

THE PHYSICOCHEMICAL, NUTRITIONAL, AND
SENSORY PROPERTIES OF COOKIES MADE WITH
PUMPKIN FLOUR

By

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Dissertation submitted in partial fulfilment of the requirements for the
Degree of Bachelor of Health Sciences (Honours)
(NUTRITION)

CERTIFICATE

This is to certify the dissertation entitled ‘THE PHYSICOCHEMICAL, NUTRITIONAL, AND SENSORY PROPERTIES OF COOKIES MADE WITH PUMPKIN FLOUR’ is the record of research work done by GAN YONG SENG, MATRIC NUMBER 159008 during period record of August 2024 until January 2025 under my supervision. I have read this dissertation and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Health Sciences (Honours) (Nutrition).

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DECLARATION

I hereby declare that this dissertation is the results of my own investigation, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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LIST OF ABBREVIATIONS

TPA	Texture Profile Analysis
ANOVA	One-Way Analysis of Variance

LIST OF SYMBOLS

H_0	Null hypothesis
H_a	Alternative hypothesis
$^{\circ}\text{C}$	Degree Celsius
$>$	Greater than
$<$	Lower than
cm	Centimeter
%	Percentage
g	Gram

Abstrak

Tabiat pemakanan memainkan peranan penting dalam kesihatan keseluruhan, namun penyelidikan mengenai kesan biskut yang kaya dengan nutrien terhadap keutamaan pengguna masih terhad. Kajian ini meneliti penggunaan tepung labu sebagai pengganti lemak untuk meningkatkan nilai pemakanan biskut sambil mengurangkan kandungan lemaknya. Tepung labu dihasilkan dan dimasukkan ke dalam formulasi biskut pada tahap penggantian sebanyak 10%, 15%, 20%, dan kumpulan kawalan. Biskut-biskut ini dianalisis dari segi sifat fizikokimia, komposisi nutrisi, dan atribut sensori melalui penilaian panel.

Penggantian mentega dengan tepung labu pada tahap 20% menghasilkan biskut dengan kandungan karbohidrat tertinggi (70.42%) dan kandungan abu (1.07%), sambil mengekalkan tahap protein yang serupa, menunjukkan potensinya untuk meningkatkan nilai pemakanan. Penggantian sebanyak 10% memberikan kesan yang minimal terhadap aroma, rasa, dan keseluruhan penerimaan, menyerupai biskut standard dengan sedikit perbezaan pada kekerasan, warna, dan penampilan, menjadikannya pilihan yang diterima baik. Selain itu, penggantian mentega dengan tepung labu secara sebahagian telah mengurangkan kandungan lemak biskut dengan ketara, daripada 37.0% dalam kumpulan kawalan kepada 29.7%, 28.25%, dan 25.62% dalam kumpulan eksperimen ($p < 0.05$), menekankan keberkesanannya sebagai alternatif pengurangan lemak dalam produk bakeri.

Kandungan karbohidrat dan serat yang tinggi secara semula jadi dalam tepung labu turut menyumbang kepada peningkatan kelembapan, abu, dan kandungan karbohidrat, sekali gus menunjukkan keberkesanannya sebagai pengganti lemak. Kesimpulannya, tepung labu boleh dimasukkan dengan berkesan ke dalam biskut sebagai pengganti lemak, meningkatkan profil pemakanan tanpa menjejaskan penerimaan sensori.

Abstract

Dietary habits play a crucial role in overall health, yet limited research has explored the impact of nutrient-dense cookies on consumer preferences. This study examined the use of pumpkin flour as a fat replacer to enhance the nutritional value of cookies while reducing their fat content. Pumpkin flour was produced in-house and incorporated into cookie formulations at substitution levels of 10%, 15%, 20%, and a control group. The cookies were analyzed for physicochemical properties, nutritional composition, and sensory attributes through panel evaluations.

Substituting butter with pumpkin flour at a 20% level resulted in cookies with the highest carbohydrate content (70.42%) and ash content (1.07%), while maintaining similar protein levels, highlighting its potential to improve nutritional value. A 10% substitution level had minimal effects on aroma, taste, and overall acceptability, closely resembling standard cookies with slight differences in hardness, color, and appearance, making it a well-received option. Additionally, partially replacing butter with pumpkin flour significantly reduced the fat content of cookies, with levels decreasing from 37.0% in the control group to 29.7%, 28.25%, and 25.62% in the experimental groups ($p < 0.05$), underscoring its effectiveness as a fat-lowering alternative in baked products.

Pumpkin flour's naturally high carbohydrate and fiber content contributed to increased moisture, ash, and carbohydrate levels, further demonstrating its effectiveness as a fat replacer. In conclusion, pumpkin flour can be effectively incorporated into cookies as a fat replacer, enhancing their nutritional profile without compromising sensory acceptability.

