Naturally Inhibited Yacon Tubers Prebiotic Syrup Increases Humoral and Cellular Immunity

Leny Yuanita[1,*] Prima R. Wikandari[2] Dewi Prastiwi[2] Raymond I. Avandi[3] Wahyu B. Sabtiawan[4] Erlix R. Purnama[5]

[1]*Departement of Chemistry, Universitas Negeri Surabaya, Indonesia*
[2]*Departement of Accounting, Universitas Negeri Surabaya, Indonesia*
[3]*Departement of Sport Science, Universitas Negeri Surabaya, Indonesia*
[4]*Departement of Natural Science, Universitas Negeri Surabaya, Indonesia*
[5]*Departement of Biology, Universitas Negeri Surabaya, Indonesia*
[*]*Corresponding author. Email: wahyusabtiawan@unesa.ac.id*

ABSTRACT
The aim of the study: to obtain yacon prebiotic syrup that can increase cellular and humoral immunity. Sixty of Rattus norvegicus aged about 40 days and weighing about 100-150 grams divided into 4 groups. Commercial FOS supplement group, non-inhibited yacon syrup, inhibited yacon syrup (FOS content ± 20% w/w diet), and water as a negative control. Induction of 2% sheep red blood cell antigen via intraperitoneal injection. Bioactive assay of FOS and chlorogenic acid in yacon syrup using HPLC and voltametry. CD4+/CD8+ cellular immunity was tested by floxytometry, while IgM and IgG humoral immunity by antibody titrant hemagglutination method. Data were analyzed statistically parametric and non parametric (α = 5%). The results showed: 1) inhibited yacon syrup had higher content of bioactive FOS and chlorogenic acid than non-inhibited yacon syrup. 2) The use of natural inhibitors is effective in maintaining the bioactive content of yacon tubers. 3) Supplementation of inhibited yacon syrup more effective in increasing cellular (CD4+/CD8+) and humoral (IgM and IgG) immunity against the control group than the non-inhibited yacon syrup.

Keywords: *Prebiotic syrup, Humoral, Cellular immunity.*

1. INTRODUCTION

Maintaining the body's immunity is one way that can be done to avoid disease, among others, through nutritional intake and consumption of bioactive supplements in order to increase the work of immune cells that function as body defenses. Various research results show that fructan/fructooligosaccharide (FOS) compounds as prebiotics can improve the body's immune system [1][2]. FOS increases the activity of cecum/colon probiotic bacteria thereby increasing the formation of short chain fatty acids (SCFA) which have the effect of stimulating immune function, the ability to fight infection and inflammation [3].

One of the main sources of good FOS prebiotics is yacon tubers [4][5][6]. Yacon tubers saccharides (mainly FOS) make up 70-80% dry weight [7]. According to Valentova et al. [8], yacon tubers consisted of fructose 350.1, glucose 158.3, sucrose 74.5, FOS (GF2-GF9) 206.4, and inulin 13.5 mg/g dry weight. FOS (fructose - 2,1glucose)n, mainly consists of 1-kestose (GF2), nystose (GF3), 1-b-D fructofuranosylnystose (GF4). According to Manrique et al.[9], 100 grams of fresh yacon tubers contain 6-12 grams of FOS. FOS prebiotics are non-digestible natural sweeteners, low in calories and maintain blood glucose (IG=1), preventing hypercholesterolemia. Besides FOS, yacon tubers also contain phenolic compounds (especially chlorogenic acid-48.5 g/g), L-tryptophan (14.6 g/g) and flavonoids which have activity to stimulate immune defense through their antioxidant, also as anti-inflammatory, anti-microbial and anti-cancer [10]. The main mineral in yacon tubers is potassium (334 mg/100g) [11], which acts as a vasodilator and helps the heart pump function thereby lowering blood pressure.

Yacon tuber bioactives, FOS and chlorogenic acid can be consumed effectively and have a longer shelf life in the form of extract, namely yacon syrup. As functional food, the bioactive FOS and chlorogenic acid of yacon tubers must be protected from condition changes that result in a decrease in their contents. Therefore in the syrup processing, it must be anticipated the bioactive components of FOS and chlorogenic acid remain stable. The main factor that directly affects the levels of bioactives in the processing and sensory acceptance of the color of yacon syrup is enzyme activity. In order to maximize the availability of bioactive components, and prevent browning from occurring, the control of related enzymes must be carried out in the production of yacon syrup. Based on the background, the research problem is how to get yacon syrup that has optimum quality to increase cellular and humoral immunity, through the use of natural enzyme inhibitors.

