Optimization of Water Kefir-based Fermentation Formula of Beetroot (*Beta vulgaris L.*), Cinnamon (*Cinnamomum verum*), and Red Ginger (*Zingiber officinale Roscoe*) for Enhanced Betalain

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Abstract. Recent trends in functional beverage development have highlighted the potential of fermented plant-based products with enhanced bioactive properties. This study explores the incorporation of beetroot (*Beta vulgaris L.*) in water kefir production and optimizes its formulation through the addition of red ginger and cinnamon to enhance antioxidant and sensory properties. The primary bioactive compound, betalain, present in beetroot contributes substantially to its antioxidant capacity. Recent research, the formulation optimization was conducted through Mixture Design D-Optimal by Design Expert, which systematically evaluated varying concentrations of beetroot (55-70%), red ginger (20-35%), and cinnamon (10-25%) to determine their optimal proportions. The optimized formulation was fermented with water kefir grains for 48 hours at 28°C, resulting in enhanced antioxidant activity through multiple pathways, such as microbial enzymatic degradation of complex compounds, generation of secondary metabolites, and improved bioavailability via cell matrix breakdown. The optimal formulation, comprising 59.528% beetroot, 30.472% cinnamon, and 10.000% red ginger, demonstrated remarkable results with a betalain content is 53.448 mg/100 g achieving a desirability value of 0.785. This research successfully establishes a novel, plant-based functional beverage with elevated antioxidant capacity, offering a viable alternative for health-conscious consumers and those with dairy sensitivities while advancing the field of functional fermented beverages.

1 Introduction

The prevalence of oxidative stress mechanisms in chronic disease pathogenesis represents a significant global health burden, with current epidemiological data indicating its involvement in 70-80% of chronic conditions. Statistical analyses demonstrate that oxidative stress-related disorders affect approximately 200 million individuals worldwide annually, with cardiovascular diseases, specifically, accounting for 33% of global mortality [1]. Furthermore, mortality data from 2022 reveals 19.8 million deaths attributed to these conditions, a figure that correlates with the expanding global population demographic. The

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development of food products based on natural ingredients with functional activities is one of the growing trends due to its health benefits [2,3]. Bioactive properties continue to be researched in the development of food products are antioxidants that are proven to minimize the aggravation of health problems [1,4].

Water kefir has emerged as a functional fermented beverage with biological activity as an anti-hyperlipidemic agent due to the metabolic potential from complex microbial interactions [5]. While water kefir demonstrates superior lipid profile improvement compared to milk kefir due to organic acids and exopolysaccharides that enhance bile salt elimination as cholesterol binders [6,7], it has limited specific bioactive compounds [8–10]. Red beetroot (*Beta vulgaris L.*), rich in betalain compounds [11–13]. along with red ginger (*Zingiber officinale var. Rosc*) and cinnamon (*Cinnamomum verum),* which contain bioactive compounds such as gingerol, shogaol, cinnamaldehyde, and polyphenols [14,15] (offer promising synergistic combinations to enhance both the antioxidant activity and sensory acceptance of water kefir products [16,17].

The development of an optimal water kefir formula incorporating red beetroot, ginger, and cinnamon requires precise composition to achieve optimal physical, chemical, microbial, sensory, and biological activity characteristics [10,18]. However traditional wet-lab methods for optimizing water kefir formulations with functional ingredients are prohibitively time-consuming and resource-intensive, making computational modeling approaches like the Design Expert 13 with Mixture Design D-Optimal (MOCD) method urgently necessary to efficiently predict optimal compositions while minimizing experimental requirements [19,20]. This research aims to obtain and analyze the optimal formula using Design Expert 13 with Mixture Design D-Optimal (MOCD) method, which provides precise formula composition predictions while reducing sample preparation requirements, thereby improving accuracy and cost-efficiency [20]. The study will explore the incorporation of beetroot (*Beta vulgaris L.*) in water kefir production and optimizes its formulation through the addition of red ginger and cinnamon to enhance antioxidant and sensory properties.

