Formulation and Validation of Probioticated Foxtail Millet Laddu as a Source of Antioxidant for Biological System

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Abstract

The present investigation deals with the formulation of Indian conventional sweet item laddu with the enriched constituents of millet and *Lactobacillus acidophilus*. The food ingredients of laddu were modelled and optimized by using Design Expert software to make optimized final testable product. The probiotic *Lactobacillus acidophilus* culture was added in three different forms namely lyophilised, gum arabic microencapsulated powder and natural curd. The formulated probiotic with foxtail laddu was selected based on the criteria of colour, odour and texture of the product. The carbohydrate, protein and fat content were estimated to be stable at 96.7g, 16.7g and 22.7g respectively. The viability test for the incorporated *Lactobacillus acidophilus* revealed that the formulated product showed a decrease in microbial count for a storage period of two months by analysing kinetic death rate. The lyophilised and microencapsulated culture showed a good viability 6.10 ± 0.09 log cfu/g and 7.43 ± 0.02 log cfu/g at 4°C when compared to storing at room temperature as 5.41 ± 0.08 log cfu/g and 6.97 ± 0.02 log cfu/g at the end of the storage period.. Probiotic millet laddu might serve as a value-added food product for the consumption of public and might improve the general health of the public.

1. Introduction

Millets are a diverse group of grasses that are widely grown all around the world as cereal crops and grains. It is used as feed for both livestock and human. Millets are highly nutritious when compared to the major and widely used food crops such as wheat and rice (Saleh et al. 2013). It is rich in dietary fibres, vitamins, iron, calcium and other minerals. (Mohan Kumar *et al*. 2012). Foxtail millet is the second most widely cultivated species of millet. According to the study of Jali (2002), foxtail millet release glucose slowly and steadily in the blood, without affecting the metabolism of the body. The prevalence of diabetes is limited among the population consuming foxtail millet (Jali, 2012). Earlier studies on household processing methods like malting of pearl millet and finger millet reduce protein content, but improves protein efficiency ratio (PER) and enhances the bioavailability of micronutrients by lowering antinutrients (Shunmukha *et al*., 2015). Finger millet in making rice ball and pearl millet in baking roti together with wheat was used (Seetha et al., 2019). Zainab *et* al., 2019 tried Foxtail millet by several workers in the development of various foods, which include bread, cakes, traditional foods, weaning foods, popped, extruded, roller-dried and flaked products, noodles. The foxtail millet rice can be used instead of rice in the preparation of all the traditional products like bisibele bath, chakkali, pongal, dosa, idli, and laddus. Suman *et al.*, 2015 formulated three products viz. laddu, halwa and biryani based on foxtail millet, barnyard millet and rice were prepared.

Probiotics are often called as good and beneficial bacteria that are naturally found in our body and also occurred in some food supplements in adequate amount confer health benefits. (Sarkar, 2013). Thus, probiotics represent a standard group of functional foods and are defined as live supplement with microorganisms, with a proven advantage to the host by improving the intestinal microbial balance of the host organism. Probiotics includes various types of bacteria which pose different benefits (Hawaz, 2014). But most of the probiotic bacteria come under two groups namely *Lactobacillus* and *Bifidobacterium*. Probiotic bacterias are most commonly found in dairy products and fermented food products. According to Divya et al. (2012) commonly claimed benefits of probiotic include strengthening of the immune system, potential antagonistic activity against pathogenic gastrointestinal microorganisms, decreasing cholesterol accumulation, improvement of bowel regularity and maintaining of individual intestinal microbiota. Probiotics improves the patient in health disorders such as diarrhea, inflammatory diseases, gastroenteritis, bowel syndrome, cancer etc., Probiotics enhances the nutritional value and the digestibility

of raw products. It improves the sensory characteristics and also enhances the functional qualities of foods and beverages. (Savita et al., 2020)

Encapsulation is a mechanical or physicochemical process mainly used in increasing the viability of bacteria by adding a protective agent. It is mainly used in food processing industries dealing with probiotics in order to increase the survival of the probiotic bacteria in various environmental conditions during the storage period (Chayarri et al. 2012). The shell material is generally made up of polysaccharides, polymers, fats and waxes. Selection of shell material is a critical factor which determines the stability of microorganisms present inside the core (Cock and Castillo, 2013).

Gum Arabic (acacia) is a complex mixture of polysaccharides and glycoproteins obtained from *Acacia senegal.* It is used in food industry for varieties of applications such as encapsulation, emulsification, gum candies, thickening agent, confectionary treats. It is highly soluble in water and does not cause any intestinal problems when consumed (Cherbut et al. 2003). Food provides the body with energy it needs for maintaining a healthy condition. Normally food supplement has to provide proper nutrients for growth and energy of our body cells. (Stubbs and Whybrow, 2004) The food habits of present scenario are becoming very dangerous leading to the deterioration of health and causing health related problems. According to this (Myles 2014) improper food habits and its supplements created the concept of unhealthy life and sources of diseases like protein-energy malnutrition, vitamin A deficiency, iodine deficiency disorders, nutritional anaemia and diet related non-communicable disease like obesity, cardiovascular disease, stroke, diabetes and other diseases. People must pay high concern towards their eating habits in order to maintain both physical and mental health. In context of health, the present study is to formulate the nutrient enriched foxtail millet based food product with probiotics as a health supplements to reduce the risk level of various kinds of diseases in individuals (Chandel, 2014).

Antioxidant is an important phenomena for controlling cell damage by various forms of free radicals produced by our biological system. Free radicals are unfilled electrons which induce normal cell damage and finally lead to degenerative diseases in human body. Millet have most phenolic compounds are present in the free form (71%). (Savita et al., 2020). The high polyphenols are phenolic acids and tannins, while flavonoids are present in small quantities; they act as antioxidant and play many roles in the body immune system (Singh and sarita, 2016). In this regard, present study utilizes the edible millet based south Indian laddu for eradicate and capture the free radicals in a system. Hence foxtail millet has been formulated with suitable probiotic culture for enhancing immune system as well as antiradical activity.

2. Materials And Methods

2.1 Formulation of foxtail laddus

The formulation of the product was done by using the following ingredients such as foxtail millet, groundnut, horse gram, jaggery, ghee, honey, cardamom and cinnamon. The ingredients were optimized and analysed for the significant level with the help of Design Expert software using One Factor, Plackett Burman and Response surface methodology. Among the ingredients used foxtail millet, ground nut, horse gram and jaggery were considered as variables whereas all other factors were considered to be constants. Analysis of four variables was done using the Design expert software, which generated 30 different experiments. All the 30 formulations of millet laddus were prepared in the laboratory for further analysis (Chen et al. 2008). The analysis was predicted by the various

responses of products. All the foxtail laddu was added with prebiotics of inulin (0.1%) to improve the growth of probiotics and as encapsulating agents in the different formulations.

2.2 Collection and maintenance of strains

Pure lyophilized cultures of probiotic microorganism *Lactobacillus acidophilus* NCIM 5306 was purchased from National Collection of Industrial Microorganisms. Pure cultures were stored at 4° C and the strains were frequently sub cultured in MRS media for maintaining the viability of the culture for subsequent usage. The strains were inoculated in 100ml of MRS media for mass production of *Lactobacillus acidophilus* strains and it was incubated for 24 hours for obtaining the log phase cultures.

