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RESEARCH ARTICLE

Phytosterol Composition in Various Kinds of Red Beans Processing (*Vigna angularis*)

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Abstract

The high level of blood cholesterol can be overcome by consuming phytosterols obtained from plants, especially nuts. One of the leading beancommodity in the society is red beans (*Vignaangularis*). This study aims to identify the content of phytosterols in red beans and the processing effect of phytosterols. Red beans sample which has been processed, was extracted with n-hexane, acetone, and hydrolyzed. The results of sample extraction were analyzed using the 6890 Series Agilent Gas Chromatography instrument with a Flame Ionization Detector (FID) and HP-5 column (5% Phenyl, 95% Methyl Siloxane). Phytosterol content in the form of campesterol, stigmasterol, and sitosterol was found in red bean samples. The most composition of stigmasterol was found in baked samples and significantly different from boiled samples ($p < 0.00$) and steamed ($p < 0.00$).Meanwhile, the most composition of campesterol is in raw samples and not significantly different from fried samples (p 0.897), roasted (p 0.996), and baked (p 0.994). The processing can increase or decrease the content of phytosterols in red beans because the release of ester-phytosterol bonds can or cannot occur. The method of processing red beans by roasting or bakingproduces the most complete composition of phytosterol compared to other processing methods.

Keywords: *Cholesterol, Food Processing, Gas chromatography, Phytosterols, Red beans.*

Introduction

Cardiovascular disease due to cholesterol buildup still needs special attention from the society[1]. High cholesterol levels is one of the highest contributors so that it has an impact to the high cost of treatment [1]. Currently there are changes in treatment guidelines from the American College of Cardiology/ American Heart Association (ACA/ AHA) in 2013 to decrease blood cholesterol levels [2].

Lifestyle arrangement such as exercise, not consuming cigarettes and alcohol, controlling body weight, changing the dietary habit become the main therapy recommended besides the use of drugs [1, 2, 3, 4]. However, there are still many cases of death foundbecause of the diseaseuntil now [5]. Based on WHO reports, cases of death due to high cholesterol levels reached a ratio of 2.6 cases per one million population in the world [5].

In 2011 to 2012, around 95 million people in the United States (> 20 years) had total cholesterol levels above 200 mg/dL and nearly 29 million people had cholesterol levels of more than 240 mg/dL [6]. 6.1% of the population in Australia (1.5 million total population) has the high cholesterol levels [7]. The cases of hypercholesterolemia as much as 29% occur in the adult population in Southeast Asia [5].

Cholesterol is a structural lipid that is important in the formation of body cell structures [8]. However, the large amounts of cholesterol can become precipitations that narrow down the diameter of the vascular wall and initiate heart disease [9, 10]. In addition to genetic factors, this is supported by the lack of physical activity and a high-fat diet [2, 3, 11]. Many research developments

have stated that high cholesterol levels can be controlled by phytosterol providing [12, 13, 14].Phytosterol reduce absorption and inhibit cholesterol synthesis so that the total cholesterol absorption decreases [15, 16]. Phytosterol are produced by plants such as milk, vegetables, garlic, and nuts [17, 18]. Beans have a composition of fatty acids, sterols, and micro also macroelements [19].

Some beans that contain phytosterol are pistachios, almonds, hazelnuts, walnuts, and peanuts [17]. However, there have not many studies discussed about the processing of phytosterol content in red beans until now. Determination of phytosterol levels qualitatively and quantitatively can be carried out in various ways one of them is using Gas Chromatography (KG) [20, 21].

Gas chromatography separates volatile solutes based on the boiling point of a compound [22]. In this study, qualitative analysis uses gas chromatography because this instrument is quite sensitive in detecting compounds, fast in the analysis process, can be combined with a mass spectrometer, has a high degree of accuracy, also reliable and simple [23, 24]. This study aims to identify the content of phytosterol in red beans and the effect of processing methods on these contents.

Method

This research is an experimental study to find out the content of phytosterol and the effect of processing to the red beans composition. The sample used was red beans (*Vignaangularis*) which had been identified in the Biology Services Unit, Faculty of Science and Technology, Universitas Airlangga. Qualitative and quantitative measurement used was the Hewlett Packard Gas Chromatograph 5890 series II instrument with a 300°C Flame Ionization Detector (FID). Gas chromatography is set with an oven temperature of 290°C for 30 minutes and an inlet temperature of 300°C; with column HP 5 (cross-linked 5% phenyl methyl silicone, 25 m x 0.32 mm x 0.17 µm film thickness), and a split ratio of 1:25.

