Research Article

Categorizing Functional Yoghurt Using Artificial Neural Network

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ABSTRACT

Yoghurt was supplemented with low molecular weight carbohydrates (LMWC) extracted from *Syzygium cumini* seeds. Total soluble solids, pH, color, titratable acidity, texture, sensory and shelf life studies were quantified in control and functional- F1 (1% LMWC) and F2 (5% LMWC) yoghurts over a period of 15 days. An artificial neural network (ANN) was developed that could classify the yoghurts with color, pH and % carbohydrate as inputs. The ANN with one hidden layer in a feed forward pyramidal framework was trained using the gradient descent algorithm to reach an MSE (Mean of Squared Errors) of 0.055314. Of the total 120 data points, 30, 60 and 30 were randomly chosen for training, testing and prediction. The ANN could classify the yoghurts with 100% efficiency (r = 0.95). This study presented a minimally invasive approach that can classify functional food products on the basis of physical and chemical properties to determine user acceptability.

Key words: Artificial Neural Network, Jamun, LMWC, Yoghurt, Sensory studies.

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INTRODUCTION

Yoghurt is the oldest known fermentable milk product with a high acceptability around the world and was therefore chosen as an affordable carrier for prebiotics. It is defined as a fermented milk product specifically characterized by the presence of the symbiotic starter cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus*.[1] Regular consumption of low-fat yoghurt can help reduce the risk of developing severe health problems like diabetes.[2] It is a rich source of lactose and casein, micronutrients like potassium, zinc, phosphorous, calcium, magnesium, vitamin A, B12, B2 and several fatty acids.[3] Fermentation by beneficial bacteria results in value addition due to production of compounds like vitamin K.[4] In the present study yoghurt has been supplemented with low molecular weight carbohydrates (LMWC) extracted from seeds of *Syzygium cumini* (Jamun). These include all those carbohydrate fractions that have a maximum molecular weight of 3500 Daltons and generally consist of monosaccharides, disaccharides, oligosaccharides (kestose, nystose, fructosylnystose, etc), polyols, uronic acid, aldols, etc. These fractions have been shown to be excellent prebiotics.[5,6] Prebiotics are defined as selectively fermentable ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health.[7] In this study, Jamun seed LMWC extracts (JSL) were added to yoghurt at 1% (F1) and 5% (F2) concentration.

After the addition of prebiotics, the yoghurt can be defined as a synbiotic since it already consists of probiotics like *Lactobacillus* and *Streptococcus*. The probiotic strains generally used in synbiotic formulations include *Lactobacilli, Bifidobacteria* spp, *S. boulardii*, *B. coagulans* etc., while the prebiotics include oligosaccharides like fructooligosaccharide (FOS), galactooligosaccharides (GOS), xyloseoligosaccharide (XOS) and polysaccharides like inulin, resistant starch, etc. The health benefits claimed by synbiotics consumption include increased probiotic counts and therefore a balanced gut microbiota, enhanced immunomodulation,

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prevention of bacterial translocation, reduced incidence of nosocomial infections, etc.[8]

Several factors like pH, H_2O_2, organic acids, oxygen, moisture stress etc. have been claimed to affect the viability of probiotics especially in dairy products like yoghurts.[9] Since the prebiotics in yoghurt will selectively promote the growth of probiotics, the physical and chemical properties of the yoghurt will be affected according to the increasing metabolic activity of the beneficial bacteria. Mac Bean[10] stated that the shelf life of yogurt products is determined by the time the product remains safe to eat, its functional claims remain true and the time its sensory properties remain acceptable to consumers. Correspondingly, in this study shelf-life, sensory and quality attributes of the functional yoghurts - F1 and F2 were analyzed for a period of 15 days in comparison with a control sample.

A neural network was designed that could predict the type of yoghurt on the basis of three inputs – pH, color (L, a*, b*) and predefined carbohydrate percentage. These inputs were chosen as they chiefly affected the difference in the prebiotic concentration and therefore probiotic composition of yoghurts. Artificial Neural Networks (ANN) are powerful tools that allow us to understand non-linear relationships between independent and dependent variables by using simple processing elements. They imitate the biological neural networks (BNN) in a way that signals are transferred from one perceptron (rudimentary artificial neuron) to another and the process can be repeated many times. Every signal has an importance (weight) on the basis of which they make a stronger or weaker connection (output). A mathematical model is developed if a suitable algorithm can calculate this weight and establish a bias (threshold value) to decide the output.

