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Technological innovation in the protection of beta carotene on MOCAF production which is rich in beta carotene

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Abstract. *Hartati, Fathoni A, Kurniawati S, Hartati NS, Sudarmonowati E. 2017. Technological innovation in the protection of beta carotene on MOCAF production which is rich in beta carotene. Nusantara Bioscience 9: 6-11.* The conversion process of high beta carotene cassava into high beta carotene MOCAF might cause a high loss of beta carotene contents during the process of flour making. This research aims to develop the most appropriate technology to protect the loss of beta carotene contents during MOCAF process. Preservation methods used consisting of four starter treatment combinations of Bimo-CF and sodium metabisulfite ($Na_2S_2O_5$) on Adira 1 variety, namely (i) 0.5 g starter/L volume of soaking solution and 0.3% of $Na_2S_2O_5$, (ii) 0.5 g starter and 0.15% of $Na_2S_2O_5$, (iii) 1 g starter and 0.3% of $Na_2S_2O_5$, and (iv) 1 g starter and 0.15% of $Na_2S_2O_5$. The results showed that $Na_2S_2O_5$ can reduce the loss of beta carotene contents during the process of flour making. Of the initial concentration of beta carotene in tubers (3.5 mg/kg), the loss of beta carotene on MOCAF control without preservation process with $Na_2S_2O_5$ reached 68.8% (1.09 mg/kg). With $Na_2S_2O_5$ application, the loss of beta carotene could be reduced to 24.3% (2.65 mg/kg). The fermentation process with Bimo-CF starter increased the protein content of MOCAF two times from the control. The best and safest protection of beta carotene for food was achieved from preservation method using the combination of 1 g/L volume of soaking solution starter and 0.15% of $Na_2S_2O_5$ which produced residue of $Na_2S_2O_5$ to 53.07 mg/kg, which means lower than the maximum residue allowed in a flour product (70 mg/kg). The MOCAF yield obtained from each combination is quite high in range of 32-38%. The highest MOCAF yield is obtained from the protection method using combination of 1 g/L volume of starter soaking solution with $Na_2S_2O_5$ 0% and 0.15%.

Keywords: Beta carotene, MOCAF, preservation, sodium metabisulfite, starter Bimo-CF

INTRODUCTION

Beta carotene or pro-vitamin A is part of the carotenoid molecule that is naturally produced by plants performing in the color of orange or yellow of tuber or fruit. Beta-carotene is very important for humans because it serves as a precursor in the synthesis of vitamin A. Vitamin A deficiency causes various diseases and blindness, and decrease the immune system of children less than five years old for up to 40%. Vitamin A is required to produce retinal, retinol and retinoic acid which are important in supporting the transmission of visual information from the eye to the brain. Vitamin A deficiency affects the production of retinol so that the transmission of visual information from the eye to the brain is interrupted. Retinol is essential for skin health and reproductive system, while retinoic acid is a hormone that regulates gene expression and protein synthesis (Mukherjee et al. 2006; Mora et al. 2008; Pino et al. 2008).

Food fortification through addition of vitamin A chemically has been used to reduce illness due to vitamin A deficiency. Plant breeders use an alternative approach to overcome the shortage of vitamin A in food crops, either through conventional breeding or the application of biotechnology to produce plant crops with high level of beta carotene contents. The improvements of beta-carotene content on cassava conventionally can be conducted by selecting and crossbreeding between targeted cassava genotypes and cassava possessing high beta carotene contents.The initial successful story of improved Golden Rice through biotechnology approach has been triggering many advanced researches focusing on the development of bulbous plants containing high beta carotene contents, such as biofortified β-carotene of cassava and improved level of orange colour in sweet potato (Egana 2009; Telengech et al. 2015). Biofortification is more profitable and sustainable technique instead of chemical food fortification and consumption of food supplements (Zimmermann and Hurrell 2002). Biofortification of beta carotene on plants can be conducted through several approaches, for instance by down regulating the gene encoding the beta carotene hydroxilase or isopentenyl diphosphate isomerase, overexpression of the gene 1 deoxy D-xylulose 5 phosphate synthase (DXS) and gene pythoene synthase (McGregor and Labonte 2006; Telengech et al. 2015). Beta carotene hydroxilase are regulator enzymes that catalyze the hydroxylation process of beta carotene into beta cryptosantin and of beta cryptosantin into zea xanthine (Kim et al. 2012). The accumulation process of provitamin A on cassava tuber (*Manihot esculenta* Cranz) is controlled by a single nucleotide polymorphism (SNP) of phytoene synthase gene (Welsch et al. 2010; Welsch 2011), therefore, overexpression of phytoene synthase genes could increase beta-carotene levels on cassava.

