

Effect of Different Rice Starches, Inulin, and Soy Protein on Microstructural, Physical, and Sensory Properties of Low-Fat, Gluten, and Lactose Free White Sauces

LUIS M. GUARDEÑO, JOSÉ L. VÁZQUEZ-GUTIÉRREZ, ISABEL HERNANDO
and AMPARO QUILES

Research Group of Food Microstructure and Chemistry, Department of Food Technology,
Polytechnic University of Valencia, Valencia, Spain

Abstract

GUARDEÑO L.M., VÁZQUEZ-GUTIÉRREZ J.L., HERNANDO I., QUILES A. (2013): **Effect of different rice starches, inulin, and soy protein on microstructural, physical, and sensory properties of low-fat, gluten, and lactose free white sauces.** Czech J. Food Sci., 31: 575–580.

The microstructural, physical, and sensory properties of low-fat sauces made with different rice starches, soy protein, and inulin were analysed in order to obtain sauces suitable for celiac and lactose intolerant consumers. Soy protein and inulin could prevent starch degradation due to their high water-binding capacity. Moreover, protein molecules could diffuse into the starch granules and soluble inulin could interact with starch polymers within the granule. Both effects would hinder amylose leaching. Inulin provides better diffusion capacity of gelatinised granules and soy protein-starch granule aggregates than sunflower oil, which helps to decrease viscosity in modified rice starch sauces. Soy protein prevents syneresis in the sauces. Inulin affects colour parameters in native rice starch sauces, probably because of inulin and retrograded amylose polymers interactions. Sauces made with sunflower oil and modified rice starch are best rated by consumers. However, according to the statistical analyses, the replacement of oil by inulin could be suitable to prepare low-fat, gluten, and lactose free white sauces when modified rice starch is used.

Keywords: low-calorie food; nutritive food; microstructure; viscosity; stability

Approximately 50% of people with celiac disease have lactose intolerance. Gluten is found in the endosperm of cereals such as wheat, barley and rye, and although it is mainly present in bakery and pasta products, it is also a component in many other foods, including white sauces. The typical ingredients of white sauce include milk, oil, flour or starch, and salt (AROCAS *et al.* 2009) cannot be consumed by celiacs and lactose intolerants. Furthermore, the demand for low-fat products is increasing every day. The white sauce typical ingredients could be substituted or reduced in order to obtain new products with appropriate and suitable nutritional profiles for consumers with

specific requirements. The properties of soy have made it an attractive ingredient for the development of functional foods (ARENDRT *et al.* 2008). Rice is one of the most widely relied upon staple foods for nearly half of the world's population. Rice flour can be used in the production of gluten-free products as well as lipid reduction through baking (JACKSON *et al.* 2006). Inulin is non-digestible fructan of interest in human nutrition due to its ability to act as dietary fibre and its prebiotic effect (ROBERFROID 2007). The objective of this paper is to obtain new formulations of gluten and lactose free white sauces and the introduction of foods with functional compounds in the diet. To

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achieve this, the microstructure of the sauces was studied using light microscopy and some sensory and physical properties (colour, viscosity, and syneresis) were examined.

MATERIAL AND METHODS

Starches. The following rice starches were used as gluten-free rice starch sources: native rice starch (RN) (Remy DR) and modified waxy rice starch [cross-linked acetylated distarch adipate (Remygel 633) (RM) from Beneo-Remy (Leuven, Belgium)].

Sample preparation. Four types of sauces were made containing: native rice starch and oil (RNO), native rice starch and inulin (RNI), modified waxy rice starch and oil (RMO) and modified waxy rice starch and inulin (RMI). The sauces consisted of water (88.86 g/100 g), starch (5 g/100 g), soy protein isolate (Vicoprot R; Trades S.A., Barcelona, Spain) (3.2 g/100 g), either sunflower oil (Coosur; Acesur, Sevilla, Spain) or inulin with an average chain length of 8–13 monomers (Frutafit HDR; Sensus, Roosendaal, the Netherlands) (2.5 g/100 g), salt (0.4 g/100 g), black pepper (0.02 g/100 g), and nutmeg (0.02 g/100 g).

The ingredients were mixed and heated up to 90°C (17°C/min at 1100 rpm) in a cooking device (Thermomix TM 31®; Vorwerk & Co. KG, Wuppertal, Germany) and kept at 90°C at the same agitation speed for 6 min (AROCAS *et al.* 2009). The samples were stored in Pyrex glass bottles (300 g) and cooled to room temperature before microstructural analysis. Viscosity, syneresis, and colour were studied on freshly made samples and after 2, 5, 9, and 15 days of storage at 4°C.

