

Development of a Functional Food Snack Product Utilizing Underexplored and Underutilized Ingredients: Spirulina Microalgae and Bilberry

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Abstract

Spirulina, a protein-rich cyanobacterium, and Bilberry, a dark berry, have the potential to be used as functional food ingredients in the food industry. These two underexplored and underutilized ingredients were used to develop an adolescent-friendly functional snack food product in the light of food industry trends. Stages of product development, shelf life/physiochemical analysis (texture, pH, color, and water activity) and sensory evaluation were utilized in developing a functional snack mini muffin containing Spirulina and Bilberry. Aqueous (AQ) and 80% ethanol (ET) extracts of mini muffin formulations (chocolate, 1% Spirulina (S) + 4% Bilberry (B), 2% Spirulina (S) + 8% Bilberry (B)) were prepared using a standard protocol. Antioxidant potential was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Potential (FRAP) assays. Utilizing a 5-point hedonic scale (1—Dislike very much, 2—Dislike a little, 3—Neither like nor dislike, 4—Like a little, 5—Like very much), 3 mini muffin formulations (chocolate, 1% S + 4% B, 2% S + 8% B), were tested among consumer panelists, with 1% S + 4% B being the most acceptable based on taste, texture, color, aroma, appearance, etc. Texture (post-peak (N) of the mini muffin did not vary between chocolate and 1 S% + 4% B formulations; however, 2% S + 8% B was 1.09 times higher compared to its counterparts. pH, color, and water activity remained constant over the 9-day shelf-life period. The Spirulina and Bilberry muffins developed exhibited antioxidant activities (highest in 2% S + 8% B), and were accepted by the sensory panelists for color, taste, mouthfeel, and aroma (panelists preferred 1% S + 4% B).

Keywords

Spirulina, Bilberry, Functional Food Product Development, Adolescents,

1. Introduction

Functional foods are gaining much importance in the food industry due to their potential benefits to promote proper growth and development and protect against disease through their bioactive compounds such as, polyphenols: flavonoids and phenolic acids [1] [2]. Natural bioactive compounds encompass phytochemicals such as polyphenols and flavonoids, categorized as secondary metabolites, along with primary metabolites like proteins, carbohydrates, lipids, and nucleic acids [3]. Polyphenols, found in plants, offer diverse health advantages, including enhancing digestion and shielding against conditions like type 2 diabetes and heart disease [2]. They are classified into many main groups, two being Flavonoids (abundant in tea (flavanols), apples, onions (flavonols), citrus fruits (flavanones), and colored fruits and vegetables (anthocyanins)); and Phenolic Acids (present in coffee, fruits, and vegetables). Consuming significant quantities of these bioactive compound-rich foods can leverage their health benefits in protecting against chronic illnesses like cardiovascular disease, type 2 diabetes, cancer, and obesity. These benefits stem from their biological properties, such as antioxidant, anti-hypertensive, and anti-cancer effects [4]. Therefore, integrating these functional foods into daily diets, especially for children, can significantly reduce the risk of obesity while promoting overall health and bridging nutritional gaps.

Consumers consume many functional foods on a daily basis, such as strawberries, blue-berries, tea, peppers and chocolate. However, with the overconsumption and utilization of many of these fruits and vegetables, such as blueberries and strawberries, it is important for the food industry to discover other alternatives to these ingredients in light of food insecurity and sustainability with the growing population. Two potential functional food ingredients that can be used and introduced into the food industry are Spirulina and Bilberry.

Spirulina, a blue-green algae, was discovered in Tenochtitlan, now Mexico City, in the 16th century [5]. This photosynthetic cyanobacterium stands out as the most widely cultivated microalgae globally, contributing to over 30% of the world's microalgal biomass production [5] [6]. Spirulina contains valuable pigmented compounds like chlorophylls and carotenoids, which not only can serve as natural food and beverage colorants in the food industry but also offer potential health benefits. Carotenoids, for instance, exhibit provitamin A activity, and their consumption has been linked to improve immune function and reduced the risk of chronic diseases. Spirulina has been deemed a "Food of the Future" by the International Association of Applied Microbiology in 1967, due to its natural blue and green pigments and its high protein content, which is about 60% - 70% of its dry weight, depending on cultivation conditions [5] [6]. Spirulina has also been certified as a Generally Recognized AS Safe (GRAS)—GRN No. 127 by the Unit-

ed States Food and Drug Administration (FDA) [5] [6]. Due to its chemical composition, Spirulina has the potential to become a “Food of the Future” for future product development [5] [6].

Bilberry, native to northern parts of the United States, Europe and Canada, is a dark berry commonly mistaken for blueberry. Previously used for its medicinal purposes such as aiding in preventing diabetes and scurvy, bilberry has the potential to be used as a natural color additive in the food industry [7]. Limited studies also suggest that the high anthocyanin content of Bilberry can lead to scavenging free radicals, reducing oxidative stress in the body [8] [9].

Functional food product development has gained a significant rise in the food industry due to the major trends surrounding the use and incorporation of functional foods into consumers’ everyday diets. Therefore, discovering new potential functional food ingredients such as Spirulina and Bilberry is important for food companies to remain competitive in the industry and meet consumer demands.

2. Methods

2.1. Product Ideation

The purpose of developing a functional snack product using Spirulina and Bilberry is to outline the applicable use of these two understudied and underutilized ingredients in the food industry space to fill the market gap in the development of functional food products for children. From chemical analysis conducted in previous research, both Spirulina and Bilberry have the potential to have functional benefits that can aid against oxidative stress and chronic diseases. Therefore, with the growing epidemic of childhood obesity, the aim of the development of the snack product was to target children with an on-the-go snack mini muffin to aid in the reduction of obesity in children.

2.2. Product Formulations

After many trials, three mini muffin formulations were developed, one control (chocolate) and two “Spilberry” (combination name between Spirulina and Bilberry) muffins: 1% Spirulina + 4% Bilberry (1% S + 4% B) and 2% Spirulina + 8% Bilberry (2% S + 8% B). The formulations can be seen in **Tables 1-3**. Staying within food industry trends, the functionality of each ingredient is found in **Table 4**, utilizing alternative flours: almond and gluten-free all-purpose flours, as consumers are pushing for more alternative flours and gluten-free products in the light of celiac disease and gluten sensitivity/gluten intolerance.

