.1	0.4	0.3	0.3	0	0.3	0.2	0.4	0.4	1.6
St*ST	0.2	0.6	4.0	4.8	15.6	4.5	1.7	8.8	3.3	1.1
R*St*ST	0.2	0.6	0.4	0.3	2.2	0.9	0.3	1.1	0.3	2.1

a_w was highly affected by R showing an increase with the decrease of sugar moving from ≈ 0.8 in FS to ≈ 0.9 in RS70. Similar trends were noticed at all storage temperatures and times. Similarly, MC was affected by recipe (R) increasing with the decreasing sugar in the recipe (from ≈ 20 g water/ 100 g sample in FS to ≈ 23 g water/100 sample in RS70 at t_0). These results were related to the water content (≈ 25 g water /100 g sample) of the semi-solid fibre syrup used to partially replace sugar and/or to its different interaction with water if compared to those sugar develops. Indeed, sugar is highly hygroscopic and has higher capacity than fibre to bind water thanks to the higher amount of available free hydroxyl group compared to long chain polysaccharides. The increase of a_w and MC when sugar is replaced by vegetable fibres was previously reported in studies conducted on cakes, muffin and fruit jellies (Milner et al., 2020; Riedel et al., 2015; Zahn et al., 2013). The increase of both parameters does not affect product shelf life because the product is acid (pH ~ 3.6) and it is stabilised with a hot filling process. MC was affected also by St which slightly fluctuate during storage probably due to a macroscopic water redistribution in the product. The storage temperature did not markedly affect neither the moisture content and the water activity with only a slightly significant difference for FS and RS30 at t_{60} .

3.3. Texture

Fruit fillings rheological properties were assessed using both empirical and fundamental techniques in order to verify agreement between the two analytical approaches providing useful information to those food industries able to implement only cheaper and easier rheological methods. Fruit fillings' hardness and adhesiveness parameters (Figure 1) allowed to investigate the mouthfeel of the product which is an important quality characteristic of semi-solid foods as fruit filling. These food materials indeed shall not be chewed and their texture features are directly perceived in the mouth by tongue receptors (Agudelo et al., 2015a).

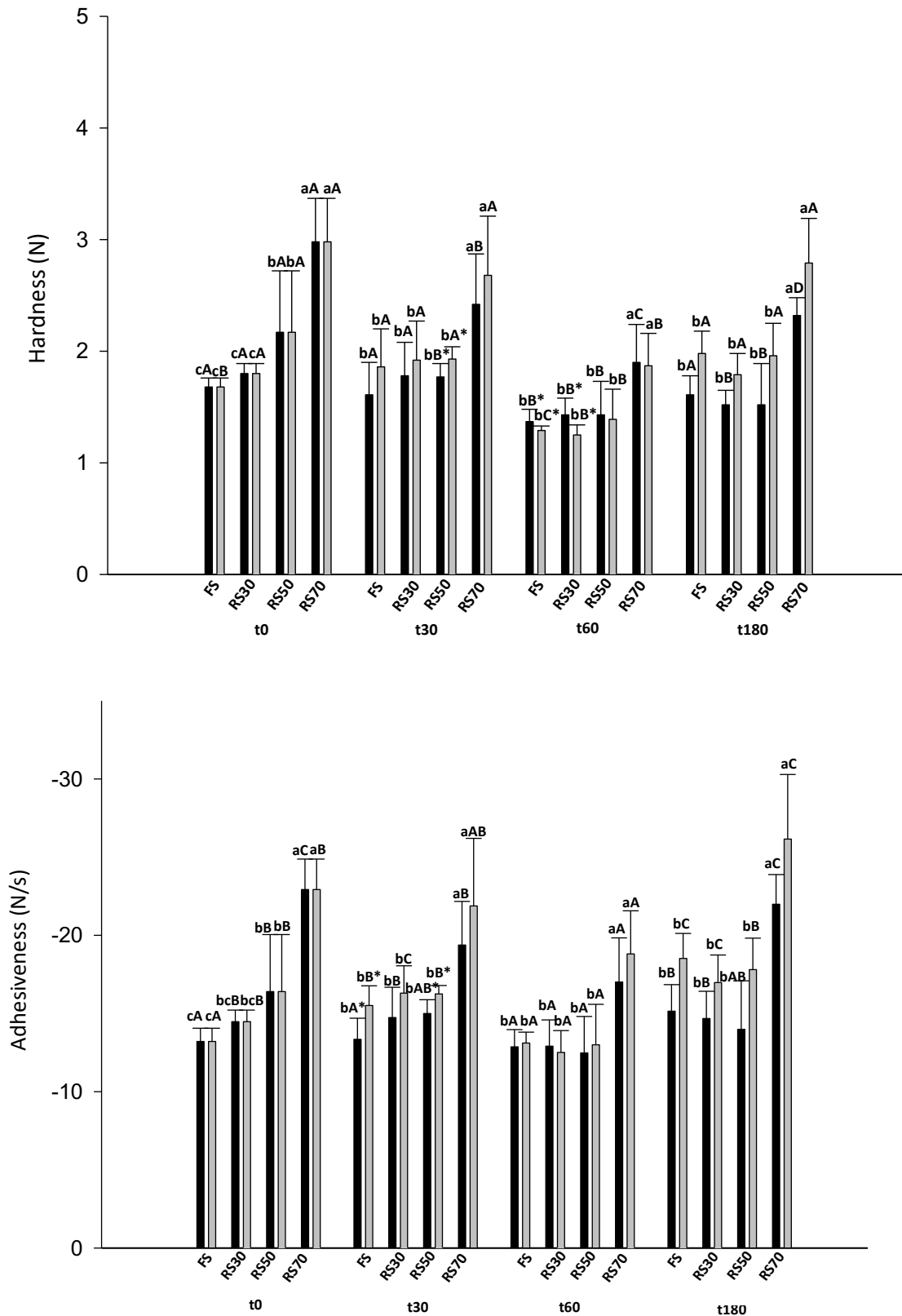


Figure 1. Hardness and adhesiveness of fruit fillings at variable sucrose content during storage at different temperatures (black: 5°C, grey: 25°C). Different letters close to number indicate significant difference among sample ($p \leq 0.05$) where the small letters due to the sugar content, capital letter due to storage time and * symbol due to storage temperature.



Three-way ANOVA (Table 4) highlighted that hardness and adhesiveness were primarily affected by R with a significant increase of both parameters with the decrease of sugar in the recipes. The increase of hardness and adhesiveness was likely affected by the increase of the semi-solid fibre syrup which bulking effect strongly impacted on the overall structure. This phenomenon is probably due to the interactions among the polysaccharide long chains present in the syrup which probably affected the macrostructure. At t30, t60 and t180, FS, RS30 and RS50 had comparable hardness and adhesiveness while RS70 was harder and more adhesive than all other samples.

Flow properties play a fundamental role in the quality characteristics of fruit filling. Its fluidity and structure may modulate the flavour perception in the mouth and therefore influence consumer acceptability; moreover, pumping and baking phase at industrial level may be strongly affected by a change in system fluidity (Agudelo et al., 2015a; Razak et al., 2018; Wei et al., 2001). Flow curves fitting allowed to calculate the non-Newtonian index (n , data not showed) and the consistency coefficient (K, Table 4, Figure 2).

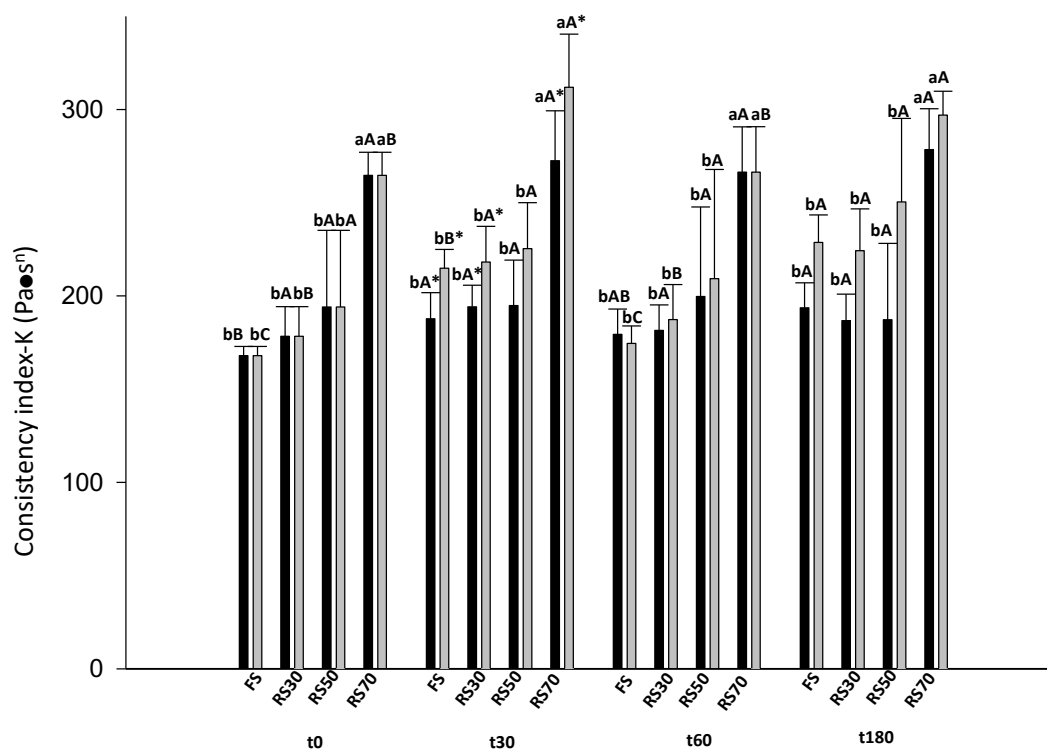


Figure 2. Consistency index of fruit fillings at variable sucrose content during storage at different temperatures (black: 5°C, grey: 25°C). Different letters close to number indicate significant difference among sample ($p \leq 0.05$) where the small letters due to the sugar content, capital letter due to storage time and * symbol due to storage temperature.

n index of all fruit fillings fell between 0.33-0.40, characterising the product as pseudo plastic fluid ($n < 1$), as previously reported in fruit filling by Wei and co-workers and Nalawade and colleagues (Wei et al., 2001, Nalawade et al., 2017). n index was more affected by St with only slight significant variation during storage time (Table 4). Consistency values K were instead strongly influenced by R showing a significantly increase with the reduction of sugar. A significantly higher K value at all times and temperatures of storage was observed for RS70 compared to all other samples. A good positive relation



($r^2 = 0.96$) was found between hardness and K, probing accordance between empirical and fundamental rheological analysis. Differently to polydextrose (E1200) which is synthetically produced for sugar reduction purpose (Schirmer et al., 2012) and its use marginally impacted on the rheological properties of fruit filling, as observed by Agudelo and co-workers (Agudelo et al., 2015a), the semi-solid fibre syrup used in this work increased macroscopic hardness and consistency coefficient K when used to reduce sugar above 30%.

