EFFECT OF DRYING PROCESS ON FUNCTIONAL PROPERTIES OF CHICKEN BREAST POWDER AND APPLICATION IN HIGH PROTEIN PANCAKE

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ผลของกระบวนการอบแห้งต่อสมบัติเชิงหน้าที่ของผงอกไก่และการประยุกต์ในแพนเค้กโปรตีนสูง

นายเกียรติ์ขจร ทัศนาสุทธิวงษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Field of Study	Food Science and Technology
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เกียรติ์ขจร ทัศนาสุทธิวงษ์ : ผลของกระบวนการอบแห้งต่อสมบัติเชิงหน้าที่ของผงอกไก่และการประยุกต์ในแพนเค้กโปรตีนสูง. (EFFECT OF DRYING PROCESS ON FUNCTIONAL PROPERTIES OF CHICKEN BREAST POWDER AND APPLICATION IN HIGH PROTEIN PANCAKE) อ.ที่ปรึกษาหลัก : รศ. ดร.ชาลีดา บรมพิชัยชาติกุล

กระบวนการอบแห้งเป็นหนึ่งในหลายวิธีที่ช่วยในเรื่องของการถนอมอาหาร แต่ถึงอย่างนั้น วิธีการอบแห้งที่ต่างกัน มีการเงื่อนไขและเทคนิคในการอบแห้งที่แตกต่างกัน ส่งผลต่อคุณภาพและสมบัติเชิงหน้าที่ของผลิตภัณฑ์อบแห้งได้ ผงอกไก่เป็นหนึ่งในผงโปรตีนทางเลือกที่เป็นแหล่งโปรตีนที่ดีสามารถใช้แทนผงเวย์ได้ โดยเฉพาะกลุ่มคนที่ไม่สามารถย่อยน้ำตาลแลคโตส โดยเฉพาะกลุ่มคนเอเชีย ในการศึกษานี้มีวัตถุประสงค์เพื่อเตรียมผงอกไก่จากเนื้ออกไก่ลอกหนัง และศึกษาสมบัติทางเคมีกายภาพ สมบัติเชิงหน้าที่ของผงอกไก่ รวมถึงการประยุกต์ผงอกไกใช้ในแพนเค้ก เมื่อเทียบกับการใช้ผงเวย์ เพื่อพัฒนาสมบัติทางกายภาพของแพนเค้ก ในการทดลองศึกษาผงอกไก่ โดยการใช้เครื่องอบแห้ง 3 ชนิด ได้แก่ เครื่องอบแห้งด้วยลมร้อน เครื่องอบแห้งด้วยสุญญากาศ และ เครื่องอบแห้งด้วยไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำ โดยคงอุณหภูมิในการอบแห้งที่ 65°C เท่ากันทุกวิธีการอบแห้ง ผลการทดลองพบว่าผงอกไก่แบบอบเครื่องอบแห้งด้วยสุญญากาศ ใช้ระยะเวลาที่สั้นที่สุด (4 ชั่วโมง) เมื่อเทียบกับการอบด้วยเครื่องอบแห้งด้วยไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำ ใช้ระยะเวลาที่ยาวที่สุด (7 ชั่วโมง) เนื่องจากการใช้ไอน้ำร้อนยิ่งยวดในระหว่างการอบแห้ง เกิดการควบแน่นของไอน้ำที่ผิวของตัวอย่าง น้ำถูกดึงกลับไปในตัวอย่าง ทำให้ใช้เวลาที่นานขึ้นในการอบแห้ง ส่งผลให้ตัวอย่างผงอกไก่ Low-pressure superheated steam dried chicken breast powder (CBL) นี้มีปริมาณค่าความชื้น (5.58±0.01) และค่ากิจกรรมของน้ำ (0.427±0.002) ที่สูงกว่าตัวอย่างอื่นอย่างมีนัยสำคัญ ซึ่งค่าความชื้นนี้ส่งผลต่อร้อยละโปรตีนในตัวอย่าง ในเรื่องของสมบัติเชิงหน้าที่ของผงอกไก่ ผลการทดลองพบว่าผงอกไก่มีความสามารถในการดูดซับน้ำและความสามารถในการดูดซับน้ำมัน ในปริมาณที่สูงกว่าผงเวย์อย่างมีนัยสำคัญ เนื่องจากโครงสร้างที่มีรูพรุนและขนาดของผงที่ใหญ่กว่า ตัวอย่าง Chicken breast powder (CB) ทั้งหมดมีดัชนีความสามารถในการละลายน้ำต่ำกว่า ดูดความชื้นต่ำกว่า และความสามารถในการละลายโปรตีนต่ำกว่าเวย์ สำหรับความสามารถในการทำให้เกิดฟอง ตัวอย่าง Chicken breast powder (CB) ทั้งหมดมีความสามารถในการทำให้เกิดฟองต่ำมาก ในขณะที่ผงเวย์แสดงความสามารถในการทำให้เกิดฟองสูงสุดและความเสถียรที่ pH เป็นกลาง ในทำนองเดียวกัน สำหรับความสามารถในการทำให้เป็นอิมัลชันและความคงตัว ตัวอย่าง CB ทั้งหมดแสดงค่าต่ำมากอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับผงเวย์ แต่ที่ค่า pH สูง ความสามารถในการทำอิมัลชันก็จะยิ่งสูงขึ้น สำหรับสูตรและการเตรียมแพนเค้ก Vacuum dried chicken breast powder (CBV) ได้รับเลือกให้แทนที่เวย์ผง 10 % ถึง 20 % ของส่วนผสมแพนเค้ก ความหนืดสูงสุดที่การทดแทนได้แก่ CB 20% เนื่องจากความสามารถในการดูดซับน้ำสูงและความสามารถในการดูดซับน้ำมันสูง การวิเคราะห์ลักษณะพื้นผิวของแพนเค้กแสดงให้เห็นว่าความเหนียวของแพนเค้กยังคงอยู่แม้จะแทนที่ CB ที่ 20% อย่างไรก็ตามพบว่ามีค่าความแข็งสูงกว่ากลุ่มควบคุมที่มีแป้งสาลีเพียงอย่างเดียวซึ่งไม่มีการแทนที่ผงโปรตีนใดๆ อย่างมีนัยสำคัญ แต่มีค่าความสปริงตัวที่ต่ำกว่าอย่างมีนัยสำคัญ ด้วยเหตุนี้ ข้อมูลที่ได้จากการศึกษานี้จึงมีความสำคัญต่อการพัฒนาผลิตภัณฑ์อกไก่แบบผงและการประยุกต์ใช้ในผลิตภัณฑ์อาหารที่มีศักยภาพต่อไป

สาขาวิชา	วิทยาศาสตร์และเทคโนโลยีทางอาหาร	ลายมือชื่อนิสิต .....
ปีการศึกษา	2564	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KEYWORD: CHICKEN BREAST POWDER, DRYING PROCESS, WHEY SUBSTITUTE, PROTEIN PANCAKE

Kiatkhajorn Thassanasuttiwong : EFFECT OF DRYING PROCESS ON FUNCTIONAL PROPERTIES OF CHICKEN BREAST POWDER AND APPLICATION IN HIGH PROTEIN PANCAKE. Advisor: Assoc. Prof. CHALEEDA BOROMPICHAICHARTKUL, Ph.D.

Drying is one of food preservative methods. However, several drying methods are applied using different drying conditions and techniques that could affect the quality and functional properties of dried products. Chicken breast powder is an upcoming alternative high protein source to replace the consumption of whey protein powder, especially Asian people who are lactose intolerant. Therefore, this presentation aimed to prepare and compare three types of dried chicken breast powder produced from different drying methods. Physical properties of low lactose and protein rich pancakes using chicken breast powder were then determined. There are three drying methods all operated at 65 °C to prepare chicken breast powder including conventional hot air drying, vacuum drying and low pressure superheated steam drying. For the result, in terms of drying time, the vacuum drying method required the shortest drying time (4 hours) compared to others, whereas, low-pressure superheated steam drying took longer drying time (7 hours) since the superheated steam continuously supplied during drying, so there is condensation on surface of product and caused moisture reuptake. In addition, low pressure superheated steam dried chicken breast powder (CBL) showed significantly higher moisture content (5.58±0.01) and higher water activity (0.427±0.002) than conventional hot air dried (CBH) and vacuum dried chicken breast powders (CBV) due to the steam involved in drying process, which also affect protein content. Furthermore, for functional properties, all CB samples have significantly higher water absorption capacity and oil absorption capacity than whey powder due to porous structure and larger particle size. Hence, all CB samples exhibited lower water solubility index, lower hygroscopicity, and definitely lower protein solubility than whey. However, for foaming capacity, all CB samples exhibited significantly very low foaming capacity, whereas whey powder showed highest foaming capacity and stability at neutral pH. Likewise, for emulsification capacity and stability, all CB samples showed significantly very low compared to whey powder. The higher the pH, the higher emulsifying capacity. For pancake formulation and preparation, CBV was chosen to replace whey powder 10 % to 20 % of pancake mix. The viscosity is highest at 20 % CB substitution due to its high water absorption capacity and oil absorption capacity. Texture profile analysis of the pancakes demonstrated that cohesiveness of the pancake is maintained even with the substitution of CB at 20%. Nevertheless, they showed significantly higher value of hardness than the control which contains only wheat flour with no substitution of any protein powder, but exhibited significantly lower value of springiness than both control and protein pancake formulation 1 (PP-1) that contains only addition of whey powder. As a result, the data obtained from this study could be crucial for further development of the chicken breast powder and application in potential food products.

Field of Study: Food Science and Technology Student's Signature .....

Academic Year: 2021 Advisor's Signature .....

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Chapter I

1.1 Introduction

Since nowadays there are many protein sources including animal protein and plant protein sources, the former tends to have a more proper amount of essential amino acids and is easier to be digested than the latter. Although one of the most popular protein powders is whey protein powder, there are still some people who are lactose-intolerant and allergic to certain whey protein. Furthermore, whey protein concentrates could also cause an increase in blood sugar level due to the fact that lactose sugar can be broken into glucose and galactose by the enzyme lactase, and also, they are usually applied in most desserts and beverages including pancake, ice cream and protein shake. Also, in terms of regulation, there is no specific level of lactose claimed as "low-lactose" product; however, there is the common threshold level for low-lactose product varying between countries which is 1 gram of lactose per 100 grams of the final product, so this could be considered and claimed as low-lactose product (Yang et al, 2019). On the other hand, according to Thai FDA, to be claimed as "high-protein" product, the food must contain 20% or more of the recommended daily intake (RDI) of protein ((No. 182) B.E. 2541 (1998) Re: Nutrition Labeling). Thus, an alternative animal protein source is recommended to be high protein, low fat, low lactose content along with high availability, quality and low cost. To clarify alternative protein sources, in terms of the availability and quality, chicken is the most commonly found in mass production in the poultry market with its high protein score of 0.95 (Boye et al., 2012). Additionally, for the cost, it also is the cheapest white meat compared to other types of white meat including turkey, goose and duck and also red meat including pork and beef. Furthermore, chicken breast is the chicken part that has lower cholesterol, lower saturated fats and higher protein content compared with other chicken edible components which are leg and wing. Hence, chicken breast has gained more popularity and interest to consumers for weight loss and muscle gain, especially those who have the problems of consuming whey protein powder. It is also high in antioxidant capacity due to histidyl dipeptides including carnosine and anserine in Thai indigenous chickens, low cost ranging 40 to

60 per kilogram, low risk of cardiovascular disease compared with red meat including pork and beef. Nevertheless, there are some limitations of chicken including strong chicken flavor, tough texture of lean meat, hormones, antibiotics and also high content of uric acid due to high consumption of chicken, especially in people with gout. Also, the drying process also definitely extends the shelf life of the product and provides convenience to consumers from a transport perspective with higher percent of protein content and less total weight. Therefore, it is crucial to compare different drying methods and investigate the effect on functional properties of chicken breast.

The United Nations (2017) reported that there are over 821 millions of people around the world who are undernourished. Thus, we need to reduce this huge number as suggested by UN Sustainable Development Goal number 2, which is Zero hunger. Moreover, one of the most crucial factors of hunger is food price because some people cannot afford it. To clarify, Natsios (2018) reported that it takes approximately 4 months to ship food aid and the shipping cost contributes more than 40 percent of the total cost. Most of the people could not afford the food cost after it surpluses in the market. Hence, if we can reduce the shipping cost by reducing the shipping food weight, this could help lower overall food price. In order to help solve this problem, one of the basic processing steps to reduce the weight of food is dehydration or drying into the dried product since drying can lower moisture content. Hence, this can also help lower the rate of quality degradation and longer shelf life. Product development needs to be developed suitable methods for the food products to have good physicochemical and functional properties.

Therefore, the main aim of my thesis is to prepare and compare three types of dried chicken breast powder including conventional hot air drying, vacuum drying, and low pressure superheated steam drying, and also to determine their proximate composition and functionalities including water holding capacity, oil absorption capacity, water solubility index, hygroscopicity, protein solubility, foaming capacity and stability, and emulsifying capacity and stability.

1.2 Objectives

- To prepare and compare three types of dried chicken breast powder including conventional hot air drying, vacuum drying, and low pressure superheated steam drying.
- To determine their proximate composition and functionalities including water holding capacity, oil absorption capacity, water solubility index, hygroscopicity, protein solubility, foaming capacity and stability, and emulsifying capacity and stability.
- To formulate a low lactose and protein rich pancake mix by substituting whey powder with chicken breast powder as an alternative protein source.
- To determine physical properties of low lactose and protein rich pancakes using chicken breast powder.

1.3 Expected output

The obtained data and information in this study could be crucial for further development for the use of chicken breast powder as a high alternative protein source by its functional properties as it can substitute whey powder in certain food product for lactose intolerant people; for instance, the application of chicken breast powder in pancake could be used to lower lactose content and improve physical and textural properties of pancake.

Chapter II

LITERATURE REVIEW

2.1 Nutrition fact of chicken breast

Based on the nutrition, fresh chicken breast will be the best part since its white meat contains slightly less cholesterol than the dark meat which are legs and wings. Therefore, for the boneless skinless breast, it will definitely have low saturated fats with a rich source of protein. According to the USDA, the nutrition fact of chicken breast of the average 4-ounce raw boneless skinless chicken breast contains approximately 110 calories, 26 grams of protein, 1 gram of fat, 75 milligrams of cholesterol, and 85 milligrams of sodium (MasterClass, 2019). Thus, it is a good source of protein that is low in fat and sodium; however, with the skin on the chicken breast, the fat content will raise up to 9.3 grams of total fat, resulting in higher calories. Since there is no carbohydrate in chicken breast, it can be considered as low-carb food, so it is commonly used to incorporate into a healthy diet to ensure the recommended daily intake of protein.

2.2 Powdered food product and benefits

Due to globalization and world change, people need something that makes their life more convenient and comfortable. Similar to the concept of medical pills or supplements that are in the form of tablet or capsule in order to keep the active ingredient stable in the solid form and improve digestibility and absorption, food also needs to be in a dry form as powder not only feature convenience, complete nutrition with the compact form, but also help extend the shelf-life of the product since the moisture content of powder is low and the water activity of the product is not enough for microorganisms to utilize and grow in those food matrix as the powder form. Furthermore, it can also help in saving the time to prepare a particular food during preparation and cooking steps, saving money of raw material and avoiding food waste, especially the leftover food on the plate. Therefore, it is quite

amazing and crucial to understand how powdered food products can benefit our life with convenience by replacing the normal role of food dish in our daily consumption to the basic innovation that can be a sustainable future of food.

2.3 Processing of chicken powder

Basically, chicken powder can be done in many processes. As shown in Figure 2.1 (Ilansuriyan et al., 2015), chicken lean meat is chopped and minced into tiny pieces of meat. The minced meat is then cooked at a high temperature to eliminate pathogens, especially by contamination of Salmonella spp. Which can be normally found in the poultry industry. In addition to microbial count reduction, cooking can also help to stop the enzymatic activity in the chicken meat. After that, the cooked meat was dried in the hot air oven to remove the water out of the sample, resulting in less moisture content and water activity of the final product. Then, the dried meat was ground by grinder until the fine powder of chicken meat was obtained. Additionally, the ground meat powder was sieved using a sieve in order to remove any contaminant, coarse and large particle size of powder or even foreign material. Lastly, it kept the air tight packaging to prevent the powder from humidity and oxygen which can affect the quality of the chicken powder and its shelf-life.