2. METHODS

Inhibited or non-inhibited yacon syrup is made from yacon tubers through an evaporation process with temperature (< 65 oC) and pH (± 5.5-7) according to the yacon syrup producing procedure [12]. Identification of FOS and saccharides (glucose, fructose and sucrose) contents of yacon syrup through HPLC Hewlett Packard Series 1050, Zorbax 300 SB-C18 column (5µm) and uv detector, while assay for chlorogenic acid contents using voltametry. Biological assay include CD4+/CD8+, IgM and IgG blood serum of experimental animals. Sixty Rattus norvegicus experimental animals were used, aged about 40 days and weighing about 100-150 grams. Divided into 4 groups, with supplementation through a nasogastric tube as follows. negative control (K-) water @ 3 ml, positive control (K+) commercial FOS @ 4 ml, P1 non-inhibited syrup @ 3 ml, and P2 inhibited syrup @ 3 ml. The FOS content in each syrup and commercial FOS is about 20%. After 30 days of treatment, intraperitoneal injection of 2% sheep red blood cell antigen (SDMD) @ 1-1.5 cc was performed. CD4+, CD8+ and IgM assay on the sixth day after induction through antigen injection, while IgG on the eleventh day. For the CD4+/CD8+, IgM and IgG assay, twenty experimental animals were prepared respectively. The CD4+ and CD8+ assay used floxytometry, while the IGM and IgG used the hemagglutination method. Agglutination reaction will be formed if the ratio of antigen and antibody is balanced or equivalent. Agglutination was facilitated by stirring or shaking, incubation at 37 oC and in Phosphate Buffer Saline solution pH 7.2. Data were analyzed statistically parametric and non parametric ($\alpha = 5\%$).

3. RESULTS AND DISCUSSION

The main components of FOS are ketose (GF2), nystose (GF3), 1-kestopentaose =1-b-D fructofuranosylnystose (GF4). The contents of FOS and saccharides of yacon prebiotic syrup (three replications) are in Table 1.

Table 1. FOS and Saccharides contents of Yacon Prebiotic Syrup (g/L)

Yacon Syrup	FOS (g/L)			∑ FOS	Saccharide (g/L)			∑ Saccharide
	GF2	GF3	GF4		Fructose	Glucose	Sucrose	
(-) inhibitor	123.236	61.541	32.455	217.23	42.248	54.476	45.194	141.918
	125.886	70.621	37.128	233.64	57.618	46.487	38.249	142.354
	112.953	65.312	34.785	213.05	59.720	45.671	32.922	138.313
Mean	**120.692**	**65.825**	**34.787**	**221.30**	**53.196**	**48.878**	**38.788**	**140.862**
(+) inhibitor	120.128	64.351	35.596	220.08	62.066	41.033	40.117	143.216
	115.745	59.895	34.780	210.42	68.513	44.923	37.950	151.387
	157.597	83.682	46.421	287.70	62.779	53.921	45.604	162.304
Mean	**131.156**	**69.309**	**38.932**	**239.40**	**64.453**	**46.627**	**41.224**	**152.302**
Significance				**0.347**	Significance			**0.29**

Table 1 shows that the total FOS content of the inhibited yacon syrup (239.4 gram/liter) was higher than that of the non-inhibited yacon syrup (221.3 gram/liter); showed that in the syrup producing process, the FOS content can be maintained; although the results of independent t test showed that the FOS content of the inhibited yacon syrup was not differs significantly from that of the non-inhibited yacon syrup. Temperature and pH highly affect FOS content; which allows it to be degraded into its components glucose and fructose or the saccharides formed will react with the amino acid groups of proteins in yacon syrup to form reactions and compounds Mailard. The enzyme 1-FEH catalyzes FOS into fructose and glucose [13], optimum at pH 4.5-5.5, 25-40 oC. Under acidic conditions, the hydrolysis of FOS was insignificant at 60ºC, but completely degraded at 90-100ºC in 1-1.5 hours. At pH 5 fructan degradation did not occur even at 100 oC heating for 55 minutes [14][15]. This means that the syrup-making process will determine the FOS content of the syrup.

3.1 Chlorogenic acid content of Yacon Prebiotic Syrup

The content of chlorogenic acid per ml of yacon syrup is shown in Table 2.

