

FORMULATION OF INULIN FIBER SUPPLEMENT PRODUCED BY HYDROLYZING *ASPERGILLUS CLAVATUS*-CBS₅ INULINASE ENZYME USING LOW-FAT SHANK GELATIN AS CHOLESTEROL BINDER

(Formulasi Suplemen Serat Inulin Hasil Hidrolisis Enzim Inulinase *Aspergillus clavatus*-CBS₅ Menggunakan Gelatin Ceker Ayam Sebagai Pengikat Kolesterol)

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ABSTRAK. Formulasi gelatin ceker ayam rendah lemak yang dihasilkan melalui proses reduksi lemak dan hidrolisat inulin sebagai fructooligosaccharides (FOS) dilakukan untuk memperoleh suplemen serat sebagai pengikat kolesterol. FOS diperoleh melalui hidrolisis enzim inulinase fungi *Aspergillus clavatus* CBS₅ pada inulin komersial. Penelitian ini bertujuan untuk mengetahui kemampuan gelatin ceker ayam rendah lemak sebagai pengikat kolesterol dalam aplikasinya sebagai suplemen serat inulin dengan gelatin komersial sebagai pembanding. Aplikasi dalam pembuatan suplemen dilakukan pada konsentrasi gelatin 30% (v/w hidrolisat) yang difortifikasi pada serat inulin dengan konsentrasi masing-masing 20, 40, 60, 80 dan 100% (v/w total fiber). Hasil penelitian menunjukkan bahwa reduksi lemak pada gelatin ceker ayam cenderung menurunkan komposisi dan kekuatan gel. Semakin tinggi konsentrasi serat inulin akan meningkatkan komposisi suplemen terhadap total padatan, total gula dan gula reduksi, namun menghasilkan optimisasi SDF, IDF dan CBC. Berdasarkan efisiensi penggunaan serat inulin dan CBC maksimal, formulasi suplemen terbaik dicapai pada serat inulin 60% yang menghasilkan formula suplemen dengan komposisi gula reduksi 99,5 mg/mL, total gula 322,18 mg/mL, total padatan 28,4%, IDF 21,783 % (berat kering), SDF 8,33 % (berat kering), CBC pH7 20,62 mg/g dan CBC pH2 16,65 mg/g. Pada kondisi optimum dengan serat inulin 60%, penggunaan gelatin ceker ayam meningkatkan CBC pH2 sebesar 11,56% dan CBC pH7 sebesar 13,71% dibandingkan dengan penggunaan gelatin komersial.

Kata kunci: serat inulin, formula, suplemen, gelatin, CBC

ABSTRACT. The formulation of low-fat shank gelatin and inulin hydrolysate as fructooligosaccharides (FOS) was conducted to obtain dietary fiber supplement as cholesterol binder. FOS was obtained through the hydrolysis of *Aspergillus clavatus* CBS₅ fungi inulinase enzyme on commercial inulin. This study aimed to evaluate the ability of low-fat shank gelatin as a cholesterol binder in its application as inulin fiber supplement with a comparison of commercial gelatin. Application in preparing supplement was carried out at gelatin concentration of 30% (v/w hydrolysate) fortified on inulin fiber with 20, 40, 60, 80 and 100% (v/w total fiber). The experiment showed that fat reduction in crude shank gelatin tends to decrease composition and gel strength. A high concentration of inulin fiber would increase supplement composition on total solids, total sugar and reducing sugar and produced optimization of Soluble Dietary Fiber (SDF), Insoluble Dietary Fiber (IDF) and Cholesterol Binding Capacity (CBC). Based on optimum inulin fiber concentration and CBC, the best supplement formulation was achieved at inulin fiber of 60% with compositions of reducing sugar 99.5 mg/mL, total sugar 322.18 mg/mL, total solids 28.4%, SDF 8.33% (dry weight), IDF 21.783% (dry weight), CBC pH 7 (20.62 mg/g) and CBC pH 2 (16.65 mg/g). In this condition, shank gelatin increased CBC pH 2 by 11.56% and CBC pH 7 by 13.71% compared to commercial gelatin.

Keywords: inulin fiber, formulation, supplement, gelatin, CBC

1. INTRODUCTION

One alternative raw materials for gelatin is chicken feet (shank), abundant poultry and chicken industry product. Currently, the shank gelatin has not been manufactured in Indonesia. Gelatin is polypeptides and protein substances found from natural collagenous derivatives by acidic and basic (alkaline) (Puspitasari & Setiani, 2013). Gelatin molecule ($C_{10}H_{151}N_{31}O_{39}$) (Santosa, H., Guyana, N. L., & Handono, 2014) has swelling property in water, resulted in functional properties as thickener, stabilizer, carrier and emulsifier agents in food additive (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011; Milani & Maleki, 2012).