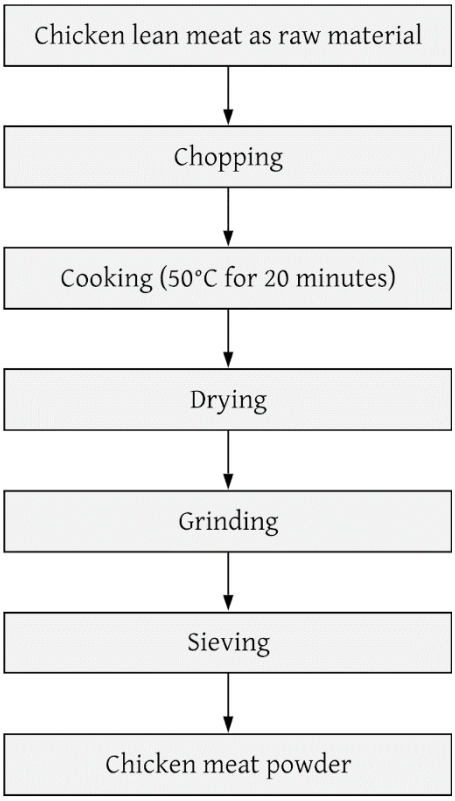

Figure 2.1 Flow chart for production of chicken drumstick powder

2.4 Effect of different drying methods on physicochemical properties of chicken powder

For the chicken breast powder, there is no study on the effect of different drying methods on the properties of the chicken breast powder. However, there is only the manufacturing of chicken powder in other parts of chicken including broiler meat (Aslam et al., 2000), chicken drumstick (Ilansuriyan et al., 2015), chicken skin (Wan Omar & Sarbon, 2016), or even chicken with the addition of herbs such as basil (Xu et al., 2021). For instance, in terms of normal broiler meat, according to Aslam et al (2000), the moisture content of chicken meat is found to be approximately 3.5 %, while the protein content is about 81% and the fat content is about 10.5%. Thus, the fat content is quite high due to the high fat content in the skin of chicken. Hence, to select one part of the chicken, according to Ilansuriyan et al (2015), they selected the chicken drumstick as the part to manufacture chicken drumstick powder

and compare them by ranging the drying temperature of 70 ℃, 75 ℃ and 80 ℃. However, the result showed that the high drying temperature of 80 ℃ could yield the high colony forming unit over 300 cfu, so it would be better to keep the operating drying temperature lower than 80 ℃ but extend longer drying time. Furthermore, for the part of chicken that contains fat, according to Wan Omar & Sarbon (2016), chicken skin can be produced into chicken skin gelatin hydrolysate. To elaborate, they focused on the effect of different drying including vacuum drying and freeze drying of chicken skin hydrolysate on its functional properties, they found out that vacuum dried chicken skin gelatin hydrolysate exhibited very low water holding capacity ranging from 8.4 to 35.1 mg/ml, whereas freeze dried chicken skin gelatin hydrolysate showed an improved water holding capacity from 27.9 to 63.7 mg/ml. Although they showed low water holding capacity and oil holding capacity, they possessed higher solubility, foaming capacity and emulsifying capacity since it is a hydrolysate which is the powder that is easily reconstituted or soluble in water. Lastly, for chicken with the addition of herbs, according to Xu et al (2021), they produced basil added chicken powder with three different drying methods including hot air drying, microwave vacuum drying and radio frequency drying, and determined functional properties and antioxidant activities. The results reported that all dried basil added chicken powder exhibited a similar trend with the decrease in solubility and increase in hygroscopicity over a period of time. For the DPPH assay, they exhibited the DPPH inhibition of 60 - 68 %, and the microwave vacuum drying showed the best antioxidant properties among all samples since it required the shortest drying time and operated under vacuum.

2.5 The role of food proteins on functional properties

To begin with, different food biopolymers have different roles on functional properties in the same food matrix. Functional properties of foods can be affected by concentration, configuration, size and polydispersity of a certain biopolymer of interest; thus, it is very critical and significant to understand those factors and how they affect the functional properties of the overall food product. To clarify, proteins are one of the food biopolymers that become more popular to be used as food ingredients due to its advantages of helping in weight loss and muscle mass gain. However, the food processing and food product formulations are very crucial and critical for improving functional properties of protein. The functional properties of protein include solubility, water absorption, oil absorption, foaming, and emulsification. The better functional properties, the more usage or application the protein will be.

To exemplify functional properties, they include water holding capacity, oil absorption capacity, water solubility index, hygroscopicity, protein solubility, foaming capacity and stability, and emulsifying capacity and stability. Usually, the proteins that were used to determine the functional properties are plant-based proteins rather than the animal-based protein. Some studies on quinoa protein isolate (Elsohaimy et al., 2015), mung bean (*Vigna radiata*) protein isolate (Brishti et al., 2020), pea protein isolate (Choe et al., 2022), soy protein (Peng et al., 2022), insect proteins (Mishyna et al., 2021), whey protein concentrate powder (Ho et al., 2021) were conducted to determine the functional properties of protein powder. According to Elsohaimy et al. (2015), the functional properties of quinoa protein isolate are affected by pH. For example, in terms of protein solubility, there was a higher protein solubility of quinoa protein isolate at stronger alkaline conditions. To clarify, the maximum protein solubility of quinoa protein isolate was observed at pH 10, whereas the minimum protein solubility was found to be in more acidic condition at pH 4. Therefore, this could exhibit that protein solubility of quinoa protein is pH-dependent. Likewise, according to Brishti et al (2020), mung bean protein also showed minimum protein solubility at pH 4.5, which could refer to the isoelectric

point of the protein and could be from the moisture resistant film by the unfolding of protein during drying. Moreover, in the study, at higher pH values of 12, there was a higher protein solubility due to the fact that there was the aggregation of water-soluble particles. Furthermore, the higher solubility of powder will result in the higher foaming capacity and emulsifying capacity since there will be more soluble protein that can be better dispersed in the solution or emulsion than the insoluble protein that could precipitate as the pellet. Similar to solubility, the higher pH will result in the higher foaming capacity and emulsification capacity since there will be more water-soluble particles to bind or aggregates to form foam or emulsion, respectively. On the other hand, the higher solubility of powder will result in the lower water holding capacity and oil absorption capacity since there will be more protein particles in the supernatant than the insoluble protein in the pellet.

When compare functional properties between animal-based protein and plant-based protein, whey protein could result in high solubility, high foaming capacity, and high emulsifying capacity as most of the plant-based protein including quinoa protein isolate, mung bean (*Vigna radiata*) protein isolate, pea protein isolate, soy protein, and etc. For instance, according to Ho et al (2021), whey protein powder has high solubility approximately up to 99 % which is quite high compared to the solubility of quinoa protein isolate which is about 75 % (Elsohaimy et al., 2015), mung bean (*Vigna radiata*) protein isolate which is up to 95 % depending on type of drying method and pH (Brishti et al., 2020), pea protein isolate which is about 98% (Choe et al., 2022), and soy protein which is up to 88 % depending on the pH and calcium content (Peng et al., 2022). Nevertheless, when comparing whey protein powder to other animal-based protein powder, it showed different results of functional properties. For example, according to Mishyna et al (2021), insect proteins exhibited very low protein solubility including cricket protein (about 28 %) and mealworm protein (about 23 %) which is significantly lower than the solubility of whey protein that is more than 90 %. On the contrary, the water absorption capacity and oil holding capacity are quite high, which are approximately 1.7 g/g and 1.4 g/g, respectively. The reason behind could be that the insect proteins could be referred to as intact protein which could have more porous, bigger particle size and higher

level of structural proteins including tertiary and quaternary protein structure that have a bigger void for the water and oil to hold inside. In contrast, whey protein powder is often processed by spray drying method that could be referred to as hydrolyzed protein which could have smaller particle size and lower level of structural proteins, mainly secondary structure of proteins including about 50 % of β-lactoglobulin and approximately 20 % of α-lactalbumin (Qi & Onwulata, 2011). that are better solubilized in the water than the insect proteins that could have insoluble fiber such as chitin in the powder as well (Ojha et al., 2021).

2.6 Factors affecting functional properties of food proteins

Basically, Haard (2001) reported that functional properties of food biopolymers mostly depend on 2 main aspects including the spatial structure of molecules and the state of association of them. For instance, in terms of the spatial structure of molecules, the partial denaturation of the protein molecule can increase the dispersion velocity due to the more molecular dimensions caused by unfolding of the protein. In addition, for the state of association of molecules, it can be the association between each single molecule or with other molecules; for example, the molecule aggregations of denatured proteins can lead to an increase of viscosity due to the higher number of smaller molecules aggregating themselves and occluding water. Furthermore, factors affecting the structure and functional properties of the food macromolecules involve the composition of the medium and the processes which alter the medium. For the composition of the medium, this can include the water content, the presence of other molecules, pH and also ionic strength. For the processes altering the medium, this can be both physical or chemical processes such as drying, heating, cooling, mechanical actions, the use of surfactants or even enzymatic modifications.

In terms of protein biopolymers, the majority of the proteins stabilized with weak bonds including hydrogen bonds can be simply degraded by chemical process or heat treatments. Mirhosseini (2012) reported that this is because the weak non-covalent bonds including hydrogen bonds, ionic bonds, and Van der Waals

attractions among many different parts of the polypeptide chain just stabilize the folded protein structure; on the other hand, strong covalent bonds are the significant bonds that link the amino acids together in the unfolded backbone structure. Thus, after applying heat to denature the protein, the proteins become unfolded because the non-covalent bonds become weaker and eventually degrade, resulting in a more flexible protein structure. Although some of them are not becoming totally unfolded, they become partially unfolded, causing the exposure of internal hydrophobic fractions due to heating processes. As a result, this is the main reason why the structure of the protein is affected directly by the drying processes.

2.7 Different types of drying methods

Vacuum drying is another drying method that is used to alleviate the drawbacks of the conventional drying method by providing higher drying rate at lower drying temperature. Jha reported that the drying temperature of vacuum drying can range from 30°C to 60°C, depending on the vacuum pressure used (Jha et al., 2016). The aim of this drying technique is to remove moisture under vacuum; thus, it operates at a lower drying temperature in an oxygen-deficient processing environment, resulting in reducing the chance of oxidation reactions in the food during the drying process. Moreover, Jiang reported that vacuum drying operated at lower drying temperature, so it could help to reduce thermal stresses and the problem of over-drying (Jiang et al., 2018). As a result, it is crucial and suitable for both thermolabile materials and oxygen sensitive food in order to enhance the quality and maintain nutritional value of those particular products. Principally, in terms of processing of vacuum drying, the surface of the vacuum dried product is heated mostly by conduction and radiation, since there is less convection in the vacuum atmosphere. To clarify, the boiling point of water is decreased due to the higher vacuum pressure, resulting in more effective hydraulic conductivity of the food matrix, less the resistance of mass transfer at the food surface, higher rate of evaporation, and less drying time. To exemplify, the use of vacuum drying at 25°C can dry the food product three times faster than the conventional method drying

with warm air at 30°C and humidity at 50%. In terms of production costs, Parikh reported that the vacuum drying method is cheaper than freeze drying and spray drying methods due to its less energy requirements (Parikh, 2015). Thus, the microwave vacuum drying is developed for extended application.

Low pressure superheated steam drying (LPSSD) is also one of the useful drying techniques that is applied in many types of food products since it is the slow drying process that operates at drying temperature lower than 100°C with the assist of reduced pressure. Unlike superheated steam drying (SSD) which applied higher pressure that could make product melt, undergo glass transition, or degrade some bioactive compounds, LPSSD can help reserve production energy, preserve food quality, and retain nutrients, especially bioactive components in heat sensitive food products. To exemplify, in terms of food quality evaluation, LPSSD exhibits higher retention of bioactive components, total phenol content and antioxidant activity in dried mango cubes than vacuum and hot air-drying methods (Sehrawat et al., 2018). Nevertheless, in case of applying a higher drying temperature, it can affect the color, appearance and texture of the food as well. For example, by comparing low pressure superheated steam drying with conventional hot air drying, LPSSD provides better quality benefits to potato chips than hot air-drying, including a lower browning index, retain product color, and also take shorter drying time (Kingcam et al., 2008). Additionally, the drying temperature and time for obtaining the desired final moisture content depends on the product size or surface area, hence chicken breast powder will definitely require lower temperature and shorter time than potato chips. Moreover, with the use of heated saturated steam and reduced pressure, it can also operate at a lower drying temperature in an oxygen-deficient processing environment, resulting in reducing the chance of oxidation reactions in the food and also yield dried product with high porosity (Devahastin et al., 2004).

2.8 Application of protein powder in pancake

For the application of protein powder in pancakes, there is one main protein powder that is used to substitute the flour, which is whey protein powder. However, with the increase of the level of whey powder substitution, it could result in the lower batter viscosity and stability, which can subsequently result in a flat pancake with higher in diameter but lower in height compared to normal pancake. To exemplify, according to Jyotsna et al (2007), the apparent viscosity of the eggless cake batter was significantly decreased from approximately 2600 mPas in the control sample to about 1400 mPas in 30% whey substituted eggless cake batter. Additionally, another study also emphasized that the other types of whey powder could reduce the ratio of height to diameter of the formulated pancake. To clarify, according to Camacho Flinois et al (2019), they reported that the implementation of yogurt acid whey could lower the the ratio of height to diameter of the pancake since it required lower kneading energy and interfered the water absorption by the flour, resulting in not reaching the desired gluten development of the dough and consequently resulting in thinner or flat pancake.

Chapter III

MATERIALS AND METHODS

Materials

Chicken breast meat

Deboned lean chicken breast meat was sourced from Tops Supermarket in Bangkok, Thailand.

Chemical reagents

All chemical reagents were analytical grade including

- Anhydrous copper sulfate (Ajax Finechem Co., Ltd, New Zealand)
- Anhydrous sodium carbonate (Lons Chemie Pvt Ltd., India)
- Boric acid (Ajax Finechem Co., Ltd, New Zealand)
- Ethanol (Qrec Chemicals, New Zealand)
- Hydrochloric acid (Qrec Chemicals, New Zealand)
- Kjeblet catalyst (Oskon Co. Ltd, Thailand)
- Methyl red indicator (Merck, Germany)
- Petroleum ether (Qrec Chemicals, New Zealand)
- Reagent grade concentrated
- Sulfuric acid (Qrec Chemicals, New Zealand)
- Sodium hydroxide (Ajax Finechem Co., Ltd, New Zealand)

Laboratory Equipment

All laboratory equipment and instrument in the experiment include

- Centrifuge (Model6000, Kubota Corporation, Japan.)
- Colorimeter (Chroma meter CR-400, Konica Minolta Sensing, Inc., Japan),
- Digestion unit (K-424) (Buchi, Switzerland)
- Distillation unit (K-324) (Buchi, Switzerland)
- Drying oven (Memmert UN 30 plus, Germany)
- Fluorescence spectrophotometer (Jasco FP-6200, Japan)
- Hardened stainless steel vernier caliper (Kanon, Japan)
- Homogenizer (IKA®T25 Digital Ultra Turrax, Guangzhou, China)
- Induction Cooker (IF-404, Thailand)
- Kitchen Aid Mixer (5KPPM5, Michigan, USA)
- Low pressure superheated steam dryer (Department of Food Engineering, King Mongkut's University of Technology Thonburi, Thailand)
- Muffle furnace (CWF 1200, Scientific Promotion Co. Ltd, Thailand)
- Seven compact pH meter (Mettler Toledo Co. Ltd, Victoria, Australia)
- Soxhlet extractor (Gerhardt, Germany)
- Vacuum oven (Model 273600, Hotpack, The United States)
- TAXT2i texture analyzer (Stable Micro systems Co; Ltd, Godalming, UK)
- Tray Dryer Oven (Thermotec2000, Auckland, New Zealand)
- Ultraviolet spectrophotometer (Evolution One, Thailand)
- Viscometer (Fungilab Premium series, Barcelona, Spain)
- Water bath (SW 23, Germany)

Methodology

3.1 Preparation of chicken breast powder

For chicken breast powder preparation processes (Figure 3.1), the deboned frozen lean chicken breast was ground by the meat mincer. After mincing, chicken meat was boiled in a pressure cooker for 15 minutes. The minced and pressure-cooked chicken meat was dried by either conventional hot air drying (at 65°C), vacuum drying (at 65°C, 0.09 MPa) (Shen et al., 2021), and low pressure superheated steam drying (at 65°C, 0.09 MPa) (Kingcam et al., 2008) with slight modifications to the dried chicken breast meat, and also the drying time is determined by the time each drying method reaching the similar level of final moisture content as the conventional method. The dried chicken breast meat was grinded into chicken breast powder, and it was sieved by using a sieve for the equal particle size of chicken breast powder.