Chapter 1

1.0 Introduction

1.1 Background of Study

Pumpkin is a gourd-like fruit native to tropical and subtropical regions. Pumpkin belongs to the genus *Cucurbita* and family Cucurbitaceae. There are three economically common species, namely, *Cucurbita maxima*, *Cucurbita pepo*, and *Cucurbita moschata*. Their major producers in the world including United States, China, Russia, and India (Hussain et al.,2022). Pumpkins are rich in various natural phytochemicals such as carotenoids, phenolics, vitamins, minerals, polysaccharides, pectins, fibers, essential oils, proteins, peptides, and amino acids (Hussain et al.,2022). Natural pigments found in pumpkins contribute to the appearance, color, and flavor of food products incorporating pumpkin, thereby enhancing their nutritional profile. Due to their nutraceutical and functional food properties, pumpkins are considered versatile food sources.

Pumpkins find application in various food products, including bakery items, pies, jams, soups, and as animal feed. Pumpkin contains 1.27% pectin with a weight of 2.4 kg. Pumpkin contains 84.4% water content, 0.1% fat, 0.9% protein, 0.4% ash, 0.3% crude fiber, and 13.9% carbohydrates (Nidhal et al.,2022). Processing pumpkin waste, comprising peel and seed byproducts, into value-added food products is an area of interest, given their nutrient-rich composition. Utilization of pumpkin peel and seeds, often discarded as waste, holds promise for economic and health benefits. These byproducts are rich in beneficial nutrients such as polysaccharides, pectin, carotenoids, and bioactive compounds. Incorporating pumpkin byproducts into feed and food products can promote health and contribute to waste reduction such as pumpkin flour.

Pumpkin flour is incorporated into various bakery products. It can replace wheat flour in the preparation of bread, cakes, and cookies. The utilization of pumpkin flour as a fat replacer is the primary focus of this study.

1.2 Problem Statement

This study aims to explore the effects of using pumpkin flour as a fat replacer on the physicochemical, nutritional, and sensory properties of cookies. While there is growing interest in healthier baking alternatives, such as pumpkin flour, there is a noticeable gap in thorough research on its impact on cookies specifically.

The key message in the Malaysian Dietary Guidelines 2020 suggests reducing the intake of foods high in fat and limiting saturated fat consumption. According to the NHMS 2019, obese Malaysians make up 19.7% of the population, while those categorized as overweight constitute 30.4% (IPH, 2020). The overconsumption of fat leads to heart-related diseases, obesity, and other non-communicable diseases. Cookies are characterized by high levels of sugar and fat. One approach to reducing fat or calories in cookies is to replace part of the fat content with carbohydrate-based fat substitutes, such as maltodextrins, modified food starches, or polydextrose compounds (Chen et al., 2020). In this study, the carbohydrate-based fat replacer, pumpkin flour, will be used to lower the fat content in cookies. Pumpkin flour contains natural nutrients and minerals that can enhance health compared to other fat replacers. Furthermore, pumpkin is highly available and cost-effective.

The optimal level of pumpkin flour substitution for traditional fat in cookies remains unclear. By systematically examining various substitution levels (10%, 15%, and 20%), this research seeks to fill this gap and understand how these changes affect the quality of cookies.

Through detailed analysis and comparison, this study intends to determine the ideal proportion of pumpkin flour that maintains or enhances cookie quality while meeting consumer preferences. By shedding light on this relationship, the findings of this research can inform the development of healthier cookie formulations that prioritize both nutrition and sensory appeal.

1.3 Research Question

- i. Is there any significant difference in the proximate analysis of cookies incorporated with pumpkin flour as a fat replacer?
- ii. Is there any significant difference in the physical properties of cookies incorporated with pumpkin flour as a fat replacer?
- iii. Is there any significant difference in the sensory evaluation of cookies incorporated with pumpkin flour as a fat replacer?

1.4 Research Objective

1.4.1 General Objective

The objective of this study is to use pumpkin flour as a fat replacer in cookies.

1.4.2 Specific Objective

- i. To produce pumpkin flour as fat replacer for butter in cookies.
- ii. To determine the proximate analysis of cookies incorporated with pumpkin flour as a fat replacer.
- iii. To determine the physical properties of cookies incorporated with pumpkin flour as a fat replacer.
- iv. To assess the sensory evaluation of cookies incorporated with pumpkin flour as a fat replacer.

1.5 Research Hypothesis

1.5.1 Null Hypothesis

H_0 = There is no significant mean difference in physicochemical, nutritional and sensorial properties between control cookies and cookies made from pumpkin flour.