Selection on the shank is performed in halal aspect for Muslim, and it has the composition of total protein (more than 80 %). However, a high content of shank gelatin (>5 %) will prohibit its application as a binder on activated components in food agent (Melanie, Susilowati, Iskandar, & Laelatunur, 2018). Low quality gelatin will facilitate rancidity (Liu, Nikoo, Boran, Zhou, & Regenstein, 2015). The fat reduction process is conducted at crude shank gelatin by adding acetic acid and decanted to filtrate as low-fat shank gelatin (Santosa, H., Guyana, N. L., & Handono, 2014).

Low-fat shank gelatin as emulsified and binder agents of cholesterol on inulin fiber-source dietary supplement will contribute to cholesterol-binding capacity (CBC) in supplement product. Supplement of inulin fiber is an alternative recovery of fiber from both direct consumptions of fiber and food source. Its presence ranges between medicine and food with a minimal nutrition claim of 25 % from Nutrition Fact (Pisoschi, Cheregi, & Danet, 2009). Selectivity of inulin fiber is based on its potency as a source of dietary.

Inulin fiber is produced through inulin hydrolysis using the inulinase enzyme of fungi of *Aspergillus clavatus*-CBS₅ (Susilowati, Lotulung, & Aristiawan, 2013). Inulin fiber is fructooligosaccharides (FOS) as (Soluble Dietary Fiber, SDF) and (Insoluble Dietary Fiber, IDF), which is non-digestive to arrange rate of cholesterol synthesis. DF from inulin is considered oligosaccharides (oligoglucose and oligofructose), while IDF is an insoluble oligosaccharide component in water, non-hydrolyzing by enzymatic or acid. SDF can directly be used to arrange cholesterol synthesis rate, whereas an extracellular enzyme of colon bacteria ferments early IDF.

In preparation of inulin fiber supplement, gelatin fortification is carried out to get fiber emulsion having CBC in digestive system, beside its resistance in acid condition. This matter is related with its preparation, in which *curing* step is important step in determining solubilization property after it is present in digestive system (Marteau et al., 2011). Emulsified gelatin in inulin fiber as stable solids due to biodegradable and cross-linking properties, and it can be modified by chemical. Functional property of gelatin is caused by its main role as collagen protein property containing amino acids with composition of glycine (2/3 from all amino acids), proline and hydroxyproline (1/3 from all amino acids) (Liu et al., 2015). The objective of this experiment was to know ability of low fat shank gelatin as cholesterol binder capacity (CBC) on its application as inulin fiber supplement.

2. METHOD

Material and Equipment

The primary materials used in this study were crude shank gelatin (Research Centre for Chemistry, LIPI), commercial inulin (Orafti, Belgium), crude inulinase enzyme of fungi of *Aspergillus clavatus*-CBS₅ (Research Centre for Chemistry, LIPI), β -Amylase enzyme (NOVO), and microfiltration membrane of 0.15 μ m (DSS, Denmark). All the chemicals were analytical grade and procured locally. The equipment utilized was general glassware (Erlenmeyer, beaker glass, cylinder glass), homogenizer tube (Ultra – Turrax, Germany), Stirred Ultrafiltration Cell (SUFC) (Model 8200, Amicon, U.S.A.), pH-meter, and spectrophotometer UV-VIS (Cary 60, Agilent Technologies, U.S.A).

Experimental Design

This experiment was carried out by using low-fat gelatin fortified (30% v/w inulin fiber) (America, 1986) at inulin fiber with concentrations of 20, 40, 60, 80 and 100% (v/w total fiber). Similar treatment is conducted by using commercial gelatin. Its concentration is similar to 30% v/w (dry weight of inulin fiber) through a dilution with water at an equal concentration of total solids to low-fat gelatin. Based on CBC, the best formulation is reached via drying under 50 °C for 24 hours, followed by capsulation. Capsulation was done by placing inulin powder from the optimum formulation into ready-made capsules. Data processed in this description were based on the average result of three replicates.

Analysis was performed on SDF and IDF (*Enzymatic-Gravimetric modification*) (McCleary et al., 2012), total solids (Gravimetric method), reducing sugar (Somogyi-Nelsen method), total sugar (Phenol-Sulphate method), dissolved protein (Lowry method) (Chemists, 1995), inulin (N. Nelson method) (Chaplin & Kennedy, 1994), Cholesterol Binding Capacity (CBC) (Q. Zhang et al., 2012), Oil Binding Capacity (OBC) (Park, 1999).

