SENSORY PROFILE ANALYSIS OF THERMALLY PROCESSED FLAVORS FROM SOY PROTEIN

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Abstract:

Thermal processing is known to trigger the development of pleasant flavors and sensory enhancement of food products. In Thailand, soymilk waste from tofu foam sheet processing plant has been simply discarded without optimized utilization. The objective of this study was to investigate flavors produced from soy protein obtained from soymilk waste by thermal process and assess aroma characteristics by descriptive sensory analysis. Soymilk waste was analyzed for protein content prior to enzyme hydrolysis by Flavourzyme® at various concentration and time. The highest degree of hydrolysis (%DH) of soymilk waste containing 43.69% of soy protein by dry weight was 9.54 and 5.76, respectively as compared to leucine and proline standard curves at 50-55°C incubation for 8 hr with 0.66 g/100 ml of enzyme added. Enzyme-treated soy protein was then undergone the flavor production by adjusting pH at different levels ranging from 3.0-8.0, with or without adding 2% of ribose sugar and subjected to heat processing at 121°C for 20, 40, 60 and 80 min. The samples were sensorially assessed by Quantitative Descriptive Analysis (QDA) using eight trained panelists recruited from undergraduate students and staffs of Department of Food Technology. Panelists were screened based on their acuity tests performance and availability. The training sessions lasted two weeks (30 hours) including aroma identification, intensity ranking and scale acquaintance of related products. The preliminary descriptors prior to term refinement ranged from 17-22 and reduced to 9-13 after refinement for each pH adjustment. Eighteen aroma descriptors were finally established according to order of appearance with respective references provided including boiled peanuts, soybean sauce, sour, seafood, sweet, mushroom, beans, alcohol, yeast, boiled sweet potato, honey, prunes, cooked rice, chocolate, malt, boiled corn, caramel and burnt. Panelists evaluated the thermally processed flavor using the established aroma lexicon and the mean scores of treated samples were subjected to Analysis of Variance (ANOVA). Results indicated that pH was the most significant factor ($p<0.05$) contributing to the aroma present during heating process. The aromas presented at pH 3-4 were alcohol, sweet potato, and prunes; at pH 3-6 were acid and soybean; at pH 3-7 were peanut and mushroom; at pH 3-8 were soybean sauce, seafood and sweet; at pH 5-8 were cooked rice; at pH 7-8 were chocolate, malt, corn, caramel, and burnt.

Introduction:

Soy consumption in Thailand has increased over the years due to the awareness of soy products as wholesome foods. More than 45% of soy production in Thailand has been processed into food products and feeds [1]. Tofu foam sheet or so called “FongTaohu” is one of the favorite soy products consumed nationwide amongst vegetarians. Especially during the religious events for people of Thai-Chinese origin the consumption rate has grown drastically. Consequently, soymilk waste from the process is tremendous and always simply discarded without proper utilization. However, soymilk is known as the excellent source of protein and essential amino acids therefore its waste is deemed to be promising for protein source as well.

Process flavors are generated from precursors via the Maillard reaction and other thermal reaction occurring during processing [2]. The type of amino acid and sugar, pH, temperature, time, moisture content have been reported as parameters influencing the overall flavors and aromas in the Maillard reaction [3]. Heat treatments given to soymilk during the production of tofu foam sheet influence basically the flavor of the milk but if heating continues excessively, destruction of amino acids and vitamins, browning and the development of cooked flavor occur [4]. A considerable number of studies including using simple model systems with amino acids have been conducted to determine the aroma compounds resulting from the Maillard reaction [5, 6]. To our knowledge, little information exists about the process flavors by thermal reaction of soymilk waste.

Sensory is one of two forms commonly used for flavor analysis apart from instrumental to help in the development and understanding the nature of products. Sensory descriptive analysis is usually used to identify the human perception of flavor.

In order to tackle this problem, we aimed to determine reaction conditions for production of process flavors by thermal reaction of soymilk waste and ribose and to characterize the aroma compounds generated by sensory descriptive analysis.

Materials and Methods:

Materials:

Soymilk waste was obtained from the local soybean processing plant in Bangkok Thailand. Flavourzyme was obtained from Brenntag Ingredients PCL (Bangkok, Thailand). L-proline, L-leucine, and D-(-)-ribose were purchased from Ajax Finechem (Scoresby, Vic, Australia). All other chemical reagents were of analytical grade.

Determination of protein contents in soymilk waste:

The nitrogen contents of the soymilk waste were determined by the standard Kjeldahl procedure of AOAC (2000) [7], using 20 mL of H_2SO_4, 12 g of K_2SO_4 and 0.05 g/mL of $CUSO_4$ as catalyst. The temperature of the hydrolysis was 410-430°C and the time 1.5 h. The nitrogen content was determined as a boric acid application and then titrated with the standardized hydrocholic acid. The crude protein was then estimated by multiplying nitrogen content by nitrogen to protein conversion factor of 5.71 for soybeans.

