

Purine Content in Various Type of Gulai as Specialty Food of West Sumatra

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Abstract – Gulai is one of the most well-known basic dishes in West Sumatra specialties made from chicken, fish, goat meat, beef, offal and vegetables. Gulai with animal ingredient is a food that generally contains high protein. Foods that have protein content, specifically have purine content which will cause hyperuricemia. The purpose of this study is to identify four purine bases (adenine, guanine, hypoxanthine and xanthine) in several types of gulai dishes typical of West Sumatra. Seven food samples were obtained from several restaurants in the city of Padang. Purine base identification is determined using the HPLC (High Performance Liquid Chromatography) method. The highest total purine content in several types of gulai dishes is found in spicy and sour fish gulai and the lowest in chicken gulai.

Keywords – Gulai, Purine, HPLC.

I. INTRODUCTION

Purines are nitrogenous bases in DNA and RNA that have a heterocyclic ring structure [1]. Purines can be in the form of adenine, guanine, xanthine, hypoxanthine which have a wide role in various biochemical processes in the body [2]. Purines are synthesized by multistep pathways known as de novo synthetic pathways, a group of purine molecules passing through several steps where they can exchange, and finally they are degraded and excreted as uric acid [3]. The structure of various purines are shown in Fig. 1.

In food, purines are bound to nucleic acids in nucleoproteins whereas in the intestine, nucleic acids are released from nucleotides by digestive enzymes, and nucleic acids are broken down into mononucleotides. Mononucleotides are hydrolyzed into absorbable nucleosides

and some are further broken down into purines and pyrimidines [4]. In humans, through metabolic processes, purine molecules are absorbed by the small intestine and then degraded and excreted as uric acid in the urine [5]. The final product of purine metabolism is gout and increased levels of uric acid in blood serum causing gout or hyperuricemia [6]. Gout is a condition of the accumulation of uric acid crystals in the joints. The buildup occurs due to excess uric acid production or suboptimal excretion of uric acid as a product of purine catabolism. Uric acid production and metabolism is a complex process involving various factors that regulate liver production, as well as kidney and intestinal excretion [7].

Gout tends to increase in the future and has entered a younger age, namely productive age which has an impact on decreasing work productivity. The prevalence of gout in

Indonesia is suffered by under 34 years of age by 32%. Risk factors that cause gout are age, excessive intake of purine compounds, excessive alcohol consumption, obesity (obesity), lack of physical activity, hypertension and heart disease, certain drugs (especially diuretics) and impaired kidney function [8]. A high purine diet associated with high protein foods can trigger gout. A condition called hyperuricemia is a risk factor for gout in which the serum uric acid concentration in the body is above 7 mg per dL, (or 420 $\mu\text{mol per L}$) [1].

According to K. Inazawa [9] in oral intake of purine in healthy subjects with hyperuricemia or gout, oral hypoxanthine, adenosine 5'-monophosphate (AMP), guanosine 5'-monophosphate (GMP), inosine 5'-monophosphate (IMP), and serum adenine uric acid levels increase, while guanine and xanthine have no effect. Adenine, hypoxanthine, AMP, GMP, and IMP have a greater hyperuricemic effect in subjects with gout compared to hyperuricemic and normouricemic controls.

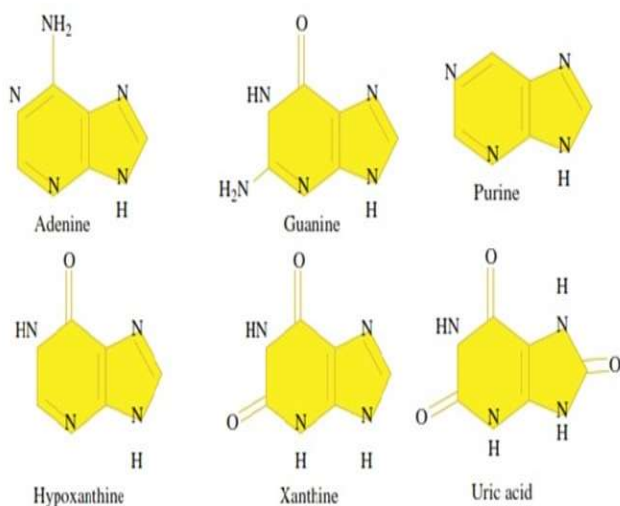


Fig. 1. Molecular structures of Various Bases (Kumari, 2017)

West Sumatra has a variety of typical foods, one of them is *gulai*. *Gulai* is a food with various types of raw materials such as beef, chicken meat, fish meat, goat meat, offal and vegetables. *Gulai* has the characteristics of dishes with spices such as turmeric, coriander, pepper, galangal, ginger, red chili, onion, garlic and others which are mashed, mixed and then cooked in coconut milk. Foods with animal ingredient have high protein content. According to Hecia and Clifford [10];[2] uric acid production will increase due to consuming protein.

