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Optimization of Formulation for Development of Amaranth Incorporated Composite Fermented Milk Drink using Response Surface Methodology

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ABSTRACT

Background: Fermented milk products are highly nutritious food containing a significant amount of macro-nutrients, micro-nutrients as well as bioactive molecules which are required for the growth and overall development of all age's people. However, they lack in fibre, vitamin C and essential mineral such as iron. Pseudo-cereal like amaranth is a principal source of energy consists of significant amount of carbohydrates, protein, fibre and some micronutrients *viz*., calcium, phosphorus, iron, zinc *etc*. Therefore, a study was undertaken to optimize the amaranth (*Amaranthus cruentus*) incorporated composite fermented milk drink (AICFMD).

Methods: In present study, the central composite rotatory design (CCRD) with response surface methodology (RSM) was used to optimize the level of various ingredients (Independent variables) *i.e*. roasted amaranth flour, sugar and curd acidity to formulate the most acceptable AICFMD.

Result: The results revealed that composite fermented milk drink prepared from toned milk 60.5%, amaranth 4%, sugar 10.5%, water 25.0% (w/w) with curd acidity 0.831% lactic acid and final total solid content maintained to 21.2%, resulted in good quality AICFMD.

Key words: Amaranth, Fermented milk drink, Optimization, Response surface methodology.

INTRODUCTION

Since ancient times, milk and pseudo-cereals have been an integral part of the human diet. These cereals are generally combined to produce composite milk products with enhanced nutritional value. Fermentation increases the digestibility and therapeutic values of food. Amaranth (*Amaranthus*) is an ancient food native to the Andean region of South America, including Argentina, Peru and Bolivia. It is a gluten-free pseudo-cereal grain that belongs to the Amaranthaceae family (Murray and Pizzorno, 2010). The Government of India has initiated a strategy to promote millets, which are drought-resistant, nutritious grains. India's campaign to make millet a global brand has got a push with the United Nations accepting India's proposal to declare 2023 as the International Year of Millets (Sharma and Philip, 2023).

In India, amaranth is popularly known as Rajgira. The well-balanced composition of amino acids, a large amount of dietary fibre, minerals, vitamins and enzymes make amaranth an important raw material for highly nutritious pseudo-cereal-based food products. RSM is commonly used for optimising ingredients level and process parameters in new product development (Yolmeh and Jafari, 2017). Hence, a study was undertaken to optimize the formulation for the development of amaranth incorporated composite fermented milk drink.

MATERIALS AND METHODS

The research was carried out at the College of Dairy Science and Food Technology, Raipur (Chhattisgarh), from 2018 to 2020.

The design matrix consisted of three factors CCRD of RSM provided by Design-Expert software was used to optimise the level of formulation variables. Based on the

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preliminary trials conducted, the level of roasted amaranth flour (4-6 %), sugar (9-11 %) and acidity of curd (0.8-1.0% LA) were selected as critical formulation variables (independent variables), whereas sensory attributes (colour and appearance, Consistency, Flavour, Sweetness, overall acceptability); acidity and viscosity of AICFMD were treated as responses (dependent variables). The CCRD of RSM proposed 20 experiments with varying levels of these three independent variables.

Considering the formulation mentioned in Table 1, AICFMD samples were prepared according to the procedure of (Shinde *et al*., 2020) (Fig 1) and subjected to sensory analysis using 9 points hedonic scale. The titratable acidity measured by titration method and viscosity by Brookfield viscometer. The experiments were replicated three times. The average value of each response was fed to the design expert software. The individual and the interactive effect of all three independent variables on seven responses were studied.

RESULTS AND DISCUSSION

Effect of the level of roasted amaranth flour, sugar and curd acidity on sensory attributes, acidity and viscosity of AICFMD

