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Ready-to-Drink Functional Drink Formulation of Black Mulberry (*Morus Nigra*) Fruit Extract with The Addition of Moringa Leaf (*Moringa Oleifera*) Extract Using D-Optimal Expert Design

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***Abstract*—This final research project aims to obtain an optimal formulation for manufacturing ready-to-drink functional drinks made from black mulberry and moringa leaf extract using the D-Optimal method of the Design Expert application. This research was carried out with the program to produce the desired chemical, physical, and organoleptic properties so that laboratory analysis results and the best formulation were obtained. Responses in this study were chemical analysis, including testing levels of vitamin C and pH, physical responses in terms of color stability, and organoleptic tests on taste, color, and aroma attributes. The results of this final project research found 15 formulations offered to produce an optimal formulation based on the desired value, which showed a value of 0.836, with 43.70% black mulberry, 33.87% moringa leaves, 5% stevia sugar, 1.42% sorbitol, and other additional ingredients as fixed variables, including 0.5% sodium benzoate 1000ppm, 0.2% CMC, 0.3% salt, and 15.00% water. The selected samples were then analyzed for antioxidant activity. Laboratory results for vitamin C were 6.43%, pH of 4.55%, color stability of -0.49, organoleptic properties of taste attribute of 4.36, color attribute of 3.36, and aroma attribute of 4.12. The laboratory analysis results are close to the predictions of the D-Optimal method on the Design Expert program. In addition, antioxidants on day 0 were 1081.41 ppm, and on day 7 were 929.79 ppm.**

***Keywords*—Back mulberry; Moringa leaves; functional drinks; design expert.**

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I. Introduction

Functional drinks are processed foods that contain one or more food components that, based on scientific research, have specific physiological functions beyond their essential functions, proven to be harmless and beneficial to health [1]–[4]. Functional drinks are one of the alternatives that are much sought after and consumed by the public. They are defined as food, either food or drink, that can be consumed as a component in the daily diet, not in the form of tablets, capsules, or powders, but in the form of liquids or drinks that have the property of preventing or curing disease in addition to the properties of the nutrients they contain [5].

Functional drinks are defined as food with natural or processed ingredients that can fulfill health benefits depending on the nutritional value of the food [6]. To be categorized as a functional drink, the food must be consumed as food and drink with sensory characteristics, such as in terms of appearance, texture, color, and taste, that are acceptable to the public and do not have side effects on the metabolism of other nutrients in the amount used. Natural materials such as plants are widely used for medicine or other purposes. The use tends to increase, especially when promoted back to nature, where people prefer non-chemical materials for various healing and therapies. The community commonly uses traditional medicines and medicinal plants in efforts to prevent disease (preventive), improve health (promotive), and restore health (rehabilitative). The use of medicinal plants and traditional medicines is relatively safer than synthetic drugs [7]–[10].

The wider community recognizes and acknowledges the tradition of consuming medicinal plants or spices in traditional herbal concoctions [9], [11]–[15]. However, not all people appreciate traditional herbal concoctions due to their bitter taste and sharp aroma, which reduces the overall appeal of the drink. Consequently, only some enjoy the benefits of these traditional herbal medicines. One of the primary factors influencing public acceptance is the sensory properties, particularly the taste and color of the ingredients. Therefore, palatability or preference holds great importance in formulating functional drinks and their nutritional and physiological aspects that can positively impact health. By incorporating food ingredients that enhance the fundamental properties of the drink, consumer acceptance can be increased. This phenomenon highlights a growing awareness among people about the significance of health, leading to the inclusion of functional beverage products in current food trends [16]. Some raw materials used in the production of functional drinks include black mulberry fruit (*Morus nigra L*) and moringa leaves (*Moringa oleifera*) [17]–[19].

Black mulberry (*Morus nigra L*) belongs to the *Moraceae* family and offers numerous health benefits [20]. It serves as a fruit plant that fulfills nutritional requirements and is a traditional medicinal ingredient with diverse properties. In China, black mulberry fruit is an effective alternative medicine due to its rich chemical content, including anthocyanins, phenolics, and other fatty acids [21]. This fruit is abundant in essential vitamins such as B1, B2, and vitamin C, along with anthocyanins, which act as antioxidants for the human body. Anthocyanins are natural water-soluble flavonoids responsible for the red, blue, and violet colors observed in various plants [22]–[25].

Despite the high production of mulberry plants, they are rarely used in processed food products. One valuable utilization of black mulberry is the creation of processed functional beverage products. In addition to black mulberry fruit, the moringa plant is another valuable ingredient for this ready-to-drink functional beverage. Moringa plant (*Moringa oleifera*) is an extraordinary plant scientifically recognized for its nutritional claims, serving as a source of protein and minerals [26]–[32]. Moringa leaves, in particular, are rich in antioxidants, with the highest concentration in the leaves. These antioxidants include tannins, triterpenoids, steroids, saponins, flavonoids, inter quinones, and alkaloids [33]. Fresh moringa leaves contain seven times more antioxidants than vitamin C [34]. The flavonoid group in moringa leaves are quercetin and kaempferol, shown to be more potent antioxidants than the traditional vitamins [35]–[37].

Moringa leaf plants serve as a valuable source of protein and minerals [34], [38]–[40]. The benefits of Moringa leaves extend to their anti-inflammatory properties, diuretic effects, and anti-allergic potential [41]. Moreover, Moringa leaves are widely utilized in traditional medicine for their anti-bacterial, anti-anemic, anti-diabetic, anti-urinary tract infections, and anti-rheumatic properties, as well as for treating external wounds, hypersensitivity, diarrhea, colitis, and dysentery [42]. Sweeteners are food additives that provide a sweet taste, often with fewer calories than regular sugar and negligible nutritional value. They can be categorized as natural sweeteners, obtained from plants like sugarcane, or artificial/synthetic sweeteners chemically synthesized. Artificial sweeteners are often recommended to individuals to lose weight [43]. Artificial sweeteners are sugar substitutes that provide high sweetening power associated with low accompanied calories [44].

The usage limits for artificial sweeteners have been regulated in the Regulation of the Minister of Health of the Republic of Indonesia No. 208/MenKes/Per/IV/1985 concerning artificial sweeteners and No. 722/MenKes/Per/IX/1988 concerning food additives. According to these regulations, special foods and beverages, particularly those low in calories and designed for people with diabetes mellitus, have specific maximum allowable levels of saccharin (300 mg/kg), cyclamate acid (11 mg/kg), and aspartame (40 mg/kg). Regulation of Minister of Health of the Republic of Indonesia, No. 033, Year 2012 on Food Additives [45] revealed that regulation concerning food additives in the Regulation of Minister of Health No. 722/Menkes/Per/IX/88 on Food Additives as already amended by Regulation of Minister of Health No. 1168/Menkes/Per/X/1999 is no longer suitable for the development of science and technology in the food sector. Regulation of Minister of Health No. 208/Menkes/Per/IV/1985 on Artificial Sweetener shall be revoked and declared inapplicable [45].

