Effect of Microwave Thawing on Microstructure and Physicochemical Stability of Low Fat White Sauces Made with Soy Protein

Luis M. GUARDEÑO[1], Amparo QUILES[1], Empar LLORCA[1], José PERTUSA[2] and Isabel HERNANDO[1]

[1]Research Group of Food Microstructure and Chemistry, Department of Food Technology, Polytechnic University of Valencia, Valencia, Spain; [2]Department of Functional Biology and Physical Anthropology, Faculty of Biological Sciences, University of Valencia, Valencia, Spain

Abstract

Guardeño L.M., Quiles A., Llorca E., Pertusa J., Hernando I. (2013): **Effect of microwave thawing on microstructure and physicochemical stability of low fat white sauces made with soy protein**. Czech J. Food Sci., **31**: 568–574.

The microstructural and physicochemical stability of white sauces made with soy protein and modified waxy maize starch was evaluated after subjecting them to a freezing-thawing process in a conventional or microwave oven. The microstructure of sauces revealed a structured matrix of soy protein and starch polymers where fat globules and swollen starch granules remain dispersed. Both thawing methods affected the fat globule size and morphology but they did not affect the starch granules. The SDS-PAGE analysis did not show any apparent changes between sauces thawed by both methods. Moreover, a similar pattern was found in the soy protein isolate used as a raw material indicating that this type of protein was also stable to the cooking process. There were no significant differences ($P > 0.05$) in the reducing power of the sauces regardless of the thawing method used. However, the acidity index and the k_{232} parameter were significantly higher ($P < 0.05$) in conventionally thawed sauces. Finally, syneresis was negligible and no significant differences ($P > 0.05$) were found among different frozen storage periods. In conclusion, the formulation of the sauce is appropriate to develop low fat, vegetarian meals which can be subjected to frozen storage and microwave reheating.

Keywords: Confocal laser scanning microscopy (CLSM); microwave heating; SDS-PAGE; soy protein isolate; starch

Demand for ready-to-eat products (RTE) has steadily increased in recent years due to new consumption habits and less available time for cooking. White sauces are frequently used in the formulation of these products not only to improve their taste but also to conduct heat during cooking (Arocas *et al.* 2009a). RTE are usually thawed and reheated in a microwave oven (MO), which has become a common appliance because of its quickness, ease of use, and energy savings in comparison with other cooking and reheating appliances such as conventional oven (CO). In fact, there is a trend to design microwavable food based on the performance of different formulated products submitted to microwave heating (Serventi *et al.* 2011). There is some research on the effect of microwave heating on the physicochemical characteristics of milk and vegetables (Villamiel *et al.* 1996; Alajaji & El-Adawy 2006), vegetable oils (Albi *et al.* 1997) and carbohydrates (Palav

Support by the Ministerio de Ciencia e Innovación, Project No. AGL2006-11653-C02, and the FPU grant awarded to L.M. Guardeño.

& Seetharaman 2007). However, there is a lack of information related to more complex food systems. Thus, the aim of this study was the analysis of physicochemical parameters and microstructure in a ready-to-eat microwavable white sauce made with soy protein after different thawing methods.

MATERIAL AND METHODS

Materials and sauce preparation. The white sauce was made from modified waxy maize starch (CHS) (cross-linked hydroxypropyl distarch phosphate, Polar Tex 06748; Cargill, Inc., Minneapolis, USA), soy protein isolate (SPI) (Vicoprot-S; Trades S.A., Tarragona, España), sunflower oil (Koipesol; SOS Cuétara S.A., Madrid, Spain), ι-carrageenan (Secovis IS; Hispanagar, Burgos, Spain), sodium chloride (Panreac Química SAU, Barcelona, Spain) and distilled water.

The white sauce consisted of CHS (4.5% w/w), SPI (3.2% w/w), sunflower oil (2.5% w/w), i-carrageenan (0.5% w/w), sodium chloride (0.4% w/w) and water up to 100% (w/w). The samples were prepared according to Arocas *et al*. (2009b), were cooled to room temperature and stored at –18°C for 168 hours.

