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USE OF MICROWAVES TO IMPROVE NUTRITIONAL VALUE OF SOYBEANS FOR FUTURE SPACE INHABITANTS

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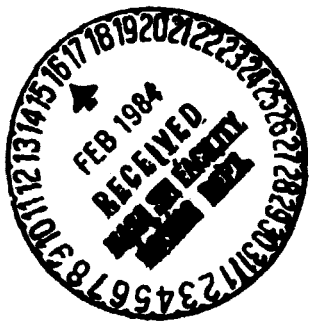

by

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ABSTRACT

Whole soybeans from four different varieties at different moisture contents were microwaved for varying times to determine the conditions for maximum destruction of trypsin inhibitor and lipoxygenase activities, and optimal growth of chicks. Microwaving 150gm-samples of soybeans (at 14-28% moisture) for 1.5 min was found optimal for reduction of Trypsin inhibitor and Lipoxygenase activities. Microwaving 1kgm samples of soybeans for 9 minutes destroyed 82% of the Trypsin inhibitor activity and gave optimal chick growth. It should be pointed out that the microwaving time would vary according to the weight of the sample and the power of the microwave oven. The microwave oven used in the above experiments was rated at 650watts @ 2450MH_Z.

Use of Microwaves to Improve the Nutritional Value of Soybeans for Future Space Inhabitants

Introduction

Soybeans are unique among plant source of proteins because they are high in essential amino acids, especially Lysine. Soybeans have also been suggested as a possible food and feed in the future space habitats. However, soybean seeds contain some antinutritional factors which can cause physiological disorders. Some of the important antinutritional factors are Trypsin inhibitors.

It is known that soybean Trypsin inhibitors can be deactivated by heating in steam or boiling water[1-3]. Milk production of cows is improved when their soybean meal is heat treated [4,5]. Protein efficiency ratio (PER) of heated soybeans fed rats is found higher than PER for raw soybeans [6]. Heating soybeans eliminates the pancreatic hypertrophy in chickens and rats, and improved growth parallels the destruction of Trypsin inhibitors [6,7,8]. These examples illustrate that deactivation of Trypsin inhibitors improves the nutritional value of soybeans.

Some recent works, including ours, show that microwave heating is an effective method of deactivating the Trypsin inhibitors [7,9]. Microwave heating has the added advantage that deactivation of Trypsin inhibitors can be accomplished without removing any water soluble nutrients as occurs in the conventional method. Moreover, this method would be ideal for space habitats where the supply of water is limited and the generation of microwaves from solar radiation is practical.

This report discusses the experiments carried out to demonstrate the destruction of Trypsin inhibitor and Lipoxygenase activities. It also describes the experiments conducted to study the growth of chicks when fed

with meal prepared from microwaved soybeans.

EXPERIMENTAL DETAILS

Microwave Oven

A commercially available domestic size microwave oven was used. The oven was rated at 650watts @ 2450MH_z. Uniformity of microwave field within the oven was investigated and found to be highly non-uniform in spite of a built-in microwave stirrer: a slowly rotating fan type reflector. The microwave reflection from the reflector blades was found to be lopsided on the input side of the waveguide. The heating effect of this inhomogeneity was smoothed out by placing the sample in a thin cylindrical shell container and rotating it slowly, counter to the rotation of the microwave stirrer. A turn table was installed for this purpose.

Determination of the Specific Heat of Soybean

In order to determine the temperature of microwaved soybeans, it is essential to have an accurate value of its specific heat. This was determined by using a vacuum jacketed calorimeter. The conventional method of mixtures[10] was used in this determination. Cold water (about 10°C below the room temperature) was taken in the calorimeter. Soybeans, at room temperature, were poured into it. The heat lost by soybeans is set equal to the heat gained by water and the calorimeter. The unknown specific heat of soybeans in this equation is calculated. Correction to the temperature of the mixture, due to the heat gained from the surrounding, is applied by observing the temperature of water for six (6) minutes before and after mixing with soybeans. Each determination was repeated for at least five times to improve the statistical

Table 1: Specific Heats of Different Varieties of Soybean

Variety	Moisture Content (% wb)	Specific Heat (cal/gm°C)
Davis	7.6	0.428
	14.3	0.494
	53.4	0.637
Essex	10.9	0.489
	23.0	0.569
	31.9	0.583
Williams	7.1	0.441
	27.3	0.486
York	8.8	0.463
	21.0	0.564
	36.2	0.622

average. Table 1 shows the value of specific heat of soybeans at different moisture contents.

