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The effect of mixing and wheat protein/gluten on the gelatinization of wheat starch☆

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Abstract

Protein was extracted with 0.1 M acetic acid and gluten was isolated from durum and Hard Red Spring (HRS) wheat. The extracted and isolated proteins were used to investigate their effect on the gelatinization of starch using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Starch and protein in different ratios were mixed with water using a spatula in test tubes and tested in a Brabender Farinograph. The data showed that mixing was an important factor, affecting starch DSC gelatinization parameters, TGA profile path and final weight loss of the blends. The starch onset and peak temperatures and ΔH of the mixed blend were compared with the unmixed blend. Mixing increased the onset and the peak temperatures of the starch gelatinization and decreased the ΔH. Higher amounts of protein in the blend increased the onset and peak temperatures and decreased the ΔH of the starch gelatinization. Protein extract and gluten were found to interact differently with starch and influence its gelatinization parameters and water evaporation, as measured by DSC and TGA, respectively.
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Keywords: Acetic acid protein extract; DSC; Onset; Peak; ΔH; TGA

1. Introduction

Starch gelatinization is influenced by the presence of other ingredients that affect water activity. Some ingredients, such as sugar, salt, and proteins, compete with starch for the available water in the system and affect the gelatinization of the starch (Wootton & Bamunuarachchi, 1980).

Starch protein interactions are a consequence of the attraction between positively and negatively charged colloids in acidic environments (Takeuchi, 1969). Dahle (1971) reported that the modification of wheat protein by heat resulted in loss of protein binding to the starch and thus diminished the interaction. The study was conducted using centrifugation of starch-protein aqueous solution at different pH and protein levels. The absorbance of the supernatant was read at 650 nm where a high absorbance value indicated low interaction. Dahle, Montgomery, and Brusco, (1975) showed that binding of wheat protein to wheat starch was diminished by disulfide splitting agent but not by sulfhydryl group blocking agent.

Eliasson (1983) used a starch–gluten system to study the gelatinization of starch in the presence of gluten from spring wheat using DSC. Wheat flour was developed into dough, and gluten was isolated by washing away non-gluten flour components and air-dried in a vacuum oven. Starch and gluten protein were blended in test tubes with a spatula at 0.9 water:starch ratio. The study concluded that the gelatinization peak temperature of the starch increased and the ΔH decreased in the presence of gluten proteins. The ΔH of gelatinization decreased as the amount of gluten proteins increased, while the peak temperature increased as the ratio of gluten:starch increased. The changes in the starch gelatinization parameters were believed to be due to less available water in the presence of the gluten.

☆ Names are necessary to report factually on available data; however, the USDA neither guarantee nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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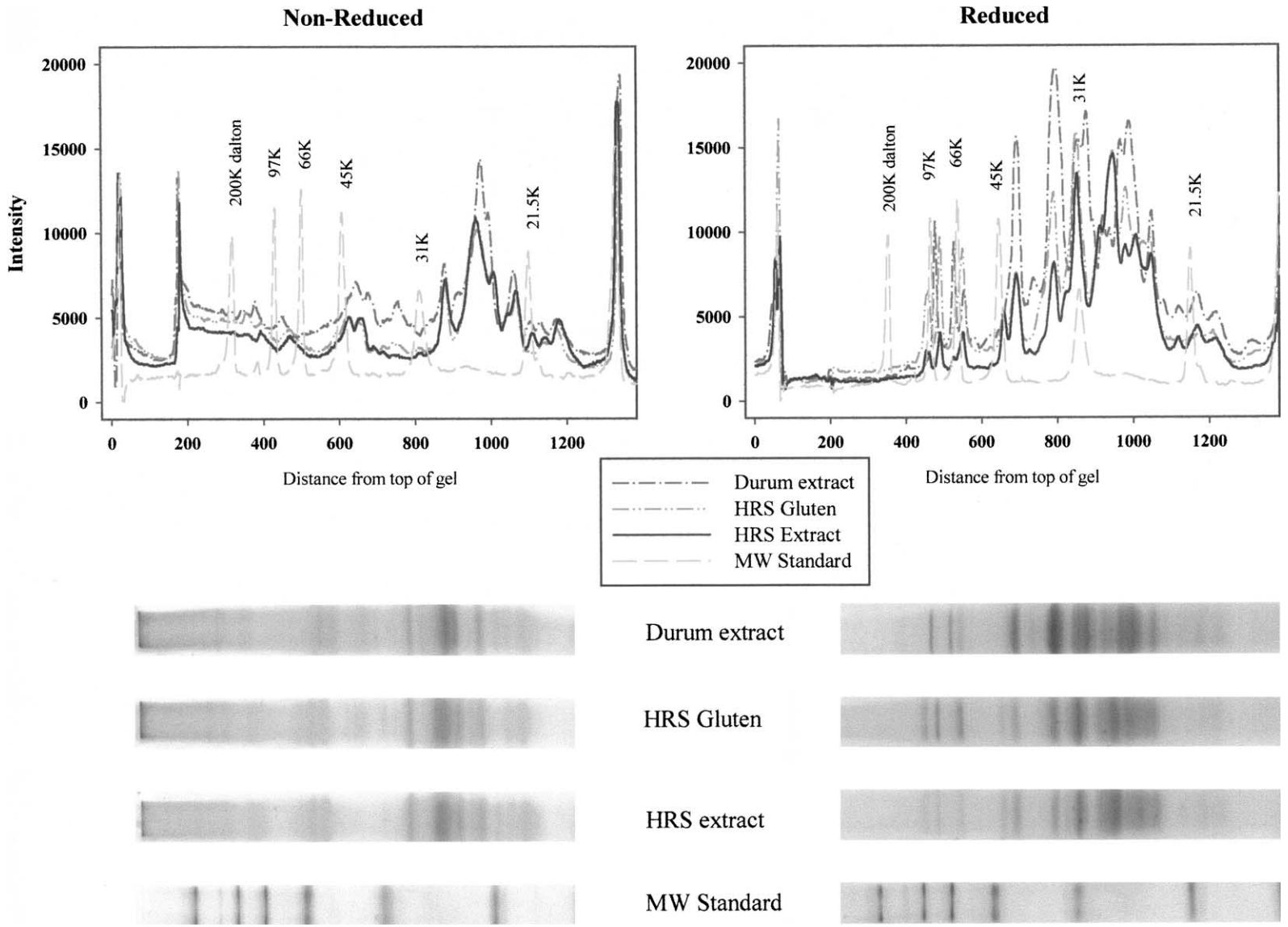

Fig. 1. SDS–PAGE profiles of durum and HRS wheat: Reduced and non-reduced.

