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NUTRITIONAL IMPROVEMENT OF SOME LEGUME PROTEINS BY

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ABSTRACT

Cowpea and Soybean flour were subjected to different heat treatments, heat alone or after addition of either sodium metabisulfite ($Na_2S_2O_5$) and reduced glutathion (GSH). Heat alone inactivated about 46 to 60% of trypsin inhibitors content. In contrast exposing Flour to the same temperature (75°C for I hr) in the presence of 0.03 M $Na_2S_2O_5$ reduced the activity of trypsin inhibitor content to about zero. Addition of 0.6 M GSH to the flour and heating to 75°C for I hr inactiviated about 91 to 93% of trypsin inhibitor.

The effect of heating treatments on chymotrypsin inhibitor activity of flour at 100°C with 0.6 M GSH or 0.03 M $Na_2S_2O_5$ for I hr reduce most of chymotrypsin inhibitor activity.

The digestability index of the raw flours was improved by increasing heating temperature, especially after treatment of flours with either 0.03 M $Na_2S_2O_5$ or 0.6M GSH and heating 75°C for I hr.

Molecular weights of flours protein subunits extracted after different heat treatments as determined by SDS-PAGE, showed that heating flours for I hr over 45°C lead to disapperrance of high molecular weight units. Addition of either 0.03 M $Na_2S_2O_5$ or 0.6 M GSH to flours and heating for I hr showed very slight reduction in some high molecular weight subunits.

INTRODUCTION

Cowpea (*Vigna unguiculate*) and soybeans (*Glycine max* L) are important source of high quality plant protein for human consumption. Legume seeds contain various antinutritional factors which elicite adverse nutritional effects. Such antinutritional factors namely e.g. trypsin inhibitors, chymotrypsin inhibitors and vicine play an important role in decreasing the nutritional value of legume foods.

Trypsin inhibitors stimulate pancreatic juice secretion and cause pancreatic hypertrophy and growth inhibition (Liener and Kakade, 1980, Liener, 1994, 1996)

Reagents such as ß-mercaptoethanol, cysteine, N-Acetyl-cysteine and reduced glutathione have been extensively used in extraction of protein and enzymes to prevent oxidation of sulfhydryl groups and decreasing the amount of protein-protein interaction (Baldry *et al.*, 1970 and Friedman *et al.*, 1982). However disulfide bridges which maintain conformational and structural integrity of proteins may be reduce by these compounds (Sondack and Light, 1971).

Rackis (1972) reported that raw soybeans, unheated beans and their products may inhibit growth, depress metabolizable energy and fat absorption, reduce protein digestibility, cause pancreatic hypertrophy, stimulate hyper- and hypo-secretion of pancreatic enzymes and reduce amino acids, vitamins and minerals availability. Also, he noticed that all of the previous factors are interrelated. Lei *et al.*, (1981) reported that cysteine facilited the heat inactivation of inhibitor activity with both purified soybean kunitz inhibitor and soybean extracts. El-Mors (1982) studied the effect of heat on field bean trypsin inhibitor which lost most of its activity after one hr. at 96°C.

Friedman *et al.*, (1982) used thiols compounds to inactivate soybean trypsin inhibitors. They found that heating in an aqueous medium in water-bath at 25 to 93°C for I hr showed very slight effect on the inactivation of inhibitor (at 55°C and 85°C inactivation was 10% and 60%, respectively). In contrast, inactivation was 70% and 94% at the same temperatures in the presence of N-acetylecysteine and proceeded more rapidly. The thiol treatment also strikingly enhanced the in-vitro digestibility of soy proteins by trypsin.

Friedman *et al.*, (1984) found that heating soy flour at 45°C in pH 8.5 tris-buffer for one hr. in the presence of cysteine or N-acetylcysteine followed by dialysis to remove unreacted thiols resulted in the introduction of new half cysteine residues and lowered trypsin inhibitor content from 37.5 to 6.8 or 4.8 mg/g respectively compared with 14.8 mg/g in blank experiment. They concluded that proteins are modified through formation of mixed disulfide bonds which leads to loss of inhibitory activity and increased protein digestibility and nutritive value.

