

PHYSICOCHEMICAL PROPERTIES AND SENSORY EVALUATION OF WHEY PROTEIN
CONCENTRATE-ENRICHED COOKIES



FoodScience
and Nutrition

By

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i3L – Indonesia International Institute for Life Sciences
School of Life Sciences

In partial fulfillment of the requirements for the Bachelor of Science in
Food Science and Nutrition

Jakarta, Indonesia

2020

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Jakarta, Indonesia
2020

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Cohort: 2014

Study Program: Food Science and Nutrition

Title of final thesis project: Physicochemical and Sensory Evaluation of Whey Protein Concentrate (WPC)-Enriched Protein Cookies

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Statement of Originality

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"Physicochemical Properties and Sensory Evaluation of Whey Protein Concentrate-Enriched Cookies"

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Abstract

Protein is a macronutrient, important for growth and muscle building in metabolism. Whey Protein Concentrate (WPC) as a high-quality protein is desirable to use in food product formulation for protein enrichment. Cookies showed an increasing consumption trend from 2013-2017, therefore it was chosen for protein enrichment with WPC. WPC-enriched cookies were made with a 0%, 5%, 10%, 15%, and 20% WPC to flour concentration (w/w). Analysis was conducted on proximate composition, texture, color, and hedonic sensory test. Proximate analysis results showed that cookies meets *Standar Nasional Indonesia* (SNI) requirements. However, only 20% WPC-enriched cookies reached the minimum protein concentration (12 g) requirements set by BPOM for food product labelling to be known as source of protein. Results showed that WPC-enriched cookies increased in hardness significantly along with the increasing WPC concentration 1485.00 gram-force (gf), 1797.00 gf, 2224.25 gf, 2701.00 gf, and 3185.75 gf at 0% to 20% of WPC concentration samples, respectively. The 0% WPC-enriched cookies showed *L* value of 67.48, *A* value of 30.03, and *B* value of 29, and 20% WPC-enriched cookies showed *L* value of 50.98, *A* value of 58.5, and *B* value of 31.95. These indicated that WPC-enriched cookies tend to have a darker and more brownish-red color compared to that of 0% sample. Changes in texture and color were attributed to increased Maillard reaction, which occurred from the presence of protein and reducing sugars in high heat condition. Hedonic acceptance results indicated that overall acceptance amongst 0%, 5%, and 10% WPC-enriched cookies do not significantly differ. Protein enrichment in cookies with WPC could be done successfully up-to 10% concentration. Further changes to the formulation however, is required to produce cookies with source of protein label, as only 20% sample met the requirement.

Keyword: Cookies, Whey Protein Concentrate (WPC), WPC-enriched cookies, Protein, Maillard reaction, Texture, Color, Sensory analysis,

Acknowledgements

Firstly I would like to raise my gratitude to God almighty for His blessings. Additionally, I also want to sincerely thank Prof. Agus Naro, Prof. Siti Muslimatun, and Mr. Yohanes Raditya. My deepest regards and apologies to them for the troubles I have caused them, more importantly also for the endless patience and support that they have provided when dealing with myself. Next is my thank you to my family, for their support, which helped push and motivate me. Many thanks as well for i3L and their staff amongst which included but not limited to lab assistants, staff in academic operations, student development staff, and lecturers. Also thanks to Saraswanti Indo Genetech laboratory for their service. Not to forget also thanks to my friends who also provided support in finishing this thesis.

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List of Abbreviations

AKG	<i>Angka Kecukupan Gizi</i>
ALG	<i>Acuan Label Gizi</i>
ANOVA	Analysis of Variance
BPOM	<i>Badan Pengawas Obat dan Makanan</i>
gf	gram force
SIG	Saraswanti Indo Genetech
SNI	<i>Standar Nasional Indonesia</i>
WPC	Whey Protein Concentrate
w/w	Weight by weight

Chapter 1

Introduction

1.1 Background

Protein is one of the macronutrients in human nutrition that is a crucial factor for growth and development. Another function of protein in human metabolism is for muscle development. Protein intake within the population can be influenced by several factors which includes age and physical routine. *Angka Kecukupan Gizi (AKG)* recommendation based on Kemenkes RI (*Kementerian Kesehatan Republik Indonesia*) for protein intake varies depending on age, gender, and pregnancy condition.

Protein requirements are higher in young people such as teenagers and young adults, male, and pregnant women. Additionally, individuals with intense or extensive workout routines are also expected to consume more protein intake to balance the protein loss from metabolism (Lin *et al.*, 2010). Increasing protein intake in a diet could be done through consuming foods enriched with protein. High protein-enriched food products are therefore desirable to those in need of a high protein diet or those seeking to build muscle mass.

One method of protein enrichment in food is through the addition of protein powder, such as whey protein, into the food product formulation. Whey protein powder is readily available and accessible as a commercial food product. Some of the recognized forms of whey protein products are concentrate, isolate, and hydrolysate. Based on the Observatory of Economic Complexity (OEC) in 2018, Indonesia is included as one of the top 10 importers of whey globally, with a trade value that reaches US\$ 141 million. In regards to accessibility, the market trend shows decreasing prices of whey products as is shown in Figure 1.1 which is reported by USDEC (United States Dairy Export Council (USDEC), 2017).

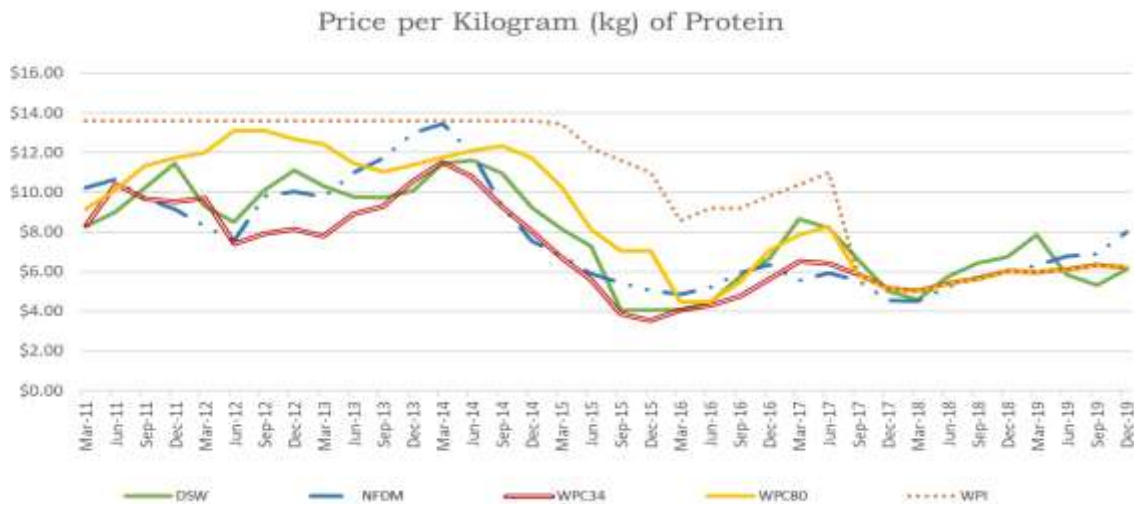


Figure 1.1 Price of Dairy Products in Dollars per Kilogram of Protein Quarterly 2011-2019