Process Step

Subsequent Hydrolysis of Inulin

A number of commercial inulin was subsequent arranged pH 4.5- 5, added β -Amylase enzyme at concentration 0.08% (v/w inulin starch) and hydrolyzed at 60 °C for 120 minutes agitating 140 rpm to get hydrolysate (I). Hydrolysate (I) was then arranged pH to 5, added inulinase enzyme of *Aspergillus clavatus* -CBS₅ at concentration 60% (v/w total sugar) and hydrolyzed at 30 °C and 100 rpm for 120 hours (Susilowati et al., 2013). Hydrolysate is the product of inulin fiber applied in the preparation of dietary fiber supplement for cholesterol binder.

Extraction and Reduction of Fat in Shank Gelatin

Extraction of shank gelatin was prepared by steaming cleaned shank at 85-90 °C for 20 minutes, soaking in acetic acid 1% (v/w shank) for 20 hours, washing to neutral pH, heating at 60 °C for 2 hours under 1 part of the shank and two parts of aquadest, and sieving through 80 mesh to obtain filtrate as gelatin (I). Gelatin (I) was heated at 100 °C for 1 hour and filtered through 80 mesh to get filtrate II as crude gelatin (Melanie, Susilowati, Iskandar, & Laelatunur, 2015). Reduction of fat was conducted by adding acetic acid 5% (v/v) on crude gelatin, decanting for 12 hours and sieving to get filtrate as low-fat shank gelatin and precipitate as shank fat.

Formulation and Drying of Fiber Supplement

Formulation of inulin fiber supplement was prepared by adding shank gelatin of low fat of 30% (v/w) on inulin fiber with concentrations of 20, 40, 60, 80 and 100% (w/w total fiber) and homogenized at 6000-8000 rpm for 30 minutes. The formulation based on optimum CBC is conducted by drying at 50 °C for 24 hours, size reducing and filling into the capsule as inulin fiber supplement.

Analysis of Soluble Dietary Fiber (SDF) and Insoluble Dietary Fiber (IDF)

The commercial MF membrane discs having a 0.15 μ m (DSS, Denmark) was placed into a dead-end Stirred Microfiltration Cell (SMFC) of 180 mL capacity and connected to a nitrogen gas cylinder. The material was added to the SMFC and operated at a stirring speed of 400 rpm and trans-membrane pressure (TMP) of 40 psia for 30 minutes. Volumetric permeate or extract flow rate was measured after each mL of permeate collected as SDF, while retentate present in SMFC is expressed as IDF (Susilowati et al., 2012). Permeate and retentate was then precipitated by adding ethanol through dissolving four parts of ethanol volume 95%, filtrated and washed with ethanol 70% (3 times), ethanol 95% (2 times) and acetone (2 times) (McCleary et al., 2012). Washed components were then dried at 50 °C for 24 – 48 hours as SDF and IDF.

Analysis of Cholesterol Binding Capacity (CBC)

Egg yolk flour (2.5 gram) and 2 gram of material sample were added with 50 mL deionization water, arranged the acidity to pH 7 and 2 (similar to pH conditions in stomach and colon), shaken at stirring speed of 80 rpm for 2 hours in a water bath at 37 °C. Then 16 mL of absolute ethanol were added and centrifuged at 4000 rpm for 20 minutes. The supernatant is taken (1 mL) and diluted five times using acetic acid 90% and vaporized using vacuum at 40 °C for 1 hour. The liquid resulted from evaporation was added with 0.1 mL of reagent of o-phthalaldehyde (50 mg in 100 mL of acetic acid 90 %) and 2 mL of concentrated sulphuric acid, homogenized and allowed for 20 minutes. Analysis was performed using spectrophotometer UV-VIS at 550 nm and compared to a blank (N. Zhang, Huang, & Ou, 2011). Cholesterol concentration in sample was determined by comparing it to the standard curve from the standard cholesterol solution. The formula is calculated as followed:

$$\text{CBC (mg/g)} = [C_1 - (C_2 - C_3) \times F_p] \times 50/W$$

in which C_1 is cholesterol concentration in egg yolk, C_2 is blank concentration in egg yolk, C_3 is egg yolk and sample concentrations, F_p is dilution factor, 50 is adsorption volume (mL), W is sample weight as dietary fiber (g).

3. RESULT AND DISCUSSION

Characteristic of Gelatin and Inulin Fiber

Crude shank gelatin due to curing for 20 hours in acetic acid 1 % was extracted at 60 °C for

2 hours and reheated at 100 °C for 1 hour. The process resulted in thick white suspension layered by fat on top suspension surface with yields ranging from 50-60%. In comparison, commercial gelatin (gelatin B) is yellowish crystalline diluted into water at total solids concentration similar to shank gelatin to obtain concentration to 30% v/w. Fat reduction is necessary to get low-fat shank gelatin as a food additive for cholesterol binder. However, the Indonesian National Standard (SNI) Certification requirements merely contain physical appearances, water and ash contents, and minerals (SNI, 1995). The composition of both types of gelatin is represented in Table 1.

