Original article

Soybean variety and storage effects on soymilk flavour and quality

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Summary Soymilk prepared from five soybean cultivars, grown in Ontario, were analysed for protein, oil, mineral composition, viscosity, colour, lipoxygenase (LOX) activities and flavour profile. Among the five soybean cultivars, the Vinton 81 variety contained the highest protein and the lowest fat. The yield of soymilk from all five cultivars was similar. Major differences were observed in viscosity and in the composition of both the soymilk and the okara. Higher protein and fat extractability was found in soymilk made from S08-80 and Vinton 81 varieties. Their okara protein contents were also among the highest. Minimum extractability was observed with S03W4 cultivar. Soymilk made from S 03W4 and Vinton 81 cultivars had the whitest colour (lowest ΔE values). Viscosity values were the highest for S08-80, FG1 and S20-20 varieties. Headspace solid-phase microextraction gas chromatography was used to analyse volatile compounds in soymilk. A total of fourteen volatiles were identified, among which aldehydes and their corresponding alcohols were the major compounds. Similar volatile compounds were identified in all the samples analysed but at different concentrations. The highest LOX activity was observed in the Vinton 81 and S20-20 soybean cultivars, which had the highest total volatile and hexanal contents. A positive correlation (correlation coefficient = 0.82) between enzymatic activity and the total volatiles was observed. Vinton 81 cultivar was subjected to storage (at 18 °C and 50% relative humidity) for a period of 10 months. Soymilk was prepared at different times during storage. The results showed that the soymilk colour, LOX and total volatiles were significantly ($P < 0.05$) affected by the storage of the soybeans over time.

Keywords Lipoxygenase activity, solid-phase microextraction-gas chromatograph/mass spectrometer identification, soybeans cultivars, soymilk, volatile compounds.

Introduction

Soybean production and use have increased dramatically in the last few decades because of its high nutritional value and low price. A variety of soyfoods can now be found in many mainstream supermarkets. One of the major ways in which soybeans are utilised is in the production of soy-based beverages. Soy-based foods are of excellent nutritional value, and soymilk, in particular, is consumed by persons of all ages, including children with intolerance to animal milk (Pereira *et al*., 2002). Acceptability of soymilk in the Occident has been somewhat restricted mainly because of its typical beany flavour when prepared using the traditional soymilk process. Developments in processing technology have helped to improve the quality of soymilk and eliminate its beany off-flavour. Examples of these modifications are hot water extraction (Wilkens *et al*., 1967), acid grinding (Kon *et al*., 1970; Al-Kishtaini, 1971) and alkali soaking (Badenhop & Hacker, 1970). While these modifications have improved the flavour of soymilk, they have generally resulted in lower protein recovery than that obtained with the traditional oriental process. Other attempts have been made to improve flavour and/or protein recovery by controlling factors such as soaking time and temperature, water-to-bean ratio, method of grinding, filtration of slurry before or after cooking, temperature and time of cooking. These techniques have all had varying degrees of success.

It is now known that a complex interaction of factors (e.g. soybean variety and quality and processing conditions) affects the quality characteristics of soymilk products. Consistent quality can only be achieved by understanding these factors and carefully controlling them during processing. Of the many factors that influence the acceptability of soymilk, flavour is one of the most important. The introduction of a new, simple

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and sensitive technique called solid-phase microextraction (SPME) developed in 1990 (Arthur & Pawliszyn, 1990) has had a tremendous impact on flavour analysis and has allowed the detection of low levels of compounds that contribute to the flavour of many foods and beverages (Coulibaly & Jeon, 1992; Marsili, 1999a,b). Typical beany-flavour-causing compounds identified in soymilk are pentanol, hexanol, heptanol, hexanal and ethyl vinyl ketone (Wang *et al*., 1998). It is also reported that saponins and isoflavones contribute to the bitterness and astringency of soybeans and soymilk (Okubo *et al*., 1992; Carrao-Panizzi *et al*., 1999).