Light microscopy. Light microscopy (LM) study was carried out according to QUILES *et al.* (2012). Distilled water solutions of iodine (10 g/l) and toluidine blue (1 g/l) were used to stain starch or proteins, respectively. A drop of the sample was placed on a slide, stained with the appropriate dye solution (20 ml) and observed in a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) using a 20× objective lens.

Apparent viscosity. The apparent viscosity (η_{app}) was measured according to QUILES *et al.* (2012) with some modifications. A viscometer (Haake Viscotester 6 R Plus; Thermo Fisher Scientific, Waltham, USA) was used. Test temperature was set at 50°C using a thermostatic bath and samples were equilibrated during 300 s prior to analysis.

A R5 spindle was immersed in the samples and a constant angular velocity of 10 rpm was applied. Values were recorded during 300 s and data obtained at 150 s after the beginning of the test were used to compare η_{app} among samples.

Syneresis. Percentage of syneresis was determined according to QUILES *et al.* (2012) with modifications. After 15 days of storage at 4°C and equilibration at room temperature (20°C), samples were centrifuged at 6000 g during 15 minutes. Water released on the top was decanted and the % of syneresis was calculated (Eq.1):

$$\text{Syneresis (\%)} = \left(\frac{\text{weight of decanted liquid}}{\text{weight before centrifugation}} \right) \times 100 \quad (1)$$

Colour measurements. CIE $L^*a^*b^*$ coordinates were measured at 20°C using a Chroma meter CR-400 (Konica Minolta Inc., New Jersey, USA) with reference to illuminant C and a visual angle of 2°. Colour difference with respect to freshly made samples, ΔE^* (Eq. 2), was calculated:

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

Sensory analysis. Untrained panellists ($n = 60$) were recruited among students and employees of the Universitat Politècnica de València. Sensory analysis were carried out in a standardised tasting room equipped with individual booths. Four ready-made samples corresponding to the different sauce formulations were maintained at 50°C and served randomly in disposable glasses coded by three digit random numbers. The test was carried out monadically in a single session following a balanced complete block experimental design. Consumer acceptance was assessed using a 5-point hedonic scale (5 = 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, USA). Tukey's HSD test was used to evaluate mean values differences ($P < 0.05$). All measurements were carried out in triplicate.

RESULTS AND DISCUSSION

Microstructure

As shown in LM micrographs, amylose and amylopectin are stained with iodine solution (Fig-

ures 1A, C, E, G). The degree of structuring in the continuous phase and the level of deterioration of the starch granules can be observed by staining with toluidine blue (Figures 1B, D, F, H).

White sauce made with a native rice starch and oil (RNO) (Figures 1A and B) is constituted by a continuous phase and a dispersed phase that differ greatly when compared (Figure 1B). The continuous phase, which can be observed as homogeneously dyed in Figure 1B, is formed of soy protein and starch polymers that have leached from the gelatinised and disintegrated starch granules. The swollen starch granules are highly dispersed in the continuous phase (Figure 1B). The dispersed phase consists of swollen starch granules and fat globules. A high proportion of the starch granules withstood heating without disintegrating and they are slightly stained by iodine solution (Figure 1A). Probably only a part of amylose has been released from inside the granules to the continuous phase. Soy protein may prevent the disintegration of granules by limiting their water absorption (GUARDEÑO *et al.* 2012). Another plausible explanation is that proteins may have been absorbed and diffused into the starch granules – thereby preventing the release of amylose (NOISUWAN *et al.* 2011). Starch granules are reported to present pits, channels, and holes at the hilum that are large enough for water or reagents to diffuse into the starch granules (BALDWIN *et al.* 1994). Although in RNO sauces the continuous phase has a high capacity for dispersion, some of the gelatinised granules that have not disintegrated during the preparation of the sauce appear to interact with each other and with soy protein to form large protein-granule aggregates. These aggregates would form part of the dispersed phase and may influence the sauce viscosity and stability. The fat globules are homogeneously distributed throughout the continuous phase and form clusters in some areas.