Whey Protein Concentrate (WPC) is available with varying protein levels, generally ranging from 34% to 80%, with WPC 80 used as a term for WPC with 80% protein level (Bulut-Solak & Akin, 2012). Whey products with 80% or more protein contents such as WPC 80 are a good choice to create high protein food due to the high protein content (de Wit, 1998). Additionally, the usage of whey in food formulation helps in color, texture, and flavor development of food products (Jeewanthi, Lee, & Paik, 2015). Cookies is one of the products that can be enriched with whey protein.

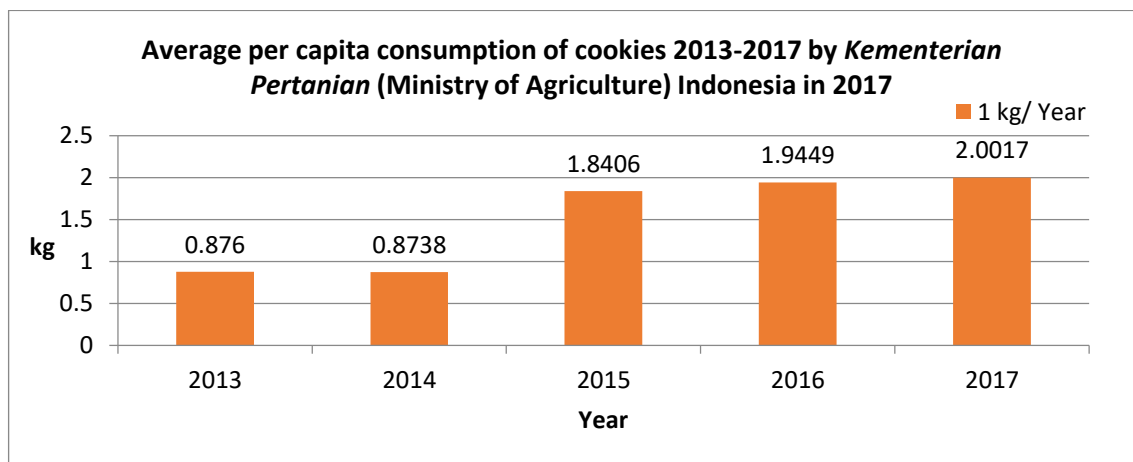


Figure 1.2 Average per Capita Consumption of Cookies for 2013-2017 in Indonesia

Cookies consumption in Indonesia has followed an increasing trend annually. Statistical data by *Sekretariat Jenderal Kementerian Pertanian Republik Indonesia* showed growth trends of cookies consumption from 2013-2017 as shown in Figure 1.2 (Komalasari *et al.*, 2017). Aside from the increasing cookies consumption in Indonesia, amongst amateur athletes and fitness enthusiast, cookies also counts as one of the food types often consumed (Çöndü, Şener, & Türkoğlu, 2019).

Depending on the quantity consumed, cookies could be categorised as either discretionary food or main food group of an individual's diet. In individuals that consumed cookies in large quantity, cookies is placed as part of the main food group because it contributes to the carbohydrate intake of the individuals (Burkhart & Pelly, 2016). Therefore using cookies for protein enrichment is a desirable option when aiming for increasing protein intake amongst young adolescence.

1.2 Objectives

This research aimed at recording and evaluating the physicochemical properties and sensorial acceptance of WPC-enriched cookies. Analysis was conducted on proximate composition, texture, color, and hedonic sensory test.

1.3 Hypothesis

Increased protein content in WPC-enriched cookies will produce a darker color and tougher cookies from Maillard reaction. These changes will produce a lower sensorial evaluation for color and texture amongst panelists, but higher sensorial evaluation in flavor and aroma.

1.4 Benefits of study

The findings of this study are expected to show the effect of adding WPC to cookies on acceptance based on the physicochemical properties and sensory evaluation. Sensory evaluation results would help in determining the acceptance of WPC-enriched cookies. This would bring new options for innovations in food industries towards consumer acceptance in WPC-added baked products, especially cookies, and the development of protein enrichment food formulations.

Chapter 2

Literature Review

2.1 Protein

Proteins are formed from groups of amino acids through peptide bonds. In nutrition, different protein has different levels of quality. Protein quality is determined through the capacity to meet protein and essential amino acid requirements, digestibility of protein, and bioavailability of the amino acids. Current protein quality evaluation uses Protein Digestibility Corrected Amino Acid Score (PDCAAS) which was suggested to be changed into using Digestible Indispensable Amino Acid Score (DIAAS) (FAO, 2011). As mentioned one of the factors affecting protein quality is the essential amino acids components, which vary depending on the protein source.

Protein sources are separated into animal and plant-based protein sources. Animal-based protein sources such as milk, meat, and eggs tend to have a high protein quality score as it contains a more complete amino acid component, specifically essential amino acids. With plant-based protein sources, it has a lower protein quality score compared to animal-based protein as they may be lacking in essential amino acids components. As essential amino acids are not reproducible by human metabolism, it is therefore necessary to obtain it from meals (Hoffman & Falvo, 2004).

In the case of whey, the protein quality score based on PDCAAS is 100, while based on DIAAS scores in the range of 90 – 110 (Rutherford, Fanning, Miller, & Moughan, 2015). Whey protein are classified as high-quality protein as they contain essential amino acids, have high bioavailability, and is also often used as for fortification in the food industry (Jooyandeh *et al.*, 2016).

2.2 Whey Protein Concentrate

Whey can be obtained as a side product during cheese-making production which can be further processed into whey protein. Trade for whey shows trends of growth, and as of 2017, The Observatory of Economic Complexity (OEC) calculated that the global market value for whey reaches 4.51 billion dollars, with countries in the EU and US being the top exporters (*Whey*, 2017). Globally, the nutritional benefits of whey protein have caused an increase in the demand for whey and are supported by the growth of cost-efficient production in the industry. Research validating the beneficial effects of whey protein towards muscle building has helped develop a market for whey in the sports sector towards athletes and casual exercisers (Lagrange, Whitsett, & Burris, 2015).