The market for soymilk and soyfoods is continuing to grow. Researchers in many institutions are undertaking intensive plant breeding programme to develop new and improved soybean varieties that have quality traits for soymilk and tofu production. It has been reported that prolonged storage of these beans can negatively affect the quality of their derived products (Lambrecht, 1991 & Thomas *et al*., 1989). Unfortunately, no studies can be found, which report on the effect of soybean storage on the flavour of soymilk. The goals of this study, therefore, were (1) to investigate the composition and quality characteristics of soymilk prepared from five different Canadian cultivars, (2) to study the flavour profile of the soymilk produced and (3) to study the effect of soybean storage (using a selected cultivar) on the colour and flavour of soymilk over a 10-month period.

Materials and methods

Materials

Five food grade soybean cultivars (S08-80, Vinton 81, FG1, S20-20 and S03W4) grown in Ontario (Canada) were supplied by Great Lakes Organics Inc. (Petrolia, ON, Canada) All cultivars had a light hilum and were chosen for their high protein content and low fat, which is suitable for soymilk and tofu production. Twenty-one volatile standards from different chemical classes including aldehydes (hexanal, heptanal, pentanal, *trans*,*trans*-2,4-decadienal, *trans*-2-heptenal), alcohols (hexanol, 2-hexanol, 1-heptanol, 1-pentanol, 2-pentanol, 1-octen-3-ol, decanol), ketones (2-heptanone, 2,3-pentanedione, 3-hydroxy-2-butanone), aromatic compounds (benzaldehyde, *n*-butyl benzene), esters (ethyl heptanoate, ethyl hexanoate, ethyl butyrate) and furans (2-pentyl furan); bovine serum albumin (BSA), linoleic acid, Tween 20, sodium tetraborate (borax) were purchased from Sigma chemical Co. (St Louis, MO, USA). The volatile standards were selected based on the reports of their presence in soymilk products. Four commercial soymilk products were purchased from a Canadian supermarket and analysed for colour for comparison purposes (for confidentiality, the samples are referred to as products A, B, C and D).

Preparation of enzymatic extracts

Lipoxygenase (LOX) extract was prepared following the method of Kumar *et al*. (2003) with some modification. About 1 g of defatted soy meal was dispersed with 25 mL of 0.2 M sodium phosphate buffer (pH 6.8). The mixture was stirred for 30 min at room temperature and then centrifuged at 13 800 g for 20 min at 4 °C. The supernatant was used as the crude extract for assaying LOX isozymes.

Substrate preparation

In 2.5 mL borate buffer (0.05 M, pH 9), 0.125 mL of Tween 20 was dissolved and 0.125 mL of linoleic acid was then added and dispersed. 0.35 mL of NaOH (1 N) was subsequently added and mixed until a translucent mixture was obtained, after which 22.5 mL of borate buffer (pH 9) was then added. The entire mixture was transferred to a glass vessel, flushed with nitrogen and the pH was adjusted to 6.3 with HCl (2 and 0.1 N). The substrate was stored in the dark at −20 °C until use. For lipoxygenase-I (LOX-I), the substrate solution was adjusted to pH 9, while for LOX-II and LOX-III, the substrate was used at pH 6.3.

Enzymatic assay

The LOX activity assay was carried out following the procedure of Garrote *et al*. (2001) with some modification. Briefly, in a 100 mL glass vessel, 27 mL of substrate solution (at room temperature) was added and N_2 flushed with continuous stirring. Three millilitres of the enzyme extract were then added. After 20 s, 1 mL of the mixture was transferred to a centrifuge tube containing 2 mL of absolute ethanol (called blank tube and considered as zero time). Then, after 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 min from the zero time, 1 mL of the reacting mixture was transferred to nine centrifuge tubes, each containing 2 mL of absolute ethanol. Seven millilitres of ethanol (60%) were added to all the centrifuge tubes and mixed. The tubes were then centrifuged for 10 min at 2000 rpm. The supernatants were transferred to quartz cuvettes, and readings were taken at 234 nm on a spectrophotometer (Cary 300 Bio; Varian Canada, Inc., St. Laurent, Quebec, Canada). LOX activity was calculated from the graph of absorbance vs. time (the reaction is of zero order), and was equal to the slope of the line (Δ absorbance units per min). The protein concentration of the enzyme extract was determined according to the method of Bradford (1976), using BSA as standard, to calculate specific enzyme activity (LOX units per mg protein).

