BIOCHEMICAL STUDIES ON IMPROVEMENT OF SOYBEAN FLOUR PROTEINS

BY

Turki, M.A.; Saad, S.M; Nadia Y. Attia
and Abd-El-Aleem, I.M.
Dept. of Agric., Biochem., Fac. of Agric., Moshtohor, Zagazig Univ.

ABSTRACT

Soybean flour obtained from Clark and Crowford soybean seeds was subjected to different heat treatments, heat alone or after addition of either sodium sulfite or cysteine. Heat alone inactivated about 64 to 70 %of the trypsin inhibitors content. In contrast exposing soy flour to the same temperature (75°C for 1hr) in the presence of 0.03 (M) sodium sulfite reduced the activity of trypsin inhibitor content to about zero . Addition of 0.128(M) cysteine to the flour and heating at 75°C for 1hr inactivated about 90 to 93% of the trypsin inhibitor.

The polyacrylamide gel electrophoresis (PAGE) protein patterns showed that most of the polypeptide destruction effect was noticed at high polarity molecules especially heat treatments without any addition. Heating soyflour for 1hr in the presence of 0.03 (M) Na_2SO_3 or cysteine showed less destruction of polypeptide chains and appearance of new bands.

Molecular weights of soybean flour protein subunits extracted after different heating treatments as determined by SDS-PAGE, showed that heating soyflour for 1hr over 45 °C lead to disappearance of high molecular weight units (between 93,000 and 60,000 D). Addition of either 0,03(M) Na_2SO_3 or 0.128 (M) cysteine to soyflour and heating for 1hr showed very slight reduction in some high molecular weight subunits.

The digestibility index of the raw soy flour was improved by increasing heating temperature, especially after treatment of soyflour with either 0.03 (M) Na_2SO_3 or 0.128 (M) cysteine and heating to 100 °C for 1hr

Amino acid patterns showed that addition of either 0.03 (M) Na_2SO_3 or 0.128 (M) cysteine to soy flour and heating prevent to some extent the destruction of some amino acids and increased the sulfur amino acids. Therefore the amounts

of essential and non-essential amino acids were nearly closed to their amount in the native soy flour especially in case of heating at 65 °C for 1hr .

INTRODUCTION

The limited supply, the balance between the world food supply and the population are one of the major problems of the Twentienth century. Due to the high cost of the animal protein, especially in developing countries, attention was directed toward plant protein.

In Egypt much attention has been paid to soybean cultivation especially in new reclaimed areas to cover the shortage in protein and edible oil consumption. In 1991 the total area cultivated with soybean was 105792 feddan.

Soybean seeds contain the highest amount of protein (35 -45%) in Leguminosae. This relatively abundant, inexpensive and good quality protein is being utilized in human food in a variety of forms including flour, soybean concentrates and soy protein isolate. While, the soybean contains a highly quality protein, it also contains various anti-nutritional factors which elicite diverse nutritional, biological and physiological response in animals (Rackis, 1972). Raw soybean inhibits growth, depress metabolizable energy and fat absorption , reduce protein digestibility, cause pancreatic hypertrophy, and reduce amino acids, vitamins and minerals availability (Rackis, 1965, 1972 and 1974).

One of the antinutritional factors which received the most investigation is the trypsin inhibitors which account for 30 - 50 % of the growth inhibitory effect of raw soybean meal and for nearly all the pancreatic hypertrophic response in affected animals.

Friedman et al. (1984) found that soyflour contained 37 ±2.66 mg /g trypsin inhibitors. Friedman and Gumbmann (1986) found that soyflour contained 50.4 trypsin inhibitor units/g samples . Safwat (1985) noted that the varieties Clark, Calland , and Colombous exhibited high effect in its antitrypsin activity. Salama (1988) studied trypsin inhibitor activities of some legume seeds and found that soybean seeds had the highest values followed by dry bean.

Birk (1961) studied the effect of heat treatments on purified trypsin inhibitor of soybean at various

temperatures for different periods. His results indicated a noticed loss of inhibitor activity after autoclaving for 20 min. at, lbs/In^2.

Friedman et al.(1982) heated soyflour in an aqueous medium from 25 to 93°C for one hr. The heat alone does not begin to inactivate the inhibitor until about 55 °C(around 10% inactivation). In contrast , inactivation in the presence of N-acetyl cysteine proceeded more rapidly, since 70% of inactivation occurred at 55°C and 94% at 85°C.

