Water Soluble Chitosan from Green Mussel (*Perna viridis*) Shells and Its Use As *Fat-Absorber* In Cookies

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Received: 31 October 2022

Accepted: 22 December 2022

Published: 30 December 2022

Academic Editor: Dr. Tatty Yuniarti

Accreditation Number:148/M/KPT/2020. ISSN: 2089-5690, e-ISSN: 2406-9272. https://doi.org/ 10.15578/squalen.731

Abstract

Green mussel chitin can be converted by H_2O_2 into water-soluble chitosan (WSC). This can subsequently be utilized for a variety of different purposes, such as a fat binder. This study examines how different H_2O_2 concentrations (13, 21.5, and 30%) affected the properties of WSC (yield, moisture content, ash content, degree of deacetylation, and solubility in water and acid). Moreover as well as how WSC (8%, 9%, and 10%) affected the hedonic scores, proximate composition, and fat binding capacity of weight-loss cookies. A single factor Completely Randomized Design and single-factor ANOVA were used to analyze the data, followed by Duncan's additional testing as necessary. The results showed that water-soluble chitosan was impacted by H_2O_2 concentration in that its yield and ash content decreased, its color changed to a brownish, and its solubility in acid and moisture content all increased. According to De Garmo's Effectiveness Index Test, 30% H_2O_2 concentration resulted in the best WSC. The addition of WSC did not affect the hedonic quality, protein, moisture, or carbohydrate contents of the cookies, but it did have an impact on the ash and fat contents. The ability of all cookie samples in all treatments to bind fat in liquified butter and peanut oil validates the use of cookies containing WSC in body weight loss research.

Keywords: water-soluble chitosan, fat-binding capacity, weight-loss cookies

Introduction

Chitin, which is found in the cell walls of fungi and the exoskeletons of insects, crustaceans, and molluscs, is the second most abundant polysaccharide in nature after celluloses. The crystallinity and insolubility of chitin limit its use in industry. Chitin, therefore, may be converted into chitosan, chitooligosaccharides, and glucosamine, which enhances its biological qualities and expands its uses in the textile, culinary, medicinal, and cosmetic industries. Due to their adaptable biological properties, chitin and chitosan are both significantly different in economic significance.

The medical and technical applications of chitosan are however restricted because of its low solubility and physiological pH (Imtihani et al., 2021). This is especially due to the large molecular weight that makes it difficult to dissolve in water (Du et al., 2009). Hydrogen peroxide (H_2O_2) could be an additive producing water-soluble chitosan. A study by Du et al. (2009), showed that hydrogen peroxide has good potential to convert crude chitosan into water-soluble chitosan. Several researchers, namely Lestari et al. (2018); Chamidah et al. (2019); Sudianto et al. (2020); and Xia et al. (2013), have used hydrogen peroxide in making water-soluble chitosan from crab and shrimp shells.

Chitosan is one of the natural ingredients that can reduce fat levels in the blood and is also used in functional food supplements for the prevention or treatment of diseases bacterial, antidiabetic, antioxidant, anticancer, anti-inflammatory, and hypocholesterolemic properties (Chiu et al.,2020). In animal studies the dietary supplemented chitosan appears to attach to negatively charged lipids, limiting their absorption through the digestive tract and lowering serum cholesterol (Zacour et al., 1992; Deuchi et al., 1995; Ormrod et al., 1998). Chitosan was assumed to be able to bind the fat and cholesterol in the body. Therefore, the blood cholesterol could decrease (Imtihani et al., 2021; Kurniasih et al., 2016; Pavinatto et al., 2005; Zhou et al., 2006; Idacahyati et al., 2020). Chitosan can also reduce the workload of the liver and reduce the work pressure of other organs due to excess fat (Pratiwi, 2014). Jin et al. (2017) in his research stated that the potential for fat binding by soluble chitosan will be greater because it is more flexible than rigid chitosan.

Epidemic overweight and obesity are major challenges to public health (Rahmad, 2019). Sety et al. (2021) explain that obesity is an excessive or abnormal accumulation of fat in the body. The mechanism of accumulation of fat in the body is caused by the unbalanced amount of energy intake and energy output (Kemenkes RI, 2018). Consumption of functional food that can reduce energy intake or improve fat metabolism is one of the methods that can be chosen for dietary purposes. One of the food products that can be used as an alternative functional food for people with obesity and diabetes mellitus is cookies (Novidahlia et al., 2015), referred to as weight loss cookies.