2.3 Probiotication and formulation of millet laddus

Millet laddus were prepared based on the optimized composition obtained from the design expert software. Wash and soak the millet and horse gram in water for overnight. Drain the water and spread them on a cloth lined plate and allow it to cool. Then millet and horse gram are dry roasted in a heavy pan on a low flame for 6–7 minutes until they when colour changes to brown. Dry roast groundnuts were added and grounded along with whole cardamom and cinnamon to a fine powder. Add melted jaggery, ghee and honey little by little until the millet mixture comes together. Finally add lyophilized culture and insulin and mix it well. Make small laddus and set aside on a plate. Store the laddus in an airtight container and use it within 3–4 days. The final volume of laddu was found to be 150g. Then the addition of probiotics was done on the basis of three different formulations to get the proper release kinetics of *Lactobacillus acidophilus.*

2.4 Addition of Free form of lyophilized *Lactobacillus acidophilus* (Formulation I)

The broth was freeze dried using a lyophilizer to obtain dried powder of the culture. The colony forming units were analysed in the dried powder by performing the serial dilution followed by pour plate techniques (Chen, 2006). The lyophilized culture of *Lactobacillus acidophilus* with a colony count of 8.52 ± 0.04 log cfu/g was added into the millet laddu in an appropriate proportion of 1g/150g of the laddu (Majeed et al. 2016). Thus the ratio of addition of lyophilized culture to the final product was 1:150 grams. The final product was packed in closed containers and stored at 4°C and at room temperature.

2.5 Addition of Microencapsulated *Lactobacillus acidophilus* (Formulation II)

Gum arabic was used as the protectant material for the encapsulation of *Lactobacillus acidophilus.* Gum arabic and sucrose in the ratio of 8:2 are taken to form a total volume of 60g were solubilised in 200ml distilled water at a temperature of 45°C. The percentage of the carrier in water is 30%. The solution is stirred until the solution is cooled down to room temperature to obtain a carrier media (Nunes and Mercadante, 2007).

L. acidophilus was cultured in 100ml of MRS broth and incubated for a period of 24 hours at room temperature. After incubation, the culture broth was centrifuged at 6000 rpm for 5 minutes. Supernatant were discarded and the pellet were washed with 50 ml phosphate buffered saline and recentrifuged. The supernatant was discarded and the pellet was resuspended in carrier media and the solution is homogenized for 30 minutes at room temperature (Yonekura et al. 2014). The carrier media containing *Lactobacillus acidophilus* was lyophilised to get a powder. The powdered samples were stored at 4°C. Probiotic millet laddu was prepared by adding 1 g of the obtained powder into the optimized concentration of millet laddu obtained from the design expert software.

2.6 Addition of probiotics in the form of curd (Formulation III)

Curd is one of the rich sources of natural probiotics and which was freeze dried to get a fine powder. Then the 1g of dried natural curd powder containing probiotics was added to the foxtail laddu with the same proportion of 1g/150g.

2.7 Evaluation of the final products

Analysis and evaluation of the final products were done on the basis of organoleptic, physical/chemical and microbiological methods. Organoleptic parameters used for analysis were appearance, taste, softness and solubility (Sasikumar, 2013). The obtained statistical values were analysed using Design Expert 7.0 in order to calculate the acceptability of the final product.

2.8 Nutritional analysis

Chemical parameters such as carbohydrate, protein, fat content as well as other nutritional contents were analysed using various experiments in the laboratory. The total carbohydrate, protein, cholesterol and fat content was analysed using anthrone method, Lowry's method, Zak's method and acid hydrolysis respectively (Lizia and John, 2014). The total dietary fibre content was analysed by McCleary method. The total sodium, potassium, calcium were estimated using flame photometry.

2.9 Stability analysis

The overall stability of the three products was also studied in order to determine the best product (Chen et al. 2008). Microbiological parameters include the analysis of total number of microbes during the storage period (Kumar et al. 2015). The prepared laddus were stored in the closed containers at 4°C and at room temperature for a period of 2 months. During the storage period the total colony forming units from both the storage conditions were analysed at an interval of one week.

2.10 Analysis of bacterial release kinetics in encapsulation

The release kinetics of probiotic bacteria from the encapsulation was studied under two different storage conditions. 1 g of laddu from each storage period was taken and was transferred into 10ml of sterile saline solution (0.9% NaCl) and it was shaken for 1 hour at 30°C. The total amount of bacteria released into the solution was determined by plate count method on MRS agar (Wu et al. 2012). The experiments were conducted in triplets to monitor the release kinetics in statistics.

2.11 Evaluation of anti-oxidant activity of formulated laddu

Determination of antioxidant activity of the formulated laddu was carried out by the following methods. 1mg/ml of the each probioticated laddu formulation was taken for the analysis of antioxidant assays. DPPH radical scavenging activity was determined for three probioticated laddus formulations using the protocol of Khalaf et al, 2008. Millet and probiotic were kept as a control. The ascorbic acid was used as a standard. FRAP assay was done using Oyaizu *et al.* 1986. Scavenging activity of hydrogen peroxide was determined using Ruch et al., 1989. Total phenolic content of the formulated laddus were determined by Folin-Ciocalteau reagent using gallic acid as a standard (Ayyappadasan et al., 2017). Statistical analysis and ANOVA were analysed by using SPSS version 25 software.

3. Results And Discussion

3.1. Formulation of organic millet laddus

The various ingredients that were used for the formulation of foxtail millet laddu include foxtail millet, ground nut, horse gram, jaggery, ghee, honey, cardamom and cinnamon. The final product composition was analysed using Design expert software, which generated 30 different experiments using four different variables namely foxtail millet, ground nut, horse gram and jaggery. The response values were shown in the Table 1. Each experiment had generated different formulations by altering the composition of ingredients involved in the product. Therefore all the formulations were prepared and subjected to further analysis based on the organoleptic parameters such as appearance, taste, softness and solubility. Statistical values and Analysis of variance were represented in the Supplementary Table 1–4. The results obtained from the experiments conducted on organoleptic properties were represented in the Table 2 and the results were fed into the software.

Table 1
Experimental Design for mixture design and responses for laddu production using Central Composite Rotatable Design (CCRD)

S.No	Std	Run	Factor 1	Factor 2	Factor 3	Factor 4	Softness	Appearance	Taste	Solubility
1	12	1	80	40	8	80	9	5	6.4	9
2	14	2	80	20	12	80	4.6	7	8	9.5
3	29	3	60	30	10	60	9	7.5	8.3	9.1
4	3	4	40	40	8	40	7	6.5	8	2.5
5	30	5	60	30	10	60	9	8.5	7.8	9.5
6	23	6	60	30	10	20	6	5.7	6.6	4.6
7	26	7	60	30	10	60	7	5.1	7	8.4
8	4	8	80	40	8	40	3	8.4	8.2	5.3
9	8	9	80	40	12	40	2	8.3	6.4	9
10	9	10	40	20	8	80	4.92	6.3	9	4.9
11	17	11	20	30	10	60	6	3.5	4.3	7
12	20	12	60	50	10	60	7	9.5	10	2
13	19	13	60	10	10	60	2.77	9	6.7	6
14	15	14	40	40	12	80	6.3	2.7	5.4	6.2
15	2	15	80	20	8	40	4.02	4.9	1.8	2.34
16	22	16	60	30	14	60	6.9	9.5	6.7	8.1
17	11	17	40	40	8	80	7.52	4.5	7.8	8.6
18	24	18	60	30	10	100	6.6	2	8	6.6
19	7	19	40	40	12	40	5	5	2	4.3
20	27	20	60	30	10	60	8	9.2	5	5.9
21	21	21	60	30	6	60	9.9	8.3	8	3.7
22	6	22	80	20	12	40	5	8.3	9	9
23	25	23	60	30	10	60	8	5.6	4	7
24	13	24	40	20	12	80	4.6	4.5	4.98	6.3
25	18	25	100	30	10	60	0	4	8	7
26	28	26	60	30	10	60	7.8	6	4	4
27	16	27	80	40	12	80	7	7.4	9.2	4.8