Determination of the Temperature of Microwaved Soybeans

Temperature is an important parameter in the process of deactivation of Trypsin inhibitors. The obvious method of sticking in the mercury thermometer does not work because the metallic part of the thermometer absorbs microwaves directly and gets overheated. Thus the thermometer will show a temperature far greater than that of the soybean sample.

A calorimetric method was applied, which relies again on the method of mixtures[10]. Conceptually the method is simple: Microwaved soybeans are quickly poured into a calibrated calorimeter containing a predetermined amount of water at a known temperature. The weight of the calorimeter is found before and after pouring soybeans to determine the weight of soybeans poured into it. These parameters provide the necessary information to compute the temperature by using the principle of heat conservation i.e. heat lost equals heat gained. Using this equality and the specific heat of soybean, the unknown temperature is calculated.

Trypsin Inhibitor and Lipoxygenase Activities Measurement

Davis, Williams, Essex, and York varieties of soybeans were soaked in water to bring the moisture content of the beans in the range of 7-47%. Samples (150gm) of the soybeans were microwaved for 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 minutes. Temperature of the microwaved soybeans was determined as described earlier. Correction to the temperature determination, resulting from loss of moisture during microwave heating, was applied. Typical temperature versus microwaving-time curves are shown in Figures 1 and 2.

Figure 1: Effect of Microwaving on Temperature of Soybeans at 25.7% Moisture.