2 Methods

The initial raw material preparation involved several extraction processes for different components, as outlined in research by multiple authors. First, red beetroot extraction was conducted by peeling and blending with water at a 1:4 w/v ratio, followed by filtration [10]. Ginger was processed by grinding into powder, with 6g macerated in 60 ml water (1:10 w/v ratio), heated at 65°C for 20 minutes, then cooled to 40°C [21]. Cinnamon powder was prepared at a 1:50 w/v ratio (1.2 g in 60 ml water) and boiled at 50°C for 5 minutes [22]. Finally, all extracts - beetroot juice, ginger extract, and cinnamon extract - were combined according to Design Expert formulations, with 8% w/v cane sugar added, followed by pasteurization at 72°C for 15 minutes [10].

Mixture Design D-Optimal (MOCD) using software Design Expert 13 (Stat-Ease, USA) was used to optimize the formulation for betalain content. The independent variables underlying the formula, such as red beetroot juice, red ginger extract, and cinnamon extract of each sample was performed by determining the upper and lower limits which served as the basis for achieving optimal response from the combination of each ingredient as shown in Table 1. A Mixture Design D-Optimal (MOCD) consisting of 17 experimental runs was applied to evaluate the effect of variables, and the response was the betalain content.

Table 1. Independent variables of red beetroot kefir formula limitations

Independent Variables	**Lower Limit (%)**	**Upper Limit (%)**
Red Beetroot Juice	55	70
Red Ginger Extract	20	35
Cinnamon Extract	10	25

The extraction and quantification of betalains from beetroot juice follows a specific laboratory procedure by Pandey [23]. The process begins by diluting 0.5 ml of beetroot juice in 10 ml of ethanol at 50% while maintaining different pH levels 5. The mixture undergoes a 10-second agitation followed by centrifugation at 6000 rpm for 10 minutes. To ensure maximum extraction, the supernatant is collected and this centrifugation process is repeated two more times. For quantification purposes, the betalain content is measured spectrophotometrically at 530 nm wavelength. The total betalain content, expressed in mg/100g, is calculated using equation of Castellar et al. (2003), which incorporates several parameters: the absorption value at 530 nm density (A), dilution volume (DF), path length of cuvette (L), molecular weight of betalains (550 g/mol), and the extinction coefficient for betalains (60000 L/mol). This comprehensive method allows for both efficient extraction and precise measurement of betalain concentration from beetroot juice samples.

3 Results and discussions

Mixture Design D-Optimal was employed to obtain the optimum formulation of water kefir with beetroot (*Beta vulgaris L.*), enhanced with red ginger and cinnamon for improved antioxidant and sensory properties. A total of 17 experimental runs were conducted with three independent variables, such as beetroot concentration (55-70%), red ginger proportion (20-35%), and cinnamon content (10-25%), evaluated at multiple levels. The optimization process considered response of betalain content, as shown in Table 3. The Design Expert software was utilized to systematically analyze these parameters and determine their optimal proportions for maximizing both functional and organoleptic characteristics of the final product. The analysis of variance (ANOVA) for each variable generated linear model as per Equation 1.

$$Y = +54.18398 + 38.87081*A - 60.31642*B - 54.95589*C \quad (1)$$

Where Y is the predicted response; A is beetroot; B is cinnamon; C is red ginger.

Table 2. ANOVA for linear model

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p value**	
Model	282.86	2	141.43	5.34	0.0189	significant
(1)Linear Mixture	282.86	2	141.43	5.34	0.0189	
Residual	371.07	14	26.51			
Lack of Fit	182.79	9	20.31	0.5393	0.8016	not significant
Pure Error	188.29	5	37.66			
Cor Total	653.93	16				

The Analysis of Variance (ANOVA) results provide compelling evidence for the effectiveness of the linear mixture model under examination. The model demonstrates statistical significance with an F-value of 5.34 and a corresponding *p value* of 0.0189, which falls well below the conventional significance threshold of 0.05. This indicates that there is only a 1.89% probability that an F-value of this magnitude could occur due to random chance alone. The model's structure consists of a Linear Mixture component that accounts for a substantial portion of the total variation, with a sum of squares of 282.86 distributed across 2 degrees of freedom, resulting in a mean square of 141.43. This relatively large mean square value, when compared to the residual mean square, suggests that the model captures meaningful patterns in the data rather than random fluctuations.

