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FLUORIDE CONTENT OF COMMON LEGUME BEANS CONSUMED IN ETHIOPIA

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ABSTRACT. The most widely used legume beans food in Ethiopia are fava beans (*Vicia faba*), green gram (*Vigna radiata*), white lupin (*Lupinus albus*), and three types of kidney beans (*Phaseolus vulgaris*). These legume crops are grown in various regions of Ethiopia and can absorb significant amounts of fluoride from air, water, and soil. This study examined the fluoride content of legume beans collected from four different regions of Ethiopia by ion selective electrode potentiometry. Fluoride concentrations in this study were found to range from 1.9 to 22.8 mg/kg. Fava bean sample from Asella was found to contain higher fluoride (22.8 mg/kg) than in the fava beans from other sites and other beans from any site. Fluoride levels (13.0 mg/kg) in kidney bean from Migira was in the middle of the range while the fluoride levels (2.5 mg/kg) in the white lupin and green gram (1.9 mg/kg) were found in the lower end of the range. This study indicates that daily consumption of 100 g of fava and kidney beans may results in health problem while that of green gram and white lupin are safe for human health (will not exceed 3 mg/day set by Food and Nutrition Board of USA).

KEY WORDS: Fava bean, Green gram, Kidney beans, White lupin, Food, Fluoride

INTRODUCTION

Fluoride is abundant in nature and can be found in all soils, water, plants, and animals. It is commonly found in plants, which can be absorbed from the soil and water. The main sources of fluoride in the atmosphere are volcanic eruptions, industrial waste gases, and coal combustion [1]. Human are exposed to fluoride through food, water, and air [2]. There are a variety of sources of fluoride, including naturally occurring fluoride in foods and water, fluoridated water, fluoride supplements, fluoride dentifrices, and professionally applied fluoride gel [3, 4].

People are exposed to fluoride through inhalation, dermal contact, food, beverages and soft drinks, dental products, and drinking water. Food preparation can also affect the amount of fluoride. Studies have been published, but there are few studies on fluoride intake from food due to its status as a minor source of fluoride [5, 6].

Fluoride ion is not considered as essential for the growth and development of human including the development of tooth and bones and it is potentially toxic substance at higher levels [7]. Prolonged exposure to high levels of fluoride can lead to severe adverse effects. The two main signs of endemic fluorosis are skeletal fluorosis and dental fluorosis, which can harm the nervous system, skeletal muscles, kidney, liver, cardiovascular system, urinary system, reproductive system, and endocrine glands. Studies suggest that fluorosis can lower a child's IQ [8]. Around 8.5 million people in Ethiopia's Rift Valley region are exposed to high levels of naturally occurring fluoride. Groundwater in Ethiopia's Rift Valley region contains high levels of fluoride, which has negatively impacted the health of the local community [9].

Leguminosae is a large and significant family of flowering plants, containing 650-750 genera and 18,000-19,000 species of herbs, climbers, shrubs, and trees. Common legumes used in Ethiopia include Alfalfa, chick peas, clovers, cowpeas, kidney beans, lentils, green grams, peanuts, peas, pigeon peas, soy beans, and vetches. Legumes are a staple in Ethiopia and have a positive effect on poverty, food security, nutrition, health, and preserving the natural resource

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base. Despite their contributions, the adoption and use of legume technologies has remained negligible [10-12].

Farmers in Sub-Saharan Africa intercropped cereals and legumes to reduce the risk of monoculture. In Sub-Saharan Africa, the most common cereal-legume intercropping systems are finger millet with pigeon pea (*Cajanus cajan*), soybean (*Glycine max*), haricot bean (*Phaseolus vulgaris*), and groundnut (*Arachis hypogaea*) [13]. This study is based on the four types of legume beans namely fava bean, green gram, kidney beans and white lupin. There general features are briefly described in the following sections.

Fava bean (*Vicia faba* L.) is a cool-season food legume crop grown in Ethiopia with production of 4.4 million tons in 2014. The Amhara region has the highest pulse area coverage and production (43.7%), followed by the Oromia region (38.0%) and contributing 39.0% of national production. The fava bean is a highly prized crop for human nutrition, with Ethiopia being the world's largest consumer and producer. It has a high protein and bioactive compound content, making it an important pulse for export and nutritional diet. In terms of exports, it ranks fourth behind France, Australia, and UK [14-16].

