

**EFFECT OF MICROWAVE HEATING AND SOAKING SOYBEAN SEEDS IN
EITHER WATER OR SODIUM SULFITE ON PROTEIN QUALITY**

BY

Shabana, M.K.S.; Nadia Y Attia,; Saad, S.M.

and Abd El-Aleem, I.M.

Biochem. Dept., Fac. of Agric., Moshtohor, Zagazig Univ.

ABSTRACT

Seeds of two soybean (*Glycine max* L) varieties, Clark and Crawford were used to study the effect of microwave heating and soaking in either water or 0.03 M sodium sulfite solution followed by heating for 1 hr. at 70 °C on trypsin inhibitor activity, protein digestibility protein sub-units and amino acids content.

Microwave heating for 9.0 min. after soaking the seeds for 1 hr. resulted in 93% inactivation of trypsin inhibitor activity. Soaking seeds for two hrs in water or in 0.03 M sodium sulfite solution followed by heating for one hr. at 70°C inactivated about 50 and 75% of trypsin inhibitor activity respectively for both Clark and Crawford varieties. Microwave heating improved protein digestibility. Soaking seeds in sodium sulfite solution showed better digestibility percentage than soaking in water. Sodium Dodecyl Sulphate-Poly-Acrylamide Gel Electrophoresis (SDS-PAGE) pattern of the extracted protein exposed to microwave heat treatment showed a great destruction effect on protein subunits especially those of high molecular weights. After 6.0 min., more than 80% of the protein subunits were disappeared. Prolonging microwave heating time showed more destruction in the total amino acids, while soaking seeds in either water or 0.03 M sodium sulfite showed better amino acids pattern, which was approximately similar to those of the control.

INTRODUCTION

Legume seeds contain a wide range of constituents which have adverse effect on enzyme activity digestibility, nutrition and health. The inhibitors of digestive enzymes are common constituents of leguminous seeds which reduce protein digestibility, depress growth and cause pancreatic hypertrophy (Liener 1976). Various methods of heating including toasting (Gardner 1975) dry roasting (Johnson *et al.* 1980) heating in boiling water (Collins and Beaty 1980) and micronization (Hutton and Foxcroft 1975) have

been used to improve the nutritional value of soybean seeds. However, little is known about the effect of microwave under different heating time and moisture content on trypsin inhibitor and nutritional value of soybean seeds protein. Youssef, et al., (1985) reported that microwave heating of soybean seeds for 9.0 min. greatly decreased its protein solubility in different solvents. They also reported that protein digestibility in-vivo were 73, 84, 87 and 81 % when the seeds were heated by microwave for 0, 9, 12 and 15 min., respectively. Hiromi and Goro (1988) showed that trypsin inhibitor in the whole soybean seeds (Okuhara variety) exposed at different levels of moisture content to microwave heat at frequency of 2450 MHz were differently inactivated trypsin inhibitor. Hewedy (1990), studied the effect of microwave on the whole soybean seeds Clark variety at different moisture content through heating for various periods (2, 4 and 6 min.). He found that microwave heating of soybean seeds for 6 min. showed an increment in trypsin inhibitor destruction. The increment of moisture content and heating time significantly decreased both nitrogen and protein solubility. Tsukamoto et al., (1983) noticed that crude extract from kintoki bean lost approximately 90% of its trypsin inhibitor activity after heating at 100 °C for 60 min. Meantime, Gamal (1989) found that heating the whole seeds in boiling water bath for 20 min. showed an inactivation of 93 and 83 % of trypsin inhibitor activity in early green mature seeds (25 days after anthesis) and dry mature seeds, 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 inhibitor 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-SO₃) which can interact further with the generated P-S to form a new disulfide bond and SO₃. The net effect of this reaction is the rearrangement of protein disulfide bonds which was catalyzed in general by SO₃ ions.

The aim of this investigation was to study the effect of microwave heating time at different moisture contents and soaking soybean seeds either in water or in 0.03 M sodium sulfite solution on trypsin inhibitor activity, digestibility index, protein subunits and amino acids content.

MATERIALS AND METHODS

The seeds of Clark and Crawford soybean varieties were obtained from Agriculture Research Center, Giza.