Friedman and Gumbmann (1986) found that treatment of raw soy flour at 75°C with 0.03 M sodium sulphite for I hr completely inactivated trypsin inhibitors leaving no sulfite residues in the soy proteins. Rat feeding study showed that protein efficiency ratio increased from 1.55 for raw flour to 2.11 for heated flour and 2.49 for heated flour in the presence of sulfite.

Sessa and Ghantous (1987) used sodium metabisulfite and glutaraldehyde alone to inactivate soybean trypsin inhibitor. El-Shakankery *et al.*, (1991) found that trypsin inhibitor activity in crude extracts of immature cowpea seeds were less thermal stable than those of dry mature seeds. After 20 min. heating the destruction of trypsin inhibitor was 70 and 47% in the extracts of samples collected at 15 and 25 days where as the crude extract of mature seed retained about 85% of its activity after the same period of heating. El-Morsi *et al.*, (1993) studied the level of trypsin inhibitor activity (TIA) in the albumins and globulins of ten cowpea varieties. Their results indicated that TIA was detected in the albumins of all cowpea varieties with amounts ranging from 2.52 to 7.36 mg/g protein. The levels of TIA in the globulins of other varieties ranged from zero to less than 10% of that reported in the albumins.

Turki *et al.*, (1993) studied the effect of heat alone or after addition of either sodium sulfite or cysteine on soybean tyrosine inhibitor. Heat alone inactivated about 64 to 70% of trypsin inhibitors content. In contrast exposing soy flour to the same temperature (75°C for 1 hr) in the presence of 0.03 M sodium sulfite reduced the activity of trypsin inhibitor to about zero. Addition of 0.128 M cysteine to the flour and heating at 75°C for 1 hr inactivated about 90 to 93% of the trypsin inhibitor.

The purpose of the present investigation is to study the improvement of cowpea flour and soybean proteins through different heat treatments for inactivation of tyrosine inhibitor, chymotrypsin inhibitors and evaluation of their nutritional value.

MATERIALS AND METHODS

Sampling:

Cowpea seeds (*Vigna unguiculates*) (Cream-7 and Black eye varieties) and soybean seeds (Williams variety) were obtained from Agricultural Research center, Giza, Egypt. The seeds were cleaned and finely ground. Hexane (B. P 40-60°C) was used for the extraction of oil from the ground seeds.

Heat treatments of flours:

Cowpea and soybean samples were heated at 45, 65, 75 and 100°C for 1 hr in the presence of 0.15, 0.30 and 0.60 M reduced glutathione (GSH). Another heat treatment under the same abovementioned temperatures in the presence of 0.015, 0.03 and 0.06 M sodium metabisulfite ($Na_2S_2O_5$).

Analytical methods:

Defatted meals were used for determining trypsin inhibitor activity (TIA) according to the method of Hamerstrand *et al.*, (1981). Chymotrypsin inhibitor activity (CIA) according Smirnoff *et al.*, (1976), total vicine (Collier, 1976), tannins and phytic acid (AOAC, 1975) and protein quality by in-vitro digestibility index (Ford and Salter, 1966).

Electrophoretic analysis:

Molecular weights of subunits of protein extracted by using (0.02 N NaoH) from different flours were determined by using sodium dodecyl sulphate-polyarylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmeli, (1970).

RESULTS AND DISCUSSION

Two cowpea varieties (Cream-7 and Black eye) and soybean variety (Williams) were subjected to chemical analysis. Data in Table (1) show that both cowpea varieties are rich sources of protein. Black eye has higher crude protein percentage (24.33%) than the other variety Cream-7 (23.25%).

