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The fate of some oligosaccharides during the preparation of wari, an Indian fermented food

H. K. Tewary* & H. G. Muller

Procter Department of Food Science, The University, Leeds LS2 9JT, UK

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The preparation of black bean and soybean wari has been standardised under controlled laboratory conditions, using *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

The flatulence-causing sugars, raffinose, stachyose and verbascose, as well as sucrose, have been determined at various stages of preparation. Paper partition chromatography and colorimetry were used. The total oligosaccharides of black bean seeds decreased from 4·4 to 0·6% in the final product, and of soybean seeds from 7·5 to 1·6%. The effects of the addition of spices, inoculum and α-galactosidase on these sugars during fermentation are discussed.

INTRODUCTION

Wari (plural: warian) is a hollow brittle cake of 2 to 30 cm^2 spread and 1 to 30 g in weight. These products have been described in a previous paper (Tewary & Muller, 1989). The present one describes the preparation of black bean and soybean warian under controlled conditions in the laboratory.

Flatulence-causing oligosaccharides, notably raffinose, stachyose and verbascose, are present in significant amounts in mature legume seeds (Reddy & Salunkhe, 1980). These sugars tend to resist human digestive enzymes and cause flatulence (Hickey *et al.*, 1972; Rathbone, 1980; Reddy *et al.*, 1984; Zapsalis & Beck, 1985). They are hydrolysed by α-galactosidase and β-fructosidase, and these enzymes are not present in the human digestive tract. The sugars can be removed by alcohol extraction and by soaking the legumes in water, by cooking or by germinating. Fermentation, enzymatic digestion and preparation of protein isolates also removes them (Rao & Belavady, 1978; Rathbone, 1980; Reddy & Salunkhe, 1980; Sathe & Salunkhe, 1984).

Gas–liquid chromatography and high pressure liquid chromatography have been used to determine these sugars (Hymowitz *et al.*, 1972; Black & Bagley, 1978). Paper partition chromatography (PPC) in conjunction with colorimetry has also been used (Dubois *et al.*, 1956; Joslyn, 1970; Reddy & Salunkhe, 1980).

In the present work, attempts have been made to reduce these sugars by fermentation or by the addition of α-galactosidase, and to determine them using PPC and colorimetry.

MATERIALS AND METHODS

Laboratory preparation of wari

Black beans and soybeans were cleaned, steeped for 20 h at 30° ± 1°C, drained and dehulled. The cotyledons were crushed using a pestle and mortar and made into a dough with spice mix, inoculation medium and some water. The spice mix contained 73·5% cumin, 7·8% cardamom seeds, 5·6% cinnamon, 3·6% cloves and 9·5% black pepper. The inoculation medium (0·1% acidity as lactic acid) contained *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in UHT milk incubated at 45°C for 5 h. The water content of all ingredients was determined and the dough water content was adjusted to 68·7%.

The dough was fermented (30° ± 1°C, 65% R.H., 20 h) in a Laboratory Thermal Equipment incubator. After fermentation the dough was briefly mixed and shaped (2·0 × 2·5 × 2·0 cm) using the moulder shown in Fig. 1. This consisted of a base plate(A), a slider(B)

* Present address: Department of Anesthesiology, University of Nebraska Medical Center, 600 South 42nd Street, Omaha, 68198 Nebraska, USA.