Research Center for Biotechnology, Indonesia Institute of Sciences (LIPI) has been collecting and identifying some potential varieties of cassava possessing high beta carotene contents, such Adira 1, Mentega 1, Mentega 2, Ubi Kuning, and FEC25 genotype derived from Somaklonal variants (Hartati et al. 2015; Hartati et al. 2015). Cassava genotypes with high beta carotene contents can be potentially used in the production of high beta carotene MOCAF (Modified Cassava Flour). Application of fermentation process during MOCAF production can change and improve the characteristics of MOCAF including the improvement of swelling power and viscosity of the paste (Misgiyarta 2009). MOCAF texture is also become softer and the cassava aroma which is less-preferred by consumer is reduced after fermentation (Subagio 2007). MOCAF has lower content of water and ash and moreover, the soluble fiber content is higher.

The production of high beta carotene MOCAF has several advantages. First, it may increase the added value of cassava as raw material for healthy food since it provides an alternative source of vitamin A and also contains gluten free. Consumption of gluten-free flour provides benefits to consumers, especially to those who are sensitive to gluten such as autism disorder. Second, the characteristics of high beta carotene MOCAF has improved and become more similar to a wheat flour characteristic. MOCAF can be widely applied to food products compared to cassava flour. The improvements on MOCAF characteristic also make it able to be applied to almost all wheat flour-based food products. Third, MOCAF'S life of shelf is longer than the cassava tubers. Its distribution is also easier to manage than that in fresh tubers. And the last, high beta carotene MOCAF can be applied in wider use.

Beta carotene is very sensitive to oxidation induced by heat, light, oxygen, acid, transition metal, or interactions with radical species (Erawati 2006; Boon et al. 2010). The processing of high beta carotene cassava tubers into high beta carotene MOCAF potentially cause a high loss of beta-carotene contents during the flour-making process, especially in the fermentation process and drying stages.

Our research aim is to develop the most appropriate technique to protect a high loss of beta carotene contents during the production of high beta carotene MOCAF.

MATERIALS AND METHODS

Materials

Adira 1 variety at age of 10 months was used as the material for production of high beta carotene and high protein MOCAF. This variety is planted in the Research Center for Biotechnology germplasm collection, Indonesian Institute of Sciences (LIPI) in Cibinong. The important characteristics of this variety is high starch and high beta-carotene content. Adira 1 cassava chips were fermented using a commercial starter BIMO-CF, which is specifically formulated for the production of MOCAF.

Methods

The effectiveness of four methods treating with different concentration of both combinations of sodium metabisulfite ($Na_2S_2O_5$) and BIMO-CFstarter were tested on the stage process of cassava chips during the production of high beta carotene and high protein MOCAF (Table 1). The lose level of beta-carotene content on MOCAF was measured by comparing it to that achieved in the control (without treatment of $Na_2S_2O_5$ and initial dried fresh tubers). We used two different concentrations of BIMO-CF starter (0.5 and 1 gram per liter volume of immersion water) and $Na_2S_2O_5$ (0.15% and 0.3%) in the fermentation process. In each treatment, 10 kg of Adira 1 cassava tubers except for combination 1 using 10.5 kg of tubers. The high beta carotene MOCAF was produced by firstly peeling cassava tubers to remove cassava peel and cortex then continued by washing them to remove mud and remaining soil. The peeled tubers were sliced into chips with a thickness of 2 mm. Cassava chips were soaked in BIMO-CF starter solution with concentration of 0.5 and 1 gram of starter per liter of water for 21 hours, and followed by soaking them in $Na_2S_2O_5$ at a concentration of 0.15% and 0.3% for 30 minutes. Sodium metabisulfite was directly added to the soaking water of fermented cassava chips, and mixed thoroughly. Cassava chips were submerged until all parts of the chips are perfectly fermented. The remaining water of fermented chips were then discarded prior to the drying process. The subsequent drying process of cassava chips was conducted either in the oven at 50° C or under the sunshine with shading net cover.

Beta carotene, protein and $Na_2S_2O_5$ residues were measured using established methods developed by the Saraswanti Indo Genentech (SIG) Laboratory, including the measurement of beta carotene based on method of 18-5-40/MU/SMM-SIG, the measurement of protein by Kjeltec method (18-8-31/MU/SMM-SIG) and the measurement of $Na_2S_2O_5$ residue with method of SNI 01-2894-1992 part 2.6. The percentage of MOCAF yield is calculated by dividing the total weight of MOCAF and peeled cassava multiplied by 100% (Amanu and Susanto 2014).

