



## **Effects of Dried Wolffia Powder on the Quality of Corn Milk Beverage Mixed with Dried Wolffia Powder**

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### **Abstract**

Wolffia is a plant-based protein alternative that has recently gained significant much attention due to its nutritional value and its ability to be processed into a variety of food products. This research aimed to study the effects of mixing different amounts of dried Wolffia powder with corn milk beverage, especially in terms of quality. This was done by analyzing important chemical components for application in the development of plant-protein beverage products with high nutritional value in order to meet the needs of today's health-conscious consumers. The development of a corn milk product mixed with dried Wolffia powder was divided into 4 treatments. The first step was to study the analysis of protein and water activity in fresh corn and dried Wolffia powder to use as basic information for research and development to improve the treatment of corn milk beverage mixed with dried Wolffia powder. It was found that the protein and water activity values were 2.71, 2.54%, 0.950 and 0.475, respectively. A physicochemical, microbiological and sensory analysis of the beverage product was conducted. The Completely Randomized Design experimental design was used for physicochemical product analysis, while the Randomized Complete Block Design experimental design was used for sensory evaluation analysis. The results showed that different amounts of dried Wolffia powder affected the physicochemical properties in all treatments, except for the total acidity percentage. The microbiological results showed that the microorganisms in all treatments were within the specified standards (Community Product Standard 124/2003). The sensory analysis results from 30 untrained panelists using the 9-point hedonic scale test found that the beverage product added with 1% of dried Wolffia powder received the highest liking score in all aspects, including appearance,



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color, odor, taste and overall acceptability. The experimental results indicated that the corn milk beverage mixed with dried *Wolffia* powder had a high overall liking level among beverage products. Therefore, the study of *Wolffia* plant-based protein is beneficial as it is another intriguing alternative use in the development of beverage products, thus increasing nutritional value for health-conscious individuals. Further, It is suitable for development into health beverage products that meet consumer demand and expand production capacity in the food industry.

### Abbreviations

ANOVA	Analysis of Variance
CC	Cocoa
CF	Instant Coffee
CM	Chufa Milk
CN	Cinnamon Extract
CO	Orange Juice
CPS	Community Product Standard
CRD	Completely Randomized Design
CS	Strawberry Juice
CT	Control
DMRT	Duncan's New Multiple Range Tests
DPPH	2,2-Diphenyl-1-Picrylhydrazyl Assay
RCBD	Randomized Complete Block Design
WPC	Whey Protein Concentrate

### Introduction

Currently, the consumption of food made from plant-based raw materials, especially plant-based protein, which is considered an alternative to replace animal protein, is increasing continuously. This is due to the impact of meat consumption in terms of ethics, animal cruelty and the environmental impact of livestock farming systems that emit relatively high carbon dioxide emissions.<sup>1</sup> Another factor is the health impact on consumers and nutritional value from the use of chemicals and antibiotics in animal farming, especially animal fats that are high in cholesterol.

Plant-based proteins are increasingly replacing animal meat in food. Plant-based proteins have flavors, textures and nutritional values similar to meat.<sup>1,2</sup> However, these are achieved with different components and ingredients that do not come from animals but rather from plants.<sup>2</sup> Consequently, plant-based protein supplement products have been developed to meet consumer demand. This is done by extracting protein from various plants, such as

pea protein, brown rice protein etc. The advantage of these plant-based protein powders is that they are allergen-free and suitable for consumers who are allergic to milk protein, lactose or egg protein.

Plant-based protein powders have the same effect on increasing muscle mass and muscle power as whey protein.<sup>3</sup> Plant protein is becoming increasingly popular in the food industry because it is an alternative source of protein for those who avoid meat. Plant-based protein products that replace meat are of interest to health-conscious consumers who do not consume animal products.

Therefore, the consumption of plant-based protein will increase nutritional value and provide the human body with all the amino acids it needs. In addition, plant-based proteins, especially beans, are rich in unsaturated fatty acids and dietary fiber, which help reduce cholesterol levels and the risk of heart disease as they contain many substances that are beneficial to health. However, the use of plant proteins in the food industry should consider the

nutritional value and functional properties of proteins that are components of food, which help increase the potential for application in the food industry.<sup>1</sup>

Water fern (*Wolffia arrhiza* (L.) Wimm) is a plant native to Thailand. It is round or almost round in shape, about 1 mm in size and green in color. It has no roots. It is a plant with high nutritional value. From the analysis of protein content in dried water fern, it was found that water fern contains a high amount of protein, approximately 35 g per 100 g of dry wt. and contains many essential amino acids such as leucine, lysine and high  $\beta$ -carotene. Most of the water fern production comes from natural water sources. Increasing the production in the farm system is crucial for both human consumption and the animal feed industry.<sup>4</sup>

Corn (maize), scientifically known as *Zea mays* L., is an important economic crop in Thailand. The total corn planting area in Thailand is roughly 7-8 million rai. Corn can be classified into seven types based on the location of each type of starch in the seed as well as the characteristics of the seed coat (glume). Different types include sweet corn, flint corn, popcorn, flour corn, dent corn, pod corn and waxy corn. Sweet corn is an important economic crop that can be grown all year round. Its benefits include a short production season and the ability to be grown in all regions of the country.<sup>5</sup>

