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Changes of Lipids in Raw and Processed Adlay Powder during Storage

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Abstract

Raw adlay powder(RAP) and processed adlay powder(PAP) were prepared and the changes of lipids in RAP and PAP during storage at 5℃ and 35℃ for six months were studied. The typical patterns of lipid oxidation were observed during the storage; the values of acid, peroxide and carbonyl in the lipids were increased and the iodine value was decreased. These changes were especially significant($P<0.05$) at higher temperature(35℃) and were more notable in the lipids from RAP than those from PAP during storage. The contents of neutral lipid, glycolipid and phospholipid were changed appreciably at 35℃, however, the changes were not significant($P<0.05$) at 5℃. Further more, triglyceride in neutral lipid, digalactosyl diglyceride in glycolipid and phosphatidyl choline in phospholipid showed respectively a tendency of decrease during storage as compared with the other contents of lipid class.

Key words: adlay lipid, cereal lipid composition, storage effect of cereal lipid

Introduction

Adlay(*Coix Lachryma-jobi Linne var. Mayuen (Roman)Stapf*) belonged to Graminaceae was used for food and medicine and its powder from is recently used as adlay tea. The lipid content of adlay represents a small fraction compared with the other components such as carbohydrate and protein. However, this lipid undoubtedly plays an important functional role in their products during processing and storage[1]. One of the major chemical reactions that takes place during processing and storage is lipid deterioration caused by lipid oxidation[2]. Most of studies for adlay lipids to date have been concerned with the fatty acid composition and the composition of lipid fractions[3,4]. At present, very little is known about the nature of the lipids in adlay during processing and storage. This study was performed to investigate the detailed changes of adlay lipids by the conditions of processing and storage. In this paper, the behaviour of lipid oxidation and change of lipid composition in raw adlay powder(RAP) and processed adlay powder(PAP) stored at 5℃ and 35℃ for six months are reported.

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Materials and Methods

Preparation of adlay powder

Adlay grown at the area of Kyung Ki Do province was used in this study. Whole grain samples of adlay were dehulled immediately after harvest. Adlay was soaked in water at 15℃ under atmospheric pressure for 8 hrs and steamed at 121℃ under $1Kg/cm^2$ for 20min. After the adlay was aerated at 30℃ until the moisture content had decreased to 30%, the steamed adlay was dried at 50℃ for 10 hrs, followed by continuous drying at 35℃ for 8 hrs up to a 12.4% moisture content.

The raw and processed adlay were milled in a Fitzmill(Model D, Fitzpatrick Company, Chicago) to pass through No. 6 standard sieve with Tyler equivalent of 80mesh. The raw adlay powder(RAP) and processed adlay powder(PAP) were stored for six months at 5℃ and 35℃ in polyethylene film bags, respectively and analyzed at one or two month interval for the various chemical parameters of quality.

Lipid extraction and purification

Crude lipids were extracted with a mixture of chloroform-methanol(2:1, v/v) as described by Folch *et al.*[5] Crude lipid extracts were freed from non-lipid contaminants by passing through a Sephadex G-25 column by the procedure of Wuthier[6]. The lipid extracts were evaporated to dryness under vacuum, weighed, redissolved in chloroform, transferred to vials, and stored at -15℃ until analysis.

Chemical characteristics of lipids

The values of acid, peroxide and iodine in the lipids extracted from RAP and PAP were measured by the AOCS Official Methods[7]. Carbonyl value was measured using the ansidine test[8].

Column chromatography

The purified lipids were subjected to column chromatography, using a silicic acid(100mesh, Mallinckrodt Chemical Works, USA) column[9]. The lipid material(0.5g) was applied in 2m*l* of chloroform onto a column containing 10g of silicic acid. Chloroform, aceton and methanol were used sequentially to elute the neutral lipid(NL), glycolipid(GL) and phospholipid(PL), respectively. After evaporation of the solvent in a Büchi rotary evaporator(Model No. 135197, Switzerland) at 35℃, the percent distribution of the three lipid fractions was determined from the weights of each fraction, transferred to vials, and stored under nitrogen at -15℃ until analysis.

