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Research Article
Adenine, Guanine, Xanthine and Hypoxanthine Content in Various Indonesian Foods

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Abstract

Background and Objective: Adenine, guanine, xanthine and hypoxanthine (inosine) are purine bases. Hyperuricemia results if blood uric acid levels are higher than normal. Arthritis or gout will occur if the blood uric acid concentration is high, causing the deposit of uric acid crystals. Each purine base has a different effect on the kidney and can increase the uric acid levels or the formation of crystals. The aim of this study was to determine the total purine, adenine, guanine, xanthine and hypoxanthine contents in various Indonesian foods as a reference for food menu preparation for hyperuricemia sufferers. **Materials and Methods:** Total purine and purine base amounts were determined by high performance liquid chromatography (HPLC) using a Hitachi Gel 43013-N column with a 4×150 mm column length and a 260 nm Ultra Violet detector. **Results:** The highest total purine content in animal source foods was found in chicken intestine, followed by cow intestine and goat intestine. For plant source foods, the highest purine content was found in string beans, followed by cauliflower and soybeans. The highest hypoxanthine content in animal source foods was observed in beef, followed by cow intestine and goat intestine. The highest adenine content was observed in chicken intestine, followed by goat intestine and cow lung. The plant source foods with the highest hypoxanthine contents were cauliflower, followed by soybeans and jengkol. The highest adenine contents were found in string beans, cauliflower and melinjo leaves. Processing decreased the purine content of foods. **Conclusion:** Foods that contain high levels of purine, such as chicken intestine, cow intestine, goat intestine, string beans, cauliflower and soybeans, should be avoided or reduced in the diets of people with hyperuricemia. Processing can be a solution to reduce the purine content in foods.

Key words: Adenine, guanine, hypoxanthine, Indonesian foods, purine, xanthine

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Adenine, guanine, hypoxanthine (inosine) and xanthine are purine bases. Purines are molecules that exist in cells; they are considered purine bases if they stand alone without binding to another molecule and purine nucleosides if they bind to a pentose sugar. If the purine base binds to a pentose sugar and phosphoric acid, it is called a purine nucleotide[1].

In food, purines are found in the nucleic acids that exist in nucleoproteins. In the intestine, the nucleic acids are released from nucleoproteins by digestive enzymes and the nucleic acids are broken down into mononucleotides. Then, the mononucleotide is hydrolyzed to a nucleoside. Nucleosides can be absorbed immediately and some are further broken down into purines and pyrimidines. Purines are oxidized and can create gout. Uric acid can be absorbed through the intestinal mucosa and excreted in the urine. In animals that have uricase enzymes, uric acid is further metabolized into allantoin[2].

The normal content of plasma sodium urate is less than 7 mg dL^{-1} [1]. Based on clinical laboratory research, normal sodium urate levels for women are 2,4-5,7 mg dL^{-1} and 3,4-7 mg dL^{-1} for men. If the sodium urate levels in the serum exceed the sodium urate solubility, also called hyperuricemia, uric acid can form crystals, called tophi and settle. Tophi can occur in the joint space, causing inflammation, swollen joints, heat, redness and pain; this is known as gout or Pirai disease. In addition to occurring in joints, tophi can also accumulate in the ears, tendons, bursa, kidneys, blood vessels and heart. In the kidney, tophi will form acidic stones and is one of the causes of kidney stones[3].

Based on research conducted by Yenrina[4], Clifford and Story[5] and Brule *et al.*[6], who treated rats with purine bases, hypoxanthine was shown to influence rat urine allantoin levels, whereas adenine bases were the metabolites responsible for crystalline formation in the kidneys. Therefore, it is necessary to conduct research to determine the type of purine base and the total purine content contained in foods. The aim of this study was to determine the contents of total purine, adenine, guanine, xanthine and hypoxanthine in various Indonesian foods.