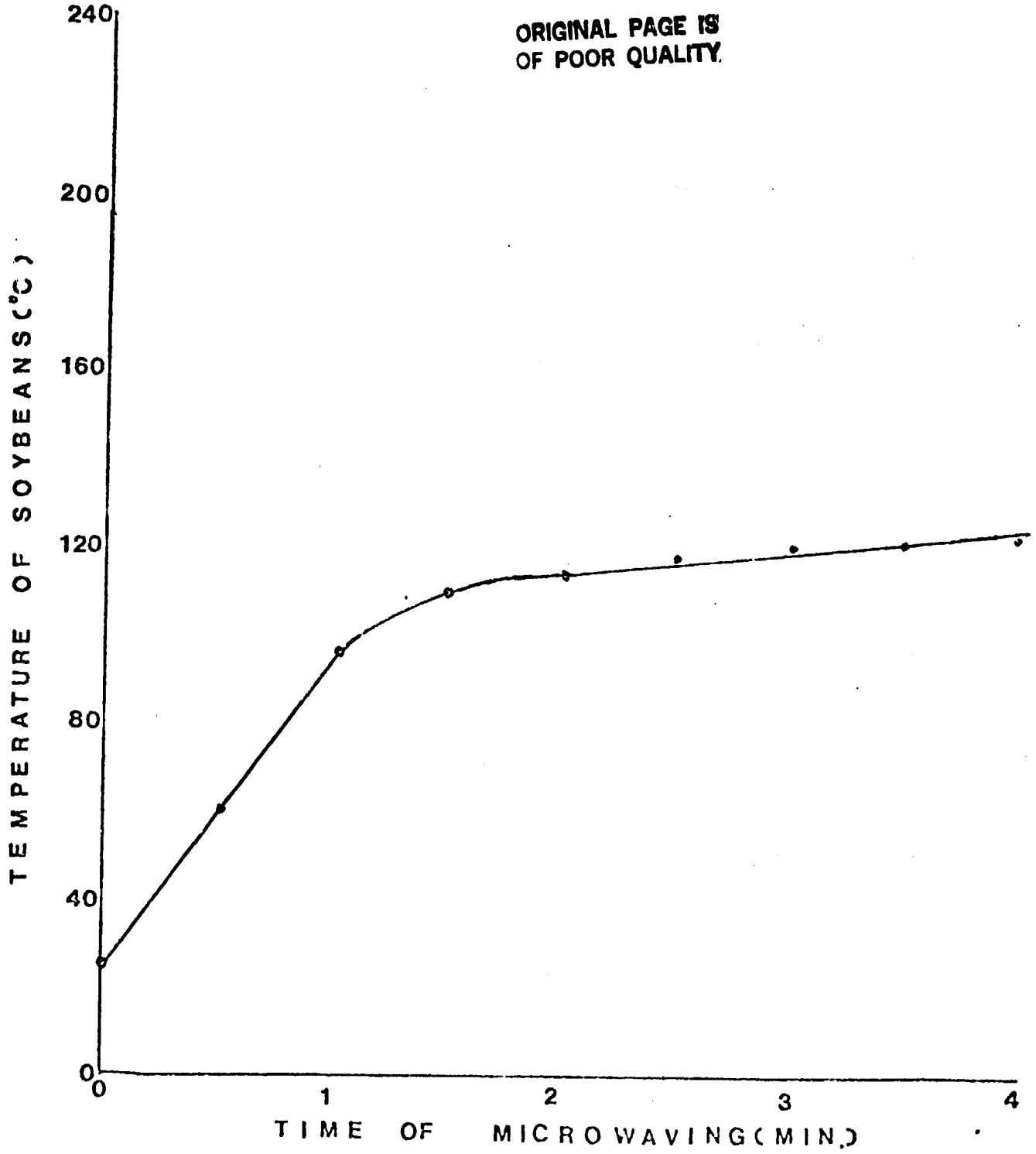
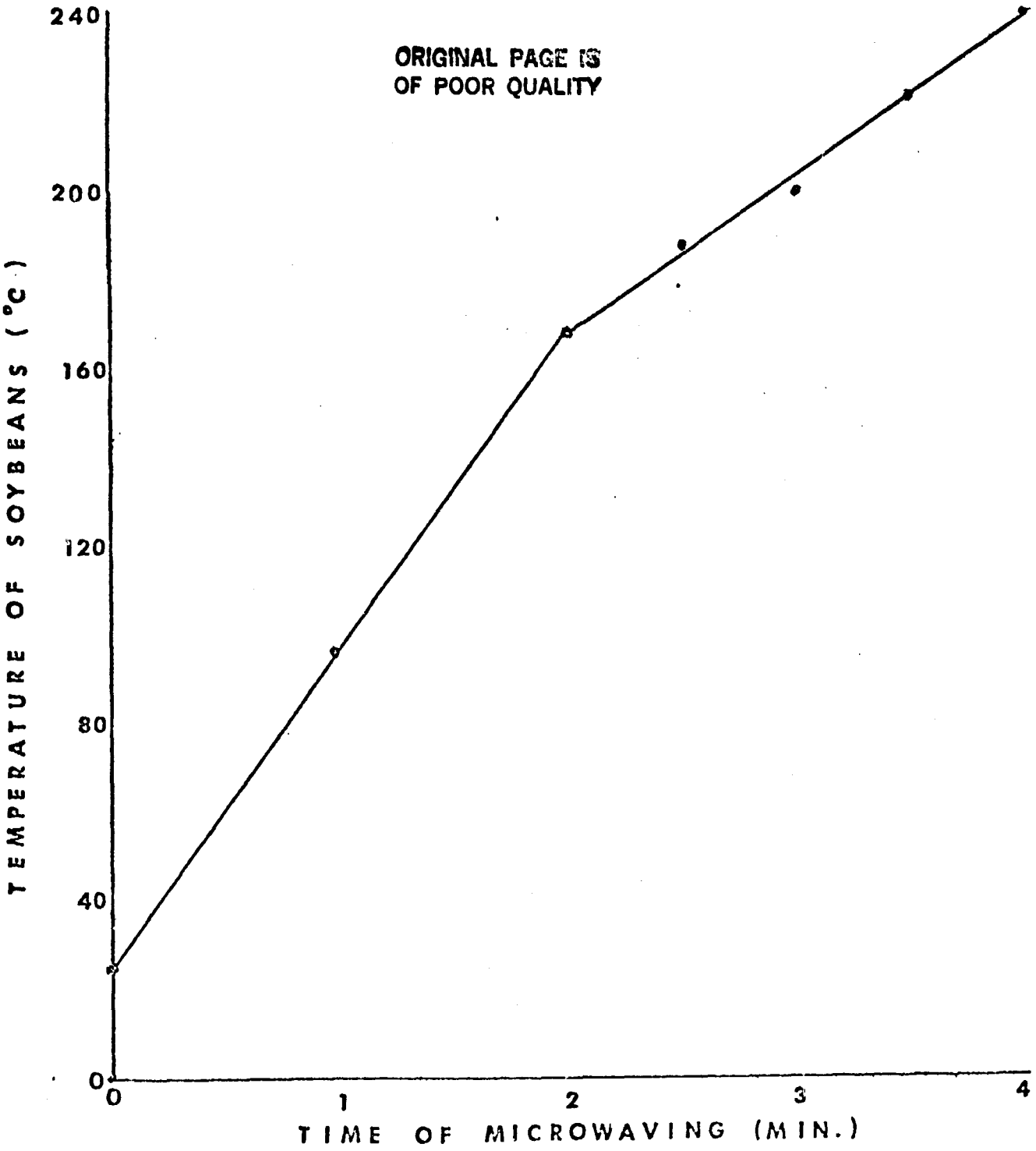


