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In Vitro Determination of Fe Levels and Fe Bioavaailabilities Prediction from Dahlia Tuber Syrup (Pinnata Cav.)

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ABSTRACT: Dahlia tubers (Pinnata Cav.) with red flowers are known to have high inulin and fructooligosaccharides (FOS) in their tubers, they also contain various minerals such as Fe and others which are needed by the human body. Therefore, this study was conducted to discuss in vitro determination of Fe levels and Fe bioavailabilities prediction from dahlia tuber syrup. The Fe content in the Dahlia tuber syrup was determined experimentally using the X-Ray Fluorescence Spectrometer (XRF) instrument. The Fe bioavailability of dahlia tuber syrup was predicted based on the standardized inulin and FOS content of dahlia tubers against yacon tubers. The results showed that the Fe content in dahlia tuber syrup was 10, 2 ppm. The bioavailability of Fe in dahlia tuber syrup in vitro is predicted to be 69.20% - 94.73%. The prediction of a fairly high Fe bioavailability indicates that dahlia tuber syrup is expected to be quite good in increasing the absorption of Fe in the body which can have health effects, among others, can play a role in improving body metabolism and being able to reduce the risk of anemia due to iron deficiency.

KEYWORDS: Bioavailability, Dahlia tubers (Pinnata Cav.), FOS, Inulin.

INTRODUCTION

Dahlia flower plants have flowers of various colors, ranging from red, white, purple, orange, and yellow to other combinations. This plant has the potential to be developed as the main ingredient in the manufacture of fructose syrup because the tuber content has very high inulin [1]. The manufacture of dahlia tuber syrup refers to the general standard, to know the Fe contained in Dahlia syrup because if the Fe exceeds the standard in the human body, it will be poisoned.

Inulin contained in dahlia tubers is a linear polysaccharide consisting of a -2, 1-linked-d-fructofuranose chain ending with -d-glucose at the reducing end. The plants rich in inulin such as chicory, dahlia, asparagus, etc., can be used as a substrate and a strong inducer for inulinase production and can also be used to produce fructooligosaccharides and high fructose syrup [2].

FOS is prebiotic so that enzymes in the small intestine cannot be digested but are fermented in the large intestine by probiotic bacteria such as Bifidobacteria and Lactobacillus to produce SCFA (Short Chain Fatty Acid) [3]. Intake of rich FOS can increase SCFA which results in a lower pH in the colon [4]. Low pH can cause the small intestine villi to be higher and denser so that the absorption surface will be wider [5]. This causes the bioavailability of Fe to increase.

Bioavailability is the proportion of a nutritional component that can be used to run and maintain normal body metabolism [6]. Minerals are bioavailable if the mineral is in the form of dissolved minerals, but not all dissolved minerals are bioavailable the dissolved mineral form is needed to facilitate the absorption of these minerals in the body [7].

The administration of dahlia tuber syrup which is known to contain Fe will result in the human body in the iron mineral needed in a certain amount and is also used to affect the nutritional content of food and bioavailability in the processing process [8]. Foods that contain high bioavailability and absorption are fish, meat, and liver. While foods that have low absorption and bioavailability are cereals, beans, rice, and vegetables. Iron anemia can also attack children with worms, malnutrition, good nutrition, and obesity [9].
In Indonesia, iron deficiency in food is a major cause of anemia. This is necessary for the formation of hemoglobin, where hemoglobin is a metalloprotein, which is a protein containing iron in red blood cells that functions as a carrier of oxygen from the lungs to the rest of the body. This disease is usually referred to as "iron deficiency anemia" [10].

Iron deficiency has an impact on growth and development in children, negative impacts on children such as decreased immunity such as infections, esophageal strictures, impaired learning achievement, or other mental disorders that can last a long time and even persist.

The positive impact is healing minor complications by giving iron [11]. So that the problems related to the need for handling various kinds of products that contain iron, one of which is dahlia syrup.

The limitation of bioavailability analysis is that only two types of enzymes are used, pepsin and pancreatin which function to break down proteins so that the bound calcium will be released and can diffuse into the dialysis bag. In the process that occurs in human digestion, there are not only two enzymes, where different enzyme activities will produce different levels of bioavailability. The existence of complex interactions between minerals, dietary fi, be,r, and other components in food also make mineral balance in humans difficult to study in vitro [12]. However, this method is considered very profitable because this method can be done quickly, practiced, ally, and cheaper so that the results can be known quickly [13].

