

Protein Characterization in Edible Coating for Snack Bar Enriched with Spirulina

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Abstract. Spirulina, renowned for its impressive protein content of 55-70% of dry weight, surpasses commonly used plant sources like soybeans, peanuts, or cereals. However, Spirulina snack bars, with their inherent texture, may face challenges like moisture absorption and microbial growth. In this context, edible coatings emerge as a solution to prevent moisture ingress, microbial growth, and preserve the bars. This study delves into analyzing the protein content of edible coatings, specifically maltodextrin with a 6% concentration and 1% gelatin, aiming to assess their efficacy in safeguarding against ambient moisture and oxygen. The findings reveal that the snack bar with the specified coating concentration showcases enhanced visual appeal and a denser texture. Protein analysis using SDS PAGE identifies bands with molecular weights of 17kDa, 25kDa, and 35kDa, likely corresponding to α -CN and β -CN proteins. Chromatogram analysis of the Spirulina snack bar using the HPX-87H column unveils a peak at 6.195 minutes in the methanol extract, indicating abundance in α -tocopherol, a vitamin E variant. This comprehensive exploration underscores the potential of edible coatings, providing insights into their role in preserving Spirulina snack bars and contributing to food industry advancements.

1 Introduction

Snack products refer to food items that are typically smaller in size compared to a regular meal portion and are taken in the intervals between meals. These products are readily accessible, extensively available, and appealing to a diverse spectrum of consumers [1]. The present shift in food consumption habits is characterized by a fast-paced lifestyle, which is made possible by the accessibility of nutritious, economical, appetizing, and convenient meal options like snacks [2]. In Indonesia, where snacks are taken in large quantities, it is important for these snacks to not only be high in energy, but also have ample amounts of protein, vitamins, minerals, dietary fiber, and other beneficial elements that positively impact health [3]. A snack bar is a safe and nutritious option for a healthy snack. A snack bar is a cylindrical food prepared by combining a variety of ingredients such as grains, fruits, and

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nuts, which are then bound together using a binder. Commonly employed binding substances encompass syrup, caramel, chocolate, and various others [4]. Food packaging serves as a physical barrier that protects food products from the external environment. This ensures the cleanliness of the food and extends the shelf life of perishable items, particularly those that are susceptible to microbial and oxidative spoilage [5]. An approach to enhance the nutritional value of snack bar products involves the incorporation of natural components, such as microalgae. Microalgae have emerged as highly promising reservoirs of chemicals due to their inherent biological activity, which can be harnessed for the production of functional products [6]. *Spirulina platensis* is a species of microalgae that has potential applications in the food industry. This organic component includes vitamins, minerals, essential fatty acids, and antioxidants. According to FDA statistics, spirulina is classified as GRAS (Generally Recognized as Safe) and is considered a nutritious product because to its bioactive components and high protein content [7-8]. Spirulina offers therapeutic advantages in conditions such as inflammation, hypercholesterolemia, hyperlipidemia, cancer, and some metabolic disorders. The majority of the Spirulina biomass currently manufactured is utilized as nutritional supplements, which are marketed as "super food" and sold in the form of dry powder, flakes, or capsules [9]. Spirulina's composition and health advantages indicate its potential to become a significant food source in the future and a valuable ingredient in the creation of functional foods [10].

However, in today's fast-paced world, consumers require not only nutritious products but also ones that have a longer shelf life. There are numerous potential methods to decrease the moisture content in food products. One method involves applying an edible coating onto the items. An edible coating is a thin layer of substance that is applied to the outside surface of food. Various types of coating agents can be utilized, including polysaccharides, proteins, resins, and lipids. Polysaccharides are mostly used as coating agents [11]. Polysaccharides such as chitosan, starch, pectin, and maltodextrin are examples of coating agents. Biopolymers-based biodegradable films are essential for reducing the environmental consequences of non-biodegradable plastic waste [12]. Protein and polysaccharides are the primary biopolymers used in the production of biodegradable films. Soy protein is a frequently investigated protein for the purpose of generating biodegradable films [13], milk protein, such as casein and whey protein [14], and gelatin [9-10]. Applying an edible coating to food products allows for the regulation of oxygen, carbon dioxide, lipid, moisture levels, as well as the scent and flavor of the food [17] and edible coating can improve the appearance of food goods and serve as a means of preservation [18]. Hence, it is imperative to conduct a study on the protein characteristics of an edible coating made from maltodextrin and gelatin, which acts as a plasticizer, for the purpose of applying it to snack bars with the inclusion of *Spirulina platensis*.

