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EXAMINATION OF DIGESIBILITY AND SENSORY PROPERTIES OF HIGH PROTEIN CREAM

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1. Introduction

Protein is an essential macromolecule made up of amino acids, which is crucial in a healthy diet (Millward, 2012). The structure of amino acids determines their digestibility and bioavailability to the human body (Ajomiwe et al., 2024). Furthermore, protein is a vital macronutrient since it helps tissue growth, enzymatic function, immune defense, maintaining muscle mass, and overall metabolism (Wu, 2016). In addition, protein directly influences the flavor of food by contributing the aroma compound and colors during the production, processing, and storage of food (Damodaran, 2008).

Nowadays, foods with high protein content are seen to be functional foods that provide health benefit (Banovic et al., 2018). Especially egg white protein, also known as albumen, is considered as a complete protein and natural functional food knowing that its superior digestibility, bioavailability (Puglisi & Fernandez, 2022,Avirineni et al., 2022). Each of proteins in egg white have a different structure and functional properties, which can play an important role in food industries such as confectionary, food cakes, and enriched high protein beverages and cream (Razi et al., 2023;Chang et al., 2018).

In recent years, especially the young generation, there are concerns a lot of the quality and nutritional value of food products. They are willing to buy a product which contains a high protein content (Baker et al., 2022). Furthermore, plant-based (white rice protein) and animal-based (whey protein concentrate and collagen peptide beef) can influence people choosing the product based on quantity and types of proteins (Rovai et al., 2025). Therefore, it is necessary to evaluate both the sensory characteristics and digestibility of this product. While digestibility guarantees that the protein is nutritional score and sensory analysis helps determine the overall impression in product for consumer.

Even though there is a growing interest in foods that are high in protein, there is limited research that focuses increasing protein on egg-white protein products. This thesis is to examine the digestibility and sensory properties of high-protein Totu cream enriched with different protein sources and evaluate protein affects the physicochemical including dry matter, pH, color, rheology and sensory, as well as to determine in-vitro protein digestibility (IVPD%). The findings are expected to contribute to the development of functional high-protein foods suitable for athletes and general consumers.

2. Literature Review

2.1 Protein source

Proteins are polymers of amino acids linked via α peptide bonds composed of small building blocks that are linked together in long, linear chains and consist of 20 kinds of amino acids. They can be represented as primary, secondary, tertiary, and even quaternary structures (Watford & Wu, 2018). Moreover, protein is an essential macronutrient for human growth, repair, and immune function (Li et al., 2007).

Different protein sources may be used to make high-protein creams, and each source has its own nutritional value, digestibility, and usefulness. The choice of protein affects not just the makeup of the amino acids but also how the texture behaves and how it affects the overall sensory quality. There are many alternative proteins, such whey protein, and are still the most common ones used in dairy products(Zeng et al., 2024). Moreover, some reference also shows how plant proteins are an option for lactose-free, vegan and allergens friendly (Liu et al., 2024).

2.1.1 Egg white protein

Eggs have found and become used in food industry, making them an essential part of the human diet worldwide. Eggs have three main components which are the shell, the egg yolk and the white (Chang et al., 2018). The white part makes up 58% of its volume, followed by the yolk (31%), and the shell (11%). The whole egg is a mixture of proteins, water, carbohydrate, fat, ash, and cholesterol (Anton, 2013).

The primary components of egg whites are water (88%), protein (10.5%), carbohydrates (0.5%), ash (0.8%), and fats (0.2%)(Campbell et al., 2003; Ma et al., 2022). Different types of proteins are present in egg white proteins, ovalbumin is one the most abundant protein found in egg white, based on highest total protein (54%), other proteins including ovotransferrin (12%), ovomucioid (11%), lysozyme and ovomucin (3.5%) (Ma et al., 2019; Razi et al., 2023).

On the other hand, egg white products are free from gluten and lactose and contain almost zero carbohydrates. These characteristics are increasing in the market of consumer who has lactose intolerant, allergic disease, or gluten free (Tóth et al., 2019). Moreover, in this studied (Schmidt et al., 2007) have said that dry heating on vitro digestibility of proteins is necessary for good nutrition. That can be proved on my results for digestion part as well.

2.1.2 Whey Protein Concentrate

Whey proteins are another major protein fraction in milk which is classified from milk by two major proteins: casein (insoluble) and whey protein (soluble) which is the liquid left after milk curdling therefore, whey is formed as a by-product in the dairy product processing (Sangwan & Seth, 2021). It consists mainly of water (94%), lactose, proteins and fats. 70–80% of all proteins found in whey mass are α-lactalbumin and β-lactoglobulin. Other components include bovine serum albumin (BSA) and bovine lactoferrin (BLF), immunoglobulins (IG) and lactoperoxidase (LP) (Madureira et al., 2007). Whey protein has been confirmed that can influence the protection of the human body against different types of cancer, such as breast, colon or prostate gland cancer. monal and cell-mediated immune reactions (Kusio et al., 2020).

There are three types of whey protein: Whey protein concentrate (WPC), whey protein isolate (WPI) and whey protein hydrolysate (WPH). Among these, whey protein concentrate (WPC) are commonly used in dairy-based products due to the balance of fat and cholesterol, higher bioactive components(Athira et al., 2013; Blome et al., 2003). Additionally, WPC is a common option for food formulations due to balanced composition, moderate taste, high soluble, and has great functional features, such as the ability to emulsify, gel, and foam (Kankanamge et al., 2015).

2.1.3 Collagen peptides beef

Collagen is the most abundant form of structural protein in the body. The primary role of collagen is to maintain connective tissue health and mechanical properties of the muscle and skin by high concentration of glycine, proline and alanine (Ricard-Blum, 2011). Collagen is hydrolyzed enzymatic it is broken down into tiny bioactive peptide that are easy for body to absorb in digestive tract (Khatri et al., 2021),

The primary source of animal protein extraction is bovine (cow or beef) because of its availability and biocompatibility(Santos et al., 2013). It can be extracting collagen from a variety of tissues, including bones, tendons, lung tissue, and even connective tissue (Darine et al., 2010; Ferraro et al., 2017). Even if collagen peptides are good for your health but overconsumed can be harmful for our health therefore concern of protein intake in dairy is the recommendation (Millward, 2002).

For that reason, animal proteins, especially bovine collagen peptide, were used in my experiment due to their excellent bioactivity (Albenzio et al., 2017).

2.1.4 Plant based protein

Plant based proteins considered as vegan, they are high in fiber, polyunsaturated fatty acids, carbohydrate and a lot of amino acids, are easily absorbed by the body, and may help cure a variety of diseases. Consequently, they are mostly linked to a decrease in cardiovascular illnesses, low-density lipoprotein (LDL) cholesterol levels, and obesity (Guasch-Ferré et al., 2019). However, they still lack some essential amino acids, for instance cereal (rice, barley, wheat) contain less lysine (Nosworthy et al., 2017).

Rice or (*Oryza sativa L.*) is a staple food and a main major protein source around the world mainly in Asian (Muthayya et al., 2014). The protein digestibility and biological value of rice have been reported to be higher than those of the other major cereals such as corn, barley and wheat (Amagliani et al., 2017).

Rice protein is gaining a lot of interest in the food industry due to its unique properties. Moreover, proteases break down the protein to produce various potential peptide sequences providing numerous functional and also enhance the antioxidative properties of native protein by attacking the peptide bonds in the interior of polypeptide chains producing a range of polypeptides that differ in molecular weight or amino acid sequences (Phongthai et al., 2017)

2.1.5 Enriched innovation of high protein

Nowadays, adding protein has become very important from now on. It aims to provide consumer nutritional advantages to those who desire to boost protein (Ding et al., 2022). Therefore, high protein products such as cream, yoghurt and milk beverages or any dairy products are gaining popular for consumers this is because of social media by online might influence the knowledge about health benefits perception, as well as the concern daily protein intake (Ortega et al., 2024; Żulewska et al., 2025). Nevertheless, besides the nutritional of high protein we still need to concern sensory in the product as well. Being that case, it is necessary to investigate the digestibility of such products along with sensory properties to guarantee that they have met the nutritional value and at the same time remain acceptable to the consumers.

