The 2nd ICOLIB

International Conference on Life Sciences and Biotechnology

Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember

(ICOLIB BIO-UNEJ 2017)

Integrated Biological Sciences for Human Welfare

PROCEEDINGS

The Panorama Hotel and Resort Jember
East Java, Indonesia
August 7 - 8, 2017

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WELCOMING ADDRESS (CONFERENCES)

The International Conference of Life Science and Biotechnology (ICOLIB) was organise by Biology department Faculty Mathematic and Basic Sciences, The University of Jember, Indonesia. This conference has been held biannually at different venues. The last one, (First ICOLIB) held in Aston Hotel Jember 2015, Indonesia. Now, we are held the 2nd ICOLIB at Panorama Hotel and Resort Jember, Indonesia. The ICOLIB is a forum for students, researchers, educators, observers and practitioners from university, research institutions, industry and general public, policy maker to exchange ideas and latest information in the field of life science and its application. The theme of the 2nd ICOLIB 2017 '**Integrated Biological Sciences for Human Welfare**' will underpin the need for collaboration and cooperation of individuals from a wide range of professional backgrounds. The scope of the 2nd ICOLIB covers several fields of studies, namely life sciences, environmental sciences, medical and pharmaceutical sciences, science of renewable energy, agricultural science and food security. This conference will also offer opportunities for discussion and sharing as well as encouraging for international research collaboration. Furthermore, the scientific articles will be peer-reviewed and published in Serial book volume publish with Cambridge Scholar Publishing UK. The selected scientific articles in the 2nd ICOLIB will be further reviewed and will also be published in Scopus-indexed Journal.

The 2nd ICOLIB have been fortunate to have Prof. Harald zur Hausen, 2008 Nobel Laureate in Physiology or Medicine for his discovery of human papilloma viruses causing cervical cancer. Prof. zurHausen and his team has made a breakthrough in 1982 and 1983 when they were able to isolate HPV 16 and HPV 18 as the virus types responsible for cervical cancer. Based on these findings, vaccines have been developed against cervical cancer, one of the most common forms of cancer among women. This work led to improved methods for predicting which women are in the risk zone. We are very honoured to present Prof. Harald zur Hausen, as a keynote speaker, and 6 distinguished scientists as invited speakers.

I sincerely hope that the results of this conference will enable all participating scientists from all over the world to have the opportunity to exchange knowledge through lectures and posters.

Purwatiningsih

Chairwoman of The 2nd ICOLIB 2017

PREFACE FROM EDITOR

Welcome to the The 2nd ICOLIB Proceedings, **Integrated Biological Sciences for Human Welfare,** The theme of this conference reflects our attention Biological science to support the human welfare across of different of fields and contexts. Indeed, the 17 contributions in these proceedings—including keynotes, invited and contributed papers—with authors from at least 6 different countries showed the great results of Biology.

All the manuscript have been peer reviewed with at least 2 people who competence with their subjects. It has been a great previlege for being Editor of the 2nd ICOLIB. Hope these output had a great support for human welfare needs in many levels. Thus enable all participating scientists from all over the world to have the opportunity to exchange knowledge through the conferencere.

Sincerely,

Editorial team of The 2nd ICOLIB

Contents

INCREASING RESISTANT STARCH TYPE 3 OF MODIFIED CASSAVA FLOUR (MOCAF) USING ONE CYCLE OF AUTOCLAVING COOLING TREATMENT FOLLOWED WITH DEBRANCHING ENZYMES PULLULANASE

