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RESEARCH ARTICLE

Development of functional golden milk by incorporating *Curcuma amada* and stevia by Response Surface Methodology

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Abstract: The objective of this study was to develop and optimize golden milk using the incorporation of *Curcuma amada* and stevia into the milk and preserve it using pasteurization. The analysis was carried out by incorporating *Curcuma amada* powder and stevia in the milk and optimizing using the Response surface methodology. A set of thirteen experiments were done in which optimized golden milk with the maximum desirability was produced using 3.43 percent *Curcuma amada* and 0.35 percent stevia, according to the outcomes of the variable optimization. The physicochemical and biochemical characteristics of the milk were also accessed. The antioxidant activity of Functional golden milk is 56.14and total phenolic content was found to be38.04 to 36.95 mg GAE. The product was accessed for its shelf life using a sensory score and found that it was stable for 7 days at 4°C.

Keywords: *Curcuma amada*, stevia, Response surface methodology, Storage life.

Introduction

Today consumers are choosing food products that strengthen their immune systems and encourage good health because they are becoming more and more aware of the significance of diet for human health. In this regard, functional foods are the best dietary choices since they enhance life quality by lowering illnesses linked to nutrition. (Janssen et al. 2021). As a result, the demand for health-promoting and immune-boosting functional foods has risen dramatically. The global functional food industry is anticipated to reach $280.10 billion by 2025. As a result, the main trend is to provide a wide range of food with health-promoting features at a low cost (Alongi and Anese; 2021).

Milk is the most acceptable nutrient-dense food, supplying energy and providing high-quality protein with a range of essential micronutrients (especially magnesium, calcium, phosphorus, potassium, and zinc) among other categories of foods taken regularly (Górska- Warsewicz et al. 2019). Milk-based beverages are proven to be excellent delivery systems for recently identified bioactive food components due to their rich flavor and nutritional content. (Sawale et al. 2020)

Curcuma amada (Mango ginger) is herb or spice used in folk medicine, culinary preparations such as preserves, sauce, pickles, candies, and the production of oleoresin, essential oil, and other products. Its extracts and powders have beneficial qualities like antioxidant, antibacterial, and antifungal properties, which are frequently used to improve the nutritional content and quality of foods (Narayanankutty et al. 2021). Rhizomes of the C. amada have long been used in Indian traditional medical systems such as Unani and Ayurveda to treat various disorders, including cancer, tumors, bronchitis, alexiteric, diuretic, skin diseases, asthma antipyretic, hiccough, and inflammation (Behera et al. 2007). Due to the presence of bioactive compounds such as curcumin, phenol, dimethoxy curcumin, bis-dimethoxy curcumin, terpenoids, and myrcene. C. amada has also shown therapeutic action (Umar et al. 2020). With the evidence that herbal milk is gaining popularity in the market, there are huge opportunities for valueaddition in milk by converting it into functional milk (Golden milk) by incorporating *C. amada*. However, introducing any herb into the milk comes with several technological obstacles, including physio-chemical, color, and flavor incompatibility, which can result in astringency, bitterness due to slow proteolysis, poor thermal stability, and change in the functional property.

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Stevia (*Stevia rebaudiana*) has been used to develop food products as a natural, low-calorie alternative for sugar.Due to the active ingredients in its leaves, the so-called steviol glycosides, stevia is the only plant in the Asteraceae family with sweetening capabilities.Stevia has also been proven in numerous research to offer antidiabetic, anti-obesity, anticancer, antihypertensive, antibacterial, anticaries, and antioxidant health effects.(Schiatti-Sisó et al. 2022)

Keeping in view the therapeutic benefits of *C. amada,* the present study was designed to develop and characterize milk-based functional golden milk incorporated with different percentages of *C. amada* (Mango ginger) powder. Optimization of *C. amada* (Mango ginger) powder level is done by response surface methodology (RSM) because it is a widely used empirical modeling methodology recognized as comprehensive, efficient, and concise methodology. The present study also analyzed golden milk's physicochemical, sensory, and nutritional potential. Furthermore, optimized golden milk was pasteurized to see the effect of heat on the golden milk.