The processed form of whey protein, such as whey protein concentrate, contains small amounts of nutrients aside from protein (Carunchia Whetstine, Croissant, & Drake, 2005). WPC with 80% protein content (WPC 80) is used exclusively for food products (de Wit, 1998). Baked goods is one of the food products that uses WPC to improve texture, flavor, and appearance (Wani, Gull, Allaie, & Safapuri, 2015). Studies on WPC have shown health benefits for several disease treatments and health problems, however direct consumption of WPC shows unappealing sensory characteristics (Parate, Kawadkar, & Sonawane, 2011). In this research, WPC 80 is the chosen option for enrichment due to its protein bioavailability and content (Hoffman & Falvo, 2004).

2.3 Cookies

Cookies by Oxford definition for North America are defined as a sweet biscuit. According to BPOM (*Badan Pengawas Obat dan Makanan*), cookies are classified as desserts or baked goods. Cookies are made from flour, sugar, and butter that are mixed following a prescribed recipe. Cookies can be identified through its high fat and sugar, and

low water content (Davidson, 2016). It is also a common practice in baking cookies to substitute, add, or both substitute and add other ingredients. In making cookies the ingredients and cooking techniques used are important as it may affect the product.

Generally all-purpose or low-protein flour is the choice for making cookies. Fat is used to help bind the ingredients together. Melted fat and solid fat when used creates different effects in cookies (Mamat & Hill, 2014). Butter is generally mixed with sugar first, and this process is called creaming. Creaming is mixing the sugar and butter thoroughly until no sugar crystals can be found, it is important in making cookies as this affects the fat structure and the texture obtained from the baking result (Jacob & Leelavathi, 2007).

Often-measured cookies parameters are physicochemical, sensorial, and textural properties; which may also include rheological properties of dough, color, cookie dimensions, and also sound properties (Munaza, Prasad, & Gayas, 2012).

SNI requirements for cookies sensory parameters are defined as normal for color, texture, and aroma. This definition by SNI standards refers to a sensory analysis done by one trained panelist or three untrained panelists which are familiar with cookies. The protein level is at a minimum of 5% weight by weight (w/w) and moisture content at a maximum of 5%. Other standards pertain to hazards within the product which encompasses microbial, chemical, and physical hazards as listed in the standard regulation SNI 2973:2011 (Standar Nasional Indonesia Biskuit, 2011).

2.3.1 Raw material

There are three basic ingredients in making cookies which are flour, sugar, and fat. Selection of specific types of these three ingredients is important in producing specific qualities in the cookies. All-purpose or low-protein flour is chosen for cookie recipe, as its lower protein content will develop enough gluten in the

dough to bind and make cookies without producing a bread-like texture or giving a chewy and tough texture (Ortolan & Steel, 2017).

For the fat, which helps bind the ingredients together, different types will affect the cookies (Mamat & Hill, 2014). Adding butter, most often used in recipes, in the melted form will lead to a chewier texture while cold and solid butter produces crispier cookies. Sugar choices are also important for the baking process. With sugars such as brown sugar or molasses it will produce darker color and impart different flavor in cookies.

2.3.2 Dough-making process

Creaming is the first step in the dough-making which is combining the butter and sugar thoroughly until a smooth texture like cream is achieved. Creaming before adding flour is important to prevent the sugar crystals from being left intact and caramelizing, which may lead to uneven texture in the cookies (Jacob & Leelavathi, 2007). Flour and other dry ingredients are the next to be added and after mixing thoroughly cookie dough will form.

A factor which differentiates cookies compared to bread is that cookie does not require kneading in its dough making process (S. P. Cauvain, 2015). Kneading, or excessive mixing, may even be undesirable in cookie making. As wheat flour contains the protein which will form gluten, kneading becomes one of the factors which lengthens and strengthens the gluten strand (Ortolan & Steel, 2017). Kneading in cookies will lead to the formation of a tough and chewy texture as opposed to a crumbly texture which is more desirable for cookies. Higher fat and sugar content will lead to weak gluten structure formation which also helps in producing crumbly cookies (Stanley P. Cauvain, 2017). Cooling the cookies dough before baking may

cause the cookie to spread less during the cooking time as the butter has been solidified.

2.3.3 Baking Process

As baked goods, cookies undergo Maillard reaction, this happens when free amino acids and reducing sugars are given enough heat and time to react and produce an array of flavor compounds which is important to food products (Gani *et al.*, 2015). In layman terms, the Maillard reaction is often called browning, as the reaction also produces brown pigments. Excess exposure to heat may lead the Maillard reaction to produce acrylamide, also known as burnt, and produces undesirable color and flavor. Aside from increasing the heat increasing free amino acids and reducing sugar content also increases the Maillard reaction.

In cookies the temperature and time control are crucial in ensuring Maillard reaction occurs, as under or over baking may lead to undesired results. Baking at a temperature that is too low or in a too short amount of time may lead to uncooked cookie dough or pale cookies, while high temperature and long baking time may cause excessive browning which may lead to the development of bitter flavor.

Chapter 3

Methodology

3.1 Material

WPC 80 used was Valens Myotein Whey Protein Concentrate and was manufactured by Bioscenerly International Sdn. Bhd. and distributed by Pharm-D Singapore Pte. Ltd. Other ingredients such as icing sugar, all-purpose wheat flour (Segitiga Biru brand), and unsalted butter (Anchor brand) were obtained from local markets. Flour to WPC 80 w/w ratio used was a 100 g to 0 g, 95 g to 5 g, 90 g to 10 g, 85 g to 15 g, and 80 g to 20 g.

Ingredients	Control	5% WPC	10% WPC	15% WPC	20% WPC
All-Purpose Flour	600	570	540	510	480
Whey Protein Concentrate 80	0	30	60	90	120
Butter	400	400	400	400	400
Sugar	200	200	200	200	200
Total Weight	1200	1200	1200	1200	1200

3.2 Cookies Preparation

Cookies were created following traditional shortbread cookies recipe made with a ratio by weight of 1 part sugar, 2 parts fat, and 3 parts flour (Roszkowska et al., 2016). WPC-enriched cookies for all concentrations used for sampling were obtained from a singular batch, the breakdown of the weight shown in Table 3.1.

Butter was first mixed with icing sugar using a stand mixer with flat beater attachment at speed 2 on a Standard Mixer KitchenAid, to emulsify the butter and sugar until smooth with no clumps of sugar left.

Flour and WPC mixture was then added to the butter and sugar, and mixed using the same flat beater attachment at the same speed setting. After properly mixed, the cookie dough mixture was rested overnight covered in plastic wrap before it was rolled out to 8 mm height and then cut using a circle-shaped 5 cm diameter cookie-cutter. A batch of cookies made an estimated 100 cookies.

