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Utilization of Inulin from Mangrove Apple and Pineapple Juice as Prebiotic Drink to Recovery Covid-19 Patients

Yoyok Budi Pramono[1]*, Sri Mulyani[1], Anang M. Legowo[1], Ahmad Ni'matullah Al-Baarri[1], Nathania Maula Bernadine[1], Daniella Olivia Afri Kusuma[1] and Angelique Gangsar Listya[1]

[1]Food Technology Study Program, Department of Agriculture, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia

Abstract:
Amidst the COVID-19 pandemic, some individuals have turned to functional foods in an effort to bolster their immune systems. One such functional option is prebiotic drinks. These beverages are crafted from fermented sugars, which serve as a carbon source for lactic acid bacteria. To enhance their functional properties, prebiotic drinks often incorporate inulin—a type of prebiotic. Traditionally, these drinks are milk-based, but for those who cannot tolerate dairy, alternatives have emerged. For instance, pineapple-based prebiotic drinks enriched with inulin from mangrove apples have gained popularity. The process involves extracting inulin from mangrove apples and combining it with pineapple to create a probiotic drink. The resulting beverage derives its sweetness from the natural sugars found in pineapples and mangrove apples. Pineapple contains approximately 2.32% glucose, 1.42% fructose, and 7.89% sucrose, while mangrove apples boast 5.08% inulin, 9.67% soluble dietary fiber, and 0.66% FOS. These sugars serve as nourishment for lactic acid bacteria, ultimately stimulating the production of IgG and IgM antibodies. In summary, the combination of inulin-rich mangrove apples and pineapple's total soluble solids can contribute to fortifying the body's immune system during the COVID-19 pandemic.

Keywords: Inulin, Pineapple, Mangrove apple, Immune

1. Introduction

The COVID-19 virus is a virus that attacks the respiratory system, but it is also possible for this virus to attack the human digestive system. COVID-19 virus in the digestive tract will cause digestive disorders such as diarrhea. During the COVID-19 pandemic, people began to consume functional food because it can increase the body's immunity. One of the functional food products is probiotic drinks. Probiotic drinks can have a good effect on body health when consumed regularly because they are made through the fermentation process of lactic acid bacteria [1]. The requirements for strains that can be used as probiotic agents are resistant to bile and acid so that they can reach the intestine and have the ability to stick to the intestinal mucosa [2]. Probiotics may serve to stimulate systemic immunity and may reduce symptoms of depression and anxiety [3]. Consumption of probiotic drinks regularly can maintain microflora balance by suppressing the growth of harmful bacteria and perfecting the human digestive process by protecting the digestive tract from pathogenic bacteria [4]. A balanced microflora in the digestive tract supports forming the body's immune system so that it is not susceptible to various viral attacks, one of which is the COVID-19 virus.

Probiotic drinks are generally made from animal-based ingredients, namely milk. Some people cannot consume cow's milk, so product diversification is carried out with fruit juices that are rich in nutrients such as rich in dietary fibre, vitamins, low in fat, and also have an attractive aroma so that they are suitable as ingredients probiotic drinks [5]. One of the tropical fruit that has the potential to be used as the base for probiotic drinks is pineapple. Pineapple (*Ananas comosus* (L) *Merr*.) is a tropical fruit and has become a commercial fruit with high nutritional value and quite an excellent sensory [6]. Pineapple is a perishable food commodity and can only last for 4-6 days because of its high water and sugar content. The water and sugar content in 100 grams of pineapple is 86 g of water and 13.12 g of carbohydrates [7]. Pineapple fruit

that is quite widely produced in Indonesia is the Queen type honey pineapple and the Cayenne type [8]. Queen honey pineapple has a yellower color and sweeter taste than Cayenne pineapple because it contains more sugar than the water, namely the amount of sugar reaches 13-18ºbrix [9]. The sugar content in pineapple is used as a carbon compound for the metabolism of lactic acid bacteria. The more sugar concentration, the total lactic acid bacteria will increase [10].