3.4. Colour

Texture and taste are not the only key quality properties of this type of food product, since consumer acceptability is also strongly influenced by the colour which is one of the most important appearance factors, influencing also the flavour perception and the overall purchase decision (Pathare et al., 2013). For this reason, all fruit fillings were analysed for their colour properties and relative results are reported in Table 5.



Table 5. Color values (L^* , a^* , b^* , ΔE) of fruit fillings at different sugar ratio during storage (t0, t30, t60, t180) at different temperatures (5°C, 25°C).

	t0		t30				t60				t180			
		ΔE	5°C		25°C		5°C		25°C		5°C		25°C	
FS	L^*	35.42 ± 1.70 aA	33.16 ± 1.27aB	33.71 ± 1.89aB	30.71 ± 1.35aC	30.58 ± 1.39aC	30.51 ± 1.16aC	28.48 ± 1.01abD						
	a^*	10.71 ± 1.28aA	8.44 ± 1.28aB	9.19 ± 1.91aB	6.53 ± 1.24aC	5.70 ± 1.53aC	6.36 ± 1.14aC*	4.87 ± 0.92aC*	-	-	-	-	-	-
	b^*	15.00 ± 2.40aA	11.80 ± 1.83aB	13.35 ± 2.72aB	8.50 ± 1.39aC	8.03 ± 1.85aC	8.27 ± 1.56aC	5.42 ± 0.99aD						
	ΔE	-	4.5	2.8	9.1	9.8	9.4	13.2						
RS30	L^*	32.40 ± 1.50bA	30.98 ± 0.42bB	30.86 ± 0.75bB	29.57 ± 0.78bC	29.43 ± 1.10bC	29.52 ± 1.24bC	27.89 ± 1.05abD						
	a^*	9.18 ± 1.78bA	7.66 ± 0.80bB	8.51 ± 1.59bA	5.25 ± 1.32bC	5.16 ± 1.94abB	6.88 ± 1.25aD*	4.10 ± 1.05aB*	5.3	3.9	2.18	1.25	1.6	
	b^*	10.85 ± 2.02bA	8.71 ± 0.76bB	9.03 ± 1.34bB	7.16 ± 0.95bC	6.03 ± 1.50bC	7.70 ± 1.47aC	4.10 ± 0.79bD						
	ΔE	-	3.0	2.5	6.1	6.9	4.8	9.6						
RS50	L^*	31.76 ± 1.01bA	29.70 ± 0.54cB	29.71 ± 0.52cB	28.97 ± 0.4cC*	28.28 ± 0.37cC*	28.28 ± 0.85cD	27.74 ± 1.14bC						
	a^*	8.56 ± 1.36bA	6.07 ± 0.90cB*	6.76 ± 0.71cB*	4.10 ± 0.62cC	4.35 ± 1.15bcC	4.78 ± 0.95bC*	4.15 ± 1.59aC*	6.8	6.3	4.0	3.77	1.7	
	b^*	9.65 ± 1.26bA	7.12 ± 0.91cB	7.10 ± 0.58cB	5.82 ± 0.64cC*	5.34 ± 0.48cC*	5.66 ± 1.04bC	4.11 ± 1.01bD						
	ΔE	-	4.1	3.7	6.5	7.0	6.5	8.1						
RS70	L^*	30.75 ± 1.12cA	30.26 ± 1.0cA*	29.53 ± 0.4cB*	28.52 ± 0.3cB*	28.17 ± 0.40cC*	28.42 ± 0.92cB	28.90 ± 2.2aBC						
	a^*	6.91 ± 1.00cA	5.51 ± 0.90cB	5.92 ± 0.57cB	3.74 ± 1.12cC	3.45 ± 0.39cC	4.13 ± 0.75bC*	2.50 ± 0.88bD*	9.2	6.1	4.7	4.38	3.4	
	b^*	8.00 ± 0.69cA	7.25 ± 1.26cB	6.68 ± 0.53cB	5.42 ± 0.73cC*	4.60 ± 0.41cC	5.13 ± 0.78bC	3.06 ± 0.81cD						
	ΔE	-	1.7	2.0	4.7	5.5	4.6	6.9						

All the data are expressed as mean ± standard deviations; different letters close to number indicate significative difference among samples ($p \leq 0.05$), where the small letters due to the sugar content, capital letter due to the time of storage and * due to temperature of storage. In horizontal are present ΔE associated to the reformulation while in horizontal ΔE associated to the storage time.



Colour values were most affected by the St (Table 4). Indeed, in all samples (more pronounced in FS) a significantly decreased of all colour parameters was noticed at both temperature of storage. A decrease of L^* , a^* and b^* during storage was observed also in previous studies on apricot (Touati et al., 2014) and strawberry jam (Wicklund et al., 2005). The darkening of jam products during storage has been associated to the fruit pigment degradation which occurs during thermal and mechanical production steps and the consequently formation of anthocyanidins from anthocyanins (the glycosylated form) which are less stable to light and oxygen and consequently more prone to browning reactions.

Colour was less affected by R (Table 4), at t0 a significative decrease of all parameters was noticed with the increase of the semi-solid fibre syrup due to its intrinsic brownish colour. The decrease of colour values caused both by time of storage and recipe was also highlighted by the increase of colour differences (ΔE). However, it is important to remark that all the formulations studied did not contain any food colouring ingredients which could be included in the formulation in the industrial scale up in order to stabilise the product during storage and to counterbalance the presence of the semi-solid fibre syrup.

3.5. Water syneresis and bake stability

Fundamental characteristic of a fruit filling is its stability at high temperature during baking (Agudelo et al., 2014; Young et al., 2003) and the absence of water syneresis which could lead to a migration of water from the fruit filling to the dough having a negative impact on the overall quality of the product that will be inhomogeneous and sticky (Cropotova et al., 2016). All different formulations prepared did not present water syneresis during storage in all times and temperature conditions considered in this study. The absence of water migration also in the RS products in which the moisture content and water activity were higher than in FS indicates that the semi-solid fibre syrup had a positive technological function avoiding water separation and acting as stabilising ingredient.

Preservation of the dimension during baking is an important trait of a fruit filling, indeed shall not modify its volume and shape to not alter the quality of the filled bakery product. Results (Table 6) indicated that bake stability was more affected by St showing a decrease in the values at both temperatures during storage in all samples.



Table 6. Bake stability of fruit filling at different sugar ratio during storage at different temperature.

		Bake stability (%)			
		t0	t30	t60	t 180
5 °C	FS	77.75 ± 0.10 bA	69.75 ± 0.12 aB	69.13 ± 0.05 aB	62.38 ± 0.09 aC
	RS30	81.58 ± 0.05 aA	75.54 ± 0.13 aB	68.63 ± 0.07 aC	60.71 ± 0.09 aD
	RS50	81.13 ± 0.05 aA	74.04 ± 0.10 aB	71.29 ± 0.10 aB*	58.46 ± 0.08 aC
	RS70	85.04 ± 0.05 aA	76.04 ± 0.10 aB	69.67 ± 0.09 aC	60.21 ± 0.07 aD
25° C	FS	77.75 ± 0.10 bA	74.67 ± 0.11 aA	69.08 ± 0.08 aB	65.33 ± 0.10 aB
	RS30	81.58 ± 0.05 abA	76.71 ± 0.07 aB	69.13 ± 0.08 aC	63.46 ± 0.09 aD
	RS50	81.13 ± 0.05 aA	76.25 ± 0.04 aB	65.29 ± 0.05 aB	56.54 ± 0.08 aB
	RS70	85.04 ± 0.05 aA	72.04 ± 0.08 aB	65.17 ± 0.08 aC	62.13 ± 0.09 aC

All the data are expressed as mean ± standard deviations; different letters close to number indicate significant difference among samples ($p \leq 0.05$), where the small letters due to the sugar content, capital letter due to storage time and * due to storage temperature.

Moreover, only at t0, formulations containing the semi-solid fibre syrup showed a significantly increase of their bake stability index if compared with FS highlighting an improvement of this quality indicator in the fresh product.



3.6. Sensory analysis

Results of the sensorial analysis of the products are reported in Figure 3.