The carrier gas is Helium 40ml/minute and the injection volume is 2 µl [25].The standards used for qualitative and quantitative analysis are cholesterol, campesterol, stigmasterol, and sitosterol (Sigma®). Samples were processed by boiling and steaming in 100°C water for 30 minutes; also fried, roasted, and baked at 180-185°C for 10-15 minutes. The samples used were 1 gram for each trial sample.

All samples were extracted into *n*-hexane fraction extracts that used forfree sterols analysis and hydrosylate fraction extracts for bound sterols analysis. This study used data analysis with the MANOVA test with a significant number (α) of 0.05 or 5%. It has carried out the normality test previously with the Saphiro-Wilk test and homogeneity test with Levene.

The first data observed was the ratio of retention time (relative retention time) of standard cholesterol, campesterol, stigmasterol, and sitosterol compared to the sample. If the sample of the relative retention time ratio is the same as the standard, then the sample contains compounds that are identical to the standard compounds.

After that, the phytosterol data in the sample is grouped to analyze the differences in the phytosterol composition and phytosterol content from various processing methods. The results of the phytosterol composition analysis can be seen from how many percentage of the phytosterol area that appear in each sample, while the phytosterol content is seen from the area of phytosterols that appear.

Results

Campesterol

The results of the campesterol test on red beans were found there was the highest composition in the raw sample (mean 3.24) followed by the roasted, baked, and steamed samples. Meanwhile, the lowest composition was found in boiled and steamed samples (mean 0.00) (see Table 1).

Table 1: Campesterol Descriptive Data

Processing Method	Solvent	Mean	SD	N
Raw	*n*-Hexane	6.47	.88	4
	Chloroform	.00	.00	4
	Total	3.24	3.51	8
Boiled	*n*-Hexane	.00	.00	4
	Chloroform	.00	.00	4

	Total	.00	.00	8
Steamed	*n*-Hexane	.00	.00	4
	Chloroform	.00	.00	4
	Total	.00	.00	8
Fried	*n*-Hexane	5.82	1.30	4
	Chloroform	.00	.00	4
	Total	2.91	3.22	8
Roasted	*n*-Hexane	6.16	1.33	4
	Chloroform	.00	.00	4
	Total	3.08	3.40	8
Baked	*n*-Hexane	6.14	.63	4
	Chloroform	.00	.00	4
	Total	3.07	3.31	8
Total	*n*-Hexane	4.10	3.07	4
	Chloroform	.00	.00	4
	Total	2.05	2.98	8

Stigmasterol

Descriptive data shows the highest mean value of stigmaterol composition found in the roasted sample (mean 36.05) followed by roasted sample (mean 33.99). The lowest stigmasterol composition was in fried sample (mean 16.88). In the boiled and steamed samples, we found the same mean value that was 18.96 (see Table 2).

Table 2: Stigmasterol Descriptive Data

Processing Method	Solvent	Mean	SD	N
Raw	*n*-Hexane	34.79	2.91	4
	Chloroform	.00	.00	4
	Total	17.39	18.69	8
Boiled	*n*-Hexane	37.92	3.39	4
	Chloroform	.00	.00	4
	Total	18.96	20.39	8
Steamed	*n*-Hexane	37.92	3.39	4
	Chloroform	.00	.00	4
	Total	18.96	20.39	8
Fried	*n*-Hexane	33.76	2.93	4
	Chloroform	.00	.00	4
	Total	16.88	18.15	8
Roasted	*n*-Hexane	35.01	.72	4
	Chloroform	32.96	.74	4
	Total	33.99	1.29	8
Baked	*n*-Hexane	36.01	3.38	4
	Chloroform	36.09	7.87	4
	Total	36.05	5.60	8
Total	*n*-Hexane	35.90	3.06	4
	Chloroform	11.51	16.90	4
	Total	23.71	17.21	8

Sitosterol

From the mean data, the highest composition of sitosterol was in boiled samples (mean 81.04) and steamed (mean 81.04). This value was followed by the roasted sample (mean 62.93) and the baked sample (mean 60.88). Meanwhile, the lowest sitosterol composition

was found in the raw sample (mean 29.37) (see Table 3).

Table 3: Sitosterol Descriptive Data

Processing Method	Solvent	Mean	SD	N
Raw	*n*-Hexane	58.74	2.50	4
	Chloroform	.00	.00	4
	Total	29.37	31.44	8
Boiled	*n*-Hexane	62.08	3.39	4
	Chloroform	100.00	.00	4
	Total	81.04	20.39	8
Steamed	*n*-Hexane	62.08	3.39	4
	Chloroform	100.00	.00	4
	Total	81.04	20.39	8
Fried	*n*-Hexane	60.42	4.13	4
	Chloroform	.00	.00	4
	Total	30.21	32.41	8
Roasted	*n*-Hexane	58.83	.67	4
	Chloroform	67.04	.74	4
	Total	62.93	4.44	8
Baked	*n*-Hexane	57.85	3.24	4
	Chloroform	63.91	7.87	4
	Total	60.88	6.44	8
Total	*n*-Hexane	60.00	57.58	4
	Chloroform	55.16	42.47	4
	Total	57.58	29.89	8

Effect of Treatment Processes on Phytosterol Content

The MANOVA test showed that the highest cholesterol composition was obtained from raw samples, but these results did not differ significantly from fried samples (p 0.897), roasted (p 0.996), and baked (p 0.994). In addition, stigmasterol was not detected in boiled and steamed samples.