***Factor 1: Foxtail millet; Factor 2: Groundnut; Factor3: Horse gram; Factor4: Jaggery. All are mentioned in gram (g)**

S.No	Std	Run	Factor 1	Factor 2	Factor 3	Factor 4	Softness	Appearance	Taste	Solubility
28	1	28	40	20	8	40	6.7	5.6	9	1
29	5	29	40	20	12	40	9	6	2	7
30	10	30	80	20	8	80	3	7.7	3.2	7
***Factor 1: Foxtail millet; Factor 2: Groundnut; Factor3: Horse gram; Factor4: Jaggery. All are mentioned in gram (g)**										

Table 2
Physical evaluation of the prepared laddu

Response	Prediction	SE Mean	95% CI low	95% CI high	SE Predicted
Softness	7.76983	0.40	6.92	8.62	1.04
Appearance	5.74265	0.56	4.56	6.93	1.45
Taste	5.72945	0.57	4.54	6.91	1.76
Solubility	5.58391	0.63	4.27	6.89	1.95

Table 3
Optimized concentration of food ingredients against different response using Design expert software

S.No	Name	Level	Low Level	High Level
1.	Foxtail Millet (g)	42.99	40.00	80.00
2.	Ground nut (g)	37.49	20.00	40.00
3.	Horse Gram (g)	10.30	8.00	12.00
4.	Jaggery (g)	60.03	40.00	80.00

3.2. Interaction of Various ingredients against the Response factor by Two factorial Interactions

The present study revolves around optimizing the medium composition by using the design expert software. The optimization is mainly based on analysing the interaction between the ingredients used and the response of the product based on the softness, appearance, solubility and taste. Use of RSM has resulted in the better understanding of the possible interaction between various ingredients used in the product. The significant interaction between variable has improved the total acceptability of the product as in the case of Pranaw et al. 2014. The study done by Lungmann *et al*. 2006, the mixture design predicted as optimized media by design expert was most superior when compared to other media in terms of final result. Similarly the optimum result from the design expert software for laddu production is preferable over other composition.

In the Fig. 1(a) represents the analysis of taste performed against foxtail millet and horse gram. From the analysis it was found that an equal proportion of foxtail millet and horse gram were required to enhance the taste of the laddu. Whereas from Fig. 1(b) it found that, there is not any notable interaction between ghee and foxtail millet in the event of enhancing the taste. With respect to the Fig. 1(c) and 1(d) it was found that a specific interaction between

jaggery and horse gram as well ground nut and jaggery is required in order to enhance the taste. But it need not to be an equal proportion, a slight range nearer to the midpoint is found to be the best.

The final predicted composition of the laddus based on analysing all the results were given in the Table 4. The final predicted composition from the software was considered as the optimum concentration and the same concentration was used for doing further experiments. The products gave a good impression which was inferred from the experimental response values. This is in parallel relationship with the study done by Aboulfazli, (2015) where the sensory analysis test was based on the attributes such as color, texture, flavour and taste and none of the product was reported as poor and all the products had a good impact on the review panel.

Table 4
Final composition of the probiotic millet laddu

S.No	Name	Level
1.	Foxtail Millet (g)	42.99
2.	Ground nut (g)	37.49
3.	Horse Gram (g)	10.30
4.	Jaggery (g)	60.03
5.	Ghee(ml)	2.75
6.	Honey(ml)	2.55
7.	Cardamom(g)	0.24
8.	Cinnamon(g)	0.235
9.	Lyoplilized culture(g)	1
10.	Inulin (g)	0.1

Softness = -1.76792 + 0.158354*Factor 1 + 0.436875*Factor 2 + 0.736458*Factor 3 -0.131313*Factor 4 + 0.001181*Factor 1 * Factor 2 + 0.001281*Factor 1 * Factor 3 + 0.002178*Factor 1 * Factor 4 -0.033687*Factor 2 * Factor 3 + 0.006381*Factor 2 * Factor 4 -0.003469*Factor 3 * Factor 4 -0.003252*Factor 1^2 -0.008296*Factor 2^2 +0.012292*Factor 3^2 -0.001190*Factor 4^2

Appearance = + 8.42083 + 0.073958* Factor 1 -0.161250* Factor 2 -2.39375*Factor 3 + 0.348125* Factor 4 + 0.001531* Factor 1 * Factor 2 + 0.015156* Factor 1 * Factor 3 + 0.000359* Factor 1 * Factor 4 -0.007187* Factor 2 * Factor 3 -0.002906* Factor 2 * Factor 4 -0.006406* Factor 3 * Factor 4 -0.002148* Factor 1^2 +0.005156* Factor 2^2 +0.107031* Factor 3^2 -0.002086* Factor 4^2

Taste = + 38.17433 – 0.531521* Factor 1 + 0.113625* Factor 2 -3.26479* Factor 3 -0.078437* Factor 4 + 0.003119* Factor 1 * Factor 2 + 0.050656* Factor 1 * Factor 3 -0.000747* Factor 1 * Factor 4 -0.026188*Factor 2 * Factor 3 + 0.000256*Factor 2 * Factor 4 + 0.013719* Factor 3 * Factor 4

Solubility = -42.30867 + 0.083854* Factor 1 + 0.585875* Factor 2 + 3.94229* Factor 3 + 0.436687* Factor 4 -0.000669* Factor 1 * Factor 2 + 0.002906* Factor 1 * Factor 3 -0.001022* Factor 1 * Factor 4 -0.055187* Factor 2 * Factor 3 -0.000269* Factor 2 * Factor 4 -0.032594* Factor 3 * Factor 4

Response surface plots on different evaluation parameters like softness, taste, appearance and solubility were shown in the Fig. 2–5. ANOVA statistical analysis for four different factors were calculated by Design expert software in Supplementary Table 1, 2,3,4 and it was found to be significant and fit model. Quadratic equation for the prediction the optimum point was obtained according to the CCRD design. Desirability value of 0.7 was obtained using CCRD model of RSM. R^2 value was found to be 0.998 and P-value of (< 0.0001) had obtained, which indicated a better agreement between the actual and predicted values of probiotic laddu physical evaluation and confirmed a significant interpretation of the mathematical model.

3.3. Probiotication of millet laddus

The final composition of the prepared laddu was given in the Table 4. The inference from the table depicts that the major nutritional content of the product was mainly offered by the four main ingredients that were taken as variables in the design expert software and the other ingredients serves as supplements that either enhances or improves the quality of the final product.

3.4. Addition of lyophilized *Lactobacillus acidophilus*

Millet laddus were prepared in the composition as mentioned in the Table 4. 1 g of lyophilised culture was added and different formulations of laddus were prepared for evaluation of nutritional and stability analysis as Fig. 7. 1g of the lyophilized sample of *Lactobacillus acidophilus* was added to 150g of the probiotic millet laddu and the final concentration of the culture in 1g of the probiotic millet laddu was found to be 10.52 ± 0.04 log cfu/g.

3.5. Addition of Microencapsulated *Lactobacillus acidophilus*

To the millet laddu composition mentioned in the Table 4, 1g of *Lactobacillus acidophilus* encapsulated with gum arabic was added, the total colony count of microencapsulated *Lactobacillus acidophilus* in 1 g of the laddu was found to be 9.97 ± 0.03 log cfu/g.