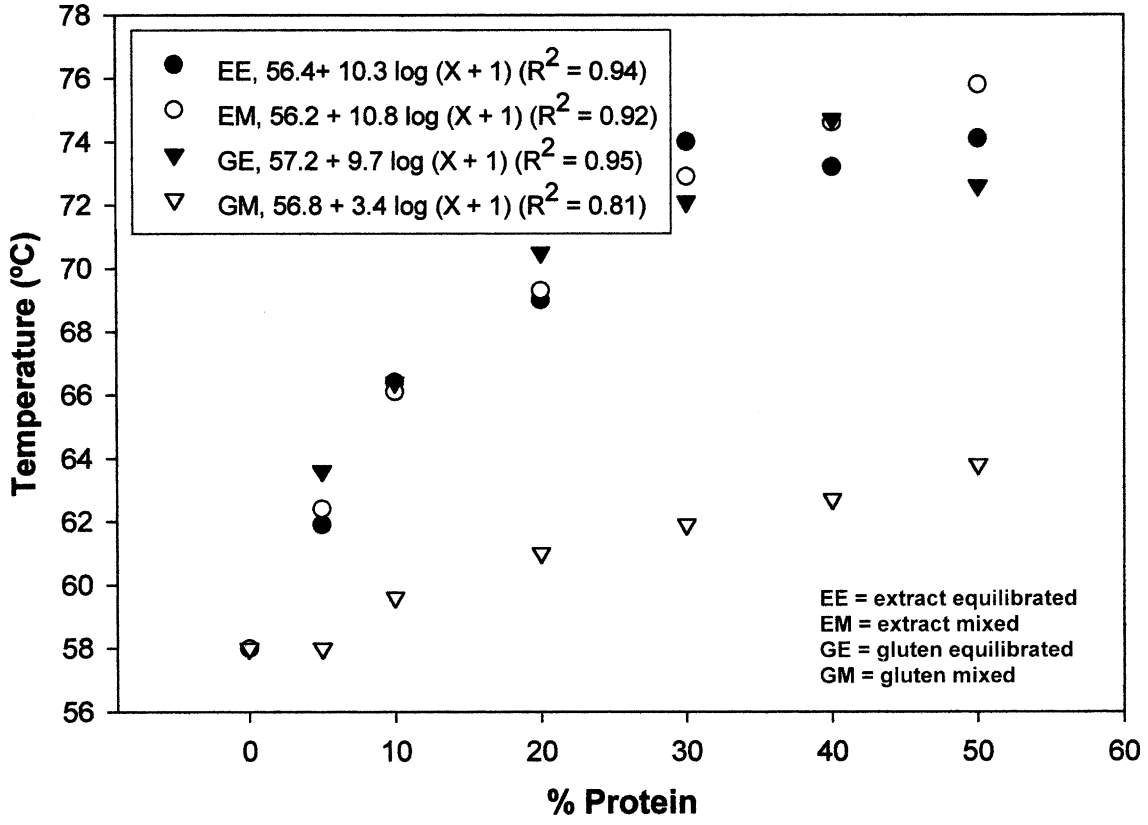

Fig. 2. Effect of durum wheat gluten and protein extract on the DSC onset temperature of durum wheat starch.

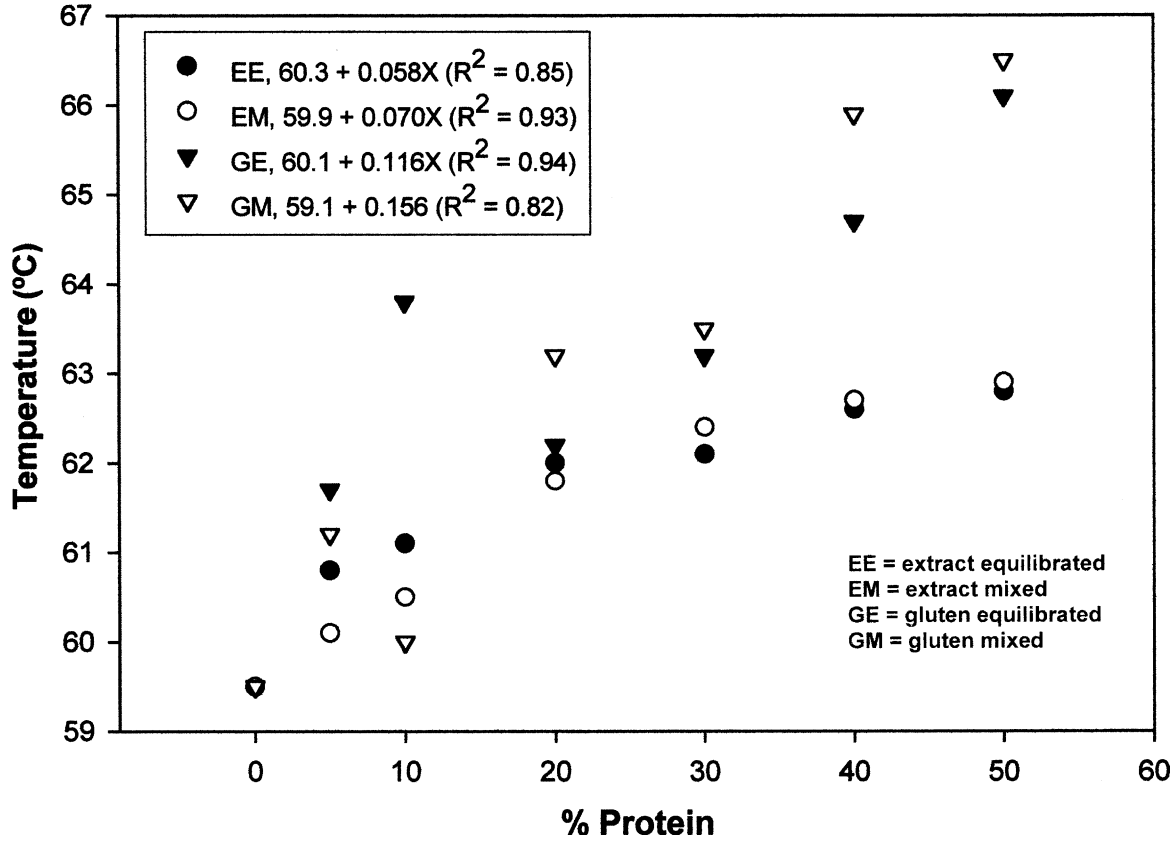

Fig. 3. Effect of HRS wheat gluten and protein extract on the DSC onset temperature of HRS wheat starch.

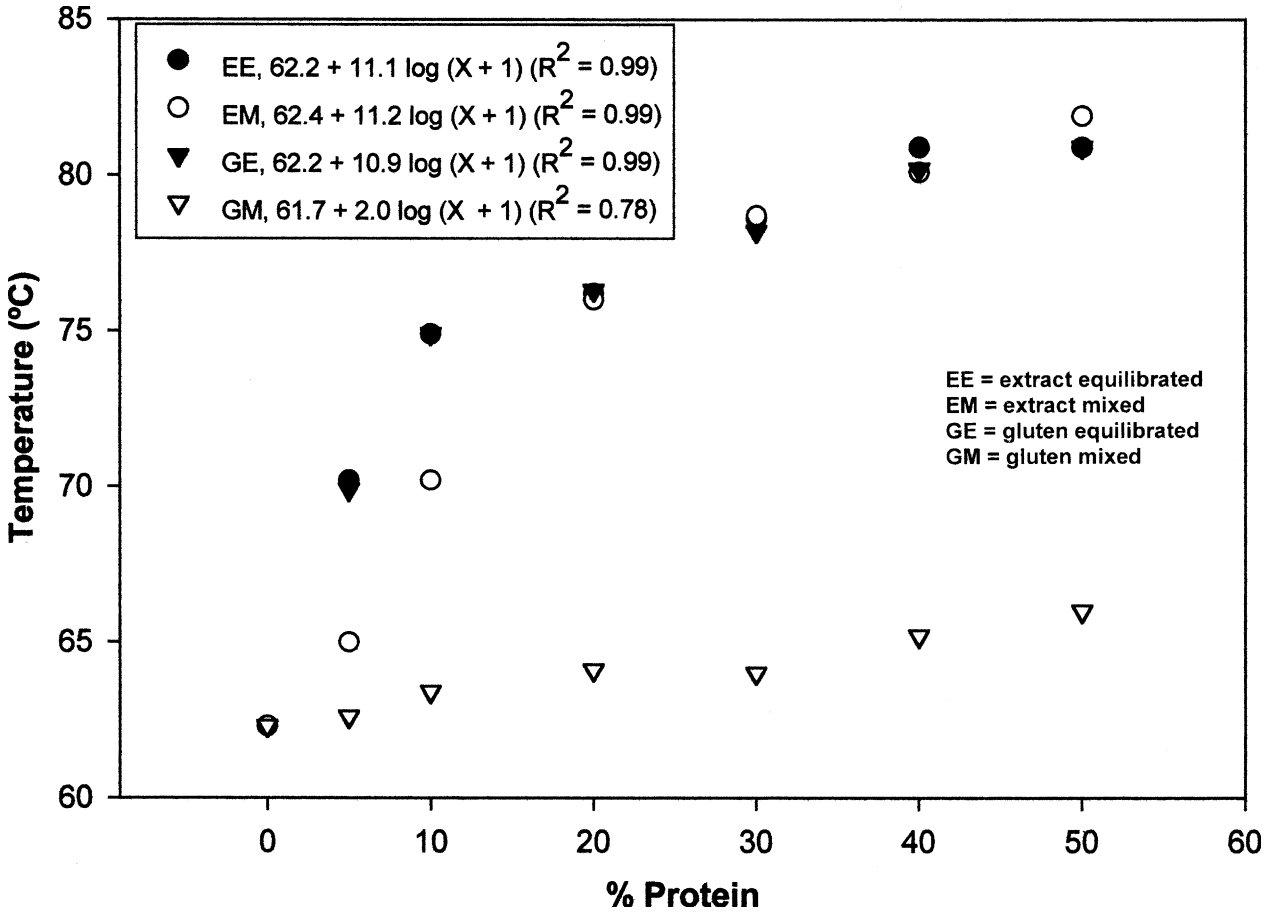

Fig. 4. Effect of durum wheat gluten and protein extract on the DSC peak temperature of durum wheat starch.