Five connected research projects focused on related products. Primarily, this research aimed to study the appropriate ratio of water to meal, the nutritional value of the food, and microbial examination. It also intended to make comparisons of consumer acceptance between a semi-finished water chestnut plant protein drink with the plant protein products in the market. The research results found that a 1:0.6 ratio of plant protein to water meal was appropriate for developing semi-finished plant protein beverage products supplemented with water meal and it was accepted at the highest level of satisfaction.<sup>6</sup>

The next step in this research was to create novel chufa milk using fruit juices or other flavoring ingredients enhanced with WPC. In addition to buffalo milk fortified with WPC, which acted as a CT, six chufa milk beverages were formulated, including plain CM, chufa milk enriched with CO or CS and chufa milk flavored with CC, CF or CN. According

to the results, the control had higher levels of ash, lactose and pH than chufa milk beverages, whereas the CM beverage had higher levels of fat, protein, sucrose and viscosity than the CT beverage. The CC beverage had the highest fat content. Additionally, the CS beverage had the highest viscosity value, while the CC and CF beverages had higher protein contents than the other flavored chufa milk beverages.<sup>7</sup>

In the next step, research was carried out to create a stable, nutrient-dense beverage based on amaranth proteins. Based on what was already known about starch separation methods and the techno-functional characteristics of amaranth proteins, the beverage was obtained. The protein extract was heated to 80°C for 20 min after gums, gellan and xanthan were added. Proteins, lipids, fiber, ashes, carbohydrate and water had compositions of  $3.42 \pm 0.08$ ;  $0.60 \pm 0.06$ ;  $1.9 \pm 0.4$ ;  $0.43 \pm 0.01$  and  $90.58 \pm 0.01\%$ , respectively, comparable to skim milk from cows. While the addition of gums resulted in a decrease in the susceptibility of proteins to heat treatment, thermal treatment led the proteins to become denatured and aggregate.<sup>8</sup>

The next step in, this research was to study the effect of the aril powder of gac fruit on the processing of tofu milk that was supplemented with 0%, 1%, 2% and 3% of aril powder. Physical properties such as brightness, color and texture, as well as appearance and chemical properties including pH, lycopene and DPPH inhibition, were examined. Sensory evaluation of the appearance, color, odor, taste, texture and overall acceptability of each product was performed by 30 untrained panelists using the 9-point hedonic scale. All treatments were found to be significantly different, except for the brightness. Chemical properties showed that they were significantly different ( $p \leq 0.05$ ). Sensory evaluation indicated that the taste, texture and overall acceptability of tofu milk products supplemented with 1% of gac fruit aril powder gave the highest scores of 7.56, 7.36 and 7.53, respectively.<sup>9</sup>

This study also investigated the optimum ratios of barley and black sesame for blended barley milk and black sesame beverage. This research consisted of four treatments. Analysis of barley and black sesame at a ratio of 400:0 and blended barley and black sesame at ratios of 390:10, 380:20 and

370:30 by wt. were performed. Physical qualities, such as appearance, color and viscosity, chemical qualities, including the pH, percentage of the total acidity, total soluble solids and the DPPH inhibition and sensory evaluation of each product's color, odor, texture, taste and overall acceptability were performed by 30 panelists using the 9-point hedonic scale. It was found that the  $L^*$ ,  $+a^*$  and  $+b^*$  of all samples were significantly different. It was shown that the chemical properties did not differ significantly different. Furthermore, sensory evaluation indicated that the samples that substituted barley with 5% black sesame gave the highest scores for texture, taste and overall acceptability of 6.77, 6.90 and 7.23, respectively.<sup>10</sup>

There are many health drinks available at present, including popular ones like tofu milk and barley milk.<sup>9,10</sup> In addition, the market is highly competitive. In order to create a beverage with a distinct flavor that benefits consumers, the idea was to combine corn milk with dried Wolffia powder to make a beverage in a ready-to-use powder form. Corn milk mixed with dried Wolffia is one type of beverage that is becoming more and more popular. The processing of dried Wolffia into semi-finished, Wolffia-enriched beverages made from plant protein represents a development aimed at diversifying alternative protein products and expanding the range of options for consumers interested in these alternatives.

According to a cursory review of the literature, no study has examined the topic of maize milk protein combined with dried Wolffia powder, despite the fact that previous research examined the process regarding specific fruits and vegetables. Corn is an important economic crop. It is easy to cultivate, takes a short time and grows year-round. Corn also contains highly-nutritious protein and it can be processed into variety of food products. For example, corn milk can help alleviate diarrhea for people who like to consume dairy products. Mixing various kinds of corn can add value to both corn and corn products, alleviating the market oversupply of corn and increasing the number of consumers. Furthermore, it can add value to corn and corn products. However, the quality of plant protein drinks must be considered first, namely the source of raw materials, nutritional value, appropriate processing and product stability.<sup>11</sup> As a result, the researchers are interested in creating a drink that also includes vitamins, antioxidants and

other minerals. In this study, sodium alginate was used as a stabilizer to stop sedimentation, which could increase its appeal to customers.