Malnutrition is a dangerous disease if not treated, one of the efforts to overcome it is by making dahlia syrup which is known to have iron bioavailability which is beneficial for the body. Thus, this article aims to determine the in vitro bioavailability of iron in dahlia syrup (Pinnata Cav.) with predictions of inulin and FOS estimates

RESEARCH METHODS

Tools and Materials

The tools and materials used are Dahlia tubers (Pinnata Cav.), which were obtained from the Wagir sub-district, Malang Regency. X-Ray Flourescene (XRF) with PANalytical Minipal4 type, Dahlia syrup, Dahlia inulin, and FOS dahlia

Procedures

To make syrup, fresh dahlia tubers are washed and then peeled and cut into small pieces, then blended (without using water) until smooth. Followed by filtering, then the sample is evaporated. Then filtered again and continued with a freeze dryer for ± 6 hours until the syrup thickens [14]. Determination of Fe content in syrup, Dahlia syrup is heated using a frying pan with a low flame and stirred until it becomes a powder, then the powder is tested using XRF instruments and the results obtained are Fe levels in the form of percent which are then converted into ppm.

Inulin Review

Dahlia tuber inulin was taken by weighing 1 kg of blood red-flowered dahlia tuber, cleaned and washed, peeled then cut and blended with the addition of 2 L of water. After that, it was heated at a temperature of 800C –900C for 30 minutes, then filtered and the filtrate was taken. Then cooled and added 30% ethanol to as much as 40% of the volume of the filtrate. The solution was stored in the freezer at 20C for 18 hours. Then the filtrate was allowed to stand at room temperature for 2 hours and then centrifuged at 1,500 rpm for 15 minutes. The sediment from this centrifuge is brownish-white (wet inulin) [15].

Fructooligosaccharide Review

The manufacture of FOS was carried out by hydrolyzing inulin flour using crude inulinase enzymes and commercial inulinase enzymes based on the method used by Singh and Singh. The substrate in the form of inulin 5% (w/v) with a pH value of 6 was put into an Erlenmeyer and added commercial inulinase 5 enzyme at a dose of 1, 3, and 10 U/g, and crude inulinase with an enzyme dose of 1, 2, and 3 U/. Hydrolysis was carried out within 24 hours at 3 hydrolysis time points (0.25; 12; and 24 hours) in a water bath at 45 oC. Each Erlenmeyer contained 20 ml of inulin substrate and inulinase enzyme at each dose and was taken at one point in hydrolysis time. During the hydrolysis process, the product was taken at 0.25 hours; 12; and 24. The product that has been taken is immediately bleached by adding 1.5% (w/v) activated charcoal and then heated at a temperature of 80-90°C for 15 minutes. Then filtered using filter paper. This direct bleaching is intended to inactivate enzymes that may be attached to the product. The amount of FOS produced can be calculated from the initial inulin content minus the remaining inulin content in the final product of hydrolysis [16].

FE Bioavailability Review

Determination of the bioavailability of Fe in dahlia tuber syrup (Pinnata Cav.) using a prediction method with comparative studies in previous studies using the Science Direct database, Google Scholar, Pubmed, Elsevier, and Researchgate. Then predicted the results of the bioavailability of Fe in dahlia syrup (Pinnata Cav.) in vitro by comparing the levels of inulin and FOS from yacon tubers.

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RESEARCH RESULTS AND DISCUSSION

Dahlia Syrup Making

Dahlia tubers used are bulbs with red flower types (Pinnata Cav.), Dahlia tubers are washed then peeled and cut into small pieces using a knife, and washed again until clean. Then weighed as much as 500 grams then put into a blender, and blended without using water.

Figure 1. Dahlia Bulb Filtrate

Followed by filtering, then the sample is evaporated. Then filtered again and continued with a freeze dryer for ± 6 hours until the syrup thickens [14].

Making FE Content in Syrup

Fe content was obtained by making dahlia syrup in powder form, then tested at 10,0562 grams using the XRF instrument, the test results can be seen in table 1. Below.