Materials and methods

Milk (Fat, 4.32%, and SNF 8.86%) was collected from the Dairy Farm of Banaras Hindu University. *Curcuma amada* and stevia (Organic India) were procured from the local market of Varanasi.

Preparation of*Curcuma amada powder* (CAP)

The fresh rhizomes of *Curcuma amada* were washed thoroughly with tap water and then placed at room temperature for 2hr to drain the excess water. After which the cleaned *C. amada* rhizomes were sliced into a thin layer and dried in a tray drier (Khera instrument, India) at 45 ± 2°C until moisture content reached below 5%. The dried rhizomes were then grounded and sieved into a fine powder.

Preparation of Functional Golden Milk (FGM)

For the preparation of FGM, RSM trials were conducted to calculate the level of *Curcuma amada* powder (CAP)and stevia. Different concentrations of *Curcuma amada* and stevia were added to the preheated milk (35-40ÚC). Samples were homogenized and filtered out to remove undissolved large particles of CAP. Figure1 illustrates the flow diagram for the preparation of golden milk using CAP. The prepared golden milk was then pasteurized using the HTST method (72 °C for 15 s), cooled, glass bottled, and stored at 5°C.

Optimization of Functional Golden Milk (FGM)

Central composite design (CCD) of the response surface methodology (RSM) technique was adopted to optimize the golden milk, Preliminary trials were conducted to select the levels of independent variables viz. *Curcuma amada*, stevia. Responses viz. sensory evaluation (color and appearance, taste, mouth feel, sweetness, flavor, and overall acceptability) were used to select the levels of independent variables for optimizing the functional golden milk formulation. Design expert software version 8.0.7.1 was used for the optimization of golden milk. A total of 13 experiments were designed according to the experimental design of RSM. A second-order polynomial equation was derived based on the chosen quadratic model as followed:

$$Y= 5Ø7ß+5Ø7ß_1 X_1+ 5Ø7ß_2X_2+5Ø7ß_{12}X_1X_2 + 5Ø7ß_{11}X_{12} + 5Ø7ß_{22}X_{22} + õ$$

Where Y is the response variable $5Ø7ß$, $5Ø7ß_1$, $5Ø7ß_2$&$5Ø7ß_{12}$ are the regression coefficient, and X_1, X_2, & X_{12} are the independent variables or the quadratic Interactive effect of chosen variables the factor, is the residual error. The error includes experimental errors and lack of fit chosen for the model. The quality of fit of the chosen model was evaluated by the coefficient of determination (R^2).

Physico-chemical analysis of the optimized functional golden milk

Proximate analysis of optimized functional golden milk

Protein content was measured using the Kjeldahl method and fat content was determined according to the Gerber method. The ash content was determined according to AOAC 923.03. Carbohydrate content was measured according to the phenol sulphuric method described by Jayathilake et al. (2022). Titratable acidity (%) of optimized functional golden milk was determined by Ranagana's (2001) method. Calibrated digital pH meter (Meter Lab, Pacific Laboratory Products; Blackburn, Vic, Melbourne, Australia) was used for pH measurements. Specific gravity and viscosity of the golden milk was measured by Brookfield viscometer (DV Next Rheometer AMETEX, Massachusetts, U.S.A) at 25°C.

Color

Color characteristics of functional golden milk were measured by colorimeter hunter lab (Color Flex EZ Spectrophotometer, Virginia, U.S.A). The following parameters were defined: L″ (lightness; black (0) to white (100)), a″ (red saturation index; +a″=red, -a″=green) and b″(yellow saturation index; +b″=yellow, -b″=blue).

Nutraceutical characteristics of Functional Golden milk

Determination of antioxidant activity

The antioxidant activity of the golden milk was determined using the DPPH inhibition technique, (Jayathilake et al. 2022)