Table 1. Control mini muffin formulation.

Ingredients	% of formulation
Whole milk	22.93
Almond flour	14.88
Gluten free all-purpose flour	14.88

Continued

Large eggs (2)	13.57
Unsalted butter	13.46
Sugar	11.91
Cocoa powder	5.95
Baking powder	1.06
Orange extract	0.50
Pure vanilla extract	0.50
Salt	0.35
Total	100.00%

Table 2. 1% Spirulina + 4% Bilberry mini muffin formulation.

Ingredients	% of formulation
Whole milk	24.34
Large eggs (2)	14.41
Unsalted BUTTER	14.28
Sugar	12.64
Almond flour	13.39
Gluten free all-purpose flour	13.39
Bilberry powder	4.00
Baking powder	1.12
Spirulina powder	1.00
Orange extract	0.53
Pure vanilla extract	0.53
Salt	0.37
Total	100.00%

Table 3. 2% Spirulina + 8% Bilberry mini muffin formulation.

Ingredients	% of formulation
Whole milk	24.33
Large eggs (2)	14.40
Unsalted butter	14.27
Sugar	12.63
Almond flour	10.91
Gluten free all-purpose flour	10.91
Bilberry powder	8.00
Baking powder	1.12
Spirulina powder	2.00
Orange extract	0.53
Pure vanilla extract	0.53
Salt	0.37
Total	100.00%

Table 4. Product ingredient functionality.

Ingredients	Functionality
Almond flour whole milk	Structure
Baking powder	Structure, texture
Bilberry powder	Color, flavor
Cocoa powder	Flavor, color, bulking, water absorption
Gluten free all-purpose flour	Structure
Large eggs (2)	Thickener, emulsifier, stabilizer, color, flavor
Orange extract	Flavor, aroma
Pure vanilla extract	Flavor, aroma
Salt	Stabilizer, structure, flavor
Spirulina powder	Thickener, emulsifier, flavor, color
Sugar	Flavor, texture
Unsalted butter	Structure

2.3. Shelf-Life Physiochemical Analysis

Product quality and safety is one of the top priorities when developing a food product in an industry to sell. Therefore, to ensure maximum safety and quality of the control and “Spilberry” mini muffin products, shelf-life studies were conducted on all three product formulations for 9 days, in 3-day increments, with the muffins left out in a room temperature environment in a clam-shell packaging to mimic a grocery store bakery muffin. The following physiochemical properties were tested: pH, water activity (aw), and color based on Hunter’s LAB color scale where L* measures darkness to lightness (0 to 100) (L*), a* measures green (-) to red (+), and b* measures blue (-) to yellow (+).

2.4. Sensory Analysis

To ensure consumer acceptability of the “Spilberry” mini muffins, a sensory panel was conducted on Alabama A&M University’s campus in the Department of Food and Animal Sciences’ Sensory Laboratory. Assessing consumer acceptability among minority adolescents, the sensory panel consisted of Black/African American (100%) males (26%) and females (74%) among the following ages: 17 (5%), 18 (47%), 19 (24%), 20 (13%), 21 (11%). The sensory participants were given a questionnaire to evaluate the three muffin formulations (control (with cocoa powder to mask color biasness), 1S + 4%B, and 2%S + 8%B) for their acceptability, beginning with demographics and a pre-screen of questions assessing their consumption of muffins and awareness of Spirulina and Bilberry. To assess consumer acceptability, the panelists were asked to rate the products using the 5-point Hedonic scale (1—Dislike very much, 2—Dislike a little, 3—Neither like nor dislike, 4—Like a little, 5—Like very much) based upon their acceptability of color, appearance, tactileness (touch), aroma, texture (mouthfeel), taste, and overall product acceptability.

2.5. Sample Preparation for SEM Analysis

Scanning Electron Microscopy (SEM) was used to examine the surface of the mini muffins to compare between subjective (consumer testing) and objective (texture analyzer) texture measurements. Proper sample preparation is important for SEM analysis. Volatile compounds such as water and oil must be removed from samples if any are present. Therefore, the mini muffin products were freeze-dried for 2 days to then be used for SEM analysis, ensuring all moisture was lost from the product, utilizing a VirTis Genesis Freeze Dryer. Reducing the particle size of each, the samples were placed on the SEM sample disk with Colloidal Silver Liquid. Afterward, the samples were coated with gold utilizing an Electron Microscopy Sciences Sputter Coating machine (Model: RV5; Code No.: A653-01-906; Serial Number: 037632871), to then be used for SEM analysis to be analyzed via micrographs of various specifications/magnifications.

2.6. Antioxidant Analysis

The antioxidant activity of the muffin products (control (without cocoa powder), 1%S + 4%B, and 2%S + 8%B) was tested using the following assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Potential (FRAP). Sample extractions were performed by mixing 100ml of 80% ethanol per 5g of the sample for 2 hours, to sonicate for 1 hour in a Bransonic M5800H Ultrasound Sonic Bath and centrifuged at 1107 g force at 4°C for 20 minutes. Following, the product supernatants were filtered using Whatman #4 filter paper, then evaporated using a rotary evaporator and reconstituted using 10ml of the 80% ethanol solution. Afterward, the samples were stored in a 4°C cooler for analysis.

2.6.1. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a deep purple free radical scavenging assay used to evaluate the antioxidant potential of mini muffin products [10]. The principle of this assay is based upon the reduction of the deep, purple-colored DPPH radical to a pale yellow or colorless 2,2-diphenyl-1-hydrazine (DPPH-H) when it is in the presence of antioxidants [11]. For analysis, diluted 80% ethanol extracts of the mini muffin were prepared and combined with the DPPH radical. The two were then incubated and read at an absorbance of 517nm at 30-minute intervals for 90 minutes [12].

