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RESEARCH ARTICLE

Optimization of Ready-to-Serve Beverages made from *Limonia acidissima* L. using CCRD design matrix of Response Surface Methodology

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ABSTRACT

The wood apple (*Limonia acidissima* L.) is a deciduous, drought-tolerant, fruit-bearing tree endemic to India and Sri Lanka. Wood apple is a low-cost, high-nutrient component that is underutilized in the food sector. With an increasing demand for traditional yet modern beverages, emerging countries are encouraged to diversify their food exports by creating novel products. The study focuses on formulating a Ready to Drink beverage from wood apple fruit pulp and optimized using CCRD of Response Surface Methodology. The RTS beverage was prepared using varied quantities of wood apple pulp, sugarcane jaggery, and carboxymethyl cellulose (CMC) as a thickening agent by keeping the center point value as 12g, 19g and 0.2g respectively. The physico - chemical (TSS, acidity, pH, viscosity, sedimentation level, Brix/Acid ratio), nutritional (total sugar, reducing sugar, and non-reducing sugar), and sensory attributes (overall sensory score based on appearance, color, flavor, sweetness, and consistency) were determined as responses for predicting best combination of wood apple pulp, sugarcane jaggery and CMC in the preparation of RTS beverage. The suggested wood apple pulp and water ratio for extraction of pulp is 1:0.5. The total cost of production was Rs. 18.00/- per 250ml of RTS beverage prepared by mixing the optimized level of extracted wood apple pulp (12.65g), sugarcane jaggery (17.832g) and CMC (0.25g).

Keywords: Wood apple, RTS, Carboxy Methyl Cellulose, CCRD design, 3D surface graphs

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INTRODUCTION

The Indian geography has such a diverse range of meteorological circumstances, a vast diversity of fruits can be grown. According to the national horticulture database, India produced 99.07 metric tonnes of fruits during fiscal year 2019-2020, accounting for 9.3% of the world's total fruit crop population (Statista, 2022). India offers a wealth of underutilized fruits with high nutraceutical value. Many of these fruits have yet to be researched for their distribution, Indigenous Traditional Knowledge (ITK), medicinal and nutraceutical properties. Wood apple is recognized by a multitude of names in different languages, such as Elephant apple (English), Aranamullu (Kannada), Cerukattunarakam (Malayalam), Kauth, Kavat, Kaveet, Kovit, Sit-ranlimbi

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(Marathi), Kapitthah, Akshasasya, Atha (Sanskrit), Narivila, Nilavila, Vilaa, Vilanga (Tamil). Embracing the titles of elephant apple, monkey fruit, curd fruit, or kath bel, this fruit is truly versatile in its identities. In the realm of botany, wood apple is scientifically known as *Limonia acidissima L*. and proudly falls under the Rutaceae family (Yadav et al., 2022). This resilient fruit crop, the wood apple, thrives across the plains of Southern Maharashtra, West Bengal, Uttar Pradesh, Chhattisgarh, and Madhya Pradesh, standing out as one of India's paramount yet underrated indigenous fruits. When compared to other overlooked fruit crops, wood apples emerge as a powerhouse of nutrition, boasting a high protein content and minimal carbohydrate levels. The tree's fruits, encompassing an edible sour-sweet pulp that is gritty, seedy, and fibrous, offer a wholesome experience for all who indulge. (Rathore, 2021). Wood apple is a low-cost, extremely nutritional, perishable, and seasonal fruit. Ripe wood apple (*Limonia acidissima*) has a hard shell, is rather large and globular in shape, and contains a fleshy, pinkish-brown aromatic edible pulp (Narain, et al. 2010). It is the ideal tree for growing in barren locations (Hemalatha, 2019; Mishra, 2020).

Flowering usually begins in July and the harvest season lasts from August to September. Fruits can occasionally be measured for maturity by bouncing them off a hard surface and recording how high they rise. Immature ones shouldn't bounce. They are maintained in sunshine for up to two weeks after harvesting to ripen (Simons, et al. 2005). Wood apple, a fruit with immense potential, serves various purposes such as nutraceuticals, medical benefits, and in traditional practices. The fruit pulp is rich in phytochemicals like amino acids, coumarins, minerals, polyphenols, phytosterols, saponins, vitamins, tri-terpenoids, tannins, and tyramine derivatives (Dar, et al. 2013). Embraced in Indian traditional medicine, it effectively addresses ailments like asthma, cardiac debility, dysentery, diarrhoea, tumours, wounds, and hepatitis (Ilango and Chitra, 2009). Undoubtedly, wood apple stands as a remarkable fruit with a plethora of uses.So yet, only a few studies have been undertaken. Thus, this crop requires complete attention to perform systematic research and development of novel products.

