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BINDERING AND THE STABILITY OF BETA CAROTEN FROM *Neurospora sitophila*

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Abstract

Neurospora sitophila, a species of fermetation fungi was reported containing the β-carotene pigments, the secondary metabolite of the yellow, orange or red-orange pigments groups, On this research, the carotenoid pigments of Neurospora sitophila has been bindered by a Oligosacharide of gelatin-maltodextrins. The bindered products were dried by a spray drier and the β-carotene powder was determined its stability to the storage influence at Relative Humidity 20-30%. Bindered product of β-carotene extract obtained the GME powder with BY value±50 %. Bindered of this extract β- carotene *was increased the water solubility and stable at Relative Humidity 20-30% condition, the stability the powder (GME) was decreased start at the third week. HPLC analysis of GME powder showed the decreasing of β-carotene about 30%, after storage for 5 weeks.*

Key words*: Bindering, β-carotene, spray-drier, stability.*

INTRODUCTION

Oncome is a food rich in carotene pigment derived from fungal fermentation process substrate oncome on solid media.

Oncome yellow to red color produced by the fungus *Neurospora sitophila*, and the strain has orange, red, and pink.

Colors are caused by the existence of a kind of secondary metabolites carotenoid called *β-carotene*.

Carotenoid compounds have a range of color variations that are very attractive, especially when used as pigments for foodstuffs, feedstuffs, cosmetics, and pharmaceuticals. But generally these secondary metabolites, sensitive to light, heat, and oxygen, as well as having properties of low water solubility.

So that the compound is water insoluble, it is necessary through binding reaction (Reaction Mailard) *β-carotene* compound with other compounds that can give the effect of increased water solubility and color stability. *B-carotene* or natural dyes produced from fermentation by molds become a promising alternative to commercial-scale production, because in addition to cultivated quickly and provide a more pure product.

Research on *β-carotene* pigment produced by fungi, has been developed in Europe, and is recommended as a safe food additive and has a beneficial effect on health (Avalos, 2003). The result of this development is further used by the industry for the production of *β-carotene* in biotechnology and the development of new natural products rich in *β-carotene*.

The active compound binding reaction carotene can be made by dissolving the active compound and copolymer (binder) in an organic solvent, and then evaporated and diluted with water. The active compound can be bound to the particles when the solvent is replaced with water (Pan, et al., 2007). Hydrophobic groups (hate water) of the protein will interact with the compound *β-carotene* hydrophobic, while the hydrophilic groups (like water) of polysaccharides going out so it can be stable in the polar atmosphere, such as water.

This study aims to perform the binding of *β-carotene* pigment mold *Neurospora sitophila* and test the stability of the resulting binding reaction products. The binding process also conducted on *β-carotene* purely as a product comparison.

MATERIALS AND METHODS

Materials

Isolates of fungi *Neurospora sitophila*, pulp, maltodextrin, gelatin, *β-carotene* standard compounds, organic solvents and other chemicals, namely HCl, trypsin, cyclohexane, Sodium Sulfate.

Tools

Analytical balance, autoclave, plastic trays (plastic tray) incubator, vacuum evaporator, separating funnel, shaker bath, Chroma meter, HPLC, optical density, oven, and spray dryer.

Fermentation procedure Neurospora sitophila

Dried tofu that has been mashed, weighed as much as 200 grams of water and added with 600 ml (1: 3). Furthermore, the substrate is sterilized by autoclaving at 121 ^{0}C for 15 minutes. The substrates were autoclaved cooled, inoculated with 50 ml spore suspension of fungi *Neurospora sitophila* (10 million spores/ml) are then transferred into 2 pieces and perforated lidded tray with a size of 35 cm × 25 cm. Incubation is carried out at 30 ^{0}C for 3-5 days. Spores are yellow-orange harvest and store in the dark for further extracted.