Determination of total phenolic content

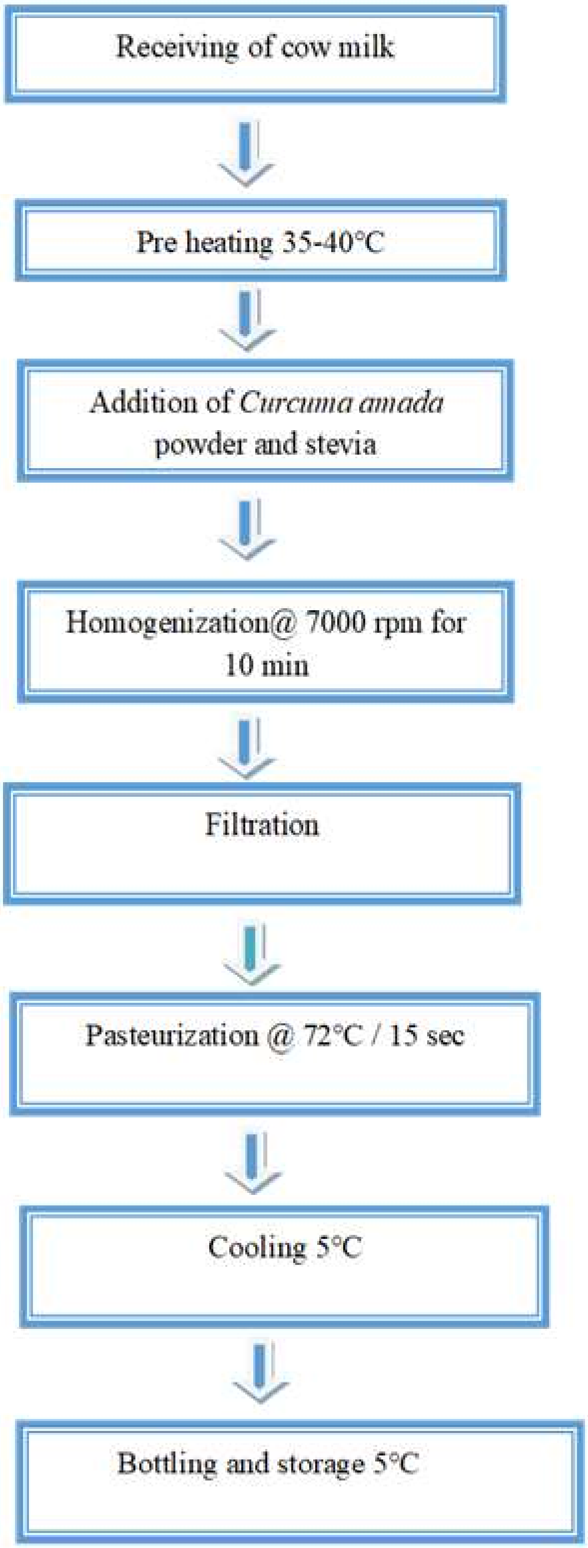

Fig 1 Flow chart of prepared Golden Milk preserved by pasteurization

The total phenolic content was determined by the Folin-Ciocalteu method with minor variations (Jayathilake et al. 2022)

Sensory evaluation

The prepared golden milk was evaluated by a panel of 10 semi-trained judges. Their response for various attributes namely color and appearance, taste, mouth feel, sweetness, flavor, and overall acceptability were recorded using 9 points hedonic scale rating ranging from 'like extremely = 9' to 'neither like nor dislike=5' to dislike extremely=1.

Results and discussion

A central composite design was employed to derive the optimum process conditions for the best composition of the functional golden milk. A set of thirteen experiments were designed to optimize the amounts of *Curcuma amada* and stevia. The experimental design and sensory score are given in Table 1.

Model fitting and interactive effects of process variables on sensory properties of Golden milk

Appearance and Color

Colour and appearance (C&A) is a common and basic sensory parameter that appeals to the consumer and influences the acceptability of any food product. Milk has yellowish color with a nice sweet flavor and no sediment while the color and appearance of flavored milk are determined by the type of flavor and color additives added to it.

The Appearance and Color of different trails of functional golden milk ranged from 5.7 to 8.8. Experiment 10 shows the lowest color, whereas experiment 8 shows the highest. In experiments 10 and 8, *Curcuma amada* and stevia levels were 0.17 percent, 0.35 percent, and 3.00 percent, 0.35 percent, respectively. The data fit the quadratic model below.