Table 1. The combination of beta carotene preservation treated with different concentration of starter and natrium metabisulphite in the production of high beta carotene MOCAF

Treatments	Tubers weight (kg)	The amount of starter (g)	The use of natrium metabisulphite (g)	Drying condition
Combination 1	10.5	10	60	Dried in the oven and under the sunshine with a shading net cover
Combination 2	10	10	30	Dried under the sunshine with a shading net cover
Combination 3	10	20	60	Dried under the sunshine with a shading net cover
Combination 4	10	20	30	Dried under the sunshine with a shading net cover
Combination 5 (Control without $Na_2S_2O_5$ treatment)	10	20	0	Dried under the sunshine with a shading net cover

RESULTS AND DISCUSSION

Beta-carotene content of Adira 1 variety

Cassava variety of Adira 1 has been evaluated to gain information about its beta carotene content. The results of our experiment showed that beta-carotene content in the dried tubers of Adira 1 apparently varied among the planted trees, which ranged from 1.00 to 8.15 mg/kg According to previous reports, level of beta carotene content in plants are influenced by several factors, including plant varieties, age of the plant, harvesting and storage, soil conditions, seasons, drought and pathogen infections (Burri 2013).

Preservation of beta carotene with sodium metabisulfite

Loss of beta carotene contents could be reduced by treating specifically the cassava chips during the fermentation and the drying process. Research Center for Biotechnology-LIPI has developed an innovative technique to protect the loss of beta carotene during the MOCAF-making process through the application of $Na_2S_2O_5$ at the end of fermentation process. Of the series treatments of concentration level of $Na_2S_2O_5$ on cassava chips, the results revealed that $Na_2S_2O_5$ treatment could significantly protect the greater loss of beta carotene content during the MOCAF production than those obtained in the control (without $Na_2S_2O_5$ treatment) (Figure 1).

The high loss of beta carotene contents in MOCAF production could be effectively hindered by treatment with the the higher application of $Na_2S_2O_5$ concentration level (with maximum concentration was 60 g starter/10 kg cassava chips/20 liters of water). The percentage of loss level of beta carotene contents in the control treatment (without preservation of $Na_2S_2O_5$) reached 68.8%. $Na_2S_2O_5$ suppresses the loss of beta-carotene content to the level of 24% (treatment 3). Our results are in agreement with those results reported by Widiyowati (2007) on sweet potatoes, which concluded that the high usage of $Na_2S_2O_5$ on immersion of sweet potatoes chips during the process of flour making reduced the possibility of high lose of beta carotene content in the flour.

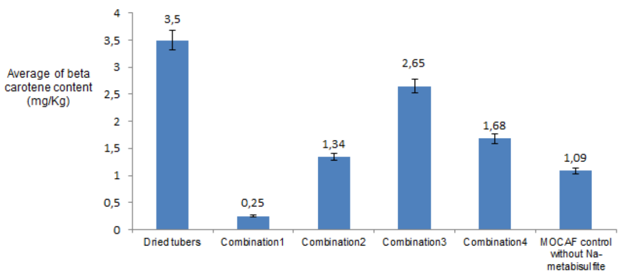

Figure 1. Analysis of beta carotene contents in MOCAF obtained from four combinations of treatments. the drying system with a combination of dried-oven and sunshine plus shading net was used in the combination 1, while sunshine drying system with shading net cover was applied in thecombination 2-4 and controls without $Na_2S_2O_5$

Sodium metabisulfite is a food additive that was used as antioxidants (Ash and Ash 2004; Tan et al. 2015), a preservative and surface protection agent (Pubchems). According to previous reports, $Na_2S_2O_5$ is applied in cassava to remove the smell and its bitter taste, to maintain the white color of cassava chips (Yusmeiarti et al. 1997) and to prevent browning in the process of tapioca production (Husniati and Adi 2010). In our work, sodium metabisulfite is applied to protect and to coat the surface of cassava chips in order to prevent the high loss of beta-carotene during the fermentation process. Polyphenols content and polyphenolase enzyme activity may determine the rate of browning in cassava. Polyphenols oxidation (PPO) catalyze the oxidation of polyphenols and transform it into brown pigments (Syarif and Irawati 1988).