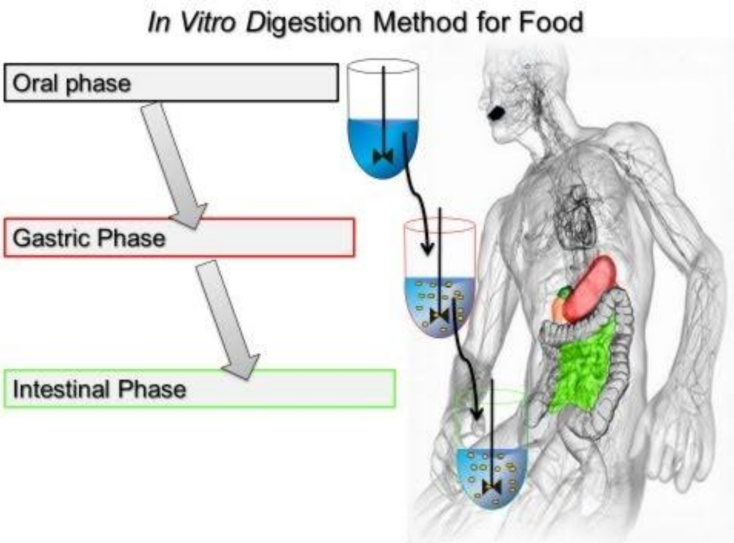
2.2 Digestibility and Bioavailability of Protein Sources

Digestibility and bioavailability are the main keys of protein quality and nutritional. Protein digestibility to ensure the amount of quality required to meet the human nutritional needs by enzymes in the gastrointestinal tract break down into amino acid and peptides in order to be absorbed (Loveday, 2023; Santos-Sánchez et al., 2024). Protein bioavailability describes how well dietary proteins can absorbed, used, and incorporated into the body metabolic functions and digestibility.(Minekus et al., 2014; Tang et al., 2009). Factors affecting protein bioavailability include protein sources, processing methods, and interactions with other dietary components. Recent investigations have investigated the bioavailability of specific proteins, including many plant-based proteins, and their potential to meet nutritional requirements. Some proteins (rice, oat, corn, and soy protein) are very low due to their particularly dense structure and stable tertiary structure, leading to a large amount of protein resources wasted (Santos-Sánchez et al., 2024; Yuanqing et al., n.d.)

2.2.1 Method for digestibility (INFOGEST)

Figure 1: Three phases of INFOGEST digestion method for food

(Source: Nature protocols, 2019)



Digestion is a breakdown of the food into small particles and the movement from the gastrointestinal into the body. Digestion of food simulations was made according to the INFOGEST protocol (Brodkorb et al., 2019). It divided it into three phases for digestive: oral, gastric and intestinal which have different conditions during each phase figure 1.

Oral phase is the first step in digestive which is preparing food for enzyme degradation by mastication and salivation. This step is the dilution of food with stimulated salivary fluid (SSF) by using with or without salivary amylase. In case of use, the limit is at pH 7 in 2 minutes at 37 °C. Secondly, **gastric phase** is an oral bonus that diluted 1:1 (vol /vol) with stimulated gastric fluid (SGF) and with enzymes such as pepsin and gastric lipase and then incubated under agitation at pH 3.0 for 2 hours at 37 °C. Lastly, the **intestinal phase** is the diluted 1:1 (vol/vol) with simulated intestinal fluid (SIF), bile salts and pancreatic enzymes and incubated at pH 7 for 2 hours at 37°C

2.2.2 Kjeldahl Analysis of protein contamination

The Kjeldahl method is one of the oldest and most accurate techniques for determining total nitrogen and calculating crude protein content in food products. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein). The Kjeldahl method is divided into three steps: digestion, distillation and titration. Digestion is a conversion of organic nitrogen to ammonium sulfate achieved by digesting the sample with concentrated sulfuric acid and it is the most consuming time in experiment. The purpose of this step is to break down the bonds that hold the polypeptides together, and convert them to simpler chemicals such as water, carbon dioxide and, of course, ammonia. The result is an ammonium sulfate solution. Distillation is to convert NH_4^+ to NH_3 by boiling and condensation of NH_3 gas in receiving solution. Titration is indicating the ammonia present in the distillate with a color change and allows for calculation of unknown concentrations by two types of titration method which are back titration and direct titration (Aguirre, 2023).

2.3 Physicochemical and Sensory Properties

Physicochemical properties describe the fundamental characteristics of food systems that can change the structure of food such as the hydration properties (water activity, water solubility), rheology behavior ,optical properties (color) can lead to influence the nutrition in food therefore, there is important parameter to know the quality of food (Igual & Martínez-Monzó, 2022). In this experiment , dry matter, pH, color, and rheological behavior were selected as keys indicators because they are the most important parameters used in the food industry to assess product quality and consistency.

Sensory evaluation is defined as a scientific field that analyzes and interprets human responses to food products as perceived through the senses of sight, smell, touch, taste, and hearing. It complements physicochemical, microbiological, and nutritional analyses to provide a more complete understanding of food characteristics and consumer preferences (Pop, 2023). Therefore a critical tool for determining the degree of acceptability in sensory and physicochemical both provide a strong agreement in scientific food safety and customer acceptance of new development in food fortification.

3. Methodology

3.1 Sensory properties

3.1.1 Pre-experiment

In the first stage of the experiment, two high proteins products which is egg white-based milk replacement “ToTu cream” produced by Capriovus (Szigetcsép, Hungary) was used as the main ingredient in our samples. A total of nine protein types were examined: collagen peptide (beef), collagen peptide (pork), branched-chain amino acids (BCAA), hemp protein powder, vegan protein powder, white rice protein, soy protein isolate, egg white protein, and whey protein concentrate.

Two types of Totu cream formulations were used, namely the standard Totu cream and the Rögös Totu cream, which differ in their ingredient composition. For the Totu cream, each sample had a total weight of 100 g and prepared according to the following recipes: 57 g of Totu cream, 25.65 g of berry juice, 12.35 g of sweetener, 5 g of protein

For the Rögös Totu cream, the total sample weight was 150 g and prepared it as following recipes: 65.25 g of Rögös Totu 21.75 g of cream, 21.75 g of sweetener, 36.25 g of mango juice and 5g of protein powder. Each of the nine protein types was incorporated into both formulations, the total was eighteen samples (nine Totu cream and nine Rögös Totu cream).

After that, a preliminary sensory evaluation was conducted in collaboration with my two supervisors. The aim of this sensory test was to select the best attributes such as texture, taste, smell, color and overall accessibility. The selected samples were then recommended for the next experiment.

Figure 2: The samples of nine different types of proteins

3.1.2 Final sample preparation

Three types of proteins that were selected from the previous experiment are collagen peptide beef, white rice protein and whey protein concentrate (WPC). The original (control) formulation consisted of 60 g Totu cream, 27 g berry fruit juice, and 13 g sugar. For the protein enriched samples, protein powders were added at different concentrations whey protein concentrate (WPC), peptide beef protein added 7.5% and white rice protein was 2.5%. To maintain a constant total weight of 100 g, the quantities of Totu cream, fruit juice, and sugar need to reduce based on the original formulation ratio (60:27:13). The protein powder replaced a portion of the original recipe by weight in table 1.

Figure 3: Totu cream

Figure 4: All ingredients used in the experiment

Table 1: Adjustment of cream samples (per 100 g)

Ingredient (g)	Original sample (g)	Whey protein sample (g)	Peptide beef sample (g)	White rice protein sample (g)
Totu cream	60	55.5	55.5	58.5
Fruit juice	27	25	25	26.3
Sugar	13	12	12	12.7
Whey protein	-	7.5	-	-
Beef peptide	-	-	7.5	-
Rice protein	-	-	-	2.5

3.1.3 Determine dry matter content

The dry matter content tells how much solid material is in the sample after the moisture has removed. or it shows the total quantity of the substance such as protein, carbohydrate, lipid and mineral that left after water evaporated.

The samples were determined approximately 1-2 g of each sample and dried in the oven at 105°C for two or three hours. Firstly, weight the empty dish and record it, secondly, add 1-2 g in each empty dish and record it and replicate it three times per sample. Lastly, put all samples into the oven and the final weight of dried sample is recorded also in order to calculate the percentage of dry matter content in following formula:

$$\text{Dry matter (\%)} = \frac{\text{Weight after drying (g)}}{\text{Intial weight of sample (g)}} \times 100$$

After that we can calculate the mean and standard deviation to show the results of the differences between three kinds of protein in Totu cream.

Figure 5: The samples after drying

3.1.4 pH measurements

The pH measurement was also used to evaluate the acidity level, which can affect microbial growth, taste, texture, protein interactions, and overall sensory perception. It also lets us know the concentration of hydrogen ion (H^+) and the scale from 0 to 14. If pH < 7 means the sample is acidic such as lemon juice and vinegar which has a citric and sour taste and, if pH > 7 means the sample is alkaline or basic such as baking soda or ammonia which has a strong chemical and pH is equal to 7 means the sample is neutral for example the pure water.

In this experiment, the pH of Totu cream samples performed using a calibrated digital pH meter. The device needs to be calibrated before using by standard buffer solutions of pH 4.00 and 7.00. The pH measurement was performed by inserting the electrode directly into the sample, and the value was recorded once a stable reading was obtained. All determinations were conducted at temperature (20–25 °C) in three replicates per each sample.

3.1.5 Color measurements

Color is the most important parameter on basic of which the quality of food material is judged and considered the most important physical attributes of food. The color of foods has been measured CIE lab or, L*a*b* it is a color space international for color measurement which stands for the commission international d'Eclairage. *L*,* the lightness or darkness of the color scale from 0-100, 0 means black and 100 means white. *a** are review green to red approximately -128 to + 127 therefore the negative value shows green color while a positive value shows red color. and *b** indicate blue to yellow so a negative value is blue, and a positive value is yellow the scale is also -128 to + 127.

