Total phenolic content and antioxidant properties of hydrophobic compounds edible coating Spirulina snack bar

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Abstract. Snack bars, known for their convenience and easy consumption, amalgamate various ingredients such as cereals, fruits, and nuts. Their portable and on-the-go nature makes them a favored choice for individuals engaged in various activities. The integration of natural additives, such as *Spirulina platensis*, to elevate the nutritional profile of snack bars represents a novel and ambitious initiative. This study delves into the innovative realm of edible coating for spirulina snack bars using maltodextrin and gelatin. The investigation explores the correlation between total phenol content and antioxidant activity through the FRAP method, employing two solvents, methanol, and n-hexane. Results demonstrate that varying concentrations of edible coating significantly enhance the appearance and texture of Spirulina Snack Bars (SSB). Notably, the SSB coated with 6% maltodextrin and 1% gelatin exhibits superior visual appeal and a firmer texture compared to its counterpart coated with 3% maltodextrin and 1% gelatin. Moreover, the n-hexane extract showcases higher antioxidant activity (19.971 mg/g) than the methanol extract (16.400 mg/g). This study underscores the effectiveness of n-hexane-based edible coatings in elevating the antioxidant potential and total phenol content of spirulina snack bars, offering essential insights for the development of functional foods with enhanced nutrition and prolonged shelf life.

1 Introduction

Presently, an increasing number of consumers are establishing a connection between their dietary habits and a healthy way of living to decrease the occurrence of chronic illnesses. Food industries have been making efforts to decrease artificial additives and create products

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that offer necessary nutrients, as well as include ingredients that promote health and enhance both physical and mental well-being [1], [2]. The present shift in food consumption habits is characterized by a fast-paced lifestyle, which is made possible by the accessibility of nutritious, inexpensive, and appetizing ready-to-eat items like snacks [3]. A snack bar is a safe and nutritious option for a healthy snack. A snack bar is a cylindrical snack prepared by combining a variety of foods such as grains, fruits, and nuts, which are then bound together using a binding agent. Commonly employed binding agents encompass syrup, caramel, chocolate, and other substances [4].

The consumption of snack bars is driven by various factors, as indicated by the International Markets Bureau. These factors include fulfilling the craving for sweets, saving time, using them as an energy source, using them for weight loss, and benefiting from their protein, fiber, and vitamin contents. According to [5], snack bars are a product that is emerging in Indonesia. These bars have distinct nutritional value, flavor, and texture, which is achieved using binders. 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 that can be harnessed as functional materials [6]. *Spirulina platensis* is a species of microalgae that has potential applications in the food industry. Spirulina is a type of cyanobacteria, specifically a non-toxic blue-green microalgae. This organic component includes vitamins, minerals, essential fatty acids, and antioxidants. The FDA data [7] confirms that spirulina is classified as (Generally Recognized as Safe (GRAS)) and is a valuable nutritional source due to its abundance of bioactive components and high protein content [8]. Most of the Spirulina biomass now produced 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 as a significant food source in the future and as an element in the creation of functional foods [10].

In the field of modern food science and nutrition, there is a growing focus on finding new ways to improve the health benefits and longevity of snack products [11]. Incorporating hydrophobic chemicals into the edible coating of snack bars is an innovative approach that enhances sensory appeal and nutritional value [12]. Phenolic chemicals are well-known for their strong antioxidant properties and are essential for protecting cellular health by counteracting free radicals. Incorporating hydrophobic chemicals into the edible coating improves texture and enhances the durability and performance of important phenolic components [13]. The correlation among hydrophobic chemicals, total phenolic content, and antioxidant activity indicates a comprehensive strategy for developing snack bars that are both appealing to the palate and beneficial for customers' health. This study aims to explore the relationship between total phenolic content and antioxidant activity in the hydrophobic compounds of edible-coating snack bars.