There are many factors that cause excess uric acid in blood plasma, namely (1) genetic factors: Lesch-Nyhan, deficiency of the glucose 6-phosphatase enzyme, (2) food consumption factors, (3) disease factors and drug side effects from diseases such as kidney disorders, hypertension, diabetes mellitus, hyperlipidemia, psoriasis, hypothyroidism, obesity and leukemia [11]; [12]; [13]; [2].

Serum uric acid levels that exceed normal are called hyperuricemia. There are two mechanisms that can cause hyperuricemia, namely over production and decreased uric acid excretion. Both can be primary or secondary Diet is also one of the factors that influence the production of serum uric acid. This is related to the increase in exogenous purines that are metabolized by the body. Carbohydrates, protein, and fat have an influence on serum uric acid levels.

One of the cause of hyperuricemia and gout is increased uric acid production in the body due to disorders of innate purine metabolism and excess consumption of high purine content, however data that directly link food intake with hyperuricemia are limited. The purpose of this study was to determine the purine content in various types of *gulai* typical of West Sumatra using HPLC.

II. MATERIALS AND METHODS

Various *gulai* are obtained from restaurants in the city of Padang. The chemicals used were adenine A8626, guanine G 0506, hypoxanthine H9377, xanthine X4002, HClO_4 , NH_4Cl , K_2HPO_4 and KH_2PO_4 . The tools used were HPLC, Waters C-18 end capped column, freeze dryer, centrifuge, filter paper, fat flask, reflux tools and others. Analytical Methods of Brulè, D., et al [14] after some modification for the Determination of the Purine Base was used.

A. Sample Preparation

Food samples were first mashed and then dried by a freeze dryer. Then the fat of the frozen material was extracted with hexane and dried. Dry and fat-free material was refined.

B. Purine extraction and separation

100 mg of the freeze-dried material was added to 4 mL HClO_4 , hydrolysis for 6 h at 105°C , adjusted to a pH of 4,8 by the addition of NH_4Cl . The solution was transferred to a 50 mL volumetric flask and then adjusted with aquadest. Then, centrifuged at 3000 rpm for 15 min. The sample were then filtered with $0.45 \mu\text{m}$ Millipore filters then considered ready for further analysis by the high performance liquid chromatography (HPLC).

C. Determination of the purine base

The measurements of total purine content used HPLC with a 254 nm ultra violet detector and a Waters C-18 column (4×150 mm column length) with a flow rate 1.0 mL/min. Adenine, guanine, hypoxanthine and xanthine and was separated by HPLC as a standard. Each sample was analysed in triplicate. Data was expressed as mean $\pm$ SD. The total purine content was calculated from the contents of adenine, guanine, hypoxanthine and xanthine.

III. RESULTS AND DISCUSSION

A. Water Content

Water is an important component in food because it can affect the appearance, texture, and taste of food. In addition, water acts as a carrier of food substances and metabolic waste, as a reaction medium that stabilizes the formation of biopolymers, and so on.

Animal and vegetable food ingredients have different amounts of water content. Moisture content was determined using AOAC 2000 [15]. The difference between fresh and dry weights is taken as the amount of water available and converted to a percentage. The results of water content analysis can be seen in Table 1.

TABLE I. AVERAGE VALUE OF WATER CONTENT IN A VARIOUS OF *GULAI* AS SPECIALTY FOOD OF WEST SUMATRA

No.	Sample	Water content (%) $\pm$ SD
1	Chicken <i>gulai</i>	71,37 $\pm$ 0,65
2	Spicy and sour fish <i>gulai</i>	68,94 $\pm$ 0,88
3	Fish <i>gulai</i>	76,73 $\pm$ 0,29
4	Intestinal <i>gulai</i>	48,01 $\pm$ 3,70
5	Cattle foot tendon <i>gulai</i>	62,90 $\pm$ 9,20
6	Cattle brain <i>gulai</i>	56,34 $\pm$ 5,25

In table 1. It shows that the fish *gulai* contains the highest water content when compared with the others which is equal to 76.73%. The lowest water content contained in the intestinal *gulai* is equal to 48.01%. High water content has an important role in metabolic reactions and helps to dissolve certain elements easily [16]. Storing power of materials, free water and bound water, wet and dry base water content, water activity, relative humidity and absolute humidity as well as the physical properties of the material are factors that influence the determination of the water content of a food.