Friedman et al. (1984) found that heating soyflour at 45°C in pH 8.5 tris buffer for 1hr, in the presence of L-cysteine and N-acetyl - L-cysteine followed by dialysis to remove unreacted thiols resulted in the introduction of new half-cystine residues and lowered trypsin inhibitor content from 37.5 to 9.8 mg/g. While, protein efficiency ratio increased from 0.95 to 2.01 or 2.2, respectively

Friedman and Gumbmann (1986) found that treatment of raw soyflour at 75°C with 0.03 (M) sodium sulfite for one hr inactivated trypsin inhibitors completely leaving no sulfite residues in the soy proteins. They added that the action of sulfite ions on the protein molecule might lead to an improve in its quality by cleaving the protein disulfide bonds to form a thiol anion (P-S) and S-sulfocysteine derivative $(P-S-S-SO_3)^-$, which can interact further with the generated $(P-S)^-$ to form a new disulfide bond and $(So_3)^{-2}$. The net effect of this reaction is the rearrangement of protein disulfide bonds which was catalyzed in general by $(So_3)^{-2}$ ions.

The aim of this research is to study the improvement of soy flour protein through different heat treatments for inactivation trypsin inhibitors and evaluation of their nutritional value.

MATERIALS AND METHODS

Materials:

Soybean seeds (Clark and Crowford varieties) were obtained from Agric Res Center Giza The seeds were cleaned and finely ground

Hexane (B.p. 40 -60°C) was used for the extraction of oil from the ground seeds by immersing in an extractor to get rid of the existed fat The solvent was removed by evaporation in a rotavapor to obtain the soyflour samples

Heat treatments on soybean flours:

Soyflour samples were heated at 45, 65, 75, and 100 °C for 1hr in the absence and presence of 0.128 (M) of cysteine according to method described by Friedman et al. (1984).

Another heat treatment under the same abovementioned temperatures and period of heating was carried out in the presence of 0.015, 0.03 and 0.06 (M) sodium sulfite, according to Friedman and Gumbmann (1986).

Extraction of proteins

Defatted soybean flour was extracted using 0.02(N) NaOH according to the method described by Melnychyn and Wollcott (1971). The supernatant was adjusted to the iso-electric point (I.E.P.) of the protein . The precipitate was washed twice by distilled water, centrifuged then dried under high vacum at 45 °C.

Analytical methods:

Determination of trypsin inhibitor activity was measured according to the method described by Hamerstrand et al. (1981) using benzoyl -DL arginie - p - nitroanilide hydrochloride (BAPA) as synthetic substrate for trypsin.

Quantitative determination of amino acids were carried out according to Moore et al. (1958) using Bechman amino acid analyzer Model 121.

Determination of (In-vitro) digestibility index for soybean flour protein was accomplished according to the method described by Ford and Salter (1966)

Electrophoretical determination for soybean flour protein of Clark and Crowford varieties were carried out by using polyacrylamide gel electrophoresis (PAGE).

Molecular weights of the subunits of protein extracted from soybean flour was determined by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli (1970) with some modifications. Slab gel was used instead of tube gel. Dimension of the slab gel was 11.5 cm (length)x11.5 cm (width) x1.0 mm (thickness). A custam - build apparatus unit which was similar to the Hoffer SE 500 vertical slab unit was used.

RESULTS AND DISCUSSIONS

I : Effect of heating treatments on trypsin inhibitor activity of soy flour:

The results concerning the effect of different heating treatments on both soy flour varieties are illustrated in Table (1).

The obtained results show that treatment of soy flour with heating alone does not inactivate all trypsin inhibitor. On the other hand , the amount of heat required to destroy trypsin inhibitors in soybean flour may destroy lysine and sulfur-containing amino acids and induce browning reactions (Rios Lriarte and Barnes 1966)

Treatment of soy flour at 100°C with 0.03 and 0.06 (M) sodium sulfite for 1hr reduced the trypsin inhibitor content to zero. Also, the results show that reduction trypsin activity by increasing the sulfite concentration up to 0.06 (M) was limited comparing with 0.03 (M). It should be noticed that a graded response to increasing sulfite concentration was not evident and the essentially full improvement in protein quality occurred with low sulfite level (0.03M). And hence , it could be concluded that heating of soyflour in the presence of sodium sulfite is highly effective in facilitating inactivation of trypsin inhibitors in soy products. The obtained results are in agreement with those reported by Friedman and Gumbmann (1986). Heat plus sulfite may act synergistically in improving the nutritional quality of soy flour Disulfide bonds of trypsin inhibitors and structural proteins in soyflour may be rearranged by the catalytic action of sulfite ions to produce new structural entities without altering the amino acid composition (Friedman 1973; Stevens 1973; Wedzicha 1984) Also, the new structures may lose their ability to complex with trypsin (Friedman and Gumbmann 1986)

The results in Table (1) illustrate that heating soy flour at 75°C and 100°C for 1hr in the presence of cysteine (0.128M) inactivated the trypsin inhibitor activity to 7.32 3.88% in the case of Clark variety and 9 38 5 21 % in Crowford variety These results prove that treatment of soy flour with cysteine form new half-cystine residues into native proteins with a corresponding improvement of nutritional quality

Table(1):Effect of heating treatments for(1hr)on trypsin inhibitor activity of soyflour.