The objectives of this study included investigating the suitability of dried Wolffia powder for making corn milk combined with dried Wolffia powder as well as the physicochemical, microbiological and sensory characteristics of a corn beverage mixed with dried Wolffia powder.

### Materials and Methods

The raw ingredients utilized in this study included fresh corn from Talad Thai in Thailand's Pathum Thani Province and dried Wolffia transported to the physicochemical laboratory from Baan Suan Noo Dee in Nakhon Nayok Province. The inquiry made use of 99.8% pure food-grade and analytical-grade substances that might be utilized in food products.

### Raw Material Preparation

#### Corn Preparation

The frozen fresh corn was placed at room temperature for 25 min, separated into the corn kernels and boiled in 100°C water for 5 min. The corn was then blended and mixed with the boiled water in a ratio of 1:2. The sample was filtered through a thin cloth and allowed to cool before mixing with Wolffia in the next step.<sup>12</sup>

### Study of the Optimum Ratio of Dried Wolffia Powder for the Development of Corn Milk Supplemented with Dried Wolffia Powder

The prepared corn milk was mixed with four treatments containing different amounts of dried Wolffia powder. Treatment 1 did not add dried Wolffia powder (0% dried Wolffia powder; control treatment), while treatments 2-4 added 1, 2 and 3% dried Wolffia powder, respectively. Sodium alginate was added to each treatment at 0.5%. Each mixture was homogenized and heated at 70°C for 20 min or until the mixture was homogeneous. After cooling all treatments to about 30°C, samples from each treatment were examined physically, chemically and microbiologically in addition to undergoing sensory analyses.<sup>13</sup>

### Physicochemical, Microbiological and Sensory Evaluation Qualities of Corn Milk Supplemented with Dried Wolffia Powder

Physically, the ( $L^*$ ), ( $+a^*$ ), and ( $+b^*$ ) were measured using a Mini Scan XE Hunter Lab model Color flex

EZ 45-0 (LAV) to analyze the characteristics of the beverage.<sup>14</sup> Furthermore, factors were assessed and assigned scores.

Chemically, pH was assessed using a pH meter, Hanna model HI98103,<sup>14</sup> while protein was assessed using the Kjeldahl method (digestion (Buchi) with model K-350. Nitrogen was examined with a distiller (Buchi) model K-350,<sup>14</sup> while total acidity (%) was checked using titration with a 0.1N NaOH method and phenolphthalein as an indicator.<sup>14</sup> Total soluble solids were examined using a hand refractometer (0-32°Brix), Atago Master-53M,<sup>14</sup> while water activity was measured with an Aqualab model 4TE.<sup>14</sup> Total chlorophyll was assessed using the calculation summation between chlorophyll a and b,<sup>15,16</sup> with chlorophyll-a using solvent extraction and the spectrophotometric method<sup>15,16</sup> and chlorophyll-b using the spectrophotometric method with Meter Tech model SP-830.<sup>15,16</sup> DPPH (mg TE/100 g) was assessed using the 2, 2-Diphenyl-1-Picrylhydrazyl assay by the spectrophotometric method with the Meter Tech model SP-830.<sup>17</sup>

Microbiological examination included total plate count using plate count agar as medium and incubator (Memmert) model UF 55 for bacterial growth,<sup>14</sup> while sensory evaluation was carried out using panelists (as determined by a 9-point hedonic scale).<sup>18</sup>

**Analysis of Data**

The study concentrated on a CRD (physicochemical analysis) rather than an RCBD (sensory evaluation), which may be used in experimental settings. ANOVA and DMRT were both employed to evaluate differences between treatments ( $p \leq 0.05$ ). Data were presented as the actual values measured as mean  $\pm$  standard deviation after each treatment, and were analyzed three times (four treatments). Data were calculated using PASW Statistics version 22.<sup>18</sup>

**Results**

Tables 1-6 display the results for each of the different dried Wolffia powder qualities.

**Table 1: Physicochemical properties of dried Wolffia powder and fresh corn**

Attributes	Value + S.D.	
	Water activity	Protein (%)
dried Wolffia powder	0.475 $\pm$ 0.07 <sup>b</sup>	2.54 $\pm$ 0.15 <sup>b</sup>
fresh corn	0.950 $\pm$ 0.02 <sup>a</sup>	2.71 $\pm$ 0.25 <sup>a</sup>

\*a-b indicates significant differences ( $p \leq 0.05$ ) in vertical line.