The model's adequacy is further supported by the analysis of residuals, which is divided into two crucial components: Lack of Fit and Pure Error. The Lack of Fit test yields an F-value of 0.5393 with a corresponding *p value* of 0.8016, which is notably not significant. This non-significance is a favourable outcome, as it indicates that the model's predicted values align well with the observed data patterns without systematic deviations. The Pure Error component, with a sum of squares of 188.29 distributed across 5 degrees of freedom (resulting in a mean square of 37.66), represents the inherent variability in repeated measurements. The total corrected sum of squares (Cor Total) of 653.93 with 16 degrees of freedom encompasses all sources of variation in the response variable. The analysis employs Type I sums of squares for inference in linear mixtures, which is appropriate for this type of experimental design as it accounts for the sequential nature of the effects being tested. The relatively small residual mean square of 26.51 compared to the model mean square suggests that the model explains a substantial portion of the variability in the data, making it a reliable tool for prediction and interpretation within the studied system.

Table 3. The response of the experimental run for red beetroot kefir formula

Independent Variables (%)				**Response Betalain (mg/100 g)**
Run	**A: Red Beetroot Juice**	**B: Cinnamon Extract**	**C: Red Ginger Extract**	
1	60.00	25.00	15.00	46.93
2	55.00	27.50	17.50	44.73
3	55.00	20.00	25.00	42.35
4	65.00	25.00	10.00	60.13
5	62.50	27.50	10.00	49.50
6	62.50	20.00	17.50	56.47
7	55.00	27.50	17.50	50.05
8	65.00	20.00	15.00	56.65
9	55.00	35.00	10.00	52.25
10	60.00	25.00	15.00	57.75
11	60.00	30.00	10.00	50.97
12	60.00	25.00	15.00	59.40
13	57.50	30.00	12.50	54.27
14	62.50	20.00	17.50	54.27
15	55.00	20.00	25.00	55.00
16	57.50	22.50	20.00	60.13
17	70.00	20.00	10.00	68.20

The Mixture Design D-Optimal optimization result indicated that the optimal formula was determined based on previously established criteria, with each ingredients are assigned different importance levels. Based on Table 4, red beetroot having a highest importance value of 1, while cinnamon have an importance level of 2 and red ginger the less importance of 3. The target betalain content, a key quality parameter, should fall between 42.35-68.2 units and shares the highest importance level of 1. All components were given equal weights of 1 for both their upper and lower limits, ensuring balanced consideration of the constraints during the optimization process. The optimal formula obtained demonstrates that the proportion of

Red Beetroot contributes the most significant portion in the formulation, followed by Cinnamon and Red Ginger, which play essential roles in enhancing the antioxidant and sensory properties of the water kefir product. The analysis was performed based on the established criteria using Design Expert, resulting in an optimal formula with response predictions that align with the optimization targets.

Table 4. The constraints of experimental red beetroot kefir formula

Name	Goal	Importance
A: Red Beetroot	is in range	1
B: Cinnamon	is in range	2
C: Red Ginger	is in range	3
Betalain Content	maximize	1

Based on Table 5, the Mixture Design D-Optimal optimization generated 4 water kefir formula solutions with 1 selected formula which have desirability value of 0.785. The optimization process resulted in a selected formulation consisting of 59.528% beetroot juice, 30.472% cinnamon, and 10.000% red ginger. This optimal mixture achieved a Betalain content of 53.448 mg/100 g and received a desirability score of 0.785, indicating that the selected formula considered moderately high appeal for a product interface based on specified constraints and requirements.