B. Purine Content

Using the modified Brulè, D., et al [14] method, purine levels in various types of *gulai* can be determined. The way HPLC works is that samples are sent through narrow columns packed with particles not larger than 50 μ m using pressure. The separation is based on the differences of the speed of migration of the sample through the column. To measure the amount of purines present, the peak area of the sample is compared to the peak area of a standard solution whose concentration is known, so that the purine content in the sample can be measured. The chromatogram of purine standar shown in Fig. 2. Based on the research of Titkova et al., [17] reverse phase HPLC has been proven to be very efficient with high column efficiency, produces good reproduction, and high speed and sensitivity for analyzing nucleic acid metabolites in biological fluids.

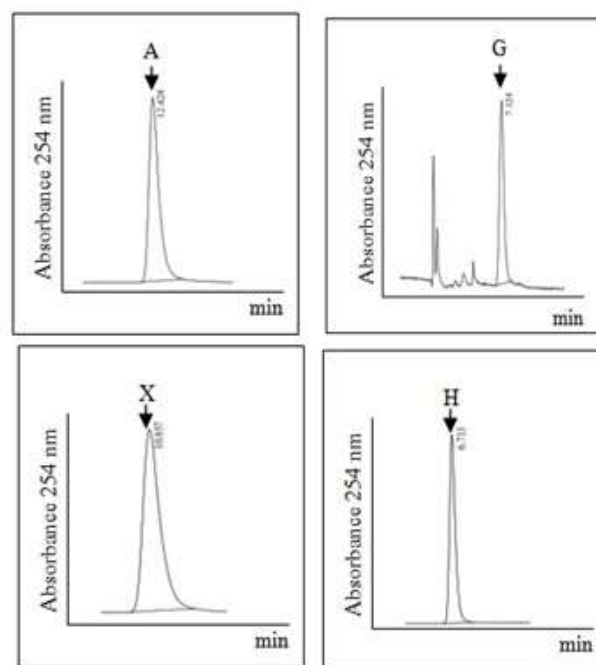


Fig 2. The chromatogram of standards purine bases

The total purines of adenine, guanine, xanthine and hypoxanthine in various types of *gulai* dishes have different amounts and with different compositions (Table 2).

TABLE III. PURINE CONTENT IN VARIOUS TYPE OF *GULAI* AS SPECIALTY FOOD OF WEST SUMATRA

N o	Food (100g db)	Adenine (mg)	Guanine (mg)	Xanthine (mg)	Hypoxanthine (mg)	Total (mg)
1	Chicken <i>gulai</i>	158,17 ± 11,56	-*	57,26 ± 15,84	-*	215,42
2	Spicy and sour fish <i>gulai</i>	168,50 ± 94,40	-*	-*	908,17 ± 426,91	1076,67
3	Fish <i>gulai</i>	103,83 ± 60,67	-*	-*	469,33 ± 281,30	573,17
4	Intestinal <i>gulai</i>	38,67 ± 11,18	425,83 ± 84,40	-*	113,33 ± 20,36	577,83
5	Cattle foot tendon <i>gulai</i>	52,36 ± 11,65	402,25 ± 115,61	-*	-*	454,61
6	Cattle brain <i>gulai</i>	-*	-*	521,17 ± 317,2	-*	521,17

*not detected

The amount of total purines was classified into five groups, the very low group: <50 mg/100 g, the low group: 50–100 mg/100 g, the moderate group: 100–200 mg/100 g, the high group: 200–300 mg/100 g, and the very high group: >300 mg/100 g [6]. Based on the results of the total purine, spicy and sour fish *gulai*, fish *gulai*, intestinal *gulai*, cattle foot tendon *gulai*, and cattle brain *gulai* included in foods that has very high purine, while chicken *gulai* is included in the high purine food group.