Preparation of enzymatic hydrolyses:

The enzyme used was Flavourzyme™ 500 LAPU/g with recommended dosage (from the manufacturer) of 0.5 g per 100g of protein. To each 100 mL of soymilk waste, the enzyme added was 0.22, 0.44, 0.66 and 1.11 g respectively based on our preliminary studies. The mixture was vigorously stirred during reaction time for 8 h at 50-55°C and aliquots (25 mL) were removed at time intervals of 2, 4, 6, 8, 10, and 12 h and immediately heated at 85°C for 30 min to terminate the enzyme reaction. The hydrolysate obtained was analyzed for the degree of hydrolysis.

Degree of hydrolysis:

Degree of hydrolysis (DH) is defined as the percentage of cleaved peptide bonds: $DH=h/h_{tot}*100\%$ where h_{tot} is the total number of peptide bonds per protein equivalent, and h is the number of hydrolyzed bonds. The expression for h is: $h=A*b/m$ where A is the absorbance of samples measured at 440 nm for proline and 570 nm for leucine respectively, b is the y-intercept of L-proline or L-leucine standard curve and m is the slope of L-proline or L-leucine standard curve. The hydrolysate was centrifuged at 4500 rpm for 20 min twice. The supernatant from this step was decanted and analyzed for DH by the ninhydrin colorimetric reaction. The leucine and proline standard were prepared in the range of 0.0-2.1 µg/mL. To calculate DH, spectrophotometer (Shimadzu UV-1601, Japan) readings of the proline and leucine standard and the test samples were carried out using diluents solution as the control.

Preparation of process flavoring samples:

5 mL of hydrolyzed soymilk waste was taken to adjust pH with 0.1N HCl and 0.5N NaOH. Each experimental sample was adjusted to obtain the pH range from 3.0-8.0 and placed in a screw-cap test tube. Deionized boiled water was added to make a final volume of 25 mL with or without 0.1 g of ribose added (depending on the treatment). Tubes were screw-capped and then placed in the autoclave at 121°C, 0.28 mPa for 20, 40, 60 and 80 min. After cooling in a water bath, samples were subjected to sensory analysis.

Sensory evaluation of process flavoring samples:

Eight panellists (1 male and 7 females, 20-22 years of age) consisting of students were selected based on their interest and availability. They were trained in performing the Quantitative Descriptive Analysis (QDA) test [8]. The training comprised fifteen 2-h sessions including aroma identification, intensity ranking and scale acquaintance of related products. Descriptors used for sensory analysis were developed during initial session in which different process flavouring samples of varying pH were presented to the panellists. The panellists were asked to describe the samples with as many descriptive terms as they found applicable. The selection of the main descriptors was performed according to ISO-11035 [9]. Specific attributes, attribute definitions, and references were developed by the panelists. Each panellist used a 15-cm unstructured line intensity scale anchored from “none” to “intense”. Descriptive sensory evaluations were conducted in partitioned booths in a sensory room for 6 consecutive days. Experimental samples were prepared the day before the test, held in a freezer, and then warmed to room temperature prior to serving to panellists. Four samples of 15 mL each were randomly served in 3-oz white plastic cup coded with 3-digit random numbers. The panellists were asked to mark the perceived intensity of the attribute by drawing a vertical line on the scale and writing the code with a 1-min break between samples. References were also provided for calibration during evaluation.

Statistical and data analysis:

Statistical significance of observed difference among means of experimental results including the sensory scores of each of the descriptors ($p<0.05$) was evaluated by analysis of variance (ANOVA) followed by a least significant difference (LSD) analysis.

Results and Discussion:

Protein content in soymilk waste:

The protein contents of soymilk waste are shown in Table 1. The protein contents of soymilk waste collected from the two processing steps; before and after production of tofu foam sheet were not significantly different. The average protein contents ranged from 43.69-49.94%. The data from Table 1 suggest that soymilk waste can serve as a good source of soy protein for process flavouring.