Green gram (*Vigna radiata* L.) is an annual herb belonging to the family of legumes and is grown in North Shewa, Debere Sina, Qallu, and South Wollo, as well as some districts in Benishangul Gumuz Regional State of Ethiopia. It is a relatively new addition to Ethiopian pulse production and is gaining popularity in Asia [17, 18]. It is a pulse crop that is being grown on a small scale in a limited area, and was recently named the sixth export commodity by the Ethiopian Commodity Exchange [19]. Green gram have higher levels of folate, iron, protein, and vitamins 20].

Kidney beans (*Phaseolus vulgaris*) are a common source of food for both people and animals due to their high flavor, protein content, and inclusion of antioxidants, minerals, and polyphenols. Kidney beans are the most significant food legume for human consumption, providing bioactive substances like lectins, phytates, and phenolics which play important roles in metabolism [21, 22]. Dark red kidney, light red kidney, black turtle soup, and small white bean are the different bean varieties [23].

Red kidney beans are a significant legume crop that is widely grown in the Asian, South American, and African continents. They are derived from vegetable proteins, carbohydrates, vitamins, dietary fiber, minerals, trypsin inhibitors, lectins, and hemagglutinins, all of which are said to hinder proteolytic enzymes and reduce the digestibility and absorption of proteins [24, 25].

White kidney beans are a widely cultivated plant grown in South American nations such as Mexico and Argentina, with South America being the largest consumer region. It is a popular raw material in the canned food industry and is usually grown for fresh and dry consumption. White kidney beans are also known as navy beans and are popular in developed countries such as the United States and the United Kingdom [26-28].

Black kidney beans are a legume widely grown in Asian, African, and Latin American nations. They have a favorable balance of amino acids, vitamins, isoflavones, flavonoids, and polyphenols. However, due to the possibility of allergies, black kidney bean protein has not been widely used. The common bean has a high protein content and many cultivars have enough essential and non-essential amino acids to meet nutritional requirements [29, 30].

White lupin (*Lupinus albus* L.) are commonly used as a supplementary food preparation and a traditional sauce in North Western Ethiopia. They have a protein content of 33-47%, higher concentrations of lysine and sulfur, and low trypsin inhibitor activity. They also help to mitigate issues related to high blood pressure, insulin resistance, or elevated cholesterol levels [31-34].

The fluoride levels of some substances like tea [35, 36], spices [37, 38], cereals [39, 40], legumes [12], alcoholic beverages [41], cabbage [42], and soft drinks [43] in Ethiopia were determined. However, there is no literature report on the fluoride levels of legume beans consumed in Ethiopia. Hence this study was aimed to determine the levels of fluoride in four types of widely used legume beans namely fava beans, green gram, kidney beans and white lupin.

The main objective of the study was to investigate the levels of fluoride in widely consumed legume beans cultivated in four different regions of Ethiopia. Specific objectives were: (i) to quantify the fluoride content in four types of widely consumed legume beans (fava beans, green gram, kidney beans and white lupin) cultivated in four different regions of Ethiopia; (ii) to compare levels of fluoride in the four types legume beans; and (iii) to compare fluoride levels in legume beans of the present study with that reported in the literature.

EXPERIMENTAL

Apparatus and instrumentation

The sample was ground into a fine powder using an electronic grinder (stone grinder) and the sample amount was measured using an electronic balance. The experiment included drying the samples in an oven (Digitheat, J.P. Selecta, Spain), fusion of the sample in the nickel crucible using a muffle furnace (Crison GLP 22 Spain), and determination of fluoride concentration in the sample using a pH/ISE meter (Orion model, EA940 Expandable ion analyzer, USA). For preparation, storage, and measurement, plastic beakers and volumetric flasks were used. The sample solution and the residue were separated using Whatman 110-mm filter paper after pH adjustment.