Thin layer chromatography(TLC) and quantitative analysis of indivisual components

Lipid fractions of the NL, GL and PL were separated by TLC on silica gel plate. For separation of NL, silica gel G plate(0.25mm) and a solvent system containing hexane-diethyl ether-acetic acid(80:20:1, v/v) were used. For separation of GL and PL, silica gel H plates(0.05mm) and solvent systems, chloroform-methanol(95:12, v/v) for GL and chloroform-methanol-water-28% aqueous ammonia(65:25:4:0.2, v/v) for PL were used. The spots were located and partially identified by spraying with 50% H_2SO_4 and subsequent charring at 120℃. The lipids separated by TLC were identified by comparing their Rf values with those of pure compounds(Sigma Chemical Co., St. Louis, USA) and using specipic sprays such as ninhydrin, molybdenum reagent for PL[10] and anthrone reagent for GL[11].

The spots separated by TLC were quantitated by TLC scanner(Shimadzu dual wavelength TLC scanner, CS-930, Japan). Operating conditions for the scanner were the same as previously described[4].

Results and Discussion

Changes in chemical characteristics of lipids

Fig. 1 and 2 show the changes of oxidation indicator index of total lipid in RAP and PAP during storage at 5℃ and 35℃. Additionally hydrolytic changes in lipid during storage were followed by determining acid value. The PAP had higher acid value than the RAP at the initial stage due to the fact that steeping prior to the processing accelerated lipolysis[12]. However, throughout the storage period, acid value of RAP stored at 35℃

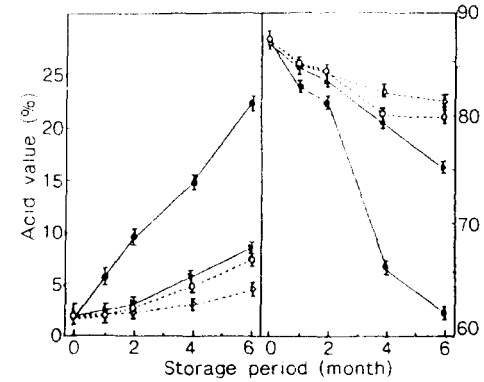

Fig. 1 Changes in acid value and iodine value of total lipids of raw adlay powder(RAP) at 5°C(○), RAP at 35°C(●), processed adlay powder(PAP) at 5°C(△) and PAP at 35°C(▲) during storage.

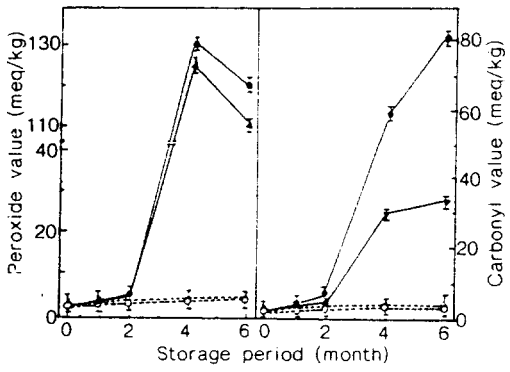

Fig. 2. Changes in peroxide value and carbonyl value of total lipids of raw adlay powder(RAP) at 5℃(○), RAP at 35℃(●), processed adlay powder(PAP) at 5℃(△) and PAP at 35℃(▲) during storage.

increased more rapidly than those of samples stored at 5℃ and those of all PAP samples. This means that not only chemical reactions generally are accelerated when temperature are increased but the action of lipase in the RAP increased at higher temperature(35℃)[13].

Peroxide value, which indicates oxidative change in lipid steadily increased early in storage, after 4 months of storage reached a maximum, and then gradually declined under storage conditions at 35℃, but was hardly changed during storage at 5℃. The PAP had higher oxidation status than the RAP at the initial stage due to the heat treatment during the processing. The higher oxidation status of the RAP may have been due to the fact that both autoxidation and lipoxygenase catalyzed oxidation were occurring in these samples stored at 35℃[14].

The samples had relatively low carbonyl value at the initial stage. The RAP appeared a higher rate of oxidation than the PAP during storage at 35℃. The higher oxidation status of the RAP may have been due to the some action of hydroperoxide lyase. Iodine value steadily decreased early in storage, and then appreciably decreased under storage conditions at 35℃. RAP had a lower iodine value than PAP as results of the more oxidation of unsaturated fatty acids in RAP and PAP during storage. In general, the changes expressed by the oxidation indicator index of the total lipid from RAP were more notable than those from PAP due to enzymes and various biological activities in the sample during storage. These changes were especially significant($P<0.05$) at higher temperature(35℃).