Soymilk preparation

Soybeans (500 g) were weighed, cleaned and soaked in distilled water (bean-to-water ratio 1:3) for 3 h at 40 °C, while maintaining the pH at 5. The soaked beans were drained, rinsed with tap water, drained again and then reweighed to determine the water uptake. Water uptake factor (WUF) was determined by dividing the drained weight of the soaked beans by the initial weight of the raw soybeans. Soymilk was prepared using a 'Prosoya' soymilk machine (ProSoya VS40 Soyacow System; ProSoya, Inc., Ottawa, ON, Canada). Soaked beans were mixed with hot water (dry beans-to-water ratio 1:7) and ground at high speed for 3 min. The slurry was continuously heated under pressure to 116 °C (15 psi) with steam infusion (steam-infusion cooking is the continuous direct injection of steam into the product to provide instantaneous heat transfer and agitation). The mixture was held for an additional 3 min at this temperature to allow for trypsin inhibitor and LOX inactivation. The soymilk slurry was then filtered and separated from the insoluble residue, known as okara.

Soybean storage

The Vinton 81 soy cultivar was selected for the storage study and was stored for 10 months at 22 °C and 30% relative humidity. Soybean samples were collected after each month during the storage period and used for soymilk preparation. The soymilk samples were analysed to determine any changes in pH, colour and volatile profile during storage.

Proximate and physicochemical analysis

Protein content was determined by measuring total nitrogen content with the Leco instrument (Leco FP 428; Leco Corp., St. Joseph, MI, USA) using the Dumas method (AOAC, 1995). A nitrogen conversion factor of 6.25 was used. Moisture content was determined by drying 0.3 g protein sample in a Fisher Isotemp Vacuum Oven (Fisher Scientific, Ottawa, ON, Canada) for 5 h at 100 °C (AACC, 1983). Ash and fat contents of beans and soymilk were determined using AACC methods 08-03 and 30-25 (2003), respectively. The pH of soymilk was measured with a pH-meter (Model AP61; Fisher Scientific, Ottawa, ON, Canada) and density was measured as the ratio of mass to volume using a pycnometer. Viscosity was measured in centipoise at 20 °C with the Cannon-Fenske viscometer (Cannon Instrument Co., State College, PA, USA), and colour measurements were made using a Labscan II colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) following the method of Chantrapornchai *et al.* (1998). ΔE (colour difference) values were calculated using the following formula: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$, where ΔL, Δa and Δb are the differences in the specified tristimulus coordinate between the sample and the cow milk used as control.

Solid-phase microextraction analysis

A Saturn 2000 gas chromatograph-mass spectrometer (Varian Analytical Systems, San Fernando, CA, USA) was used for the analysis. The gas chromatograph was equipped with a split/splitless model CP-3800 injector. The mass spectrometer (MS) detector was used in the electron impact mode, with a mass range of 40–300 m/z. The SPME fibre used was 85 μm Carboxen-PDMS (Supelco, Bellefonte, PA, USA). Five millilitres of soymilk sample were incubated for 20 min at 40 °C, and the adsorbed volatiles were desorbed in the injector port in splitless mode at 300 °C for 3 min. Volatiles were eluted with helium gas in a wall-coated open tubular fused silica 30 m × 0.25 mm column coated with 0.25 μm of chemically bonded polysiloxane low bleed phase (CP-SIL 8 CB Low Bleed/MS, Varian Canada Inc., St. Laurent, Quebec, Canada). The temperature was programmed as follows: initial temperature was kept at 35 °C for 3 min and then increased to 210 °C at 6 °C min^{-1}, and held for 10 min. Compounds were identified based on National Institute of Standards and Technology database through Saturn mass spectra library search. The identities of compounds were further confirmed by comparing their mass spectra and retention times with those obtained for the respective standards.

Statistical analysis

Data were statistically analysed by one-way analysis of variance using the PRISM software, version 3.02 (Graph Pad Software, Inc., San Diego, CA, USA). Significant differences between mean values were determined by Tukey's multiple comparison test procedure at the 5% significance level. Correlation coefficient between LOX activity and total volatiles was determined using Pearson's correlation.

