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Technical paper

Changes in Lipid Components during Peanut Tofu Production

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The present study clarified the changes of lipid components, which are considered to be most involved in physiological properties, stability against oxidation, and flavor formation during peanut tofu production. The chemical characteristics of lipids were nondrying oil and within the range of those in peanut oil. POV and AV level was high in roasted peanut, suggesting that peanuts were susceptible to thermal oxidation during the roasting process. Lipid composition was 97.8–98.6% NL, 0.73–1.01% GL and 0.6–1.2% PL. In NL and PL, triacylglycerol and phospholipids decomposition occurred, decreasing levels to 90.8%, 80.7% in dried peanut tofu and 83.1%, 41.6% in roasted peanut tofu, respectively. Among the main fatty acid components in TL, NL, and PL, C18: 1 was the greatest, followed by C18: 2 and C16: 0. Long-chain fatty acids, such as C22: 0 and C24: 0, were also detected. Among the unsaponifable matter, α-Toc, which is physiologically active, and γ-Toc, which is highly antioxidative, were detected. The level of β-sitosterol was highest at approximately 75% of 4-Desmethylsterol.

Keywords: peanut tofu, peanut, dried and roasted, lipid, fatty acid, tocopherol, sterol
Abbreviations: POV: peroxide value, AV: acid value, TL: total lipid, NL: neutral lipid, GL: glycolipid, PL: phospholipid, Toc: tocopherol

Introduction

Peanuts have a unique flavor and aroma upon being roasted. In order to utilize these qualities, peanuts have been widely used for snacks and luxury foods, such as roasted peanuts, butter-coated peanuts, and peanut butter. However, only a few processed food products and food items made with peanuts are consumed on a daily basis, like those made with soybeans (Watanabe, 2000). Since peanuts have also been revealed to contain oleic acid, which lowers blood cholesterol levels (Taira, 1985; Kaneko and Fuziwara, 2001; Igarashi and Yasuda, 2000), vitamin E, which has antioxidant and anti-aging properties (Fukuba *et al.*,1985), and resveratrol, which is contained in seed coats of peanut polyphenols and possesses a strong antioxidant activity (Ogaki and Sagawa, 2003; Sakai, 2004), it is desirable that they should be utilized in home cooking and health foods. Peanut tofu is one of the few food items that utilizes peanuts; however, it is a local culinary item rather than a regular food (Takahashi, 1989). It is produced by a more complex method than other similar food items such as sesame tofu (Sato *et al.*, 1995; 1999), which is produced by boiling down ingredients such as arrowroot starch and potato starch. As no detailed description of the production method for peanut tofu is available, no analysis of the lipid characteristics in the production process of peanut tofu has been performed. Therefore, the present study conducted detailed investigations on the changes of lipid characteristics, such as lipid composition, fatty acid composition, sterol and tocopherol compositions, that are considered to occur in peanut tofu, thereby affecting quality, stability against oxidation, flavor, and taste.

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Materials and Methods

Process of peanut tofu production The outline of process of peanut tofu production is shown in Figure 1. Two types of peanuts of average grain weight 0.8〜1.0 g with pellicles, dried and roasted, (hereafter referred to as DP, dried peanuts, and RP, roasted peanuts) were used for the experiment. The peanut variety was subsp. *hypogaea* var. *hypogaea* (Virginia type) of *Arachis hypogaea L.* grown in Yachimata, Chiba, Japan. Peanuts were roasted in a roaster over a low gas flame with continuous, slow rotation for approximately 1 h (highest temperature 160℃). DP was soaked in water of approximately 20℃ for 20 h to ensure maximum water absorption, resulting in an approximately 1.7-fold increase in weight. The soaked peanuts were homogenized in an ultrahomogenizer at 5000 rpm for 10 min. A volume of water approximately 3 times that of the peanut material was added to the homogenate, and the mixture was heated at 90–95℃ for 10 min. After filtering the mixture with a nylon cloth, DP milk was obtained. For RP, the soaking step was omitted. The same processes described above were performed to obtain RP milk. To coagulate the milk, gelatin was added to both milk

