Journal section: Food Chemistry.

Microstructural, physical, and sensory impact of starch, inulin, and soy protein in low-fat gluten and lactose free white sauces.

Luis M. Guardeño*, Isabel Hernando, Empar Llorca, María Hernández-Carrión, Amparo Quiles

Microstructure and Chemistry of Food Research Group. Department of Food Technology. Universitat Politècnica de València. Camino de Vera s/n, 46022 Valencia, Spain.

*corresponding author: Luis M. Guardeño,
e-mail address: luiguaex@upvnet.upv.es
Camino de Vera s/n, 46022 Valencia (Spain)
Tel: +34 96 387 70 00 (Ext. 78230) Fax: +34 96 387 93 60

Word count of the body text: 4981 words.

Short version of title: Characterization of low-fat white sauces…

ABSTRACT

The microstructural, physical, and sensory properties of low-fat sauces made with different starches, soy protein, and inulin as a fat replacer were analyzed. Gluten-free waxy starches -rice and corn- were selected as well as soy protein in order to obtain sauces suitable for celiac and lactose intolerant consumers. Light microscopy was used to visualize the swollen starch granules dispersed in a protein-amylopectin-inulin phase. Inulin seemed to limit protein network development, which was related with a higher dispersion of starch granules within the sauce matrix. Therefore, the sauces made with inulin had a lower apparent viscosity (η_{app}) values ($P < 0.05$) in comparison with oil sauces. The sauces made with rice starches also exhibited a lower viscosity ($P < 0.05$) since these granules did not swell as corn granules do. All the sauces had a remarkable physical stability since there were no syneresis phenomena and color did not change significantly ($P < 0.05$) after 15 days of refrigeration storage (4 ºC). Finally, the sensory test suggests that oil could be substituted by inulin in the preparation of low-fat sauces since no significant differences ($P < 0.05$) in texture and flavor were found. These results encourage further research to optimize the formulations of these types of alternative white sauces.

Keywords: inulin, starch, soy protein, microstructure, white sauces.

Practical application: nowadays there is a great demand of ready-to-eat products due to new consumptions habits. In this context, it would be interesting to develop low fat sauces with inulin that could be used in this type of products improving their nutritional profile. The requirement of processed food for specific groups of population, such as celiac and lactose intolerant consumers, makes it necessary to use gluten free starches and soy protein in the formulation of sauces. The characterization of structure, physical and sensory properties is required to understand the product acceptability and its behavior during its shelf life.

Introduction

Consumers are increasingly demanding high quality, ready-to-eat food products designed not only to feed them but to contribute to improve their health. In order to meet these demands, food industry is developing innovative products which may be an alternative to traditional ones, especially for specific groups of population with certain health requirements. In this context, celiac disease is one of the few genetic disorders with well-defined environmental factors, that have a definite impact on the disease, because it is an immune response to gluten in the diet of genetically susceptible individuals (Niewinski 2008). Gluten is found in the endosperm of cereals such as wheat, barley, and rye (Jabri and others 2005) and, although it is mainly present in bakery and pasta products it is also a component in many other foods, including white sauces (Nishimura and others 2001).

The typical ingredients of white sauce include milk, oil, flour or starch, and salt (Arocas and others 2009). The most common problem with this type of blends is destabilization after preparation and/or during storage. There are two types of problems with destabilization: those associated with the emulsion (Ostwald ripening, mixing, flocculation, and coalescence of fat); and those problems caused by the interaction of two or more ingredients in the sauce. The milk proteins and starch act as emulsifiers and stabilizers in this type of products (Mandala and others 2004; Walstra and van Vliet 2007). However, the use of dairy ingredients with high lactose content is unsuitable for celiacs because many of them also suffer from lactose intolerance (Murray 1999). Moreover, celiac consumers often find that the range of products suitable for their condition is limited and many products are viewed as being of inferior quality (especially sensory) compared with their traditional, i.e. non gluten-free, counterparts (Kelly and others 2008).

There are different functional ingredients that have given technological, nutritional and tasty solutions to these products. Along this line, the properties of soybean have made it an appropriate substitute of milk since its proteins improve the structure of gluten-free products and its isoflavones lower the risk of cardiovascular disease and breast cancer (Arendt and others 2008). On the other hand, the use of modified gluten-free starches such as corn and rice ones is commonly used in dietetic products. Modified corn starch is mainly used because of its appropriate rheological behavior after being subjected to different heat and agitation conditions (Mason 2009), and rice starch not only is hypoallergenic, colorless, and relatively tasteless but it decreases the absorption of fat and the peroxidation during frozen storage of fried products (Jackson and others 2006). Inulin is other well-known ingredient which has been reported to have several benefits to human health (Roberfroid 2007). It is a non-digestible polysaccharide that consists of a chain of fructose molecules with a terminal glucose molecule that has been used as a sweetener in low-caloric products; as a substitute for fat; and as a texture modifier (Guggisberg and others 2009).

Recent research related to physical and rheological properties of soy protein-starch (Ribotta and others 2007; Lim and Narsimhan 2006) and inulin in low-fat semisolid desserts and yogurt (Bayarri and others 2011; Paseephol and others 2008; Zimeri and Kokini 2003; Guggisberg and others 2009) has been carried out. In this work, new formulations of low-fat or inulin white sauces made with soy protein and gluten–free starches were prepared. To the best of our knowledge, there is no research studying a food system containing such a mixture. Thus, the aim was to analyze the impact of the different ingredients on the microstructural, physical, and sensory characteristics in order to assess their stability and consumer acceptability.

Materials and methods

Starches

Modified waxy corn starch (crosslinked hydroxypropyl distarch phosphate, C Polar Tex 06741®) from Cargill, Inc (Minneapolis, Minn., U.S.A) and modified waxy rice starch (crosslinked acetylated distarch, Remygel 663®) from Beneo-Remy (Leuven, Belgium) were used. The starches were selected in order to compare different botanic sources which were gluten-free and gave an adequate viscosity according to previous assays.

