ACID HYDROLYSIS OF SOYBEAN OLIGOSACCHARIDES AT THE ISOELECTRIC POINT OF SOYBEAN GLOBULINS*

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Introduction

In processing defatted soybean meal or flakes the so-called isoelectric treatment is often applied to remove sugars and other components with a minimal loss of proteins. By isoelectric treatment is meant the treatment of meal or flakes with a slightly acid solution of pH 4.3, which is the isoelectric point of the soybean globulin as a whole.

The value of the isoelectric point differs in a narrow range according to the methods of determination and of preparation of proteins. CIRCLE[1] discussed this problem in the classical monograph on soybeans published in 1950, giving the values 4.1—5.5. ISHII, IWAMOTO, and KAWAMURA[2] reported that it was about pH 4.4 by observing the point of maximum precipitation of proteins by adding sulfuric acid to the extract obtained with 0.4% sodium sulfite, and it was pH 4.6 by observing the point of minimum solubility of acid-precipitated soybean protein dispersed in various buffer solutions (MCILVAINE).

Now from the practical viewpoint the isoelectric point of soybean globulins is assumed to be pH 4.3 by many later experiences and studies.

Originally it was aimed that the isoelectric treatment would afford a suitable method for sugar extraction from defatted soybean meal. However, this treatment gave very low sugar content when determined by the anthrone method. As reported earlier,[3] "For example the anthrone method gave the value 13—14% of total sugar in case of extraction with 50% or 80% ethanol, while it gave the values of only 2—4% of total sugar in case of extraction at pH 4.3. Moreover, the value of reducing sugar by the SOMOGYI colorimetric method was higher (e. g. 1.7%) in case of isoelectric extraction than in case of alcoholic extraction (e. g. 0.1—0.2%)."

In 1949 KAWAMURA[4] reported that soybean whey, i. e. the waste supernatant from precipitated soybean protein, contained a considerable amount of reducing sugar which increased by allowing the whey to stand in an ice box (7°) at pH 4.3, e. g. from about 0.3 to 0.5 g/dl.

Soybean oligosaccharides are considered to be relatively unstable, as they were actually changed to reducing sugars in the course of protein precipitation with acid after dispersion with 0.4% sodium sulfite.

Some experiments were made to reexamine the hydrolysis of oligosaccharides in soybeans at pH 4.3.

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Experimental

Two series of experiments were made. It was considered convenient to describe them separately.

(I) Decomposition of Oligosaccharides of Defatted Soybean Meal at pH 4.3

1. Materials and Methods

Sample The air-dry cotyledon (moisture 11.05%) of the Harosoy soybeans was used after defatting with ether at room temperature. This contained 0.35% reducing sugar and 15.85% total sugar. The latter was determined by extraction with hot 80% ethanol and cold water as usual.

Method Weigh 2.000 g of the above-described sample into a 100 ml Erlenmeyer flask. Add 10 parts dilute hydrochloric acid solution to make the pH of the mixture 4.30 (measured by a pH-meter). Put the flask in a thermostat kept at 20, 30, or 40° ± 0.1° for 1, 2, 5, 10, or 24 hours. Cover the flask with paraffin paper.

The mixture kept after definite hours at a definite temperature was put to analysis. pH, reducing sugar, and total sugar were determined. Sugars were determined by the SOMOGYI colorimetric method after centrifugation from the residue.

2. Results

The results are shown in Table 1.

Table 1 pH and sugar contents of defatted soybean meal kept at pH 4.3

		1	2	5	10	24 hrs.
20°	pH	4.33	4.48	4.50	—	—
	Reducing sugar	—	0.55	0.70	0.96	1.35
	Total sugar	10.53	10.65	10.63	11.51	—
30°	pH	4.34	4.45	4.40	4.40	—
	Reducing sugar	0.53	0.75	1.06	1.27	—
	Total sugar	—	—	—	—	—
40°	pH	—	4.50	4.50	4.40	4.50
	Reducing sugar	0.86	1.42	1.62	2.07	2.77
	Total sugar	14.17	—	15.19	15.33	16.13

Thus pH tended to increase but only a little by the buffer action of soybean protein. Reducing sugar (0.35% in the original sample) clearly increased by keeping defatted soybean cotyledon in dilute hydrochloric acid at pH 4.3–4.5. As expected, higher temperature had higher effect on this increase. Total sugar of the original sample as determined by the SOMOGYI colorimetry after extraction under the standard condition was 15.85 %. At 20° it was only 10.5–11.5%, while at 40° it was 14.2–15.3%. The ineffectiveness of isoelectric treatment in extracting sugar may be in the lower temperature compared to the standard conditions where hot 80% ethanol is used.