Chevallier and Colonna (1999) studied a gluten–starch blend (1:9) with different water contents. The water was added to the blend without mixing. Due to the low moisture (23%) of the blend, starch peak temperature was reported to be 130 °C while the same temperature was 128 °C when wheat flour was analysed at 23 °C. They concluded that a 10% protein content did not alter starch thermal properties. They also noticed that starch transition concealed the exothermic transition that existed when pure gluten was analysed.

Eliasson and Tjerneld (1990) studied starch–protein interaction by measuring the amount of protein adsorbed into starch granules. A low molecular weight wheat protein fraction was found to have low adsorption. The high molecular weight wheat fraction had a high level of adsorption to starch granules. Most of the work reported in the literature regarding starch thermal properties, in the presence of proteins was done without considering mixing as a factor. In this work, which is one part of a number of studies on starch–protein interaction, the effects of a 0.1 M acetic acid extract from durum and Hard Red Spring (HRS) wheat on the onset, peak temperature, and ΔH of the starch were investigated. Acetic acid is shown to extract a representative sample of wheat protein fractions, i.e. glutenin, gliadin in their native form. Acetic acid extraction of wheat protein was used to allow the investigation of the effect of disulfide bond formation in the presence of starch during mixing. This may show differences in the interaction between starch and gluten (because disulfide bonds formed during the gluten isolation step) and between starch and wheat protein void of disulfide bonds (because disulfide bonds will form in the presence of starch). Mixing is an important step of dough formation. This work was also meant to study the effect of mixing on the thermal properties of starch. Since wheat flours have different protein contents, the effect of wheat protein content on starch thermal properties was also studied. The equilibrated samples were used to allow water to move between the components (starch, gluten, and protein extract) without mixing.

Hagerdale and Martens (1976) reported that the denaturation temperature of myoglobin increased with increase in water content up to 30% while the transition heat decreased with increase in water content. It is well established that the addition of alginate decreases product expansion during soy protein extrusion, as a result of viscosity reduction (Berrington, Imeson, Ledward, Mitchell, & Smith, 1984; Imeson, Richmond, & Smith,

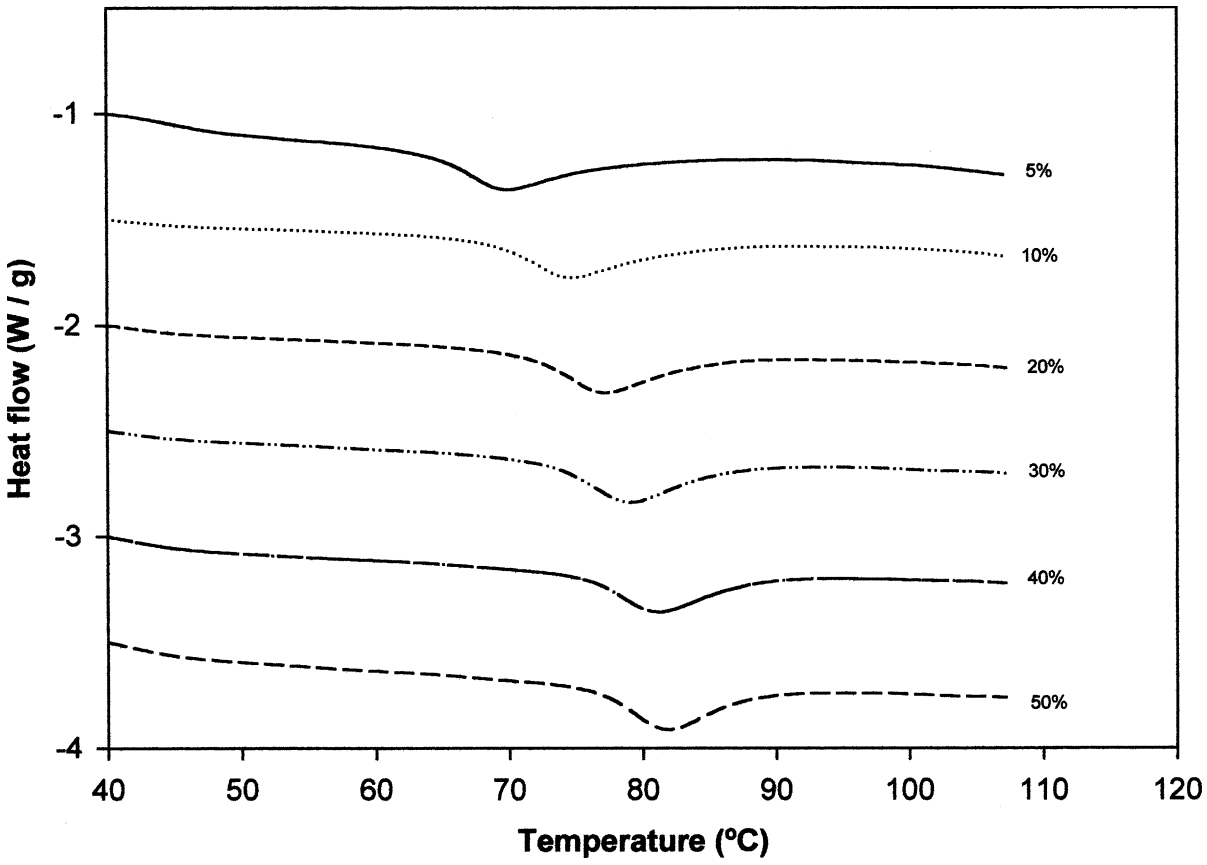

Fig. 5. DSC profiles of HRS wheat protein extract and starch blends.

1985). Oates, Ledward, and Mitchell, (1987) reported that alginate increases the water binding ability of soy protein during heating and after denaturation. This effect could be the cause of lower viscosity of the system during extrusion. In general, the amount of free water and water migration within the system are considered important factors that affect protein–carbohydrate blend behaviour as studied by DSC (Donovan & Beardslee, 1975).

Thermogravimetric analysis is used to show how water evaporates from a system or to show the mechanism by which a material loses weight as a result of controlled heating. The weight loss profile can show if there is a difference in the behaviour of the components of the blend. In the current report this technique will help to explain the effect of mixing (and the type of protein) on water evaporation from the starch–protein system as a result of controlled and systematic heating.

The objectives of this study were to study the effects of wheat protein extract and gluten, from durum and hard red spring wheat, on the gelatinization of starch using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). It is also intended to show the difference between mixed and equilibrated (unmixed) starch–protein blends on the gelatinization of starch.

2. Materials and methods

2.1. Protein extraction

The protein was extracted from a strong gluten variety of durum wheat (*Triticum turgidum* var. *Renville*), obtained from the North Central Research and Extension Center (Minot, ND), and Hard red spring wheat commercial blend, obtained from Sands Tylor and Woods Co., Norwich, Vermont 05055. Protein was extracted from semolina and HRS flour according to the modified method of Patey and Shearer (1980). Sample (10 g) was mixed with 0.1 M acetic acid (100 ml), homogenized for 5 min at low speed (5D45 Polytron Homogenizer, Minneapolis), allowed to stand 1 h, and centrifuged (6000 × *g*, 30 min, 10 °C). The pH of the supernatant was adjusted to 6.5 using a 1.0 M NaOH solution and the supernatant was freeze-dried.