Sample preparation

Four types of sauces were made containing: corn starch and oil (CSO), corn starch and inulin (CSI), rice starch and oil (RSO), and rice starch and inulin (RSI). The sauces consisted of starch (5 g/100 g), sunflower oil (Coosur®, Acesur, Sevilla, Spain) or inulin with an average chain length of 8-13 monomers

(Frutafit HD®, Sensus, Roosendaal, The Netherlands) (2.5 g/100g), soy protein isolate (Vicoprot®, Trades S.A., Barcelona, Spain) (3.2 g/100 g), salt (0.4 g/100 g), black pepper (0.02 g/100 g), nutmeg (0.02 g/100 g) and water 88.86 g/100 g w/w. The samples were prepared according to Arocas and others (2009). Briefly, all ingredients were placed into a cooking device (Thermomix TM 31®, Wuppertal, Germany) heated up to 90 ºC (17 ºC/min) at 1100 r/min and kept at 90 ºC at the same agitation speed for 6 min. The samples were stored in Pyrex glass bottles (300 g) and the analyses were performed after cooling them to room temperature. The study of viscosity, syneresis and color throughout storage were carried out on refrigerated (4 ºC) samples at days 2, 5, 9 and 15 after preparation. All measurements were carried out in triplicate.

Microstructural analysis

Light Microscopy (LM)

A light microscope (Nikon Eclipse E800® microscope, Nikon, Tokyo, Japan) was used. Two different dyes were dissolved in distilled water: iodine (1 g/L) to stain starch and toulidine blue (10 g/L) to stain proteins. A drop of the sample was placed on a slide, stained with the appropriate dye solution (20 μL) and visualized using a 20x objective lens.

Confocal laser scanning microscopy (CLSM)

A Nikon confocal microscope C1 unit that was fitted on the Nikon Eclipse E800 was used. An Ar laser line (488 nm) was employed as light source to excite fluorescent dyes rhodamine B and Nile red. Rhodamine B (Fluka, Sigma-Aldrich, St. Louis, Mo., U.S.A.) with $\lambda_{ex\ max}$ 488 nm and $\lambda_{em\ max}$ 580 nm was dissolved in distilled water at 1 g/L. This dye was used to stain proteins and starch. Nile red (Fluka, Sigma-Aldrich) with $\lambda_{ex\ max}$ 488 nm and $\lambda_{em\ max}$ 515 nm was dissolved in PEG at 0.1 g/L. This dye was used to stain fat. A drop of the sample was put on a slide, stained with 20 µL of the rhodamine B solution and 20 µL of the Nile red solution, and visualized using an oil immersion objective lens (40x).

Image analysis

The LM and CLSM images were acquired with a 1024 x 1024 pixel resolution and were analyzed using the software ImageJ v.1.43s (National Institute of Health, Bethesda, Maryland, U.S.A.). Starch granule swelling was assessed from 15 randomly acquired LM images. The granules were manually labeled and their area (µm^2) measured from each image. On the other hand, 15 randomly acquired CLSM images were binarized after grayscale threshold segmentation. Fat globule area (µm^2), was automatically determined from the binarized images. Since fat globule area did not show a normal distribution in every sauce analyzed, the 80th percentile (P_{80}) of the globule area was used as the parameter which better represented the fat globule size. P_{80} indicated that 80% of the globules in each image had an area below this value.

Apparent viscosity

The apparent viscosity (η_{app}) of the samples was studied using a viscometer (Haake Viscotester 6 R Plus, Thermo Fisher Scientific, Waltham, U.S.A.). The samples were placed in a thermostatic bath in order to maintain test temperature (50 ºC). A R5 spindle was immersed in the samples and remained 300 s as equilibration time prior to analysis. A constant share rate of 10 rpm was applied and values were registered during 300 s. Data at 150 s after the beginning of the test were used to compare η_{app} among samples.

Syneresis

Syneresis was determined according to Heyman and others (2010) with modifications. Subsamples were introduced in centrifuge tubes and stored at 4 ºC during 15 days. After equilibration to 20 ºC, the samples were centrifuged during 15 min at 6000 g. The quantity of water released on the top was decanted and the % of syneresis was calculated (Eq.1).

% Syneresis = (weight of decanted liquid / total weight before centrifugation) x 100 (1)

Color measurements

CIE L*a*b* coordinates were measured at 20 ºC using a Chroma meter CR-400 (Konica Minolta Sensing Americas, Inc. USA) with reference to illuminant C and a visual angle of 2°. L* denotes lightness on a 0 to 100 scale from black to white; a*, (+) red or (-) green; and b*, (+) yellow or (-) blue. Color difference with respect to freshly-made samples (ΔE^*) (Eq. 2), chrome ($C_{ab}*$) (Eq. 3) and hue (h_{ab}) (Eq. 4) were calculated throughout storage period.

$$\Delta E^*=[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2} \quad (2)$$

$$C^*_{ab}=[(a^*)^2+(b^*)^2]^{1/2} \quad (3)$$

$$h_{ab}=\arctan (b^*/a^*) \quad (4)$$

Sensory analysis

Consumers were recruited among students and employees of the Universitat Politècnica de València. A total of 52 untrained panelists aged 22-63 were used for the study. The samples were assessed in a standardized tasting room equipped with individual booths. White sauces were made and kept in a thermostatic bath at 50 ºC. Each consumer received four disposable glasses of the different formulations of the sauces monadically in a single session following a balanced complete block experimental design. The glasses were coded by three digit random numbers and served in random order. Consumer acceptance testing was done using a successive-category scale to score the "appearance", "texture" and "flavor". The scale was a 7-point hedonic scale (7= like extremely, and 1= dislike extremely).

Statistical analysis

Analysis of variance (ANOVA) was performed on the data using the Statgraphics Plus 5.1 software package (Statistical graph Co., Rockville, Md, USA). Least Significant Difference (LSD) Fisher's test was used to evaluate mean values differences ($P < 0.05$).

