

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Evaluation of the chemical quality traits of soybean seeds, as related to sensory attributes of soymilk

Lei Ma¹, Bin Li¹, Fenxia Han, Shurong Yan, Lianzheng Wang, Junming Sun*

The National Key Facility for Crop Gene Resources and Genetic Improvement, MOA Key Laboratory of Soybean Biology (Beijing), Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China

ARTICLE INFO

Article history:

Received 4 June 2014

Received in revised form 13 October 2014

Accepted 17 October 2014

Available online 25 October 2014

Keywords:

Soybean (*Glycine max* L. Merr.)

Soymilk

Sensory attributes

Seed chemical quality

ABSTRACT

The soybean seed chemical quality traits (including protein content, oil content, fatty acid composition, isoflavone content, and protein subunits), soymilk chemical character (soluble solid), and soymilk sensory attributes were evaluated among 70 genotypes to determine the correlation between seed chemical quality traits and soymilk sensory attributes. Six sensory parameters (i.e., soymilk aroma, smoothness in the mouth, thickness in the mouth, sweetness, colour and appearance, and overall acceptability) and a seven-point hedonic scale for each parameter were developed. Significant positive correlations were observed between overall acceptability and the other five evaluation parameters, suggesting that overall acceptability is an ideal parameter for evaluating soymilk flavour. The soymilk sensory attributes were significantly positively correlated with the characteristics of the glycinin (11S)/beta-conglycinin (7S) protein ratio, soluble solid, and oil content but negatively correlated with glycitein and protein content. Our results indicated that soymilk sensory attributes could be improved by selecting the desirable seed chemical quality traits in practical soybean breeding programs.

© 2014 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

1. Introduction

The soybean has long been a staple of the human diet in Asia, especially the soyfood such as soymilk or tofu (Liu, 1997). Soy protein is the most inexpensive source of high-nutritional quality protein and therefore is the world's predominant commercially available vegetable protein. Additionally, several putative health-beneficial substances (e.g., isoflavone, saponin, oligosaccharide, phospholipid, polypeptide and dietary fibre) have been identified in soybeans, leading to an increased interest in and demand for soybean and soy-based products. Soymilk is a popular beverage with abundant vegetable protein in Asian countries. As a nutrient-rich beverage, soymilk consumption has sustained a growth rate of 21% per year in the U.S. (Wrick, 2003). However, soymilk is still considered unpleasant to teenagers and Western consumers due to its off-flavour, especially its bitter taste, as well as its beany and rancid flavour (Damondaran & Kinsella, 1981; Wrick, 2003).

Two types of off-flavour in soymilk have been reported. The volatile beany and herbal flavour is composed of the aldehydes, alcohols, ketones, and furans (Kaneko, Kumazawa, & Nishimura, 2011; Wang, Dou, Macura, Durance, & Nakai, 1998; Wilkens &

Lin, 1970), whereas the nonvolatile bitterness and astringency consist of phenolic acid, isoflavone, saponin, tetro, and other substances (Heng et al., 2006; Kudou et al., 1991). The off-flavour development in soymilk is primarily due to the lipoxygenase or the oxidative rancidity of unsaturated fatty acids (Gardner, 1985; Lee, Min, Choe, & Min, 2003; Wolf, 1975). It was reported that plant lipids are sequentially degraded into volatile and nonvolatile compounds by a series of enzymes via the lipoxygenase pathway, which catalyses the hydroperoxidation of polyunsaturated fatty acids containing a 1,4-cis,cis-pentadiene structure to form the medium-chain-length aldehyde and alcohols that are responsible for the grassy-beany flavour (Iassonova, Johnson, Hammond, & Beattie, 2009; Moreira, Tavares, Ramos, & De Barros, 1993; Wolf, 1975). Otherwise, singlet oxygen oxidation could also cause off-flavours due to the oxidation of polyunsaturated fatty acids, as well as the decomposition of vitamin D, riboflavin, and ascorbic acid in foods (Jung, Yoon, Lee, & Min, 1998; Lee et al., 2003; Min & Boff, 2002). Singlet oxygen oxidation is notably rapid in foods containing compounds with double bonds due to the low activation energy for the chemical reaction (Min & Boff, 2002). In addition, singlet oxygen oxidation with linoleic acid is approximately 1,450 times faster than ordinary triplet autooxidation with linoleic acid (Bradley & Min, 1992). Unfortunately, the off-flavour compounds are highly difficult to remove from soymilk processing due to these compounds' high affinities with the soy protein

* Corresponding author. Tel./fax: +86 10 8210 5805.

E-mail address: sunjunming@caas.cn (J. Sun).

¹ These authors contributed equally to this work.

(Gkionakis, David Anthony Taylor, Ahmad, & Heliopoulos, 2007; O'Keefe, Resurreccion, Wilson, & Murphy, 1991; Zhou, Boatright, Johnson, & Reuber, 2002).

The flavour property of soymilk is affected by many factors, such as the genotype of soybean cultivars, the processing method, and environmental conditions. Moreover, the soybean seed chemical quality properties—including protein and oil content, fatty acids, isoflavones, saponins, oligosaccharide and peptides—can affect the soymilk flavour attributes significantly (Kudou et al., 1991; Min, Yu, Yoo, & Martin, 2005; Terhaag, Almeida, & Benassi, 2013). Owing to soymilk's off-flavour, many efforts have been taken to improve soymilk flavour based on the selection of soybean cultivars and enhancement of the processing technology (Hildebrand & Hymowitz, 1981; Kwok, Liang, & Niranjana, 2002; Suppavorasatit, Lee, & Cadwallader, 2013). However, the adjustment of processing may lead to a risk of protein denaturation and nutrition destruction in soymilk (Kwok et al., 2002). Therefore, it is necessary to select specific soybean cultivars suitable for soymilk processing in soybean breeding programs.

Taken together, Soymilk is a popular beverage in Asian countries. Additionally, soymilk and its products are regarded as nutritious and cholesterol-free health foods, with considerable potential application. However, information regarding soymilk sensory evaluation and the effect of soybean seed chemical quality traits on soymilk sensory attributes were notably limited (Poysa & Woodrow, 2002; Terhaag et al., 2013). As a result, it is difficult to select suitable cultivars for soymilk processing. Therefore, the objectives of this study were the following: (1) assess the soymilk flavour attributes based on the soymilk sensory evaluation method among 70 soybean genotypes; (2) analyse the correlations between the soymilk flavour attributes and seed chemical quality traits (i.e., protein, oil, storage protein subunits, isoflavones and fatty acids); (3) develop the regression equations for soymilk sensory attributes using soybean seed chemical quality traits; and (4) identify the breeding indexes related to soymilk flavour attributes for soybean quality breeding. This study will improve the standardisation of the soymilk flavour evaluation method and stimulate soybean breeding for improving soymilk flavour.

