Conformational changes of polymers in model batter systems

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Abstract

Cake batters - made of flour, egg, sugar and fat - are complex systems. Ingredients interactions and their impact on protein secondary structure and starch conformational structures were studied in model batter systems using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy. The results showed the possibility of using the pregelatinized starch ~~for improving the texture of the cake~~ without affecting protein conformation. The estimation of protein secondary structure highlighted the prevalence of α-helical structures in the model batter system, while β-sheets are predominant in flour systems as known in dough systems. The protein conformation in batter system are related to fat-protein interactions and could explain fat functionality in the final product. Starch crystallinity increased when each ingredient - except for pregelatinized starches - was added to the flour. Changes in starch conformation could be related to the redistribution of water between the batter ingredients. The overall results highlighted the importance of ingredients on the structural conformation of the batter polymers - starch and proteins - which could be the key factor to understand the functional properties of the batter.

Keywords: cake batter; pregelatinized starch; protein secondary structure; starch structure; ATR-FTIR spectroscopy

1. Introduction

Quality control has become a highly important topic in food industry. The quality of cakes depends on the balanced formulas, aeration of cake batters, stability of fluid batters in the early stage of baking, and thermal-setting stage (Gomez et al., 2007). In addition, macromolecular interactions are highly considered to influence the baked-products quality (Bruun, 2006). Cakes - as all baking products - are basically composed of molecular and colloid dispersions of biopolymers and their complexes (Bennion & Bamford, 1997). Starch and protein - the major two biopolymers - are fundamental to the structure, rheology, and other physical properties, as well as the sensory perception of these products. Water and lipids, bound to other components or acting as solvents, are important factors as well (Bruun, 2006).

Starch represents an important constituent acting in two ways: during batter mixing, starch with the other components of flour hinders fat coalescence by increasing the viscosity of the aqueous phase (Shepherd & Yoell, 1976), while during baking starch is responsible for the transformation of an aqueous, fluid batter into a solid, porous cake structure (Donovan, 1977). As regards gluten, full development of gluten into a continuous visco-elastic structure such as it occurs in bread making does not happen in cakes due to the formulations and mixing procedures (Donelson & Wilson, 1960). Although the development of a gluten network is limited in cake batter, gluten proteins may become important for cake structure during baking (Wilderjans et al., 2008).

Egg proteins, during mixing, are responsible for the formation of a stable emulsion (Wilderjans et al., 2013). Mine (2002) assumed that yolk proteins adsorb at the oil-water interface in the cake batter which contributes to film formation that stabilizes the fat coalescence.

Fats retain the gas cells at room temperature during mixing, making them immobile and hence stable (Delcour & Hoseney, 2010). These gas cells are then used as nuclei during cake baking.

Sugar has an abrasive effect on the fat and promotes the breakdown of crystal aggregates during mixing into smaller size crystals which are the most effective for air incorporation (Shepherd & Yoell, 1976). It also competes with gluten proteins for water, promoting a weaker gluten development (Donelson & Wilson, 1960).

Finally, recently it was demonstrated that adding pregelatinized starch improves cake softness and softness retaining during storage (Sozer et al., 2011, Hesso et al., 2014).

From the above, it is clear that the cake batter functional properties reflect the physico-chemical properties of both the complexed and the individual macromolecules, which contribute to the diversity of structures (Bruun, 2006). One way to understand these functionalities is to study the chemical interactions taking place between its ingredients during mixing. Kaddour et al. (2008) showed that during batter mixing chemical interactions take place between the ingredients and induce conformational changes in proteins and starch structures. These chemical changes can be assessed by spectroscopic investigations as FTIR spectroscopy, which is a particularly powerful tool for probing the secondary structures and conformations of protein/polysaccharides/lipid/water (Subramanian & Rodriguez-Saona, 2009; Sivam et al., 2012; Sivam et al., 2013). Moreover in combination with new methods of sample presentation such as Attenuated Total Reflectance (ATR) - which enables analysis of food matrix directly in the solid or liquid state without further preparation - FTIR allows the acquisition of high-quality spectra with high reproducibility from previously quite intractable materials (Wilson et al., 1988; Van de Voort et al., 1992). ATR-FTIR spectroscopy has been used to probe gluten secondary structure in dough under various conditions and formulations (Li et al., 2006; Wellner et al., 2005, Kaddour et al., 2008; Meziani et al., 2011; Sivam et al., 2012; Bock & Damodaran, 2013; Bock et al., 2013; Wang et al., 2014; Jazaeri et al., 2015). More recently, the poor quality of bran-enriched bread was explained by the conversion of β-turn structure into β-sheet and random structures when bran is added to the gluten or to wheat (Bock et al., 2013; Bock and Damodaran, 2013).

As regards starch, previous works have used this technique to estimate the amount of ordered or crystalline domains of starch or to study the starch gelatinization and retrogradation (Wilson et al., 1987; Wilson et al., 1988; Wilson & Belton, 1988; Van Soest et al., 1995; Flores-Morales et al., 2012; Ambigaipalan et al., 2014).

Despite the few studies carried out on ingredients functionalities in cakes systems (Wilderjans et al., 2008; Wilderjans et al., 2010), all of them were performed only on starch and gluten, neglecting the interactions between macromolecules that occur in the original batter. The aim of the present study was to investigate the role of ingredients interactions on cake batter directly by using ATR-FTIR spectroscopy. The present study explores the conformational changes in wheat proteins and starch in model batter systems prepared from batter ingredients (pregelatinized wheat starch, ~~pregelatinized maize starch,~~ eggs, fat or/and sugar) to reach the final batter system.

2. Materials and methods

2.1. Materials

Wheat flour (protein: 11.6%, fat: 1.3%, starch: 83 %, ash: 0.5 % ~~ash~~; all on dry basis) was supplied by Giraudineau (France). Whole liquid eggs were purchased from Cargill kitchen solutions Inc., Monticello (MN, USA). Sugar was supplied by United Sugar Corporation (MN, USA). Fat consisted of rapeseed oil (70 %) and anhydrous milk fat (30 %) and was supplied by Corman (Belgium). ~~Sodium bicarbonate was supplied by ARM & HAMMER, Princeton (New Jersey, USA).~~ Pregelatinized wheat starch (PWS) ~~and pregelatinized maize starch (PMS) were~~ was supplied by Roquette (France).

2.2. Model batter systems preparations

The reference batter was a pound cake recipe (Hesso et al., 2014): 29.5% wheat flour, 25% sucrose, 25% whole liquid eggs, 20% fat and 0.5% sodium bicarbonate. 20% of flour was replaced by pregelatinized starch for the formula containing PWS.

Model systems were prepared by hand mixing for 5 min the ingredients (flour, PWS ~~or~~ ~~PMS~~, eggs, sugar or/and fat) in the same proportion as present in reference batter, starting with the flour (S1) till reaching a complete and complex model with all ingredients in limited water content ranging from 23 to 39% (Table 1). The water content was chosen depending on the water content in the real batter (around 23%) as in the final model system (S6). For the flour systems (S1 and S2), the addition of water was essential for creating a homogenous mixture. While for flour + egg system (S3), the water was brought by adding liquid egg (77% water content). Freshly prepared batter was covered with a plastic paraffin film to prevent moisture loss. Each system was prepared in duplicate.

