



Development, evaluation and optimization of Ber (*Ziziphus mauritiana* Lam.) based Kiwifruit jam

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Abstract

Response surface methodology (RSM) was used for the development and optimization of ber based kiwifruit jam, where the effect of kiwifruit pulp (90-100g/100g), lyophilized pulp (5-15g/100g) and sugar (50-65g/100g) on the various responses like sensory, total soluble solids, polyphenolic content, antioxidant activity and energy is studied. The physical chemical characteristics of mixed fruit jam is also determined. The optimum conditions obtained from response surfaces analysis was 100g kiwifruit pulp, 15g lyophilized ber and 65g sugar per 100 g. under these optimal conditions, the sensory score of 6.74 (9 point hedonic scale), total polyphenol of 14.87mg/100g, antioxidant capacity of IC50 value 36.16 mg/ml and high energy of 187.88 kCal/100g was obtained. The optimized mixed fruit jam was having a good antioxidant capacity that is helpful for those who are having a problem of motion sickness or sea sickness. The antioxidant rich jam can able to control these kind of stresses.

Keywords: antioxidants, RSM, lyophilised, optimization, IC50, Sea sickness

1. Introduction

Travel sickness, also known as motion sickness, is indeed not a life-threatening condition. But, of course, it has the power to spoil your journeys. As we all know, motion sickness is a very common illness caused by repeated unusual movements during travelling. Resulting from the imbalance between the body's sensory parts, motion sickness causes the symptom of feeling nausea, sweating and headaches, among others. In short, it is capable enough to ruin your trip, be it a short or long one. Similarly, seasickness, which is a form of motion sickness, is perhaps the most common sickness to hit any person while at sea. Seasickness is a condition wherein the motion sense of the vestibular system and the motion perceived visually do not concur, causing nausea, fatigue and dizziness etc. Seasickness is very common in first time passengers of any ships or boats or even ferries. This problem has roots in the signals to the brain and therefore should be dealt with great sense of care and interest. However, the problem lays in motion therefore ones the motion stops the patient slowly returns to normal.

Ziziphus species (Rhamnaceae) are widely used as medicine predominantly in Asian countries, particularly in Taiwan and China for the treatment of allergies, constipation, urinary troubles, depression, chronic bronchitis, insomnia and liver diseases (Li *et al.*, 2005). Indian jujube or ber (*Ziziphus mauritiana* Lam.) belongs to the family Rhamnaceae and considered to be an indigenous fruit crop of India. It is an economically important arid fruit tree, which is grown all over the drier parts of the country as it can withstand long periods of drought. Green kiwifruit (*Actinidia deliciosa*) is unusual in many aspects in comparison with other fruits, including its flavour, colour, aroma, shape and nutritional content (Ahmet *et al.* 2007; Nishiyama 2007). Kiwifruits possess high levels

of antioxidants (Szeto *et al.* 2002), vitamin C (Rasam and Laing 2005; Ahmet *et al.* 2007), and dietary fibre (Chan *et al.* 2007; Nishiyama 2007), and have been proposed to promote a wide range of health benefits (Rush *et al.* 2002; Duttaroy and Jørgensen 2004; Chan *et al.* 2007; Philpott *et al.* 2007). Few types of processed kiwifruit food products are commercially available to consumers. Kiwifruits are mainly eaten as whole fruits, because of technical issues such as nutrient and colour loss encountered in processing and storage (Lodge and Robertson 1990).

The fruits which are rich in antioxidants will help to control the stresses as mentioned earlier. Keeping these things in mind, the objectives were framed for development and optimization of antioxidant and polyphenol rich fruit jam by utilizing lyophilized ber and kiwifruit using response surface methodology (RSM).

2. Materials and Methods

2.1 Raw materials

2.1.1 Fruit

Kiwifruit (*Actinidia deliciosa* var. Hayward) and Indian jujube/Ber fruit (*Ziziphus mauritiana* Lam.) from the city of Mysuru is purchased from a local market. The ber fruit is cut into small pieces, grind and freeze dried. The outer peel of the fresh kiwifruit is peeled.