**Table 2: Appearance of product characteristics of corn milk mixed with dried Wolffia powder**

Dried Wolffia powder (%)	Appearance	Color	Odor	Taste
0.0	few corn sediment	light brown, cloudy	corn	little sweet
1.0	few corn and Wolffia sediment	light brown, cloudy	Wolffia and corn	little sweet and bitter
2.0	few corn and Wolffia sediment	dark brown cloudy	Wolffia and corn	little sweet medium bitter
3.0	few corn and medium Wolffia sediment	dark brown, cloudy	Wolffia and corn	little sweet medium bitter

**Table 3:  $L^*$ ,  $+a^*$  and  $+b^*$  of corn milk mixed with dried Wolffia powder**

Attributes	Dried Wolffia powder (%)			
	0.0	1.0	2.0	3.0
$L^*$	28.59± 0.17 <sup>a</sup>	26.53±0.29 <sup>a</sup>	25.45±0.74 <sup>ab</sup>	23.11±0.39 <sup>b</sup>
$+a^*$	12.54±0.27 <sup>a</sup>	10.46±0.56 <sup>ab</sup>	9.67±0.25 <sup>ab</sup>	8.31±0.77 <sup>b</sup>
$+b^*$	11.87± 0.49 <sup>a</sup>	10.23±0.43 <sup>ab</sup>	9.33±0.38 <sup>ab</sup>	8.09±0.66 <sup>b</sup>

\*a-b indicates significant differences ( $p \leq 0.05$ ) in horizontal line.

**Table 4: Chemical components of corn milk mixed with dried Wolffia powder**

Chemical components	Dried Wolffia powder (%)			
	0.0	1.0	2.0	3.0
Protein (%) <sup>*</sup>	2.71±0.25 <sup>b</sup>	3.05±0.28 <sup>a</sup>	3.18±0.54 <sup>a</sup>	3.22±0.17 <sup>a</sup>
Total soluble solid (°Brix) <sup>*</sup>	16.60±0.35 <sup>a</sup>	12.93±0.14 <sup>b</sup>	12.46±0.40 <sup>c</sup>	12.16±0.00 <sup>d</sup>
pH <sup>*</sup>	6.55±0.28 <sup>a</sup>	6.41±0.17 <sup>b</sup>	6.24±0.15 <sup>c</sup>	6.10 <sup>d</sup> ±0.08
Total acidity (%) <sup>ns</sup>	0.35±0.28	0.38±0.25	0.36±0.47	0.31±0.28
Total chlorophyll (mg/L) <sup>*</sup>	43.25±0.02 <sup>b</sup>	46.49±0.31 <sup>ab</sup>	48.04±0.18 <sup>a</sup>	49.09±0.28 <sup>a</sup>
Chlorophyll-a (mg/L) <sup>*</sup>	13.14±0.01 <sup>b</sup>	14.25±0.23 <sup>ab</sup>	14.93±0.58 <sup>ab</sup>	15.21±0.21 <sup>a</sup>
Chlorophyll-b (mg/L) <sup>*</sup>	30.11±0.01 <sup>b</sup>	32.24±0.18 <sup>ab</sup>	33.11±0.45 <sup>a</sup>	33.88±0.65 <sup>a</sup>
DPPH (mg TE/100 g) <sup>*</sup>	45.84±0.01 <sup>d</sup>	48.61±0.01 <sup>c</sup>	51.91±0.02 <sup>b</sup>	54.14±0.17 <sup>a</sup>

\*a-d indicates significantly different ( $p \leq 0.05$ ) in horizontal line., ns indicates non significant ( $p > 0.05$ ) in horizontal line.

**Table 5: Microbiological properties of corn milk mixed with dried Wolffia powder**

Dried Wolffia powder (%)	Values (CFU/mL)
0.0	<1×10 <sup>4</sup>
1.0	<1×10 <sup>4</sup>
2.0	<1×10 <sup>4</sup>
3.0	<1×10 <sup>4</sup>

**Table 6: Sensory evaluation of corn milk mixed with dried Wolffia powder**

Dried Wolffia powder (%)	Likability scores				
	appearance <sup>*</sup>	color <sup>*</sup>	odor <sup>*</sup>	taste <sup>*</sup>	overall acceptability <sup>*</sup>
0.0	5.40±1.2 <sup>c</sup>	5.26±1.1 <sup>c</sup>	5.23±1.1 <sup>c</sup>	5.90±1.1 <sup>d</sup>	5.00±1.0 <sup>d</sup>
1.0	7.00±1.1 <sup>a</sup>	7.76±1.4 <sup>a</sup>	6.90±1.4 <sup>a</sup>	6.83±1.2 <sup>a</sup>	6.26±0.9 <sup>a</sup>
2.0	6.76±1.1 <sup>b</sup>	6.53±1.2 <sup>b</sup>	5.33±1.3 <sup>b</sup>	5.43±1.2 <sup>b</sup>	5.03±1.1 <sup>b</sup>
3.0	5.60±1.0 <sup>c</sup>	5.70±1.1 <sup>c</sup>	5.43± 1.3 <sup>b</sup>	5.66±1.1 <sup>c</sup>	5.70±1.1 <sup>c</sup>

\*a-d indicates significant differences ( $p \leq 0.05$ ) in vertical line.