Table 1. Preference-acceptance test for white sauces – 5-point hedonic scale (5 = like extremely, and 1 = dislike extremely) (mean \pm SD)

Sauce	Appearance	Texture	Flavour
RMI	2.76 \pm 0.70 ^a	3.00 \pm 0.77 ^{ac}	2.24 \pm 0.83 ^a
RMO	3.75 \pm 0.78 ^b	3.60 \pm 0.99 ^a	3.00 \pm 1.30 ^b
RNI	1.71 \pm 0.78 ^c	1.71 \pm 0.78 ^b	2.52 \pm 1.17 ^{ab}
RNO	2.67 \pm 1.42 ^a	2.62 \pm 1.36 ^c	2.85 \pm 1.28 ^{ab}

^{a-c} values in the same column without a common letter are significantly different ($P < 0.05$)

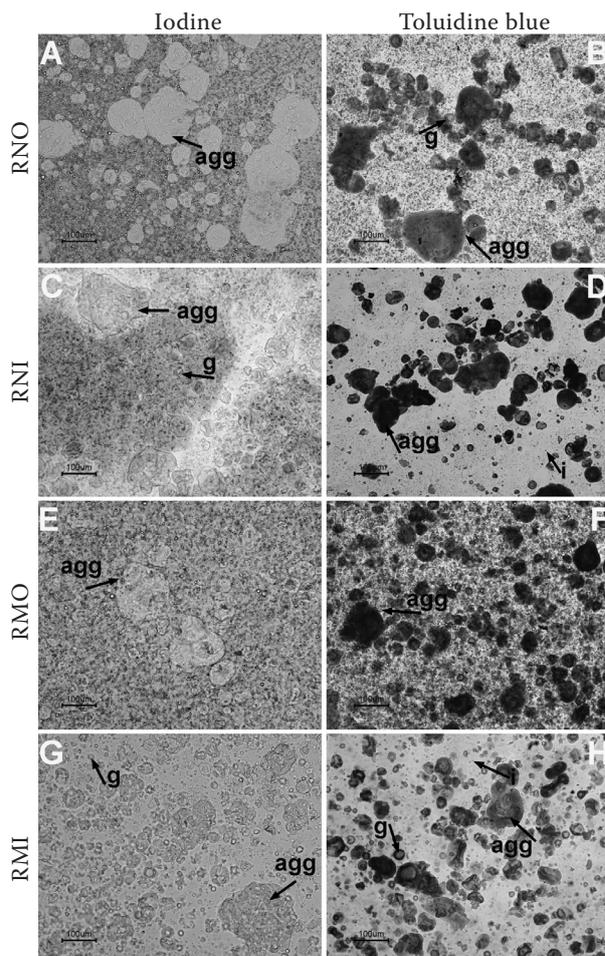


Figure 1. Light microscopy of the sauces. Staining with iodine and toluidine blue (agg – aggregates; g – starch granule; i – inulin)

In the white sauce made with native rice starch and inulin (RNI), the continuous phase consisting of inulin, soy protein, and starch polymers appears to be lighter and improves the diffusion of the gelatinized starch granules (Figure 1D) compared to the RNO sauce. Inulin binds water molecules and so favours homogeneous diffusion of soy protein and starch polymers. Like in the RNO sauces, a high proportion of starch granules resist the heat process without disintegrating. These granules appear swollen and slightly more stained by iodine solution (Figure 1C) than the RNO sauces (Figure 1A). This is probably because less amylose is released. Inulin also appears to have a protective effect on the granules, which show reduced swelling capacity and greater resistance to degradation. This may be partially due to competition between inulin and starch to bind with the water. Moreover, soluble inulin (in addition to protein) may disseminate within a granule and by interacting with

amylopectin it may hinder the release of amylose. Although the continuous phase shows a high diffusion capacity (Figure 1C), the gelatinised granules interact with soy protein to form protein-granule aggregates which seem to be more scattered than in the RNO sauces. Small crystals of inulin form part of the dispersed phase and are distributed throughout the continuous phase (Figure 1D).

When the white sauce is prepared with modified rice starch and sunflower oil (RMO) (Figures 1E and F), the continuous phase consisting of soy protein and starch polymers shows a good dispersal of granules of gelatinised starch. There is no observable tendency to form networks or branches between the components of the continuous phase in the RMO sauce (Figure 1E). The starch granules, which together with the fat globules constitute the dispersed phase, are highly heat resistant (Figure 1F). The RMO sauces also tend to form soy protein aggregates and gelatinised starch granules in the same way as RNO and RNI sauces. The RMO sauces generally present a continuous phase that disperses the granules, although these sauces also contain a high proportion of swollen granules. These two characteristics could establish an adequate viscosity-stability relationship in the sauces. The fat globules homogeneously distributed throughout the continuous phase form clusters like in the RNO sauce.