Heating treatment	Clark variety					Crowford variety				
	Heating without any additatives	Heating in the presence of sodium sulfite (Na_2SO_4)			Heating in the presence of 0.128 M cysteine	Heating without any additatives	Heating in presence of sodium sulfite (Na_2SO_4)			Heating in the presence of 0.128 M cysteine
		0.015 M	0.03 M	0.06 M			0.015 M	0.03 M	0.06 M	
	% Trypsin inhibitor activity (TI activity)									
Room temperature (control)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Heating (45 °C)	68.97	42.54	32.26	29.94	22.87	62.52	36.96	30.77	30.54	20.94
Heating (65 °C)	51.72	29.58	10.22	9.6	9.73	46.89	27.35	10.68	10.84	14.26
Heating (75 °C)	44.92	15.79	0.13	0.12	7.32	40.38	13.12	0.098	0.11	9.38
Heating (100 °C)	36.51	11.28	0.00	0.00	3.88	29.47	9.44	0.00	0.00	5.21

From the economical point of view, heating of soy flour at 75°C with 0.03(M) sodium sulfite for 1hr is the most effective and suitable treatment since cysteine is more expensive and less effective.

II: Effect of soyflour heating on PAGE patterns of soybean protein extraction:

Soybean protein Clark and Crowford varieties under investigation were extracted by NaOH (0.02N) and separated electrophoretically. Polyacryalmide gel electrophoresis (PAGE) separated each protein to 12 staining bands. The effect of heat treatments at 45 , 65, 75 and 100°C for 1hr on the extracted proteins are illustrated in Fig. (1,A) which show a gradual reduction in the number of the separated bands by increasing heat temperature up to 75 °C.

Samples heated at 45°C showed the presence of 10 bands and decreased 6 bands by heating at 65°C. At 75°C and 100°C only 4 stable bands were noticed (bands 6,7,9 and 10) in both Clark and Crowford varieties. This observation may be due to the destructive effect of heat treatments, on high molecular weight peptides, to yield a lower molecular weight peptide chains, because, no destructive effect was noticed in the low molecular weight molecules.

Also, the effect of different heat temperatures for 1hr in the presence of 0.03 (M) Na_2SO_3 or 0.128 (M) cysteine on the extracted soybean protein was electrophoretically studied and illustrated in Fig. (1, B.C).

The obtained results indicate that heating soy flour for 1hr in the presence of sodium sulfite (0.03M) at 65 °C showed the presence of 7 bands Fig. (1,B). In addition, it was noticed that some bands were stable to heat treatments. New bands were formed and others disappeared. This observation may be attributed to that structural proteins in soy flour may be rearranged by the catalytic action of sulfite ions to produce new structural entities without altering amino acids composition (Friedman 1973 and Wedzicha ,1984).

Polyacrylamide gel electrophoresis patterns of soybean flour protein extraction after heat treatments in the presence of cysteine (0.128 M) indicated the presence of 10 bands at 45 °C (Fig .,1,C) . The number of bands decreased to 8 and 7 when the samples were heated at 65 75 and 100°C Results also show that addition of cysteine to soy

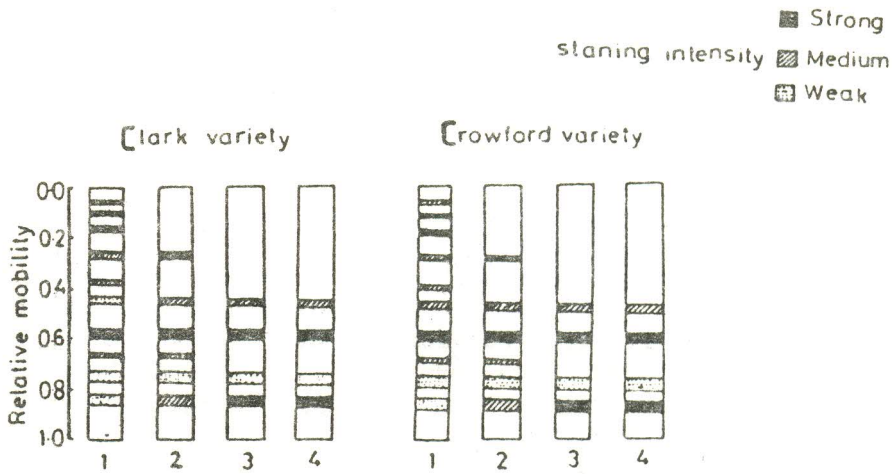

Fig. (1 ,A) : Heating only .

