Effect of extraction conditions on lecithin from rice bran gum and soybean gum

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Abstract

The objective of this study was to study the optimum conditions for the extraction of lecithin from rice bran and soybean gums. The extraction study used de-oiled gum:solvent ratio of 1:1 (w/v) and 4 types of solvents including ethanol (ET), ethanol:isopropanol (ET:IP), isopropanol:ethanol (IP:ET) and isopropanol (IP) at 4 ratios of 100:0, 60:40, 60:40 and 0:100 (v/v). It was found that the extracted lecithin from both types of gum using ET showed the highest phosphatidylcholine (PC) content for both upper and lower layers, therefore it was selected for further study. The selected solvent (ET) was applied in the extraction of lecithin by using three ratios of de-oiled gum:solvent at 1:3, 1:5 and 1:7 (w/v). Even though the chemical properties of all three ratios of de-oiled gum:solvent were better than using ratio 1:1 in the first experiment, it had a lower PC content. Therefore, lecithin extracted with the ratio of de-oiled gum:solvent at 1:1 (w/v) using ET was compared with the commercial soybean lecithin. It was shown that the chemical properties of commercial soybean lecithin were better than the extracted lecithin. However, the PC content of the commercial soybean lecithin was lower than the extracted lecithin. The optimum condition for the extraction of lecithin from rice bran and soybean gums in this study showed high PC content, but an improved purification method should be adopted to improve the chemical properties of the extracted lecithin.

Keywords**:** *Rice bran gum, Soybean gum, De-oiled gum, Solvent extraction, Lecithin, Phosphatidylcholine*

1. Introduction

Gum is a by-product of the water degumming step in rice bran oil (RBO) and the soybean oil (SBO) refining process. The rice bran gum (RBG) and soybean gum (SBG) contain small amounts of phospholipids, water, oil and suspended matter (van Nieuwenhuyzen and Tomás, 2008). Rice bran contains about 53-55% and soybean contains about 58-65% of phospholipids (PLs). The important PL components of lecithin are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA) (Ng, 2011). The main component of lecithin is PC that accounts for about 19-22% of rice bran lecithin and 13-18% of soybean lecithin (List, Avellameda, & Mounts, 1981; Pragasam, Indira, & Krishna, 2002).

Lecithin is obtained by removing oil, water and other components such as glycolipids from gum (van Nieuwenhuyzen and Tomás, 2008). The most important step of fractionation in lecithin enrichment is the separation of neutral lipids from polar lipids. The separation of polar lipids like glycolipids and phospholipids from lecithin is based on insolubility in cold acetone (Aneja, Chadha, & Yoell, 1971; Flider, 1985). The high purity of lecithin can be obtained by fractionation of phospholipids, but before that the crude lecithin has to go through a de-oiling process in which the neutral and polar lipids are separated (Schneider, 1989). Lecithin has been extensively used in its individual capacity. The fractionation of lecithin by extraction with alcohol depends on several extraction conditions including extraction time, solvent volume, mixing intensity and solvent polarity (Adhikari and Adhikari, 1986).

Some quality parameters are usually used to determine the specifications during lecithin production, including acetone insoluble (AI) matter, toluene insoluble (TI) matter, acid value (AV), moisture content (MC), color, peroxide value (PV), hexane insoluble (HI) matter, consistency, clarity and microbiology (van Nieuwenhuyzen and Tomás, 2008). The higher AI is, the better the approximate indication for the amount of phospholipids, glycolipids, and carbohydrates present is, while the oil and fatty acids dissolve in acetone.

Therefore, high AI indicates high phospholipids or lecithin. On the contrary, the lower HI/TI is, the better the amount of impurities such as residual, fiber, protein, and dirt are dissolved in hexane/tolueine. Therefore, lower HI/TI shows lower impurities in lecithin (Van Nieuwenhuyzen, 2015). In addition, the lower MC is, the better AV and PV are. Lower MC makes the lecithin inappropriate for microbial growth and reduces oxidation that has a relation with AV and PV (Szujah, 1989). The AV indicates acidity with phospholipids and free fatty acids. Most liquid lecithin is caused by the addition of free fatty acids for the viscosity of the product, which is not more than 36 mg KOH/g. The PV is a result of oxidation and present in propagation process, so high PV may also show any residual peroxide left in the product (Van Nieuwenhuyzen, 2010).