Extraction of Carotenoids

Extract was filtered using filter paper of medium size. This extraction step is repeated until the entire pigment residues lifted and pale yellow spores. Further pigment extract was concentrated by means of a vacuum evaporator at 40 ^{0}C.

Extraction of carotenoids in the product GMB and GME follow the method Xiao Yun et al. (2007) were modified. The powder containing the carotenoid dispersed with 1 ml of HCl 0.1M and added trypsin (0.2 mg/ml). Fluid further incubated at 37 ^{0}C for 1 day.

Hydrolysis of protein molecules (trypsin) was stopped with 0.5 ml of acid solution (0.2M HCl). Cyclohexane is added at a certain ratio and shaken for 1 hour. The mixture is separated using a separating funnel, cyclohexane phase (top) is separated, added sodium sulfate dried and evaporated.

Carotenoids by HPLC analysis

Analysis of carotenoids was performed using HPLC following the method Sandman et al (2008) were modified, with a UV detector measured at a wavelength of 450 nm, mobile phase of acetonitrile: methanol: 2-propanol (85: 10: 5), a flow rate of 2 ml/minute, temperature 26 ^{0}C, type C18 column (Waters Symmetry size of 150 x 3.9 mm) and time analysis for 20 minutes. *Β-carotene* Type II (Sigma-Aldrich) was used as the standard compound HPLC.

Making copolymer through Maillard Reaction

Making copolymer based on the principle of Maillard reaction, following the method of Pan et al, (2007) were modified. In making this copolymer used gelatin (Merck), maltodextrin with a DE value of 13 - 17. The copolymer is made from a mixture of gelatin-maltodextrin with a ratio of 4: 1. The Maillard reaction is carried out at a temperature of 50 ^{0}C, 100 rpm for 4 hours.

Carotenoids Bindering process

Pulverizing carotenoids performed based on the method of Chen and Tang (1998) were modified. A total of 1.3 grams of carotenoid extract is diluted with a little technical ethanol. Gelatin-maltodextrin copolymer is prepared by mixing 40 grams of gelatin and 10 grams of maltodextrin, then dissolved in 1 liter of distilled water. Copolymerization reaction performed on a shaker apparatus with a temperature of 50 ^{0}C, 100 rpm, for 4 hours. Encapsulation of carotenoids GME (gelatin-maltodextrin-extract) is done by adding a carotenoid extract that has been dissolved in 1 liter of ethanol into the solution. Copolymer gelatin-maltodextrin that has been cooled. Furthermore, the solution is homogenized using a magnetic stirrer for 30 minutes. The powder is made by drying the carotenoid GME, GME suspension by means of spray-drier at an inlet temperature of 160 ^{0}C conditions, the outlet 70 ^{0}C, a pressure of 1 bar with an average flow rate of 8 ml/min. Samples are stored in a desiccator with a humidity of 20% - 30%, temperatures between 25 ^{0}C - 28 ^{0}C.

Results of the binding reaction (Bindering yield / BY) calculated by the equation:

$$\% \text{ BY} = \text{MSC x } 100 / \text{MSB}$$

MSC = Total mass of aggregate pigments after copolymerization

MSB = Total mass of aggregate pigments before copolymerization

GMB and GME powder and tested its stability against the solubility properties of carotenoids (OD.450), the intensity of the color by means of Chroma meter, and water content (Sudarmadji et al., 1997)

RESULTS AND DISCUSSIONS

Manufacture of gelatin-maltodextrin copolymers made through the Maillard reaction which is the reaction between the protein molecules with molecular saccharides. Copolymer casein-g-dextran has been made through the Maillard reaction is a reaction of transplant (grafting) dextran molecules with molecules of casein.

The copolymer is soluble in water at pH 7.0, but not soluble in ethanol. Meanwhile, *β-carotene* hydrophobic, solubility increases in ethanol (Pan, et al., 2007).

On this basis, the reaction bindering carotenoid extract *Neurospora sitophila* with gelatin-maltodextrin copolymer made to improve the solubility of the carotenoid in the water.

