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Physicochemical Properties and Flavor Compounds of Soy Milk Yogurt

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A novel soy milk yogurt (SMY32) prepared from mixture of pure soy milk and recombined milk with the ratio of 3:2 (v/v) was subjected to physicochemical and flavor compounds analysis using milk yogurt (MY) as a control. Compared with MY, SMY32 had higher water holding capacity and apparent viscosity, and had no significant difference in the total count of lactic acid bacteria. Protein and amino acid contents of SMY32 were remarkably higher than those of MY. Sensory evaluation revealed that sensory texture score of SMY32 was quite similar to that of MY, and its sensory appearance, flavor and overall acceptability scores were slightly lower. Twenty six flavor compounds were found to have a major impact on the aroma of SMY32, mainly including: ketone, acid, aldehyde, alcohol and ester. Furthermore, 2-pentyl furan was detected out in SMY32 but not in MY. These results indicated that SMY32 is a novel yogurt with excellent quality.

Key words: Lactic acid bacteria, Amino acid, Sensory evaluation.

Yogurt is usually made from fresh or reconstituted milk by fermentation with lactobacillus. For many years, some researchers have been preparing sorts of yogurt by utilizing peanut, maize, soybean and hawk tea, etc[1,2,3,4]. to reduce material costs or strengthen certain function. Besides researchers found that soy yogurt possessed effective cholesterol reduction, oxidation resistance and cancer cells restriction in some extent [5,6].

Soybean, seed of papilionaceae glycine wild annual herbs, is rich in such components as protein and flavones, etc. And soybean protein is the best utilizable resource of plant resources[7]. Pure soy milk, i.e. the water extract of soybean, is an applicable food for lactose-intolerant consumers, vegetarians and milk-allergy patients[8]. The original beany flavor and non-digestible oligosaccharides (e.g. stachyose and raffinose) contained in soy milk, however, limit the wide consumption of soymilk and other soybean products[9]. In order to overcome these restrictions, scholars have tried fermenting soy milk with some microorganisms, for instance, lactobacillus, and studied component changes of the fermented soy milk[10] and survival of lactobacillus in soy yogurt, etc.[8,11], but little concern has been paid to flavor compounds of soy yogurt.

Presently, gas chromatography (GC), gas chromatogramphy-mass spectrometry (GC-MS) and SPME-GC-MS have been commonly used to conduct qualitative and quantitative analysis[12]. And combination of SPME and GC-MS, characterized by convenient, quick, high selectivity and sensitivity, have been widely used for volatile component analysis of milk products[13,14,15].

This study was intended to prepare SMY32 by adopting pure soy milk and recombined milk as major raw materials and fermenting with *Streptococcus thermophilus* and *Lactobacillus*

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bulgaricus, and disclosed the difference of physicochemical property and flavor compounds between SMY32 and milk yogurt (M Y).

MATERIALS AND METHODS

Materials

High-quality soybean with full seed and free from insects and molds was purchased from Carrefour (Hefei, China). Skim milk power (Guangming), full cream milk powder (Nestle) and sucrose were purchased from Carrefour (Hefei, China). The starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was provided by Microbial Resources and Application Laboratory, Hefei University of Technology (Hefei, China).

Methods

Preparation for milk

At 25°C, soybean was soaked in the 0.5 % sodium bicarbonate solution for 14 h, then was peeled, the mixture of water and peeled soybean with the ratio of 5:1 was grinded at 80°C with colloid mill for 5 min, and then got pure soy milk (solid contents about 12 g/100 g) by sifting with a 150 mesh sift.

Full cream milk powder and skim milk power were mixed with the ratio of 5:1, added to 42°Cwarm water, and stirred continuously until all dissolved into recombined milk with total solid content about 12 g/100 g.

Preparation for starter culture

Freeze-dried yogurt starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were revived separately by adding into 12 g/100 g skim reconstituted milk, and repeated activation 3 times, and then *S. thermophilus* and *L.bulgaricus* with the ratio of 1:1 were added into 12 g/100 g skim reconstituted milk, refrigerated at 4°C for further use.

Preparation for SMY32

Pure soy milk and reconstituted milk (V/V) were mixed with the ratio of 10:0, 9:1, 8:2, 7:3, 6:4 respectively, and 6 g sucrose was added as sweetener into 100 mL mixing milk, and the blend was homogenized at 25 MPa for 10 min (Homogenizer, (JHG-Q954)-P(60), Shangahai, China), then sterilized 25 min at 95°C, after that, the sterilized milk was inoculated with 4 mL/100 mL yogurt starter (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*; 1:1) when it was cooled to 43°C, the product was refrigerate in 4°C after being fermented at 42°C for 6 h. Titrable acidity was took as evaluation index, only the acidity of SMY32 prepared by mixing pure soy milk and reconstituted milk (V/V) with the ratio of 6:4 was no less than 70 °T, the prepared SMY32 was deemed meeting the requirement of yogurt acidity in GB 19302-2010[16] , so it was confirmed to prepare SMY32 (SMY32) by mixing pure soy milk and reconstituted milk (V/V) with the ratio of 3:2.