Therefore, formulation development is of paramount importance in producing food products that are well-received by the current community. The combination of black mulberry fruit extract and moringa leaf extract in the formulation of ready-to-drink functional drinks can significantly influence the characteristics of the final product. The Design Expert program is crucial in optimizing products or processes by analyzing the primary responses caused by several variables. It offers various design options, including Mixture Design, which helps determine the optimal formulation. This research has identified that manufacturing ready-to-drink functional drinks using black mulberry and moringa leaf extract can be optimally formulated using the D-Optimal mixture method in the Design Expert program. Kahfi et al. [46] used design expert d-optimal for formula optimization of functional drink enriched with moringa leaf extract (*Moringa oleifera*).

II. Materials and Method

The method used in this study consists of several stages: treatment plan, experimental, analysis, and response design.

A. Treatment Plan

In the treatment design, sorting, washing, trimming, size reduction, crushing, filtration, weighing, mixing, pasteurization, tempering, packaging, and testing are carried out. The procedure for using the Design Expert software using the D-Optimal method is as follows:

a. The raw materials added are the changing variables in the mixture component, including black mulberry fruit, moringa leaves, stevia sugar, and sorbitol. These variables constitute 84% of the total ingredients, as evident from the remaining fixed variables.
b. In the low and high columns, enter the upper and lower limits using raw materials (changing variables), including black mulberry fruit, moringa leaves, stevia sugar, and sorbitol.
c. Enter the number of responses to be analyzed in a required unit.
d. Based on the results of the data described above, 15 formulations were produced with four changing variables, including black mulberry, moringa leaves, stevia sugar, and sorbitol.
e. The analysis results are entered in the empty table column, including vitamin C levels, color stability, pH, and organoleptic quality attributes of color, taste, and aroma.

B. Experimental, Analysis, and Response Design

The research consisted of preliminary research, primary research, analytical design, and response design, including chemical, physical, and organoleptic responses. The D-Optimal equation is obtained from three processes, including based on the sequential model sum of squares [Type 1] for the model that has a value of "Prob < F" smaller or equal to 0.05 (significant), lack of fit test for the model that has a value of "Prob > F" higher than or equal to 0.05 (not significant), and a statistical summary model. The best model can be determined with the maximum adjusted R-squares and Predicted R-squared parameters. The overall response design carried out in this study consisted of chemical responses, physical responses, and organoleptic responses.

III. Results and Discussion

A. Determination of Fixed and Changing Variables

Determining fixed and changing variables is conducted to identify materials that can be used as fixed variables and materials that can be used as changing variables. The selection of fixed and changing variables is based on various research needs. Several factors were considered in this study, including the functional content of the material, the material's characteristics, the desired product characteristics, and the associated costs. Following a thorough literature review and careful consideration of the desired criteria, the fixed and changing variables for the research are presented in Table 1.

TABLE I

RESULTS OF ANALYSIS OF FIXED VARIABLES AND CHANGING VARIABLES

Fixed Variables	Changing Variables
Sodium Benzoate 1000ppm	Black Mulberry
CMC	Moringa Leaves
Salt	Stevia Sugar
Water	Sorbitol

The next stage involves running the data using the D-Optimal method in the Design Expert software. The input data will include the changing variables: black mulberry fruit, moringa leaves, stevia sugar, and sorbitol. This process will generate 15 recommended formulations provided by the Design Expert. Subsequently, chemical, physical, and organoleptic tests will be conducted on these formulations. The 15 ready-to-drink black mulberry and moringa leaf functional drink formulations are listed in Table 2.

TABLE II

FUNCTIONAL FORMULATION OF BLACK MULBERRY AND MORINGA LEAVES

Formulation	Black Mulberry (%)	Moringa Leaves (%)	Stevia Sugar (%)	Sorbitol (%)
1	53.667	25.333	3	2
2	57	20	5	2
3	47.333	30.667	5	1
4	43	36	3	2
5	50	28	4	2
6	49.333	30.667	3	1
7	52.333	25.333	5	1.333
8	41	36	5	2
9	45.75	32	4.5	1.75
10	55.25	24	3.5	1.25
11	58.667	20	4.333	1
12	59.333	20	3	1.667
13	52	28	3	1
14	42	36	5	1
15	42.5	36	4	1.5

B. Chemical, Physical, and Organoleptic Data Analysis

This research involved creating ready-to-drink functional drinks using black mulberry and moringa leaves, with 15 formulations generated using the D-Optimal method in the Design Expert software. Subsequently, chemical analysis was performed to determine vitamin C levels and pH, while physical analysis focused on color stability. Additionally, organoleptic analysis assessed attributes such as taste, color, and aroma. The collected data were processed and analyzed, and samples were obtained for further evaluation.

The D-Optimal method of the Design Expert is a quantitative analysis technique for process optimization that uses a mathematical model. The D-Optimal Design Expert software aims to determine the optimal formulation of a product that complies with existing standards and can be accepted by consumers [47]. The chemical, physical, and organoleptic analysis results can be seen in Table 3.

TABLE III

RESULTS OF CHEMICAL, PHYSICAL, AND ORGANOLEPTIC ANALYSES

Run	Component 1 A: Black Mulberry	Component 2 B: Daun Kelor	Component 3 C: Gula Stevia	Component 4 D: Sorbitol	Response 1 Vitamin C mg/100 gram	Response 2 pH	Response 3 Kestabilan Warna	Response 4 Rasa	Response 5 Warna	Response 6 Aroma
1	53.6667	25.3333	3	2	6.7	4.61	-1.04	3.36	5.04	3.52
2	57	20	5	2	6.3	4.38	-1.26	3.32	5.08	3.68
3	47.3333	30.6667	5	1	7.33	4.36	-0.49	2.52	3.72	3.04
4	43	36	3	2	5.87	4.55	-0.05	2.28	2.8	2.8
5	50	28	4	2	7.06	4.26	-0.48	3.6	3.44	3.04
6	49.3333	30.6667	3	1	8.6	4.31	-0.88	3	4.8	3.28
7	52.3333	25.3333	5	1.33333	7.14	4.31	-1.01	3.6	5.4	4.12
8	41	36	5	2	8.84	4.43	0.28	3.32	4.44	3.76
9	45.75	32	4.5	1.75	7.33	4.3	-0.01	3.28	4.6	3.8
10	55.25	24	3.5	1.25	6.43	4.15	-0.56	3.2	5.08	3.64
11	58.6667	20	4.33333	1	5.91	4.06	-0.75	3.4	5.16	3.28
12	59.3333	20	3	1.66667	6.15	4.13	-0.71	3.24	5.2	3.16
13	52	28	3	1	7.31	4.27	-0.5	3.28	5.28	3.28
14	42	36	5	1	8.81	4.26	-0.08	3.32	4.52	3.2
15	42.5	36	4	1.5	7.4	4.3	0.04	3.16	4.2	3.24

1) Chemical Analysis

a. Determination of Vitamin C Levels

Vitamin C is an essential nutrient our bodies require as it plays a crucial role in forming collagen, fiber, and protein structures. Collagen is necessary for bone and teeth formation, as well as for repairing scar tissue. Vitamin C also strengthens the body's immune system against infections and aids in iron absorption [48]. UV-VIS spectrophotometry is conducted to determine vitamin C levels in ready-to-drink functional drinks made from black mulberry fruit and moringa leaf extract. UV-VIS spectrophotometry is a method used to measure the amount of light absorbed at different wavelengths in the ultraviolet and visible regions. In this device, a light beam is split, and some light is passed through a transparent cell containing the solvent. When electromagnetic radiation in the UV-VIS region passes through a compound containing double bonds, the compound absorbs some of the radiation. This radiation absorbance is typically caused by a reduction in the light energy as the electrons in the excited regions of the compound occupy high-energy orbitals [49]. The ANOVA data for determining vitamin C levels in black mulberry and moringa leaf functional drinks using UV-VIS spectrophotometry are presented in Table 4.