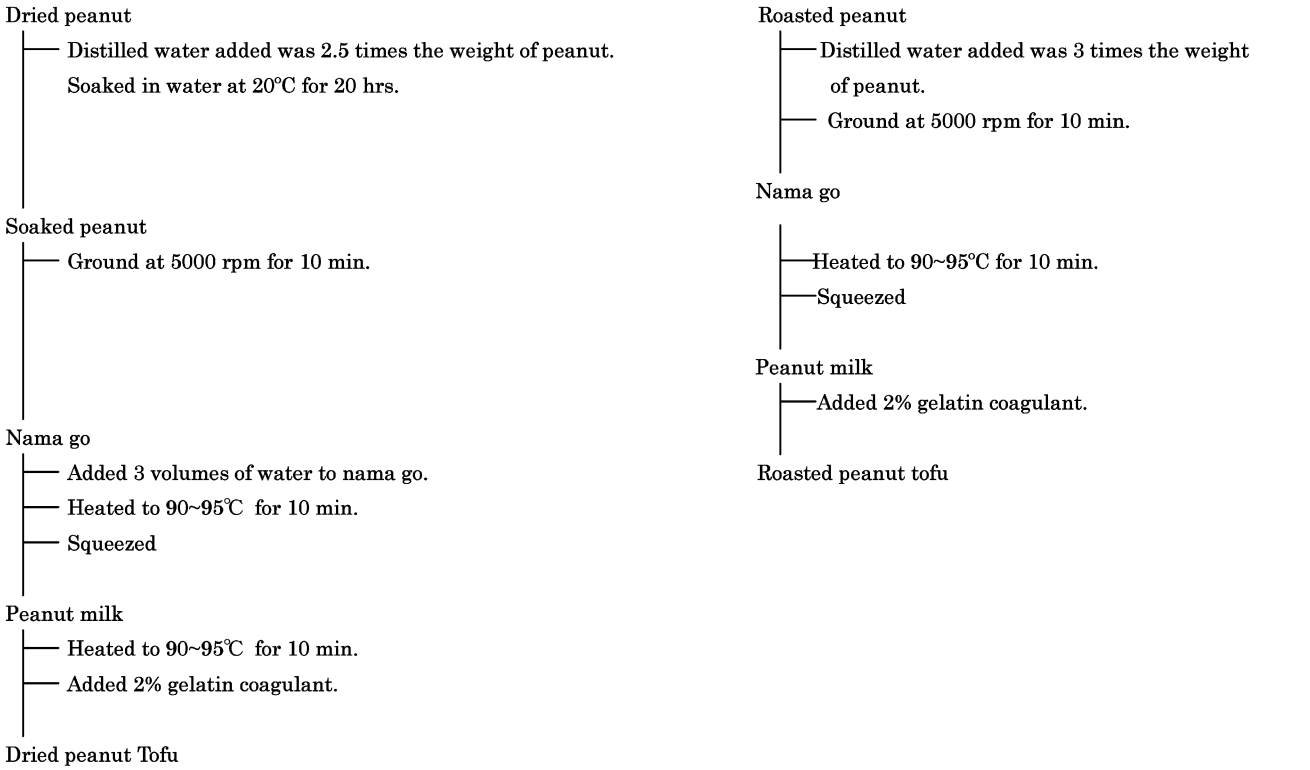

Fig. 1. Process of peanut tofu production.

samples at a concentration of 2%, which scored highest on both physical measurement and sensory evaluation (Tokue and Kataoka, 1999), and two types of peanut tofu, dried peanut tofu (DP Tofu) and roasted peanut tofu (RP tofu) were obtained.