3.6. Addition of probiotics in the form of curd

The third method probiotics in the form of curd was directly incorporated into the final product, the total colony count of curd incorporated laddu was found to be 10.34 ± 0.03 log cfu/g.

3.7. Evaluation of the prepared laddu against nutritional and stability

Analysis and evaluation of the final product was done on the basis of organoleptic, physical/chemical and microbiological methods.

3.8. Nutritional analysis

Nutritional content of the 4 different kinds of laddu including conventional laddus were given in the Table 5. The formulated millet laddus were found to contain major nutritional components that could satisfy all the requirements for a healthy diet. Appreciable quantity of protein and mineral found in the product could increase the nutritional value of the product. The conventional laddu showed very high content of carbohydrate and cholesterol as 101.44g and 30.23g respectively whereas other important nutrition's were presented in very less content than the foxtail laddu. Overall mean among the nutrient composition of the three different formulations of laddus were found to be 18.196. Comparison of the mean difference among the different groups was mentioned in Table 6. Lizia and John (2014) developed a millet based high fibre biscuits and nutritional properties for the same was analysed. The total protein, carbohydrate, fat and fibre of the high fibre biscuit was tested and it was found to be 8.62 g, 68.05 g, 13.10g

and 19.2 g per 100 g of the product, whereas the probiotic millet laddu have nearly the same nutritional composition as that of high fibre biscuits except for the fibre content. The total protein, carbohydrate, fat and fibre of the millet laddus was 11.133 g, 64.466 g, 15.133 g and 5.06 g per 100 g of the product. The amino acid profile is balanced and the dietary fiber content is very high compared to other cereals. Nutritional composition of Foxtail millet per 100 gm is fat (4.3 gm), minerals (3 gm), protein (12.3 gm), calcium (31 mg %), carbohydrate (60.9 gm), phosphorous (290 mg%) and dietary fibre (14 gm) was found in Zainab et al., 2019. The nutritional analysis of the pearl millet laddu showed that the moisture 12.6 ± 0.2%, protein 9.9 ± 2.8 g, fibre 2 ± 2.6 g, fat 4.2 ± 0.5 g, carbohydrate 69 g was slightly similar to that of Uttara et al., 2017. The total protein, carbohydrate and fat content of the millet laddu were similar to the fibre biscuits with little variation in the composition which is acceptable. Verma et al., 2015 explained that the foxtail and barnyard millet laddu had comparatively high protein content, 5.00 and 3.41%, respectively and low carbohydrate content in both Foxtail and barnyard millet based laddu.

Table 5
Analysis of nutrient composition of the three different formulations of laddus

Nutrition	**Conventional Laddu (g)**	**Lyophilized probiotic Laddu (g)**	**Probiotic Encapsulated Laddu (g)**	**Curd incorporated laddu (g)**	**Mean**	**SD**
Carbohydrates	101.44 ± 2.8	96.7 ± 1.4	97.5 ± 0.8	97.01 ± 1	98.16	2.21
Proteins	14.39 ± 0.2	16.7 ± 2	16.8 ± 1.6	16.75 ± 0.4	16.16	1.18
Fats	23.18 ± 1.2	22.7 ± 0.4	22.9 ± 2.2	22.74 ± 1.2 g	22.88	.013
Cholesterol	0.030 ± 0.0008	0.0039 ± 0.0005	0.0040 ± 0.0007	0.0040 ± 0.0014	.01048	.614
Fibre	6.95 ± 1.6	7.6 ± 0.4	8.45 ± 0.4	7.61 ± 0.7	7.6525	.6148
Sodium	0.041 ± 0.0004	0.0256 ± 0.0003	0.0295 ± 0.0004	0.0260 ± 0.0008	.03053	.0072
Potassium	0.545 ± 0.0012	0.6251 ± 0.0006	0.627 ± 0.0008	0.6266 ± 0.002	.60592	.0407
Calcium	0.029 ± 0.0002	0.0841 ± 0.0013	0.0951 ± 0.0014	0.0853 ± 0.0004	.07338	.0299
Mean	18.325 ± 0.73	18.054 ± 0.53	18.301 ± 0.63	18.106 ± 0.413	18.196	
Std. Dev.	34.634 ± 1.05	32.940 ± 0.76	33.177 ± 0.85	33.046 ± 0.49		31.79

* Composition /150g of the laddu

Table 6
Comparison between the groups of different laddu samples with respect to nutrition

S.No	Samples		Mean difference
1	Conventional Laddu	Lyophilized probiotic Laddu	0.271
2	Conventional Laddu	Probiotic Encapsulated Laddu	0.024
3	Conventional Laddu	Curd incorporated Laddu	0.2191
4	Lyophilized probiotic Laddu	Probiotic Encapsulated Laddu	0.2458
5	Lyophilized probiotic Laddu	Curd incorporated Laddu	0.0516
6	Probiotic Encapsulated Laddu	Curd incorporated Laddu	0.1942
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3.9. Stability analysis

The laddu incorporated with lyophilized culture of *Lactobacillus acidophilus* was analysed for the total colony forming units during the storage at 4°C and at room temperature. Figure 6. depicts the total colony forming units over the period of two months during the two different storage conditions. During the initial storage period the total cfu in both the conditions were same and it was around 8.20 ± 0.1 log cfu/g, but once the storage time increases there is a significant decrease in the colony count. On storing the product up to three weeks there is a slight difference in the viability between the two storage conditions. After three weeks there is a great difference in the colony count between two storage conditions. At the last week the total cfu at 4°C was 6.10 ± 0.09 log cfu/g and cfu at room temperature was around 5.41 ± 0.08 log cfu/g. The loss in viability of the lyophilised *Lactobacillus acidophilus* culture during the storage period of two months was explained in the Fig. 6. From the result it is obvious that storing the product at 4°C was found to be the best condition for maintaining the viability of the freeze dried product when compared to storing the product at room temperature. On storing the product at 4° and room temperature in closed container, comparatively a high rate of survival of probiotic microorganism *Lactobacillus acidophilus* was observed at 4°C. The obtained result was in accordance with that of the Chen et al. (2008) where storing the product at 4° and 25°C in laminated pouch and glass bottle, the best result was obtained when the product is stored at 4°C in glass bottle. In accordance to the Selvi et al., 2013, The total plate count showed slight elevation on storage and ranged between 2.4 to 3.4 * $10^{4,}$ 0.7 to 1.2 * 10^{4} at the end of the storage period of 180 days.