TABLE IV
ANOVA DATA OF VITAMIN C LEVELS

Source	Sum of Squares	df	Mean Square	F-Value	P-Value	
Model (1)	7.19	3	2.40	4.18	0.0334	Significant
Linear Mixture	7.19	3	2.40	4.18	0.0334	
Residual	6.31	11	0.5734			
Cor Total	13.49	14				

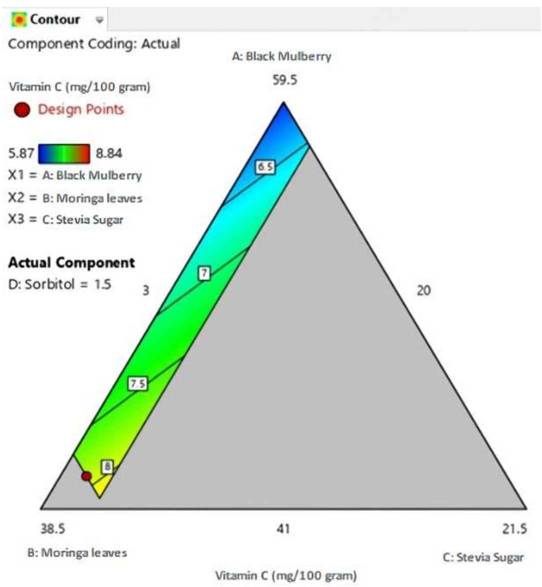

Fig. 1 Contour Plot Graphical Model for the Response Determination of Vitamin C Levels

The Table 4 depicts the ANOVA data for the 15 formulations used as the initial model for data analysis. The results significantly affected chemical analysis, specifically in determining vitamin C levels. The low value of "Prob < F" (less than 0.05) indicates that the 15 formulations had a significant influence on the response, specifically in determining vitamin C levels using the UV-VIS spectrophotometry method. The "Prob < F" value resulting from the modeling of the 15 initial formulations was 0.0334 in response to determining vitamin C levels using the UV-VIS spectrophotometry method, with an effect size of 3.34%. The values of "Prob < F" less than 0.05 indicate a significant influence between the different models for determining vitamin C levels in the ready-to-drink functional drink made from black mulberry fruit and moringa leaf extract as shown in Figure 1

The contour plot graph above illustrates the range of vitamin C levels from the lowest to the highest, with colors indicating the variation. Dark blue represents the lowest range, while green represents the highest range of vitamin C levels. The green dots on the graph indicate the presence of the 15 formulations used in determining the vitamin C content. The graph shows the results of the response to the determination of vitamin C content in the 15 formulations, ranging from 5.87 mg/100 g of material to 8.84 mg/100 g of material. The green color on the graph predicts the percentage response for determining the maximum vitamin C levels in the ready-to-drink functional drink made from black mulberry fruit and moringa leaf extract.

Figure 2 presents a three-dimensional graph depicting the interaction relationships between the components. The difference in surface height reveals distinct response values for each combination of formula components. The highest area represents the maximum vitamin C level, while the lowest area indicates the lowest vitamin C level among the 15 formulations.

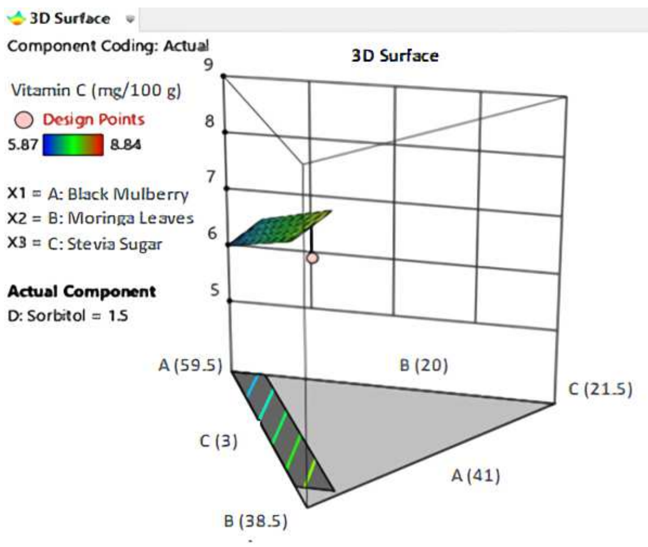

Fig. 2 Three-Dimensional Graphical Model for Determining Vitamin C Levels

The analysis of vitamin C levels is crucial for various reasons, including assessing food ingredients, composition, quality standards, and quality control. The two graphs above show that the vitamin C content in the ready-to-drink black mulberry and moringa leaf functional drinks increased with these two ingredients. Vitamin C, ascorbic acid, is a non-enzymatic water-soluble antioxidant. It plays a vital role in the body's defense against reactive oxygen compounds in plasma cells. Szent-Gyorgyi first isolated this compound in 1928 [50], [51]. Based on the findings from the preliminary and main research on the determination of vitamin C levels, it can be concluded that the rawer materials added to the production of black mulberry and moringa leaf functional drinks, the higher the levels of vitamin C in the final product.

b. pH

A pH meter is used to analyze the ready-to-drink functional drinks made from black mulberry fruit and moringa leaf extract. The pH meter is an electronic measuring instrument specifically designed to measure the pH level (acidity or alkalinity) of liquids, and in some instances, it can also be used to calculate the pH level of semi-solid substances. Generally, a pH meter consists of a specialized measuring probe (glass electrode) connected to an electronic meter that can measure and display the pH reading [52]. Table 5 shows the ANOVA data for the pH analysis of the functional drinks made from black mulberry fruit and moringa leaf extract obtained using the pH meter.