There are now just a few processed items or beverages made from wood apple. Fruit loss could be considerably reduced if proper processing technology can be developed, made available, and implemented to make use of excess wood apples during peak season. With these considerations in mind, the current study was conducted to determine the robust protocol to extract the pulp in pulp extractor from fiber mesh and development of ready-to-serve (RTS) beverages using wood apple pulp, sugarcane jaggery, and carboxymethyl cellulose (CMC), as proposed by the CCRD design matrix of Response Surface Methodology.

MATERIALS AND METHODS

Sample Collection

The matured wood apple (*Limonia acidissima*) fruits was procured from Salem, Dharmapuri and Krishnagiri local markets by adopting the traditional bouncing technique. The fruit was round 5-12.5cm wide with a hard woody grayish brown scurfy rind about ¼ inch (6mm) thick, pulp dotted with countless tiny white seeds which was dark, mealy, odorous, resinous, astringent, acidic and sweet-tasting.

Pulp Extraction

The fresh, fully ripe and sound wood apple was cut into two halves to extract pulp. The pulp with seed was scooped and placed into glass bottles with 2-3cm (1") headspace, screw-capped, pasteurized, and stored in a refrigerator at 4℃ for further investigation. The pulp yield per 100g fruit was determined and reported.

Preparation and Characterization of RTS Beverage from Wood Apple Pulp

Thermally processed, bottled and hermetically sealed RTS beverage from wood apple pulp was prepared with the aim of having 10% fruit pulp, 10% total soluble solids and 0.3% acidity as revealed by FOOD SAFETY AND STANDARDS (FOOD PRODUCTS STANDARDS AND FOOD ADDITIVES) REGULATIONS, 2011 (Chapter 2, clause 2.3.10) (https://www.fssai.gov.in/upload/uploadfiles/files/Food_Additives_Regulations.pdf).

Selection of best suited sugar for the preparation of RTS beverage

The suitable type of sugar to prepare the RTS beverage from wood apple pulp was selected by preparing RTS beverage with varied combination of different types of sugar (Table 1) in concentration of sugar syrup between 10 and 40 %. The scooped wood apple fruit pulp was mixed with potable sterilized water in the ratio of 1:0.5, mixed thoroughly and passed through 12 mesh size test sieves to exclude the fibrous matter and seed portion of the scooped pulp. The extracted pulp was homogenized and blended with sugar syrup prepared with different types of sugar in varied concentration. Carboxy Methyl Cellulose (CMC) at 0.25% was added to the syrup to stabilize the beverage. The suitable type of sugar and its concentration was finalized based on the mean overall acceptability score of the RTS beverage determined by evaluating the appearance, colour, flavor, taste and consistency as its sensory attributes using a 5 point hedonic rating scale.

Table 1: Variation on types of sugar and mean overall acceptability score of the prepared RTS beverage

Trial No.	Sugar type	Concentration of sugar in syrup (%)	Level of wood apple pulp (g)	Mean overall acceptability score
1.	Sugarcane Jaggery (Gur)	10	10	15.1±4.012
		20	10	16.5±4.696
		30	10	18.65±4.184
		40	10	17.45±3.517
2.	Palm Jaggery (Karuppatti)	10	10	13.8±4.549
		20	10	14.8±4.142
		30	10	17.5±3.052
		40	10	16.9±4.128
3.	Powderd Jaggery (Nattu Chakkarai)	10	10	13.85±5.274
		20	10	15.65±4.545
		30	10	17.6±4.112
		40	10	16.95±4.358
4.	Sucrose (Common sugar)	10	10	14.8±4.323
		20	10	14.85±4.392
		30	10	17.95±5.186
		40	10	17.8±3.381
5.	Palm Candy (Panagkalkandu)	10	10	14.5±4.872
		20	10	15.45±4.273
		30	10	18.85±4.056
		40	10	18.4±4.070

Mean overall acceptability score was the average of 20 determinants.