Figure 2: Effect of Microwaving on Temperature of Soybeans at 9.1% moisture.



Fat from 1.5 g of 60 mesh ground soybeans was extracted with petroleum ether. Trypsin inhibitor activity of the defatted samples was determined by the method of Kakade et al.[11] using α-N-benzoyl-DL-arginine-p-nitroanlide as the substrate for Trypsin. One Trypsin unit (TU) is defined as an increase of 0.01 abosrbance units at 410 nm per 10 ml of the reaction mixture under the conditions defined herein. Trypsin inhibitor activity is defined as the number of Trypsin units inhibited (TUI).

The Lipoxygenase activity was assayed using linoleic acid as a substrate. A modification of the spectrophotometric method of Surrey[12] was used. 0.4 g of the defatted sample was agitated for 30 min with 40 ml of deionized water, and then centrigfuged for 15 min at 5,000 rpm. Borate buffer, pH 9, was used to bring 2 ml of the supernatant up to 100 ml. The substrate, 0.05 ml linoleic acid, was mixed in an equal amount of 90% ethanol, and the volume brought up to 50 ml with deionized water for a stock solution. The working substrate was 5 ml of the stock linoleic acid solution brought up to 30 ml with pH 9.0 borate buffer. A reaction mixture, 2.4 ml of borate buffer and 0.5 ml of substrate working solution, was brought to 23.5°C in a cuvette with waterjacket. The reaction was started by the addition of 0.1 ml sample. One Lipoxygenase unit is defined as that amount which causes an increase of 0.001 absorbance unit per minute at 234 nm under the stated conditions.

Feeding Chicks With Microwaved Soymeal

One kilogram batches of Williams soybeans were microwaved in the same manner as previously described. The larger sample size necessitated longer microwaving times: 0, 9, 12 and 15 minutes. The soybeans were ground through 0.25 inch mesh on a hammer mill and the oil removed by extraction with hexanes in a 2.2 liter capacity soxhlet. Diet was made with this soymeal as the

Table 2. Composition of Diets

Component	Amount (g)
Defatted Soybean Meal	537.0
Cornstarch	169.0
Dextrose	150.0
Briggs Mineral Mix	55.0
Alphacel	50.0
Soy Oil	30.0
A I N vitamin mixture '76	4.5
D - L Methionine	3.0
Choline Chloride	1.5

Table 3: The Effect of Microwave on the Trypsin Inhibitor in Williams Variety of Soybeans.

Microwave Time (min.)	MOISTURE CONTENT								
	7.1%			14.0%			27.3%		
	Temperature[1] °C	TIA[2]/mg sample ± SEM	%TIA[3] destroyed	Temperature °C	TIA/mg sample ± SEM	% TIA destroyed	Temperature °C	TIA/mg sample ± SEM	% TIA destroyed
0.0	24.2	48.6 ± 3.01	0.0	24.5	42.7 ± .54	0.0	25.0	43.2 ± .55	0.0
0.5	n.t.[4]	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
1.0	89.0	43.8 ± .28	10.0	115.0	37.0 ± .38	13.3	114.0	16.5 ± 2.50	61.8
1.5	n.t.	n.t.	n.t.	102.4	8.9 ± .56	79.1	n.t.	n.t.	n.t.
2.0	163.3	16.6 ± .51	65.8	156.0	8.5 ± 1.38	80.1	130.4	3.3 ± .27	92.4
2.5	175.7	13.7 ± .09	71.8	116.4	13.0 ± .89	69.6	125.0	3.5 ± .45	91.9
3.0	188.0	16.0 ± .78	67.1	182.8	16.2 ± .22	62.1	126.5	4.9 ± 1.19	88.8

[1] Temperature of soybeans at end of microwaving time

[2] Trypsin Inhibitor Activity, as measured by the number of trypsin units inhibited per mg defatted soybean sample

[3] Percent trypsin inhibitor activity destroyed by using 0 time as the control

[4] not tested

Table 4. The Effect of Microwave on the Trypsin Inhibitor in Essex Variety of Soybeans.