Appearance & Color = + 8.49 + 0.93 * A + 0.029 * B – 0.40 * A * B-0.79* A2 – 0.24 * B2

The coefficient of determination (R^2) was 0.9537 for the quadratic model. The adequate precision was 16.266. The "pred R- squared" is in reasonable agreement with the "adj R- squared". The model can be used to navigate the design space. Model F value of 29.48 implied that the model was significant. The response surface plot for the color and appearance as influenced by the level of *Curcuma amada* and stevia is given in Fig 2. It can be observed that with the increase in the level of *Curcuma amada,* the acceptability of color and appearance of functional golden milk increased. Unlikely Sawale et al. (2020) reported that an increased level of addition of herb (0.1 to 0.5%, *Pueraria tuberosa*) into milk resulted in decrease in color and appearance, and mouthfeel scores.

Taste

Taste plays a paramount role in the evaluation of the quality of food products. The taste ranged from 5.00 to 8.89 on a scale of one to ten. Experiment 1 yielded the lowest taste score, whereas experiment 8 yielded the highest flavor score. In experiments 1 and 8, *Curcuma amada* and stevia levels were 5.83 percent,0.35 percent, and 3.00 percent,0.35 percent, respectively. The data fit into the quadratic model below.

Taste = + 8.17 – 0.49 * A – 0.19* B + 0.13 * A * B – 1.45 * A2 – 0.71 * B2

Table1 Experimental design and sensory score of functional golden milk with different levels of *Curcuma amada* and stevia

Run	*Curcuma amada A (%)*	Stevia B (%)	Color and appearance	Taste	Mouth feel	Sweetness	Flavor	Overall acceptability
1	5.83	0.35	8.20	5.00	6.10	5.50	5.5	7.0
2	3.00	0.35	8.67	7.94	8.04	7.20	7.03	6.4
3	3.00	0.35	8.09	8.27	8.70	8.00	7.49	8.5
4	1.00	0.60	6.84	6.19	5.12	6.37	6.25	6.5
5	5.00	0.60	7.98	5.03	5.76	5.90	5.75	6.0
6	3.00	0.35	8.09	7.99	7.58	7.25	7.88	8.5
7	5.00	0.10	8.75	5.38	5.09	5.60	5.83	8.2
8	3.00	0.35	8.80	8.89	8.90	8.67	8.17	8
9	3.00	0.35	8.78	7.78	8.66	8.27	8.15	8.4
10	0.17	0.35	5.70	5.75	5.13	6.61	5.70	7.8
11	3.00	-0.00	7.99	6.96	5.45	5.15	5.25	7.0
12	1.00	0.10	6.02	7.04	5.87	5.08	6.01	8.8
13	3.00	0.70	8.12	6.74	5.11	5.05	5.95	6.8

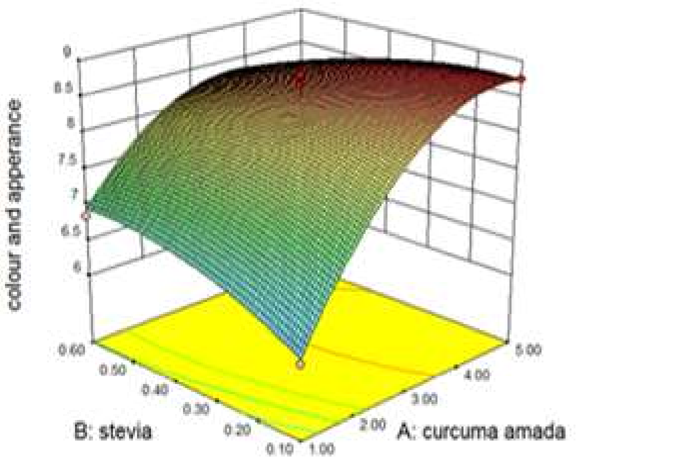

Fig 2. 3D Surface plot showing the interaction of *Curcuma amada* and stevia on the color and the appearance of functional golden milk

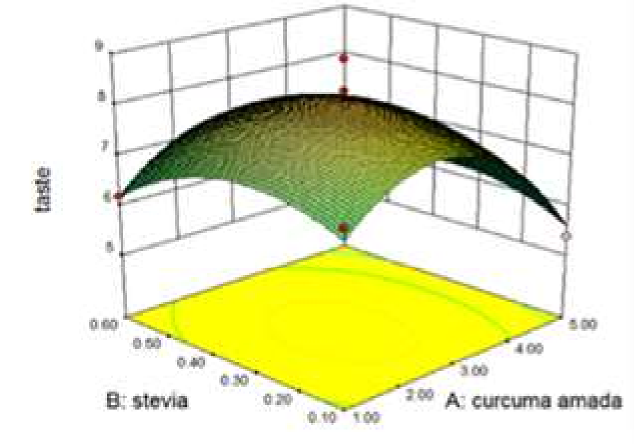

Fig 3. 3D Surface plot showing interaction of *Curcuma amada* and stevia on the taste of functional golden milk