The level of TIA in cowpea seeds was lower than that recorded for soybean and of the two cowpea varieties cream-7 was characterized by lower content TIA than black eye sample (Table 1). Phytic acid content was studied because the nutritional importance of phytic acid lies in its ability to chelate several mineral elements, especially the divalent metals such as Ca, Fe, Zn, Mn, Cu, Mg and Mo and there by reduce their availability in the intestinal tract (Maga, 1982, Beleia *et al.*, 1993). It forms a complex with proteins and inhibits the enzymatic digestion of ingested protein (Nolan and Duffin, 1987). For cowpea seeds used in this work the phytic acid content were higher than that recorded by Fawzy (1998) but lower than the value reported by Ismail *et al.*, (1995). Differences in phytic acid content may be caused by phosphorus utilization efficiency of each cultivars, which then determines phytic acid content of the seeds. This assumption is supported by the suggestion of Bhatty and Cherdkiatgumchai (1990) that the difference in phytic acid is due to total phosphorous content of cultivars. It is also possible that phytic acid concentration is differentially affected by environment among cultivars. Ockenden *et al.*, (1997) observed marked year to year variation in phyate content despite the fact that the plants were grown in the same area and the growth conditions were kept as similar as possible. These variation were found to be correlated with differences in environmental conditions, particularly the yearly differences in temperature.

Tannin contents shown in Table (1) indicated that the levels of which varied from 0.63 to 0.82% in the three legume samples and the highest value was recorded in soybean and the lowest in Black eye cowpea seeds.

The effect of heating legume flour of three samples in the absence or present of different concentrations of sodium metabisulfite and reduced glutathione on TIA is presented in Fig. (1) and Table (2). The results indicated inactivating more TIA with increasing the temperature but the flours retained about 40% their TIA even after heating at 100°C For one hour without any additive (Fig. 1).

Table (1): Chemical composition of two varieties of cow pea seeds and soy bean seeds, (calculated on dry weight basis).

Varieties	Protein %	Fat %	Ash %	Carbohydrate %	Trypsin inhibitor mg/g	Protein digestability %	Phytic acid mg/g	Vicine mg/g	Tannins mg/100g
Cream-7	23.25	3.08	3.80	69.66	9.53	79.33	7.21	3.50	0.76
Black eye	24.33	3.45	3.42	68.32	10.91	78.21	7.43	3.70	0.63
Williams	44.65	25.50	5.60	24.25	28.31	74.82	10.15	5.53	0.82

Table (2): Effect of heating treatments for I hr on trypsin inhibitors activity.

Heat treatment	Cream-7							Black eye							Williams						
	without any additatives	$Na_2S_2O_5$			GSH			without any additatives	$Na_2S_2O_5$			GSH			without any additatives	$Na_2S_2O_5$			GSH		
		0.015	0.03	0.06	0.15	0.30	0.60		0.015	0.03	0.06	0.15	0.30	0.60		0.015	0.03	0.06	0.15	0.30	0.60
Without heating	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
heating 45°C	75.31	45.33	35.62	30.59	51.92	48.91	40.60	79.17	46.91	35.93	31.29	50.17	45.93	41.70	71.41	45.60	33.71	27.13	24.93	42.11	40.39
heating 65°C	64.63	31.52	12.62	10.11	40.32	36.72	24.65	66.78	33.11	13.97	12.31	35.82	33.94	29.38	58.13	30.14	15.24	10.81	30.39	23.78	20.90
heating 75°C	54.29	16.73	0.00	0.00	33.12	30.10	8.13	59.27	16.93	0.00	0.00	30.72	23.12	7.61	40.93	17.03	7.04	6.82	28.46	19.81	18.42
heating 100°C	43.47	14.36	0.00	0.00	18.83	15.92	3.66	47.62	14.92	0.00	0.00	20.36	18.26	3.95	38.18	10.18	0.00	0.00	14.96	9.24	7.03