Paper chromatography was applied to the concentrated solution removed from the insoluble residue by centrifugation after treatment at pH 4.3 at 40°. Identification of monosaccharides was unsatisfactory. Raffinose spot decreased by the time and could not

be detected after 10 hours of treatment at pH 4.3. Stachyose spot also decreased. Verbascose spot could not be detected, though it was detected in the original sample.

The fraction eluted with water after carbon column chromatography gave 5 fractions.

Paper chromatography with butanol-pyridine-water (6 : 4 : 2) as solvent system and anisidine hydrochloride as spray reagent gave the evidence for presence of fructose, glucose, an unidentified spot with R_f between glucose and sucrose, and 3 unidentified spots with R_f between sucrose and raffinose.

3. Discussion

When defatted soybean meal is treated with dilute hydrochloric acid at pH 4.3 (isoelectric point of soybean globulin), some of higher oligosaccharides decrease and eventually disappear and reducing sugar increases, even at 20°, but naturally more prominently at 40°.

The reducing sugars formed during isoelectric treatment of defatted cotyledon were identified as fructose and glucose together with some unidentified sugars.

Fructose may be freed during this mild acid treatment, since fructoside linkage is susceptible to acid. If sucrose loses fructose residue, there should be found glucose. If raffinose loses fructose, there should be melibiose, a reducing disaccharide; similarly stachyose would give manninotriose, a reducing trisaccharide.

(II) Changes of Oligosaccharides of Raw and Autoclaved Defatted Soybean Meal at pH 4.3

1. Materials and Methods

The Merit soybean was defatted by the plant process with hexane. The flakes were pulverized with an electric mixer to pass a 32-mesh sieve. Some part of flakes was autoclaved at 20% moisture at 120° for 10 min. The autoclaved flakes were dried in a vacuum drier at 50° for 12 hours and pulverized as above.

Air-dry flakes (raw or autoclaved) (10 g) were put in an Erlenmeyer flask with 100 ml McIlvaine buffer solution (0.1 *M* citric acid plus 0.2 *M* Na_2HPO_4) and the pH was adjusted to 4.30 with a pH-meter. The flakes were maintained at 30° for 2 hours or at 40° for 10 hours. The temperature was constant at the degree of ± 0.1°. After acid treatment pH was measured and the mixture was neutralized at pH 6.8 with 0.2 *M* Na_2HPO_4.

As the control, alcoholic treatment was applied. Defatted soybean meal (10 g) was refluxed with 100 ml 80% ethanol for 1 hour.

The sugar extracts (after acid or alcoholic treatment) were purified with lead acetate and sodium carbonate to remove protein and with ion-exchange resins to remove ions.

Paper chromatography and the Somogyi method were applied as usual.

2. Results

When 1 part of the McIlvaine buffer of pH 4.30 was added to 10 parts of defatted soybean meal, the mixture showed the pH 4.56-4.73. Citric acid was added to this mixture to make the pH exactly 4.30. After standing at 30° or 40°, the final mixture showed the pH 4.37-4.39.

Table 2 Sugar contents (for air-dry defatted soybean meal) by isoelectric treatment

		Alcoholic treatment	Isoelectric treatment	
			30°, 2 h.	40°, 10 h.
Raw	Total sugar	8.57	7.38	6.36
	Reducing sugar	0.34	0.96	1.60
	Nonreducing sugar	8.23	6.42	4.76
Autoclaved	Total sugar	7.60	8.23	6.45
	Reducing sugar	2.04	2.13	3.58
	Nonreducing sugar	5.56	6.10	2.87

Table 3 Quantitative paper chromatography of sugars (% of air-dry defatted soybean meal) in the course of isoelectric treatment

(1) Raw defatted meal

	Alcoholic treatment	Isoelectric treatment	
		30°, 2 h.	40°, 10 h.
Reducing sugars:			
Fructose	—	0.12	0.22
Glucose	0.34	0.33	0.56
Galactose	—	0.39	0.74
Melibiose	—	0.12	0.08
Total reducing	0.34	0.96	1.60
Nonreducing sugars:			
Sucrose	2.61	3.75	3.58
Raffinose	0.55	0.64	0.26
Stachyose	5.04	2.01	0.89
Verbascose	±	±	±
Total nonreducing	8.23	6.42	4.76
Total sugar	8.57	7.38	6.36

(2) Autoclaved defatted meal

	Alcoholic treatment	Isoelectric treatment	
		30°, 2 h.	40°, 10 h.
Reducing sugars:			
Fructose	±	0.55	1.63
Glucose	0.86	0.65	0.78
Galactose	1.16	0.51	0.73
Melibiose	±	0.42	0.44
Total reducing	2.04	2.13	3.58
Nonreducing sugars:			
Sucrose	2.32	3.40	1.45
Raffinose	0.92	0.73	0.32
Stachyose	2.32	1.93	1.08
Verbascose	±	±	±
Total nonreducing	5.56	6.10	2.87
Total sugar	7.60	8.23	6.45

Table 2 shows the result of sugar determinations by the SOMOGYI method.