Measurement of chemical composition and extraction and fractionation of lipids On the basis of a previous report (Tokue *et al.,* 1987), chemical composition was examined by analyzing each sample 3 times, and the average of the 3 measurements was calculated. Measurements of chemical characteristics of lipid and lipid composition were performed in the same manner. Extraction and purification of lipids were conducted the according to the methods of Folch *et al.* (1957). Total lipid (TL) was fractionated according to the methods of Rouser *et al.* (1967). A mixture of silicic acid, Unisil (Clarkson Chemical Co.), and diatomaceous earth (2: 1) was suspended in chloroform and packed into a glass column (2 cm × 40 cm). Two hundred miligrams of TL was dissolved in 5 ml of chloroform, and applied to the packed column. The column was then washed with 175 ml of chloroform to elute neutral lipids (NL), with 700 ml of acetone to elute glycolipids (GL), and then with 175 ml of methanol to elute phospholipids (PL).

Iodine value (IV), saponification value (SV), acid value (AV), peroxide value (POV), carbonyl value (COV), and unsaponifiable matter were measured. These measurements were conducted according to standard methods for the analysis of oils and fats, and related materials (Japan Oil Chemists' Society, 1986).

Measurement of lipid composition In the identification of lipid composition of NL and PL, each component was identified using thin-layer chromatography (TLC) (Rouser *et al.,* 1967) by referring to the standards in terms of Rf value and reaction to various color reagents, and the results of previous studies (Wada and Sugano, 1972; Fujino, 1978; Moris, 1975). The level of spot concentration was determined by densitometry using a Shimadzu TLC scanner CS-920, and the composition ratio was calculated. For NL composition identification, the prepared sample was applied to a silicagel-70 plate (Wako Pure Chemical Industries Ltd.), and the plate was developed with a solvent mixture containing hexane/diethy ether/acetic acid (80: 20: 1, v/v/v). For PL, the sample was applied to a Kieselguhr-60 plate (Merck & Co., Ltd.), and the plate was developed with a mixture of chloroform/methanol/acetic acid/water (65: 25: 8: 4, v/v/v/v). Standard lipids were the same as those used in the previous report (Wada and Sugano, 1972), and phospholipids were identified by coloring reagents.

Measurement of fatty acid composition Fatty acids were methylated using the methanol-HCl method (Fujino, 1978) and applied to GLC analysis under the following conditions: GC-7A (Shimadzu) with a glass column (3 mm × 2 m) packed with Chromosorb W (AW-DMCS) 80–100; 15% DEGS for a liquid phase. Quantification was conducted using a SIC Chromatocoder 12 (System Instruments Co., Ltd.), and the average value of the 3 measurements was calculated. Measurements of tocopherol, sterol composition were performed in the same manner.

Quantification of tocopherol (Toc) homologues Quantification of Toc homologues in TL was conducted using

Table 1. Chemical composition (%) during peanuts tofu production.
( )=dry base percentage.

	Dried		Roasted	
Component	Peanut	Peanut tofu	Peanut	Peanut tofu
Moisture	7.2	80.3	3.3	80.4
Protein	26.3	5.8	27.4	4.8
	(28.3)	(29.4)	(28.3)	(24.5)
Lipid	44.5	8.4	46.1	7.8
	(47.9)	(42.6)	(47.1)	(39.8)
Total sugar	13.7	4.7	14.7	5.8
	(14.8)	(23.9)	(15.3)	(29.6)
Fiber	6.1	0.1	6.3	0.1
	(6.6)	(0.5)	(6.5)	(0.5)
Ash	2.2	0.7	2.2	1.1
	(2.4)	(3.6)	(2.2)	(1.1)

the HPLC method (JASCO-PU-980) based on the method of Katsui *et al.* (1975), under the following conditions: Senshu pack Silica-1251-N column; hexane/1, 4-dioxan/2-propanol (97.7: 1.9: 0.4, v/v/v) for the mobile phase; an elution rate of 1.4 mL/min; and JASCO-FP-550 spectrofluorometer (EX 298 nm, EM 325 nm) for detection.