The suspension of the carotenoid extract mixture with gelatin-copolymer maltodextrin, dried by means of a spray dryer to obtain powder carotenoid GME (gelatin-maltodextrin-extract). As a comparison, the same encapsulation process is carried out using pure *β-carotene* (Sigma), thus obtained GMB powders (gelatin-maltodextrin- β-carotene).

Drying results obtained in this study were obtained powder GMB value BY 60%, and powder GME with value BY (Bindering Yield) 50%. The results of the spray drying process depends on the configuration tool, while bindering reaction efficiency is strongly associated with physio-chemical properties of the core material and a binder. The results were not much different from that reported by Nunez and Mercadante (2007) to the binding reaction lycopene compound with *β-cyclodextrin*, providing product acquisition by 51 ± 1%. Chen and Tang (1998) reported a 67% by values for binding results carotenoids from carrot pulp with gelatin-sucrose.

Binding reaction products (Mailard Reaction) copolymer carotenoids with hydrophilic (water loving) compounds such as starch group can improve solubility in water.

The core of the particle hydrophobic (hates water) will act as storage space, while the outer shell (shell) that will stabilize hydrophilic particles in a water molecule dispersed water conditions (Pan et al., 2007).

In this study, solubility and stability of carotenoids and GME GMB powder in water determined value of absorbance at a wavelength of 450 nm (OD.450).

The powder stability testing performed until week 5. Analysis of the second powder, a decline in the absorbance began at week 3. The statistical test result, a decline in the average value OD.450 on GME powder 2.5% and amounted GMB powder 0.76%.

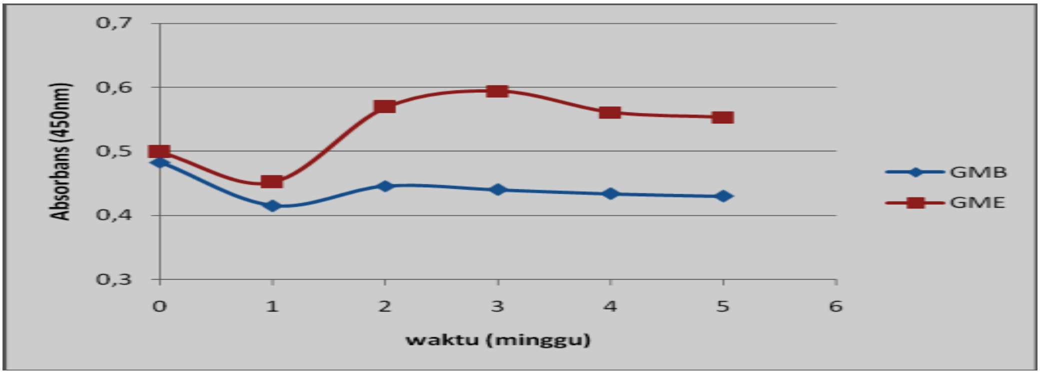

Figure 1. Stability Testing Results Carotenoids (OD.450) on Suspension GMB and GME

Increasing the value of OD occurred at weeks 1, especially on sample GME. This happens because a significant antioxidant response of *Neurospora sitophila* extract carotenoids which are a mixture of several carotenoids, and the response decreases after week 3. The study conducted by Priatni et al. (2010) there are at least five carotenoid compounds that have antioxidant properties of extracts of *Neurospora sitophila*, among others: lycopene, neurospore, *γ-carotene, β-carotene* and *phytoene*.