Similarly, MY was prepared as control yogurt by taking reconstituted milk (about 12 g/100 g solid content) as the fermentation substrate.

Lactic acid bacteria number

Lactic acid bacteria number of SMY32 and MY were measured with the agar medium MRS by dilution-plate method, the plate was put into Forma 1029 Anaerobic System (Thermo Electron Corp., Waltham, MA, USA), and cultured at 37°C for 48 h. The colony count on each plate was recorded.

Water-holding capability

After weighing and loading the sample W_0 with centrifugal tube, the tube was put into centrifuge with 15 min of centrifugation at 3000 r/min. After that, it was took out and positioned it in quiescence for 10 min, then removed supernatant fluid and measured residue matters mass W, water-holding capability (WHC) of yogurt was calculated as

$$\text{WHC}\,(\%) = \frac{W}{W_0} \times 100$$

Apparent viscosity

The apparent viscosity of sample was measured by using Brookfield DV I+ viscosity meter (Brookûeld Engineering Laboratory Inc., Stoughton, MA) with rotational speed of 10 rpm at 10°C.

Component analysis

Total solids, ash, crude protein and fat contents of milks and yogurts were determined according to AOAC methods [17].

Amino acid composition

Measurement of amino acid composition was referred to methods of Joel Isanga and Zhang[2] with slight modification. The accurately weighed samples were put into digestion tube and then 10 mL of 6 N Hydrochloric acid were added to them for hydrolysis at 135°C for 6 h. After that, the acid leaching sample was switched to evaporating dish and evaporated at 60°C water for 3 h. 5 mL of 0.02 N hydrochloric acid were put in the beakers to dissolve the amino acids. 20 µl of the resulting solution was injected into the amino acid automatic analysis meter (L-8800, HITICHI Company, Japan) to conduct measurement.

Measurement of flavor compounds

Extraction of flavor compounds: 5 mL yogurt sample were added into 20 mL glass bottles (Supelco, Bellefonte, PA, USA), which were sealed on the top. A syringe with a CAR/PDMS fiber needle (Supelco, Bellefonte, PA, USA) coated with 75 µm coating was used to penetrate the seal for extraction at 60 °C for 30 min.

GC-MS analysis of flavor compounds: the extracted components by solid phase micro-extraction was identified with C4000 gas chromatograph-mass spectrometer (Varian Inc., Walnut Creek, CA, USA). Injection temperature was 250 °C, introduction mode was splitless, and column was DB-5MS gas chromatography column (30 m×0.25 mm×0.25 µm). The oven was held at 45°C for 5 min, then heated to 80°C at a rate of 10°C min^{-1}, and then further heated to 240 °C at the rate of 5 °C min^{-1}. Velocity of helium gas was 1 mL min^{-1}. The temperature of the transmission line was 250 °C. Samples were ionized by electron impact at 70 eV, with the mass/charge (m/z) range of 30–400 and scanning rate of 4.5 scans s^{-1}.

Flavor compounds were determined by comparing the retention time with that in Mainlib mass spectra database, and volatile flavor substance contents were represented by the relative peak area of each substance.

Sensory evaluation

After 14 h of refrigeration at 2-4 °C, 10 reviewers who had knowledge of food science and sensory quality of yogurt evaluated the yogurt from its appearance/color, texture/mouth feel, flavor and overall acceptability[18] .

Statistic analysis

All above experiments were repeated three times with each test carried out in triplicate. ANOVA model of SAS (SAS Institute Inc., Cary, NC) was used for analysis of variance for all data, and significance of differences between the means was determined with Duncan's multiple comparison test at the significance level of $P<0.05$. All results are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Number of lactic acid bacteria, water-holding capability and apparent viscosity

Positive function of yogurt can be attributed to nutrient components and survival probiotics count in yogurt. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* show good cooperative effect in yogurt fermentation[19]. Lactic acid bacteria number of SMY32 (8.68 log cfu/mL^{-1}) approximated to that of MY (8.72 log cfu/mL^{-1}), no conspicuous difference between them ($P>0.05$), which indicated that the two lactobacillus grew well in SMY32.