TABLE V
ANOVA pH ANALYSIS DATA

Source	Sum of Squares	df	Mean Square	F-Value	P-Value	
Model	0.1433	3	0.0478	3.42	0.0561	Not Significant
(1) Linear Mixture	0.1433	3	0.0478	3.42	0.0561	
Residual	0.1534	11	0.0139			
Cor Total	0.2966	14				

Table 5 shows the ANOVA data for the 15 formulations used as the initial model for data analysis. The results indicate no significant effects on chemical analysis, specifically pH levels, from the 15 formulations produced. This conclusion is drawn because the "Prob > F" value is higher than or equal to 0.05, indicating a non-significant response (no effect). The "Prob > F" value resulting from modeling the 15 initial formulations for pH response is 0.0561, with an effect size of 5.61%. The "Prob > F" values that are higher than or equal to 0.05 indicate a lack of significant modeling (no effect) between the different models for the pH of the ready-to-drink functional drink made from black mulberry fruit and moringa leaf extract.

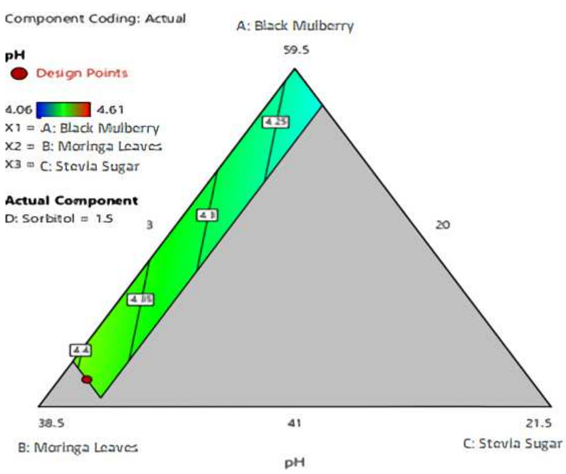

Fig. 3 Graphical Model of pH Analysis

The contour plot graph above illustrates the range of pH numbers from the lowest to the highest. Light blue represents the lowest range, while dark green indicates the highest range of pH values. The dark green dots on the graph represent the 15 formulations used in the pH analysis. The graph displays the results of the pH analysis for the 15 formulations, ranging from 4.06 to 4.61. The dark green indicates the predicted maximum pH analysis percentage for the ready-to-drink black mulberry and moringa leaf functional drinks produced.

Figure 4 presents a three-dimensional graph showing the interaction relationship between components. Differences in surface heights reveal different response values for each combination of formula components. The highest area corresponds to the highest acidity test (pH) response value, while the lowest area corresponds to the lowest acidity test (pH) value among the 15 formulations.

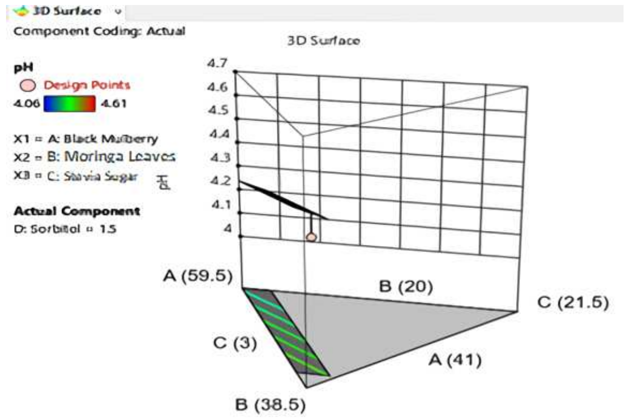

Fig. 4 Three-Dimensional Graphical Model of pH Response Determination

The results showed that the pH value of ready-to-drink black mulberry and moringa leaf functional drinks ranged from 4.06% to 4.61%. The more mulberry is added to manufacture functional drinks, the higher the degree of acidity (decreasing pH).

2) Physics Analysis

a. Color Stability

The color analysis of the ready-to-drink functional drinks made from black mulberry and moringa leaf extract was conducted using a colorimeter, which provided values for L, a, b, and Hue. The L value indicates brightness, while a and b are chromaticity coordinates, with a representing green (negative values) to red (positive values) and b representing blue (negative values) to yellow (positive values). The hue value indicates the resulting sample's color [53] Table 6. presents the ANOVA data for color analysis on the ready-to-drink functional drinks made from black mulberry fruit and moringa leaf extract, obtained using the colorimeter.

TABLE VI
ANOVA COLOR STABILITY ANALYSIS DATA

Source	Sum of Squares	df	Mean Square	F-Value	P-Value	
Model	1.96	3	0.6543	7.65	0.0049	Significant
(1) Linear Mixture	1.96	3	0.6543	7.65	0.0049	
Residual	0.9405	11	0.0855			
Cor Total	2.90	14				

Table 6 presents the ANOVA data for the 15 formulations utilized as the initial model for data analysis. The results indicate a significant influence on the physical analysis of the ready-to-drink functional drinks, precisely the color stability. This conclusion is supported by the low “Prob < F” value (less than 0.05), signifying a significant response (has an influence). The “Prob < F” value resulting from modeling the 15 initial formulations for color stability response using a colorimeter is 0.0049, with an effect size of 0.49%. The values of “Prob < F” less than 0.05 indicate significant modeling (has an influence) between different models on the color stability response of the ready-to-drink black mulberry and moringa leaf functional drinks produced.

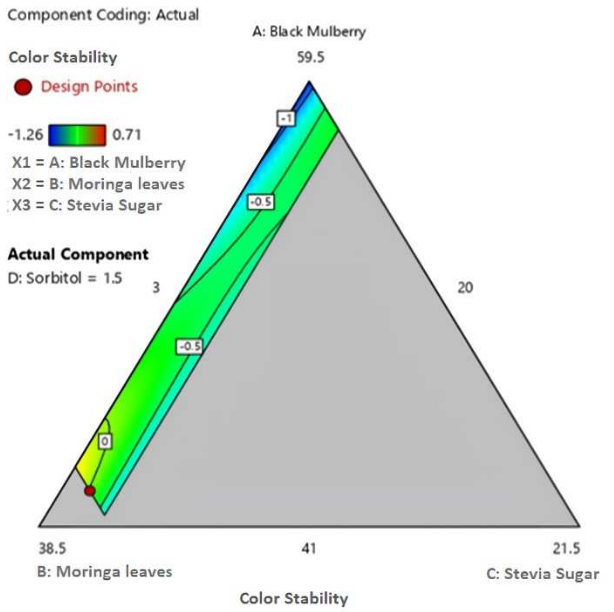

Fig. 5 Graphical Model of Color Stability Analysis

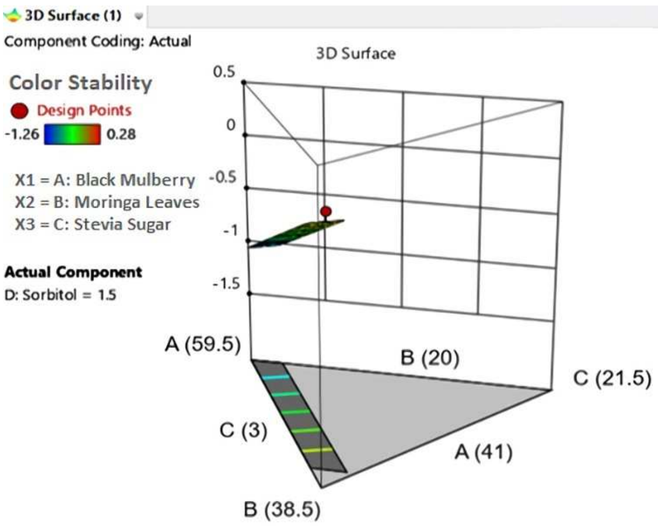

Fig. 6 Three-Dimensional Graphical Model for Color Stability Response Determination

The contour plot graph above illustrates the range of color stability numbers, with dark blue representing the lowest range and green indicating the highest range. The green dots on the graph represent the 15 formulations used in the color stability analysis. The graph displays the results of the color stability analysis for the 15 formulations, ranging from -1.26 to 0.71. The green color on the graph predicts the maximum color stability percentage analysis for the ready-to-drink functional drink made from black mulberry and moringa leaves.