Results and discussion

Composition and physicochemical properties

The protein and fat contents of the five cultivars studied are presented in Table 1. Within the cultivars, the Vinton 81 had the highest protein content (45.7%) and one of the lowest lipid content (20.4%). The S08-80, FG1, S20-20 and S03W4 varieties all had lower protein contents with no significant ($P < 0.05$) difference in their fat contents, except for S08-80 variety. In general, the higher the protein in the soybean, the higher the protein in the soymilk and tofu; high protein varieties are, therefore, preferred for soymilk production (Cai *et al.*, 1997; Khatib *et al.*, 2002).

Under identical conditions of extraction, the yield of soymilk from the five cultivars was similar (3.5 L soymilk per 500 g dry beans). The WUF were similar for all the varieties, ranging from 2.25 to 2.32 (Table 2). There were also no significant ($P < 0.05$) differences in the pH and densities of the soymilk. Major differences were observed in viscosity and in the composition of both the soymilk and okara. Higher protein and fat extractability was observed in soymilk made from S08-80 and Vinton 81 varieties compared with other soymilk (Table 2). Their okara protein contents were also among the highest. Minimum extractability was observed with S03W4 cultivar. The protein content of soymilk prepared using the 'Prosoya' soymilk machine showed higher values (3.29–4.16%) than those reported by Thomas *et al*. (1989) and Saxena & Singh (1997), who found contents of 2.82–3.45% and 2.04–2.93%, respectively. These differences may be attributed to varietal differences, storage and extraction conditions. Viscosity values were higher for S08-80 (2.55 cP), FG1 (2.23 cP) and S20-20 (2.04 cP) among the soybeans cultivars studied. These values are much lower than those reported by Reddy & Mital (1992) and Saxena & Singh (1997) for various Indian varieties. The discrepancies among various studies might be partly because of the varietal variations and processing conditions.

The mineral content in soymilk prepared from different cultivars varied considerably (Table 3). Vinton 81 contained the highest quantity of calcium and magnesium, whereas phosphorus and potassium were the highest in soymilk prepared from S08-80 cultivar.

The results of colour difference (ΔE), calculated from the L, a, b tristimulus values, are presented in Table 4. Generally, the lower the ΔE, the whiter the colour of the soymilk sample. Soymilk made from S03W4 and Vinton 81 cultivars showed the lowest ΔE values and, therefore, had whiter colour compared with soymilk made from other soybean cultivars. The main colouring compounds in soybeans have been reported to be polyphenols and total carotenoids (Taira *et al*., 1985; Katayama & Tajima, 2003). As an example, lutein (the major carotenoid) in soybean extracts when present in high amounts could potentially affect the colour of soymilk processed from such beans. Lutein content has been found to depend on factors such as the maturation stage of the beans (Monma *et al*., 1994), variety and type of growing soil (Saito *et al*., 2004). Polyphenol oxidase (PPO) and peroxidase (POD) are two enzymes that could also contribute to colour and flavour development in soy products. PPO could be a cause of enzymatic browning and loss of nutritive value of protein (Nakabayashi, 1968), while POD generally has little

Table 1 Proximate analysis of soybeans cultivars

Cultivar	Protein (%)[a]	Fat (%)[a]
S08-80	42.7 ± 0.5	22.3 ± 0.4
Vinton 81	45.7 ± 0.7	20.4 ± 0.1
FG1	42.3 ± 0.3	20.2 ± 1.0
S20-20	42.0 ± 0.9	21.5 ± 0.4
S03W4	42.8 ± 0.5	21.2 ± 0.8

[a]Dry basis.