In this experiment, the colorimeter is CR-400 is a handheld portable measurement instrument was performed. First of all, calibrate it in the buffer solution at pH 4 and pH 7 to ensure accurate reading value. After that, cover the instrument head with plastic wrapping paper and then place it directly into the surface of samples of Totu cream. Each sample measured replicate five times. The corresponding will display L*, a*, and b* values.

3.1.6 Sensory evaluation

The sensory evaluation is used to analyze based on human sensory perception which is carried out under double blind test by evaluating taste, texture, color, aroma and overall acceptability. In the enriched protein like Totu cream, the different types of protein that added might influence taste perception due to amino acid composition, flavor compounds or the bitterness from peptide breakdown.

The sensory evaluation was carried out by coding 231 (Whey protein concentrate 7.5%), 357 (collagen peptide beef 7.5%), and 961 (white rice protein 2.5%) using 9-point scales, where 1 represented *"dislike extremely"* and 9 represented *"like extremely"*. The four panelists participated in the evaluation. The samples were presented in a randomized order to avoid bias and to rate the color, smell, fruit taste, sweet taste, texture and overall impression.

The scores from four panels were collected and will be illustrated in the results section while using Radar graph to analyze which protein source from Totu cream is the most favorable.

3.1.7 Rheological properties

The rheological characteristics examine how materials deform and flow when influenced by an external force. In the food scientific terms, rheology shows how a product reacts to shear stress, which shows its viscosity, flow behavior, and texture. These qualities are very important for quality of a product, how well it works in processing, especially for semi solid samples like creams, yogurts, and spreads. Moreover, the different types of proteins in Totu cream with different concentrations, or how they interact with other elements like lipids or polysaccharides may change the viscosity or flow of a substance because the rheological behavior provides consistency and structure stability.

In this experiment, the samples determined by using Anton Paar MCR 92 rheometer at temperature 25 ± 1 °C. Anton Paar RheoCompass software (v 1.21.852) was used to control the measurements. The temperature of the rheological experiment was kept constant at 15°C. Shear stress was measured in increasing and decreasing shear rate intervals between 10 and 1,000 s^{-1} for 31 measurement points in each interval with a period of 3 second.

The Herschel-Bulkley model was used to analyze the flow curves (shear rate-shear stress diagrams) data of decreasing shear rate interval were analyzed using Excel solver.

Three samples were tested: whey protein concentrate (WPC, 7.5%), collagen peptide beef (7.5%), and white rice protein (2.5%). Each sample was analyzed 3 parallels per sample approximately 2–3 g of each cream sample was placed on the parallel plate geometry (50 mm diameter)

Figure 6: Anton Paar MCR 92 rheometer

All data of shear rate ($\dot{\gamma}$) and shear stress (τ) were exported from the rheometer software into Excel. The results were processed according to the Herschel–Bulkley model, as shown below:

$$\tau = \tau_0 + K \cdot \dot{\gamma}^n$$

where:

τ = shear stress (Pa)

τ_0= yield stress (Pa)

K= consistency coefficient (Pa·s^n)

n= flow behavior index (dimensionless)

If $n < 1$, the sample acts like cream and is shear-thinning (pseudoplastic); if $n > 1$, it is shear-thickening (dilatant); and if $n = 1$, it acts like a Newtonian fluid.

The Rheology Evaluation Process 2025 guideline used to evaluate the data. The Excel Solver tool was used to find the τ_0, K, and n values for three replicates by minimizing the sum of the squared discrepancies between the observed and model- predicted shear stress values. Then, the mean and standard deviation were found from three measurements.

The obtained parameters were used to describe the flow behavior of the Totu cream samples. The flow curves (shear rate vs. shear stress) were plotted to visualize the rheological response and to compare the influence of three protein and in the result part we can illustrate all results flow curve and viscosity curve in three different proteins samples.

3.2 Digestibility

The *in vitro* digestionsimulation wasconducted to evaluat the protein digestibility of Totu cream with three different types of proteins sources added: whey protein concentrate (WPC), collagen peptide beef and white rice protein. This method simulates enzymatic condition of the human gastrointestinal tract and reveals the digestibility of proteins.

3.2.1 Sample preparation

For the digestibility experiment, four samples were prepared as follows: a control sample without added protein, 7.5% whey protein concentrate (WPC), a sample with 7.5% beef peptide, and a sample with 2.5% white rice protein. Before digestion test, we figured out how much protein was in each formulation and wrote it down in grams per 100 g of product (g/100 g).

Noted that protein from Totu cream is 19 g and protein from powders are 80%. To standardize the protein input for the digestion experiment, each sample needs to be weighed to provide exactly 40 mg (0.04 g**)** of protein. After that the corrected protein was calculated by protein from Totu + protein from the protein powder as shown in (Table 2).

Table 2: Summary proteins of Totu + three different proteins powders

Sample	Totu cream (g)	Protein added in sample (g)	Protein from Totu cream (g)	Protein from Powder (g)	Corrected protein (g/100g)	Sample weight for digestion (g)
WPC	55.5	7.5	10.55	6	16.55	0.240
Peptide beef	55.5	7.5	10.55	6	16.55	0.240
White rice protein	58.5	2.5	11.12	2	13.12	0.230
Control	60	0	11.4	0	11.4	0.350

The required amount of each sample was weighed. To ensure a uniform total sample weight of 1.000 g, the remaining mass was adjusted by adding distilled water. The calculated sample and water masses are shown in (Table 3).

Table 3: Total amount of three proteins to be added in digestion

	m_{sample} (g)	To 1g (V_{water})
WPC (7.5%)	0.240	0.760
Peptide beef (7.5%)	0.240	0.760
White rice protein (2.5%)	0.300	0.700
Control (0%)	0.350	0.650

3.2.2 In Vitro digestion simulation

For oral phase, to the 1g of the sample, 0.80 mL of SSF (Stimulated Salivary Fluid), 5 µL of $CaCl_2(H_2O_2)$, and 0.195 mL of distilled water were added. Homogenized samples were incubated in an overhead shaker (Heidolph Reax 2, Heidolph Instruments, Schwabach, Germany) fitted inside a preheated drying cabinet (Memmert UNE300, Memmert GmbH, Schwabach, Germany) for 2 min at 37 °C. The total value should be 2 mL.

In the gastric phase, to the 2 mL of the sample, 1.28 mL of SGF (stimulated gastric fluid), 1 µL of $CaCl_2(H_2O_2)$, 0.32 mL of pepsin solution (2500 U/mL) and 0.300 mL of water was added, adjust in pH 3 and, the mixture was incubated in the overhead shaker at 37 °C for 2 hours and the total amount should be 4 mL.

For the small intestine phase, to the 4 mL of the sample, 2.70 mL of SIF (Stimulated Intestinal Fuild), 8 µL of $CaCl_2(H_2O_2)$, 8 µL of bile extract solution (160 mM in SIF), 1 mL of pancreatin solution (800 U/mL), required and 0.792 mL of water was added, adjust pH at 7 and mixture was incubated in the overhead shaker at 37 °C another 2 h, total amount should be 8 mL. After small intestinal digestion phase was completed.

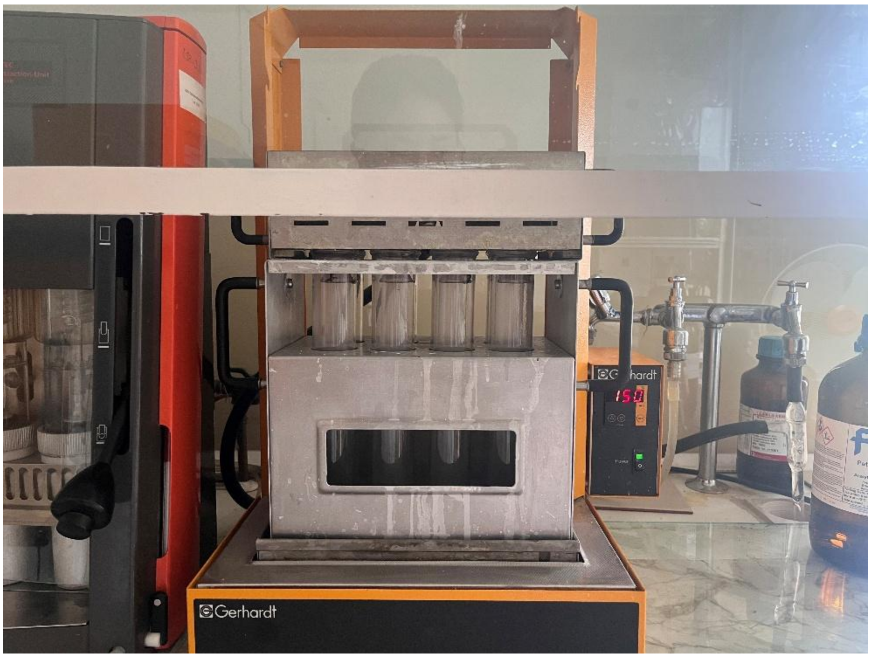
3.2.3 Isolation of the digested proteins

After compleeting the *in vitro* digestion 32 ml of methanol was added to all samples to precipitate undigested protein. The samples were handled by centrifugation in order to separate the soluble (bioaccessible) and insoluble (undigested) protein fractions (6000 rpm, 20 min, 4 °C). From the supernatant (soluble part) I transferred 30 ml of the solution into the new tube. The insoluble pellets (undigested part) were washed with 5 ml of methanol and centrifuged again at 6000 rpm for 10 minutes at 4 °C and repeat it one more time to ensure the complete remove of the soluble residue. Afterwards, pellets were dried.