Lecithin increases high-density lipoprotein (HDL), decreases low-density lipoprotein (LDL) cholesterols and also reduces the risk of cardiovascular diseases. In addition, lecithin is a precursor of choline that helps to nourish the brain and associate with better memory (Dov, 2019). Lecithin is considered an excellent source of choline for supplement and helps the texture of food. It acts as an emulsifying agent in chocolate, caramels and chewing gum (Prosise, 1985). In the cosmetic industry, lecithin is an ingredient that helps to form emulsion by reducing the surface tension of substances to be emulsified (Maron et al, 2007). In present, both RBG and SBG have been mixed with acid oil and sold for animal feed with the price of only 20-30 baht/kg while lecithin is worth 600-4,000 baht/kg depending on its purity. Since lecithin is widely used as emulsifier and antioxidant in various industries and there has not been any lecithin from RBG or from rice available, the extraction of lecithin from available RBG and SBG would add value to these underutilized gums.

2. Objectives

The objectives of this study were to find the optimal conditions for the extraction of lecithin from gum that is a by-product of rice bran oil and soybean oil production and to compare the properties of the extracted lecithin with commercial soybean lecithin.

3. Materials and Methods

3.1 Materials and Chemicals

Rice bran gum (RBG), soybean gum (SBG) and commercial soybean lecithin used in this study were obtained from Surin Bran Oil Co., Ltd. (Surin, Thailand), P. A. S. Export & Silo Co. Ltd. (Sukhothai, Thailand) and Union Science (Thailand), respectively. Phosphatidylcholine (99%) was purchased from Sigma Aldrich (USA). All chemicals and solvents were of analytical and HPLC grades.

3.2 De-oil processing

The de-oiled gum was obtained by removing the oil from the gum using hexane extraction (gum/hexane ratio of 1:5 w/v) for 30 min and centrifugation at 4500 rpm for 10 min. The soluble layer was collected and hexane was removed using vacuum conditions in a rotary evaporator. The remaining oil was extracted by cold acetone (gum/acetone ratio of 1:1.5 w/v) for 30 min. Insoluble matter (phospholipids) was collected and the acetone was removed in a hot-air oven at 70°C.

3.3 Effect of solvent type and ratio on lecithin extraction

De-oiled gum (5 g) was weighed in a centrifuge tube and heated to 50°C for 15 min after which 5 mL of various types of solvent (ethanol, ET and isopropanol, IP) at 1:1 w/v was added. The ratio of mixed solvent was 100:0 (ET), 60:40 (ET:IP), 60:40 (IP:ET) and 100:0 (IP). Then, it was vortexed for 3 min and centrifuged at 4500 rpm for 10 min. The soluble matter (upper layer) and insoluble matter (lower layer) were collected. The solvent was removed by the hot-air oven at 70°C. The dried sticky mass of PC from both soluble and insoluble fractions was analyzed for chemical properties. The analysis of lecithin was performed using HPLC–UV. The optimal condition based on PC content was selected for further extraction.

3.4 Effect of de-oiled gum and solvent ratio on lecithin extraction

De-oiled gum (5 g) was weighed in a centrifuge tube. The solvent selection from section 3.3 was added in the ratios of 1:3, 1:5 and 1:7 (w/v) of de-oiled gum: solvent. Then, it was vortexed for 3 min and centrifuged at 4500 rpm for 10 min. The soluble matter was collected. The solvent was removed by the hot-air oven at 70°C. The dried sticky mass of PC rich fraction was analyzed for chemical properties. The analysis of lecithin was performed using HPLC–UV. The optimal condition based on PC content was selected to compare with the commercial soybean lecithin.

3.5 Analysis of extracted lecithin

Chemical properties i.e. moisture content (MC), acid value (AV), hexane insoluble matter (HI) and acetone insoluble matter (AI) of the extracted lecithin were determined using the AOCS official methods (AOCS, 2017). The PC content was determined by HPLC–UV following the method of Rehman et al, (2017)

3.6 Statistical Analysis

The experimental design used was complete randomized design (CRD). The data were presented as the mean values ± standard deviation. Statistical analyses were carried out using the IBM SPSS statistics 22 software using one-way ANOVA and Duncan's multiple range tests. The results were considered statistically significant at a 95% confidence interval ($p<0.05$).

4. Results and Discussion

4.1 The effect of solvent type and ratio on lecithin extraction

The effect of solvent type and ratio on extraction of RBL are presented in Table 1. The upper layer of IP and lower layer of IP:ET showed significantly lower MC (2.8±0.1% and 2.9±0.2%) than the other samples. The lower layer of ET had lower AV than the other conditions, except the lower layer of IP. The HI matter in the upper layer was better than that of the lower layer for all conditions while AI matter was found the highest in the lower layers of IP.