Moisture content (Table 1) of each sample was measured by drying the sample at 130°C for 40 min by an infrared balance (MB 45, OHAUS, Parsippany, NJ, USA).

2.3. ATR-FTIR spectroscopy

ATR-FTIR spectroscopy was used to collect spectral data for protein secondary structures and starch crystalline and amorphous structures analysis. A Bruker Tensor 37 (Bruker Optics, Inc., Billerica, MA, USA) was used with a horizontal multi-reflectance zinc selenide (ZnSe) crystal accessory. The instrument housed a deuterated tri-glycine sulfate (DTGS) detector. Spectra were collected in the 4000-600 cm^{-1} infrared spectral range at room temperature. Each spectrum was an average of 32 scans at 4 cm^{-1} resolution. A background spectrum of the empty trough sampling plate was collected before each sample. Spectra were collected within 3 minutes after batter preparation and a minimum of 4 spectra for each sample was used for spectral analysis. The sample was pressed firmly onto the crystal to eliminate air and to achieve better contact. Spectral analysis was performed as described by Bock et al. (2013) using OPUS software v. 7.0. Reference H_2O – D_2O spectra (at 25, 30, and 37% water content) were collected and used to correct the sample moisture content for protein analysis. All spectra were vector-normalized to correct any differences in sample penetration depth. Vector-normalized spectra were subsequently offset corrected before digital subtraction of H_2O – D_2O reference spectra representing the same moisture

154 content. For ingredients spectra, air compensation, base line correction and smoothing were
155 done before normalization.
156
157 *2.3.1. Protein secondary structure estimation*
158 The quantitative estimation of proteins secondary structure in different model systems
159 was determined from second-derivative spectra of a 5-point Savitsky-Golay function
160 according to Bock et al. (2013), in order to facilitate white-noise removal and resolve the
161 individual band component corresponding to specific secondary structure (Kong and Yu,
162 2007). The difference spectra resulting from digital subtraction were second derivated before
163 the analysis of the amide I band (1600 – 1700 cm^{-1}). The characteristic mean absorption
164 frequencies of the secondary structural elements in proteins are listed in Table 2. The
165 secondary structure estimate was determined from the relative area of the peaks centered at
166 these absorption bands (Bock et al., 2013). Each structure was expressed as percentages of
167 the proteins total secondary structures.
168
169 *2.3.2. Starch crystalline and amorphous structures*
170 To study the effect of the ingredients interactions on the starch amorphous and
171 crystalline structures, the region 800-1200 cm^{-1} of normalized spectra was used. The amount
172 of short-range ordering of the starch samples can be expressed by the intensity ratio of the
173 bands most characteristic of crystalline at 1047 cm^{-1} and amorphous at 1022 cm^{-1} starch R
174 (1047/1022) or by using the intensity ratio of the band characteristic of crystalline starch at
175 1047 and 1035 cm^{-1} R' (1047/1035) (Van Soest et al., 1995), where 1035 is the valley
176 between the two bands (1047 and 1022 cm^{-1}). These two ratios could explain the crystallinity
177 in two ways: R (1047/1022) explains the changes due to the modifications in starch
178 crystalline structure and amorphous form, while R'(1047/1035) explains the changes related
179 just to crystalline structure.
180
181 *2.4. Statistical analysis*

Each model system was prepared in duplicate, and at least 4 replicates were carried out for each sample, so that at least eight replicates were performed for each model system. Differences amongst model systems were assessed at the 5% level, using analysis of variance (ANOVA), which was performed by using Microsoft Excel (V. 2013).

3. Results and Discussion

3.1. ATR-FTIR spectra for batter ingredients

Fig.1 shows the ATR-FTIR spectra of all the ingredients. Flour, PWS, ~~PMS~~ and sugar were analyzed in the dry state. The spectra of the ingredients were similar to those reported in previous works (Wilson et al, 1988; Meziani et al., 2011; Sivam et al., 2012 and 2013). The band in the OH stretch region (3000-3700 cm^{-1}) for flour, pregelatinized starches and egg spectra is mostly related to water (Sivam et al., 2013). Wheat flour showed a broad bimodal peak band centered at 3410 cm^{-1} and 3242 cm^{-1}. The former is attributed to small hydrogen bonded clusters, the latter to extensively hydrogen-bonded water and associated chains of water (Jain et al., 1989; Sutandar et al., 1994).The bimodal distribution could be explained by the possibility that not all the water is tightly associated with flour components (Bock et al., 2013). While PWS ~~and PMS~~ showed only one peak related to the OH stretch at 3325 cm^{-1} suggesting that the majority of water could be associated to starch (Bock et al., 2013).The broad peak in the OH stretch region in eggs is related to the high moisture content of this sample (77%). The absorption band in both flour and eggs at 1600-1700 cm^{-1} is attributed to amide I (80% C=O stretch and 10% N-H bending), which is the most sensitive spectral region to the protein secondary structural components (Sivam et al., 2013). The peaks at 800– 1200 cm^{-1} in flour and PWS ~~and PMS~~ spectra are characteristic for starch crystalline and amorphous structures and showed bands at 1077, 1047, 1022, 994, and 928 cm^{-1} (COH bending and CH_2 related modes) (Wilson et al., 1988; Van Soest et al., 1995). Flour spectra exhibited an intense absorption signal at 1019 cm^{-1} – which is characteristics of starch - and weaker features at 1529 (amide II) and 1640 (amide I) cm^{-1} - characteristic bands of gluten – in agreement with relative proportions of starch (62–72%) and gluten (6–13%) in wheat flour.

Eggs showed also a weak absorption intensity in the 3000-2800 cm^{-1} region that corresponds to lipids, which is more intense in fat spectra. Fat spectra exhibited high absorption intensities at 3013 cm^{-1}, 2937 cm^{-1}, and 2856 cm^{-1}, which are associated with the asymmetric stretch of CH_3, the asymmetric stretch of CH_2, and the symmetric stretch of CH_2 of aliphatic fatty acid chains, respectively. It showed an intensive absorption at 1743 cm^{-1} attributed to C=O ester groups and peaks at 1240 cm^{-1}and1195-1129 cm^{-1} are related to C-O stretching (Yang et al., 2005).

Finally, sugar showed intense and characteristic bands in the region between 1200 and 900 cm^{-1} and bands between 2800 and 3700 cm^{-1}. The first bands are assigned to deformation of –CH2 and angular deformation of C–C–H and H–C–O, while the others are related to water (Bureau et al., 2009).

In this study, attention has been paid on the amide I region (1600-1700 cm^{-1}) and starch region (800-1200cm^{-1}) in order to investigate the impact of ingredients addition on gluten secondary structure and starch conformation in cake batter.