3.2.4 Kjeldahl protein determination

The protein content of both supernatant and pellet was determined by using Kjeldahl method. Weight the samples from the supernatants and pellets 0.5 - 1 mL into digestive tubes, add 1 mL of $CuSO_4$, homogenize of K_2SO_4 as a catalyst, 20 mL of H_2SO_4 using cylinder. After that, place all the samples into Gerhardt (digestion heating) at different times and temperatures at following temperature: 50°C (5 min), 150°C (20 min), 250°C (20 min), 370°C (120 min). Tubes must be cooled down after finishing for 45 minutes.

Figure 7: Gerhardt (digestion heating)



Digested samples were distilled by using Gerhardt Vapodest 45s. Add distilled water 40 mL for all each digestive tube.

Figure 8: Gerhardt Vapodest (distillation)

Fill (0.05M H_2SO_4) into burette and add some drop of mix indicator (the color should be green) and then titrate it until the color changes purple and then record the V value to calculate IVPD% by following this formula:

$$\text{IVPD\%} = \frac{\text{Protein content in supernatant}}{\text{Protein content (supernatant + pellet)}} \times 100$$

Moreover, calculate RSD% or relative standard deviation to show how high or low in the results while replicates by following this formula:

$$\text{RSD\%} = \frac{\text{Standard deviation (SD)}}{\text{Mean}} \times 100$$

Figure 9: Color after adding mix indicator

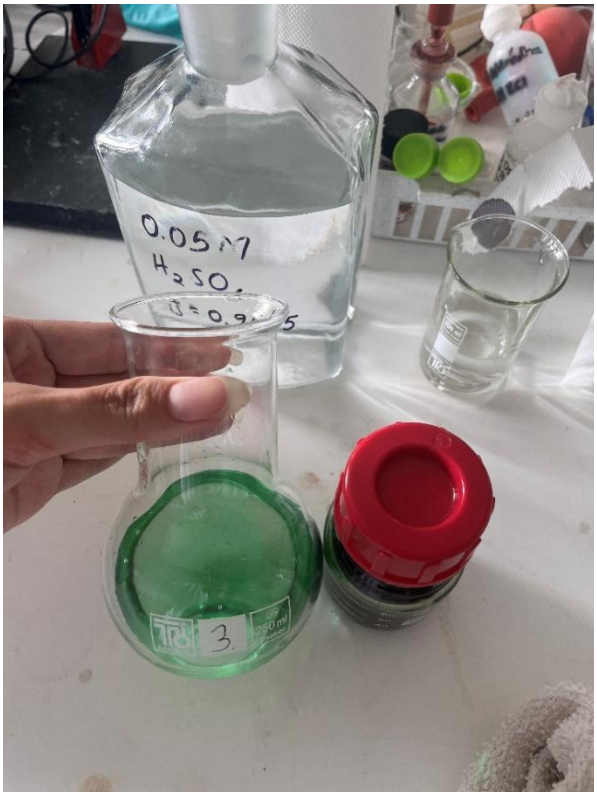


Figure 10: Color after titration with 0.05M $\mathbf{H_2SO_4}$

4. Result

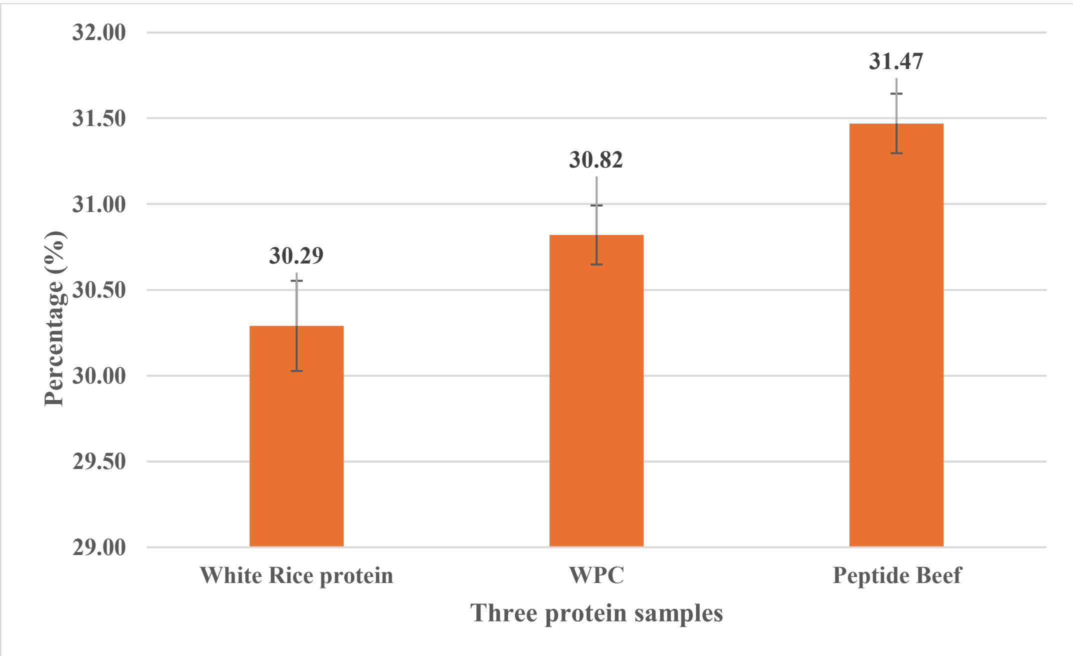
This chapter shows the results of the physicochemical, sensory, rheological, and *in vitro* digestibility tests on Totu cream samples that were made with three different protein sources. The results show an average and standardization of dry matter, the pH, the color parameters (L*, a*, b*), the sensory evaluation scores, the rheological behavior, and the *in vitro* protein digestibility (IVPD %) as well as replicate in every experiment to check the accurate.

4.1 Determine Dry matter content %

Table 4: Mean and SD of dry matter (%)

Sample	(Mean ± SD)
White rice protein	30.29 ± 0.26
Whey protein concentrate	30.82 ± 0.17
Collagen peptide beef	31.47 ± 0.17

Figure 11: Comparison of dry matter content of three samples



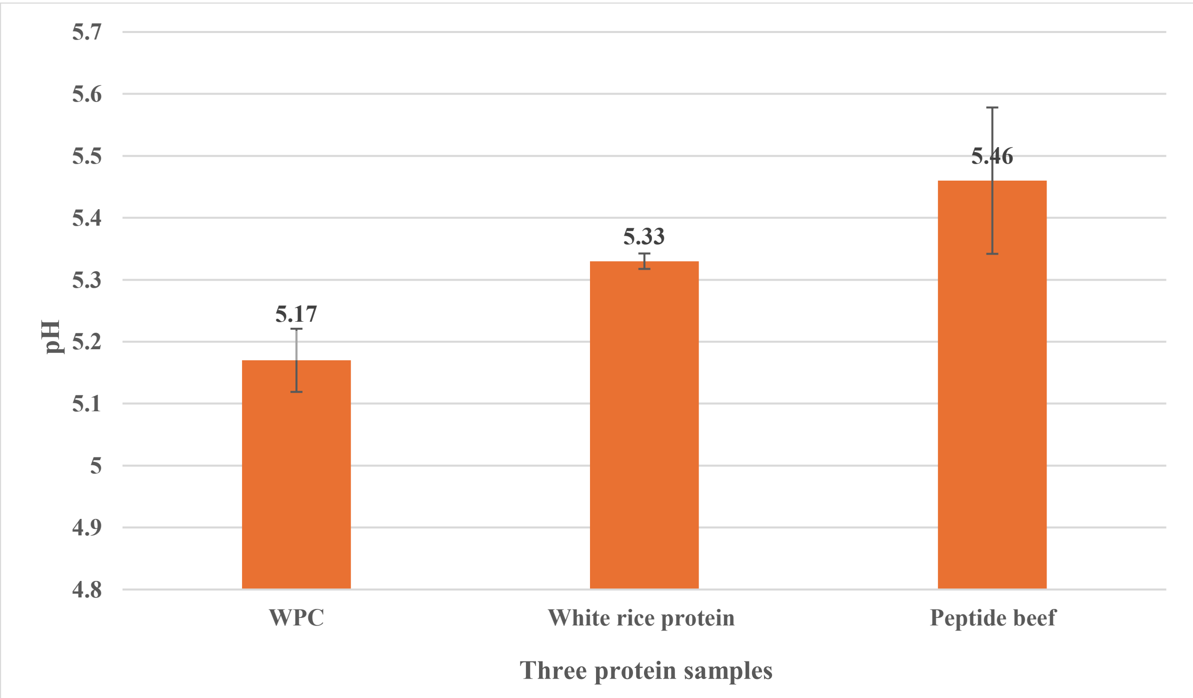
As a result, the highest dry matter content is collagen peptide beef (31.47 ± 0.17), followed by whey protein concentrate (30.82 ± 0.17) and the lowest is white rice protein (30.29 ± 0.26). The means of the collagen peptide beef contains a little more solid material and slightly less water than the others. Even though the values all seem close, all samples have a dry matter content around 30-31% which shows that the cream when added were quite similar and stable. The standard deviation (SD) ranging from 0.17 to 0.26, indicates that the results were consistent across replicates. Error bars in the graph represent the SD of each sample, confirming minimal variability among the triplicate measurements.