Thawing methods. A batch of sauces was thawed in a microwave oven at 700 W for 6 min, and another batch was thawed in a conventional oven at 220°C for 30 minutes. The thawing conditions were selected in order to completely thaw the samples, i.e. the temperature was at least 1°C at the centre of the bottles.

Temperature measurements. A hole was drilled to the centre of frozen samples. The temperature during microwave or conventional thawing was measured by positioning the tip of an ST3 optical fibre probe connected to a FOTEMP1-OEM R signal conditioner (OPTICOM, Dresden, Germany) or positioning the tip of a type K thermocouple connected to a thermometer (TES-1300; Electrical Electronic Co., Taipei, Taiwan), respectively.

Microstructural analysis. A Nikon confocal microscope C1 unit that was fitted on a Nikon Eclipse E800 microscope was used (Nikon, Tokyo, Japan). An AR laser line (488 nm) was employed to excite the fluorescent dyes rhodamine B and Nile red. Fifteen randomly acquired images were taken per sample at 1024 × 1024 pixel resolution. Stacks of images were also obtained by automatically scanning 20 mm depth through the sample in order to create 3D reconstructions.

Images were binarised after greyscale threshold segmentation and analysed using the ImageJ software (National Institutes of Health, Bethesda, USA). The 80th percentile (P_{80}) of the fat globule area was measured; this parameter indicates that 80% of the globules had an area below this value. Fat globule circularity was also determined according to Equation 1:

$$\text{Circularity} = 4\pi \frac{\text{area}}{\text{perimeter}^2} \quad (1)$$

Physico-chemical analysis

Lipid fraction. Lyophilised samples were subjected to a lipid extraction using a semi-automatic extractor (Soxtec 2055; Foss, Höganäs, Sweden). The acidity index was determined in accordance with AOAC (1998). The K_{232} and K_{270} parameters were determined by spectrophotometric analysis (UNE 1973).

Water-soluble protein fraction. Lyophilised and defatted samples (2 g) were mixed with distilled water (15 ml) and centrifuged at 15 000 rpm (4°C) for 15 minutes. Finally, the supernatant was transferred to a volumetric flask. This process was repeated twice more. The extracted water-soluble fraction was quantified by the Kjeldahl method of AOAC (1998).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out. The previously lyophilised and defatted sample (1 g) was thoroughly mixed with 20 ml of extraction buffer (60mM tris-HCl, pH 8). The sample was then centrifuged at 15 000 rpm for 15 minutes. Aliquots were then mixed with non-reducing (2% SDS, 60mM tris-HCl, pH 6.8) or reducing (2% SDS, 60mM tris-HCl, 0.1M dithiotreitol pH 6.8) buffers. Electrophoresis was performed using 12.5% polyacrylamide precast gels (ExcelGel SDS Homogeneous; GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

Carbohydrate fraction. The measurement of reducing sugars was carried out using the Shaffer-Somogyi iodometric method (AOAC 1998). Anhydrous glucose was used as standard and the results were expressed as mg glucose/g sample (dry basis).

Syneresis. Syneresis was measured in freshly made and frozen-stored samples (48, 96, 168 h). Samples were centrifuged at 6000 *g* during 15 min

after equilibration at 20°C. The quantity of water released on the top was decanted and the % of syneresis was calculated (Eq. 2):

$$\% \text{ syneresis} = (\text{weight of decanted liquid/total weight before centrifugation}) \times 100 \quad (2)$$

Statistical analysis. The physico-chemical analysis were measured in triplicate. The statistical analysis was carried out by ANOVA and the least significant differences (*LSD*) were calculated at a $P < 0.05$ significance level. The statistics software Statgraphics Plus version 5.1 (Manugistics, Inc., Rockville, USA) was used.