2 Methods

2.1 Snack bar preparation

The Spirulina Snack Bar (SSB) was created through the addition of 2% Spirulina powder (commercially from PT. Algae Park Indonesia). The composition of the spirulina snack bar includes almonds, a binding agent, rice balls and spirulina powder. The variety of nuts, including cashews, almonds, sesame seeds, pumpkin seeds, and sunflower seeds, together with the binding agents such as honey (Lentera), palm sugar (Enau), maize sugar (Mulyot),

and lemon juice (Dari Bumi), were accurately measured by weight. Subsequently, the nuts were subjected to a drying process in the oven at a temperature of 125ºC for a duration of 15 minutes. The mixture of honey, palm sugar, maize sugar and lemon juice was cooked on the fire and continuously stirred until thoroughly blended. The desiccated nuts were incorporated into the adhesive and agitated until thoroughly mixed. 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 in terms of weight. A mixture of corn sugar, lemon juice and spirulina were combined and then incorporated with rice puffs through stirring. Next, the dough was placed onto the initial dough and shaped into parts within a rectangle tray to create a snack bar. Subsequently, the dish is cooked in the oven at a temperature of 125ºC 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. The obtained snack bars were divided into three different groups. Two groups were used in coating experiments while the other one was evaluated as control group.

2.2 Formation of edible coating

The edible coating is dissolved in distilled water according to the specifications and blended thoroughly to form a uniform solution. The edible coating's functionality is improved by subjecting the solution to a temperature of 90°C for 30 minutes using a water bath. Cool the solution to room temperature by submerging it in chilled water. Two distinct treatments were administered using 3% and 6% maltodextrin solutions. 1% gelatin was added to each treatment as plasticizer and mixed thoroughly. The solutions will be reheated to 50°C for 10 minutes and then cooled back to room temperature. After cooling of the solutions to room temperature, the snack bars were coated by dipping into the solution for 5 s. Both sides of the bars were dried for 24 hours at room temperature under shade.

2.3 Total phenolic content

The products' total phenolic content (TPC) was assessed using the Folin –Ciocalteu method as described in [14]. Prior to the evaluations, samples were obtained using the methodology outlined by [15], with minor adjustments. The specimens (3.5 ± 0.1 g) were freeze-dried, finely pulverized and subjected to extraction with 80% methanol (100 mL) for a duration of 2 hours at a temperature of 20°C. Various samples were prepared using N hexane as the organic solvent. The total phenolic content was determined by quantifying the reduction of Folin–Ciocalteu reagent complexes, measured at a wavelength of 725 nm.

2.4 Antioxidant activity

Preparing the antioxidant assay by using the FRAP method. Sodium acetate trihydrate, TPTZ, and FeCl3.6H2O were individually weighed at 187 mg, 150 mg, and 270 mg, respectively. A solution was prepared by combining 0.3 M acetate buffer at pH 3.6, 0.01 M TPTZ in 0.04 M HCl, and 0.02 M FeCl3.6H2O to form the FRAP reagent. The ratio of TPTZ, FeCl3.6H2O, and acetate buffer was 10:1:1. Three milliliters of the FRAP reagent, six milliliters of distilled water, and five milliliters of the sample were combined to assess absorbance. The sample-reagent mixture was vortexed to homogenize and then incubated at 37°C for 30 minutes. The absorbance was measured at a wavelength of 593-595 nm at that juncture. The standard solution consisted of hydrated ferrous sulphate (FeSO4.7H2O) dissolved in distilled water.

The FRAP method was selected because it can quantify antioxidant capacity in μmol Fe (II) per gramme of extract [16].

3 Results and discussion

3.1 Dipping coating of spirulina snack bar

Maltodextrin is often used as a wall material due to its effectiveness, cost-effectiveness, neutral taste and odor, and high solubility in water. An edible coating is a thin layer of substance put to the outside surface of food. Multiple coating agents can be used, such as polysaccharides, proteins, resins, and lipids. Polysaccharides are mostly used as coating agents [17]. Chitosan, starch, pectin, and maltodextrin are examples of polysaccharides that can be used as coating agents. Figure 1 illustrates the dipping coating procedure of SSB with 6% Maltodextrin and 1% gelatin [18].