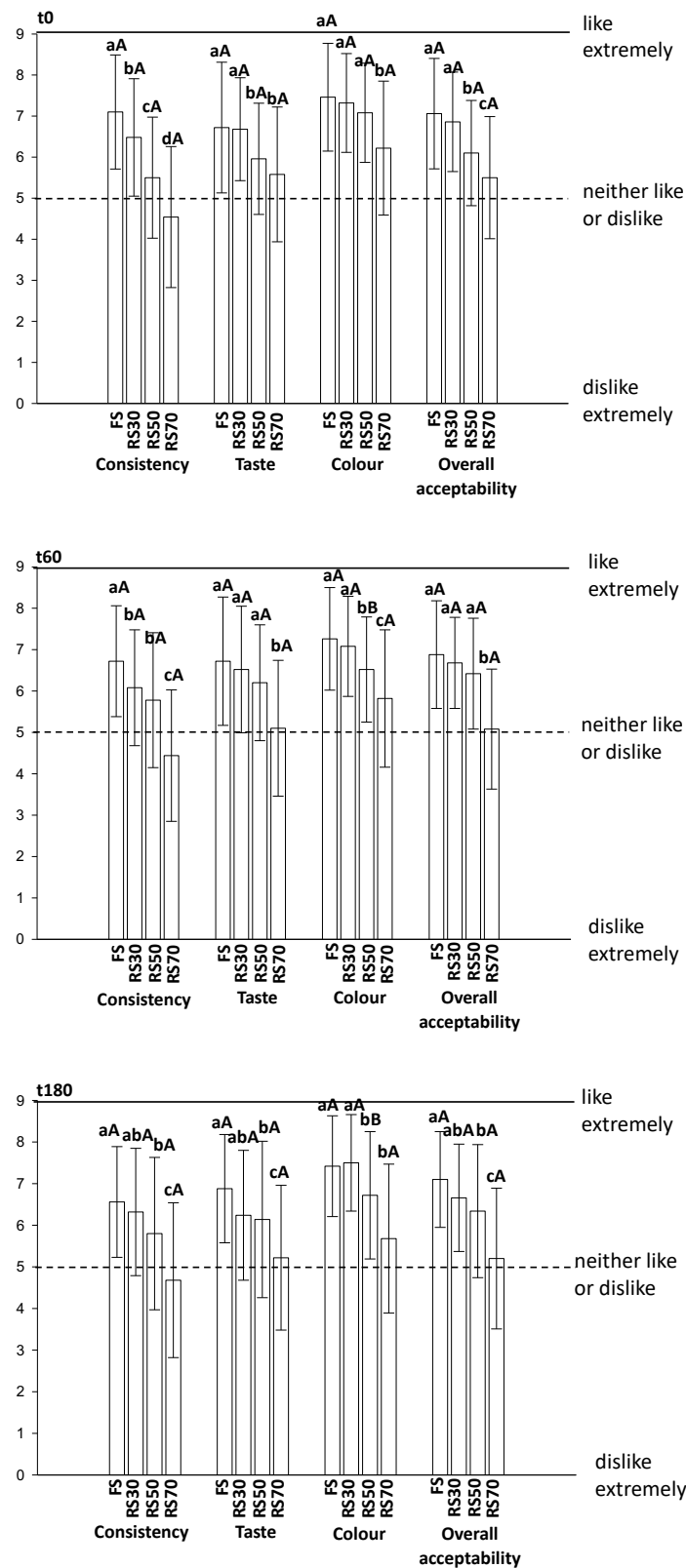


Figure 3. Sensory scores for consistency, taste, colour and overall acceptability of fruit filling at variable sucrose content. Different letters close to number indicate significant difference among sample ($p \leq 0.05$) where the small letters due to the sugar content, capital letter due to storage time.



All the fruit fillings were considered acceptable by the consumers (overall acceptability scores higher than 5 in all cases) with FS and RS30 considered the most preferred (≈ 7 the average score). Indeed, RS30 showed the highest and comparable scores with FS for all attributes. The lowest scores were attributed to RS70, in particular for consistency which was evaluated with a score below 5 indicating a worsening of the fruit filling structure probably due to its higher hardness, adhesiveness and K compared to the other formulations. No significative differences able to modify the sensorial results were noticed during storage. These findings are encouraging considering that the stability of sensory properties of the product for long lasting shelf-life is a relevant product quality feature. A better elucidation of the consumers perception of the fruit fillings was assessed using a CATA test. The results obtained by CATA test were analysed using a correspondence analysis. “IDEAL” fruit filling and the different samples were graphically represented on a factor plane. In the factor plane representing the CATA test conducted at t0 (Figure 4), the two dimensions explained $\approx 93\%$ of the variance, in particular dimension 1 explaining ≈ 80 and dimension 2 explaining ≈ 13 .

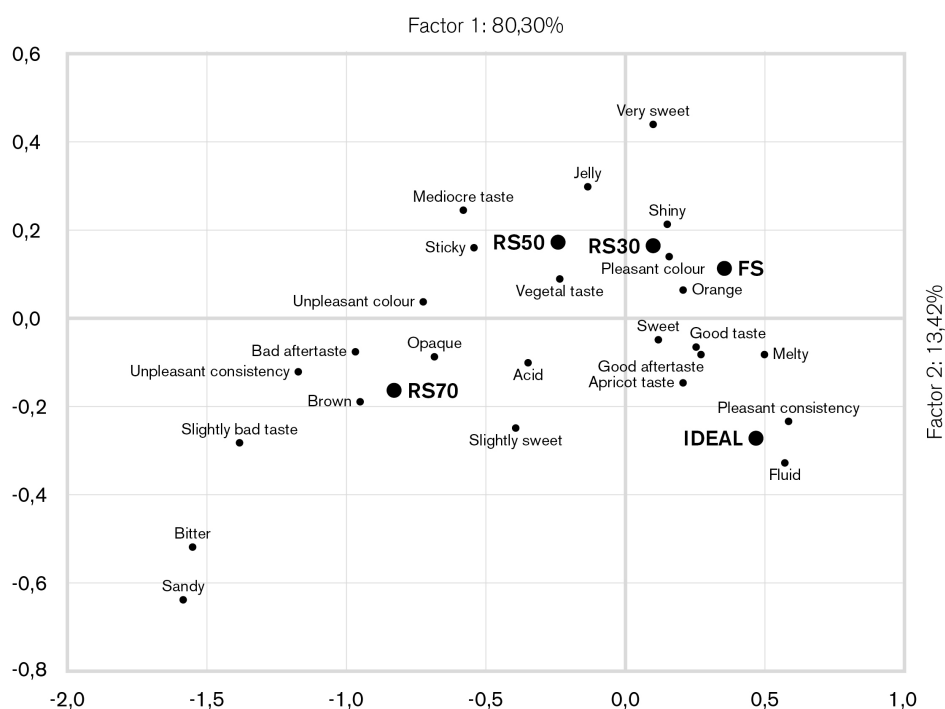


Figure 4. Correspondence analysis of the CATA test data of fruit filling prepared with different sucrose content.

“IDEAL” fruit filling was described with the following attributes: fluid, pleasant consistency, melty, sweet, apricot taste, good aftertaste, good taste. Similar attributes for an “IDEAL” fruit filling were observed in a study of Agudelo and co-workers (Agudelo et al., 2015b), in particular for the term “melty”, “sweet” and “fruity”. FS and RS30 had been described by similar attributes as pleasant colour, orange, shiny, very sweet. On the opposite, RS50 and RS70 were characterised by negative attributes as jelly, vegetal taste, mediocre taste, sticky and unpleasant colour for RS50, and opaque, acid, slightly sweet, bad aftertaste, unpleasant consistency, brown, bad taste, sandy and bitter for RS70. The consistency negative attributes



for RS50 and RS70 highlighted that the use of the semi-solid fibre syrup in high amount led to a detrimental of the product texture increasing the jelly perception that is the opposite of the fluid attribute indicated for the IDEAL product. Consumers' appreciation towards a fluid consistency for fruit filling was also highlighted in a consumer test on fruit filling performed by Agudelo and co-workers (Agudelo et al., 2015b) which observed that less consistent fruit fillings were the most appreciated. Overall, RS30 was the most appreciated reduced-sugar formulation on the basis of both acceptability and CATA test in particular for its texture properties. During storage no remarkably differences on samples attributes were noticed confirming the results of the acceptability test.

4. Conclusions

Sugar reduced" and "high in fibre" claims could be used to label reformulated fruit fillings by means of the use of a semi-solid syrup based on chickpea and maize fibre. The use of the semi-solid fibre syrup increased the water activity, moisture content as well as hardness, adhesiveness, K-consistency coefficient and bake stability in reformulated samples, while colour coordinates L^* , a^* , b^* decreased. The storage time mainly affected the colour and the bake stability of fruit fillings while the storage temperature presented a little or negligible effect on all studied parameters. When sugar was reduced by 30% the overall physicochemical and sensorial properties of the reformulated fillings resulted similar to the full-sugar counterpart. The use of the semi-solid-fibre syrup was therefore found to be a valuable ingredient on a technological standpoint, allowing to eliminate an E-number ingredient and obtain a clean-label product.