Water-holding capability of protein gels is the critical parameter in yogurt preparation[20], the intrinsic factors affecting food's protein water-holding capability include amino acid composition, protein constitution and surface hydrophility/hydrophobility[21]. Water-holding capability of SMY32 (43.8 %) was relatively higher than that of MY (39.3 %), probably because of richer contents of amino acid and protein in SMY32. Furthermore,

Table 1. Proximate composition of milk, soy milk, milk yogurt and soy milk yogurt 32

Composition	Milk		Yogurt	
(g/100g)	SM	M	SMY32	MY
Total solids	12.59±0.18 ^{b}	12.45±0.10 ^{b}	14.88±0.15 ^{a}	14.73±0.21 ^{a}
Ash	0.47±0.04 ^{c}	0.76±0.03 ^{b}	0.66±0.05 ^{b}	0.97±0.04 ^{a}
Solids-not-fat	9.61±0.19 ^{b}	9.26±0.12 ^{b}	12.33±0.14 ^{a}	11.96±0.17 ^{a}
Crude protein	4.68±0.23 ^{a}	3.02±0.11 ^{b}	4.53±0.28 ^{a}	2.92±0.22 ^{b}
Fat	2.98±0.12 ^{ab}	3.19±0.07 ^{a}	2.55±0.09 ^{b}	2.77±0.08 ^{ab}

Values with different superscript letters within a row differ significantly ($P<0.05$).

water-holding capability of yogurt can be improved by adding stabilizers, since stabilizers have two main functions in a yogurt type product: firstly to bind water and secondly to reduce water flow in the matrix space. Moreover, stabilizers interact with milk constituents such as proteins and can increase their level of hydration[22].

Whey isolation is closely related to gel stability of yogurt system, and apparent viscosity could be one of the non-uniformity indexes in yogurt gel system. The higher the total solid content (especially the protein content) in yogurt is, the lighter the whey isolation degree will be[23], it is reported that viscosity of yogurt is relevant to arrangement of protein and cross-linking among proteins[24]. Apparent viscosity of SMY32 (5.14 Pa.s) was slightly higher than that of MY (5.02 Pa.s), this was attributed to higher protein content in SMY32 than that in MY (Table 1).

Analysis of proximate composition in SMY32

Total solid contents in SMY32 and MY were respectively higher than ($P < 0.05$) that of soy milk (SM) and milk (M) (Table 1), which was mainly attributed to the addition of 6 g/100 mL sucrose, however, total solid contents of SM and M had no evident difference, totally approximated to 12 g/100 g which was similar to that of pure milk 12 g/100 g [2]. Protein contents in SMY32 and SM (4.53 g/100 g and 4.68 g/100 g) were respectively higher than that in MY and M (2.92 g/100 g and 3.02 g/100 g), and ash contents in SMY32 and MY were respectively lower than that in SM and M (Table 1). After fermentation, fat contents of soy milk and milk reduced, which was basically in accordance with the reported results of Isanga and Sunny-Roberts[2,25].

Amino acid composition

Essential amino acids composition is the major index to evaluate protein quality, the good quality of protein have complete range and sufficient amount of essential amino acids. The essential amino acids, critical to synthesis of body protein, can only be acquired from diet. Nine kinds of essential amino acids were detected out in SMY32 and MY, of which eight kinds of essential amino acids contents in SMY32 such as leucine, lysine, threonine, valine, isoleucine, phenylalanine, arginine and histidine were all higher than those in MY, and contents of isoleucine, histidine and arginine (1.73 mg/ g; 1.21 mg/ g; 2.19 mg/ g) were significantly higher than those in MY (1.10 mg/ g; 0.68 mg/ g; 0.87 mg/ g). Arginine is extremely useful in enhancing the immune system in all animals, and infants have to get arginine in their feed to meet their requirements[26]. In SMY32, the contents of four non-essential amino acids (Asp, Gly, Ala, Cys) were conspicuously higher than those in MY, only methionine content in SMY32 was lower than that in MY, as methionine in soybean is the restricted amino acid with little quantity[27]. Table 2 indicated that total amino acid content in SMY32 was significantly higher than that in MY, besides the total content of essential amino acids was equally higher than that of MY, indicating the SMY32 with superior quality of protein composition.