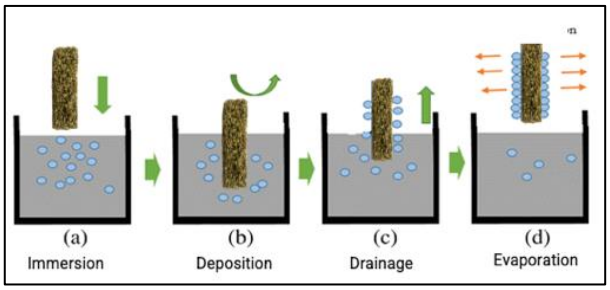

Fig. 1. The dipping coating process of SSB with MD and Gelatin

Figure 2 demonstrates that applying edible coating with two different concentrations on the SSB resulted in better appearance and a more solid texture for the SSB with 6% maltodextrin and 1% gelatin compared to the SSB with 3% maltodextrin and 1% gelatin. Edible coating has been conclusively demonstrated to enhance the visual appeal of food products and act as a method of preservation [19].

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

Edible coatings can extend the shelf life of food products by reducing moisture loss, solute migration, gas exchange, and physiological issues. Edible coatings have great potential in controlling browning, discoloration, off-flavors, microbial growth, and prolonging the shelf life of food products [20-21]. Plasticizers are tiny chemicals used with protein coating

material to enhance and alter its structural capabilities [22]. The dipping method is a frequently used technique for applying edible coatings to food items. During this process, the meals are submerged in a coating solution for a period of 5 to 30 seconds [23].

3.2 Total phenolic content (TPC)

Using gallic acid as a reference component, the Folin-Ciocalteu technique was used to determine the total phenol concentration. Measurement of the gallic acid reference compound resulted in a linear regression with the equation y = 0.0636x – 0.1057 and an R^2 value of 0.9148, as seen in Figure 3.

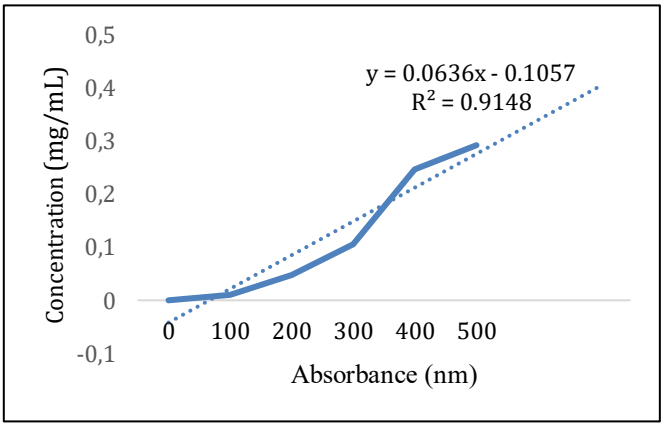

Fig 3. Standard Curve of Gallic Acid

The measurement results indicate that the phenolic component concentration in both methanol and hexane solvent is 6.131 and 9.742 mg GAE/g, respectively (Figure 3). Refers to the number of phenolic compounds present in the sample. Phenolic compounds are a class of chemical compounds with phenol rings, often found in plants and known for their antioxidant properties [24]. Two different solvents, methanol, and hexane, were used for the extraction process. Solvents are substances used to dissolve or extract components from a sample. These values indicate that the phenolic component concentration is higher when using hexane as the solvent compared to methanol [25]. The results suggest that hexane is more efficient in extracting phenolic compounds from the snack bar.

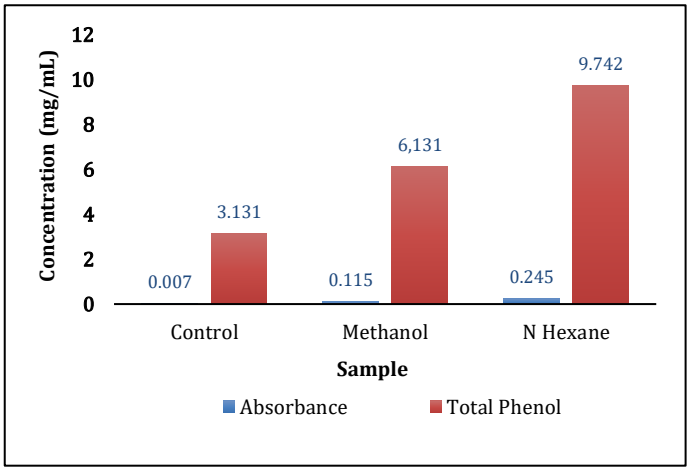

Fig. 4. Total Phenol Content

Moreover, figure 4 indicates that the bars coated with maltodextrin and gelatin as plasticizer, along with n-hexane as the organic compound, exhibited the highest overall phenolic levels. In contrast, the control sample has the lowest concentration of total phenolics. The results unequivocally shown that the application of an edible coating effectively safeguards against the deterioration and oxidation of phenolic compounds. The edible coating provides protection by utilizing the barrier qualities of the coating components against oxidative elements such as light, oxygen, and humidity [26].