Table 5. The solutions of final red beetroot kefir formula

Red Beetroot (%)	**Cinnamon (%)**	**Red Ginger (%)**	**Betalain Content (mg/100 g)**	**Desirability**	
59.528	30.472	10.000	53.448	0.785	**Selected**
62.500	27.500	10.000	56.396	0.666	
55.000	23.691	21.309	49.563	0.399	
63.161	20.000	16.839	57.417	0.119	

The Normal Probability Plot of Residuals (**Fig. 1**) shows externally studentized residuals plotted against normal percentiles (1-99%), with a red diagonal reference line representing perfect normality. The data points (green and black squares) closely follow this line, particularly in the central region (-1.0 to +1.0), with only minor deviations at the extreme tails, suggesting that the residuals are sufficiently normally distributed and the underlying statistical model's assumptions are met for valid analysis. The analysis of the mixture experiment, conducted using Mixture Design D-Optimal methodology, examines the effects of three components (Beetroot Juice, Cinnamon, and Red Ginger) on Betalain concentration. The ternary plot displays the relationship between these components, (**Fig 2)** where the highest Betalain values are clearly observed in the region dominated by Beetroot Juice (maximum point at 70.00). The experimental points were strategically selected using D-optimal criteria to provide the most informative data while minimizing the number of required experiments.

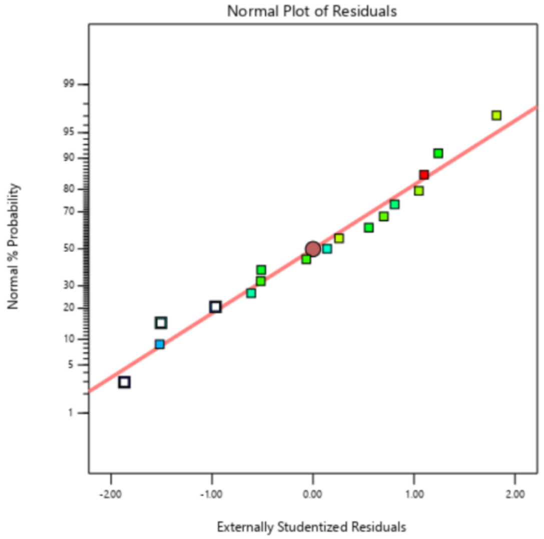

Fig. 1. Normal plot of residuals

The three-dimensional representation further supports these findings, showing a distinct peak in betalain concentration when beetroot juice content is high. The analysis reveals that Beetroot Juice has the strongest positive influence on betalain concentration, followed by a moderate effect from cinnamon, while red ginger shows the least impact. The smooth contour patterns across the design space indicate well-behaved mixture interactions, suggesting that optimal formulations should prioritize beetroot juice content while carefully balancing the proportions of cinnamon and red ginger. This efficient experimental design approach successfully identified the key relationships between components, providing valuable insights for formulation optimization while maintaining statistical rigor.

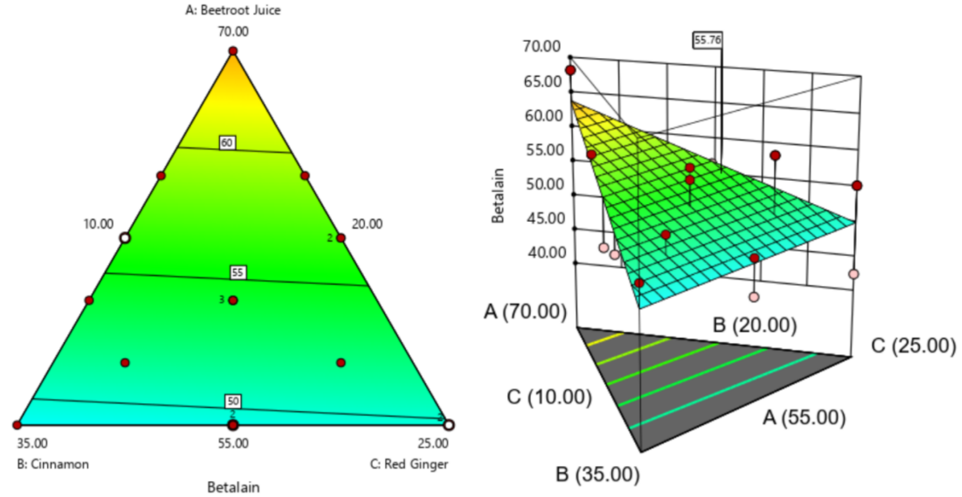

Fig. 2. Interaction between red beetroot juice (%), cinnamon extract (%), red ginger extract (%) and betalain content (%)