Table 1. XRF Instrument Testing Results

Komponen	Unit
P	4%
S	0,93%
K	58,7%
Ca	32,5%
Fe	0,75%
Cu	1,4%
Zn	0,37%
Rb	0,86%
Sr	0,44%
Zr	0,1%

Based on table 1. The results obtained are levels of Fe that are equal to 0.75% and in the form of ppm that is equal to 10.2 ppm and other components.

The known Fe content in dahlia tubers is then compared with the Nutrition Label Reference (ALG) of BPOM RI to determine the feasibility of using standard syrup, which can be seen in

Table 2. Below: Comparison of Common Syrup Levels

No		Berat	Fe	Ppm
1	BPOM	100 gr	15%	3.9
2	Umbi Dahlia	20 gr	0.75%	10.2

In table 2, it is known that the comparison does not exceed the minimum regulation from BPOM RI in 2011 which states that the content of processed food in liquid form is said to be high in Fe content if it has levels of at least 15% of the Nutrition Label

Reference (ALG) per 100 ml [17]. so that the reference value of Fe content in food products is 10.0562 mg. It means that at 15% the Fe content of ALG is 1.50843 mg. This shows that dahlia syrup (Pinnata Cav.) does not exceed the levels of the Nutrition Label Reference so that it can be consumed directly.

Inulin Review

Inulin, a natural fructose polymer, Inulin in dahlia tuber roots is a polysaccharide belonging to the Compositae and Gramineae families. Inulin serves as reserve storage for carbohydrates [18]. The structure can be seen in Figure 2. below

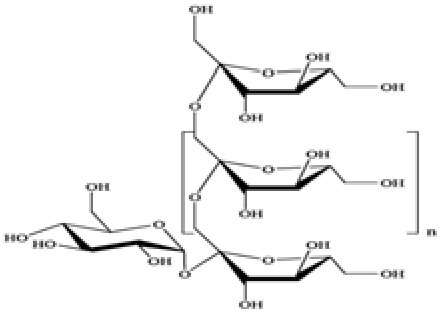

Figure 2. Inulin structure [19].

From the picture above, it can be seen that Inulin is a linear polysaccharide consisting of -2, 1-linked-d-fructofuranose chains ending with -d-glucose at the reducing end. The structure of inulin from dahlia tubers has the molecular formula (C6H10O5)n. Inulin dahlia contains a water content of 3% dry weight and ash content of 0.2 - 4.5% dry weight [20].

Plants rich in inulin such as chicory, dahlia, asparagus, etc., can be used as substrates and strong inducers for inulinase production and can also be used to produce fructooligosaccharides and high fructose syrup [2].

Enzymatic production of fructose syrup usually uses the basic ingredients of potato, cassava, starch, corn, sweet potato, sago, and dahlia. Dahlia tubers are known to contain inulin which functions as a natural sweetener polymer. The manufacture of fructose syrup requires Inulin as a substrate, if the yield of fructose produced is higher (it can reach 92-98%), while starch is only 45%; shorter bioconversion time between 10-12 hours, while starches 3-3.5 days; requires only one step an of an enzymatic reaction, namely with inulinase, starch requires three stages, namely liquidation, saccharification and isomerization with three types of enzymes, namely -amylase, amyloglucosidase and glucoisomerase [18].

Hydrolysis of inulin into its monomer fructose can be carried out by hydrolyzing it using acid catalysts such as hydrochloric acid, citric acid, oxalic acid, and an enzyme catalyst, namely inulinase. The production of fructose syrup uses inulin with a small degree of polymerization so that the hydrolysis process can take place quickly. In addition, inulin can be used as a prebiotic. Prebiotics are dietary supplements that function as substrates for the intestinal microflora. Inulin is a food that is preferred by colonic microflora and can improve the balance of good bacteria in the intestine [21].

From previous studies, the highest levels of inulin in dahlia tubers (Pinnata L.) were found in dahlia tubers with an orange crown of 82.8%, while a red crown of 4.4% was the best yield and a light purple crown of 41.7% was the highest yield of inulin. extracted pure inulin. So dahlia tubers with bloodbloodpompon have the potential as a source of prebiotics [1]. Dahlia tuber inulin has a high dietary fiber content of about 65.7% dry weight [22] and 93.5% commercial inulin content. Dietary fiber can pass intact to the large intestine and is fermented by probiotic bacteria to produce compounds that are good for the human body. Such as bile salt hydrolysis (BSH) which can reduce bad cholesterol levels in the blood because it prevents the absorption of cholesterol in the intestines [23].