2.2. Gluten isolation

Gluten was isolated from durum and HRS wheat flour as described by Ann-Charlotte Eliasson and Kare Larsson with the exception that the isolated gluten was freeze-dried instead of oven-dried. Flour was hand-mixed with water to form dough as a result of gluten

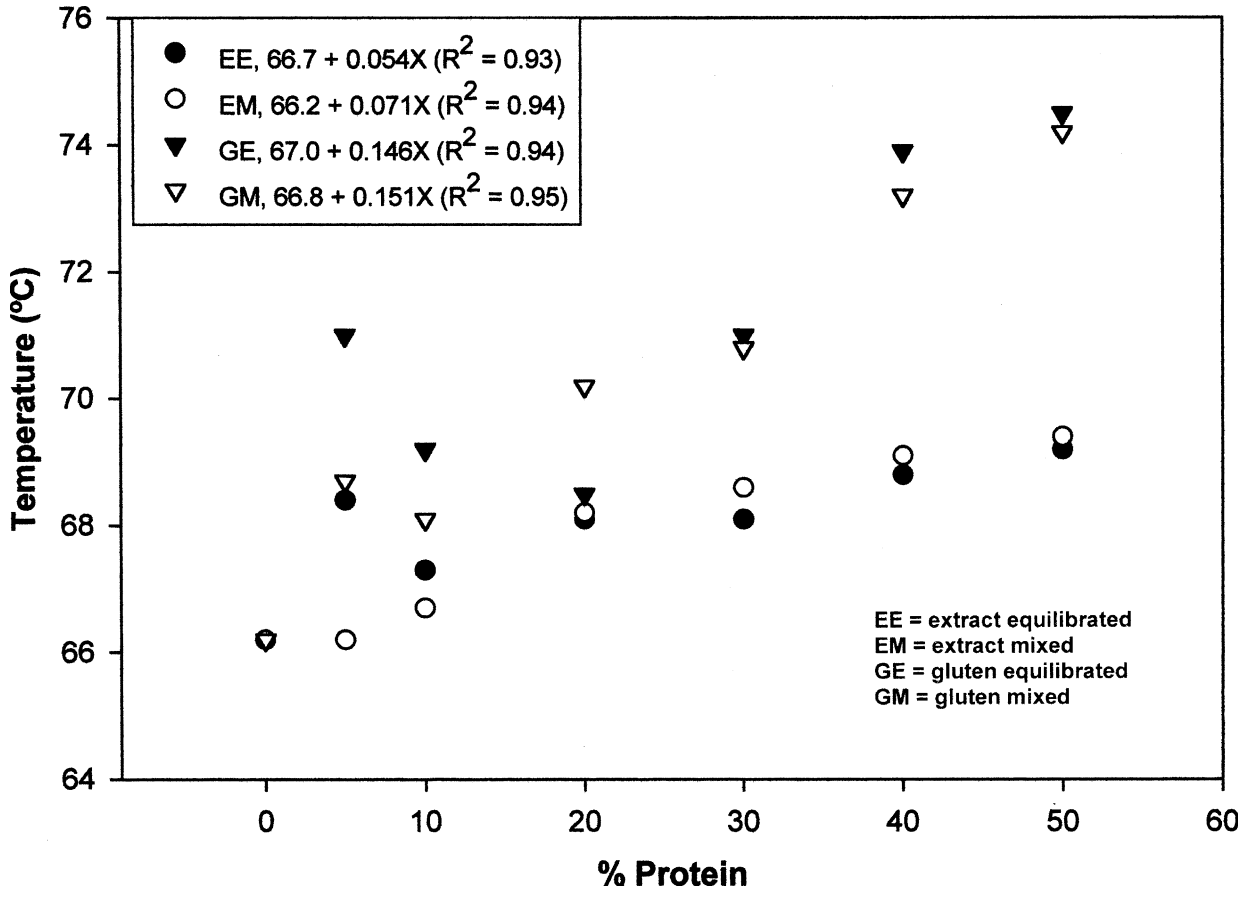

Fig. 6. Effect of HRS wheat gluten and protein extract on the DSC peak temperature of HRS wheat starch.

development. The dough was washed with water to remove the starch and the water-solubles. The washing continued until no more starch was released.

2.3. Starch isolation

The starch in the precipitate of the protein extraction step was washed three times with distilled water and centrifuged (1000 × *g*, 20 min, 10 °C). After each wash, the protein layer on the surface was scraped off with a spatula. The starch was air-dried and ground to pass through a 70-mesh sieve. The remaining protein in the starch was determined by the nitrogen combustion method using a LECO CHN-2000 instrument (3000 Lakeview Ave, St. Joseph MI 49085). Protein content is total nitrogen times 5.7.

2.4. Total carbohydrates of the protein extract and gluten

The phenol sulfuric acid method, described by Dubois, Gilles, Hamilton, Rebes, and Smith, (1956), was used to determine the total carbohydrate content of the protein extract. Five grammes of freeze-dried protein samples were milled to pass through a 40-mesh sieve and suspended in 25 ml of distilled water. The suspension was diluted 1:25 with distilled water and aliquots (1.0 ml) were used for analysis. Anhydrous glucose was used at 10, 20, 30, 50, 100, and 150 μg/ml as a standard curve.

2.5. SDS–PAGE

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was used to examine possible differences between developed gluten and acetic acid wheat protein extract. SDS–PAGE was performed according to Laemmli (1970), as described by Khan, Tammiga, and Lukow, (1989) with 11.8% acrylamide and 0.1% bis-acrylamide for the separating gel. The stacking gel was prepared with 4.5% acrylamide and 0.1% bis-acrylamide. Samples were reduced with dithiothreitol (DTT) to test whether proteins formed aggregates involving disulfide bonds.

2.6. Sample preparation for DSC

The freeze-dried protein (extract and gluten) was thoroughly mixed with a spatula in test tubes in a dry state at 5, 10, 20, 30, 40, and 50% levels of the starch total weight. Isolated durum and HRS wheat starch dry powder was used as a control (2% protein). The amount

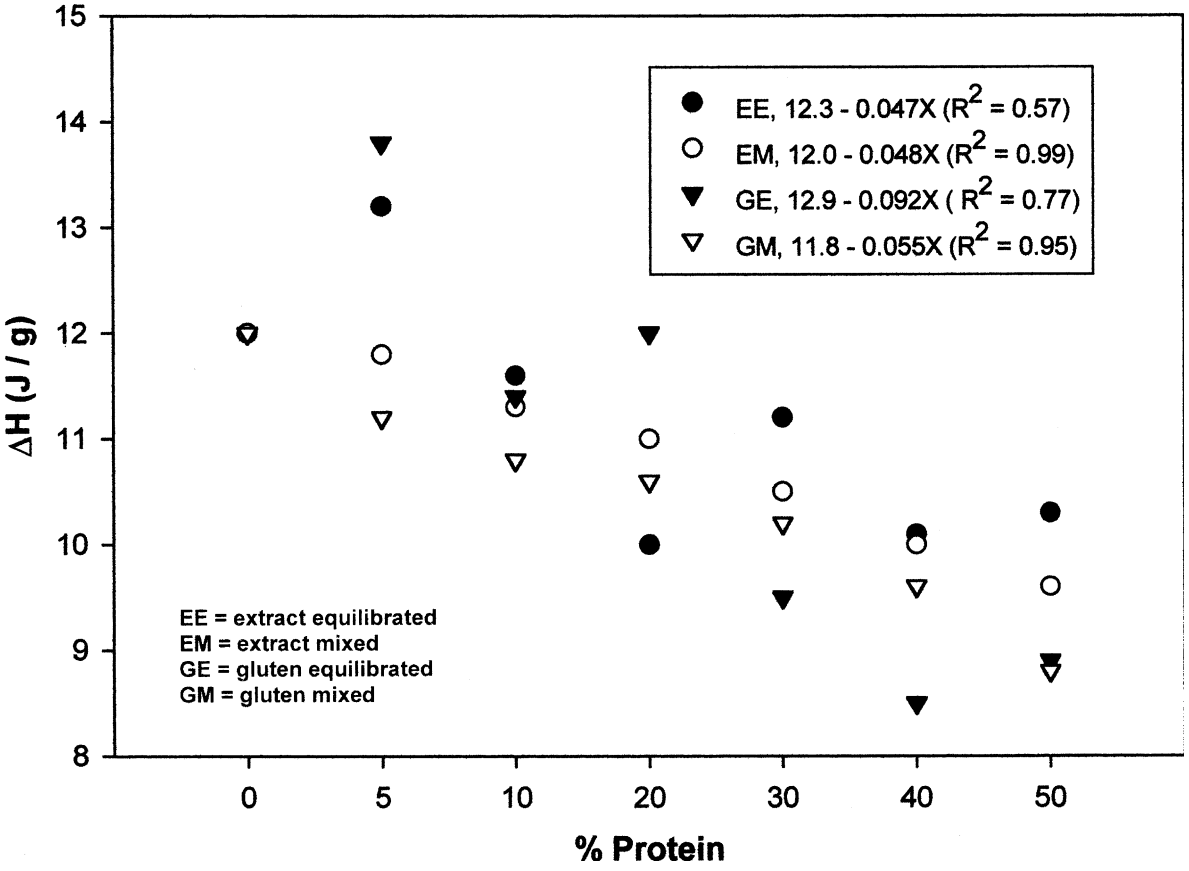

Fig. 7. Effect of durum wheat gluten and protein extract on the DSC ΔH of durum wheat starch.