3.2. ATR-FTIR spectra for model batter systems

3.2.1. Effect of ingredients interactions on protein conformational structure

The ATR-FTIR absorbance spectra for the batter model systems prepared with flour (S1) and pregelatinized starch (S2) are shown in Fig. 2a. As expected, the intensity of the OH stretch band (3000-3700 cm^{-1}) increased when water was added to flour and flour + PWS systems. In addition, the intensity of the absorption band in the amide I region (1600-1700 cm^{-1}) increased, suggesting changes in protein secondary structure. The relative amount of secondary structures in wheat flour (in dry state) followed the order β-sheet (59.9%) > random (31%) >β-turn (7.8%) >α-helix (1.3%) (Fig. 2b). Water addition to flour (S1) - together with mixing step - changed this order. The secondary structure contents of protein estimated for S1 followed the order β-sheet (48.7%) > random (26%)>α-helix (15.7) >β-turn (9.6%) (Fig. 2b) indicating that water addition rearranged the protein molecules but kept the β-sheet structure as the preferred secondary structure of wheat flour, in agreement with previous

238 studies carried out on wheat flour at various hydration states (35-50% moisture content)
239 (Bock et al., 2013). However, after water addition and mixing, the structure changed from an
240 ordered structure (β-sheets) to an unordered structure (α-helix). The low percentage of β-
241 turns in system 1 is consistent with the fact that the development of a gluten network is
242 limited in batters (Huebner et al., 1999). The effect of gluten development on β-turns
243 structures has been well documented in previous studies (Bock & Damodaran, 2013): the β-
244 turn increase at the expense of the β-sheet and the random structures as the water content
245 increased.

246 The effect of pregelatinized starch on protein secondary structure is shown in Fig. 2b.
247 The positive effect of pregelatinized starches on cakes texture has been proved (Sozer et al.,
248 2011, Hesso et al., 2014), while this is the first attempt to investigate their effect on the
249 protein structure. In this study, pregelatinized wheat starch (PWS) was tested in batter model
250 systems. No significant difference of their impact on protein secondary structure was found.
251 Consequently, only the results of PWS will be presented and discussed. System 2 (Fig. 2)
252 was prepared by the partial substitution of the flour with PWS at the same moisture content
253 as S1 (35%). The protein secondary structure estimated for the S2 followed the same order
254 as the S1: β-sheet (53.2%) > random (23.8%) >α-helix (14.5%) >β-turn (8.6%), with a slight
255 but significant ($P<0.05$) increase in β -sheets structure at the expense of unordered
256 structures (Fig. 2b). This change could be related to the competition between the PWS and
257 wheat flour protein for water, as described by Bock et al. (2013) for bran. Components able
258 to absorb water - such as pregelatinized starches, hydrocolloid or fiber - affect the water
259 distribution among dough components promoting a partial dehydration of gluten and the
260 collapse of β-spirals (consecutive β-turns) into β-sheet structures (Sivam et al., 2012; Bock et
261 al., 2013).

262 The effect of eggs, fat, sugar and their combination on protein conformation is shown
263 in Fig. 3. Eggs addition to flour (S3) increased β-sheet structure from 48.7% to 56% at the
264 expense of unordered structure which decreased from 26% to 17%. These changes reflect
265 the protein secondary structure in liquid egg: 74.1% β -sheet, 13.8% α-helix, 9% random and

266 3.1% β-turns. According to Bruun (2006), the β-sheet structure is the most important
267 structure for protein-protein interaction. Therefore, it is possible that β-sheets structures built
268 up in the network between flour protein and egg proteins when the flour was mixed with other
269 batter components such as eggs due to eggs proteins-gluten interaction.

270 The addition of the fat to flour in system 4 (S4) promoted the formation of α-helical
271 structures- which increased from 16% to 35% - and the decrease in β-sheet structure (from
272 49% to 22%) confirming previous findings on a bread system (Sivam et al., 2012). The
273 change in conformation in presence of lipids could be related to the decrease of the
274 interactions among hydrophilic molecules; it has been hypothesized that in presence of oil,
275 water molecules move less freely and that, consequently, the gluten-water interaction is
276 limited/restricted (Sivam et al., 2012). They showed that the presence of the oil in bread
277 dough formulation decreased the β-sheet structure, promoting fewer intermolecular hydrogen
278 bondings.

279 Adding sugar to flour (system S5) increased the β-sheet from 48.7% to 55% and
280 decreased the α-helix structure from 15.7% to 10 % (Fig. 3). Sugar competes with flour
281 proteins for water and also may attach to the hydrophobic pockets of gluten side chains,
282 which led to more intermolecular hydrogen bondings promoting more β-sheets (Sivam et al.,
283 2012). Sivam et al. (2012) showed that the sugar addition to a bread formulation led to a
284 decrease in α-helices (at 1653 cm^{-1}) in amide I band. The sugar seems to act contrary to the
285 fat concerning the intermolecular hydrogen bonds.

286 Protein conformation of batter model system (S6) - which includes all the ingredients -
287 was characterized by 22.5% β-sheet, 41% α-helix, 33.5% random and 3% β-turns. A general
288 look at the protein conformational changes resulted from each ingredient addition (Fig.3),
289 showed that fat addition (S4) promoted the most important conformational change in the final
290 batter system. These results confirmed that the fat is the functional ingredient in the cake
291 batter, and as in S4, it decreased the intermolecular β-sheets between gluten and egg
292 protein which un-stabilized and decreased the hydrogen bonding of β-turns (Sivam et al.,
293 2012). Meziani et al (2011) demonstrated that the β-sheets structure is the preferred

294 secondary structure of gluten in sweet doughs. In the present study, the β-sheets were the
295 dominant structure of flour model systems (S1, S3 and S5). This was not the case for the
296 systems containing fat (final batter system and S4) where α-helix structure dominated. These
297 conformational changes in proteins structure between the flour system (S1) and the final
298 batter system (S6) could explain the importance of fat in the batter ingredients interactions
299 which led to a weak gluten development which was already affected by water content and
300 sugar presence. Changes in protein conformation in system S6 due to fat addition could be
301 explained by two mechanisms, and taking into consideration that in S6 proteins from wheat
302 and egg contribute to the final network. As regards flour proteins, the presence of fat reduced
303 water mobility limiting protein accessibility to water (Sivam et al., 2012). While egg proteins
304 could be adsorbed on the surface of fat - as in emulsions (Graham and Phillips, 1979) -
305 stabilizing the emulsion in the batter system and improving its structure homogeneity, and
306 likely affecting the cake functionality.
307 The PWS was studied in the different systems with batter ingredients. The presence of PWS
308 did not affect dramatically the protein conformation (data not shown) with a slight but
309 significant ($P<0.05$) increase in β-sheets compared to the flour alone.
310
311 *3.2.2. Effect of ingredient interactions on starch amorphous and crystalline structures*
312 As mentioned above, batter properties could be affected not only by protein but also
313 by starch (Bruun, 2006). This consideration led us to the second objective of this study which
314 concerns the effects of batter ingredients on starch conformational changes in the crystalline
315 and amorphous structures. The FTIR spectra of flour (S1) and Flour + PWS (S2) reported in
316 Fig. 2a showed a broad envelope at 1200– 800 cm^{-1} with bands at 1047, 1022 and 994 cm^{-1}
317 - related to crystallinity and to vibrational modes within the amorphous domains of starch
318 granules (Van Soest et al., 1995). These bands were previously identified in wheat, potato,
319 maize, waxy maize and amylomaize starches (Wilson et al., 1988). The intensity ratios R
320 (1047/ 1022) and R'(1047/ 1035) were used to express the amount of ordered crystalline
321 domains with respect to amorphous domains in native starch gels (Wilson et al., 1987 et