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Chapter 6

The reduction of sugar with a semi-solid fibre syrup: the ripple sauce case study

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Abstract

A consumer recognizable semi-solid fibre syrup was used as bulking agent in a sugar reduction reformulation strategy to satisfy the consumer needs of healthier and clean label products. A high sugar ripple sauce was used as standard to realize with the use of the above-mentioned syrup three sugar reduced recipes (SR30%, SR50%, SR70%). The technological functionality of the semi-solid fibre syrup was tested analysing the ripple sauces on their physicochemical, stability, rheological and sensorial attributes during 60 days of storage at two temperatures (5°C and 25°C). The water content carried by the semi-solid fibre syrup increase the water activity and the moisture content of the reformulated ripples but despite this no water synthesis phenomena occurred during storage. The industrial scalability was confirmed for the ripple sauces in which sugar was reduced by 30% as they were similar at rheological level to the full sugar counterpart also during storage. At colour level the reformulated ripples were similar to the standard thanks to the use of a colouring agent even if a darkening effect on ripples was noticed during storage. The consumer acceptability of ripples in which sugar was reduced by 30% furthermore confirm the possibility to use the semi-solid fibre syrup as sugar substitute high sugar products.

Keywords

clean label; sugar reduction; dietary fibre; semi-solid fibre syrup; ripple



1. Introduction

The development of new food products (able to incorporate the consumer needs) is an essential strategy for the food company to differentiate themselves and to obtain a competitive advantage in the actual complex global food market (Agudelo et al., 2015a).

Modern consumers are interested by different aspects of products. Indeed, the purchase of a food is no longer just driven by taste and price, but other aspects such as for example the nutritional quality, are nowadays of particular attractiveness for the consumer (Annunziata & Vecchio, 2011). One of the major trend is the attention on health concerns related to food consumption primarily due to the increase of knowledge of the strong connection between dietary habits and health (Annunziata & Vecchio, 2011; Kearney, 2010; Roberfroid, 2000). One of the main concern is surely associated to sugar overconsumption which is linked to obesity, metabolic syndrome and diabetes (Schwingshackl et al., 2017; Te Morenga et al., 2012; Yang et al., 2014). Contrast the epidemic spread of non-communicable disease associated to sugar overconsumption is indeed a pillar of the WHO guideline (World Health Organization, 2015) which recommend to reduce the sugar intake to less than 10% of the total energy intake. International institution and governments promoted awareness campaign to push consumers to decrease the sugar daily intake (Piernas et al., 2013). As a result, consumers showed great interest on reduced sugar products which become one of the top European sales trends, as evidenced by the 2020 market analysis presented at Food Ingredient Europe conference (FIE Global, 2020). Only in Italy, products with the claim “reduced sugar” increased their sales volume by 7.6% during 2019 (Osservatorio Immagino GS1 Nielsen, 2020).

In recent years, consumers have also showed a strong interest in the “naturalness” of the product purchasing products with a “clean label” and avoiding food additives (Battacchi et al., 2020). A specific definition of a “clean label” product does not exist so far, but it is associated with foods in which the consumer can find ingredients that can be considered healthy, familiar and easily accessible in their kitchen cupboard (Asioli et al., 2017). In Italy, foods with the claim “free from additives” had an increase of their sales of 2.7% during the 2019 (Osservatorio Immagino GS1 Nielsen, 2020).

Satisfying both consumer needs (reduced sugar and clean label) can be particularly successfully for companies. The use of “sugar substitute” ingredients is the most used strategies studied to reduce sugar, especially when a high amount of sugar needs to be replaced (Hutchings et al., 2019). Some products as jam, jellies, filling and ripple sauces typically may contain up to 60% of sugar (Di Monaco et al., 2018). Sugar imparts both the sweetness flavour to the product, which role can be also given by non-caloric sweetener, and a mass body which effect should be replicated through the use of bulking agent ingredients (Agudelo et al., 2015a). The bulking effect is difficult to replace with ingredients that are at the same time clean label. In confectionary products which have a high consumption level and in which high quantity of sugar shall be replaced, polyols (which are the most used sugar substitutes) cannot be used due to their laxative effect above 10% concentration (Riedel et al., 2015). Therefore, the most



common bulking agent ingredients studied are non-digestible carbohydrate such: inulin, polydextrose, fructooligosaccharide and modified starches (J. L. Buttriss, 2017; Di Monaco et al., 2018). Usually, confectionary foods are multicomponent products and the sugar derives from different sources. For example, in industrial ice cream, a high source of sugar is represented by the ripple sauces which are used as soft flavouring ingredients and for decoration purposes imparting a distinctive and unusual appearance to the product. (Goff & Hartel, 2005; Hui, 2006; Jiang & Lei, 2014; Kilara & Chandan, 2009). Ripple sauces are mainly made of sugar, water, fruit puree, a stabiliser (usually pectin), citric acid, colouring and flavouring agents (Hui, 2006; Pilgrim et al., 1991). The presence of a topping as the ripple sauce increases the sugar intake of the ice creams making them unhealthier (An & Jiang, 2017). To the authors' knowledge no studies for the sugar reduction of this product category have been performed so far.

Therefore, the aim of this study was the development of ripple sauces for ice cream with reduced sugar contents using a semi-solid fibre syrup (MELTEC®). Formulations obtained were characterized for their physicochemical properties and sensorial attributes during a storage period of 60 days at 5°C and 25°C.



2. Materials and Methods

2.1. Materials

MELTEC® was supplied by HI-FOOD S.p.A. (Parma, Italy). MELTEC® is a semi-solid fibre syrup based on corn/chickpea fibres and water; its consistency is similar to honey but the sugar present is below 1% and the moisture content is $\approx 25\%$ (g water/100 g sample). The other ingredients used for the preparation of the ripple sauces were: crystalline sucrose (British sugar, Peterborough, UK), crystalline fructose (Omnia Nisasta, Adana, Turkey), inverted sugar syrup (Lambrè, Bolzano, Italy), frozen strawberry puree (Rogelfrut, Cuneo, Italy), low-methoxyl pectin Grinsted® Pectin Prime (DuPont™, Melle, France), citric acid (Brenntag S.p.A., Milano, Italy), potassium sorbate (Giusto Faravelli, Milano, Italy), strawberry flavour “Strawberry Juicy Flavouring NATLQ11247” (International Taste Solution Ltd, Newbury, UK), food colour E129 “Allura Red AC85%” (Fiorio Colori, Milano, Italy).

2.2. Ripple sauces manufacturing

Full sugar (FS) and sugar reduced (SR) ripple sauces were prepared on the basis of an industrial recipe (Table 1).

Table 1. Ripple sauces recipes (% wt). FS: full sugar; SR30: sugar reduced of 30%; SR50: sugar reduced of 50%; SR70: sugar reduced of 70%

	FS	SR30	SR50	SR70
Sucrose	25.5	25.5	17.3	9.1
Fructose	9	9	6.3	3.2
Inverted sugar	25	-	-	-
MELTEC®	-	25	35.9	47.2
Water	15.3	15.2	15.18	15.13
Strawberry puree	24.5	24.5	24.5	24.5
L-M Pectin	0.4	0.4	0.4	0.4
Citric acid	0.16	0.26	0.28	0.33
Potassium sorbate	0.05	0.05	0.05	0.05
Strawberry flavour	0.07	0.07	0.07	0.07
Red colouring agent	0.02	0.02	0.02	0.02

SR ripple sauces were obtained replacing all inverted sugar and increasing level of crystalline fructose and sucrose with MELTEC® in a 1:1 ratio to reduce sugar of 30% (SR30), 50% (SR50) and 70% (SR70). All ripple sauces (FS and SRs) were manufactured using a bowl chopper (Polyfunctional QB 8-3, Roboqbo, Bologna, Italy). Firstly, a blender (Minipimer MQ5035, Braun, Germany) was used to dissolve pectin and crystalline sucrose in pre-heated water (80°C) at 1:4.5 ratio at 13500 rpm for 5 minutes. Only



half of the water present in the recipe was used for this step. Secondly, crystalline sucrose, crystalline fructose, inverted sugar, MELTEC® (SR recipes), strawberry puree, and the remaining water in which was dissolved potassium sorbate and citric acid were mixed in the bowl chopper at 500 rpm and heat treated at 70°C until reaching 66 °Brix. Thirdly, the sucrose:pectin solution was introduced in the bowl chopper and all the ingredients were mixed and heated at 500 rpm and 90°C under vacuum (1 bar). Once reached 67 °Brix and a 3.3 pH, strawberry flavour and E129 were added. As the last step, the obtained strawberry ripple was cooled under vacuum (1 bar) at 300 rpm until reaching the temperature of 50°C. All produced ripple sauces were then moved to a plastic container to be analysed after reaching the room temperature (t₀) or stocked at 25°C and 5°C for analysis after 30 and 60 days (t₃₀, t₆₀). Two batches of ripple sauces of each formulation were produced in two different days.

2.3. Ripple sauces characterisation

2.3.1. pH and °Brix

pH value was determined in triplicate at 25°C with a potenziometer pH7+ DHS Food (XS Instruments, Modena, Italy). °Brix was determined in triplicate at 25°C using a portable refractometer HB 95 (Lega Italy, Ravenna, Italy). Both analyses were also performed as control step during the manufacturing process.

2.3.2. Nutritional label

The macronutrients of FS and SR ripple sauces were calculated using the nutritional information reported in the technical data sheet of the ingredients. All macronutrients were multiplied for their energy factors [in specific: carbohydrate = 17 kJ (4 kcal); protein = 17 kJ, (4 kcal); fat = 37 kJ, (9 kcal); fibre = 8 kJ, (2 kcal)] to obtain the energy values (kJ, kcal) of all the samples.

2.3.3. Water activity and moisture content

Water activity was measured at 25 °C with an Aqualab 4 TE (Decagon Devices Inc. WA, USA). Moisture content (MC, g of water/100 g of sample) was measured by weight loss by drying in a forced-air oven (M120-TBR, MPM Instruments Srl, Milano, Italy) at 70 °C to constant weight. At least three determinations were performed for each sample for a total of six determinations.