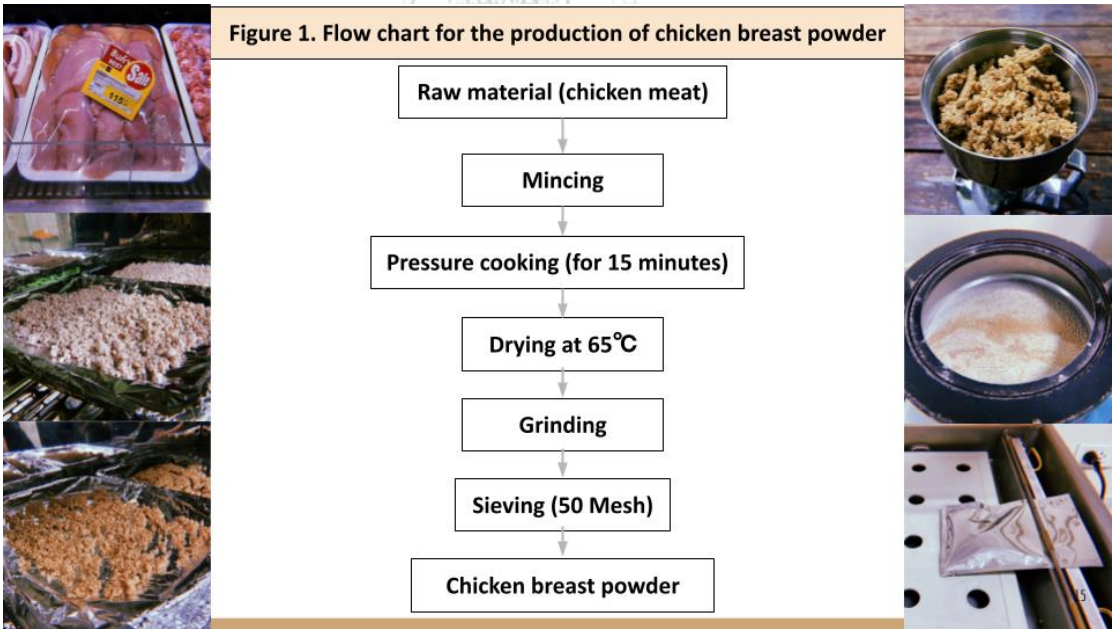

Figure 3.1 Flow chart for the production of chicken breast powder

3.2 Determination of drying time and temperature

The determination of drying time and temperature for cooked chicken breast meat will be performed and followed by the drying methods including conventional hot air drying (at 65°C), vacuum drying (at 65°C, 0.09 MPa) (Shen et al., 2021), and low pressure superheated steam drying (at 65°C, 0.09 MPa) with slight modifications, and also the drying time is determined by the time each drying method reaches the similar level of final moisture content as the conventional method to compare the effect of those drying techniques on chicken breast powder in further steps. The target final moisture content in this experiment should be controlled to be ranged from 4±2% since the maximum moisture content in powder is 8% according to Regulation (EC) No 1069/2009 and Regulation (EU) No 142/2011 of the food standard (Official Journal of the European Union, 2017). Furthermore, in accordance with international standards, in terms of microbiological standard, *Salmonella* (ISO 6579), *enterobacteriaceae* (NEN-EN-ISO 21528-2), *Escherichia coli* (ISO 16649-2), yeasts and molds (ISO 7954) need to be limited to be lower than standards limits by ISO methods.

3.3 Determination of physicochemical properties

3.3.1 Moisture content determination

Moisture content determination is determined by conventional oven drying method at 105°C (AOAC, 2006).

Figure 3.2 Moisture content determination of chicken breast powder

3.3.2 Water activity determination

The water activity of chicken breast powder was analyzed by using AquaLab Dew point Water Activity Meter 4TE. 1 gram of chicken breast powder was placed in a sample cup covered with the lid and ensure that the powder covers all the surface area in the bottom as possible to maximize the water level coming out from the samples. The analysis was done in triplicate and reported in mean.

Figure 3.3 Water activity determination of chicken breast powder

3.3.3 Protein determination

Protein content is determined by Kjeldahl method (AOAC, 2006)

Figure 3.4 The chicken breast powder sample in distillation unit

3.3.4 Fat determination

Fat content is determined by Soxhlet method (AOAC, 2006)

Figure 3.5 The chicken breast powder samples in the thimbles in a Soxhlet extractor

3.3.5 Ash determination

Ash content is determined by the method of AOAC (2006)

Figure 3.6 The chicken breast powder samples in the crucibles in a muffle furnace

3.3.6 pH determination

The pH of chicken breast powder was analyzed by using a pH meter. 15 grams of chicken breast powder was used and diluted in 30 ml of distilled water in %w/v (1:2).

Figure 3.7 pH determination of chicken breast powder using seven compact pH meter

3.3.7 Color determination

The color of chicken breast powder was analyzed by using the chroma meter. The colorimeter was calibrated using a white reference plate before chicken breast powder measurement. The results were done in triplicate and shown as the value of L*, a*, and b*. Additionally, chroma (c*), hue angle (h*), whiteness index (WI), yellowness index (YI), and brownness index (BI) were also calculated according to the equations as shown in Table 3.1 (Pankaj et al., 2013).

Figure 3.8 Kinoca Minolta CR-400 chroma meter

Table 3.1 Quantification of colour and equations (Pankaj et al., 2013)

Quantification of colour	Equations
Chroma (c^*)	$c^* = \sqrt{a^2 + b^2}$
Hue angle (h^*)	$h^* = \tan^{-1}(\frac{b^*}{a^*})$
Whiteness index (WI)	$WI = \mathrm{L} - 3\mathrm{b} + 3\mathrm{a}$
Yellowness index (YI)	$YI = \frac{142.86b^*}{L^*}$
Brownness index (BI)	$BI = 100 \times \left(\frac{X-0.31}{0.17}\right)$, where, $X = \frac{(a+1.75\,L)}{(5.645L+a-3.012b)}$

3.4 Determination of functional properties

3.4.1 Water holding capacity

For water holding capacity, followed by Elsohaimy method with some changes, whey protein concentrate and chicken breast powder were used in water holding capacity analysis in order to compare its holding capacity. 5 grams of the sample and 10 ml of distilled water were weighed and put in a 50-ml centrifuge tube, then thoroughly mixed and allowed to stand for 30 minutes. The centrifuge tubes were centrifuged at 4°C for both whey protein concentrate and chicken breast powder, at 7000 rpm for 30 minutes each. The supernatant or liquid part was poured from the tube and weighed (Elsohaimy et al., 2015).

3.4.2 Oil absorption capacity

For oil absorption capacity (OAC), followed by Elsohaimy method with some changes, whey protein concentrate and chicken breast powder were used in oil absorption capacity analysis in order to compare its absorbing capacity. 5 grams of the sample and 10 ml of soybean oil were weighed and put in a 50-ml centrifuge tube, then thoroughly mixed and allowed to stand for 30 minutes. The centrifuge tubes were centrifuged at 4°C for both whey protein concentrate and chicken breast powder, at 9000 rpm for 30 minutes each. The supernatant or liquid part was poured from the tube and weighed (Elsohaimy et al., 2015).

3.4.3 Water solubility index

For water solubility index (WSI), followed by Jafari method with some slight modifications, whey powder and chicken breast powder were used in water solubility analysis. 1 gram of the sample and 10 mL of distilled water were weighed and put in a 50-mL centrifuge tube, then the suspension was stirred in a vortex mixer for 1 minute. After that, they were placed in a water bath at 37 °C for 30 minutes, and then centrifuged at 3500 rpm and 4°C for 20 minutes. The liquid supernatant was poured into a pre-weighed dish and dried at 105°C to a constant weight (Jafari et al., 2017).

For the WSI was calculated by the equation below:

$$WSI\ (\%)\ = \frac{Dried\ supernatant\ weight}{Initial\ sample\ weight}\ x\ 100$$

3.4.4 Hygroscopicity

For the hygroscopicity, followed by Teng method with some slight changes, whey powder and chicken breast powder were used in hygroscopicity analysis to compare the ability to absorb moisture exposed to high humidity conditions. Approximately 0.3 gram of powder were put into a pre-dried aluminum tray, and it was placed in a desiccator where saturated NaCl solution was placed. For the storage, the desiccator was put in room temperature for 7 days. For the result, the moisture content of chicken powders was measured at day 0 and 7 (Teng et al., 2019).

The hygroscopicity of samples was calculated according to the following equation:

$$H\ (\%)\ =\ \frac{m_2\ -\ m_1}{m_0}\ x\ 100$$

where: H (%) is for hygroscopicity of powder samples; m_0 (g) is the weight of chicken powders; m_1 (g) and m_2 (g) are the weight of the box and chicken powders before and after absorbing water, respectively.

3.4.5 Protein solubility

For protein solubility, followed by Shen method with some slight modification, the pH of whey protein concentrate or chicken breast powder was used and adjusted using 1 M NaOH or HCl to get the desired pH level ranging from pH 3 to pH 9. After that, at room temperature, the suspension was further stirred for 30 minutes and centrifuged at 4000 g for 30 minutes. Consequently, protein concentration in the solution was measured by Biuret method and analyzed using a spectrophotometer at 540 nm absorbance (Shen et al., 2021).

3.4.6 Foaming capacity and stability

For foaming capacity and stability, followed by Shen method with some slight modifications, 0.5 gram of whey protein concentrate or chicken breast powder was dispersed into 50 mL DI water in a beaker. Then, a high-performance disperser was used to homogenize the suspension for 2 minutes at 20,000 rpm to create foam. After that, the foam is immediately transferred to a graduated cylinder, and volume of foam is recorded (Shen et al., 2021).

3.4.7 Emulsifying capacity and stability

For emulsifying capacity and stability, followed by Shen method with some modifications, 2 grams of whey protein concentrate or chicken breast powder was homogenized with 25 mL deionized water for 30 seconds using a blender. Respectively, 25 mL of soybean oil was added to the suspension and then homogenized for another 30 seconds. After that, the emulsion was centrifuged at 1500 g for 5 minutes (Shen et al., 2021).

3.5 Determination of amino acid composition

For amino acid profile, followed by Sá method with slight modifications, total amino acids of the chicken breast powder were determined and performed by using reverse-phase column chromatography in a HPLC. In order to release individual amino acid, 6 M of hydrochloric acid and phenol solutions were used for acid hydrolysis at 110°C for 24 hours. After the hydrolysis, in terms of quantification of total amino acids, α-aminobutyric acid is added as an internal standard. In addition, for identification of the amino acids, an external standard is used to compare individual amino acids (Sá et al, 2021).

3.6 Determination of antioxidant activities

The total phenolic content and antioxidant activity assays including DPPH, and FRAP will be determined in all dried chicken breast powder samples from different drying methods (Kopec et al., 2020). The supernatant was prepared followed by the method of Jang et al. (2008) with some slight modifications. 1 gram of chicken breast powder was mixed with 9 ml methanol. Then, the solution was homogenized for 1 minute and stirred for another 1 minute. After that, the homogenate samples were centrifuged at 10,000 g and 4°C for 20 minutes, and the supernatant filtered through Whatman filter paper (Number 1) with 11 µm pore filter. Consequently, the extract for determination of antioxidant activities was obtained and stored in the amber glass bottle in order to be protected from the exposure to light (Echegaray et al., 2021).

3.6.1 Total phenolic content: Folin-Ciocalteu method

The determination of total phenolic content will be done by Folin-Ciocalteu assay with some slight changes (Singleton & Rossi, 1965).

3.6.2 Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power assay at wavelength 593 nm will be conducted to measure the ferric potential in samples through the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by reducing antioxidants present in the samples, resulting in blue complex (Benzie & Strain, 1996).

3.6.3 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH scavenging activity assay at wavelength 517 nm will be performed followed by the method of Brand-Williams (1995) with some slight changes to determine the ability to scavenge DPPH, resulting in purple color.

3.7 Formulation and preparation of chicken breast powder fortified pancake

As the use of whey protein concentrate can make the pancake to be thinner, the dried chicken breast powder that has overall better functional properties from the proper drying technique will be selected to use as the alternative protein powder to substitute whey protein concentrate as a proportion in traditional protein rich pancake recipes. For the protein rich pancake mix formulations, as shown in Table 3.2, it followed the recipe of Texanerin Baking (2022) with some modifications to make pancakes without milk, so water is added instead of milk to reduce the lactose content. Subsequently, the type of protein powder in the formulations will be varied including PP-1 (0% chicken breast powder, 20% whey protein concentrate), PP-2 (10% chicken breast powder, 10% whey protein concentrate) and PP-3 (20% chicken breast powder, 0% whey protein concentrate). Subsequently, the formulated powder samples will be mixed with water or lactose-free milk in order to make the pancake as the following step. For the preparation steps, all dry ingredients were gradually mixed with the mixture of wet ingredients to create the viscous pancake batter, and mixed by hand for 30 seconds with a whisk. Then, for the pancake cooking method, followed by the method of Finnie et al. (2006), with slight modifications, approximately 40 grams of pancake batter was poured to the Tefal pan using the induction cooker with the surface pan temperature at 140°C. After cooking the first side of the pancake for 90 seconds and the pancake batter got some bubbles, the pancake was once flipped and cooked for additional 90 seconds for the second side.

Figure 3.9 Ingredients for making pancake samples

Figure 3.10 Preparation of dry and wet ingredients for pancake sample

Figure 3.11 Pancake sample cooked until bubbles formed and flipped

Table 3.2 Pancake formulation

Formulation	Standard formula		CT	PP-1	PP-2	PP-3
	(g)	(Bakers %)	(Bakers %)			
All-purpose flour	161.11	80.40	80.00	60.00	60.00	60.00
Whey powder	-	-	0.00	20.00	10.00	0.00
Chicken breast powder	-	-	0.00	0.00	10.00	20.00
Caster sugar	29.16	14.55	15.00	15.00	15.00	15.00
Baking powder	6.50	3.24	3.50	3.50	3.50	3.50
Salt	3.62	1.81	1.50	1.50	1.50	1.50
Total dry ingredient	200.39	100.00	100.00	100.00	100.00	100.00
Water	142.08	58.06	60.00	60.00	60.00	60.00
Oil	33.67	13.76	14.00	14.00	14.00	14.00
Egg	55.47	22.67	22.00	22.00	22.00	22.00
Vanilla	13.50	5.52	6.00	6.00	6.00	6.00
Total wet ingredient	244.72	100.00	100.00	100.00	100.00	100.00

Note: CT: Control or standard formulation, PP-1: formulation with 0 % chicken breast powder, PP-2: formulation with 10% chicken breast powder, PP-3: formulation with 20% chicken breast powder

3.8 Determination of pancake properties

3.8.1 Determination of pancake batter viscosity

Pancake batter viscosity was determined by using Fungilab premium viscometer at room temperature (25±1°C). The pancake batter viscosity was immediately measured after mixing all the dry and wet ingredients into the batter. The viscosity was measured with a spindle probe No. R4 with different speed in rpm regarding significant differences in viscosity between the samples including Spindle R4 used at 12 rpm for CT and PP-1, 6 rpm for PP-2, 1.5 rpm for PP-3. The analysis was done in triplicate, recorded as centipoises, and converted to $N.s.m^{-2}$.

Figure 3.12 Pancake batter viscosity determination using Fungilab premium viscometer

3.8.2 Determination of pancake quality

After the cooked pancake is cooled, both diameter and height of each pancake sample were measured using a 7” hardened stainless steel vernier caliper (Kanon, Japan) (Cho et al., 2019). The analysis was done in triplicate, recorded as cm.