The laddu incorporated with microencapsulated *Lactobacillus acidophilus* were also analysed at both the storage conditions. The analysis of total colony count was done by studying the release kinetics of encapsulated bacteria at these storage conditions and it is represented in the Fig. 6. The initial count of bacteria in carrier incorporated laddu was found to be 9.97 ± 0.03 log cfu/g. The study on release kinetics of encapsulated bacteria revealed that the initial viable colony count of 9.97 ± 0.03 log cfu/g in laddu gradually declined and reached a value of 7.43 ± 0.02 log cfu/g at 4°C and 6.97 ± 0.02 log cfu/g at room temperature. The result obtained is in concordance with Talebzadeh and Sharifan 2016 where alginate-chitosan encapsulated *Lactobacillus acidophilus* showed better viable number of bacteria at 7°C when compared to 25°C in probiotic jellies. The total colony count obtained for the microencapsulated laddu was far higher than that of the laddu incorporated only with lyophilised *Lactobacillus acidophilus.* The result revealed that encapsulation of *Lactobacillus acidophilus* using gum arabic before lyophilisation provides protection of the organism during the storage period. It is inferred that the total amount of

bacteria retained in the laddu can be protected by microencapsulation before lyophilisation. Thus, instead of simple lyophilisation microencapsulation of *Lactobacillus acidophilus* before lyophilisation could aid in the protection of bacteria from various stresses during the storage period and could possibly increase the life span of incorporated probiotics. The number of free cells were $6.4 * 10^7$ at first and $4.6 * 10^4$ after last day. Encapsulated were $6.8 * 10^7$ at first and $5.7 * 10^5$ after last day Microencapsulation plays protective role and the bacterial strains is survive longer than the free coating cells. (Farzad, 2020)

3.10. Antioxidant activity

The result of formulated probioticated laddu was analysed for various antioxidant capacity. DPPH radical activity revealed that probiotication of laddu showed moderate capacity of capturing hydrogen free radicals were listed in Table 7. The different formulation of probiotication was performed in which encapsulated laddu predicted the highest percentage of antioxidant potential of 25.39 ± 0.47% followed by curd incorporated and Lyophilized probiotics. Hydrogen free radicals might be captured in highest content by slow release of encapsulation process as well as phenolic substances present in the bacteria and foxtail millet. Ferric reducing anion power assay has carried out to identify the potential of reducing capacity of radical ions in to neutral. The similar trend was also obtained for FRAP assay also compared to other formulation. Highest absorption of reducing capacity was exhibited in encapsulated probiotic laddu as 23.78 ± 0.43%. H_2O_2 assay was performed to analyse the capturing ability of hydroxyl ions in which curd incorporated laddu showed much potential followed by encapsulated and lyophilized probiotics. Total phenolic content of all the formulations were studied to predict the correlation among them. There was a strong significant correlation was observed in antioxidant capacity as well as phenolic substances in the laddu which indicates the phenolic content is much responsible for producing antioxidant activity. Since the laddu is an eatable, the main focus is towards the taste. Therefore all the ingredients were analysed to test their effect on the taste using two factorial interaction method of design expert software. The products gave a good impression which was inferred from the experimental response values. Comparison between the groups of different laddu formulations with respect to antioxidant activity was represented in Table 8. This is in parallel relationship with the study done by Aboulfazli et al., 2015 where the sensory analysis test was based on the attributes such as color, texture, flavour and taste and none of the product was reported as poor and all the products had a good impact on the review panel. Similarly Savita et al., 2020 has shown that major free phenolic acid among vanillic, ferulic, gallic and caffeic acid is protocatechuic acid (45 mg/100 g) Ofosu et al., 2020 reported that among the millet grains evaluated, barnyard and finger Italian millet exhibited the highest DPPH radical scavenging activity of 359.6 and 436.25 µg/mL respectively.

Table 7
Antioxidant activity of various formulation of probioticated laddu

Antioxidant assays	Control	Foxtail Millet	Probiotic	Lyophilized probiotic Laddu	Probiotic Encapsulated Laddu	Curd incorporated Laddu	Mean	SD
% Inhibition								
DPPH	8.23 ± 0.43	12.34 ± 0.18	9.34 ± 0.24	15.45 ± 0.23	30.12 ± 0.87	17.12 ± 0.63	15.43	7.96
FRAP	6.23 ± 0.12	6.59 ± 0.22	12.73 ± 1.3	22.12 ± 0.12	23.78 ± 0.43	18.38 ± 0.54	14.97	7.64
H_2O_2	11.13 ± 0.3	13.17 ± 0.67	12.34 ± 0.366	18.24 ± 0.32	25.39 ± 0.47	27.12 ± 0.21	17.89	6.93
Mean	8.53	10.7	11.47	18.6	26.43	20.87	16.1	
SD	2.46	3.58	1.85	3.34	3.29	5.44		7.19
N	3	3	3	3	3	3		
Total phenolic (µg)	120	140	135	134	178	123	138.33	20.86

Table 8
Comparison between the groups of different laddu formulations with respect to antioxidant activity

Samples		Mean difference
Control	Foxtail Millet	2.17
Control	Probiotic	2.94
Control	Lyophilized probiotic	10.07
Control	Probiotic Encapsulated	17.9
Control	Curd incorporated laddu	12.3
Foxtail Millet	Probiotic	0.77
Foxtail Millet	Lyophilized probiotic	7.9
Foxtail Millet	Probiotic Encapsulated	15.73
Foxtail Millet	Curd incorporated laddu	10.17
Probiotic	Lyophilized probiotic	7.13
Probiotic	Probiotic Encapsulated	7.82
Probiotic	Curd incorporated laddu	2.27
Lyophilized probiotic	Probiotic Encapsulated	7.82
Lyophilized probiotic	Curd incorporated laddu	2.27
Probiotic Encapsulated	Curd incorporated laddu	5.55

During the storage period, the laddus incorporated with curd showed least stability and are highly susceptible to contamination as they developed white patches on the surface during both the storage conditions. The white patches might have been developed due to the increased proliferation of microorganisms during the storage period. Since the curd incorporated laddus contains more colony forming units of microorganisms than the recommended dose of probiotics, they are further not used as the probiotic food as they might affect the consumers. This was similar to the study by Yuvarani and Anitha, 2016. The cooked multigrain ladoo contains 618 µg/100g of antioxidant content. The raw ingredients which were used to prepare multigrain ladoo and their antioxidant level are as follows; finger millet 268 µg/100g, foxtail millet 340 µg/100g, wheat 197 µg/100g, cardamom powder 182 µg/100g, ghee 80.1 µg/100g, horse gram sprouted and green gram sprouted together 662 µg/100g, jaggery 115 µg/100g.

4. Conclusions

Foxtail millet and Lactobacillus acidophilus have a very good potential have a synergistic beneficial effect to serve as a wholesome food. As the millet is rich in the fibre, catechin and quercetin it controls cholesterol and blood sugar level and also boosts the function of the liver and kidney. The probiotic bacteria incorporated foxtail millet has a good potential to serve as a functional food. Thus, the Probiotic millet laddu might serve as a value-added food product for the consumption of public and might improve the general health of the public. The physical evaluation has done with the formulated food product, and it showed a prominent softness, appearance, taste and solubility at

7.769, 5.742, 5.729, 5.583, respectively. It has exhibited that better physical evaluation was attained at a concentration of 42.99g foxtail millet, 37.49 g of groundnut, 10.30g of horse gram and 60.03g of jaggery. It was identified that the four variables demonstrated a notable effect on the formation of antioxidant-rich probiotic laddu using foxtail millet and has noticed positive relationships with all the variables. The mathematical model with an R^2 value of (0.998) and P-value of (< 0.0001) had obtained, which indicated a better agreement between the actual and predicted values of probiotic laddu physical evaluation and confirmed a significant interpretation of the mathematical model. The microencapsulated *L. acidophilus* incorporated laddu indicated the eminent stability of 7.43 ± 0.02 log cfu/g at 4°C than other methods of probiotic addition. The DPPH radical activity affirmed the potential antioxidant activity of 25.39 ± 0.4 % at microencapsulation method. Thus the present study concluded that it has more potential to use in the traditional food products. It can be produced in large scale for commercial sales as probiotic snack which also abides to the current trend of health-conscious society of switching over to balanced nutritional food diet.