Table 1 The chemical properties of extracted rice bran lecithin in the upper and lower layers by using ratio of de-oiled gum:solvent at 1:1 (w/v)

Layer	Solvents	MC (%)	AV (mg KOH/g)	HI (%)	AI (%)
Upper	ET	4.1±0.1^{b}	27.5±0.2^{a}	2.0±0.1^{c}	46.6±0.2^{e}
	ET:IP	3.9±0.1^{b}	24.6±0.5^{b}	2.1±0.2^{c}	33.3±0.1^{f}
	IP:ET	3.5±0.1^{c}	22.5±0.2^{c}	2.3±0.2^{c}	32.8±0.4^{f}
	IP	2.8±0.1^{d}	22.1±02^{c}	2.3±0.3^{c}	23.0±0.3^{g}
Lower	ET	3.3±0.1^{c}	17.5±0.2^{d}	3.4±0.4^{b}	54.6±0.1^{d}
	ET:IP	4.6±0.2^{a}	16.5±0.1^{e}	4.7±0.1^{a}	56.5±0.5^{c}
	IP:ET	2.9±0.2^{d}	16.2±0.1^{e}	4.7±0.3^{a}	66.5±0.5^{b}
	IP	4.9±0.1^{a}	16.4±0.8^{e}	4.9±0.1^{a}	73.8±0.4^{a}

The means with different small letters in the same column were significantly different ($p<0.05$)

The results of extracted SBL are presented in Table 2. The lower layer had lower MC and AV than that of the upper layer. The lower layer of IP showed the lowest MC which was not significantly different from the lower layer of ET. In addition, the lower layer of IP and its mixture showed the highest AI matter. Nevertheless, it had the highest HI matter. The upper layer of ET showed the lowest HI matter (0.4±0.1%) which was not significantly different from the upper layer of ET:IP and the lower layer of ET.

Table 2 The chemical properties of extracted soybean lecithin in the upper and lower layers by using ratio of de-oiled gum:solvent at 1:1 (w/v)

Layer	Solvents	MC (%)	AV (mg KOH/g)	HI (%)	AI (%)
Upper	ET	9.6±0.3^{a}	20.7±1.9^{b}	0.4±0.1^{d}	45.1±0.3^{c}
	ET:IP	7.9±0.7^{b}	25.0±0.6^{a}	0.5±0.2^{cd}	32.3±0.5^{d}
	IP:ET	7.3±0.1^{bc}	22.4±0.3^{b}	0.7±0.2^{bc}	27.4±1.1^{e}
	IP	6.2±0.4^{c}	25.7±0.3^{a}	0.9±0.1^{b}	21.9±0.4^{f}
Lower	ET	4.6±0.9^{d}	20.4±1.6^{abc}	0.5±0.1^{cd}	74.3±0.1^{b}
	ET:IP	6.5±0.6^{c}	19.6±0.2^{cde}	1.5±0.1^{a}	80.1±0.4^{a}
	IP:ET	6.6±0.4^{bc}	18.4±0.3^{de}	1.5±0.2^{a}	79.7±0.2^{a}
	IP	4.8±0.8^{d}	17.6±0.5^{e}	1.7±0.4^{a}	80.7±0.7^{a}

The means with different small letters in the same column were significantly different ($p<0.05$)

The lower layers of all solvents showed a lower AV than the upper layer. Fatty acids found in the upper layer was higher than the lower layer and, therefore, contributed to the higher AV of the upper layer. The HI matter in the upper layer was lower than that of the lower layer for all treatments. The upper layer contained triglycerides and fatty acids that dissolved well in hexane and, therefore, contributed to lower values of HI matter in the upper layer (Parkinson, 1966). On the other hand, AI matter was found higher in the lower layer than the upper layer. This was because the lower layer contained phospholipid and glycolipids that contributed to the higher AI matter. The lower layer of IP had the highest value. However, all samples had MC and HI matters higher than the standard. There might be water and some impurities in the lecithin. Further improvement in extraction method and purification is recommended.