2 Material and methods

2.1 Snack bar preparation

The Spirulina Snack Bar (SSB) was created by incorporating 2% Spirulina powder. The composition of the spirulina snack bar includes almonds, a binding agent, rice balls and spirulina powder. The assortment of nuts, including cashews, almonds, sesame seeds, pumpkin seeds, and sunflower seeds, as well as the binding agents, such as honey, palm sugar, maize sugar, and lemon juice, were measured by weight. Subsequently, the nuts were subjected to a drying process in the oven at a temperature of 125°C for a duration of 15 minutes. The mixture of honey, palm sugar, maize sugar and lemon juice was heated on the fire and continuously stirred until thoroughly combined. The desiccated nuts were

incorporated into the binder and agitated until thoroughly combined. The dough is put onto a baking pan that has been prepared with baking paper. Subsequently, the rice puffs, spirulina powder, corn syrup and lemon water were measured by weight. A mixture of corn sugar, lemon juice and spirulina were combined and then incorporated into rice puffs through stirring. Next, the dough was poured over the initial dough and shaped into parts on a rectangular tray to create a snack bar. Subsequently, the dish is cooked in the oven at a temperature of 125°C for a duration of 20 minutes. Subsequently, the sample was allowed to cool at ambient temperature for a duration of 15 minutes and subsequently placed in the refrigerator for subsequent analysis.

2.2 Formation of edible coating

According to the specified criteria, the edible coating is dissolved in distilled water and carefully mixed with 6% Maltodextrin to create a homogeneous solution. To enhance the functionality of the edible film, the solution is subjected to denaturation at a temperature of 90°C for a duration of 30 minutes using a water bath. The solution should be cooled to room temperature by immersing it in chilled water. Add 1% gelatin to the solution as a plasticizer and stir it completely. Subsequently, the produced solution will be rewarmed to a temperature of 50°C for a duration of 10 minutes, followed by a subsequent cooling to the ambient temperature.

2.3 Preparation and characterization of protein extract from SSB

In order to prepare and analyze the protein extracted from the bars, the SSB (specifically, soluble soybean) was dehydrated and pulverized into a fine powder (80-mesh, 0.2 mm) using a laboratory grinder known as the Universal TM-767. The sample powder was combined with methanol in a ratio of 1:3 (w/v) and stirred continuously at room temperature for 3-4 hours. The methanol was isolated using vacuum filtration, and samples devoid of fat were desiccated inside a fume hood at room temperature for 24 hours to eliminate any remaining methanol. Deionized water was added to the defatted sample at a ratio of 1:10 (weight/volume). The mixture was mixed for 2 hours and then centrifuged using a Hettich Centrifuge Universal 32 R model at a speed of 10,000 times the force of gravity for 30 minutes at a temperature of 4°C. The protein, which cannot dissolve in solution, was separated, and collected by causing it to form a solid at its isoelectric point, which is pH 4.6. This was achieved by adding a 1 M HCl solution and subjecting the mixture to centrifugation at a force of 10,000 times the acceleration due to gravity for a duration of 30 minutes at a temperature of 4°C. The protein that was precipitated at its isoelectric point was dissolved again using deionized water and its pH was adjusted to 7.0 using a 1 M solution of sodium hydroxide. The protein, which was insoluble and precipitated at its isoelectric point, was reconstituted, freeze dried, and stored at a temperature of 4°C. It was then submitted to electrophoresis.

2.4 Characterization of proteins in SSB by electrophoresis

The sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) technique employed a 4% stacking slab gel and a 17% resolving gel. The protein solutions were placed onto a discontinuous buffer system consisting of SDS-tris-glycine, with a constant electric field of 40 mA. The electrophoresis was conducted for a duration of 90-120 minutes using a consistent power source provided by Bio-Rad Laboratories. The proteins in the standard marker solution exhibited a wide variety of molecular sizes, ranging from 11 to 180 kilodaltons (kDa). The staining solution used to determine the molecular size of each protein in the bar samples was Coomassie brilliant blue, which was prepared as a 0.1% solution in a

mixture of acetic acid, ethanol, and water (10/40/50, v/v/v).