Table 1. Protein contents of soymilk waste before and after tofu foam sheet production

Sample	Stage	Soymilk protein %[a]
Soymilk waste	Before	49.94±0.75
	After	43.69±0.57

[a] Average value, measured in triplicate

Degree of hydrolysis:

The time courses for hydrolysis of soymilk waste using L-leucine or L-proline as standards are shown in Table 2. There was a steady increase in DH% with increase time for all samples. The DH% at 8 h of hydrolysis with 1.11% enzyme added was 9.54%. However, a similar result was obtained when 0.66% enzyme was used indicating that substrate in the soymilk waste was sufficient for enzyme activity. When L-proline was used as a standard, the DH values also increased with the hydrolysis time in a similar fashion. The DH values obtained in this study were lower that those reported by Hrčková et al [10] who used Flavourzyme™ 1000 LAPU/g (Leucine amino peptidase units per gram). Flavourzyme™ 500 LAPU/g employed in our study was probably less efficient than that one for hydrolysis. Consequently, the condition using 0.66% enzyme concentration at 50-55°C for 8 h gave the highest DH value.

Table 2. DH determination of soy protein hydrolysate measured by ninhydrin colorimetric reaction with L-leucine (A) and L-proline (B) as standard amino acids.

Time of hydrolysis (h)	Standard amino acid	DH (%)			
		0.22 % (w/v)	0.44 % (w/v)	0.66% (w/v)	1.11% (w/v)
0	A	$3.18\pm0.33^{c,e}$	$3.18\pm0.33^{b,e}$	$3.11\pm0.37^{a,e}$	$3.32\pm0.49^{a,e}$
2		$3.07\pm0.16^{c,d}$	$4.15\pm0.33^{b,d}$	$7.97\pm0.12^{a,d}$	$8.18\pm0.80^{a,d}$
4		$3.40\pm0.16^{c,cd}$	$4.18\pm0.64^{b,cd}$	$8.40\pm0.33^{a,cd}$	$8.68\pm0.85^{a,cd}$
6		$3.61\pm0.34^{c,bc}$	$5.11\pm0.06^{b,bc}$	$8.90\pm0.67^{a,bc}$	$9.29\pm0.27^{a,bc}$
8		$5.40\pm0.12^{c,a}$	$5.86\pm0.34^{b,a}$	$9.54\pm0.49^{a,a}$	$9.54\pm0.19^{a,a}$
10		$5.11\pm0.27^{c,abc}$	$5.58\pm0.19^{b,abc}$	$8.93\pm0.22^{a,abc}$	$9.36\pm0.33^{a,abc}$
12		$5.22\pm0.34^{c,ab}$	$6.11\pm0.32^{b,ab}$	$9.18\pm0.41^{a,ab}$	$8.79\pm0.37^{a,ab}$
0	B	$0.78\pm0.12^{c,e}$	$1.00\pm0.12^{c,e}$	$0.50\pm0.12^{b,e}$	$1.07\pm0.21^{a,e}$
2		$2.42\pm0.33^{c,d}$	$2.35\pm0.21^{c,d}$	$2.70\pm0.12^{b,d}$	$3.84\pm0.00^{a,d}$
4		$3.27\pm0.25^{c,c}$	$3.06\pm0.25^{c,c}$	$3.34\pm0.12^{b,c}$	$4.55\pm0.65^{a,c}$
6		$3.91\pm0.25^{c,b}$	$4.34\pm0.33^{c,b}$	$5.19\pm0.12^{b,b}$	$5.40\pm0.69^{a,b}$
8		$4.34\pm0.25^{c,a}$	$4.55\pm0.12^{c,a}$	$5.76\pm0.56^{b,a}$	$5.90\pm0.33^{a,a}$
10		$3.91\pm0.12^{c,b}$	$4.34\pm0.12^{c,b}$	$5.40\pm0.12^{b,b}$	$5.19\pm0.12^{a,b}$
12		$4.05\pm0.21^{c,ab}$	$4.90\pm0.77^{c,ab}$	$5.40\pm0.25^{b,ab}$	$5.33\pm0.37^{a,ab}$

Sensory analysis:

The initial working list includes 30 terms (Table 3). By concensus, the redundant and vague terms were discarded. Twelve of the 30 terms attributes were excluded from the analysis because they were not detected or detected by a few panellists as confirmed by a lower M value (geometric average) (bamboo shoot aroma, barbecue aroma, rancid, roasted peanut aroma, flour aroma, chrysanthemum aroma, cinnamon aroma, raisin aroma, pineapple aroma, tea aroma, fermented soybean and milky aroma) [9]. This type of reduction is normal during initial training sessions [8]. The final list of sensory attributes and the standard references are presented in Table 4. To assess the importance of the components in the generation of aromas, the sensory aroma profiles were determined for the process flavoring under made conditions. The effects of pH, time, and sugar on processed flavor notes were evaluated using the established aroma descriptors and the mean score were subjected to Analysis of Variance (ANOVA). It was found that pH was the highly significant factor ($p<0.001$) affecting the generation of perceived aromas during heating process. Time was found to significantly affect the production of sweet and honey aroma ($p<0.01$) whereas soy sauce aroma, seafood aroma, sweet aroma, yeast aroma, cooked rice aroma were affected by ribose added. The aromas presented at pH 3-4 were alcohol, sweet potato, and prunes; at pH 3-6 were acid and soybean; at pH 3-7 were peanut and mushroom; at pH 3-8 were soybean sauce, seafood and sweet; at pH 5-8 were cooked rice; at pH 7-8 were chocolate, malt, corn, caramel, and burnt. (Table 5.) Our results corresponded with those reported by Hofmann and Schieberle [11].