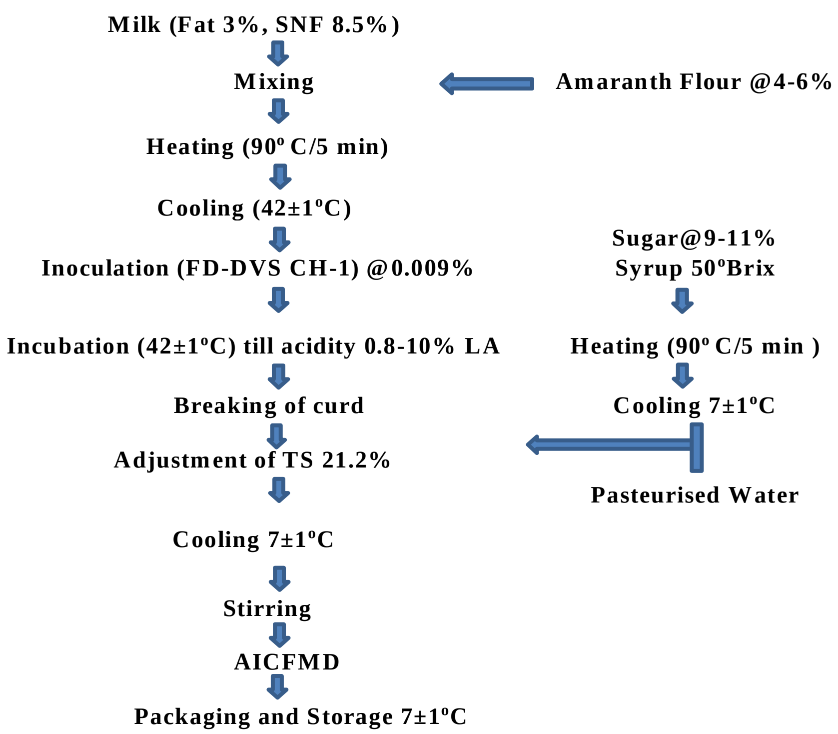
Colour and appearance

Colour and appearance (C and A) are the first characteristics of visual perception seen by the user before ingestion and essentially determine the quality of the product (Mistry, 2016). As per Table 1, the average colour and appearance score of AICFMD ranged from 6.00 to 7.93. The AICFMD prepared from a formulation with a curd acidity of 0.9 per cent LA, sugar of 10 per cent and amaranth flour level of 3.318 per cent recorded the highest score of 7.93.

The quadratic response surface model was selected for the CandA of AICFMD. There were 97.91 per cent chances that the C and A score of AICFMD were affected by formulation variables. Table 2 revealed that the level of amaranth flour and sugar had a highly significant negative effect on the colour and appearance of AICFMD at the linear term ($p<0.01$) and a significant adverse effect at the quadratic term ($p<0.05$). This might be due to roasted amaranth flour's red and yellow tint. The results of the present study are in agreement with Boyapati (2019), who reported that an increase in the level of quinoa flour decreased the C and A score of quinoa-based ready-to-serve dairy beverages. The curd acidity showed non-significant positive effect on the C and A of AICFMD at linear as well as at the quadratic term ($p>0.05$).

Flavour

Flavour is characterised as a combination of mouthfeel, texture, taste and aroma. Flavour plays a significant role in any product's acceptability (Ganguly, 2013). Table 1 indicated that the flavour score of AICFMD ranged from 6.79 to 8.29. The highest score, 8.29, was perceived for AICFMD prepared with a formulation with a curd acidity of 0.9 per cent LA, sugar at 10 per cent and amaranth at 3.318 per cent.



(Shinde *et al*., 2020)

Fig 1: Flow chart for the preparation of AICFMD.

Table 1: Experimental design matrix and responses for AICFMD.