4.2 pH measurements

Table 5: Mean and SD of three sample in pH

Sample	(Mean ± SD)
Whey protein concentrate	5.17 ± 0.05
White rice protein	5.33 ± 0.01
Collagen peptide beef	5.46 ± 0.11

Figure 12: Comparison of three different proteins in pH



The pH readings of all the samples were between 5.17 and 5.47, which means that the formulations were only slightly different from each other. The collagen peptide beef sample had the greatest pH (5.47 ± 0.12), followed by the white rice protein sample (5.34 ± 0.01). The whey protein (5.17 ± 0.05).

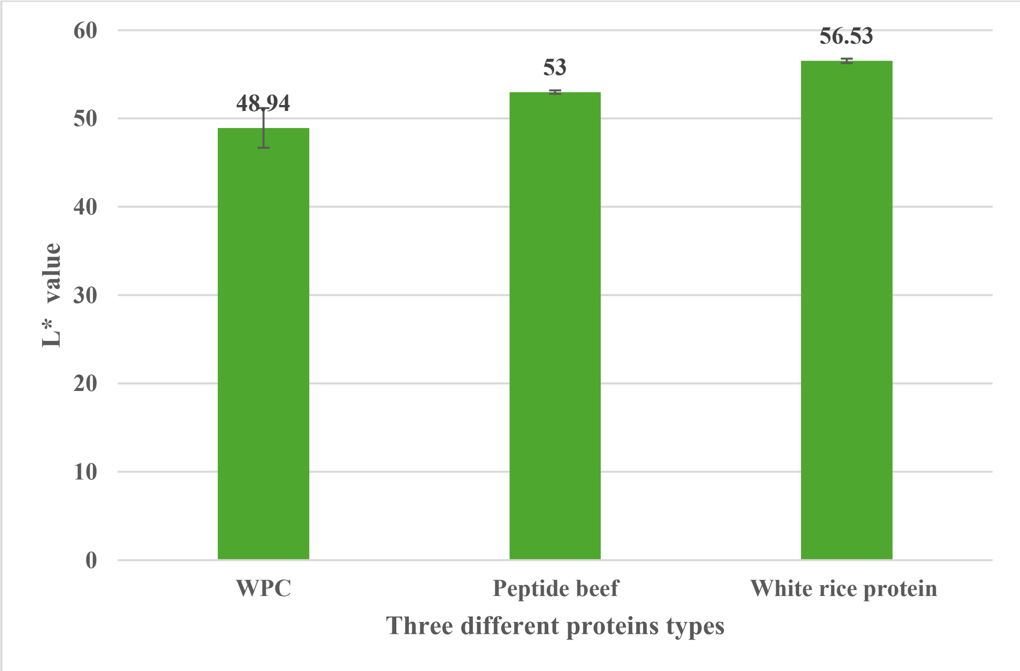
These findings show that adding various forms of protein had a little effect on the acidity of the Totu cream. In general, a pH value between 5 and 5.5 means that the product is acidic. This is common for creams and emulsions that are based on protein. The WPC sample lower pH might be because it contains acidic amino acids or leftover lactic acid, which are prevalent in whey-based foods. The peptide beef and rice protein samples, on the other hand, had somewhat higher pH values. This might be because peptides act as buffers and their composition has less acidic residues. The tiny standard deviations (0.01–0.12) show that the measurements were the same for all of the tests.

4.3 Color measurements results

Table 6: Mean and SD of color measurement (L* a* b*)

Sample	**(Mean ± SD) L***	**(Mean ± SD) a***	**(Mean ± SD) b***
Whey protein concentrate	48.94 ± 2.24	14.28 ± 0.40	0.75 ± 0.03
Collagen peptide beef	53 ± 0.19	14.02 ± 0.17	0.69 ± 0.03
White rice protein	56.53 ± 0.25	13.63 ± 0.08	1.06 ± 0.06

Figure 13: Comparison L* value of three proteins

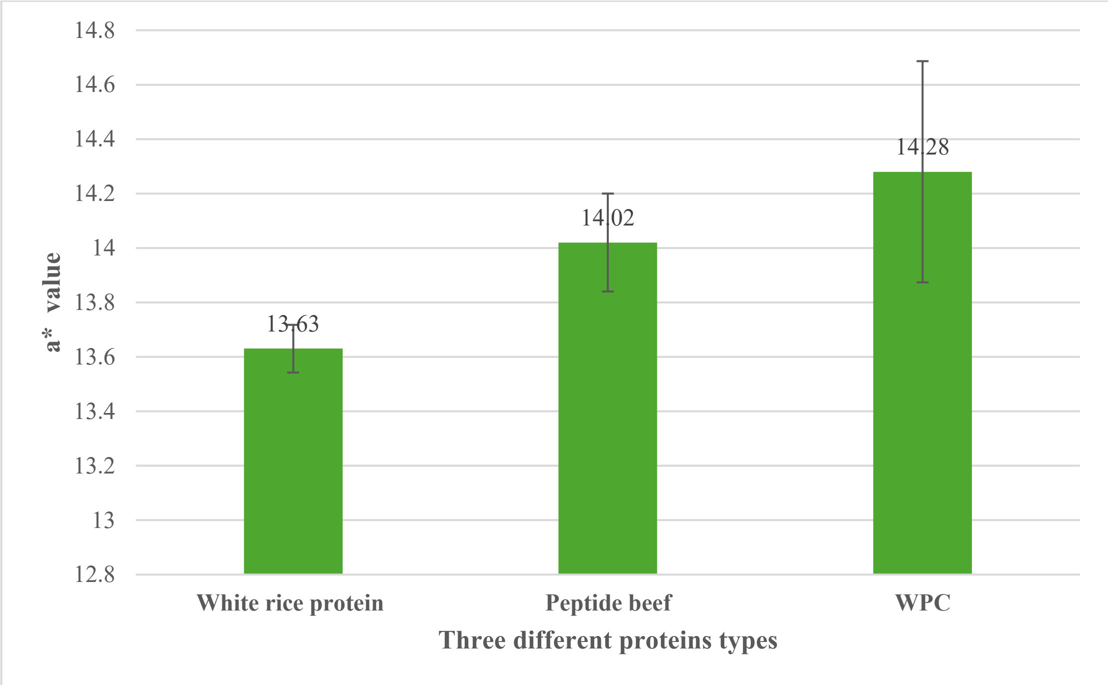


The L* value indicates how light or dark our samples. In this figure: The white rice protein sample showed the highest of the lightness (56.53 ± 0.25), followed by collagen peptide beef (53.01 ± 0.19), and the whey protein concentrate sample showed the lowest of the lightness (48.94 ± 2.24).

The white rice protein cream appears lighter, due to the natural pale color of rice protein powder. The collagen peptide beef protein sample showed moderate lightness, perhaps because of the slightly beige tone of hydrolyzed peptides. In contrast, the whey protein concentrate cream showed darker, which might be related to protein sugar interactions, as whey proteins are more reactive with reducing sugars.