Chemicals and reagents

All the reagents used in this study were of the analytical grade. For the preparation of reagents and standard solutions, distilled-deionized water was used. The fluoride stock standard solution was prepared using sodium fluoride (99.0% NaF, BDH Chemicals Ltd., Poole, England). Glacial acetic acid (99.5%, BDH Chemicals Ltd., Poole, England), sodium chloride (Scharlau, European Union), sodium citrate (Research-Lab Fine Chem Industries Mumbai, India) and EDTA (Reagent grade, Spain) were used to prepare total ionic strength adjustment buffer (TISAB) solution. Sodium hydroxide (Scharlau, European Union) solution was used to adjust pH of TISAB solution and hydrochloric acid (Scharlau, European Union) to adjust pH of the sample solution.

Sample collection

The fava beans, kidney beans and white lupin samples (500 g from each site) were collected from the local markets while visiting the representative region, which included Bure, Kosober, and Gojam Merawi in the Amhara Region, Asella, Adama Migera, Sebeta, and Guder in the Oromia Region, and Bensa in the Sidama Region. The green gram samples were purchased from two different supermarkets of Addis Ababa. Random sampling method was used. The samples were collected in the same harvesting season (November – December 2022). The study areas were selected based on the higher production of the legume beans.

Sample pretreatment

The collected samples of fava beans, green gram, white lupin, and three types of kidney beans were transported to the laboratories for analysis. The samples were washed with tap water and rinsed with deionized water. Washed samples were air dried at room temperature for one week. The dried samples were ground in a stone grinder and placed in plastic sample holder tubes until digestion.

Sample preparation for fusion

Each sample was individually placed in a 50 mL nickel crucible with an accurately weighted 0.5 g powdered sample before 6 mL of 9 M NaOH solution was added and thoroughly mixed. The mixture was heated to 150 °C for two hours in an oven to dry it. The residue was placed in a furnace and heated to 570 °C for two hours after drying it in an oven [44].

pH calibration

The fused sample was dissolved in de-ionized water. The prepared solution was extremely basic, necessitating pH adjustment to reduce hydroxide's interference with the fluoride ion during measurement. The pH of the sample solution was adjusted using the pH meter. Before measuring the pH of the sample solution and the blank solution, the pH meter was calibrated using a buffer solution of pH 4, 7, and 10.

Determination of fluoride in the legume bean samples

The ion-selective electrode (ISE) was used to determine the fluoride in this study. The fusion cake was allowed to cool to room temperature before being placed on a hot magnetic plate with 15 mL of deionized water. The solution was then transferred to a 50 mL plastic beaker, and the sample solution was treated with concentrated and dilute HCl to lower the pH to 5.0-5.4 using continuous string and pH control. The concentration of fluoride ions was determined by adding 5 mL of sample solution and 5 mL of TISAB to a homogeneous solution using a magnetic stirrer. Finally, the fluoride concentration in the solution was measured in triplicate for each sample with continuous strings and magnetic stirrers using an Orion ion selective electrode meter and an expandable electrode [44].

Fluoride standard solution

Standard fluoride solutions with concentrations of 0.1, 0.5, 1, 5, and 10 mg/L were prepared by appropriate dilution from 1000 mg/L of fluoride standard solution (2.21 g of NaF was dissolved in 1000 mL of deionized water to produce the standard solution) for calibrating ion selective electrode instruments. The calibration curve for the fluoride determination is shown in Figure 1. The detection limit of the fluoride ion selective electrode potentiometry used was 0.02 mg F/L and limit of quantification for the legume sample was found to be 0.06 mg F/kg.

Method validation

Sample prepared by alkali fusion and spiked with known concentrations of sodium fluoride was used in this study's analysis of fluoride concentration. Similar procedures were used to prepare the sample for the spiked samples, which were then prepared by adding a known quantity of fluoride standard solution to the sample of legume beans. The sample was then tested for fluoride levels to determine the recovery percentage. The fluoride content of the legume sample that was spiked was nearly equal to 100% of the fluoride content of the original (unspiked) samples. By comparing the outcomes between the fluoride found and the fluoride added, the percent recovery was thus determined [45].