The oven was preheated to 176 degrees Celsius for top heat and 172 degrees Celsius for bottom heat. Cut pieces of cookie dough were placed onto a baking tray that has been lined with parchment paper. Cookies were baked for 20 minutes and left to cool for 20 minutes before being stored in a zip-lock plastic bag. Cookies were stored for at least one day before further measurements and analysis were taken.

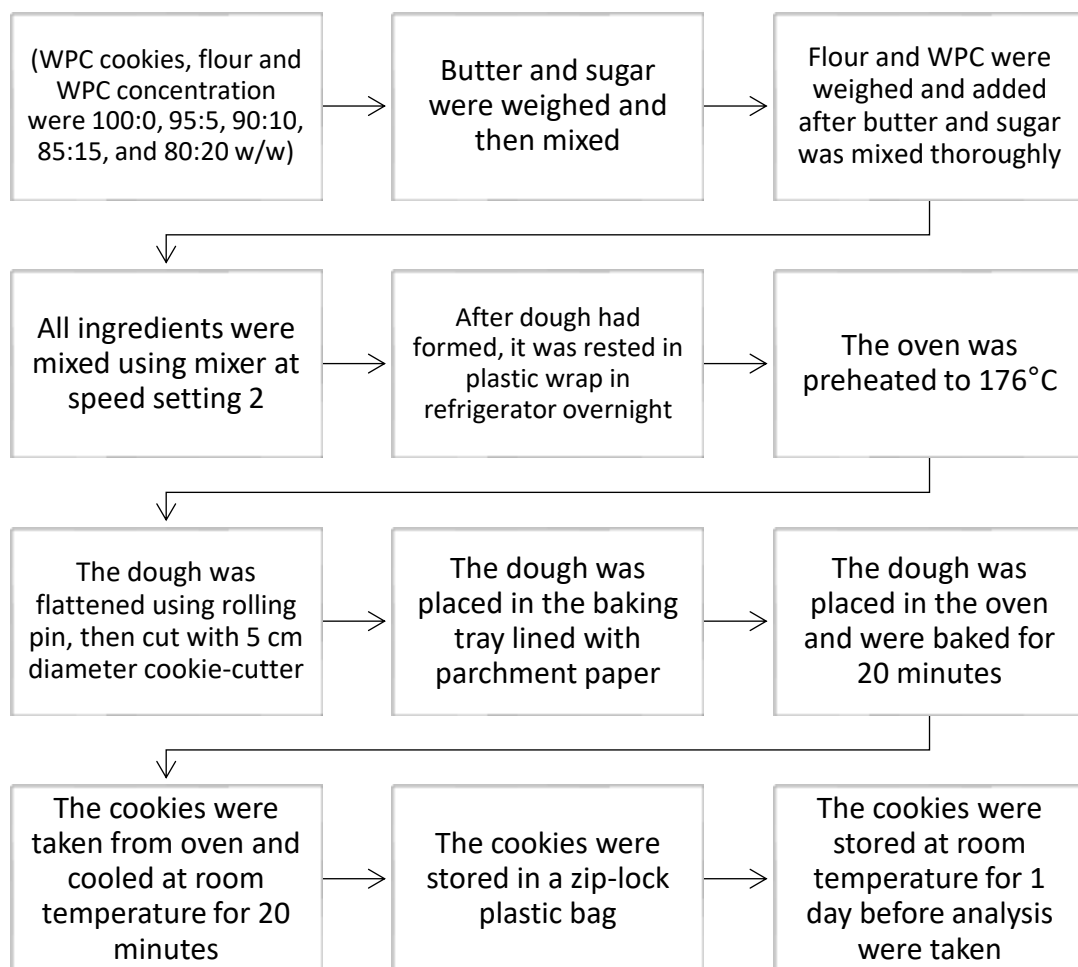


Figure 3.1 Flowchart of WPC Cookies Production

3.3 Analysis

3.3.1 Proximate Analysis

Proximate analysis of the WPC cookies was conducted based on several official methods which were SNI, Kjeldahl, Weibull, and SIG. The proximate analysis measured the total calorie, ash, moisture, carbohydrate, total fat, and protein content and was conducted by a third-party lab, Saraswanti Indo Genetech (SIG) laboratory. Triplicate data were obtained from 500 g random samples of the same batch.

3.3.1.1. Total Calorie

The total calorie content of a food sample was obtained by using the formula below: $Energy\ value\ \left(\frac{Kcal}{100g}\right) = (2.62 \times \%protein) + (8.37 \times \%fat) + (4.2 \times \%carb.)$

3.3.1.2. Ash Content

Ash content analysis followed the SNI 01-2891-1992, 6.1 method. In the ashing process, organic substances broke down into water and CO₂; however, inorganic substances did not and remained as ash. The sample was weighed carefully to 2-3 g into a weighed evaporating dish. The sample was placed in an electric furnace at 550°C until the ashing process was completed, approximately 3-4 hours. The sample and evaporating dish was removed from the oven and placed in the desiccator to cool until all the heat was absorbed, and then weighed. Ash content was calculated with the following formula:

$$Ash\ content = \frac{W1 - W2}{W} \times 100\%$$

3.3.1.3. Moisture Content

Moisture content analysis followed the SNI 2973 -2011 point A.3 method. Moisture content was calculated based on the weight lost during the heating process in the oven. The weighing bottle and the lid were heated in the oven at 130°C for one hour and cooled in a desiccator for 30 minutes before it is weighed (W_0). A total of 2 g of sample was put into the weighing bottle, covered then weighed (W_1). The weighing bottle containing the sample was heated in an open state in the oven at 130°C for one hour. While still in the oven the weighing bottle was closed, then immediately transferred to the desiccator and cooled for thirty minutes and weighed (W_2). Moisture content was then calculated with the formula below:

$$\text{Moisture content} = \frac{W_1 - W_2}{W_1 - W_0} \times 100\%$$

3.3.1.4. Carbohydrate

Carbohydrate analysis was calculated through the difference method (18-8-9/MU/SMM-SIG). The carbohydrate content of the sample was calculated by subtracting 100% as the nutrient content of the sample with water content, ash content, protein content, fiber content, and fat content. The value was determined using the following formula:

$$\begin{aligned} \text{Carbohydrate content (\%)} \\ = 100\% - \text{levels (water + ash + protein + fat)} \end{aligned}$$

3.3.1.5. Total Fat Content

Total Fat Content analysis followed the Soxhlet method (18-8-5/MU/SMM - SIG, Weilbull). It was performed by grinding approximately 30 grams of the sample with pestle and mortar until uniform. Pre-dried

cellulose extraction thimbles were removed from the desiccator and accurately weighed.