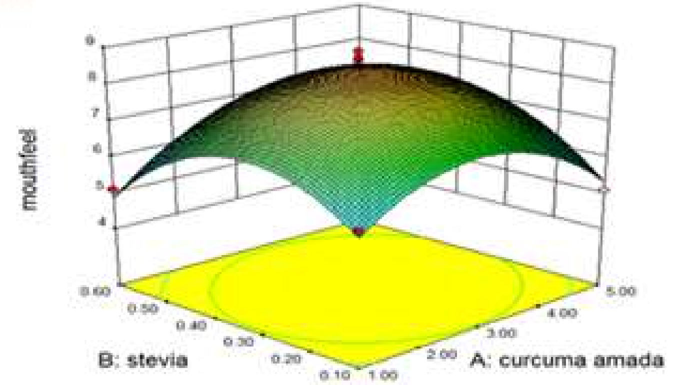

Fig 4. 3D surface plot showing interaction of *Curcuma amada* and Stevia on the mouthfeel of functional golden milk

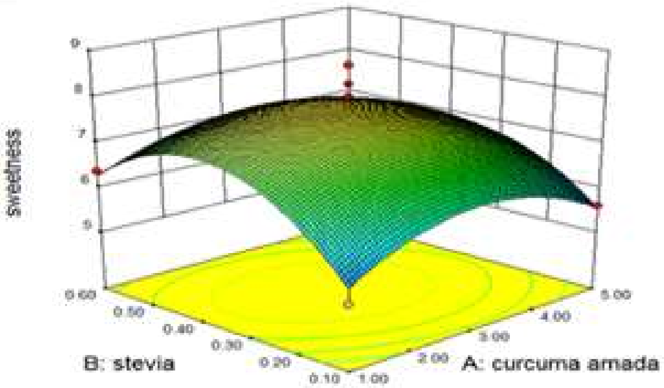

Fig 5. 3D surface plot showing the interaction of *Curcuma amada* and Stevia on the sweetness of functional golden milk

The coefficient of determination (R^2) was 0.9338 for the quadratic model. The adequate precision was 12.095. the pre-R- squared is in reasonable agreement with the Adj R-Squared. The response surface plot for the taste as influenced by the level of *Curcuma amada* and stevia is given in Fig 3. The model can be used to navigate the design space. Model F value of 19.74 implied that the model was significant.

Mouthfeel

Mouthfeel plays an important role in examining the body and texture characteristics. The pressure between the teeth and jaws determines the hardness, chewiness, and gumminess.

The mouthfeel ranged from 5.09 to 8.89 on a scale of one to ten. Experiment 7 yielded the lowest taste score, whereas experiment 8 yielded the highest flavor score. In experiments 7 and 8, *Curcuma amada* and stevia levels were 5.00 percent, 0.10 percent, and 3.00 percent, 0.35 percent, respectively. The data fit into the quadratic model below.

Mouthfeel = + 8.37 + 0.15 * A – 0.070 * B + 0.36 * A * B – 1.38 * A2 – 1.54 * B2

The coefficient of determination (R^2) was 0.9472 for the quadratic model. The adequate precision was 12.095. the pre-R- squared is in reasonable agreement with the Adj R-Squared. The model can be used to navigate the design space. Model F value of 5.09 implied that the model was significant and the lack of fit was insignificant.

The response surface plot for the mouthfeel as influenced by the level of *Curcuma amada* and stevia is given in Fig 4. It can be observed that as the level of *Curcuma amada* increased, the sensory score for mouthfeel also increased.