1988, Van Soest 1995), starch granules (Sevenou et al., 2002), doughs (Meziani et al.,2011; Sivam et al., 2013), bread (Sivam et al., 2013), and tortillas (Flores-Morales et al., 2012). However, since the penetration depth of the IR beam in the ATR-FTIR system is about 2 μm, the organization investigated by ATR-FTIR is limited to the regions near the granule surface (Ambigaipalan et al., 2014). The ATR-FTIR spectra of starch structures related regions for flour, PWS and flour + PWS are shown in Fig. 4. The ratio R' (1047/1035) was 0.90±0.01, 0.88±0.01 and 0.83±0.01 for flour, flour + PWS and PWS respectively, while no significant changes were observed for R, suggesting a general increase in the spectra intensity with the PWS. The ratio R (1045/1022) for native wheat starch (0.63) given by Sevenou et al. (2002) was in agreement with these results. The decrease in crystalline ratio R' with the addition of pregelatinized starch was caused by the increase in starch amorphous structure when the PWS was added, which can be clearly observed in the PWS specter alone (Fig. 4). Investigating the starch gelatinization and retrogradation, Wilson et al. (1988) found that the bands at 1046 cm^{-1} and 1000 cm^{-1} – which represent the starch crystalline structure - were disappeared during gelatinization but gradually reformed during storage. It is consistent with the fact that the gelatinized starch gave more disordered structure and less crystalline structure as in the case of the PWS used in batter systems. ~~Since the effects of both PWS and PMS on starch conformational changes were quite similar (data not shown), only the role of PWS in batter system was presented and discussed.~~ Fig.4 showed a shift of the crystalline peak towards higher frequency for the PWS alone. This should be related to the establishment of hydrogen bonds between neighboring helices during growth of the crystal structure, in another word the complex amylose-lipids which is already formed in the PWS (Rappenecker & Zugenmaier, 1981).

When water was added to flour and flour + PWS mixtures (S1 and S2), the normalized absorbance at 1047 and 1022 cm^{-1} were smaller than in the dry state (Fig. 5), possibly suggesting a more ordered saccharide conformation with fewer conformations which give smaller distribution of bond energies in dry state. Sivam et al. (2013) found that interestingly the 1100–900 cm^{-1} envelope was weaker as the water content in bread dough

350 increased. Van Velzen et al. (2003) explained that an increase in available water thus may
351 have changed the bonding arrangement and diluted the signal. On the other hand, no
352 changes in the ratios were detected after adding water to the flour (S1) compared to flour
353 (dry state) (Table 3). The decrease in crystallinity ratio R' and even in R by the PWS in
354 system 2 (Table 3) could be due to the interaction between water and the PWS promoting
355 more amorphous structure. This was confirmed by analyzing the pregelatinized wheat starch
356 with the same water content as in systems S1 and S2 (data not shown).

357 Starch region ATR-FTIR spectra and intensity ratios for flour with batter ingredients
358 addition (egg, fat or/and sugar) are shown in Fig.6. Both ratios R and R' increased when the
359 egg was added to flour (S3). This indicates an increase in the intensity of the band at 1046
360 cm^{-1} and a decrease in the intensity of the band at 1022 cm^{-1}. The valley formed between the
361 two bands is growing and therefore its intensity decreases. The changes in the intensity of
362 these bands by egg addition reflected an increase in crystallinity ratio that could probably be
363 explained by the water availability in the matrix as water provided into the system by the egg
364 will be more easily available for egg proteins than for starch. Fat addition to flour (S4)
365 increased the ratios, due to similar changes in the intensity of the bands as described for
366 eggs addition. The changes with fat addition could be explained by slowing down the
367 hydration of starch by its hydrophobic effect (Wilderjans et al., 2013). Bogracheva et al.
368 (2001) studied the effect of water content on the ordered/disordered structures in starches.
369 They concluded that the proportion of the ordered structure depended on the water content.
370 With low water content, the proportion of the ordered structures was significantly reduced.
371 This means that the presence of any batter ingredients would prevent the access of water to
372 starch granules affecting its crystalline and ordered structures, as the intensity ratios
373 changed by fat or/and egg addition (Fig. 6). The same tendency was found with sugar
374 addition to flour in system 5 with more important increase in R as in S3 or S4. One of the
375 reasons of the crystalline increase could be the competition for water between sugar and
376 starch ~~as in the case of egg addition~~. On the other hand, sugar has a crystalline peak (Fig. 1)
377 which could interfere with starch structures and results in the important increase in crystalline

intensity values (Bureau et al., 2009). Even in a solution, the sugar showed an absorbance at the bands characteristic to starch (results not shown).

When all ingredients were added (egg, fat and sugar), an increase in crystalline to amorphous ratios was observed (Fig. 6: S6) compared to flour model system (S1). The model batter system (S6) crystalline ratio R was in agreement with previous work on a sweet dough by Meziani et al. (2011), with the R (1047/1022) value of 0.898±0.016. These conformational changes in the amorphous and crystalline structure were due to a combined effect of all ingredients which defined the final batter structure. This increase should be related to the interaction between ingredient and the availability of water for starch (Hedley et al., 2001). Van Soest et al. (1995) showed an increase in R' with the increase in water content for potato starch. They showed that the short-range order in starch is sensitive to changes in water content.

The effect of the PWS was studied on starch structures for all model systems (Table 4). In general, the PWS slightly decreased the crystalline ratio for the S3, S4 and S5 with the PWS addition, which confirmed the previous results on flour alone (S2). The flour + PWS mixtures had more amorphous structure that the flour mixtures (S1, S3 and S5). However, this was not found in the final batter systems (S6 + PWS), where the starch structure ratios were similar to the S6 (without PWS presence) as shown in Table 4. This could be due to the combined effect of all ingredients (fat, sugar and egg) which was more pronounced than the effect of PWS addition. PWS addition should be considered as a positive point, since the cake with PWS will have the same ratio of starch amorphous and crystalline structures - or decreased crystalline structure as found in some studied model systems (S2 or S3+PWS). The pregelatinized starch used in this study did not show a crystalline structure likely due to the retrogradation of amylose and/or amylopectin (data not shown). Therefore, adding PWS in cake formulation would not affect the batter crystallinity and would not promote a fast staling after baking compared to the reference cake (Hesso et al. 2014).

4. Conclusions

406 The present study investigated the effect of ingredients on the proteins secondary
407 structures and starch conformational structure taking place during batter preparation by ATR-
408 FTIR. The PWS addition to the batter did not dramatically affect the protein secondary
409 structures or the starch crystallinity. This positive result highlights the possibility of using
410 pregelatinized starches in cake formulation to inhibit the staling phenomenon without
411 affecting the protein secondary structure and likely the quality of the final product. α-helix
412 structure is the predominant protein conformational structure in flour + fat system and cake
413 batter, while the β-sheet structures are predominant in flour and dough systems from the
414 literature. These results highlighted the limited development in gluten network in cake batter,
415 since β-turn structures are predominant in well-developed gluten network. Moreover, fat has
416 a key role in cake batter and its functionality is likely the most important in batter. The need
417 to produce cakes with low fat content leads to the research for a fat-replacer which is able to
418 keep the α-helix structure as the main protein structure in the final product.
419
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423
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536

Table 1.