Artificial Neural Networks have been used for various applications in food science like determination of authenticity of low-fat yoghurts[11] and predicting the acceptability of ice-cream variants.[12] For a classification task such as the one aimed in this study, a pre-defined model (unlike BNN where neurons can make or break new connections) was used to calculate the difference between (error, E) the networks' expected and generated output. If a trained model with lowest error is also the best one for validation and testing, one can assume that it is a good model for future forecasting. In this work, the ANN was built based on simple input parameters to classify functional yoghurts with the least error possible. Information of functional yoghurts incorporated with JSL and their identification using non-destructive methods is not available in literature. Therefore, the objectives of this work were to provide an extensive shelf-life study on the functional yoghurts and investigate the efficiency of ANN to predict the total user acceptance of the product. Results from this research are of paramount importance to dairy industries because development of new products is driven by user acceptance. The procedures described here can be used as a basis for preparation and identification of functional products that promise maximal user preference.

MATERIALS AND METHODS

Preparation of yoghurts

For preparing yoghurt, toned milk was collected from Aavin, Chennai with a composition of fat 3 g, solid not fat 8.5 g, protein 3.2 g and carbohydrate 4.7 g (according to manufacturer description). The bacterial strains *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were purchased from MTCC (IMTECH, India) and their cultures were maintained to achieve a bacterial count of 10^6 CFU/mL. The cultures were activated at 42°C for 15 min before use. Milk was heated to 90°C for 15 min and then cooled to 30°C and poured into 50 mL containers inside a laminar flow chamber.

Jamun seeds were separated, washed and dried in a hot air oven till the seeds attained a constant weight. They were powdered to a particle size of 0.6 mm and extracted with 50 % ethanol-water mixture as solvent for a period of 83-84 h. The extracts were lyophilized and stored as powders until further use.[13] Yield was calculated as follows:

Yield (% fresh weight) = (weight of lyophilized powder/ weight of dried seed powder) * 100

To prepare functional yoghurts, JSL extracts were added to milk at a concentration of 0.4 g (F1) and 2 g (F2) representing 1 % and 5 % concentrations respectively. Milk was homogenized and 1.5% (g / 100 mL) by weight of bacterial cultures was added and incubated at 30°C for 4–6 h. After the formation of a firm coagulum, yoghurt was cooled and stored under refrigeration conditions (4–7°C) until further studies. All samples of yoghurts were prepared to provide enough sample volume for further tests to be performed in triplicates.

Analysis of yogurts

The first hour in which milk samples were completely curdled was considered as the start of day 1.

Proximate Analysis

Moisture, ash and crude fiber were determined according to AOAC.[14] Crude protein was estimated by using micro-kjeldahl method using the factor 6.25

for converting nitrogen content into crude protein. Determination of milk fat in yoghurt was done by Rose-Gottlieb method.[15] Amount of carbohydrates was calculated as the difference between 100 and the sum of all other proximate components[14] (AOAC, 1995).

Microbial Tests

Broths and agars used in the study were prepared according to manufacturers' (Himedia, India) instructions.

Total plate count otherwise called as total viable count was done to check the microbiological quality of yoghurts. Serial dilution was done in peptone water and the 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} dilutions were selected and plated on plate count agar and mixed properly so that the colonies can grow individually. The plates were incubated at 37°C for 24 h and calculation was done as follows:

$$\text{Plate count (CFU/g)} = \frac{\text{No. of colonies x Dilution factor}}{\text{Volume of Sample plated (0.1 ml)}}$$

To test the presence of *Escherichia coli* (EC) in yoghurt, 10 mL of EC broth was prepared in a test tube. A Durham's tube was placed in the test tube. One mL of the sample was inoculated into the test tube and incubated at 45°C for 24 h. After incubation, when gas production was observed, a loopful of colonies from EC broth were streaked on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h.

To test for the presence of coliforms MacConkey broth was prepared and 1 mL of sample was added and incubated at 37°C for 24 h. A Durham's tube was kept in the test tube to check for gas production. Ten µL from MacConkey broth was added to Brilliant green bile lactose broth (BGLP broth) and the tubes were incubated at 37°C for 24 h. This was followed by streaking 100 µL of sample from BGLP broth on MacConkey agar. To test for presence of yeasts and molds, diluted sample was plated on yeast glucose chloramphenicol agar. The plates were incubated at 25°C for 5 days and enumerated.

Sensory Study

The yoghurts were evaluated by trained panelists for sensory characteristics at the Food Analysis Laboratory, Center for Food Technology, Anna University, Chennai. In order to test panelists in first training session, three coded samples were given in which two of them were alike and one was different. Those who could recognize the difference were chosen for the sensory evaluation of yoghurts. Five 30-min training sessions were held over a period of 1 month. In these sessions, definition of attributes and assessment technique were introduced and sample evaluation was done practically.[12] Finally, 20 panelists, 11 females and 9 males, all between the ages of 20 and 24 were selected. All samples were served in 50 mL lidded plastic containers and evaluation was done at 29 ± 1°C in plain view under white lights. Water was provided to cleanse the palate in between samples. Sensory evaluations of appearance, flavor, taste, color and texture and total acceptance were performed using the 9-point scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Four panel sessions were established and two or three samples were assessed in each one. The samples were presented in triplicates and in random order.