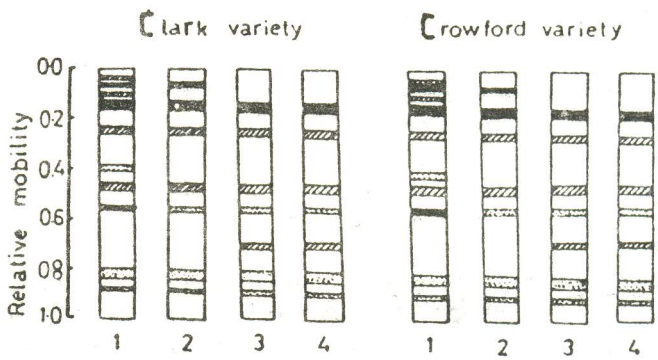

Fig. (1 , B) : Heating in the presence of 0.03 mol Na_2SO_3

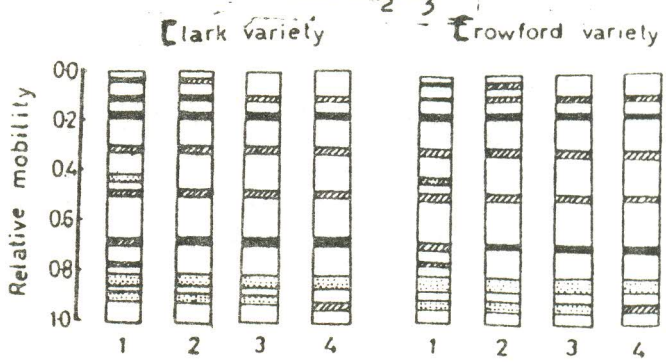

Fig. (1 , C) : Heating in the presence of 0.128 mol cysteine .

Fig. (1) : PAGE patterns of soybean flour protein extraction after different heating treatments for 1hr .

1) Heating at $45^{0}C$ 2) Heating at $65^{0}C$
3) Heating at $75^{0}C$ 4) Heating at $100^{0}C$

flour decreased the destructive effect of heat on proteins . This may be according to the fact established before by Friedman et al (1984) that heating soy flour in the presence of cysteine modified the proteins through the formation of mixed disulfide bonds which leads to less destruction of the polypeptide chains and appearance of new bands.

III: Effect of heating soy flour on the extracted protein subunits

Polyacrylamide gel electrophoresis in the presence of the detergent sodium dodecyl sulfate (SDS-PADGE) was used to determine the subunits molecular weights of the protein extracted from soybean flour after different heat treatments. The SDS-PAGE polypeptide patterns of the overall polypeptides in soybean protein together with protein standards are shown in Fig. (2).

The extracted protein of control samples (unheated Clark and Crowford soy flour proteins) showed the presence of 12 subunits with molecular weight ranging from 89,000 to 18,000 (D) in Clark flour variety and 93,000 to 18,000 (D) in Crowford variety. The staining intensity of these bands were stable when heated at 45°C for 1hr except band number 1 in case of Crowford soy flour protein which disappeared.

Table (2) and Fig. (2,A) illustrate the effect of heating temperatures i,e. 45, 65, 75, and 100 °C for 1hr on molecular weights of soy bean flour protein subunits. The obtained results show that noticeable reduction in the number of the extracted protein was observed when the soy flour was heated to temperatures more than 45 °C . The SDS- PAGE patterns illustrate that heating soy flour over 45 °C caused a reduction in the intensity of some bands and disappearance of high molecular weight subunits (between 93,000 and 60,000 D). This is due to the destruction of high molecules (Mw) to lower molecules (Mw).

The data concerning the effect of different heat temperatures (45, 65, 75 and 100 °C for 1hr) on Clark and Crowford soybean flour protein subunits in the presence of (0.03M) Na_2SO_3 are shown in Table (3) and illustrated in Fig. (2,B). By comparing the SDS-PAGE pattern of this heat treatment with that obtained in heating alone (Fig. 2,A; and Table 2) , it was noticed that addition of Na_2SO_3 (0.03M) stop to some extent the destruction effect of heat on protein molecule. Therefore high molecular weight subunits (between 90,000 and 60,000D) were very slightly affected.

Table (2) Molecular weights of soybean protein subunits extraction at different heating for 1 hr.

Band	Clark variety					Crowford variety				
	control	heating				control	heating			
		45 °C	65 °C	75 °C	100 °C		45 °C	65 °C	75 °C	100 °C
1	89000	89000	----	----	----	93000	---	----	----	----
2	83000	83000	----	----	----	89000	89000	---	----	----
3	79000	79000	----	----	----	81000	81000	----	----	----
4	72000	72000	----	-----	----	75000	75000	----	----	----
5	69000	69000	----	----	----	70000	70000	----	----	----
6	60000	60000	58000	---	----	63000	63000	----	----	----
7	53000	53000	57000	57000	57000	53000	53000	----	----	----
8	39000	39000	41000	41000	41000	41000	41000	41000	41000	41000
9	37000	37000	30000	30000	30000	38000	38000	39000	39000	39000
10	22000	22000	22000	22000	22000	25000	25000	22000	22000	22000
11	19000	19000	19000	19000	19000	19000	19000	19000	19000	19000
12	17000	17000	----	----	----	17000	17000	----	----	----

Table (3) Molecular weights of soybean protein subunits extraction at different heating with 0.03 mol Na_2SO_3.