	Flour (g)	PWS (g)	Egg (g)	Fat (g)	Sugar (g)	Moisture content (%)
S1: Flour	100	-	-	-	-	35
S2: Flour + PWS	80	20	-	-	-	35
S3: Flour + egg	100	-	85	-	-	39
S4: Flour + fat	100	-	-	67	-	29
S5: Flour + sugar	100	-	-	-	85	29
S6: Flour + egg + fat + sugar	100	-	85	67	85	23

Table 2.

Mean frequencies (cm^{-1})	Secondary structure assignment
1620-1641	β-sheets
1643-1651	unordered (random)
1653-1659	α-helix
1660-1684	β-turns

Table 3.

	R' (1047/1035)	R (1047/1022)
Flour (dry state)	0.90±0.01[c]	0.70±0.01[b]
Flour + PWS (dry state)	0.88±0.01[b]	0.70±0.01[b]
PWS (dry state)	0.83±0.01[a]	0.69±0.01[b]
S1: Flour	0.91±0.02[c]	0.69±0.06[b]
S2: Flour + PWS	0.84±0.02[a]	0.64±0.04[a]

Table 4.

	R' (1047/1035)	R (1047/1022)
S1: Flour	0.91±0.02[b]	0.69±0.06[a]
S2: Flour + PWS	0.84±0.02[a]	0.64±0.04[a]
S3: Flour + egg	0.98±0.01[c]	0.85±0.01[b]
S3 + PWS	0.92±0.01[b]	0.82±0.03[b]
S6: Flour + egg + fat + sugar	1.10±0.01[d]	1.17±0.02[c]
S6 + PWS	1.11±0.01[d]	1.17±0.02[c]

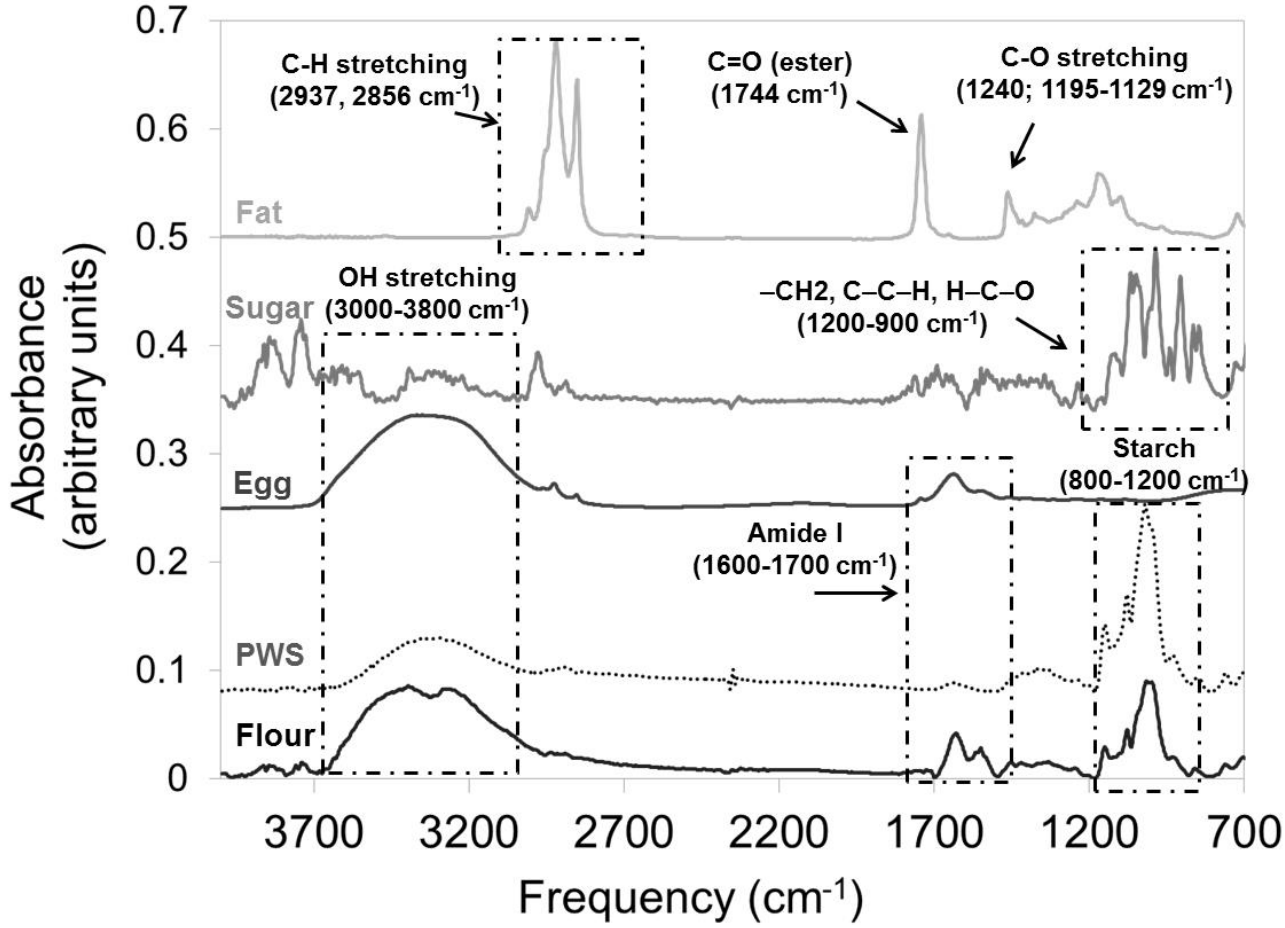
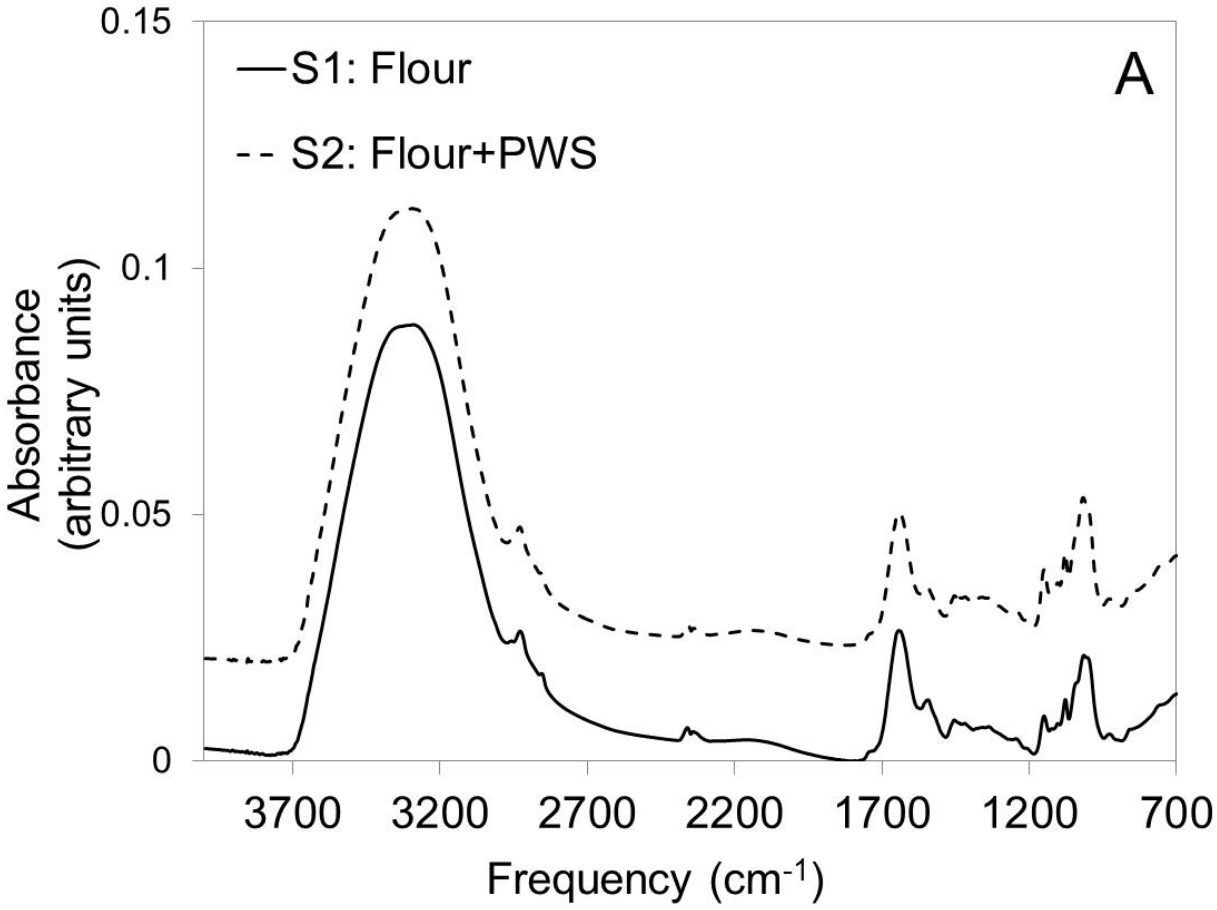