1.5.2 Alternative Hypothesis

H_a = There is significance mean difference in physicochemical, nutritional and sensorial properties between control cookies and cookies made from pumpkin flour.

Chapter 2

2.0 Literature Review

2.1 Cookies

Cookies, made primarily from wheat, have become increasingly popular as a convenient, ready-to-eat, and easily storable snack. They are widely available in various types and are affordable (Krajewska, 2023). Typically, cookies are made from a blend of butter, sugar, and flour, which, while contributing to their desirable texture, also makes them calorie-dense.

A cookie is a type of baked snack or dessert, typically hand-held and made from flour, sugar, eggs, and butter, with additional ingredients such as nuts, chocolate chips, and dried fruits. The texture of cookies can vary, being either crisp or soft, depending on the recipe and preparation methods. The term "cookie" is used differently in various countries. For example, in England and Australia, cookies are referred to as "biscuits," while in Malaysia, they are often called "biskut" or "roti" in some states. Historically, cookies originated as "test cakes" in culinary practices, where a small portion of cake batter was baked to check the oven temperature. The term "cookie" itself is derived from the Dutch word *koekje*, meaning "small cake," and from the Latin *bis coctum*, meaning "twice baked."

Soft cookies often result from an excessive amount of flour, sugar, and butter. Excess flour interacts with the water in the butter to form a gluten network, which increases chewiness as more gluten forms. Sugar competes with flour for available water, making the cookies softer. Additionally, butter functions as a lubricant, enhancing the softness and texture of the cookies (Friday et al., 2016).

2.2 Utilization of Pumpkin Flour

Pumpkin powder is commonly used as a coloring, thickening, or flavoring agent (Hussain et al., 2022). The primary carotenoids found in pumpkin are lutein and β -carotene, which are responsible for the vibrant yellow, orange, and red colors in the

fruits, seeds, and peels of pumpkins (Grassino et al., 2023). When used in cooking, pumpkin powder provides a natural coloring option, appealing to manufacturers aiming to avoid artificial colorings and meet consumer demand for natural ingredients.

In addition to its coloring properties, pumpkin flour is an excellent thickening agent due to its rich fiber content. It helps achieve the desired texture and consistency in food preparations such as soups, sauces, and gravies. Its binding properties also act as stabilizers, preventing ingredients from separating and enhancing the overall stability of the product.

For individuals with gluten sensitivities or those following a gluten-free diet, pumpkin flour serves as a great alternative to traditional wheat flour. It can replace wheat flour partially or entirely in bakery recipes, offering a unique flavor and providing nutritional benefits. Its subtle sweetness enhances a variety of baked goods, including bread, muffins, cookies, and pancakes, while improving moisture retention and prolonging shelf life.

The carotenoid content of pumpkin flour can be increased by including the peel in its preparation. However, removing the pumpkin peel may eliminate some fat, protein, and fiber, thus reducing the nutritional value of the flour (Aziah et al., 2009). Additionally, pumpkin seeds and shells are rich in antioxidant compounds, such as polyphenols, and serve as valuable raw materials for enriching food products with high antioxidant activity (Shevchenko et al., 2022). By utilizing pumpkin seeds and peels, the nutritional value of pumpkin flour can be enhanced, while also reducing food waste.

In a study by Scarton (2021), pumpkin flour was partially substituted for wheat flour in muffins. The results suggested that pumpkin flour could be produced and used as a regional, enriched, and natural-colored ingredient for muffins or other bakery products. Similarly, in the research by Indrianti (2021), the use of pumpkin flour improved the color, antioxidant activity, and texture (stickiness) of flat tuber noodles.

2.3 Benefits of Pumpkin Flour

Pumpkin flour is free from gluten, making it suitable for individuals with gluten sensitivities, celiac disease, or those adhering to a gluten-free diet. A study by Goni et al. (2021) highlights that pumpkin flour is an excellent substitute for wheat flour in gluten-free baking, providing both texture and nutrition without the risk of gluten-related adverse reactions. Substituting traditional wheat flour with pumpkin flour allows individuals to enjoy a diverse range of baked goods, such as cookies, muffins, and bread, without the risk of adverse reactions associated with gluten consumption. This makes pumpkin flour an ideal ingredient for the growing gluten-free market, offering a nutritious, alternative option for individuals with dietary restrictions. (Goni et al., 2021)

Pumpkin flour is abundant in essential minerals, providing a rich source of copper, calcium, potassium, phosphorus, and magnesium (Hussain et al., 2022). These minerals are crucial for various bodily functions, including bone health, muscle function, nerve transmission, and maintaining electrolyte balance. For instance, calcium and magnesium play a key role in bone density and muscle contractions, while potassium aids in regulating blood pressure. Research by Liu et al. (2020) further confirms that pumpkin flour's high mineral content contributes significantly to overall health, as it can help reduce the risk of mineral deficiencies, especially in populations with limited access to other mineral-rich foods. By incorporating pumpkin flour into recipes, individuals can enhance their mineral intake, promoting overall health and well-being, particularly for those who have limited dietary options for meeting their mineral needs. (Liu et al., 2020)