These functional properties can be achieved by substituting the main ingredients, namely wheat flour with other ingredients that have high fiber such as oatmeal, use low-calorie sugar, and ingredients that have fat absorption capacity such as chitosan. According to Jin et al. (2017), water-soluble chitosan was an excellent ingredient for functional food to treat obesity due to its ability to absorb, bind and trap cholesterol, fat, sterols, and triglycerides in the diet. In clinical trials, chitosan was found to have anti-obesity benefits and to be able to lower blood pressure, cholesterol levels, and body weight in obese/overweight people as well as in animal models fed a high-fat diet (Huang et al., 2019). In addition, Liu et al. (2021) demonstrated that high-molecular-weight chitosan was able to increase lipid excretion and inhibit lipid absorption in induced obese rats. A meta-analysis of human trials using chitosan revealed better weight loss with chitosan than with a placebo (Ernst & Pittler, 1998). Bahijri et al. (2017) reported that chitosan improved lipid profile, insulin sensitivity, and oxidative stress and might be useful in controlling the weight of Wistar rats fed with a high-fat/high-cholesterol-containing chitosan diet. There are, however, debates in the literature regarding the ability of chitosan to absorb fat or to reduce weight (Mhurchu et al., 2005).

Green mussels (*Perna viridis*) are one of the Indonesian fishery commodities that have high economic value and are abundantly found in coastal waters, mangrove areas, and river estuaries. The Indonesian mussels (blood clams, green mussels, simping oysters, pearl mussels, and mussels) production in 2020 was 34,427 tons valued at Rp. 30,256,914,000 (KKP, 2020). Mussels shells, including those from green mussel shells, contain chitin (Danarto & Distantina, 2016; Wulandari et al., 2020), and can be further converted into chitosan using hydrogen peroxide and used for certain health purposes. However, there is limited information available in the literature on the studies of the conversion of their chitin to water-soluble chitosan using hydrogen peroxide. The purpose of this study was to examine the effects of various H_2O_2 concentrations on the properties of water-soluble chitosan derived from green mussel shells and to examine the possible use of water-soluble chitosan as a fat absorber in weight-loss cookies.

Material and Methods

Materials

The green mussel shells were collected at a green mussel meat picking operation in Ketapang, Tangerang, and were transported to the laboratory of AUP Polytechnic in South Jakarta which took 2 hours driving. The shells were cleaned upon arrival, dried, and ground to 200 mesh. Other materials used for producing water-soluble chitosan (WSC) and cookies include calico fabric sheet with a dimension of 150 cm x 60 cm and 232 g/m density, 60 cm x 60 cm filter paper with a maximum pore size of 10-15 m, Hydrogen peroxide 50% (Supelco Merck brand), Acetic acid (Merck brand), Iodized table salt (Dolphin brand), Palm sugar (Nourish Indonesia brand), Backing powder (KoePoe brand), Baking soda (KoePoe brand), n-Hexane for analysis, Sulfuric acid 95-97%, Sodium hydroxide (Merck brand), Methyl red indicator, Hydrochloric acid 37% for analysis (Merck brand), Boric acid (Merck brand), di-Sodium tetraborate decahydrate for analysis, Peanut oil (Golden nut brand), Butter (Menara brand), Ethanol 96% and Diethyl ether (Merck brand).