1- Microwave heating:

The seeds were cleaned and divided into three groups according to their soaking time in water (0, 30 and 60 min. at about 25 °C) then air dried at room temperature for 30 min. Each group was divided into four sub-groups which were microwave heated for 3, 6, 9 and 12 min. A Moulinex microwave oven (serie FMI) generating 0.5 KW power at 2450 MHZ was used in this study according to the method of Hiromi and Goro (1988).

2- Soaking soybean seeds either in water or in sodium sulfite:

Soybean seeds were soaked in water and in 0.03 M sodium sulfite in the ratio 1 : 5 (w/v), for 0, 30, 60 and 120 min. then heated at 70°C for one hr. and air dried.

The seeds from the previous treatments were ground and the oil was extracted by hexane, filtered then air dried and subjected to the following determinations.

- 1- Trypsin inhibitor activity was determined as described by Hamerstrand *et al.*, (1981) using benzoyl-DL-arginine-P-nitroanilide hydrochloride (BAPA) as synthetic substrate for trypsin.
- 2- Digestibility was carried out according to the method described by Ford and Salter (1966) and digestibility index was determined as follows:

$$\text{Digestibility index} = \frac{\text{mg nitrogen in the supernatant}}{\text{Total nitrogen in the sample}} \times 100$$

- 3- Soybean protein was extracted from the defatted seeds by using NaOH (0.02N). Soluble soyprotein was examined electrophoretically through Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then it was performed to determine the molecular weight of the polypeptide chains according to Laemmli (1970) with some modifications. Slab gel was used instead of tube gel. Dimension of the slab gel was 11.5 cm x 11.5 cm x 1.0 mm (thickness). A custom-build apparatus unit which was similar to the Hoffer SE 500 vertical slab unit was used.

The Pharmacia low Molecular weight (LMW) calibration kit provides six protein standards covering subunits molecular weight range from 14.000 to 94.000 KD was used for constructing the calibration curve.

- 4- Quantitative determination of the amino acids was carried out according to Moore *et al.*, (1958) using Bechman amino acid analyzer Model 121.

RESULTS AND DISCUSSION

Trypsin inhibitor activity, digestibility index, protein subunits and amino acids content are factors affecting soybean protein quality. The effect of microwave heating and soaking either in water or in sodium sulfite followed by heating on each of these factors was studied herein in an attempt to identify the suitable treatment for improving the nutritional value of soybean protein.

1- Trypsin Inhibitor Activity:

The effect of microwave heating on trypsin inhibitor activity of soybean seeds is shown in Table (1). Results show that microwave heating for 3 min. decreased trypsin inhibitor rapidly in all treatments. After 6 min. notable reduction in trypsin activity was observed in the seeds of both Clark and Crawford varieties. Trypsin inhibitor was also decreased as soaking time increased and nine min. microwave heating (after soaking for one hr.) inactivated about 93% of the trypsin inhibitor. Microwave heating for high moistured seeds (1 hr soaking) caused an increment in inhibitor destruction, which indicate that hydroxyl radicals are very efficient in inactivating trypsin inhibitor. These results are in agreement with those reported by Hiromi and Goro (1988) and Hewedy (1990).

The changes in soybean seeds trypsin inhibitor activity occurred by soaking the seeds either in water or in 0.03 M Na_2SO_3 followed by heating for one hr at 70°C are also presented in Table (1). As soaking time progressed, gradual reduction in trypsin inhibitor activity was noticed. Trypsin inhibitor in the two varieties lost about 50 and 75 % of its activity after soaking the seeds either in water or in sodium sulfite solution, followed by heating for on hr at 70°C, respectively.

Therefore, those results suggest that soaking soybean seeds in sodium sulfite solution is more effective for trypsin inhibitor inactivation than soaking in water.