Results and discussion

Microstructure

Figure 1 shows the images obtained by light microscopy (LM) from the different sauces. The iodine solution stains amylose in blue and amylopectin in brown or violet (Conde-Petit and others 1998). On the other hand, staining with toluidine blue allows us to visualize the protein network throughout the continuous phase. The starch granules morphology became irregular as they swell so it was difficult to accurately assess the extent of their swelling. However, as a rough estimate, the mean area of swollen granules sections was measured.

The CSO sauces (Figure 1A, B) consisted of a complex matrix containing two different phases: a dispersed phase of swollen starch granules, that have partially resisted the cooking process, and fat globules stabilized by a continuous phase of soy protein and starch polymers which have leached out from the granules during gelatinization. Figure 1A shows the continuous phase stained in brown since amylopectin was the main polymer leached from the waxy modified starch granules. The soy protein is shown in Figure 1B forming a blue network throughout the sample. The CSO starch granules -with a mean area of 245 μm^2- were strongly swollen but not completely disintegrated, which indicated their high resistance to heat and shear given by the chemical modification. This fact was also stated by Arocas and others (2009). Moreover, soy protein could have a protective effect on starch granule disintegration by limiting the absorption of water. As it can be observed, the CSO starch granules did not disperse easily in the continuous phase and in some areas they aggregated. In addition, the contact between granules, which is likely to determine the viscosity and stability of CSO sauces, could act as a barrier by limiting further swelling and disintegration. Fat globules were homogeneously distributed within the soy protein-starch polymer matrix.

The CSI sauces containing inulin instead of oil (Figure 1C, D) showed a continuous phase that could not be clearly distinguished. This was the result of interaction between soy protein, inulin, and starch polymers. The replacement of oil, which is a component of the dispersed phase, with inulin, which is a component of the continuous phase, may prevent the formation of soy protein networks and soy protein-amylopectin networks by acting as a binding element for the continuous phase components. Moreover, the presence of some inulin crystals indicated that inulin did not solubilized completely in the continuous phase. Zimeri and Kokini (2003) also found crystals in water solutions of 5% inulin and suggested that this fact was the result of either insolubility or recrystallization after cooling. In the CSI and RSI sauces, the soy protein and starch polymers in the continuous phase could limit inulin solubilization and therefore favor crystallization. Most of this phase appeared covered by gelatinized swollen granules (Figure 1C) that sometimes interacted with each other to form aggregates. In the CSI sauces, the gelatinized granules -with mean area of 220 μm^2- seemed to have a lower degree of swelling and deterioration than in the CSO sauces. Inulin could have a protective effect against granule degradation- limiting granule swelling- by competing with starch polymers to bind water molecules or interacting directly with starch polymers.

The microstructure of RSO sauces is shown in Figure 1E, F. The continuous phase of soy protein and starch granule polymers appeared to have a greater capacity to disperse starch granules and less tendency to form networks between proteins and between soy protein and starch polymers (Figure 1F) than in CSO sauces (Figure 1B). The RSO starch granules - with mean area of 208 μm^2- showed a higher degree of integrity and lesser swelling and aggregation tendencies in comparison with the CSO ones. The fat globules were homogeneously distributed throughout the continuous phase as in CSO sauces.

The microstructure of the RSI sauce is shown in Figure 1G, H. The swollen starch granules had a mean area of 199 μm^2 and were more dispersed than those in CSI sauces. The rice granules in this sauce had the least tendency to form aggregates among all the other starches and showed a low degree of deterioration after cooking. Rice starch granules have been reported to be smaller than corn starch ones after being subjected to high temperatures (Arocas and others 2010). This fact, along with the higher swelling power of hydroxypropyl substitution (in corn starch) in comparison with acetylation (in rice starch), could explain the observed microstructure.

Figure 2 shows micrographs of the sauces acquired using confocal laser scanning microscopy (CLSM). This technique allowed us to obtain accurate, single optical sections, and a better visualization of sauce components by fluorescent labeling. The protein, starch granules, and solubilized granule components were stained red by rhodamine and fat globules were stained green by nile red. The CSO and RSO images (Figure 2A, C) show gelatinized starch granules and fat globules embedded in a continuous red phase of soy protein and starch polymers, which confirms the observations made by LM. The CSI sauces exhibited many tightly packed granules (Figure 2B), whereas the granules seemed to be more dispersed in RSI sauces (Figure 2D). When comparing sauces made with oil (CSO and RSO) with those made with inulin (CSI and RSI), the latter showed a less cohesive and compact continuous phase. These results are consistent with the research by Guggisberg and others (2009) in which an increase in inulin concentration caused an slightly less cohesive protein network in low-fat yogurt.

Figure 3 shows representative micrographs of sauces made with oil (CSO and RSO) and their respective frequency histograms of fat globules areas. The fat globules had a well defined spherical morphology in this type of sauces

(Guardeño and others 2011) and hence the mean area of many optical sections could be used as an approach of the fat globule size. Both CSO and RSO sauces exhibited high frequencies in small globules, i.e. the 73 % of the fat globules had an area smaller than 10 μm^2, and there were no significant differences ($P < 0.05$) between the mean values of the P_{80} in both CSO and RSO sauces (Figure 3C, D). As it can be noticed the fat globules were stabilized by the network of protein and starch polymers which led to a homogeneous distribution within the continuous phase. This fact, together with small size of the globules could limit destabilization –e.g. coalescence- during further processing or storage.

In brief, rice starch in RSO and RSI sauces showed a greater degree of dispersion in the continuous phase, less capacity to swell and less tendency to aggregate than corn starch in CSO and CSI. Therefore, it is to be expected that RSO and RSI sauces have a lower viscosity. The presence of inulin in the CSI and RSI sauces seemed to preserve the integrity of the starch granule, reducing its swelling capacity and binding the continuous phase which was less compact and, therefore, the starch granules were more easily dispersed.