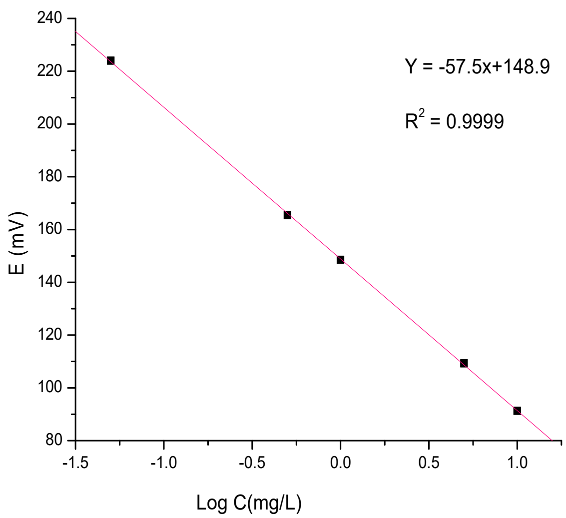

Figure 1. Calibration curve for fluoride determination.

RESULTS AND DISCUSSION

Recovery results of fluoride determination

The percentage recovery of the method was evaluated by calculating the percentage recovery (% R). Recoveries for legume beans samples were found in the range 91.8–108% (Tables 1–4).

Table 1. Recovery test results of fluoride levels in fava beans.

Sample site	Sample code	Amount in unspiked sample (mg/kg)	Amount added (mg/kg)	Amount in spiked sample (mg/kg)	Recovery (%)
Sebeta	S-1	3.4	3.35	6.7	98.5
	S-2	3.3	3.1	6.2	93.5
	S-3	3.2	3.2	6.4	100
Guder	G-1	3.4	3.35	6.7	98.5
	G-2	3.3	3.05	6.1	91.8
	G-3	3.4	3.35	6.7	98.5
Asella	A-1	24	24.04	48.08	94.7
	A-2	22	21.6	43.35	100.1
	A-3	22.1	21.7	43.55	99.3

Sample code S-1, S-2, S-3 represents sample 1, 2, 3 from Sebeta; G-1, G-2, G-3 represents sample 1, 2, 3 from Guder; A-1, A-2, A-3 represents sample 1, 2, 3 from Asella.

Table 2. Recovery test results of fluoride level in kidney beans.

Sample site	Sample code	Amount in unspiked sample (mg/kg)	Amount added (mg/kg)	Amount in spiked sample (mg/kg)	Recovery (%)
Bensa	B-1	3.2	3.1	6.2	96.8
	B-2	3.1	3	6	96.6
	B-3	3	2.95	5.9	98.3
	W-1	3.7	3.6	7.28	99.4
	W-2	4	3.93	7.86	98.2
	W-3	3.7	3.6	7.27	99.2
	R-1	3	2.95	5.9	98.3
	R-2	2.8	2.7	5.5	100
	R-3	2.9	2.85	5.7	98.2
Bura	R-1	2.5	2.45	4.9	97.9
	R-2	2.4	2.3	4.6	97.8
	R-3	2.4	2.35	4.7	97.8
	W-1	2.8	2.74	5.48	97.8
	W-2	2.6	2.54	5.09	98.0
	W-3	3	2.9	5.88	99.3
Migera	W-1	13.3	13	26	97.6
	W-2	13.5	13.25	26.5	98.1
	W-3	13.1	12.6	25.3	96.8
	R-1	11.4	11.15	22.3	97.7
	R-2	11.4	11	22	96.3
	R-3	11.6	11.5	22.6	95.6

Sample code B-1, B-2, B-3 represents black kidney bean sample 1, 2, 3; W-1, W-2, W-3 represents white kidney bean sample 1, 2, 3; R-1, R-2, R-3 represents red kidney bean sample 1, 2, 3 from respective sample site given in column 1.

Table 3. Recovery test results of fluoride level in green gram.

Sample site	Sample code	Amount in unspiked sample (mg/kg)	Amount added (mg/kg)	Amount in spiked sample (mg/kg)	Recovery (%)
Abader supermarket	GA-1	3	2.9	5.8	96.5
	GA-2	2.8	2.8	5.6	100
	GA-3	3	2.85	5.7	94.7
Belonyas supermarket	GB-1	2	1.9	3.8	94.7
	GB-2	1.8	1.75	3.5	97.1
	GB-3	2	1.9	3.8	94.7

Sample code GA-1, GA-2, GA-3 represents green gram sample 1, 2, 3 from Abader supermarket and GB-1, GB-2, GB-3 represents green gram sample 1, 2, 3 from Belonyas supermarket.

Table 4. Recovery test results of fluoride level in white lupin.