Changes in content of total lipid fraction

Table 1 shows the changes in the lipid content and lipid fraction of RAP and PAP during storage. The compositions of lipid fraction; neutral lipids(NL), glycolipid(GL) and phospholipid(PL) accounted for 87-88%, 8-9% and 3-4%, respectively.

Throughout the storage period, it was observed that the contents of NL, GL and PL were changed appreciably when the samples were stored at 35℃, however, the changes were not significant at 5℃. The RAP also showed greater changes in the contents of NL, GL and PL than the PAP. Moreover, the contents of GL and PL in total lipids decreased more rapidly than those of NL during storage at 35℃.

These results indicate that the oxidation and lipolysis of adlay powder lipid depended on the storage temperature and conditions of processing.

Changes in composition of neutral lipids

The neutral lipids from samples were resolved by TLC as shown in Fig. 3. The components were identified with the aid of pure standards such as cholesteryl palmitate, tripalmitin, linoleic acid, cholesterol and 1,3-dipalmitin. Triglycerides(TG, 90.6%) were the major fraction of neutral lipids. Minor fractions such as diglycerides(DG), free sterol(FS), free fatty acid(FFA) and esterified sterol(ES) were also present(Table 2). The neutral lipid compositions of the RAP and PAP were qualitatively similar to those studied on other low

Table 1. Changes in lipid composition of total lipids in raw adlay powder(RAP) and processed adlay powder (PAP) during storage at 5°C and 35°C

	Temperature (°C)	Time (month)	Lipid content (%)[a]	Lipid fraction (%)		
				Neutral lipid	Glycolipid	Phospholipid
RAP	5	0	3.7 ± 0.2[b]	87.3 ± 0.2	8.4 ± 0.1	3.5 ± 0.3
		2	3.7 ± 0.1	87.4 ± 0.1	8.5 ± 0.1	3.4 ± 0.2
		4	3.7 ± 0.3	87.6 ± 0.1	8.4 ± 0.2	3.4 ± 0.1
		6	3.7 ± 0.2	87.8 ± 0.3	8.4 ± 0.1	3.4 ± 0.1
	35	0	3.7 ± 0.2	87.3 ± 0.2	8.4 ± 0.1	3.5 ± 0.3
		2	3.7 ± 0.0	88.0 ± 0.4	8.2 ± 0.1	3.4 ± 0.2
		4	3.4 ± 0.3	88.9 ± 0.1	7.5 ± 0.2	2.9 ± 0.1
		6	3.2 ± 0.2	89.9 ± 0.1	6.9 ± 0.2	2.6 ± 0.1
PAP	5	0	3.7 ± 0.2	87.3 ± 0.2	9.2 ± 0.1	4.1 ± 0.1
		2	3.7 ± 0.1	87.3 ± 0.1	9.2 ± 0.2	4.1 ± 0.1
		4	3.7 ± 0.1	87.3 ± 0.3	8.9 ± 0.2	4.0 ± 0.1
		6	3.6 ± 0.2	87.3 ± 0.2	9.0 ± 0.2	4.0 ± 0.2
	35	0	3.7 ± 0.2	87.3 ± 0.2	9.2 ± 0.1	4.1 ± 0.1
		2	3.7 ± 0.1	87.4 ± 0.1	9.1 ± 0.2	4.0 ± 0.1
		4	3.5 ± 0.2	87.8 ± 0.1	8.4 ± 0.1	3.1 ± 0.1
		6	3.4 ± 0.2	89.0 ± 0.3	7.9 ± 0.2	2.9 ± 0.1

[a]g per 100g of adlay

[b]Values are mean±standard deviation of three determinations.