Table 1. Characteristic of crude shank gelatin and commercial gelatin (B).

Components	Crude Shank Gelatin	Commercial Gelatin
Total solids (%)	7.15	92.54
Fat (%)	6.89	0.44
CBC (mg/g)	6.80	6.60
Soluble Protein (mg/mL)	0.44	2.20
Bloom strength	300	180

The characteristic of gelatin seemed that the main difference lies in gel strength (bloom

strength), in which crude shank gelatin shows bloom strength of 300 g bloom, which is higher than commercial gelatin (B) of 180 g bloom. This difference is possibly caused by the curing process and amino acids composition supporting functional gelatin properties (Eastwood, 2013).

Inulin fiber as fructooligosaccharides (FOS) is produced through hydrolysis of commercial inulin using β -amylase enzyme, followed by inulinase enzyme of fungi of *Aspergillus clavatus*-CBC5. Its composition showed concentrations of total solids of 42.52 %, total sugar of 327.96 mg/mL, reducing sugar of 76.66 mg/mL, SDF of 5.68%, IDF of 28.2%, and inulin of 37.38% (dry weight). Figure 1 illustrated crude shank gelatin (a), commercial gelatin (b) in water (total solids concentration is equivalent to shank gelatin, 7.15%) and inulin fiber (c) from hydrolysis using inulinase enzyme of fungi of *Aspergillus clavatus*-CBC5.

Such composition indicates the difference in inulin composition before the hydrolysis process, particularly at higher concentration of inulin (68.2% dry weight) (Susilowati et al., 2013). For example, the hydrolysis process using the inulinase enzyme of *Aspergillus clavatus*-CBS5 produced inulin 30.82% before hydrolysis due to various factors, such as process conditions (hydrolysis time, activity of inulinase enzyme).

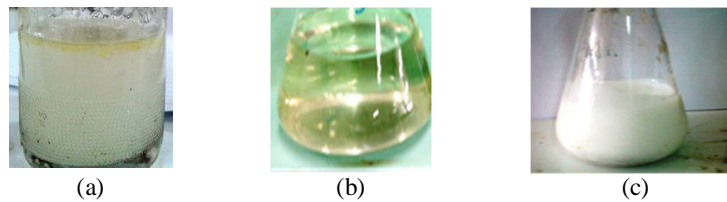


Figure 1. Crude shank gelatin (a), commercial gelatin (b), inulin fiber as a result of hydrolysis using *Aspergillus clavatus*-CBC5 (c)

Effect of Fat Reduction in Shank Gelatin

Crude gelatin resulting from the extraction of shank through curing process (acetic acid of 1% v/v) is a mixture of flavorless colloidal suspension with turbid white color and fat layer on the top

suspension surface. Process of fat reduction in crude gelatin through adding acetic acid of 5% (v/v) and decanting for 12 hours yields precipitate as gelatin suspension, while filtrate as fat, as shown in Figure 2.



Figure 2. Precipitated shank gelatin of low fat (a) and shank fat (b) as a fat reduction at crude shank gelatin

The composition of low-fat shank gelatin shows concentrations of fat of 0.96%, dissolved protein of 0.22 mg/mL, Oil Binding Capacity (OBC) (Park, 1999) of 6.33 mg/g, total solids of 7.56% and *gel strength (bloom strength)* of 77.41 gram bloom (America, 1986). In this process, acetic acid hydrolyzed collagen protein so that it occurred denaturation. Simultaneously, fat linked to collagen protein will be coagulated with protein and other components. The whole processes for fat reduction of crude shank gelatin affect the composition of decantation result. It has been known that the composition of the shank (1 piece of shank is equivalent to 73 calories) was 63% fat, 0% carbohydrate, 37% protein or equivalent to double saturated fat of 1.013 gram and single fat of 187 gram (Sun, Xiang Dong, 2014). A decrease of fat in this process has occurred because in shank is more saturated fat. In addition, 5% acetic acid will hydrolyze fat to form simpler fatty acids, causing a drop of fat concentration from concentration 6.89% (before reduction) to 0.96% (after reduction). This process also decreased dissolved protein from 0.44 mg/mL to 0.22 mg/mL and Oil Binding Capacity (OBC) from 6.8 mg/g to 6.33 mg/g, but it increased total solids from 7.15% to 7.56%. At the coagulation of collagen, the composition of gelatin

protein is lower, which enables to decrease gel strength (*bloom strength*) 74.2% from 300 gram bloom to 77.41 gram bloom. Gel strength was one of the main characteristics in evaluating gelatin quality (Balti et al., 2011). However, gel strength quality regulation was compatible with application product for many food types (America, 1986). Compared with commercial gelatin composition, there was a significant difference, particularly in gel strength, dissolved protein and total solids. Gel strength is relating to dissolved protein due to the functional property of gelatin as collagen.