RESULTS AND DISCUSSION

Microstructure

Freshly made sauces (Figure 1A) show a matrix composed of a continuous phase of soy protein, i-carrageenan and starch polymers leached out of the swollen starch granules that act as a dispersed phase. These starch granules have been chemically modified (crosslinking and substitution) in order to reduce retrogradation and to prevent disruption under heat and shear conditions and so they have partially resisted the cooking process. Some starch granules seem to be brighter, which could be related to the presence of soy protein on the granule surface as Noisuwan *et al.* (2011) observed in milk protein-rice starch mixtures. Alternatively, these brighter areas could correspond to a lower degree of gelatinisation in contrast to other starch granules that seem to be empty and appear darker. Soy protein could compete for water with starch granules and so a high amount of starch polymers could remain inside them giving this brighter appearance. Fat globules were dispersed through the continuous phase mainly associated to protein.

Table 1. Mean values of the 80th percentile of fat globule area (μm^2) of freshly made and thawed sauces (mean ± SD)

Treatment	Area (μm^2)	Circularity
F	11.74 ± 2.75^a	0.874 ± 0.057^a
MO	26.75 ± 6.06^b	0.832 ± 0.040^b
CO	26.34 ± 4.79^b	0.839 ± 0.027^b

F – freshly made sauces; MO – sauces thawed in microwave oven; CO – sauces thawed in conventional oven; ^{a,b}values with different letters within the same column are significantly different ($P < 0.05$) according to the *LSD* multiple range test

In thawed samples (Figures 1B and C), it can be seen that protein tended to aggregate. As fat globules were stabilised by the protein phase, they approximated to each other forming fat globule clusters that finally coalesced. There were significant differences ($P < 0.05$) in the P_{80} and circularity values between freshly made and both thawing methods, but no differences were found between conventional and microwave thawing (Table 1). These results were in accordance with previous works on sauces made with skimmed milk where the fat globule area increased after thawing due to coalescence (Guardeño *et al.* 2009). Regarding starch granules, there was no apparent increase in granule swelling after thawing. This fact could be related to a high water-binding capacity of soy protein that could limit further starch swelling once it is cooked. The high water-binding capacity of soy protein has also been stated in starch-soy

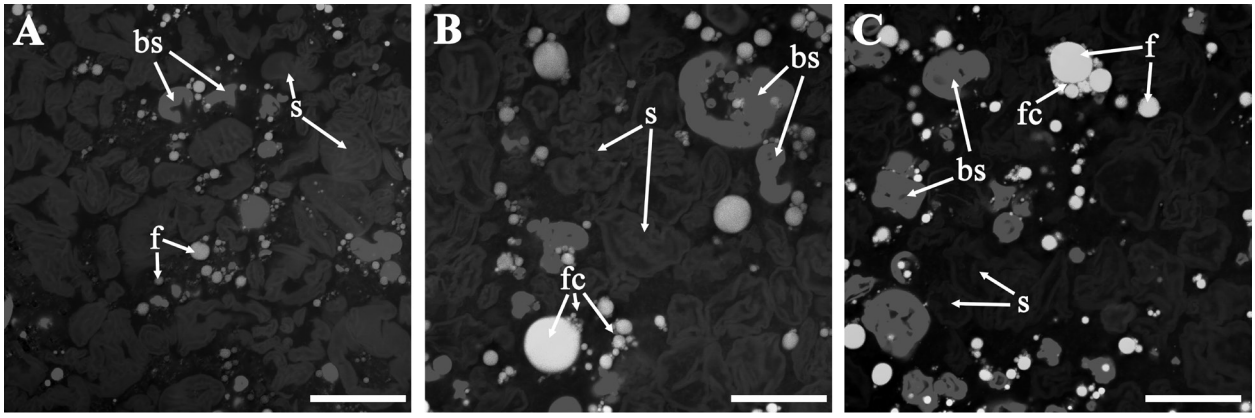

Figure 1. CLSM micrographs: (A) freshly made sauces, (B) sauces thawed in a microwave oven, and (C) sauces thawed in a conventional oven (f – fat globule; fc – fat globule cluster; s – starch granule; bs – bright starch granule; bar 50 μm)