2.6.2. Ferric Reducing Antioxidant Potential (FRAP)

Ferric Reducing Antioxidant Potential (FRAP) is an antioxidant test used to assess the reducing power of antioxidants present in various samples, including the mini muffins in the present study. Through a colorimetric reaction, the principle of the test is that in the presence of an antioxidant, the sample will reduce from ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The FRAP assay involves the use of a FRAP reagent, which consists of a ferric chloride solution, a tripyridyltriazine (TPTZ) solution, and a buffer solution, typically an acetate buffer. The FRAP reagent

reacts with the ferrous ions produced from the reduction of ferric ions, resulting in a blue-colored complex that absorbs light at 593 nm. The intensity of this blue color is directly proportional to the reducing power of the antioxidants in the sample. Therefore, in the procedure, the diluted mini muffin samples, extracted with 80% ethanol, were mixed with deionized water and the FRAP reagent to be incubated at 37°C, and read at an absorbance of 593 nm [13].

3. Results and Discussion

3.1. Shelf-Life

The shelf life of the control and “Spilberry” mini muffins was conducted over a 9-day period, with data taken in 3-day increments. **Figure 1** shows the pH and water activity, and **Figure 2** shows the color analysis of the top, side, and bottom of each product.

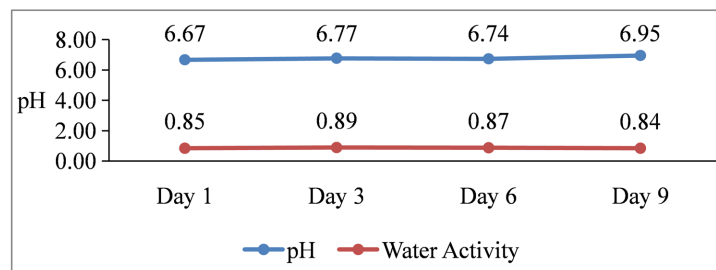


Figure 1. pH and water activity (n = 3) of control muffin (9-day shelf-life).

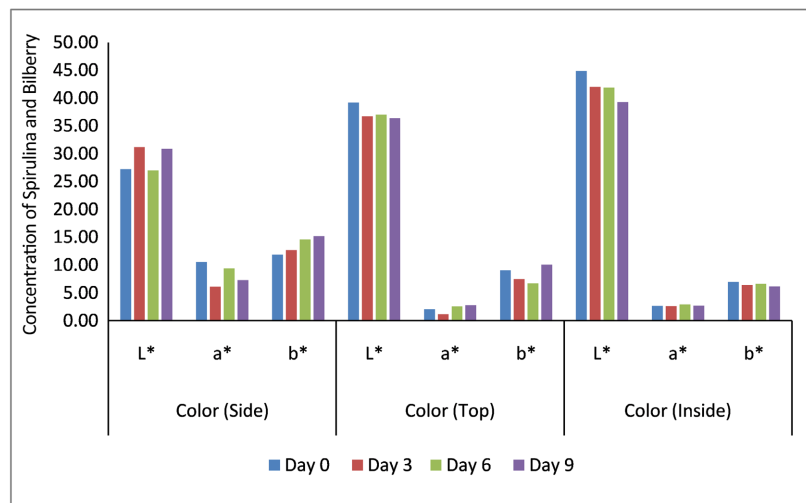


Figure 2. Color analysis (n = 3) of control muffin (L*a*b*) (9-day shelf-life).

Over the 9-day period, the pH and water activity remained constant, with the pH ranging from 6.67 - 6.95, and water activity ranging from 0.84-0.89Aw. Color analysis of the top, side, and inside of the muffin product was conducted by using L*a*b* values to determine its color with L* measuring darkness to lightness (0 to 100) (L*), a* green (-) to red (+), and b* blue (-) to yellow (+). Overall, the L*

seemed to decrease in lightness over the 9-day period, whereas the a^* and b^* value increased indicating a more of a red and yellow hue. The reason for the muffin's darkening in color is oxidation during the shelf-life period, when left out in the room temperature environment within the clam shell packaging. The exposure to oxygen, light, and moisture could have contributed to the color change as well.

The pH of the 1% Spirulina + 4% Bilberry muffin increased from 6.62 (Day 1) to 6.90 (Day 9), whereas the water activity fluctuated between 0.82 - 0.86Aw (**Figure 3**). The top, side, and inside of color of the muffin had slight changes during the shelf-life (**Figure 4**). Low values of L^* indicate the darkness of the product caused by the mixture between the dark green Spirulina powder and the dark purple-red Bilberry powder. Low a^* values were present within the muffin product, which indicates the "redness" of the product which is produced by the higher amount of Bilberry to Spirulina in the formulation, and low levels of b^* indicating the "bluer" hue of the muffins. Overall, the colors slightly changed over time with the a^* and b^* values increasing over time.

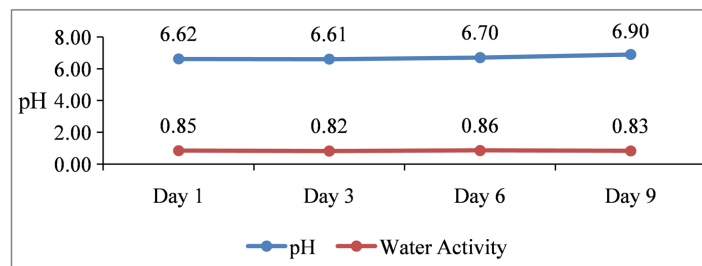


Figure 3. pH and water activity ($n = 3$) of 1% Spirulina + 4% Bilberry muffin (9-day shelf-life).