2.1.2 Sucrose, gelling agent and other ingredients

Food grade commercial sucrose was used to prepare jams. A sucrose syrup is prepared and added during jam preparation. Food grade carrageenan is used as a gelling agent. An artificial green food colour (Fast Green FCF – 80ppm), mixed fruit essence is also used to improve colour (as chlorophyll degrades on heating) and smell of jam respectively.

2.2 Formulation of Ber based Kiwifruit Jam

The ber based kiwi fruit jam was formulated by using lyophilised ber fruit powder and fresh kiwi fruits along with the other ingredients as follows:

Table 1: Developed formula for ber based kiwi fruit jam

Ingredients	Amount (g/100g)
Lyophilised Ber Fruit	Variable*
Kiwi fruit	Variable*
Sugar	Variable*
Water ^b	-
Citric acid	0.8
Carrageenan (Food grade)	0.9
Food Colour	80 ppm
Essence	Mixed Fruit

* Amount varied according to the experimental design (Table 2), b – water added approximately 100ml.

2.3 Jam preparation procedure

The procedure for preparation of jam is followed as described by the Indian quality norms (R. P. Srivastava and K. Sanjeev, 2002) with little modifications. The conventional method is used for preparing jam. Firstly, the kiwifruit pulp and lyophilised ber fruit powder is mixed in a different proportion with respect to the different runs of central composite design (Table 2), grinded with addition of required quantity of water (about 50ml) and start to heat by using a stainless steel utensil at medium flame. The sugar is added as a syrup for ease in mixing and stirred continuously. The carrageenan is first dissolved in water (about 50 ml) with little heat as it will become solidify quickly. Then the dissolved carrageenan is mixed well with the jam, green food colour and mixed fruit essence is also added at this stage. The final TSS is checked with a digital hand refractometer to be 65-68° Brix. The whole content is poured in to sterilized glass bottle and kept for setting and cooling at room temperature about 10-15 minute and capped tightly. The same procedure is followed for all the treatments with different quantities of variables i.e., lyophilized ber, kiwifruit pulp and sugar.

2.4 Analysis

2.4.1 Sensory evaluation

A panel of 20 tasters, 12 men and 8 women which includes scientists and food project students (at Defence Food Research Laboratory, Mysuru, India), carried out a sensory analysis of different samples of ber based kiwifruit jams. The age of the panellists ranged from 21 to 50 years old. The panellist carried out a sensory analysis based on the 9-point hedonic scale (ranging from Extremely dislike-1 to Extremely like-9) for all the 9 sample including a control in which there is no lyophilized ber fruit. The attributes evaluated were appearance/colour, taste, consistency/texture, aroma and overall acceptability. Samples were served at room temperature in transparent plastic glass coded with three digit random numbers. Each panellist tasted approximately the same amount of each sample and mineral water was provided to the assessors to rinse their mouth.

2.4.2 TSS

The total soluble solids were measured at room temperature with the help of digital hand refractometer equipped with a per cent scale and was recorded and expressed as degree Brix (°B) after making necessary temperature corrections.

2.4.3 Total polyphenols

The effect of variables i.e., lyophilised ber fruit and kiwifruit on final product is analysed by Folin-Ciocalteu method described by Makkar (2003). Initially, pipette out 0.2, 0.4, 0.6, 0.8 and 1 mL aliquots of working standard solution into the series of test tubes marked S1, S2, S3, S4 and S5, respectively. Then, take 50 µL of phenolics extract of sample into series of test tubes. Perform the analyses in triplicates. Make up the contents of all the test tubes to 1 mL with distilled water. Another test tube marked 'B' with 1 mL of distilled water serves as the blank. Then, add 0.5 mL of Folin-Ciocalteu reagent (1 N) to each test tube including the blank. Vortex all the test tubes well and allow to stand for 5 min at room temperature. After that add 2.5 mL of 5 % sodium carbonate to all the test tubes including the blank. Vortex the test tubes again and incubate in the dark at room temperature for 40 min. Measure the absorbance of the blue colour developed against the reagent blank at 725 nm using spectrophotometer. Draw a standard graph by plotting concentration of gallic acid on x-axis and respective absorbance on y-axis. Finally, calculate the amount of total phenolics in the sample and express as mg gallic acid equivalents/g sample.