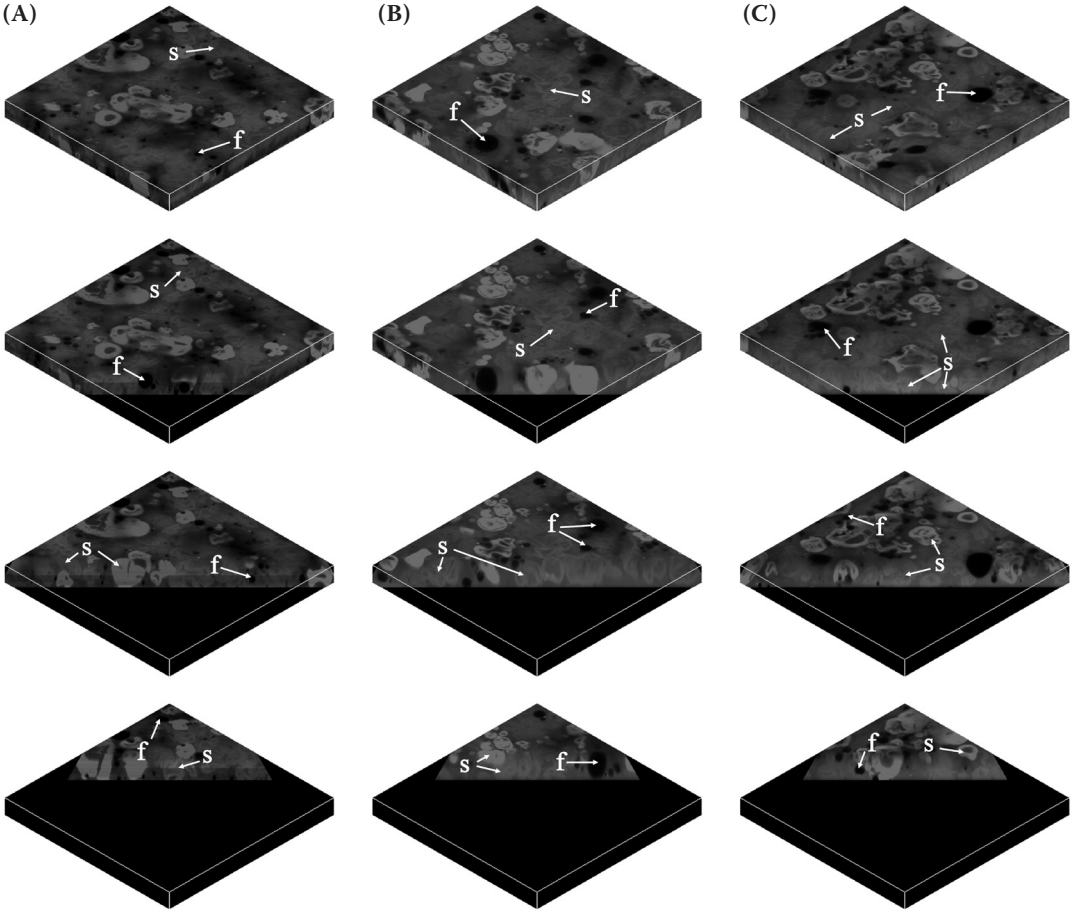

Figure 2. 3D reconstructions: (A) freshly made sauces, (B) sauces thawed in a microwave oven, and (C) sauces thawed in a conventional oven (f – fat globule; s – starch granule)

protein doughs (Serventi *et al.* 2011). 3D image reconstruction (Figure 2) enabled a detailed analysis of the irregular morphology of starch granules which did not apparently change after thawing.