Pumpkins are naturally packed with beta-carotene, a powerful antioxidant that the body converts into vitamin A. Beta-carotene plays a crucial role in neutralizing harmful free radicals, protecting cells from oxidative damage, maintaining healthy vision, supporting immune function, and promoting skin health. According to a study by Williams et al. (2022), pumpkin flour provides an abundant source of beta-carotene, with concentrations that can support the prevention of age-related macular degeneration and enhance skin elasticity. By using pumpkin flour in cooking and baking, individuals can boost their beta-carotene intake, reaping its numerous

health benefits, including improved vision, stronger immune defense, and healthier skin. Additionally, incorporating pumpkin flour into regular meals offers a practical and delicious way to increase antioxidant intake, contributing to the prevention of chronic diseases linked to oxidative stress. (Williams et al., 2022)

2.4 Fat replacer

Many baked products, with the exception of bread (e.g., cakes, cookies, laminated pastries), generally contain high levels of fat in their formulations. These products require different types of bakery fats, which impart specific quality characteristics to the products through their various functionalities (Yazar, 2023). Although fat is essential for the quality of baked goods, strategies have been developed to replace fat in recipes due to the health risks associated with excessive fat intake, such as obesity, diabetes, and cardiovascular diseases.

Fat replacers are widely utilized in the food industry to lower calorie intake by partially or fully replacing the fat content in food products while preserving their desirable sensory and functional qualities (Anand et al., 2022; Saeed et al., 2023). These fat replacers mimic the physical and sensory attributes of fat, enhancing texture, mouthfeel, and overall consumer acceptability. Beyond serving as fat substitutes, they also perform additional roles as stabilizers, thickeners, gelling agents, and emulsifiers. Some fat replacers can further enhance sweetness, color, or texture, making them versatile ingredients in food formulation.

Replacing fat with these alternatives can help curb excessive fat consumption, which is linked to health issues such as obesity, cardiovascular diseases, and metabolic disorders. Fat replacers are derived from a wide array of sources and exhibit diverse sensory, functional, and physiological properties. Based on their chemical composition and function, fat replacers are classified into two primary categories: fat substitutes and fat mimetics (Gao et al., 2024).

Fat substitutes are typically lipid-based compounds that closely replicate the physical and chemical properties of natural fats. They are commonly used in

products where the structural role of fat is essential, such as baked goods and spreads. Fat mimetics, in contrast, are protein- or carbohydrate-based substances that mimic the creamy or moist sensation of fats without necessarily reproducing their chemical properties. Due to their distinct chemical nature, protein- and carbohydrate-based fat replacers cannot replicate fats at the molecular level.

Fat replacers are further categorized based on their composition into protein-based, carbohydrate-based, lipid-based, or complex fat replacers, each with specific properties and applications. Protein-based fat replacers often enhance creamy texture and emulsification, while carbohydrate-based fat replacers, such as starches and gums, contribute viscosity and gelling capabilities. Lipid-based fat replacers, being more similar to fats, offer comparable melting and lubricating properties. However, the selection of fat replacers can significantly influence the sensory characteristics, shelf stability, and appearance of food products.

The use of fat replacers can result in noticeable changes to the structural and sensory characteristics of foods. For instance, reducing fat in solid or semi-solid foods often increases their elasticity and crunchiness, resulting in greater hardness, elasticity, and reduced meltability (Bourouis et al., 2023). These textural modifications can affect the overall acceptability of the product, highlighting the importance of optimizing formulations to balance health benefits with sensory appeal.

2.5 Sensory evaluation

Sensory evaluation is a technique used to assess the development process of food products in the food industry. It helps measure the perception of various food attributes. The key factors to focus on during sensory evaluation include the attributes to be measured, such as taste, aroma, texture, and appearance. Several tests can be conducted in sensory evaluation, including descriptive tests, discriminative tests, scale methods, and affective tests.

Descriptive tests were not utilized in this study as they require trained panelists. Discriminative tests can be used to assess the impact of ingredient changes on

acceptability; however, they are typically used for testing two samples. For example, in the triangle test, panelists are given two identical samples and one different sample, and they must identify the odd one. In the paired comparison test, two samples are provided, and panelists choose the most preferred one.

The scale method is commonly used for consumer testing and measuring acceptance. An example of this is the 7-point hedonic scale. Affective tests, on the other hand, are used to measure consumer preferences and emotional responses to a product. They are particularly suitable for consumer studies aimed at determining market acceptability and preferences.