2-3 grams of samples were placed in the thimble, then weighed and recorded. Thimbles with the sample were then placed in the Soxhlet extraction unit. Flask was added with 350ml of ether and several glass boiling beads, extraction would then be held for 4-6 hours. A 250ml beaker glass was placed below the sample on the Soxhlet extraction. Thimbles were removed from the Soxhlet extractor and air-dried overnight at 150°C then dried in a vacuum oven at 70°C for 25 minutes. The dried sample would then be cooled down in the desiccator and it would be then weighed and recorded. The fat content was calculated using the formula below:

$$\begin{aligned} & \textit{Fat content} \\ & = \frac{(\textit{sample + thimble weight}) - (\textit{dried sample + thimble weight})}{\textit{wet sample weight}} \\ & \times 100\% \end{aligned}$$

3.3.1.6. Protein Content

Protein Content analysis followed the Kjeldahl method (18-8-31/MU/SMM - SIG, Kjeltec).

3.4.1.6.1. Sample Preparation

Each sample was weighed accurately for 1.0 gram, the sample was placed into the Kjeldahl digestion tube. Catalyst tablet and 12 ml of concentrated sulfuric acid were added to each Kjeldahl tube, also another tube with a blank which only contained the catalyst tablet. The digestion tube was placed on a digestion block

then the digestion block was covered, and the exhaust system turned on.

The solution would change into transparent which indicates that the digestion process was completed. The sample was then cooled down at room temperature. Each tube of the digest was diluted with distilled water and swirled.

3.4.1.6.2. Distillation Process

Boric acid was dispensed into the receiving flask then placed in a distillation system and ensure the sample was submerged. The sample was placed in a tube and distillation proceeded until complete.

3.4.1.6.3. Titration Process

Distillate which was contained in the Erlenmeyer was then titrated on a magnetic stirrer using HCl solution until discoloration to gray color occurred. A blank determination was also carried out.

Nitrogen and protein content was obtained from the formula below:

$$\%N = \text{normality HCL} + \frac{\text{corrected acid volume (ml)}}{\text{sample}} \times \frac{14 \text{ g N}}{\text{mol}} \times 100$$

$$\%Protein = \%N \times \text{protein factor conversion (6.25)}$$

3.3.2 Texture Analysis

TVT 6700 Texture Analyzer was used to measure the hardness of the WPC-enriched cookie. Break Probe 70mm Aluminum was used for the probe and three-point bend rig for the base of the cookie.

The settings used for the experiment were adjusted to an initial speed of 1.0 mm/s, test speed of 3.0 mm/s, retract speed of 10.0 mm/s, trigger force of 15 g, a data rate of 2pps, sample height was set on 55mm, starting distance was set to 5.0 mm, and compression was set to 15%. Data were obtained from randomly sampling 4 cookie pieces for each measurement. Data output was in gram-force (gf) unit.

3.3.3 Color Analysis

The color of the cookie was measured with Kingwell JZ300 Colorimeter. The measurement of the color was taken after cookies were rested. The colorimeter was calibrated using white paper. Data were obtained from randomly sampling 4 cookie pieces for each measurement.

The surface of the cookies was measured with the *LAB* values recorded. *L* value measured for lightness when + and darkness when -, *A* value measured for red when + and green when -, *B* value measured for yellow when + and blue when -. Delta E or total color difference would be obtained from the following formula:

$$\Delta E = \sqrt{(l_2 - l_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

3.3.4 Hedonic Sensory Test

One method of sensory testing used to measure acceptance based on human senses is the hedonic sensory test. The sensory analysis used in this study was a hedonic test with a scale of 1 (Dislike Extremely) – 9 (Like Extremely). Hedonic testing was conducted due to its simplicity, precision, and accuracy of consumer liking. Sensory attributes that were measured were on appearance, aroma, color, texture, sweetness, and overall acceptance.

Untrained panelists were chosen as the hedonic test did not require trained panelists. The age range of the panelists was restricted to be between 18 years old

and 35 years old. Samples were coded with random three-digit codes. Volunteers were gathered from i3L campus area, which included staff members and students, were asked to participate in the sensory test as panelists.

Panelist first received a questionnaire that described 9-point scale as a reference. One of each sample with the code was then provided to the panelist. Water was provided for the panelists to rinse the mouth for in-between samples. An example of the hedonic test questionnaire is provided in the appendix A.

3.3.5 Statistical Analysis

Statistical analysis was done using data analysis in Microsoft Excel. Experiments were conducted with a minimum of triplicate data for each result. Standard deviation, comparison of mean and One-way Analysis of Variance (ANOVA) was used to find the significant difference among all the WPC cookies samples with the control, with a p-value < 0.05. A T-test was conducted to determine if the data is statistically significant when compared with control. Further analysis of the data to compare samples result was done with Tukey's Kramer HSD analysis.

Chapter 4

Results

4.1 Proximate Analysis

Results of the proximate analysis were shown in Table 4.1 and ANOVA analysis of all attributes, were found to be significantly different. The 20% WPC-enriched cookies had the highest value for total calorie, ash, total fat, and protein content. The 10% WPC-enriched cookies had the highest moisture content, while 0% WPC-enriched cookies (control) had the highest carbohydrate content. A visible trend can be observed in ash, carbohydrate, and protein content. In ash and protein content a positive trend was observed in the results, however, carbohydrate content showed a negative trend.