2. Materials and methods

2.1. Plant materials and field experiments

Seventy soybean genotypes of diverse origins were used in this study, which included 23 Chinese leading cultivars, 14 lines selected from two sets of near-isogenic lines with or without lipoxygenase isozymes (NILs Suzuyutaka from Japan and NILs Century from USA), and 33 advanced lines from representative soybean-producing regions (Table S1). These cultivars were planted at the Changping experimental station (N40°13' and E116°14') of the Institute of Crop Science, Chinese Academy of Agricultural Sciences, in 2010 and 2011. Soybean samples were sowed and harvested at the same time. At the experiment's onset, soil pH, all nitrogen, phosphorus, potassium and organic matter levels were 8.22, 80.5 mg kg⁻¹, 68.7 mg kg⁻¹, 14.58 g kg⁻¹ and 12.31 g kg⁻¹, respectively. A randomised complete block design in triplicate was employed and the test plots were managed according to the local cropping practice with a row length of 3 m, row spacing of 0.5 m and plant spacing of 0.1 m. Plots were fertilised with 15 t ha⁻¹ organic fertilizer, 30 kg ha⁻¹ of nitrogen and sufficient phosphorus and potassium during field preparation. Weeds were controlled by the post-emergence application of 2.55 L ha⁻¹ of acetochlor, as well as hand weeding during the growing season. Plots were harvested manually when the plants reached physiological maturity. Samples of each soybean genotype were harvested

from three plots and analysed for their soymilk flavour attributes and other seed chemical quality traits. Weather data during both years' growing seasons were retrieved from a nearby weather station (Table S2).

2.2. Preparation of soymilk

The soymilk preparation equipment was made of either stainless steel or plastic. The flow diagram of the soymilk preparation process followed the method described by Min et al. (2005). As shown in Fig. S1, 25 g of soybean seeds were rinsed and soaked in 250 mL of distilled water for 10 h at room temperature. The soaked soybean seeds were drained, rinsed, and ground in a Phillips blender (HR2003, Phillips Hong Kong Limited, China) for 1.0 min at high speed with corresponding water to make a total of 500 g of soybean slurry. The ratio of dry soybean seeds to water was 1:20 (w:w). The soybean slurry was then filtered through a Phillips filter screen and approximately 400 mL of soymilk was isolated. The soymilk was boiled for 10 min and then served at 70 °C in glass cup for sensory evaluation. This temperature was selected according to the drinking habit for soymilk in China. Generally, Chinese people prefer hot soymilk to cold one, which is similar to the drinking habits for coffee or tea.

2.3. Sensory attributes evaluation of soymilk

For the sensory evaluation, the soymilk samples prepared from six soybean genotypes were tested in duplicate at each panel session and the cultivar ZH13 was used as a control; cv. ZH13 is a leading soybean cultivar in the Yellow and Huai valley region of China. This cultivar exhibited a high content of protein and a relatively good soymilk quality score in a preliminary sensory test. The procedure for the sensory evaluation is shown in Fig. S2. The sensory evaluation was performed by at least eight trained panelists (25–30 years of age) from the Institute of Crop Science, Chinese Academy of Agricultural Sciences. Each panelist received 6 h of training sessions and practice in soymilk evaluation. During the training, panelists evaluated and discussed soymilk sensory attributes by comparing to cv. ZH13. Specific attributes, attribute definitions, and references were developed by the panelists (data not shown). Panelists compared six parameters—including colour and appearance, aroma, sweetness, thickness in the mouth, smoothness in the mouth, and overall acceptability—and assigned a score to each sample based on a 7-point hedonic scale (1–7) for soymilk flavour sensory evaluation: 1 = 'strongly disliked'; 2 = 'moderately disliked'; 3 = 'slightly disliked'; 4 = 'indifferent'; 5 = 'slightly liked'; 6 = 'moderately liked'; and 7 = 'strongly liked' (Robinson, Chambers, & Milliken, 2005). To adapt to a traditional taste style, the soymilk was kept at approximately 70 °C before sensory evaluation. The analysis of variance (ANOVA) indicated that the panel and panelists could consistently use the attributes to differentiate the soymilk samples.

For the soymilk flavour evaluation, the basic panel procedures followed the previous method (Chambers, Jenkins, & McGuire, 2006). The panel tasted one sample at a time. The flavour and mouth feel attributes were recorded 60 s after swallowing. The panel openly discussed each soymilk sample to reach a consensus concerning the flavour and mouth feel properties.

2.4. Determination of protein and oil content

The protein and oil content could be estimated by near-infrared spectroscopy (Hymowitz, Dudley, Collins, & Brown, 1974). In this study, 50 g of soybean seeds for each sample were analysed by transform near-infrared absorption spectroscopy (Bruker Fourier, Germany). The spectrum value of each sample represented the

average value of triplicate and the absorption ranged from 4000 to 8000 cm^{-1} . The collected spectra were transferred to the protein and oil content by the Quant 2 method of Bruker's OPUS 4.2 software.

2.5. Relative content of subunits 11S and 7S

It is reported 11S/7S ratio can be used as a criterion of indirect selection for high quality protein (Sharma, Kaur, Goyal, & Gill, 2014). For determination of the 11S/7S ratio, the storage protein subunits glycinin (11S) and β -conglycinin (7S) were quantified by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Bradford, 1976). Ten milligrams of soybean flour for each sample were extracted with 500 μL extraction solution (0.05 M Tris buffer, pH 8.0, 0.01 M β -mercaptoethanol, and 2% SDS) for 1 h at 4 $^{\circ}\text{C}$. Samples were then centrifuged at room temperature at 12,000 rpm for 15 min. The supernatant contained the total soybean proteins. Next, 5.0 μL of supernatant was loaded onto a gradient gel containing 5–12% polyacrylamide. SDS-PAGE was performed in a vertical electrophoresis unit DYY-8C (Beijing Liuyi Instrument Factory, Beijing, China) at 80 V constant voltage for 40 min, followed by 120 V constant voltages until the tracking dye migrated to the bottom edge of the gel (approximately 5 h). Gels were stained with Coomassie Brilliant Blue R-250 (0.05%, w/v) in a staining solution containing 45% (v/v) methanol and 10% (v/v) acetic acid and then destained in a destaining solution containing 10% (v/v) methanol and 10% (v/v) acetic acid.