Drying system

Beside the application of $Na_2S_2O_5$ had a positive outcome in the preservation of beta carotene contents in MOCAF production (Figure 1), the selection of an appropriate drying system is also a crucial factor affecting the preservation of beta carotene content during the production of high beta carotene MOCAF. Our results showed that the best drying system used for the production of high beta carotene MOCAF was by using sunshine with shading net cover. The highest loss of beta carotene contents in MOCAF of all treatments combination has been found in the drying system using both oven and sunshine performed in treatment 1.

Higher loss of beta carotene content in the combination 1 is affected by the unstable temperature in oven during the drying process. We found that the temperature in the oven higher than 50°C, even the regulator was set on 50°C. The double bond in the chemical structure of beta-carotene is susceptible to oxidation induced by air, light, metal, peroxide, and heat (Erawati 2006). The ideal temperatures for the drying process of high beta carotene MOCAF are 40^o-50^o C (Fathoni 2012, unpublished data). On a drying system with stable temperature or in a drying process with sunshine with shading net cover, the protection system of beta-carotene with $Na_2S_2O_5$ could be maximized.

Analysis of sodium metabisulfite residue for food safety

Based on the regulation of the Minister of Health of the Republic of Indonesia, the allowed dosage of $Na_2S_2O_5$ for the daily intake is 70 mg per kg of body weight. MOCAF products which are safe for daily consumption and meet the standart of food safety are MOCAF generated from combinations 1 and 2 (Figure 2). In this treatment, residue of $Na_2S_2O_5$ is under 70 mg/kg. High application of sodium metabisulfite which did not pass to the requirement of food safety would be prohibited due to it has high risk possibility for the consumer health especially to those who have the high sensitivity to sulfites.

Utilization of sodium metabisulfite as food additives should meet standarts and requirements noted in the Indonesian food codex released by the Minister of Health of the Republic of Indonesia No. 722/Menkes/Per/IX/1988 (Permenkes 1988) and No. 33 of 2012 (Permenkes 2012). The analysis of $Na_2S_2O_5$ residu in MOCAF shows that the combination of $Na_2S_2O_5$ and BIMO-CF starter had impact on the level of residue contents of $Na_2S_2O_5$ in the product. Application of higher quantity of $Na_2S_2O_5$ provided a better protection from the loss of beta carotene content, but it also caused the increased level of $Na_2S_2O_5$ residues on MOCAF. Higher quantity of $Na_2S_2O_5$ residue on MOCAF is apparently affected by the use of the large amount of starter. Utilization of higher Bimo-CF increase the number of broken cassava cells leading to the more sodium metabisulfite absorbed by MOCAF.

BIMO-CF, a commercial starter generated from consortium of lactic acid bacteria and others, produces several types of enzymes including cellulolytic and pectinolytic enzymes. These two enzymes digest the cellulose and pectin in the cell walls of cassava, and affect the release of starch granules from cassava cell. Lactic acid bacteria also produces enzymes that hydrolyze starch into sugars and subsequently convert it into organic acids, including lactic acid. The lactic acids imbibe into MOCAF chips together with $Na_2S_2O_5$, lead to the reduction of the aroma and flavor of cassava, which is unlikely preferred by consumers (Subagio 2007).

Protein

Application of microbial starter in the fermentation process of cassava chips increased the protein content of MOCAF. The highest protein content is obtained in MOCAF produced from the treatment 2 with the percentage of protein 2.19% (Figure 3). By comparing the protein content of MOCAF from each treatment to that of Adira 1 control tubers, it is noted that the protein content of MOCAF increased two times from the control. According to previous reports, in general, the total percentage of protein content of flour is 1% (Ministry of Industry 1990).

The starter increased the protein content of MOCAF through the production of single cell protein (SCP) during fermentation process. Lactic acid bacteria contained in BIMO-CF reached exponential growth phase after it has been fermented for 12 hours. In this phase, bacteria produced higher single cell protein. The type of starter used in fermentation process had effect on the enhancement of protein content in MOCAF. Previous experiments showed *Lactobacillus plantarum* could increase the protein content of MOCAF from 2.78% to 3.39% after being fermented for 72 hours (Tandrianto et al. 2014). *Streptococcus thermophilus* increased the protein content from 6.58% to 7.58% after chips have been fermented for 24 hours (Puspitojati and Santoso 2014).