FOS REVIEW

FOS, Fructooligosaccharides

Inulin and oligofructose, also called fructooligosaccharides (FOS), belong to the class of fructan carbohydrates. FOS is known to prevent human intestinal colonization by pathogenic microorganisms. Bifidobacteria and lactobacilli are known to produce a wide range of antimicrobial agents from short-chain fatty acids to peptides [24] which is why there is much interest in the potential use of prebiotics to reduce the risk of infection. I. FOS is more effective in inhibiting colonization by Clostridium difficile [25] than Listeria monocytogenes and Salmonella typhimurium [26]. Fructans have also been evaluated for their ability to act synergistically with probiotics to inhibit pathogens [27]. The role of FOS in diabetes control has also been the subject of research by many workers [28]. It has been reported that daily consumption of 20 g of FOS reduces basal hepatic glucose production in healthy sub-groups [29].

FOS also has many other health-promoting functions [30]. One of the important nutritional attributes of inulin and FOS is their action as dietary fiber. FOS resists digestion and absorption in the human stomach and small intestine [31]. They affect bowel function by increasing stool frequency, especially in constipated patients[32], and/or causing softer stools [33]. Inulin and FOS are known to increase daily stool output [34]. The increase in fecal output can be ascribed to a significant increase in fecal bacterial mass and a significant increase in water content[35]. It is well known that FOSs, in addition to their effects on the gastrointestinal tract, are also capable of exerting systemic effects by modifying hepatic lipid metabolism in many animal models [36]. Colonic fermentation of FOS results in the synthesis of short-chain fatty acids, which affect lipid metabolism in humans [37].

It has been reported in an animal study in obese Zucker fa/fa rats that dietary enrichment with FOS can reduce the development of fat mass and the occurrence of hepatic steatosis [38]. In a comparative study of the effects of FOS on lipid metabolism in humans and animal models, it has been shown that FOS has triacylglycerol-rol (TAG) and cholesterol-lowering effects in rodents [39]. They may also have a protective effect on TAG accumulation in the liver and the development of steatosis in animals. The results in humans are more contradictory. Of the nine reviewed studies on blood lipid responses to inulin and FOS [37], three showed no effect on blood cholesterol or TAG levels, three showed a significant reduction in TAG, while four showed a modest reduction in total. and LDL cholesterol. Animal studies provide strong evidence that FOS inhibits the secretion of TAG-rich very-low-density lipoprotein (VLDL) particles through de novo inhibition of fatty acid synthesis [38].

Short-chain fructans have been shown to decrease total serum and LDL cholesterol in non-insulin-dependent diabetic patients, but not in healthy subjects. There is a lot of interest in the potential of probiotics to increase the absorption of minerals from the gut. Fermentation of FOS into short-chain fatty acids reduces the pH in the large intestine and this facilitates the absorption of mineral ions from the intestine, especially calcium and magnesium [21]. Most animal studies show that fructan consumption increases calcium absorption [40-43]. Although the mechanism of the effect of fructans on calcium is not known with certainty, several hypotheses have been proposed [44]. In an animal study, administration of cellulose/FOS significantly increased the absorption and retention of Ca, Mg, Zn, and Fe in rats [45].

From previous studies, FOS Dahlia was obtained from inulin through hydrolysis, the inulin used was 5%, the comparison of the results from the hydrolysis of the commercial inulase enzyme with doses of 1, 2, 3, 5, 7.5 & 10 U/gram, which obtained a FOS value of 10 .79%, 9.36%, 7.89%, 24.34%, 21.74, 29.62%. Meanwhile, crude inulase with doses of 1, 2, and 3 U/gram obtained FOS of 22.46%, 15.29%, and 29.15% [16].