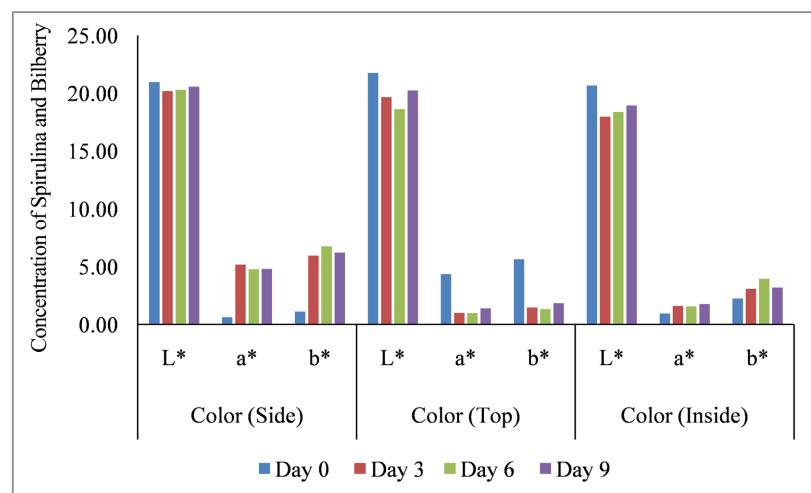


Figure 4. Color analysis ($n = 3$) of 1% Spirulina + 4% Bilberry muffin (9-day shelf-life).

The pH of the 2% Spirulina + 8% Bilberry varied between 6.63 - 6.84, whereas the water activity decreased from 0.84 to 0.81, which could have been due to its exposure to oxygen, causing a loss in moisture. Increasing the Spirulina and

Bilberry powder percentages in the 2% Spirulina + 8% Bilberry muffin formulations resulted in a rich dark purple hue, indicated by the low L*a*b* levels compared to the others. The reason for this could be the increased concentration of Spirulina and Bilberry interacting with each other to develop a rich dark purple/brown color. (Figure 5, Figure 6)

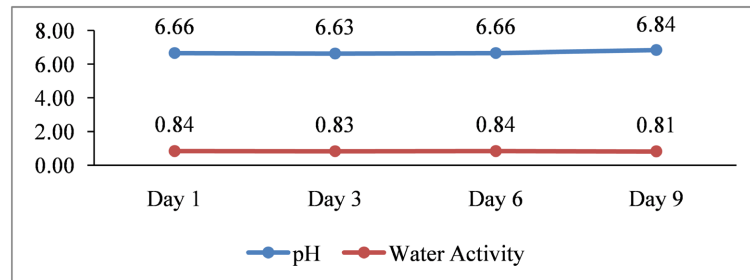


Figure 5. pH and water activity (n = 3) of 2% Spirulina + 8% Bilberry muffin (9-day shelf-life).

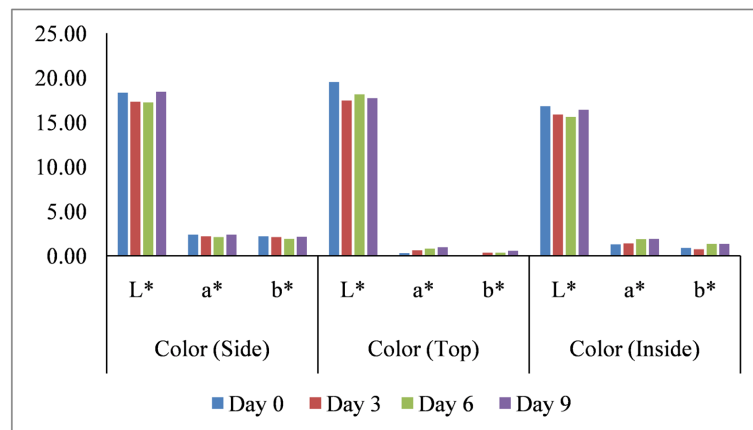


Figure 6. Color analysis (n = 3) of 2% Spirulina + 8% Bilberry muffin (9-day shelf-life).

3.2. Texture Analysis

One of the most important attributes of food products is its texture, which can be measured subjectively (human subjects) or objectively (instrumentation) [14]. Using objective measurements, Table 5 shows the results of the texture analysis of the three mini muffin formulations measuring post peak (N) hardness, integral (J), and slope (N/mm).

Table 5. Texture analysis of muffin formulations (control, 1% Spirulina + 4% Bilberry, 2% Spirulina + 8% Bilberry).

Muffin Formulations	PostPeak (N)	Integral (J)	Slope (N/mm)
Control	152.33 ± 3.67 ^a	1.49 ± 0.15 ^b	11.36 ± 0.48 ^a
1 S% + 4 B%	157.00 ± 3.79 ^a	1.52 ± 0.18 ^b	10.40 ± 1.69 ^a
2 S% + 8 B%	170.00 ± 5.13 ^a	2.48 ± 0.24 ^a	8.15 ± 1.23 ^a

Means ± standard error (n = 3). Significant differences ($p \leq 0.05$) of muffin texture properties, shown in columns, indicated by letters “abc”.

Post peak (N) is the amount of force involved in compressing the muffin sample. Comparing the three mini muffins, there were no significant differences among the three. However, the highest post peak value was seen in the 2 S% + 8 B% (170.00 ± 5.13 N), whereas the lowest was found in the control (152.33 ± 3.67 N). The reasoning behind this can be due to high amounts of Spirulina and Bilberry incorporated into the product, specifically incorporating higher amounts of the Spirulina microalgae. Integral (J) calculates the work per unit fracture surface area of the product [15]. The integral range of the muffin formulations ranged from 1.49 ± 0.15 to 2.48 ± 0.24 (control - 2S%+8B%) with 2 S% + 8 B% (2.48 ± 0.24 N) being significantly higher than control (1.49 ± 0.15 N) and 1 S% + 2 B% (1.52 ± 0.18 N). Opposite results were found in the slope (N/mm), where the control (11.36 ± 0.48 N) was higher compared to 1 S% + 2 B% (10.40 ± 1.69 N) and 2 S% + 8 B% (8.15 ± 1.23 N) muffins.

3.3. Sensory Analysis

Snacking is very common among college students. With their busy schedules, it is important for them to consume snacks that are not only tasty but filling and provide them with satiety and energy throughout their productive days. Therefore, accessing college students was important for this study, as well as with childhood obesity and other chronic diseases being the more prominent in minority adolescents, accessing consumer acceptability among these individuals was crucial. Therefore, consumer acceptability of the product was accessed using 38 minority panelists, whom were asked to give their acceptability of color, appearance, tacitleness (touch), aroma, texture (mouthfeel), taste, and overall product acceptability based upon a 5-Point Hedonic scale (1—Dislike very much, 2—Dislike a little, 3—Neither like nor dislike, 4—Like a little, 5—Like very much). The results for each muffin formulation can be seen in Figures 7-9.