Figure 6 shows a three-dimensional graph showing the interaction relationship between components. Differences in surface height show different response values for each combination of formula components. The highest area shows the highest color stability test response value, while the lowest area shows the lowest color stability test value for 15 formulations.

The results showed that the color stability values of the ready-to-drink black mulberry and moringa leaf functional drinks ranged from -1.26 to +0.28. The blacker mulberry and moringa leaves are added, and the (+) color stability is yellow or brown.

3) *Organoleptic Test Results*

a. Taste Attribute

Consumer acceptance of food is also determined by its taste. Taste is formed from a combination of the ingredients used in a food product. The taste of a food ingredient results from the collaboration of several senses: sight, smell, hearing, and touch or multisensory approach [54], [55]. ANOVA data from organoleptic test results on taste attributes can be seen in Table 7.

TABLE VII
ANOVA DATA FOR THE TASTE ATTRIBUTE OF THE ORGANOLEPTIC TEST

Source	Sum of Squares	df	Mean Square	F-Value	P-Value	
Model	1.79	13	0.1376	34.38	0.1328	Not Significant
(1) Linear Mixture	0.4545	3	0.1515	37.86	0.1188	
AB	0.0322	1	0.0322	8.06	0.2157	
AC	0.2423	1	0.2423	60.56	0.0814	
AD	0.0009	1	0.0009	0.2162	0.7229	
BC	0.2386	1	0.2386	59.63	0.0820	
BD	0.0005	1	0.0005	0.1128	0.7937	
CD	0.1299	1	0.1299	32.46	0.1106	
ABC	0.1311	1	0.1311	32.76	0.1101	
ABD	0.0077	1	0.0077	1.93	0.3972	
ACD	0.1393	1	0.1393	34.82	0.1069	
BCD	0.1414	1	0.1414	35.32	0.1061	
Residual	0.0040	1	0.0040			
Cor Total	1.79	14				

Table 7 displays the ANOVA data for the 15 formulations used as the initial model for data analysis. The results indicate no significant effects on the organoleptic analysis, specifically the taste attribute, from the 15 formulations produced. This conclusion is drawn because the “Prob > F” value is higher than or equal to 0.05, indicating a non-significant response (no effect). The “Prob > F” value resulting from modeling the 15 initial formulations for the taste attribute in the organoleptic response is 0.1328, with an effect size of 1.33%. Values of “Prob > F” higher than or equal to 0.05 indicate a lack of significant modeling (no effect) between different models on the taste attribute of the ready-to-drink functional drink made from black mulberry and moringa leaf extract.

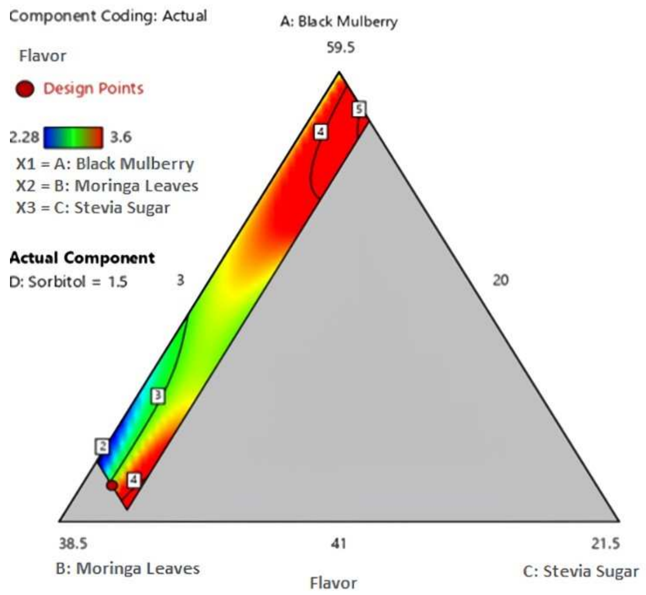

Fig. 7 Graphical Model for the Taste Attribute of the Organoleptic Test

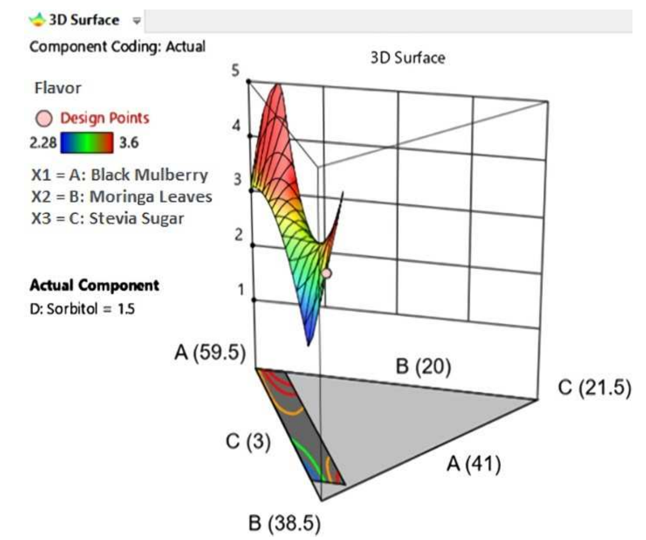

Fig. 8 Three-Dimensional Graphical Model of the Taste Attribute for the Organoleptic Test Response

The Design Expert graph above demonstrates the range of organoleptic numbers for the taste attribute, varying from the lowest to the highest. The color spectrum ranges from dark blue, representing the lowest range, to red, representing the highest range of numbers. The red dots on the graph indicate the presence of 15 formulations of the organoleptic attributes of taste. The graph displays the results of the organoleptic taste attributes for these 15 formulations, ranging from 2.28% to 3.6%. The red color on the graph represents the predicted percentage for the maximum taste attribute in the organoleptic analysis of the ready-to-drink functional drinks made from black mulberry fruit and moringa leaf extract. Figure 8 presents a three-dimensional graph, revealing the interaction relationship between the components. The difference in surface height indicates different response values for each combination of formula components. The highest area represents the highest organoleptic taste attribute test response value, while the lower area corresponds to the lowest taste attribute of organoleptic test value among the 15 formulations.