Table 2 Physicochemical properties of soymilk produced from different soybean cultivars

		Soymilk						Okara
Cultivars	WUF	PH	Density (g mL^{-1})	Viscosity (cP)	Protein (%)	Fat (%)	Particle size (μm)	Protein (%)
S08-80	2.28	6.77	0.99	2.55	4.2	1.9	0.78	31.0
Vinton 81	2.36	6.69	0.98	1.90	4.2	1.8	0.80	34.8
FG1	2.32	6.66	0.99	2.23	3.9	1.8	0.85	30.6
S20-20	2.26	6.76	0.99	2.04	3.9	1.7	0.71	30.7
S03W4	2.25	6.75	0.99	1.77	3.3	1.6	0.42	31.9

Table 3 Mineral content in soymilk prepared from different soybean cultivars

	mg per 250 mL				
Soybean cultivars	Ca	K	Mg	Na	P
S08-80	44.5 ± 0.3	368.8 ± 0.0	51.5 ± 0.3	7.0 ± 0.5	114.8 ± 2.2
Vinton 81	60.7 ± 0.5	302.4 ± 13.5	63.8 ± 0.3	6.0 ± 0.8	97.9 ± 0.8
FG1	46.5 ± 1.1	340.2 ± 8.6	55.2 ± 2.3	7.3 ± 0.2	85.5 ± 2.4
S20-20	49.3 ± 0.6	312.6 ± 0.9	54.5 ± 0.1	9.2 ± 0.2	92.1 ± 1.6
S03W4	53.2 ± 0.3	317.5 ± 1.5	55.4 ± 0.5	6.2 ± 0.5	95.6 ± 0.6

impact on enzymatic browning, but contributes more to production of off-flavour (Flurkey *et al.*, 1978; Rackis *et al.*, 1979). However, these enzymatic effects were not of concern, as our beans were soaked under acidic conditions (pH 5) and the soymilk was processed at very high temperatures, which were out of the PPO and POD optimum pH (> 7.5) and temperature (40–50 °C) ranges (Toiguchi *et al.*, 1989).

The colour of soymilk can also be affected by protein aggregates, droplet size concentration and lipid content (Chantrapornchai *et al.*, 1998; Chanamai & McClements, 2001). Soymilk samples prepared from S08-80, Vinton 81, FG1 and S20-20 soybean cultivars all showed particle size distribution around 0.8 μm, except for S03W4 with particle size distribution of 0.4 μm. The lower protein and fat contents of soymilk prepared from this cultivar (Table 2) may have resulted in smaller particles and its whiter colour (lowest ΔE). As colour is an important parameter for consumer acceptability of soymilk product, the soymilk products were compared with commercially available soymilk and the results obtained are shown in Table 4.

Table 4 Hunterlab colour values of soymilk made from five different cultivars

	Colour values			
Product	***L***	***a***	***b***	**ΔE**
Standard				
Cow milk (Parmalat 3.25%)	96.06	−2.31	8.07	0
Commercial soymilk				
Product A	72.98	1.09	13.45	23.94 ± 1.0
Product B	78.60	−1.29	10.00	17.60 ± 0.7
Product C	84.30	−1.20	15.31	13.85 ± 0.4
Product D	84.50	−0.46	15.45	14.44 ± 0.1
Soymilk made from cultivars				
S08-80	83.07	−0.93	13.97	14.33 ± 0.2
Vinton 81	87.12	−1.28	15.86	11.90 ± 0.5
FG1	85.17	−0.44	14.84	12.96 ± 0.1
S20-20	83.55	−0.72	16.33	15.08 ± 0.3
S03W4	86.38	−0.44	14.44	11.74 ± 0.2

The '*L*' scale denotes lightness-to-darkness in 100–0 units. The '*a*' scale represents redness (+*a*) vs. greenness (−*a*) and the '*b*' scale represents yellowness (+*b*) vs. blueness (−*b*). The *L, a, b* values are referred to as tristimulus values for the Hunter *L, a, b* solid.

Solid-phase microextraction analysis of volatile compounds in soymilk samples

Table 5 shows the relative percentages of the major compounds identified in soymilk samples prepared from the five soybean cultivars. Identities of the soymilk volatile compounds were confirmed on the basis of the retention times of pure standards and MS spectral libraries. The main constituents identified were hexanal, hexanol, pentanol, 1-octen-3-ol, 2-pentyl furan, octanone, octanal and nonanal. Other compounds present, but at lower concentrations, included heptanal, benzaldehyde, heptanol, 2-heptanone, 5-ethyl cyclopentene carboxaldehyde and pentadecanol.

