The Effect of Heat Treatment of Flour on the Extractability Characteristics of Wheat Protein

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ABSTRACT

The effect of heat treatment of wheat flour on the protein components by monitoring changes in protein extractability, SDS sedimentation volume and electrophoretic patterns was investigated. In this study the moisture content of flour was adjusted to 6% and heated in a convection oven at 75, 100 or 125°C for 30 mins. The results showed that heat treatment of wheat flour at 75°C had no effect on the total amount of protein extractability. When flour was heated at 100 and 125°C the total amount of protein extractability decreased. The significant effect ($P<0.05$) of heat treatment was observed on albumins and SDS-extractable glutenins comparing to globulins and gliadins. Heating at 100 and 125°C increased the amount of protein extracable by SDS and β-mercaptoethonal.

The results also showed that the SDS-sedimentation volume of wheat flour increased after heating at 100 and 125°C due to the aggregation of protein induced by heating. SDS-PAGE showed that heat treatment resulted in changes in the total amount of the extractability of protein from wheat flour with the most significant effect being occur after heating at 125°C . Also SDS-PAGE confirmed that gliadin fraction was less sensitive than SDS-extractable glutenin proteins.

Keywords: wheat flour, protein extractability, SDS-sedimentation.

INTRODUCTION

The quantity and quality of proteins in wheat flour are important in determining their functionality. Heat treatment is used to modify the functionality of wheat flour for use in specific applications e.g. soups and sauces. In addition, the baking performance of flour in high ratio cakes improves when the flour is heated. Johnson and Hoseney (1980) reported that heating wheat flour at 56°C for 20-240 h improved the crumb grain in high ratio cakes and prevented collapse during baking. Thomasson *et al.* (1995) reported that heating wheat flour (7% moisture) at 125°C for 30 min produced cakes with a greater volume than cakes made from unheated flour. Fustier & Gelinas (1998) reported that heating flour at 125°C for 1 hr. resulted in increase of cake batter viscosity.

Most of the studies on the effect of heat treatment of wheat flour have focused on the starch component. Several investigations have also been carried out on the effects of heat treatment of wheat starch on its physicochemical properties. The effect of heat treatment on isolated gluten has also received much attention. Jeanjean *et al.* (1980) reported that the amount of salt and ethanol extractable protein decreased after heating wheat gluten (71% moisture) at 100°C for 7 min. They also reported that the viscoelastic properties of wheat gluten were affected by heating resulting in a decrease in compressibility and an increase in firmness. Weegels *et al.* (1994) observed that heat treatment of gluten at 80°C for 30 min (at >20% moisture) was accompanied by a decrease in protein extractability and an increase in apparent viscosity.

Schofield *et al.* (1983) reported that the extractability of gluten proteins in SDS and in aqueous propan-1-ol decreased after heating wet wheat gluten at 100°C for 10 min. These changes can be attributed mainly to the increased protein aggregation involving S-S bonds. Hay and Every (1990) reported that when heat treated wheat gluten (90°C for 1 h) was added to white flour, loaf volume decreased. He & Hoseney (1991) also studied the effect of heat treatment at 100°C for 6 min on the viscosity of wheat protein that had been extracted into 1% SDS and reported that the viscosity of the protein solution decreased after heating.

The effects of heating isolated gluten at high moisture levels cannot be directly related to effects of heat treatment on wheat proteins when flour is heated. The aim of this study was to investigate the effect of heat treatment of flour on the protein components by monitoring changes in proteins extractability, SDS sedimentation volume and electrophoretic patterns.

MATERIAL AND METHODS

Chemicals and reagents

Sodium chloride (NaCl), sodium dodecyl sulphate (SDS), β-mercaptoethanol, N,N,N,N tetramethylethylenediamine (TEMED), acrylamide, bisacrylamide and ammonium persulphate were purchased from Sigma Chemical Co. (Saint Louis, Missouri, USA). All other chemicals were obtained from commercial sources and were of the highest quality available.

Wheat flour

Soft white wheat flour was obtained from Odlum Group Limited (Alexandra Road, Dublin1, Ireland). It contained 13% moisture, 74% starch, 9.1%, protein, 2.8% fat and 0.26% ash.

Adjustment of flour moisture

The moisture content of flour was adjusted to 6% where necessary by freeze drying, according to the method of Thomasson *et al.* (1995).