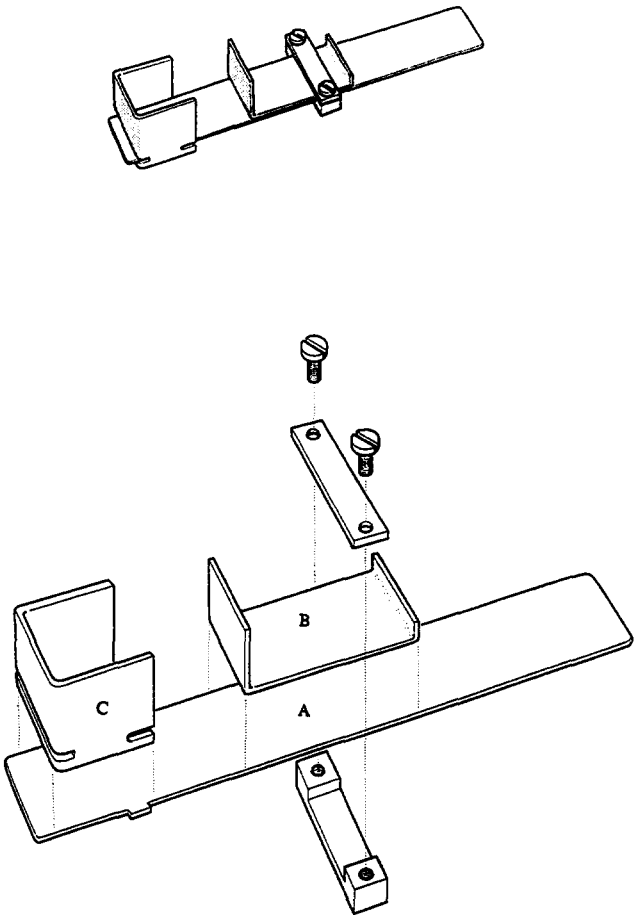

Fig. 1. The wari moulder.

and a box open on three sides(C). In use, the box was engaged into the base plate and the slider was moved to close the box which remained open at the top. Using a spatula the box was now filled with the dough, avoiding the trapping of air bubbles. The box was then taken apart and the dough piece placed on a platform to dry in a standard manner (Tewary, 1989). The heat source was a mercury tungsten bulb (500 W, 240–250 V; Thorn EMI Lighting Ltd) designed to imitate sunlight as closely as possible (Nkama *et al.*, 1987). It was used to maintain a temperature of 50° ± 1°C. Drying was continued for 12 to 18 h until the moisture content was *c.* 4–5%.

Tabie 1. Some physical characteristics of warian made from black beans and soybeans (n=4)

Physical characteristics	Black beans	Soybeans
Cake weight (g)	3·7	2·8
Spread (cm^2)	9·9	4·6
Volume (ml)	4·7	5·7
Cavity/weight(ml/g)	0·85	0·19
Volume/weight(ml/g)	1·28	2·04
Density (g/ml)	2·39	0·54

Table 2. $R_f \times 100$ of oligosaccharides using the two methods of elution (n=6, see text)

Sugars	Black beans (Method 1)	Soybeans (Method 2)
Sucrose	95·3	86·2
Raffinose	88·6	64·3
Stachyose	72·9	40·2
Verbascose	56·8	36·7

The physical characteristics defined previously (Tewary & Muller, 1989) are given in Table 1.

Sugar analysis

For the sugar analysis the methods of Shallenberger & Moores (1957), Joslyn (1970) and Hymowitz *et al.* (1972) were used. For the quantitative analysis of the sugars, descending PPC at 19° to 20°C, with multiple solvent systems and multiple unidirectional development, was used. Two spots each at 1 cm distance from the paper's edges, but 3 cm apart, were applied to Whatman No. 3 mm Chromatography paper strips (30 cm × 5 cm). These were then dried. Three solvent systems were used: (A) acetone–water (9:1), (B) acetone–water–chloroform–methanol (7·5:0·5:1:1) and (C) acetone–water–chloroform–methanol (7:1:1·5:1·5). Two methods were used. The black bean samples were irrigated six times with solvent system (A) and thrice with (C). This was Method 1. The soybean samples were irrigated four times with (A) and twice with (B). This was Method 2. The $R_f \times 100$ values are given in Table 2.

The different methods of elution had to be used for the different beans because black beans contained five sugars, viz. sucrose, raffinose, stachyose, verbascose and an 'unknown', whereas soybeans contained only three, viz. sucrose, raffinose and stachyose, and a clear separation was essential for further quantitative analysis. After irrigation and drying, the paper strips were cut longitudinally into two halves. One half was sprayed with diphenylamine–aniline and phosphoric acid. Yellowish-brown spots were obtained after heating at 70°C for 10 min (Stahl, 1969). The other half was used with the method of Dubois *et al.* (1956). Sugars give an orange-yellow colour when reacted with concentrated sulphuric acid in the presence of phenol. The intensity of the colour is directly proportional to the quantity of the sugars and is measured with a spectrophotometer. The standard sugars were obtained from Sigma Chemicals, except the verbascose, which came from the AFRC Food Laboratory, Norwich. The unknown sugar was determined in terms of verbascose, because its molecular weight was higher than that of verbascose as shown during the qualitative analysis. α-Galactosidase originated from Boehringer, London. Five units of the commercial enzyme were used per 10 g

Table 3. Quantitative analysis of oligosaccharides and lactic acid of black bean (traditional) and soybean (commercial, extruded) warian (g/100g, % dry wt basis, n=2)