Neural Network

Artificial neural networks (ANN) are mathematical models that mimic the learning and prediction ability of the human nervous system. Each element of the ANN is named an artificial neuron and many such neurons constitute the network architecture in three interconnected layers – input, hidden and output. The input and output data were normalized using the Microsoft Excel Add-in 4CastXL to obtain the values within the range of 0 to 1 that represent the actual values between minimum and maximum, respectively.

A feed forward network was used where the calculations and analyses move in forward direction only. Each neuron in a layer collects a summated signal from the previous layer of neurons based on weights and bias. Simultaneously, weights are also assigned to represent relative importance or prejudice of each input. The net input is calculated according to Eq. 1, where y_k represents the net input to node k, N is number of nodes, $w_{k,j}$ are associated weights of each node and b_k is bias associated at node k. Only when the weighted sum of the inputs is more than an arbitrary threshold Θ (theta), the output is set as 1 (Eq. 2).

To convert the final summated input variable to the corresponding output, a transfer function was chosen based on type of internal activation. Internal activation is a binary representation of the neuron firing. It defines the corresponding output at each node for its input or a set of inputs. A logistic sigmoid function with values from range 0 to 1 was used in this ANN (Eq. 3).

$$y_k = f[\Sigma_{j=1}^{N}(x_j w_{k,j}) + b_k] \quad [1]$$

y_k = net input to node k

N = number of nodes

= value at each node

$w_{k,j}$=associated weights of each node

b_k = bias associated at node k

$y = 1$ if $\sum_{i=0}^{n} x_i * w_i \geq 0$ [2]

$y = 0$ if $\sum_{i=0}^{n} x_i * w_i < 0$

y = net output

$\sum_{i=0}^{n} x_i * w_i$ = total sum of weight at each node

$$f(x) = \frac{1}{1+e^{-\beta x}} \quad [3]$$

A multilayer perception network (Figure 2) was trained using the Gradient Descent iterative back propagation learning algorithm. It is a first order optimization algorithm and was used to find the local minimum of the function and quantify output of the neuron in the output layer. The weights and biases were adjusted to minimize the error in difference between predicted and observed values. Root mean square error (RMSE) was calculated to identify accuracy of network prediction. Model with the least RMSE was chosen. To validate the model, coefficient of correlation (r) was determined. A value of r closer to 1 indicated that the model has learned successfully.

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n} e_i^2}$$

where,

n = number of data points

e represents error for every i^{th} sample fed into the network

$$r^2 = 1 - \left(\frac{\sum_j (f_i - y_i)2}{\sum_j y_i^2}\right)$$

where f_i is the i^{th} predicted value and y_i is the i^{th} actual value

Hundred twenty data were collected, randomized and initially 40 were used for training, 40 for testing and 40 for prediction. This number was later varied to minimize error in activation transfer function.

Shelf-life Studies

The pH of the samples was measured using a digital potentiometer (IR 501A, Infra Digi, India). The electrode was directly dipped into homogenized samples and the reading was noted.

For measuring color, a Hunter Colorimeter (UltraScan VIS, USVIS1217, Hunter Lab, Reston, USA) was used that employs the three-dimensional scales CIE L a* b*, to quantify color values. This scale defines color as follows: L (lightness) axis: 0 to 100 (complete absorbance represented by black to complete reflectance represented by white); a* (red - green) axis: positive values are red, negative values are green and 0 is neutral; b* (yellow – blue) axis: positive values are yellow, negative values are blue and 0 is neutral (Table 1). The measurements were conducted under constant lighting conditions using reflectance mode at room temperature (25 ± 2°C) with white tile as control (L* = 98.76, a* = 0.04, b* = 2.01). All samples were placed in the sample holder and the reflectance was auto-recorded.

Total Milk Solids (TMS) was calculated as the difference between total solids (TS) and added sugar (AS). Total solids were determined by sodium hydroxide method (IS 12333: 1997), where volumetric determination of NaOH required to neutralize the acidity in sample was followed by maintaining the sample at 100 ± 2°C to allow evaporation and estimate the total solids. Sucrose content was determined by volumetric Lane – Eynon method.[16] Texture analyzer (TA.XT Plus, Stable Microsystems, Godalming, Surrey GU7 1YL, UK) was used to physically deform the test samples in a controlled manner and measures their response. The texture analysis was performed using the probe to make a 10 mm penetration with a speed of 5 mm/s. Firmness, consistency, cohesiveness and viscosity were determined by the software. The characteristics of the force response are as a result of the sample's mechanical properties, which correlate to specific sensory texture attributes. Cohesiveness was measured as the ratio of the positive force area during the second compression to that of the first compression.

The Sag test is done for the semisolids like yoghurt to test the gel strength. The samples are inverted directly on to a flat surface and checked for extent of deformation of the food sample.

Statistical Analysis

The significant differences in composition among the control and functional yoghurts were evaluated using one way ANOVA with Tukey's test using IBM SPSS 20.0 for Windows. The results were presented as mean ± SE. Values with $P \leq 0.05$ were considered as statistically significant at 5% level.