The reported chemical analysis of lecithin (Pragasam, Indira, & Krishna, 2002) showed lower MC (0.7%) and AV (20.9 mg KOH/g) than this present study. Nevertheless, the value in this report showed better AI matter (43.5%) in the upper layer of RBL. The food grade SBL must contain HI matter of not more than 0.3%. Protein fraction of lecithin would reside in this insoluble material (Food and Agriculture Organization of the United Nations, 2007). However, sunflower lecithin contained HI matter of not more than 1% (The United States Pharmacopeia Convention, 2014). Pranali and Amit (2017) reported the similar AV (22.1 mg KOH/g) of hydroxylated SBL to this research.

The PC content in both layers of the extracted RBL and SBL was ranged between 19.3±0.2 – 4.7±0.6% as shown in Figure 1. The upper layer had significantly higher PC content than the lower layer. The ET presented the highest PC content than other solvents (18.9±0.3% of RBL and 19.3±0.2% of SBL). For the lower layer, ET:IP presented the highest PC content (5.6±0.2%) for RBL ($p<0.05$) whereas IP showed the highest PC content for SBL (9.1±1.3%) which was not significantly different to IP:ET. The upper layer of ET showed the highest PC content. This was probably because most phospholipids dissolve well in ethanol. Phospholipids are polar ;therefore, they dissolved or liked polar solvents. Ethanol is more polar than isopropanol and was, therefore, able to provide a higher yield and PC content than isopropanol (Wu and Wang, 2004).

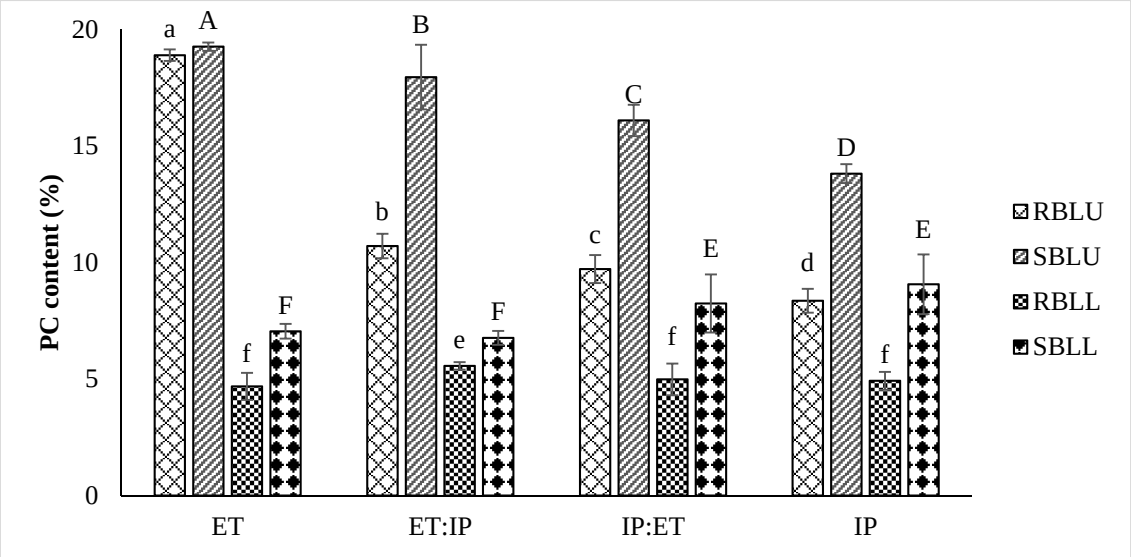

Figure 1 The PC content was measured at different ethanol to isopropanol ratios in the upper and lower layers by using ratios of de-oiled gum: solvent at 1:1 (w/v). RBLU is rice bran lecithin in upper layer, SBLU is soybean lecithin in upper layer, RBLL is rice bran lecithin in the lower layer and SBLL is soybean lecithin in lower layer. The mean with different small letters and capital letters was significantly different for RBL and SBL ($p<0.05$), respectively.

Vilas, Revanappa, & Bhaskar (2010) reported the extraction of PC content from de-oiled lecithin. They used different combinations of solvent and different lecithin to solvent ratios. It was found that the highest PC content was obtained using ethanol that is similar to that used in this present study. Pojanapornpan (2012) extracted soybean lecithin with different solvent concentrations and found that the percentage of PC in the upper layer (64.8%) was higher than the lower layer (17.16%). However, the studies by Flider (1985) and Aneja, Chadha, & Yoell, (1971) reported that the PC content in RBL and SBL were 22% and 18%, respectively.