Declarations

Further Studies:

More stability and more traditional recipes need to be studied using the different millet and cereals to meet the challenges of modern industries. This study can be carried out with a different ingredients combinations. Optimization using nutritional and stability studies using RSM need to be done for further improvement. Assessment of the product should be studied for the longer duration of time with more sample size.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Figures

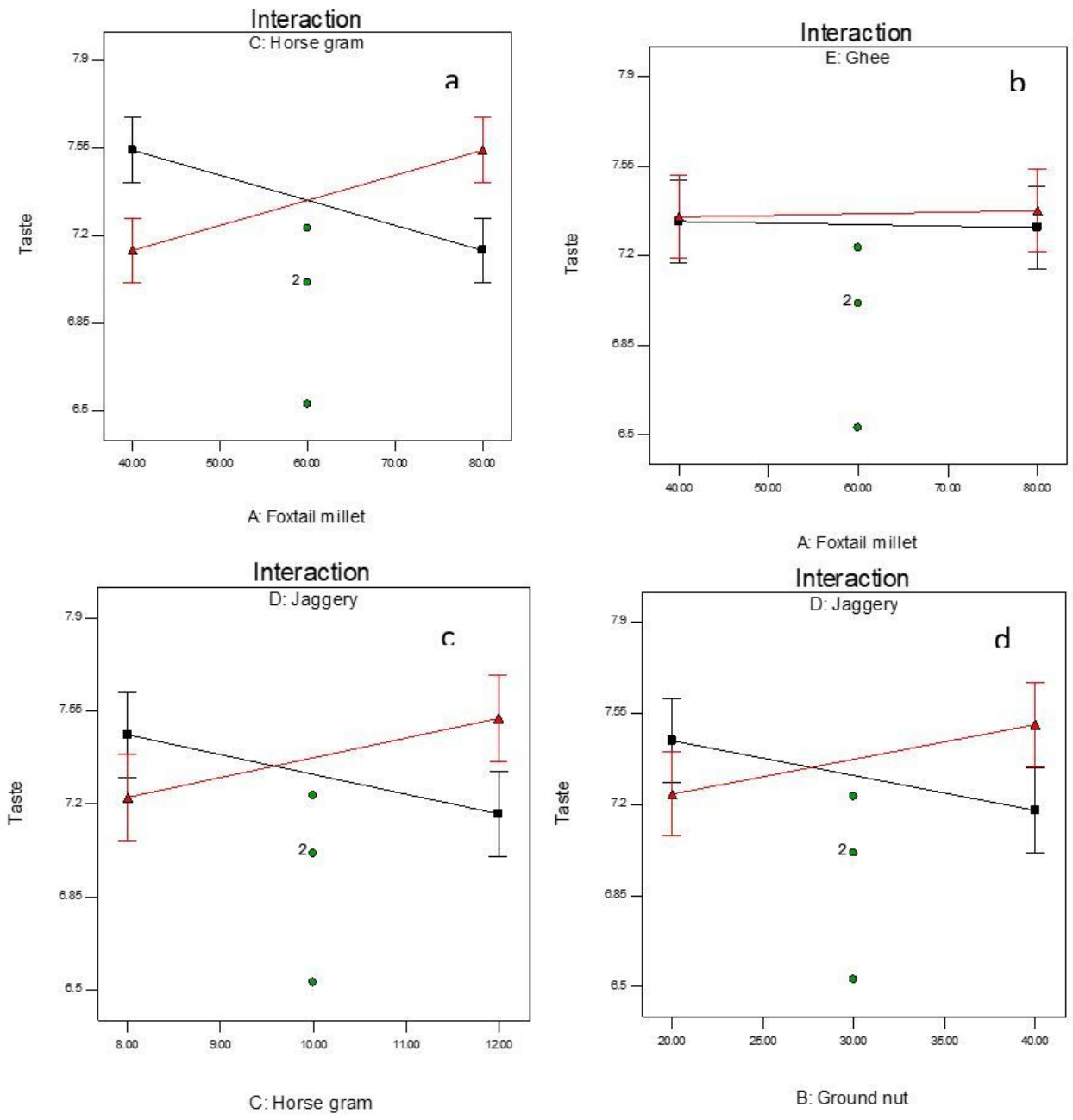

Figure 1

Interaction of Various ingredients against the Response factor by two factorial Interactions a) foxtail millet and horse gram. b) ghee and foxtail millet c) jaggery and horse gram d) jaggery and ground nut