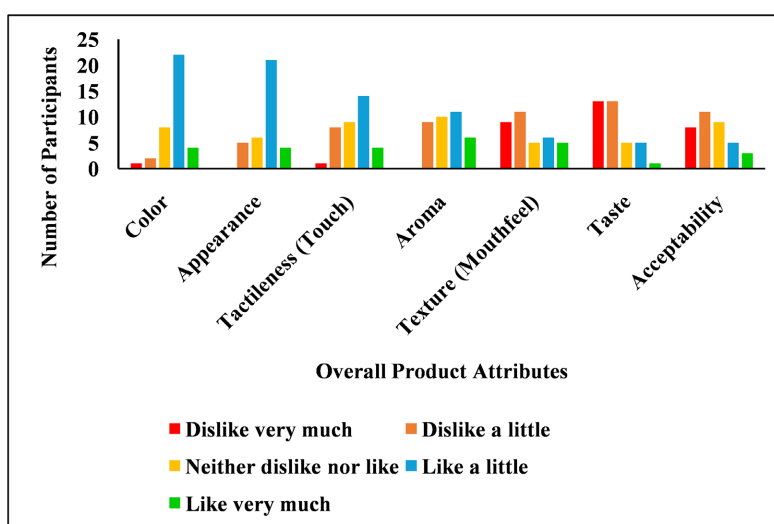


Figure 7. Product attributes scores of control muffin based on 5-point hedonic scale (n = 38).

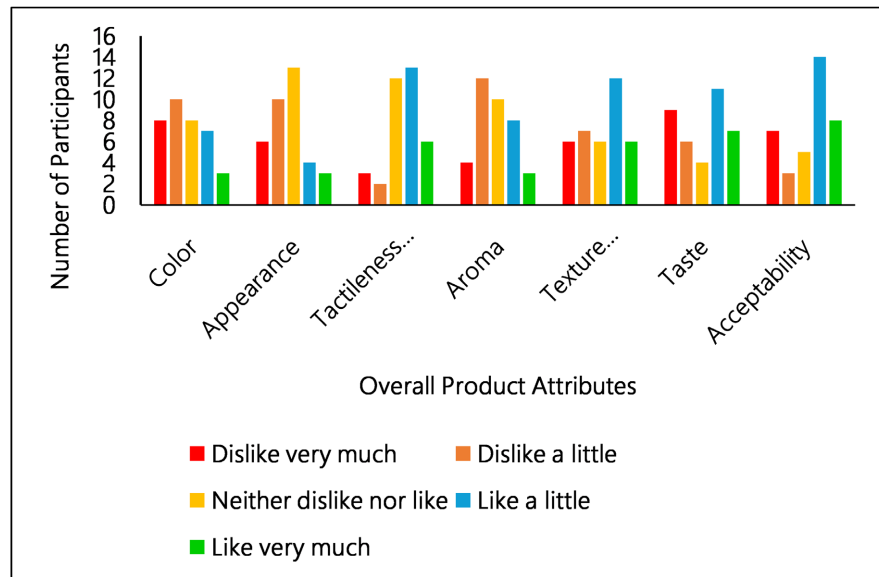


Figure 8. Product attributes scores of 1% Spirulina + 4% Bilberry (1%*s*+4%*b*) mini muffin based on 5-point hedonic scale (n = 38).

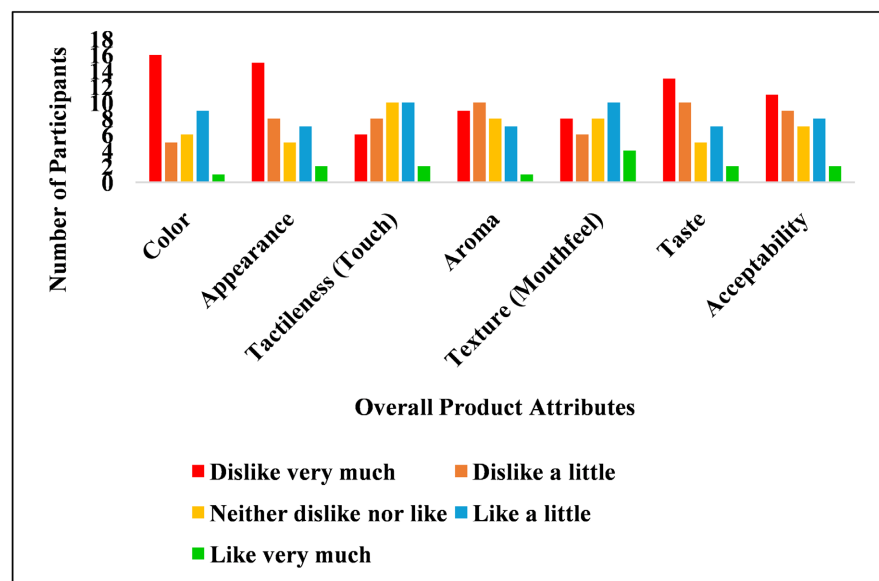


Figure 9. Product attributes scores of 2% Spirulina + 8% Bilberry (2%*s*+8%*b*) mini muffin based on 5-point hedonic scale (n = 38).