Sweetness

Sweetness is an attribute of perceived taste. The sweetness varied from 5.05 to 8.67. The minimum sweetness was obtained from experiment no 13 and the maximum sweetness score was

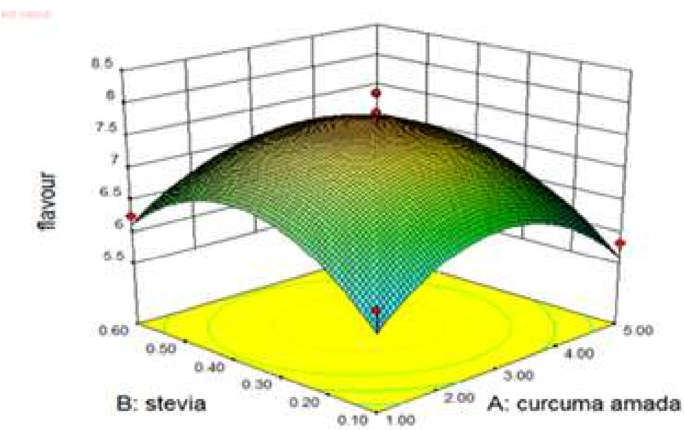

Fig 6. 3D surface plot showing interaction of *Curcuma amada* and stevia on the flavour of functional golden milk

obtained from experiment no 8. The level of *Curcuma amada* and stevia in experiments no 13 and 8 were 3.00%, 0.70%, and 3.00%,0.35% respectively. The data fitted the following quadratic model.

Sweetness = + 7.88 – 0.19 * A + 0.18 * B – 0.25 * A * B – 0.87 * A2 – 1.35 * B2

The coefficient of determination (R^2) was 0.8753 for the quadratic model. The adequate precision was 7.420. the "Pred R-Squared" is in reasonable agreement with the "Adj R- Squared". The model can be used to navigate the design space. Model F value of 9.83 implied that model was significant. The response surface plot for the sweetness as influenced by the level of *Curcuma amada*, stevia is given in Fig 5. The coefficient of estimation of *Curcuma amada* incorporated in functional milk golden showed that the level of *Curcuma amada* and stevia both have a positive effect on the score of sweetness.

Flavor

Flavor, as an attribute of foods, beverages, and seasonings, has been defined as the sum of perceptions resulting from stimulation of the sense ends that are grouped together at the entrance of the alimentary and respiratory tracts.

The flavor intensity ranged from 5.25 to 8.17. Experiment 11 yielded the lowest flavor score, whereas experiment 8 yielded the highest flavor score. In experiments 13 and 8, the levels of *Curcuma amada* and stevia were 3.00 percent, -0.00 percent, and 3.00 percent,0.35 percent, respectively. The data fit into the quadratic model below.526

Flavour = + 7.74 – 0.12 * A + 0.14 * B – 0.080 * A * B – 0.98 * A2 – 0.98* B2

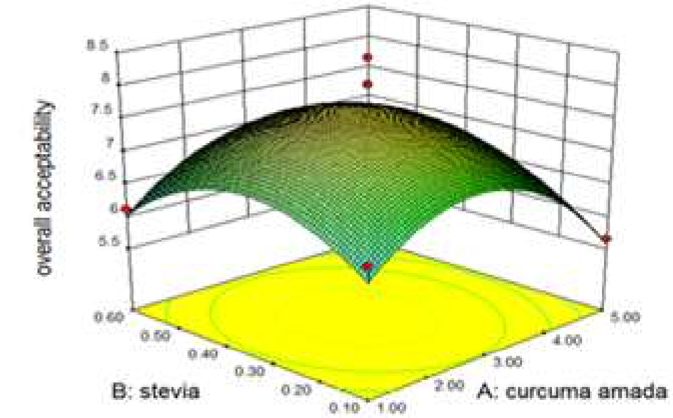

Fig 7. 3D surface plot showing the interaction of *Curcuma amada* and stevia on the overall acceptance of functional golden milk

The coefficient of determination (R^2) was 0.9033 for the quadratic model. The adequate precision was 7.391. The pre R^2 is in reasonable agreement with the Adj R-Squared. The model can be used to navigate the design space. Model F value of 13.07 implied that the model was significant.

The response surface plot for the flavor as influenced by the level of *Curcuma amada*, stevia is given in Fig 6. It can be observed that as level of *Curcuma amada* increased, sensory score for flavor also increased. The increase in the level of stevia also depicts increase in score of flavors remarkably.