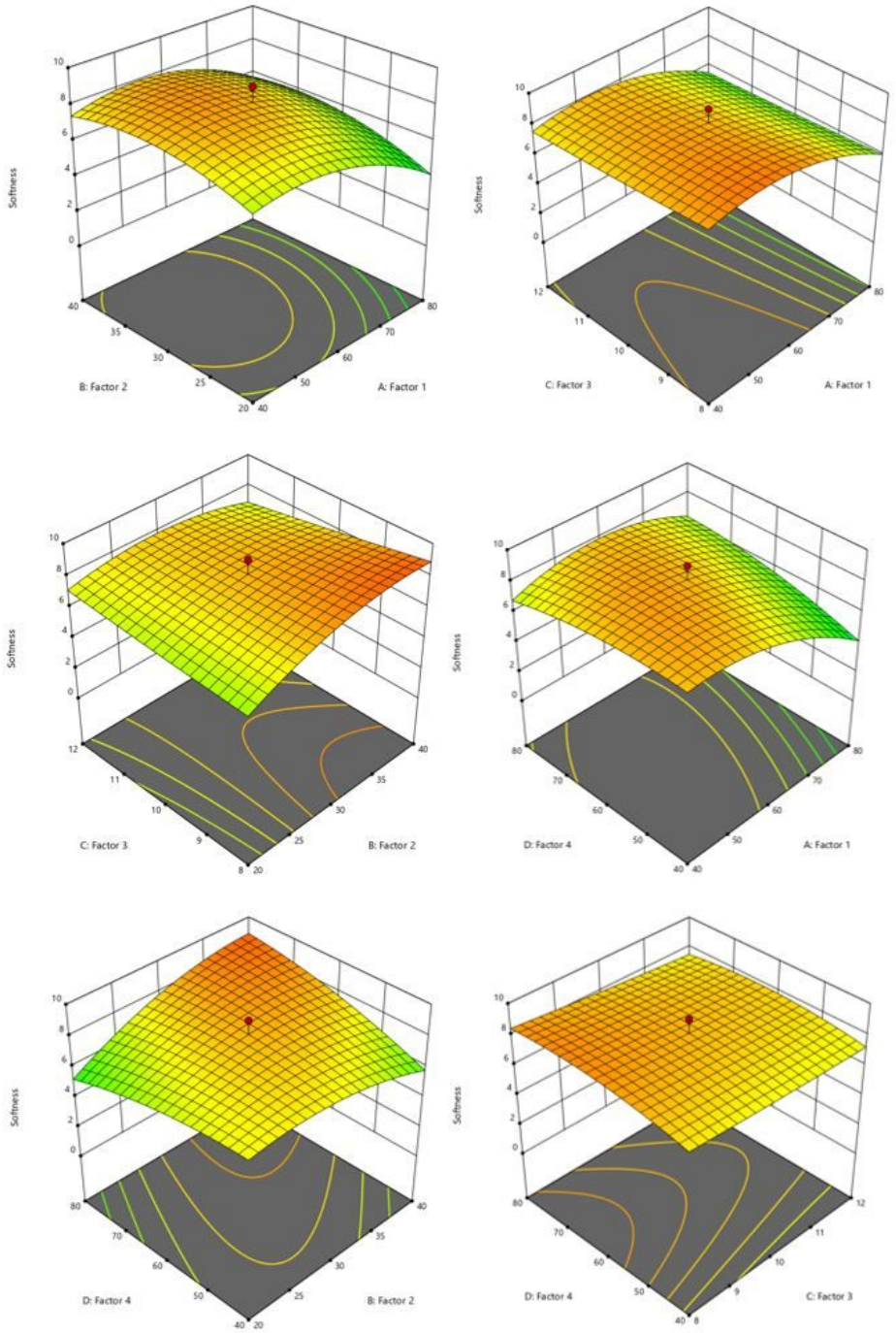

Figure 2

Response surface plot showing the effects on softness of probiotic millet laddus

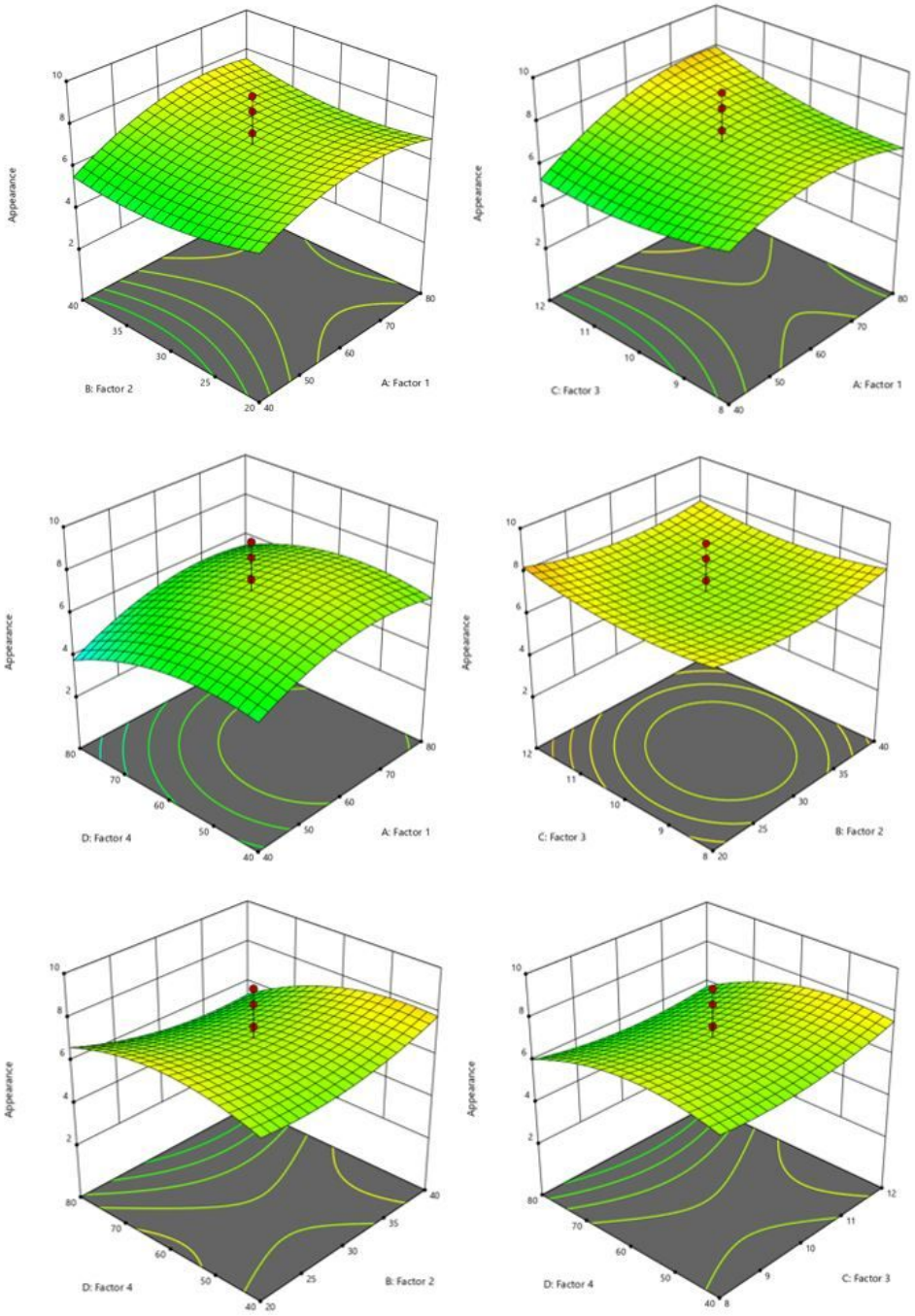

Figure 3

Response surface plot showing the effects on appearence of probiotic millet laddus