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Abstract

Modified Cassava Flour (Mocaf) is widely used as food ingredients either because they have good functional values such as rich in fiber and contain no gluten that believed related to the occurrence of certain diseases such asautism. By increasing the content of resistant starch (RS) in Mocaf we can increase its role as aprebiotic. One type of RS that widely used is RS type3 (RS3) which formed through retrogradation process using heat and cooling (autoclaving- cooling) treatment. Previous study by Asbar *et al*, demonstrated that 3 cycle of autoclaving-cooling treatment on Mocaf can increase the levels of RS3 as much as 8.73 percent. In the other study conducted by Zahruniya et al, demonstrated that by adding debranching enzyme pullulanase after one cycle of autoclaving-cooling treatemment can increase levels of RS3 incassava starch by 87.64%. The purpose of this study is to increase the levels of RS3 in Mocaf by using one cycles autoclaving-cooling treatment followed with debranching enzyme pullulanase. The results demonstrate that this method can increase the levels of RS3 in Mocaf from 9.66% to 60.87 %.

Keywords: Modified Cassava Flour (Mocaf), Resistant Starch type 3, autoclaving cooling, Pullulanase.

1. Introduction

Currently Modified Cassava Flour (Mocaf) is widely used as food ingredients such as a flour substitute either because they have good functional values such as rich in fiber and contain no gluten that believed related to the occurrence of certain diseases such as autism. By increasing the content of resistant starch (RS) in Mocaf we can increase its role as a prebiotic. RS is part of starch that resistant to hydrolysis process from digestive enzyme such as amylases of that it become poorly digested and cannot be absorbed in the small intestine. In the colon, RS is then fermented by intestinal microflora to produce short chain fatty acids (SCFA) [4] Many research suggests that by consuming food that contain RS can improve glucose metabolism and improve insulin sensitivity. Short chain fatty acids (SCFA) from fermented RS inside the colon will increasethe expression of glucagon like peptide- 1 (GLP-1) gene of Lcell in the intestine wall so that it can increase the production and secretion of GLP-1 in the blood plasma. Glp-1 is a proinsulin peptide. Increasing concentration of GLP-1 in the plasma will also increase the production of insulin in the pancreatic cell. Thus foods containing RS has a great potential in the prevention and treatment of type 2 diabetes mellitus [2, 6, 7] One type of RS that widely

used is RS type (RS3) wich formed through retrogradation process using heating treatment with an autoclave (121°C) followed by cooling at low or at room temperature (autoclaving-cooling). Retrogradation occurs through a process of reassociation (realignment) hydrogen bonding between short chain amylose formed after the heating process [5].

Increasing the content of the RS3 is not enough if only processed using the method of autoclaving-cooling alone. In a study by Asbar et al, demonstrated that 3 cycle of autoclaving- cooling treatment of Mocaf can only increase the levels of RS3 as much as 8.73 percent. By adding starch hidrolizing enzymes such as Pullulanase that hidrolize the α-1,6 branch of amylopectin (debranching) will increase the content of the RS3 even with one cycle of autoclaving-cooling treatment. The longer of the debranching process more short chain amylose will be produced which can multiply the opportunities of RS3 formation. In a study conducted by Zahruniya et al, demonstrated that one cycle of autoclaving-cooling treatemment followed with debranching process for 24 hours using pullulanase 1.04 U/g can increase RS3 in cassava starch up to 88.64% [1,9].

Research on modification of Mocaf to increasethe levels of RS3 with autoclaving-cooling followed with debranching process using pullulanase yet ever done. The purpose of this study is to increase the levels of RS3 in Mocaf by using one cycles of autoclaving-cooling treatment followed with debranching enzyme pullulanase.

This study conducted in April 2017. The modification and analysing process of Mocaf were conducted in several laboratories; The Laboratory of Biochemistry Faculty of Medicine University of Jember and Integrated Analysis Laboratory Faculty of Engineering of Agricultural Products University of Jember. There are three stages in this study: (1) characterization and analysis of native Mocaf (2) Increasing of RS3 in Mocaf using one cycle of autoclaving-cooling treatment followed with debranching process (3) characterization and analysis of RS3 in Mocaf after treatment.