Figure 3.13 Determination of diameter and height of pancake samples using Kanon hardened stainless vernier caliper

3.8.3 Texture Profile Analysis (TPA)

Texture Profile Analysis (TPA) of pancake CT, PP-1, PP-2, and PP-3 were performed and analyzed at room temperature by using TA-XT2i texture analyzer (Stable Micro Systems Co; Ltd, Godalming, UK). The sample dimension is in cylinder shape with a diameter of 30 mm. The samples were compressed by using the 100mm compression plate (P/100). Two cycles as the double bite compression were applied, with 5 g trigger force, at constant test speed of 10.00 mm/s, until strain of 50% reached the target (Finnie et al., 2006). In terms of adjusting beam, the locate base is at 30 mm. The load-time curve and texture attributes including hardness, springiness, and cohesiveness were obtained through “Exponent” software.

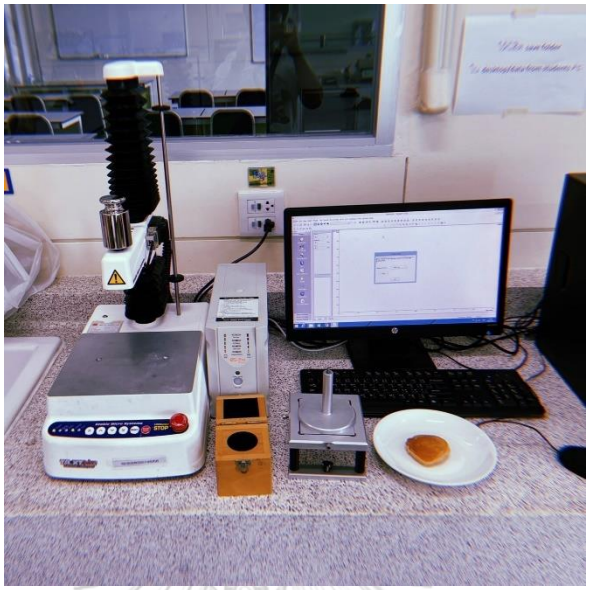

Figure 3.14 Texture Profile Analysis (TPA) of pancake sample at room temperature by using TA-XT2i texture analyzer

3.8.4 Color determination

The color of the pancake was analyzed by using the chroma meter. The colorimeter was calibrated using a white reference plate before chicken breast powder measurement. The results were done in triplicate and shown as the value of L*, a*, and b*. Additionally, chroma (c*), hue angle (h*), whiteness index (WI), yellowness index (YI), and brownness index (BI) were also calculated according to the equations as shown in Table 3.1 (Pankaj et al., 2013).

3.9 Statistical analysis

Statistical analysis will be performed by using SPSS for Windows with the Duncan test at 95% confidence to compare means. Also, analysis of variance (ANOVA) will be used to determine the significant differences from the testing.

Chapter IV

RESULTS AND DISCUSSION

4.1 Preparation of chicken breast powder

4.1.1 Yield of dried chicken breast powder samples

According to Table 4.1, as compared the yield by percentage after processing steps, chicken breast powder dried by low pressure superheated steam dryer (CBL) gives higher yield than both powders dried by vacuum dryer (CBV) and conventional hot air dryer (CBH), respectively. This might be due to higher moisture content in CBL remaining in the sample. The reason behind this is that low pressure superheated steam drying can reduce the degree of shrinkage and also improve shrinkage by simultaneously involving vapor inside the sample and expanding into the cell, leading to uniform shrinkage (Kerdpiboon & Devahastin, 2007). Since the drying chamber in low pressure superheated steam dryer is more humid than vacuum dryer and conventional hot air dryer, this milder condition could prevent case hardening in the sample. To exemplify, in case of vacuum and conventional hot air drying, the outer surface of the sample tends to be dried and rigid faster than the center, taking surface water by the dry air, resulting in the non-uniform shrinkage blocking the surface to absorb vapor from the surrounding.

Figure 4.1 Dried chicken breast meat from different drying methods: conventional hot air drying (left), vacuum drying (middle), and low pressure superheated steam drying (right)

Table 4.1. % Yield of three types of chicken breast powder

Types of drying method	Weight of raw chicken breast (g)	Weight of pressure-cooked chicken breast (g)	Weight of chicken breast powder (g)	Yield (%)
Conventional hot air drying (CBH)	920.2	551.11	154.95	16.84
Vacuum drying (CBV)	829.8	501.10	136.25	16.42
Low pressure superheated steam drying (CBL)	766.7	462.70	163.11	21.27

4.1.2 Determination of drying time and temperature

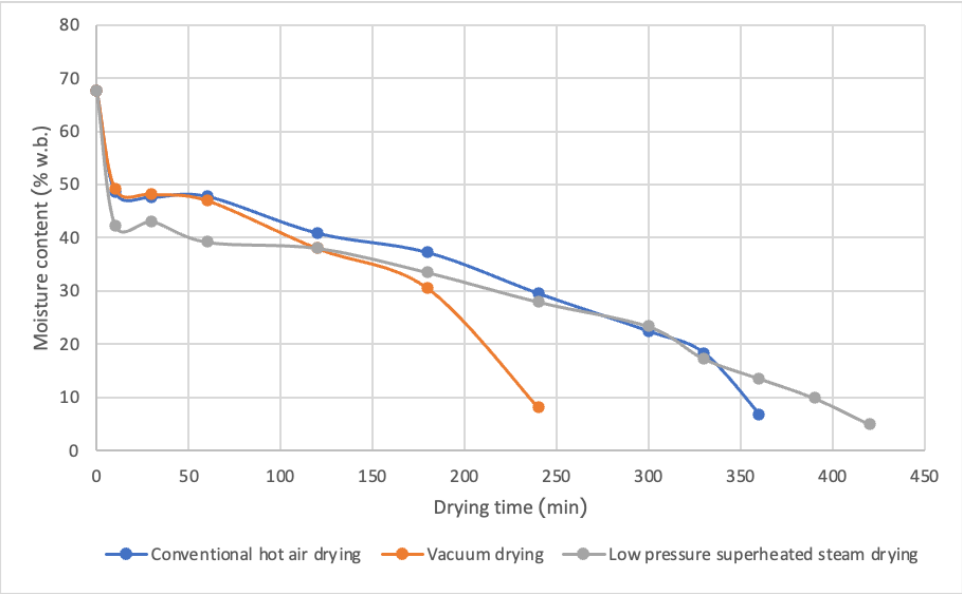

Figure 4.2 Drying rate curve of chicken breast powder (Moisture content vs. Drying time)

For the determination of drying time and temperature, all chicken breast samples were dried at 65°C. In addition, to determine drying time of each drying method, the powder needed to reach the similar level of final moisture content as the conventional method. As the chicken breast powder dried by conventional hot air oven method is dried for 6 hours, vacuum dried and low pressure superheated steam dried chicken breast powders required about 4 hours and 7 hours, respectively. In terms of drying curve, as shown in Figure 4.2, by determining the drying time until the weight of cooked chicken breast tends to remain constant, the vacuum drying method requires the shortest drying time than others because it is dried under vacuum providing higher drying rate at lower drying temperature. Unlike vacuum drying, low pressure superheated steam drying requires the longest drying time than others due to the fact that the superheated steam continuously input during drying, so there will be the cycle of some moisture reuptake of the sample and vapor dried out during drying, even though it is a slow drying process that

operates at drying temperature lower than 100°C with the assist of reduced pressure similar to vacuum drying method.

4.2 Determination of chicken breast powder physicochemical properties

4.2.1 Determination of chemical properties

In terms of physicochemical properties, as shown in Table 4.2, the pH of chicken breast powder from all drying methods are about 6.3 which is almost neutral and lower than the whey protein powder which is 6.80±0.02, and the percent yield of chicken breast powder is lost due to loss of weight of water, fat and some of the irregular coarse chicken breast powder during the process of preparation including pressure cooking, drying and sieving respectively. Thus, the moisture content (% w.b.) and water activity (a_w) of chicken breast powder are dependent on those changes mainly during drying. As a result, as shown in Table 4.2, CBL has significantly higher moisture content (5.58 ±0.01) and water activity (0.427±0.002) than both CBV (3.62±0.64), (0.280±0.002) and CBH (3.92±0.24), (0.288±0.012) respectively due to the steam continuously involved in low pressure superheated steam drying chamber. Consequently, in CBL, as the moisture content increases, the water activity increases. Therefore, the reason why the results are compared based on different final moisture contents of the product is that the humidity in the drying chamber of a low pressure superheated steam dryer is possibly higher than others because of the continuous steam input in the chamber during drying. To compare with the commercial whey powder, the moisture content of chicken breast powder is higher than commercial whey powder which is 2.03±0.13, and the water activity of chicken breast powder is also higher than commercial whey powder which is 0.196±0.005. The reason behind this is that moisture content of the food products is usually measured by heating the material to dry and recording the weights of the product before and after the drying process. Nevertheless, since the moisture content of chicken breast powder is higher, this might be due to the bigger particle

size of the chicken breast powder compared with the commercial whey powder, in which it could hold more moisture in the cell. For the water activity, it is a measurement of free water that is available to react with itself or another material. In addition, for the long time of storage, the food products can reuptake the moisture from the surrounding which can be referred as moisture sorption isotherm. Therefore, since the chicken breast powder was passed through the process of drying in either conventional hot air oven, vacuum oven or low pressure superheated steam oven and cooled down before packing and further analysis, so it should have more water activity compared with the commercial whey powder because it has larger particle size to rehydrate during cooled down and storage, resulting in the absorption of moisture from the surrounding with higher humidity to the product.

For the nutrients in chicken breast powder, as shown in Table 4.2, CBV mainly contains the highest protein as a major source which is 94.56±0.55 % in dry weight basis, with lowest 7.66±0.16 % of fat and highest 2.67±0.47 % of ash content since it has the lowest moisture content among all samples since moisture content can further affect other compositions in dry basis. Nevertheless, for the commercial whey powder, the protein content of the concentrate is approximately 70%.

4.2.2 Determination of color properties

In terms of color, the color profile of both chicken breast powder was shown in Table 4.2. The lightness (L*) of the chicken breast powder from conventional hot air drying (CBH) (77.60±0.24) has the darkest sample which is significantly lower L* than CBV (78.53±0.45) and (CBL) (80.06±0.06), respectively. To clarify, the darker color of CBH could come from the longer drying process and the use of air movement with low humidity compared with vacuum and low-pressure superheated steam drying, respectively. Moreover, another reason for the darker color of CBH than CBV and CBL is that the presence of oxygen in hot air drying causes the surface temperature

of the product to be quite high, so this could further result in promoting oxidation and other chemical reactions of the heat sensitive materials, resulting in further Maillard browning reaction on the surface of the product that could affect the color during drying. Hence, since there was a vacuum applied in CBV and CBL, the absence of oxygen would inhibit the oxidation reaction of color, resulting in better retaining of original color. On the other hand, in terms of redness (a*) of the chicken breast powder, CBL (0.40±0.01) significantly redder than CBV (-0.56±0.05) and CBH (-0.89±0.05). Furthermore, for the yellowness (b*) of the chicken breast powder, CBL is also (17.88±0.16) significantly more yellowish than CBV (16.48±0.19) and CBH (15.33±0.13). To elaborate, the more red and yellowish color of CBL could come from the browning reaction during drying since it has higher water activity, so it could increase the brownish rate. Moreover, the color profile of both chicken breast powder and commercial whey powder were shown in Table 4.2 and 4.3 respectively. The lightness (L*) of the commercial whey powder (96.60±0.33) is higher than all types of dried chicken breast powders, whereas the yellowness (b*) of the chicken breast powder is much higher than the commercial whey powder (1.61±1.54). To clarify, the yellowness of the chicken breast powder could come from the browning reaction during the process of pressure cooking and drying. However, in terms of redness (a*), CBL shows a higher value than others.

As a result of this, all the L*, a*, b* values can be proved and calculated for the quantification of color in several values including chroma (c*), hue angle (h*), whiteness index (WI), yellowness index (YI), and brownness index (BI), and those results corresponded to the CIELAB color space. To illustrate, as CBL exhibited the highest a* and b* values, so it gives more redness and yellowness in color, resulting in higher chroma (c*) and hue angle (h*) that is closer to 90° which is represented in yellow color. In addition, like chroma and hue angle, CBL also showed the highest in yellowness index (31.91±0.33) and brownness index (25.06±0.21) values since it took longer drying and steam involved, so this might extend the browning

reaction during drying, resulting in generating more brownish and yellowish pigments. On the other hand, in terms of whiteness (WI), it exhibited contrast to the lightness of the powder in this case. To explain, whiteness index can be measured to correlate the result with the preference of the consumers for white color or the absence of color, whereas lightness corresponds to the ability to reflect light off of white surface, usually blue light. The higher the blue color, the higher lightness in the food product. Consequently, as shown in Table 4.2, CBH has significantly higher whiteness index value (28.92±0.34) than others since it has significantly lower b* value corresponding to bluer color for higher light reflection. Likewise, even though CBL has the highest L*, it exhibited lower whiteness index value since CBL possessed more yellowish and brownish color. Subsequently, as the consumers possibly see in Figure 4.3, they might see that CBL looks darker than CBH and whey powder.

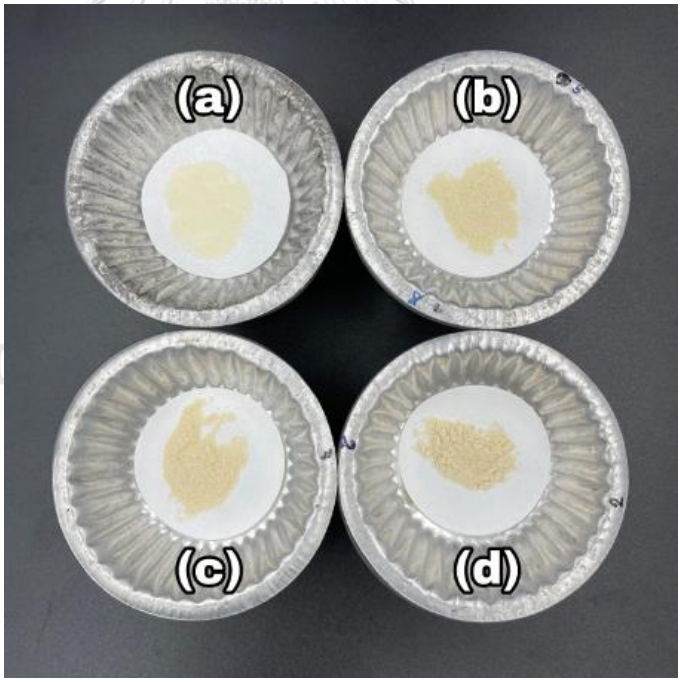

Figure 4.3 Colors of different types of powder: (a) Commercial whey powder, (b) Hot air-dried chicken breast powder, (c) Vacuum dried chicken breast powder, and (d) Low pressure superheated steam dried chicken breast powder

Table 4.2 Proximate compositions of dried chicken breast powder samples (db.)