Heat treatment and changes in moisture content

A 0.5 cm layer of flour (25 g, 13% moisture content) was spread evenly on a sheet of aluminium foil and the foil was sealed. The flour was heated in a convection oven (Sanyo Gallenkamp PLC, Loughhborough, Leics, UK) at 75, 100 or 125°C for 30 min. After heating, the flour was allowed to cool to ambient temperature. Before and after heating, the moisture content of the flour was determined by oven-drying, according to AOAC (1995).

Sequential protein extraction

Proteins were extracted from flour using sequential extraction at room temperature, according to a modified Osborne procedure as follows: albumins and globulins were extracted using distilled water and 0.4 M NaCl, respectively. Gliadins were extracted with 70% ethanol. The residue was then extracted sequentially with 1.5% SDS and a mixed solution of 1.5% SDS and 1% β-mercaptoethanol. Two successive extractions were made with each solvent using 10 g of solvent per gram of flour. The suspensions were stirred for 30 min using a magnetic stirrer and centrifuged at ~3000 μg for 30 min. The protein content of the supernatants was determined by the Kjeldahl method (AOAC, 1995) and the supernatants were freeze dried.

SDS-sedimentation volume test

The SDS-sedimentation volume of the control and heated flours was determined using ICC methods (1995).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out using a 12% separation gel and 4% stacking gel according to Laemmli (1970). Freeze dried supernatants of wheat protein extracts (10 mg) were dissolved in 1ml of buffer solution, containing 3.8 ml of distilled water, 1.0 ml of 0.5 M Tris-HCL (pH 6.8), 0.8 ml of glycerol, 1.6 ml of SDS (10% w/v), 0.4 ml of β-mercaptoethanol and 0.4 ml of 0.05% (w/v) bromophenol. Samples were heated for 5 min in a boiling water bath and 10 μl of sample was applied to the gel. Each gel was placed overnight in staining solution consisting of 15 ml of Coomassie Brilliant Blue R-250 (4 g dissolved in 1 L of 95% ethanol), 25 ml of 60% trichloroacetic acid (TCA), and 210 ml of distilled water. Gels were then washed several times with distilled water. The molecular weights of the protein bands were estimated using protein standards (Sigma, No. M4038).

Statistical analysis

Statistical analysis was carried out using the statistical software SPSS 9.0 for Windows. One way ANOVA test was performed and followed by least significant difference (LSD) comparison test for means.

RESULTS AND DISCUSSION

Protein extractability

The effect of heat treatment on proteins extractability is shown in Table (1). The total amount of proteins extracted from control flour was ~93%, which confirms the effectiveness of the extraction process used in this study. This protein recovery rate compares favourably with that reported by Duviau *et al.* (1996) who recovered 87% of the total proteins from wheat flour using sequential extractions with NaCl, ethanol, sodium tetradecanoate and β-mercaptoethanol. Heating flour at 75°C had no significant ($P > 0.05$) effect on the total amount of proteins extracted. However, when flour was heated at 100 and 125°C, the total amount of protein extracted decreased significantly ($P<0.05$) to ~90 and 88%, respectively. These results indicate

Table 1: The effect of heat treatment on the extractability of wheat proteins with various solutions

Tempera-ture °C	Moisture (%)	Water	NaCL (0.4 M)	Ethanoal (70%)	SDS (1.50%)	SDS &-Mc (1.50% & 1%)	Total
20	6	22.2±0.2[a]	9.4±0.3[a]	23.5±0.2[a]	19.2±0.2[a]	19.2±0.2[a]	93.0±0.4[a]
75	6	26.6 ±0.2[a]	9.6±0.2[a]	23.8 ±0.2[a]	18.0±0.3[a]	19.1±0.1[a]	93.0±0.4[a]
100	6	18.9 ±0.2[b]	9.5±0.2[a]	23.1±0.2[a]	15.8±0.1[b]	23.5±0.3[b]	90.9±0.3[b]
125	6	11.2±0.2[c]	5.5±0.2[b]	20.4±0.4[b]	10.1±0.2[c]	40.6±0.2[c]	88.0±0.3[c]
125	13	10.9±0.3[c]	5.4±0.2[b]	20.2±0.2[b]	10.2±0.2[c]	40.9±0.2[c]	87.9±0.2[c]

Values in a column not sharing the same letter are significantly different at P<0.05.

that protein aggregation occurred during heating, particularly at 125°C and that this process could not be totally reversed by using reducing agents.

