EFFECT OF AUTOCLAVING ON SUGARS OF DEFATTED SOYBEAN FLAKES FROM SELECTED VARIETIES

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Introduction

Since the kind and amount of main sugars present in the defatted soybean flakes were elucidated as reported in the preceding papers,[1,2] the changes of such sugars by heating are to be examined.

First the suitable conditions for heating soybean flakes were examined from the ratio of water-soluble nitrogen to total nitrogen (**I**). The autoclaving of defatted flakes (moisture 20%) at 120° for 10 min seemed to be suitable, and these heating conditions were applied to defatted flakes (extracted by the plant process) of the 6 American and 3 Japanese varieties. Quantitative paper chromatography was applied to these autoclaved as well as the raw defatted flakes after sugar extraction according to the experimental conditions established formerly (**II**). An additional experimet (**III**) is described on sugar determination after autoclaving the residue of soybean flake from which soluble sugars have been removed.

I Wate-soluble nitrogen content of defatted flakes by heating

Some experimetns were made to see the decrease in water-soluble nitrogen content according to heating. It was aimed to get the conditions where water-soluble nitrogen became about 10-20% of total nitrogen.

Materials and Methods

As the sample was used defatted soybean flake extracted at room temperature from mixed soybeans at the Mizushima Factory of Nippon Kôyu Kôgyô Co., Kurashiki, Okayama-ken.

Methods of heating (a) Open dry heating and (b) autoclaving were applied.

(a) Open dry heating. Add water to the defatted soybean flake by spraying so as to make the water content 20%. Allow to stand for some hours in a desiccator without desiccating agent. Heat under various conditions, as shown in Table 1. This experiment was made as follows. Spread 30 g sample flake (moisture 11.0%) in a Petri dish. Spray water so as to make the content 20%. Control the temperature of an electric air thermostat at 100°. Put the dish containing the wet flake to get to 100°. Then allow to stand at 100° for 30 min. This is shown as "100°, 30 min" in the table. Other conditions of higher temperatures or longer duration are shown similarly in the table, e.g. 100°, 60 min; 120°, 60 min.

(b) Autoclaving. The moisture content of the flake was adjusted to 20% by spraying water over the flake. Two vessels were used: one was a Petri dish with a loose lid of a

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larger Petri dish and the other was a 500 ml beaker, in which the moistened flake was covered by a piece of gauze. Each 20 g flake was used. The heating process was as follows. The open autoclave was heated by propane gas firing. The temperature reached 100° after about 12 min. Then the samples in a Petri dish and a beaker were put into the autoclave. On heating for about 4 min up to the time of blowing, the autoclave was closed. It took about 5 min to reach the predetermined pressure (1 atmosphere above atmospheric, or 120°), which was maintained for 10 min. The autoclave was gradually opened and cooled down and the samples were taken out.

Methods of analysis Air-dry the heated samples. Pulverize with an electric mixer. Sift to pass a 32-mesh sieve. Analyze the original unheated and heated samples (both after pulverization) for moisture, total nitrogen (only in case of the original sample), and water-soluble nitrogen.

To determine the water-soluble nitrogen, stir a mixture of 0.5 g sample and about 20 ml distilled water for 30 min with a magnetic stirrer, centrifuge, and decant. Then centrifuge again the mixture of the residue with some additional distilled water, and decant. Repeat until no precipitate is obtained in the aqueous extract with the Nessler reagent. Collect the aqueous extracts and determine nitrogen in the collected extracts.

Results

(a) Open dry heating. As shown in Table 1, the solubility of nitrogen did not decrease much at 100° even after 60 min (only from 77.6 % to 72.6 %). Open heating at 120° for 60 min gave 34.6 % water-soluble nitrogen and heating at 130° for 60 min gave 18.1 % water-soluble nitrogen. This shows that some denaturation progressed during storage of the sample deftted at room temprature.

(b) Autoclaving. As shown in Table 2, the autoclaved or steamed sample showed about 11% water-soluble nitrogen/total nitrogen.

Thus further autoclaving was made with Petri dishes mainly from the uniform appearance

Table 1. Water-soluble nitrogen content of heated flakes

Heating	Moisture, %	Water-sol. N on dry basis, %	$\frac{\text{Water-sol. N}}{\text{Total N}}$ %
Unheated	11.00	6.89	77.6
100°, 30 min	5.40	6.65	74.8
100°, 60 min	4.63	6.45	72.6
115°, 30 min	6.73	6.15	69.2
120°, 60 min	4.18	3.09	34.6
130°, 60 min	4.48	1.61	18.1

Table 2. Water-soluble nitrogen content of steamed flakes

Vessel	Water-sol. N on dry basis, %	$\frac{\text{Water-sol. N}}{\text{Total N}}$ %	Appearance of steamed flakes
Petri dish	0.99	11.2	Uniformly wet
Beaker	0.98	11.0	Water drops on some part

of the product, since nitrogen solubility was similar.