In this study, the color, aroma, hardness, taste, and overall acceptability of the cookies were measured. According to the research by Fathonah (2020), cookies with higher pumpkin content exhibited a deeper yellow-orange color, indicating a higher beta-carotene content. The cookies with 20% pumpkin flour had the highest beta-carotene content (12,712 μg), while cookies with 15% pumpkin flour achieved the highest sensory quality and acceptability (9,796 μg). The aroma of the cookies is detected by olfactory receptors and can influence their taste perception. The perception of taste often begins with visual evaluation, which determines whether the food appears edible (Aminah, 2000).

The sensory evaluation form was designed to evaluate the cookies' color and appearance, aroma, hardness, taste, and overall acceptability. Panelists first observed the samples visually, then smelled them. Next, they bit into the samples to evaluate hardness, followed by taste evaluation. Finally, they provided an overall acceptability rating.

During the sensory evaluation sessions, five panelists were assessed in different time slots. A master sheet was prepared to organize the panelists into groups. The samples were rearranged before being presented to the panelists using permutation (1–4). Each sample was labeled with a random three-digit number before distribution. This randomization of sample numbers and arrangements aimed to minimize bias during sensory evaluation.

Chapter 3

3.0 Research Methodology

3.1 Study Design

The type of study applied in this research was an experimental design. The cookies were produced with 10%, 15%, and 20% of pumpkin flour as a fat replacer. These cookies were analyzed in terms of physicochemical, nutritional, and sensory properties to determine which cookies were the best. The substitution levels of 10%, 15%, and 20% pumpkin flour were selected based on findings from the Fathonah study that demonstrated these levels as optimal for balancing nutritional enhancement with acceptable sensory and functional properties. Preliminary testing also indicated that substitution levels above 20% significantly affected the texture and flavor of the cookies.

3.2 Preparation and Processing of Raw Material

In this study, the cookies were prepared using ingredients purchased from Mydin Kubang Kerian. The cookies were made in the kitchen of Universiti Sains Malaysia (Health Campus), 16150 Kubang Kerian, Kota Bharu, Kelantan. This location was selected due to its accessible cooking equipment and facilities. The pumpkin dehydration process was carried out in the Functional Food Laboratory.

3.3 Preparation of Pumpkin Flour

1. The pumpkin was washed properly. All parts of the pumpkin were used, not only the flesh but also the seeds and peel.
2. The damaged spots, holes, bruises, or soft spots on the outer surface were removed.
3. The pumpkin was cut into small pieces and placed in the oven at 60°C for 18 hours.
4. The dehydrated pumpkin was ground in a grinding machine at 2500 RPM for 30 seconds.

5. The pumpkin flour was sifted to remove large particles and make the flour nice and fluffy.
6. The flour was stored in a sealed container.



Figure 3.1 : Dehydration of pumpkin

3.4 Preparation of Cookies

3.4.1 Recipe of Cookies.

The recipe was modified from Fathonah et al.,(2020).

In this study , pumpkin flour was used to replace the butter by 10%,15% and 20%

Ingredients (g)	Sample			
	Control	10%	15%	20%
Pumpkin Flour	0	35	52.5	70
Butter	350	315	297.5	280
Wheat Flour	450	450	450	450
Corn Starch	50	50	50	50
Refined Sugar	180	180	180	180
Egg Yolk	32	32	32	32
Baking Powder	5	5	5	5

Table 3.1 Recipe of cookies

3.4.2 Procedure

1. The flour, baking soda, baking powder, and cornstarch were mixed and set aside.
2. Butter and sugars were creamed together until combined.
3. Egg yolk was added and beaten for 1 minute.
4. The dry ingredients were mixed until combined.
5. 6 grams of dough was shaped using a mold and evenly spaced on the prepared cookie sheets.
6. The dough was baked in the oven at 150 degrees Celsius on both upper and bottom flames for 18 minutes. It was taken out when it was just barely starting to turn brown.
7. The cookies were placed on the baking pan for 2 minutes before being removed to a cooling rack.



Figure 3.2 : Cookies doughs

3.5 Proximate Analysis

3.5.1 Moisture

The moisture content in the cookies was determined using the oven method. Firstly, the empty dish was dried in a 105°C oven overnight. After overnight drying, the dish was placed in a desiccator to cool before being weighed. Then, 5g of the homogenized sample was weighed and dried overnight at 105°C in the oven. After drying, the dish containing the sample was placed in the desiccator to cool until a consistent weight was achieved. The moisture content of the sample was calculated as the difference between the constant end weight and the initial weight after drying. The following formula was used to calculate the moisture content:

$$\text{Moisture (\%)} = \frac{w_1 - w_2}{w_1} \times 100$$

where,

w1 = weight (g) of sample before drying

w2 = weight (g) of sample after drying

3.5.2 Ash

The ash content of the cookies was determined using the dry ashing method. Firstly, a shallow crucible was dried for 3 hours in an air oven set to 105°C. After drying, the crucible was cooled in a desiccator, and once it reached room temperature, the weight of the crucible was measured and designated as W1.