Taste is a significant factor in determining the overall appeal of a food product. The desired taste of a food item depends on its constituent compounds. The taste assessment is based on the response to chemical stimulation by the taste buds on the tongue. However, the overall taste of a food product is influenced not only by its taste but also by the interplay of aroma, texture, and other sensory experiences [56]–[61]. Flavor or taste can be defined as the sensory perception created by the ingredients consumed, particularly perceived by the taste and smell senses, along with other stimuli such as touch and the perception of heat in the mouth. It is the sensation that results from the combination of ingredients and their composition in a food product, detected by the sense of taste. Taste is an essential quality attribute of a product and is often a decisive factor for consumers when selecting a particular product.

b. Color Attribute

Food quality is determined by several factors, including taste, texture, color, nutritional value, and microbiological properties. Color is one of the primary attributes assessed when evaluating a food product. It is a material property resulting from the light spectrum's dispersion. Color, however, is not a tangible substance or object; instead, it is a sensation experienced by an individual due to the stimulation of the eyes by radiant energy. The ANOVA data for color analysis on the ready-to-drink functional drinks made from black mulberry and moringa leaves, obtained using a colorimeter, can be found in Table 8. This data is essential in assessing the color quality of functional drinks and understanding how different formulations and ingredients may affect their visual appeal.

TABLE VIII
ANOVA COLOR STABILITY ANALYSIS DATA

Source	Sum of Squares	df	Mean Square	F-Value	P-Value	
Model	3.87	3	1.29	3.42	0.0562	Not Significant
(1) Linear Mixture	3.87	3	1.29	3.42	0.0562	
Residual	4.15	11	0.3769			
Cor Total	8.02	14				

Table 8 displays ANOVA data for 15 formulations used as the initial model for data analysis. The analysis aimed to assess the impact of these formulations on organoleptic attributes, particularly focusing on color. The results showed no significant effects on the color attributes, suggesting that the 15 formulations had no notable impact on the organoleptic analysis of color attributes. This conclusion is drawn from the fact that the "Prob > F" value is higher than or equal to 0.05, indicating a non-significant response (no effect).

Prob > F resulting from the modeling of the 15 initial formulations, equal to 0.0562 in the organoleptic response to the color attribute, while the effect is 0.56%. The values of "Prob > F" are more than or equal to 0.05, indicating not significant modeling (no effect) between one model and the other models on the color of the ready-to-drink black mulberry and moringa leaf functional drinks produced.

The Design Expert graph above illustrates a range of organoleptic color attribute values, with colors representing the spectrum from the lowest to the highest values. Dark green indicates the lowest range, while red indicates the highest range of numbers. Each red dot on the graph represents one of the 15 formulations' organoleptic color attributes. Furthermore, the graph showcases the results of the organoleptic taste attributes for the 15 formulations, ranging from 2.8% to 5.4%. The red color in the graph represents the predicted maximum percentage for the color attribute in the organoleptic analysis of ready-to-drink functional drinks made from black mulberry fruit and moringa leaf extract.

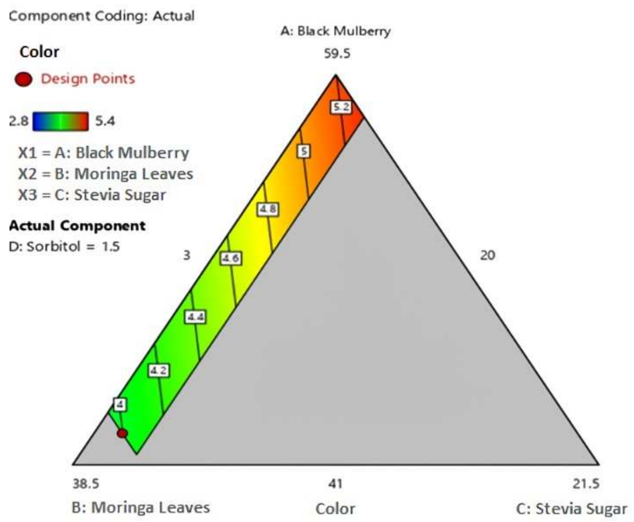

Fig. 9 Graphical Model for the Color Attribute of the Organoleptic Test

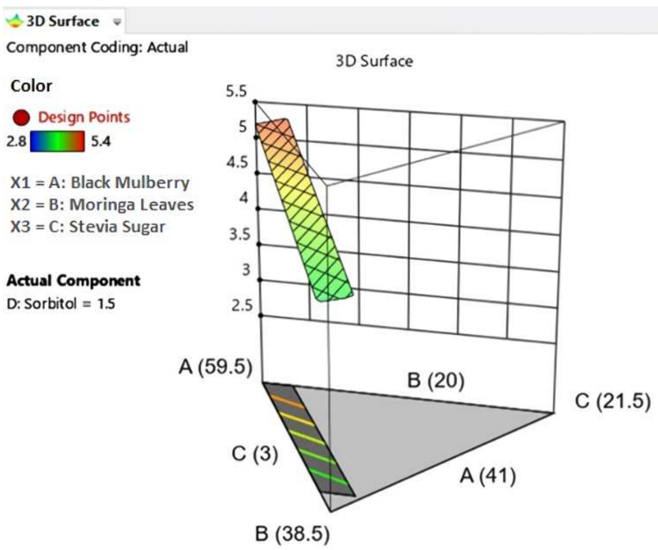

Fig. 10 Three-Dimensional Graphical Model for the Color Attribute of the Organoleptic Test Response

Figure 10 shows a three-dimensional graph showing the interaction relationship between components. Differences in surface height show different response values for each combination of formula components. The highest area shows the highest color attribute organoleptic test response value, while the lower area shows the lowest color attribute organoleptic test value for the 15 formulations.

Color assessment in organoleptic testing has a vital role in visual product acceptance. This can be caused by psychological influences that can interfere with the sensitivity of a panelist, such as affecting concentration or something that prevents individuals from relaxing [62]. These things include a state of depression, frustration, excessive sadness, turbulent joy, hurry, and stress.

c. Aroma Attribute

An aroma is something that can be observed with a sense of smell. To produce aroma, aroma substances must be able to evaporate, slightly soluble in water, and somewhat soluble in fat. In the food industry, aroma testing is considered very important because it can quickly provide results of an assessment of product acceptance [63]. ANOVA data from organoleptic test results on the aroma attribute can be seen in Table 9.