Thus by autoclaving total sugar decreased and reducing sugar increased. By isoelectric treatment of both the raw and autoclaved meal, total sugar and reducing sugar changed their contents similarly.

The result of quantitative paper chromatography is shown in Table 3.

3. Discussion

In case of autoclaved meal similar changes occurred in sugars with the raw meal during isoelectric treatment. Thus these changes, namely hydrolysis of oligosaccharides, is not enzymatic and is due to acidity.

Eight sugars were detected by paper chromatography. Two other reducing sugars, i. e. manninotriose and verbascotetraose, respectively from stachyose and verbascose, could not be detected.

Verbascose was present in traces throughout the original and treated meals. It may have decreased, but nothing could be said.

Stachyose decreased considerably by isoelectric treatment. The most probable products are fructose and manninotriose, but some raffinose might be produced also. Raffinose increased by isoelectric treatment at 30° for 2 hours.

Melibiose is absent in soybeans, but it appeared by isoelectric treatment. Raffinose or higher oligosaccharides may have given this reducing disaccharide.

Sucrose increased a little. It may be due to formation from higher oligosaccharides, though it should be hydrolyzed to glucose and fructose.

Monosaccharides identified include fructose, glucose, and galactose. It is clear that they were formed by hydrolysis of oligosaccharides. Some monosaccharides formed may have decomposed to form hydroxymethylfurfural, levulinic acid, formic acid, etc.

Conclusion

Soybean oligosaccharides are *relatively unstable to dilute acid such as pH* 4.3 or isoelectric point of soybean globulin. Reducing sugars increase and nonreducing sugars decrease. Sucrose is easily hydrolyzed to fructose and glucose. Raffinose is hydrolyzed to fructose and melibiose (and perhaps slightly also to sucrose and galactose). Stachyose may be hydrolyzed to fructose and manninotriose or to galactose and raffinose or in other ways. By paper chromatography *glucose, fructose, galactose, and melibiose were identified as reducing sugars*. Experiments with autoclaved soybean meal show that these changes are *not enzymatic*. This point may be of interest, since isoelectric treatment is sometimes practiced to recover sugars from defatted soybean flakes.

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大豆グロブリンの等電点における大豆少糖類の酸加水分解

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要　　旨

脱脂大豆の処理においてしばしば大豆グロブリンの等電点 pH 4.3 における酸処理が行なわれる．これはタンパク質をなるべく損失することなく，糖類などの可溶性成分を除去するためである．この等電点[1]は測定法やタンパク質の調製法により異なるが，石井，岩本，川村 (1953)[2]は2法により求めて pH 4.4 と 4.6 との値をえた．しかし現在工業的には pH 4.3 とされているので，それに従った。すでに川村 (1949)[4] は大豆ホエー，すなわちタンパク質を沈でんさせた母液の中で還元糖が増加することを発見している．

この報告では脱脂大豆粉の中の少糖類が pH 4.3 でどのように変化するかを調べた。その結果，やはり大豆少糖類は pH 4.3 のようなうすい酸性でもかなり不安定であること，還元糖が増加することを再確認した。β-フルクトシド結合がとくに弱いので，フルクトースが切れると，サッカロース，ラフィノース，スタキオース，ベルバスコースからは，それぞれ，グルコース，メリビオース，マンニノトリオース，ベルバスコテトラオースができるはずで，これらはすべて還元糖である．なおα-ガラクトシド結合もわずかに切れるとも推定された。というのは検出されたものに，フルクトース，グルコース，メリビオースのほかにガラクトースも含まれたこと，処理の途中でサッカロースが一時増加したらしいことによる。しかしこれらはペーパークロマトグラフィーによる推定である。また脱脂大豆粉という複合系を用いているので，別に純粋な糖により実験も行なっている(別報の予定)。なおこの加水分解が酵素作用によると考えられないことは加熱した脱脂大豆粉の場合にも pH 4.3 で還元糖増加を認めたことによりほぼ明らかである。

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