RESULTS

Addition of JSL to yoghurt conferred a grainy mouth feel that was described as user-perceived chewiness. A radar chart was used to display the attributes on a predefined scale to create a visual representation of the user response to the products. The graph helps in easy understanding of all parameters that are linked together (Figure 1).

The total plate count of the F2 yoghurt showed the highest bacterial count of 136 ± 11 (Table 1). This also describes the lowest pH in F2 (4.3 ± 0.02) due to the innate ability of the bacteria to convert lactose of milk to lactic acid. The variation in pH was prominent from day 1 to day 9 after which it remained steady till day

15 (Table 2). The table also shows increase in titratable acidity (TA) of all yoghurt variants from day 1 through day 13. In control, syneresis was observed on day 9 that resulted in complete separation of whey from gelled curd. Syneresis was observed in F1 yoghurt on day 11. No syneresis of any proportion was seen in F2 yoghurt up till day 15. The functional yoghurts were firmer than the control yoghurt (Table 3). This was also confirmed by the sag test (Figure 2).

The CIELAB scale is a uniform scale for mechanical approximation of color. The JSL had the characteristic purple to bluish color that was dulled to an extent after adding into yoghurt. Table 2 gives the color measurement output for each of the samples. There was a significant difference between Control, F1 and F2 yoghurts on any particular day, but the variation within each sample over the duration of study was very high, especially from day 9 (Table 2 and Figure 2).

To develop the ANN (Figure 3), 30 (25 %) results of the total data (120 experimental results) were randomly chosen for training and 60 (50 %) for testing/validation the model. Of the former, 12 were control, 10 F1 and 8 F2. The remaining 30 (25 %) results of the total data were used for prediction. The accuracy of prediction (confidence level) for Control (98.51 %) and F2 (96.18 %) was higher than that of F1 (87.14 %). The maximum deviation was obtained for F1 samples as shown in Figure 4b. Here, a scatter plot of target and predicted values of 18 randomly chosen (6 each from Control, F1 and F2) samples is shown where, 1, 0.5 and 0.1 represent Control, F2, F1 yoghurts respectively. To quantify the concurrence between predicted and expected values, correlation coefficient (r) was calculated as described previously. A value of 0.95 showed a high correlation between the values and therefore suitability of the model (Figure 4a).

DISCUSSION

There is an increasing interest in developing functional foods that provide benefits beyond basic nutrition. Prebiotics with plant origin that selectively improve the growth of probiotics are perceived as vital food ingredients. Seeds of *Syzygium cumini*, a traditional Indian plant known for its medicinal properties served as an excellent source of LMWC with a yield of 24%. Crude extracts from Jamun seeds introduce off-flavors and appalling mouths feel after consumption due to high amount secondary metabolites like tannins. Similar sensory attributes were reported by Monika *et al.*[17] when they incorporated crude jamun seed powder into noodles. The acceptability of noodles decreased with the increasing percentage of the seed powder, the least accepted being 10 % Jamun noodles. In the present study therefore isolated JSL were added to yoghurt in only 1 % and 5 % concentrations. Additional raise in concentration of the extracts was not done as the sensory studies reported a decreased user acceptability of F2 yoghurts (Figure 1). Further, there are reports that higher concentrations of polysaccharides can lead to separation of milk into two phases: a polysaccharide-enriched and casein-enriched phase.[18] Heat treatment of milk and the action of starter bacteria during yogurt production cause the breakdown of milk protein, leading to increased level of soluble proteins, free amino acids and non-protein nitrogen.[19]

Sensory Study

Sensory evaluation of foods represents interpretation of responses by the evaluator and has an impact on scaling up pilot samples to large-scale manufacturing. Analytical tests with a low sensory threshold may be ineffective in determining the presence of disagreeable flavors. In general, substitution with prebiotic ingredients has a greater influence on texture and aroma, whereas substitution with probiotic products has a greater effect on flavor and aroma. When a prebiotic ingredient is incorporated into the product matrix it enforces the existent bonding between different components of the food and sometimes substitutes for fat that may result in softness and creaminess of the food. Furthermore in the latter, metabolic end products of an added probiotic culture can sometimes result in the so-called probiotic off-flavor. *Bifidobacterium* species for example, produce acetic acid as a by-product of their metabolism that adds a vinegary flavor to the product affecting its sensory assessments.[20] Many studies reported that addition of prebiotic changes the sensory attributes of yogurt such as aroma, taste and mouth-feel.[21-23]Appearance reflects visual perception of the end user that may/may not stimulate appetite. A positive response from end user may result if the food product they see is better than or similar to what they might have perceived in mind. The three esters that produce the characteristic flavor perceived by olfactory receptors in *Syzygium cumini* are dihydrocarvyl acetate, geranyl butyrate and terpinyl valerate.[24] The puckering effect of Jamun fruit on tongue and gums due to the high amount of tannins could have reduced the taste score of F2 yoghurt considerably in comparison to the other two samples. Texture is the most vital attribute of a food and is dependent on mouth-feel, consistency, firmness, viscosity and chewiness. It creates a perceptible image in the mind of the consumer for the next eat. From these

sensory studies, we could conclude that F1 yoghurts had the highest acceptability at 8.6 ± 0.09 on a 9-point hedonic scale (Figure 1).