The complex interplay between betalain content and the optimized formulation in water kefir production reveals fascinating biochemical interactions supported by extensive research

literature. Studies have demonstrated that betalains, the water-soluble pigments predominantly found in red beetroot, exhibit enhanced stability and bioavailability when combined with complementary ingredients containing phenolic compounds and natural antioxidants [24]. Betalains function as efficient antioxidants by donating hydrogen to reactive species, thereby delaying or preventing developing of free radical [13,25]. Research by [26]. highlights the synergistic relationship between betalains and the natural antioxidants found in cinnamon and ginger, where the phenolic compounds and gingerols create a protective environment for betalain molecules. This synergy is further enhanced by the pH-stabilizing properties of cinnamon and the organic acids in red ginger, which maintain the optimal pH range of 4-6 necessary for betalain stability. The presence of these complementary ingredients also provides improved thermal stability during processing, as demonstrated by Panghal [27]
where the antioxidant compounds act as natural preservatives against thermal degradation.

The formulation's effectiveness is further explained by its impact on betalain bioavailability and preservation. The gingerols from red ginger have been shown to enhance betalain absorption, while cinnamon's polyphenols provide protection against oxidative stress during digestion. Additionally, the natural fibers and compounds present in both cinnamon and ginger create a protective microencapsulation effect around betalain molecules, shielding them from environmental factors that could lead to degradation. This comprehensive protection system explains why the optimal formulation achieves a stable betalain content of 53.448 mg/100 g, demonstrating the successful integration of traditional ingredients with modern optimization techniques in functional beverage development. The balanced proportions of ingredients not only maximize betalain stability but also enhance the overall functional and sensory properties of the water kefir product, creating a synergistic blend that optimizes both nutritional value and consumer acceptance.

4 Conclusions

By Mixture Design D-Optimal, 17 formulas were generated using Design Expert software and a linear model was fitted to the experimental results of the determined runs. The model was verified statistically. The ANOVA table demonstrated that both the model and the parameters of the model are significant with F-value of 5.34 and *p value* of 0.0189, indicating good model fit. The optimal formulation for water kefir production containing beetroot (*Beta vulgaris L.*) was determined to be 59.528% beetroot, 30.472% cinnamon, and 10.000% red ginger, demonstrated remarkable results with a betalain content is 53.448%. This formulation represents a balanced composition that maximizes the stability of betalain of the final product showed by solutions results that suggested from Design Expert. The model's reliability is supported by the non-significant lack of fit (*p value* = 0.8016), suggesting that the predicted values align well with the experimental data. The successful optimization demonstrates the effectiveness of the Mixture Design D-Optimal approach in developing functional beverages with enhanced nutritional and sensory characteristics.

Fundings
None.

Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contribution statement
Tri Dewanti Widyaningsih: Conceptualization, Methodology, Investigation, Writing- Original draft preparation. Siti Qodriyatus Solikhah: Data curation, Writing- Reviewing and Editing. Erryana Martati: Validation, Writing- Reviewing and Editing.