The presence of $Na_2S_2O_5$ or GSH in legume increased the effectiveness of heat treatment in destroying the TIA but sodium metabisulfite was more effective than glutathion. Heating at 75°C for one hour in the presence of 0.03 M $Na_2S_2O_5$ resulted in complete inactivation of TIA in cowpea samples but soybean flour retained about 7% of its original level under the same conditions (Fig. 1). All TIA of soybean samples were inactivated by heating at 100°C for one hour in the presence of 0.03 M $Na_2S_2O_5$. To achieve inactivating most of TIA in three legume by heating them in the presence of glutathione this would require heating at 100°C for one hour in the presence of 0.6 M of this additive (Fig. 1 and Table 2).

Under the same conditions chymotrypsin inhibitory activitys in the three legume samples were more resistant to heat treatment compared to TIA and non of the treatments applied in this work resulted in complete inactivation of such inhibitor (Fig. 2 and Table 3). The presence of either sodium metabisulfite or glutathion decreased the resistance of chymotrypsin inhibitor to heat treatment but the former compound was more effective than the second one (Fig. 2 and Table 3). Our data on heat inactivation of the inhibitors in the presence of sulfite are in a good agreement with those reported by Friedman and Gumbmann (1986) and (Mahmoud, 1993). Several workers have attributed the inactivation of protease inhibitor by heat treatment in the presence of sulfite and reduced glutation to the destruction of disulfide bridges of the inhibitors and structural proteins in flour and subsequent rearrangement of protein structures with the formation of new structural entities without altering the amino acid composition (Friedman, 1973; Wedzicha, 1984; Mahmoud, 1993). These new structures which may loose their ability to bind to proteolytic enzymes.

Effect of heat treatment on protein digestibility:

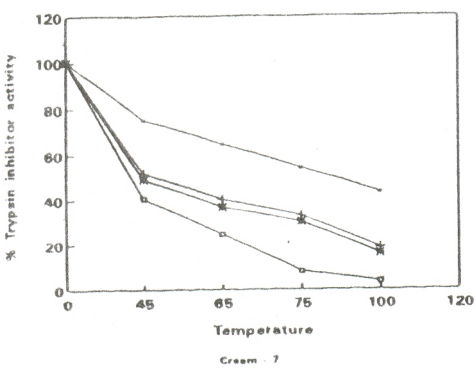
In-vitro protein digestibility of raw and treated flour was performed and the results are shown in Table (4). Heat treatment resulted in improvement of protein digestibility which were calculated to be 80.2%, 80.5% and 80.7% in flour samples of cowpea cream-7, Black eye and soybean, heated 100°C for one hour, compared with 77.1%, 76.7% and 74.8% for the same raw samples. The presence of $Na_2S_2O_5$ or GSH resulted in further increase in protein digestibility of the three samples used in this work. Factors suggested to limit the digestibility and reduced the nutritional quality of legume proteins include the presence of antinutritional factors, the structure of protein and complexing of the protein with starch, hemicellulose, minerals and other proteins (Deshpande and Nielsen, 1987a) while heating of legume seeds is known to inactivate preatease inhibitors (Fig. 1 and 2) some concern has been raised about the presence of heat-stable trypsin, chymotrypsin inhibitors in dry beans (Despande and Nielsen, 1987 b). According to Salunkhe and Kadam (1989) cooking improved the protein digestibility which may result from protein denaturation and inactivation of protease inhibitors by heat treatment. Several workers (Metry *et al* 1985 Barampama and Simard, 1994; Ismail *et al* 1995 and Zaki 1996) have

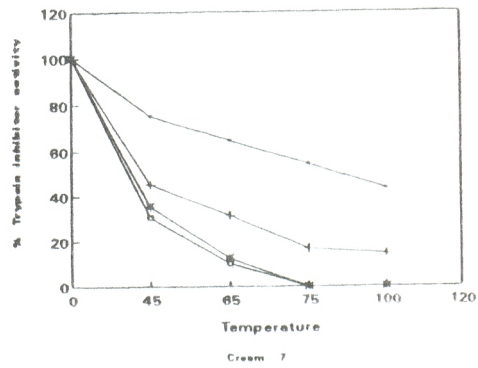
Table (3): Effect of heating treatments for I hr on chymotrypsin inhibitors activity.