## Discussion

The development of the dried Wolffia beverage mixed with corn milk is aimed at the development of a health drink product that can be considered an alternative for those who do not consume meat.<sup>1</sup> Panyoyai<sup>19</sup> stated that blending various plant-based proteins is a way to enhance protein quality. These plant-based proteins differ in molecular structure, with their amino acid composition and quantity affecting protein function. In particular, solubility determines their utilization properties. Plant-based proteins are readily available in the market and are a global driving force for the food industry in the future.<sup>20-23</sup>

This aligns with Cheewapruk and Limsangouan<sup>1</sup>, who stated that the food industry is conducting research and development on raw material sources and appropriate processing practices to manufacture products that meet market demands in terms of taste, texture and nutritional value. The growing popularity of plant-based protein products has attracted increased interest from entrepreneurs, as has the development of these products for the market, both in terms of nutritional value and safety. From the experimental results in Table 1, it was found that the difference in water activity values between dried Wolffia was 0.475, which was significantly lower than fresh corn with a water activity of 0.950 ( $p \leq 0.05$ ). The low water activity value in dried Wolffia indicates that the drying process reduces the amount of water in the system, resulting in low moisture content and stability during storage, reducing the chance of microbial growth, deterioration or chemical and biological changes. On the contrary, the high water activity value in fresh corn indicates a higher amount of water in the system, which is suitable for microbial growth and may cause rapid deterioration. This result demonstrates the importance of controlling the water activity value in food products by reducing the water activity value, such as by drying, which can extend the shelf life and reduce the risk of microbial contamination.<sup>24</sup>

The initial protein values of dried Wolffia and corn were equal to 2.54% and 2.71%, respectively. Protein value was inconsistent with the research of Doungtip *et al.*,<sup>25</sup> which reported the analysis of dried protein at 23.25%. This may have been due to differences in the species, season and environment used for cultivation, causing the measured values

to vary. For protein values, a report by the USDA<sup>26</sup> noted that fresh corn had 3.27% protein, which was slightly higher than the researched value, possibly due to differences in the species, season and environmental conditions.

The amounts of dried Wolffia powder blended in the corn milk beverage were 0.0, 1.0, 2.0 and 3.0% (Table 2), according to the investigation of ready-to-drink maize protein integrated with different dried Wolffia milk beverage products. The appearance of a beverage product greatly influences consumer acceptance. It is well known that sedimentation is a common physical defect in beverages.<sup>27</sup>

Gravity causes Wolffia and corn of a particular weight and size to settle organically in the suspension system.<sup>27</sup> All treatments were found to have comparable values; the appearance showed a tiny amount of maize and dried Wolffia sediments that increased as the amount of dried Wolffia powder increased until the treatment had more than 2%. The powdered (dried) Wolffia was dark brown and precipitated when heated. It can be shown that the incidence of sedimentation is positively correlated with the 0.5% addition of sodium alginate (as a preservative).<sup>28</sup> Their capacity to network with one another and with the components of the Wolffia protein may be the cause of this influence.<sup>29</sup> The collected data revealed some ambiguous separation. This outcome is in line with research by Tantidetpaisarn *et al.*,<sup>11</sup> The product will have a scent similar to that of Wolffia. As the quantity of dried Wolffia protein powder rises, the flavor of the product will become slightly sweet and bitter.

Color is another critical factor and the first quality aspect that consumers assess since it influences their choices and decisions, even before the product is consumed. Color and appearance are both related to sensory evaluation.<sup>30</sup> As additional amounts of dried Wolffia powder were added, the  $L^*$ ,  $+a^*$  and  $+b^*$  values of all treatments fell, according to the physical property study of corn protein combined with dried Wolffia powder (Table 3). In this research Chroma ( $C^*$ ) and Hue value were in the range of 28.87 and 0.98, respectively.

The measured color values in each treatment were different due to the different amounts of dried Wolffia powder added ( $p \leq 0.05$ ) and the main pigment found

in Wolffia is chlorophyll. In corn, it is carotenoids, which contain a large number of double bonds. The powdered (dried) Wolffia serves as an ingredient or element that is crucial to the formation of a dark brown hue.<sup>31</sup>

Since the primary structure of chlorophyll is a ring with  $Mg^{+2}$  in the center (porphyrin ring), a Maillard reaction occurs between reducing sugars when heated by drying porphyrin and blended with corn in the beverage manufacturing process. The sugars include glycoside or glucosamine molecules, which comprise the main structural components of corn sugars and amino acids, which are components of porphyrin and corn protein. When the mixture is pasteurized with heat, the Maillard reaction is accelerated further. This is in line with the results of the study of the Maillard reaction between reducing sugars in sugar and amino acids, which found that the reaction increases when the temperature is higher.<sup>32</sup>