Table 2. Chlorogenic acid content of Yacon Prebiotic Syrup (ppm/ml)

Yacon syrup	Chlorogenic acid (ppm)	Mean ± SD
(-) inhibitor	356.796	
	396.504	
	382.877	
	377.659	
	417.036	
		386.174±22.390
(+) inhibitor	499.676	
	503.569	
	546.371	
	536.070	
	465.935	
		510.324±31.983
Significance (p)		0.000

The results of the assay for chlorogenic acid content of yacon syrup showed that the chlorogenic acid content of the inhibited yacon syrup was significantly higher than the non-inhibited yacon syrup. This means that the use of natural inhibitors, pH and temperature regulation is able to maintain the bioactive content of chlorogenic acid in inhibited yacon syrup. Without inhibitor, chlorogenic acid forms hydroquinone and polymerizes to form brown melanoidin. This reaction depends on the polyphenol oxidase enzyme with copper (Cu) cofactor and O2 as electron donors. Chelating agent will chelate copper resulting in enzyme inactivation; while the reducing agent will reduce the quinone to chlorogenic acid again. The oxidation reaction of chlorogenic acid is as follows [16]:

Chlorogenic acid + [O] → Quinone (by enzyme)

Quinone + H_2O → Hydroxychlorogenic acid

Quinone + Hydroxychlorogenic acid → Hydroxyquinone + Chlorogenic acid

Chlorogenic acid + [O] → Quinone (by enzyme)

Hydroxyquinone → polymerization to Quinone.

3.2 Cellular Immunity: CD4+/CD8+

The antigen used in the induction of antibody production is sheep red blood cells. SDMD is a polyvalent antigen, a protein with a greater potential determinant than a monovalent antigen. When exposed to antigens or foreign proteins, the natural immune system which is the body's first defense system assists the recognition of these antigens and plays an important role in the activation of the adaptive immune system. However, if the natural immune response is not sufficient to overcome the pathogen, the body must activate an adaptive immune response mediated by T lymphocytes and B lymphocytes. T-helper (Th) cells stimulate other T cell responses, T helper cells stimulate a humoral immune response called CD4 cells; while cytotoxic T cells (Tc) are called CD8 cells (killer cells).

The results of the CD4+/CD8+ ratio on experimental animals in the K (-), K(+), P1 and P2 groups are shown in Table 3.

Table 3. CD4+/CD8+ of experimental animals

Groups*)	Mean of CD4+/ CD8+	SD	Significance
K-	0.7200	0.146	A
K+	1.2240	0.242	B
P1	0.8720	0.034	Ac
P2	1.0000	0.007	Cd
Significance	F=11.234; p=0.000		

*) Supplements in the group:
K (-): water, K(+): commercial FOS, P1: non-inhibited syrup, P2: inhibited syrup

Supplementation of inhibited yacon syrup increased cellular immunity (CD4+/CD8+) against the control group; while non-inhibited yacon syrup is not significant. A low CD4+/CD8+ ratio indicates a decrease in resistance to infection, while an inverse ratio (< 1) indicates a disorder of the immune system. The mean of CD4+/CD8+ for K(-) group =0.7200, P1=0.8720. This is due to the presence of SDMD foreign protein antigens. The experimental animals in the K(-) group did not receive immunostimulator supplements, while the non-inhibited yacon syrup less functional as an immunostimulator for the experimental animals in the P1 group. The highest ratio was found in the K(+) group due to the administration of commercial FOS and followed by P2. In addition to FOS as a prebiotic capable of increasing the cellular immune system, inhibited yacon syrup is better for the cellular immune system than non-inhibited yacon syrup. Inhibited yacon syrup contains immunostimulator compounds, namely FOS, also higher flavonoid and chlorogenic phenolic compounds and has a higher antioxidant activity than non-inhibited yacon syrup [17].

FOS prebiotics play a role in improving the immune system through the formation of short chain fatty acids that have receptors on immune cells GALT (Gut-associated lymphoid tissue), and it is hypothesized that carbohydrates in prebiotics interact with receptors on immune cells [3]. FOS modulates the immune system directly through the lymphoid tissue associated with the gastrointestinal tract; activate immune cells macrophages, dendritic cells, lymphocytes, neutrophils [5]. There was an increase in the production of IL-10 from Peyer's path and mesenteric lymph nodes, and IFN- from Peyer's path in mice with supplementation of inulin enriched with FOS [18]. Intake of 3% yacon and 5% FOS for 30 days, increased mucosal immunity IgA in intestinal tissue, reduced the inflammatory cytokine IL-1β in macrophages thereby preventing the risk of autoimmune and metabolic disorders [6]