Most stigmasterol compositions were obtained from baked samples and were not significantly different from raw samples (p 0.994), fried (p 0.995), and roasted (p 1,000). However, the stigmasterol composition of the baked sample was significantly different from the boiled sample (p <0.00) and steamed (p <0.00). Most sitosterol compositions were obtained from boiled and steamed samples which were significantly different from roasted and baked samples.

Discussion

The identification results show that campesterol, stigmasterol, and sitosterol are free phytosterols which found in raw, fried, roasted, and baked samples. Meanwhile, free phytosterols in steamed and boiled samples are stigmasterol and sitosterol. This is due to the phytosterol ester bond of the bound phytosterol which has not been released in the boiled and steamed samples so that free phytosterol cannot be detected. The ester-phytosterol bond from the bound phytosterol can be released by hydrolysis using water but this process is very slow. Phytosterol esters are more susceptible to oxygen and temperature than free phytosterols [23]. At temperatures of 160-200^{o}C, phytosterol bounded grade up to 1.3%, while degradation up to 2.5% occurs at temperatures of 300-310^{o}C [23]. Stigmasterol and sitosterol found as bound phytosterol to roasted and baked samples. In boiled and steamed samples, researchers identified there was sitosterol as bound phytosterol.

However, the bound phytosterol was not found in raw and fried samples. This can be caused by the bonding of phytosterol esters from raw and fried samples that cannot be released so that no bound phytosterol is detected. Identification of each compound was carried by comparing the retention time of the sample with the standard cholesterol, campesterol, stigmasterol, and sitosterol mixed.

The mixed standards are injected three times, at the beginning, middle and the end, so that if there is a shift in retention time, the nearest standard can be used as a reference. In addition to the retention time, the relative retention time of the sample and standard are calculated to be matched with each other.

The calculation of the relative retention time used sitosterol as a reference because there is sitosterol in all samples. The difference in relative retention time is only allowed up to

the 3% RSD limit. In the score plot, it was showed that the fried sample is located closer to the oil compared to the other samples, so it can be concluded that the cooking oil affects the composition of the phytosterols obtained, while the samples that are close together/ in groups, it can be concluded that the composition of the phytosterol is similar.

In loading the plot it was found that cholesterol, campesterol, stigmasterol, and sitosterol were influenced by the way of processing because cholesterol, campesterol, and stigmasterol showed values approaching +1 while sitosterol showed values approaching -1. The processing of red beans such as soaking and temperature when processing affects the texture of red beans and their composition [26].

Similar to peanuts which show that the boiling process increases phytosterol levels 2 to 3 times compared to raw peanuts [27]. Increased levels of phytosterols also occur in cabbage, cucumbers, celery, garlic, and boiled red peppers [18]. However, the processing had no effect on the composition of phytosterols in karabenguk beans [28]. Phytosterols can be degraded due to oxidation even in small amounts [23]. Based on the results of the descriptive sample test and the MANOVA test, the most cholesterol composition was found in the raw sample, but not significantly different from the fried, roasted, and baked samples.

The most stigmasterol compositions were found in baked samples, followed by raw, fried and roasted samples. Meanwhile, stigmasterol was not detected in boiled and steamed samples. The most phytosterol composition was found in boiled and steamed samples. Meanwhile, the smallest amount of phytosterol was in roasted and baked samples. From this explanation, it can be concluded that the composition of most campesterol was found in raw samples, the highest composition of stigmasterol was in baked samples, and the highest composition of sitosterol was in boiled and steamed samples.

Conclusion

Red beans (*Vignaangularis*) contain of phytosterols. There are 3 types of free phytosterols which found in raw, fried, roasted, and baked samples namely campesterol, stigmasterol, and sitosterol. Meanwhile, free phytosterols found in the steamed and boiled samples that are two kinds namely stigmasterol and sitosterol. There are two types of bound phytosterols which found in roasted and baked samples, namely stigmasterol and sitosterol, in boiled and steamed samples there is only sitosterol, whereas in raw and fried samples no bound phytosterols are detected. The processing also has the effect of the phytosterol composition in red beans (*Vignaangularis*).

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