For quantification of the 11S and 7S fractions and their respective subunits, the gels were rinsed and scanned by the GelDoc EZ imager (Bio-Rad laboratories, Inc., Hercules, CA, USA) after destaining. The protein bands representing the 11S and 7S fractions were quantified by densitometric analysis using the Gel-Pro Analyzer 4.0 software (Media Cybernetics, Inc., Rockville, MD, USA). The protein ratio of subunit 11S/7S was subsequently calculated.

2.6. Fatty acid determination

The seed fatty acid composition was determined using Gas Chromatography (GC) of the methyl ester method (Sun, Han, Yan, Yang, & Tetsuo, 2008). Next, 0.5 g of soybean seed powder for each sample was mixed with 1.5 mL hexane overnight and the mixture was centrifuged at 7000 rpm for 5 min. The supernatant was collected and added to 350 μL of sodium methoxide solution. After vortexing, the mixture was shook for 1 h. After centrifugation at 7000 rpm for 5 min, the supernatant was filtered into the special sample bottle for GC detectors. The GC analysis was performed on a RTX-Wax Column (30 m \times 0.25 mm \times 0.25 mm, Germany) with nitrogen, hydrogen and air as the carrier gases for 20 min. The injection volume was 1 μL . The area normalisation method was used to calculate the percentage of five fatty acid components—palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid—on a GC2010 workstation (Shimadzu, Japan).

2.7. Isoflavone extraction and HPLC assay

The isoflavone concentration was analysed with the High Performance Liquid Chromatography (HPLC) method (Sun, Sun, Han, Yan, Yang, & Kikuchi, 2011). Approximately 20 g of soybean seeds were ground using a cyclone mill (Retsch ZM100, Φ = 1.0 mm, Rheinische, Germany). Next, 0.1 g of this powder was added to 5 mL of extraction solution containing 0.1% (v/v) acetic acid and 70% (v/v) ethanol. The mixture was shaken at room temperature for 12 h. After centrifugation at 5000 rpm for 5 min, the supernatant was filtered using 0.2 μm nylon syringe filters. Next, 10 μL of the filtrates was subjected to High Performance Liquid Chromatography (HPLC) on an Agilent 1100 series system. Quantitative analyses were per-

formed on the YMC Pack, ODS-AM-303 column (250 mm \times 4.6 mm i.d., S-5 μm , 120 Å , YMC Co., Kyoto, Japan) at 35 $^{\circ}\text{C}$, using a 70-min linear gradient of 13–35% acetonitrile in aqueous solution containing 0.1% acetic acid. The solvent flow rate was 1.0 mL min^{-1} , and the UV absorption was measured at 260 nm.

Twelve standards of isoflavone components, including daidzin, glycitin, genistin, malonyldaidzin, malonylglycitin, malonylgenistin, acetyldaidzin, acetylglycitin, acetylgenistin, daidzein, glycitein, and genistein, were provided by Dr. Akio Kikuchi (National Agricultural Research Center for Tohoku Region, Japan). Separate standard stock solutions were made for all of 12 isoflavone forms and stored at 4 $^{\circ}\text{C}$.

According to the retention time and the maximum UV absorbance for the 12 standards, we accurately detected all forms of isoflavone components based on the UV absorption value at 260 nm. The various components of isoflavones, the aglycone form of isoflavone and the total isoflavone content in soybean seeds were calculated as described by Sun et al. (2011).

2.8. Soluble solids analysis

Soluble solids content is an important parameter for beverage evaluation in food industry. Therefore, the soluble solids of soymilk were determined using a Digital Handheld "Pocket" Refractometer PAL-1 (ATAGO Co., LTD, Tokyo, Japan) at room temperature in three replicates before heating. The results were expressed as degrees Brix at 20 $^{\circ}\text{C}$.

2.9. Statistical analysis

The plots of each experiment were arranged in a randomised complete block design with three replicates. All data were subjected to an ANOVA using the general linear model (GLM) procedure of the SAS 9.2 software for Windows (SAS Institute, 2009) to identify significant treatment effects. Comparisons among means were made using the Least Significant Difference (LSD) test at α = 0.05 or less when ANOVA indicated that model and treatment were significant. Pearson correlation coefficients for seed quality traits and soymilk sensory attributes were calculated based on genotypic means across the years using the correlation procedure (PROC CORR) of SAS 9.2. Moreover, a Principal Component Analysis (PCA) of the correlation matrix was performed for ranking sum values of sensory attributes using the SAS 9.2 software. Stepwise regression was performed with soymilk sensory parameters and soybean seed chemical traits using SAS 9.2 software. All proceeding treatments were duplicated and field treatments were triplicated.

3. Results and discussion

3.1. Genetic and environmental effects on seed chemical quality traits

ANOVA showed significant differences in protein and oil contents, fatty acid composition, isoflavone content, the ratio of 11S/7S, and soluble solid among 70 soybean genotypes (Table 1). This is consistent with previous studies (Poysa & Woodrow, 2002; Yoshikawa, Chen, Zhang, Scaboo, & Orazaly, 2014). Moreover, the variance for each seed quality trait spanned a wide range among 70 genotypes in this study. Protein content ranged from 37.04% in HF48 to 47.87% in 09P-21; oil content ranged from 16.97% in LD4 to 22.88% in ZH31; the protein ratio of 11S/7S subunit ranged from 0.99 in SuN to 8.28 in JD12; and isoflavone content ranged from 769.55 $\mu\text{g g}^{-1}$ in 09J-28 to 2558.56 $\mu\text{g g}^{-1}$ in 09P-1. The wide variance of seed chemical quality traits suggested an abundant genetic diversity among the 70 soybean genotypes.

Table 1
ANOVA for soybean chemical quality traits in 70 soybean varieties.

Main quality trait	Variation source					
	Cultivar			Repeat in field		
	df ^a	Sum of square	Pr>F	df	Sum of square	Pr>F
Protein	69	939.41	<0.0001	2	0.72	0.9363
11S/7S	69	232.75	<0.0001	2	4.60	0.1795
Oil	69	272.36	<0.0001	2	2.52	0.4481
Palmitic acid	69	85.44	<0.0001	2	2.76	0.0563
Stearic acid	69	54.16	<0.0001	2	1.84	0.0423
Oleic acid	69	1880.20	<0.0001	2	5.99	0.7490
Linoleic acid	69	1046.41	<0.0001	2	19.27	0.2021
Linolenic acid	69	357.46	<0.0001	2	0.78	0.8079
Daidzein	69	7142946.18	<0.0001	2	507510.55	0.0021
Glycitein	69	445044.04	<0.0001	2	33146.24	0.0022
Genistein	69	7341430.02	<0.0001	2	1912400.37	<0.0001
Total isoflavone	69	26026203.78	<0.0001	2	5172739.39	<0.0001

^a Degrees of freedom.