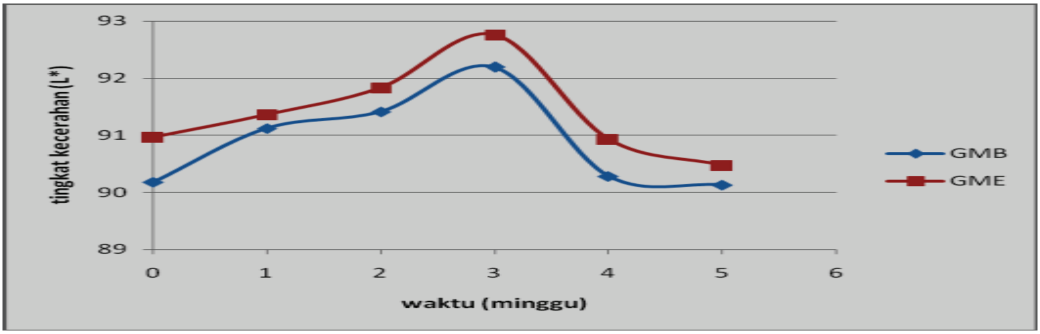

Figure 2. Stability Testing Results Color Brightness (L *) on the powder GMB and GME

Analysis of the powder GME showed a decline in the level of color brightness (L* value) average of 0.84% and a decrease in yellow (b* value) average of 8.64%. While the analysis of the powder GMB showed a decline in the level of color brightness (L* value) average of 0.76% and a decrease in yellow (b* value) average of 5.24%. Studies conducted Chen and Tang (1998) that the decline in the value of L*, a* and b* in powder carotenoid declines with increasing storage time.

Decrease yellow carotenoids during storage of the powder can be caused by the degradation of trans-*β-carotene* and formation to form the cis isomer. It has been widely reported that the formation of cis on a carotenoids may reduce the intensity of the color. As stated by Natalia et al. (2009) that were causing color changes in carotenoid compounds as a result of their defense mechanism by carotene due to the heat radiation so that the carotenoid isomers shaped-trans transformation changed into a form of transformation-cis.

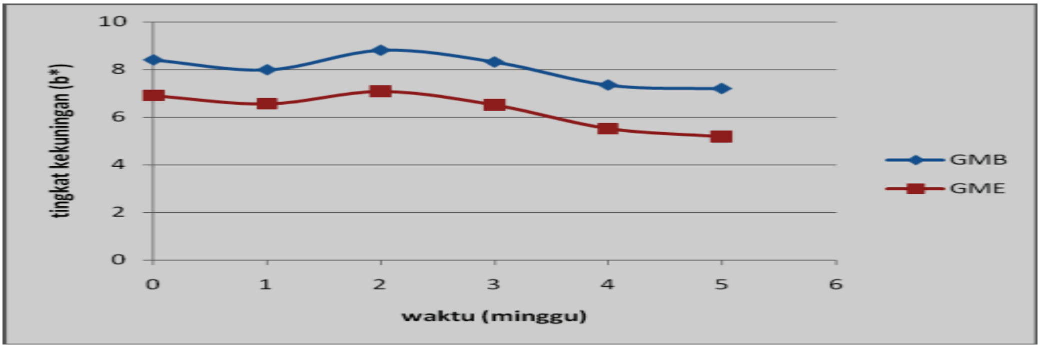

Figure 3. Stability Testing Results Level Yellowish (b *) on the powder GMB and GME

During stability testing, the GMB and GME powder placed in a eksikator with humidity (% RH) between 20% - 30% and temperatures between 25 ^{0}C - 28 ^{0}C. In the controlled humidity and temperature, changes in the water content of the powder results copolymerization (Reaction Mailard) was observed during storage until week five (5).