II Effect of autoclaving on sugars of defatted soybean flakes from 9 varieties

Defatted soybean flakes obtained by the plant process[2] were subjected to autoclaving at 120° for 10 min at moisture content of 20% in Petri dishes, since it was ascertained by other experiments that this toasting was suitable for feeds.

Materials and Methods

Nine (6 American and 3 Japanese) varieties of soybeans were used. Autoclaving of 9 samples was made simultaneously. Autoclaved defatted flakes were dried to about 10% moisture by a drier below 40°. They were pulverized to pass a 30-mesh sieve by an electric mixer. Sugar extraction was carried out as usual by refluxing with 80% ethanol for 1 hour followed by extensive extraction and washing with water at room temperature. The volume of washings was 500-800 ml (till negative anthrone reaction). The mixed extracts and washings were deproteinized, concentrated, passed through ion-exchange resins, concentrated, and made to 25 ml.

The analyses of defatted soybeans were reported earlier.[2] However, 7 out of the 9 varieties were again analyzed, since several months passed.

Results and Discussion

The results are tabulated (Tables 3-11). In case of Hawkeye and Shirasaya No. 1 earlier data were used for unheated flakes. Only the total sugar was determined by anthrone colorimetry, while in other cases reducing and total sugars were determined by the Somogyi method.

When the data for nonreducing sugar were available, the result of quantitative paper chromatography were recalculated to make the sum of chromatographically estimated values for oligosaccharides equal to the nonreducing sugar content. When only the data for total sugar were available, the results of quantitative paper chromatography were recalculated to make the sum of chromatographically estimated values equal to the total sugar content.

These results in the Tables 3-11 are not consistent. They may be summarized as

Table 3. Changes of sugars on autoclaving, Ani

	Raw	Autoclaved
Glucose	—	—
Sucrose	6.3	7.1
Raffinose	0.8	0.4
Stachyose	3.0	2.5
Verbascose	—	—
Reducing sugar	0.16	0.30
Nonreducing sugar	10.08	9.98
Total sugar	10.24	10.28

Table 4. Changes of sugar on autoclaving, Chippewa

	Raw	Autoclaved
Glucose	—	+
Sucrose	5.7	7.3
Raffinose	0.6	1.1
Stachyose	2.6	2.4
Verbascose	—	—
Reducing sugar	0.17	0.33
Nonreducing sugar	8.82	10.78
Total sugar	8.99	11.11

follows.

The total sugar value determined by the anthrone colorimetry was always higher than that determined by the Somogyi method. In general the former was more similar to the sum of individual sugars determined by densitometry.

In general reducing sugar increased by autoclaving. The sugar detected was glucose and in one case (Shirasaya No. 1) fructose also was noted on paper chromatogram.

Table 5. Changes of sugars on autoclaving, Hampton

	Raw	Autoclaved
Glucose	+	−
Sucrose	4.7	7.4
Raffinose	1.1	0.9
Stachyose	2.0	2.6
Verbascose	−	−
Reducing sugar	0.97	0.35?
Nonreducing sugar	7.73	10.85
Total sugar	8.70	11.20

Table 6. Changes of sugars on autoclaving, Harosoy

	Raw	Autoclaved
Glucose	−	+
Sucrose	5.8	4.4
Raffinose	0.?	0.6
Stachyose	3.4	3.0
Verbascose	+	−
Reducing sugar	0.12	0.43
Nonreducing sugar	10.09	7.96
Total sugar	10.21	8.39

Table 7. Changes of sugars on autoclaving, Hawkeye

	Raw	Autoclaved
Glucose	−	+
Sucrose	5.6	5.1
Raffinose	1.5	1.4
Stachyose	5.0	2.7
Verbascose	−	−
Reducing sugar	...	0.42
Nonreducing sugar	...	8.74
Total sugar	12.56	9.18

Table 8. Changes of sugars on autoclaving, Lee

	Raw	Autoclaved
Glucose	+	+
Sucrose	5.3	5.0
Raffinose	1.5	1.2
Stachyose	3.4	3.2
Verbascose	+	−
Reducing sugar	0.17	0.23
Nonreducing sugar	10.22	9.38
Total sugar	10.39	9.61

Table 9. Changes of sugars on autoclaving, Merit

	Raw	Autoclaved
Glucose	−	+
Sucrose	5.5	5.9
Raffinose	0.5	0.7
Stachyose	4.0	4.1
Verbascose	−	−
Reducing sugar	0.08	0.27
Nonreducing sugar	10.01	10.68
Total sugar	10.09	10.95

Table 10. Changes of sugars on autoclaving, Shirasaya No. 1

	Raw	Autoclaved
Glucose (+Fru?)	−	+
Sucrose	6.3	4.4
Raffinose	1.8	1.2
Stachyose	5.4	2.5
Verbascose	−	+
Reducing sugar	...	1.02
Nonreducing sugar	...	7.14
Total sugar	13.49	8.16

Change of total sugar content was rather inconsistent. It decreased in majority of cases (Harosoy, Hawkeye, Lee, Shirasaya No. 1, and Tokachi Nagaha), it remained similar in 2 cases (Ani and Merit), and it increased in the other 2 cases (Chippewa and Hampton).