Table 4.1 Results of WPC-Enriched Cookies Proximate Analysis

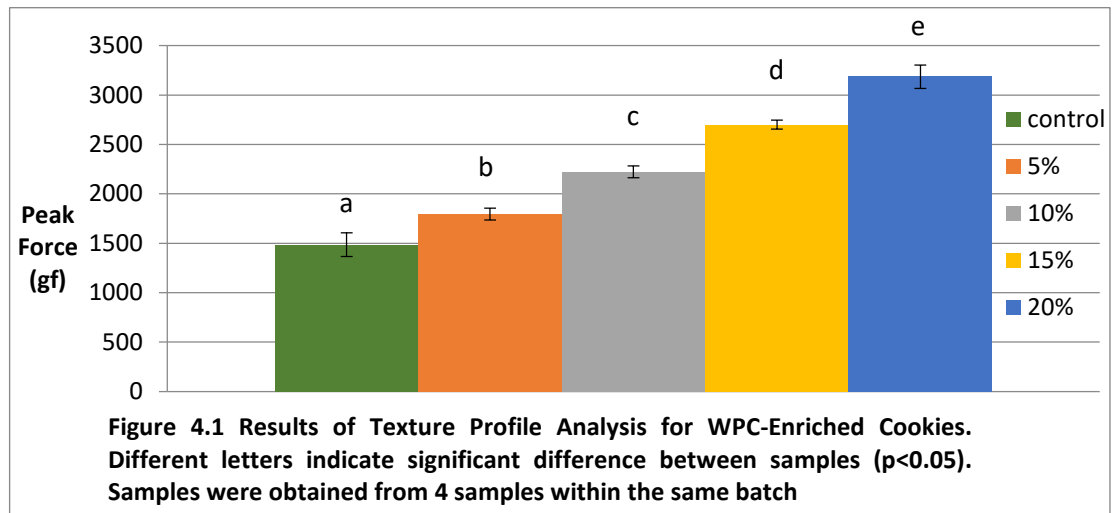
WPC (%)	Attributes					
	Total Calorie (Kcal/100g)	Ash (%)	Moisture Content (%)	Carbohydrate (%)	Total Fat (%)	Protein (%)
0 (control)	537.52±3.03 ^a	0.39±0.03 ^a	3.15±0.06 ^a	59.53±0.42 ^a	30.34±0.34 ^a	6.59±0.12 ^a
5	530.31±3.68 ^b	0.43±0.01 ^b	3.42±0.13 ^b	58.65±0.59 ^a	29.15±0.41 ^b	8.35±0.04 ^b
10	533.66±0.77 ^{bc}	0.49±0.01 ^c	4.15±0.03 ^c	55.03±0.27 ^b	30.44±0.08 ^{ad}	9.88±0.16 ^c
15	535.55±2.50 ^a	0.59±0.01 ^d	3.61±0.06 ^d	53.41±0.14 ^c	30.47±0.28 ^{ade}	11.92±0.28 ^d
20	540.34±3.27 ^{ac}	0.61±0.01 ^d	3.45±0.02 ^{bd}	51.79±0.26 ^d	31.31±0.36 ^{ce}	12.84±0.14 ^e

**Same superscript letter indicates no significant differences between samples*

4.2 Texture Analysis

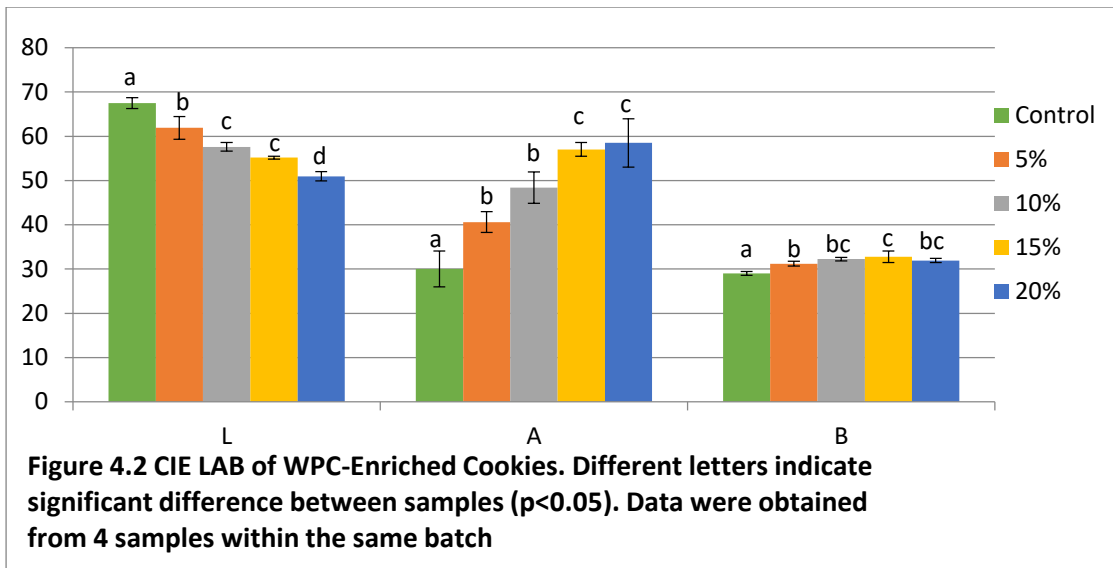
Results from the texture analysis in Figure 4.1 showed that 20% WPC concentration had the highest peak force followed by 15%, 10%, 5%, and control having the smallest peak force as shown in Figure 4.1. ANOVA of the results showed that the p-value was 1.74×10^{-13} ,

which indicated that the peak force between the WPC-enriched cookies samples had very high significant difference. Post-hoc analysis of the results also showed that between each samples was significantly different indicated by the letters in Figure 4.1.



4.3 Color Analysis

ANOVA of L , A , and B values indicated that results were significantly different among samples. As seen in Figure 4.2, all samples were significantly different in all three color parameters. The L values between all samples were found to be significantly different. The A values between samples were not significantly different between 5% and 10% WPC-enriched cookies, and 15% and 20% WPC-enriched cookies. The B values were not significantly different for 5%, 10% and 20%, and for 10%, 15% and 20% WPC-enriched cookies. Colorimeter identified the color of the WPC-enriched cookies as dark reddish-brown for all samples.



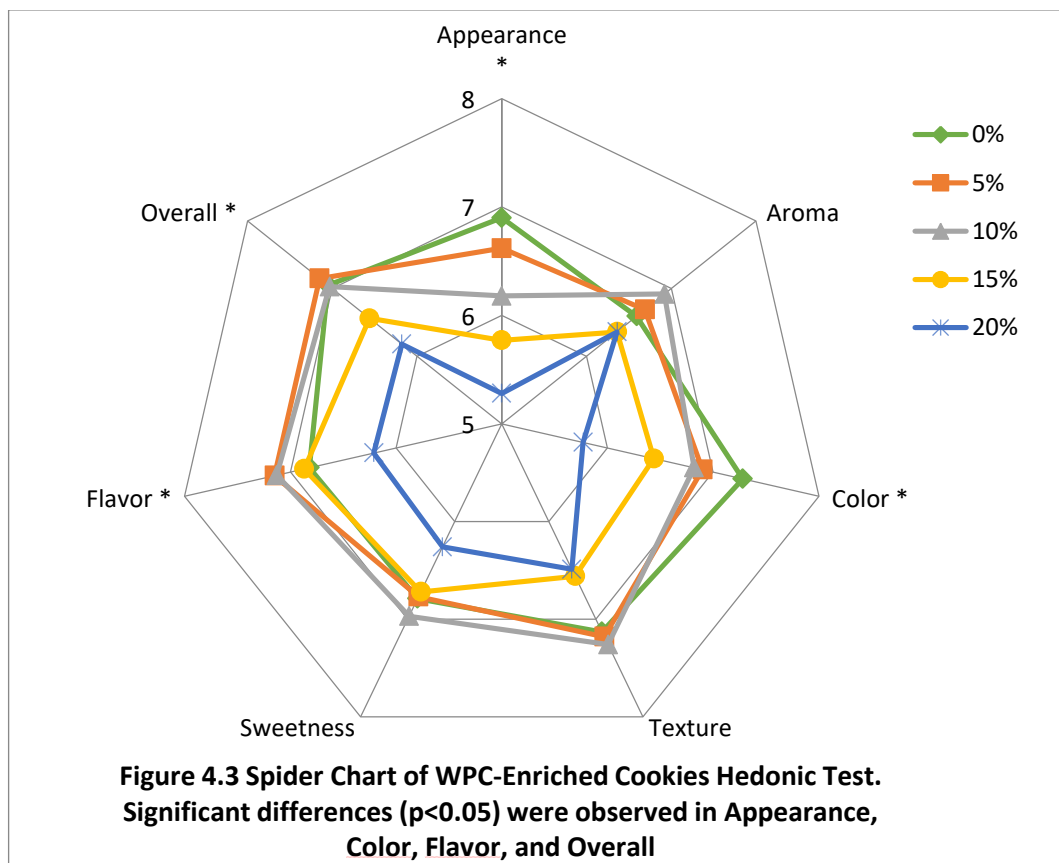
Delta E value was obtained by comparing other WPC concentration samples with 0% WPC-enriched cookies (control). The obtained results indicated that the measured changes in the color difference of WPC-enriched cookies were present when compared to control. Delta E standard values were measured from 0 to 100, with 0 means no change in color and 100 means the opposite or a total change in color as shown in Table 4.2. The results also indicated that as WPC concentration increased so did the changes in color difference.