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance (arbitrary units)
C-H stretching (2937, 2856 cm-1)
C=O (ester) (1744 cm-1)
C-O stretching (1240; 1195-1129 cm-1)
Fat
OH stretching (3000-3800 cm-1)
Sugar
–CH2, C–C–H, H–C–O (1200-900 cm-1)
Starch (800-1200 cm-1)
Egg
Amide I (1600-1700 cm-1)
PWS
Flour
3700
3200
2700
2200
1700
1200
700
Frequency (cm-1)

Fig. 1


0.15
0.1
0.05
0
Absorbance (arbitrary units)
S1: Flour
S2: Flour+PWS
A
3700
3200
2700
2200
1700
1200
700
Frequency (cm-1)

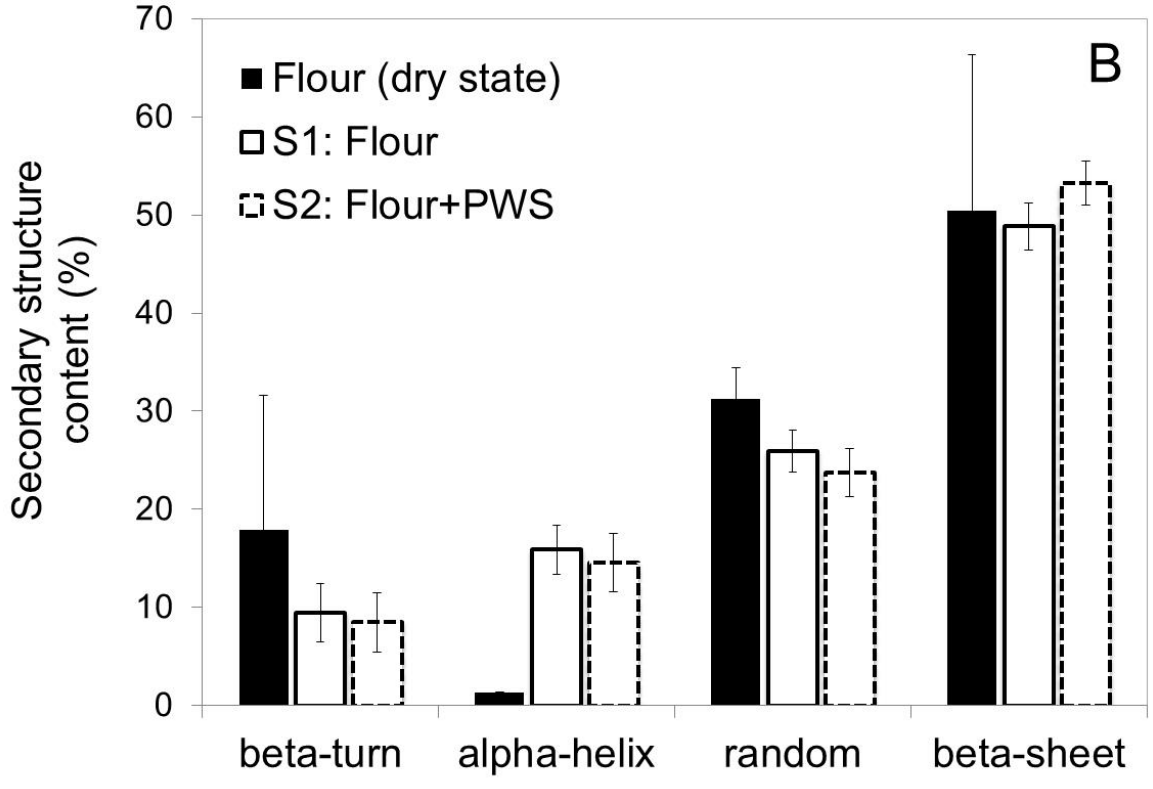

70
60
50
40
30
20
10
0
Secondary structure content (%)
Flour (dry state)
S1: Flour
S2: Flour+PWS
B
beta-turn
alpha-helix
random
beta-sheet