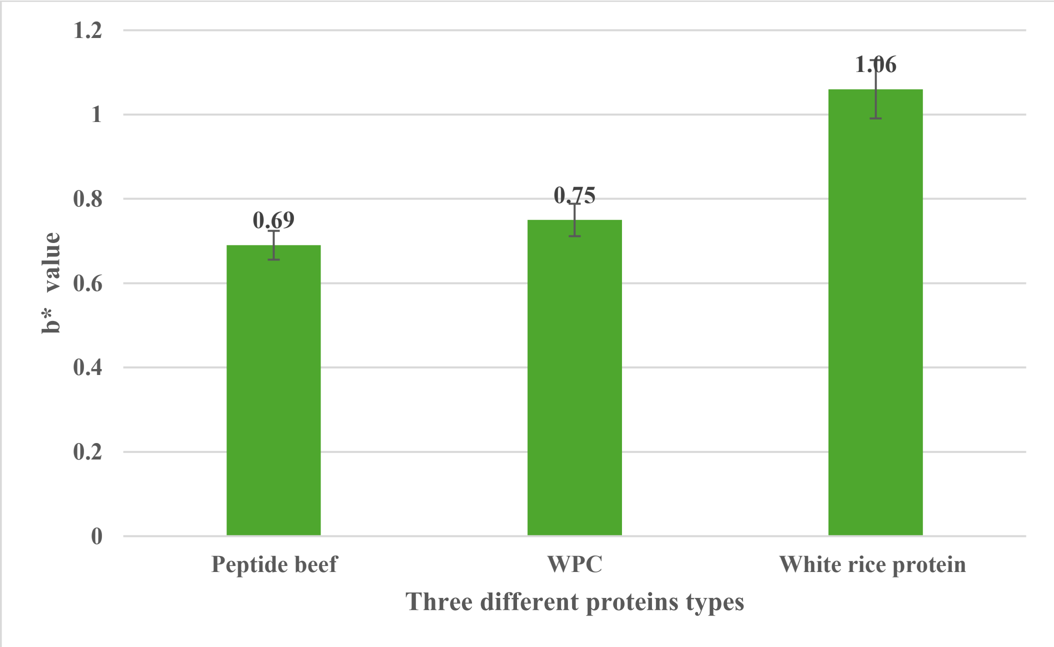
The standard deviations (0.19–2.24) indicate that the measurements were consistent. However, whey protein concentrate (WPC) has greater fluctuation because the sample might not be uniform enough.

Figure 14: Comparison a* value of three proteins



The a* parameter represents the color spectrum from green (negative values) to red (positive values). As shown in Table 6 and Figure 14, all samples exhibited positive a* values, indicating a slightly reddish tone in the Totu cream samples. In this figure, the whey protein concentrate (WPC) sample had the greatest value (14.28 ± 0.41), followed by the collagen peptide beef (14.02 ± 0.17) and the lowest value is white rice protein (13.63 ± 0.08). Therefore, the whey protein concentrate (WPC) is redder than the white rice protein and peptide beef due to the increased redness in the WPC sample could be attributed to Maillard reactions between whey proteins and residual lactose or sugars during preparation, which produce redness. On the other hand, the white rice protein has the least reaction with sugar, so the color seems lighter or less red pigment. The standard deviations (0.09–0.41) show that the color measurements were mostly the same in all replicates five times.

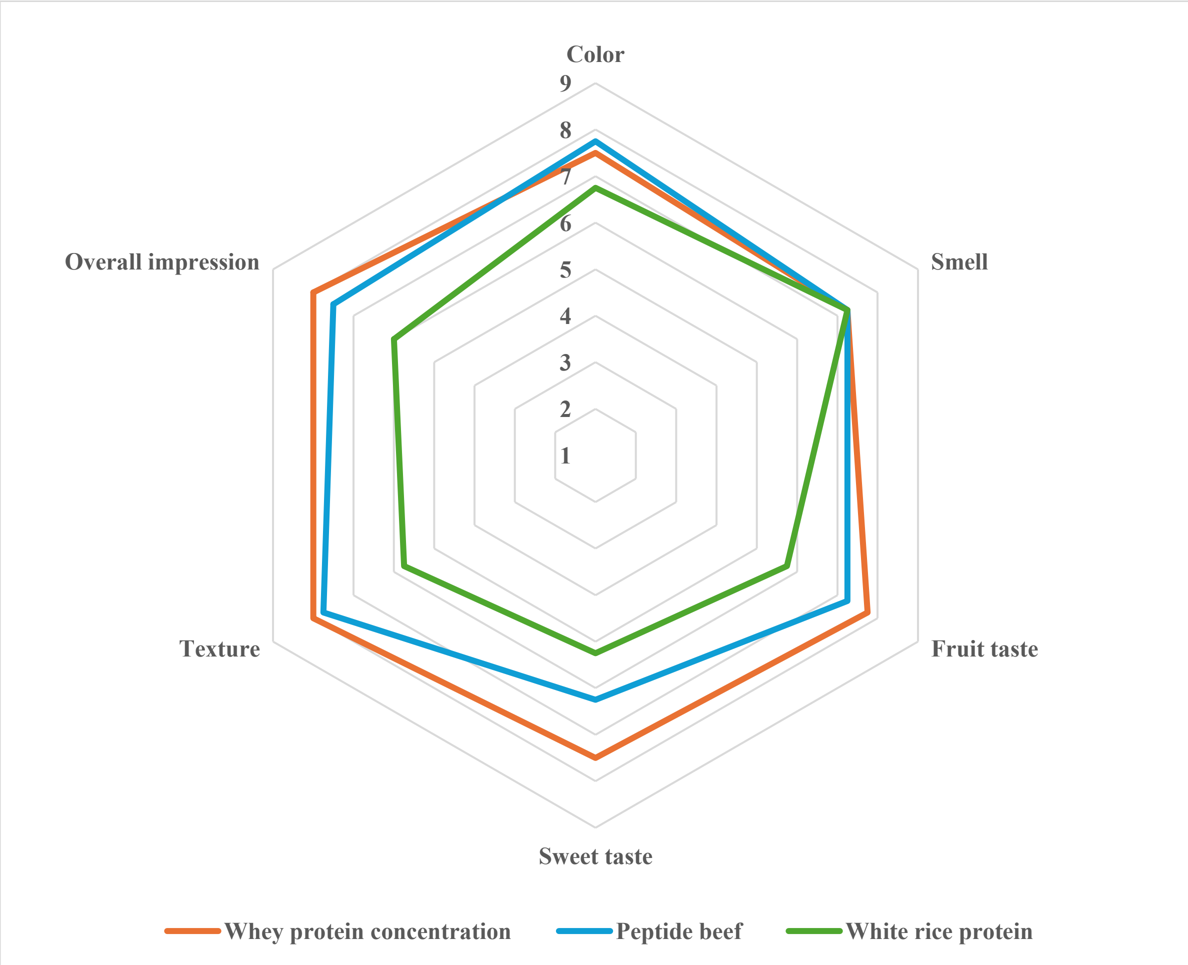
Figure 15: Comparison b* value of three proteins



All the samples had positive b* values which means all samples had a yellow color rather than blue color. In this figure the highest value is the white rice protein has the brightest yellow (1.06 ± 0.06), followed by whey protein concentrate had the medium yellowness (0.75 ± 0.0) and the lowest is the collagen peptide beef (0.69 ± 0.03). These findings indicate that the protein source affected the yellow pigment of the Totu cream. The white rice protein sample is higher yellow hue maybe because rice protein powder is naturally yellow and has a surface that reflects light, making it brighter. The peptide beef sample's lower b* value might be because the peptides are darker or because hydrolyzed amino acids give the peptides a brownish tint. The tiny standard deviations (0.03–0.07) show that all the color measurements were the same and could be repeated.

4.4 Sensory analysis results

Figure 16: Sensory evaluation of three proteins



A sensory evaluation was carried out to assess the consumer acceptability of Totu cream samples enriched with different protein sources. Four trained panelists evaluated each sample based on color, smell, fruit taste, sweet taste, texture, and overall impression using a 9-point scale (1 = dislike extremely, 9 = like extremely). The mean scores of each attribute are represented in figure: The radar chart shows that all samples sensory scores, with only minor differences between protein types. Overall, the whey protein concentrate (WPC) and collagen peptide beef samples achieved slightly higher overall impression and texture scores, while the white rice protein sample was rated lower in flavor and texture attributes.