Protein quality of microwaved & soaked soybean 291

Table (1): Effect of microwave heating and soaking soybean seeds in water and in 0.03 M Na₂SO₃ solution followed by heating at 70°C for one hr on trypsin inhibitor activity (TI) and protein digestibility

Treatment	TI activity %		Digestibility %		
	Clark variety	Crawford variety	Clark variety	Crawford variety	
Untreated seeds (control)	100.00	100.00	72.00	71.70	
Microwave heating:					
1- Heating only for;	3 min.	43.34	47.80	81.50	80.55
	6 min.	30.64	28.88	83.91	83.12
	9 min.	9.99	9.77	85.31	84.31
	12 min.	3.87	6.19	86.17	85.81
2- Soaking in H ₂ O for 30 min. followed by heating for:	3 min.	33.00	44.29	82.31	82.03
	6 min.	18.06	27.65	84.91	85.13
	9 min.	6.77	9.12	85.90	86.12
	12 min.	3.22	5.54	86.98	87.21
3- Soaking in H ₂ O for 60 min. followed by heating for:	3 min.	26.91	35.07	82.44	83.17
	6 min.	23.91	26.31	85.11	84.93
	9 min.	6.06	6.81	85.61	86.22
	12 min.	2.90	2.87	87.13	87.51
Unsoaked and heated		61.55	71.99	74.93	73.22
Soaked and heated:					
1- Soaked in H ₂ O for:	30 min. and heated	71.32	78.44	74.93	73.22
	60 min. and heated	58.07	56.46	74.94	73.43
	120 min. and heated	53.85	51.81	75.90	74.67
2- Soaked in 0.03 M Na ₂ SO ₃ for:	30 min. and heated	42.96	48.52	77.13	74.81
	60 min. and heated	35.41	39.40	78.04	76.23
	120 min. and heated	25.81	29.37	80.03	78.94

2- Protein Digestibility:

The effect of microwave heating on protein digestibility of soybean seeds is shown in Table (1). Results show that increasing microwave heating time improved protein digestibility for both varieties. Soybean seeds with high moisture content (soaked seeds) exhibited higher protein digestibility. This may be due to that the increment in seed moisture content showed an increment in trypsin inhibitor destruction and consequently improved digestibility. On the other hand, microwave heating caused denaturation of the protein which also increased its digestibility. These results are in agreement with those of Youssef *et al.*, (1985) and Hewedy (1990).

Data presented in Table (1) also show that soaking the seeds of the two varieties in water for 60 and 120 min. slightly increased digestibility percentage while, soaking seeds in 0.03 M Na_2SO_3 solution for the same periods resulted in notable increment in digestibility especially Clark variety. The increment in protein digestibility is parallel to inactivation of antitrypsin destruction. Therefore, soaking followed by heating treatments, reduced inhibitor activity and increased protein digestibility and its nutritive values. The obtained results agree with those reported by Friedman and Gumbman (1986) on soyflour protein.

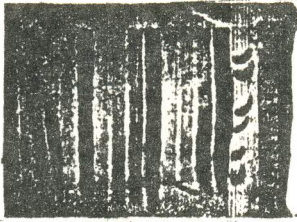
3- Protein Subunits:

PAGE pattern of the soybean protein extraction of soybean seeds microwave heated gave unsatisfied results. The protein bands were intersectioned to each other and all samples gave only one tailing band from the anode to the cathode. This may be due to the destruction of the protein chains. As shown in Table (2) and illustrated in Fig. (1), the SDS-PAGE of the extracted proteins of the unheated seeds of Clark variety showed the presence of 12 subunits with molecular weights ranged from 89.000 to 18.000 KD. On the other hand, microwave heated seeds for 3 min. showed 6 subunits with molecular weights ranged from 79.000 to 19.000 KD. As microwave heating time progressed up, the number of subunits was reduced till only one subunit at 12 min. microwave heating time. Soaking soybean seeds in water for 30 and 60 min. and microwave heated showed also a reduction in the number of the detected subunits.

Proteins of the unheated Crawford seeds variety had 12 subunits with molecular weights ranged from 93.000 to 18.000 KD. Remarkable reduction in the number of the separated

clark variety

crowford variety



1211109837654321



1211109837654321

Fig. (1) : SDS-PAGE patterns of soybean protein extraction after microwave heating for clark and crowford varieties.

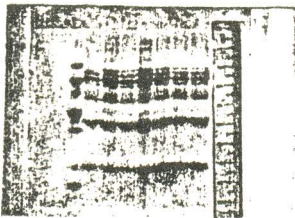
1,2,3,4 : Heating for 3,6,9,12 minutes .