Among the five soybean cultivars, only soymilk made from S20-20 variety showed significant ($P < 0.05$) difference (highest) in its total volatiles compared with other soymilk (Fig. 1). Soymilk sample with the lowest level of total volatiles was from S03W4 variety, followed by soymilk samples from FG1, S08-80, Vinton 81 and S20-20 cultivars. It is widely accepted that the principal

Table 5 Identification of volatile compounds in soymilk prepared from different cultivars using SPME–GC–MS

	Total volatile (%)					
Volatiles	**S08-80**	**Vinton 81**	**FG1**	**S20-20**	**S03W4**	**MS identification[a]**
Pentanol	5.2	6.5	8.1	7.3	7.2	Standard
Hexanal	49.7	61.2	66.0	58.1	58.5	Standard
Hexanol	22.6	13.6	14.6	17.8	10.5	Standard
2-Heptanone	0.6	0.5	0.6	0.5	1.4	Standard
Heptanal	1.2	1.0	0.9	0.9	2.4	Standard
Benzaldehyde	0.9	1.4	1.3	1.1	2.2	Standard
Heptanol	1.0	1.0	0.7	0.4	0	Standard
Octen-3-ol	7.5	4.2	3.1	2.6	2.2	Standard
Octanone	2.8	2.0	2.1	1.8	1.8	Standard
2-Pentylfuran	4.5	4.6	5.4	5.7	8.5	Standard
Octanal	0.7	1.1	3.2	0.8	1.5	Standard
Nonanal	1.4	1.8	1.9	1.8	2.1	Standard
5-Ethylcyclopent-1-ene carboxaldehyde	1.2	1.2	1.2	1.1	1.7	Library
Pentadecanol	0.6	0	0	0	0	Library

[a]The volatiles were either positively identified using MS of pure standards or identified using the GC–MS spectra library.

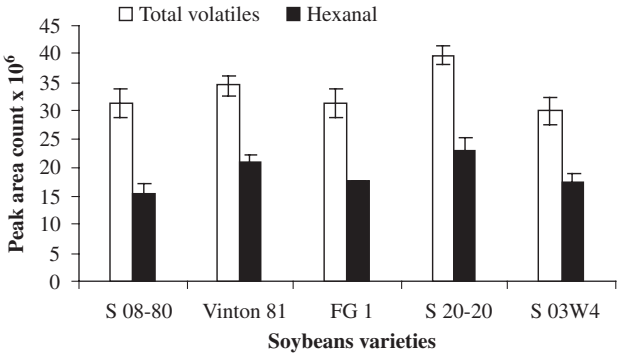

Figure 1 (□) Total volatiles recovery (MS response-peak areas) and (■) percent ratio of hexanal to total volatiles recovery of soymilk prepared from different soybean cultivars.

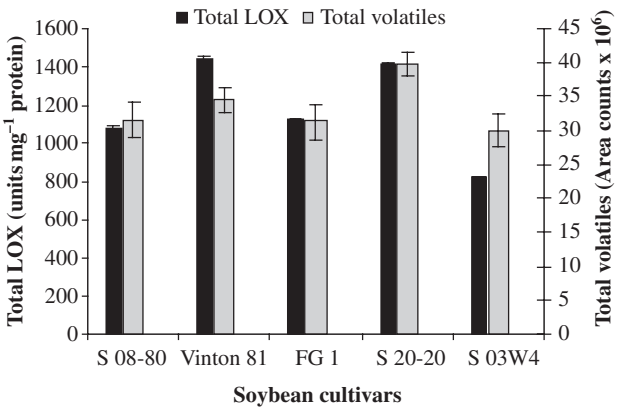

Figure 2 LOX activity relationship to total volatiles compounds in different soybean cultivars.

contributors to off-flavours in soymilk are the volatile carbonyl compounds, particularly hexanal (MacLeod & Ames, 1988). Figure 1 also shows the percentage of hexanal relative to total volatiles in the soymilk prepared from different cultivars. Significant ($P < 0.05$) differences in hexanal contents were observed among the different soymilk samples. Soymilk made from S08-80 variety had the lowest hexanal followed by soymilk made from S03W4, FG1, Vinton 81 and S20-20 cultivars.