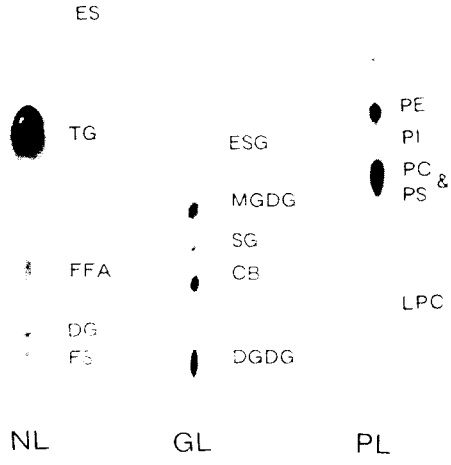

Fig. 3. Typical patterns of thin layer chromatographic separation of neutral lipid(NL), glycolipid(GL) and phospholipid(PL) in raw adlay powder.
The spots were identified as follows: DG, diglycerides; FS, free sterol; FFA, free fatty acid; TG, triglycerides; ES, esterified sterol; DGDG, digalactosyl diglycerides; CB, cerebrosides; SG, steryl glycosides; MGDG, monogalactosyl diglycerides; ESG, esterified steryl glycosides; LPC, lysophosphatidyl choline; PS, phosphatidyl serine; PC, phosphatidyl choline; PI, phosphatidyl inositol; PE, phosphatidyl ethanolamine.

fat cereal, wheat[15] and millet[16], but quantitatively not similar to those of other cereal grains. The PAP had lower TG and higher FFA contents than the RAP at the initial stage. This may have been due to the lipolysis during the processing.

Throughout the storage periods, the contents of TG clearly decreased, but those of FFA relatively increased in samples stored at 35℃. In the case of samples stored at 5℃, however, a slight decrease in the TG occurred. The decrease in the content of the TG was mainly caused by degradation of TG to DG, MG and FFA during the period of storage, their degree being much more striking when the storage temperature was 35℃. Especially, the RAP had a higher rate of degradation of TG than the PAP. This may have been due to the more active action of lipase during storage at 35℃.

Changes in composition of glycolipids

The glycolipids from samples were separated into six distinct spots by TLC(Fig. 3). The components were identified by co-chromatography with glycolipid standards such as esterified steryl glycoside(ESG), monogalactosyl diglyceride(MGDG), steryl glycoside(SG), cerebroside(CB) and digalactosyl diglyceride(DGDG). The DGDG con-

Table 2. Changes in lipid composition of neutral lipid fraction in raw adlay powder(RAP) and processed adlay powder(PAP) during storage at 5°C and 35°C

(Area %)

Sample	Temperature	Neutral lipids	Storage period (month)			
			0	2	4	6
RAP	5°C	ES	2.4 ± 0.4[a]	2.2 ± 0.1	2.6 ± 0.3	2.1 ± 0.6
		TG	90.6 ± 0.5	88.8 ± 0.1	85.7 ± 0.1	78.0 ± 0.4
		FFA	2.9 ± 0.4	3.6 ± 0.1	6.2 ± 1.1	11.0 ± 1.0
		FS	3.0 ± 0.1	3.4 ± 0.4	3.2 ± 0.2	3.3 ± 0.5
		DG	1.3 ± 0.2	2.9 ± 0.5	2.5 ± 0.5	6.0 ± 0.8
	35°C	ES	2.4 ± 0.4	2.5 ± 0.5	2.5 ± 0.6	2.4 ± 0.4
		TG	90.6 ± 0.5	81.9 ± 1.0	71.5 ± 1.5	61.3 ± 2.6
		FFA	2.9 ± 0.4	7.4 ± 0.6	16.9 ± 1.3	26.4 ± 1.8
		FS	3.0 ± 0.1	3.6 ± 0.8	2.4 ± 0.6	2.9 ± 0.2
		DG	1.3 ± 0.2	4.8 ± 1.8	6.9 ± 0.1	6.8 ± 0.3
PAP	5°C	ES	2.6 ± 0.2	2.6 ± 0.5	2.6 ± 0.1	2.8 ± 0.5
		TG	89.3 ± 1.2	88.0 ± 1.4	86.3 ± 1.6	82.8 ± 1.0
		FFA	4.3 ± 0.2	4.6 ± 1.2	4.9 ± 1.1	7.2 ± 1.0
		FS	2.7 ± 0.6	2.6 ± 0.4	2.7 ± 0.8	2.9 ± 1.0
		DG	1.8 ± 0.2	3.4 ± 0.1	3.7 ± 0.1	4.4 ± 1.4
	35°C	ES	2.6 ± 0.2	3.1 ± 0.6	3.3 ± 0.8	3.0 ± 0.6
		TG	89.3 ± 1.2	84.2 ± 1.4	82.0 ± 0.7	74.1 ± 0.6
		FFA	4.3 ± 0.2	5.1 ± 1.0	7.2 ± 1.0	14.7 ± 0.6
		FS	2.7 ± 0.6	3.6 ± 0.2	3.1 ± 0.8	4.1 ± 0.8
		DG	1.8 ± 0.2	3.6 ± 0.6	3.1 ± 0.4	4.1 ± 1.3