of water added to each starch–protein mixture was 60% of total sample weight. The moistures of the protein and the starch were measured and included in the total amount of water added to the dry mixture. Each dry mixture was separated into two parts. One part was used to weigh 6 mg into the DSC pans, 60% (w/w) water was added to it and it was followed by a 3-h resting (equilibration) period without mixing before analysis. The other part was premixed for 3 min with a spatula in the test tubes at 60% water content. Mixing was done this way to make possible the comparison with the data in the literature, where samples were mixed in the same way. The premixed protein–starch sample was weighed and sealed in aluminium DSC pans and allowed a 1-h resting period before analysis. Samples were analysed using a Seiko DSC-220C equipped with automatic cooling (SEIKO Instrument Inc., Tokyo, Japan) and TA Instrument 2920 (TA Instruments Thermal Analysis and Rheology, New Castle, DE 19720). Samples were heated in the range 30–110 °C with a heating rate of 10 °C/min. All analyses were done on one replicate with three sub-samplings. HRS sets of samples with 10, 20, and 50% gluten or extract were mixed in a Brabender® Farinograph to simulate what may be happing during baking. Samples were mixed for 3 min with 60% water absorption. Samples were let to rest for 1 h before DSC analysis.

2.7. Sample preparation for thermogravimetric analysis

Samples were prepared for TGA as they were prepared for DSC with the exception that, samples were loaded in TGA 2050 (TA Instruments Thermal Analysis and Rheology, New Castle, DE 19720) in an open platinum pan and heated from 30 to 150 °C with 10 °C/min heating rate and holding for 15 min at 150 °C. The equilibrated samples were covered with parafilm to eliminate water evaporation. About 25 mg of sample were used for analysis.

2.8. Statistical analysis

Regression equations were developed for characterizing three response variables, onset temperature, peak temperature, and ΔH, as a function of protein, for four treatment combinations for HRS wheat and as a function of log(protein + 1) for the four treatment combinations for durum wheat. The treatment combinations were Extract–Equilibrium (EE), Extract–Mixed (EM), Gluten–Equilibrium (GE), and Gluten–Mixed (GM).

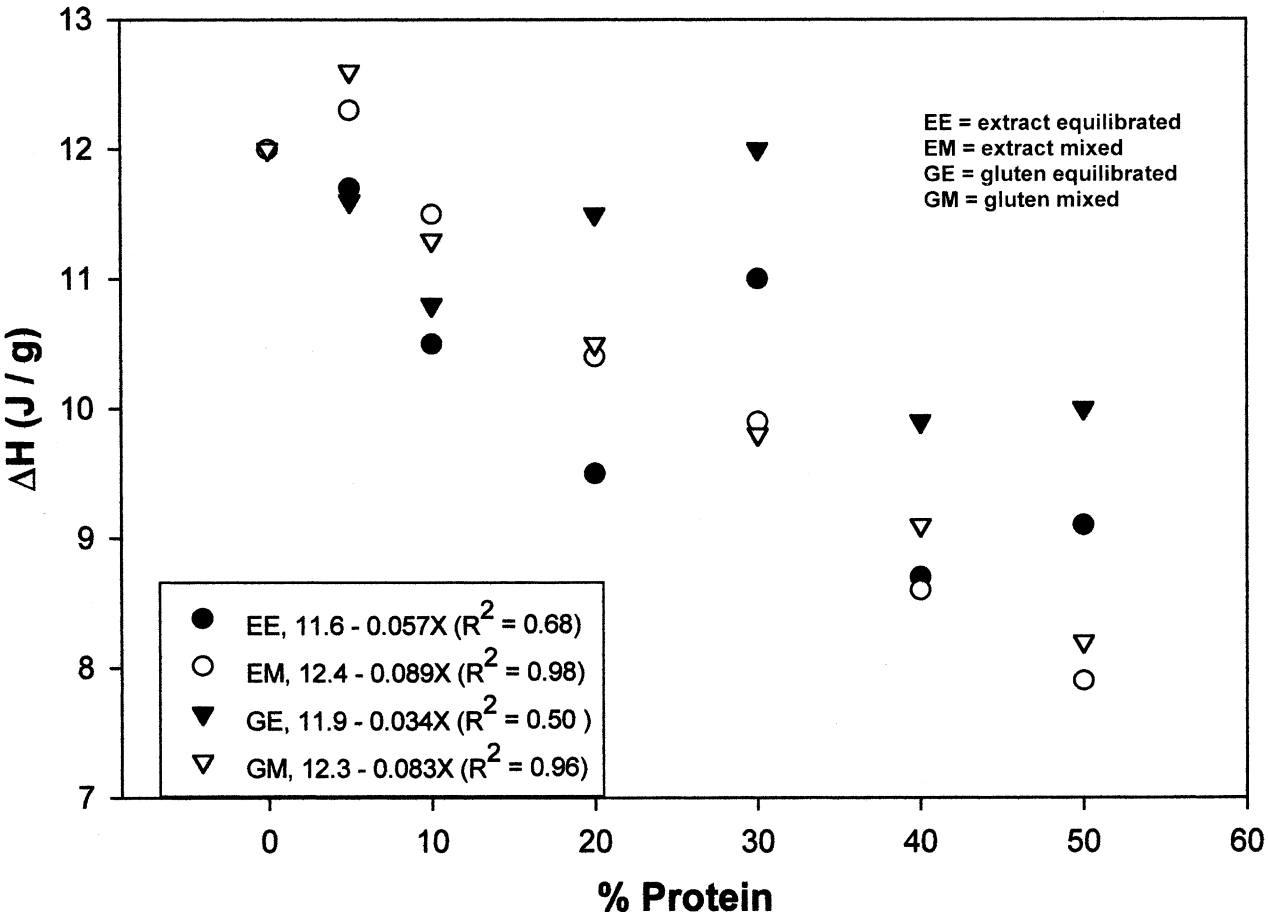

Fig. 8. Effect of HRS wheat gluten and protein extract on the DSC ΔH of HRS wheat starch.

For each wheat type and response variable, the four treatment combination regressions were compared using a general linear model *F*-test (SAS Institute, 1990). If a significant *F*-test was found, slope comparisons were made to determine which equations were different from the others. Comparisons of the mean predicted values of the four treatment combinations were made at each protein level (0, 5, 10, 20, 30, 40, and 50%) by comparing the 95% confidence limits for each wheat type and response variable. If there is any overlap of the confidence intervals, then the treatments are not significantly different.

3. Results and discussion

Since most wheat flour is used for products that require the presence of water and mixing, it is relevant to investigate the effect mixing on wheat flour. Starch is a component of wheat flour affected by the amount of available water and the mixing process. Starch has a clear and measurable thermal transition that can be altered by mixing and water availability. The protein used in this work was 0.1 M acetic acid extract of durum and HRS wheat, containing 2.8 and 3.2% carbohydrates, respectively, and isolated gluten, with 4 and 4.6% carbohydrate contents. Isolated starch contained 2% protein. The gel electrophoresis of the acetic acid extract of durum and HRS showed similarity in the bands present (Fig. 1). The durum wheat gluten gel electrophoresis is not included to avoid showing a crowded graph. The expected difference between the two types of proteins is that, unlike gluten, the extract has few if any disulfide bonds formed due to the non-mixing method of isolation. The amount of water added for DSC testing was 60% of the blend weight. After a trial, the equilibration (resting) times for the mixed sample was determined to be 1 h, since no difference was noticed between 1 and 3 h resting time. The unmixed samples showed no difference only around 3 h of equilibration. This clearly showed that, with the mixing process, a homogeneous hydration would be obtained in a shorter time (1 versus 3 h).

The pre-developed gluten has an extended structure where most of the side chains are exposed and disulfide bonds are formed. The extract, on the other hand, makes the protein starch blends more like flour and is representative of what would happen when flour is mixed with water. Despite the difference in the protein isolation method, extract versus gluten, and wheat type, durum versus HRS between this work and the work of

Eliasson (1983), The assumption was made that any significant effect due to mixing should be noticed.