Physical properties	Conventional hot air drying	Vacuum drying	Low pressure superheated steam drying
Moisture content (% w.b.)	3.92±0.24^{b}	3.62±0.09^{b}	5.58±0.01^{a}
Water Activity	0.288±0.012^{b}	0.280±0.002^{b}	0.427±0.002^{a}
pH	6.28±0.008^{b}	6.19±0.012^{c}	6.33±0.005^{a}
Protein content (%)	91.88±0.45^{b}	94.56±0.55^{a}	89.91±0.12^{c}
Fat content (%)	8.59±0.28^{b}	7.66±0.16^{c}	9.81±0.38^{a}
Ash content (%)	2.00±0.10^{b}	2.67±0.58^{a}	1.86±0.15^{b}
Color			
L*	77.60±0.24^{c}	78.53±0.45^{b}	80.06±0.06^{a}
a*	-0.89±0.05^{c}	-0.56±0.05^{b}	0.40±0.01^{a}
b*	15.33±0.13^{c}	16.48±0.19^{b}	17.88±0.16^{a}
c*	15.36±0.16^{c}	16.49±0.24^{b}	17.89±0.20^{a}
h*	86.67±0.21^{c}	88.05±0.20^{b}	88.73±0.02^{a}
WI	28.92±0.34^{a}	27.42±0.53^{b}	27.60±0.50^{b}
YI	28.23±0.22^{c}	29.97±0.29^{b}	31.91±0.33^{a}
BI	20.62±0.21^{c}	22.45±0.21^{b}	25.06±0.21^{a}

*Mean values ± standard deviation (n=3) with different superscript letters (a-c) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

Note: WI = Whiteness Index

YI = Yellowness Index

BI = Brownness Index

Table 4.3 Proximate compositions of commercial whey powder

Physical properties	Whey protein powder
Moisture content (% w.b.)	2.03±0.13
Water Activity	0.196±0.005
pH	6.80±0.02
Color	
L*	96.60±0.33
a*	0.17±0.38
b*	1.61±1.54
h*	82.08±0.15
c*	17.39±0.22
WI	27.70±0.26
YI	28.42±0.15
BI	19.54±0.10

Note: WI = Whiteness Index
YI = Yellowness Index
BI = Brownness Index

4.3 Determination of chicken breast powder functional properties

Table 4.4 Functional properties of dried chicken breast powder samples

Functional properties	Conventional hot air drying	Vacuum drying	Low pressure superheated steam drying
Water absorption capacity (g H_20/ g protein)			
pH 5	2.54±0.025^{f}	2.59±0.031^{de}	2.69±0.017^{c}
pH 6	2.31±0.005^{h}	2.35±0.012^{h}	2.39±0.024^{g}
pH 7	2.57±0.021^{ef}	2.63±0.022^{d}	2.91±0.021^{b}
pH 8	2.98±0.025^{a}	2.71±0.012^{c}	2.71±0.021^{c}
Oil absorption capacity (g oil/ g protein)	1.70±0.002^{c}	1.80±0.008^{b}	1.99±0.015^{a}
Emulsion capacity (%)			
pH 5	8.10±0.67^{ef}	6.19±0.67^{f}	8.10±1.78^{ef}
pH 6	12.86±1.17^{de}	11.90±0.67^{ef}	10.48±0.67^{ef}
pH 7	18.10±3.56^{d}	47.62±4.42^{c}	44.29±3.50^{c}
pH 8	60.95±4.86^{a}	54.76±2.94^{b}	56.67±2.94^{ab}
Emulsion stability (%)			
pH 5	2.38±0.67^{f}	3.33±0.67^{ef}	5.24±0.67^{cde}
pH 6	4.29±1.17^{cde}	6.67±1.35^{cde}	8.27±1.17^{bcd}
pH 7	7.62±1.35^{de}	13.81±1.35^{cd}	13.81±1.35^{b}
pH 8	6.67±1.78^{cde}	30.95±2.43^{a}	12.86±3.50^{bc}

*Mean values ± standard deviation (n=3) with different superscript letters (a-h) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

Table 4.5 Functional properties of commercial whey powder

Functional properties	Whey powder
Water absorption capacity (g H_20/ g protein)	
pH 5	1.04±0.041[a]
pH 6	0.91±0.024[b]
pH 7	0.73±0.033[c]
pH 8	0.56±0.025[d]
Oil absorption capacity (g oil/ g protein)	1.13±0.05
Emulsion capacity (%)	
pH 5	81.90±3.56[c]
pH 6	93.81±2.43[b]
pH 7	98.57±1.17[ab]
pH 8	99.52±0.67[a]
Emulsion stability (%)	
pH 5	62.38±3.75[c]
pH 6	74.29±3.50[b]
pH 7	92.38±2.94[a]
pH 8	93.33±1.35[a]

*Mean values ± standard deviation (n=3) with different superscript letters (a-d) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

4.3.1 Water absorption capacity

Moreover, as shown in Table 4.4, in terms of water holding capacity, CBL could hold more water than CBV and CBH at almost all the pH since the uniform porous structure of CBL could rehydrate and absorb water into the cell. However, lower water absorption capacity (WAC) value could be observed if the adjusted pH is close to the pI or the isoelectric point of the protein. Therefore, as in all samples, at pH 6, they all showed the lowest WAC values, so this can imply that the isoelectric point chicken breast powder is around pH 6. For instance, the lowest water holding capacity of each type of chicken breast powder sample was seen for pH 6 in all CBH, CBV and CBL, while the highest water holding capacity for both CBH and CBV is at pH higher than 7. Furthermore, in case of CBL, the highest water holding capacity was seen on pH 7 since the porous uniform structure could reuptake moisture and rehydrate well at the pH close to water; however, if the pH is adjusted to be lowered by the HCl or to be higher by NaOH, there might be slight protein denaturation to partially unfold the protein, causing in significantly lower water holding capacity of CBL at pH 8. Therefore, all in all, if we adjust the pH to be away from pH 6, the water absorption capacity of the chicken breast powder tends to increase respectively, resulting in absorbing and holding more moisture in itself. Furthermore, the degree of water absorption capacity might be affected by the drying time as well. The longer drying time like drying in CBH, the less porous structure inside the cell due to case hardening on the surface. Hence, CBV could absorb and hold more water than CBH since it is dried in shorter drying time with the assistance of vacuum pump, resulting in less denaturation and non-uniform shrinkage during drying to cause case hardening. To compare with whey protein powder, all chicken breast powders have higher water holding capacity than whey protein powder which is since whey usually has finer particle size than chicken breast powder, so it could not absorb and hold water in itself well due to the fact that whey is also soluble well in water.

When comparing among whey protein samples at different pH, as shown in Table 4.5, it exhibited that the water holding capacity significantly increases as the pH decreases. The reason behind this is that the isoelectric point of whey protein is less than 5.5, so if the pH is lower than 5.5, the protein is more likely to precipitate at pI, resulting in less solubility in supernatant and higher in water holding capacity.

4.3.2 Oil absorption capacity

Furthermore, for oil absorption capacity, as shown in Table 4.4, CBL (1.99±0.015) also could absorb and hold more oil than CBV (1.80±0.008) and CBH (1.70±0.002), respectively. Since CBL involved superheated steam in the drying process, it could lower the degree of case hardening and reduce the degree of protein denaturation on the surface. Thus, like freeze-dried protein, low-pressure superheated steam dried protein could have significantly higher oil absorption than vacuum and conventional hot air-dried proteins due to possessed possibly higher surface hydrophobicity (Shen et al., 2021) and a bigger porous structure to better absorb oil. To compare with whey protein powder, all chicken breast powders have higher oil absorption capacity than whey protein powder which is 1.13±0.05 (Table 4.5) since whey usually has smaller particle size than chicken breast powder, so it could absorb and hold oil well due to possibly less space inside the cell.

4.3.3 Water solubility index

Table 4.6 Water solubility index (%) of different protein powder samples

Functional properties	CBH	CBV	CBL	Whey
Water solubility index (%)	4.02±0.30^{b}	3.89±0.12^{b}	2.86±0.18^{c}	107.16±3.97^{a}

*Mean values ± standard deviation (n=3) with different superscript letters (a-c) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

Additionally, for water solubility index, as shown in Table 4.6, CBL (2.86±0.18%) exhibited lower water solubility index than CBV (3.89±0.12%) and CBH (4.02±0.30%), respectively. This results conversely to the water absorption capacity. The more water-holded pellets after centrifuge, the less water solubility in the supernatant for the analysis. Moreover, to compare with whey powder (107.16%), since chicken breast exhibited higher water absorption capacity than whey, it resulted in lower water solubility as less supernatant and more water-holded pellets remained in the centrifuge tube. The reason behind this could be due to the fact that whey powder usually has smaller particle size than chicken breast powder since it was usually spray-dried, so it better solubilizes in solution well due to possibly more surface area inside the cell. Also, according to Jafari et al (2017), lower density powder could cause a slight increase of water solubility since the smaller particles float on the water surface, while the larger particles sink or hold water inside the cell. Therefore, since there might be more uniform porous structure and less chance of case-hardening in CBL, it could rehydrate and absorb water into the cell, resulting in sinking in the water (less floating on the water surface) and less water solubility in the supernatant.

4.3.4 Hygroscopicity

Table 4.7 Hygroscopicity (%) of different protein powder samples

Functional properties	CBH	CBV	CBL	Whey
Hygroscopicity (%)	0.31±0.12[b]	0.36±0.04[b]	0.25±0.07[b]	0.59±0.11[a]

*Mean values ± standard deviation (n=3) with different superscript letters (a-c) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

For the hygroscopicity, as shown in Table 4.7, whey powder possessed higher hygroscopicity than all chicken breast powders since whey powder can be considered as hydrolysate powder, so it tends to be more hygroscopic and thermoplastic compared to powders that contain muscle proteins or intact proteins, resulting in poorer stability (Hogan & O'Callaghan, 2013). Thus, chicken breast powder contains more muscle proteins including actomyosin, so it is definitely less hygroscopic than whey, resulting in better stability when exposed to a high humidity environment. With increase in hygroscopicity, solubility also increases. Furthermore, as shown in Figure 4.4, there are also brownish spots observed in whey powder, so this might be due to the reaction of whey powder and water from the mixture. Consequently, these results correlated to the water solubility and contrasted to the water absorption capacity.

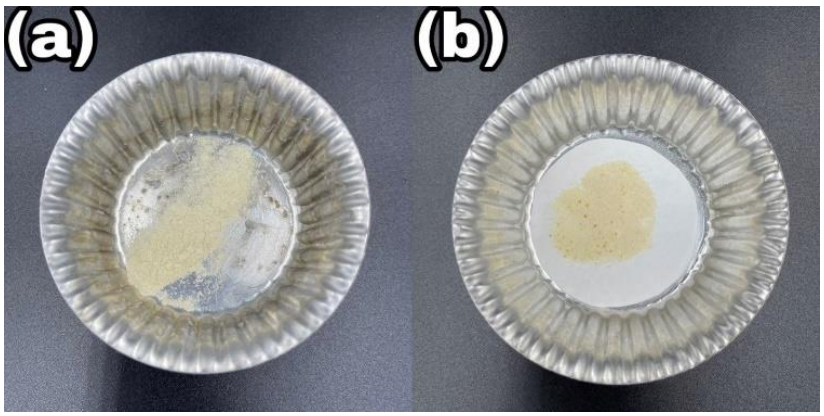

Figure 4.4 Hygroscopicity of chicken breast powder sample (a) and whey sample with observed brownish spots (b)

4.3.5 Protein solubility

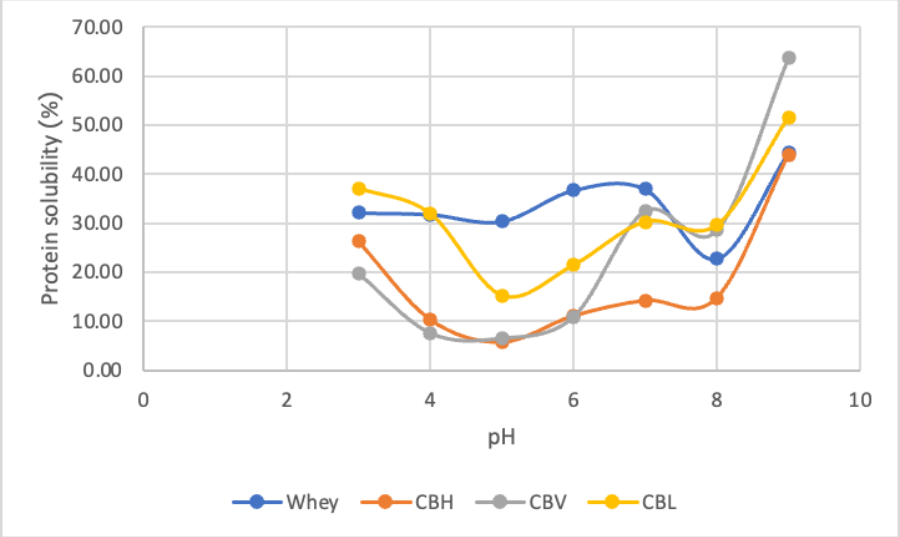

Figure 4.5 Protein solubility (%)

In terms of protein solubility, as shown in Figure 4.5, chicken breast protein showed minimum protein solubility in isoelectric range with pH ranging from 5.0 to 6.0. On the other hand, whey protein possesses better solubility than chicken breast in low pH and has isoelectric range with pH ranging from 4.0 to 5.0, usually pI is at the pH of 4.5. As a result, as pH increases or decreases away from the isoelectric point (pI), protein solubility gradually increases, related to the alteration of the electrical charge distribution and net charge values with the certain pH of the sample.

4.3.6 Foaming capacity and stability

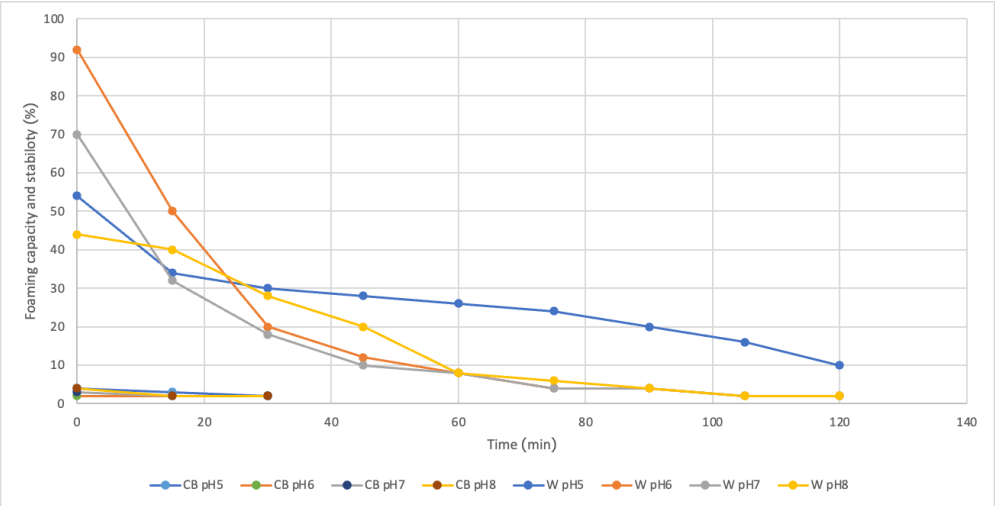

Figure 4.6 Foaming capacity and stability (%) of chicken breast powder and whey powder

For foaming capacity and stability, as shown in Figure 4.6, whey protein possessed significantly higher foaming capacity and stability because enzymatic hydrolysis by rennet could reduce the molecular size and increase the flexibility of the protein to form interfacial membranes. In contrast, chicken breast powder has high water and oil absorption capacity, so it might need to be adequately unfolded and molecularly flexible to form interfacial membranes in order to create foam. In addition, since chicken breast powder contains muscle proteins or intact proteins including actomyosin, the density is higher than whey powder which are hydrolyze powder, resulting in sinking of high-density intact protein at the bottom of the solution, not floating or dispersing well to create foam. Moreover, the lower foaming capacity observed around the isoelectric point is attributed to the low protein solubility. Subsequently, the closer the pH to the isoelectric point, the lower foaming capacity of the powder due to protein precipitation and zero net charge of protein in the aqueous solution. However, at pH 5, although it exhibited foaming capacity than neutral pH, the foaming stability slightly decreased and was quite stable compared to others. The reason behind this

is that an increase in net charge density may interrupt and prevent protein-protein interactions in the foam lamellae, resulting in the destabilization of the foam and poorer foaming stability (Townsend & Nakai, 1983).

4.3.7 Emulsifying capacity and stability

For emulsifying capacity and stability, like foaming capacity and stability, when the pH is close to the isoelectric point, it results in protein aggregation, and lower solubility and emulsifying properties. Nevertheless, if the pH value increases to be higher than the isoelectric point, it greatly enhances protein-water interaction and results in higher solubility; therefore, the emulsifying properties increases. To illustrate, as shown in Table 4.4 and 4.5, the emulsifying capacity increases as the pH increases from pH 5 to pH 8. To clarify on the effect of pH on emulsifying capacity and stability, for the acidic condition, the emulsifying capacity is lower since the protein precipitates as it is fully protonated. On the contrary, for the basic condition, with the increased emulsion pH, smaller size of emulsion droplets are stabilized as the droplets dispersed well in high pH emulsion, which is independent from the precipitation. Like water solubility and protein solubility, the higher solubility correlated to the higher emulsifying capacity and stability since the protein can better disperse in the emulsion, contribute less precipitation, and associate with the oil-water interface well. To further elaborate on chicken breast powder samples, CBH showed highest emulsifying capacity due to its higher value in solubility, whereas CBV exhibited highest emulsifying stability, due to its higher value in holding capacity, resulting in possibly less net charge. As chicken breast powder has high water and oil absorption capacity, it might need to be adequately unfolded and open more hydrophobic sites in order to bind both oil and water phases to increase its emulsifying capacity. Hence, when compared with whey powder, this is the reason why the chicken breast powder samples exhibited significantly very low emulsifying capacity and stability due to its higher holding capacity in itself and less dispersed in the solution.