Effect of Gelatin Fortification on the Characteristic of Inulin Fiber Supplement

CBC pH 2 and CBC pH 7 (mg/g)

The formulation process using shank gelatin with low fat and commercial gelatin (B, cow skin) at a fixed concentration (30%, v/w total fiber) generates an increase of formula with CBC pH 7 relating to increasing of inulin fiber concentration. Using shank gelatin at CBC pH 2 will increase CBC, but the use of commercial gelatin decreases CBC associating with an increase of inulin fiber concentration, as displayed in Figure 3.

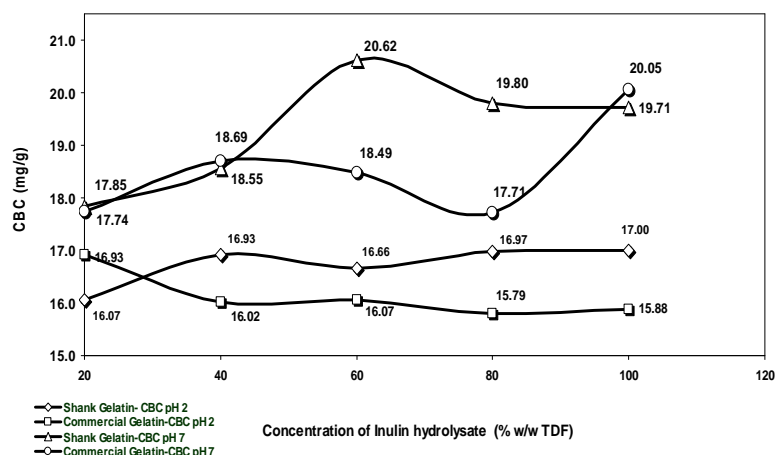


Figure 3. Relationship between inulin fiber concentration and type of gelatin on CBC pH 2 and CBC pH 7 in inulin fiber supplement

Based on efficiency, the optimization condition was reached by shank gelatin of 20.62 mg/g at inulin fiber concentration of 60% (v/w, total fiber) higher than commercial gelatin use of 20.05 mg/g inulin fiber concentration of 100% (v/w, total fiber). The different trend showed at CBC with pH 2, as commercial gelatin was more efficient and resulted in optimal CBC (16.93 mg/g) at inulin fiber

20% (v/w, total fiber) compared with shank gelatin had CBC 17 mg/g at inulin fiber 100% (v/w, total fiber). In other words, commercial gelatin is more efficient to bind cholesterol in the colon (pH 2) because it needs less inulin fiber concentration (20%) to get optimal CBC (16.93 mg/g). This possibly caused by a facilitating to bind cholesterol at neutral condition (pH 7) compared with acid

condition (pH 2) so that there is no barrier in developing gel (Eastwood, 2013). Dietary fiber works based on gel formation, water binding capacity and cation exchange (Eastwood, 2013). This cholesterol-binding capacity is related to swelling property and gel strength to contribute to CBC. The whole processes, optimal formula condition on CBC at pH 7 was reached by using shank gelatin with inulin fiber concentration 60% (w/w, total fiber), increasing CBC of 69.3% from before formulation of 6.33 mg/g to 20.62 mg/g. In

this condition, the use of shank gelatin increases CBC by 11.56% compared with commercial gelatin of 18.49 mg/g at CBC with pH 2 and 13.71% at CBC with pH 7.

Reducing sugar and total sugar (mg/mL)

The formulation process generates fiber supplement formula with total sugar and reducing sugar increasing, as shown in Figure 4.

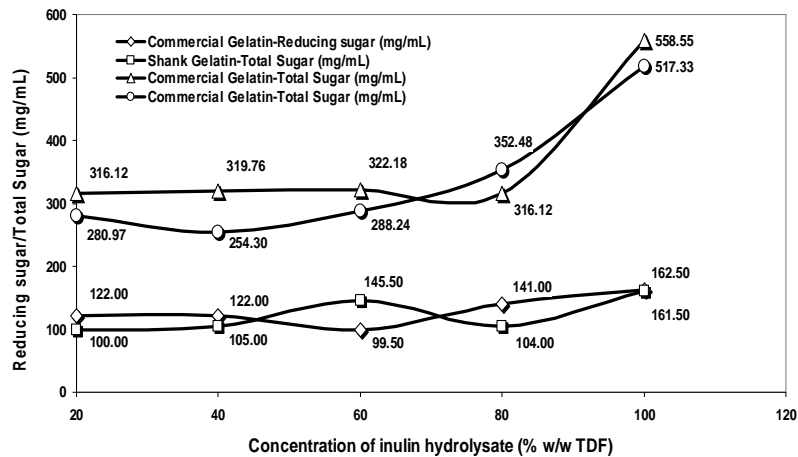


Figure 4. Relationship between inulin hydrolysate concentration and type of gelatin on total sugar and reducing sugar in inulin fiber supplement