There was no significant difference (P>0.05) in the extractability of water-soluble albumins between the control and flour heated at 75°C. However, flour treated at 100°C and 125°C showed significant decreases (P<0.05) in extractability of albumins of ~15% and 50%, respectively. Extractability of salt soluble globulins appeared to be less sensitive to heating than albumins. Heating flour at 75 and 100°C showed no significant (P>0.05) effect on the extractability of globulins. However, when flour was heated at 125°C globulins extractability decreased by ~40%. These results are inconsistent with the earlier observation by Jeanjean *et al.* (1980) who reported that the extractability of albumins and globulins decreased after heating wheat gluten (71% moisture) at 100°C for 7 min.

Proteins extracted in 70% ethanol are mainly referred to as the gliadin fraction (Schofield *et al.*, 1983, Weegels & Hamer 1998). It is evident (Table 1) that there was no significant difference (P>0.05) in gliadin extractability between control and flour heated at both 75 and 100°C. However, the extractability of the gliadin fraction was reduced by ~13% when flour was heated at 125°C for 30 min. Geddes (1930) reported that heating of wheat flour at 77°C had no effect on the extractability of gliadins. In addition, Schofield *et al.* (1983) showed that gliadin extractability was unaffected by heating wet wheat gluten at temperatures up to 75°C but decreased on heating at 100°C.

The amount of protein extracted by SDS decreased by ~ 17.7 and 47% after heating flour at 100 and 125°C, respectively. These results appear to indicate that glutenin proteins extractable by SDS were more heat sensitive than gliadin proteins. Schofield *et al.* (1983) reported that gliadin proteins were more heat stable than glutenin proteins when wet wheat gluten was heated up to 100°C. They also reported that heat treatment of wheat gluten causes conformational changes, particularly in the glutenin proteins, such that disulphide linked protein aggregates are formed, resulting in tough gluten that is difficult to deform during the baking process. In addition, studies on the effect of heat treatment on wheat gluten have shown that disulphide bonds play a role in developing the final gluten structure (Jeanjean *et al.* 1980, Lavelli *et al.*,1996). Weegels *et al.* (1994) found that the reduction in the solubility of wheat proteins in SDS after heating wheat gluten at 80°C for 30 min at moisture 6.8% was due to a decrease in the solubility of the glutenin fractions.

No significant differences (P<0.05) in the amount of proteins extracted using SDS and β-mercaptoethanol were observed between the control and flour heated at 75°C. Extractability of proteins in SDS and β-mercaptoethanol was significantly (P<0.005) increased after heating at 100°C and 125°C. These results show that some proteins rendered insoluble in other solvents by heating at 100 and 125°C are extracted by SDS and β-mercaptoethanol, indicating the importance of disulphide bonds in the formation of heat-induced protein crosslinks. These results are inconsistent with those of a previous study by Guerrieri & Cerletti (1996) who reported that more than 95% of wheat protein were solubilized by SDS and reducing agent such as DTT when wheat flour (13% moisture) was heated at ~90°C for 10 min. Guerrieri *et al.* (1996) also reported that ~94% of the total gluten protein was extracted by 2% SDS under reducing condition when wheat gluten (60% moisture) was heated at 45, 65, 90 and 110°C for 1 h.

Weegels *et al.* (1994) demonstrated that the effect of heat treatment on the properties of wheat gluten depends on moisture content. When wheat

gluten is heated at 80°C for 30 min at moisture contents ranging from 6% to 60%, the most pronounced effects of heat treatment on the extractability of protein in SDS occurred at >20% moisture. The moisture content is also an important parameter influencing the effects of heat treatment on flour functionality. Russo & Doe (1970) demonstrated that moisture content of flour during heat treatment affects its baking performance in high ratio cakes. They reported that the optimum effects of heat treatment were obtained at moisture contents below 7%. Thomasson *et al.* (1995) found that decreasing the moisture content of flour to 7% before heat treatment improved cake volume. In this study it was found that when the moisture content of flour was increased from 6 to 13% no significant ($P<0.05$) change occurred in the amount of protein insolubilization which occurred on heating at 125°C.