Band	Clark variety					Crowford variety				
	Control	Heating+0.03M Na_2SO_3				Control	Heating+ 0.03M Na_2SO_3			
		45 °C	65 °C	75 °C	100 °C		45 °C	65 °C	75 °C	100 °C
1	89000	----	----	----	----	93000	-----	-----	-----	-----
2	38000	83000	83000	83000	83000	88000	88000	88000	88000	88000
3	79000	79000	79000	79000	79000	81000	81000	81000	81000	81000
4	72000	72000	72000	72000	72000	75000	75000	75000	75000	75000
5	69000	----	-----	-----	----	70000	-----	----	----	-----
6	60000	57000	57000	57000	57000	63000	65000	65000	65000	65000
7	53000	50000	50000	50000	50000	53000	53000	55000	53000	53000
8	39000	----	-----	-----	----	41000	-----	-----	----	----
9	37000	36000	36000	36000	36000	38000	38000	38000	38000	38000
10	22000	23000	23000	23000	23000	25000	25000	25000	25000	25000
11	19000	20000	20000	20000	20000	19000	20000	20000	20000	20000
12	17000	-----	-----	-----	----	17000	-----	-----	-----	----

I : Clark variety II: Crowford variety

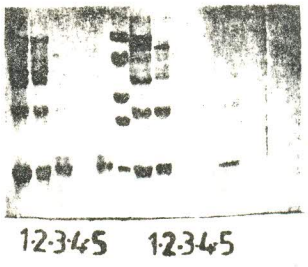

Fig. (2 , A) Heating only .

1) Sample without heating .
2) Heating at $45^{0}C$.
3) Heating at $65^{0}C$.
4) Heating at $75^{0}C$.
5) Heating at $100^{0}C$.

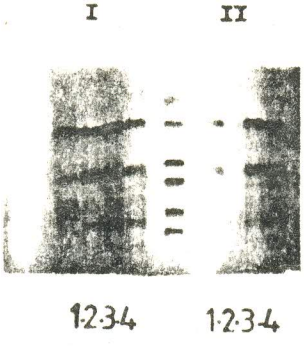

Fig. (2 , B) Heating in the presence. of 0.03 mol. Na_2SO_3 .

1) Heating at $45^{0}C$.
3) Heating at $75^{0}C$.

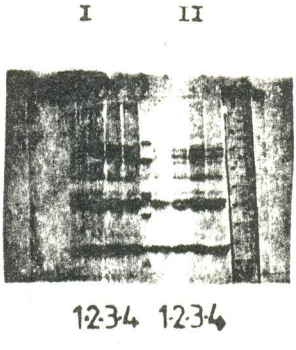

Fig. (2 , C) Heating in the presence of 0.128M cyateine

2) Heating at $65^{0}C$.
4) Heating at $100^{0}C$.

Fig. (2 , A ,B ,C) : SDS - PAGE patterns of soybean flour proteins ext-extraction after heating treatments for 1hr.

Data in Table (4) and Fig.(2, C) show the effect of heating soy flour Clark and Crowford varieties in the presence of (0.128 M) cysteine at different heat temperatures on the extracted protein subunits. The results indicate that the heated samples had 8 subunits with molecular weights ranging from 87,000 to 20,000 (D) for both varieties . On other words Clark and Crowford proteins showed a disappearance of four subunits i.e. 4,7,11 12,for the former and 1,7,10,12 for the latter ones. Molecular weights of other subunits were decreased owing to the formation of new half cystine peptide residues. These results confirm the idea that heating soy flour in the presence of cysteine modified the structure (Friedman et al. 1984).

IV: Effect of heat treatments on soybean flour protein digestibility:

Data in Table (5) show the effect of different heating treatments for 1hr on soy flour protein digestibility index.

The obtained results indicated that digestibility index of the native flour were 72 (Clark) and 71.7 (Crowford) then were improved by increasing heat temperature. Therefore, in both varieties the highest digestibility index i.e. 80.10 and 78.23 were observed after heat treatment at 100°C for 1hr . Such improvement could be partially attributed to protein denaturation which improves protein susceptibility to enzyme attack. Furthermore, inactivation of the trypsin inhibitors would certainly improve in- vitro protein digestibility (Metry et al.,1985). Such results are in agreement with that reported by Sathe and Salunkhe (1981).