It is noteworthy that isoflavone components were also significantly different among field experiment repeats, whereas no significant difference was observed in other chemical quality traits (Table 1). This indicates that in addition to genetic factors, environmental factors also have a great effect on seed isoflavone concentrations, which is consistent with previous reports (Seguin, Zheng, Smith, & Deng, 2004; Zhang et al., 2014).

3.2. Evaluation of soymilk sensory attributes

The soymilk sensory attributes were analysed by the sensory evaluation method, as described in Fig. S2. The coefficient of variance for soymilk sensory attributes ranged from 4.68% to 11.94% (Table 2). Large variances were observed in soymilk colour and appearance, sweetness and overall acceptability. Their coefficients of variance were 11.94%, 7.42% and 8.72%, respectively (Table 2).

Soybean genotypes and environments had significant effects on soymilk sensory attributes. Highly significant differences were observed among various soybean genotypes for soymilk colour and appearance, smoothness in the mouth, sweetness, and overall acceptability parameters (Table S3), suggesting that the sensory property was mainly determined by genotypic factor. Conversely, the soymilk aroma parameter had significant variances among replicates in the field, replicates in the lab and years (Table S3),

indicating that it was mainly affected by environmental conditions. Other parameters of sensory attributes were affected by both genotypic and environmental factors (Table S3), implying that the soymilk sensory was a complex quality trait. Noticeably, the overall acceptability was merely affected by genotypes and independent of two environments in this study, which implied that it could be a stable parameter in soymilk sensory evaluation among soybean genotypes. Owing to the significant genotypic effects for most soymilk sensory attributes, we confirmed that genetic factor plays an important role in soymilk sensory attributes, as was reported by previous studies (Min et al., 2005; Poysa & Woodrow, 2002).

The correlation coefficient (*r*) from the averaged data of triplicates showed that the overall acceptability was significantly positively associated with other soymilk sensory parameters (Table 3). This suggested once more that as an important sensory attribute, the overall acceptability may be an ideal indicator for soymilk sensory evaluation.

3.3. Effects of protein content and 11S/7S ratio on soymilk sensory attributes

Soluble proteins are the main components of soymilk, which consist of glycinin (11S) and β -conglycinin (7S) subunits. The two types of protein components represent more than 70% of the total

Table 2
The sensory evaluation scores for soymilk flavour quality in 70 Chinese soybean varieties.

Sensory index	No.	Min	Max	Mean	Std. deviation	Variance	CV% ^a
Aroma	70	4.25	5.83	4.85	0.28	0.08	5.86
Smoothness in the mouth	70	4.33	5.67	5.06	0.27	0.07	5.30
Thickness in the mouth	70	4.53	5.50	5.03	0.23	0.05	4.48
Sweetness	70	2.50	4.17	3.50	0.31	0.09	8.72
Colour and appearance	70	3.50	5.83	4.54	0.54	0.29	11.94
Overall acceptability	70	3.92	5.50	4.87	0.36	0.13	7.42

^a Coefficient of variation.

Table 3
Correlation coefficients among soymilk sensory parameters.

Sensory attribute	Aroma	Smoothness in the mouth	Thickness in the mouth	Sweetness	Colour and appearance
Smoothness in the mouth	0.043				
Thickness in the mouth	0.307**	−0.185			
Sweetness	0.301*	0.308**	0.295*		
Colour and appearance	−0.017	0.168	0.073	0.209	
Overall acceptability	0.269*	0.384**	0.277*	0.538**	0.291*

* Represent the significance levels at $P < 0.05$.

** Represent the significance levels at $P < 0.01$.

Table 4
Correlation analysis between soymilk sensory attributes and soybean seed chemical quality traits.

Seed quality trait	Aroma	Smoothness in the mouth	Thickness in the mouth	Sweetness	Colour and appearance	Overall acceptability
Protein content	0.091	-0.111	0.024	-0.185	-0.215	-0.305*
11S/7S	0.390**	0.112	0.242*	0.204	0.171	0.272*
Oil content	0.030	0.152	0.166	0.015	-0.026	0.298*
Soluble solid	0.330**	0.151	0.410**	0.173	0.062	0.427**
Palmitic acid	-0.350**	-0.060	-0.143	0.098	0.405**	-0.008
Stearic acid	-0.236*	-0.092	-0.293*	0.144	-0.133	-0.060
Oleic acid	0.213	0.253*	0.086	-0.067	-0.122	0.084
Linoleic acid	-0.058	-0.179	0.070	-0.110	-0.101	-0.139
Linolenic acid	-0.120	-0.206	-0.130	0.237*	0.302*	0.072
Daidzein	-0.072	-0.138	-0.109	-0.116	-0.053	-0.089
Glycitein	-0.086	-0.244*	-0.069	-0.302*	-0.420**	-0.375**
Genistein	-0.021	-0.043	-0.018	-0.212	-0.226	0.014
Total of isoflavone	-0.060	-0.127	-0.076	-0.212	-0.201	-0.088

* Represent the significance levels at $P < 0.05$.

** Represent the significance levels at $P < 0.01$.

soy proteins (Liu, 1997). Glycinin is in hexameric form, and each monomer unit consists of an acidic and a basic polypeptide linked together by a disulphide bond (Nielsen et al., 1986). Generally, glycinin subunits could be divided to three groups: group I (A1aB1b, A1bB2, and A2B1a), group IIa (A5A4B3), and group IIb (A3B4). Another main component of soluble proteins, β -conglycinin, which belongs to the trimeric glycoprotein, includes three subunits— α' , α , and β —linked by hydrophobic interactions and hydrogen bridging (Liu, 1997). It has been previously demonstrated that the soymilk flavour attributes are affected not only by processing and environmental conditions but also by protein composition (Nik, Tosh, Woodrow, Poysa, & Corredig, 2009; Poysa & Woodrow, 2002). The ratio of glycinin to β -conglycinin has an important effect on soymilk quality and could be used as a criterion of indirect selection for high quality protein (Sharma et al., 2014; Tezuka, Taira, Igarashi, Yagasaki, & Ono, 2000; Tezuka, Yagasaki, & Ono, 2004). As an example, soymilk containing group I subunits (A1, A2) of glycinin has more particles than those without group I (Nik et al., 2009). In our study, significant positive correlations were observed between subunit ratio of 11S/7S and soymilk aroma ($r = 0.39^*$), thickness in the mouth ($r = 0.242^*$), and overall acceptability ($r = 0.272^*$) (Table 4), indicating a high ratio of 11S/7S benefits soymilk sensory. This may be due to the higher content of sulphur-containing amino acids and more particles containing in glycinin compared to β -conglycinin. In contrast, a significant negative correlation was observed between seed protein content and soymilk overall acceptability ($r = -0.305^*$) (Table 4), which suggested that high protein content may not benefit soymilk flavour. This could be explained by the unfavorable bitter tastes produced in the hydrolysis of polypeptides, as well as the unfavorable colour and appearance caused by the Maillard Browning reaction (Kwok, MacDougall, & Niranjana, 1999). Moreover, it has been reported that the protein content is positively correlated with soymilk's beany odour content, which affects the flavour of soymilk (Min et al., 2005; Yuan & Chang, 2007).