Microwave Time (min.)	MOISTURE CONTENT								
	10.9%			23%			31.9%		
	Temperature[1] °C	TIA[2]/mg sample ± SEM	% TIA[3] destroyed	Temperature °C	TIA/mg sample ± SEM	% TIA destroyed	Temperature °C	TIA/mg sample ± SEM	% TIA destroyed
0.0	25.0	52.6 ± .67	0.0	24.0	47.7 ± .22	0.0	24.5	56.4 ± .33	0.0
0.5	65.7	47.2 ± .62	10.3	66.1	47.4 ± .86	0.1	65.1	34.3 ± .17	39.2
1.0	104.8	46.1 ± .19	12.4	103.9	32.3 ± .64	32.3	98.1	12.6 ± .73	77.7
1.5	142.1	24.8 ± .59	52.8	124.1	4.7 ± .35	90.2	117.4	9.1 ± .61	71.5
2.0	153.3	15.6 ± .71	70.3	128.0	4.2 ± .65	91.2	120.9	11.4 ± .55	79.8
2.5	160.2	17.9 ± .47	65.9	129.5	6.9 ± .27	85.5	121.5	13.7 ± .53	75.7
3.0	171.5	23.2 ± .28	55.9	134.7	8.3 ± .14	82.6	122.0	16.6 ± .65	70.6

[1]Temperature of soybeans at end of microwaving time

[2]Trypsin Inhibitor Activity, as measured by the number of trypsin units inhibited per mg defatted soybean sample

[3]Percent trypsin inhibitor activity destroyed by using 0 time as the control

protein source and other nutrients were added to meet NRC (1977) recommendations for poultry (Table 2).

The soymeal was analyzed for browning index and Trypsin inhibitor activity. The nonenzymatic browning of free-protein extract was determined by the method of Fishwick and Zmarlicki[13] with modification by Rhee and Rhee[14]. The results are expressed as absorbance at 400 nm x 100 / g sample. The Trypsin inhibitors were measured by the method as described previously.

One day old male white hubard chicks were placed for one week on commercial chick starter. Then, they were divided into four dietary teatments, twenty chicks for each treatment. Bodyweights and food consumption were measured for each group. The animals from each group were sacrificed after 14 days. Pancreas was removed from each animal, and the net weight was recorded. The difference in body weight and pancreas weight as percent body weight were tested by Duncan's Multiple Range Test[15].

RESULTS AND DISCUSSION

Trypsin Inhibitor and Lipoxygenase Activities

Temperature of the soybeans increased with an increase in microwaving time (Tables 3 and 4). Rate of temperature increase was greater during the first two minutes of microwaving time than the second two minutes. At lower moisture contents the rate of temperature increase was greater after the first minute than in soybeans of higher moisture contents. In the later, temperature tended to a plateau. The lower the moisture content of the soybeans, the higher the temperature for the same microwaving time. These results were consistent across all four varieties studied. (Figures 1 & 2).

Table 5: The Effect of Microwaveson Lipoxygenase Activity

Variety	Moisture Content	Liposygenase Activities/min./mg Defatted Sample ± SEM				
		Microwave Time (min.)				
		0.0	0.5	1.0	1.5	2.0
Essex	10.9%	10465±493	7523±319	2766±370	0	0
	23.0%	7453± 87	5994± 86	1015± 52	0	0
	31.9%	7132±200	3010±193	988± 0	700±26	0
York	8.9%	8791± 0	2554±182	1469± 2	0	0
	21.0%	8533± 50	1795± 10	744± 10	0	0
	36.2%	8714±157	8098±520	744± 0	0	0
Davis	7.6%	7100±12?	n.t.[1]	1479± 0	n.t.	0
	14.2%	5508±114	n.t.	0	0	n.t.
	46.9%	6315±131	4399±215	n.t.	0	n.t.