2.4.4 Antioxidant Activity

The in vitro antioxidant activity i.e., DPPH radical scavenging activity is carried out (as described by Blois 1958a, b) for all the sample of jam including control in order to study the effect of variable ingredients. Take different concentrations of the extracts and make the volume to 100 µL with methanol. Add 5 mL of 0.1 mM methanolic solution of DPPH· and incubate for 20 min at 27 °C. Measure the absorbance of the solution at 517 nm. Methanol alone will serve as blank. A test tube with 100 µL of methanol and 5 mL of DPPH solution serves as negative control. The mixture of methanol, DPPH and standard (BHT, BHA, quercetin and α-tocopherol) will serve as positive control. The reduction in purple colour of the DPPH solution to pale yellow will give the percentage of inhibition. Calculate the percentage inhibition from the absorbance of sample and negative control using the equation,

$$\% \text{ Inhibition} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Calculate the IC50 (Inhibitory Concentration 50 %) from the concentration and percentage inhibition graph.

2.4.5 Energy

In order to calculate the energy obtained by consumption of 100g of sample, the physiochemical analysis is carried out i.e., per cent of water, protein, fat, fibre, total ash and carbohydrate. As we know that 1g of protein and carbohydrate produces about 4 kCal of energy each and 1g of fat produces about 9 kCal of energy. This concept is utilised for the

calculation of energy per 100g of product. The estimation of protein is done by micro-kjeldahl method, for fat by soxhlet extraction method, crude fibre by loss on ignition of diared residue remaining after sequential digestion of sample with 1.25 % H₂SO₄ & 1.25% NaOH solution specific conditions and moisture content by using a vaccum dryer. All these analysis is done for all the treatments in triplicates.

2.5 Experimental Design and statistical analysis

In designing this experiment by response surface methodology (RSM) using Design Expert ver. 11, a central composite design was employed. Three quantitative controllable factors (independent variables) were used: level of Kiwi fruit pulp (A), level of lyophilised ber fruit powder (B), and level of sugar (C). Five dependent variables were selected as responses for representing the main parameters of ber based kiwi fruit jam quality: Sensory score/Overall acceptability (OAA) (R1), TSS (R2), Total Polyphenols (R3), Antioxidant activity i.e., DPPH scavenging activity (R4) and Calories (R5).

After preliminary experiment, the upper and lower limits for the independent variables were established. Lyophilised ber fruit powder levels were from 5-15 g/100 g, kiwi fruit pulp levels were 90 - 100 g/100 g and Sugar level from 50-65 g/100 g. Three levels of each variable were chosen and nine jam making trials were performed for the evaluation of the optimized formulation while keeping a control in which no

lyophilised ber fruit powder was added. Six replicates at the centre of the design were used to allow for estimation of the pure error at sum of the square. The experimental data for each response variable were fitted to the 2FI and Linear Model. An analysis of variance (ANOVA) was performed to determine the lack of fit and the effect of linear, 2FI and interaction terms on various responses. The analysis of data and the optimizing process were generated using Design Expert v.11 (Stat-Ease Inc.).

3. Results and discussion

The analyses of variance were performed to determine the lack of fit and the significance of the linear, quadratic and interaction effects of the independent variables on the dependent variables. The lack of fit test is a measure of the failure of a model to represent data in the experimental domain at which points were not included in the regression. Coefficient of determination is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for good fit model. The results showed that the models for all the response variables were highly adequate because they have satisfactory levels of R² of more than 80% and lack of fit is insignificant. The regression coefficients are shown in the equations for each of the response variables could be derived from the predicted values of each response variable.

Table 2: Experimental design and response

Run	A:Kiwi Pulp (g)	B: Lyophilised Ber (g)	C: Sugar (g)	OAA	TSS (Brix)	TPC (mg/100g)	Antioxidant DPPH (IC50)	Calories (kCal)
1	90	15	50	6.26	68.8	15.64	14.95	184.96
2	95	10	57.5	6.4	67	12.74	54.09	183.88
3	90	5	65	6.3	66.1	10.29	79.21	207
4	100	15	50	6.78	67.3	15.02	45.46	184.08
5	100	5	65	5.83	66.7	12.7	73.32	227
6	90	15	65	6.45	65.5	18.17	25.58	224.16
7	100	5	50	6.13	66.2	12.63	54.48	186
8	100	15	65	6.74	68.2	14.87	36.16	201.84
9	90	5	50	6.39	65.9	11.67	53.94	187.88