Apparent viscosity

Figure 4A shows the time dependence of η_{app} of the freshly-made sauces which was more evident in the corn starch sauces than in rice starch ones. In order to assess viscosity changes during refrigeration storage at 4 ºC, the η_{app} values at a specific time (150 s) were recorded and compared among samples and storage periods (Figure 4B). The CSO sauces showed the highest η_{app} values whereas the RSI ones showed the lowest values during storage period. As shown by LM and CLSM, the CSO sauce showed large swollen starch granules and the greatest tendency to form aggregates; while the RSI sauce showed less swollen starch granules -being more dispersed due to a less degree of cohesion in the continuous phase-.

The sauces made with corn starch (CSO and CSI) had higher η_{app} values (P < 0.05) throughout all storage period than the sauces made with rice starch (RSO and RSI). This fact was related with the microstructural analysis where the higher swelling power –due to hydroxypropylation- and size of corn starch in comparison to acetylated rice starch was observed. Singh and others (2003) explained that the viscosity of starch blends was directly related to the size and interaction of the starch granules.

On the other hand, the sauces made with inulin (CSI and RSI) had lower values (P < 0.05) than those made with oil (CSO and RSO). LM revealed that inulin preserves the integrity of the granules and could limit the formation of the protein-starch polymers network in the continuous phase, which in turn, was less compact and more able to disperse starch granules. In this line, other authors have suggested that inulin molecules could interfere with the protein matrix formation and be responsible for a lower firmness in yogurt (Paseephol and others 2008). Alternatively, Bayarri and others (2011) reported an increase in most rheological parameters when long-chain inulin was used in low-fat dairy desserts, which was attributed to the presence of inulin aggregates. However, they state that the addition of native inulin –with a similar average chain length of the one used in this study- hardly modified the rheological properties of the samples. In our study, both soy protein and starch concentrations were the same in all the sauces analyzed but inulin replaced oil. Thus, the decrease in

η_{app} values could be due to, on the one hand, the interaction of inulin and starch polymers which limits starch granule swelling, on the other hand, the inulin capacity to bind water which could hinder the proper development of a protein-amylopectin matrix in the continuous phase.

Figure 4B shows a general storage-time independence regarding apparent viscosity. However, there was an increase in η_{app} at day 2 of storage which was significant ($P < 0.05$) in the corn starch sauces (CSO and CSI). This fact was not expected because hydroxypropylation in the corn starch sauces is considered to impart a greater freeze-thaw stability than acetylation in the rice starch sauces (Mason 2009). All in all, the apparent viscosity did not change from day 2 to 9 for every type of sauce and it decreased to the values of freshly-made sauces at day 15 for CSO sauces. In general, the use of waxy modified starches was appropriate to maintain the viscosity throughout cold storage.

Syneresis

Products that contain starch release water mainly due to retrogradation of amylose. In this study, waxy starches (very low amylose content) have been used in order to minimize this phenomenon. Moreover, the crosslinking and substitution had an extra protective effect against retrogradation because these modifications decreased the starch polymer leaching from the granules. After 15 days of storage at 4 °C none of the sauces showed syneresis, and therefore all the formulations were stable to cold storage. These results are consistent with those observed by Arocas and others (2009) in their studies of white sauces prepared with various corn starches, which concluded that syneresis does not occur when using modified starches. In addition, presence of the hydrophilic groups of soy protein along with the presence of inulin could improve the water retention capacity.

Color

The color evolution during 15 days of storage at 4 °C is shown in Figure 5. The values of lightness (L*) were significantly higher (*P* < 0.05) in the sauces made with oil (CSO and RSO) than in those made with inulin (CSI and RSI). On the other hand, L* did not change (*P* > 0.05) from the day 2 of storage for CSO sauces and from the day 0 for CSI, RSO and RSI ones (Figure 5A).

Figure 5B shows the evolution of chroma (C^*_{ab}) during storage period. There were significant differences (*P* < 0.05) in C^*_{ab} among all types of formulation used, but no significant differences in C^*_{ab} (*P* > 0.05) occurred for any of the sauces throughout the storage period. Sauces with oil (CSO and RSO) revealed significantly higher values of C*ab (*P* < 0.05) within the storage period than those made with inulin (CSI and RSI).

Figure 5C shows the evolution of the hue (h_{ab}) values. Sauces with oil (CSO and RSO) exhibited significantly lower h_{ab} values (*P* < 0.05) than sauces made with inulin (CSI and RSI). All the sauces revealed stable h_{ab} values throughout the storage period.

Finally, Figure 5D shows the evolution of the color difference (ΔE *) of the samples at different days of storage, regarding the freshly-made ones (storage time = 0 d). The values ranged from 0.29 to 2.14, being the CSO sauce the one which exhibited the higher ΔE* value (*P* < 0.05). However, color differences values were below 3 in every sauce analyzed which was not considered to be detectable for the human eye (Francis and Clydesdale 1977). In summary, inulin was the most important component regarding sauce color since the sauces in which it was used showed lower values of L^* and C^*_{ab} and higher values of h_{ab} in comparison to sauces without inulin. In general, color stability during refrigeration storage is achieved.

Sensory analysis

The consumer acceptability testing scores for appearance, texture, and flavor of the different formulations studied are shown in table 1. As it can be seen, there were no significant differences ($P > 0.05$) between the sauces made with oil (CSO and RSO) and those made with inulin (CSI and RSI) for texture and flavor. Sauces made with oil were better rated in appearance than those containing inulin, with RSO sauce being the best and CSI sauce being the worst evaluated. A statistical analysis of the results shows that oil could be substituted by inulin in the preparation of low-fat white sauces that contained neither gluten nor lactose as far as texture and flavor go.