4.4 Amino acid composition of chicken breast powder

In terms of amino acid composition, Table 4.8 shows the amino acid profiles of three different chicken breast powder samples. In addition, the total amino acid content of CBH, CBV, and CBL were 91.89, 87.99, and 90.86 g/ 100 g of sample, respectively. In terms all chicken breast powder samples showed lowest in hydroxylysine, hydroxyproline (< 500 mg/100 g), and cysteine. On the other hand, for essential amino acids, they exhibited high levels of glutamic acid, aspartic acid, and alanine which ranged from 5.33 to 13.52 g/ 100 g sample. Additionally, in terms of non-essential amino acids, they showed high levels of leucine, lysine, and arginine which ranged from 5.74 to 8.72 g/ 100 g sample. To compare all three chicken breast powder samples, CBV showed quite lower methionine, tyrosine, phenylalanine, lysine and arginine compared the other two methods. To clarify, according to Supreetha et al. (2009), in terms of lower lysine content in vacuum drying, this might be due to the drier heat treatment could result in greater loss of lysine when compared to moist heat-treated conditions. Therefore, as vacuum pumps the air and vapor out the chamber, the drying chamber in vacuum dryer might have lower humidity or be more dried than conventional hot air drying and especially low-pressure superheated steam which has steam involved during the drying process. For CBL, the loss of lysine could come from the Maillard reaction and longer drying time, resulting in darker color of powder. On the other hand, the loss of lysine in CBV could cause by the combination effect of both Maillard reaction and the dry heat-treated condition. However, the amount of lysine in all CB samples is still higher than the minimum daily requirement of 30 mg/kg (Tomé & Bos, 2007), so all CB samples are in a good range and not over-processed. Moreover, when compared to whey powder, according to Banaszek et al. (2019), chicken breast powder has quite higher in some amino acid content including alanine, arginine, aspartic acid, glycine, histidine, isoleucine, phenylalanine, tyrosine and valine (Table 4.9).

Table 4.8 Amino acid profiles of different dried chicken breast powder samples

Amino acid profiles (in g/ 100 g)	CBH	CBV	CBL
Alanine	5.33	5.55	5.51
Arginine	6.44	5.74	6.41
Aspartic acid	9.06	9.07	8.85
Cystine	1.01	0.96	1
Glutamic acid	13.52	13.4	13.14
Glycine	4	3.89	3.92
Histidine	3.04	2.69	2.91
Hydroxylysine	Not detected	Not detected	Not detected
Hydroxyproline	< 0.5	< 0.5	< 0.5
Isoleucine	4.64	4.59	4.52
Leucine	8.27	8.26	8.13
Lysine	7.55	6.71	7.41
Methionine	2.84	2.5	2.86
Phenylalanine	4.14	3.19	4.19
Proline	3.4	3.55	3.49
Serine	3.99	3.97	4.01
Threonine	4.51	4.52	4.53
Tryptophan	1.04	1.07	0.92
Tyrosine	3.54	2.82	3.61
Valine	5.07	5.01	4.95

Table 4.9 Amino acid composition in g/100 g of whey protein (Banaszek et al., 2019)

Amino acid profiles (in g/ 100 g)	Whey Protein	Pea Protein
Alanine	3.5	4.3
Arginine	2.3	8.7
Aspartic acid	8.4	11.5
Cystine	1.7	1
Glutamic acid	13.3	16.8
Glycine	1.4	4.1
Histidine	1.6	2.5
Isoleucine	4.6	4.5
Leucine	8.8	8.4
Lysine	7.5	7.2
Methionine	1.6	1.1
Phenylalanine	2.6	5.5
Proline	6.6	4.5
Serine	4.6	5.3
Threonine	4.5	3.9
Tryptophan	1.3	1
Tyrosine	2.3	3.8
Valine	4.4	5

4.5 Antioxidant activities

Table 4.10 Antioxidant properties of different dried chicken breast powder samples

Antioxidant properties	CBH	CBV	CBL
TPC (mg GAE/L)	1.82±0.15[a]	2.73±1.79[a]	2.16±0.33[a]
FRAP (μM trolox equivalent/ml of sample)	129.33±12.26[c]	248.19±6.00[a]	162.95±7.54[b]
DPPH (TEAC) (μM trolox equivalent/ml of sample)	99.13±1.67[b]	112.13±4.36[a]	88.69±8.80[c]

*Mean values ± standard deviation (n=3) with different superscript letters (a-c) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

4.5.1 Total Phenolic Content

The total phenolic content of chicken breast powder samples were reported as shown in Table 4.10. The total phenolic content (TPC) of CBH, CBV, and CBL were 1.82±0.15, 4.95±3.19, and 2.16±0.33 mg GAE/L, respectively. The results showed that total phenolic content found in all chicken breast powder samples were not significantly different ($p<0.05$). However, when deeply looking at the number, CBV has higher TPC value than others with higher standard deviation, so this might be due to the shorter drying time that could retain some of the phenolic content, while prolonged drying process could further destroy them.

4.5.2 Ferric Reducing Antioxidant Power

For the ferric reducing antioxidant power (FRAP), as shown in Table 4.10, the significantly higher FRAP was found in CBV (248.19±6.00 μM trolox equivalent/ml of sample), compared to CBL (162.95±7.54 μM trolox equivalent/ml of sample) and CBH (129.33±12.26 μM trolox equivalent/ml of sample), respectively ($p<0.05$). For the reaction, as it is measured by the

capacity of the antioxidant peptides in reducing TPTZ–Fe(III) complex to TPTZ– Fe(II) complex, CBH and CBL exhibited lower ferric reducing antioxidant power since longer drying time could damage some antioxidative peptides, resulting in lower metal-chelating ability. Moreover, as shown in Table 4.10, CBV has lowest total free amino acid, so this could explain that the antioxidative peptides in CBV is less damaged due to less drying time, resulting in less conformational change of peptides and the highest ferric reducing antioxidant power compared to other drying methods.

4.5.3 DPPH Free Radical Scavenging Activity

In terms of DPPH free radical scavenging activity, this mechanism could help determine the radical scavenging activity in inhibiting lipid oxidation to evaluate its antioxidant activity. As shown in Table 4.10, the significantly higher DPPH (TEAC) was found in CBV (112.13±4.36 μM trolox equivalent/ml of sample), compared to CBH (99.13±1.67 μM trolox equivalent/ml of sample) and CBL (88.69±8.80 μM trolox equivalent/ml of sample), respectively ($p<0.05$). Similar to FRAP, these results also showed that CBV has the highest DPPH (TEAC) value since there is less detrimental effect on the antioxidant peptides due to shorter drying time. Additionally, another reason is that the absence of oxygen in vacuum drying could help retain the antioxidant, bioactive compounds, and oxygen sensitive materials to be less oxidized, resulting in higher antioxidant activity. However, even though low-pressure superheated steam drying operates under vacuum, there are still oxygen molecules from the superheated steam that could react or oxidize those active compounds, resulting in lower antioxidant activity than vacuum drying. Although some of the amino acids in CBV are less than others; however, the effect of antioxidant activity by antioxidant peptides might be more significant in this case. To explain, since chicken breast powder is quite an intact protein powder, it could rely more on the effect of the antioxidant activity on antioxidant peptides rather than free amino acids as in hydrolysate

protein powder. Therefore, according to Samaranayaka & Li-Chan (2011), their result also correlates and emphasize that the antioxidative activity of peptides is higher than the antioxidative activity of free amino acids Furthermore, for CBH and CBL that have longer drying time, the antioxidant peptides usually react with reducing sugar under thermal process undergoing Maillard reaction, so this could alter the functions of antioxidant peptides to be less antioxidant.

4.6 Physical properties of pancake

4.6.1 Pancake batter viscosity

The viscosity of different pancake batter was measured by using a Fungilab Premium Viscometer with the same spindle No. R4 but different rpm since their viscosity is much different from each other. Therefore, different test speeds of rpm were adjusted to give the % torque to be ranged from 50 - 80 %. As shown in Table 4.11, batter viscosity of the pancakes significantly exhibits different values for the formulas that substitute wheat flour or whey powder with chicken breast powder. As the percentage of chicken breast powder substituted in the pancake formula increases, the thickener the batter will be. However, when comparing the control (CT) with the pancake formula that substitutes wheat flour with only whey powder (PP-1), the viscosity is not significantly different. Therefore, the reason why the higher percentage of chicken breast powder yields higher viscosity of pancake batter is that chicken breast powder has a very high-water absorption capacity and oil absorption capacity when compared to whey, so it could absorb more water and oil as in the suspension in the batter, resulting in thicker pancake batter especially in PP-3 (105.16±2.70) as shown in Figure 4.7.

Table 4.11 Viscosity of pancake batter

	Pancake formulations			
	CT	**PP-1**	**PP-2**	**PP-3**
Batter viscosity ($N.s.m^{-2}$)	12.94 ± 0.37^{c}	10.18 ± 2.56^{c}	26.93 ± 4.49^{b}	105.16 ± 2.70^{a}

Spindle R4 used at 12 rpm for CT and PP-1, 6 rpm for PP-2, 1.5 rpm for PP-3

*Mean values ± standard deviation (n=3) with different superscript letters (a-c) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

Figure 4.7 Different pancake batter samples and observed viscosities

4.6.2 Pancake quality

Table 4.12 Physical pancake quality of different pancake formulations

Physical attributes	**CT**	**PP-1**	**PP-2**	**PP-3**
Diameter (cm)	7.83 ± 0.25^{b}	8.63 ± 0.12^{a}	8.13 ± 0.41^{ab}	6.83 ± 0.34^{c}
Height (cm)	0.93 ± 0.05^{b}	0.63 ± 0.05^{c}	1.03 ± 0.12^{b}	1.47 ± 0.21^{a}

*Mean values ± standard deviation (n=4) with different superscript letters (a-c) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

For pancake quality, physical attributes including diameter and height are determined using a 7" hardened stainless steel vernier caliper (Kanon, Japan). As shown in Table 4.12, the result showed that PP-1 and PP-2 samples have significantly higher values of diameter than PP-3 and CT samples, which are the samples that include only chicken breast powder and only flour, respectively. Consequently, this could be stated that addition of

whey powder in pancakes results in a flatter pancake. Moreover, in terms of height, the result also showed that PP-1 (0.63±0.05) has a significantly lower value than all other samples, whereas PP-3 (1.47±0.21) exhibited a significantly higher value than all other samples. This could be due to the high-water solubility of whey powder that could make the pancake batter waterier and flatten; in contrast, high water absorption capacity of chicken breast powder could yield stickier and more viscous pancake batter. According to Cho et al. (2019), their study was also emphasized as the pancake diameter was negatively correlated with the solvent retention capacity value; in the other word, this solvent retention capacity could refer to the water absorption or water holding capacity in this case. As a result of this, the functional properties of protein powder, especially water solubility and water absorption capacity, could have a significant effect on pancake quality and its physical characteristics.

4.6.3 Texture Profile Analysis of pancake

Table 4.13 Texture attributes of different pancake formulations

Textural properties	CT	PP-1	PP-2	PP-3
Hardness	5496.79±192.82^{b}	13274.35±204.64^{a}	11505.36±535.18^{a}	12291.48±1916.39^{a}
Springiness	0.996±0.003^{a}	0.961±0.040^{a}	0.884±0.017^{b}	0.846±0.041^{b}
Cohesiveness	0.869±0.061^{a}	0.875±0.052^{a}	0.817±0.077^{a}	0.751±0.087^{a}

*Mean values ± standard deviation (n=4) with different superscript letters (a-b) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

In terms of texture profile analysis, the textural attributes include hardness, cohesiveness, and springiness. In terms of hardness, the result shows that there are no significant differences between PP samples; however, the hardness of all PP samples are significantly higher than the control (CT). As shown in Table 4.13, with the replacement of wheat flour with protein

powder either chicken breast powder or whey powder, it results in higher hardness. This could be explained by the increase of protein content in the pancake that contributes to the harder texture, and also the water absorption of the protein powder that could interfere and lower the water absorption by the wheat flour, which could also result in less gluten development. As a result of this, with the increase of protein content by protein powder, this could replace the decrease in gluten in the pancake by creating the network itself with the available polysaccharides in the mixture (Sun et al., 2019). Although the fibrous structure of protein in the control sample could be found to be stronger and harder in the PP sample with the globular structure of animal-based protein, there is a small amount of protein in the all-purpose flour in the formula. Subsequently, this can explain the reason why PP-1, PP-2, and PP-3 are significantly harder the control since they are high in protein content, and the PP-1 with 20% whey substitution has highest value of hardness could due to the tightly packed of small particle size of protein, which is different from chicken breast powder that has larger particle size and porous structure to absorb water that could yield lower hardness value. Moreover, with the increase of protein powder, this could indicate the increase in firmness of the pancake as well. Additionally, for the springiness, it could refer to the elasticity and flexibility of the pancake determined by the recovery of its texture after the first and the second compression of the sample. As shown in Table 4.13, the result exhibits that CT and PP-1 samples are significantly springier than PP-2 and PP-3 samples, which are the samples that include chicken breast powder. To clarify, this might be due to the higher water absorption and oil absorption capacity of chicken breast powder, since it might absorb more water and keep the texture more stable, less flexible, and likely to remain unchanged when compared to whey that is more soluble in the batter. Moreover, in terms of cohesiveness, it could refer to the internal resistance of the pancake structure determining its texture after the first and the second compression of the sample. For the result, as shown in Table 4.13, although the cohesiveness value seems to show that the samples

with chicken breast powder are less cohesive, it showed that there is no significant difference among the cohesiveness for all the samples from control up to 20% of chicken breast powder substitution. This could be discussed by the equalization of the higher gluten development creating a potential gluten network in the control pancake sample and also the potential protein network formed by the higher protein content in the protein powder fortified pancake samples. With the increase of chicken breast powder in the pancake, it could be enough to create a potential protein network that has an internal resistance against the compression as resistant as the control sample.

4.6.4 Color properties of pancake

Table 4.14 Color attributes of different pancake formulations

Physical properties	CT	PP-1	PP-2	PP-3
Color				
L*	62.37±0.91^{a}	38.52±0.94^{d}	42.96±0.48^{c}	55.10±1.24^{b}
a*	11.45±0.47^{c}	19.65±0.48^{a}	18.88±0.43^{a}	13.99±1.38^{b}
b*	38.78±0.23^{a}	38.56±1.32^{a}	40.78±1.59^{a}	39.68±1.41^{a}
c*	40.44±0.42^{c}	43.28±1.47^{ab}	44.94±1.96^{b}	42.08±2.18^{ab}
h*	73.56±0.68^{a}	62.97±1.18^{d}	65.14±0.61^{c}	70.62±1.42^{b}
WI	19.63±0.32^{a}	18.19±4.14^{a}	22.74±4.74^{a}	21.96±1.55^{a}
YI	88.86±2.01^{c}	142.95±1.75^{a}	135.61±6.78^{a}	102.99±6.99^{b}
BI	105.25±4.01^{d}	240.95±4.13^{a}	217.46±18.75^{b}	134.24±15.36^{c}

*Mean values ± standard deviation (n=4) with different superscript letters (a-d) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

In terms of color, the color profile of all pancake samples was shown in Table 4.14. The lightness (L*) of the control sample (CT) (62.37±0.91) has the lightest sample which is significantly higher L* than all PP samples including PP-3 (55.10±1.24), PP-2 (42.96±0.48), and PP-1 (38.52±0.94), respectively. To clarify, the darker color of PP samples could come from the higher protein content in the sample that could cause browning as the Maillard reaction occurs with the presence of sugar and protein under heat, so this could cause them to be darker as they are more brownish. On the other hand, in terms of redness (a*), PP-1 (19.65±0.48) and PP-2 (18.88±0.43) significantly redder than PP-3 (13.99±1.38) and CT (11.45±0.47), respectively. This could be due to the fact that both PP-1 and PP-2 contain whey powder in the pancake, so lactose present in the whey powder might be an additional sugar to bind with the protein that could cause further browning reaction to occur than other samples with less available sugar. Furthermore, for the yellowness (b*), there is no significant difference among all the samples from control up to 20% of chicken breast powder substitution.