3.1 Response surface plots

3.1.1 Effect of independent variables on overall acceptability

The data pertaining to the sensory evaluation of ber based kiwifruit jam is presented in table 2. All the treatments were found statistically significant. The sensory characteristics of the jam like appearance/colour, flavour, taste, texture/consistency and overall acceptability were evaluated. The relationship between the independent variable and overall acceptability is shown in the fig. 1. The sensory attribute

aroma has no significant difference among the different treatments as it has same smell because of external addition of mixed fruit essence. Jam containing higher level of independent variables were rated high due to their good taste. Response surface plot (Fig. 4.6) shows that up to a certain limit acceptability increased as the independent variable interacted among each other. The combination of medium or slight high levels of both factors resulted in the highest scores in terms of overall acceptability.

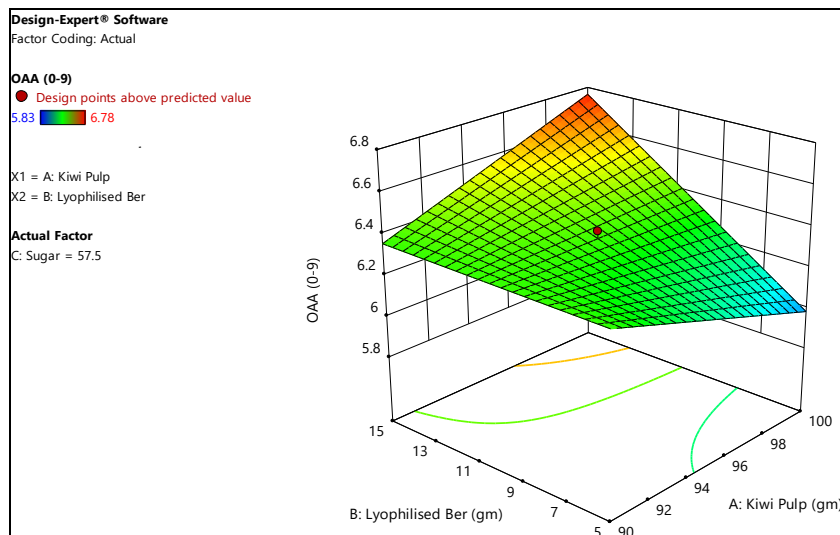


Fig 1: Response surface plot showing the effect of independent variables A, B and C on over all acceptability.

$$\text{Overall acceptability} = +6.34778 + 0.009333 * KP - 0.795500 * LB + 0.117333 * S + 0.007700 * KP * LB - 0.001467 * KP * S + 0.001800 * LB * S \dots\dots\dots (1)$$

Analysis of variance for each response showed that a significant effect ($P < 0.05$) was found for overall acceptability, with regard to lyophilized ber fruit, kiwifruit pulp and sugar. All the three variables exhibited positive 2FI and negative quadratic effect as seen from the equation.

3.1.2 Effect of independent variables on total soluble solids

The data pertaining to the total soluble solids of ber based

kiwifruit jam is presented in table 2 and expressed in °Brix. There is not significant difference among the variables as shown in the fig. 2, but there is higher level of TSS as the variables increases.

The specification of jam preparation should have a TSS of minimum 65 °Brix and a maximum of 68 °Brix. In the preparation of ber based kiwifruit jam does not have significant difference among them with respect to the level of ingredients. The difference in shown in the data is based on the cooking time, this may lead to change in the TSS of the final product.