3.4. Effects of isoflavone components on soymilk sensory attributes

Soymilk is an unpleasant beverage for teenagers and Western consumers because of its bitter, beany and rancid flavour, which consists of volatile and nonvolatile compounds (MacLeod, Ames, & Betz, 1988). Isoflavones—the main nonvolatile off-flavour compounds in soymilk—are believed to be responsible for the bitter and astringent flavours (Aldin, Reitmeier, & Murphy, 2006; Matsuura, Obata, & Fukushima, 1989). In our study, as a bitter taste factor, the contents of individual isoflavone components were measured for all 12 forms of isoflavones found in the soybean seed.

Because isoflavones are absorbed by the human body mainly in the aglycone form, the total concentration of isoflavones in soymilk should be expressed as the arithmetic sum of the adjusted sums of total genistein, total daidzein, and total glycitein (Murphy et al., 1999). As expected, negative correlations between isoflavone components and all soymilk sensory attributes were observed (Table 4). In particular, glycitein was significantly negatively correlated with soymilk smoothness in the mouth ($r = -0.244^*$), sweetness ($r = -0.302^*$), colour and appearance ($r = -0.420^*$), and overall acceptability ($r = -0.375^*$) (Table 4), suggesting glycitein is a typical substance adversely affecting soymilk flavour. This may be due to the least taste threshold value of glycitein (Kudou et al., 1991). Moreover, as a type of natural pigment, the high content of glycitein was also unfavorable for the soymilk colour attribute ($r = -0.420^*$) (Table 4).

3.5. Effects of oil content and fatty acid components on soymilk sensory attributes

The volatile off-flavour problems associated with soymilk have been characterised mainly as green, beany and grassy. The off-flavour development in soymilk is primarily due to the lipoxygenase or the oxidative rancidity of unsaturated fatty acids (Wolf, 1975). Therefore, soybean oil content and fatty acid composition play important roles in the flavour attributes, despite their limited amounts in soymilk. In our study, a significant positive correlation between oil content and soymilk overall acceptability was observed ($r = 0.298^*$) (Table 4), suggesting the oil content benefits the soymilk flavour property. However, for fatty acid composition, the correlations were considerably complicated (Table 4). For instance, significant negative correlations were observed between soymilk aroma and saturated fatty acids (i.e., palmitic acid ($r = -0.350^*$) and stearic acid ($r = -0.236^*$)), whereas significant positive correlation of colour and appearance with palmitic acid ($r = 0.405^{**}$) and linolenic acid ($r = 0.302^*$) were observed (Table 4). Oleic acid and linolenic acid were significantly positively correlated with smoothness in the mouth and sweetness ($r = 0.253^*$ and $r = 0.237^*$, respectively), whereas stearic acid was significantly negatively correlated with thickness in the mouth ($r = -0.293^*$) (Table 4). Moreover, as the most important sensory parameter, the overall acceptability failed to correlate with any fatty acid components (Table 4). It has been reported that soybean lipoxygenases catalyse the oxidation of polyunsaturated fatty acids, forming hydroperoxide derivatives, which undergo a scission and dismutation reaction, resulting in the development of off-flavours (Iassonova et al., 2009; Wolf, 1975; Moreira et al., 1993). Especially, the beany flavour that makes soymilk taste unpleasant

to Westerners may be due to 2-pentylfuran, which is mainly formed from linoleic acid by singlet oxygen (Min et al., 2005). Moreover, free linoleic acid and linolenic acid in soymilk present bitterness and beany odour (Stephan & Steinhart, 2000). Our results also suggested an important role of fatty acid composition in soymilk sensory attributes, however, the effect of fatty acid composition on soymilk sensory attributes were considerably complicated.

3.6. Effects of soluble solids on soymilk sensory attributes

Soluble solids content is an important parameter for beverage evaluation in food industry. High soluble solids content was desirable soymilk characters for consumers (Lim, Deman, Deman, & Buzzell, 1990). Moreover, the soluble solids content was significantly affected by soybean cultivars (Aziadekey, 2001). Therefore, the soluble solids content was determined as a soymilk chemical character in this study. According to our results, soluble solids content was positively correlated with all of soymilk sensory attributes (Table 4). Especially, significant positive correlations were observed between soluble solids content and soymilk aroma ($r = 0.330^{**}$), thickness in the mouth ($r = 0.410^{**}$), and over acceptability ($r = 0.427^{**}$) (Table 4). This suggested a trend that superior soymilk lines had higher total soluble solids content than the inferior lines, which was consistent with previous reports (Poysa & Woodrow 2002).

3.7. Effects of lipoxygenase on soymilk sensory attributes

Soymilk flavour is formed by a complex combination and interaction of multiple chemical compounds. To improve the soymilk flavour, soybean lines lacking one or more lipoxygenase isozymes had been developed and the aroma constituents of soymilk were analysed (Kobayashi, Tsuda, Hirata, Kubota, & Kitamura, 1995). In these lines, although the yields of volatile compounds were greatly decreased, the chemical compounds responsible for the beany flavours still remained (Kobayashi et al., 1995; Torres-Penaranda & Reitmeier, 2001). In our study, we also detected the soymilk flavour attributes in two series of near isogenic lines with or without lipoxygenase isozymes. Unfortunately, no significant correlation between the lipoxygenase-lacking lines and soymilk flavour parameters was observed (data not shown). This implied that there may exist an oxidative rancidity of unsaturated fatty acids in soymilk (Wolf, 1975), in addition to lipoxygenase mediated oxidation.