Overall acceptability

The overall acceptance ranged from 5.25 to 8.42 on a scale of one to ten. Experiment 1 yielded the lowest overall acceptance score, whereas experiment 9 yielded the highest overall acceptability score. In experiments 13 and 8, *Curcuma amada* and stevia levels were 5.83 percent, 0.36 percent, and 3.00 percent, 0.35 percent, respectively. The data fit into the quadratic model below.

Overall acceptability= + 7.70 – 0.25 * A – 0.028 * B + 0.060 * A * B – 1.06 * A2 – 0.78 *B2

The coefficient of determination (R^2) was 0.8630 for the quadratic model. The adequate precision was 7.188. The "Pred R-Squared' is in reasonable agreement with the "Adj R- Squared". The model can be used to navigate the design space. Model F value of 8.82 implied that the model was significant.

The response surface plot for the overall acceptability as influenced by the level of *Curcuma amada*, stevia is given in Fig 7.

The coefficient of estimation of *Curcuma amada* functional golden milk showed that the level of *Curcuma amada* had a positive effect on the score of overall acceptability but stevia had a slightly negative effect on the score of overall acceptability. In contrast to this result, it was observed that increasing the addition of the herb *Pueraria tuberosa* (0.1 to 0.5%) in milk decreased its flavor score and overall acceptability score. (Sawale et al. 2020)

Analysis of regression of sensory characteristics

Coefficients of determination (R^2) for appearance and color, taste, mouth feel, sweetness, flavor, and overall acceptance of functional golden milk were 0.9537, 0.9338, 0.9472, 0.8753,0.9033, and 0.8630, respectively, according to the analysis of regression as presented in the data of Table 2, and the test for 'lack of fit ' was non-significant, suggesting that the model was accurate enough to predict these sensory features of functional golden milk created with any combination of components lies within the tested level range.

For appearance and color, taste, mouthfeel, sweetness, flavor, and overall acceptance of functional golden milk, the precised adequate values are determined as 16.266, 12.095, 11.086, 7.420,7.391, and 7.188, respectively.

The signal-to-noise ratio is calculated with adequate precision. It is preferable to have a ratio of four or greater. The ratio of all sensory ratings shows that the signal is adequate. The design space may be navigated using this concept.

Optimized product

To discover the best combination of ingredient-based variables, the Design Expert 8.0.6 optimization command was utilized. The responses were chosen based on sensory evaluations that influenced the ultimate product's acceptance. The responses were chosen based on sensory evaluations that influenced the ultimate product's acceptance. Appearance and color, taste, texture, flavor, and overall acceptance were all preserved at their highest levels, while sweetness was kept in check during optimization.

Functional golden milk was prepared using the optimized combination as given in Table 3 and subjected to evaluate its physicochemical properties after preserving the optimized product.

Proximate analysis of functional golden milk

Table 4 shows the ash, total solids, protein, fat, carbohydrates, and energy content of pasteurized functional golden milk. (Palthur et al. 2014) showed somewhat comparable results for total solids, protein, and fat. Milk produced with wheatgrass juice had a calorific value of 64.43 Kcal, which was lower than our data, (Kumar et al. 2017). (Palthur et al. 2014) found a specific gravity of 1.078, which was lower than our results in table 4.

The prepared functional golden milk viscosity was measured at 25 °C using a Brookfield viscometer. The viscosity of functional golden milk was 4.12Cp at 25°C.

Antioxidant activity of functional golden milk

Table 2 Curcuma amada and stevia impact on sensory attributes of functional golden milk: Regression Coefficient and ANOVA of the quadratic model

Factor	Colour& appearance	Taste	Mouth feel	Sweetness	Flavor	Overall acceptance
Intercept	8.486	8.17	8.38	7.88	7.74	7.70
Curcuma amada (A)	0.93	0.49	0.15	-0.19	0.12	-0.25
Stevia(B)	0.029	-0.19	-0.070	0.18	0.14	-0.028
AB	-0.40	0.12	0.36	-0.25	-0.080	0.060
A^2	-0.79	-1.45	-1.38	-0.87	-0.98	-1.06
B^2	-0.24	-0.71	-1.54	-1.35	-0.98	-0.78
R^2	0.9537	0.9338	0.9472	0.8753	0.9033	0.8630
Adequate precision	16.266	12.095	11.086	7.420	7.391	7.188
PRESS	1.10	5.23	4.06	7.94	4.06	3.53
Model F- value	29.48	19.74	25.10	9.83	13.07	8.82
Lack of fit	N S	N S	N S	N S	N S	N S