Figure 7 represents the product attribute scores of the control mini muffin based upon consumer acceptability. Out of the 38 participants, the majority of the individuals liked the color, appearance, and touch, but disliked the texture, taste, and overall acceptability of the chocolate mini muffin. Whereas the two “Spiriberry” mini muffins showed opposite results. Panelists disliked the color and aroma, but liked the overall touch, texture, taste, and overall acceptability of the 1% S + 4% B mini muffin. Consumers did not respond as well to the 2% S + 8% B compared to the 1% S + 4% B in regard to color, appearance, and taste. However,

some of the individuals liked the color and appearance better than the 1% S + 4% B but disliked the taste and overall acceptability more. Overall, consumers accepted each formulation in terms of different product attributes, mainly taste, texture, and touch. A few consumers were not pleased with the color of the two “Spilberry” formulations, due to the mixture of the extremely dark green and dark red colors that caused a dark green-blue hue (1% S + 4% B) and a dark red-purple hue (2% S + 8% B). Both are rather acceptable on their own; however, in combination, the off-green and dark purple colors are not acceptable. The reason for this is due to consumer perception and what they previously were accustomed to. Consumers are not accustomed to seeing green-blue and red-purple cake, bread-like products, unless it is artificial colored icing. Therefore, perception is very key when it comes to sensorial results.

Overall, the results of the product development suggested that Spirulina and Bilberry, are acceptable to be utilized in food products to increase their use in the food industry for functional food health benefits among adolescent nutrition product development.

3.4. SEM Texture Analysis

Figures 10-13 show the micrograph images from the SEM of the chocolate control, 1% S + 4% B, and 2% S + 8% B muffin products. The purpose of using SEM was to analyze the texture and air pocket formations within the product to compare and understand why some consumers accepted the texture of one muffin (1% S + 4% B) over the other (2% S + 8% B). **Figure 10** shows major air pocket formation within the chocolate muffin, which could be caused by the leavening agent used, overmixing, aeration, etc. These air pockets are often associated with a lighter, more open crumb structure. **Figure 11** shows very nice structural formations of the 1% S + 4% B muffin product, which shows a well-defined and consistent microstructure with evenly distributed air pockets and a uniform crumb texture. This suggests that the lower concentration of Spirulina and Bilberry may have contributed to a more desirable texture by maintaining better batter consistency and air incorporation. In contrast, **Figure 12** shows some differences in texture across the surface of the 2% S + 8% B muffin. It can be observed that the product has a crumbly and compact texture in comparison to the other two muffins in **Figure 13**. This uneven texture is indicative of a denser and more compact structure. The reasoning behind this discovery could be the increased concentration of Spirulina. Spirulina is a microalga and can be used as a thickening agent in the product. During preparation of the raw muffin batter, the texture of the 2% S + 8% B muffin batter was thicker compared to the other two products, which resulted in a thicker more compact texture after baking. This increased viscosity led to a denser batter that did not rise as much during baking, resulting in a more compact and crumblier final product. Thus, the higher Spirulina content appears to have significantly impacted the texture of the muffins, influencing consumer preference to prefer the 1% S + 4% B muffin over the 2% S + 8% B muffin.

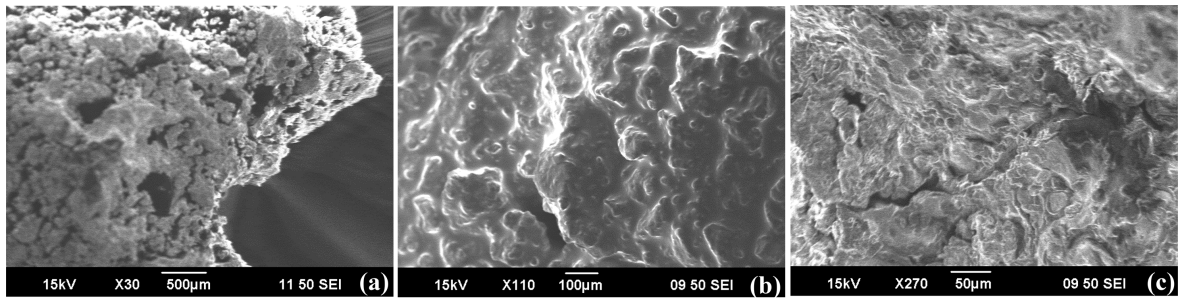


Figure 10. Micrographs (30×, 110×, 270×) of chocolate control muffin.

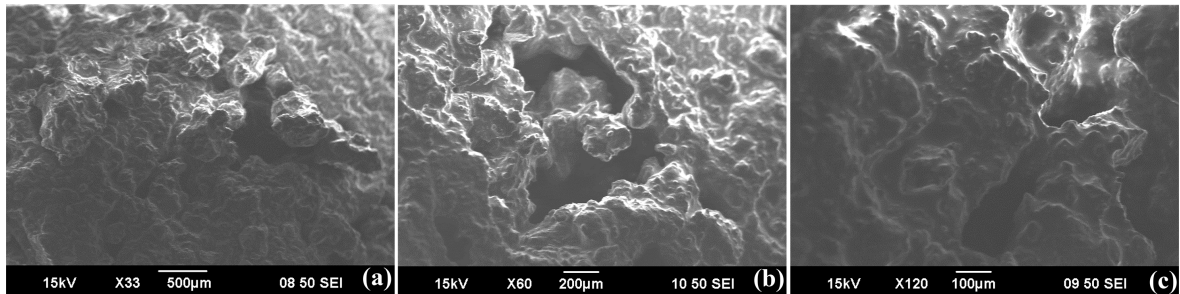


Figure 11. Micrographs (33×, 60×, 120×) of 1% spirulina + 4% bilberry muffin.