Loss of moisture during heat treatment

The moisture content of flour samples was measured before and after heat treatment (30 min). The results are shown in Table (2). For flour with an initial moisture content of 6.0%, the final moisture content decreased progressively with increasing the temperature of heat treatment, from 5.8% at 75°C to 5.3% at 125°C. Heating at 125°C caused a much greater decrease of initial moisture content of 13%, which dropped to 8.6% moisture after heating.

Table 2: Moisture content of wheat flour before and after heat treatment

Treatment	Moisture (%) Before	Moisture (%) After
Flour heated at 75°C	6	5.8
Flour heated at 100°C	6	5.4
Flour heated at 125°C	6	5.3
Flour heated at 125°C	13	8.6

SDS-Sedimentation volume

The effect of heat treatment on the sedimentation volume of wheat flour is shown in Table (3). The SDS sedimentation volume test was similar for the control and flour heated 75°C. However, heating at 100 and 125°C increased the sedimentation volume, which may reflect protein aggregation induced by heating. Veraverbeke *et al.* (1997) reported that the SDS-sedimentation volume increased when dry wheat gluten was heated at 80°C for 24 hr or at 90 or 105°C for ~60 min. Similar SDS sedimentation volumes were found for flour with 6% and 13% moisture heated at 125°C.

Table 3: SDS-Sedimentation volume of wheat flour before and after treatment

Treatment	Moisture (%)	Sedimentation Volums (ml)
Unheated flour	6	18
Flour heated at 75°C	6	18
Flour heated at 100°C	6	22
Flour heated at 125°C	6	33
Flour heated at 125°C	13	32

SDS-PAGE

The results of SDS-PAGE show that changes in wheat protein induced by heating were qualitative as well as quantitative. Figures (1 and 2) show the electrophoretic patterns for the water and salt extractable fractions of wheat proteins before and after heat treatment. The water-extractable fraction of the control gave 11 bands with Mr ranging from ~16 to 64 kD while 13 bands with Mr values ranging from ~20 to 67 kD were observed in the salt extractable fraction of the control. Duviau *et al.* (1996) reported that 10 bands with Mr values ranging from 14 to 67 kD were observed in the water extractable fraction of wheat flour.

Heating flour for 30 min at 75°C had no major effect on the electrophoretic patterns of the water

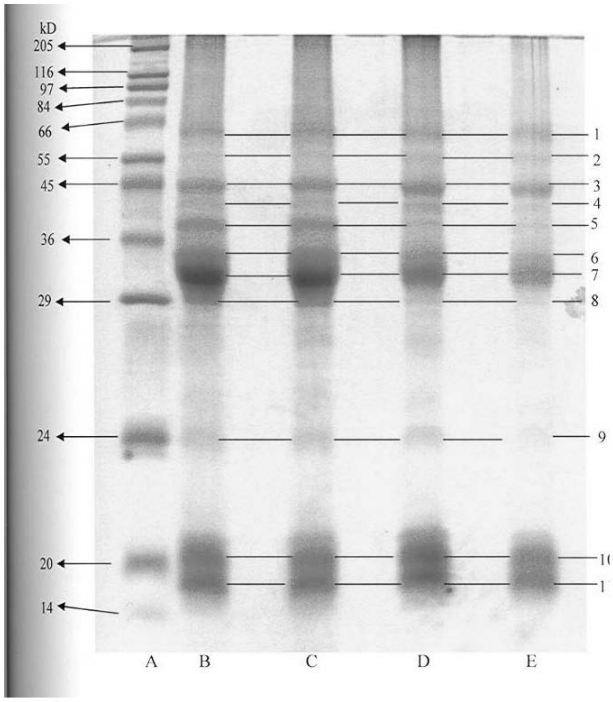

Fig. 1: SDS-PAGE profile of water soluble fractions; A: standard; B: control; flour heated at C: 75: D: 100 and E: 125ºC