[1] not tested

The Trypsin inhibitor activity decreased as microwaving time increased with most inhibitor destruction occurring during the first 1.5 min. of microwaving. The greatest amount of Trypsin inhibitor activity destruction occured in samples which had 14-28% moisture content. At high moisture contents (above 30%), microwave heating was less effective in destroying Trypsin inhibitor activity. The lower moisture (below 14%) soybeans had the highest temperatures and the lowest Trypsin inhibitor activity destruction. These results were true for all varieties studied. These data agree with McNaughton and Reece[8] findings that at increased moisture content (up to 25%) there is increased Trypsin inhibitor destruction.

The Lipoxygenase activity of the soybeans decreased rapidly with an increase in microwaving time. In most cases, 1.5 minutes of microwaving (of 150gm sample) inactivated Lipoxygenase completely (Table 5). The time necessary to deactivate Lipoxygenase decreased with increased moisture content, as also reported by Rice, et.al [16].

Growth of Chicks

Growth of chicks improved when the feed was prepared with microwaved soybeans. Greatest weight gain occurred when soybeans were microwaved for either 9 or 12 minutes of 1kg samples (Table 5). There was no significant difference in the weight gains of animals fed soymeal microwaved for 9 or 12 minutes. Weight gain of animals fed soymeal microwaved for 15 minutes was significantly lower than for either 9 or 12 minutes. Feed efficiency ratio was improved in microwaved soymeals. The lowest pancreas weight as precent body weight occurred when the soymeal was microwaved for 12 minutes. There was no significant difference in the pancreas weights of animals fed soymeal microwaved for 9 or 15 minutes (Table 6).

Table 6 : The Effect of Microwave Heating of soybean meal on Body Weight Gain, Feed Efficiency Ratio, and Pancreas Weight of Growing Chicks.[1]

Microwave Time (min)	Body Weight Gain (g) [3]	Feed Efficiency Ratio[2]	Pancrease Weight % Body Weight[3]
0	85.21 ± 6.63[a]	0.27	1.269 ± 0.089[a]
9	205.73 ± 7.78[b]	0.64	0.484 ± 0.001[b]
12	209.43 ± 8.78[b]	0.58	0.382 ± 0.043[c]
15	179.05 ± 7.90[c]	0.56	0.550 ± 0.072[b]

[1] Whole soybeans were microwaved in 1 kg batches.

[2] Grams of Body Weight gain/ Grams of feed consumed.

[3] Data are reported as Mean ± Standard Error of the Mean, values followed by same letter are not significantly different ($P>0.05$), values followed by different letters are significantly different ($P<0.05$).

Table 7: The Effects of Microwave Heating of soybeans on Trypsin Inhibitor Activity and Browning Index.[1]

Microwave Time (min)	Browning Index	TIA/mg sample[2] ± SEM
0	16.4	50.5 ± 0.54
9	113.5	9.0 ± 0.23
12	168.5	12.7 ± 0.18
15	193.3	13.4 ± 0.27

[1] Whole soybeans were microwaved in 1 kg batches.

[2] Trypsin Inhibitor Activity, as measured by the number of trypsin units inhibited per mg defatted sample.

Browning index increased with increasing microwaving time (Table 7). Microwaving soybeans for 15 minutes decreased the body weight gain (Table 6), that may be attributed to the increase in the browning index. Rhee and Rhee[14], observed decreased digestibility of protein in vitro with increased browning. Separate experiments conducted in this laboratory found that browning substances formed during the boiling of small peptides (from the digestion of soybean protein) and reducing sugars (glucose, galactose, fructose of lactose) had Trypsin inhibitor activity. The Trypsin inhibitor activity of the browning products from the Mailliard reaction may explain the increase in Trypsin inhibitor activity and decrease in feed efficiency ratio above 9 minutes of microwaving.

Optimal growth of rats occurs when 79-87% or more of the Trypsin inhibitor activity is destroyed[3]. Under the conditions of this experiment, microwaving 150g samples of soybeans for 1.5 minutes after soaking to 14-28% moisture content should be optimal. This treatment destroyed greater than 79% of the Trypsin inhibitor activity and all Lipoygenase activity. Microwaving 1 kg samples of soybeans for 9 minutes destroyed 82% of the Trypsin inhibitor activity and gave optimal growth. It should be pointed out that the microwaving time would vary according to the weight of the sample to be microwaved and the power of the oven.

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