2.5 HPLC for bioactive compounds of SSB

The Shodex RI-87H column is a high-performance liquid chromatography (HPLC) column that utilizes refractive index (RI) detection. It is specifically designed for the study of a wide range of substances, such as sugars, organic acids, and polymers. Optimization was performed on the flow rate, pressure, temperature, and injection volume. The mobile phase consisted of ultrapure water and a solution of 0.01 N H₂SO₄, which was used in conjunction with the HPX-87H column. The flow rate of the mobile phase, the volume of the injection, and the temperature of the column oven were optimized. The residence periods and peak resolution are greatly influenced by these chromatographic parameters. Band broadening may be caused by the longitudinal diffusion of the solute in the mobile phase and limited mass transfer between the solute and the mobile phase. Analysis was performed by HPLC (equipment described above) using a Refractive Index (RI) detector (Hitachi, Chromaster 5210, Japan). However, a mutually agreeable solution was reached for each approach, resulting in effective differentiation of peaks within acceptable time frames.

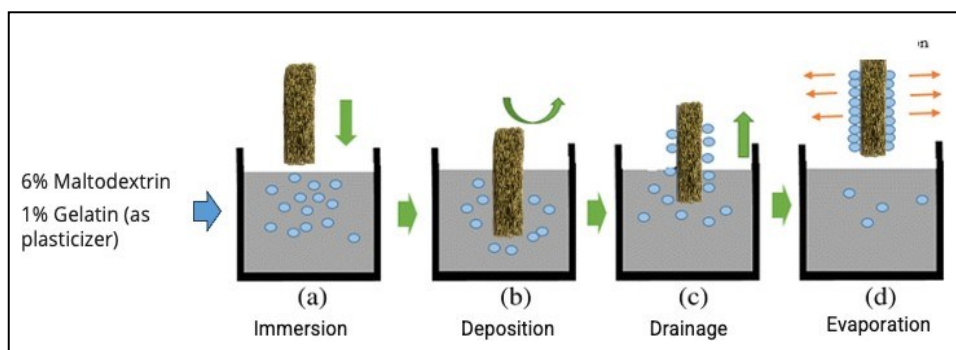


Fig. 1. The dipping coating process of SSB with MD and Gelatin.

3 Results and discussion

3.1 Spirulina snack bar with dipping coating

Maltodextrin (MD) is commonly employed as a wall material primarily because of its efficacy, affordability, lack of distinct taste and smell, and excellent ability to dissolve in water [14-15]. An edible coating is a thin layer of substance that is applied to the outside surface of food. Various coating agents can be utilized, including polysaccharides, proteins, resins, and lipids. Polysaccharides are mostly utilized as coating agents [21]. Polysaccharides such as chitosan, starch, pectin, and maltodextrin are examples of coating agents. In figure 1 showed the process of dipping coating process of SSB with 6% MD and 1% gelatin [22].

According to the result of dipping coating with maltodextrin, as shown in figure 2, by applying edible coating on the SSB with two different concentrations, which is B (SSB with 3 % MD and 1% Gelatin) and C (SSB with 6 % MD and 1% Gelatin), it showed that the SSB with 6 % MD and 1% Gelatin have better appearance dan the texture more solid compare to SSB with 3 % MD and 1% Gelatin. Edible coating has been definitively proven to improve the appearance of food products and also serve as a means of preservation [23].



Fig. 2. A (control), B (SSB with 3 % MD 1% Gelatin), C (SSB with 6 % MD 1% Gelatin)

Edible coatings can prolong the shelf life of food goods by minimizing moisture loss, solute migration, gas exchange, and physiological problems. Edible coatings possess significant promise for managing browning, discoloration, off-flavors, microbiological activity, and extending the shelf life of food products [24-25]. Plasticizers consist of small molecules that are combined with protein coating material to improve and modify its structural capacity [26]. The dipping method is a commonly employed technique for the application of edible coatings onto food items. In this process, the meals are immersed in a coating solution for aduration of 5 to 30 seconds [27].

3.2 Protein characterization

Protein in sample (extract of SSB with methanol) can be analysis after electrophoresis. According to the electrophoretograms of SSB, bar #1 (control), bar #2 (SSB extract), and bar#3 (SSB extract) are shown in Figure 3 along with the standard marker (180 kDa –11 kDa). The bands sample showed bands between 17 - 63 kDa. The three dense bands at the positions of 17–35 kDa may represent the α s-CN and β -CN, respectively. The third very thin band below these two protein bands indicated the κ - CN [28]. The band at the position of 35 kDa could be the combination of two proteins (α s1-CN and α s2-CN) in the form of α s-CN as [29] stated SDS-PAGE could not separate these two bands because of similar molecular weights. The bands near 25 kDa could be from β - lactoglobulin and light chain of immunoglobins (29 kDa), as confirmed by [30]. Three bands close to 17 kDa, 25 kDa and 48 kDa are also visible in bars #2 and #3, which could be the protein bands from SSB. Nevertheless, gel electrophoresis may occasionally provide feeble bands or the absence of bands due to improper sample preparation, low protein content, inadequate electrophoresis settings, or issues with the gel or buffer.