Delgano et al. results showed that the use of yacon increased the production of Th/CD4 cell cytokines namely IL-10, IFN-α and IL-4, in the cells of baby mice [6]. The use of yacon tubers flour increases the intestinal immune system by increasing IgA and cytokines; T cells are activated and are able to induce the production of IL-1 and IFN-ϒ. The use of yacon tubers flour for a long time (75 days) will maintain intestinal homeostasis without inflammatory effects, mainly due to the regulatory effects of IL-10 and IL-4 cytokines [29]. Polyphenols significantly increased IL-21 and decreased to release IL-1β and IL-6 [20]. The polyphenolic compound chlorogenic acid inhibits the release of pro-inflammatory mediators TNFα and IL-1β through increased expression of the anti-inflammatory cytokine IL-10 [21]. According to [22], the effect of flavonoids as immunomodulators on the immune system is through the suppression of mTOR activity and consequently is able to induce regulatory T cells.

3.3 Humoral Immunity: IgM and IgG

Antibodies or immunoglobulins are proteins that formed by plasma cells (B cell proliferation) due to contact with antigens. IgM and IgG antibodies are produced by B cells. The success of B cells against microorganisms is assisted by The cells. IgM is an immunoglobulin that is first produced in response to an immune response to an antigen, followed by a switch to the production of IgG or another class of antibodies. The interaction between antigen and antibody will produce in the formation of an agglutination reaction. Agglomerates are formed between antigens and specific anti-serums that coalesce and precipitate.

Observation of the activity of IgM and IgG immunoglobulins was carried out by looking at the antibody titrant, which was the highest dilution of the solution that still showed agglutination reactions. The results of the calculation of the IgM and IgG immunoglobulin titrants were obtained by converting the antibody titrant values with the formula [2 log(titrant) + 1], are shown in Tables 4 and 5.

Table 4. IgM Antibody Titrant

Groups	IgM Titrant	SD	Significance
K-	1.709	0.348	a
K+	3.696	0.269	b
P1	2.792	0.291	c
P2	3.597	0.406	bd
	Kruskal Wallis; Chi sq = 22.260; p=0.000		

*) Supplements in the group:
K (-): water, K(+): commercial FOS, P1: non-inhibited syrup, P2: inhibited syrup

Table 5. IgG Antibody Titrant

Groups	Titrant IgG	SD	Significance
K-	1.408	0.492	a
K+	3.515	0.348	b
P1	2.461	0.425	c
P2	3.386	0.670	bd
	One Way Anova; F=16.356; p=0.000		

*) Supplements in the group:
K (-): water, K(+): commercial FOS, P1: non-inhibited syrup, P2: inhibited syrup

The results showed that IgM and IgG antibodies in the inhibited yacon syrup supplement group as good as the commercial FOS supplement group; while the IgM and IgG of the non-inhibited yacon syrup supplement group were significantly lower than the commercial FOS group. The use of inhibited yacon syrup supplement can enhance IgM and IgG humoral immunity as in the commercial FOS supplement group; showed that the contents and roles of FOS and other bioactives in inhibitor syrup could be maintained so that they were close to the IgM and IgG values of the commercial FOS group. Non-inhibited yacon syrup is less able to maintain contents and roles of FOS and other bioactives that can act as immunostimulators for the adaptive immune response of T cells and B cells.

The role of FOS on B lymphocyte cells was found by [23] that there was an increase in the number of B cells in Peyer's path after FOS supplementation in experimental rat animals. The percentage of blood B cells increased with prolonged FOS supplementation [24]. The use of 1.25 g/L FOS for 7 weeks in rat experimental animals increased serum IgA and IgG [25]; while the use of 0.1% oligofructose in pregnant experimental animals during lactation, increased IgM in colostrum and milk [26]. Saeed et al revealed that yacon tubers modulates the immune system by regulating the secretion of IgA and interferon IFN-γ in the gut which further increases resistance to infection and allergic reactions [27].

4. CONCLUSION

In conclusion, there are two statements representing the findings: (1) In the process of making yacon syrup, natural inhibitors and regulation of pH and evaporation temperature have a very important role in maintaining the contents and functions of the bioactive components of FOS and phenolic compounds of yacon tubers; and (2) Cellular and humoral immunity of the inhibited yacon syrup supplement group was higher than the non-inhibited syrup supplement group. Inhibited yacon syrup is effective in increasing cellular and humoral immunity of experimental animals.

AUTHORS CONTRIBUTION

All authors conceived and designed this study. All authors contributed to the process of revising the manuscript, and at the end all authors have approved the final version of this manuscript.

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