GC-MS analysis of flavor compounds

Aroma compounds of yogurt can be divided into four categorized: non-volatile acid (lactic acid or pyruvic acid), volatile acid (butyric acid, acetic acid etc.), carbonyl compound (acetaldehyde and butanedione) and other kinds of compounds such as amino, etc.[19]. Twenty six kinds of volatile components were identified in

Table 2. Amino acid composition of MY and SMY32

Amino acids	Amounts (mg/g) MY	SMY32
Aspartate(Asp)	2.11±0.11 ^{b}	3.84±0.28 ^{a}
Threonine(Thr)*	1.19±0.15 ^{a}	1.64±0.13 ^{a}
Serine(Ser)	1.42±0.16 ^{a}	1.78±0.20 ^{a}
Glutamate(Glu)	5.21±0.47 ^{a}	5.62±0.52 ^{a}
Glycine(Gly)	0.47±0.09 ^{b}	1.13±0.10 ^{a}
Alanine(Ala)	0.71±0.06 ^{b}	1.24±0.05 ^{a}
Cystein(Cys)	0.16±0.01 ^{b}	0.33±0.02 ^{a}
Valine(Val)*	1.07±0.17 ^{a}	1.55±0.14 ^{a}
Methionine(Met)*	0.49±0.08 ^{a}	0.27±0.05 ^{b}
Isoleucine(Ile)*	1.10±0.16 ^{b}	1.73±0.17 ^{a}
Leucine(Leu)*	2.70±0.14 ^{a}	3.35±0.11 ^{a}
Tyrosine(Tyr)	1.39±0.20 ^{a}	1.67±0.17 ^{a}
Phenylalanine(Phe)*	1.41±0.28 ^{a}	1.94±0.27 ^{a}
Lysine(Lys)*	2.17±0.27 ^{a}	2.74±0.31 ^{a}
Histidine(His)*	0.68±0.18 ^{b}	1.21±0.15 ^{a}
Arginine(Arg)*	0.87±0.16 ^{b}	2.19±0.23 ^{a}
Proline(Pro)	2.42±0.32 ^{a}	2.26±0.18 ^{a}
Total amino acid	25.57±0.18 ^{b}	34.59±0.16 ^{a}
Essential amino acid	11.68±0.15 ^{b}	16.62±0.17 ^{a}

*Essential amino acid
Values with different superscript letters within a row differ significantly (P<0.05).

SMY32, mainly including ketone, acid, aldehyde and alcohol. 2-pentyl furan, being detected out from SMY32, was mainly from pure soy milk and possessed beany flavor which formed from linoleic acid by singlet oxygen[28]. In MY, except 2-pentyl furan, other twenty five kinds of volatile components in SMY32 were identified and parts of them were identical to the report by Ye *et al.*[4].

Ketone substances mainly included 2,3-diacetyl, 3-hydroxyl-2-butanone, 2-hydroxyl-3-pentanone, 1-hydroxyl-2-acetone, 2-heptanone, 2-nonanone and acetone. Butanedione, the main part of yogurt flavor[14], is metabolic transformed mainly from lactobacillus by utilizing lactose through EMP route[29]. In SMY32, 2, 3-butanedione content was lower than that of MY ($P < 0.05$), probably attributed to the lower content of lactose that could be metabolic transformed by lactobacillus in soy milk than in milk. Contents of 2-nonanone and acetone in SMY32 were considerably higher than those in MY ($P < 0.05$), and contents of other ketone substances were lower than those in MY (Table 3).

Acids are the major components causing the sour taste of yogurt. In SMY32, contents of caproic acid, heptylate acid and pelargonic acid were significantly higher than those in MY($P < 0.05$), while contents of acetic acid, butanoic acid and benzoic acid were significantly lower than those in MY ($P < 0.05$).

Aldehydes is one of the important substance for the classic flavor of yogurt, and acetaldehyde is one of the critical element[30], which can be produced from the metabolism of lactose by lactobacillus through EMP, also be produced from decomposition of threonine by threonine aldolase[31]. Hexanal, aldehyde and nonanal contents