Shelf-life studies

The rationale for preparing such a formulation was to harness the probiotic properties of bacterial strains for a longer time as their sustenance could be met by LMWC in the mixture. Low digestible carbohydrates like palatinose, inulin and α-cyclodextrin have been shown to increase the count and antibacterial activity of *Lactobacillus* spp. and *Lactococcus* spp. when used to create synbiotic yogurt.[25,26] Viability of *L. casei* was improved when fat free plain yoghurt was supplemented with inulin.[27] Capela *et al*.[28] recorded similar observations with starter cultures of *L. acidophilus* and *B. longum* when whole milk yoghurt was incorporated with FOS. Our study corroborates these findings.

The increase in fiber, ash and carbohydrate content of F2 yoghurt can be attributed to the added JSL (Table 1). It has been reported[29] that TA of yogurt stored under refrigeration conditions increased significantly with increasing storage period. Such an increase was highest in F2 yoghurt especially by day 13 at 0.89 ± 0.07 owing to a higher LMWC concentration that can support higher bacterial populations. Fermentation is generally monitored by measuring pH as a function of time. From the results described here, it can be suggested that the time taken for yoghurt formation can be decreased by addition of suitable prebiotics in milk that could be financially advantageous to manufacturers. The steady state of pH from day 9 to day 15 suggests a bacteriostatic condition in yoghurt either due to depletion of metabolizable nutrients or increased acidity due to bacterial activity.

Since the JSL were added as lyophilized powders, they were able to efficiently absorb moisture content in F1 and F2 yoghurts in comparison to control. The specific rheological and 3D textural properties of yoghurt are a result of the aggregation of casein micelles and denatured whey proteins via hydrophobic and electrostatic bonds. It behaves as a weak gel that shows a time-dependent and shear-thinning flow behavior.[30] A good quality yogurt is characterized by strong curd integrity without shrinkage and whey separation. Yogurt gel contraction and innate instability of protein gel causes the production of free whey during storage. Absence of syneresis in F2 yoghurt could be due to the significantly higher protein contents in F1 (4.0 ± 0.28) and F2 (4.3 ± 0.24) when compared to control (3.8 ± 0.14).

Milk solids (TMS) are the non-water components of milk and consist of casein, lactose and minerals. These are sometimes referred to as solids not fat (SNF) content and when the fat is included it is called total solids (TS) content. Though the values remained steady over the period of study, there was a significant increase on Day 7 in control (33.23 ± 0.47) and F1 (38.81 ± 0.18) yoghurts (Table 2). This could be an internal starting point for the start of syneresis in these samples. Reduction in water content due to syneresis could have proportionally increased the protein content in samples.[31] Visibly, there was no water seen in F2 yoghurts and there was only a marginal increase in TS. This could be due to binding effect of JSL (Figure 2).

There is a marked decrease in the firmness of control from Day 1 (64.20 ± 0.05) to Day 15 (48.33 ± 0.08). This could be attributed to the interaction between milk protein and the carbohydrates of the prebiotic that provided rigidity to the product. The cohesive ability of the yoghurt(s) to resist disintegration and hold together under mechanical pressure was decreased due to the addition of LMWC. This could have led to faster disintegration of the F2 yoghurts under pressure.

Neural Network

A neural network was developed to identify the F1 yoghurts from the lot as they had maximal user

Table 1: Analysis of yoghurts.

Sample	Moisture	Crude Protein	Crude Fat	Crude Fibre	Ash	Carbohydrate	TPC CFU/mL
Control	92 ± 0.18^{a}	3.8 ± 0.14^{q}	2.8 ± 0.14^{f}	0.2 ± 0.01^{t}	0.6 ± 0.04^{w}	0.4 ± 0.01^{c}	119 ± 12^{c}
F1	86 ± 0.36^{b}	4.0 ± 0.28^{r}	1.5 ± 0.23^{d}	0.9 ± 0.01^{g}	0.9 ± 0.05^{j}	6.7 ± 0.04^{d}	128 ± 9^{d}
F2	78 ± 0.47^{c}	4.3 ± 0.24^{d}	0.9 ± 0.01^{e}	1.4 ± 0.02^{k}	1.1 ± 0.04^{n}	14.3 ± 0.03^{r}	136 ± 11^{e}

Functional yoghurt with 1% LMWC (F1), Functional yoghurt with 5% LMWC (F2). TPC = Total Plate Count. Different superscripts are representing values in each column subjected to Tukey test that differ significantly at $P<0.05$

Table 2: Variation in pH, titratable acidity, color and total milk solids of the samples over a period of 15 days.