Heat plus sulfite increased the protein digestibility and improved the nutritional quality of soy flour. Increasing sulfite concentration showed slight increment in digestibility index. The most suitable improvement in protein quality occurred with the sulfite level of (0.03M) at 75°C for 1hr for both Clark and Crowford varieties. These results may be attributed to the proteins modification through a rearrangement of protein disulfide bonds catalyzed by $SO_3^=$ ions. This modification leads to loss inhibitory activity and increased protein digestibility and nutritive values (Friedman et al .,1984). These results are in agreement with those reported by Friedman and Gumbman (1986).

Heat plus cysteine treatment of soy flour Clark and Crowford varieties increased protein digestibility comparing with heat treatments alone The optimum conditions for

Table(4) Molecular weights of soybean protein subunits extraction at different heating treatments with 0.128 mol cysteine

Band	Clark variety					Crowford variety				
	Control	Heating+0.128M cysteine				Control	Heating+ 0.128M cysteine			
		45 °C	65 °C	75 °C	100 °C		45 °C	65 °C	75 °C	100 °C
1	89000	87000	87000	87000	87000	93000	-----	-----	-----	-----
2	83000	83000	83000	83000	83000	89000	87000	87000	87096	87000
3	79000	75000	75000	75000	75000	81000	83000	83000	83000	83000
4	72000	-----	----	-----	-----	75000	75000	75000	75000	75000
5	69000	67000	67000	67000	67000	70000	67000	67000	67000	67000
6	60000	56000	56000	56000	56000	63000	56000	56000	56000	56000
7	53000	-----	----	-----	-----	53000	-----	-----	-----	-----
8	39000	39000	39000	39000	39000	41000	39000	39000	39000	39000
9	37000	38000	38000	38000	38000	38000	38000	38000	38000	38000
10	22000	19000	19000	19000	19000	25000	-----	-----	-----	-----
11	19000	-----	----	----	-----	19000	19000	19000	19000	19000
12	17000	-----	----	----	-----	17000	----	-----	-----	-----

Table(5): Effect of the heating treatments(1hr) on soyflour protein digestibility index .

Heat treatment	Clark variety					Crowford variety				
	Without any additatives	Na_2SO_3 0.015 M	Na_2SO_3 0.03 M	Na_2SO_3 0.06 M	Cysteine 0.128 M	Without any additatives	Na_2SO_3 0.015 M	Na_2SO_3 0.03 M	Na_2SO_3 0.06 M	Cysteine 0.128 M
Without heating	72.00	- - -	- - -	- - -	- - -	71.70	-			- - -
Heating (45 °C)	73.30	80.09	82.03	82.03	82.03	72.00	77.83	78.13	79.00	83.11
Heating (65 °C)	75.90	81.73	82.51	83.19	83.19	73.21	80.81	81.71	81.75	84.41
Heating (75 °C)	77.50	83.14	84.90	84.93	84.93	74.91	82.14	83.83	84.28	85.03
Heating (100 °C)	80.10	84.15	85.11	84.95	84.95	78.23	84.21	85.19	85.31	85.81

Table (6) : Effect of the heating treatments for(1hr)on the protein amino acids percentage(g/100g protein) of soyflour(Clark variety)

Amino acids	Without heating	Heating		Heating in the presence of (0.03 M) Na_2SO_3		Heating in the presence of (0.125 M) cysteine.	
		65 °C	75 °C	65 °C	75 °C	65 °C	75 °C
E.A.A.							
Lys	5.5	4.71	4.43	4.69	4.45	5.01	4.95
Leu.	6.3	6.33	5.56	6.38	5.88	6.24	5.89
Isoleu.	4.52	3.39	3.19	3.40	3.35	3.19	3.45
Cys + Met.	0.14+1.69	0.10+0.98	0.06+0.85	0.16+0.96	0.16+0.86	1.44+1.78	1.40+1.44
Phe. +Tyr.	5.15+4.21	4.78+3.93	4.55+3.91	4.92+4.19	4.42+3.95	4.93+3.81	4.82+3.62
Thr.	3.83	3.74	3.71	3.83	3.76	3.83	3.66
Val.	5.02	4.86	4.45	5.11	4.88	5.21	4.36
His.	3.40	3.12	3.09	3.33	3.21	3.31	3.10
T.E.A.A.	39.81	35.94	34.60	36.97	35.72	38.57	36.71
N.E.A.A.							
Arg.	5.82	3.36	5.31	5.68	5.50	5.56	5.36
Asp.	10.41	9.71	9.63	10.99	9.99	10.89	10.18
Glu.	15.25	19.99	13.81	15.39	14.49	15.41	14.56
Ser	4.68	4.50	4.49	4.66	4.48	4.65	4.50
Pro.	9.22	9.88	9.74	10.11	9.79	9.85	9.79
Gly	4.09	3.87	3.84	3.95	3.82	3.87	3.84
Ala.	4.41	4.33	4.21	4.35	4.26	4.34	4.30
T.N.E.A.A	53.88	52.64	52.03	55.15	52.33	54.37	52.63
T.A.A.	93.69	88.58	86.63	92.12	88.05	93.14	89.34

maximum digestibility was noticed at 75°C for 1hr . These results 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).