Jangle, Magar, & Thorat (2013) studied the effect of the presence of IP along with ethanol as a mixed solvent. The ratio of 4:3 showed high purity PC and the corresponding yield obtained from different combinations of ethanol and IP on the extraction. In addition, higher ethanol concentrations decreased the purity of PC. The combination of different solvents causes different forms of polarity, which causes changes in the percentage of PC in the extraction (Vilas, Revanappa, & Bhaskar, 2010). Jangle, Magar, & Thorat (2013) reported a similar result. They showed the combinations of ET:IP; when the ethanol concentration was higher, the PC content was also higher and 4:3 ratio of ET:IP showed the highest PC content. Due to the highest PC content, ET was selected as the extraction solvent for lecithin and the upper layer of the extract was selected for further studies.

4.2 Effect of de-oiled gum to solvent ratio on the lecithin extraction

The effect of de-oiled gum: solvent ratios on the lecithin extraction is presented in Table 3. The 1:7 ratio of gum:ET showed better MC (4.2±0.1%), AV (20.5±1.2 mg KOH/g) and HI matter (1.3±0.1%). Nevertheless, the 1:3 ratio had the highest AI matter in RBL. The chemical properties of SBL were determined. It was found that the MC (5.4±0.4%), AV (21.8±1.5 mg KOH/g) and HI matter (1.5±0.1%) of the ratio of 1:7 were better than the other conditions. However, its AI matter (43.1±1.9%) was the lowest.

The de-oiled gum: solvent ratio of 1:1 from the previous part showed higher PC content than other ratios. The results of this study showed conflicts with the theory. It was probably due to, when using more solvents, other substances were extracted together with the PC such as PE and PI. These substances are weakly polar and can be extracted by ethanol. Therefore, it decreased the PC content (Pojanapornpan, 2012).

Table 3 The chemical properties of extracted rice bran lecithin and soybean lecithin in the upper layer by using various ratio of de-oiled gum: ethanol (w/v)

Sources	Ratio (gum:ethanol)	MC (%)	AV (mg KOH/g)	HI (%)	AI (%)
Rice bran	1:3	5.8±0.1^{a}	25.5±0.1^{a}	1.8±0.1^{a}	52.3±0.8^{a}
	1:5	5.9±0.1^{a}	21.6±0.2^{b}	1.6±0.3^{ab}	48.7±1.4^{b}
	1:7	4.2±0.1^{b}	20.5±1.2^{b}	1.3±0.1^{b}	42.7±0.8^{c}
Soybean	1:3	6.8±0.2^{A}	31.9±0.1^{A}	1.9±0.5^{A}	55.7±1.0^{A}
	1:5	5.5±0.5^{B}	24.4±0.2^{B}	1.6±0.3^{AB}	52.4±0.7^{B}
	1:7	5.4±0.4^{B}	21.8±1.5^{B}	1.5±0.1^{B}	43.1±1.9^{C}

The means that with different small letters and capital letters in the same column were significantly different ($p<0.05$)

The PC content at different de-oiled gum: ethanol ratios is shown in Figure 2. It was found that the PC contents of RBL at the ratio of 1:3 (10.2±0.3%) and 1:5 (9.9±0.1%) were not statistically different. In SBL, the ratio of 1:3 showed the highest PC content (17.9±0.3%).