Table 3 Based on RSM analysis, suggested solutions for the components of functional golden milk, as well as their quantities and expected scores

No.	*Curcuma amada*(A)	Stevia (B)	Colour and appearance	Taste	Mouth feel	Sweetness	Flavour	Overall acceptability	Desirability
1	3.36	0.35	8.62522	8.04072	8.3597	7.81751	7.69236	7.62186	0.782

Table 4 Physiochemical, Proximate and biochemical analysis of the optimized functional Golden milk

Parameters	Optimized Product
L^*	79.01±0.4
a^*	0.61±0.21
b^*	72.95±0.83
Specific Gravity	1.12 @ 25°C
Viscosity	4.12Cp @ 25°C
Total Solids (%)	15.72±0.17
Protein (%)	3.93±0.10
Fat (%)	4.6±0.10
Ash (%)	0.74±0.12
pH	6.3
Acidity (%)	0.10±0.21
Carbohydrates (%)	15.09±0.80
Energy (Kcal)	117.48
Antioxidant (% DPPH activity)	56.14±0.12
Total Phenolic GAE/100 g Content	38.04 ± 0.61mg

Table 5 Mean sensory score of pasteurized functional golden milk

Duration	Color & Appearance	Flavor	Texture	Taste	Sweetness	Overall acceptability
0 DAY	8.5±0.2	7.9±0.5	7.7±0.2	7.9±0.4	7.5±0.2	8.02±0.9
3rs DAY	8.4±0.9	7.9±1.0	7.7±0.4	7.8±0.3	7.5±1.0	7.9±1.1
5th DAY	7.4±0.7	7.1±0.6	7.0±1.0	7.2±0.2	7.0±0.5	6.7±0.3
7th DAY	6.9±0.4	6.5±0.3	5.5±0.06	6.1±0.7	5.3±1.0	5.6±0.5

The antioxidant activity of Functional golden milk is 56.14. Similarly, the antioxidant activity of milk was made by the partial substitute of *Ocimum sanctum* powder using the DPPH technique and discovered 40% DPPH activity. (Palthur et al. 2014)

Total phenolic content of functional golden milk

Functional golden milk has a total phenolic concentration of 38.04 to 36.95 mg GAE (Gallic acid equivalent)/100 g. According to Tyagi et al. 2020, because phenolic components are active hydrogen donors and powerful antioxidants, total phenols and antioxidant activity are interrelated.

Variation in the sensory score during the storage period

The shelf life of a product may be defined as the time period within which it is found to be fit for consumption under defined conditions of storage and distribution. Shelf life depends on several intrinsic (water activity, pH, acidity, buffering power, redox-potential, inhibitory substances, etc.) and extrinsic product parameters (storage temperature, Relative Humidity, or RH, packaging material, etc.) The final optimized functional golden milk was packaged in sterilized bottles. The pasteurized sample was stored at 5 °C, sensory scores were assessed every 2 days. Pasteurized functional golden milk was stable for 7 days at 4°C as given in Table 5.

Conclusions

Based on the results of sensory and Physico-chemical analysis of the Golden milk formulated using different levels of *Curcuma amada* and Stevia, a combination of both the ingredients with desirable attributes were obtained. 3.36 percent *Curcuma amada* powder and 0.35 percent stevia can be used to make high-quality functional golden milk.This functional golden milk obviously has more nutritious characteristics than plain milk because it includes the optimal quantity of antioxidants and total phenolic content.Consequently, *Curcuma amada*'s application may be promoted to all aspects of human health. *Curcuma amada* has a cooling impact on our bodies and can be utilized by persons who live in hotter climates. With the result of this research, it may be concluded that *Curcuma amada* is beneficial and indeed be effectively and feasibly used in milk to make functional golden milk, which is very nutritious, inexpensive, and has several advantages.

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