The addition of sodium alginate, which is a yellow-brown powder and sodium alginate as a hydrocolloid that is a heteropolysaccharide, increases the Maillard reaction. Heteropolysaccharide is a polymer of sugar derivatives, namely mannuronic acid, (D-mannuronic acid) guluronic acid, which is a reducing sugar, increases the Maillard reaction.<sup>9-11,25,32</sup> For the mechanism, sodium alginate can form a covalent conjugation with dried Wolffia protein and corn protein through the Maillard reaction. It can also react with polyphenol oxidase, so the oxidation reaction can occur. When the enzyme polyphenol oxidase acts as a catalyst to bring phenolic chemicals and oxygen into contact, the colorless monophenol is oxidized to colorless diphenol and further oxidized to o-quinone, which reacts with amino acids or proteins to form a brown substance. They will combine to form large, brown polymers. Heat causes a structural change in the carotenoids, resulting from the isomerization of trans to cis. Moreover, a non-enzymatic caramelization reaction influences the color change of the product.<sup>9-11,25,32</sup>

Table 4 revealed that, except for the total acidity percentage, all of the chemical properties of corn beverages combined with dried Wolffia powder beverages using four different levels of dried Wolffia powder content at 0.0% (control treatment), 1.0, 2.0 and 3.0% showed statistically significant differences

( $p \leq 0.05$ ). The highest protein percentage, 3.22%, was discovered to be with 3.0% dried Wolffia powder. This was a result of the largest amount of dried Wolffia powder being added during this treatment.<sup>6,25,32</sup> According to the experimental findings, the protein content tends to rise as the amount of dried Wolffia powder increases.<sup>6,25,32</sup> It was also shown that the amount of protein in the corn milk combined with dried Wolffia protein powder affected the  $L^*$ ,  $+a^*$  and  $+b^*$  values. The protein value increases as the amount of dried Wolffia powder increases.

Generally, the quality of the final product is influenced by the quality of the raw materials. In terms of protein and sugar concentration, both Wolffia and corn milk impact the Maillard reaction.<sup>6,10-11,25,27,32</sup> This is because the heating procedure breaks down the bioactive ingredient in both Wolffia and corn milk, producing aldehydes that aid in the reaction.<sup>6,10,11,25,27,32</sup> Arnoldi *et al.*,<sup>33</sup> stated that protein will oxidize and create aldehydes and ketones when heated. The Maillard process alters the color of proteins by causing fructose and amino acids to form the brown chemical known as melanoidin.<sup>6,25,27,33</sup> Another reason is that the added sodium alginate is extracted from the cell walls of brown algae, which already has a brownish-yellow powder.

Furthermore, sodium alginate is a hydrocolloid that is a heteropolysaccharide, which is a polymer of sugar derivatives, namely mannuronic acid (D-mannuronic acid) and guluronic acid, which are reducing sugars.<sup>11,33</sup>

According to the analysis of total soluble solids, four different levels of dried Wolffia powder were combined in the corn milk and served as a ready-to-drink product. Significant differences ( $p \leq 0.05$ ) were found between the quantities of dried Wolffia powder at 0.0, 1.0, 2.0 and 3.0%, which were 16.60, 12.93, 12.46 and 12.16°Brix, respectively.<sup>28</sup> It was discovered that the amount of total soluble solids tended to decrease when the amount of dried Wolffia powder increased.

The findings of the experiment demonstrated that the pH was impacted by the quantity of dried Wolffia powder supplied. When the pH of the beverage items was measured, the results fell between 6.10 and 6.55. Microorganisms cause it to spoil easily

because it is a low-acid product and needs to be stored at low temperatures.<sup>28</sup> The pH regulators were most likely added to the corn milk beverages mixed with dried *Wolffia* powder to maintain a slightly acidic pH of 5–6 and increase shelf-life stability as well as reduce the alkalinity or bitter flavor characteristics.<sup>28</sup> When dissolved in water, these acidic substances release H<sup>+</sup> ions and interact with water molecules. The pH decreases and more H<sup>+</sup> ions are produced when the substance is more acidic.<sup>29</sup>

Additionally, the pH and percentage of total acidity change due to the heat treatment used in pasteurization.<sup>18,28</sup> Compared to pH, the total acidity of a food system is a better indicator of how an acid will affect flavor because it shows the overall acid concentration in food. The concentration of hydroxonium ions in the food system determines whether microbes can grow in a particular diet.<sup>34</sup> The percentages of total acidity, which were 0.35, 0.38, 0.36 and 0.31%, respectively, did not differ substantially ( $p > 0.05$ ). It was discovered that, as the amount of dried *Wolffia* powder increased by more than 1.0%, the percentage of overall acidity tended to decrease.<sup>33</sup>

However, when considering the relationship between pH and total acidity percentage, it was found that there was a relationship only when adding dried *Wolffia* powder. The values of treatments without adding dried *Wolffia* powder were lower than when adding 1.0, 2.0 and 3.0% of dried *Wolffia* powder, but not significantly. The difference may be due to the low acidifying component or the decrease in the value during the heating process.<sup>33</sup>