5,6,7,8 : Heating for 3,6,9,12 minutes after 30 min. soaking in H_2O .

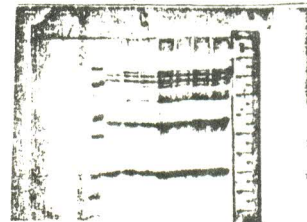
9,10,11,12 : Heating for 3,6,9,12, min. after 1hr soaking in H_2O

clark variety

crowford variety



1234567



1234567

Fig. (2) : SDS-PAGE patterns of soaking soybean seeds in tap water or Na_2SO_3 solution and heating at $70^{\circ}C$ for 1hr.

1 : Unsoaked sample

2,3,4 : Soaking in H_2O for 30,60,120 min.

5,6,7 : Soaking in Na_2SO_3 for 30 ,60 ,120 min.

subunits was shown as microwave heating time increased. The soaked and heated seeds for different periods were affected by microwave heating especially the samples soaked for one hr and heated for 6 min. In general, it could be concluded that microwave heating causes a great destructive effect on protein subunits especially those of high molecular weights which were destructed to lower molecular weight subunits. Also, results show that after 6 min. of microwave heating in different treatments, more than 80% of the protein subunits were disappeared. The time of microwave heating was the main factor affecting protein subunits while, soaking time was not effective.

Data concerning the effect of soaking soybean seeds (Clark and Crawford varieties) for 0, 30, 60 and 120 min. in both water and sodium sulfite 0.03 M solution followed by heating at 70 °C for one hr on protein subunits are shown in Table (3) and illustrated in Fig. (2). The extracted protein of the unsoaked unheated (control) Clark seeds indicate the presence of 12 subunits in its SDS-PAGE pattern. While, the extracted protein of the unsoaked and heated seeds showed the presence of 9 subunits with molecular weights ranged from 85.000 to 20.000 KD. Also, there were similarity in the SDS-PAGE patterns of the extracted proteins from the soaked seeds in either water or sodium sulfite solution for different periods and heated for one hr at 70 °C .

The SDS-PAGE pattern of the extracted protein from Crawford seeds (control) indicated the presence of 12 subunits. The unsoaked and heated seeds protein had remarkable reduction in the number of subunits. It had 8 subunits with molecular weights ranged from 79.000 to 20.000 KD while, the soaked seeds in water and in sodium sulfite solution and heated showed the presence of 11 subunits with molecular weights ranged from 83.000 to 20.000 KD. Therefore, it could be concluded that soaking soybean seeds followed by heating for one hr at 70°C caused mild reduction in the molecular weights of the extracted protein subunits. Also, heat is the main factor affecting the protein subunits.

4- Amino Acids Content:

The results concerning the effect of microwave heating at different times on amino acid composition of soybean seeds Clark variety are shown in Table (4) Results indicated that prolonging microwave heating time increased the destruction of both essential and non-essential amino acids. Also, sulfur amino acids decreased by increasing

Table (3): Molecular weights of soybean protein subunits soaked in water and in Na_2SO_3 and heated at 70°C for one hr (as determined by SDS-PAGE, KD).

Variety	Band	Control	Un-soaked and heated	Soaked in H_2O for:			Soaked in 0.03 M Na_2SO_3 for:		
				30 min. (and heated)	60 min. (and heated)	120 min. (and heated)	30 min. (and heated)	60 min. (and heated)	120 min. (and heated)
<u>Clark</u> (KD)									
	1	89	--	--	--	--	--	--	--
	2	83	85	85	85	85	85	85	85
	3	79	79	79	79	79	79	79	79
	4	72	75	75	75	75	75	75	75
	5	69	70	70	70	70	70	70	70
	6	60	64	64	64	64	64	64	64
	7	53	54	54	54	54	54	54	54
	8	39	48	48	48	48	48	48	48
	9	37	39	39	39	39	39	39	39
	10	22	20	20	20	20	20	20	20
	11	19	--	--	--	--	--	--	--
	12	18	--	--	--	--	--	--	--
<u>Crawfoed</u>									
	1	93	--	--	--	--	--	--	--
	2	88	--	83	83	83	83	83	83
	3	81	79	79	79	79	79	79	79
	4	75	72	72	72	72	72	72	72
	5	70	--	69	69	69	69	69	69
	6	63	64	64	64	64	64	64	64
	7	53	60	60	60	60	60	60	60
	8	41	56	56	56	56	56	56	56
	9	38	40	40	40	40	40	40	40
	10	25	26	26	26	26	26	26	26
	11	20	20	20	20	20	20	20	20
	12	18	--	--	--	--	--	--	--