Table 3. Preliminary descriptors

Burnt aroma	Alcohol aroma	Peanut aroma
Soysauce aroma	Malty aroma	Honey aroma
Sweet aroma	Chocolate aroma	Cinnamon aroma
Prunes aroma	Boiled corn aroma	Raisin aroma
Soybean aroma	bamboo shoot aroma	Yeast aroma
Acid aroma	Barbecue aroma	Cooked rice aroma
Seafood aroma	Rancid	Pineapple aroma
Boiled sweet potato aroma	Roasted peanut aroma	Fermented soybean aroma
Caramel aroma	Flour aroma	Tea aroma
Mushroom aroma	chrysanthemum aroma	Milky aroma

Table 4. Sensory attributes employed in descriptive analysis

Descriptor	Definition	Reference sample
Peanut aroma	aromatic associated with boiled peanut	Boiled peanut
Soy sauce aroma	aromatic associated with soy sauce	NguanChiang®, soy sauce
Acid aroma	aromatic associated with fermented dairy products	Dutchie®, plain yogurt
Seafood aroma	aromatic associated with fish maw	Boiled fish maw
Sweet aroma	sweet aromatic associated with sucrose	5% sucrose in water
Mushroom aroma	aromatic associated with mushroom	Cooked straw mushroom
Soybean aroma	aromatic associated with cooked soybean milk	Soymilk
Alcohol aroma	aromatic associated with alcohol	70% ethyl alcohol
Yeast aroma	sharp aromatic associated with yeast	Perfect®, dried yeast powder
Sweet potato aroma	aromatic associated with boiled sweet potato	boiled sweet potato
Honey aroma	aromatic associated with honey	Chitralada, honey
Prunes aroma	aromatic associated with dried prunes	Sunsweet® , prunes
Cooked rice aroma	aromatic associated with cooked rice	cooked white rice
Chocolate aroma	aromatic associated with chocolate drink	Hershey's®, milk chocolate
Malty aroma	aromatic associated with malt	Ovaltine®, malt drink
Corn aroma	aromatic associated with boiled corn	boiled sweet corn
Caramel aroma	aromatic associated with caramel	Imperial® topping, caramel
Burnt aroma	aromatic associated with burnt	burnt toast

Table 5. Range of sensory scores (0-15) and significant levels for the sensory evaluation (18 attributes) of 6 process flavouring samples (pH 3-8)

Descriptor	Sensory scores[a]		pH	Time	Sugar	Panelist
	A	B				
Peanut aroma	2.9-7.1	3.2-6.2	***	NS	NS	***
Soy sauce aroma	2.7-6.1	1.8-5.6	***	NS	**	***
Acid aroma	1.2-4.9	1.7-3.5	***	NS	NS	***
Seafood aroma	2.3-7.0	1.9-6.1	***	NS	**	***
Sweet aroma	3.3-7.5	2.5-6.6	***	*	**	***
Mushroom aroma	2.9-6.2	2.4-6.0	***	NS	NS	***
Soybean aroma	2.8-5.4	3.6-5.2	***	NS	NS	***
Alcohol aroma	1.9-2.6	1.8-2.9	***	NS	NS	***
Yeast aroma	2.3-8.3	2.5-6.2	***	NS	**	***
Sweet potato aroma	3.9-6.7	3.9-6.2	***	NS	NS	***
Honey aroma	1.4-3.2	1.1-3.3	***	*	NS	***
Prunes aroma	2.0-2.9	2.2-3.7	***	NS	NS	***
Cooked rice aroma	1.8-3.5	1.8-6.3	***	NS	**	***
Chocolate aroma	3.5-4.9	4.0-5.0	***	NS	NS	***
Malty aroma	3.6-6.8	4.2-5.8	***	NS	NS	***
Corn aroma	2.0-3.2	2.2-4.1	***	NS	NS	***
Caramel aroma	2.0-3.5	1.8-3.4	***	NS	NS	***
Burnt aroma	3.2-4.7	2.7-3.6	***	NS	NS	**

[a] A=sugar added, B=No sugar added;
***, **, * Significantly different at P<0.001, P<0.01, P<0.05 respectively.

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