Then, 0.500 ± 0.001g of the food sample was weighed into the crucible and labeled as W2. The dried sample was charred on an electric coil heating rack until it stopped smoking.

Next, the crucible was transferred to a cold muffle furnace and heated to 550°C to ash the food sample until it turned pale or greyish. Once ashing was complete, the

crucible was removed, cooled in desiccators, and weighed as soon as it reached room temperature. This weighed crucible was labeled as W3.

The muffle furnace was replaced, and heating continued until a steady weight was achieved. The total ash content of the food sample was calculated using the following formula:

$$\begin{aligned}\% \text{ Ash} &= \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100 \\ &= \frac{(W3-W1)}{(W2-W1)} \times 100\end{aligned}$$

3.5.3 Protein

The protein concentration in the cookies will be determined using the Kjeldahl method, which evaluates the total nitrogen content.

Reagent preparation

Sodium hydroxide, NaOH (40% w/v)

A volumetric flask with a volume of 1000 ml was used to dissolve 400 g of NaOH in 700 ml of distilled water. Subsequently, the mixture was further diluted with distilled water to reach a total volume of 1000 ml.

Boric acid solution (4% w/v)

In a volumetric flask with a capacity of 1000 ml, 40 g of boric acid was dissolved in 700 ml of distilled water. Following this, the solution was diluted to a total volume of 1000 ml with additional distilled water.

Hydrochloric acid, HCL (0.1N)

A 37% HCl solution (8.88 ml) was introduced into a 1000 ml volumetric flask containing 700 ml of distilled water. Subsequently, the mixture was diluted to a total volume of 1000 ml with additional distilled water.

Procedure

Firstly, 1.000 ± 0.001 g of the homogenized sample was precisely weighed into a Kjeldahl digestion flask. Then, two tablets of catalyst were slowly poured into the digestion flask, followed by 20ml of sulfuric acid. For the blank, only catalyst and sulfuric acid were added, without the sample.

The mixture was heated in an inclined position on an electric coil heating rack in a fume cupboard. After the initial frothing subsided, the liquid was heated more vigorously for about 60 minutes at 400°C until it boiled at a moderate rate.

The digestion unit was then turned off once the liquid became clear and colorless. It was left to cool for about 30 minutes or until it reached room temperature. Subsequently, the digestion flask and receiver flask were transferred to the distillation unit.

Steam was passed through the distillation unit for three to four minutes before stopping. The contents of the receiver flask were titrated back to the original purplish color using 0.1N hydrochloric acid from the burette. These procedures were repeated for the blank digest. Finally, the protein content was calculated using the following formula:

$$\% \text{ Protein} = \frac{(\text{ml HCL} - \text{ml HCL blank}) \times 14.008 \times 0.1 \text{NHCL} \times \text{protein factor}}{\text{Weight in mg of the sample}} \times 100$$

3.5.4 Fat

The Soxhlet method was used to determine the fat content in the cookies. In a dry thimble, 3.000 ± 0.0001 g of the sample was weighed. The thimble was subsequently inserted into the extraction equipment. The extract collection jar, which had been dried to a constant weight and designated as W1, was weighed and incorporated into the extraction equipment. Petroleum ether (80ml) was added as the solvent. The extraction unit was closed, and the cooling water flow and heating were initiated. When the solvent began to boil, the thimble was submerged by moving the slider to the "Immersion" position. After 30 minutes, the thimble was extracted from the solvent by moving the slider to the "Washing" position. Following another 30 minutes of reflux washing, the stopcock under the water-cooled condenser was closed, and the solvent evaporated entirely from the extraction vessel. The extract collecting vessel was then placed in an oven for 45 minutes at a high temperature to completely evaporate the solvent residues. Finally, the extract collecting vessel was cooled in a desiccator, weighed, and labeled as W2. The final fat content of the food sample was estimated using the following formula:

$$\begin{aligned}\% \text{ Fat} &= \frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} \times 100 \\ &= \frac{(W2-W1)}{\text{Weight of sample (g)}} \times 100\end{aligned}$$

3.5.5 Carbohydrate

To obtain the carbohydrate content in cookies, we use $100\% - (\text{Fat percentage, ash percentage, protein percentage and moisture})$

3.5.6 Determination of Calorie

The samples were compressed using a pelleting press. Exactly 1.000 ± 0.001 g of the tablet was weighed directly into a pre-dried crucible. To access the decomposition vessel, the union nut was unscrewed, and the cover with the handle was removed. A cotton thread was attached to the filament, with its ends placed in the crucible to act as a fuse. The length of the thread remained consistent for each burn to ensure uniform heat production, and the combustion heat of the thread was excluded from the calculations.