Methods

Green mussel shell meal was initially converted into crude chitosan using Danarto and Distantina's method, (2016), and then converted into water-soluble chitosan (WSC) following the method of Du et al. (2009). The modification was on the concentrations of hydrogen peroxide. A 2% acetic acid solution was used to dissolve the chitosan in a 1:20 ratio, and different amounts of H_2O_2 (13, 21.5, and 30%) were added in a 1:2 ratio. The mixture was then heated for four hours at 40 °C in a water bath. The solution was subsequently neutralized with 10% NaOH, filtered through the calico fabric, and the filtrate was then added with twice as much 96% ethanol. This mixture was then incubated at 10 °C for 24 hours before drying in an oven at 50 °C for three hours. The experiments were replicated three times. The weight-loss cookies were made based on

Table 1. Weight-Loss Cookies Formulation

Ingredients	Proportion (g)			
	W0	W8	W9	W10
Oatmeal	25.07.00	25.07.00	25.07.00	25.07.00
Low-protein wheat flour	12.09	12.09	12.09	12.09
Palm sugar	14.03	14.03	14.03	14.03
Butter	21.04	21.04	21.04	21.04
Egg	15.06	15.06	15.06	15.06
Skim Milk	07.01	07.01	07.01	07.01
Vanilla extract	00.05	00.05	00.05	00.05
Salt	00.05	00.05	00.05	00.05
Baking powder	00.05	00.05	00.05	00.05
Baking soda	00.05	00.05	00.05	00.05
WSC	0	8	9	10

Note: W0: 0% WSC/control; W8: 8% WSC; W9: 9% WSC; W10: 10% WSC WSC: Water soluble chitosan

the method of Zakiyah & Handayani,(2021), while the addition of WSC (0, 8, 9, and 10 g) to 100 g of cookie dough referred to Maezaki et al., (1993). The formulation of cookies is depicted in Table 1.

The WSC was analyzed for yield, degree of deacetylation using potentiometric titration procedure no III of Czechowska-Biskup et al. (2012), solubility in water and acid (Tungtong et al., 2012), moisture content (BSN, 2015a), and ash content (BSN, 2010). All tests were done in triplicates. The properties of weight-loss cookies tested in the study included hedonic (BSN, 2015b), moisture-content (BSN, 2015a), ash content (BSN, 2010), protein content using the Kjeldahl method (BSN, 2006), fat content (BSN, 2017), carbohydrate content (by difference), and fat binding capacity (Jin et al., 2017) by simulating gastrointestinal conditions in human using peanut oil and liquified butter as standard fats. Cookies with and without the addition of WSC from mussel shells were tested. The calories (kcal) in the cookies were calculated per 100 grams using the Atwater method (4-9-4) as described by Lestari et al. (2017).

Data Analysis

One Way ANOVA and Duncan's Advanced Test (SPSS IBM 25 software) were used to analyze the effect of hydrogen peroxide concentration on the properties of water-soluble chitosan, and to analyze the effect of water-soluble chitosan on the properties of weight loss-friendly cookies. Determination of the best concentration of water-soluble chitosan was carried out using the Effectiveness Index Test method (De Garmo et al., 1984), by which the parameters included in the analysis were solubility in water and acid, fat binding capacity, degree of deacetylation and yield.

Results and Discussion

Properties of WSC from Green Mussel Shells

The properties of WSC for each treatment are presented in Table 2.

Appearance

The appearance of water-soluble chitosan in each treatment was relatively the same. However, regarding the color in Figure 1, the control was more white. This may be caused by the Maillard reaction during the chitosan depolymerization process as is also reported by Tian et al. (2004) that in the FTIR spectrum of water-soluble chitosan there is an absorbance at 777.5 cm^{-1} which indicates a Maillard reaction between -CHO_2, 5-anhydro-D-mannose and –NH2 of chitosan.

The Chitosan depolymerization process began with the dissolution of chitosan in a 2 % acetic acid solution and was carried out in a water bath. This led to an increase in pH due to the binding of H^+ in solution with NH23 (nucleophile) to form NH $^+$ (electrophile). Referring to Hodge (1953) in Yue, (2014) the increase in the reactivity of the amine group by deprotonation in a higher pH solution can lead to the Maillard reaction. However, the protonated free amino group can also enhance the steric barrier effect for the Maillard reaction. In addition, oxygen due to the open reaction is also able to slow down and even inhibit the Maillard, reaction (Katsuno et al., 2013). Therefore, the color of water-soluble chitosan in each treatment was not profound and only produced a slightly brownish color.