References

[1] WHO, WHO Guidelines on physical activity and sedentary behaviour, 2016).
[2] D. Granato, F.J. Barba, D. Bursać Kovačević, J.M. Lorenzo, A.G. Cruz, P. Putnik, Functional Foods: Product Development, Technological Trends, Efficacy Testing, and Safety. Annual Review of Food Science and Technology 11, 93–118 (2020). https://doi.org/10.1146/annurev-food-032519-051708.
[3] M.M. Essa, M. Bishir, A. Bhat, S.B. Chidambaram, B. Al-Balushi, H. Hamdan, N. Govindarajan, R.P. Freidland, M.W. Qoronfleh, Functional foods and their impact on health. Journal of Food Science and Technology 60, 820–834 (2023). https://doi.org/10.1007/s13197-021-05193-3.
[4] M. Kurek, N. Benaida-Debbache, I.E. Garofulić, K. Galić, S. Avallone, A. Voilley, Y. Waché, Antioxidants and Bioactive Compounds in Food: Critical Review of Issues and Prospects†. Antioxidants 11, 1–23 (2022). https://doi.org/10.3390/antiox11040742.
[5] S. Wijaya, Development of A Combination of Red Ginger, Cinnamon, and Honey as Antioxidant. Sport and Nutrition Journal 6, 66–78 (2024). https://doi.org/10.1016/j.sasoi.2013.12.010.
[6] M.E.C. Moreira, M.H.D. Santos, G.P.P. Zolini, A.T.B. Wouters, J.C.T. Carvalho, J.M. Schneedorf, Anti-inflammatory and cicatrizing activities of a carbohydrate fraction isolated from sugary kefir. Journal of Medicinal Food 11, 356–361 (2008). https://doi.org/10.1089/jmf.2007.329.
[7] A. Rocha-Gomes, A. Escobar, J.S. Soares, A.A. da Silva, N.A.V. Dessimoni-Pinto, T.R. Riul, Chemical composition and hypocholesterolemic effect of milk kefir and water kefir in Wistar rats. Revista de Nutricao 31, 137–145 (2018). https://doi.org/10.1590/1678-98652018000200001.
[8] O. Corona, W. Randazzo, A. Miceli, R. Guarcello, N. Francesca, H. Erten, G. Moschetti, L. Settanni, Characterization of kefir-like beverages produced from vegetable juices. Lwt 66, 572–581 (2016). https://doi.org/10.1016/j.lwt.2015.11.014.
[9] F. Afiati, S. Fitri, G. Priadi, Characterization of curd kefir milk with the addition of beetroot (Beta vulgaris). Jurnal Biodiv Indonesia 4, 270–273 (2018). https://doi.org/10.13057/psnmbi/m040230.
[10] X. Wang, P. Wang, Red beetroot juice fermented by water kefir grains: physicochemical, antioxidant profile and anticancer activity. European Food Research and Technology 249, 939–950 (2023). https://doi.org/10.1007/s00217-022-04185-7.
[11] D. dos S. Baião, D.V.T. da Silva, V.M.F. Paschoalin, Beetroot, a remarkable vegetable: Its nitrate and phytochemical contents can be adjusted in novel formulations to benefit health and support cardiovascular disease therapies. Antioxidants 9, 1–36 (2020). https://doi.org/10.3390/antiox9100960.
[12] M. Nowacka, S. Tappi, A. Wiktor, K. Rybak, A. Miszczykowska, J. Czyzewski, K. Drozdzal, D. Witrowa-Rajchert, U. Tylewicz, The impact of pulsed electric field on the extraction of bioactive compounds from beetroot. Foods 8, (2019). https://doi.org/10.3390/foods8070244.