Sugars	Traditional black bean warian[a]	Extruded soybean warian[a]
Sucrose	0·4 ± 0·06	3·6 ± 0·05
Raffinose	0·0	1·2 ± 0·07
Stachyose	0·0	3·4 ± 0·04
Verbascose	0·0	0·0
Total:		
(with sucrose)	0·4	8·2
(without sucrose)	0·0	4·6
Lactic acid		
D-lactic acid	1·08	0·02
L-lactic acid	1·25	0·00
Total:	2·3	0·02

[a] Mean ± standard deviation.

of dry matter of the fermenting dough (pH 5·8, 20 h, 30°C). This dough was then kept at 70°C for 24 h to stop enzymatic activity. All other chemicals were of AnalaR grade.

RESULTS AND DISCUSSION

In a previous paper, 16 samples of warian were analysed (Tewary & Muller, 1989). Two of these showed extreme values of both physical characteristics and of L- and D-lactic acids. One sample of black bean wari had been fermented and contained the highest level of lactic acid i.e. 2·3%. The other, an extruded soybean wari, was unfermented and contained the lowest level, i.e. 0·02%. On sugar analysis (Table 3), the first sample contained only 0·4% sucrose, whereas the soybean sample contained 3·6% sucrose, 1·2% raffinose and 3·4% stachyose, a total of 8·2%. These results confirmed that the black bean warian had been fermented and the soybean sample had not.

Black bean wari

Table 4 gives the quantitative analysis of the oligosaccharides during black bean and soybean wari preparation.

The raffinose family oligosaccharides have α-1,6-galactosidic linkages, which, when acted upon by α-galactosidase, result in the production of the next lower member of the family (Florkin & Stotz, 1963; Percival, 1962).

Hence, although the quantity of verbascose in the black bean and its crushed cotyledons was identical at 1·8% after 20 h of steeping, the amount of the 'unknown' sugar had decreased. Therefore, some verbascose or another lower oligosaccharide may have been degraded during the various stages of processing, but replenished through the breakdown of the 'unknown' or a higher oligosaccharide. The loss of sugars during steeping might be caused by diffusion from the cell wall or by microbial activity. Because there was a small decrease of 0·12% in the raffinose family sugars between the seed and the crushed cotyledon stages, this would suggest that microbial or enzymatic activities would not be very vigorous during steeping. The inoculation medium did not contain any of the relevant sugars. The spice mix contained 0·8% raffinose, but no stachyose or verbascose. The amount of sucrose in the spice mix was regarded as negligible.

The raffinose in the dough mix (0·18%) included the amount of raffinose contributed both by the crushed cotyledons (0·03%) and the spice mix (0·15%). The values of the other sugars contained in the crushed cotyledons, i.e. at 65·5% moisture, decreased in the dough mix because of the increase in moisture to 68·7%. On dough making, the crushed cotyledons contributed 1·93% of the flatulence-causing sugars and the spice

Table 4. Quantitative analysis of oligosaccharides during black bean and soybean warian preparation (g/100g, % dry wt basis, n=2)

	Black beans[a]					Soybeans[a]				
Sugars	Seed	Crushed cotyledons	Dough mix[b]	Fermented dough	Wari	Seed	Crushed cotyledons	Dough mix[b]	Fermented dough	Wari
Sucrose	1·6 ± 0·05	0·6 ± 0·04	0·44	0·3 ± 0·10	0·3 ± 0·05	3·9 ± 0·04	3·3 ± 0·05	2·41	0·5 ± 0·06	0·5 ± 0·05
Raffinose	0·07 ± 0·02	0·05 ± 0·02	0·18	0·70 ± 0·05	0·30 ± 0·05	0·8 ± 0·05	0·6 ± 0·06	0·58	0·4 ± 0·02	0·6 ± 0·04
Stachyose	0·4 ± 0·05	0·7 ± 0·03	0·51	0·0	0·0	2·8 ± 0·04	1·8 ± 0·04	1·32	0·8 ± 0·04	0·5 ± 0·06
Verbascose	1·8 ± 0·10	1·8 ± 0·10	1·32	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Unknown	0·5 ± 0·05	0·1 ± 0·02	0·07	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Total:										
(with sucrose)	4·37	3·25	2·52	1·00	0·60	7·5	5·7	4·31	1·7	1·6
(without sucrose)	2·77	2·65	2·08	0·70	0·30	3·6	2·4	1·9	1·2	1·1

[a] Mean ± standard deviation.
[b] Calculated values.