Lipid fraction

Table 2 shows the mean values of the lipid parameters. The acidity index and K_{232} values were significantly higher in conventionally thawed sauces than in both freshly made and microwave-thawed sauces. This fact could be related with the more prolonged thawing time in CO in comparison with MO to reach the same temperature at the centre of the sample (Figure 3). As CO-thawed samples are thawed during 30 min, the temperature in the outer areas of the bottles will be significantly higher than at the centre and thermal lipolysis and oxidation could occur. Albi *et al.* (1997) concluded

Table 2. Means of the chemical parameters. freshly made sauces (F), microwave-thawed sauces (MO), and conventionally thawed sauces (CO) (mean ± SD)

Sauce	Parameters				
	acidity index	K_{232}	K_{270}	soluble protein (g/100g)	reducing power (mg glucose/g sample dry basis)
F	$0.50^{a} \pm 0.04$	$5.32^{a} \pm 0.21$	$1.96^{a} \pm 0.18)$	$0.49^{a} \pm 0.06$	$2.34^{a} \pm 0.34$
MO	$0.51^{a} \pm 0.04$	$5.54^{a} \pm 0.32$	$1.98^{a} \pm 0.08$	$0.53^{ab} \pm 0.04$	$2.61^{a} \pm 0.28$
CO	$0.61^{b} \pm 0.07$	$6.39^{b} \pm 0.32$	$2.10^{a} \pm 0.16$	$0.56^{b} \pm 0.03$	$2.44^{a} \pm 0.29$

[a]values with the same letter are not significantly different ($P < 0.05$) according to the *LSD* multiple range test

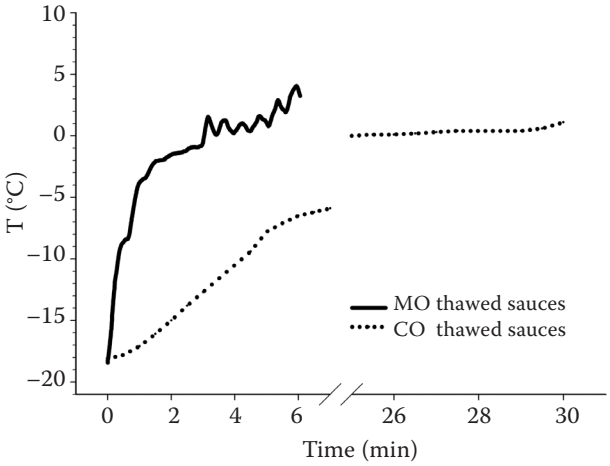

Figure 3. Temperature profiles during thawing (MO – microwave oven; CO – conventional oven)

that the values of K_{232} and K_{270} in different fats were affected by the increase in the temperature rather than by microwave effects.

Protein fraction

The conventionally thawed sauce had significantly higher values of water soluble protein than the freshly made one (Table 2). This fact was not expected since heating processes lead to protein denaturation which in turn should decrease the amount of soluble protein fraction (Sorgentini *et al.* 1995). However, this type of sauce is a complex system where protein could interact with leached starch polymers and swollen starch granules that have not been totally disrupted during gelatinisation. It has been stated that soy protein could adsorb to starch granules by hydrophobic or hydrogen bonds depending on the characteristics of the granule surface components (Ryan & Brewer 2007). Thereby, it could be possible that in the freshly made sauce some protein could remain adsorbed to the starch granules and so it was not extracted in the supernatant after centrifugation. Afterwards, when samples were subjected to a long thawing process, i.e. conventional thawing, these starch-protein interactions could be weakened and higher soluble protein content could be achieved.

Figure 4A shows the electrophoretic profile of samples in the presence of dithiotreitol (DTT). There was a group of bands between 48 and 78 kDa which correspond to β-conglycinin subunits: α' (77 kDa), α (71 kDa), and β (46 kDa). The two main polypeptides forming the glycinin subunits can also be seen – acid polypeptide A (38 kDa) and basic B (23 kDa). These molecular weights are in agreement with those reported by other authors (Li *et al.* 2007).