Erdogdu, Czuchajowska, and Pomeranz, (1995) reported that gluten did not show any distinct DSC transition at 2:1 water:gluten, which is consistent with our findings. Figs. 2–8 shows varying amounts of a 0.1 M acetic acid durum and HRS wheat protein extract and gluten added to their respective starch. The effect of proteins on starch gelatinization parameters, onset, peak and ΔH are shown in separate figures. The equilibrated and mixed samples are compared within the same figure. The regression equations and R^2 values are also noted in each figure. The limits on statistical mean predicted value differences for all DSC parameters are shown in Table 1.

Generally, the onset temperature of starch was increased by the presence of both protein types (extract and gluten) and sample preparation (mixed versus equilibrated). The regression data of the onset temperature showed that EE and EM, and GE have R^2 values higher than GM (Fig. 2). The better regression fit, indicated by the high R^2, may be a result of less variation between the values. The slope is also an indication of difference as is clear in the slope of GE. Fig. 2 shows a significant onset temperature difference between durum GE and the other samples ($P \leqslant 0.05$). When the R^2 value and the slope of GE combined together are shown in Fig. 2, it is clear that pre-isolated gluten and the mixing process are major factors that influence starch, protein and water interaction. The formation of the disulfide bonds, prior to mixing with starch, seemed to limit protein water absorption. That is noticeable on the lower durum GM R^2 values, which show that water migration between the starch and the protein is affected by the type of protein and the mixing process. Samples with less water available for starch gelatinization due to protein water absorption showed higher peak temperatures. The equilibrated durum extract samples showed higher R^2 values, indicating that water migrated evenly between the starch and the durum protein extract. That allowed less water available for starch gelatinization, as reported by Wootton and Bamunuarachchi (1980). The HRS wheat showed a different picture with respect to the onset temperature (Fig. 3 and Table 1). Either, extract or gluten, equilibrated or mixed samples showed no significant differences between the regression slopes up to 10% protein content (Table 1). The presence of the protein at 20% or higher, appears to be the determining factor of the protein influence on the starch gelatinization. Figs. 2 and 3 and Table 1 show a difference between mixed and equilibrated and between extract or gluten within and between wheat types. The high R^2 values of durum EM and HRS EM in Figs. 2 and 3 showed that mixing brought consistency to the blends in comparison to the equilibrated samples. Mixing of pre-isolated gluten and starch lowered the R^2 values of both samples, indicating different behaviour of the water and the starch in the presence of pre-isolated gluten (Figs. 2 and 3).

Durum sample peak temperatures (Table 1, Fig. 4) displayed similar slopes to the onset temperatures but the R^2 values indicated similar fits for both protein type and sample preparation except for GM of durum, where $R^2 = 0.78$ (Fig. 4). The HRS data reported here and shown in Fig. 6 agrees with Eliasson's (1983) work, showing a linear relationship ($R^2 = 0.996$) of the developed gluten for the increase of peak temperature of starch gelatinization. The data reported here (HRS extract and starch) reflect similar effects of protein in a dough-like system, unlike a pre-developed gluten system, where mixed and equilibrated samples showed similar slopes and R^2 values. The peak temperature of the starch gelatinization showed a trend to increase with the increase in the amount of HRS protein extract added to the starch. The effects of 0, 5, and 10% protein extracts were not significantly different when compared to each other, while 20, 30, 40, and 50% were not significantly

Table 1
95% Confidence limits on mean predicted values

%Protein	Durum				HRS[a]			
	EE[b]	EM[c]	GE[d]	GM[e]	EE	EM	GE	GM
Onset								
0	a	a	a	a	a	a	a	a
5	a	a	a	b	a	a	a	a
10	a	a	a	b	a	a	a	a
20	a	a	a	b	ab	b	a	ab
30	a	a	a	b	b	b	a	ab
40	a	a	a	b	bc	c	a	ab
50	a	a	a	b	b	b	a	a
Peak								
0	a	a	a	a	a	a	a	a
5	a	a	a	b	a	a	a	a
10	a	a	a	b	ab	b	a	a
20	a	a	a	b	b	b	a	a
30	a	a	a	b	b	b	a	a
40	a	a	a	b	b	b	a	a
50	a	a	a	b	b	b	a	a
ΔH								
0	a	a	a	a	a	a	a	a
5	a	a	a	a	a	a	a	a
10	a	a	a	a	a	a	a	a
20	a	a	a	b	a	a	a	a
30	ab	a	ab	b	ab	b	a	ab
40	ab	a	ab	b	ab	b	a	b
50	a	a	a	a	ab	b	a	b

[a] HRS, Hard red spring wheat; at $\alpha = 0.05$, rows followed by the same letter(s) are not statistically different.
[b] EE, Extracted equilibrated.
[c] EM, Extracted mixed.
[d] GE, Gluten equilibrated.
[e] GM, Gluten mixed.

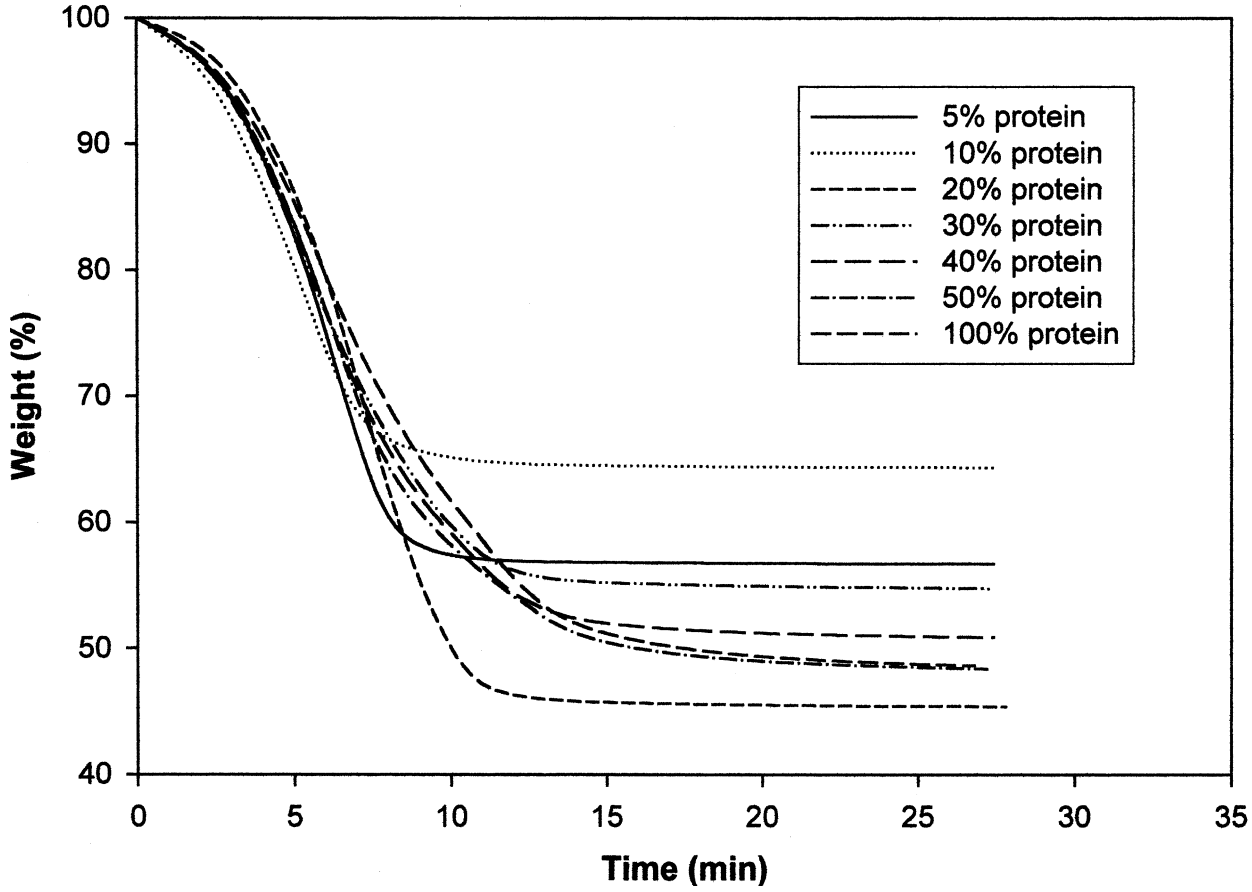

Fig. 9. TGA profile of equilibrated durum wheat protein extract and starch blends.

different when compared with each other, but the two groups were significantly different ($P \leqslant 0.05$).