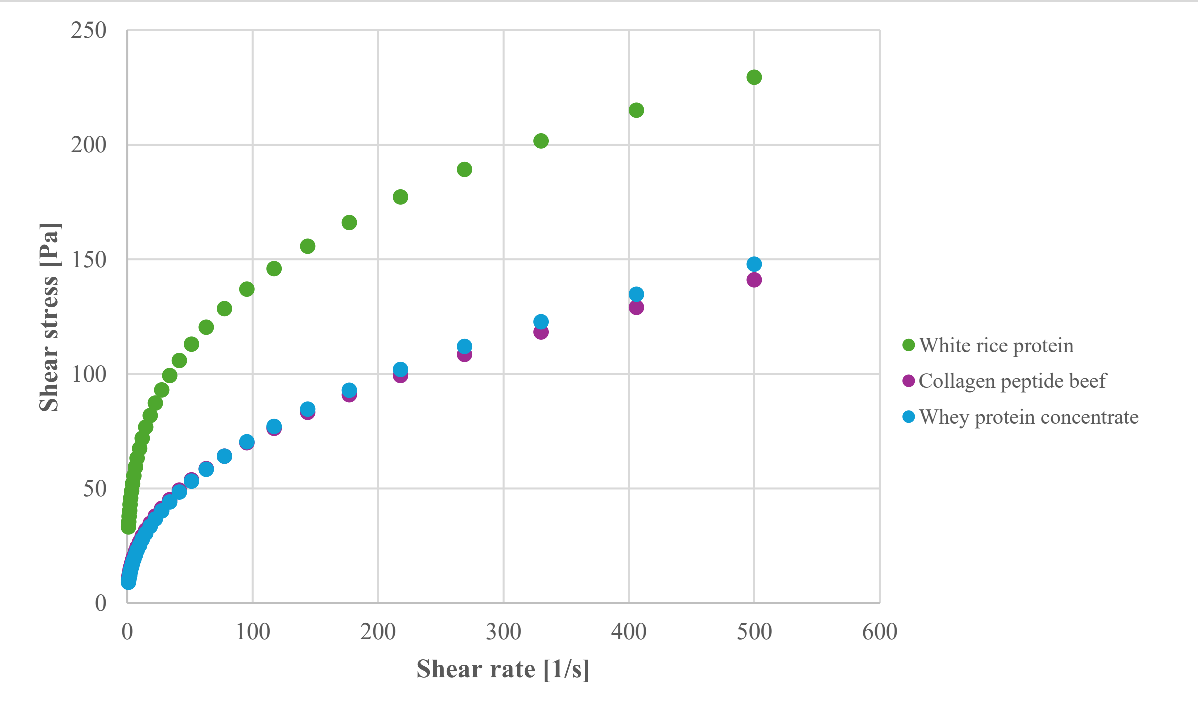
In color scored, all samples scored between 7 to 8 which means the color are acceptable. For the smell scores were relatively close 6 to 7. The peptide beef received a bit lower score due to the strong peptide aroma from beef compared to the whey protein concentrate and white rice protein scent. For fruit taste and sweet taste, the highest is the whey protein concentrate (7 to

8), which means smoother, while the white rice protein showed slightly bland taste (around 6). In case of the texture, whey protein concentrate also received the highest score of 7 to 8, while the white rice protein has the lowest score it might because of the thicker texture from the rice protein which might affect the mouthfeel while eating. Finally, the overall impression scored rate by WPC > Peptide beef > White rice protein. Therefore, the whey protein added in Totu cream was the most liked by panelists.

4.5 Rheology model variables

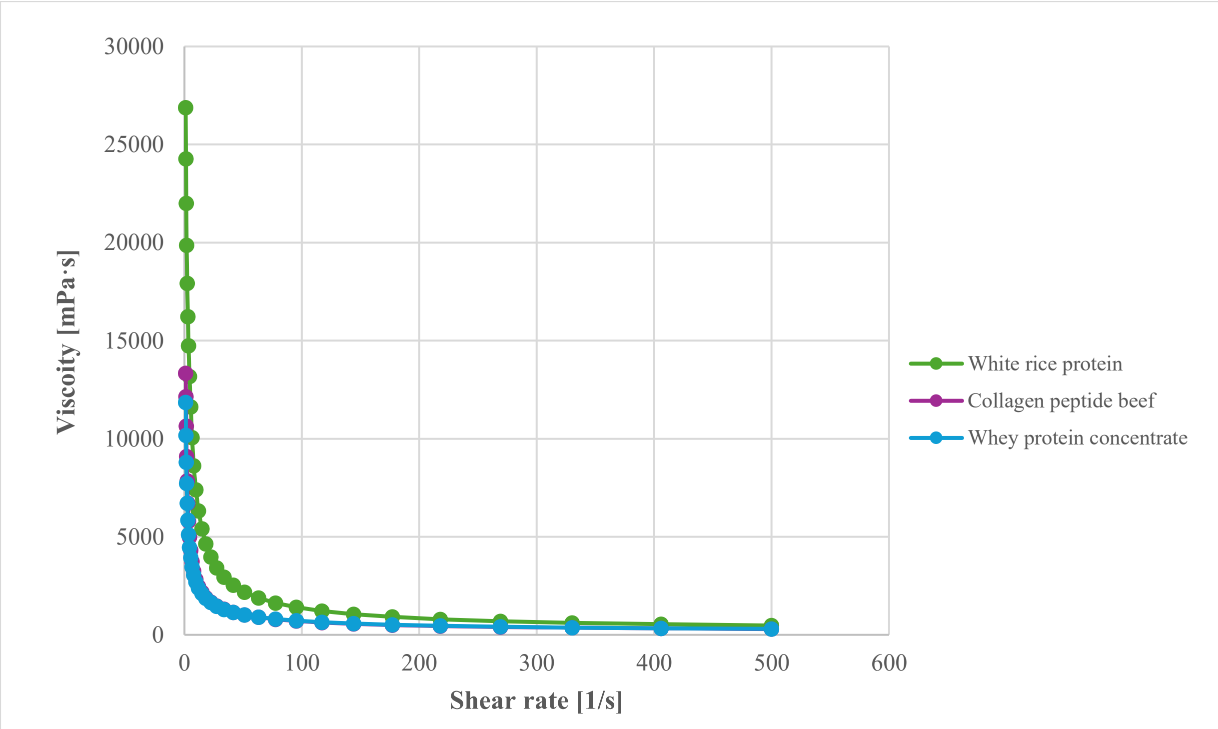
Rheological properties describe how the Totu cream behaves under applied force, providing information about its flow and texture characteristics. The flow curve (relationship between shear stress and shear rate) for the three protein-enriched samples is shown in Figure 17.

Figure 17: Comparison of flow curve in three proteins



This figure shows the shear stress (Pa) went up when the shear rate (1/s) went uo for all sample. This means that all the samples are non-Newtonian. Therefore, the viscosity of cream decreased when shear rate increased. This is common property of semi solid as a dairy product. The white rice protein (green curve color) has the highest apparent viscosity and the thickest consistency. The peptide beef (purple curve) had moderate viscosity that is lower than white rice protein but higher than whey protein concentrate. WPC or Whey protein concentrate (blue curve) has the lowest shear stress, which means the most fluid and easiest to spread.

Figure 18: Comparison of viscosity in three proteins



In this figure, the graph shows the viscosity of all samples dropped quickly as the shear rate went up which means a non-Newtonian, shear thinning (pseudoplastic). The white rice protein (red curve) shows the highest viscosity throughout the whole shear rate, indicate a thicker and less to flow easy. The collagen peptide beef (blue curve) showed moderate viscosity which means lower than white rice protein but greater than whey protein concentrate. Lastly, the whey protein concentrate (green curve) was the least viscous, which means it is the most fluid and easiest to spread. The graph illustrates the flow curve, and viscosity results that protein sources have a huge effect on rheological quality of the Totu cream such as texture, consistency and spread ability.

Table 7: Comparison of different proteins of the model Herschel-Bulkley

Sample	(Mean ± SD) τ_0 (Pa)	(Mean ± SD) K (Pa·s^n)	(Mean ± SD) n
Whey protein concentrate	0.11 ± 0.006	9.09 ± 0.57	0.44 ± 0.09
Collagen peptide beef	0.11 ± 0.004	10.19 ± 2.06	0.42 ± 0.019
White rice protein	0.22 ± 0.01	33.15 ± 1.96	0.31 ± 0.003

The white rice protein shows the highest yield stress (τ_0= 0.22 ± 0.01), suggesting that more force was required to start flow compared with whey protein (0.11 ± 0.006) and collagen peptide beef (0.11 ± 0.004). This implies a stronger internal structure or network formation within the cream matrix when white rice protein was added.

The consistency coefficient (K) of white rice protein (33.15 ± 1.96) was much higher than the other samples, indicating greater apparent viscosity and thicker texture. The whey protein and collagen peptide beef samples were lower (9.09 ± 0.57) and (10.19 ± 2.06) showing less viscous behavior.

For the flow behavior index (n), all samples had values less than 1 confirming that the creams behaved as pseudoplastic (shear-thinning) fluids.

4.6 Digestibility results

This chapter presents the results of the corrected protein content, *in vitro* protein digestibility (IVPD%) and relative standard deviation (RSD%) of Totu cream samples with different protein sources such as whey protein concentrate (7.5%), collagen peptide beef (7.5%), white rice protein (2.5%) and the control sample (no added protein powder). The IVPD% values were calculated from supernatant and the total protein (supernatant + pellet) that was obtained by the Kjeldahl method. The RSD% values are used to assess the precision of replicate measurements.

Table 8: *In vitro* protein digestibility results of four samples

Sample	Corrected protein content (g/100g)	IVPD%	RSD %
Whey protein concentrate	16.55	97.04 ± 0.21	0.22
Collagen peptide beef	16.55	96.2 ± 0.12	0.13
White rice protein	13.12	96.6 ± 0.04	0.04
Control	11.4	94.01 ± 0.91	0.96

Table 8 shows that the whey protein concentrate sample had the greatest digestibility value (97.04 ± 0.21%), followed by the white rice protein (96.6 ± 0.04%) and the collagen peptide beef (96.2 ± 0.12%). The control sample has the lowest digestibility value (94.01 ± 0.91%). Based on the statistical analysis (ANOVA), there was no significant difference for all samples ($p = 0.00026 < 0.05$) meaning that adding different protein types affected how well the cream could be digested. The control sample was significantly lower in digestibility compared with three fortified creams, while there was no major difference among the three fortified types themselves.