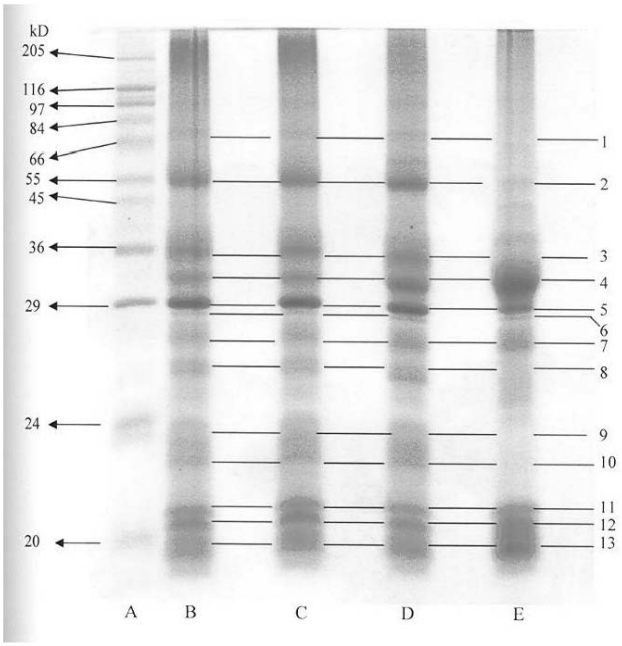

Fig. 2: SDS-PAGE profile of salt soluble fractions; A: standard; B: control; flour heated at C: 75: D: 100 and E: 125ºC

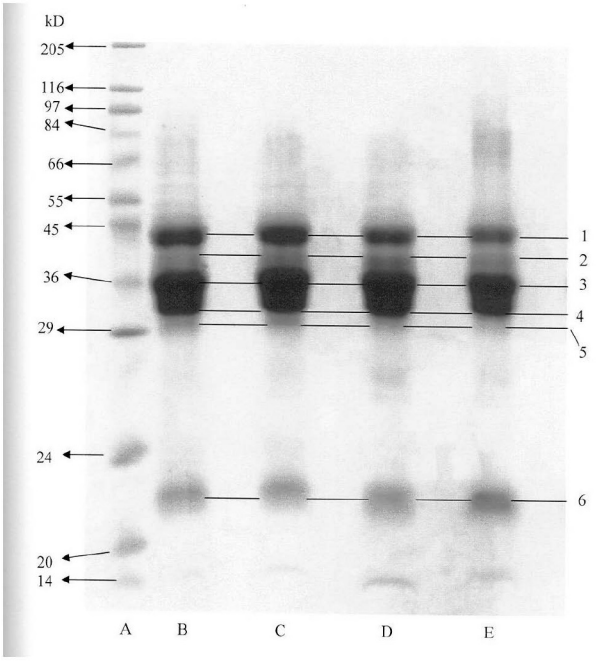

Fig. 3: SDS-PAGE profile of ethanol soluble fractions; A: standard; B: control; flour heated at C: 75: D: 100 and E: 125ºC

and salt extractable fractions. However, after heating at 100°C the intensity of bands number 5, 6 and 7 of the water- extractable fraction with Mr in the 30-40 kD region decreased and band number 8 with Mr of ~29 kD disappeared while no difference was observed for the salt-soluble fraction except a band with Mr of ~60 kD became evident. For flour heated at 125°C bands number 4, 5 and 8 of the albumin fraction with Mr ranging from ~29 to 40 kD disappeared and the intensities of all other bands decreased. Bands number 1, 2, 3, 8, 9 and 10 of the globulin fraction with Mr values ranging from 23 to 67 kD disappeared after heating at 125°C.

Figure (3) illustrates the electrophoretic pattern of wheat proteins extractable in 70% ethanol. Control flour displayed 6 bands with Mr values ranging from ~22 to 45 kD. The high molecular weight band (Mr ~45 kD) corresponds to ω- type gliadins and α, β, γ- type gliadins are the bands in the region Mr ~29 to 36 kD. Lavelli *et al.* (1996) observed that gliadin fraction contains α and γ - gliadins with Mr values from 30 to 40kD and ω-type gliadins with Mr 55 kD. Heating flour at 75 and 100°C showed no effect on the electrophoretic patterns of this fraction. Heating at 125°C caused a minor change in electrophoretic pattern with band number 5 (Mr ~30 kD) disappearing.