3.3 Antioxidant activity

Figure 5 shows the linear regression equation derived from the antioxidant activity testing data using the FRAP method. The equation is $y = 0.0013x + 0.198$ with a R^2 value of 0.9058 at the peak wavelength of 795 nm. This specific wavelength is likely relevant to the absorption or activity of the compounds being analyzed, and it represents the point where the antioxidant activity is assessed. The linear regression equation and R^2 value provide a quantitative representation of the relationship between the FRAP parameter and antioxidant activity, suggesting a significant correlation [27].

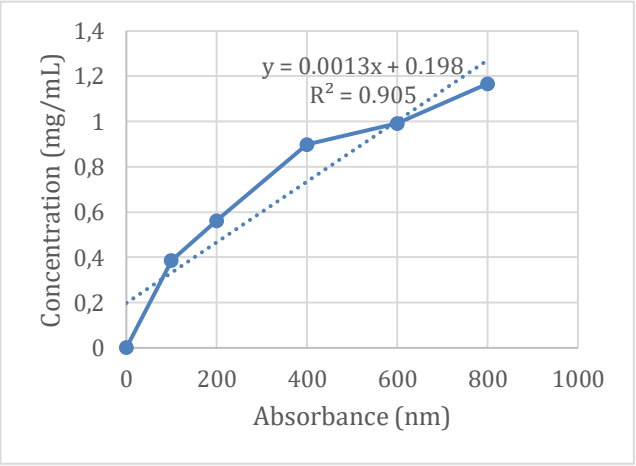

Fig. 5. Standard Curve of FRAP

After graphing the sample amounts against the linear regression equation, the antioxidant activity of both extracts was determined and is shown in Table 1.

Table 1. Antioxidant Activity Test Results

No	Sample	Absorbance (nm)	Antioxidant Activity (mg/gram)
1	Methanol	0.030	16.400
2	N Hexane	0.035	19.971

The extract produced on the hexane showed higher antioxidant activity compared to the extract produced on the methanol solvent, with an equivalency value of 19.971 mg/g and 16.400 mg/g of the sample, respectively. Electron transfer occurs between the antioxidant and the Fe^{3+}-TPTZ complex molecule in the assessment of antioxidant activity using the Ferric Reducing Antioxidant Power (FRAP) approach. The Fe3+-TPTZ molecule is a

potentially hazardous oxidizing compound that may be found in the body and could harm cells. The Fe^{3+} ion is reduced to Fe^{2+} under these conditions, causing the solution to change to a blue colour. The experiment took place in an acidic environment with a pH of 3.6 to guarantee the iron ions' solubility. Antioxidant activity is assessed by the FRAP method by measuring the absorbance changes at 795 nm in the sample solution and comparing it to a reference solution with known amounts of Fe2+ ions [28].

4 Conclusion

Spirulina Snack bars (SSB), being a convenient and popular choice, are adeptly positioned to receive a nutritional boost through innovative approaches such as edible coatings. The incorporation of maltodextrin and gelatin in this study successfully elevated the aesthetic and textural qualities of SSB. The findings highlight the notable superiority of the SSB coated with 6% maltodextrin and 1% gelatin, showcasing enhanced visual appeal and a firmer texture compared to its counterpart with a lower coating concentration. Furthermore, the exploration of solvents revealed that n-hexane, when employed for the extraction process, yielded an extract with significantly higher antioxidant activity compared to methanol. The hexane-extracted sample exhibited significantly greater antioxidant activity than its methanol-extracted counterpart, boasting an equivalency value of 19.971 mg/g compared to 16.400 mg/g. These results offer valuable insights for the development of functional foods, emphasizing the potential for enhanced nutrition and extended shelf life in response to consumer demands for healthier and more sustainable snack options.

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