The crucible was then placed on the crucible holder, and the lid was pressed onto the lower section until it contacted the stop piece. The union nut was manually tightened to secure the setup.

To begin the measurement, the decomposition vessel was inserted into the filler head of the open measuring cell cover and locked into place. The process was initiated by pressing the Start button. Upon completion or termination of the measurement, the measuring cell cover was opened, and the contents of the inner vessel were drained. When the message "Bomb increase arrow" appeared, the decomposition vessel was promptly removed.

Pressure within the decomposition vessel was carefully relieved using a laboratory discharge hood equipped with a venting button. A small valve was then opened to equalize the internal and external pressures. Afterward, the chamber was unlocked, and the crucible and combustion chamber were examined for any signs of incomplete combustion. If unburned organic material was detected, the experiment was repeated.

The combustion chamber's top was cooled with running water and dried to prepare it for subsequent measurements. The results, originally recorded in calories (Cal), were converted into kilocalories per 100 grams (kcal/100g) using the formula:

$$kcal/100g = \frac{cal}{1000} \times 100g$$

This ensured the data was presented in the desired unit of kcal/100g.

3.6 Physicochemical Analysis

3.6.1 Texture Profile Analysis

Texture is a key quality attribute of cookies, and the texture of the cookies was evaluated using a Texture Analyzer TA.XTplus. The analyzer was equipped with the Texture Exponent Software package and the necessary accessories for the analysis.

Cookies samples were prepared, baked, and keep in a seal container on the day to testing. Before beginning the evaluation, the probe was calibrated following the manufacturer's guidelines. The cookies was carefully positioned center beneath the probe to ensure same part of the cookies tested in order to make accurate comparisons.

A 2mm cylinder probe, fitted with a 5 kg load cell and heavy duty platform with a hole plate were employed to measure the cookies' hardness and fracturability.

Table 3.2 : Operating setting for Texture Profile Analysis (TPA)

Test mode	Compression
Mode	Measure force to compression
Option	Return to start
Pre-test speed	1.0 mm/s
Test speed	0.5 mm/s
Post-test speed	10.0 mm/s
Distance	2 mm
Trigger force	Auto - 5g
Tare mode	Auto
Data acquisition rate	400 pps

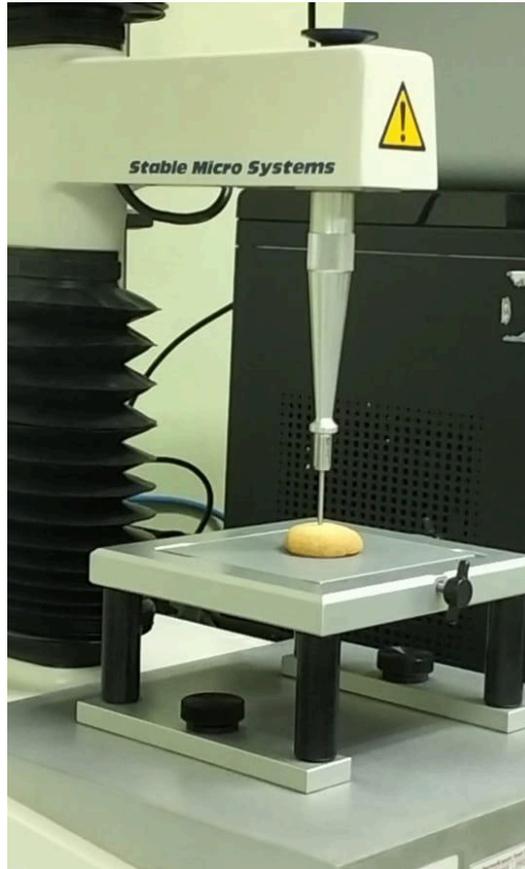


Figure 3.3 : TPA on cookies

3.6.2 Physical Properties

The physical properties (weight, colour, diameter, thickness, and spread ratio) of the cookies were tested. Four replicates for each sample were measured for weight, diameter, and height (thickness). The colour of the cookies was measured using a colorimeter. The spread ratio was calculated as diameter/height (Besaar et al., 2008). The method of Zoulias et al. (2002) was used for the measurement of biscuit diameter.