Day	Sample	pH	Titratable Acidity	Colour Specifications			Total Solids (%)
				L*	a*	b*	
1	Control	4.6 ± 0.05^{a}	0.72 ± 0.14^{a}	88.65 ± 0.13^{a}	-0.95 ± 0.02^{a}	9.14 ± 0.05^{a}	29.83 ± 0.26^{a}
	F1	4.5 ± 0.03^{c}	0.75 ± 0.12^{b}	85.66 ± 0.19^{b}	-0.19 ± 0.03^{d}	10.64 ± 0.06^{b}	37.32 ± 0.26^{b}
	F2	4.3 ± 0.02^{d}	0.77 ± 0.09^{ab}	80.49 ± 0.21^{c}	1.58 ± 0.04^{c}	10.68 ± 0.01^{f}	39.39 ± 0.14^{f}
3	Control	4.6 ± 0.10^{a}	0.72 ± 0.11^{a}	88.67 ± 0.20^{a}	-0.91 ± 0.01^{g}	9.03 ± 0.08^{b}	29.83 ± 0.55^{a}
	F1	4.5 ± 0.02^{c}	0.75 ± 0.09^{b}	86.13 ± 0.14^{b}	-0.20 ± 0.04^{d}	10.41 ± 0.04^{ab}	37.32 ± 0.29^{b}
	F2	4.3 ± 0.05^{d}	0.77 ± 0.14^{ab}	80.51 ± 0.17^{c}	1.51 ± 0.01^{ac}	10.62 ± 0.01^{f}	39.39 ± 0.18^{f}
5	Control	4.5 ± 0.01^{c}	0.72 ± 0.08^{a}	88.69 ± 0.23^{a}	-0.89 ± 0.01^{g}	9.01 ± 0.07^{b}	29.83 ± 0.24^{a}
	F1	4.4 ± 0.02^{ab}	0.76 ± 0.13^{b}	86.14 ± 0.17^{b}	-0.22 ± 0.02^{e}	10.21 ± 0.04^{bc}	37.32 ± 0.29^{b}
	F2	4.2 ± 0.05^{ce}	0.79 ± 0.11^{c}	80.53 ± 0.26^{c}	1.49 ± 0.01^{de}	10.51 ± 0.05^{d}	39.39 ± 0.34^{f}
7	Control	4.5 ± 0.01^{c}	0.78 ± 0.12^{ab}	88.71 ± 0.19a	-0.86 ± 0.02^{g}	8.97 ± 0.08^{b}	33.23 ± 0.47^{d}
	F1	4.4 ± 0.02^{ab}	0.76 ± 0.11^{b}	86.16 ± 0.22^{b}	-0.25 ± 0.03^{e}	10.12 ± 0.06^{e}	38.81 ± 0.18^{c}
	F2	4.2 ± 0.02^{ce}	0.79 ± 0.09^{c}	80.57 ± 0.27^{c}	1.47 ± 0.04^{de}	10.32 ± 0.06^{g}	41.95 ± 0.27^{ab}
9	Control	4.3 ± 0.05^{d}	0.80 ± 0.12^{c}	89.09 ± 0.17^{d}	-0.83 ± 0.04^{g}	8.17 ± 0.07^{c}	33.23 ± 0.59^{d}
	F1	4.2 ± 0.03^{ce}	0.82 ± 0.13^{ac}	86.90 ± 0.18^{b}	-0.27 ± 0.01^{e}	10.09 ± 0.05^{ae}	38.81 ± 0.47^{c}
	F2	4.0 ± 0.01^{b}	0.84 ± 0.11^{ac}	80.76 ± 0.21^{ab}	1.43 ± 0.02^{de}	10.29 ± 0.04^{g}	41.95 ± 0.18^{ab}
11	Control	4.3 ± 0.05^{d}	0.81 ± 0.10^{ac}	89.17 ± 0.15^{d}	-0.81 ± 0.01^{g}	8.11 ± 0.08^{c}	34.27 ± 0.37^{ae}
	F1	4.2 ± 0.01^{ce}	0.83 ± 0.09^{ac}	86.78 ± 0.19^{b}	-0.28 ± 0.04^{e}	10.01 ± 0.07^{bc}	39.28 ± 0.28^{f}
	F2	4.0 ± 0.03^{b}	0.88 ± 0.08^{d}	81.89 ± 0.22^{ac}	1.42 ± 0.02^{de}	10.24 ± 0.09^{g}	41.56 ± 0.36^{bc}
13	Control	4.2 ± 0.05^{ce}	0.83 ± 0.07^{ac}	89.22 ± 0.21^{d}	-0.81 ± 0.03^{g}	8.03 ± 0.07^{d}	34.27 ± 0.54^{ae}
	F1	4.1 ± 0.05^{b}	0.84 ± 0.04^{ac}	86.66 ± 0.17^{b}	-0.29 ± 0.01^{e}	9.89 ± 0.07^{bc}	39.28 ± 0.48^{f}
	F2	4.0 ± 0.05^{b}	0.89 ± 0.07^{d}	83.61 ± 0.16^{ed}	1.41 ± 0.04^{de}	10.21 ± 0.06^{g}	41.56 ± 0.41^{ab}