Sample site	Sample code	Amount in unspiked sample (mg/kg)	Amount added (mg/kg)	Amount in spiked sample (mg/kg)	Recovery (%)
Merawi	WM-1	2.4	2.6	5.2	107.6
	WM-2	2.4	2.5	5	104.0
	WM-3	2.6	2.7	5.4	103.7
Kosober	WK-1	2.6	2.7	5.4	103.7
	WK-2	2.7	2.8	5.7	107.1
	WK-3	2.4	2.55	5.1	105.8

Sample code WM-1, WM-2, WM-3 represents white lupin sample 1, 2, 3 from Merawi and WK-1, WK-2, WK-3 represents white lupin sample 1, 2, 3 from Kosober.

Comparison of the fluoride levels in each sample based on the differences in their areas

The fluoride contents of legume beans collected from different regions of Ethiopia are compared with respect to their cultivars and area of cultivation. The results are summarized in Tables 5 and 6. The comparison of fluoride levels in the three varieties of kidney beans showed that samples from Migera content much higher amount (11.5-13.0 mg/kg) of fluoride than the samples from other two sites which ranged (2.4-3.1 mg/kg). The levels of fluoride in the individual varieties (red, white and black) of kidney beans are comparable irrespective of the three sampling sites. One-way analysis of variance (ANOVA) indicated that there is no significant difference at 95% confidence level ($p = 0.5$) in the levels of fluoride in the red, white and black kidney beans from the three sampling sites. The level of fluoride (22.8 mg/kg) was found to be highest in the fava bean collected from Assela while Sebeta sample has a lowest concentration (3.3 mg/kg) of fluoride. ANOVA indicated that there is significant difference at $p = 0.5$ in the levels of fluoride in the fava beans from the three sampling sites. The fluoride levels in the green gram samples, which were purchased from two different supermarkets, ranged (1.9-2.9 mg/kg). ANOVA indicated that there is significant difference at $p = 0.5$ in the levels of fluoride in the green gram collected from the two supermarkets. White lupin samples collected from two different sites contained roughly similar concentration of fluoride (2.5-2.6 mg/kg). ANOVA indicated that there is no significant difference at $p = 0.5$ in the levels of fluoride in the white lupin collected from the two sites.

In general, the fluoride levels in legume beans depends on several factors such as genetic variations (type of legumes), geographical locations, fluoride content and pH of soil and surrounding water bodies, climatic conditions and environmental factors [48].

Table 5. The distribution of fluoride (mean ± SD, mg/kg) in kidney beans from different areas.

Sample site	Kidney beans		
	Red	White	Black
Bensa	2.9 ± 0.1	3.8 ± 0.17	3.1 ± 0.1
Bure	2.4 ± 0.06	2.8 ± 0.2	-
Migera	11.5 ± 0.12	13.0 ± 0.2	-

Table 6. The distribution of fluoride (mean ± SD, mg/kg) in fava bean, green gram and white lupin.

Sample site	Fava beans	Green gram	White lupin
Guder	3.4 ± 0.17		
Sebeta	3.3 ± 0.1		
Asella	22.8 ± 1.4		
Belonoyas supermarket		1.9 ± 0.12	
Abader supermarket		2.9 ± 0.12	
Kosober			2.6 ± 0.15
Merawi			2.5 ± 0.12

The comparison of amount of fluoride in fava beans, green gram, white lupin, and three types of kidney beans are shown as a bar graph in Figure 2. As can be seen from the figure, fava beans from Asella contains the much higher amount of fluoride (roughly 8-10 times) than the other beans except the kidney beans from Migera. The red and white kidney beans from Migera also content higher amount (about 4-5 times) of fluoride than the remaining legumes which contain much lower amount (1.9-3.5 mg/kg) of fluoride.