For this research, three values of chlorophyll were analyzed: total chlorophyll, chlorophyll-a and chlorophyll-b. For the analyzed values, there was a tendency in the same direction ( $p \leq 0.05$ ) when adding more dried *Wolffia* powder,<sup>35</sup> resulting in an increase in the analyzed chlorophyll value. The addition of 3.0% dried *Wolffia* powder resulted in the highest total chlorophyll, chlorophyll-a and chlorophyll-b, which were 15.21, 33.88 and 49.09 mg/L, respectively. Chlorophyll is a primary pigment found in green vegetables and important to the body for many reasons, such as cancer prevention and antioxidant effects. The chemical structure of chlorophyll consists of a head and a tail. The head is a porphyrin ring with a magnesium (Mg<sup>2+</sup>) atom

in the middle and the tail is a long chain called phytol. The porphyrin ring is made up of four pyrrole rings connected by a methene bridge.<sup>36</sup> There are several types of chlorophyll, but the basic structure is the same: a porphyrin ring with magnesium and a phytol tail. Chlorophyll-a and -b are the main structures found in green plants. Solvent extraction and spectrophotometric calculation by equation were utilized for the analysis of chlorophyll-a, b, as well as the total chlorophyll of *Wolffia* and products.<sup>15,16</sup>

The structural difference is that chlorophyll a has a methyl group (-CH<sub>3</sub>) at the carbon position 3 of the porphyrin ring, which absorbs blue and red light well. Chlorophyll-b has an aldehyde group (-CHO) at the carbon position 3 of the porphyrin ring. The total chlorophyll content is the sum of primary chlorophylls (-a and -b) and a small amount of secondary chlorophylls. The total chlorophyll contents, chlorophyll-a and chlorophyll-b, are directly proportional to leaf greenness, i.e., chlorophyll-a increases with increasing leaf greenness.<sup>37</sup> The environmental and plant-growing circumstances have an impact on the amount of chlorophyll in plants. Furthermore, the release of magnesium from the chlorophyll molecule occurs when direct sunlight and oxygen reach the material. Hydrogen then begins to replace magnesium, resulting in the formation of pheophytins and their derivatives, followed by pyropheophytins and pheophorbides.<sup>38</sup> Numerous assays, including DPPH, can be used to evaluate the antioxidant qualities of maize and *Wolffia*, which are significant for beverage products. *Wolffia* also contains luteolin-7-O- $\beta$ -D-glucoside, ferulic acid,  $\beta$ -carotene, kaempferol and chlorophyll, all of which have antioxidant qualities.<sup>39</sup> The DPPH approach was chosen for this study,<sup>37</sup> preventing free radicals from stealing hydrogen atoms from antioxidant chemicals, which allows them to steal one electron from those compounds.<sup>37</sup> One stable free radical with a purple hue is DPPH.<sup>39</sup> Spectrophotometric measurement of antioxidant activity revealed a decrease in the intensity of the purple color in the sample solution, which suggested a decrease in DPPH free radical.<sup>40</sup> The decreased intensity of the purple color is due to free radicals absorbing electrons, which renders them non-radicals since the electrons have been paired. The color shift corresponds to the quantity of electrons captured.<sup>41</sup> The type of solvent, i.e., water and methanol, affected the solubility of polyphenolic

compounds. Methanol has less polarity than water. Methanol, which dissolves anthocyanin, terpenoid, saponin, tannin, flavone and polyphenol, was the solvent employed in this investigation.<sup>42</sup> The amount of Wolffia powder added lifted the DPPH value, according to the analytical data. The experiment with the highest DPPH result, 54.14 mg TE/100 g, had 3.0% dried Wolffia powder and compared to Wirivutthikorn<sup>43</sup> reported DPPH (%) in corn: brown jasmine rice ratio 100:60 had the highest value (34.21%).<sup>44-46</sup>

The amount of dried Wolffia powder applied, the type of dried Wolffia and the time of year it was bred, as well as the surrounding conditions, affected the various values.<sup>31</sup> It has been shown that several of the bioactive substances in Wolffia have antioxidant properties that enable them to scavenge peroxide and free radicals, thereby halting oxidation.<sup>31</sup> As a result, the non-enzymatic reaction known as the Maillard reaction, which is triggered by exposure to high temperatures during the heating process, might produce intermediate molecules that have an impact on antioxidant activity.<sup>30</sup> Since the resultant intermediate chemicals, such pyrazinium and pyridinium, are reactive, antioxidant activity will decrease as reactive compounds are formed with longer heating times and higher temperatures.<sup>9-11,25,32</sup>

The microbiological analysis results of the four experimental products (Table 5) showed that there were fewer than  $1 \times 10^4$  CFU/g of sample. The product requirement for maize milk for industrial use,<sup>47</sup> which stipulates that the total number of microorganisms in corn milk products shall not exceed  $1 \times 10^4$  CFU/g of sample, was compared with the results of the bacteria identified in the corn milk mixed with dried Wolffia powder.<sup>47</sup> It was shown that every beverage product used in the experiment was pasteurized to a temperature high enough to kill certain germs. Plant-based beverages that have undergone pasteurization can eliminate the majority of non-pathogenic and pathogenic bacteria, but they still include trace amounts of heat-resistant bacteria and spores, which shortens their shelf life.<sup>48</sup>

Nonetheless, some microbes that can withstand high temperatures can proliferate. GMP<sup>9,10</sup> guarantees the safety, hygiene and cleanliness of the production procedures and methods. The results of this study corroborated those of Wirivutthikorn,<sup>9</sup> who found that

the bacteria in maize milk beverages supplemented with various gac fruits aril were less than 1 CFU/sample in all treatments. Yodpradit *et al.*,<sup>6</sup> reported that the bacteria in plant protein drink products supplemented with water meal semi-finish amounted to  $3.6 \times 10^2$  CFU/sample.