Conclusions

Soy protein white sauces made with rice or corn modified waxy starches, and with oil or inulin, were physically stable to refrigeration storage. The presence of inulin as a fat replacer caused a decrease in apparent viscosity. This fact was related to a decrease in starch granule swelling and water competition between inulin and the protein-starch polymer network in the continuous phase. Moreover, the apparent viscosity and the color parameters analyzed did not change significantly during storage and there were no syneresis phenomena throughout 15 days of storage at 4 ºC. Sensory test revealed a good acceptability of soy protein-based sauces. In the light of these results, the use of inulin as a fat replacer would be appropriate to develop sauces addressed to celiac and lactose intolerants consumers.

Acknowledgements

The authors are grateful for the FPU grant awarded to L.M Guardeño and the economic support received from Universitat Politècnica de València -project PAID-06-09-2871- and Generalitat Valenciana – project GV-2010/038-.

References

Arendt EK, Morrissey A, Moore MM & Dal Bello F. 2008. Gluten-free breads. In: Elke, K. A. & Fabio Dal, B., editors. Gluten-free cereal products and beverages. San Diego: Academic Press. p. 289-319.

Arocas A, Sanz T & Fiszman SM. 2009. Influence of corn starch type in the rheological properties of a white sauce after heating and freezing. Food Hydrocolloids 23(3):901-907.

Arocas A, Sanz T, Hernández-Carrión M, Hernando M & Fiszman S. 2010. Effect of cooking time and ingredients on the performance of different starches in white sauces. European Food Research and Technology 231(3):395-405.

Bayarri S, GonzáLez-Tomás L, Hernando I, Lluch MA & Costell E. 2011. Texture perceived on inulin-enriched low-fat semisolid dairy desserts. Rheological and structural basis. Journal of Texture Studies 42(3):174-184.

Conde-Petit B, Nuessli J, Handschin S & Escher F. 1998. Comparative characterisation of aqueous starch dispersions by light microscopy, rheometry and iodine binding behaviour. Starch - Stärke 50(5):184-192.

Francis FJ & Clydesdale FM. 1977. Food colorimetry: Theory and applications. Westport, Connecticut: The Avi Publishing Company, Inc.

Guardeño LM, Sanz T, Fiszman SM, Quiles A & Hernando I. 2011. Microwave heating effect on rheology and microstructure of white sauces. Journal of Food Science 76(8):E544-E552.

Guggisberg D, Cuthbert-Steven J, Piccinali P, Bütikofer U & Eberhard P. 2009. Rheological, microstructural and sensory characterization of low-fat and whole milk set yoghurt as influenced by inulin addition. International Dairy Journal 19(2):107-115.

Heyman B, Depypere F, Delbaere C & Dewettinck K. 2010. Effects of non-starch hydrocolloids on the physicochemical properties and stability of a commercial béchamel sauce. Journal of Food Engineering 99(2):115-120.

Jabri B, Kasarda DD & Green PHR. 2005. Innate and adaptive immunity: the Yin and Yang of celiac disease. Immunological Reviews 206(1):219-231.

Jackson V, Schilling MW, Coggins PC & Martin JM. 2006. Utilization of rice starch in the formulation of low-fat, wheat-free chicken nuggets. The Journal of Applied Poultry Research 15(3):417-424.

Kelly AL, Moore MM & Arendt EK. 2008. 18 - New product development: The case of gluten-free food products. In: Elke, K. A. & Fabio Dal, B., editors. Gluten-free cereal products and beverages. San Diego: Academic Press. p. 413-431.

Lim HS & Narsimhan G. 2006. Pasting and rheological behavior of soy protein-based pudding. LWT - Food Science and Technology 39(4):344-350.

Mandala IG, Savvas TP & Kostaropoulos AE. 2004. Xanthan and locust bean gum influence on the rheology and structure of a white model-sauce. Journal of Food Engineering 64(3):335-342.

Mason WR. 2009. Starch use in foods. In: James, B. & Roy, W., editors. Starch (Third Edition). San Diego: Academic Press. p. 745-795.

Murray JA. 1999. The widening spectrum of celiac disease. The American Journal of Clinical Nutrition 69(3):354-365.

Niewinski MM. 2008. Advances in celiac disease and gluten-free diet. Journal of the American Dietetic Association 108(4):661-672.

Nishimura K, Goto M, Higasa T, Kawase S-i & Matsumura Y. 2001. Aggregation behaviour of bovine serum albumin as a cause of sauce liquid separation by heating. Journal of the Science of Food and Agriculture 81(1):76-81.

Paseephol T, Small DM & Sherkat F. 2008. Rheology and texture of set yogurt as affected by inulin addition. Journal of Texture Studies 39(6):617-634.

Ribotta PD, Colombo A, León AE & Añón MC. 2007. Effects of soy protein on physical and rheological properties of wheat starch. Starch - Stärke 59(12):614-623.

Roberfroid MB. 2007. Inulin-type fructans: functional food ingredients. The Journal of Nutrition 137(11):2493S-2502S.

Singh N, Singh J, Kaur L, Singh Sodhi N & Singh Gill B. 2003. Morphological, thermal and rheological properties of starches from different botanical sources. Food Chemistry 81(2):219-231.

Walstra P & van Vliet T. 2007. Dispersed systems: basic considerations. In: Damodaran, S., Parkin, K. L. & Fennema, O. R., editors. Food Chemistry. 4 ed. New York: CRC Press.

Zimeri JE & Kokini JL. 2003. Morphological characterization of the phase behavior of inulin–waxy maize starch systems in high moisture environments. Carbohydrate Polymers 52(3):225-236.

Table 1. Mean values of the sensory scores for the attributes analyzed in the consumer (n= 52) acceptance testing.

	Sensory attribute*		
Sauce	Appearance	Texture	Flavor
CSO	5.1[a] (1.5)	5.2[a] (1.3)	4.5[a] (1.1)
CSI	3.8[b] (1.7)	4.5[a] (1.7)	4.0[a] (1.3)
RSO	5.4[a] (1.1)	5.0[a] (1.3)	4.5[a] (1.4)
RSI	4.0[b] (1.6)	4.5[a] (1.6)	4.0[a] (1.5)

*Results from a 7-point hedonic rating test.