Run	Independent variables (Factor)			Dependent variables (Responses)						
	Curd acidity	Amaranth flour	Sugar	Colour and appearance	Flavour	Consistency	Sweetness	Overall acceptability	Acidity	Viscosity
	(% LA)	(%)	(%)	Score out of 9					(% LA)	(cP)
	A	B	C	X_1	X_2	X_3	X_4	X_5	X_6	X_7
1	0.8	6	11	6.07	7	6.43	7.86	6.84	0.434	377
2	1	6	11	6.14	6.93	6.36	7.64	6.77	0.524	363
3	0.8	6	9	6.79	7.07	6.86	6.79	6.88	0.569	410
4	0.9	5	11.68	6.43	7.64	7.00	8.00	7.27	0.51	235
5	0.8	4	9	7.79	7.93	7.86	6.64	7.55	0.686	195
6	0.9	6.682	10	6.00	6.79	6.14	7.43	6.59	0.48	470
7	0.732	5	10	7.36	7.71	7.36	7.57	7.5	0.515	269
8	1	6	9	6.64	6.86	6.79	6.21	6.63	0.699	400
9	0.9	3.318	10	7.93	8.29	7.93	7.21	7.84	0.726	110
10	1.068	5	10	7.43	7.14	7.07	6.93	7.14	0.722	260
11	1	4	11	7.64	8.07	7.71	7.71	7.79	0.668	143
12	0.9	5	10	7.07	7.36	7.36	7.43	7.3	0.623	267
13	0.9	5	8.32	7.57	7.36	7.43	5.86	7.05	0.749	305
14	1	4	9	7.86	7.93	7.79	6.29	7.46	0.839	207
15	0.9	5	10	7.29	7.57	7.21	7.29	7.34	0.618	240
16	0.9	5	10	7.21	7.21	7.21	7.5	7.29	0.609	280
17	0.9	5	10	7.07	7.36	7.21	7.21	7.21	0.623	274
18	0.9	5	10	7.36	7.5	7.29	7.43	7.39	0.618	262
19	0.9	5	10	7.21	7.43	7.29	7.5	7.36	0.618	255
20	0.8	4	11	7.57	8.21	7.5	7.79	7.77	0.542	160

The linear response surface model was selected for the flavour of AICFMD. There were 93.82 per cent chances that the flavour of AICFMD was affected by formulation variables. Table 2 revealed that curd acidity had a significant negative effect ($p<0.05$) and the level of amaranth flour showed a highly significant negative effect ($p<0.01$) on the flavour of AICFMD at linear term. This negative effect on flavour score might be due to the typical nutty aroma of amaranth flour (Murray and Pizzorno, 2010). The results are in conformity with the report of Bianchi (2015), who reported that an increase in the level of quinoa flour decreased the flavour score of potentially synbiotic fermented beverages.

Consistency

Consistency describes the substance's physical nature in terms of thick or thin, smooth or lumpy, convenient to pour or not. Sweet fermented milk drinks should have a smooth and uniform consistency and be neither too thick nor too thin (Khurana, 2006). Table 1 represents average consistency score of AICFMD varying from 6.14 to 7.93. The maximum score was obtained for AICFMD formulations having curd acidity of 0.9 per cent as LA, 3.318 per cent amaranth flour and 10 per cent sugar.

The quadratic response surface model was selected for the consistency of AICFMD. There were 98.69 per cent possibilities that the consistency of AICFMD was affected by formulation variables. Table 2 showed that the level of amaranth flour had a highly significant negative effect ($p<0.01$) at linear term and a significant negative effect ($p<0.05$) at quadratic term. The level of sugar also showed a highly significant negative effect ($p<0.01$) at the linear term. Contradictory to this, the curd acidity showed non-significant negative effect on the consistency of AICFMD at the linear and quadratic terms ($p>0.05$).

Sweetness

Sweetness is one of the primary constituents of taste. During sensory analysis of products, sweetness plays a prominent role. It can be observed from Table 1 that the average sweetness score of AICFMD varied from 5.86 to 8. The maximum score was obtained for formulations having curd acidity of 0.9 per cent, 5.0 per cent amaranth flour and 11.68 per cent sugar.

The quadratic response surface model was selected for the sweetness of AICFMD as there were 98.62 per cent chances that various formulation variables would affect the sweetness of AICFMD. Table 2 revealed that curd acidity had a highly significant negative effect ($p<0.01$) on the sweetness of AICFMD at linear term and a significant negative effect ($p<0.05$) at quadratic term. The level of sugar showed a highly significant positive effect ($p<0.01$) on the sweetness of AICFMD at the linear term, whereas a highly significant negative effect ($p<0.05$) at the quadratic term. The curd acidity and sugar level showed a significant effect ($p<0.05$) on the sweetness of AICFMD at interactive term. The level of amaranth flour did not show any significant effect ($p>0.05$) on the sweetness of AICFMD either at linear or at interactive or quadratic terms.

Overall acceptability

Overall acceptability (OA) of AICFMD reflected the panellist's collective choice based on colour, appearance, flavour, consistency and sweetness (Ahuja, 2015). Table 1 indicated that the average OA score of AICFMD ranged from 6.59 to 7.84. The highest score of 7.84 was recorded for formulations having curd acidity of 0.9 per cent, amaranth flour at 3.318 per cent and sugar at 10.0 per cent.