MATERIALS AND METHODS

Using a descriptive exploratory design, foodstuffs were selected based on a survey of hyperuricemia sufferers in the Padang city. The results of the previous survey showed that the types of food that could increase uric acid levels in the blood were animal source foods, nuts and green vegetables. Various types of food: chicken intestine, cow intestine, goat intestine, tripe, cow lung, beef, squid, full cream milk, kikil, egg white, egg yolk, string bean, cauliflower, soybean, melinjo leaf, pea, kale, spinach, peanut, melinjo fruit, cowpea, red bean, jengkol, green bean, tempeh and tofu were used in this study. Foodstuffs were purchased from several traditional markets. The chemicals used were a purin standard (adenine A8626, guanine G 0506, hypoxanthine H9377, xanthine×4002), $HClO_4$, KOH, NH_4CL, K_2HPO_4 and CH_3CN. The tools used were HPLC, Hitachi Gel column 43013-N, freeze dryer, centrifuge, filter paper, fat flask, reflux tools and others. Analytical Methods of Young[7] after some modification for the Determination of the Purine Base was used.

Sample preparation: Before analysis, the foodstuffs were first mashed and then dried by a freeze dryer. After drying, the fat of the frozen material was extracted with hexane and dried and the resulting dry and fat-free material was refined.

Purine extraction and separation: One gram of the freeze-dried material was added to 10 mL $HClO_4$, refluxed for 1 h at 100°C, adjusted to a pH of 11 by the addition of KOH and centrifuged at 3000 rpm for 10 min. The supernatant was taken to 20 mL and filtered with 0.45 µm millipore. The sample was then considered ready for further analysis.

Determination of the purine base: The analysis of the measurements of total purine content and purine bases used HPLC with a 260 nm ultra violet detector and a Hitachi Gel 43013-N column (4×150 mm column length) with a flow rate 1.0 mL min^{-1} and a column temperature of 70°C. The purin standard consisted of adenine, guanine, hypoxanthine and xanthine and was separated by HPLC as a standard. The total purine content was calculated from the contents of adenine, guanine, hypoxanthine and xanthine.

RESULTS AND DISCUSSION

Based on previous research, some foods commonly consumed by Indonesian people are suspected to cause a recurrence of hyperuricemia. Purine and purine bases have not been included in the list of food ingredients issued by the Indonesian Ministry of Health, making it difficult for hyperuricemia sufferers to adjust their diet. In this study, foodstuffs were divided into several categories: Animal, plant source foods, meat and processed meat and soybeans and processed soybeans. Table 1-4 present the results of the analysis of purine base content in the various foods.

Table 1: Purine bases and purine content of animal source foods

Food (100 g db)	Hypoxanthine (mg)	Guanine (mg)	Adenine (mg)	Xanthine (mg)	Purin (mg)
Chicken intestine	179.22	318.64	249.27	107.18	854.310
Cow intestine	220.55	448.51	-	182.58	851.630
Goat intestine	180.82	249.35	165.32	107.10	702.590
Cow rumen	137.51	297.99	9.24	25.16	469.900
Cow lung	24.57	196.04	148.86	28.65	398.120
Beef	237.79	87.14	43.57	17.13	385.640
Squid	39.50	73.97	62.35	13.10	188.910
Full cream milk	10.10	24.00	3.90	50.30	88.301
Kikil	11.37	42.02	24.26	4.01	81.660
Egg white	6.50	12.10	2.40	10.70	31.700
Egg yolk	10.61	2.70	-	1.35	14.660

Table 2: Purine bases and purine content of vegetable source foods

Food (100 g db)	Hypoxanthine (mg)	Guanine (mg)	Adenine (mg)	Xanthine (mg)	Purine (mg)
String bean	94.89	182.51	523.37	62.57	863.34
Cauliflower	139.34	243.43	215.31	105.95	704.04
Soybean	133.90	140.09	111.99	58.04	444.04
Melinjo leaf	53.52	169.84	142.84	-	366.20
Pea	23.63	167.00	142.48	-	333.12
Kale	8.26	83.27	68.70	138.18	298.41
Spinach	51.15	118.19	110.00	11.08	290.42
Peanut	55.54	89.64	48.69	41.64	235.50
Melinjo bean	7.92	30.72	25.22	158.84	222.71
Cowpea	-	69.31	50.31	97.07	216.69
Red bean	19.82	72.89	45.42	56.21	194.35
Jengkol	56.12	68.36	66.27	-	190.75
Green bean	53.49	62.07	44.41	11.55	171.51