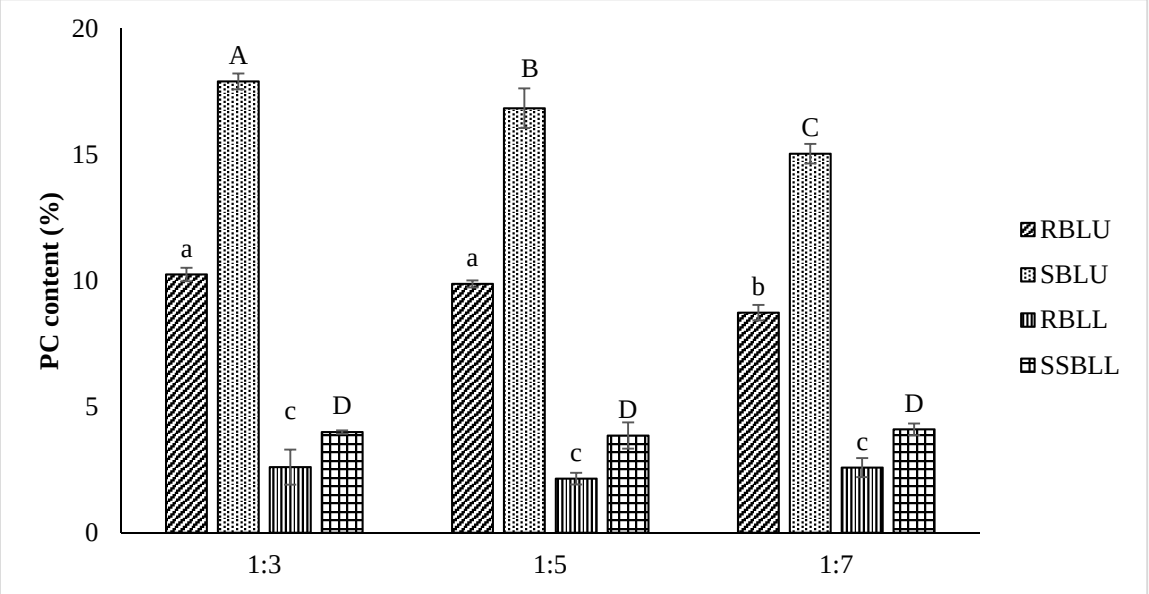

Figure 2 The PC content was measured at different de-oiled gum to ethanol ratios in the upper layer, RBLU is rice bran lecithin in the upper layer and SBLU is soybean lecithin in the upper layer. The means with different small letters and capital letters were significantly different for RBL and SBL ($p<0.05$)

The report on extraction of SBL was conducted by Pojanapornpan (2012). It was found that increasing the ethanol to gum ratio reduced the PC content. The ratio of 1:1 presented the highest PC content as shown in Figure 1. However, the report by Jangle, Magar, & Thorat (2013) found that as the de-oiled gum to ethanol ratio increases, the percentage purity of PC also increases. The different ratios of de-oiled gum to ethanol were studied and, at the ratio of 7:1, the percentage purity of PC obtained was the highest of the combinations studied in this experiment. Similar results were reported by Vilas, Revanappa, & Bhaskar (2010) on the percentage of PC extracted at different de-oiled gums to ethanol ratios. It can be seen that the percentage of PC in the extracted phase increases with the increase in lecithin to ethanol ratio up to 1:7.

4.3 The comparison of extracted lecithin to commercial soybean lecithin

The condition that provided the highest PC content was selected to compare with the commercial SBL. The results are shown in Table 4. Commercial SBL presented better MC (3.4±0.1%), HI matter (0.3±0.1%) and AI matter (96.6±0.1%) than the extracted lecithin. No significant difference was found for AV for all samples. Nevertheless, the extracted lecithin had better PC content than the commercial soybean lecithin.

Table 4 The chemical properties of extracted lecithin and commercial soybean lecithin

Properties	Extracted lecithin		Commercial
	RBL	SBL	
MC (%)	4.1±0.1^{b}	9.6±0.3^{a}	3.4±0.1^{b}
AV (mg KOH/g)	27.5±0.2^{ns}	20.7±1.9	26.8±4.0
HI (%)	2.0±0.1^{a}	0.4±0.1^{b}	0.3±0.1^{b}
AI (%)	46.6±0.2^{b}	45.1±0.3^{c}	96.6±0.1^{a}
PC (%)	18.9±0.3^{a}	19.3±0.2^{a}	16.6±0.1^{b}

The means with different small letters in the same row were significantly different ($p<0.05$), ns, not significantly different ($p>0.05$)

The food grade lecithin or food additive meets the following specifications. MC was not more than 4%, AV was not more than 40 mg KOH/g, HI matter was not more than 0.3% and AI matter (phosphatides) was not less than 50% (Food and Drug Administration, 2013). The report on the determination of the commercial lecithin was conducted by Penci, Constenla, & Carelli (2010). They found that it had MC of 2.84%, AV of 25.7 mg KOH/g, HI matter of 0.29% and AI matter of 93.4% which is similar to this present research, but the PC content (39.0%) was higher than the present study.

5. Conclusion

The extraction of lecithin from RBG and SBG using the water degumming process was achieved. Ethanol was suitable for the extraction of RBL and SBL. The upper layer showed higher PC content than the lower layer. The ratio of gum to ethanol at 1:1 showed a reasonable PC content and higher PC content than commercial soybean lecithin. Nevertheless, the commercial SBL had chemical properties conform to the standard whereas those for RBL and SBL were above the standard. Further improvement in extraction must be carried out in order to reduce the MC of the lecithin and to make the lecithin more purified: further purification is necessary.

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