Measurement of sterol composition The levels and composition of sterols were analyzed according to the method developed by the Small Committee for Gas Chromatographic Data (methods for fats and oils, and related products section, Ito *et al.,* 1981). Unsaponifiable matter was applied to a silica gel plate for TLC (Wakogel B10), and the plate was developed with a solvent mixture containing hexane/diethyl ether (70: 30, v/v). A 4-desmethylsterol (DeMS) fraction (Rf 0.35–0.42) was detached from the plate, and then was subjected to GLC analysis for determination of sterols using cholesterol as an internal standard. GLC measurement was conducted under the following conditions: a glass column (3 mm×2 m); Gas-Chrom Q (100–120 mesh); 2% Silicon OV-17 for the liquid phase.

Results

Chemical composition of peanut tofu Chemical compositions of peanut tofu prepared for the present study are shown in Table 1. The moisture content of DP tofu and RP tofu was approximately 80%; thus, they were harder and more likely to maintain shape than *momen* tofu. The level of lipids and dietary fiber was decreased in the process of peanut tofu production, while that of carbohydrates and ash was increased.

Chemical characteristics of lipids in peanut tofu Chemical characteristics of lipids are shown in Table 2. Both lipids were yellow liquid at room temperature, and IV and SV were equivalent to those of nondrying oil and within the range observed for peanut oil. POV, which indicates oxidative deterioration, was 3.24 for DP tofu, which was slightly higher than 0.72 for DP. POV of RP was 15.6; thus oxidation was caused by heating. POV of RP tofu was 18.3; hence, little oxidation occurred in the production of both kinds of tofu.

Lipid composition of total lipids Changes in the lipid composition of TL are shown in Table 3. The proportions of NL, GL, and PL were 97.8–98.6%, 0.73–1.01%, and 0.6–1.2%, respectively; thus, no significant changes were observed in composition, although lipid content was decreased in the process of peanut tofu production.

Table 2. Chemical characteristics of total lipids during peanut tofu production.

Characteristics	Dried		Roasted	
	Peanut	Peanut tofu	Peanut	Peanut tofu
Iodine value (g/100 g)	102.1	99.8	98.2	96.1
Saponification value (mg/100 g)	189.2	188.0	187.6	186.3
Acid value (mg/g)	0.50	3.21	12.01	14.45
Peroxide value (meq/kg)	0.72	3.24	15.61	18.31
Carbonyl value	3.17	4.50	6.78	7.12

Table 3. Lipid class composition of total lipids during peanut tofu production (mg/g).

Lipid	Dried		Roasted	
Fraction	Peanut	Peanut tofu	Peanut	Peanut tofu
Neutral lipids	468	418	468	392
	(97.8)	(98.2)	(98.2)	(98.6)
Phospholipids	5.75	4.64	3.82	2.39
	(1.2)	(1.1)	(0.8)	(0.6)
Glycolipids	4.79	3.11	4.82	3.18
	(1.0)	(0.7)	(1.0)	(0.8)
Total lipids	479	426	477	398

( )=dry basepercentage.

Table 4. Composition of neutral lipids during peanut tofu production (mg/g).

Lipid class	Dried		Roasted	
	Peanut	Peanut tofu	Peanut	Peanut tofu
Triacylglycerol	439	380	399	326
1,2-Diacylglycerol	9.36	10.51	16.40	11.40
1,3-Diacylglycerol	7.50	7.94	10.80	6.71
Sterol	6.10	5.85	11.71	11.01
Free fatty acid	3.28	10.01	25.30	28.11
Sterol ester	2.35	4.18	4.68	9.11
Total	468	418	468	392

Lipid composition of NL and PL. In NL (Table 4), triacylglycerol decomposition occurred, decreasing levels to 90.8% in DP tofu and 83.1% in RP tofu. While the intermediate degradation products, 1,2- and 1,3-diacylglycerols, were increased in DP tofu, these products were decreased in RP tofu, and the level of the final free fatty acid product was approximately 3 times higher in RP tofu than in DP tofu. Therefore, a decrease of triacylglycerols and an increase of free fatty acids is considered to contribute to the increase of AV. This was assumed to be due to enhanced oxidation in the roasting process. In PL (Table 5),

phospholipids decomposition occurred, decreasing levels to 81% in DP tofu and 42% in RP tofu. Phosphatidylethanolamine was the component with the highest concentration, followed by phosphatidylinositol and then phosphatidylcholine. In DP tofu, these 3 phospholipids comprised 75% of lecithins, whereas in RP tofu, the percentage was decreased to approximately 60%, and the degradation product of phosphatidic acid was increased.