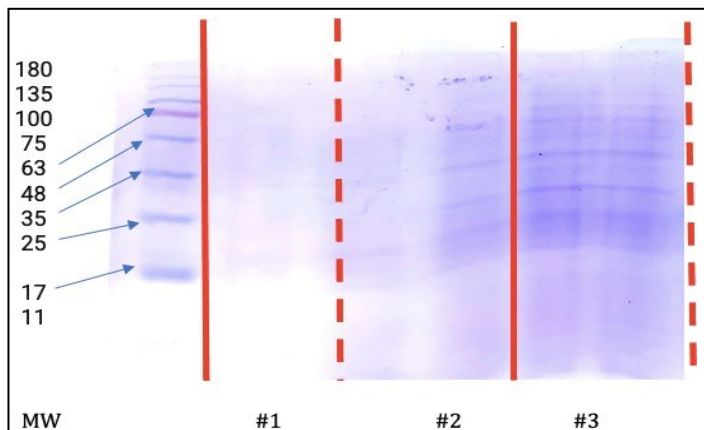


Fig. 3. Protein molecular bands of Spirulina Snack Bar (SSB) by SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE). MW: molecular weight standard. Bar #1 (control), Bar #2 (SSB extract), and Bar #3 (SSB extract)

3.3 Bioactive Compounds selection by HPLC

HPLC data is utilized for quality control objectives to verify that the snack bars adhere to specific requirements and laws. Through the examination of the chemical components and their respective concentrations, we can observe the durability of the product as it progresses and evaluate its longevity. According to the sharpness of the peaks, Rt min, and concentration were documented as shown in figure 4. The concentration of glucose, succinic acid and other organic acids were determined by HPLC equipped with refractive index detectors. According to the chromatogram result of SSB by using HPX-87H column was eluted isocratically with 0,01 N H₂SO₄ the peak showed at 6.195 minute of real time in the methanol extract. The Spirulina Snack Bar (SSB) contains an extract from *Spirulina platensis* that is rich in α -tocopherol, a form of vitamin E [31]. Regarding this result, *Spirulina platensis* can be regarded as a natural reservoir of medicinal substances due to its substantial levels of β carotene, inositol, niacin, α tocopherol, and polyunsaturated fatty acids (PUFA) [32-33]. These molecules are widely used as functional ingredients in food, pharmaceutical, and cosmetic preparations [17].

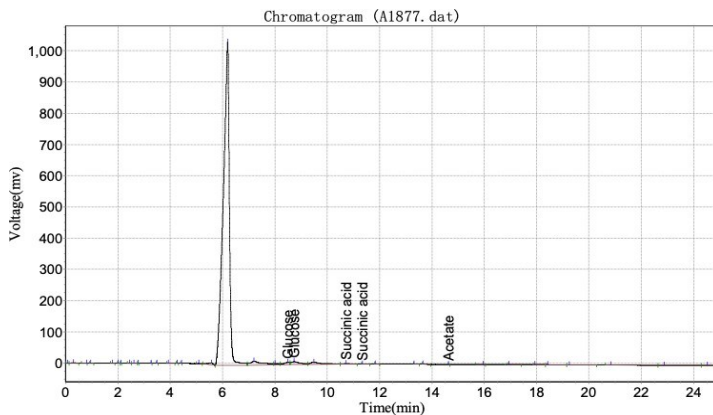


Fig. 4. Chromatogram of the SSB using the HPX-87H column

4 Conclusion

Spirulina platensis is now one of the most widely used products in the food sector because of research showing the advantages of consuming *Spirulina* Snack Bars to human health. The study successfully achieved the characterization of protein stability and bioactive components in *Spirulina* Snack Bar with the use of maltodextrin and gelatin covering. The nutritional components were enhanced by maltodextrin and gelatin as a coating. Therefore, these microalgae and their bioactive components can be employed for the advancement of bioactive substances.

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