2.3.4. Syneresis degree

Ripple sauces syneresis was analysed with the method described by Crototova and co-workers (Crototova et al., 2009). 5 g of sample (F₀) were placed into a 50 ml falcon tube, closed up with plastic cap and centrifugated at 3000 rpm for 20 min (centrifuge 5910R, Eppendorf, Milan, Italy). The separated liquid (F₁) was weighted and grade of syneresis was obtained as percentage (%) = (F₁/F₀). Three analysis were performed for each formulation for a total of six determinations.



2.3.5. Rheological properties

A stress-controlled rheometer (MCR 702 twin drive, Anton Paar, Graz, Austria) with 50 mm parallel plate was used to analyse the rheological properties of the ripple sauces at 25°C. Samples were placed between the plates with a gap of 1 mm, and paraffin oil was arranged on the exposed surface. After sample loading, sample went through a resting time until axial force reached about 0 N prior to start experiment to allow for temperature equilibration and dough relaxation.

Flow curves were acquired increasing shear rates from 1 to 100 s⁻¹ and fitted with the Power law equation:

$$\sigma = K\gamma^n$$

where σ is the Shear stress (Pa), γ is the shear rate (s⁻¹), K is the consistency coefficient (Pa·sⁿ) and n the flow behaviour index (dimensionless).

Flow curves were acquired in triplicate for each formulation for a total of six determinations.

2.3.6. Bostwick consistency

Bostwick consistency was measured using a Bostwick consistometer (LS100, Laboscintifica, Parma, Italy). The Bostwick consistometer chamber was filled with 100 ml of ripple sauce, and the distance (cm) travelled by the sample after the release of the chamber gate was recorded after 1 min (running distance). Three measurements were performed for each formulation for a total of six determinations.

2.3.7. Surface adhesiveness

Surface adhesiveness was determined using a TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with a P/40 probe. Cylindrical container (58 X 70 mm) were filled to the brim with the ripple sauces. The probe applied a compression force of 6 g at 1 mm/s on the surface of the sample with a holding time to 2 s, and then returned (8 mm/s) in the starting rest position. The texturogram obtained was analysed with a Texture Expert software (Stable Micro Systems, Godalming, UK) to determine the adhesiveness (peak force, N) as the maximum force required to separate the probe from the sample surface. Five replicates were taken for each formulation for a total of ten determinations.

2.3.8. Colour

The colour of different ripple sauces samples was measured using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65 and a 10° position of the standard observer. The results were expressed in accordance with the CIE Lab system. The parameters determined were: L^* [0 (black) - 100 (white)], a^* ($-a^*$ =greenness and $+a^*$ =redness) and b^* ($-b^*$ =blueness and $+b^*$ =yellowness). ΔE (Limbo & Piergiovanni, 2006) was obtained using FS as the



reference sample. At least ten determinations were performed for each formulation for a total of at least twenty determinations.

2.3.9. Sensorial analysis

Sensorial properties of ripple sauces were studied using an acceptability test and a rapid profiling method check-all-that-apply (CATA) on both fresh and stored sample (t0, t60). Ripple sauce samples were identified with a three-digit random number and introduced to 50 untrained judges. In the acceptability test a 9-points hedonic scale was used, (1=dislike extremely, 2=dislike very much, 3=dislike, 4=dislike slightly, 5=neither like nor dislike, 6=likes slightly, 7=like, 8=like very much and 9=like extremely). Judges were allowed to drink water between the samples to cleanse the palate. Overall, an ANOVA test was performed to evaluate significant differences of the scores among samples. In CATA, test judges had to indicate all the attributes applying in the ripple sauces and in the ideal version. The attributes, reported randomly in the questionnaire, were: pleasant colour, unpleasant colour, dull colour, bright colour, opaque, shiny, acid, bitter, sweet, very sweet, slightly sweet, good taste, bad taste, mediocre taste, vegetal taste, strawberry taste, good aftertaste, bad aftertaste, pleasant consistency, unpleasant consistency, sticky, fluid.

2.4. Statistical analysis

A three-way ANOVA test was carried out using three fixed factors: recipe (R), storage time (St) and storage temperature (ST). The study of each factor and their interactions in the variability of each parameter was the result of the partition of total variance of sum square (SS%). Significant differences ($p \leq 0.05$) among different samples were assessed by one-way-analysis of variance (ANOVA) with a Duncan post-hoc test using an IBM SPSS statistical software (Version 24.0, SPSS Inc., Armonk, New York, USA). The contingency table of CATA dataset was realised on the basis of samples and attributes. A correspondence analysis was performed to summarize the relationships between samples and attributes with the use of the software Statistica (Version.13.3, TIBCO Software Inc.).

3. Results

In order to be aligned with the industrial practice for this product category, pH and °Brix values of reduced sugar ripple sauces samples were checked during manufacturing in order to keep them equal to those of FS. Especially, a pH range of 3.3 ± 0.1 and a °Brix range of 67 ± 1 were established and reached in all recipes prolonging the cooking step or adding citric acid when necessary. Additionally, all samples were analysed for both parameters also during storage; all values obtained were in the established range (data not shown) and only significant but slight variations, were observed.



3.1. Nutritional label information

The nutritional label of the ripple sauces is reported in Table 2.

Table 2. Nutritional label on the basis of Reg EU 1169/2011 of ripple sauces. FS: full sugar; SR30: sugar reduced of 30%; SR50: sugar reduced of 50%; SR70: sugar reduced of 70%

Average for 100g	FS	SR30	SR50	SR70
Energy (kJ)	963	840	737	630
Energy (kcal)	230	201	176	151
Fat (g)	0	0	0	0
-of which saturated (g)	0	0	0	0
Carbohydrates (g)	57	40	30	20
-of which sugars (g)	57	39	28	17
Fibre (g)	0	17	24	31.5
Protein (g)	0	0	0	0
Salt (g)	0	0	0	0

The energy values of the reformulated ripple sauces diminished accordingly to the sugar reduction and the increase of the semi-solid fibre syrup. In details, they moved from 963 kJ/ 230 Kcal of the FS recipe to 840 kJ/201 kcal of SR30, 737 kJ/176 kcal of SR50 and 630 kJ/151 kcal of SR70. At macronutrient level, the sugar content moved from 57 g/ 100 of FS to 39 g/100 g, 28 g/100 g, and 17 g/100 g of SR30, SR50 and SR70, respectively. In other words, sugar was reduced by 30%, 50% and 70% (SR30, SR50 and SR70, respectively). Furthermore, the dietary fibre increased from 0 g/100 g of the FS recipe to 17 g/100 g, 24 g/100 g and 31.5 g/100 g of SR30, SR50 and SR70, respectively.



3.2. Moisture content, water activity and water syneresis

Water activity (a_w) and moisture content (MC) are reported in Table 3 while the effect of all single factors and their interactions in the variability of each parameter are presented in Table 4.

Table 3. Moisture content (MC, g water/100 g sample), water activity (a_w), n values of ripples at different sugar ratio during storage at different temperature. FS: full sugar; SR30: sugar reduced of 30%; SR50: sugar reduced of 50%; SR70: sugar reduced of 70%

		Water activity (a_w)		
		t0	t30	t60
5 °C	FS	0.803 ± 0.003 dB	0.804 ± 0.002 dB	0.807 ± 0.002 dA*
	SR30	0.862 ± 0.002 cA	0.861 ± 0.002 cA	0.862 ± 0.003 cA*
	SR50	0.881 ± 0.002 bA	0.877 ± 0.010 bA	0.880 ± 0.001 bA*
	SR70	0.906 ± 0.005 aA	0.905 ± 0.002 aA	0.907 ± 0.002 aA
25° C	FS	0.803 ± 0.003 dA	0.801 ± 0.003 dA	0.803 ± 0.002 dA*
	SR30	0.862 ± 0.002 cA	0.861 ± 0.003 cA	0.859 ± 0.002 cA*
	SR50	0.881 ± 0.002 bA	0.881 ± 0.001 bA	0.878 ± 0.001 bB*
	SR70	0.906 ± 0.005 aA	0.906 ± 0.003 aA	0.906 ± 0.003 aA
		Moisture content (MC) g H ₂ O/ 100 g sample		
		t0	t30	t60
5 °C	FS	26.02 ± 0.01 cC	28.13 ± 0.01 cB	29.50 ± 0.01 bA*
	SR30	27.58 ± 0.01 bC	29.38 ± 0.01 abB	30.17 ± 0.01 abA*
	SR50	27.92 ± 0.01 bB	29.13 ± 0.01 bcAB	30.17 ± 0.01 abA
	SR70	29.05 ± 0.01 aB	30.37 ± 0.01 aA	30.67 ± 0.01 aA
25° C	FS	26.02 ± 0.01 cB	27.54 ± 0.01 bA	28.33 ± 0.01 aA*
	SR30	27.58 ± 0.01 bB	29.25 ± 0.01 aA	29.00 ± 0.01 aA*
	SR50	27.92 ± 0.01 bA	29.60 ± 0.01 aA	29.83 ± 0.02 aA
	SR70	29.05 ± 0.01 aA	29.79 ± 0.02 aA	30.17 ± 0.01 aA

All the data are expressed as mean ± standard deviations; different letters close to number indicate significative differences among samples ($p \leq 0.05$), where the small letters due to the sugar content, capital letter due to the storage time and * due to storage temperature.