The electrophoretic patterns of SDS extractable fraction before and after heat treatment are shown in Figure (4). Control flour displayed 14 bands with Mr values ranging from ~16 to 115 kD. This fraction includes the low molecular weight (30 to 55 kD) and high molecular weight glutenin subunits (60 to 115 kD). Guerrieri *et al.* (1996) reported that 14 bands Mr value ranging from 14 to 116 kD were observed in the SDS extractable fraction of wheat gluten. Apichartsrangkoon *et al.* (1998) reported that 12 bands with Mr value ranging from 20 to 116 kD were apparent when wheat gluten was extracted using SDS (2%).

Similar electrophoretic patterns were observed for the control and flour heated at 75°C. However, heating flour at 100°C resulted in a decrease in the intensity of all bands. When flour was heated at 125°C, most of the bands disappeared apart from bands number 5, 7, 9, 11 and 13 with Mr ~58, 43, 35, 29 and 18 kD which were very faint. These results indicate that heat treatment makes glutenin proteins less extractable in SDS due to formation of protein cross-links. Lavelli *et al.* (1996) reported that some bands of SDS extractable fraction disappeared when wheat gluten was heated at 65 to 110°C (60% moisture).

Figure (5) shows the electrophoretic patterns of the fraction extractable in SDS and β-mercaptoethanol. Control flour showed 6 bands with Mr values ranging from 36 to 116 kD. The electrophoretic

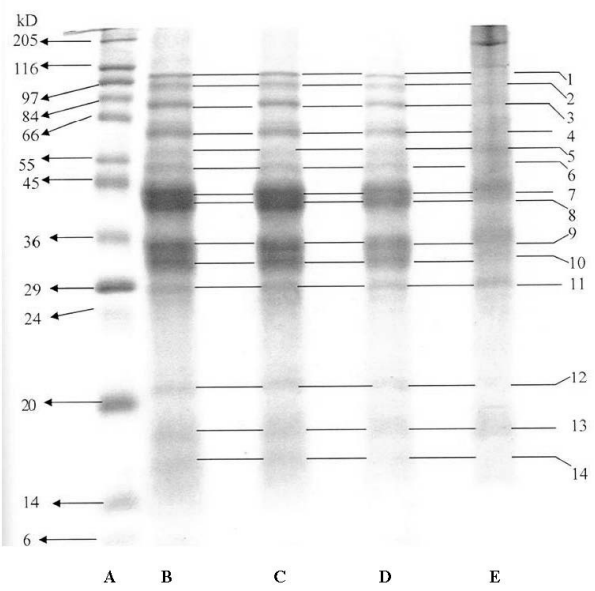

Fig. 4: SDS-PAGE profile of SDS soluble fractions; A: standard, B: control; flour heated at C:75: D: 100 E: 125°C

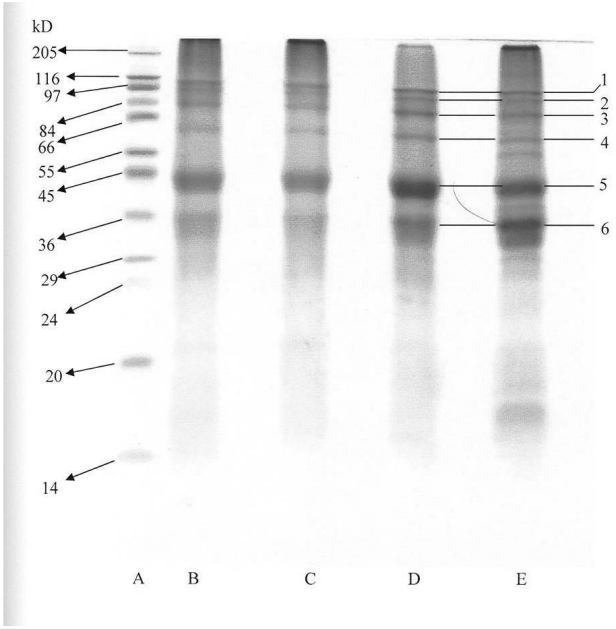

Fig. 5: SDS-PAGE profile of SDS and B-mercaptoethanol soluble fractions; A, standard; B: control; flour heated at C: 75: D: 100 and E: 125ºC

pattern of flour heated at 75°C was similar to that of the control. However, the intensity of all bands increased when wheat flour was heated at 100°C. For flour heated at 125°C, 4 additional bands with Mr values of ~16, 38, 55 and 64 kD were apparent. The appearance of these bands provides evidence for the formation of S-S linked protein aggregates during heating at 125°C. Schofield *et al.* (1983) reported that disulphide bonds were involved in protein polymerisation after heating wet wheat gluten at 100°C. Jeanjean *et al.* (1980) reported that the ability of SDS to disrupt heat induced aggregates of gluten proteins was more significant under reducing conditions.