TABLE IX
ANOVA DATA FOR THE AROMA ATTRIBUTE OF THE ORGANOLEPTIC TEST

Source	Sum of Squares	df	Mean Square	F-Value	P-Value	
Model	1.74	13	0.1340	1174.71	0.0228	Significant
(1) Linear Mixture	0.4876	3	0.1625	1424.83	0.0195	
AB	0.0004	1	0.0004	3.15	0.3265	
AC	0.0080	1	0.0080	70.26	0.0756	
AD	0.5797	1	0.5797	5081.07	0.0089	
BC	0.0091	1	0.0091	79.47	0.0711	
BD	0.5942	1	0.5942	5208.10	0.0088	
CD	0.0478	1	0.0478	419.20	0.0311	
ABC	0.0006	1	0.0006	4.93	0.2693	
ABD	0.0482	1	0.0482	422.47	0.0309	
ACD	0.0375	1	0.0375	328.77	0.0351	
BCD	0.0375	1	0.0375	328.93	0.0351	
Residual	0.0001	1	0.0001			
Cor Total	1.74	14				

Table 9 displays ANOVA data for 15 formulations used as the initial model for data analysis, which revealed significant results for the organoleptic attributes related to aroma. Therefore, it can be concluded that these 15 formulations had a noticeable effect on the organoleptic perception of the aroma attribute. This conclusion is drawn from the fact that the "Prob < F" value has a very low value (less than 0.05), indicating a highly significant response. The "Prob < F" resulting from the modeling of the 15 initial formulations is reported to be 0.0228 for the organoleptic response in the aroma attribute, with an effect size of 2.28%. The values of "Prob < F" being less than 0.05 indicate significant modeling (effect) between the different models on the organoleptic response regarding the aroma attribute of the ready-to-drink functional drinks made from black mulberry and moringa leaves.

The Design Expert graph above illustrates the range of organoleptic numbers of the aroma attribute, with colors representing the spectrum from the lowest (dark blue) to the highest (red) values. Each red dot on the graph indicates one of the 15 formulations concerning the organoleptic attributes of aroma. The graph presents the organoleptic results of the aroma attribute for the 15 formulations, falling within the range of 2.8% to 4.12%. The red color on the graph represents the predicted maximum percentage for the organoleptic analysis of aroma attributes in the ready-to-drink functional drinks made from black mulberry and moringa leaves. Figure 11 displays a three-dimensional graph of the interaction relationship between components, showing different response values for each combination of formula components. The highest area corresponds to the highest value of the organoleptic test for the aroma attribute. In contrast, the lower area corresponds to the lowest value of the organoleptic test among the 15 formulations for the aroma attribute.

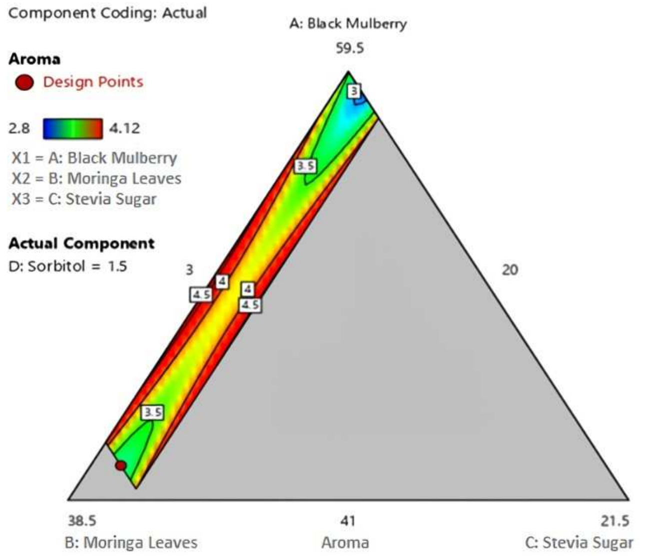

Fig. 11 Graphical Model for the Aroma Attribute of the Organoleptic Test

The Design Expert graph above illustrates the range of organoleptic numbers of the aroma attribute, with colors representing the spectrum from the lowest (dark blue) to the highest (red) values. Each red dot on the graph indicates one of the 15 formulations concerning the organoleptic attributes of aroma. The graph presents the organoleptic results of the aroma attribute for the 15 formulations, falling within the range of 2.8% to 4.12%. The red color on the graph represents the predicted maximum percentage for the organoleptic analysis of aroma attributes in the ready-to-drink functional drinks made from black mulberry and moringa leaves. Figure 12 displays a three-dimensional graph of the interaction relationship between components, showing different response values for each combination of formula components. The highest area corresponds to the highest value of the organoleptic test for the aroma attribute. In contrast, the lower area corresponds to the lowest value of the organoleptic test among the 15 formulations for the aroma attribute.

Aroma is a crucial parameter in determining the quality of a food product. The unique aroma of a food item is perceived through the sense of smell, which is influenced by the ingredients used in its preparation. Aromas typically originate from volatile aroma-producing substances, such as volatile compounds, as well as slightly water-soluble and fat-soluble compounds like essential oils [64]. During the organoleptic test of the aroma attribute, fluctuating results were observed. This variation could be attributed to a psychological error made by the panelists, known as the error of central tendency. This error occurs when panelists tend to give ratings around the middle of the scale and hesitate to assign the highest score. Consequently, this error leads the panelists to assume that all tested samples are nearly identical. It's important to consider and address such psychological errors in sensory evaluation to obtain more accurate and reliable results for assessing the aroma attributes of food products.

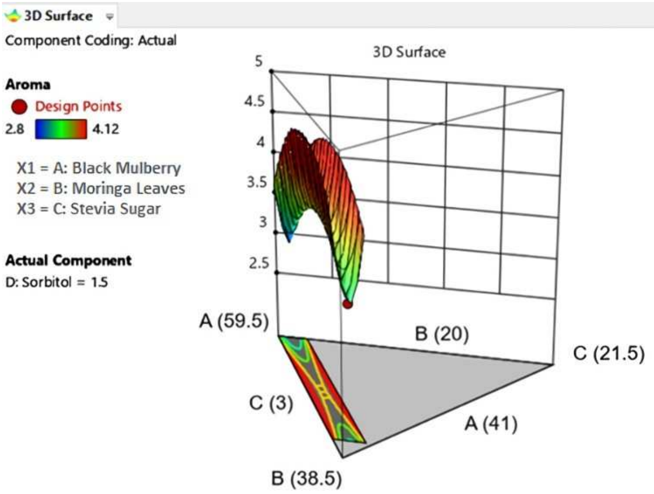

Fig. 12 Three-Dimensional Graphical Model for the Aroma Attribute of the Organoleptic Test Response

C. Selected Formula

The selected formula is the optimal solution or formulation predicted by the D-Optimal method of the Design Expert based on the results of an analysis of the chemical response (Determination of vitamin C content and pH), physical response (Color stability), and organoleptic response (taste, color, and aroma attributes). The selected formula for ready-to-drink black mulberry and moringa leaf functional drink is presented in the following Table 10 and Table 11.