Table 4. Partition of the total variance of sum square (SS%) of each single factor (R: recipe, St: storage time; ST: storage temperature) and their interactions for each parameter studied.

	a_w	MC	n	K	Botswick Running distance	Surface adhesiveness	L*	a*	b*
R	84.2	15.2	85.1	63.2	82.1	17.1	11.4	5.5	5.6
St	1.4	66.2	3.0	3.4	2.1	64	55.7	71.1	70.7
ST	0.7	0.4	0	3.8	2.2	0	10.3	5.3	9.5
R*St	4.2	3.5	3.6	23.3	8.4	13.9	13.9	8.5	5.9
R*ST	2.3	2.6	3.3	1.9	1.4	1.1	0.2	0.5	0.4
St*ST	1.4	0.6	0.8	2.2	2.3	2.0	7.9	8.5	7.8
R*St*ST	5.8	11.5	4.2	2.2	1.4	1.9	0.5	0.6	0.2

a_w was highly affected by R as it moved from ≈ 0.8 of FS to ≈ 0.9 of SR70 at all times and temperatures of storage. Only significant but slight differences due to the temperature of storage were noticed for FS, SR30, SR50 at t60. MC was instead more affected by St with an increase during storage. It was also observed (especially at t0) the reformulation impact on the MC with an increase of its values with the



decrease of sugar. All the ripple sauces (FS and SRs) did not present any water syneresis at all studied storage conditions.

3.3. Colour

All colour parameters (L^* , a^* , b^*) were mainly affected by St with a darkening of ripple sauces during storage, in particular at 25°C (Table 4, Table 5). It was indeed observed a colour difference (ΔE) between 3 (slightly distinguishable) and 7.5 (more than distinguishable) for samples with the same sugar level during storage.



Table 5. Color values (L^* , a^* , b^* , ΔE) of ripple sauces at different sugar ratio during storage at different times and temperatures.

		t0			t30			t60			
				ΔE			ΔE			ΔE	
				5°C		25°C	ΔE	5°C		25°C	ΔE
FS	L^*	26.83 ± 1.87 aA		25.69 ± 0.21 bB		25.62 ± 0.21 aB		26.38 ± 0.98 aAB*		25.52 ± 0.10 aB*	
	a^*	11.77 ± 3.19 aA	-	5.92 ± 0.59 aB	-	6.06 ± 0.49 aB	-	6.98 ± 2.75 aB*	-	4.60 ± 0.26 aC*	-
	b^*	3.58 ± 1.15 aA		2.28 ± 0.23 aB		2.19 ± 0.27 aB		2.22 ± 0.58 aB*		1.67 ± 0.11 aC*	
	ΔE	-		6.1		6		5.0		7.5	
SR30	L^*	26.98 ± 1.11 aA		25.83 ± 0.23 aB		25.69 ± 0.21 aB		26.26 ± 0.61 aB*		25.49 ± 0.06 aB*	
	a^*	10.88 ± 2.47 aA	0.9	5.68 ± 0.58 aB	0.3	5.67 ± 1.08 aB	0.4	6.97 ± 2.03 aB*	0.1	4.32 ± 0.33 bC*	0.3
	b^*	3.34 ± 0.89 abA		2.22 ± 0.21 aB*		2.00 ± 0.36 bB*		2.30 ± 0.45 aB*		1.58 ± 0.12 bC*	
	ΔE	-		5.4		5.5		4.1		6.7	
SR50	L^*	26.04 ± 1.13 aA		25.67 ± 0.14 bA		25.57 ± 0.17 aAB		26.13 ± 0.89 aA*		25.49 ± 0.07 aB*	
	a^*	10.16 ± 2.11 aA	1.8	5.22 ± 0.30 bC	0.7	5.08 ± 0.35 bB	1.0	6.67 ± 2.00 aB*	0.4	3.96 ± 0.19 cC*	0.7
	b^*	3.36 ± 0.68 abA		2.07 ± 0.14 bB*		1.87 ± 0.17 bcB*		2.37 ± 0.50 aB*		1.45 ± 0.06 cC*	
	ΔE	-		5.1		5.3		3.6		6.5	
SR70	L^*	26.00 ± 1.01 aA		25.73 ± 0.12 abA*		25.58 ± 0.12 aB*		25.95 ± 0.60 aA*		25.45 ± 0.09 aB*	
	a^*	8.32 ± 1.37 bA	3.7	5.19 ± 0.39 bB*	1.5	4.70 ± 0.21 bB*	1.5	6.04 ± 1.76 aB*	1.1	3.77 ± 0.14 dC*	0.9
	b^*	2.73 ± 0.56 bA		1.99 ± 0.15 bB*		1.70 ± 0.09 cB*		1.98 ± 0.36 aB*		1.42 ± 0.06 cC*	
	ΔE	-		3.2		3.8		2.4		4.7	

All the data are expressed as mean ± standard deviations; different letters close to number indicate significative differences among samples ($p \leq 0.05$), where the small letters due to the sugar content, capital letter due to the storage time and * due to storage temperature. In horizontal are present ΔE associated to the reformulation while in horizontal ΔE associated to the storage time



3.4. Ripple sauce rheology

Non-newtonian index (n) was mainly affected by R with a slight but significant increase with the sugar reduction (from ≈ 0.4 of FS to ≈ 0.5 of SR70, data non showed) at all storage times and temperatures. Consistency coefficient (K) values were more affected by R and the interaction R*St (Table 4). Indeed, the reduction of sugar from 50% to 70% (SR50 and SR70) significantly increased K (Figure 1) starting from 30 days of storage (t_{30}), while for FS and SR30 no differences were found during the entire storage time.

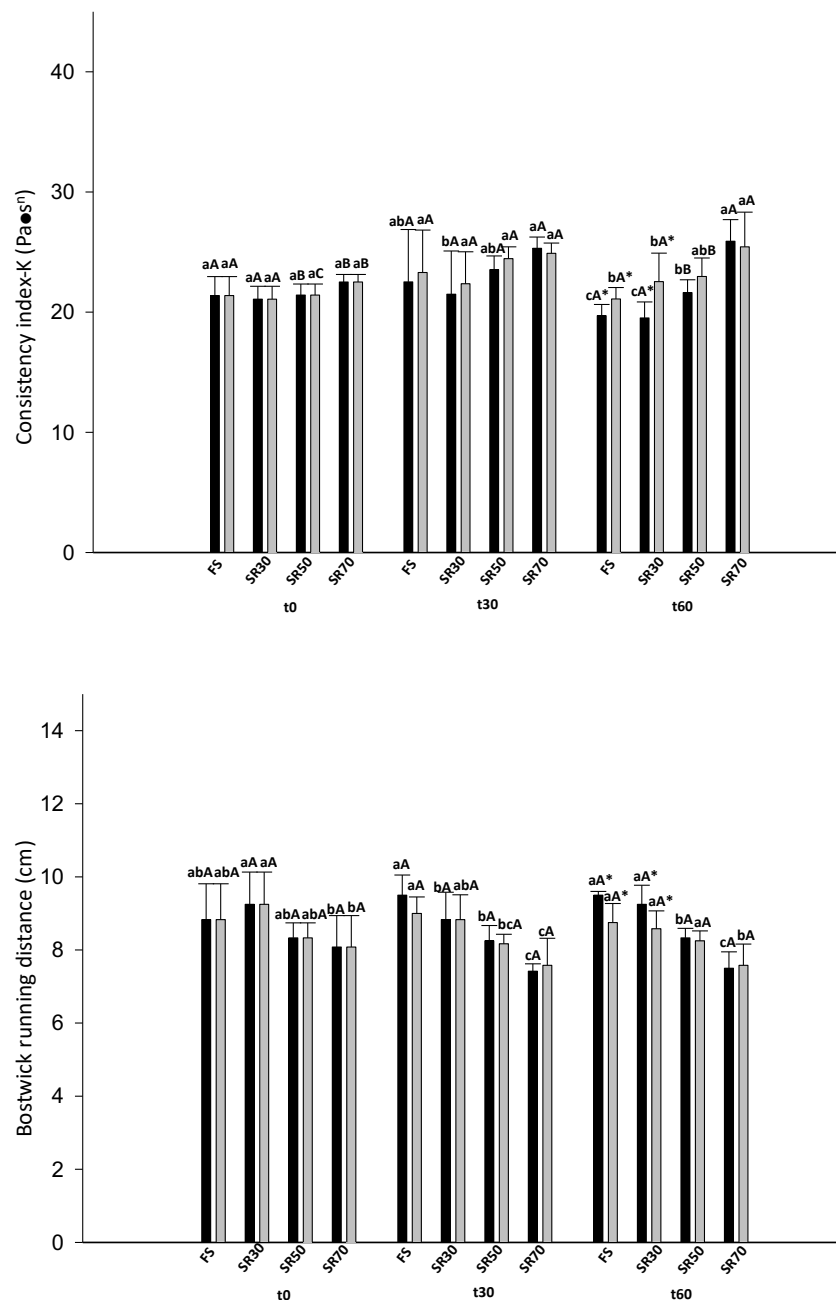


Figure 1. Consistency index and Bostwick running distance of ripple sauces at different sugar content during storage at different temperatures (black: 5°C, grey: 25°C). Different letters close to number indicate significant differences among samples ($p \leq 0.05$) where the small letters due to the sugar content, capital letter due to the storage time and * due to storage temperature.



Only slight significant differences were noticed for FS and SR30 due to the temperature of storage. A decrease in the Bostwick running distance (Figure 1, Table 4) at all times and temperatures of storage was found with the sugar reduction. Surface adhesiveness (Figure 2, Table 4) was highly affected by St decreasing during storage for both storage temperatures; conversely, an increase of the surface adhesiveness with sugar reduction at all times and temperatures of storage was also found. The temperature of storage had only a little impact on the surface adhesiveness with only slight significant variations.