In Figure 4B, the electrophoretic profile of samples in the absence of DTT is shown. It is similar

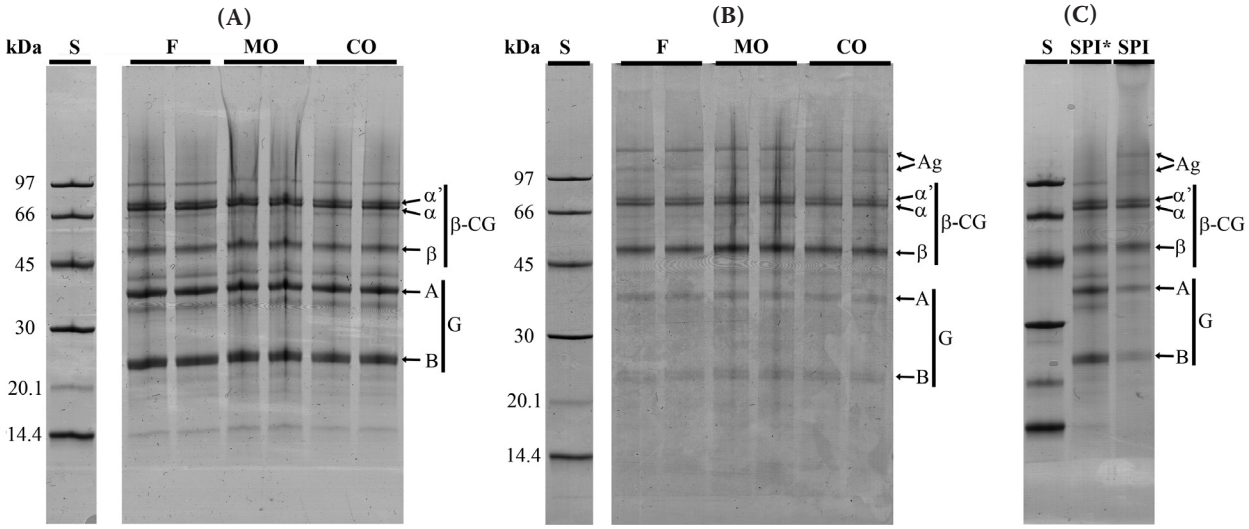

Figure 4. SDS-PAGE: (A) freshly made and thawed samples under reducing conditions, (B) freshly made and thawed samples under non-reducing conditions, and (C) electrophoresis of soy protein isolate under both reducing and non-reducing conditions (S – low molecular weight standard; F – freshly made sauces; MO – microwave-thawed sauces; CO – conventionally thawed sauces; SPI* – soy protein isolate under reducing conditions; SPI – soy protein isolate under non-reducing conditions; β-CG – β-conglycinin; α', α, β – β-conglycinin subunits; G – glycinin; A, B – glycinin polypeptide chains; Ag – protein aggregate)

Table 3. Mean values of syneresis during frozen storage (mean ± SD)

Frozen storage period (h)	0	48	96	168	336
Syneresis (%)	0.39 ± 0.08[a]	0.40 ± 0.18[a]	0.45 ± 0.17[a]	0.40 ± 0.18[a]	0.41 ± 0.17[a]

[a]values with the same letter are not significantly different ($P < 0.05$) according to the *LSD* multiple range test

to that obtained under reducing conditions and the bands corresponding to the β-conglycinin are also observed. However, there are bands (100 kDa) which were not observed in the electrophoresis conducted in reducing conditions. This fact indicates the presence of disulphide bonds. Li *et al*. (2007) reported that the formation of these aggregates implied covalent and no covalent unions between a and α' subunits of β-conglycinin and A and B glycinin subunits. In fact, a faint staining was observed in the latter proteins in comparison with those in Figure 4A corresponding to reducing conditions.

The freshly made sauces had the same electrophoretic profile (Figure 4C) as the SPI used as raw material, which indicated that the cooking process did not cause any detectable protein changes by SDS-PAGE. Moreover, the thawing method did not affect the electrophoretic profile of the different samples since there were no noticeable changes in the profiles corresponding to MO-thawed samples and CO-samples and between these ones and the freshly made samples.

Carbohydrate fraction

Starch, although having terminal oxidable groups, has such a molecular size that the reducing power of the molecules is negligible. Thus, an increase in the reducing power could only occur in the case of starch degradation and release of sugars during cooking or thawing. There were no significant differences in the reducing power values among the different sauces, which indicated the stability of the carbohydrate fraction in relation to both thawing methods (Table 2).