Values between parentheses are the standard deviations.

For each attribute means without the same letter reveal significant difference (P < 0.05) according to the LSD multiple range test.

CSO: sauce made with corn starch an oil; CSI: sauce made with corn starch and inulin; RSO: sauce made with rice starch and oil; RSI: sauce made with rice starch and inulin.

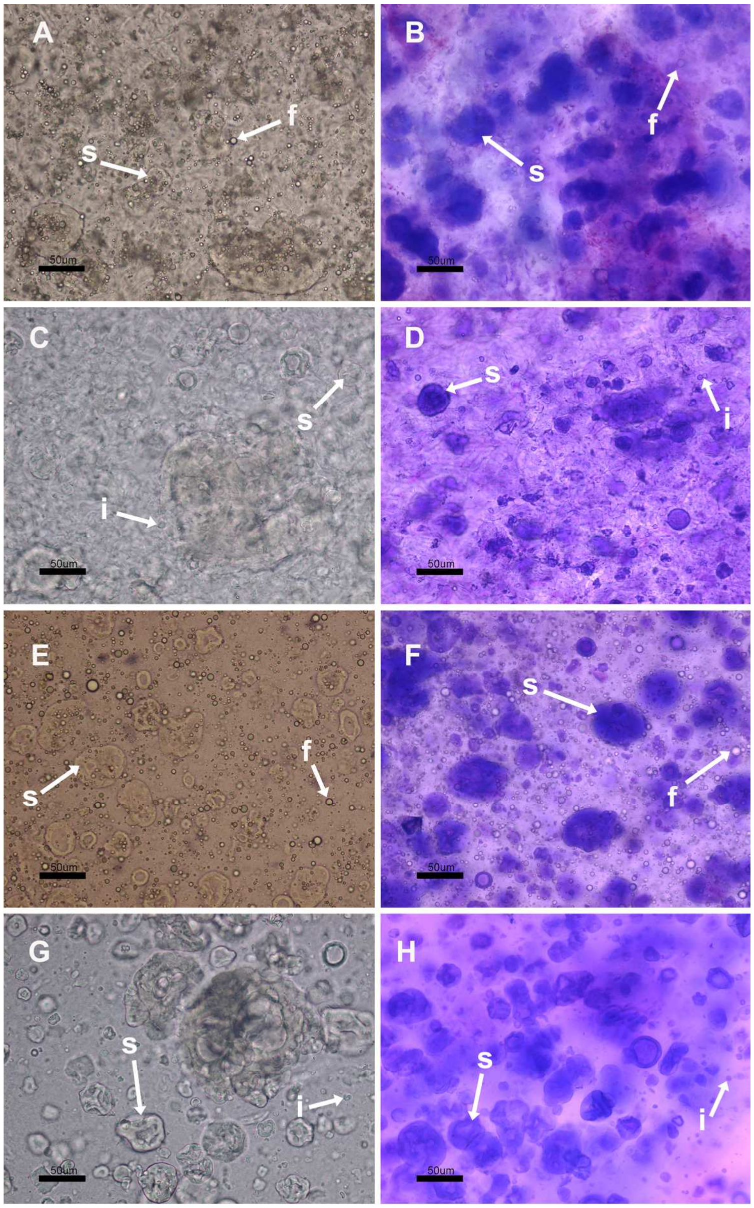

Figure 1. Light microscopy (LM) micrographs of the different sauces stained with iodine (A, C, E and G) and toluidine (B, D, F and H) solutions. A and B: CSO sauce made with corn starch and oil; C and D: CSI sauce made with corn starch and inulin; E and F: RSO sauce made with rice starch and oil; G and H: RSI sauce made with rice starch and inulin. i: inulin crystal; f: fat globule; s: starch granule. Scale bar: 50 μm.
140x224mm (300 x 300 DPI)