V: Effect of heat treatments on the amino acids of Clark soybean flour:

Abd-El-Aleem (1992) indicated that there is no differences in both varieties , i.e. Clark and Crowford, seeds and protein isolate in their total , aromatic and basic amino acids . On the other hand , Clark protein isolate contains higher amount of essential amino acids . Amino acids content of Clark soybean flour before and after different heat treatments were determined and tabulated in Table (6).

Results show that heating soy flour (without any additatives) caused a slight decrease on essential and non-essential amino acids . Although heating the flour at 75°C was not noticed to have more destructive effect on the amino acids than heating at 65°C.

The obtained results show that heating of flour in the presence of 0.03 (M) Na_2SO_3 prevented to some extent the destruction of some amino acids (Table, 6). Consequently , the amount of the total essential amino acids was improved and became similar to the amount in the native flour . Also , heat treatment of soyflour at 65° C in the presence of sodium sulfite (0.03M) yields a product better in its amino acids content than that at 75°C. These results are in agreement with that reported by Friedman (1973) , Wedzicha (1984), and Metry et al. (1985) on chick pea proteins.

Results in Table (6) illustrate that addition of cysteine to soy flour improved the amounts of sulfur amino acids in the flour. The amounts of both cysteine and methionine increased from (0.10 and 0.98) to (1.44 and 1.78) and from (0.06 and 0.85) to (1.40 and 1.44) on heating at 65°C and 75°C , respectively. Also, the amounts of the essential amino acids is higher after cysteine treatment than the heat treatment only (38.57 and 36.71% for the former, 35 .94 and 34.60 % for the latter). On the other hand , the amounts of essential and non-essential amino acids are closed to its amount in the native soy flour protein It can be concluded that addition of cysteine to soy flour protect protein amino acids destruction under heat treatment and enrichment the sulfur amino acids of the flour These

results are in agreement with those reported by Friedman (1973) and Wedzicha (1984).

REFERENCES

Abd-El-Aleem, I.M. (1992) : Chemical studies on soybean protein; Ph.D. Thesis, Fac. of Agric., Moshtohor, Zagazig Univ.

Birk, Y. (1961): Purification and some properties of a highly active inhibitor of trypsin and chymotrypsin from soybean; Biochim. Biophys. Acta., 54, 378-381.

Ford, J.F. and Salter, D.N. (1966): Analysis of enzymically digested food proteins, J. Nutr., 20,483.

Friedman, M. (1973): The chemistry and biochemistry of the sulfohydryl group in amino acids, peptides and proteins; p.199, Pergamon Press Oxford, England .

Friedman, M., Grosjean, O.K.and Zannley C.J. (1982): Inactivation of soybean trypsin inhibitor by thiols; J.Sci . Food Agric., 33, 165-172.

Friedman, M. , Gumbmann, M.R., and Grosjeon, O.K. (1984): Nutritional improvement of soy flour; J. Nutr. ,114, 2241-2246.

Friedman, M. and Gumbmann, M.R. (1986): Nutritional improvement of soy flour through inactivation of trypsin inhibitors by sodium sulfite; J. Food Sci., 51, 1239 - 1241.

Hamerstrand, G.E.; Black, L.T.; and Glover, J.D. (1981): Trypsin inhibitors in soy products , modification of the standard analysis procedure; Cereal Chem. 58, 42-45.

Laemmli, U.K. (1970): Cleavage of structral proteins during the assembly of the head of bacteriophage T4; J. Nutr., 227, 680-685

Melnychyn, P. and Wolcott, J.M. (1971): Isolated soy protein, U.S. Patent; 3,630 -753.

Metry, S.W., El-Morsi, E.A., and El-Dein, M.A (1985): Effect of roasting on chemical composition amino acid content, trypsin inhibitor and in-vitro protein digestibility of chick pea seeds 2 nd Agric. Conf. Bot. Sci., 21-24 Sept. Mansoura Univ., 363 - 378 (1985)

Moore, S. Spachman, D.H., and Stein, W. (1958): Chromatography of amino acid on sulphonated polystyrene resins, Anal. Chem; 30 , 1185- 1190

Rackis, J.J. (1965): Physiological properties of soybean trypsin inhibitors and their relationships to pancreatic hypertrophy and growth inhibition of rats, Fed, Proc. Am. Soc. Exp. Biol., 24, 1488- 1497.