Increasing of RS3 in Mocaf using one cycle of autoclaving-cooling treatment followed with debranching process

In the early stages, Mocaf gelatinized at high temperature using an autoclav. A total of 200 g of Mocaf were suspended with 300 mL of aquadest in Erlenmeyer flasks (20%w/v). Samples were then heated in an autoclave at 121° C for 1 hour. This process will make the starch granules swell and make amylose out from starch granules. Furthermore, the starch is incubated at-20°C for 6 hours. Then proceed with the debranching process. As much as 25 grams sample in 100 ml of acetate buffer pH5.2 heated at 95 °C for 10 minutes, then resuspended in 125 mL of acetate buffer pH 5.2 and heated in an autoclav at121 °C for 30 minutes, after the temperature down to 50 °C. Sample were hydrolyzed by adding pullulanase 1.04U/g. Samples were then incubated at 50°C for 24 hours while shaken at a speed of 160 rpm. Termination of the enzymatic reaction carried out by heating the sample in an autoclav for 1 hour and then dried in an oven at 50°C and then analyzed (Zahruniya 2014)

2. RESULTS

Characterization And Analysis Of Native Mocaf

Analysis showed the water content of native Mocaf was 14.87 %, the starch content was 53.23%. Native Mocaf contain amylose as much as 27.29%. Digestibility analysis showed that native Mocaf digestibility was 15.32%. Furthermore, the analysis showed RS content in native Mocaf was 9.66%, Rapid digestable starch (RDS) content was 43.19% and Slow digestable starch (SDS) was 47.16%.

Increasing of RS3 in Mocaf using one cycle of autoclaving-cooling treatment followed with debranching process

After heated with an autoclave at 121°C for one hour, sample then cooled at low temperature of 20 °C for 6 hours followed with debranching process using pullulanase for 24 hours then driyed at 50 °C in an oven. The starch inside the sample were retrograded. Retrogradation occurs through a process of

reassociation (rearrangement) of hydrogen bonds between short chain amylose that is formed after the heating process [5]. The morphology of RS3 can be seen using electron microscope as seen in Figure 1.

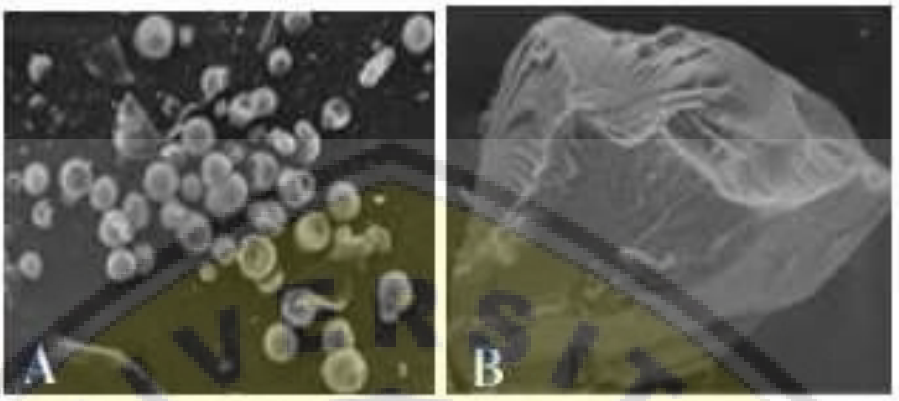

Figure 1. Morphology of starchgranule usingelectron microscope600x:A.NativeMocaf..B.RS3 Mocaf.

Using an electron microscope on the natural starch we would seem starch granules are spherical. While RS3 has been turned into an amorphous shape. Between another starch, RS3 is preferrable because it is more stable against heating so it is not damaged when processed into food products [8]

Characterization and analysis of RS3 in Mocaf after treatment

After the modification process of native Mocaf with one cycle ofautoclaving-cooling followed with debranching process using pullulanase the analysis showed that the water content was 71.09%, the starch content was 3.72%. Amylose content was 7.05%. The result of digestibility analysis was 11.66%. RS level analysis shows that with one cycles autoclaving cooling treatment followed with debranching process using pullulanase produce RS3 as much as 60,88%, and as much as 18.18 % of RDS and 20.94 % of SDS.