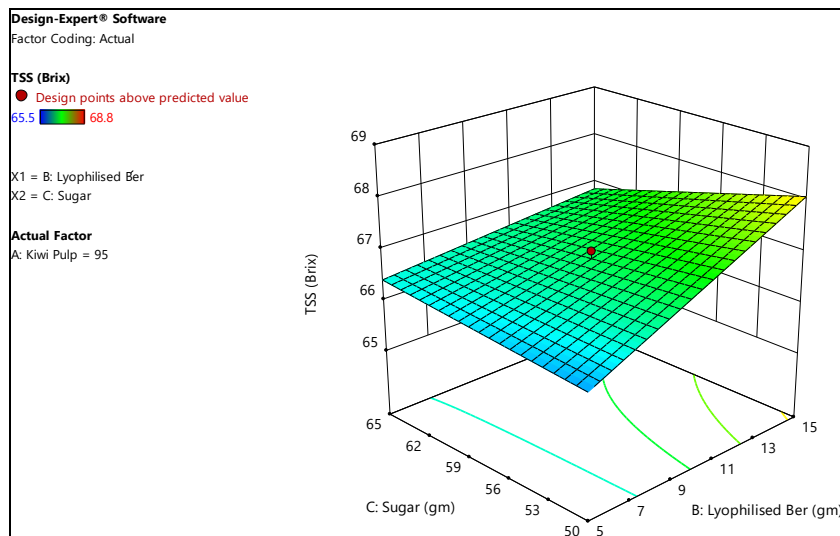


Fig 2: Response surface plot showing the effect of independent variables A, B and C on total soluble solids

$$\text{Total soluble solids} = +139.69306 - 0.825000 * KP + 0.574167 * LB - 1.35000 * S + 0.001500 * KP * LB + 0.015000 * KP * S - 0.010333 * LB * S \dots\dots\dots (2)$$

3.1.3 Effect of independent variables on total polyphenols

Polyphenolic compounds have a wide range of protective effects such as anti-inflammatory responses, prevention of low density lipoprotein oxidation and anti-hypertensive, anti thrombic and carcinostatic actions. Therefore, it is necessary

to estimate their total amount in the sample chosen for extraction. The content of total polyphenols in the different combinations of jujube based kiwi jam was determined using the Folin-Ciocalteu assay, calculated and expressed as Gallic Acid Equivalent (GAE). The results of polyphenol content of the different treatment run of jam were depicted in Table 2. The polyphenol content varies in relation to the independent variables A and B i.e., kiwifruit and lyophilised ber and there is no relation with sugar. As shown in the fig 4.8, the

polyphenol content in the jam were tend to increase with the increase in the levels of kiwifruit and lyophilised ber, apart from this heating also affect the polyphenol content in the final jam. As heating time increases the polyphenol content in the jam decreases because of the enzymatic and non-

enzymatic interaction with in the content of jam. A control with no lyophilised ber jam shows a very less content of polyphenolic content of 7.10 ± 0.18 mg/100g of jam. This implies that the addition of lyophilised ber will increase the total polyphenol content to several extents.

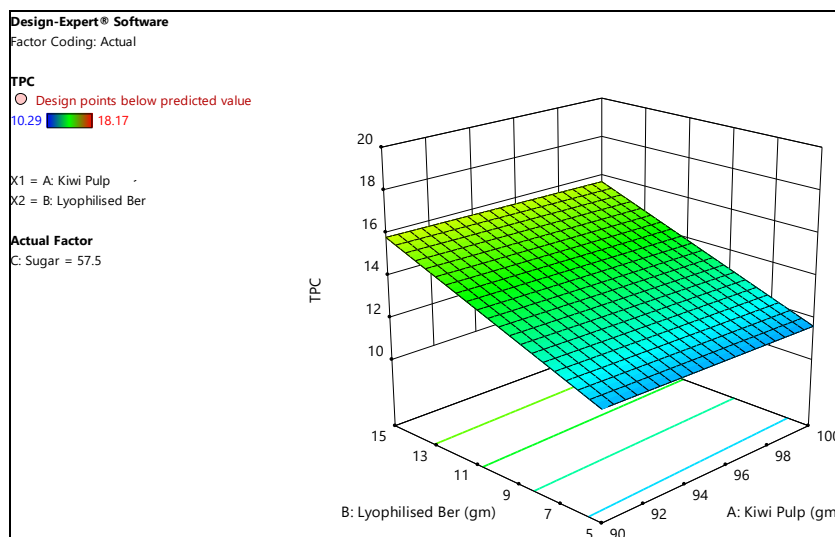


Fig 3: Response surface plot showing the effect of independent variables A and B on total polyphenol content

$$\text{Total polyphenol content} = +9.92611 - 0.013750 * KP + 0.410250 * LB + 0.017833 * S \quad (3)$$