Syneresis

There were no significant differences ($P < 0.05$) in syneresis values during the storage period while the amount of the liquid released after centrifugation was negligible. The use of a waxy starch, with low amylose content, limited the retrogradation and thus the syneresis phenomena. In addition, modification of the starch by means of chemical substitution interrupts the association of starch polymers having an extra protective effect against retrogradation. Finally, the soy protein could improve the water retention capacity of the sauce and favour the freezing-thawing process.

CONCLUSIONS

White sauces formulated with modified maize starch and soy protein were physico-chemically stable to different thawing methods and microstructure analyses revealed no differences between conventionally and microwave-thawed sauces. This sauce formulation is appropriate for microwave thawing which can be preferable to conventional thawing due to energy savings, speed and convenience. Besides, the use of soy protein and this type of starch in the formulation make this sauce suitable for vegetarian, low-fat meals that can be consumed by celiacs.

References

Alajaji S.A., El-Adawy T.A. (2006): Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. Journal of Food Composition and Analysis, **19**: 806–812.

Albi T., Lanzón A., Guinda A., Leon M., Perez-Camino M.C. (1997): Microwave and conventional heating effects on thermoxidative degradation of edible fats. Journal of Agricultural and Food Chemistry, **45**: 3795–3798.

AOAC (1998): Official Methods of Analysis. Association of Official Analytical Chemists International, Arlington.

Arocas A., Sanz T., Fiszman S.M. (2009a): Improving effect of xanthan and locust bean gums on the freeze-thaw stability of white sauces made with different native starches. Food Hydrocolloids, **23**: 2478–2484.

Arocas A., Sanz T., Fiszman S.M. (2009b): Influence of corn starch type in the rheological properties of a white sauce after heating and freezing. Food Hydrocolloids, **23**: 901–907.

Guardeño L.M., Llorca E., Pérez-Munuera I., Quiles A., Hernando I. (2009): Chemical and structural changes in white sauces thawed by microwave or conventional oven. Czech Journal of Food Sciences, **27**: 28–30.

Li X., Li Y., Hua Y., Qiu A., Yang C., Cui S. (2007): Effect of concentration, ionic strength and freeze-drying on the heat-induced aggregation of soy proteins. Food Chemistry, **104**: 1410–1417.

Noisuwan A., Hemar Y., Wilkinson B., Bronlund J.E. (2011): Adsorption of milk proteins onto rice starch granules. Carbohydrate Polymers, **84**: 247–254.

Palav T., Seetharaman K. (2007): Impact of microwave heating on the physico-chemical properties of a starch-water model system. Carbohydrate Polymers, **67**: 596–604.

Ryan K.J., Brewer M.S. (2007): *In situ* examination of starch granule-soy protein and wheat protein interactions. Food Chemistry, **104**: 619–629.

Serventi L., Sachleben J., Vodovotz Y. (2011): Soy addition improves the texture of microwavable par-baked pocket-type flat doughs. Journal of Thermal Analysis and Calorimetry, **106**: 117–121.

Sorgentini D.A., Wagner J.R., Anon M.C. (1995): Effects of thermal treatment of soy protein isolate on the characteristics and structure-function relationship of soluble and insoluble fractions. Journal of Agricultural and Food Chemistry, **43**: 2471–2479.

UNE (1973): Fats. Spectrophotometric measure of absorption in the ultraviolet area. Asociación Española de Normalización y Certificación (AENOR).

Villamiel M., Corzo N., Martínez-Castro I., Olano A. (1996): Chemical changes during microwave treatment of milk. Food Chemistry, **56**: 385–388.

Received for publication November 18, 2012
Accepted after corrections May 24, 2013

Corresponding author:

Luis Miguel Guardeño, PhD, Universidad Politécnica de Valencia, Department of Food Technology, Research Group of Food Microstructure and Chemistry, Camino de Vera s/n, 46022 Valencia, Spain; E-mail: luiguaex@upvnet.upv.es