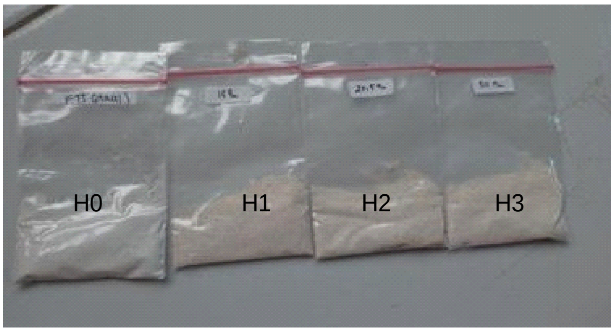

Figure 1. Chitosan (H0/control) and Water Soluble Chitosan (H1-H3) (H0: H_2O_2 0%/control; H1: H_2O_2 13%; H2: H_2O_2 21.5% and H3: H_2O_2 30%)

Table 2. Properties of WSC from Green Mussel Shells

Parameters	H0	H1	H2	H3	SNI 7949-2013
Appearance	Brownish white	Brownish white	Brownish white	Brownish white	Pale brown to white
Yield	-	44.79±1.44a	37.40±0.14b	33.13±1.06c	-
Degree of deacetylation	78.01±0.35d	72.37±0.07c	73.59±0.14b	75.08±0.12a	min 75%
Solubility:					
· Acid	92.92±0.11d	93.59±0.09c	95.77±0.56b	97.27±0.27a	min 99%
· Water	21.77±0.03d	76.39±0.58c	86.92±0.23b	91.72±0.40a	-
Moisture Content	0.41 ± 0.04d	2.20 ± 0.09c	2.59 ± 0.03b	3.71 ± 0.07a	max 12%
Ash content	4.54 ± 0.03a	4.19 ± 0.06b	4.15 ± 0.08b	4.11 ± 0.07b	max 5%
Fat binding capacity:					
· Peanut oil	4.73 ± 0.34d	5.61 ± 0.02c	8.30 ± 0.35b	9.18 ± 0.13a	-
· Butter	4.10 ± 0.04d	4.35 ± 0.15c	6.37 ± 0.06b	7.60 ± 0.09a	-

Note: H0: H_2O_2 0%/control; H1: H_2O_2 13%; H2: H_2O_2 21.5% and H3: H_2O_2 30% *different letters that follow the figures in the same row denote a significant difference ($p<0.05$)

Yield

The yield of water-soluble chitosan for each treatment was calculated based on the percentage of final weight after precipitation by ethanol to the initial weight (5 g) as shown in Table 2. The results of the ANOVA test and Duncan's test showed that differences in hydrogen peroxide concentration resulted in significantly different yields ($p<0.05$), where the higher the concentration, the lower the yield. According to Tian et al. (2004), the molecular weight of chitosan decreases as the concentration of hydrogen peroxide used increases, and the lower the molecular weight of chitosan, the lighter and smaller the molecular size. This prevents chitosan from being precipitated by ethanol and released during the filtration process (Tanasale, 2019).

Degree of Deacetylation

The percentage loss of acetyl groups in chitosan is indicated by the degree of deacetylation. The proportion of D-glucosamine and N-acetyl-D-glucosamine in chitosan determines the degree of deacetylation (Saleh, 2017). Table 2 displays data on the degree of deacetylation of water-soluble chitosan for each treatment. The ANOVA test results revealed that differences in hydrogen peroxide concentration resulted in significantly different degrees of deacetylation ($p<0.05$), with the higher concentration resulting in a lower degree. When the reaction is carried out under acidic conditions, more amino groups become protonated groups, increasing chitosan solubility and assisting in raising the pH of the solution. This shows that the more free amino groups in the polysaccharide chain, the easier –NH2 reacts with H_2O_2 to break the chitosan chain (Tian et al., 2004).