Fatty acid composition of TL, NL, and PL. Regarding the fatty acid composition of TL and NL (Table 6), oleic acid (18: 1) was the most frequent (approximately 50%), followed by linoleic acid (18: 2), and palmitic acid (16: 0). These 3 occupied more than approximately 80%. Unusually, long-chain fatty acids such as behenic acid (22: 0) and lignoceric acid (24: 0), were observed. In peanut tofu, the number of types of unsaturated fatty acids (USFA), such as palmitoleic acid (16: 1) and linolenic acid (18: 3) was decreased. In PL, the level of 18: 1 was decreased, while that of SFA (16: 0) was increased. While the ratio of SFA to USFA in TL and NL was found to be approximately 19: 81, that of SFA in PL was higher at approximately 32: 68. The high content of 18: 1, and the low content of 18: 3, which are indicators of superior flavor stability, are considered significant.

Amount and composition of Toc homologues The amount of Toc homologues (Table 7) was highest in DP, followed by DP tofu. α-Toc had the highest concentration, followed by γ-Toc, while only a little β- and δ-Toc was present. RP and RP tofu were found to contain a high level of γ-Toc, but α-Toc, which has a high physiological activity (Igarashi, 1978) was diminished in the roasting process. γ-Toc, which has a high antioxidative action, did not decrease upon roasting and processing.

Sterol composition in unsaponifiable matter. Amounts and composition of sterols are shown in Table 8. The amount of unsaponifiable matter was 0.43–0.61%. Regarding the contents of 4-desmethylsterol in unsaponifiable matter, the level of β-sitosterol was highest at approximately 75%, followed by campesterol and stigmasterol. These 3 compounds accounted for more than 94% of 4-desmethylsterol. β-sitosterol is known to lower blood cholesterol level (Oka *et al.*,1972; Goto *et al.*, 1999), and such phytosterols have recently received much attention.

Table 5. Composition of phospholipids during peanut tofu production (mg/g).

Lipid class	Dried		Roasted	
	Peanut	Peanut tofu	Peanut	Peanut tofu
Phosphatidyl-ethanolamine	2.33	1.74	1.25	0.75
Phosphatidyl-inositol	1.21	0.94	0.67	0.39
Phosphatidyl-choline	1.00	0.84	0.62	0.37
Phosphatidic acid	0.31	0.38	0.41	0.46
Others	0.90	0.74	0.87	0.42
Total	5.75	4.64	3.82	2.39

Discussion

It was found that the levels of lipids and dietary fiber in the peanuts decreased during peanut tofu production. The decrease in lipids was assumed to be due to the decomposition of triacylglycerols, which account for approximately 98% of total lipids, into intermediate products, such as 1,2- and 1,3-diacylglycerol, and final products like free fatty acids were the primary reason for the decline in lipid levels (Table 4). The decrease of dietary fiber was assumed to be due to filtration using a nylon cloth.

The chemical characteristics of lipids in *momen* Tofu (Tokue and Kataoka, 1997) and DP tofu were as follows: 1.31 and 3.24, respectively, for POV; and 1.65 and 3.21, respectively for AV. Although these values were slightly higher in DP tofu, it was assumed that little oxidation occurred. In contrast, both POV and AV values in RP tofu were high, indicating that oxidation increased. Such differences were assumed to be due to low values of POV and AV in DP for DP tofu production, and high values in

Table 6. Fatty acid composition (%) of total lipids, neutral lipids and phospholipids during peanut tofu production.