Determination of lipoxygenase activity

Lipoxygenase isozyme activities were assayed using linoleic acid as substrate at pH 6.3 for LOX-II and LOX-III and at pH 9.0 for LOX-I. The results as presented in Table 6 showed that the total LOX activity was significantly ($P < 0.001$) different among the different soybean cultivars, except between Vinton 81 and S20-20 cultivars. The LOX-I activities for all cultivars were higher than the activities of LOX-II and LOX-III. Similar results were reported by Marczy *et al.* (1995) for other soybean varieties. Other authors have reported that LOX isozyme activities in soybean seeds are influenced significantly by the growing locations (Kumar *et al.*, 2003) and by both cultivar and climatic conditions (Marczy *et al.*, 1995).

It is well established that the beany flavour associated with various soy-based foods results from the formation of volatile carbonyl compounds during catalysis of polyunsaturated fatty acids by LOX (Rackis *et al.*, 1979). Moreover, the isozymes (LOX-II and LOX-III) catalyse the formation of volatile compounds much more intensively than does LOX-I (Marczy *et al.*, 1995). The data reported in Fig. 2 showed that soybean cultivars (Vinton 81 and S20-20) had the highest LOX activity that corresponded to a greater content of total volatiles, whereas with S03W4, the low enzymatic activity resulted in lower total volatiles. It appears, therefore, that there is a positive correlation (correlation coefficient = 0.82) between the total volatile compounds observed and the LOX activity, which confirms the involvement of this enzyme in the biogenesis of the volatile compounds (Ridolfi *et al.*, 2002).

Effect of soybean storage

Because of its higher protein, lower fat contents and better colour characteristics, which are highly recom-

Table 6 Lipoxygenase (LOX) isozymes activities[a] (units per mg protein) in various soybean cultivars

Cultivars	Protein (mg)	LOX-I	LOX-II + LOX-III	Total LOX activity
S08-80	2.28	1012.8 ± 12	66.3 ± 0.5	1079.1 ± 12.4[a]
Vinton 81	2.36	1203.4 ± 8	242.7 ± 2.5	1446.1 ± 11.3[b]
FG1	2.32	996.7 ± 8.5	127.8 ± 4.6	1124.5 ± 1.9[c]
S20-20	2.26	1232.2 ± 4	186.3 ± 1.0	1418.5 ± 5.2[b]
S03W4	2.25	732.1 ± 4	90.2 ± 0.7	822.3 ± 5.2[d]
Stored Vinton 81	1.97	939.2 ± 6	211.9 ± 1.0	1161.1 ± 7.9

Mean values within the same column followed by different superscript letters are significantly different ($P < 0.05$).

[a]Values are average of duplicates ± SD.

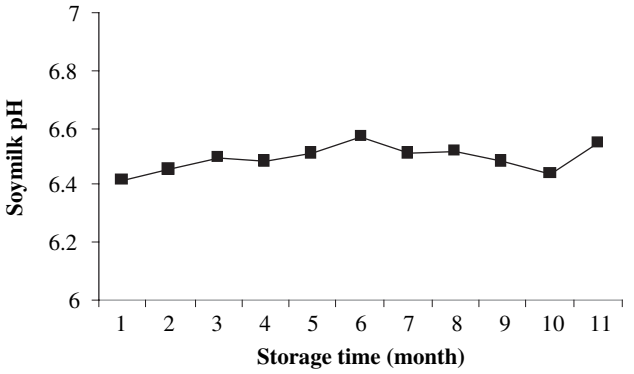

Figure 3 Soybean storage effect on the pH of soymilk over a period of 10 months.

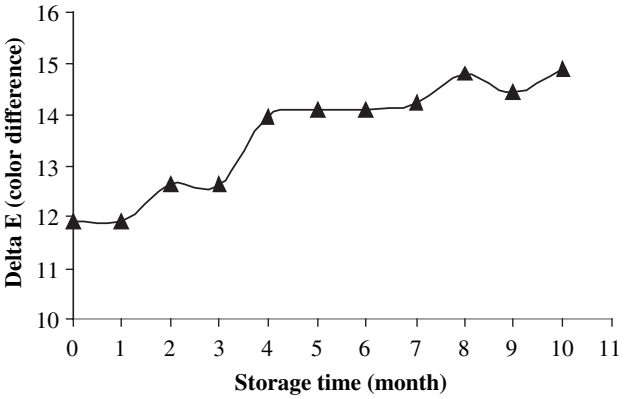

Figure 4 Soybean storage effect on soymilk colour difference (ΔE) over a period of 10 months.