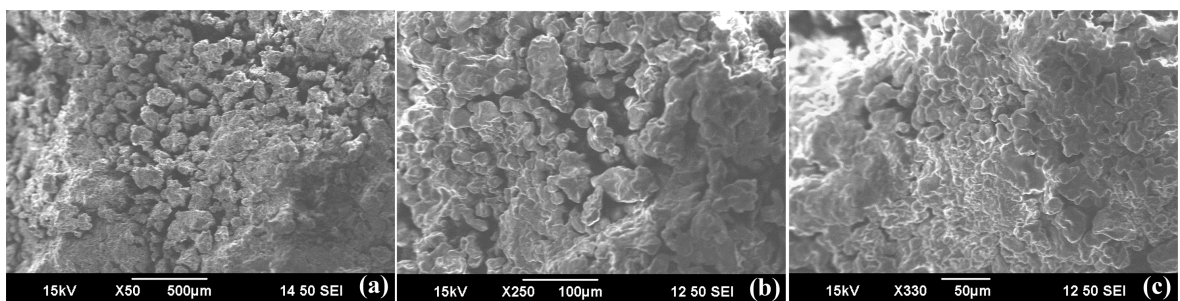
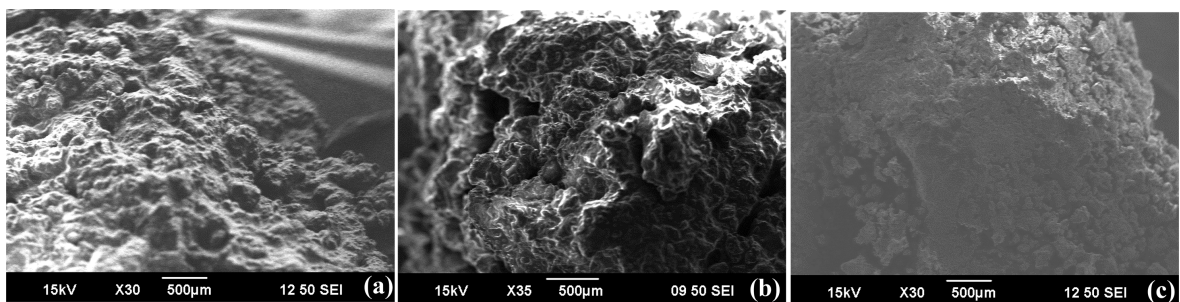


Figure 12. Micrographs (50×, 250×, 330×) of 1% spirulina + 4% bilberry Muffin.



(a): Chocolate muffin (30× Micrograph); (b): 1% spirulina + 4% bilberry (35× Micrograph); (c): 2% spirulina + 8% bilberry (30× Micrograph).

Figure 13. Micrographs of control and “Spilberry” muffins.

3.5. Antioxidant Analysis

The results for DPPH and FRAP of the control (without cocoa powder) and “Spilberry” muffin samples can be seen in [Table 6](#) and [Table 7](#). The purpose of the

control without cocoa powder was to have a pure control to measure the antioxidant activity of the Spirulina and Bilberry without the interference of the bio-active compounds present in cocoa powder.

The DPPH radical exhibits a deep purple color due to the delocalization within its aromatic rings. When the DPPH radical accepts an electron from an antioxidant, it becomes neutralized, causing the color to change from deep purple to pale yellow. The radical scavenging assay was conducted using an absorbance of 517 nm [11]. **Table 6** presents the DPPH antioxidant activity of control and “Spilberry” muffins extracted with 80% ethanol.

Table 6. 2,2-diphenyl-1-picrylhydrazyl (DPPH) % inhibition by control and “Spilberry” muffin 80% ethanol extracts.

Control & “Spilberry” Muffin Extracts	DPPH (% Inhibition) 30 g/DW
Control	46.75 ± 2.49 c
1% Spirulina + 4% Bilberry (1% S + 4% B)	66.49 ± 2.06 b
2% Spirulina + 8% Bilberry (2% S + 8% B)	89.54 ± 2.06 a

*Data was averaged in triplicates (n = 3). Data based on serving size of 2 muffins (30 g). Significant differences ($p \leq 0.05$) of muffin extracts indicated by letters “abc”.

DPPH ranged from 47% to 90% % inhibition with the 2%S + 8%B muffin being 1.35 - 1.91 higher compared to the 1% S + 4% B and control muffins. In order for the muffin products to be considered inhibiting the DPPH radical as an antioxidant, the % inhibition must be above 50%. Fortunately, both muffins containing Spirulina and Bilberry showed an overall inhibition greater than 50%, suggesting that Spirulina and Bilberry are two powerful antioxidants that can be used in a functional food product.

The Ferric Reducing Antioxidant Potential (FRAP) assay measures the reduction of ferric-tripyridyltriazine [$\text{Fe}^{\text{III}}(\text{TPTZ})$]³⁺ to the ferrous complex [$\text{Fe}^{\text{II}}(\text{TPTZ})$]²⁺ by antioxidants, resulting in a dark blue color [16]. **Figure 6** illustrates the FRAP values for the control and “Spilberry” muffins.

Table 7. Ferric reducing antioxidant potential (FRAP) of control and “Spilberry” muffin 80% ethanol extracts.

Control & “Spilberry” Muffin Extracts	FRAP (mM Fe (II)/30 g DW)
Control	73.76 ± 3.00 c
1% Spirulina + 4% Bilberry (1% S + 4% B)	125.42 ± 0.97 b
2% Spirulina + 8% Bilberry (2% S + 8% B)	177.08 ± 0.26 a

*Fe (II): Ferric iron; Data was averaged in triplicates (n = 3). Data based on serving size of 2 muffins (30 g). Significant differences ($p \leq 0.05$) of muffin extracts indicated by letters “abc”.

Following similar trends from the DPPH antioxidant assay, FRAP was significantly higher in the 2% S + 8% B (177.08 ± 0.26) formulation compared to 1% S + 4% B (125.42 ± 0.97) and the control (73.76 ± 3.00) muffin which was a 2.4 fold increase. Suggesting that as concentrations of both Spirulina and Bilberry increased, antioxidant potential/power increased as well.

Overall, the DPPH and FRAP increased as Spirulina and Bilberry concentrations increased from 1% Spirulina + 4% Bilberry to 2% Spirulina + 8% Bilberry.