Table 3. Analytical results of flavor compounds in MY and SMY32

	Compound	Relative content/%	
		MY	SMY32
Ketones	2,3- diacetyl	19.35±0.28 ^{a}	10.30±0.39 ^{b}
	3-Hydroxy-2-butanone	7.56±0.45 ^{a}	5.43±0.45 ^{b}
	2-Hydroxy-3-pentanone	1.66±0.13 ^{a}	1.07±0.08 ^{b}
	1-Hydroxy-2-acetone	1.32±0.13 ^{a}	1.01±0.02 ^{b}
	2-Heptanone	0.18±0.05 ^{a}	0.16±0.04 ^{a}
	2-Nonanone	1.55±0.11 ^{b}	2.26±0.10 ^{a}
	Acetone	1.65±0.09 ^{b}	2.30±0.18 ^{a}
Acids	Acetic acid	10.40±0.28 ^{a}	7.81±0.31^b
	Butanoic acid	2.96±0.21 ^{a}	0.13±0.06 ^{b}
	Hexanoic acid	3.05±0.23 ^{b}	4.00±0.17 ^{a}
	Benzoic acid	1.97±0.13 ^{a}	1.12±0.10 ^{b}
	Octanoic acid	1.34±0.08 ^{a}	1.33±0.12 ^{a}
	Heptanoic acid	0.14±0.04 ^{b}	0.28±0.06 ^{a}
	Nonanoic acid	0.07±0.03 ^{b}	0.14±0.01 ^{a}
	Decanoic acid	2.38±0.46 ^{a}	2.29±0.05 ^{a}
Aldehydes	Hexanal	0.05±0.01 ^{b}	0.34±0.05 ^{a}
	Benzaldehyde	0.61±0.08 ^{a}	0.59±0.05 ^{a}
	Aldehyde	1.80±0.11 ^{b}	2.13±0.05 ^{a}
	Furaldehyde	0.69±0.09 ^{a}	0.62±0.06 ^{a}
	Nonanal	0.39±0.08 ^{b}	0.97±0.06 ^{a}
Alcohols	Hexanol	1.17±0.13 ^{a}	0.77±0.06 ^{b}
	3-Pentanol	2.44±0.16 ^{a}	1.28±0.15 ^{b}
	Phenethylalcohol	0.15±0.03 ^{b}	0.28±0.05 ^{a}
Esters	Ethyl butyrate	0.82±0.07 ^{a}	0.77±0.05 ^{a}
	´-decalactone	0.29±0.11 ^{a}	0.14±0.04 ^{b}
Else	2-pentyl furan	ND	1.22±0.29

ND, not detected. Values with different superscript letters within a row differ significantly(P<0.05) of SMY32 were significantly higher than those of MY.

Content of phenethylalcohol in SMY32 was significantly higher than that in MY, while contents of hexanol and 3-pentanol were significantly lower than those in MY. Furthermore, esters contained in SMY32 mainly included ethyl butyrate and δ-decalactone, and the latter was considerably lower than that in MY (Table 3).

Sensory evalution

From the sensory evaluation on SMY32 and MY by 10 reviewers, the results of appearance, texture, flavor and overall acceptability of two yogurts (Fig. 1.) indicated that average value of each evaluation index for two yogurts were within the commercially acceptable range (4-9 points)[18].

SMY32 and MY showed no evident variance in texture, which represented good texture states, but MY had slightly higher scores than SMY32 in appearance, flavor and overall acceptability, ($P > 0.05$) (Fig. 1.). In the case of appearance/color, more reviewers favorred pure white of traditional yogurt, meanwhile, did not reject the yellowish of SMY32. A few of reviewers suggested to add some scent substance (e.g. juice) to increase its acceptability for its light beany flavor.

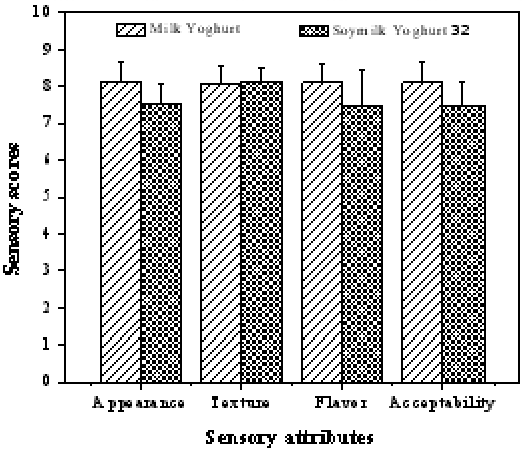

Fig. 1. Scores of different sensory attributes of MY and SMY32

CONCLUSIONS

The study revealed that water-holding capability and apparent viscosity of SMY32 were all higher than those of MY, and contents of protein and total amino acid were higher. What's more, total content of essential amino acids was also considerably higher, while with slightly lower lactic acid bacteria number than that of MY, but it was still consistent with the national standards.

Twenty six kinds of flavor compounds were detected out in SMY32, and contents of 2-nonanone, acetone, caproic acid, heptylate acid, pelargonic acid, hexanal, acetaldehyde, nonanal, and phenethylalcohol were noticeably higher than those of MY. Furthermore, 2-pentyl furan was detected out in SMY32 but not in MY. Sensory texture score of SMY32 was approximated to that of MY, and its sensory appearance, flavor and overall acceptability scores were slightly lower. These results indicated that, compared with MY, SMY32 was richer in nutrition; it could be widely spread and consumed for its superior quality.

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