3.1.4 Effect of independent variables on antioxidant activity

The antioxidant activity of different extracts was calculated as their capacity to scavenge free radicals of DPPH, which has been widely used to evaluate the antioxidant activity of natural products (Blois 1958a, b). Although radical scavenging activity should not be considered as being synonyms with

antioxidant activity, it is a fact that almost all of the powerful natural antioxidants, such as tocopherol, carnosal and ascorbic acid are also strong scavengers of the DPPH radical. Thus, good activity in this test is also an indication of the presence of possible antioxidants. The extracts of all the treatment including control used for DPPH analysis and IC₅₀ values were calculated using regression equations derived from graph. A control jam with no lyophilised ber is also prepared with check the IC₅₀ value that how lyophilised ber as an ingredient will affect the DPPH scavenging activity.

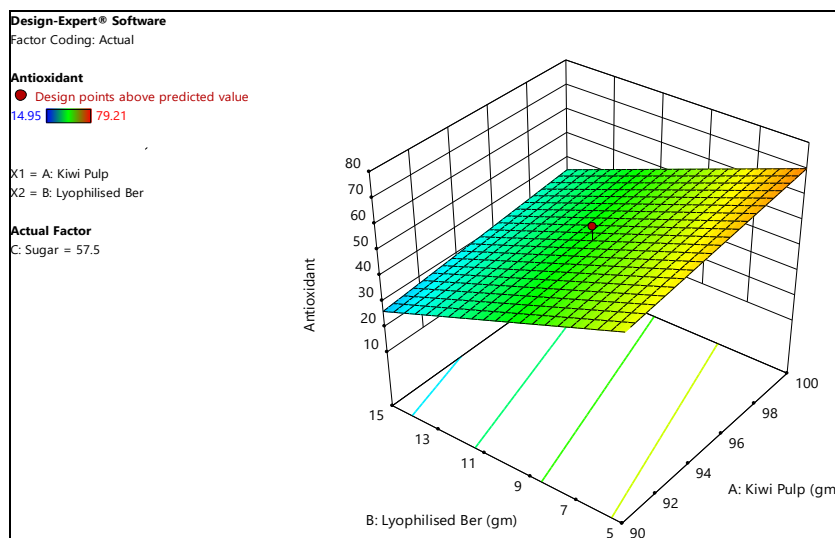


Fig 4: Response surface plot showing the effect of ingredients A, B and C on Antioxidant activity (DPPH scavenging activity)

$$\text{Antioxidant activity} = -45.15250 + 0.893500 * KP - 3.47000 * LB + 0.757333 * S \quad (4)$$

The IC₅₀ values of respective jam samples are also shown in the figures. The better IC₅₀ value is having sample 1 i.e.,

14.95 mg/ml than followed by sample 6 i.e., 25.58 mg/ml, sample 8 i.e., 36.16 mg/ml and so on as shown in the fig. 5. The lower the Ic₅₀ value the better the scavenging activity. The control is having higher IC₅₀ value of 125.16 mg/ml

which is less scavenging activity while the sample 1 is having smaller IC50 value which is affected by the addition of lyophilised jujube.

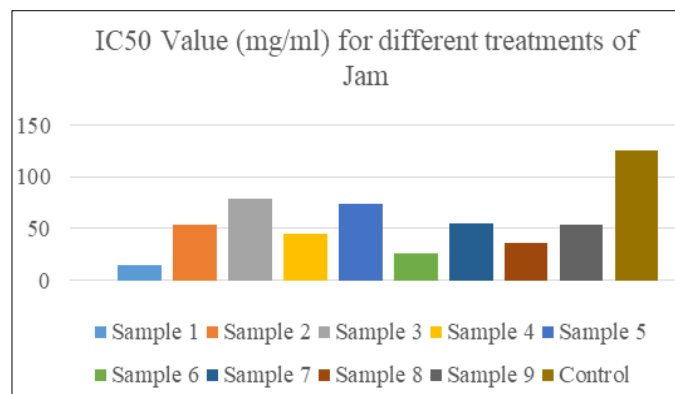


Fig 5: IC50 values (mg/ml) for different treatments of Jam

The relationship between the variables and antioxidant activity

by using DPPH assay can be seen from the fig 4.11. analysis of variance shows that a significant effect ($P < 0.05$) is found for antioxidant activity, with regard to all the three variables. This implies that the lower the value in the graph, the better the antioxidant capacity.