This matter is caused by inulin fiber as fructooligosaccharides (FOS), in which high concentration will increase recovery of total sugar and reducing sugar. Reducing sugar is a parameter in which inulin fiber as the primary material is a monosaccharide, particularly fructose or glucose and its derivative, that can decrease according to Somogyi Nelson method (Chemists, 1995).

Utilising both gelatins did not indicate a significant difference. Optimization condition is reached at inulin fiber 100% (v/w, total fiber). However, shank gelatin resulted in both higher concentrations of total sugar (162.5 mg/mL) and reducing sugar (558.54 mg/mL) when compared to commercial gelatin with total sugar (161.5 mg/mL) and reducing sugar (517.33 mg/mL) at inulin fiber 100% (v/w, total fiber). Gelatin does not contribute to total sugar and reducing sugar due to no carbohydrate (0%) in its composition. However, the composition of gelatin consists of C (50.5%), H (6.8%), N (17%), O (25.2%) as C102H151N31O39, so that recovery of total sugar and reducing sugar are yielded from inulin fiber.

SDF and IDF (% dry weight)

The formulation process of inulin fiber indicates its effect on IDF and SDF, as shown in Figure 5. Increasing inulin fiber concentration increases optimal IDF at inulin fiber 60%, which is reached by shank gelatin of 21.78% (dry weight), higher when compared with commercial gelatin of 20.17% (dry weight) at inulin fiber 100%. However, a different trend seems on SDF. Optimization of shank gelatin and commercial gelatin uses obtained at inulin fiber concentrations of 40% and 80% with SDF of 24% and 24.59% (dry weight), respectively. IDF in inulin has a larger molecular weight because it is a polymer of fructose that not all of them are hydrolyzed by the inulinase enzyme. In contrast, SDF in inulin is a polymer of fructose. Therefore, SDF is soluble in water with a lower MW. This formulation condition is possibly predicted by interaction between molecules of amino acids in shank gelatin and fructose polymer components compared with a molecule of amino acids in cow skin collagen. It has been known that commercial gelatin is type B gelatin from calfskin. Gelatin is a mixture of amino acids bonded in peptide with a molecular weight of 15,000 – 400,000 Da. (Maidin,

2014). Due to the swelling property in water (Keenan, 2000), the formulation is easier and more efficient using shank gelatin to achieve optimal IDF (21.78% dry weight) and SDF (24% dry weight) with inulin fiber 60% and 40%. In comparison,

commercial gelatin with inulin fiber concentration 100% and 80% (w/w total fiber) attained optimum IDF and SDF of 20.17% and 24.59% (dry weight). At the same time, the optimisation difference in SDF tends to be no significant.

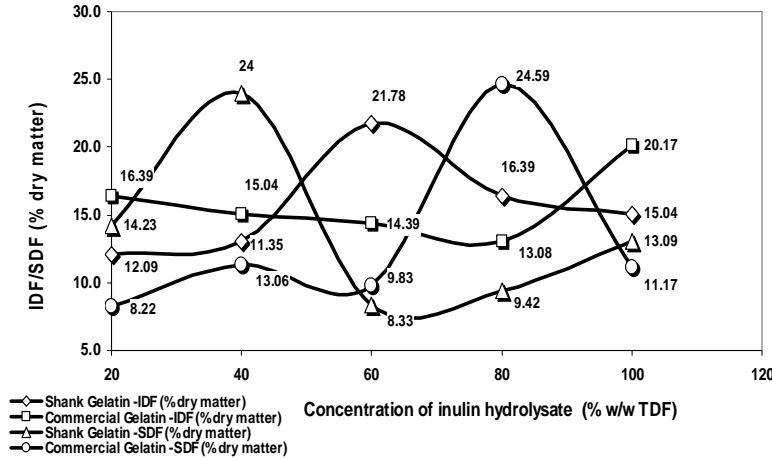


Figure 5. Relationship between inulin hydrolysate concentration and type of gelatin on SDF and IDF in inulin fiber supplement