Based on the results, the highest purine content food were spicy and sour fish *gulai* with total purines 1076.67 mg / 100g db and the lowest purine content food were chicken *gulai* with total purines 215.42 mg / 100g db. Spicy and sour fish *gulai* have high levels of adenine and hypoxanthine compared to other types of *gulai*. The level of adenine in spicy and sour fish *gulai* is 168.50 mg / 100 g db and hypoxanthine levels 908.17 mg / 100g db. Chicken *gulai* adenine levels were 158.17 mg / 100g db and xantine levels 57.26 mg / 100g db. Adenine levels of *gulai* fish are 103.83 mg / 100 g and hypoxanthine 469.33 mg / 100 g db. Adenine intestinal *gulai* levels are 38.67 mg / 100 g, guanine is 425.83 mg / 100 g and hypoxanthine 113.33 mg / 100 g. Adenine cattle foot tendon *gulai* content is 52.36 mg / 100 g and guanine 402.25 mg / 100 g. In cattle brain *gulai* only xanthine is detected which is 521.17 mg / 100 g.

In this study guanine and xanthine levels are difficult to detect, this is because the pH of the mobile phase used is 4.8 whereas according to Pratiwi's research [18], the guanine and xanthine bases cannot dissolve in the mobile phase which has a pH of 4. The results of the experiments conducted Pratiwi [18] showed that guanine and xanthine can dissolve at an alkaline pH that is at a pH between 12-13. According to Clifford's research [19] in Ellington [1] adenine and hypoxanthine are known to have more impact on the

accumulation of uric acid in the body than the other two types of purine bases. Other studies also indicate that because adenine and hypoxanthine metabolized differently, they may have a different impact on uric acid levels than the other purine bases.

Based on the Indonesian Food Composition Table [20] chicken meat has a high protein content compared to other animal meat that is 18.2g / 100g ingredients, while protein of fish is 13.7g / 100g and beef is 17.5 / 100g ingredients. The product of protein metabolism contributes to gout biosynthesis, but it affects less uric acid production compared to purine nucleotides.

The main function of protein nucleotides together with pyrimidine nucleotides is as a precursor to the formation of nucleic acids namely deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In addition, protein nucleotides act as components of high-energy molecules, namely adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and guanosine monophosphate (GMP), guanosine diphosphate (GDP) and guanosine triphosphate (GTP).

Based on the results of the study it was found that the highest purine levels are *gulai* made from fish which have low protein levels. This may be due to the use of spices used in spicy and sour fish *gulai* dishes. According to K Sayuti et al [4] purine content in food will be reduced in the presence of processing, such as boiling, fermenting, and frying.

Purine content in raw materials and processed foods is different. In previous studies, the value of purine content in raw chicken was 582.34 mg / 100g db, after being processed into chicken *gulai* the value of purine level was reduced to 215.43 mg / 100 g db. Based on the results of the purine content of fresh food from the Kesuma S 2018, it was found that the total purine in the *kikil* as a raw material for making

cattle foot tendon *gulai* is 81.66 mg / 100 g and then the purine content after processed to 215.43 mg / 100 g. In raw intestine as raw material for making intestine *gulai* that is 851.63 mg / 100g db and after processing the purine level becomes 577.83 mg / 100g db

Food processing, cooking and storage are factors that can change the purine content [21], [22],[23] [24], [5]. Temperature and time are the main factors that influence the purine content during storage. Nucleotide pathways and purine base content patterns will slow due to decreased storage temperatures reducing enzyme activity [24];[5].

Although there is no intake of purines, uric acid still forms in substantial amounts because in the body the purine cycle occurs continuously along with the synthesis and decomposition of RNA and DNA [25], [26].

According to Passmore and Easwood [12] in Yenrina [2], there are ways that can be done to reduce uric acid levels in blood plasma that is medically, with drugs that can inhibit the work of xanthine oxidase enzymes so that the formation of uric acid can be reduced and by regulating diet. In addition, uric acid levels in the blood can be reduced by adjusting the pH of urine, an increase in urine pH will increase the solubility of uric acid, in this case commonly used is sodium bicarbonate, trisodium citrate or potassium salt [2].

According to Kaneko et al [6], the limit of daily purine intake for the treatment of hyperuricemia in Japan is ≥ 400 mg. To control uric acid levels, foods with a higher total purine amount and uricogenic purine base content must be avoided.

IV. CONCLUSIONS

Various types of *gulai* dishes as typical food of West Sumatra, have a high purine content. The highest total purines are found in spicy and sour fish *gulai*. Consuming foods with purine levels of >200 are considered high risk for people with hyperuricemia. Foods that contain not only high amounts of total purines but also high levels of adenine and hypoxanthine need to be avoided.

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