Solubility

The WSC solubility was expressed in two parameters, namely solubility in acid and solubility in water. The data presented in Table 2 shows that differences in hydrogen peroxide concentration resulted in significantly different solubility in acid and water ($p<0.05$). It might be due to the HCl being the corrosive acid so the chitosan was easily soluble. Whenever more acetyl groups in WSC were cut or deacetylated, the molecular weight decreased causing chitosan more soluble in water (Tian et al., 2004). Therefore the value of the solubility of chitosan will be directly proportional to the degree of deacetylation. Based on Table 2, it can be seen that the highest solubility of WSC at the degree of deacetylase was 75.08%. Depolymerization decreases molecular weight and shortens chitosan molecular chains (Tanasale et al., 2016). Santoso et al. (2020) state that an increase in the degree of deacetylation may contribute to a reduction in the molecular weight of chitosan. According to Chamidah et al. (2019). chitosan with low molecular weight makes chitosan have a high solubility in water. Meanwhile, according to Lu et al. (2004), the degree of deacetylation of chitosan will determine the solubility of chitosan in water and chitosan will dissolve in water with a degree of deacetylation of 46.7-64.4%.

Moisture and Ash Contents

The moisture content of water-soluble chitosan is presented in Table 2. The addition of hydrogen peroxide significantly ($p<0.05$) affected the moisture content. The moisture content of chitosan is influenced by the relative humidity of the air around its storage area because chitosan is easy to absorb moisture from the surrounding air. Chitosan polymer groups (amine, N-acetyl, and hydroxyl groups) will form hydrogen bonds with H_2O from the air (Dompeipen et al., 2016). Table 2 also shows that the addition of hydrogen peroxide significantly ($p<0.05$) reduced the ash content compared to the original chitosan, while the effect of hydrogen peroxide concentrations was not significant. The ash content of water-soluble chitosan in each treatment was relatively lower when compared to that of the original chitosan. The organic materials used in the process were thought to affect the ash content of water-soluble chitosan. The acetic acid was used to bind the amine groups of chitosan, hydrogen peroxide for depolymerization of chitosan, NaOH to neutralize the solution, and ethanol to precipitate the filtrate. According to Basmal et al. (2007), the ash content of water-soluble chitosan is influenced by the number of organic materials that react with chitosan during the process of converting water-soluble chitosan.

Fat Binding Capacity

The molecular weight of water-soluble chitosan will change depending on the hydrogen peroxide concentration. The molecular weight of chitosan decreases with increasing hydrogen peroxide concentration. The molecular dimensions and shape of chitosan must be able to capture fat or oil droplets (Muzzarelli, 1996). This is due to a high likelihood of failing to capture fat droplets of bigger size, small particles with short polymer chains are less effective at binding fat, making the chitosan-lipid complex less durable. A higher percentage of fat molecules can be maintained in the particles and medium-sized polymer chains are more flexible. Due to its high degree of molecular affinity and crystallinity, the ability of chitosan to bind fat will decrease as polymer chains get longer. Additionally, the capacity to bind fat may be diminished as a result of the potent intramolecular interactions and the creation of particle aggregates.

The ability of different chitosan chain lengths to form micelles and capture the oil phase in precipitation varied (Czechowska-Biskup et al., 2005). Due to their relative rigidity, long chains are not appropriate for producing micelles. The ability to trap fat molecules may be improved by shorter chains with more mobility. At neutral pH, very short chains will have a higher level of solubility. As a result, chitosan will be more likely to form stand-alone dissolved molecules than to bind fat molecules.

In addition to molecular weight, another characteristic of chitosan that can affect fat-binding capacity is the degree of deacetylation. To ascertain the relationship between the level of deacetylation and the ability of water-soluble chitosan to bind fat, Pearson analysis was used. For peanut oil and butter, the coefficient of determination (R^2) was respectively 0.9329 and 0.9695. The capacity of chitosan to bind fat increases with deacetylation level, resulting in a more linear relationship between the two (Dimzon et al., 2013). Chitosan had a higher level of deacetylation than the three samples of water-soluble chitosan, but it had a lower potential for binding to fat. This is probably because chitosan has a more rigid structure, making it less effective at trapping fat droplets. In contrast, water-soluble chitosan has a shorter chain with higher mobility, making it more successful at doing so.