Table 3: Purine base and Purine content of beef and processed beef

Food (100 g db)	Hypoxanthine (mg)	Guanine (mg)	Adenine (mg)	Xanthine (mg)	Purine (mg)
Raw beef	237.79	87.14	43.57	17.13	385.84
Boiled beef	131.87	51.81	45.14	33.11	261.92
Fried beef	194.46	114.29	45.30	30.56	384.61

Table 4: Purine base and Purine content of soybeans and processed soybeans

Food (100 g db)	Hypoxanthine (mg)	Guanine (mg)	Adenine (mg)	Xanthine (mg)	Purine (mg)
Raw soybeans	133.90	140.09	111.99	58.04	444.02
Raw tempeh	16.74	54.83	36.72	33.39	141.48
Raw tofu	15.43	57.81	29.48	4.84	107.55

Animal and plant source foods contain total purine adenine, guanine, xanthine and hypoxanthine at different amounts and with different compositions. Most beef and beef products contained a higher amount of hypoxanthine than adenine and guanine but the plant source products contained higher amounts of adenine and guanine than hypoxanthine (from 50.8-80.9% for processed meat)[8]. Based on the purine content, foodstuffs can be grouped into group A, which contains ingredients with high purine concentrations (150-1000 mg/100 g); group B, which contains ingredients with medium purine concentrations (50-150 mg/100 g) and group C, which contains ingredients with low purine concentrations (0-50 mg g^{-1})[9,10].

In boiled beef, there was a decrease in the total purine content from 385.64-261 mg/100 g db. This result occurred because of the release of purine, especially hypoxanthine and guanine, which are some of the purine bases that turn into xanthine, into the boiled water. According to Young[7], research comparing the purine levels in raw and roasted chicken indicates that adenine and guanine levels increased after processing due to water content and fat losses during roasting but the hypoxanthine content decreased due to it dissolving in water. According to Fardiaz[11], several nucleotide components can dissolve in water, especially at the time of boiling, which usually occurs in boiling meat, fish or shrimp that are more known as broth. Levels of purine in fried foods are not much different than those in raw meat and this result is thought to occur because purines do not dissolve well in fat or oil.

Soybeans processed into tempeh showed a reduced purine content from 444.02-141.48 mg/100 g db. This result occurs because purine is lost through seepage into the

water or through disposal along with soybean skin during the boiling stage of tempeh production. Tempeh has a higher purine content because of the purine contribution derived from the mold used as an inoculum in the process of making tempeh. The manufacture and coagulation of soy milk is thought to release purine buried in the soybean pulp. Dwiyanti[12] increased the adenine picrate and tempeh RNA through fermentation processes. Tofu has lower purine levels than soybean tempeh because of this stage.

Data on the purine content and purine bases obtained from this study can be used to complete the list of food ingredients in Indonesia. This composition list can be used to determine the food menu for people in Indonesia, especially those who suffer from hyperuricemia. However, this study measured the purine content and purine bases in only a few foodstuffs shown to increase blood uric acid levels. For patients with hyperuricemia, in it recommended to reduce the consumption of high purine foods.

CONCLUSION

Foodstuffs that contain high amounts of purines are derived not only from animals but also from plant source foods. The highest total purine content in animal source food was found in chicken intestine, followed by cow intestine and goat intestine. For plant source foods, the highest purine content was found in string beans, followed by cauliflower and soybean. The highest hypoxanthine content was observed in beef, followed by cow intestine and goat intestine. The highest adenine content was found in chicken intestine, followed by goat intestine and cow lung. The vegetables with the highest hypoxanthine contents were cauliflower, followed by soybeans and jengkol. The highest adenine contents were found in string beans, cauliflower and melinjo leaves. Processing methods, such as boiling, fermenting and frying, can decrease the purine content of foods. Foods that contain high purine contents should be avoided or reduced in the diets of hyperuricemia sufferers.

SIGNIFICANCE STATEMENTS

This study found complete data on the purine content and purine bases in some foodstuffs and processed foods commonly consumed by Indonesian people, which can be a useful guide for food menus for hyperuricemia sufferers. This study enables the addition of purine contents and purine bases to the database of Indonesian food ingredients, which other researchers have never examined before. Thus, nutritionists and the general public can regulate their diets to prevent or treat hyperuricemia.

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