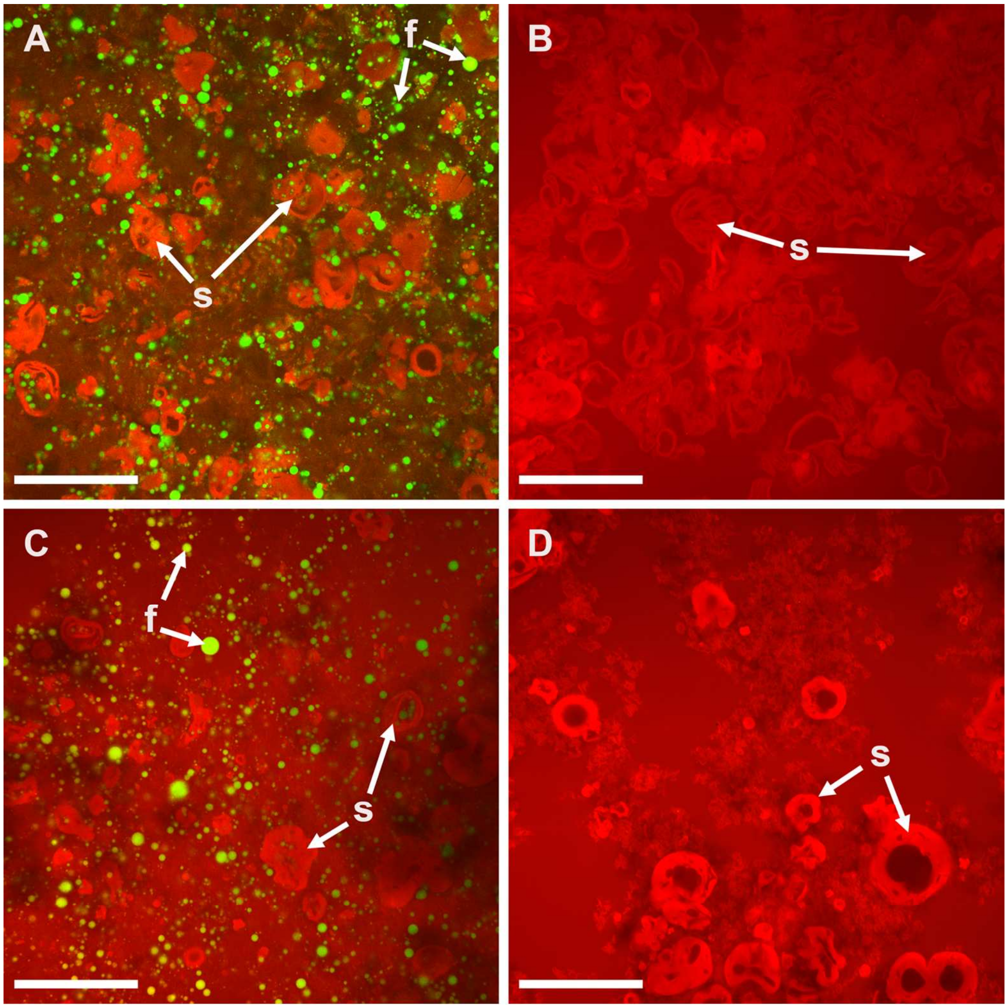

Figure 2. Confocal laser scanning microscopy (CLSM) micrographs of the different sauces. A: CSO sauce made with modified corn starch and oil; B: CSI sauce made with modified corn starch and inulin; C: RSO sauce made with modified rice starch and oil; D: RSI sauce made with modified rice starch and inulin. f: fat globule; s: starch granule. Scale bar: 80 μm.
140x140mm (300 x 300 DPI)

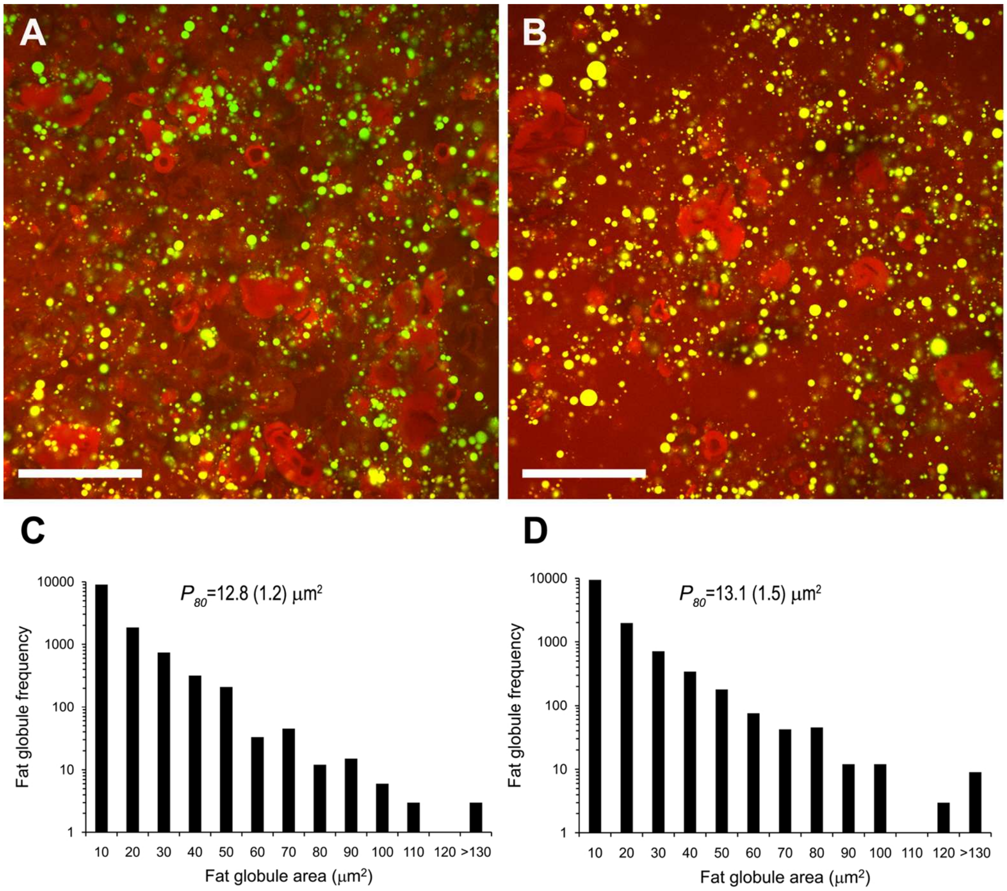

Figure 3. Confocal laser scanning microscopy (CLSM) micrographs of the sauces made with oil and frequency histograms of fat globule areas. A: CSO sauce made with modified corn starch and oil; B: RSO sauce made with modified rice starch and oil. Frequency histogram and the 80th percentile (P_{80}) of fat globule area for CSO sauce (C) and RSO sauce (D). Values between parentheses are the standard deviations. Scale bar: 80 μm.
140x126mm (300 x 300 DPI)