3.1.5 Effect of independent variables on Energy

The content of available carbohydrate is determined by difference, i.e., by subtracting from 100 the sum of the values (per 100g) for moisture, protein, fat, ash and crude fibre.

The energy or calories of the product can be calculated by using the amount of energy released during the complete digestion of carbohydrate, protein and fat. The energy released by complete digestion of 1g of carbohydrate is about 4 kCal, for protein 4 kcal and for fat 9 kCal. The total energy or calories obtained by complete digestion of 100g of product is calculated and expressed in terms of kilocalories.

The proximate analysis of the different treatments/samples and control is given in the table 3. and the energy or calories for the different treatments/samples is given in the table 4.

Table 3: Proximate analysis of different samples of ber based kiwifruit jam

Sample	Moisture Content (%)	Ash Contet (%)	Crude fibre content (%)	Protien (%)	Fat content (%)	Total Carbohydrate (%)
Sample 1	50	0.9	2.86	1.4	0	44.84
Sample 2	50	0.5	3.53	1.4	0	44.57
Sample 3	45	0.5	2.75	1.05	0	50.7
Sample 4	50	0.5	3.48	1.05	0	44.97
Sample 5	40	0.35	2.9	1.05	0	55.7
Sample 6	40	0.75	3.21	1.4	0	54.64
Sample 7	50	0.4	3.1	1.05	0	45.45
Sample 8	45	1.05	3.49	1.4	0	49.06
Sample 9	49.5	0.85	2.68	1.05	0	45.92
Control	25.5	0.45	2.25	1.05	0	70.75

Table 4: Energy or Calories of different samples of ber based kiwifruit jam

Sample	Energy (kCal)
Sample 1	184.96
Sample 2	183.88
Sample 3	207
Sample 4	184.08
Sample 5	227
Sample 6	224.16
Sample 7	186
Sample 8	201.84
Sample 9	187.88
Control	287.2

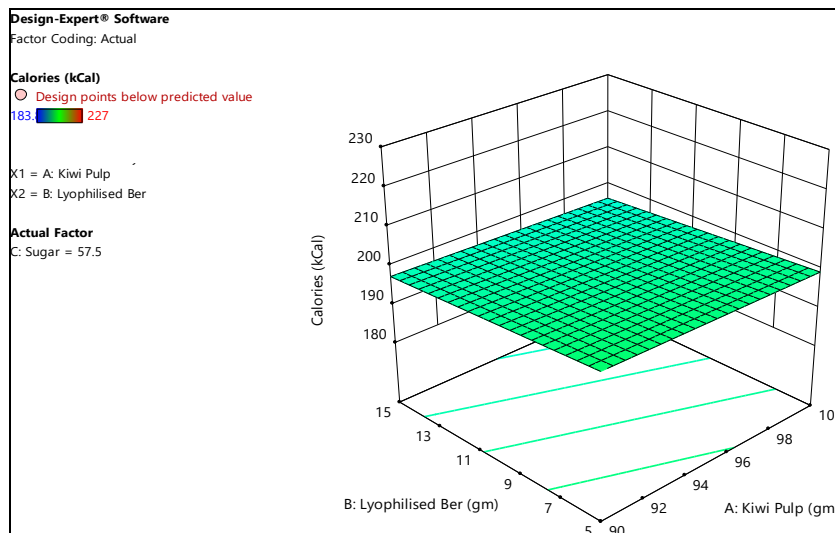


Fig 6: Response surface plot showing the effect of ingredients A, B and C on Calories/Energy

The highest energy is from the sample 6 i.e., 224.16 kCal followed by sample 8 i.e., 201.84 kCal and so on. The relationship between lyophilised ber and kiwifruit and sugar does not show significant difference among the samples of ber based kiwifruit jam.