The trend of increase in the onset temperature was more obvious than the trend of increase shown at the peak temperature. At the peak temperature, starch granules absorb more water. These physical changes improve the chance for starch–protein interaction. Beside water migration, starch–protein interaction can be in the form of adsorption of protein on the starch granules (Eliasson & Tjerneld, 1990). Gluten is rich in glutamine, 41% of which is a nonpolar amino acid (Bushuk & Wrigley, 1974). Hydrogen bonding may take place between the amino group of the glutamine and the second or the third hydroxyl of the glucose molecules of the starch after the gelatinization of the starch. The second and third hydroxyl groups of the glucose unit have higher probabilities of forming hydrogen bonding due to the open area around them, unlike the other free hydroxyl group.

The increase in the amount of the protein extract generally decreased the ΔH (Figs. 7 and 8). Eliasson (1983) reported that the effect of gluten on the gelatinization of the starch was due to the migration of water from the starch to the protein. The lesser amount of water available for the starch decreased the degree of starch gelatinization and thus lowered the ΔH. No significant differences in ΔH of starch gelatinization were observed between durum wheat starch samples with 0, 5, 10, and 20% protein (Table 1). Differences between equilibration and mixing were apparent in the slope value of the durum samples, for both extract and gluten. The R^2 values for EE and GE of durum samples were 0.57 and 0.77, respectively, indicating a fit with high variation. This may be due to protein availability and ability to absorb water in unmixed samples, where water can migrate with different rates between starch and protein without the physical action of mixing. The outcome of mixing of the blends is the development of the gluten, i.e. extension of the polypeptides and the formation of disulfide bonds. These changes increase the chances for interaction between the gluten different fractions. The lower ΔH values could mean that the starch in the blend has less available water due to protein competition, as reported earlier. Mixing, due to interaction between protein and starch, may restrict water migration. The same phenomena are true for HRS samples where the effect of mixing can be seen as a factor that brings consistency and homogeneity to the blend and thus changes the level of interaction, as reflected in the minimal variation in ΔH values. The change in the enthalpy was inversely proportional to the protein concentration.

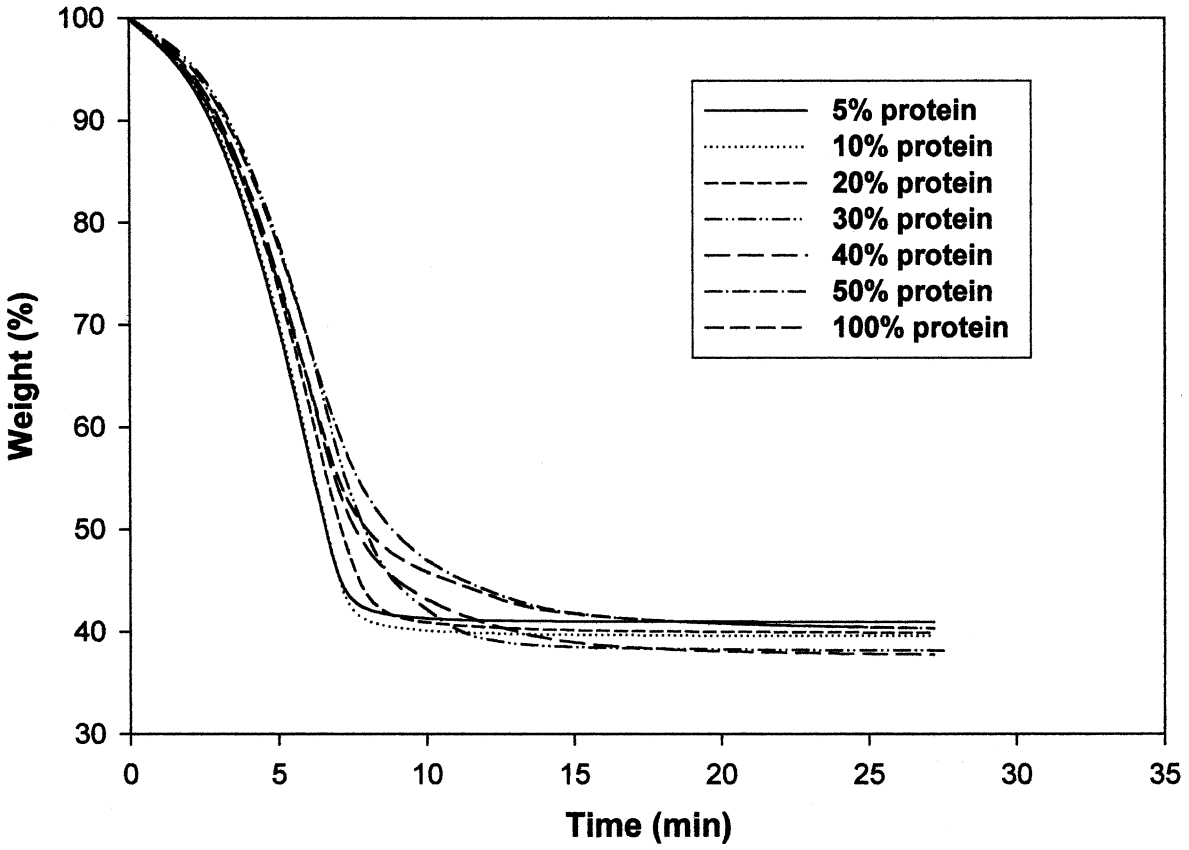

Fig. 10. TGA profile of mixed durum wheat protein extract and starch blends.

Protein extract increased the onset and peak temperatures and lowered the ΔH while mixing seemed to bring consistency to the effect of both durum and HRS wheat flour extracts represented in the R^2 values of the ΔH. A probable explanation for the difference could be that with mixing, a network is formed between the gluten proteins, which might have resulted in the alignment and the covering of the starch granules along the gluten network. This might increase the onset and peak temperatures of starch as a result of bringing starch granules into the protein network and restriction of water movement within the network. The formed network seemed to start to collapse soon after the starch peak temperature. The collapse appeared to happen rapidly when the starch granules lost the stability conferrred by the protein network formation. The increase in the protein concentration allowed the construction of a stronger network and limited water migration to the protein at the expense of the starch. Higher onset and peak temperatures were observed at higher protein contents, which in turn, lowered the ΔH values, indicating incomplete starch gelatinization. The Farinograph mixed samples behaved differently, based on the type of protein used. The extract and gluten increased the onset and peak temperatures, while the ΔH decreased. The Brabender-mixing showed a similar effect to the hand-mixing. The difference between the Brabender-mixing and the hand-mixing was apparent at the lower ΔH values for both gluten (5.0) and the extract (5.8) while the values for the hand-mixed samples were 12.4 and 12.3, respectively. The onset and the peak temperatures were comparable to the hand-mixed samples. The linear regression equations for the Brabender-blended samples are; ΔH, EM $5.8-0.01x$ ($R^2=0.72$) and GM $5.0-0.03x$ ($R^2=0.92$). The onset temperature, EM $65.6+0.17x$ ($R^2=0.83$) or GM $59.7+0.01x$ ($R^2=0.51$), while the peak temperature showed, EM $74.2+0.12x$ ($R^2=0.91$) or GM $66.0+0.5x$ ($R^2=0.56$). The regression data indicate the effect of the type of mixing on the gelatinization of the starch. The lower ΔH and R^2 values could be because Brabender-blending facilitated more water absorption by the gluten leaving less water for the starch. A microscopic test confirmed that some starch granules were still intact.

Thermogravimetric analysis is a technique where the mass of a material is measured as a function temperature while the material is subjected to controlled heating. The data can be reported as mass loss as a function of temperature or time. The application of TGA technique to the blends is meant to provide information about how water evaporation is influenced by the type and the varying level of protein content in the blends.