Considering that heat treatment has been postulated as an alternative technology to chlorination to modify functionality of wheat flour it is interesting to compare effects of heating as obtained in this study to those reported for chlorination by Duviau *et al.* (1996). Chlorination also modifies the extractability of protein in wheat flour. The extractability of the albumin and globulin fractions decreases after chlorination of wheat flour while the amount of protein extracted by 70% ethanol increases. Chlorination appeared to have little effect on the extractability of high molecular weight glutenins. Low molecular weight proteins with Mr between 9 and 15 kD were also affected by chlorination of wheat flour while free SH groups decreased slightly in chlorinated flour. The authors concluded that the amount of S-S bonds was not affected by chlorination. The results of this present study suggest that heat treatment has considerably different effects on the extractability of wheat proteins compared with chlorination.

CONCLUSION

The main conclusions from this study can be summarized as follows.

1- Heat treatment of wheat flour at 75°C had no effect on the total amount of proteins extracted from the flour using a modified Osborne fractionation procedure. However, heating at 100 and 125°C resulted in a decrease in total proteins extractability.

2- Albumins and SDS-extractable glutenins were more sensitive to heating than globulins and gliadins.

3- The amount of proteins extractable by SDS and β-mercaptoethanol increased when flour was heated at 100 and 125°C, indicating that heat-induced protein aggregation occurred through the formation of disulphide bonds.

4- The moisture content of the flour (6 and 13%) prior to heating did not appear to significantly affect the amount of protein insolubiliation induced by heating.

5- The SDS-sedimentation volume increased when wheat flour was heated at 100 and 125°C.

6- SDS-PAGE showed that heat treatment caused qualitative changes in the extractability of proteins from wheat flour, with the most significant effects being apparent after heating at 125°C and with the SDS-extractable glutenin proteins being more sensitive to heat than the gliadin fraction.

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تأثير المعاملة الحرارية علي صفات مستخلص بروتين دقيق القمح

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تمت دراسة تأثير المعاملة الحرارية على صفات بروتينات دقيق القمح من خلال ملاحظة التغيرات التي تحدث على البروتين المستخلص والحجم المرسب ب SDS ونماذج البروتين المفصولة بالهجرة الكهربائية (SDS-PAGE) .

تم تعديل المحتوى الرطوبي للدقيق قيد الدراسة إلى ٦٪ ثم تم تسخين الدقيق على درجات حرارة ٧٥، ١٠٠، ١٢٥°م لمدة ٣٠ دقيقة.

أوضحت نتائج الدراسة أن معاملة دقيق القمح على درجة ٧٥°م ليس لها تأثير معنوي على كمية البروتينات المستخلصة بينما أدي تسخين الدقيق على درجات حرارة و ١٢٥°م إلى تقليل كمية البروتين المستخلص.

و قد تمت ملاحظة التأثير المعنوي للمعاملة الحرارية عند مستوى معنوية معنوية ٠,٠٥٪ على الألبومينات والجلوتينات المستخلصة ب SDS مقارنة بالجلوبيولونات والجلادينات.

أدي التسخين على درجة ١٠٠ و ١٢٥°م إلى زيادة كمية البروتينات المستخلصة باستخدام β-mercaptoethanol and SDS.

بينت النتائج أن كمية الحجم المرسب ب SDS زادت بعد التسخين على درجة حرارة ١٠٠ و ١٢٥°م وقد يرجع ذلك إلى حدوث تجمع لجزيئات البروتين المحفز بالحرارة.

أظهرت نماذج SDS-PAGE أن المعاملة الحرارية أدت إلى حدوث تغيرات في الكمية الكلية لمستخلص بروتينات دقيق القمح وكان التأثير الأهم معنويا عند درجة حرارة ١٢٥°م كذلك بينت نتائج هذه الدراسة أن SDS-PAGE على إن جزيئات الجلادين كانت اقل حساسية للحرارة مقارنة ببروتينات الجلوتينات باستخدام SDS.