The quadratic response surface model was selected for the OA of AICFMD. There were 98.23 per cent probabilities that the OA of AICFMD was affected by formulation variables. Table 2, revealed that the amaranth flour level had a significant negative effect ($p<0.01$). In contrast, a level of sugar showed a significant positive effect ($p<0.05$) on the OA of AICFMD at linear term and a significant negative effect ($p<0.05$) at quadratic term. The curd acidity showed a significant negative effect ($p<0.05$) on the OA of AICFMD at linear term.

The amaranth flour and sugar level showed a significant negative effect ($p<0.05$) on the OA of AICFMD at interactive term. Because of the absence of published literature, the results of the present findings could not be compared.

Acidity

Acidity is the chemical property of a fermented milk drink, which specifies the product's final acceptability (Modha, 2006). Table 1 showed that the average acidity of AICFMD expressed as per cent lactic acid (LA) ranged from 0.434 to 0.839. The highest OA score was obtained for the formulations having curd acidity of 1.0 per cent, amaranth flour at 4.0 per cent and sugar at 9.0 per cent.

The quadratic response surface model was selected for the acidity of AICFMD. There were 99.66 per cent chances that the acidity of AICFMD was affected by formulation variables. Table 2 revealed that at linear term, the level of amaranth flour and sugar had a highly significant ($p<0.01$) negative effect, whereas curd acidity positively affected the acidity of the AICFMD. The curd acidity, level of amaranth flour and sugar showed significant negative interactive effect ($p<0.05$) on the acidity of AICFMD. In the absence of pertinent literature, the results of this study could not be compared.

Viscosity

A fermented milk beverage's consistency and flow behaviour are directly associated with its viscosity. From Table 1, the average viscosity of AICFMD ranged from 110 to 470 cP (Table 1). The highest viscosity of 470 cP was recorded for AICFMD formulation having curd acidity of 0.9 per cent, amaranth flour at 6.682 per cent and sugar at 10 per cent. From Table 2, the quadratic response surface model was selected for the viscosity of AICFMD. There was a 99.24 per cent chance that formulation variables influenced the viscosity of AICFMD. Table 2 revealed that at linear term,

Table 2: Regression coefficient and ANOVA for sensory attributes, acidity and viscosity of AICFMD.

Factor		Response variable						
		C and A	Flavour	Consistency	Sweetness	OA	Acidity (% LA)	Viscosity (cP)
Intercept	β_0	7.20	7.47	7.26	7.39	7.32	0.6181	262.80
A-Curd acidity	β_1	0.0130 ^{NS}	-0.1009*	-0.0357 ^{NS}	-0.1689**	-0.0729*	0.0620**	-3.23 ^{NS}
B-Amaranth flour	β_2	-0.6199**	-0.4981**	-0.5441**	0.0322 ^{NS}	-0.4066**	-0.0676**	106.21**
C-Sugar	β_3	-0.2619**	0.0652 ^{NS}	-0.1481**	0.6348**	0.0747*	-0.0752**	-20.99**
AB	β_{12}	-0.0275 ^{NS}	-	-0.0350 ^{NS}	-0.0463 ^{NS}	-0.0312 ^{NS}	-0.0074*	-2.38 ^{NS}
AC	β_{13}	0.0275 ^{NS}	-	0.0350 ^{NS}	0.0788*	0.0363 ^{NS}	-0.0084*	-4.13 ^{NS}
BC	β_{14}	-0.0975 ^{NS}	-	-0.0525 ^{NS}	-0.0087 ^{NS}	-0.0562*	0.0006 ^{NS}	3.62 ^{NS}
A^2	β_{11}	0.0573 ^{NS}	-	-0.0136 ^{NS}	-0.0584*	-0.0015 ^{NS}	0.0008 ^{NS}	1.86 ^{NS}
B^2	β_{22}	-0.0948*	-	-0.0772*	-0.0337 ^{NS}	-0.0386 ^{NS}	-0.0047 ^{NS}	10.88*
C^2	β_{33}	-0.0824*	-	-0.0136 ^{NS}	-0.1716**	-0.0580*	0.0047 ^{NS}	3.81 ^{NS}
Model selected		Quadratic	Linear	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic
Model F-value		45.31**	81.00**	83.55**	79.22**	61.57**	330.17**	145.95**
R^2		0.9761	0.9382	0.9869	0.9862	0.9823	0.9966	0.9924
Std.Dev		0.1268	0.1215	0.0773	0.0949	0.0674	0.0081	11.12
Lack of Fit F-value		1.38 ^{NS}	0.9048 ^{NS}	2.10 ^{NS}	0.2923 ^{NS}	1.25 ^{NS}	3.99 ^{NS}	0.2115 ^{NS}