Table 3. Result of the Effectiveness Index Test

Parameters	Effectiveness Index			
	H0	H1	H2	H3
Solubility in water	0	0,119444444	0,142361111	0,152777778
Solubility in acid	0	0.015	0.034	0.048
Fat binding capacity	0,013	0	0,082638889	0,229166667
Degree of deacetylation	0,18	0.068	0.022	0
Yield	0,2	0.036	0.014	0
Total	0,393	0,202083333	0,272916667	0,415277778

Note: H0: H_2O_2 0%/control; H1: H_2O_2 13%; H2: H_2O_2 21.5% and H3: H_2O_2 30%

Selection of Best H_2O_2 Concentration

Compared to SNI 7949-2013 for chitosan (BSN, 2013), specifications for moisture and ash contents were met by all samples, while for the degree of acetylation, only H0 and H3 met the specification. To select the best H_2O_2 concentration for the current condition, Effectiveness Index Test (De Garmo et al.,1984), was used. Each parameter was given weighted scores for importance, followed by a series of calculations to result in an effectiveness index for each parameter which was the total ranked. The results are presented in Table 3, showing that H3 (WSC produced with 30% H_2O_2) obtained the highest total value, ie. 0.598. In the second experiment, weight-loss cookies were made using this WSC (H3).

Properties of Weight-Loss Cookies

The properties of the cookies tested included hedonic scoring, proximate composition, and calories; all were compared to SNI 01-2973 (BSN, 1992) for appropriate parameters, of which the results are presented in Table 4.

Table 4. Properties of weight-loss Cookies

Properties	Treatment				
	W0	W8	W9	W10	SNI 01 - 2973
Hedonic score (9-scale)	7.17±0.69a	7.37±0.55a	7.30±0.69a	7.13±0.99a	-
Proximate composition (%):					
· Moisture	4.24±0.1a	4.14±0.04a	4.38±0.07a	4.19±0.04a	Max 5 %
· Ash	0.35±0.01c	0.84±0.03b	0.85±0.02bc	0.92±0.01a	Max 1.6 %
· Protein	6.66±0.21a	6.80±0.06a	7.00±0.03a	6.90±0.01a	Min 9 %
· Fat	11.29±0.13a	12.00±0.01a	10.73±0.19b	10.96±0.02b	Min 9.5 %
· Carbohydrate	76.95±0.08a	76.22±0.02b	77.01±0 .23a	77.03±0.02a	Min 70 %
Total calories (kcal/100g):	441.47±0.95	440.145±0.29	432.69±1.01	434.425±0.35	Min 400
- Protein	26.66±1.16	27.22±0.37	28.02±0.20	27.6±0.06	
- Carbohydrate	307.8±0.45	304.88±0.11	308.06±1.27	308.14±0.08	
- Fat	107.01±1.65	108.05±0.19	96.62±2.48		
Fat binding capacity :					
• Peanut oil	4.15±0.01d	9.26±0.04c	12.22±0.03b	13.16±0.04a	
• Liquified butter	4.36±0.11d	8.24±0.06c	10.36±0.04b	11.10±0.05a	

Note: W0: 0% WCS/control; W8: 8% WCS; W9: 9% WCS and W10: 10% WCS; * different letter following figures in the same row indicates a significant difference

Hedonic Scores

The average hedonic scores of cookies as presented in Table 4 are the average scores of appearance, flavor, taste, and texture. As shown in the table, WSC did not affect the scores significantly ($p<0.05$). However, the scores were increased when the concentration of WCS was added at the level of 8% compared to the control treatment (WCS 0%) and then decreased as the concentration increased. Similarly to this, Ghoshal & Mehta, (2019) found that as chitosan levels grew from 0.1 to 2% w/w, bread's sensory scores decreased. Since chitin and chitosan are known to have inherent astringency (Luck et al., 2015 and Wang et al., 2021) it could be possible that the hedonic ratings of cookies in this study may have declined as the WSC increased.