Process protocol optimization for the preparation of RTS beverage from wood apple pulp

The process for the preparation of RTS beverage from deseeded extracted wood apple pulp was optimized by optimizing the level of ingredients and process protocol for the preparation of RTS beverage from wood apple pulp.

Optimization of Level of Ingredients

The level of addition of fruit pulp, sugarcane jaggery and CMC in 100ml of RTS beverages was optimized using central composite rotatable design (CCRD) of Response Surface Methodology (RSM). The 5 point design matrix (-2, -1, 0, 1, 2) in CCRD and levels of variables in coded and uncoded form is depicted in Table 2.

Table 2: Coded and Uncoded Levels of Independent Variables for 20 Experimental Runs

Experimental Runs	Coded levels			Uncoded level		
	X_1	X_2	X_3	Pulp (g)	Sugarcane Jaggery (g)	CMC (g)
1	-1	-1	-1	11	17	0.15
2	1	-1	-1	13	17	0.15
3	-1	1	-1	11	21	0.15
4	1	1	-1	13	21	0.15
5	-1	-1	1	11	17	0.25
6	1	-1	1	13	17	0.1
7	-1	1	1	11	21	0.25
8	1	1	1	13	21	0.25
9	-2	0	0	10	19	0.2
10	2	0	0	14	19	0.2
11	0	-2	0	12	15	0.2
12	0	2	0	11	23	0.2
13	0	0	-2	12	19	0.1
14	0	0	2	12	19	0.3
15	0	0	0	12	19	0.2
16	0	0	0	12	19	0.2
17	0	0	0	12	19	0.2
18	0	0	0	12	19	0.2
19	0	0	0	12	19	0.2
20	0	0	0	12	19	0.2

Responses for Optimization

The developed RTS beverage based on 20 experimental runs (Table 2) were analyzed for total soluble solids (TSS) ranges between 20-36% acidity between 0.1 - 0.2% pH between 3.98 - 4.29 Brix / Acid ratio between 151.3 - 250; viscosity between 67.5 - 190. 5 cP; sedimentation value between 0.3 - 0.83% total sugar between 9.0-17.0% reducing sugar between 4.0-12.6% non reducing sugar between 2.4-11.8% and total sensory score between 15.66-19.96.

The titrable acidity total soluble solids and pulp level all experiments runs was according to the limit prescribed by FPO specification and FSSAI, 2011 (TA - 0.3% Maximum; TSS-10% minimum and pulp level - 10% minimum) as quality responses for optimization. The sensory attributes for assessing the acceptability score were appearance, color, flavor, sweetness, and consistency. The sensory evaluation was done by 30 semi-trained panel members from the Department of Food Science and Nutrition, Periyar University, Salem, Tamil Nadu, India.

Point Prediction and Numerical Optimization

A second order quadratic polynomial regression equation model was fitted to the data of all responses for prediction. The proposed model was

$$Y=\beta_0+ \beta_1+X_1+ \beta_2X_2 + \beta_3X_3+\beta_{12}X_1X_2+ \beta_{13}X_1X_3+ \beta_{23}X_2X_3+ \beta_{11}X_1X_1+ \beta_{22}X_2X_2+ \beta_{33}X_3X_3$$

Where β_0, β_1, β_2, β_3, β_{11}, β_{22}, β_{33}, β_{12}, β_{13}, β_{23} were the regression coefficients, X_1, X_2, and X_3 were the independent variables and Y was the dependent variable. To assess the effect of independent variables on RTS beverage quality, counter plots and 3D surface graphs for each quality measure (Responses) were constructed as a function of two variables while the other variables remain constant. The levels of each independent variable were forecasted into the current model by computing the expected responses and associated 95% confidence intervals using the prediction equation. The quality of fit of the second order quadratic polynomial regression equation was expressed by the coefficient of determination R^2, model 'p' value, lack of fit 'p' value, Coefficient of variation (CV%) and adequate precision value.

The best level of each independent variable was chosen using numerical multi-response optimization. The set goal of each response (Table 3) for optimization of independent variable levels was according to the quality parameters of commercial RTS. The predicted response levels for optimized levels of independent variables were validated through experimentation.