FE Bioavailability Results

Bioavailability can be defined as the number of minerals in food that can be absorbed and used by the body [21]. Measurement of iron bioavailability using in vitro can measure iron bioavailability by determining the amount of iron and food with fatty acids or separating iron ions from food with metal binders based on food digestion or food testing with pepsin, hydrochloric acid, and digestive enzymes. followed by the separation of soluble diacrylate or iron. In vitro measurement of iron bioavailability generally gives the same value as humans, although it underestimates the absorption of components with low bioavailability. This indicates that the large variation in the biological availability of iron in vitro differs from that in vivo [25].

Factors from food components that can affect iron absorption are acids (ascorbic acid, citric acid, lactic and tartaric acid), sugar, protein, and mucin. While the food component factors that can inhibit the absorption of iron are polyphenols (tannins), oxalic acid, phytate, fosvitin, and nutrients such as calcium, phosphate, zinc, magnesium, and nickel. Based on this, it can be seen that some food components that inhibit iron absorption can chelate with insoluble iron. Although these results show different results, for example, oxalate was first reported to have the potential to inhibit iron absorption, the results showed neutral in terms of iron absorption in humans and a positive effect in experimental mice. Fiber and its components show varying effects on the biological availability of iron. If hemicellulose and lignin inhibit iron absorption in humans, cellulose and pectin show opposite effects. This can be seen which is a factor that can cause differences in results in research, namely the presence or absence of chelating, different components used, and the effect of pH [46].

It is known from experiments from previous studies that regarding the bioavailability test of yacon tubers of the type with blackish green (H) flowers in syrup in vitro, with samples of yacon tubers with a Fe content of 9.7 ppm, the results are shown in the following table.

Table 3. The results of FOS Fe, Yakon tuber syrup, blackish green flower type (H)

	Total
Fe(mg/L)	100
H1	78.06
H2	72.4
H3	74.95
H4	75.033
H5	59.18
Average	71.9246

Table 4. Results FOS of Fe syrup extracted from yellow (K) yacon tubers

	Total
Fe(mg/L)	100
K1	80.12
K2	73.52
K3	114.62
K4	195.54
K5	129.59
Average	118.678

In table 3. Fos Fe syrup on Yakon tubers with blackish green flower types, the average yield is 71.92% and in table 4. Fos Fe syrup on Yakon tuber yellow extract obtained an average yield of 99.46%

Table 5. Comparison of Fe, Inulin, and Bioavailability levels

Bulb Type	Fe	Inulin	Fe/Inulin	Fe Bioavailability
Yakon H	9.7 ppm = 0.00097%	70-80% = 75.5%	0.00001284768%	71.92%
Yakon K	9.7 ppm = 0.00097%	70-80% = 75.5%	0.00001284768%	118.68%
Dahlia H	10.2 ppm = 0.00102 %	65.7-93.5% = 79.6%	0.00001281407%	69.20%
Dahlia K	10.2 ppm = 0.00102 %	65.7-93.5% = 79.6%	0.00001281407%	94,73%

Table 6. Comparison of Fe, FOS, and Bioavailability levels

Bulb Type	Fe	FOS	Fe/FOS	Fe Bioavailability
Yakon H	9.7 ppm = 0.00097%	22.13%.	0.0000438319%.	71.92%
Yakon K	9.7 ppm = 0.00097%	22.13%.	0.0000438319%.	118.68%
Dahlia H	10.2 ppm = 0.00102 %	29.62%	0.00003443619%	65.13%
Dahlia K	10.2 ppm = 0.00102 %	29.62%	0.00003443619%	80,34%

In table 5. Comparison of Fe, inulin, and Fe bioavailability values which are known from the predicted value of Fe bioavailability in dahlia tuber syrup against Yakon tuber syrup H blackish-green extract) is 69.20% while Yakon tuber syrup K (blackish yellow extract) is 94.73%.

In table 6. Comparison of Fe levels, FOS, and Fe bioavailability it is known that the predicted value of Ca bioavailability in dahlia tuber syrup against Yakon tuber syrup H (blackish-green extract) is 60.13%, while from Yakon tuber syrup K (blackish yellow extract) is 80.34% [47].

From the results of this comparison, it can be concluded that the bioavailability of Fe in dahlia tuber syrup against yacon tubers with a ratio of inulin is in the range of 65.13-80.34%. Meanwhile, the bioavailability of Ca in dahlia tuber syrup to Yakon tuber with a FOS ratio was in the range of 69.20-94.73%.