Fatty acid	Total lipids				Neutral lipids				Phospholipids			
	A	B	C	D	A	B	C	D	A	B	C	D
C16:0	11.1	12.1	11.5	12.8	10.0	11.1	10.2	11.5	25.4	24.3	24.8	25.9
C16:1	0.2	-	0.2	-	0.1	-	0.1	-	1.0	-	0.7	-
C18:0	2.9	3.5	3.0	3.8	3.0	3.4	2.9	3.5	3.5	3.7	3.2	3.7
C18:1	48.1	50.4	48.2	49.0	50.0	51.0	49.9	50.9	31.1	34.0	31.4	33.6
C18:2	30.8	30.5	29.4	31.0	29.5	31.0	30.0	30.6	31.9	35.9	32.5	34.8
C18:3	0.2	-	0.3	-	0.3	-	0.4	-	0.2	-	0.3	-
C20:0	0.9	-	1.0	-	0.8	-	1.1	-	1.3	-	1.5	-
C20:1	1.2	-	1.3	-	1.3	-	1.2	-	1.1	-	1.0	-
C22:0	2.7	3.5	3.0	3.4	3.0	3.5	3.0	3.5	2.5	2.1	2.2	2.0
C22:1	0.5	-	0.6	-	0.7	-	0.6	-	0.5	-	0.6	-
C24:0	1.4	-	1.5	-	1.3	-	1.6	-	1.5	-	1.8	-
Saturated	19.0	19.1	20.0	20.0	18.1	18.0	18.8	18.5	34.2	30.1	33.5	31.6
Unsaturated	81.0	80.9	80.0	80.0	81.9	82.0	81.2	81.5	65.8	69.9	66.5	68.4

A: Dried peanut; B: Dried peanut tofu; C: Roasted peanut; D: Roasted peanut tofu.

Table 7. Tocopherol content during peanut tofu production (mg/100 g lipids).

Tocopherol	Dried		Roasted	
	Peanut	Peanut tofu	Peanut	Peanut tofu
α-tocopherol	6.24	4.17	3.21	1.52
β-tocopherol	0.30	tr	tr	tr
γ-tocopherol	4.74	4.09	4.06	3.65
δ-tocopherol	0.62	tr	tr	tr
Total	11.90	8.62	7.27	5.17

tr: trace.

Table 8. Content of 4-desmethylsterol fraction in total lipids during peanut tofu production (mg/100 g lipids).

4-Desmetylsterol	Dried		Roasted	
	Peanut	Peanut tofu	Peanut	Peanut tofu
Campesterol	14.6	9.9	14.0	9.7
Stigmasterol	8.8	6.8	8.8	6.4
Sitosterol	81.9	60.4	80.5	57.8
Isofucosterol	3.9	2.1	3.2	1.6
7-stigmasterol	0.9	0.3	0.6	0.2
Unknown	1.4	2.7	0.4	1.8
Total	111.5	82.2	107.5	77.5
Unsaponifiable (%)	0.61	0.46	0.59	0.43

RP for RP tofu production. It was therefore suggested that peanuts were susceptible to thermal oxidation during the roasting process. Yamaguchi *et al.* (1999) reported that the stability against oxidation was improved by controlling the roasting temperature. It is thus necessary to examine the roasting method.

Regarding sensory evaluations, the highest mean scores for all categories, including appearance, texture, flavor, and overall evaluation, were observed in the sample to which 2% gelatin added, followed by the sample to which 15% starch was added, and the sample to which 1% agar was added, respectively. The highest mean score for all categories except appearance, including flavor, texture, and overall evaluation, was observed in RP tofu. The aroma of roasted peanuts was found to be preferred. Furthermore, POV and AV values, which were high in RP tofu, did not affect the flavor or texture of tofu. In consideration of occurrence of lipid oxidation, since only a limited difference was observed in properties of RP tofu and DP tofu, it may be more suitable to use DP for peanut tofu production.

Peanut tofu containing high levels of oleic acid and β-sitosterol, which lowers blood cholesterol levels, should be recognized as a food item in with significant nutritional advantages.

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