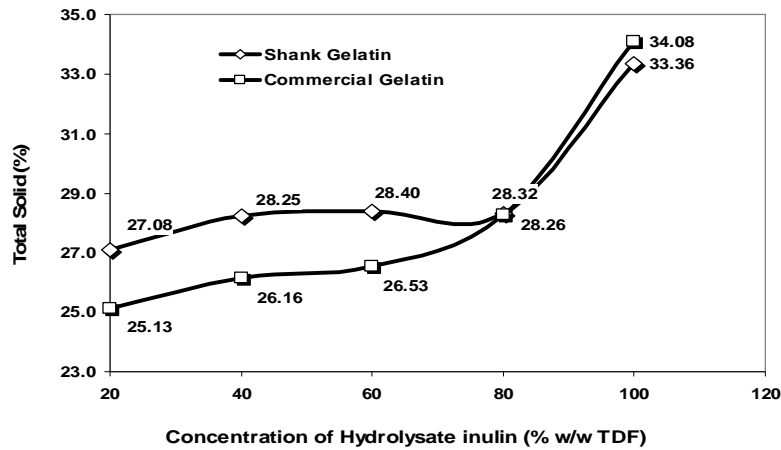


Figure 6. Relationship between inulin fiber concentration and type of gelatin on total solids in inulin fiber supplement

Total solids (%)

The supplement formulation from the inulin fiber showed interaction effect between gelatin and inulin fiber concentration on total solids, as shown in Figure 6. Increasing total solids concentration using both shank and commercial gelatins, the highest concentration of inulin fiber (100% w/w total fiber) reached maximum condition, 33.36 and 34.08%. In other words, commercial gelatin yields supplement with higher total solids than that shank gelatin for all treatment of inulin fiber. This difference is possibly caused by the initial higher

composition of commercial gelatin than that shank gelatin. However, this formulation was carried out at a similar concentration based on total solids of (30% v/w total fiber or 2.14% w/w total fiber). In addition, higher gel strength of commercial gelatin (180 gram bloom) than gel strength of shank gelatin (77.41 gram bloom) indicated that total solid is higher for all treatments because total protein is the highest components of gelatin.

From the whole treatments and based on the optimal CBC, the best formulation of inulin fiber supplement was reached by using shank gelatin at

inulin fiber 60% (w/w total fiber). This formulation is then conducted drying process at 50 °C for 24 hours to produce powder inulin fiber and then to be capsulated. Figure 7 displayed the best formulation

of inulin fiber supplement using shank gelatin, inulin fiber formula at dry condition and after capsulation.

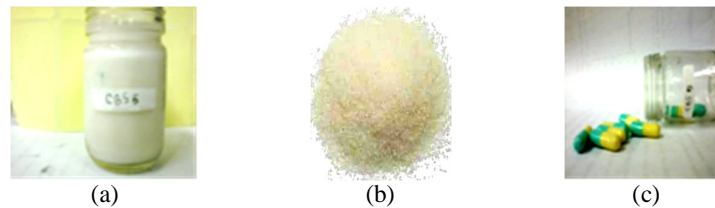


Figure 7. Supplement formula at inulin fiber concentration of 60 % (w/w, total fiber) with the fortification of shank gelatin (30 % v/w total fiber) (a), supplement formula of dry inulin fiber (powder) (b) and inulin fiber supplement in capsule (c)

4. CONCLUSION

Experiment results showed that fat reduction of crude shank gelatin resulted in a separation between gelatin and fat, but it decreased composition and gel strength. A high concentration of inulin fiber on supplement formulation increased compositions of total solids, total sugar and reducing sugar. At the same time, SDF, IDF and CBC tend to increase up to the optimal limit, followed by dropping their concentrations relating to increased inulin fiber concentration. Based on optimum CBC, the best supplement formulation was reached at inulin fiber 60%, resulting in supplement formulation of inulin fiber with compositions of reducing sugar 99.5 mg/mL, total sugar 322.18 mg/mL, total solids 28.4%, SDF 8.33% (dry weight), IDF 21.783% (dry weight) and CBC pH 7 20.62 mg/g, CBC pH 2 16.65 mg/g. As expected, the use of shank gelatin increased CBC of 11.56% compared with commercial gelatin (18.4856 mg/g) at CBC pH 2 and 13.71% at CBC pH 7, respectively.

REFERENCES

America, G. M. I. of. (1986). *Standard Methods for the Sampling and Testing of Gelatins*. Gelatins manufacturing Institute of America.

Balti, R., Jridi, M., Sila, A., Souissi, N., Nedjar-Arroume, N., Guillochon, D., & Nasri, M. (2011). Extraction and functional properties of gelatin from the skin of cuttlefish (*Sepia officinalis*) using smooth hound crude acid protease-aided process. *Food Hydrocolloids*, 25(5), 943–950.