LAB in large quantities can provide a beneficial effect for those who consume it. Lactic acid bacteria, as probiotic agents, can inhibit pathogenic bacteria's growth and function as immunomodulators [11]. Immunomodulators are divided into immunostimulants and immunosuppressants that play a role in suppressing the immune system. Immunomodulators improve the immune system by stimulating (immunostimulants) or suppressing abnormal immune reactions [12]. Immunomodulators are needed, especially in decreased immune system status conditions, so people are very susceptible to diseases caused by bacteria, fungi, or viruses. Increasing age can reduce the immune system and the growth of mucosal immune responses to antigens. Other factors that can affect the immune system are changes in the environment and microflora and the presence of inflammatory diseases [13]. The immune system is controlled by B cells produced by the spinal cord and T cells produced by the thymus [14]. Immunomodulators can stimulate the development and activity of T cells.

To add functional value, probiotic drinks will be better if given the addition of prebiotics that can increase body immunity. The prebiotics most often added to low pH drinks, such as probiotic drinks, are inulin, FOS, GOS, and resistant starch [15]. Inulin can be found in several types of root crops, dandelions, onions, raw oats, and barley. Inulin also can be obtained from local food plants, which is apple mangrove extract. Apple mangrove (*Soneratia caseolaris*) is a mangrove plant that generally grows in the tropics and belongs to the *Sonneratiaceae* species [16]. Mangrove apples, both in fruit and extracts, also contain 5.08% inulin, 9.67% soluble dietary fibre from inulin and inulin fructans, and 0.66% FOS [17]. In mangrove apples, the content of inulin, soluble fibre, and FOS can increase body immunity during the COVID-19 pandemic. The body's immunity during COVID-19 can be increased by adding inulin to the product through a direct response to carbohydrate receptors on intestinal epithelial cells and immune cells or indirectly through changes in the composition of the intestinal flora and the production of short-chain fatty acids from the inulin fermentation process in the product and the body. As an immune regulator, it can increase the body's immunity and improve the digestive system [18].

1.1. The Process of Making Mangrove Apple Inulin Extract

The process of making mangrove apple inulin is first washing the mangrove apples, then separating from the seeds and cutting them into small pieces. The small mangrove apples were then extracted at 90°C for 60 minutes with 1:4 w/v. The extracted filtrate was then precipitated with 70% ethanol and frozen at -18°C for 6 hours. The frozen filtrate was then thawed again at room temperature and centrifuged for 5 minutes at 5000 rpm then the supernatant from the filtrate was discarded. Mangrove apple inulin was then dried using a cabinet dryer at 50°C for 12 hours. The dried mangrove apple inulin was then mashed using a blender and then sieved using a 60mesh sieve. The finished mangrove apple inulin powder is then stored in an airtight container.

1.2. The Process of Making Pineapple Probiotic Drink with The Addition of Mangrove Apple Inulin

The process of making pineapple fruit probiotic drinks with the addition of inulin is the honey queen pineapple juice which has been sterile inoculated with pure *L. plantarum* starter and 6% (v/v) yogourmet starter which previously added 3% mangrove apple inulin (v/v). Honey pineapple probiotic drink which has been added with starter and inulin then fermented at 42°C for 4 hours and can be stored for 28 days at 4°C.

2. Literature review

Probiotic drinks are drinks made from a fermentation process by lactic acid bacteria (LAB) and have a beneficial effect on the host to improve the balance of intestinal microbes. Probiotic bacteria in probiotic drinks can increase the nutritional value by increasing the production of vitamins, calcium, iron, manganese, copper, phosphorus, and protein and fat digestibility [19]. Probiotic drink products deserve to be considered functional drinks if they contain good microorganisms, especially bacteria in the genus Lactobacillus and

Bifidobacterium. These two bacteria are rarely found naturally in food, so they are usually found in starter cultures [20]. The main requirement for probiotic drinks is that they contain >106 good bacteria cells in the drink [21]. These bacteria must be resistant during processing, resistant to bile salts, able to pass gastric acid with a pH ranging from 3-to 5, and survive in the digestive tract to provide sound health effects for the body [22].