3.2 Optimization and validation of regression model

The result obtained from the RSM plot indicated the dependence of combination of independent factor on the quality attributes of ber based kiwifruit jam. Response surface plots were taken into account in the optimization, considering that the optimal solution arises from an interaction among the different responses. The criteria of optimization were selected on the basis of for widely accepted jam quality criteria like good flavour, nutritive, energy and high overall acceptability. Thus considering the above mentioned approach, the objective was to maximize the responses sensory score, TSS, polyphenol content and energy and to minimize IC50 value. Optimization was performed on the basis of a multiple

response method called desirability. This optimization method incorporates desires and priorities for each of the variables. This procedure involved transforming scores on each of the dependent variables into desirability scores that could range from 0.0 for undesirable to 1.0 for very desirable. After transforming the predicted values of the dependent variables at different combinations of levels of the predictor variables into individual desirability scores, the overall desirability of the outcomes at different combinations of levels of the predictor variables can be computed as the geometric mean of the individual desirabilities.

As a result of the optimization step, the best conditions, which were attained for the expected response value were, kiwi pulp =100%, lyophilized ber =15% and sugar =65%. The calculated desirability for this formulation was 0.662 and resulted jam of good quality, which was subsequently analysed in order to compare predicted responses to measured values. The response variables of the bread can be predicted by using the achieved mathematical models.

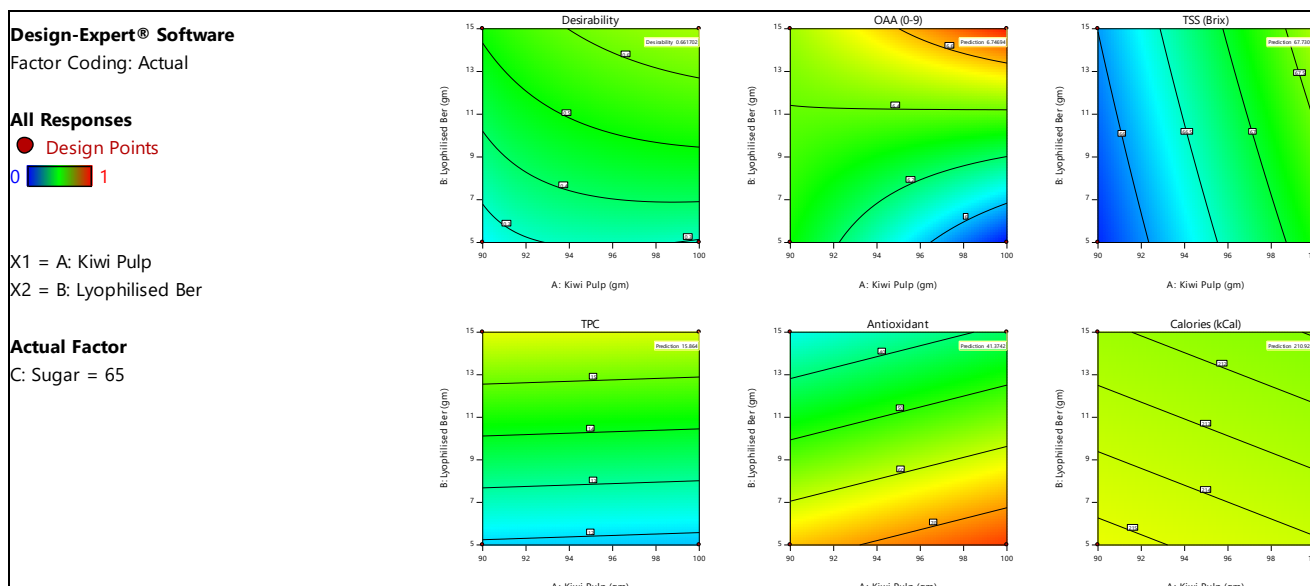


Fig 7: Optimization and validation of regression model

4. Conclusion

Response surface methodology was successfully applied to optimize kiwifruit pulp, lyophilized ber and sugar in antioxidant rich jam. The three variables employed in the study had a great effect on the quality of jam. As the addition of lyophilized ber increases, the total phenolic content and antioxidant capacity also increases. Modelling of experimental data allowed the generation of useful equations for general use, to predict the behavior of the system under different factor combinations. Hence the lyophilised ber fruit can be utilize in the mixed fruit jam preparation with good quality and higher nutritive value which can tackle various stress related problems.

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