As a result of this, all the L*, a*, b* values can be proved and calculated for the quantification of color in several values including chroma (c*), hue angle (h*), whiteness index (WI), yellowness index (YI), and brownness index (BI), and those results corresponded to the CIELAB color space. To illustrate, although control sample (CT) has the highest L*, it exhibited the lowest a* value, so it gives less yellowness and brownness in color, resulting in hue angle (h*) that is closer to 90° which is represented in yellow color and significantly less chroma (c*) compared to other pancake samples. Unlike CT, even though PP-1 has the lowest L*, it exhibited the highest a* value, so it gives more redness in color, resulting in hue angle (h*) that is closer to 0° which is represented in the red color compared to other samples. Furthermore, this could also contribute to the highest brownness value of PP-1; consequently, this can confirm that the lactose present in the whey powder might be an additional sugar to bind with the protein in the pancake that could cause further browning reaction to occur than other

samples with less available sugar. Therefore, lactose, which is the simple sugar, can further form complexes with the amino acids in the pancake such as melanoidins, resulting in distinct brownish color and flavor development. On the contrary, in terms of whiteness (WI), there is no significant difference among the whiteness for all the samples from control up to 20% of chicken breast powder substitution since it correlates the preference of the consumers for white color or the absence of color that consumers see (Figure 4.8).

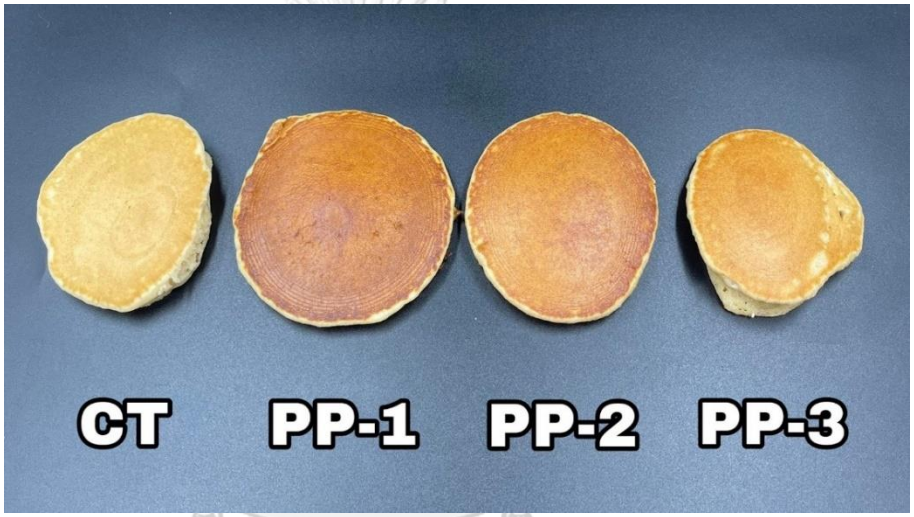

Figure 4.8 Color of fortified pancake samples including CT (Control), PP-1, PP-2, and PP-3

Chapter V

5.1 Conclusion

In conclusion, vacuum drying can be considered as a better choice compared with other drying methods. It requires the lowest drying time, and gives the lowest moisture content and water activity, resulting in higher protein content on a dry basis. For the color, CBL exhibited the most yellowish and brownish color due to longer drying time and steam involved, resulting in extension of browning reaction during drying process. For water absorption capacity, chicken breast powder all showed the lowest WAC values at pH 6. Low pressure superheated steam maintains uniform porous structure and exhibits higher oil absorption capacity. As a result, correlated to absorption capacity, all CB samples exhibited lower water solubility index, lower hygroscopicity, and definitely lower protein solubility than whey due to the higher density of the powder, larger particle size of intact muscle proteins compared to hydrolysate powder, more porous structure, less surface area to disperse in the solution. For foaming and emulsifying, chicken breast powder significantly exhibited very low value compared to whey powder since it requires more protein unfolding or denaturation to be more flexible to form interfacial membranes to create and to open more hydrophobic site to help emulsify oil-water phases. In the future, this study could guide the possibility of chicken breast powder to be further investigated for other potential functional properties that can help improve functions in a particular food product as an alternative protein powder.

For the application of chicken breast powder in pancake, for the PP-3 which has the highest chicken breast powder substitution of 20%. Each cooked pancake was approximated to be 40 grams in weight, so 8 grams of chicken breast powder could additionally yield at least 7.2 grams of protein found in the powder. Subsequently, according to Thai Dietetic Association. (2020), since the average protein requirement per kg/day is 1.00 g, at least seven PP-3 (280 g of pancake batter) could be sufficient for the recommended daily intake (RDI) for an adult with an average weight of 50 kg. For the texture profile analysis, PP-3 sample does not

show significant difference in hardness and cohesiveness between protein pancake (PP) samples, so it could be a good protein powder to substitute whey powder to maintain some of texture attributes as in protein pancake. In terms of color, unlike PP samples with the addition of whey powder that seems to be too dark brown in color due to Maillard reaction, PP-3 seems to be lighter and less brownish to be more golden-brown pancake, which could be more preferable and more correlated with the control (CT) sample. As a result of all, the obtained data or information gained from this study could be beneficial for further development of the chicken breast powder with drying techniques and further enhancing the application of the powder in potential food products that require alternative protein sources that contain good amino acid content.

5.2 Recommendations for the future work

This study serves the effect of the drying process on functional properties of chicken breast powder and application in protein pancakes to be used as alternative protein powder instead of whey powder in lactose intolerant people and could be also further implemented in developments of potential food products. Therefore, it is recommended that:

1. Further in-depth studies of the quality of proteins that exist in CB samples such as digestibility scores should be further studied.
2. Besides, the microbiological analysis of the CB samples such as microbial count should be evaluated to ensure its safety.
3. Additionally, sensorial properties, sensory perception, and consumer preference towards the fortified pancakes should be evaluated.
4. Lastly, the examination of the effects of chicken breast powder on physiological adaptations of high-intensity functional training (HIFT) with a certain period of time to follow up should be determined in order to compare the outcomes with whey powder on body composition, muscle, force, strength and performance of human body, especially athletes or bodybuilders.

Appendix A

1. Protein Determination by using Kjeldahl method

Table 1. Standardization of HCl solution

	Trial	Weight of sample (g)	Volume of HCl solution used (ml)	Normality of HCl solution (N)	% Protein (Dry weight basis)
Conventional hot air drying	1	1.0067	10.2	1.02 x 10-2	91.64
	2	1.0020	10.2	1.02 x 10-2	92.51
	3	1.0075	10.2	1.02 x 10-2	91.49
	Average	1.0054	10.2	1.02 x 10-2	91.88
Vacuum drying	1	1.0028	10.5	1.05 x 10-2	94.82
	2	1.0014	10.5	1.05 x 10-2	95.07
	3	1.0081	10.5	1.05 x 10-2	93.79
	Average	1.0041	10.5	1.05 x 10-2	94.56
Low pressure superheated steam drying	1	1.0061	9.8	0.98 x 10-2	89.74
	2	1.0044	9.8	0.98 x 10-2	90.03
	3	1.0051	9.8	0.98 x 10-2	89.96
	Average	1.0052	9.8	0.98 x 10-2	89.91

Protein Analysis

Appendix 1: Calculation of percent nitrogen and percent protein using Kjeldahl method

CBH Trial 1

Percent nitrogen

- % N = $\frac{Normality\ HCl \times corrected\ acid\ volume\ (ml) \times 14\ g\ (N) \times 100}{g\ of\ sample}$
- Corrected acid volume = (ml standard acid for samples) - (ml standard for blank)
- Corrected acid volume = 10.2 - 0 = 10.2 ml
- Mole of HCl used

  1000 ml = 1 mole

  10.2 ml = X mole

  Thus, X = $\frac{10.2}{1000} \times 1 = 1.02 \times 10^{-2}$

% N = $\frac{1.02 \times 10^{-2}\ mole}{1.0067\ g\ of\ sample} \times \frac{14\ g\ N}{mole} \times 100$ = 14.18 %

Percent Protein

- ❑ % Protein = $\% N \times Protein\ factor$
- ❑ Protein factor for meat = 6.25
- ❑ Moisture content = 3.92 %

% Protein on wet weight basis

% Protein = 14.18 % ✕ 6.25 = **88.63 % wet weight basis**

% Protein on dry weight basis

- ❑ Moisture content = 3.92%
- ❑ Dry weight basis = $\frac{100-3.92}{100} \times 1.0067\ g\ of\ wet\ basis\ sample$

= 0.9672 g

% Protein = 88.63 % / 0.9672 g dry solids

% Protein = **91.64 % dry weight basis**

CBH Trial 2

Percent nitrogen

- % N = $\frac{Normality\ HCl \times corrected\ acid\ volume\ (ml) \times 14\ g\ (N) \times 100}{g\ of\ sample}$
- Corrected acid volume = (ml standard acid for samples) - (ml standard for blank)
- Corrected acid volume = 10.2 - 0 = 10.2 ml
- Mole of HCl used

  1000 ml = 1 mole

  10.2 ml = X mole

  Thus, X = $\frac{10.2}{1000} \times 1 = 1.02 \times 10^{-2}$

% N = $\frac{1.02 \times 10^{-2}\ mole}{1.0020\ g\ of\ sample} \times \frac{14\ g\ N}{mole} \times 100$ = 14.25 %

Percent Protein

- ❑ % Protein = $\% N \times Protein\ factor$
- ❑ Protein factor for meat = 6.25
- ❑ Moisture content = 3.92 %

% Protein on wet weight basis

% Protein = 14.25 % ✕ 6.25 = **89.06 % wet weight basis**

% Protein on dry weight basis

- ❑ Moisture content = 3.92%
- ❑ Dry weight basis = $\frac{100-3.92}{100} \times 1.0020\ g\ of\ wet\ basis\ sample$

= 0.9627 g

% Protein = 89.06 % / 0.9627 g dry solids

% Protein = **92.51 % dry weight basis**

CBH Trial 3

Percent nitrogen

- % N = $\frac{Normality\ HCl \times corrected\ acid\ volume\ (ml) \times 14\ g\ (N) \times 100}{g\ of\ sample}$
- Corrected acid volume = (ml standard acid for samples) - (ml standard for blank)
- Corrected acid volume = 10.2 - 0 = 10.2 ml
- Mole of HCl used

  1000 ml = 1 mole

  10.2 ml = X mole

  Thus, X = $\frac{10.2}{1000} \times 1 = 1.02 \times 10^{-2}$

% N = $\frac{1.02 \times 10^{-2}\ mole}{1.0075\ g\ of\ sample} \times \frac{14\ g\ N}{mole} \times 100$= 14.17 %

Percent Protein

- ❑ % Protein = $\% N \times Protein\ factor$
- ❑ Protein factor for meat = 6.25
- ❑ Moisture content = 3.92 %

% Protein on wet weight basis

% Protein = 14.17 % ✕ 6.25 = **88.56 % wet weight basis**

% Protein on dry weight basis

- ❑ Moisture content = 3.92%
- ❑ Dry weight basis = $\frac{100-3.92}{100} \times 1.0075\ g\ of\ wet\ basis\ sample$

= 0.9680 g

% Protein = 88.56 % / 0.9680 g dry solids

% Protein = **91.49 % dry weight basis**

CBV Trial 1

Percent nitrogen

- % N = $\frac{Normality\ HCl \times corrected\ acid\ volume\ (ml) \times 14\ g\ (N) \times 100}{g\ of\ sample}$
- Corrected acid volume = (ml standard acid for samples) - (ml standard for blank)
- Corrected acid volume = 10.5 - 0 = 10.5 ml
- Mole of HCl used

  1000 ml = 1 mole

  10.5 ml = X mole

  Thus, X = $\frac{10.5}{1000} \times 1 = 1.05 \times 10^{-2}$

% N = $\frac{1.05 \times 10^{-2}\ mole}{1.0014\ g\ of\ sample} \times \frac{14\ g\ N}{mole} \times 100$= 14.68 %

Percent Protein

- ❑ % Protein = $\% N \times Protein\ factor$
- ❑ Protein factor for meat = 6.25
- ❑ Moisture content = 3.62 %

% Protein on wet weight basis

% Protein = 14.68 % ✕ 6.25 = **91.75 % wet weight basis**

% Protein on dry weight basis

- ❑ Moisture content = 3.62%
- ❑ Dry weight basis = $\frac{100-3.62}{100} \times 1.0014\ g\ of\ wet\ basis\ sample$

= 0.9651 g

% Protein = 91.75 % / 0.9651 g dry solids

% Protein = **95.07 % dry weight basis**

CBV Trial 2

Percent nitrogen

- % N = $\frac{Normality\ HCl \times corrected\ acid\ volume\ (ml) \times 14\ g\ (N) \times 100}{g\ of\ sample}$
- Corrected acid volume = (ml standard acid for samples) - (ml standard for blank)
- Corrected acid volume = 10.5 - 0 = 10.5 ml
- Mole of HCl used

  1000 ml = 1 mole

  10.5 ml = X mole

  Thus, X = $\frac{10.5}{1000} \times 1 = 1.05 \times 10^{-2}$

% N = $\frac{1.05 \times 10^{-2}\ mole}{1.0028\ g\ of\ sample} \times \frac{14\ g\ N}{mole} \times 100$= 14.66 %

Percent Protein

- ❑ % Protein = $\% N \times Protein\ factor$
- ❑ Protein factor for meat = 6.25
- ❑ Moisture content = 3.62 %

% Protein on wet weight basis

% Protein = 14.66 % ✕ 6.25 = **91.63 % wet weight basis**

% Protein on dry weight basis

- ❑ Moisture content = 3.62%
- ❑ Dry weight basis = $\frac{100-3.62}{100} \times 1.0028\ g\ of\ wet\ basis\ sample$

= 0.9664 g

% Protein = 91.63 % / 0.9664 g dry solids

% Protein = **94.82 % dry weight basis**

CBV Trial 3

Percent nitrogen

- % N = $\frac{Normality\ HCl \times corrected\ acid\ volume\ (ml) \times 14\ g\ (N) \times 100}{g\ of\ sample}$
- Corrected acid volume = (ml standard acid for samples) - (ml standard for blank)
- Corrected acid volume = 10.5 - 0 = 10.5 ml
- Mole of HCl used

  1000 ml = 1 mole

  10.5 ml = X mole

  Thus, X = $\frac{10.5}{1000} \times 1 = 1.05 \times 10^{-2}$

% N = $\frac{1.05 \times 10^{-2}\ mole}{1.0081\ g\ of\ sample} \times \frac{14\ g\ N}{mole} \times 100$= 14.58 %

Percent Protein

- ❑ % Protein = $\% N \times Protein\ factor$
- ❑ Protein factor for meat = 6.25
- ❑ Moisture content = 3.62 %

% Protein on wet weight basis

% Protein = 14.64 % ✕ 6.25 = **91.13 % wet weight basis**

% Protein on dry weight basis

- ❑ Moisture content = 3.62%
- ❑ Dry weight basis = $\frac{100-3.62}{100} \times 1.0081\ g\ of\ wet\ basis\ sample$

= 0.9716 g

% Protein = 91.13 % / 0.9716 g dry solids

% Protein = **93.79 % dry weight basis**

CBL Trial 1

Percent nitrogen

- % N = $\frac{Normality\ HCl \times corrected\ acid\ volume\ (ml) \times 14\ g\ (N) \times 100}{g\ of\ sample}$
- Corrected acid volume = (ml standard acid for samples) - (ml standard for blank)
- Corrected acid volume = 9.8 - 0 = 9.8 ml
- Mole of HCl used

  1000 ml = 1 mole

  9.8 ml = X mole

  Thus, X = $\frac{9.8}{1000} \times 1 = 0.98 \times 10^{-2}$

% N = $\frac{0.98 \times 10^{-2}\ mole}{1.0061\ g\ of\ sample} \times \frac{14\ g\ N}{mole} \times 100$ = 13.64 %

Percent Protein

- ❑ % Protein = $\% N \times Protein\ factor$
- ❑ Protein factor for meat = 6.25
- ❑ Moisture content = 5.58 %

% Protein on wet weight basis

% Protein = 13.64 % ✕6.25 = **85.25 % wet weight basis**

% Protein on dry weight basis

- ❑ Moisture content = 5.58%
- ❑ Dry weight basis = $\frac{100-5.58}{100} \times 1.0061\ g\ of\ wet\ basis\ sample$