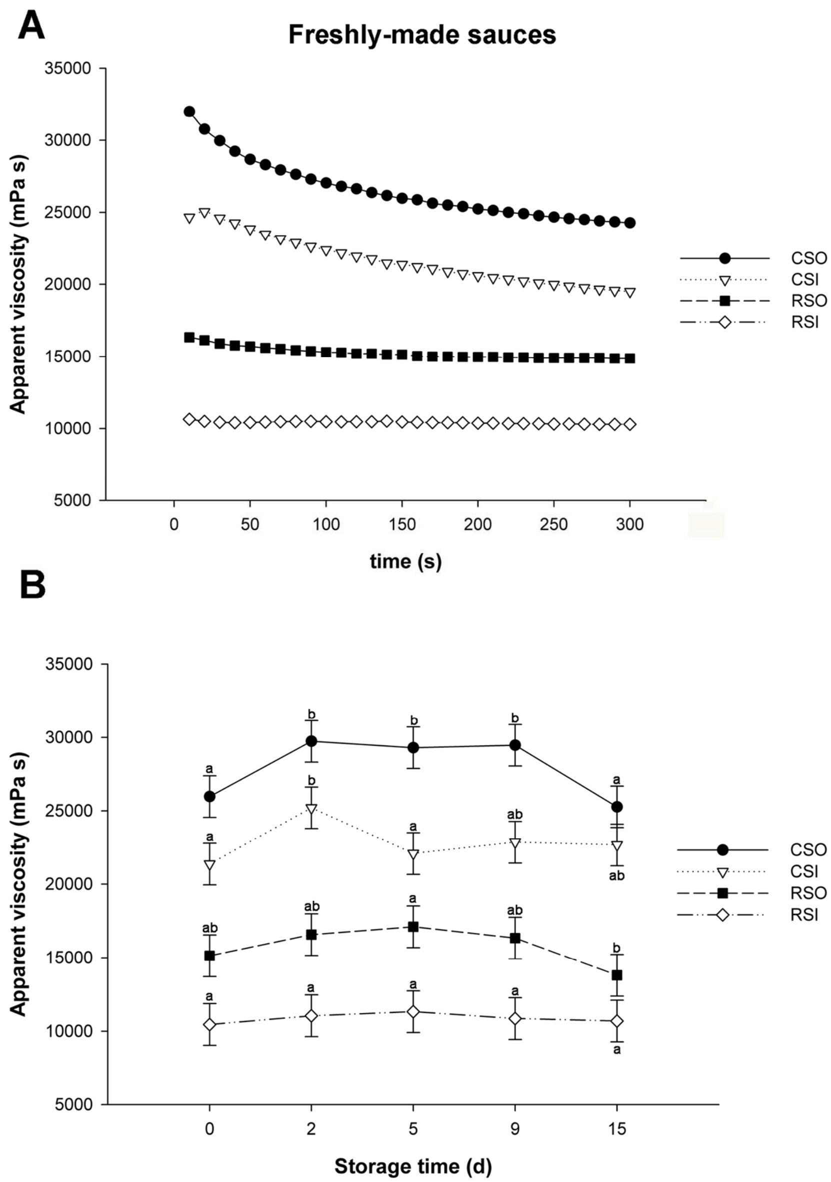

Figure 4. Apparent viscosity of the sauces. A: thixotropy plot (time versus apparent viscosity) of the freshly-sauces subjected to a constant shear rate (10 rpm) during 300 s at 50 ºC. B: evolution of the apparent viscosity during storage at 4 ºC, interactions and mean plots with LSD intervals between type of sauce and storage time (days). Different letters within the same type of sauce reveal significant differences ($P < 0.05$). CSO: sauces made with corn starch and oil; CSI: sauces made with corn starch and inulin; RSO: sauces made with rice starch and oil; RSI: sauces made with rice starch and inulin.
127x180mm (300 x 300 DPI)

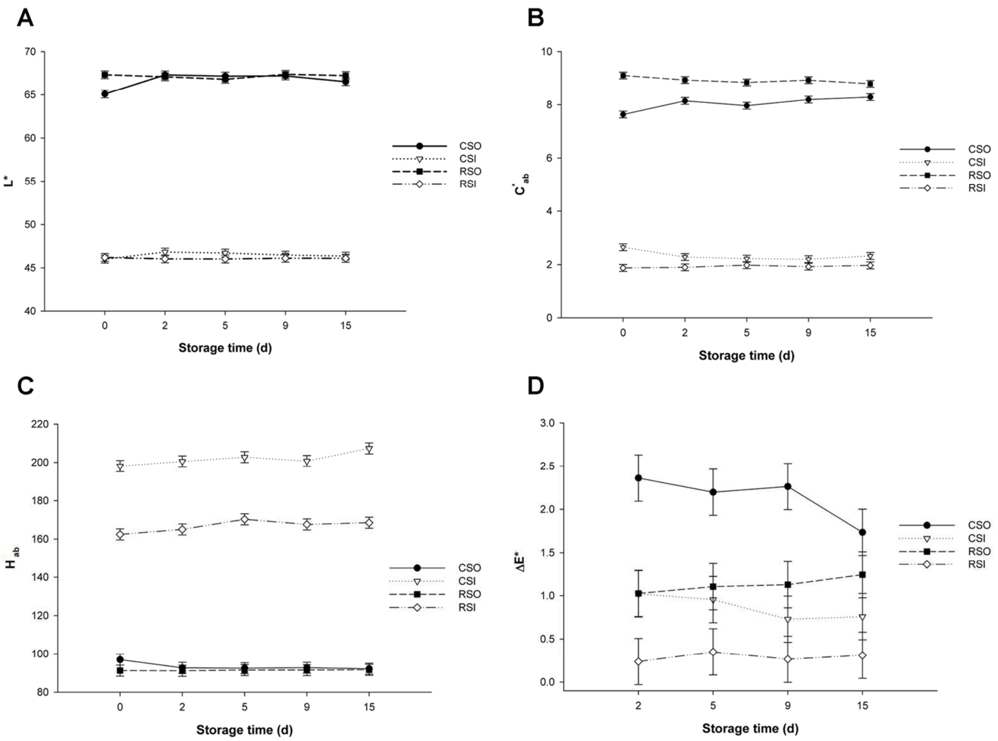

Figure 5. Colour parameters evolution during storage at 4 ºC. Interactions and mean plots with LSD intervals (*P* < 0.05) between type of sauce and storage time (days) for lightness, L* (A); chroma, C^*_{ab} (B); hue, h_{ab} (C) and colour difference, ΔE (D). CSO: sauces made with corn starch and oil; CSI: sauces made with corn starch and inulin; RSO: sauces made with rice starch and oil; RSI: sauces made with rice starch and inulin.
140x105mm (300 x 300 DPI)