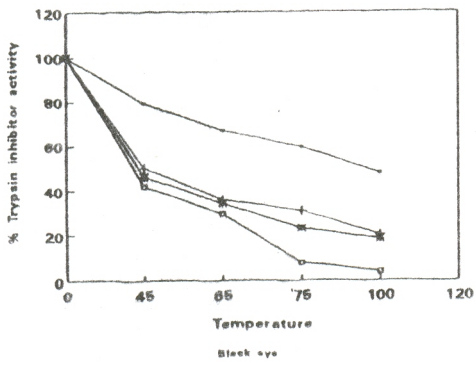
Heat treatment	Cream-7							Black eye							Williams						
	without any additatives	$Na_2S_2O_5$			GSH			without any additatives	$Na_2S_2O_5$			GSH			without any additatives	$Na_2S_2O_5$			GSH		
		0.015	0.03	0.06	0.15	0.30	0.60		0.015	0.03	0.06	0.15	0.30	0.60		0.015	0.03	0.06	0.15	0.30	0.60
Without heating	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
heating 45°C	91.20	70.92	55.21	45.31	81.32	73.26	65.28	93.51	72.13	57.91	47.13	83.14	75.45	69.13	88.14	73.12	59.72	48.30	83.71	69.31	63.22
heating 65°C	88.34	58.44	40.11	36.44	73.45	58.64	45.71	88.97	60.15	44.82	37.18	75.61	58.92	47.22	82.45	59.71	45.18	35.91	76.12	54.19	42.03
heating 75°C	79.39	39.72	28.24	20.53	59.87	42.51	33.14	81.23	43.36	31.71	20.88	63.17	44.81	36.18	73.14	45.34	31.12	23.71	54.81	37.81	31.14
heating 100°C	65.42	23.63	13.12	10.62	48.41	35.72	23.91	69.86	26.42	18.33	13.43	52.39	40.13	27.21	63.62	28.76	18.87	14.91	42.13	25.81	19.40

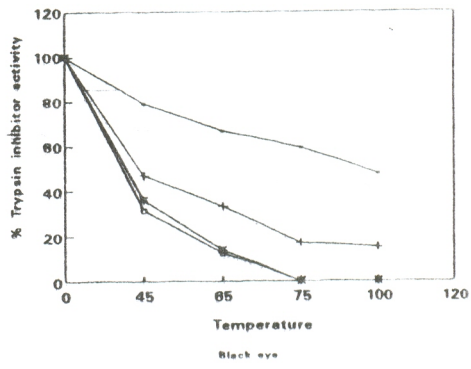
Table (4): Effect of heating treatments for I hr on flour protein digestibility index.