Whey protein concentrate (WPC) is known to contain easily hydrolysable peptides and proteins that can be efficiently broken down during enzymatic digestion, which could contribute to its higher IVPD% compared with other protein sources. Collagen peptide beef and white rice protein both had good digestibility ratings there are very close value, which means that peptide hydrolysates and plant-based proteins may be easily digested in egg white cream. The control sample is lower IVPD% may be because it has less total protein (11.4 g/100 g) and compare with the enriched formulations (13.12–16.55 g/100 g). This means that adding protein made the overall protein concentration and the digestible percentage better.

The relative standard deviation (RSD%) showed the value between 0.22% to 0.96%, which means that the results were consistent across the three samples. There was no significant difference ($p > 0.05$) which mean, all three samples were similarly digestible.

5. Discussion

In this thesis, all results that I enriched egg white protein cream with three different proteins source like whey protein concentrate (WPC), collagen peptide beef and white rice protein are given positive results in every experiment.

The dry matter content values are between (30.29–31.47%) shows that all formulations had comparable solid content and moisture stability. The slightly higher dry matter of collagen peptide beef (31.47 ± 0.17%) suggests stronger water-holding capacity due to the small molecular peptides that form a denser protein network, in agreement with findings by Albenzio et al. (2017).

The pH of all samples ranged from 5.17–5.46. Whey protein showed slightly lower pH (5.17 ± 0.05), possibly due to residual lactic compounds from the dairy origin, while collagen peptide beef had the highest (5.46 ± 0.11) means more neutral formulation that may enhance storage stability.

The white rice protein sample was the lightest (L* = 56.53 ± 0.25), reflecting the naturally pale color of rice protein, while WPC is the lowest lightness (48.94 ± 2.24) and the most reddish (a* = 14.28 ± 0.41), which could be attributed to Maillard reactions during preparation. The b* values indicated that all samples tended toward yellowish tones, with white rice protein being the brightest due to its native pigment and reflective structure.

All samples demonstrate non - Newtonian, shear-thinning (pseudoplastic) It is typical of semi-solid product. The white rice protein is the highest viscosity and yield stress (τ_0 = 0.225 Pa; K = 33.16 Pa·s^n), indicating a stronger internal gel network and thicker consistency. Collagen peptide beef showed moderate viscosity, while WPC displayed the lowest viscosity (K = 9.09 Pa·s^n), meaning it was the most spreadable and fluid. These rheological differences reflect how protein structure affects particle interactions hydrolyzed peptides form loose networks, while plant proteins tend to increase thickness due to insoluble fiber fractions.

The sensory results confirmed that WPC enriched cream was the preferred sample. It achieved the highest scores in texture, sweetness, and overall impression (average = 8.25 / 9), indicating that WPC improved smoothness and palatability. Collagen peptide beef was acceptable but had a slightly noticeable smell, it might be from peptide hydrolysates. White rice protein, despite its bright color, scored lower (6.85 / 9) in texture and taste due to its thicker and grainier mouthfeel, which is consistent with common sensory limitations of plant proteins.

Last experiment was digestibility. The *in vitro* protein digestibility (IVPD%) analysis revealed that all enriched formulations had high digestibility > 96%, confirming their suitability as functional high-protein foods. WPC showed the highest digestibility (97.04 ± 0.21%), attributed to its solubility and balanced amino acid profile. White rice protein and collagen peptide beef had similarly high values (96.6% and 96.2%), demonstrating efficient enzymatic breakdown. The control (94.01%) the lowest therefore it can be guaranteed that protein fortification increased total digestible protein. The RSD% values (10.72–16.06%) indicate acceptable repeatability by replicates. Due to similar values for both IVPD% and RSD% therefore there was no significant ($p = 0.00026 < 0.05$).

As I mentioned above, all three proteins that are used in the experiment have ability to develop protein fortification products in the future. Whey protein concentrates is the most effective with both sensory and digestibility.

However, there remains a gap that could be worked on in the future. In the sensory evaluation since I only have few people participated. For more efficient and focus more on the consumer we need more than 20 participants and also concerned about micro contamination.

Moreover, plant based is a good idea to use, but my idea is what if we could change the typical protein to edible insects' protein source product it is innovation is unique, and it can be sustainable food source in the future.

6. Conclusion

To sum up, this thesis work is to investigate the sensory properties and digestibility of Totu cream (mainly consist of egg white protein similar texture as butter cream) with three different types of proteins such as whey protein concentrate (7.5%), collagen peptide beef (7.5%) and white rice protein (2.5%). Firstly, physicochemical experiments that I measured for dry matter content, pH, color and rheology among three proteins sample and evaluated of in vitro protein digestibility (IVPD%).

In case of the dry matter contents, the collagen peptide beef is the highest dry matter (31.25 ± 0.09%) which means that low molecular weight of peptides could help the cream to hold the water. In pH, it shows that all three sample are very similar values range between (5.17–5.46). However, the highest pH is the white rice protein (5.46 ± 0.03) and lowest pH is WPC (5.17 ± 0.02) due to residual lactic compounds from whey.

Color measurements, White rice protein showed the highest L* or lightness (91.24 ± 0.18) that provide the brightest color, while the lowest was the collagen peptide beef (87.35 ± 0.22) because original beef pigment is dark compared to white rice pigment. The a* spectrum from green (negative values) to red (positive values) in the result all samples were giving a positive value. The highest is WPC (14.28 ± 0.41) because of Maillard reaction during preparation and lowest value is white rice protein (13.63 ± 0.08) has the least reaction with sugar. All the b* samples were positive which means all samples had a yellow color rather than blue color. The highest value is the white rice protein has the brightest yellow (1.06 ± 0.06) because it is naturally yellow and has a surface that reflects light, making it brighter, and the lowest is the collagen peptide beef (0.69 ± 0.03) it might be the peptides are darker.

The sensory evaluation conducted with four trained panelists evaluated each sample based on color, smell, fruit taste, sweet taste, texture, and overall impression using a 9-point scale (1 = dislike extremely, 9 = like extremely). The whey protein concentrate (WPC) got higher overall impression (8.25/9) while the white rice protein was the lowest (6.85/9).

Furthermore, the rheological behavior which is followed a pseudoplastic pattern that illustrate all samples were non - Newtonian means semi-solid in cream. The highest was white rice protein that has apparent viscosity and thickest consistency and the lowest shear stress, which means the most fluid and easiest to spread was the WPC we can see the graph in figure 17 and figure 18.

Over and above that, the digestibility experiment was carried out by INFOGET protocol. for *in vitro* protein digestibility (IVPD%) all three fortified had high digestibility. WPC showed the highest digestibility (97.04 ± 0.21%), White rice protein and collagen peptide beef had similarly high values (96.6% and 96.2%), demonstrating efficient enzymatic breakdown. The control (94.01%) the lowest therefore it can be guaranteed that protein fortification increased total digestible protein. The RSD% values 0.22% to 0.96% indicate acceptable repeatability by replicates. Due to similar values for both IVPD% and RSD% therefore there was no significant ($p = 0.00026 < 0.05$).

In conclusion, it can confirm that the protein fortification Totu cream with adding more nutritious, sensory varieties, and digestibility among all three different protein samples was suitable. Whey protein concentrate (WPC) was the best overall performance. It had a smooth texture, great overall impression by panelists, and excellent digestibility. Therefore, it can be considered as a new product development with high protein content and easy to digest in the human body.

7. Acknowledge

Working on this thesis is one of best achievements that I spent all my time and passion during two semesters researching and doing experiments in the laboratory. It is not only a thesis work experience but also a personal journey of growth, discovery, and exploring real life work experiences in the future as a food engineer.

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10. Declaration

DECLARATION

the public access and authenticity of the thesis

Student's name:	Chanphengxay Malisa
Student's Neptun code:	KF0CVM
Title of thesis:	The Examination of Digestibility and Sensory Properties of High Protein Cream
Year of publication:	2025
Name of the consultant's institute:	Tóth Dr. Adrienn Vargáné Dr. Judit Tormási
Name of consultant's deparment:	Department of Livestock and Technology and Department of Food Chemistry and Analysis

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Name of the student:	Chanphengxay Malisa
Neptun ID:	KF0CVM
Level of program (mark with X):	X BSc/BA ☐ MSc/MA ☐ Doctoral School (PhD) ☐ Other: ………………………
Name and code of the subject*:	Bachelor's degree
Title of the work:	The Examination of Digestibility and Sensory Properties of High Protein Cream

* Not required to be completed in the case of a doctoral dissertation.

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I, the undersigned, fully aware of my ethical responsibility, make the following declaration:

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