In Indonesia, pineapple (*Ananas comosus* (L.) *Merr*) is very abundant and easy to obtain because it has a harvest season that does not know season. A pineapple fruit has easily damaged properties and rots quickly [23]. The types of pineapple that are widely grown in Indonesia are the queen and cayenne types. Queen honey pineapple has a small size of 10-16 cm, weighs 300-600 grams, is yellow-orange, has a sweet taste, and has a fragrant aroma [24]. Honey pineapple has a sweeter flavor because the sugar content is relatively high compared to the water, reaching 13-18 Brix [9]. Types of sugar content in honey pineapple are sucrose sugar and reducing sugar. The total sugar content in honey pineapple is glucose 2.32%, fructose 1.42%, and sucrose 7.89% [25]. The sugar content in honey pineapple can be called total dissolved solids because the component of the total dissolved solids, as much as 85%, is sugar [26]. Glucose, fructose, sucrose, and pectin are sugar components that affect the value of total dissolved solids because they are soluble in water. The high sugar content in pineapple can be a medium for lactic acid fermentation through the glycolysis process [27]. Lactic acid bacteria in fermenting sugar produce primary and secondary metabolites. Increased body immunity can be caused by secondary metabolites resulting from bacterial metabolism, one of which is caused by exopolysaccharides (EPS) produced by LAB [28]. Exopolysaccharide is a polysaccharide secreted from lactic acid bacteria (LAB) released in the extracellular around cells [29]. Exopolysaccharides have an immunomodulatory potential by stimulating IgG production, and IgM has functional properties, including enhancing the immune system [30]. The high antioxidant activity of EPS indicates that EPS can be used as a promising natural supplement or antioxidant to be added to the human diet [31].

The mangrove apple fruit plant is one of the plants that make up mangrove forests on the coast with low salinity and is a habitat for fireflies. The productivity of mangrove apples is relatively high, producing the fruit of as much as 2 kg/day. It does not require special handling to make a food product that can be enjoyed because it is delicious and delicious. Mangrove apples can be made into lunkhead, syrup, jam, juice, instant drinks, candy, and flour as the essential ingredients for making cakes because of their carbohydrate content. [32]. The content of 100 grams of mangrove apple is 84.76% water, 77.57% carbohydrates, 9.21% protein, 8.4% ash, 4.82% fat, vitamin A 221.97 IU, vitamin B 5.04 mg, vitamin B2 7.65 mg, and vitamin C 56.74 mg [33]. Mangrove apples also contain inulin with a relatively high fibre of 63.70%, of which 9.8% is water-soluble dietary fibre, so mangrove apples act as natural prebiotics [34]. Inulin can be divided into two categories. DP is the number of fructose monomers in polysaccharides. The chain length includes long-chain inulin with a DP of 10-65 units and FOS with a DP of 2-10 units [35]. Therefore, the mangrove apple extract contains 5.08% inulin, 9.67% soluble dietary fibre from the combination of inulin and inulin fructans, and 0.66% FOS [15] and in probiotic drinks, inulin with a low DP will be fermented by lactic acid bacteria in the product compared to a large DP. A large DP in long-chain inulin can reach and be fermented by probiotic bacteria in the large intestine.

Inulin is a natural linear polymer composed of monomers in the form of fructose then linked by -2,1-glycosidic bonds and bound by glucose at the end of inulin [36]. Inulin as a food additive plays a role in spurring the activity of probiotic bacteria but does not provide flavor to food [37]. The characteristic of inulin is that it is easily soluble in water but challenging to hydrolyze by enzymes in the digestive tract to enter the large intestine with an intact size. In the large intestine, inulin will then be fermented by probiotic bacteria in the intestine into short-chain fatty acids and some specific microflora that produce lactic acid. The bacteria that can ferment inulin are *L. bulgaricus, L. Plantarum,* and *S. thermophilus* by breaking down inulin into its constituent monomers fructose and glucose, with the enzyme β-fructofuranoside [38]. The total sugar content in inulin is 95.53%, consisting of 90.81% fructose and 4.71% glucose [39]. Sugar in this simple form is used as a carbon source during the inulin fermentation process. Later, the total soluble solids in probiotic beverage products decrease, and the metabolic effects of fermentation in the form of lactic acid and short-chain fatty acids can positively impact body health.