Taken together, our study demonstrated that, as a comprehensive evaluation index, overall acceptability is the most important parameter for soymilk sensory evaluation due to the significant correlation with other flavour indexes and seed chemical quality parameters (Tables 3 and 4). Therefore, this parameter could be used to select soybean cultivars with good soymilk flavour attributes.

3.8. Principle Component Analysis for soymilk sensory attributes

SAS 9.2 software was used to analyse the soymilk sensory attributes using Principal Component Analysis (PCA). PCA is a widely used multivariate analytical statistical method, which could reduce the set of dependent variables to a smaller number based on the original variables' correlation pattern (Lawless & Heymann, 1998). In this study, six principle components (PCs) were identified and the first four PCs could explain 85.03% of the total variance. As shown in Fig. 1, the first component (PC1) explaining 36.86% of the total variance was designated as the soymilk overall flavour factor, as it was mainly associated with soymilk overall acceptability ($r = 0.557$) and sweetness ($r = 0.540$). The second component (PC2) explaining 21.90% of the total variance was designated as

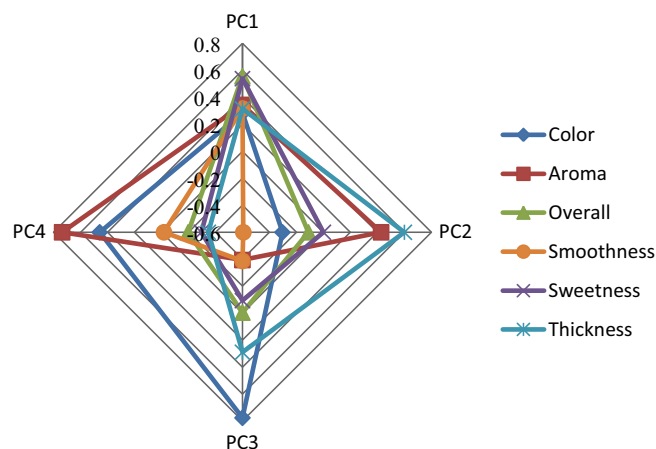


Fig. 1. Eigenvectors for soymilk flavour quality. "PC" represents the principle component; "colour" stands for colour and appearance; "overall" stands for overall acceptability; "smoothness" stands for smoothness in the mouth, and "thickness" stands for thickness in the mouth.

the soymilk taste factor, as it was primarily associated with soymilk thickness in the mouth ($r = 0.600$) and smoothness in the mouth ($r = -0.593$). The third component (PC3) explaining 15.42% of the total variance was designated as the soymilk appearance factor for its strong association with soymilk colour and appearance ($r = 0.776$). The fourth component (PC4) explaining 10.85% of the total variance was designated as the soymilk aroma factor for its primary association with soymilk aroma ($r = 0.737$). The above results were mainly based on the preference of soymilk for Chinese consumers. However, for Western consumers, owing to the different consumption habits, the first component was mainly associated with soymilk colour and thickness (Villegas, Caronell, & Costell, 2009). This implied that the most important attribute for Western consumers was soymilk colour and appearance. In contrast, for Chinese consumers, the mouth feeling of soymilk was the most important attribute. Therefore, it would be possible to improve the sensory attributes of soymilk according to the different consumers' habits through practical soybean breeding programs.

3.9. Screening the breeding indexes for soymilk sensory attributes

The stepwise regression was also performed and the regression equations for six soymilk sensory parameters were obtained (Table 5). By combining the stepwise regression and Principle Component Analysis results, seven seed chemical quality traits—

Table 5

The stepwise regression analysis for soymilk sensory attributes in soybeans.

Regression equation	Correlation coefficient (r)
$y_1 = -0.0040x_6 + 0.2650x_9 + 2.3287$	0.524 ^{**}
$y_2 = 0.1013x_3 - 0.1454x_9 + 6.2187$	0.515 ^{**}
$y_3 = 0.9493x_4 - 0.0710x_{12} + 0.1082x_{13} + 4.1743$	0.640 ^{**}
$y_4 = 0.44851x_4 - 0.0022x_6 + 0.2941x_{10} + 1.0263$	0.497 ^{**}
$y_5 = 0.3239x_4 + 3.826$	0.410 ^{**}
$y_6 = 0.0224x_{11} + 4.5175$	0.253 [*]

The standardised vectors $y_1 - y_6$ were nominated as corresponding soymilk colour and appearance (y_1), aroma (y_2), overall acceptability (y_3), sweetness (y_4), thickness in the mouth (y_5), and smoothness in the mouth (y_6), respectively. The standardised vectors $x_3, x_4, x_6, x_9, x_{10}, x_{11}, x_{12}$ and x_{13} were defined as the ratio of 11S/7S (x_3), soluble solid (x_4), glycitein (x_6), palmitic acid (x_9), stearic acid (x_{10}), oleic acid (x_{11}), linoleic acid (x_{12}) and linolenic acid (x_{13}), respectively.

* Represent the significance levels at $P < 0.05$.

** Represent the significance levels at $P < 0.01$.

the subunit ratio of 11S/7S, glycitein, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid—and one soymilk chemical parameter, soluble solids content, were significantly associated with the soymilk sensory attributes. In particular, soluble solids content, glycitein, and palmitic acid play more important roles in soymilk sensory attributes. This result suggested that the soymilk flavour attributes could be predicted and evaluated based on these chemical quality traits in the soybean breeding programs for improving soymilk flavour. As far as this study was concerned, for the overall soymilk flavour, soybean cultivars with a high ratio of 11S/7S, high contents of soluble solids and oil, plus relative low contents of glycitein and protein are desirable for soymilk processing in China.

In this study, we observed a correlation between soymilk sensory attributes and soybean seed chemical quality traits and provided evaluation parameters for soymilk sensory attributes, which will facilitate developing specific soybean cultivars for soymilk. However, a dilemma exists obviously between better soymilk flavour and rich nutritional value. For instance, glycitein, which is one of the soybean isoflavone components and a typical antitumor compound, was unfavorable to soymilk flavour attributes. As another example, linolenic acid, which is beneficial to human health, was negatively correlated with soymilk sensory attributes. As a result, if we decrease the contents of these substances to improve soymilk's flavour attributes, the nutritional and health values of soymilk will decrease simultaneously. Therefore, the concentration thresholds of these substances affecting soymilk flavour properties should be determined and a balance between better flavour properties and rich nutritional value should be achieved in the soybean breeding practice.