C and A= Colour and Appearance; OA=Overall Acceptability; *Significant ($p \leq 0.05$), **Highly Significant ($p \leq 0.01$); ^{NS}= Non-Significant; β_0 = Regression coefficient for constant term; β_1, β_2, β_3, = Regression coefficient for linear terms; β12, β13, β14 = Regression coefficient for interactive terms; β11, β22, β33 = Regression coefficient for quadratic term.

Table 3: Levels of factors fixed for the optimization of AICFMD.

Name	Goal	Lower limit	Upper limit
A: Curd acidity % LA	Is in range	0.8	1
B: Amaranth flour %	Is in range	4	6
C: Sugar %	Is in range	9	11
Colour and appearance	Is in range	7	7.93
Flavour	Maximize	7	8.29
Consistency	Is in range	7	7.93
Sweetness	Is in range	7	8
Overall acceptance	Maximize	7	7.84
Acidity % LA	Is in range	0.6	0.839
Viscosity cP at 20°C	Is in range	110	470

Table 4: Comparison of predicted against actual values of sensory attributes, acidity and viscosity of AICFMD (T_1).

Name	Predicted results*	Actual results**	t-value***
Colour and appearance	7.62	7.74±0.11	1.114
Flavour	8.07	7.86±0.14	-1.463
Consistency	7.66	7.92±0.16	1.631
Sweetness	7.64	7.9±0.15	1.714
Overall acceptance	7.75	7.86±0.12	0.879
Acidity (% LA)	0.6	0.605±0.01	0.461
Viscosity (cP, at 20°C)	159.11	152.8±2.48	-2.547

*Predicted value by design-expert software.

**Actual results value (mean of 5 replications) of optimised AICFMD.

***t- Value found non-significant at 5 per cent level of significance. Table value of t (0.05) = 2.776.

the level of amaranth flour had a highly significant ($p<0.01$) positive effect, whereas the sugar level negatively affected the viscosity of AICFMD. The level of amaranth flour showed a significant positive effect ($p<0.05$) on the viscosity of AICFMD at the quadratic term. It might be due to leaching and gelatinisation of amaranth starch during pre-heat treatment of amaranth incorporated milk mass. No reports are available on consistency of amaranth incorporated composite fermented milk drink; however, the results are in line with Mlakar *et al.* (2009), who reported increase in the level of amaranth flour increased the viscosity of bread loaf.

Optimisation of AICFMD formulations

The data generated during 20 runs is again fed to RSM software and based on these data upper and lower limits for factor and responses were set for obtaining the best combination (Table 3).

The RSM software provided 79 solutions, out of which the solution with the highest desirability, 0.86 (Amaranth flour 4%, Sugar 10.50% with curd acidy 0.831% LA), was selected for optimisation. Considering this formulation, the AICFMD was prepared and subjected to sensory analysis, acidity and viscosity measurement. The experiment was replicated five times. The actual mean value for all responses was compared with the expected values by applying a one-sample t-test using Web Agri Stat Package 2.0 (WASP 2.0).

It was observed that for the optimized sample there was no significant difference between the predicted and actual values of responses at a 5 per cent significance level (Table 4). The amaranth-incorporated composite fermented milk drink prepared from optimized formulation showed the optimum sensory score for colour and appearance, flavour, consistency, sweetness and overall acceptance as 7.62, 8.07, 7.66, 7.64 and 7.75, respectively. The optimum value of acidity and viscosity was 0.6% LA and 159.11 cP at 20°C, respectively.

CONCLUSION

The present study revealed that composite fermented milk drink prepared from 60.5% toned milk, 4% amaranth, 10.5% sugar, 25.0% water (w/w) with curd 0.831% acidity and final total solid content of 21.2%, resulted in good quality AICFMD.

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Conflict of interest

There is no conflict of interest for any authors for the article.

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