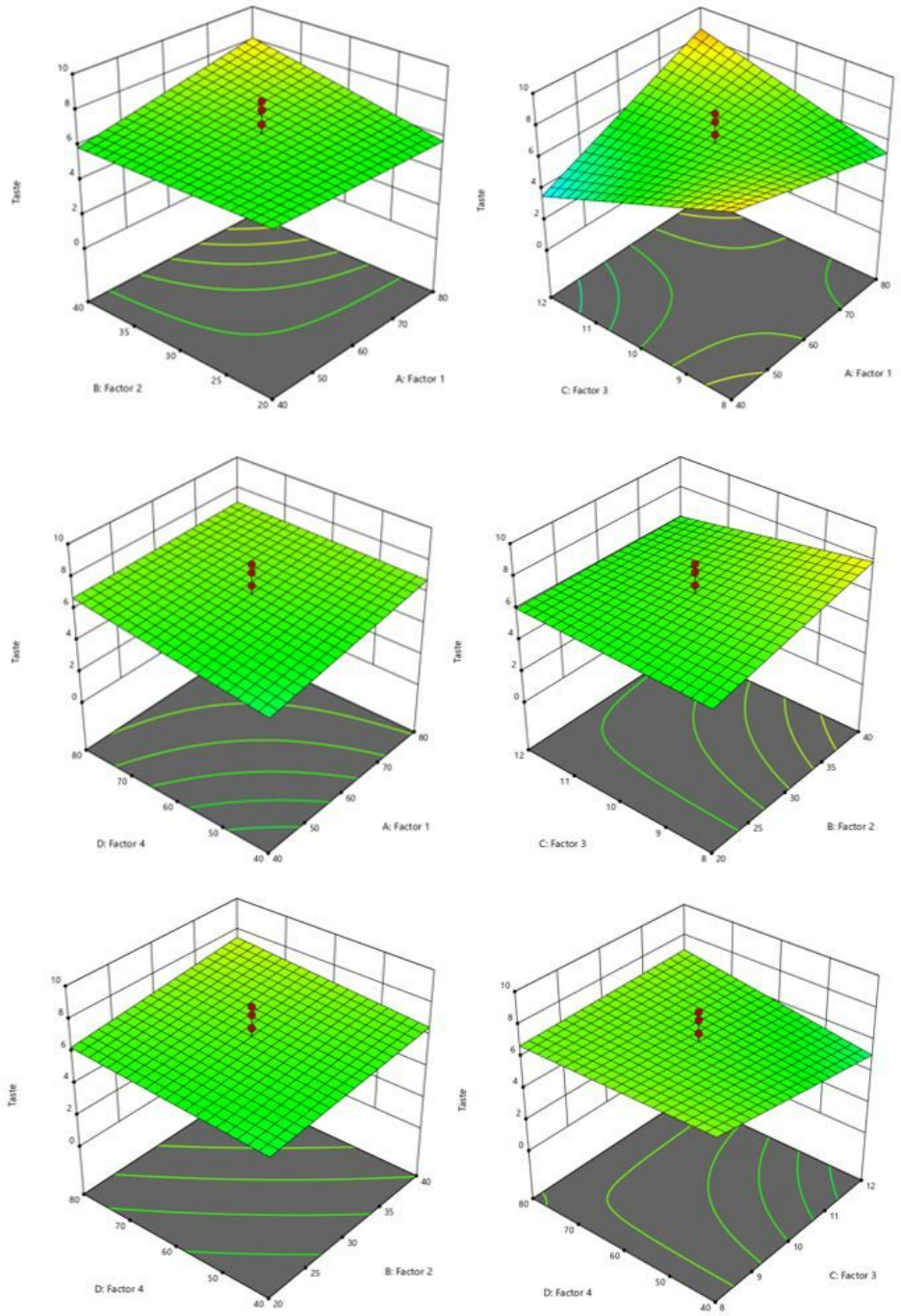

Figure 4

Response surface plot showing the effects on taste of probiotic millet laddus

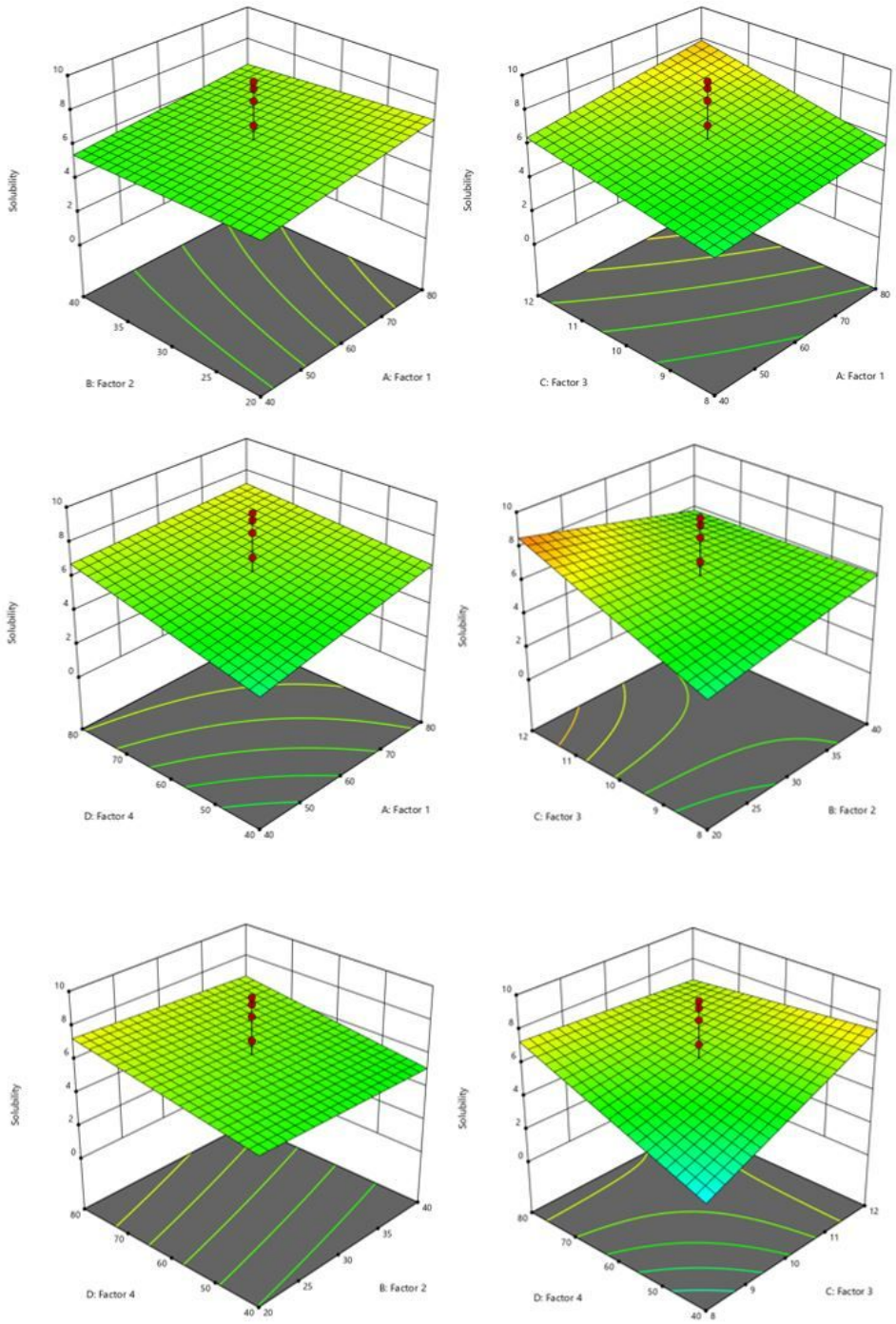

Figure 5

Response surface plot showing the effects on solubility of probiotic millet laddus

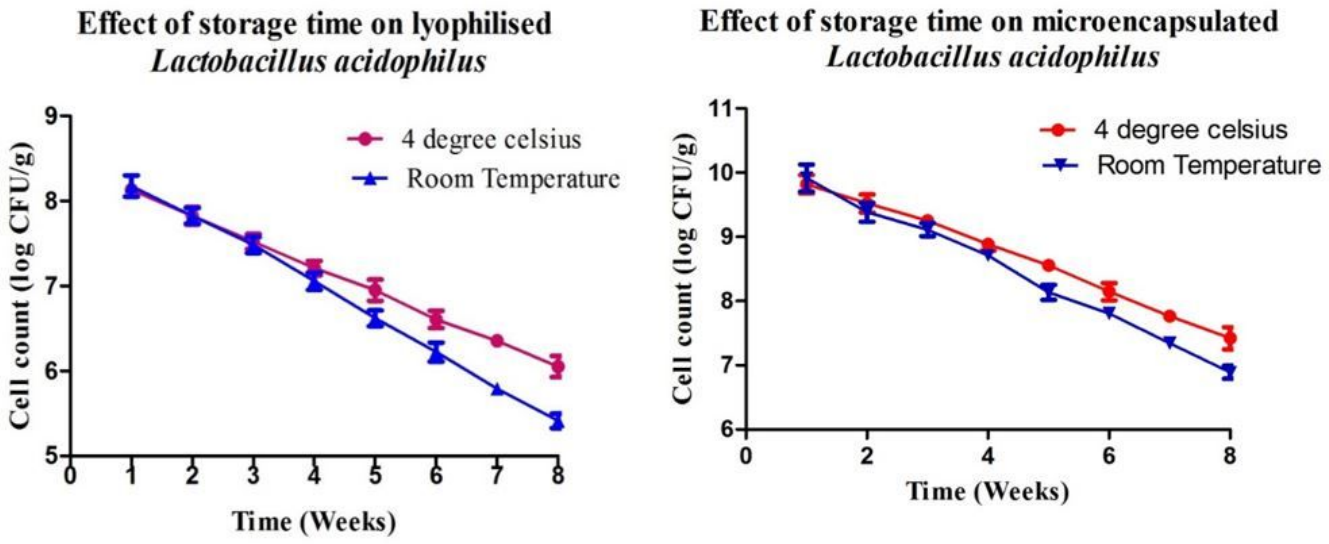

Figure 6

Effect of storage time on a) lyophilised *Lactobacillus acidophilus* incorporated laddu b) microencapsulated *Lactobacillus acidophilus* incorporated laddu

Figure 7

Different formulations of millet laddus for evaluation of nutritional and stability analysis.