MOCAF yield

Detailed information on how much the MOCAF could be produced per kilogram of peeled cassava tubers is important to determine MOCAF yield value. The selected cassava genotypes used as material for MOCAF production should be the one with MOCAF yield above 30% in order to give high profit for production. Our results showed that the level of MOCAF yield generated from each treatment was high in ranged of 32 to 38%. The highest yield of MOCAF is obtained from the combinations of treatments 4 and 5 which are using starter 1 g/L soaking solution and $Na_2S_2O_5$ 0% and 0.15% (Figure 4). This result indicates that Adira 1 is ideal to use as material for the production of high beta carotene MOCAF. Beside high starch and high beta carotene content, Adira 1 are also early bulking and mature, and has the low HCN (Howeler 1987).

The commercial prospect of high beta carotene MOCAF

The commercial prospect of high beta carotene MOCAF in Indonesia is very promising. The first reason, based on the market and raw material availability, cassava production in Indonesia is abundant. Therefore, MOCAF is expected to be a tight competitor of wheat flour with some added value. Second, the substitution ability of MOCAF to wheat flour can reach 30-100%. Third, high content of beta-carotene and protein will increase the value of food products made from MOCAF.

In addition, innovation of cassava product into high beta carotene MOCAF will provide great benefits to household consumer and food industries that rely on flour as the basic ingredient, especially when observed from the aspect of health and substitution ability (Ruriano et al. 2013; Hartati et al. 2015). Based on information gained from home industries that has been used our high beta carotene MOCAF as basic ingredients, the usage of high beta carotene MOCAF made from Adira 1 increased the crispness of fried snack. Furthermore, this product innovation will influence the cassava farmers around the MOCAF flour mill. The welfares of farmers who supply materials of cassava, either in raw form or in the form of fermented chips, will increased.

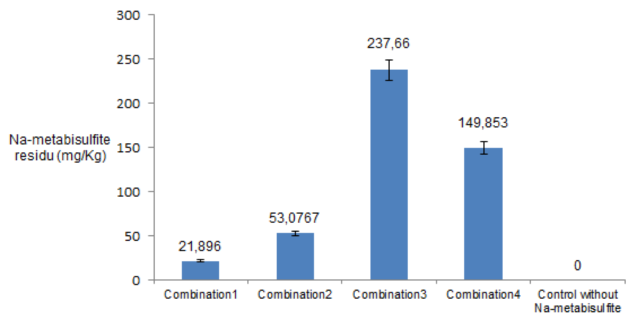

Figure 2. The analysis of $Na_2S_2O_5$ residue on MOCAF obtained from 4 combinations treatment

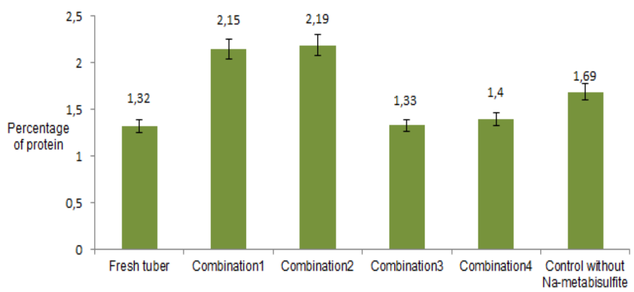

Figure 3. Average percentage of protein on Adira 1 fresh tubers and MOCAF obtained from four combinations

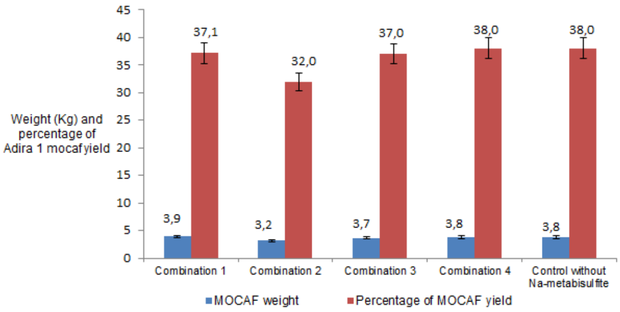

Figure 4. The weight and the percentage of Adira 1 MOCAF yield obtained from 4 combinations.

In conclusion, we suggest to use combination 2 as a protocol to produce high beta carotene MOCAF since this method provide a good protection to the loss of beta carotene content. The application of sodium metabisulfite in the production of high beta carotene MOCAF has proven gives a good protection to high loss of beta carotene content during the process of flour making. Moreover, through combination 2 method, the protein content in MOCAF has been increased up to 2,16% and sodium metabisulfite residu still meet food safety. Sodium metabisulfite content obtained from combination 2 was 53.07 mg/kg, while the maximum limit allowed in flour was 70 mg/kg. The yield of MOCAF obtained from the combination 2 was also high.

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