Sensory acceptability of four treatments of corn milk beverage mixed with dried Wolffia powder, with adjusted proportions of main components, dried Wolffia powder and corn milk, was assessed by 30 untrained panelists using a 9-point hedonic scale to analyze preferences in various aspects, including appearance, color, odor, taste and overall acceptability. The panelists provided scores according to established standards. The results of the sensory acceptability assessment for all four treatments showed statistical differences across all analyzed values, as detailed below. Table 6 compares the likability scores between the control treatment and the sensory evaluation of appearance, color, odor, taste and overall acceptability ( $p \leq 0.05$ ) (0.0% of dried Wolffia powder) for the corn milk mixed with 1.0, 2.0 and 3.0% dried Wolffia powder. In the experimental results, it was found that using 1% dried Wolffia powder resulted in the highest acceptance scores in terms of appearance, color, smell, taste and overall acceptability for all tested products. Odor seemed to be highly influential to overall liking, although few additives (sodium alginate) were used in the beverage.<sup>34</sup> Adding excessive amounts of dried Wolffia powder decreased the overall acceptance scores in all aspects, which may be because dried Wolffia powder contains chlorophyll as the main pigment, which has a distinct odor and bitterness.<sup>34</sup> Adding excessive amounts of dried Wolffia powder affects the flavor and aroma of the beverage, making it unacceptable to consumers. The synthesis of furfural and the breakdown of ascorbic acid may be the cause of the minor loss of flavor, taste and general acceptability.<sup>34</sup>

When the beverage products are heated, structural changes occur in the form of porphyrin, enzymatic and non-enzymatic reactions (Maillard reaction).<sup>11,25,32</sup> Their concentration in Wolffia is also one of the most important factors because some polyphenols, antioxidants, amino acids and chlorophyll are carriers of astringency and bitterness.<sup>34-35</sup> All sensory scores dropped when increasing amounts of Wolffia powder were added.

As more dried Wolffia powder was added, the panelists' acceptance of the corn-based Wolffia drink likewise decreased.<sup>11,31</sup> This might be a result of the abundance of bioactive compounds. The genetics, cultivar, growing techniques, breeding season and environmental factors of different dried Wolffia powders are explained.<sup>11,31,34,35</sup> Yet, the inclusion of dried Wolffia powder raises the nutritional value of the beverage in proportion to the higher quantity of dried Wolffia powder. Wolffia contains  $\beta$ -carotene, ascorbic acid, amino acids, ferulic acid and luteoline-7-O- $\beta$ -D-glucoside, which can help boost the immune system, heal damaged body parts, and nourish the eyes, brain and heart.<sup>3,8,13</sup> Furthermore, a non-enzymatic browning reaction occurs in maize milk and Wolffia powder because of the heating procedure.<sup>14,19</sup> According to the findings of the study, adding more than 1.0% of dried Wolffia powder caused all product approval metrics to drop. This research result is inconsistent with Yodpradit *et al.*<sup>6</sup> This may be because the addition of a high amount of dried Wolffia powder has a certain odor, which is the smell of chlorophyll or the added amount may affect sensory acceptance.<sup>49,50</sup>

### Conclusion

This study concerned the development of corn milk beverage products mixed with dried Wolffia powder using different ratios of dried Wolffia powder, divided into 4 levels by considering the results of physicochemical analysis, including color, total soluble solids, pH, protein, antioxidants, total chlorophyll, chlorophyll-a, chlorophyll-b, total acidity percentage, microbiology and sensory evaluation quality. The experimental results showed that the treatment with 3% dried Wolffia powder had the highest amounts of protein, antioxidants, total chlorophyll, chlorophyll-a and chlorophyll-b. The microbiological analysis found that bacteria did not exceed the specified standard (CPS.124/2003). In terms of sensory quality, the treatment with 1% dried Wolffia powder received the highest liking score in all aspects, including appearance, color, odor, taste and overall acceptability. The results of this study also showed that the treatment adding 1% dried Wolffia powder was the most suitable in all aspects for use in the production of corn milk mixed with dried Wolffia powder. In future research, the researcher aims to launch new products cover with increased nutritional value as well as good odor

and taste, making them appropriate for development into health supplement products. Other forms of plant protein and appropriate packaging, as well as shelf life, could be studied to meet the needs and requirements of today's consumers.

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### Conflict of Interest

The author does not have any conflict of interest.

### Data Availability Statement

This manuscript incorporates all datasets examined throughout this research study.

### Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

### Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

### Clinical Trial Registration

This research does not involve any clinical trials.

### Permission to Reproduce Material from Other Sources

Not applicable.

### Author Contributions

The sole author was responsible for the conceptualization, methodology, data collection, analysis, writing and final approval of the manuscript.

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