Based on Tables 5 and 6, it is known that the Yakon tuber has an average Fe bioavailability value of around 71.92-118.68%. with Fe content of 9.7 ppm, The average inulin content was 75.5% and the FOS in the yacon syrup was around 22.31%. This applies to dahlia tuber syrup which has a Fe content of 10.2 ppm, an average inulin content of 79.6% and the yield of FOS which is hydrolyzed from 5% inulin is about 29.6%. The predicted Fe bioavailability from the ratio of yacon tuber syrup is around 65.13-85.118.68%. This high bioavailability of Fe may also be influenced by the content of inulin and FOS in dahlia tubers, by research that has occurred that inulin and FOS can increase the bioavailability of minerals including iron. Inulin can increase the absorption or absorption of iron thereby reducing the risk of iron deficiency anemia (ADB).

The FOS compound present in the inulin structure, which is a specific structure (1-2 bond) causes an increase in the bioavailability of Fe minerals. Biologically, FOS compounds cannot be digested in the small intestine but are fermented by anaerobic microbes such as Bifidobacteria and Lactobacillus in the large intestine. -fructofuranosidase enzyme produced by Bifidobacteria and Lactobacilli will degrade FOS into glucose and fructose. The degraded monosaccharides then enter the Embden-Meyerhof-Parnas pathway or commonly called glycolysis and the pentose-phosphate pathway to be converted into phosphoenol pyruvate (PEP) [48]. Furthermore, the formed pyruvate will be converted into SCFA through 3 main pathways, namely the Acrylate Pathway, Succinate Pathway, and Wood-Ljungdahl Pathway. The final product resulting from the FOS fermentation process is SCFA (Short Chain Fatty Acid) such as acetate, propionate, and butyrate [49]. SCFA in the colon, especially butyrate, causes the proliferation of intestinal epithelial cells which affects the small intestinal villi to become taller and denser, resulting in an expansion of the absorption surface [50-51]. According to Freitas [52], SCFA also causes the pH in the colon to drop, making it difficult for Fe to form complexes with antinutrient substances and SCFA can maintain Fe minerals in solution form. This causes Fe more easily absorbed by the colon. Iron minerals that have been absorbed can be stored in ferritin or can also be transported into blood plasma via basolateral transport proteins [53]. Based on Tako's research [54] it is also stated that consuming prebiotics can increase the activity of iron transporter and receptor genes such as ferritin, DMT1, Dcytb, ferroportin, and transferrin receptor (Tfr).

This occurs because the villi of the small intestine become taller and denser as a result of increased SCFA production resulting in an expansion of the absorption surface [55]. SCFA also lowers the pH in the large intestine which makes it difficult for Fe to form complexes with antinutrient substances such as polyphenols, calcium, and phosphorus [52]. This causes Fe to be more easily absorbed so that its bioavailability increases. According to Miller [5], Fe bioavailability can be affected by all conditions in the period of feeding to contact with the mucosa, but pH is the main factor affecting Fe bioavailability. So that the bioavailability of Fe is very important for health and reduces the risk of iron deficiency anemia (ADB).

CONCLUSIONS AND SUGGESTIONS

Conclusion

Based on the research and literature studies that have been carried out, it can be concluded that this study was conducted to discuss the determination of Fe levels and prediction of Fe bioavailability in vitro from dahlia tuber syrup. The Fe content in Dahlia tuber syrup was determined experimentally using an X-Ray Fluorescence Spectrometer (XRF). The Fe bioavailability of dahlia tuber syrup was predicted based on the standardization of inulin and FOS content of dahlia tubers on yacon tubers. The results showed that the Fe content in dahlia tuber syrup was 10.2 ppm. The bioavailability of Fe in dahlia tuber syrup in vitro is predicted to be 69.20% - 94.73%. The prediction of a fairly high Fe bioavailability indicates that dahlia tuber syrup is expected to be quite good at increasing the absorption of Fe in the body which can have health effects, among others, can play a role in improving body metabolism and being able to reduce the risk of anemia due to iron deficiency.

Suggestions

Researchers who are interested in continuing the results of this article are expected to conduct research on in vivo testing.

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