Table 4.2 Measure of Change in Color Difference of WPC-Enriched Cookies Samples Compared to 0% (Control) WPC Enriched Cookies

WPC Concentration (%)	Total Color Difference (ΔE)
5	12.18
10	21.12
15	29.93
20	33.04

4.4 Sensory Analysis

A visualization of the results obtained can be found in Figure 4.3. Statistical analysis of the results showed that no significant differences were found for texture, aroma, and sweetness parameter. Significant differences were observed in appearance, color, flavor, and overall acceptance as indicated by the asterisk mark in Figure 4.1.



Based on results as shown in Table 4.3, 0% WPC-enriched cookies had achieved the highest average score for appearance and color attributes, and the average scores for both attributes decreased as WPC concentration increased. The 5% WPC-enriched cookies had the highest average score for flavor and overall acceptance. The 10% WPC-enriched cookies had the highest average score in aroma, texture, and sweetness attributes.

WPC cookies sensory test was conducted with the help of 41 untrained panelists in the sensory lab. Data from a total of 39 panelists were valid and used in this study and data from 2 of the panelists were not valid, as some parts of the questionnaire were left empty. Useable data came from 14 male panelists and 25 female panelists.

Table 4.3 Results of WPC-Enriched Cookies Hedonic Sensory Test

WPC (%)	Attributes			
	Appearance	Color	Flavor	Overall
0	6.90± 1.33 ^a	7.28±1.36 ^a	6.82±1.60 ^{ab}	7.05±1.30 ^a
5	6.62± 1.37 ^{ab}	6.90±1.44 ^a	7.15±1.31 ^a	7.15±1.04 ^a
10	6.18±1.71 ^{abc}	6.82±1.60 ^a	7.13±1.26 ^a	7.03±1.11 ^a
15	5.77±1.44 ^{bc}	6.44±1.48 ^{ab}	6.87±1.10 ^{ab}	6.56±0.99 ^{ab}
20	5.28±1.82 ^c	5.77±1.78 ^b	6.21±1.45 ^b	6.18±1.27 ^b

**Same superscript letter indicates no significant differences between samples*

Chapter 5

Discussion

5.1 Proximate Analysis

Proximate analysis results for protein content showed that all WPC-enriched cookies that were produced fulfilled the SNI protein requirements for cookies, which was a minimum of 5% protein w/w. The protein content of the cookies as shown in Table 4.1 increased as the WPC content increased, with the 20% WPC-enriched cookies nearly doubled in the protein content when compared to control. This showed that there was a positive trend in protein content, which was the increase in protein content as the WPC concentration increased. Food labelling in BPOM for source of protein label in solid food requires the ALG (*Angka Label Gizi*) to reach a minimum 20% from the recommended daily intake for protein (60 g), and this was calculated into 12% protein w/w. From the obtained result, only the 20% addition of WPC into cookies could therefore be labeled as source of protein. Aside from protein, the other results that showed a trend were in ash content and carbohydrate content.

Ash content which showed a positive trend was observed to have increased as WPC concentration increased. Results for carbohydrate content showed a decrease as WPC concentration increased. Changes towards protein, ash, and carbohydrate content of WPC-enriched cookies could be attributed to the substitution of flour with WPC. As WPC contains higher mineral levels compared to flour, the ash content of WPC-enriched cookies should increase, as was observed in this study. Additionally the substitution of flour, which was low in protein and high in carbohydrate, with WPC, which was high in protein and low in carbohydrate, would reduce the carbohydrate content to increase protein content in WPC-enriched cookies as the results of Table 4.1 showed.

With total calorie, moisture, and total fat content the results did not show any particular trends amongst WPC-enriched cookies. The moisture content of the WPC-enriched cookies still meets the SNI requirement which was under 5% w/w. Increase in WPC concentration would suggest changes in nutritional content such as protein, carbohydrate and ash content of the cookies, and constant results in others (Munaza, Prasad, & Gayas, 2012). Fluctuation in the total calorie and total fat content had occurred from the butter not thoroughly mixed with the flour during dough formation. The unevenness which occurred during the dough formation could be attributed to the minimal mixing during dough formation, which was done to prevent a bread-like texture from developing due to gluten content.

5.2 Texture Analysis

As shown in Figure 4.1 increased WPC concentration in cookies also showed an increase in the hardness of the cookies, denoted by the peak force data. This result showed that protein increase in cookies would result in harder textured cookies, which was appropriate with the observation that an increase in cookies hardness incorporated with WPC occurred when increasing WPC concentration from 4% to 6% (Wani et al., 2015). The increased fracture force of the WPC-enriched cookies and its hardness could be attributed to increased protein content which affected the cookie during the dough formation and also the baking process (Gallagher, Kenny, & Arendt, 2005).

One of the observations found during the dough making was that increasing WPC content in the formulation dried out the dough, which made the dough more crumbly and harder to mold. This can be attributed to the lack of water within the formulation that would have helped the WPC to bind with the water. Although increased WPC produced drier dough, this did not influence the moisture content of the cookies as Table 4.1 showed. With

the increasing hardness in increased WPC concentration, it could be attributed to the Maillard reaction during the baking of WPC-enriched cookies.