TABLE X
SELECTED FORMULA OF BLACK MULBERRY AND MORINGA LEAF FUNCTIONAL DRINK (1)

Component	Name	Level	Low Level	High Level	Std. Dev	Coding
A	Black Mulberry	50.50	41.00	60.00	0.00000	Actual
B	Daun Kelor	28.00	20.00	36.00	0.00000	Actual
C	Gula Stevia	4.00	3.00	5.00	0.00000	Actual
D	Sorbitol	1.50	1.0000	2.00	0.00000	Actual
	Total	84.00				

TABLE XI
SELECTED FORMULA OF BLACK MULBERRY AND MORINGA LEAF FUNCTIONAL DRINK (2)

Response	Predicted Mean	Predicted Median	Observed	Std. Dev	SE Mean	95% CI Low for Mean	95% CI High for Mean	95% TI Low for 99% Pop	95% TI High for 99% Pop
Vitamin C	7.08585	7.08585		0.757231	0.196364	6.65366	7.51805	3.70869	10.463
pH	4.30693	4.30693		0.118081	0.0306206	4.23954	4.37433	3.78031	4.83356
Color stability	-0.533332	-0.533332		0.292397	0.075824	-0.700219	-0.366444	-1.83739	0.770724
Taste	3.28756	3.28756		0.0632598	0.0636904	2.4783	4.09683	0.271548	6.30358
Color	4.62454	4.62454		0.613959	0.159211	4.27412	4.97497	1.88636	7.36273
Aroma	3.76638	3.76638		0.0106809	0.0107537	3.62974	3.90301	3.25714	4.27561

Two-sided Confidence = 95% Population = 99%

The response variable values obtained from each formulation of black mulberry and moringa leaf functional drinks are fed into the Design Expert 12 program. The software processes these response variables for each formulation and proposes solutions that align with the specified optimization targets. These optimization targets are quantified by a value known as the desirability value, which ranges from zero to one. A desirability value close to one indicates that the formulation is optimal based on the desired response variables [65]. A desirability index close to zero suggests that the formulation is suboptimal for the specified response variables.

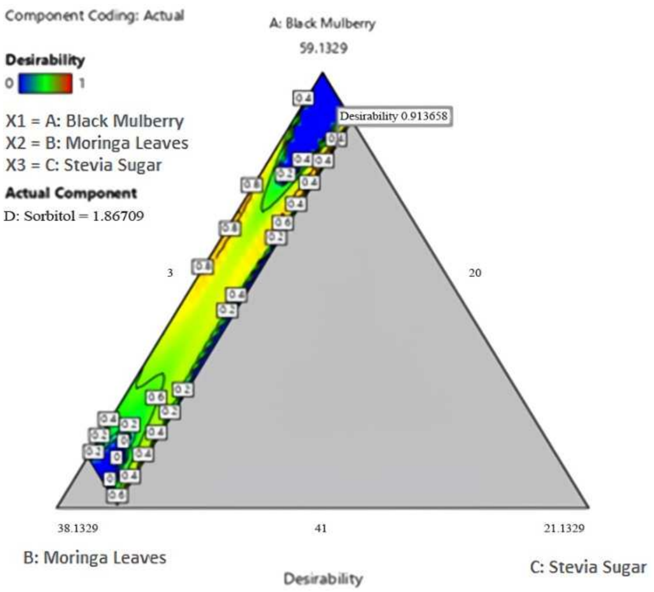

Fig. 13 Contour Plot Graph of the Optimal Formula

The selected formula comprises 50.50% black mulberry, 28.00% moringa leaves, 4.00% stevia sugar, and 1.50% sorbitol. This formula achieved a high desirability value of 0.914, indicating that it aligns well with the optimization target of 91.40%. This formula is predicted to yield products with the following characteristics: vitamin C content of 6.42 mg / 100 grams, a pH value of 4.28, a color stability value of -0.910, and organoleptic scores of 3.60 for taste, 5.03 for color, and 4.12 for aroma. The contour plot figure and three-dimensional cross-section of this formula can be found in Figure 7 and Figure 8, respectively. In the contour plot graphic, lines formed by dots represent various combinations of the three components and their quantities, resulting in different desirability values. Higher areas indicate higher desirability values, while lower areas indicate lower desirability values.

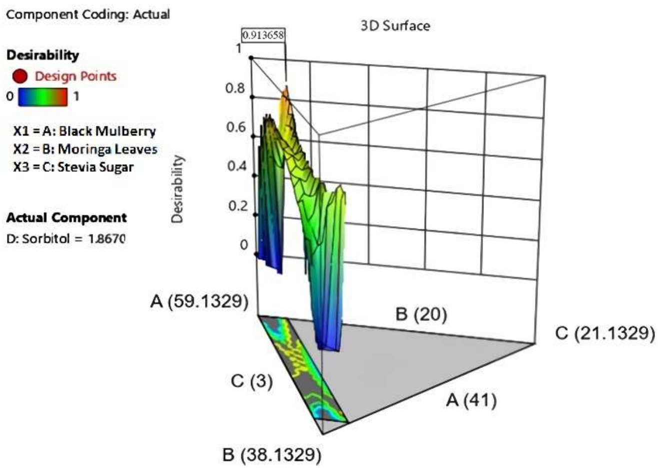

Fig. 14 Three-Dimensional Graphical Model of the Optimal Formula

D. *Verification of the Optimization Result Formula*

The optimal (selected) formula generated by Design Expert 12 software is compared with the laboratory analysis results. The software provides both a confidence interval and a prediction interval for each response prediction value at a significance level of 5%. The confidence interval represents a range that indicates the expected average of the following measurement results at a certain significant level, in this case, 5%. On the other hand, the prediction interval represents a range that shows the expectation of the following response measurement result under the same conditions at a particular significance level, also 5%. The verification stage results and the predictions for each response are presented in Table 12.

TABLE XII
RESULTS OF VERIFICATION STAGES ALONG WITH PREDICTIONS OF EACH RESPONSE

Response	Results		95% CI low	95% CI high	95% TI low	95% TI high
	Predictions	Lab				
Vitamin C	7.98	6.54	7.24	8.73	4.35	11;61
pH	4.33	4.55	4.21	4.45	3.76	4.90
Color stability	-0.153	- 0.49	- 0.440	0.134	- 1.555	1.249
Taste	3.77	4.36	2.92	4.63	0.60	6.94
Color	4.26	3.36	3.65	4.86	1.31	7.20
Aroma	4.12	4.12	3.97	4.26	3.58	4.65

Notes: CI = Confidence Interval: TI = Tolerance Interval

Based on the verification conducted, it is evident that the laboratory analysis results align closely with the predictions made by Design Expert 12 software, as the differences between the two sets of results are minimal. This consistency is further supported by the response variables, such as vitamin C, color stability, and organoleptic attributes (taste, color, and aroma), which are considered reasonable due to their values falling within the range of the low and high confidence interval values. However, the pH response is deemed poor because it exceeds the high confidence interval limit. Moreover, it is essential to note that all the tested responses are still considered good and within an acceptable tolerance, as their values fall between the low and high Tolerance Interval values previously predicted by Design Expert 12 software.

IV. Conclusion

The Design Expert program selected 1 formulation to prepare a ready-to-drink black mulberry and moringa leaf functional drink. The chosen formula is composed of 50.50% black mulberry, 28.00% moringa leaves, 4.00% stevia sugar, 1.50% sorbitol, 0.50% sodium benzoate of 1000ppm, 0.20% CMC, 0.30% salt, and 15.00% water.

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