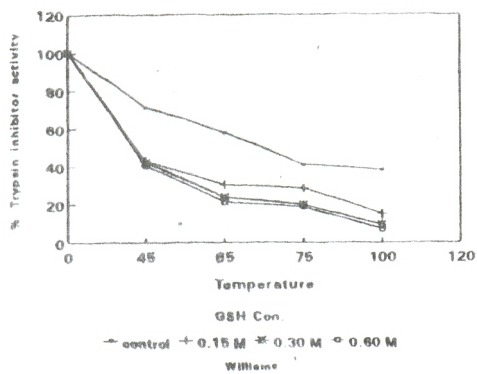
Heat treatment	Cream-7							Black eye							Williams						
	without any additatives	$Na_2S_2O_5$			GSH			without any additatives	$Na_2S_2O_5$			GSH			without any additatives	$Na_2S_2O_5$			GSH		
		0.015	0.03	0.06	0.15	0.30	0.60		0.015	0.03	0.06	0.15	0.30	0.60		0.015	0.03	0.06	0.15	0.30	0.60
Without heating	77.14	-	-	-	-	-	-	76.72	-	-	-	-	-	-	74.82	-	-	-	-	-	-
heating 45°C	77.43	78.70	81.62	82.31	78.13	82.51	82.55	77.13	78.42	81.91	82.17	77.83	80.72	80.72	75.13	80.13	81.23	81.95	77.14	82.23	82.34
heating 65°C	78.22	81.71	82.23	82.75	79.61	82.90	83.41	78.34	79.33	83.71	83.75	78.65	82.12	82.14	76.72	81.43	82.52	83.71	82.19	84.11	83.91
heating 75°C	78.81	82.13	84.81	84.84	80.72	83.14	83.96	80.12	81.22	84.88	84.79	80.41	82.68	82.91	79.41	83.11	83.82	84.36	83.91	84.82	84.73
heating 100°C	80.22	83.41	85.13	85.71	82.14	84.71	85.13	80.56	82.70	84.90	85.13	81.56	83.82	84.23	80.71	83.71	84.03	84.91	84.72	85.01	84.93











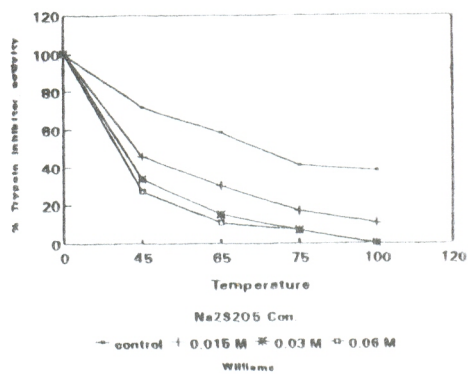
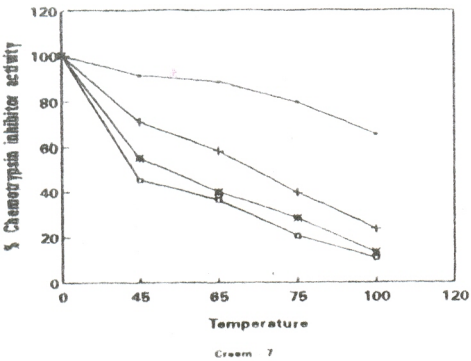
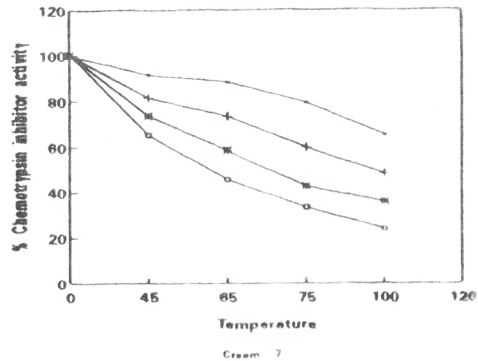
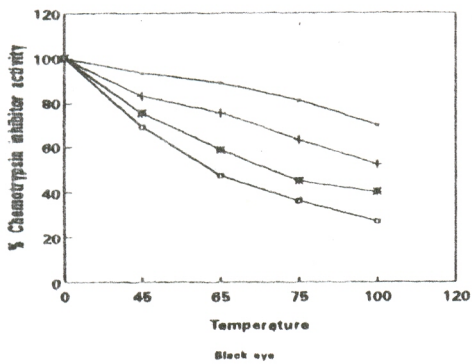
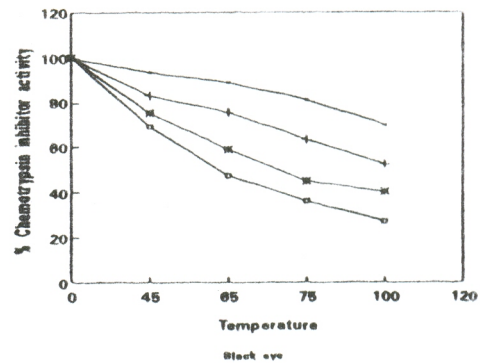