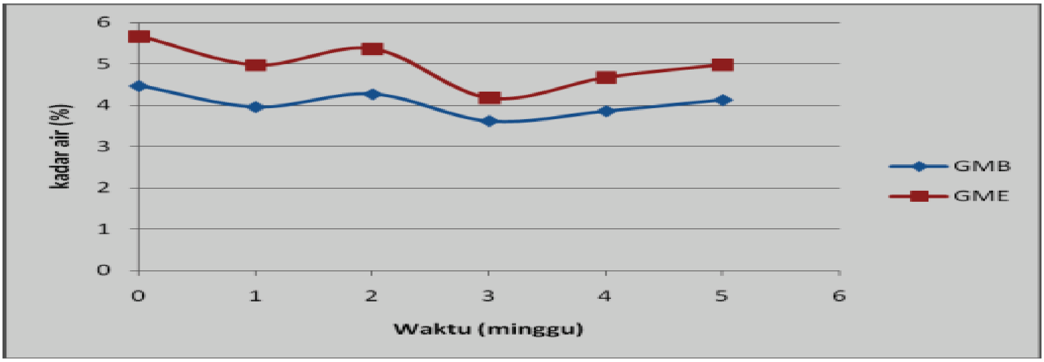

Figure 4. The results of the analysis of the water content of the powder GMB and GME

Results of statistical analysis of the powder showed elevated levels of water GME average of 5.48% and the powder GMB water levels increase an average of 4.09%. Increased levels of water, clearly occurred after week 3. According Onwulata, et al. (1998) have been used for some disaccharide binder. Various volatile substances have been successfully trapped in sucrose, maltose or lactose via freeze drying. The molecular structure of amorphous-shaped sugar molecules are transformed into crystalline (thin surface more stable). Surface membrane of the disaccharide is watertight with high resistance against diffusion style at low water levels that are dispersed to form more complex molecules.

The binding process can improve the ability of blocking duplicate water into the particles. The hitch can be formed through a combination of polymerization between lipids and disaccharide substance, so as to form a double wall. Reduction of water absorption is very important for the stability and effectiveness of the binding powders of carotenoids.

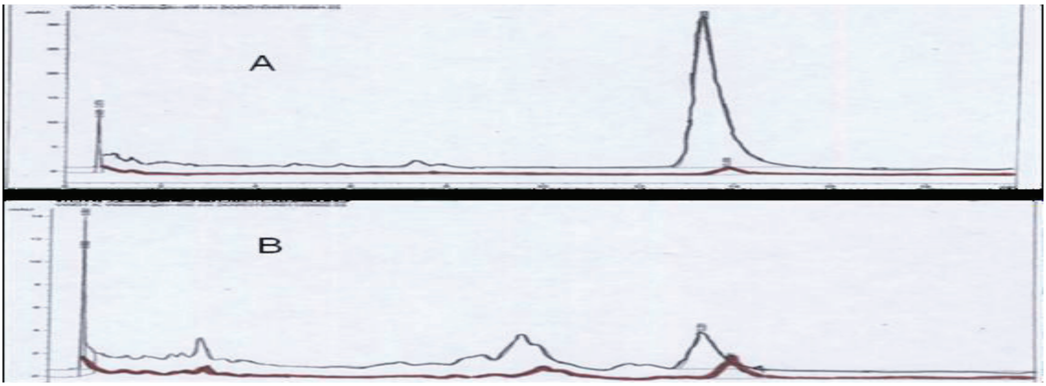

Figure 5. HPLC Analysis of Stability of Carotenoids in the GMB powder (A) and GME (B),
1: peak of *β-carotene* 0 Sunday, 1 ': *β-carotene* 5 Sunday

Results of HPLC analysis, showed a decrease in the content of *β-carotene* from 0.0090 ug be 0.0035 mg per gram powder and 0.178 g GME be 0.0075 mg per gram of powder GMB, after storage in eksikator in week 5. The degradation of the *α* and *β-carotene* follow first-order reaction kinetics. According to Robert (2003) during storage powder carotenoid pigments of carrots with maltodextrin encapsulation yields, the inlet temperature to the spray dryer greatly affect the acquisition of pigment. Higher drying temperatures (>150 ^{o}C) may cause decomposition of carotenoid compounds.

CONCLUSIONS

The process of binding of *β-carotene* pigment *Neurospora sitophila* with gelatin-maltodextrin copolymer (GME) can increase the solubility in water and is stable at 20-30% RH storage for 3 weeks.

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