Diameter

The diameter of the cookies obtained by:

- 1) Placing 4 cookies adjacent in a line.
- 2) Measure the first total length of the cookies, l. Record the data.
- 3) Rotate the cookies 90⁰ and measure the second total length, L. Record the data.
- 4) Calculate the average diameter of the cookies using the formula:

$$\text{average diameter of cookies} = \frac{(l+L)/2}{4}$$

Colorimeter

Using a colorimeter for cookie analysis involves a systematic method to measure and interpret their color. After baking the cookies according to a standardized recipe, they are cooled to room temperature. The colorimeter is calibrated using a reference standard to ensure accuracy. Cookie samples are then placed in the instrument's sample holder, and the measurement function is activated. The device provides numerical data for color parameters, such as lightness (L*), redness (a*), and yellowness (b*) (Wrolstad et al., 2017). These values are analyzed to evaluate the cookies' quality, including browning levels and color uniformity.

For this study, the color intensity of both the top surface and the surface in contact with the baking tray was measured using the colorimeter. The instrument was calibrated with a white tile before obtaining the L*, a*, and b* values. Triplicate readings were taken for each cookie formulation to ensure reliability and accuracy. Comparing these measurements across batches or formulations provides insights into ingredient variations and baking conditions.

3.7 Sensory Evaluation

Sensory evaluations will be undertaken using a 7-point hedonic scale, with higher scores indicating greater acceptance (1, dislike very much, and 7, like very much) (Aminah , 2000). Sensory attributes such as appearance, aroma, taste, texture, colour and overall acceptability will be evaluated. Each product will be reviewed independently by 50 healthy panellists who will participate as volunteers and score each product based on their likeness. Staff and students from the School of Health Sciences at Universiti Sains Malaysia will be chosen at random as volunteers based on their willingness to participate in the test. The duration of each participant to conduct this sensory evaluation is about 15-20 minutes. Before the sensory evaluation session, the selected participants will be given a consent form and a briefing.

3.7.1 Inclusion criteria

- Undergraduate students of health campus USM/staff of health campus USM
- Year 1 until year 5 students
- Available in campus

3.7.2 Exclusion criteria

- Allergic to eggs and other ingredients in cookies
- Sick or ill that can interfere with ability to taste and smell

In case that any volunteers experience symptoms or an allergic response subsequent to testing the duck sausage, they will promptly be directed to the medical team stationed in the emergency department of USM hospital. The sensory evaluation performance was authorised by the USM Human Ethics Committee (USM/JEPeM/KK/24060508) on July 14, 2024.

3.8 Data Analysis

All collected data were organized systematically in a Microsoft Excel table for efficient data management. Subsequently, the data were analyzed using IBM SPSS Statistics, version 29. A One-Way Analysis of Variance (ANOVA) was performed, followed by Duncan's post-hoc test for multiple mean comparisons. Cookies with varying levels of pumpkin flour substitution as a fat replacer were produced and analyzed in this study to evaluate their proximate composition, physicochemical properties, and sensory attributes. The results were presented as mean values with standard deviations (SD) based on four replicates, except for the sensory evaluation, which included 50 participants (n=50). The significance level was set at $p < 0.05$.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Proximate Analysis

Result of proximate composition presented in Table 4.1. The result showed that there was significant difference ($p < 0.05$) of moisture, ash, fat, protein and carbohydrate between cookies with different percentage of pumpkin flour as fat replacer.

The moisture content of cookies showed a significant difference ($p < 0.05$) among the control, 10%, 15%, and 20% levels of pumpkin flour used as a fat replacer. The cookies with 20% pumpkin flour had the highest moisture content (2.59%) compared to the control (1.07%), 10% (1.63%), and 15% (1.67%). This difference can be attributed to the gelatinization process of wheat and pumpkin flour (Dwiloka, 2019).

Gelatinization occurs when starch granules transition from a crystalline to an expanded state through heating and water absorption. Two key starch components influence this process: amylose and amylopectin. Amylose strengthens the gel structure, while amylopectin absorbs water and facilitates the expansion of starch granules. Amylopectin draws water from the raw ingredients and the surrounding air, trapping it within the granules and thereby increasing the moisture content in the cookies. Additionally, the fiber content in pumpkin flour binds water, helping the cookies retain more moisture.

The ash content of cookies showed a significant difference ($p < 0.05$) among the control, 10%, 15%, and 20% levels of pumpkin flour used as a fat replacer. Cookies with 20% pumpkin flour had the highest ash content (1.07%) compared to the control (0.82%), 10% (0.90%), and 15% (0.98%). Ash content represents the total mineral content in a product. The addition of pumpkin flour influenced the ash content of the cookies, as it contains essential minerals such as calcium, magnesium, potassium, sodium, phosphorus, and trace elements like zinc and iron. The fiber in pumpkin