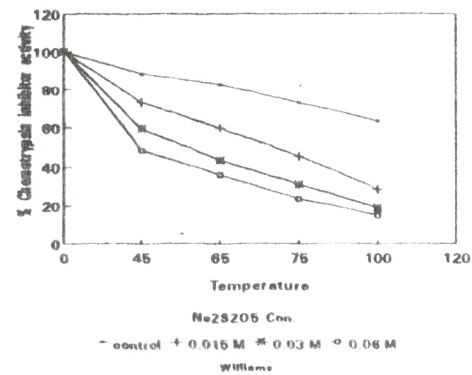
Fig (1) Effect of heating treatments for 1hr on trypsin inhibitors activity.











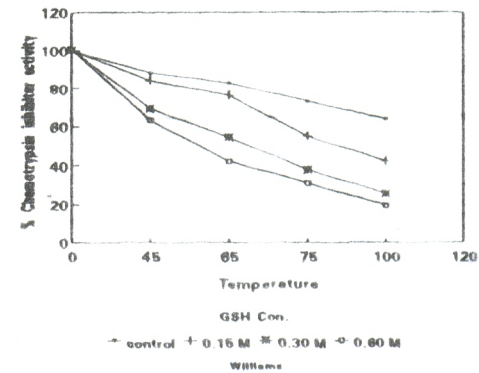


Fig (2) effect of heatin treatments for 1 hr on chemotrypsin inhibitors activity

reported improved the digestibility of legume protein after heat treatment which are in a good agreement with our data shown in Table (4).

Our results showed that metabisulfite level (0.03 M) at 75°C for 1 hr showed the most suitable improvement in protein quality. These results may be attributed to the proteins modification through a rearrangement of protein disulfide bonds. This modification leads to loss inhibitory activity and increased protein digestibility and nutritive values (Friedman *et al.*, 1984). Heat plus reduced glutathione treatment of flour increased protein digestibility comparing with heat alone. The optimum condition for maximum digestibility was noticed at 75°C for 1 hr which may be due to the formation new half-cysteine residues into native proteins which leads to loss its inhibitory activity and increased protein digestibility and nutritive value (Friedman *et al.*, 1984).

Polyacrylamide gel electrophoresis in the presence of the detergent sodium dodecyl sulfate (SDS-PAGE) was used to determine the effect of different heat treatment on the subunits molecular weights of the protein extracted from Black eye and Williams flour which revealed the presence 11 and 12 subunits, respectively with molecular weight ranging from 80.0 to 13.5 KD in Black eye flour and 90 to 17 KD in Williams variety, (Table 5). Heating flour devoid any additive revealed the disappearance of about half of the subunits of the protein of the two Legumes and all of the disappeared subunits were those of high molecular weights (Table 5). The presence of $Na_2S_2O_5$ (0.03 M) and GSH (0.06 M) in flour during heat treatment played a role in subunit structure of the protein and prevent the disappearance of high molecular weight subunit during heating.