[a]Values are mean±standard deviation of three determinations.
Abbreviations are the same as in Fig. 3

tent was 31.7-36.2% of the total sugar of the glycolipids(Table 3). Although DGDG would contain two moles of sugar compared to one in the other glycolipid, the DGDG had a higher molar concentration than the others and it was the dominant in the grain. This contrasts with photosynthetic tissue, such as chloroplast, where MGDG predominates[17]. In grain, MGDG ranked second. Also, the glycolipid contained ESG, SG, CB and unidentified lipid as minor components. The PAP seemed to have lower DGDG and higher MGDG content than the RAP at the initial stage due to the degradation of DGDG to MGDG during the processing.

Throughout the storage periods, the compositions of glycolipids were hardly changed at 5℃, whereas it was significantly changed at 35℃. DGDG slightly decreased early in storage and after 4 months of storage, the contents of DGDG in RAP markedly decreased during storage at 35℃. The decrease in the content of the DGDG was mainly caused by degradation of DGDG to MGDG during storage. The SG and ESG increased slightly, but were relatively minor components of the glycolipid during the period of storage.

Changes in composition of phospholipids

The phospholipids from RAP and PAP were resolved by TLC as shown in Fig. 3. The components were identified by co-chromatography with lysophosphatidyl choline(LPC), phosphatidyl choline(PC), phosphatidyl serine(PS), phosphatidyl inositol(PI) and phosphatidyl ethanolamine(PE). The spot representing PC was the largest in this lipid fraction. PE and LPC ranked second and third, respectively. These components were the major phospholipids. The overlapping of PC and PS was confirmed by the use of ninhydrin spray reagent and authentic standards. Lesser amounts of PI, PS and an unknown phospholipid were also present(Table 4). Phospholipids have a role in membrane development, fatty acid and phytin

Table 3. Changes in lipid composition of glycolipid fraction in raw adlay powder(RAP) and processed adlay powder(PAP) during storage at 5°C and 35°C

(area %)

Sample	Temperature	Glycolipids	Storage period (month)			
			0	2	4	6
RAP	5°C	DGDG	36.2 ± 1.3[a]	35.4 ± 1.6	33.6 ± 1.3	32.3 ± 1.8
		CB	15.7 ± 1.6	14.7 ± 1.4	13.0 ± 1.2	11.6 ± 1.6
		SG	4.6 ± 0.5	5.6 ± 0.2	2.4 ± 0.7	3.1 ± 0.1
		MGDG	22.2 ± 0.9	23.0 ± 1.4	21.0 ± 1.6	22.7 ± 0.9
		ESG	14.6 ± 0.7	13.9 ± 1.2	16.0 ± 0.4	15.8 ± 0.6
	35°C	DGDG	36.2 ± 1.3	33.6 ± 1.6	28.4 ± 1.6	23.6 ± 1.4
		CB	15.7 ± 1.6	14.3 ± 1.2	12.2 ± 1.3	11.5 ± 1.4
		SG	4.6 ± 0.5	4.7 ± 0.3	4.5 ± 0.7	4.0 ± 0.4
		MGDG	22.2 ± 0.9	20.5 ± 0.5	20.2 ± 0.7	21.4 ± 0.6
		ESG	14.6 ± 0.7	13.2 ± 0.4	15.1 ± 0.4	14.6 ± 0.7
PAP	5°C	DGDG	31.7 ± 2.0[a]	30.7 ± 1.2	29.7 ± 2.2	28.4 ± 1.8
		CB	15.4 ± 2.1	16.1 ± 1.7	16.0 ± 2.8	16.3 ± 1.6
		SG	5.8 ± 0.2	4.0 ± 0.5	6.0 ± 0.3	6.3 ± 0.8
		MGDG	26.3 ± 0.6	25.7 ± 0.8	25.5 ± 1.3	26.5 ± 0.9
		ESG	18.1 ± 1.1	16.8 ± 0.7	18.3 ± 0.5	18.6 ± 0.5
	35°C	DGDG	31.7 ± 2.0	30.0 ± 1.3	26.3 ± 1.6	22.7 ± 1.8
		CB	15.4 ± 2.1	16.1 ± 1.4	14.8 ± 1.6	15.1 ± 1.6
		SG	5.8 ± 0.2	3.6 ± 0.3	4.3 ± 0.5	5.6 ± 0.2
		MGDG	26.3 ± 0.6	23.2 ± 0.4	23.1 ± 0.5	24.3 ± 0.5
		ESG	18.1 ± 1.1	19.3 ± 0.6	17.8 ± 0.6	17.3 ± 0.7

[a]Values are mean±standard deviation of three determinations.
Abbreviations are the same as in Fig. 3.