When the sauce is made with modified rice starch and inulin (RMI), the continuous phase (consisting of soy protein and polymers of starch and inulin) appears very light and the granules seem to have a greater dispersion capability (Figure 1H) than in the RMO sauces (Figure 1F). Inulin limits the formation of networks between the protein and the starch polymers and so a homogeneous continuous phase can be observed. Few inulin crystals distributed throughout the continuous phase can be observed (Figure 1H). Like in the RMO sauce, a high proportion of the starch granules is swollen but not disintegrated. Aggregates of soy protein and soy protein-gelatinised starch granules can also be observed; however, the latter appear in smaller numbers and are more dispersed.

Apparent viscosity (η_{app})

The sauces do not experience significant differences ($P > 0.05$) in the η_{app} values during the first 5 days of storage (Figure 2). At the beginning of storage, RMO sauces present significantly ($P <$

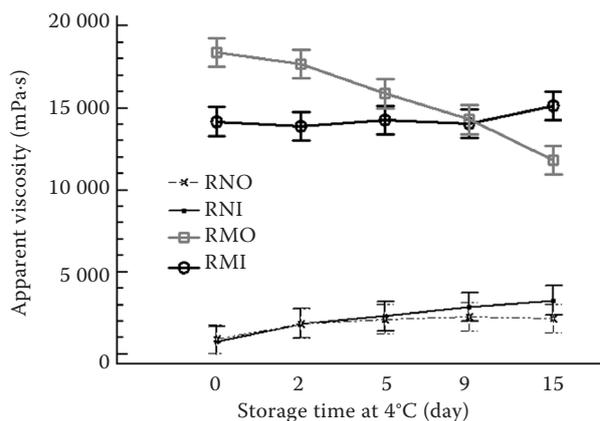


Figure 2. Type of sauce-storage time interaction plot for η_{app} of the sauces

0.05) higher η_{app} values than the RMI sauces. As observed in the microstructural study, RMO sauces show a lower dispersal of gelatinised granules and soy protein-granule aggregates than the RMI sauces. A tendency of η_{app} to diminish becomes significant ($P < 0.05$) after day 9 of storage in the RMO sauce, while the η_{app} values for the RMI sauces remain constant throughout the storage period. This may be due to a higher proportion of non-disintegrated gelatinised starch granules in the modified starch sauces. The sauces prepared with RM (RMO and RMI) maintained significantly ($P < 0.05$) higher η_{app} values than the sauces made with native rice starches (RNO and RNI) throughout the entire storage period. As observed in the microstructural study, RMO and RMI sauces show a high percentage of swollen starch granules that have not disintegrated. Sauces made with RN maintained constant η_{app} values during the storage period overall. Since inulin preserves the integrity of the granule and generates a more homogeneous continuous phase, sauces containing inulin instead of sunflower oil generally maintain more stable η_{app} values throughout the storage period.

Synaeresis

None of the studied sauces showed synaeresis during 15 days of storage at 4°C and, therefore, they are stable when refrigerated. Complex interactions between amylose chains from the swollen starch granules and the continuous phase may prevent the rearrangement of the amylose chains. The presence of soy protein-starch granule aggregates, observed in all the studied sauces, may favour

their stability by preventing the rearrangement of the amylose chains (in the case of RN) and amylopectin (in the case of RM). The use of soy protein seems suitable to make sauces that resist syneresis regardless of the rice starch type. The replacement of oil by inulin does not seem to influence the sauce stability.

Colour

Figure 3a shows the evolution of lightness (L^*) during 15 days of storage at 4°C. The RNO sauce shows the significantly ($P < 0.05$) highest values of L^* during storage. The sauces made with sunflower oil (RMO and RNO) generally show significantly ($P < 0.05$) higher L^* values than those made with inulin (RMI and RNI) throughout the entire storage period. Both RNO and RMO sauces show stable L^* values without significant differences ($P > 0.05$) during the 15 days of storage. The RMI sauce shows significantly ($P < 0.05$) lower L^* values during storage. These values remain stable until the 9th day, but a significant ($P < 0.05$) increase of L^* is seen on the 15th day of storage. It seems that sunflower oil has the greatest influence on the parameter values of L^* . The RNI sauce reveals a significant ($P < 0.05$)

decrease of L^* on the second day of storage and the values remain stable from this day onwards.