The addition of inulin as a prebiotic in pineapple fruit probiotic drinks can increase body immunity during COVID-19. Inulin tested in mice was shown to provide protection against influenza A infection by

producing Ly6C cells that patrol the production of white blood cells monocytes and T CD8+ cells [40]. The increase in body immunity that occurs with the addition of inulin is through the regulation of intestinal mucosa and epithelial cells and can also induce the protection of humoral immunity and cellular immunity [41]. These mechanisms occur because of the lactic acid bacteria in inulin fermentation produced lactic acid and short-chain fatty acids. One humoral response the addition of inulin can induce is the Ig A antibody which can bind to pathogenic bacteria and toxins to optimize the immune response [42]. The presence of lactic acid and short-chain fatty acids resulting from inulin fermentation will provoke an adhesin substance derived from bacterial cell surface molecules so that it can increase specific IgA. Adhesin is a particular protein that stimulates NCR (Creatin Reactive Protein) as an immunogen that increases IgA production. Immunogens are antigens that enter the respiratory tract, digestive tract, food, drink, eyes, and reproductive organs. This collection of antigens will stimulate T cells, then T cells will become blast cells; these blast cells will divide to become activated T cells to produce lymphocytes sensitized to antigens or antibodies produced with antibodies different types for defense against disease [43]. Therefore, the inulin fermentation process both in the body and in products can increase the body's immunity during the COVID-19 pandemic.

3. Conclusion

Inulin from mangrove apples and pineapple juice as prebiotic drinks can improve the body's immune system of COVID-19 patients. These total soluble solids of pineapple can improve the body's immune system by stimulating the production of IgG and IgM. The inulin's mangrove apple can increase the body's immune system with direct response through carbohydrate receptors on intestinal epithelial cells, immune cells, and induced IgA and with indirect responses by the production of short-chain fatty acids as an immune regulator.

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Author Profile

Dr. Yoyok Budi Pramono Lecture in the Food Technology study program at Diponegoro University. His undergraduate degree was obtained from the Faculty of Animal and Agriculture Science. The Master degree and the Doctoral degree was obtained from Gadjah Mada University. He focuses on functional food science. He was published many of books and scientific works have been published in Scopus and Sinta-Indexed journals.

Dr. Sri Mulyani Lecture in the Food Technology program at Diponegoro University. She current position is secretary of food technology study program. Her undergraduate degree was obtained from Jendral Soedirman University. The master degree and the Doctoral degree was obtained from Gadjah Mada University. She focuses on development of food products and she has been published several scientific works in Scopus and Sinta-Indexed journals.

Prof. Dr. Anang M. Legowo Prof. at Faculty of Animal and Agriculture Science, Diponegoro University and as a lecture at food technology study program. The undergraduate program was obtained in Gadjah Mada University. The master degree was obtained in Kagawa University and the Doctoral degree was obtained in Ehime University. He focuses on food analyst and he has been published several scientific works in Scopus and Sinta-Indexed journals.

Ahmad Ni'matullah Al-Baarri Ph.D. He current position is Head of the food technology study program, Diponegoro University and as a lecture at food technology study program. The undergraduate program was obtained in Gadjah Mada University and the Doctoral degree was obtained in Kagawa University. He focuses on food process chemistry and he has been published several scientific works in Scopus and Sinta-Indexed journals.

Nathania Maula Bernadine was born in Semarang on April 6, 2000. She was accepted as a student in the Study Program Food Technology, Diponegoro University in 2018. She has become an alumnus of Food Technology in 2022.

Daniella Olivia Afri Kusuma was born in Semarang on August 27, 2000. She was accepted as a student in the Study Program Food Technology, Diponegoro University in 2018. She has become an alumnus of Food Technology in 2022.

Angelique Gangsar Listya was born in Kebumen on October 30, 2003. She was accepted as a student in the S-1 Study Program Food Technology, Diponegoro University in 2021. She is currently pursuing her undergraduate degree at Food Technology and she is writing a book on the theme of taste.