Table 4. Changes in lipid composition of phospholipid fraction in raw adlay powder(RAP) and processed adlay powder(PAP) during storage at 5°C and 35°C

(area %)

Sample	Temperature	Phospholipids	Storage period (month)			
			0	2	4	6
RAP	5°C	LPC	4.2 ± 0.9[a]	5.1 ± 1.2	6.9 ± 0.4	8.5 ± 0.9
		PC & PS	67.3 ± 1.8	67.2 ± 1.1	65.1 ± 1.3	63.7 ± 1.1
		PI	1.5 ± 0.4	1.9 ± 0.6	1.7 ± 0.6	2.2 ± 0.8
		PE	27.1 ± 0.6	25.9 ± 0.6	26.3 ± 0.3	25.7 ± 0.6
	35°C	LPC	4.2 ± 0.9	6.5 ± 0.9	11.1 ± 0.3	14.2 ± 1.3
		PC & PS	67.3 ± 1.8	64.0 ± 1.3	59.8 ± 1.1	56.7 ± 1.1
		PI	1.5 ± 0.4	3.2 ± 0.4	4.0 ± 0.1	5.5 ± 0.3
		PE	27.1 ± 0.6	26.3 ± 0.2	25.3 ± 0.5	23.7 ± 0.6
PAP	5°C	LPC	4.2 ± 1.0	4.5 ± 0.9	5.7 ± 1.1	7.5 ± 1.8
		PC & PS	65.5 ± 1.9	65.6 ± 1.8	64.3 ± 1.5	63.6 ± 1.6
		PI	2.3 ± 0.6	2.5 ± 0.2	2.0 ± 0.3	1.9 ± 0.6
		PE	28.1 ± 0.4	27.6 ± 0.6	17.1 ± 1.5	27.1 ± 0.8
	35°C	LPC	4.2 ± 1.0	6.4 ± 1.1	7.7 ± 1.1	9.2 ± 0.9
		PC & PS	65.6 ± 1.9	64.6 ± 1.0	62.9 ± 1.1	61.4 ± 1.0
		PI	2.3 ± 0.6	3.3 ± 0.8	3.8 ± 0.4	4.1 ± 0.4
		PE	28.1 ± 0.4	25.8 ± 0.9	35.7 ± 0.4	25.5 ± 0.4

[a]Values are mean±standard deviation of three determinations.
Abbreviations are the same as in Fig. 3.

synthesis[17]. The PAP seemed to contain lower PC than the RAP at the initial stage due to the degradation of PC during the processing.

Throughout the storage period, PC and PE had the higher rate of oxidation than the other components in the samples stored at 35℃. The relative contents of LPC was found to increase in relation to the decrease of PC. The changes in composition of phospholipid in RAP were more remarkable than those of the PAP due to the action of phospholipase during storage at 35℃.

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율무가루 저장중 지방질 조성의 변화

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생 율무가루와 가공된 율무가루를 5℃ 및 35℃에서 6개월 동안 저장하면서 지방질 조성의 변화와 산화 양상을 조사하였다. 저장중 총 지방질의 산값, 과산화물값, carbonyl 값은 증가하였으나 요오드 값은 감소하였고,

이러한 변화는 35℃로 저장했을 때 현저하였으며 생 율무 가루에서 더 큰 변화를 나타내었다. 중성, 당 및 인지방질의 함량은 5℃ 보다 35℃에서 저장하였을 때 더 큰 변화를 보였으며, 특히 생 시료의 변화가 더욱 현저하였다. 중성 지방질에서의 triglyceride 및 인지방질에 있는 phosphatidyl choline의 함량은 저장동안 다른 지방질 성분보다 더욱 현저히 감소되었으며, 상대적으로 free fatty acid 및 lysophosphatidyl choline의 함량이 증가하였다.