Table (5): Molecular weight of Black eye and Williams protein subunits (KD) extraction at different heating with 0.03 $Na_2S_2O_5$ and 0.6 GSH (SDS-PAGE)

	Black eye													Williams											
	control	Heating				$Na_2S_2O_5$				GSH				Control	Heating				$Na_2S_2O_5$				GSH			
Bands		45°C	65°C	75°C	100°C	45°C	65°C	75°C	100°C	45°C	65°C	75°C	100°C		45°C	65°C	75°C	100°C	45°C	65°C	75°C	100°C	45°C	65°C	75°C	100°C
1	80	-	-	-	-	-	-	-	-	-	-	-	-	90	-	-	-	-	-	-	-	-	-	-	-	-
2	72	72	-	-	-	72	-	-	-	75	-	-	-	83	83	-	-	-	83	-	-	-	83	83	83	83
3	69	69	-	-	-	69	69	69	69	70	70	70	70	80	80	-	-	-	80	81	81	81	-	-	-	-
4	68	68	-	-	-	68	67	67	67	68	68	68	68	73	73	-	-	-	73	73	73	73	75	75	75	75
5	57	57	-	-	-	-	-	-	-	57	57	57	57	69	69	-	-	-	69	69	69	69	69	69	69	69
6	50	50	50	-	-	55	55	55	55	-	-	-	-	61	61	-	-	-	61	61	61	61	60	60	60	60
7	42	42	43	43	43	43	43	43	43	43	43	43	43	55	55	55	-	-	55	54	54	54	51	51	51	51
8	31	31	35	35	35	30	30	30	30	33	33	33	33	41	41	41	45	45	40	39	39	39	42	42	42	42
9	22	22	30	30	30	24	24	24	24	24	24	24	24	37	37	37	38	38	36	32	32	32	-	-	-	-
10	14	14	19	19	19	15	15	15	15	15	15	15	15	25	25	25	23	23	25	25	25	25	22	22	22	22
11	13.5	13	13	13	13	-	-	-	-	13	13	13	13	18	18	18	18	18	20	20	20	20	18	18	18	18
12														17	17	17	-	-	-	-	-	-	-	-	-	-

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تحسين القيمة الغذائية لبروتين بعض البقوليات

فرحات فوده على فوده ، إبراهيم محمد عبد العليم

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يهدف هذا البحث إلى تحسين خواص بروتينات اللوبيا لصنفى كريم ٧ و بلاك إى وبروتينات فول الصويا صنف وليامز بإستخدام المعاملات الحرارية المختلفة (التسخين فقط وكذلك التسخين بعد إضافة صوديوم ميتابيرسلفيت والجلوتاثيون).

وقد أوضحت النتائج أن التسخين فقط أدى إلى تثبيط فعل مثبطات إنزيم التربسين بحوالى ٤٦%-٦٠% بينما عند تعريض دقيق البقوليات على نفس درجة الحرارة ٧٥°م لمدة ساعة فى وجود صوديوم ميتاسلفيت (٠ر٠٣ مول) أدى إلى تخفيض نشاط مثبط إنزيم التربسين إلى صفر بينما فى حالة الجلوتاثيون (٠ر٠٦ مول) وتحت نفس الظروف أدى إلى تخفيض النشاط بمسنة ٩١-٩٣% مقارنة بالكونترول.

وقد أدى التسخين فى وجود صوديوم ميتاسلفيت (٠ر٠٣ مول) والجلوتاثيون (٠ر٠٦ مول) إلى إزالة معظم مثبطات إنزيم الكيموتربسين عند درجة الحرارة ٧٥°م لمدة ساعة.

وقد أوضحت النتائج أن معامل الهضم قد تحسن مع أزدياد درجة حرارة التسخين وخاصة فى وجود صوديوم ميتابيرسلفيت (٠ر٠٣ مول) والجلوتاثيون (٠ر٠٦ مول) على درجة الحرارة ٧٥°م لمدة ساعة.

وقد وجد بإستخدام التفريد الكهربي بإستخدام SDS-PAGE أن التسخين على درجة حرارة أعلى من ٤٥°م لمدة ساعة أدى إلى إختفاء بعض وحدات البروتين ذات الأوزان الجزيئية العالية بينما إضافة صوديوم ميتاسلفيت وكذلك الجلوتاثيون أدى إلى نقص بسيط فى وحدات البروتين ذات الوزن الجزيئى العالى.