mended for soymilk production, the Vinton 81 variety was selected for follow-up studies on the effect of soybean storage (10 months) and on physical properties and flavour characteristics of soymilk. In an earlier study, which compared the quality and yield of soymilk and tofu made from thirteen soybean varieties, Cai *et al.* (1997) also reported that Vinton was one of the most popular commercial varieties used in the soymilk and tofu industry.

No differences were observed in WUF of soybeans during the 10 months of storage under the applied conditions. The amount of water imbibed by soybeans during soaking is important in the processing of soybeans into soymilk and tofu, as it reduces the amount of energy required to grind them and increase the subsequent rate of nutrient extraction (Shurtleff & Aoyagi, 1990). Other authors have reported that water uptake decreased when seeds were stored at higher temperatures and higher relative humidities (Ohta *et al.*, 1979; Lambrecht *et al.*, 1995).

Storage of the soybeans did not also have a significant ($P < 0.05$) impact on the pH of the prepared soymilk (Fig. 3). The pH values of the soymilk remained between 6.4 and 6.6. However, the colour of the soymilk prepared from the stored soybeans became increasingly darker over the 10-month storage period. This can be seen by the increase in ΔE values from 11.9 to 14.9 over the period of storage (Fig. 4). In their study, Narayan *et al.* (1988a,b) reported earlier that the colour of stored soybeans changed from creamy yellow to brown with the increase in storage period and the intensity of the colour increased at ambient temperature during storage.

The effect of soybean storage on the flavour profile of soymilk is represented in Fig. 5. Storage resulted in a significant ($P < 0.05$) decrease in the total volatiles recovery over time. The sharpest decrease in total volatiles was observed during the initial months of storage (88% loss after 3 months). Beyond the third month, total volatiles recovery remained fairly constant.

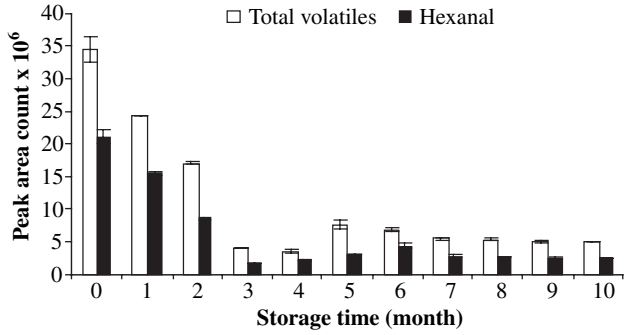

Figure 5 Soybean storage effect on the total volatiles (□) recovery (MS response-peak areas) and per cent ratio of hexanal (■) to total volatiles recovery of soymilk over a period of 10 months.

This decrease in total volatile corresponds with the 20% decrease in the total LOX activity observed during storage. Similar findings were also reported by several authors (Saio *et al.*, 1980; Narayan *et al.*, 1988a; Thomas *et al.*, 1989; Lambrecht *et al.*, 1995). This finding suggests that soymilk prepared from stored soybeans may result in better soymilk flavour and quality. The higher level of total volatiles in the soymilk samples for the control and during the first month of storage could have been influenced by the intrinsic properties of soybeans at the time of their harvesting (i.e. moisture content, chlorophyll (pigment) concentration, LOX activity and chemical reactions between soybean constituents).

Conclusions

The work reported in this article has shown that in addition to variety, storage affects the quality of the soybeans used for making soymilk, particularly its flavour. Some researchers have also reported a decrease

in protein extractability as a result of storage (Saio *et al.*, 1980; Thomas *et al.*, 1989). The LOX activity of the different soybean cultivars was well correlated with the formation of volatile compounds. Further studies on flavour, quality and yield using a wider variety of soybean stored under different conditions (temperature, relative humidity, light/dark) for varying periods will be useful to determine the optimal conditions of storage of soybeans for soymilk production.

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