Table (4): Effect of microwave heating and soaking soybean seeds (Clark variety) in water and in 0.03 M Na₂SO₃ solution followed by heating at 70°C for one hr on amino acid content (g/100 g protein).

Amino acids	Without heating	Microwave treatments				Soaking treatments	
		Unsoaked		Soaked for 30 min.		Heated at 70°C for one hr after 30 min. soaking in H ₂ O	Heated at 70°C for one hr after 30 min. soaking in Na ₂ SO ₃
		Heated 3 min.	Heated 12 min.	Heated 3 min.	Heated 12 min.		
E.A.A.							
Lys.	5.55	4.91	3.93	4.99	3.52	5.16	4.99
Leu.	6.3	6.26	6.14	6.57	6.22	5.96	6.10
Isoleu.	4.52	4.50	4.34	4.53	4.40	4.45	4.55
Cys. + Met.	0.14+1.69	0.16+0.96	0.06+0.85	0.16+1.10	0.06+0.91	0.06+1.22	0.10+1.63
Phe. + Tyr.	5.15+4.21	4.36+3.58	4.16+3.48	5.00+4.20	4.91+3.99	5.00+4.15	4.96+4.19
Thr.	3.83	3.80	3.42	3.88	3.30	3.66	3.65
Val.	5.02	5.02	4.85	5.13	4.55	5.22	5.29
His.	3.40	3.33	2.97	3.41	3.11	3.32	3.27
T.E.A.A.	39.81	36.88	34.20	38.97	34.97	38.20	38.73
N.E.A.A.							
Arg.	5.82	5.25	4.55	4.90	4.36	4.83	4.89
Asp.	10.41	10.72	8.86	11.03	9.67	10.45	10.03
Glu.	15.25	15.56	15.06	15.80	15.30	15.66	15.51
Ser	4.68	4.55	3.77	4.65	4.10	4.76	4.58
Pro.	9.22	10.03	9.88	9.92	10.11	10.17	10.21
Gly.	4.09	3.46	3.41	3.72	3.99	4.00	4.01
Ala.	4.41	4.30	3.92	4.31	4.00	4.00	3.93
T.N.A.A.	53.88	53.87	49.45	54.33	51.53	53.87	53.21
T.A.A.	93.69	90.75	83.65	93.30	86.50	92.07	91.94

microwave heating time from 3 to 12 min. Microwave heating after soaking the seeds for 30 min. in water showed favourable effect in protecting the amino acids which were slightly affected. Therefore, high amount of amino acids content was observed in the samples soaked in water for 30 min. followed by microwave heat for 3 min.

Data in Table (4) indicate that soaking soybean seeds either in water or in sodium sulfite followed by heating at 70 °C had slight effect on amino acid content. Also, the amount of essential and non-essential amino acids are similar to that of the control.

Soaking seeds in sodium sulfite for one hr show an improvement in the amount of sulfur amino acids than soaking in water. As a result, it could be concluded that soaking the seeds in either water or in 0.03 M sodium sulfite solution had better amino acids pattern than that of microwave heat treatments.

REFERENCES

- Collins, J.L. and Beaty, B.F. (1980):* Heat inactivation of trypsin inhibitor in fresh green soybeans and physiological responses of rats fed the beans. *J. Food Sci.*, 45: 542.
- Ford, J.F. and Salter, D.N. (1966):* Analysis of enzymically digested food proteins. *J. Nutr.* 20:843.
- Frideman, M. and Gumbman, M.R. (1986):* Nutritional improvement of soyflour through inactivation of trypsin inhibitors by sodium sulfite. *J. Food Sci.*, 51: 1239.
- Gamal, F.A. (1989):* Biochemical studies on trypsin inhibitors in some local legumes. M.Sc. Thesis, Fac. of Agric., Minia Univ., Egypt.
- Gardnar, H.W. (1975):* Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with non enzymic. *J. Agric. Food Chem.* 23: 129.
- Harmestrand, G.E.; Black, L.T. and Glover, J.D. (1981):* Trypsin inhibitors in soyproducts, modification of the standard analysis procedure. *Cereal Chem.* 58:42.