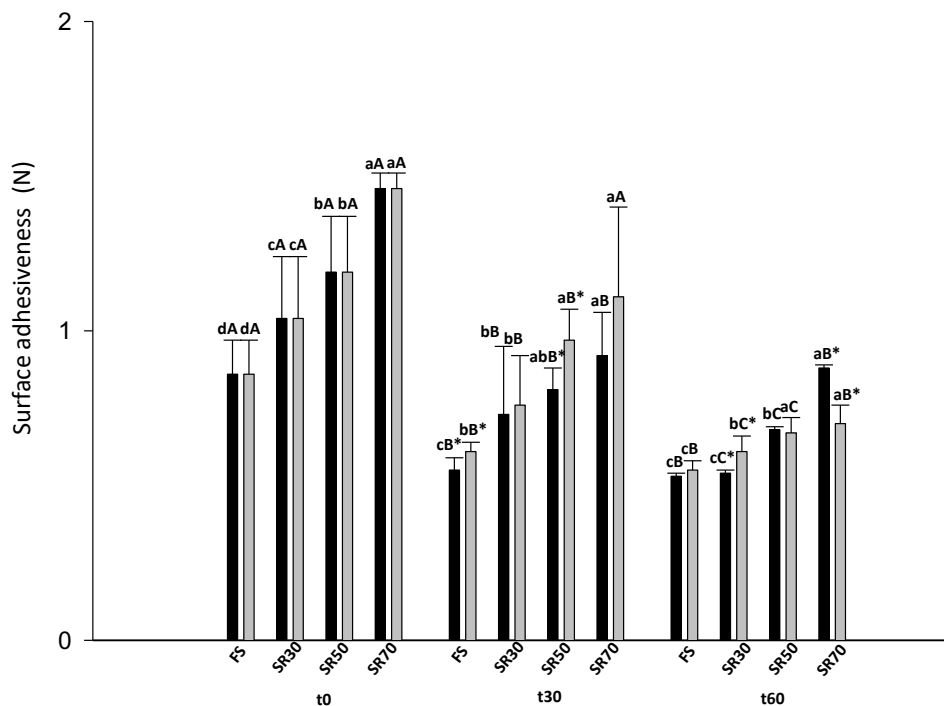


Figure 2. Surface adhesiveness of ripple sauces at different sugar content during storage at different temperatures (black: 5°C, grey: 25°C). Different letters close to number indicate significant differences among samples ($p \leq 0.05$) where the small letters due to the sugar content, capital letter due to the storage time and * due to storage temperature.

3.5. Sensory analysis

The sensorial analysis results are described in Figure 3.

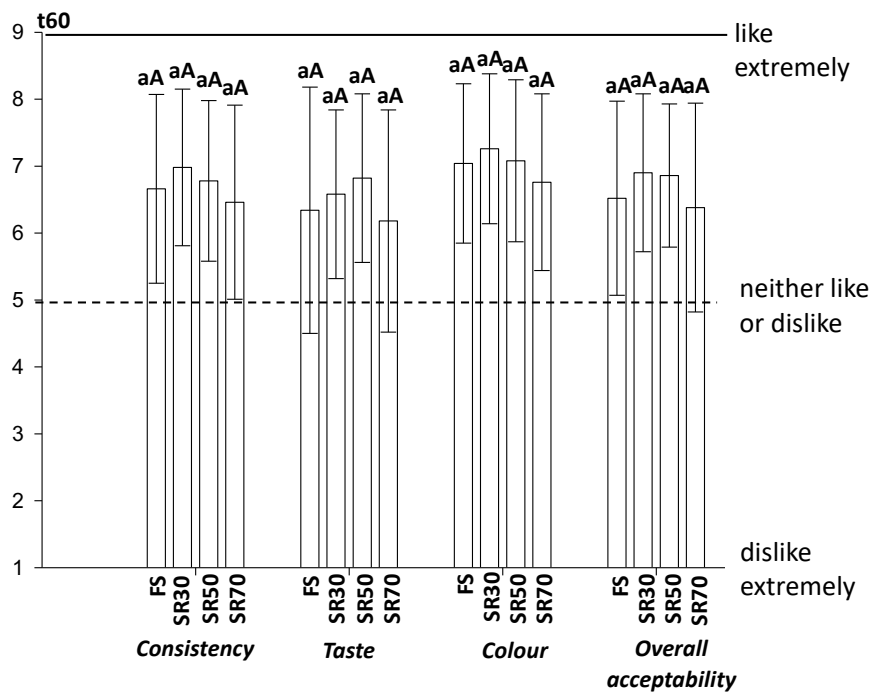
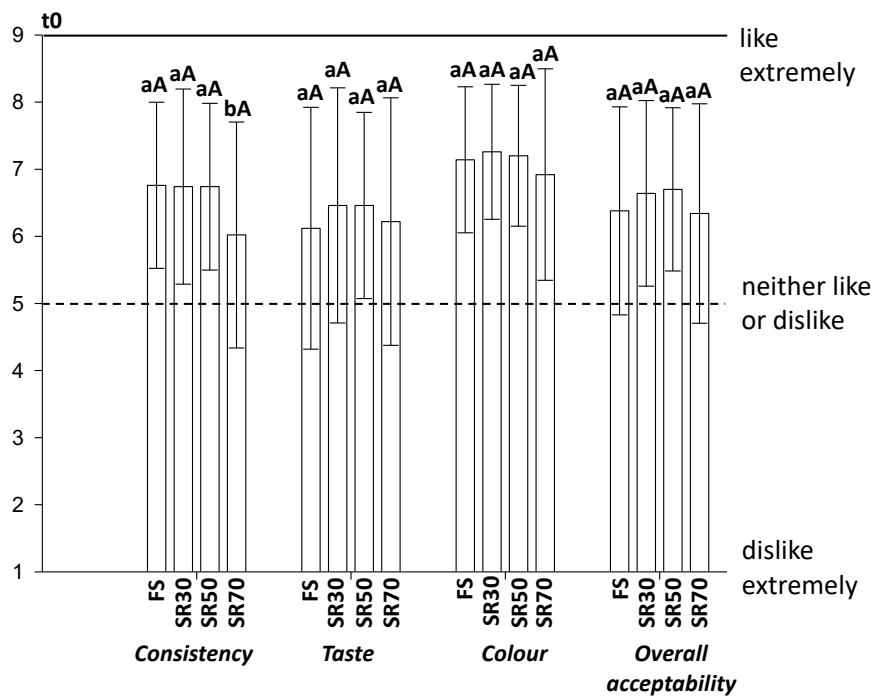


Figure 3 Sensory scores for consistency, taste, colour and overall acceptability of ripple sauces at different sugar content. Different letters close to number indicate significant difference among sample ($P \leq 0.05$) where the small letters due to the sugar content, capital letter due to storage time.



All the ripple sauces tested were appreciated by the consumer with comparable scores for different parameters tested (between 6 - likes slightly, and 7 - like). Only a significant lower score was observed for the consistency of SR70 at t0 compared to all the other samples. The CATA test results of the “IDEAL” and the ripple sauces samples are reported as a graphical representation on a factor plane (Figure 4).

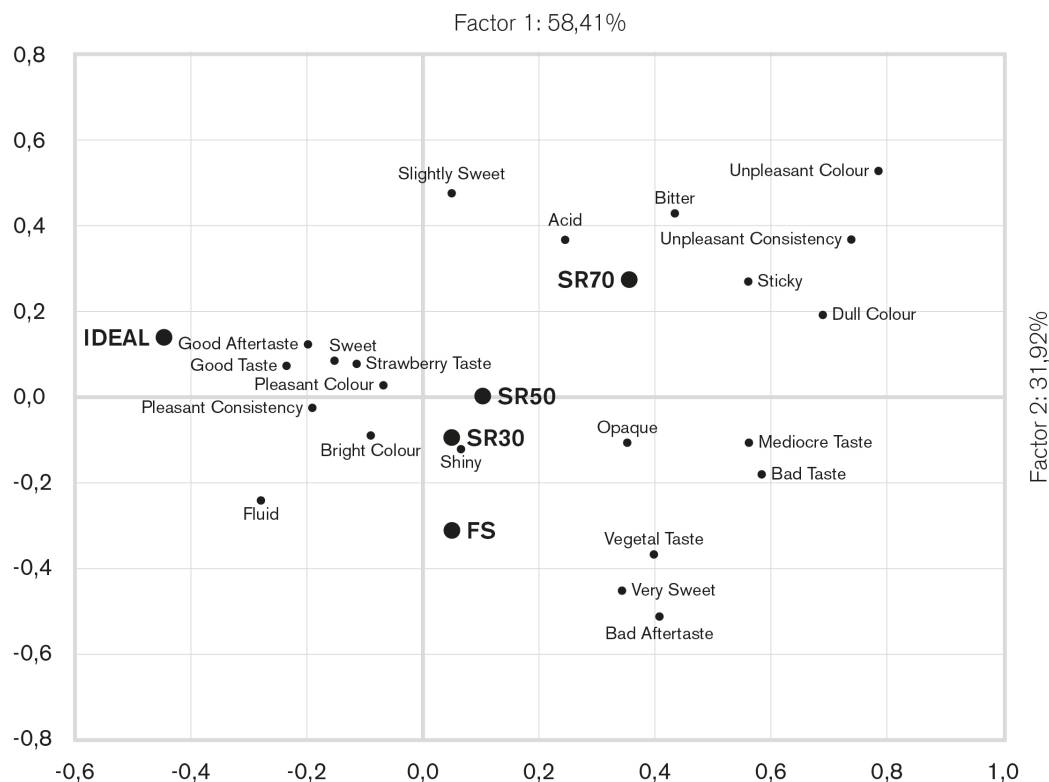


Figure 4: Factor plane of the correspondence analysis for the CATA test data of ripple sauces at different sugar content.