Functional yoghurt with 1% LMWC (F1), Functional yoghurt with 5% LMWC (F2). ΔL* = (L* sample - L* standard) = difference in lightness and darkness (+ = lighter, - = darker), Δa* = (a* sample - a* standard) = difference in red and green (+ = redder, - = greener), Δb* = (b* sample - b* standard) = difference in yellow and blue (+ = yellower, - = bluer). Mean ± Standard
Deviation (SD) values of triplicates. All the obtained data were subjected to univariate with Duncan's multiple range test. Values in a single column followed by the same superscripts are not significantly different at 0.05 LSD.

Table 3: Texture Attributes of the yoghurts on 1st, 7th and 15th day.

Day	Sample	Firmness (g)	Consistency (gsec)	Cohesiveness (g)	Index of viscocity (gsec)
1	Control	64.20 ± 0.05^{a}	3056.37 ± 0.24^{t}	-76.70 ± 0.19^{j}	-142.32 ± 0.02^{g}
	F1	84.49 ± 0.07^{b}	3929.48 ± 0.21^{y}	-84.53 ± 0.24^{j}	-131.17 ± 0.17^{t}
	F2	88.24 ± 0.12^{ab}	4131.10 ± 0.19^{ty}	-87.49 ± 0.17^{ji}	-113.16 ± 0.16^{gt}
7	Control	62.17 ± 0.14c	2239.20 ± 0.27^{u}	-72.04 ± 0.27^{v}	-139.36 ± 0.24^{ni}
	F1	82.68 ± 0.09^{d}	3852.42 ± 0.34^{h}	-79.76 ± 0.16^{x}	-129.50 ± 0.11^{nj}
	F2	86.94 ± 0.11^{cd}	4027.76 ± 0.24^{uh}	-82.10 ± 0.25^{vx}	-109.39 ± 0.16^{g}
15	Control	48.33 ± 0.08^{e}	1662.82 ± 0.17^{k}	-68.93 ± 0.18^{r}	-132.39 ± 0.17^{bi}
	F1	81.88 ± 0.21^{f}	3699.95 ± 0.28^{l}	-77.03 ± 0.27^{s}	-116.56 ± 0.19^{hi}
	F2	85.15 ± 0.19^{ef}	3959.24 ± 0.17^{kl}	-79.19 ± 0.13^{rs}	-93.64 ± 0.05^{nl}

Functional yoghurt with 1% LMWC (F1), Functional yoghurt with 5% LMWC (F2). All values in each column were subjected to Tukey test and differ significantly at $P<0.05$ and are represented with different supercripts.

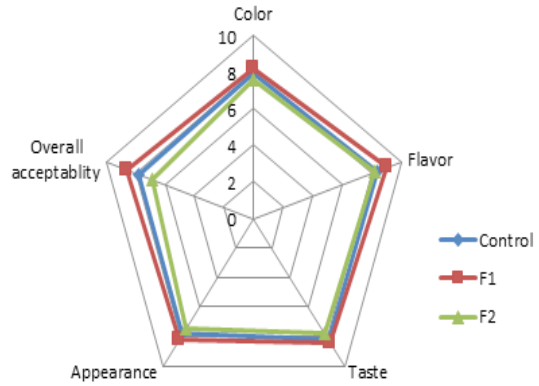


Figure 1: Sensory attributes of the yoghurt. Functional yoghurt with 1% LMWC (F1), Functional yoghurt with 5% LMWC (F2). User-defined sensory attributes on a 9 point hedonic scale.

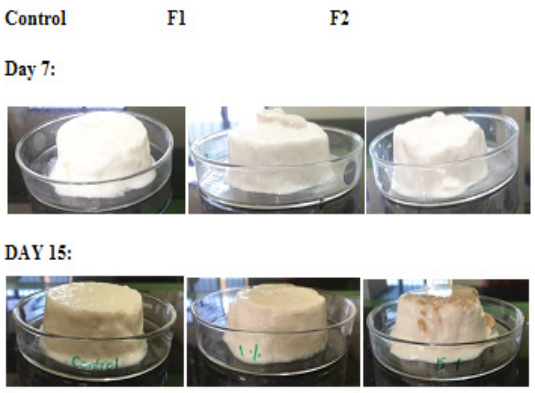


Figure 2: Stability of yoghurts.