- Hewedy, M.F. (1990):* Electrophoretic studies on the proteins and trypsin inhibitor of microwave soybeans. *Minofiya, J. Agric. Res.* 15(2): 3033.
- Hiromi, Y. and Goro, K. (1988):* Effect of microwave treatment on the trypsin inhibitor and molecular species of triglycerides in soybeans. *J. Food Sci.*, 53: 1756-1760.
- Hutton, K. and Foxcroft, P.D. (1975):* Effect of processing temperature on some indices of nutritional significance for micronized soya beans. *Proc. Nutr. Soc.* 34: A49.
- Johnson, L.A.; Deyoe, C.W.; Hoover, W.J. and Schwenke, J.R. (1980):* Inactivation of trypsin inhibitors in aqueous soybean extracts by direct steam infusion. *Cereal Chem.* 57: 376.
- Laemmli, U.K. (1970):* Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *J. Nutr.* 227: 680-685.
- Liner, I.E. (1976):* Legume toxin in relation to protein digestibility. *J. Food Sci.*, 41: 1076.
- Moore, S.; Spachman, D.H. and Stein, W. (1958):* Chromatography of amino acid on sulphonated polystyrene resins. *Anal. Chem.*, 30: 1185.
- Tsukamoto, I.; Miyoshi, M. and Hamaguchi, Y. (1983):* Heat inactivation of trypsin inhibitor in kintoki bean (Phaseolus vulgaris). *Cereal Chem.* 60: 194-198.
- Youssef, S.H.; Ali, I.M.; Fawzy, M.H. and Gurbav, S. (1985):* Effect of microwave heating on solubility, digestibility and metabolism of soyprotein. *J. Food Sci.*, 50: 415-417.

تأثير التسخين بالميكرويف ونقع

بدور فول الصويا فى الماء أو فى كبريتيت الصوديوم على نوعية البروتين

ناديه يحيى أحمد عطيه

مصطفى كمال صبرى شبانه

ابراهيم عبدالعليم

صلاح مصطفى سعـد

استخدمت بذور فول الصويا صنفى كلارك وكرافورد لدراسة تأثير الميكرويف والنقع فى الماء أو فى محلول كبريتيت الصوديوم (٣رمول) ثم التسخين على درجة ٧٠م لمدة ساعة على كل من نشاط مثبت التربسين ، هضم البروتين ، السلاسل البيبتيدية للبروتينات ومحتوى الاحماض الامينية .

وقد أدى تسخين البذور التى تم نقعها لمدة ساعة فى فرن الميكرويف لمدة ٩دقائق الى تثبيط نشاط الانزيم بنسبة ٩٣٪ بينما أدى نقع البذور لمدة ساعتين فى الماء أو فى محلول كبريتيت الصوديوم (٣ممول) ثم التسخين لمدة ساعة على ٧٠م الى تثبيط ٥٠٪ ، ٧٥٪ من نشاط مثبطات التربسين فى كلا من الصنفين (كلارك ، كرافورد) .

وقد تحسن هدم البروتين باستخدام التسخين بواسطة الميكرويف . كذلك تحسنت نسبة الهضم بنقع البذور فى محلول كبريتيت الصوديوم بدرجة أعلى منها عن النقع فى الماء مقارنة بالكنترول .

وقد أظهر التفريد الكهربائى للبروتين المستخلص بعد التسخين بواسطة الميكرويف أن هناك هدم لسلاسل البروتين خاصة ذات الوزن الجزيئى المرتفع مما يوضح أن لهذه المعاملة تأثيرا واضحا على المكونات البورتينية . كذلك تأثرت الاحماض الامينية الاساسية والغير أساسية . بينما أد النقع فى الماء أو فى محلول كبريتيت الصوديوم الى الحصول على محتوى من الاحماض الامينية مماثل تقريبا العينة المقارنة .