Fig. 2

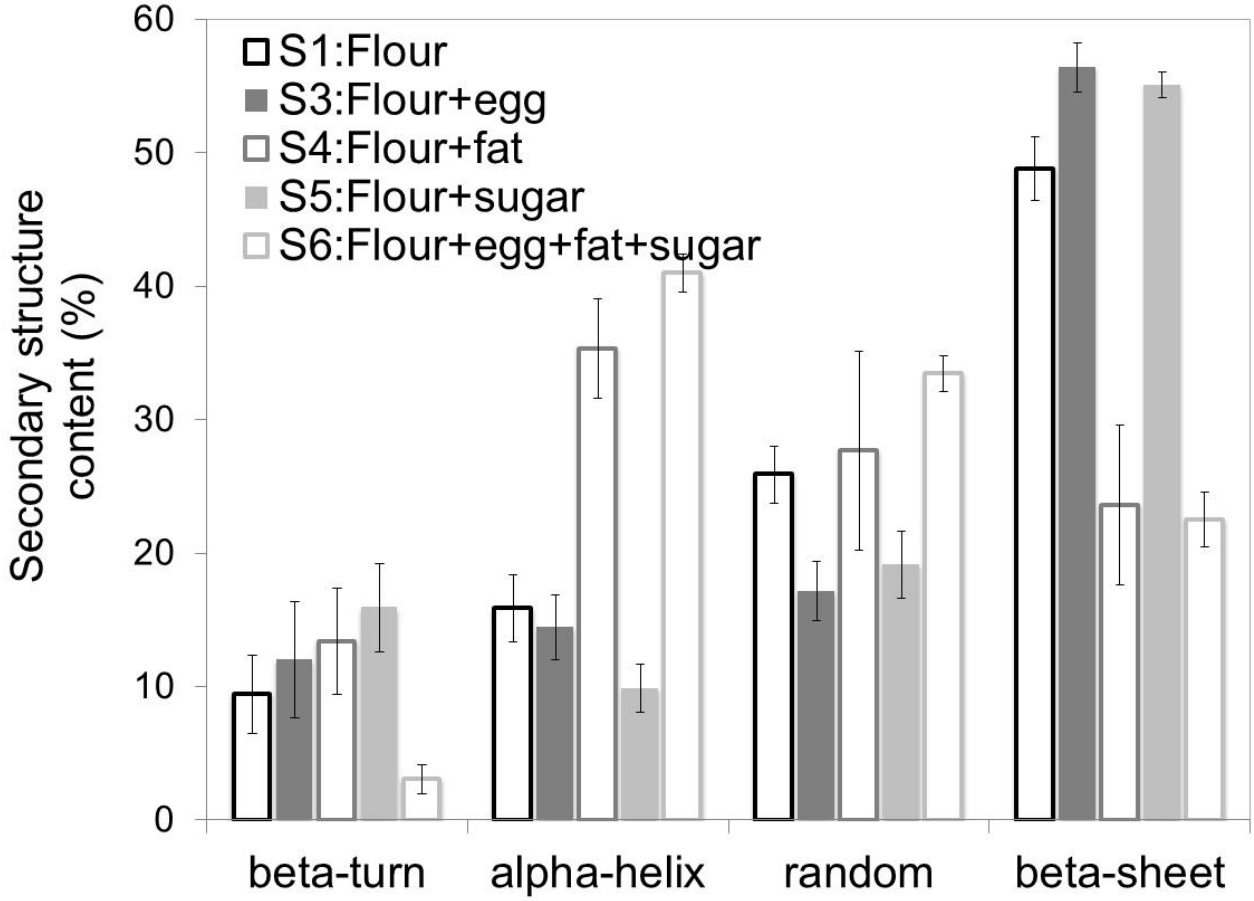

60
50
40
30
20
10
0
Secondary structure content (%)
S1:Flour
S3:Flour+egg
S4:Flour+fat
S5:Flour+sugar
S6:Flour+egg+fat+sugar
beta-turn
alpha-helix
random
beta-sheet

Fig. 3

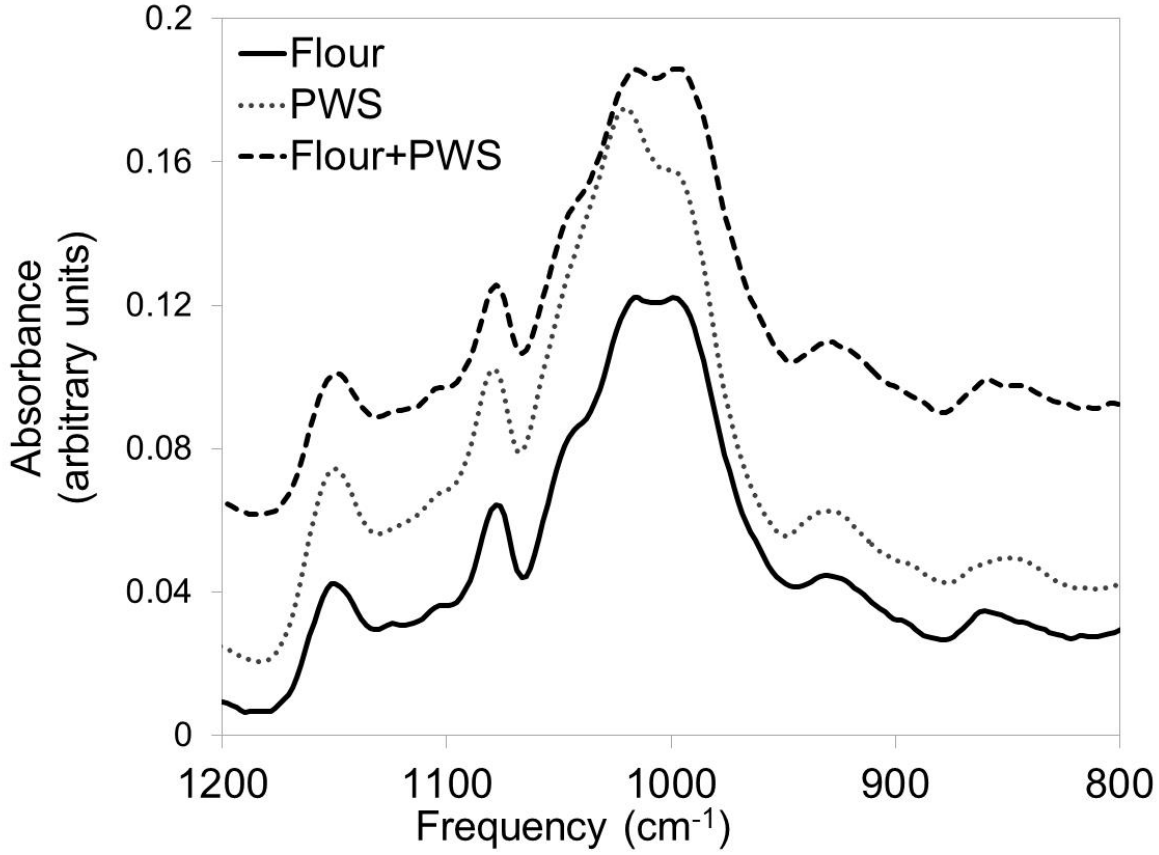

0.2
0.16
0.12
0.08
0.04
0
Absorbance (arbitrary units)
Flour
PWS
Flour+PWS
1200
1100
1000
900
800
Frequency (cm-1)