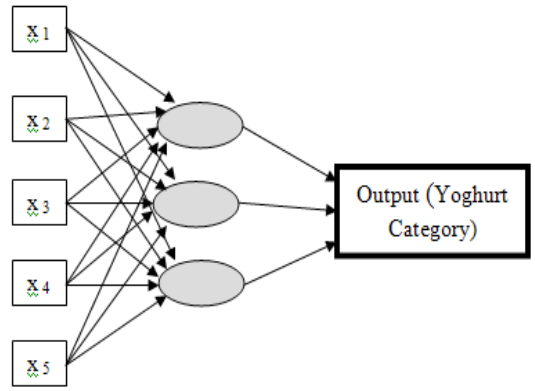
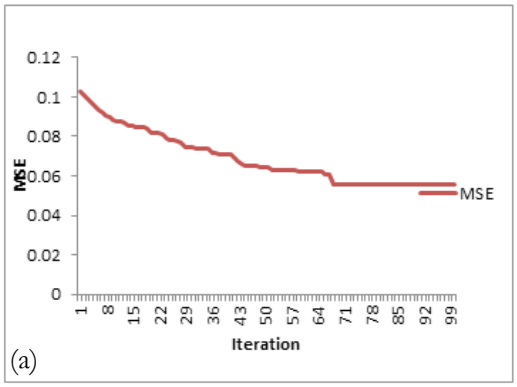


Figure 3: Multilayer model of the neural network used in the study.
A feed forward three-layer network where x1 = pH, x2 = L*, x3 = a*, x4 = b*, x5 = % carbohydrate.
represents the Hidden Layer with three nodes

acceptability. The Non-linear Activation Functions are the most used activation functions because linear functions do not help with the complex parameters of usual data that is fed to the neural networks. The



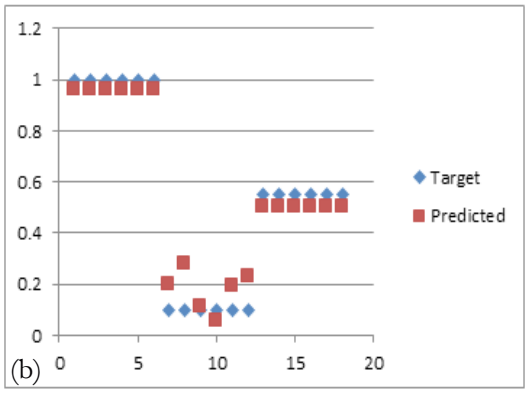


Figure 4: Suitability of the Network Model.
(a) Training mean square error (MSE) as a function of number of iterations;
(b) Target (actual) and the neural model prediction (predicted) of the yoghurt categories for a sample set. 0.1 = F1 yoghurt, 0.5 = F2 yoghurt, 1 = Control yoghurt.

network model should be suited to understand the range of acceptability / unacceptability. So, a non-linear sigmoidal function that predicts the output as a range from 0 to 1 was chosen to predict the type of yoghurt and therefore its acceptability. Every true input value is scaled by the software and is given an arbitrary value with 1 for highest and 0 for least in the range.

A non-recurrent multi-layer feed forward network having more than one weighted layer with all nodes in a layer connected with the nodes of the previous layers was developed. The signal will only flow in one direction from input to output without any feedback loop (Figure 3). A pyramidal scheme (5-3-1) was used to select the number of nodes for the hidden layer.[32] Initially, each connection has different weights upon them that are randomly assigned by the software. The set of weights that minimizes the error between predicted and a target output was identified by an algorithm that searches (training) over multiple discrete steps to reach a minimal loss function by trial and error method. In this study, gradient descent optimization algorithm was used to train the ANN. It calculates the

error (slope) and continues to do so by moving down along the slope till a minimum error level is reached. The weights of the connections are continuously changed during training until a point where the error is minimal whereby the performance of the model is at its best. The back apropagation algorithm enables changing of model parameters (or weights) on the basis of change in error. A set of improved internal model parameters is then established that perform well to reduce the mean squared error, the most popularly used error function.[33] With 100 epochs, the training method found a MSE of 0.055314 for 87 training pairs (Figure 4a). The prediction MSE was 0.055481. This showed that the accuracy of prediction of all yoghurts including F1 (87.14%) was well within the acceptable range of the network.

CONCLUSION

In the present study we were able to estimate the effect of incorporation of JSL on physical and chemical attributes of yoghurt over a period of two weeks. JSL pose as affordable sources of prebiotics that can be packaged in yoghurt medium to achieve maximal user acceptance. The F1 yoghurts showed highest user acceptability. The addition of JSL reduced the time required to achieve user acceptable pH and taste. The application of ANN to predict the type of yoghurt and therefore acceptance by user was established. The results show that a 5-3-1 pyramidal model could be a perfect fit for such a prediction (r = 0.95). pH, color and % carbohydrate have been shown as suitable inputs to achieve an ANN with lowest error. This study model could be applicable for categorizing commercially produced functional food products.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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