Rackis, J.J. (1972): Biologically active components in soybean chemistry and technology, Ed. Smith , A.K. and Circle , S.J. Vol., 7 P, 158, Avi Publ. Co., Westport, Conn.

Rackis , J.J. (1974): Biological and physiological factors in soybeans, J,Am. oil chem., Soc. , 161A- 174A.

Rios Lriarte , B.J. and Barnes (1966): The effect of over heating on certain nutritional properties of the protein of soybeans , Food Technol., 20, 835- 838,

Safwat , H. A. (1985): Biochemical studies on soybean proteins , M.Sc . Thesis , Fac. of Agric. , Ain Shams Univ.

Salama, H.D. (1988): Proteinous evaluation of some leguminous seeds, Ph. D. Thesis, Fac. of Agric. , Ain Shams Univ.

Sathe, S.K. and Salunkhe, D.K. (1981): Studies on trypsin and chymotrypsin inhibitor activities., Hemagglutiating activity, and sugars in the Great Nothern Beans (Phaseolus Vulgaris L.),J. Food Sci., 46, 626-629.

Stevens, D.L. (1973): Reaction of wheat protein with sulfite. III. Measurement of labile reactive disulfide bonds in glidin and in the protein of aleurone cells , J. Sci. Food Agric. , 16, 279.

Wedzicha, B.I. (1984): Chemistry of sulfur dioxide in foods, Applied Science Publishers, P 69 El-Sevier London and New York

دراسات كيميائيه حيويه عن تحسين بروتين فول الصويـــــــــا

منير عبدالعظيم تركى . صلاح مصطفى سعد ، ناديه يحى أحمدعطيه . ابراهيم محمدعبدالعليم

يهدف هذا البحث الى تحسين خواص دقيق فول الصويا لصنفى كلارك وكروفورد باستخدام المعاملات الحرارية المختلفة (التسخين فقط وكذلك التسخين بعد اضافة كبرتيت الصوديوم أو السستئين) وقد أوضحت النتائج أن التسخين فقطأدى الى تثبيط فعل مثبطات أنزيم التربسين (TI) بحوالى ٦٤ - ٧٠٪ بينما عند تعريض دقيق فول الصويا على نفس درجة الحرارة (٧٥°م) لمدة ساعة فى وجود كبرتيت الصوديوم (٣رمول) أدى الى تخفيض نشاط مثبطات أنزيم التربسين الى درجة الصفر تقريبا - أما فى حالة اضافة السستئين (١٢٨رمول) وتحت نفس الظروف أدى الى تخفيض النشاط بنسبة ٩٠ - ٩٣٪ مقارنة بالكنترول .

باستخدام التفريد الكهربائى (PAGE) اتضح ان اكثر سلاسل البروتين التى حدث لها تكسير هى الجزئيات التى لها درجة قطبية عالية خاصة فى حالة المعاملات الحرارية التى بدون أى اضافات أما بالنسبة للمعاملات الحرارية فى وجود كبرتيت الصوديوم (٣ر مـــول) أو السستئين أظهرت أقل تكسيرا فى سلاسل البروتين .

وقد وجد باستخدام التفريد الكهربائى فى وجود مادة صوديوم دوديسيل (SDS-PAGE) أن تسخين دقيق فول الصوياعلى درجة حرارة أعلى من درجة ٤٥°م لمدة ساعة ، أدى ذلك الى اختفاء بعض وحدات البروتين (Subunits) ذات الاوزان الجزيئية التى تتراوح ما بين ٩٣ر٠٠٠ الى ٦٠ر٠٠٠ دالتون وفى حالة اضافة كبرتيت الصوديوم بتركز ٣رمول أو السستئين بتركز ١٢٨رمول أدى ذلك الى نقص بسيط فى بعض وحدات البروتين ذات الوزن الجزيئى العالى.

وقد أوضحت نتائج معامل قابلية البروتين للهضم أن الدقيق الخام لفول الصويا لكـــلا الصنفين (كلارك ، كروفورد) قد تحسن مع ازدياد درجه حرارة التسخين وخاصة التسخين فى وجـــود كبرتيت الصوديوم (٣رمول) أو السستئين (١٢٨رمول) على درجة حرارة ١٠٠°م لمدة ساعة.

أوضحت أنظمه الاحماض الاميبيه لبروتينات دقيق فول الصويا (صنف كلارك) أنه باضافـــة كبرتيت الصوديوم (٣رمول) أو السستئين (١٢٨رمول) والتسخين أدى الى عدم التكسير الـــى حد ما فى بعض الاحماض الاميبية الكبرتية وكذلك فان كميات الاحماض الاميبية الاساسية والغيـــر أساسية أصبحت قريبه من الدقيق الغير معامل حراريا خاصة التسخين على درجه حراره ٦٥°م لمـــدة ســـــاعة.