3. Discussion

The use of native starch is currently very limited because of the physical and chemical properties that are less appropriate in wide spread use. It is therefore necessary modifications to the starch have added value. Modifications can be done physically, chemically, or a combination of both. One type of modified starch is Resistant Starch (RS) which is resistant to the hydrolyzing process of digestive enzymes [3, 9]

Starch can be classified based on its digestibility by amylase and of the method in producing them. Based on the digestibility by amylase, starch can be classified into 3 namely starches that can be digested quickly or rapidly digestible starch (RDS), slowly digestible starch (SDS), and cannot digested or resistant starch (RS). Based on the way of producing, RS grouped into 5 groups one of which is the RS3. RS3 formed through a process of retrogradation by heating with an autoclave at 121°C, followed by cooling at low temperature orat room temperature. Retrogradation occurs through a process of reassociation (rearrangement) of hydrogen bonds between short chain amylose that is formed after the heating process and form double helix structure that will inhibit water absorption and enzymatic process [5]

The morphology of RS3 can be seen using electron microscopy. Using an electron microscope on the natural starch we would seem starch granules are spherical. While RS3 has been turned into an amorphous shape. Between another starch, RS3 is preferrable because it is more stable against heating so it is not damaged when processed

into food products (Kouamé et al, 2015; Vatanasuchart et al, 2010)

Several in vivo studies conducted on animals and humans indicate that RS has the potential to support human health one of them is as a prebiotic ingredient. Research on RS3 indicate that the granules of RS3 starch form a pattern of attachment specifically in the upper intestines that it can become an attachment for probiotic bacteria.(Brighenti et al,2006)

The fermentation of RS3 by probiotic bacteria result in the form of short chain fatty acids (SCFA) such as acetate, propionate and butyrate. Acetate and propionate has a role in increasing the expression of precursor gene of GLP-1. By getting a lot of content RS3 in food consumed the higher levels of GLP-1 in the intestinal L cell will produced and the higher levels of GLP-1 secreted in blood plasma. Increased levels of GLP-1 will induce pancreatic β cell proliferation, increases the production and secretion of insulin and glucagon to control glucose concentration in the blood and in muscle cells. That is way Food containing RS3 has a potential role as an important nutrient in the prevention and treatment of type 2 diabetes mellitus. (Brighenti et al.2006)

Increasing the content of the RS3 is not enough if only processed using the method of autoclaving-cooling by autoclav alone. The addition of starch hydrolyzing enzymes will increase the content of the RS3. The enzyme frequently used to hydrolyze the starch is pullulanase. Pullulanase has a spesific acts that is hydrolized the α-1,6 branch of amylopectin (debranching).(Zahruniya 2014)

This study has prove that by using one cycles of autoclaving-cooling treatment followed with debranching the α-1,6 bond of amylopectin on Mocaf using pullulanase 1.U/g can increase the levels of RS3 in Mocaf as much as 60.88 % or 6 times higher than the levels of RS in native Mocaf. The comparation between native Mocaf before and after treatment can be seen in table1. Further research related to the type of SCFA formed, the type of probiotic bacteria grown in the colon due to consumption of foods containing RS3 Mocaf as well as its role in the prevention and treatment of type2 diabetes mellitus or another diseases were still need to be done.

Table1. Comparation between native Mocaf and RS3 Mocaf

Parameters	Native Mocaf	RS 3 Mocaf
Starch Content	53.23%	37.2%
Amylose	27.29%	7.05%
RS	9.66%	60,88%
SDS	47.16%	20.94%
RDS	43.19%	18.18%
Digestibility	15.32%	11.66%

4. Conclusion

From these results it can be concluded modification of Mocaf with one cycle of autoclaving-cooling treatment followed with debranching process using pullulanase successfully increase the levels of RS3 in Mocaf as much as 60,88 % or 6 times higher than the RS levels in native Mocaf.

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