Three proposed ways on Maillard reactions effect on texture development consists of protein cross-linking formation, emulsions properties, and protein-polysaccharides conjugates formation (Starowicz & Zieliński, 2019). As the formulation of WPC-enriched cookies did not include water and used temperature above the WPC denaturation temperature, emulsion properties and protein-polysaccharides conjugation formation from Maillard reaction were unlikely to affect WPC-enriched cookies texture for this study.

There were minimal studies on the specific method of Maillard reaction effects on texture, baked goods were speculated to be influenced by protein cross-linking formation. Therefore specific to this study on WPC-enriched cookies, the proposed mechanism affecting the texture of the cookies was through protein cross-linking formation during the Maillard reaction (Charissou, Ait-Ameur, & Birlouez-Aragon, 2007).

5.3 Color Analysis

The results from Figure 4.2 showed a decrease in the lightness of cookies with increasing WPC concentrations in line with the hypothesis. The *A* and *B*, which indicated red and yellow values when positive, increased with the increase of WPC concentration. Whereas the color differences between each cookie sample in comparison to control can be seen through the Delta E values. Shown in Table 4.2, the increased WPC concentration also increased the Delta E value which represented the total color difference (Gani, *et al.*, 2015).

As cookies with increased protein content so did the Maillard reaction, which produces melanin a pigment for brown colors, also increased and produced the darker coloring in the food. Results from WPC-enriched cookies showed darkening in color as WPC concentration increases, which indicated an increase in the Maillard reaction. Table 4.2 however, showed that the increase in total color difference from 15% to 20% was not as

large as the other WPC-enriched cookies percentages. This means a further increase in WPC concentration higher than 20% may produce minimal changes to the color of the cookies.

5.4 Sensory Analysis

Hedonic sensory test of WPC-enriched cookies results showed average scores that were between neither like or dislike, and like moderately. WPC-enriched cookies amongst all WPC concentration were therefore still counted as liked or positively evaluated by the panelists. As the hedonic test relied on preference individual data showed the variation in sensory evaluation by panelist towards the cookies. This was also further supported by the histogram of the data spread.

Statistically, the sensorial evaluation of control cookies, found to be less hard in texture as shown in Figure 4.1, was not significantly different from the harder 20% WPC cookies. This was also observed in the individual data obtained, where several panelists scored highly on both control and 20% samples. From Figure 4.3 results, it could be seen that although appearance and color perception worsens as it becomes darker, the rest of the parameters were less impacted by increased WPC content, especially between 0%, 5%, and 10% concentration. Several panelists indicated bitterness was present in 20% concentration which suggested an excessive Maillard reaction, and the milk-like flavor was also present with WPC-enriched cookies (Sarabhai *et al.*, 2015). The Presence of WPC in cookies was identified to produce a milk powder taste noted by the panelists.

As shown in Figure 4.3, three parameters that did not have significant differences amongst samples were aroma, texture, and sweetness. Although statistically there were no significant differences in texture, results for 15% and 20% had lower average scores than control, 5%, and 10% as seen in Figure 4.3. In conjunction with the results for the hardness of WPC-enriched cookies, it showed an indication that the cookies texture after a certain

level of hardness would produce a lower average score in the texture attribute for the hedonic sensory test. In this study, the cut-off for the negative impact of hardness in cookies for the hedonic sensory result was between 10% and 15% WPC-enriched cookies.

In the attributes for appearance and color, results showed a decrease as WPC content increased, these results together with the color analysis indicated that darker colored cookies would produce a lower hedonic score for both attributes. Unlike with color and appearance attributes, the flavor and overall acceptance attributes did not decrease with increasing WPC concentration. Average scores of flavor parameters showed that it increases from control to 10% but then decreased with 15% and 20% WPC concentration.

Flavor score of 5%, 10%, and 15% WPC-enrich cookies were however higher than 0% WPC-enriched cookies. This indicated that the increased Maillard reaction does influence the sensorial perception of the WPC-enriched cookies for flavor and color. Excessive Maillard reaction, however, would produce bitter flavor and darker colors which negatively affects the result as seen in 20% WPC-enriched cookies for flavor, appearance, and color parameters.

The overall acceptance score of all samples indicated that the WPC-enriched cookies were accepted as cookies by the untrained panelists. The scores for the attributes had therefore fulfilled the SNI requirements for cookies which required a normal evaluation for cookies on aroma, taste, and color of cookies. Further statistical analysis indicated that the acceptance level of 0%, 5%, and 10% WPC-enriched cookies were not significantly different in all attributes, as seen by the Tukey-Kramer analysis on the result in Table 4.3.

These results showed that the hypothesis on WPC effect was proven for color attribute, but for only partially proven on texture, aroma and flavor attribute. In texture and aroma attributes results were not statistically different, which disprove the hypothesis.

However 15% and 20% WPC-enriched cookies did result in lower average scores. With flavor attribute 20% WPC-enriched cookies was not as hypothesized. Therefore the hypothesis could only apply to WPC-enriched cookies with 5% and 10% concentration.

Chapter 6

Conclusion and Recommendations

6.1 Conclusions

In conclusion, the substitution of flour with WPC at all concentrations did result in changes to the physicochemical and sensorial properties of the WPC-enriched cookies. Proximate analysis and the sensorial evaluation result of the WPC-enriched cookies have met SNI requirements and could therefore be categorized as cookies. The physicochemical properties of the cookies, except for color, had values that were positively affected; with color a darkening resulted in lower LAB values. As a result of the sensory evaluation, the formulated WPC cookies were well accepted by the panelists mostly were from the 5% and 10% WPC-enriched cookies concentration.

The experiment however, also showed that to meet the BPOM definition of source of protein, a minimum of 20% of WPC needs to be incorporated into the cookies formula. Therefore, although the study revealed that cookies can be formulated with WPC successfully, to produce cookies with specific nutritional labeling further study is required for 20% WPC-enriched cookies.

6.2 Recommendations

Further studies are needed to optimize and characterize the sensory profile of WPC-enriched foods, especially to formulate food products with food labeling following BPOM requirements. Additionally further studies of WPC incorporation into food formulation in more varied baked goods other than cookies can also be done. For cookie making, increased WPC concentration produced drier and crumbly dough which presented a challenge in molding the cookies. A side observation made in this study and several panelists were the milky aroma, flavor, and dryness within higher WPC concentrations.

Improvement for future studies to formulate WPC-enriched cookies with source of protein labeling, can therefore focus on the flavor profile observed in WPC cookies and to prevent dried out cookie dough from high WPC concentration. Another suggestion for future study is to use different cookie formulation either from ingredients, temperature, or time used. Incorporations of different ingredients in a cookie recipe may help in combining the milk-like flavor noted by panelists into the WPC-enriched cookies flavor profile. Changing the temperature and time in baking the cookies may help with overcoming the bitterness noted by panelists, which was identified as being caused by excessive Maillard reaction.

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