Since water migration between the starch and the protein is shown to have a major effect on starch

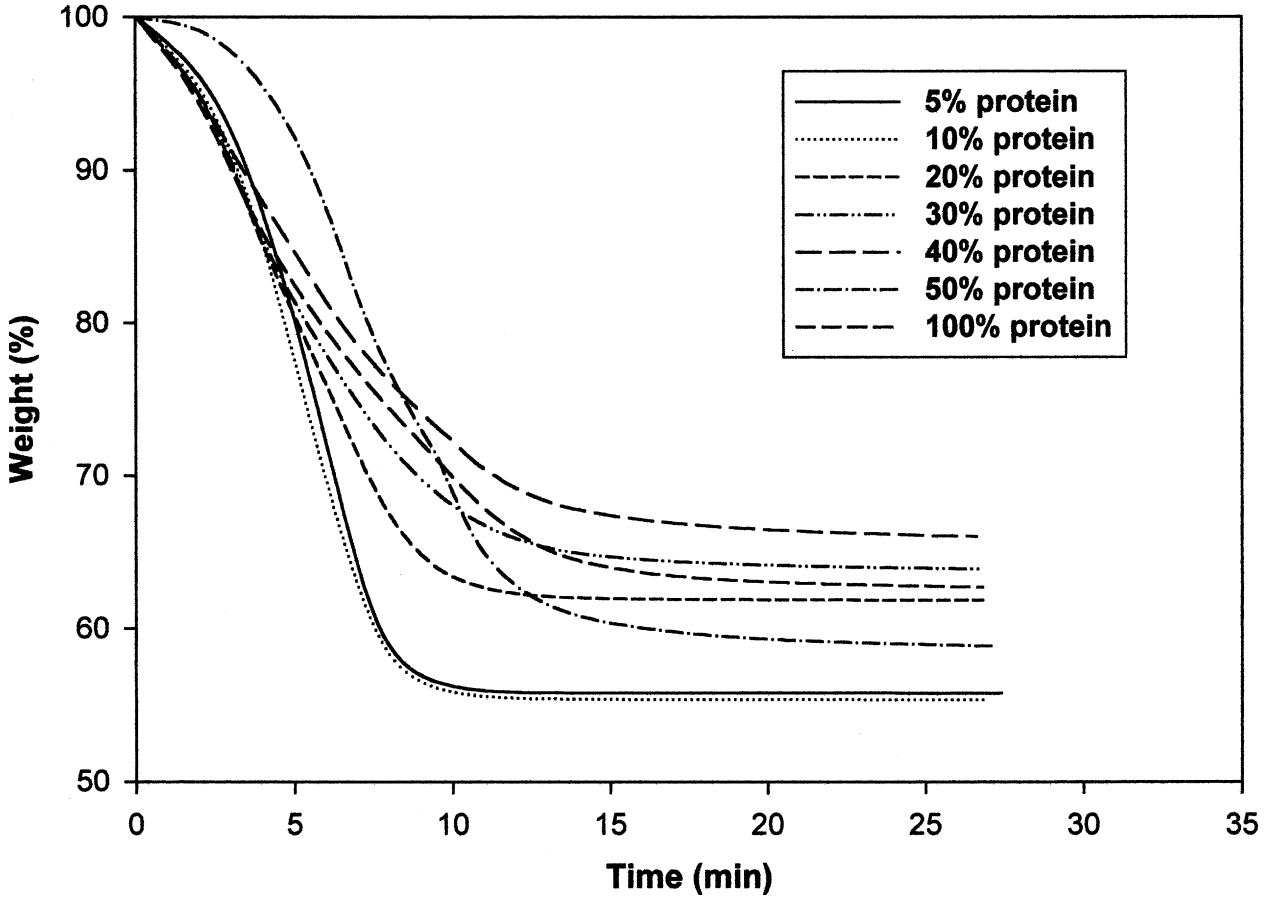

Fig. 11. TGA profile of equilibrated durum wheat gluten and starch blends.

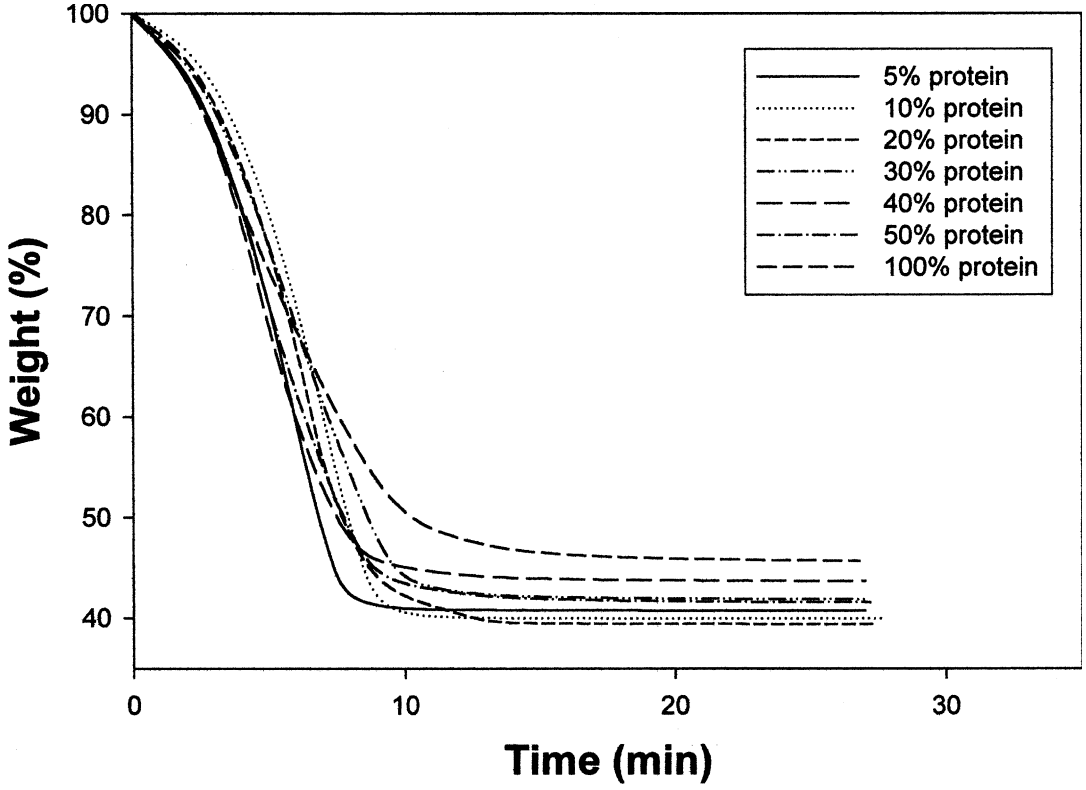

Fig. 12. TGA profile of mixed durum wheat gluten and starch blends.

gelatinization, it is relevant to investigate the way in which water evaporates from the system. TGA will allow for such investigation under different protein levels or type and equilibrated versus mixed samples.

Since the trends of water evaporation from durum and HRS are similar, only durum wheat results are presented here and the HRS data will be mentioned in the text. The data in Figs. 9–12 show the profiles of weight loss as a function of time. The durum EE samples showed more final weight variation than mixed samples (Fig. 9). It is clear that durum EM samples lost similar amounts of water with less variation within protein content, indicating the consistency brought by mixing while, in the case of the equilibrated samples, water migrated freely (Fig. 10). The difference between the weight-loss of the mixed and equilibrated samples is noticeable, i.e. at 10 min, mixed samples lost similar weights, while the equilibrated showed variation in their weight-loss values (Figs. 9 and 10). The HRS wheat samples, prepared with protein extract, were similar to durum samples. Samples with higher protein content finished with lower percentage of weight-loss, indicating that there is an amount of water remaining, possibly trapped by the protein.

The mixing process had a similar effect on the weight-loss profile regardless of protein type. Samples prepared with gluten protein displayed a water evaporation curves less homogeneous than those prepared with protein extract. This difference could be credited to the formation of disulfide bonds prior to blending protein with starch. Protein structure affects the way that protein interacts with water, in that respect, wheat protein and wheat gluten have different interactions with water. The long extended peptide chains of gluten are compared with shorter peptides of protein extract. Durum samples prepared with gluten showed less water loss than those prepared with extract. Water seemed to be held more strongly with pre-developed gluten. The mixing process did not close the gap between samples prepared with gluten and those prepared with protein extract, but it did close the gap between samples of the same type containing varying amounts of protein. It is interesting to notice that at specific times, samples show different TGA weight-loss profiles, i.e. at 5 min most durum EE samples lost approximately 25%, EM 30%, GE 10–15%, and GM 10–15%. Most variations in water loss curves within protein type and among protein contents took place around 10 min or at 100 °C (Figs. 9–12). The rate of the water loss was influenced by the amount of protein. The TGA data showed that high protein content delayed water evaporation in both mixed and equilibrated types. The mixed samples showed higher rate of water loss when compared to the equilibrated. This is further evidence of the effect of mixing on the interaction between starch, protein and water.

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