The sum of the two dimensions explained $\approx 90\%$ of variance with dimension 1 explaining $\approx 58\%$ and dimension 2 explaining $\approx 32\%$. The “IDEAL” ripple sauce was described by the following attributes: “good aftertaste”, “good taste”, “sweet”, “strawberry flavour” and “pleasant colour”. It can be observed that the attributes chosen for the “IDEAL” product were very generic, probably because the consumer is not used to consume ripple sauces without the ice cream. FS and SR30 were characterised by similar attributes: “shiny”, “very sweet”, “bad aftertaste”, mediocre taste”, “bad taste” and “vegetable taste”. SR50 was not discriminated by any attributes while SR70 was described by the attributes “unpleasant consistency”, “sticky consistency”, “acid”, “bitter”, “unpleasant colour” and “slightly sweet”.



4. Discussion

Sugar, with its high amount due to the presence of sucrose, fructose and inverted sugar, is the main component of the ripple sauces used in the food industry as topping for ice cream. The main objective of this work was the gradual reduction of the sugar present in the recipe with the use of a clean label ingredient able to satisfy the needs of the modern consumer. It was therefore used a consumer recognisable semi-solid fibre syrup which can at the same time (i) accomplish the necessary technological functionality (bulking effect), (ii) contribute to clean the label and enhance the nutritional value. The improvement of the nutritional profile was pointed out by the reduction of the energy and sugar as well as the increase of dietary fibre in the SR ripple sauces formulations. Moreover, the product reformulation by means of the semi-solid fibre syrup allows to use a double nutritional claim “reduced in sugar” and “high in fibre” on the basis of EU Regulation on nutritional and health claims (Regulation (EC) No1924/2006).

At industrial level, a ripple sauce used as topping for ice cream must comply with specific quality properties which shall be kept in mind during a reformulation process. In particular, a ripple sauce (i) it has to maintain its particular shape on the ice cream avoiding any form of water syneis, (ii) it has to have e maintain a proper consistency and adhesiveness to avoid the mixing with the ice cream (Grujić et al., 2014; Hull, 2010), (iii) it shall be easily pumped by the variegating pump and finally, (iii) it shall impart a distinctive colour and flavour to attract the consumer (Douglas Goff & Hartel, 2013; Pathare et al., 2013). Therefore, to evaluate the impact of the reduction of sugar by means of the use of a semi-solid fibre syrup on product quality, it was necessary to take into consideration the physicochemical, stability, rheological and sensorial properties of the SR ripple sauces.

The semi-solid fibre syrup has a moisture content ≈ 25 g water /100 g sample and a different intrinsically ability to bind water, due to very different chemical structure and properties if compared with sugar. Generally, fibre shows weaker water interaction ability than sugar which is characterized by high hygroscopicity (Milner et al., 2020). These factors affected the a_w and MC of RS ripple sauces resulting higher than the full sugar counterpart. Similar results were also observed in a previous study on sugar reduction in a fruit filling (Chapter 5) in which it was reported an increase of a_w and MC associated to the increase of the semi-solid fibre syrup in the reduced sugar recipes. Moreover, an increase of a_w and MC was also reported when vegetable fibres (apple, pea, oat, wheat fibres) were used in sugar reduced products as cakes, muffin and fruit jellies (Milner et al., 2020; Riedel et al., 2015; Zahn et al., 2013). The higher a_w and MC observed in the RS ripples anyhow did not jeopardize their shelf life stability at microbiological level since pH and the hot filling treatment allows the product stability at R/T. Instead, the increase of the macroscopic water parameters in the RS ripples may modify their stability, general appearance and colour, and flowability. However, syneresis degree results indicated that the semi-solid fibre syrup was able to avoid water separation since water syneis did not occur neither during storage, as also previously observed in fruit filling (Chapter 5).



As previously reported, the distinctive colour of the ripple sauce is an important consumer attractive characteristic. In this study, the use of a colouring agent helped to reduce the colour differences (ΔE) between reduced sugar samples and full sugar counterpart, that can be observed as a result to the use of the brownish semi-solid fibre syrup (Chapter 5). Anyway, a darkening effect during storage was noticed in all samples. This darkening effect was instead caused by the removal of the sugar molecules from the anthocyanins of the strawberry during the production step and the consequent formation of anthocyanidins. The anthocyanidins are more susceptible compounds to light and oxygen degradation and consequently to browning reaction. Similar results were observed by Grujić and co-worker on raspberries ice cream topping (Grujić et al., 2014), Touati and co-workers on apricot jam (Touati et al., 2014) and Wicklund and co-workers on strawberry jam (Wicklund et al., 2005).

At rheological level, the n values of all samples (FS and SR) classify them as pseudo plastic fluid ($n < 1$), as previously reported for similar products by Nalawade and colleagues (Nalawade et al., 2017). K values indicated a similarity only between FS and SR30 on fresh product and during storage at both temperatures. The similarity between FS and SR30 were also observed when rheological properties were assessed with Bostwick consistometer (running distance) while a slight significant difference was noticed with Texture Analyzer (Adhesiveness). Both techniques were used because more usable at industrial level. A 30% sugar reduction can therefore be reached with the use of the semi-solid fibre syrup without strongly alter the rheological properties e.g., the processability of the ripple sauces. Sensorial evaluation had also pointed out that all the ripple sauces tested by the consumers were appreciated with comparable overall acceptability scores higher than 5 and presented a comparable score for all the parameters tested (consistency, taste, colour). SR70 was the only sample with a significant lower score on consistency at t_0 probably due to the highest K and adhesiveness parameters for this sample highlighted with analytical techniques. Noteworthy, especially for long lasting shelf life products, no relevant changes during storage were noticed by the consumers. The IDEAL attributes associated to the ripple sauce were very generic probably because the consumer is not used to consume ripple sauces without the ice-cream. In general, the results obtained indicate a similarity of the FS and SR30 ripple sauces in agreement with the rheological tests, indicating that higher MC and a_w in SR ripple sauces as a result of the use of the semi-solid fibre syrup, did not affect the mouthfeel of the product if used within a certain amount. Indeed, higher content than those used to reduce sugar to 30% leads to a detrimental of the texture characteristics as observed by the negative texture attributes of SR70.

5. Conclusions

A clean label chickpea and maize based semi-solid fibre syrup was successfully used to reduce sugar by 30% in a ripple sauce. The obtained ripple sauces had comparable rheological and sensorial properties than the full sugar counterpart also during storage. The colour differences between samples



were minimised with the use of a colouring ingredient while the differences on a_w and MC can be considered as negligible at industrial level due to the production method applied.

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Chapter 7

Overall Conclusions and Future Perspective

Consumer interest on food increased constantly over the years, in particular considering the request for food products which can be considered healthier, familiar and natural.

Satisfy the consumer needs can be highly challenging for the food companies which necessarily need to invest in product reformulation to differentiate their products and increase their sales volumes. The potentiality of new functional vegetable ingredients for reformulation purpose has been pointed out by this industrial PhD thesis.

In the first part, a physically modified corn flour obtained through a heating-extrusion process has been extensively characterised for its physicochemical properties. In particular a multilevel and multi-analytical approach has been carried out to characterise the flour-water interaction of this new ingredient, highlighting its higher capacity in the interaction of water compared to a heated and a native corn flour. The physical treatment to which the flours undergone were pointed out at molecular level and corroborated at macroscopic and mesoscopic level. Subsequently, its effectiveness as clean label thickening agent was positively proven in a carrot soup, a tomato sauce and a meat patty. To combine the need of clean label and healthier food needs the physically modified flour has been further investigated in reduced-fat mayonnaise. The flour has been successfully used to produce a fat replacer gel which led to the production of reduced-fat mayonnaise which texture, rheological properties and stability were comparable to the full-fat counterpart. The differences noticed for colour and sensorial properties can be easily overcome with slight recipe modification during the industrialisation step.

In the second part of the thesis an innovative semi-solid fibre syrup based on chickpea and maize has been used as bulking agent to enhance the nutritional profile of food products, reducing their sugar and increasing their dietary fibre content. Initially, its functionality has been tested in short bread cookies. Interestingly, the use of the semi-solid fibre syrup did not jeopardize the dough workability and above all permitted a partial preservation of the short bread cookies structure and to enhance their nutritional profile, thus their consumer acceptability. The semi-solid fibre syrup has been therefore used in high sugar products, in particular a fruit filling and a ripple sauce. In both applications, the use of the semi-solid fibre syrup permitted to obtain satisfactory up to 30% of reduced sugar recipes which were considered acceptable by the consumers and similar to the full-sugar counterpart at rheological level also during storage. Instead, high values of the semi-solid fibre led to a general detrimental of the products due to a massive modification of the original structure.

The studies reported in this thesis are only a small piece of the wide world of the vegetable functional ingredients which is day by day evolving trying to follow, but primarily anticipate, the new request of the market. New technologies of extrusion and smart combination of vegetable ingredients will be central in

the new product development of meat and fish substitutes which probably soon will become predominant in our daily diet. HI-FOOD continues its expansion also in this sector and it will continue to assist the food companies in their new future development.

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- internal auditing
- suppliers and toll manufacturer audit
- delivery of policies, procedures and best practices
- data analysis for the management review of annual objectives
- HACCP plant review
- traceability test
- training to staff
- development and maintenance of Kosher, Halal, Organic certification
-

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-suppliers audit

-delivery of policies, procedures and best practices

-data analysis for the management review of annual objectives

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A1	A1	A1	A1	A1

Levels: A1/A2: Basic user - B1/B2: Independent user - C1/C2 Proficient user

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Driving licence	B
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