4. Conclusions

In this study, we developed six parameters—soymilk aroma, smoothness in the mouth, thickness in the mouth, sweetness, colour and appearance, and overall acceptability—and a seven-point hedonic scale to rate each parameter during the evaluation of soymilk sensory attributes. Owing to the genotypes' significant effects, plus the insignificant effects of year and replicate on the sensory attributes, we confirmed that genetic factor plays the most important role in soymilk sensory attributes. Based on the significant positive correlations, we suggested that the overall acceptability is an ideal parameter for soymilk flavour attributes evaluation. Correlation analysis and principal components analysis (PCA) demonstrated that seed chemical quality traits and soymilk chemical character were significantly correlated with soymilk sensory attributes among 70 soybean genotypes, suggesting that seed chemical quality traits and soymilk chemical character could be used as an indirect evaluation and selection index for soybean genotypes with better soymilk flavour in soybean breeding programs. Moreover, owing to the different dietary habits, there were different preferences for soymilk flavour attributes between Western and Chinese consumers. Overall, high yield breeding lines with a relatively high ratio of 11S/7S, high content of soluble solids and oil, plus relative low content of glycitein and protein will have the best chance of being accepted by a soymilk processing company in China.

Acknowledgements

This work was supported by the National Nature Science Foundation (No. 31171576), the Genetically Modified Organisms Breeding Major Projects (No. 2011ZX08004-003), the National Science and Technology Pillar Program during the Twelfth Five-Year Plan Period of China (Nos. 2011BAD35B06-3, 2014BAD11B01) and the CAAS Innovation Project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2014.10.096>.

References

- Aldin, E., Reitmeier, H. A., & Murphy, P. (2006). Bitterness of soy extracts containing isoflavones and saponins. *Journal of Food Science*, *71*, S211–S215.
- Aziadekey, M. (2001). Effects of soybean cultivars on soymilk quality. *Tropicicultura*, *19*, 203–205.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254.
- Bradley, D. G., & Min, D. B. (1992). Singlet oxygen oxidation of foods. *Critical Reviews in Food Science and Nutrition*, *31*, 211–236.
- Chambers, E., Jenkins, A., & McGuire, H. B. (2006). Flavor properties of plain soymilk. *Journal of Sensory Studies*, *21*, 165–179.
- Damondaran, S., & Kinsella, J. E. (1981). Interaction of carbonyls with soy protein: Conformational effects. *Journal of Agricultural and Food Chemistry*, *29*, 1253–1257.
- Gardner, H. W. (1985). Flavor and bitter tastes from oxidation of lipids by enzymes. In B. D. Min & T. H. Smouse (Eds.), *Flavor chemistry of fats and oils* (pp. 189–206). Champaign, Illinois: The American Oil Chemists' Society.
- Gkionakis, G. A., David Anthony Taylor, K., Ahmad, J., & Heliopoulos, G. (2007). The binding of the flavour of lactones by soya protein, amino acids and casein. *International Journal of Food Science Technology*, *42*, 165–174.
- Heng, L., Vincken, J. P., van Koningsveld, G., Legger, A., Gruppen, H., Boekel, T., et al. (2006). Bitterness of saponins and their content in dry peas. *Journal of the Science of Food and Agriculture*, *86*, 1225–1231.
- Hildebrand, D. F., & Hymowitz, T. (1981). Two soybean genotypes lacking lipoxygenase-1. *Journal of the American Oil Chemists' Society*, *58*, 583–586.
- Hymowitz, T., Dudley, J. W., Collins, F. I., & Brown, C. M. (1974). Estimations of protein and oil concentration in corn, soybean, and oat seed by near-infrared light reflectance. *Crop Science*, *14*, 713–715.
- Iassonova, D. R., Johnson, L. A., Hammond, E. G., & Beattie, S. E. (2009). Evidence of an enzymatic source of off flavors in "lipoxygenase-null" soybeans. *Journal of the American Oil Chemists' Society*, *86*, 59–64.
- Jung, M. Y., Yoon, S. H., Lee, H. O., & Min, D. B. (1998). Singlet oxygen and ascorbic acid effects on dimethyl disulfide and off-flavor in skim milk exposed to light. *Journal of Food Science*, *63*, 408–412.
- Kaneko, S., Kumazawa, K., & Nishimura, O. (2011). Studies on the key aroma compounds in soy milk made from three different soybean cultivars. *Journal of Agricultural and Food Chemistry*, *59*, 12204–12209.
- Kobayashi, A., Tsuda, Y., Hirata, N., Kubota, K., & Kitamura, K. (1995). Aroma constituents of soybean [*Glycine max* (L.) Merrill] milk lacking lipoxygenase isozymes. *Journal of Agricultural and Food Chemistry*, *43*, 2449–2452.
- Kudou, S., Fleury, Y., Welti, D., Magnolato, D., Uchida, T., Kitamura, K., et al. (1991). Malonyl isoflavone glycosides in soybean seeds [*Glycine max* (L.) Merrill]. *Agricultural and Biological Chemistry*, *55*, 2227–2233.
- Kwok, K. C., Liang, H. H., & Niranjan, K. (2002). Optimizing conditions for thermal processes of soy milk. *Journal of Agricultural and Food Chemistry*, *50*, 4834–4838.
- Kwok, K. C., MacDougall, D. B., & Niranjan, K. (1999). Reaction kinetics of heat-induced colour changes in soymilk. *Journal of Food Engineering*, *40*, 15–20.
- Lawless, H. T., & Heymann, H. (1998). *Sensory evaluation of food: principles and practices*. New York: Chapman & Hall, pp. 606–608.
- Lee, J. Y., Min, S., Choe, E. O., & Min, D. B. (2003). Formation of volatile compounds in soy flour by singlet oxygen oxidation during storage under light. *Journal of Food Science*, *68*, 1933–1937.
- Lim, B. T., Deman, J. M., Deman, L., & Buzzell, R. I. (1990). Yield and quality of tofu as affected by soybean and soymilk characteristics. Calcium sulfate coagulant. *Journal of Food Science*, *55*, 1088–1092.
- Liu, K. (1997). Chemistry and nutritional value of soybean components. In K. Liu (Ed.), *Soybeans: chemistry, technology and utilization* (pp. 25–113). New York: Chapman & Hall.
- MacLeod, G., Ames, J., & Betz, N. L. (1988). Soy flavor and its improvement. *Critical Reviews in Food Science & Nutrition*, *27*, 219–400.
- Matsaura, M., Obata, A., & Fukushima, D. (1989). Objectionable flavor of soy milk developed during the soaking of soybeans and its control. *Journal of Food Science*, *54*, 602–605.
- Min, D. B., & Boff, J. M. (2002). Chemistry and reaction of singlet oxygen in foods. *Comprehensive Reviews in Food Science and Food Safety*, *1*, 58–72.
- Min, S., Yu, Y., Yoo, S., & Martin, S. S. (2005). Effect of soybean varieties and growing locations on the flavor of soymilk. *Journal of Food Science*, *70*, C1–C7.
- Moreira, M. A., Tavares, S. R., Ramos, V., & De Barros, E. G. (1993). Hexanal production and TBA number are reduced in soybean [*Glycine max* (L.) Merr.] seeds lacking lipoxygenase isozymes 2 and 3. *Journal of Agricultural and Food Chemistry*, *41*, 103–106.
- Murphy, P. A., Song, T., Buseman, G., Barua, K., Beecher, R. G., Trainer, D., et al. (1999). Isoflavones in retail and institutional soy foods. *Journal of Agricultural and Food Chemistry*, *47*, 2697–2704.