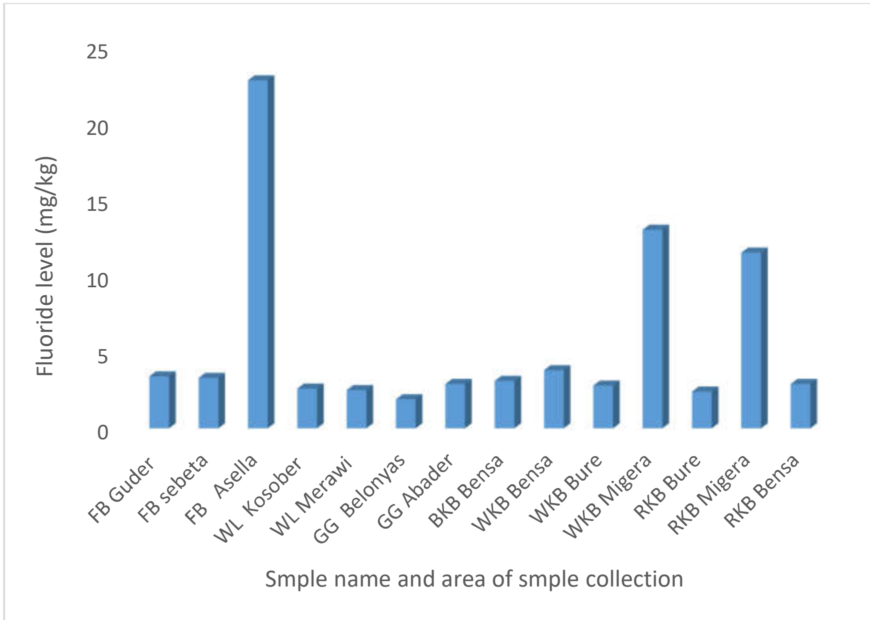

Figure 2. Comparison of levels of fluoride in legume samples (where, FB - fava beans, BKB - black kidney beans, WL - white lupin, WKB - white kidney beans, GG - green gram, RKB - red kidney beans).

Comparison of fluoride levels of this study with literature values

In this study, the mean fluoride concentration in the kidney beans was ranged (2.4-13.0 mg/kg) which are much lower than the fluoride level (31.7 mg/kg) reported in the kidney beans from Pakistan [46]. The fluoride level in green gram in this study was in the range (1.9-2.9 mg/kg) which is comparable to that from India (2.5 mg/kg) [47] but much lower than the fluoride levels in green gram from Pakistan (164 mg/kg) [46]. Fava beans in this study contained (3.3-22.8 mg/kg) fluoride which are much higher than that from India (0.015 mg/kg) [46]. The fluoride level in white lupin in this study raged (2.5-2.6 mg/kg). There is no study reported the fluoride content in white lupin from any other country. Hence, no comparison is done. Table 7 compares fluoride levels in legume beans to previously reported values.

Table 7. Comparison of fluoride levels in legume beans with previously reported values (mg/kg).

Legume bean	Concentration in mg/kg	Origin	Reference
Kidney bean	31.7	Pakistan	[46]
	2.4-13.0	Ethiopia	This study
Green gram	164	Pakistan	[46]
	2.5	India	[47]
	1.9-2.9	Ethiopia	This study
Fava bean	0.015	India	[47]
	3.3-22.8	Ethiopia	This study
White lupin	2.5-2.6	Ethiopia	This study

In general, the fluoride levels in legume beans depends on several factors such as geographical locations, fluoride content and pH of soil and surrounding water bodies, climatic conditions and environmental factors [48].

CONCLUSION

This study investigated the level of fluoride in legume beans including fava bean, green gram, kidney beans, and white lupin collected from four different regions of Ethiopia. The amount of fluoride in each samples varies significantly at p = 0.05 with sampling areas (as indicated by ANOVA test) which might be due to differences in the level of fluoride in groundwater, soil types, or soil pH. The fluoride concentration ranged from 1.9 to 22.8 mg/kg. Samples from Asella and Migera, both in the Oromiya region, have the higher fluoride levels, at 22.8 and 13.0 mg/kg, respectively. Because this region is in the Ethiopia's rift valley, the levels of fluoride in legume beans are found to be higher than legume beans from other regions. Samples from Kosober (2.6 mg/kg), Merawi (2.5 mg/kg) and Bure (2.4 mg/kg) have lower levels of fluoride. The mean fluoride levels in legume beans were in the order: fava bean (9.8 mg/kg) > kidney beans (5.6 mg/kg) > white lupin (2.55 mg/kg) > green gram (2.4 mg/kg) in the range. In general, the present study indicated that daily consumption of 100 g of fava and kidney beans may results in health problem while that of green gram and white lupin are safe for human health (will not exceed 3 mg/day set by Food and Nutrition Board of USA) [49, 50].

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