Concerning individual sugars, higher oligosaccharides (raffinose and stachyose) decreased by autoclaving in 7 varieties out of the 9, but sucrose apparently decreased in 4 varieties, remained similar in 1 variety, and contrary to expectation apparently increased in 4 varieties.

Table 11. Changes of sugars on autoclaving, Tokachi Nagaha

	Raw	Autoclaved
Glucose	−	−
Sucrose	5.0	4.2
Raffinose	1.2	0.3
Stachyose	2.7	2.4
Verbascose	+	−
Reducing sugar	0.52	0.32?
Nonreducing sugar	9.90	6.96
Total sugar	10.42	7.28

III An additional experiment on the determination of sugar after autoclaving the residue of soybeau flake from which soluble sugars have been removed.

It is well understood that by heating treatment the reducing sugar increased and total sugar decreased, since heating might cause decomposition of higher oligosaccharides not only hydrolytically, but also degradatively. Sugars may be degraded to lower molecules, or may be condensed to higher molecules, which do not give sugar properties such as reducing power after hydrolysis.

However, it is not easily understood that total sugar apparently increased in some varieties by autoclaving.

An additional experiment was carried out to see if any sugars were extracted by the usual method, when the residue of original flake after removal of sugars was autoclaved.

Such newly obtained sugars might have been in polysaccharide form in original flake and they might have been solubilized by autoclaving. Thus this experiment was carried out.

Materials and Method

As the sample was used defatted flake of the Hampton soybean, in which former result (II) showed that total sugar increased by autoclaving.

Reflux 5.000 g Hampton defatted flake with 80% ethanol. Treat the residue with water at room temperature. Wash until negative anthrone reaction. Collect the extracts and washings. Deproteinize, concentrate, and determine reducing and total sugar by the Somogyi method.

Dry the residue of soybean flake, from which soluble sugars have been entirely removed, by use of absolute alcohol. Autoclave the air-dry residue as usual at 120° for 5 and 10 min. Extract sugars from the autoclaved sample similarly as before.

Results

As shown in Table 12, the residue, from which sugars have been removed, gave by autoclaving 10 min about 0.1% reducing sugar and about 0.5% nonreducing sugar.

Table 12. Sugars newly formed by autoclaving the sugar extraction residue (% on dry basis)

	Raw	Residue autoclaved for 5 min	10 min
Reducing sugar	0.72	0.10	0.07
Nonreducing sugar	7.34	0.37	0.55
Total sugar	8.06	0.47	0.62

Though it is not clear whether total sugar actually increases by autoclaving in case of the Hampton soybean flake, such an increase in total sugar, if any, might be due to easier extraction of some sugar, which might have been originally in the form of difficultly soluble polysaccharide.

We wished to identify the apparently newly extracted sugars by paper chromatography. If they were arabinose, galactose, etc., or oligosaccharides of different types from sucrose, raffinose, and stachyose, the newly obtained sugars should have been components of polysaccharides. But we could not identify such different sugars. Rather, sugars probably of ordinary raffinose family were detected.

Thus the possibility of polysaccharide solubilizing as imagined may be small. Then one possibility is the incompleteness of sugar extraction in case of the raw or unheated soybean flakes.

Summary

In general total sugar decreased, reducing sugar increased, and nonreducing sugar decreased on autoclaving defatted soybean flakes. The main oligosaccharides generally decreased, and glucose, fructose, and galactose increased or appeared on autoclaving.

Acknowledgments

This paper corresponds to the first 2 sections of Chapter 4 (The changes of sugars of defatted soybeans by heating) of the final report of research conducted under grants (grant number FG-Ja-105) authorized by Public Law 480 for the United States Department of Agriculture.

Thanks are due to the members of Northern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, for their encouragements.

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脱脂大豆の糖類に及ぼす加熱の影響

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要　旨：室温抽出法で圧延，脱脂した大豆を空気浴中とオートクレーブ中で加熱を行なった結果，100°の加熱では水溶性窒素の割合は73～75%で原料の78%と大差がなかったが，空気溶中での100°以上の加熱により水溶性窒素の割合は69%から18%と減少した．次にオートクレーブ処理（120°，10分）により水溶性窒素の割合は11%に減少した．米国産大豆6品種と日本産大豆3品種についてオートクレーブ処理について検討したところ，一般に還元糖は増加し，全糖と非還元糖は減少の傾向を示した．脱脂大豆から完全に糖を抽出した残留物をオートクレーブ処理したところ0.1%の還元糖と0.5%の非還元糖が抽出されるようになった．これはオートクレーブ処理によって多糖類が分解したのではなくて，原料中の糖の抽出が不完全であったためと考えられる．

この研究はアメリカ農務省の委託による．

実験に協力された本田章子さんに感謝する．

(Received Oct. 31, 1966)

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