- Nielsen, N. C., Floener, L., Evans, R. P., Scallan, B. J., Dickinson, C., & Cho, T. J. (1986). The structure and expression of glycinin genes from soybean. *Journal of the American Oil Chemist's Society*, 63, 458.
- Nik, A. M., Tosh, S. M., Woodrow, L., Poysa, V., & Corredig, M. (2009). Effect of soy protein subunit composition and processing conditions on stability and particle size distribution of soymilk. *LWT-Food Science and Technology*, 42, 1245–1252.
- O'Keefe, S. F., Resurreccion, A. P., Wilson, L. A., & Murphy, P. A. (1991). Temperature effect on binding of volatile flavor compounds to soy protein in aqueous model systems. *Journal of Food Science*, 56, 802–806.
- Poysa, V., & Woodrow, L. (2002). Stability of soybean seed composition and its effect on soymilk and tofu yield and quality. *Food Research International*, 35, 337–345.
- Robinson, A. L., Chambers, E., & Milliken, G. (2005). Just-About-Right (JAR) scales and hedonic scales provide different results. *IFT Annual Meeting, Session 99-F*, abstract 6, 2p. Louisiana.
- SAS Institute (2009). The SAS system for Windows, Release 9.2. SAS Inst. Inc., Cary, NC.
- Seguin, P., Zheng, W., Smith, D., & Deng, W. (2004). Isoflavone content of soybean cultivars grown in eastern Canada. *Journal of the Science Food Agriculture*, 84, 1327–1332.
- Sharma, S., Kaur, M., Goyal, R., & Gill, B. S. (2014). Physical characteristics and nutritional composition of some new soybean (*Glycine max* (L.) Merrill) genotypes. *Journal of Food Science and Technology*, 51, 551–557.
- Stephan, A., & Steinhart, H. (2000). Bitter taste of unsaturated free fatty acids in emulsions: contribution to the off-flavour of soybean lecithins. *European Food Research and Technology*, 212, 17–25.
- Sun, J., Han, F., Yan, S., Yang, H., & Tetsuo, S. (2008). Rapid determination of fatty acids in soybeans [*Glycine max* (L.) Merr.] by FT-Near-Infrared reflectance spectroscopy. *Spectroscopy and Spectral Analysis*, 28, 1290–1295.
- Sun, J., Sun, B., Han, F., Yan, S., Yang, H., & Kikuchi, A. (2011). Rapid HPLC method for determination of 12 isoflavone components in soybean seeds. *Agricultural Sciences in China*, 10, 101–105.
- Suppavorasatit, I., Lee, S. Y., & Cadwallader, K. R. (2013). Effect of enzymatic protein deamidation on protein solubility and flavor binding properties of soymilk. *Journal of Food Science*, 78, C1–C7.
- Terhaag, M. M., Almeida, M. B., & Benassi, M. D. T. (2013). Soymilk plain beverages: Correlation between acceptability and physical and chemical characteristics. *Food Science and Technology (Campinas)*, 33, 387–394.
- Tezuka, M., Taira, H., Igarashi, Y., Yagasaki, K., & Ono, T. (2000). Properties of tofus and soy milks prepared from soybeans having different subunits of glycinin. *Journal of Agricultural and Food Chemistry*, 48, 1111–1117.
- Tezuka, M., Yagasaki, K., & Ono, T. (2004). Changes in characters of soybean glycinin groups I, IIa, and IIb caused by heating. *Journal of Agricultural and Food Chemistry*, 52, 1693–1699.
- Torres-Penaranda, A. V., & Reitmeier, C. A. (2001). Sensory descriptive analysis of soymilk. *Journal of Food Science*, 66, 352–356.
- Villegas, B., Caronell, I., & Costell, E. (2009). Acceptability of milk and soymilk Vanilla beverages: Demographics consumption frequency and sensory aspects. *Food Science and Technology International*, 15, 203–210.
- Wang, Z. H., Dou, J., Macura, D., Durance, T. D., & Nakai, S. (1998). Solid-phase extraction for GC analysis of beany flavours in soymilk. *Food Research International*, 30, 503–511.
- Wilkens, W. F., & Lin, F. M. (1970). Gas chromatographic and mass spectral analyses of soybean milk volatiles. *Journal of Agricultural and Food Chemistry*, 18, 333–336.
- Wolf, W. J. (1975). Lipoxygenase and flavor of soybean protein products. *Journal of Agricultural and Food Chemistry*, 23, 136–141.
- Wrick, K. (2003). The U.S. market – an update and outlook. *Nutraceuticals World*, 22, 36–40.
- Yoshikawa, Y., Chen, P., Zhang, B., Scaboo, A., & Orazaly, M. (2014). Evaluation of seed chemical quality traits and sensory properties of natto soybean. *Food Chemistry*, 153, 186–192.
- Yuan, S., & Chang, S. K. C. (2007). Selected odor compounds in soymilk as affected by chemical composition and lipoxygenases in five soybean materials. *Journal of Agricultural and Food Chemistry*, 55, 426–431.
- Zhang, J., Ge, Y., Han, F., Li, B., Yan, S., Sun, J., et al. (2014). Isoflavone content of soybean cultivars from maturity group 0 to VI grown in northern and southern China. *Journal of American Oil Chemist's Society*, 91, 1019–1028.
- Zhou, A., Boatright, W. L., Johnson, L. A., & Reuber, M. (2002). Binding properties of 2-pentyl pyridine to soy protein as measured by solid phase microextraction. *Journal of Food Science*, 67, 142–145.