4. Conclusions

Functional foods are increasingly important in the food industry due to their potential health benefits, including promoting growth, development, and protection against diseases. Bioactive compounds like polyphenols, flavonoids, and phenolic acids play a significant role in these benefits. Integrating functional foods into daily diets, especially for children, can help reduce the risk of obesity and improve overall health. Spirulina and Bilberry are two understudied and underutilized ingredients that have the promising potential of being functional food ingredients for the food industry. Spirulina, a blue-green algae, is rich in proteins and pigments like chlorophylls and carotenoids, offering health benefits and serving as natural food colorants. Bilberry, a dark berry, is known for its high anthocyanin content, which can reduce oxidative stress. Developing functional snacks using these ingredients can fill market gaps and address childhood obesity. The study focused on developing three mini muffin formulations: a control (chocolate) and two “Spilberry” muffins with varying Spirulina and Bilberry percentages (1% Spirulina + 4% Bilberry and 2% Spirulina + 8% Bilberry). The muffins were evaluated for shelf life based on their physiochemical properties: color, pH, and water activity. Consumer acceptability was assessed using a 5-point Hedonic scale among minority panelists, in which both “Spilberry” muffins were acceptable to consumers in regard to color, texture, taste, appearance, and aroma, with the 1% S + 4% B being the most accepted. In regard to the texture being accepted by the consumers, SEM analysis conducted showed that the 1% S + 4% B muffin had a well-defined and consistent microstructure, while the 2% S + 8% B muffin had a denser and more compact texture, , giving a better understanding of why 1%S + 4%B was accepted more than 2%S + 8%B. This difference in texture was attributed to the higher concentration of Spirulina, which acted as a thickening agent. The antioxidant assays (DPPH and FRAP) demonstrated that both “Spilberry” muffins had significant antioxidant activity, with the 2% S + 8% B muffin showing higher activity than the 1% S + 4% B and control muffins. This indicates that increasing the concentrations of Spirulina and Bilberry enhances the antioxidant potential of the muffins.

In conclusion, the study supports the use of Spirulina and Bilberry in functional food product development, particularly for their health benefits and potential to address and help prevent childhood obesity and other chronic diseases. The findings highlight the importance of consumer perception and acceptance, as well as

the health benefits of functional foods in product development and suggest that Spirulina and Bilberry can be effectively utilized in the food industry.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Cory, H., Passarelli, S., Szeto, J., Tamez, M. and Mattei, J. (2018). The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. *Frontiers in Nutrition*, **5**, 87. <https://doi.org/10.3389/fnut.2018.00087>
- [2] Williamson, G. (2017) The Role of Polyphenols in Modern Nutrition. *Nutrition bulletin*, **42**, 226-235. <https://doi.org/10.1111/nbu.12278>
- [3] Mondal, S., Soumya, N.P.P., Mini, S. and Sivan, S.K. (2021) Bioactive Compounds in Functional Food and Their Role as Therapeutics. *Bioactive Compounds in Health and Disease*, **4**, 24-39. <https://doi.org/10.31989/bchd.v4i3.786>
- [4] Konstantinidi, M. and Koutelidakis, A.E. (2019) Functional Foods and Bioactive Compounds: A Review of Its Possible Role on Weight Management and Obesity's Metabolic Consequences. *Medicines*, **6**, Article No. 94. <https://doi.org/10.3390/medicines6030094>
- [5] Lafarga, T., Fernández-Sevilla, J.M., González-López, C. and Acien-Fernández, F.G. (2020) Spirulina for the Food and Functional Food Industries. *Food Research International*, **137**, Article ID: 109356. <https://doi.org/10.1016/j.foodres.2020.109356>
- [6] Costa, J.A.V., Freitas, B.C.B., Rosa, G.M., Moraes, L., Morais, M.G. and Mitchell, B.G. (2019) Operational and Economic Aspects of Spirulina-Based Biorefinery. *Biore-source Technology*, **292**, Article ID: 121946. <https://doi.org/10.1016/j.biortech.2019.121946>
- [7] NCCIH (2020) Bilberry. National Center for Complementary and Integrative Health. <https://www.nccih.nih.gov/health/bilberry>
- [8] Chu, W., Cheung, S., Lau, R. and Benzie, I. (2011) Bilberry (*Vaccinium myrtillus* L.). In: Benzie, I.F.F. and Wachtel-Galor, S., Eds., *Herbal Medicine: Biomolecular and Clinical Aspects*, CRC Press, 55-71. <https://doi.org/10.1201/b10787-5>
- [9] Ozawa, Y., Kawashima, M., Inoue, S., Inagaki, E., Suzuki, A., Ooe, E., *et al.* (2015) Bilberry Extract Supplementation for Preventing Eye Fatigue in Video Display Terminal Workers. *The Journal of Nutrition, Health and Aging*, **19**, 548-554. <https://doi.org/10.1007/s12603-014-0573-6>
- [10] Kedare, S.B. and Singh, R.P. (2011) Genesis and Development of DPPH Method of Antioxidant Assay. *Journal of Food Science and Technology*, **48**, 412-422. <https://doi.org/10.1007/s13197-011-0251-1>
- [11] Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R.P., *et al.* (2022) Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus Religiosa. *Molecules*, **27**, Article No. 1326. <https://doi.org/10.3390/molecules27041326>
- [12] Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995) Use of a Free Radical

- Method to Evaluate Antioxidant Activity. *LWT—Food Science and Technology*, **28**, 25-30. [https://doi.org/10.1016/s0023-6438\(95\)80008-5](https://doi.org/10.1016/s0023-6438(95)80008-5)
- [13] Benzie, I.F.F. and Strain, J.J. (1996) The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. *Analytical Biochemistry*, **239**, 70-76. <https://doi.org/10.1006/abio.1996.0292>
- [14] Chen, L. and Opara, U.L. (2013) Texture Measurement Approaches in Fresh and Processed Foods—A Review. *Food Research International*, **51**, 823-835. <https://doi.org/10.1016/j.foodres.2013.01.046>
- [15] Bai, Q. and Bai, Y. (2014) Fatigue and Fracture. In: Bai, Q. and Bai, Y., Eds., *Subsea Pipeline Design, Analysis, and Installation*, Elsevier, 283-318. <https://doi.org/10.1016/b978-0-12-386888-6.00012-2>
- [16] Bibi Sadeer, N., Montesano, D., Albrizio, S., Zengin, G. and Mahomoodally, M.F. (2020) The Versatility of Antioxidant Assays in Food Science and Safety—Chemistry, Applications, Strengths, and Limitations. *Antioxidants*, **9**, Article No. 709. <https://doi.org/10.3390/antiox9080709>