Table 5. Quantitative analysis of oligosaccharides during soybean wari preparation (standard composition, but without spices, g/100g, % dry wt basis, n=2)

Sugars	Dough mix[a]	Fermented dough[b]	Wari[b]
Sucrose	2·95	0·4 ± 0·05	0·3 ± 0·05
Raffinose	0·53	0·3 ± 0·05	0·2 ± 0·10
Stachyose	1·61	0·0	0·0
Total:			
(with sucrose)	5·09	0·7	0·5
(without sucrose)	2·14	0·3	0·2

[a] =Calculated values.
[b] =Mean ± standard deviation.

mix 0·15%, a total of 2·08%. This was reduced by 1·38% during fermentation and by a further 0·4% during drying. This would indicate that microbial or enzymatic activity played a part during drying.

The traditional black bean wari (Table 3) contained no raffinose while the laboratory produced one (Table 4) contained 0·3%. This would indicate greater degradation of sugars during longer drying in the former.

Soybean wari

Table 4 also gives the quantitative oligosaccharide analysis during soybean wari preparation. These beans did not contain verbascose or any other higher oligosaccharide. Steeping and dehulling reduced the total oligosaccharides by 1·8% and the flatulence-causing sugars by 1·2%. A greater loss of the total oligosaccharides indicated that there was a greater loss of sucrose during steeping and dehulling than of the other sugars. The dough contained 1·9% of the flatulence-causing oligosaccharides; 1·75% were contributed by the crushed cotyledons and 0·15% by the spice mix.

Table 6. Quantitative analysis of oligosaccharides of fermented doughs of black bean and soybean in the presence of α-galactosidase (g/100g, % wt dry basis, n=2, 5 units of enzyme/10 g of dough (dry basis), incubation 30°C, 20 h)

Sugars	Black beans[a] (standard composition)	Soybeans[a]	
		Standard composition	Standard composition, no spices
Sucrose	0·5 ± 0·05	1·5 ± 0·05	2·4 ± 0·05
Raffinose	0·7 ± 0·02	1·3 ± 0·03	1·3 ± 0·06
Stachyose	0·3 ± 0·04	0·6 ± 0·04	1·6 ± 0·05
Verbascose	1·0 ± 0·03	0·0	0·0
Unknown	0·0	0·0	0·0
Total:			
(with sucrose)	2·5	3·4	5·3
(without sucrose)	2·0	1·9	2·9

[a] Mean ± standard deviation.

Fermentation reduced these sugars by 0·7%. Drying did not have an appreciable effect on sugar breakdown.

The difference between the fates of the oligosaccharides during black bean and soybean processing might be due to different biochemical composition or to the type of indigenous microflora (Tewary & Muller; 1989).

Effect of spices on soybean fermentation

In the absence of spices, the crushed cotyledons only contributed to the flatulence-causing sugars in the dough. A comparison of the dough with and without spices is given in Table 4 and 5. Although the spices themselves contributed raffinose, they tended to inhibit microbial or enzymatic activities. Without spices, the drying period could be reduced from 12 to 10 h. In spite of 2 h less drying, there was a greater loss of total oligosaccharides.

Enzymatic degradation of oligosaccharides

Table 6 shows the effect of added α-galactosidase on the fermenting black bean and soybean doughs, the latter with and without spices. A comparison of Tables 4, 5 and 6 shows that the addition of the enzyme caused changes in the individual oligosaccharides.

Genetically, an enzyme is known as repressive, if its synthesis is inhibited when its reaction products are readily available to microorganisms (Lehninger, 1982). Such a repressive effect of β-galactosidase has been found on the fermenting microorganism used for the breakdown of lactose (Sastry, G.R.K., 1989, pers. comm.). The results in Table 6 seem to show a similar repressive effect with α-galactosidase.

Comparison of the fermented and unspiced soybean, with and without enzyme (Table 5 and 6), shows changes in all the other sugars except stachyose. The increase of raffinose and decrease of sucrose in the enzyme containing dough could be caused by *trans*-glycosylation reactions (Florkin & Stotz, 1963).

It has been shown that black beans and soybeans contain flatulence-causing oligosaccharides. These can be reduced substantially by steeping and controlled fermentation. Addition of α-galactosidase also reduced some of these oligosaccharides.

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