Fig. 4

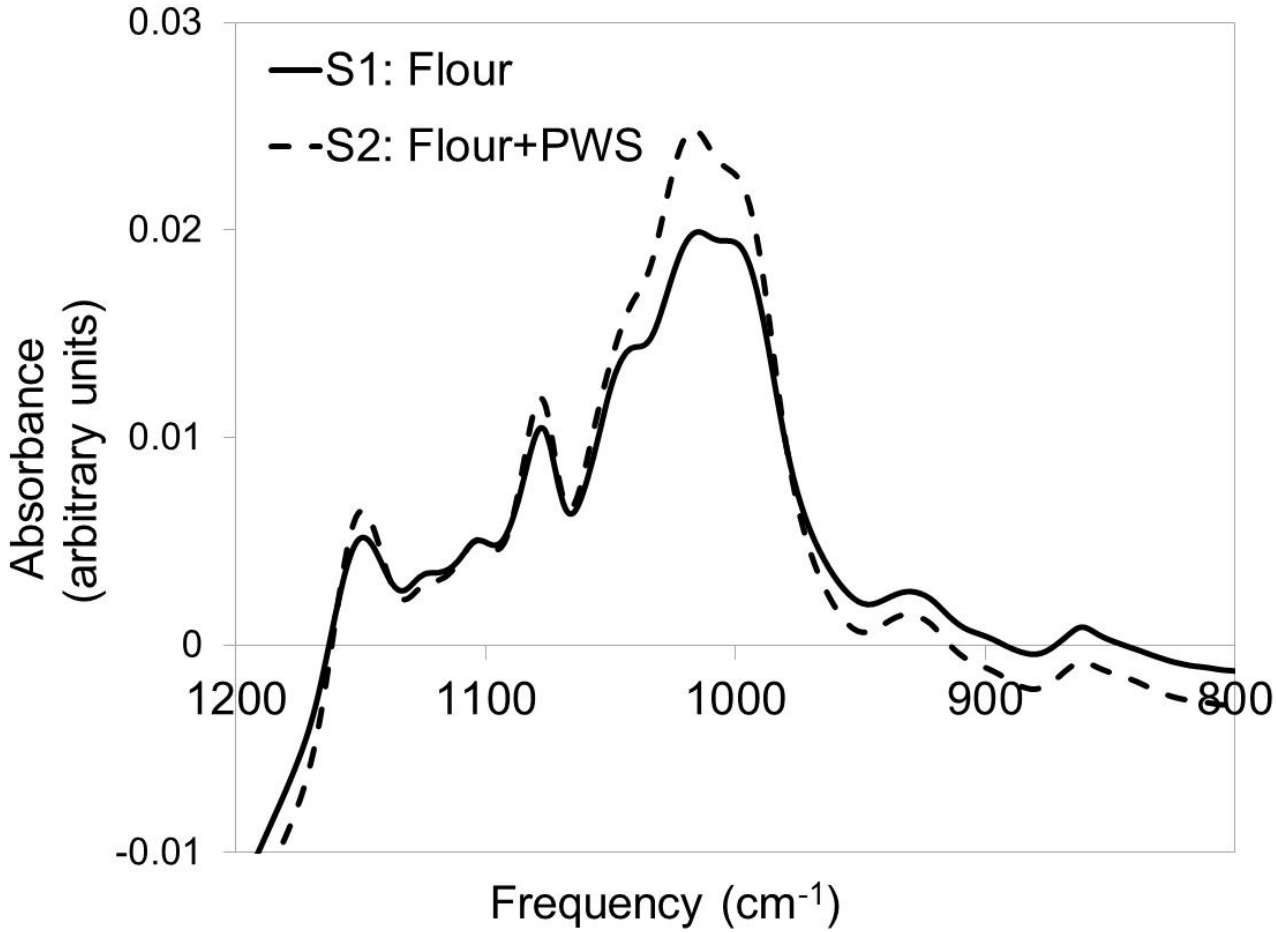

0.03
0.02
0.01
0
-0.01
Absorbance (arbitrary units)
S1: Flour
S2: Flour+PWS
1200
1100
1000
900
800
Frequency (cm-1)

Fig. 5

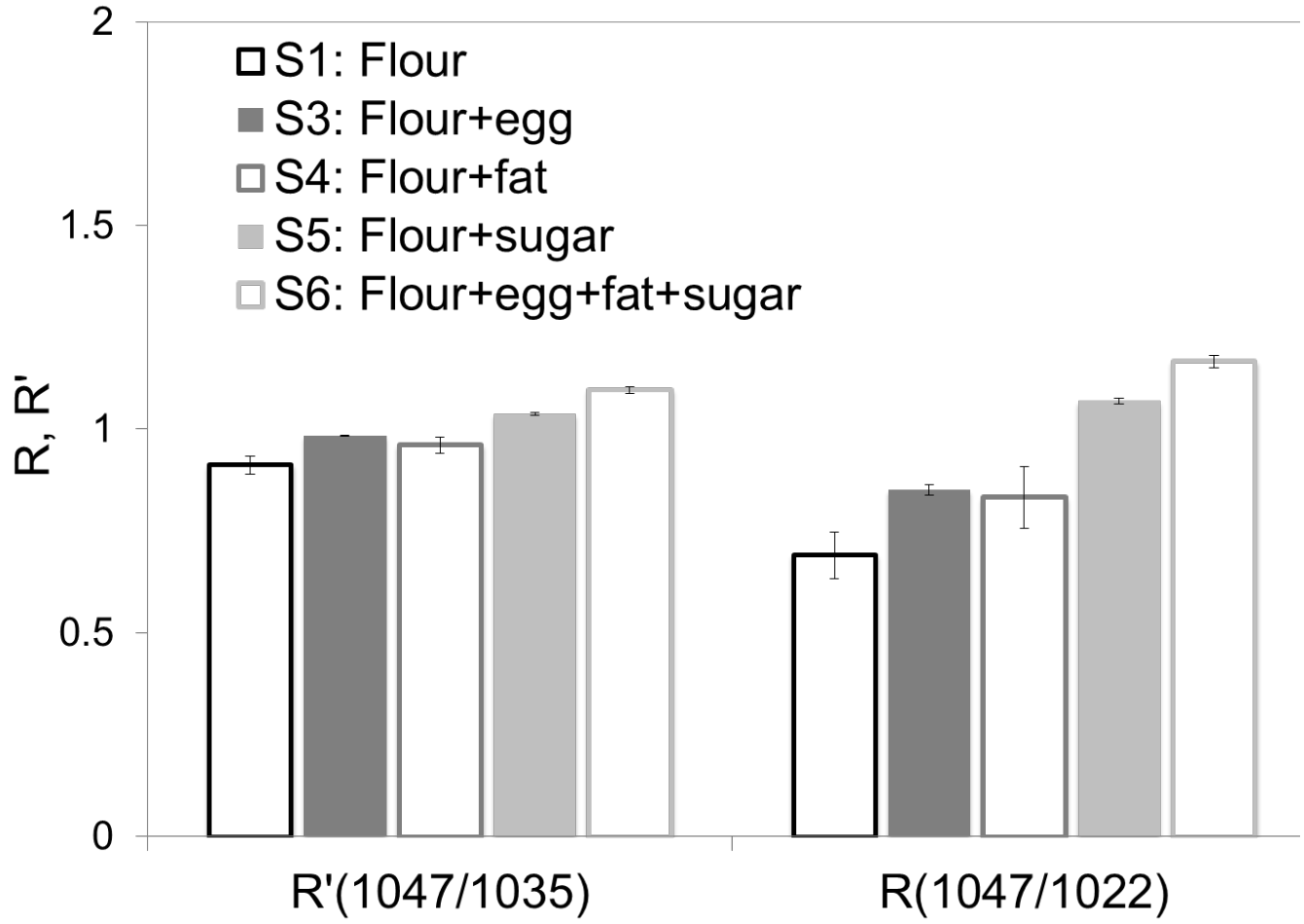

2
1.5
1
0.5
0
R, R'
S1: Flour
S3: Flour+egg
S4: Flour+fat
S5: Flour+sugar
S6: Flour+egg+fat+sugar
R'(1047/1035)
R(1047/1022)

Fig. 6