Chaplin, M. F., & Kennedy, J. F. (1994). *Carbohydrate analysis: a practical approach.. ed. 2*. IRL Press

Ltd.

Chemists, A. of O. A. (1995). *Official methods of analysis of AOAC International*.

Eastwood, M. A. (2013). *Principles of human nutrition*. Springer.

Gómez-Guillén, M. C., Giménez, B., López-Caballero, M. E. al, & Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocolloids*, 25(8), 1813–1827.

Keenan, T. R. (2000). Gelatin. *Kirk-Othmer Encyclopedia of Chemical Technology*.

Liu, D., Nikoo, M., Boran, G., Zhou, P., & Regenstien, J. M. (2015). Collagen and gelatin. *Annual Review of Food Science and Technology*, 6, 527–557.

Maidin, R. A. (2014). *Extraction and characterization of gelatine from chicken skin*. UMP.

Marteau, P., Jacobs, H., Cazaubiel, M., Signoret, C., Prevel, J.-M., & Housez, B. (2011). Effects of chicory inulin in constipated elderly people: a double-blind controlled trial. *International Journal of Food Sciences and Nutrition*, 62(2), 164–170.

McCleary, B. V., DeVries, J. W., Rader, J. I., Cohen, G., Prosky, L., Mugford, D. C., ... Okuma, K. (2012). Determination of insoluble, soluble, and total dietary fiber (CODEX definition) by enzymatic-gravimetric method and liquid chromatography: collaborative study. *Journal of AOAC International*, 95(3), 824–844.

Melanie, H., Susilowati, A., Iskandar, J. M., & Laelatur, M. (2015). Fat Reduction in Improving the Quality of Chicken Feet Gelatine for Functional Food Application. *Jurnal Kimia Valensi*, 1(1), 12–19.

- Milani, J., & Maleki, G. (2012). Hydrocolloids in food industry. *Food Industrial Processes—Methods and Equipment*, 2, 2–37.
- Park, Y. W. (1999). *Cholesterol contents of U . S . and imported goat milk cheeses as quanti ® ed by different colorimetric methods*. 32, 77–82.
- Pisoschi, A. M., Cheregi, M. C., & Danet, A. F. (2009). *Total Antioxidant Capacity of Some Commercial Fruit Juices: Electrochemical and Spectrophotometrical Approaches*. 480–493. <https://doi.org/10.3390/molecules14010480>
- Puspitasari, D. A. P., & Setiani, V. P. B. B. E. (2013). *SIFAT- SIFAT GEL GELATIN TULANG CAKAR AYAM Geling Properties of Chicken Shank Bone Gelatin*. 04(07).
- Santosa, H., Guyana, N. L., & Handono, S. F. D. (2014). Hidrolisa Kolagen dalam Ceker Ayam Hasil Perendaman dengan Asam Asetat pada Proses Pembuatan Gelatin. In *Gema Teknologi* (Vol. 20). Vocational School Diponegoro University.
- Standar Nasional Indonesia (SNI) 06-3735. (1995). Mutu dan Cara Uji Gelatin. Badan Standardisasi Nasional, Jakarta.
- Sun, Xiang Dong, and R. A. H. (2014). Factors influencing gel formation by myofibrillar proteins in muscle foods. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 10). Wiley Online Library.
- Susilowati, A., Lotulung, P. D., & Aristiawan, Y. (2013). Potensi umbi dahlia merah (Dahlia spp.) lokal dalam perolehan serat inulin sebagai pangan fungsional melalui hidrolisis enzimatik. *Prosiding Seminar Nasional XX Jaringan Kerjasama Kimia Indonesia (JASAKIA)*, Yogyakarta, 3.
- Susilowati, A., Melanie, H., Aspiyanto, Y. M., & Maryati, Y. (2012). Pengembangan pangan fungsional berbasis inulin dari umbi dahlia (Dahlia spp.) Sebagai serat pangan larut air (SDF) untuk anti kolesterol. *Program Tematik, Kedepujian IPT, Pusat Penelitian Kimia-LIPI, Serpong, Tangerang*.
- Zhang, N., Huang, C., & Ou, S. (2011). In vitro binding capacities of three dietary fibers and their mixture for four toxic elements, cholesterol, and bile acid. *Journal of Hazardous Materials*, 186(1), 236–239.
- Zhang, Q., Wang, T., Li, B., Jiang, T., Ma, L., Zhang, X., & Liu, Q. (2012). Aqueous phase reforming of sorbitol to bio-gasoline over Ni / HZSM-5 catalysts. *APPLIED ENERGY*. <https://doi.org/10.1016/j.apenergy.2011.12.044>