Proximate Composition

Water in food is a medium for bacteria (microbes) and fungi to grow and develop (Natalia et al., 2019). The way to preserve food is to reduce the moisture content in foodstuffs. Overall, the moisture content in each treatment has met the standard for biscuits (BSN, 1992), which is a maximum of 5%. The ANOVA showed that there was no effect of the addition of water-soluble chitosan on the moisture content of cookies. Ash content is a mixture of inorganic or mineral components contained in a food ingredient (Novidahlia et al., 2015). Overall, cookies in each treatment have met the standard limit of ash content for biscuits (BSN,1992), which is a maximum of 1.5%. However, cookies with the addition of water-soluble chitosan have a relatively high ash content. The ash content detected in the cookies most likely came from the added water-soluble chitosan. Chemically, the added quality of WSC contained 4.11% ash. In addition, the large proportion of oatmeal in the cookie is also a contributing factor. Oatmeal contains fiber consisting of the basic elements that make up plant cell walls and contain inorganic ions. Fiber can act as a mineral and electrolyte binder because of the presence of free carboxyl groups in the glucuronic acid that makes up hemicellulose (Schneeman, 1986, Kumalasari, 2018). Therefore, the higher the fiber content in cookies, the higher the ash content. The ANOVA showed that there was a very significant difference in the addition of WSC to the ash content of cookies. The greater the mass of the addition of water-soluble chitosan in cookies, the greater the ash content of cookies. This is due to the ash content in the water-soluble chitosan itself which can increase the ash content of cookies.

Overall, the protein content in cookies for each treatment did not meet the standard for biscuits (BSN, 1992), which was a minimum of 9%. The low content is related to protein loss due to heating during processing, which caused the Maillard reaction. The Maillard reaction occurs at a temperature above 115 °C, while cookies are baked at a temperature of

160 °C for 10 minutes (Widiawati & Anjani, 2017). In addition, the use of low-protein flour can also be a factor causing the low protein content in cookies. The ANOVA results showed that there is no significant difference in the addition of WSC to the protein content of cookies.

Based on a standard for biscuits (BSN, 1992), the standard carbohydrate content in cookies is at least 70%. So it can be concluded that cookies that have met the standard are those with the addition of 9 and 10 grams of WSC. Oats and wheat flour are ingredients that contribute to the carbohydrate content of cookies. Oats are whole grains, which include complex carbohydrates so they take longer to be digested by the body (Utami, 2020). The ANOVA shows that there is a significant effect ($p<0.05$) of the addition of water-soluble chitosan on the carbohydrate content of cookies

Calories of Cookies

The calorie of *cookies* is calculated as data to support the creation of *weight-loss-friendly cookies*. Although the weight-loss cookies produced are low in calories, the quality still meets the standards of oatmeal cookies as a product with ideal nutrition which must contain a minimum of 400 kcal/100 g of energy (van Tongeren & Jansen, 2020). Table 4 shows the total calories in cookies having met the minimum energy of cookies.

Fat Binding Capacity

The test results of fat binding capacity (Table 4) show that all samples can have the ability to bind fats, both fats from peanut oil and liquified butter. The addition of WSC to cookies has significantly affected the fat-binding capacity of cookies to both peanut oil and liquified butter fats ($p<0.05$). The greater the concentration of water-soluble chitosan, the greater the fat-binding capacity. These results are consistent with a study conducted by Maezaki et al., (1993), that the addition of 99.2 grams of chitosan in 1 kg of biscuit dough was able to reduce LDL levels, triglycerides, and increase HDL levels in the respondents' blood. Based on Table 4 it can be concluded that the fat-binding capacity of the sample added with WSC is greater in peanut oil than in liquified butter. Peanut oil contains more monounsaturated and polyunsaturated fatty acids (Sinaga, 2018), while butter contains more saturated fatty acids (Li et al., 2018). The interaction of chitosan and fatty acids will be different depending on the type of acid as stated by (Wydro et al., 2007) that the binding power or trapping power of chitosan to monounsaturated fatty acids and polyunsaturated fatty acids is stronger than that of saturated fatty acids.

Conclusion

Water-soluble chitosan is affected by H_2O_2 concentration in that the color turns brownish, the yield and ash content decrease, and the increasing of a degree of deacetylation, solubility in acid and water, and moisture content. When hydrogen peroxide is used to produce WSC, a 30% concentration produced the best WSC. The hedonic results of quality, protein, water, and carbohydrate contents of cookies were unaffected by the addition of WSC. However, the ash and fat contents were affected. All cookie samples in all treatments demonstrated the ability to bind fat in liquified butter and peanut oil, which justifies the use of cookies containing WSC in body weight reduction research. Further research is needed to be carried out related to the fat-binding capacity of cookies using the in vivo method in test animals (pure strain white mice) or humans.

Supplementary Material

Supplementary material is not available for this article.

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