Regarding the ΔE^* values (Figure 3b), they are significantly greater than those ($P < 0.05$) for the RNI sauce from day 2 of storage, and so this difference would be noticeable to the human eye (FRANCIS & CLYDESDALE 1977). From this day onwards no significant changes in ΔE^* occur during the 15 days of storage. However, the colour differences in RMI, RMO, and RNO sauces would not be noticeable to the human eye during the entire storage period. It seems that both inulin and the type of starch influence ΔE^* , this effect being more pronounced when RN is used with inulin.

In summary, inulin affects the values of L^* and ΔE^* when RN is in the formulation, probably because there are interactions between inulin and retrograded amylose polymers that do not occur when RM is used.

Sensory analysis

When evaluating appearance, there are no significant differences ($P > 0.05$) between the RMI and RNO sauces, but that there are significant differences ($P < 0.05$) between these sauces and the RMO and RNI sauces. The sauces made with oil and RM (RMO) are the best evaluated for appearance. The RNI sauce is the worst evaluated for appearance, and is also the sauce that shows the greatest changes in colour. Replacing inulin by sunflower oil positively influences appearance when the sauce is made with RM. No significant differences ($P > 0.05$) in texture exist between the RMI and RMO sauces, or between the RMI and RNO sauces. The best texture is generally evaluated in the sauces made with RM (RMO and RMI), regardless of whether they are made with inulin or sunflower oil. There are significant differences between RNI and RNO sauces, and the worst texture evaluations are given to those made with inulin and RN (RNI). When the sauce is made with RN, inulin negatively affects texture. In terms of flavour, the best evaluated sauce is the RMO sauce, while the worst evaluated is the RMI sauce. However, there are no significant differences ($P > 0.05$) between those made with RMO, RNI, and RNO. In general for the three attributes, the sauces made with oil and modified rice starch are the best evaluated. However, according to the statistical analysis, replacing oil with inulin may

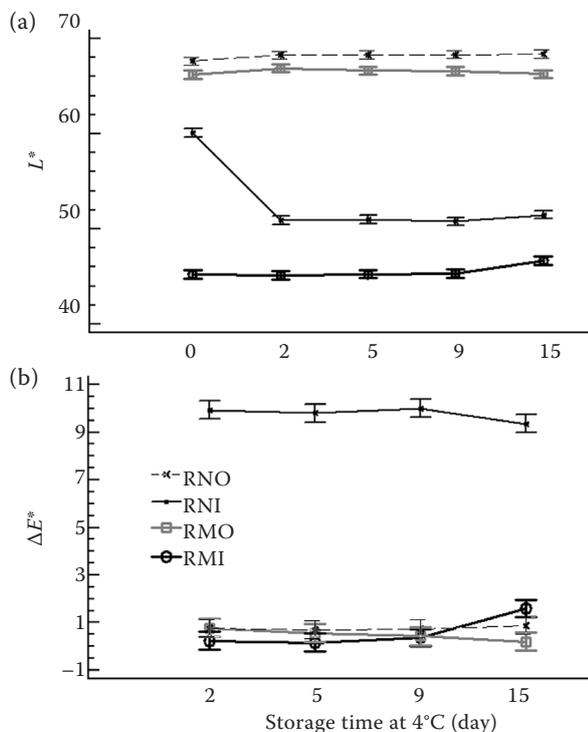


Figure 3. Type of sauce-storage time interaction plot for L^* (a) and ΔE^* (b) of the sauces

be adequate when making gluten and lactose-free low-calorie white sauces with RM.

CONCLUSIONS

Inulin favours the dispersion of gelatinized granules and soy protein-starch granule aggregates and this reduces the viscosity of modified starch sauces. However, inulin maintains viscosity stable during refrigerated storage. The use of soy protein produces sauces without syneresis, regardless of the type of rice starch used and the use of sunflower oil or inulin. The most significant colour changes occur in sauces made with native starch and inulin. This fact together with their lower viscosity produces higher rates of rejection during sensory evaluations. In contrast, the use of modified rice starch with inulin and soy protein is accepted by consumers and is suitable for formulating gluten and lactose-free sauces that are stable when stored in refrigerators.

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Corresponding author:

Dr AMPARO QUILES, Universitat Politècnica de València, Departamento de Tecnología de Alimentos, Camino de Vera s/n, 46022 València, Spain; E-mail: mquichu@tal.upv.es