= 0.9500 g

% Protein = 85.25 % / 0.9500 g dry solids

% Protein = **89.74 % dry weight basis**

CBL Trial 2

Percent nitrogen

- % N = $\frac{Normality\ HCl \times corrected\ acid\ volume\ (ml) \times 14\ g\ (N) \times 100}{g\ of\ sample}$
- Corrected acid volume = (ml standard acid for samples) - (ml standard for blank)
- Corrected acid volume = 9.8 - 0 = 9.8 ml
- Mole of HCl used

  1000 ml = 1 mole

  9.8 ml = X mole

  Thus, X = $\frac{9.8}{1000} \times 1 = 0.98 \times 10^{-2}$

% N = $\frac{0.98 \times 10^{-2}\ mole}{1.0044\ g\ of\ sample} \times \frac{14\ g\ N}{mole} \times 100$ = 13.66 %

Percent Protein

- ❑ % Protein = $\% N \times Protein\ factor$
- ❑ Protein factor for meat = 6.25
- ❑ Moisture content = 5.58 %

% Protein on wet weight basis

% Protein = 13.66 % ✕6.25 = **85.38 % wet weight basis**

% Protein on dry weight basis

- ❑ Moisture content = 5.58%
- ❑ Dry weight basis = $\frac{100-5.58}{100} \times 1.0044\ g\ of\ wet\ basis\ sample$

= 0.9484 g

% Protein = 85.38 % / 0.9484 g dry solids

% Protein = **90.03 % dry weight basis**

CBL Trial 3

Percent nitrogen

- % N = $\frac{Normality\ HCl \times corrected\ acid\ volume\ (ml) \times 14\ g\ (N) \times 100}{g\ of\ sample}$
- Corrected acid volume = (ml standard acid for samples) - (ml standard for blank)
- Corrected acid volume = 9.8 - 0 = 9.8 ml
- Mole of HCl used

  1000 ml = 1 mole

  9.8 ml = X mole

  Thus, X = $\frac{9.8}{1000} \times 1 = 0.98 \times 10^{-2}$

% N = $\frac{0.98 \times 10^{-2}\ mole}{1.0051\ g\ of\ sample} \times \frac{14\ g\ N}{mole} \times 100$ = 13.66 %

Percent Protein

- ❑ % Protein = $\% N \times Protein\ factor$
- ❑ Protein factor for meat = 6.25
- ❑ Moisture content = 5.58 %

% Protein on wet weight basis

% Protein = 13.66 % ✕6.25 = **85.38 % wet weight basis**

% Protein on dry weight basis

- ❑ Moisture content = 5.58%
- ❑ Dry weight basis = $\frac{100-5.58}{100} \times 1.0051\ g\ of\ wet\ basis\ sample$

= 0.9490 g

% Protein = 85.38 % / 0.9490 g dry solids

% Protein = **89.96 % dry weight basis**

2. Fat Determination by using Soxhlet method

Table 2. *Percent fat of Soxhlet extracted contents from chicken breast powder after extraction for 6 hours, air dry for 24 hours, and oven at 100 °C for 2 hours done in duplicate*

Sample	Blank	CBH	CBV	CBL
Average %Fat	-	8.59±0.28	7.66±0.16	9.81±0.38

Note: "-" represents no obtainable data

*Weight of water in chicken breast powder is excluded

Fat Analysis

Appendix 2: Raw data of fat analysis using Soxhlet extraction method

Table 3. *The weight of each component before Soxhlet extraction (CBH)*

Content	Weight of CBH Trial 1 (g)	Weight of CBH Trial 2 (g)	Weight of CBH Trial 3 (g)
Thimble	3.8057	3.3817	3.3896
Thimble + Powder	6.8348	6.4135	6.4127
Powder (wet basis)	3.0291	3.0318	3.0231
Water in powder (3.92%)	0.1187	0.1188	0.1185
Powder (dry basis)	2.9104	2.9130	2.9046
% Fat	8.93	8.24	8.61

Table 4. *The weight of each component before Soxhlet extraction (CBV)*

Content	Weight of CBV Trial 1 (g)	Weight of CBV Trial 2 (g)	Weight of CBV Trial 3 (g)
Thimble	4.0756	2.9124	2.8281
Thimble + Powder	7.1007	5.9343	5.8542
Powder (wet basis)	3.0251	3.0219	3.0261
Water in powder (3.62%)	0.1095	0.1093	0.1095
Powder (dry basis)	2.9156	2.9126	2.9166
% Fat	7.55	7.55	7.89

Table 5. *The weight of each component before Soxhlet extraction (CBL)*

Content	Weight of CBL Trial 1 (g)	Weight of CBL Trial 2 (g)	Weight of CBL Trial 3 (g)
Thimble	3.3895	3.3820	3.8055
Thimble + Powder	6.4356	6.4318	6.8822
Powder (wet basis)	3.0461	3.0498	3.0767
Water in powder (5.58%)	0.1700	0.1702	0.1717
Powder (dry basis)	2.8761	2.8796	2.9050
% Fat	9.39	9.72	10.32

Table 6. *The weight of each component after Soxhlet extraction for 4 hours and air dry oven at 105 °C for 2 hours (CBH)*

Conventional hot air oven drying	Content	Weight of CBH Trial 1 (g)	Weight of CBH Trial 2 (g)	Weight of CBH Trial 3 (g)
Petroleum ether with fat round bottom flask	Round bottom flask	166.83	170.03	150.74
	Round bottom flask + fat	167.09	170.27	150.99
	Fat	**0.26**	**0.24**	**0.25**

Table 7. *The weight of each component after Soxhlet extraction for 4 hours and air dry oven at 105 °C for 2 hours (CBV)*

Vacuum drying	Content	Weight of CBV Trial 1 (g)	Weight of CBV Trial 2 (g)	Weight of CBV Trial 3 (g)
Petroleum ether with fat round bottom flask	Round bottom flask	172.61	153.95	169.13
	Round bottom flask + fat	172.83	154.17	169.36
	Fat	**0.22**	**0.22**	**0.23**

Table 8. *The weight of each component after Soxhlet extraction for 4 hours and air dry oven at 105 °C for 2 hours (CBL)*

Vacuum drying	Content	Weight of CBL Trial 1 (g)	Weight of CBL Trial 2 (g)	Weight of CBL Trial 3 (g)
Petroleum ether with fat round bottom flask	Round bottom flask	170.03	172.61	169.13
	Round bottom flask + fat	170.30	154.89	169.43
	Fat	**0.27**	**0.28**	**0.30**

Appendix 3: Calculation of fat analysis using Soxhlet extraction method

Calculation of components' weight before Soxhlet extraction

- Weight of powder (wet basis) (g.) = Weight of thimble with powder (g) - Weight of thimble (g)
- Weight of water in powder (g) = $\frac{3.92}{100}$ ×Weight of powder (wet basis) (g.)
- Weight of powder (dry basis) (g.) = Weight of powder (wet basis) (g.) - Weight of water in powder (g)

Calculation of components' weight after Soxhlet extraction

- Weight of powder in thimble-with-powder beaker

  Powder (g.) = Weight of powder with beaker and thimble (g) - Weight of beaker (g) - Weight of thimble (g.)

- Weight of fat in petroleum-ether-with-fat beaker

  Fat (g) = Weight of fat with beaker (g) - Weight of beaker (g)

Calculation of % Fat content

- % Fat content as dry weight basis from weight of chicken breast powder with beaker

  % Fat =

$$\frac{Initial\ dry\ weight\ of\ powder\ with\ beaker\ (g) - Final\ weight\ of\ powder\ with\ beaker\ (g)}{Initial\ dry\ weight\ of\ powder\ with\ beaker\ (g)} \times 100$$

- % Fat content from weight of extracted fat

$$\% \text{ Fat} = \frac{Weight\ of\ fat\ from\ solvent\ (g)}{Initial\ dry\ weight\ of\ powder\ (g)} \times 100$$

3. Ash Determination

Table 9. *Raw weight on both wet and dry basis before and after ashing method at 550 °C for 24 hours, moisture content, and average percent ash on dry basis of triplicate chicken breast powder (CBH)*

CBH	Trial 1		Trial 2		Trial 3	
	Before	After	Before	After	Before	After
Weight of sample (g)	2.5172	0.0520	2.6525	0.0543	2.6100	0.0491
% Ash	2.738		2.48		2.648	
Average % Ash ±SD	2.00±0.10					

Table 10. *Raw weight on both wet and dry basis before and after ashing method at 550 °C for 24 hours, moisture content, and average percent ash on dry basis of triplicate chicken breast powder (CBV)*

CBV	Trial 1		Trial 2		Trial 3	
	Before	After	Before	After	Before	After
Weight of sample (g)	2.6831	0.0868	2.6448	0.0715	2.6787	0.0556
% Ash	3.235		2.703		2.076	
Average % Ash ±SD	2.67±0.58					

Table 11. *Raw weight on both wet and dry basis before and after ashing method at 550 ^{o}C for 24 hours, moisture content, and average percent ash on dry basis of triplicate chicken breast powder (CBL)*

CBL	Trial 1		Trial 2		Trial 3	
	Before	After	Before	After	Before	After
Weight of sample (g)	2.6262	0.0457	2.5476	0.0462	2.5545	0.0520
% Ash	1.740		1.813		2.036	
Average % Ash	1.86±0.15					

Appendix 4: Ashing calculation

- % Ash

$$\% \text{ Ash} = \frac{Weight\ of\ sample\ after\ ashing\ (g)}{Initial\ weight\ of\ dry\ sample\ (g)} \times 100$$

- Average % Ash

$$\text{Average \% Ash} = \frac{\%\ ash\ 1 + \%\ ash\ 2 + \%\ ash\ 3}{3}$$

4. Antioxidant activities (Standard curves)

4.1 Total phenolic content

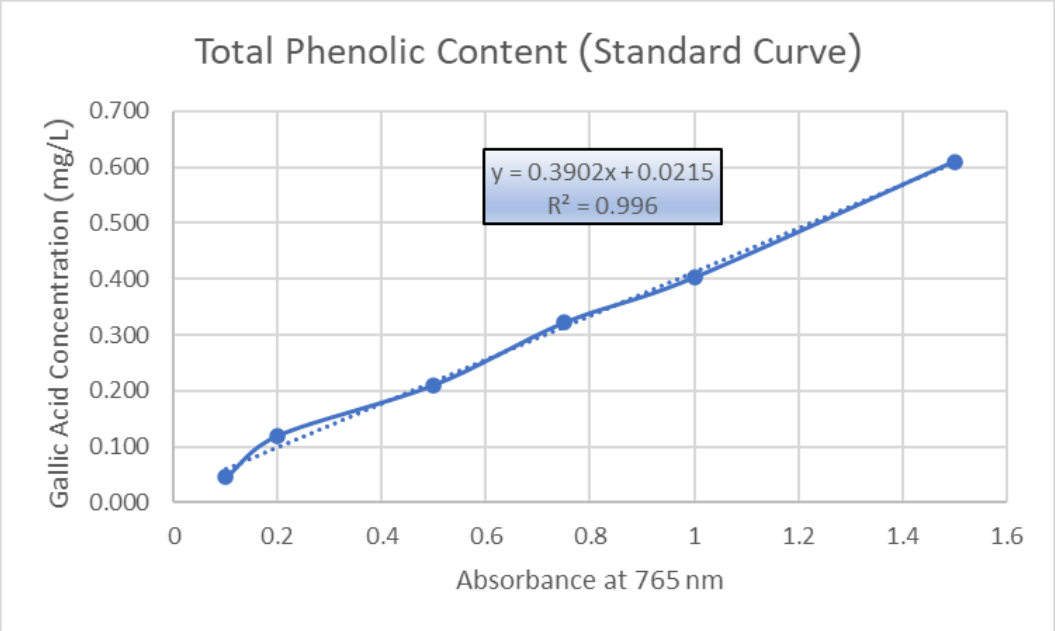

Figure A-1. Standard curve of total phenolic content using gallic acid

4.2 Ferric reducing antioxidant power (FRAP)

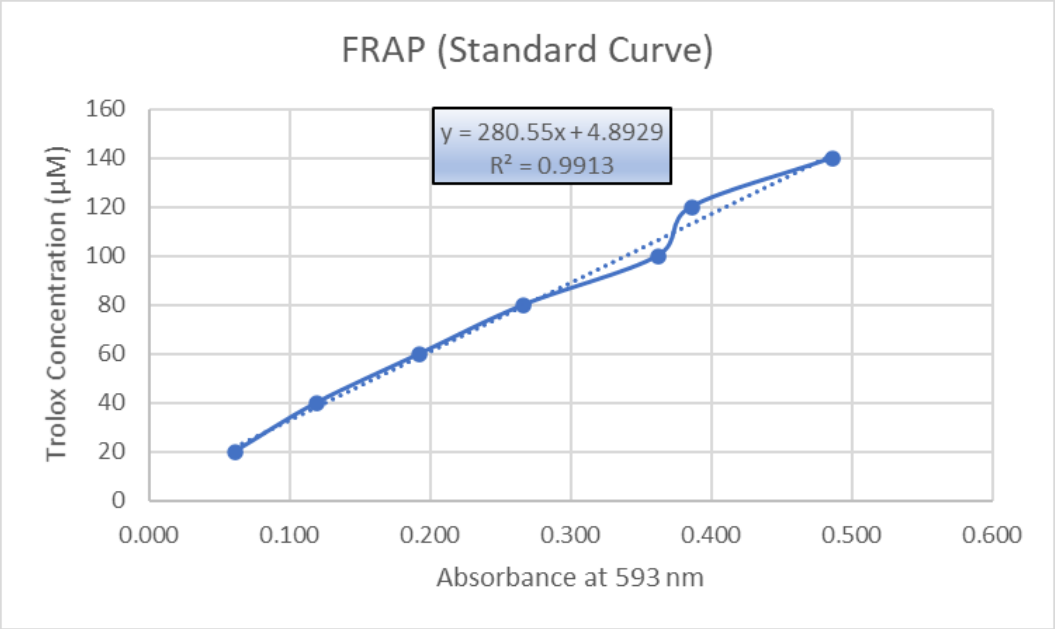

Figure A-2. Standard curve of FRAP assay

4.3 2,2-diphenyl-1-picrylhydrazyl (DPPH)

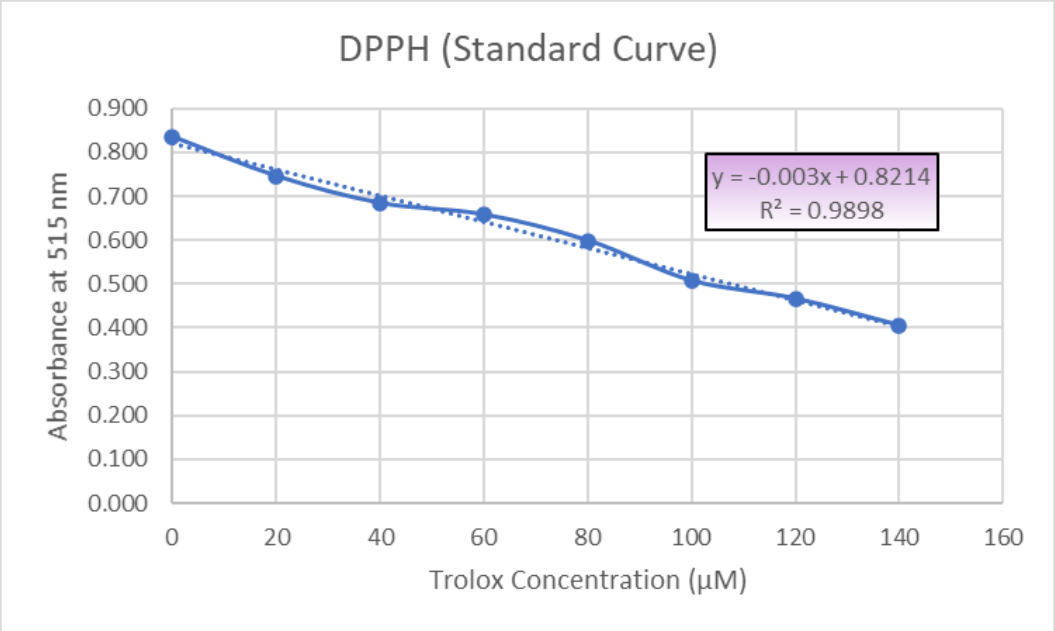

Figure A-3. Standard curve of DPPH assay

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