[13] N. Chhikara, K. Kushwaha, P. Sharma, Y. Gat, A. Panghal, Bioactive compounds of beetroot and utilization in food processing industry: A critical review. Food Chemistry 272, 192–200 (2019). https://doi.org/10.1016/j.foodchem.2018.08.022.
[14] L. Ma, L. Hu, X. Feng, S. Wang, Nitrate and nitrite in health and disease. Aging and Disease 9, 938–945 (2018). https://doi.org/10.14336/AD.2017.1207.
[15] N. Mahmudati, P. Wahyono, D. Djunaedi, Antioxidant activity and total phenolic content of three varieties of Ginger (Zingiber officinale) in decoction and infusion extraction method. Journal of Physics: Conference Series 1567, (2020). https://doi.org/10.1088/1742-6596/1567/2/022028.
[16] N.S. Ismail, Protective Effects of Aqueous Extracts of Cinnamon and Ginger Herbs Against Obesity and Diabetes in Obese Diabetic Rat. World Journal of Dairy & Food Sciences 9, 145–153 (2014). https://doi.org/10.5829/idosi.wjdfs.2014.9.2.1137.
[17] S. Novakovic, V. Jakovljevic, N. Jovic, K. Andric, M. Milinkovic, T. Anicic, B. Pindovic, E.N. Kareva, V.P. Fisenko, A. Dimitrijevic, J. Joksimovic Jovic, Exploring the Antioxidative Effects of Ginger and Cinnamon: A Comprehensive Review of Evidence and Molecular Mechanisms Involved in Polycystic Ovary Syndrome (PCOS) and Other Oxidative Stress-Related Disorders. Antioxidants 13, (2024). https://doi.org/10.3390/antiox13040392.
[18] C. Ebere, I.S. Lemon, Quality Characteristics of Beetroot Juice Treated with Indigenous Spices Quality Characteristics of Beetroot Juice Treated with. (2016). https://doi.org/10.5923/j.food.20160601.03.
[19] R. Sarteshnizi, H. Hosseini, D. Bondarianzadeh, F.J. Colmenero, R. Khaksar, Optimization of prebiotic sausage formulation: Effect of using β-glucan and resistant starch by D-optimal mixture design approach. Lwt 62, 704–710 (2015). https://doi.org/10.1016/j.lwt.2014.05.014.
[20] E. Niño, J.F. Rosas, S.A. Bonet, N. Ramírez, M. Cabrera-Ríos, Multiple objective optimization using desirability functions for the design of a 3D printer prototype. IIE Annual Conference and Expo 2015 1294–1303 (2015).
[21] J. Kusnadi, A.R. Tirtania, E.L. Arumingtyas, Tropical Journal of Natural Product Research Antioxidant Activity , Physicochemical Characterisation and Antibacterial Properties of Caspian Sea Yoghurt Enriched with Ginger and Sappanwood Extracts. 7, 2536–2539 (2023).
[22] E. Zubaidah, R. Aulia Salafy, D. Widya Ningtyas, A.C. Denty Wiryawan, Antioxidant and antibacterial activity of sappan wood (Caesalpinia sappan L.) kombucha. Advances in Food Science, Sustainable Agriculture and Agroindustrial Engineering 7, 10–19 (2024). https://doi.org/10.21776/ub.afssaae.2024.007.01.2.
[23] G. Pandey, J. Singh, G. Thomas, Analysis of beetroot (Beta Vulgaris L.) juice processing effect for maximum Betalain extractability and optimum antioxidant activity. Plant Archives 17, 798–802 (2017).
[24] K. Zeka, K. Ruparelia, R. Arroo, R. Budriesi, M. Micucci, Flavonoids and Their Metabolites: Prevention in Cardiovascular Diseases and Diabetes. Diseases 5, 19 (2017). https://doi.org/10.3390/diseases5030019.
[25] A. Dhiman, R. Suhag, D.S. Chauhan, D. Thakur, S. Chhikara, P.K. Prabhakar, Status of beetroot processing and processed products: Thermal and emerging technologies intervention. Trends in Food Science & Technology 114, 443–458 (2021). https://doi.org/10.1016/J.TIFS.2021.05.042.
[26] A. Gengatharan, G.A. Dykes, W.S. Choo, Betalains: Natural plant pigments with potential application in functional foods. Lwt 64, 645–649 (2015). https://doi.org/10.1016/j.lwt.2015.06.052.

[27] A. Panghal, K. Virkar, V. Kumar, S.B. Dhull, Y. Gat, N. Chhikara, Development of probiotic beetroot drink. Current Research in Nutrition and Food Science 5, 257–262 (2017). https://doi.org/10.12944/CRNFSJ.5.3.10.