Table 3: Goals set for each Responses on pair with Commercial Juice

Responses	Lower limit	Upper limit	*Commercial* juice
TSS	20	36	15.5
pH	3.98	4.29	3.82
Acidity	0.104	0.18	0.046
Viscosity	67.5	197	96.75
Sedimentation	0.33	0.83	0.01
Total sugar	9	18.6	4.0
Reducing sugar	4	12.6	3.75
Non reducing sugar	2.4	11.8	0.75

Process Protocol for the Preparation of RTS Beverage

The process protocol for the preparation of RTS beverage by mixing the optimized level of ingredients was defined by referring to the scale up process adopted in the manufacture of RTS beverage using mango pulp.

Statistical Analysis

Design Expert software version 7.1.6 was used to optimize the level of ingredients. Every response was tested four times, average being used for optimization. Using the Microsoft Excel data analysis tool, the mean, standard deviation, and mean difference testing were performed on the gathered data.

RESULTS AND DISCUSSION

Optimized level of Ingredients for the Preparation of RTS Beverage

The level of each ingredient was decided based on the numerical optimization level predicted using the CCRD design of the Response Surface Methodology.

Response Levels for Experimental Runs

Ready to serve beverage from thermally processed wood apple pulp was prepared using varying proportions of wood apple pulp, sugarcane jaggery and carboxymethyl cellulose (CMC) as a thickening agent proposed by CCRD design matrix of Response surface model. The estimated levels of responses for 20 experimental runs as per CCRD design matrix are presented in Table 4. The total soluble solids was ranged between 20 and 36%, acidity between 0.1 and 0.2%, pH between 3.98 and 4.29, Brix / Acid ratio between 151.3 and 250; viscosity between 67.5 and 190.5 cP; sedimentation value between 0.3 and 0.83%, total sugar between 9.0 and 17.0%, reducing sugar between 4.0 and 12.6%, non-reducing sugar between 2.4 and 11.8% and overall sensory score between 15.66 and 19.96 out of 20. The titrable acidity, total soluble solids and pulp level in all experimental runs was according to the limit prescribed by FPO and FSSAI, 2011 (TA-0.3% Maximum; TSS-10% minimum and pulp level - 10% minimum) for RTS beverages.

Influence of Independent Variables on Responses

The coefficients for the proposed second order quadratic polynomial regression equation in terms of actual variables and the regression equation are given in table 5 and 6 respectively. At linear level, the TSS, acidity, viscosity, sedimentation level and overall sensory score were increased with increased level of wood apple pulp, while pH, total sugar, reducing sugar and non-reducing sugar were decreased. The addition of sugarcane jaggery significantly increases the pH and sedimentation level at $p<0.01$. While increasing the level of sugarcane Jaggery, the TSS, pH, acidity, viscosity, sedimentation level were increased, but the total sugar, reducing sugar, non – reducing sugar were decreased. This may be due to sucrose; invert sugar and moisture present in sugarcane jaggery.

The level of addition of carboxymethyl cellulose as thickening agent showed negative influence on pH (significant at $p<0.01$), sedimentation level, total sugar, reducing sugar (significant at $p<0.01$), and overall sensory score and positive influence on TSS, acidity, viscosity and non reducing sugar levels.

At quadratic level, wood apple pulp had positive significant influence on TSS, pH, acidity at $p<0.01$) and negative significant influence on total sugar and non-reducing sugar at $p<0.05$. A positive significant influence was imposed on pH ($p<0.01$) acidity ($p<0.05$) and negative significant influence on non-reducing sugar ($p<0.01$) by CMC. The interaction effect of independent variables on each response was visualized through interactive response 3D surface graphs (Fig. 1 to 9).

Table 4: Estimated Response Levels of 20 Experimental Runs

Runs	TSS (%)	Brix/acid	pH (%)	Acidity (%)	Viscosity (cP)	Sedimentation (%)	Total Sugar(g)	Reducing Sugar(g)	Non-reducing Sugar (g)	Total Mean Sensory Score
1	25±0.0	190.8± 5.344	4.20±0.005	0.131±0.003	101±5.657	0.33±0.016	12.0±0.006	6.8±0.005	5.2±0.0045	19±0.3153
2	24±0.5	190.4±12.381	4.11±0.005	0.126±0.006	89±1.414	0.33±0.040	16.0±0.003	11.1±0.003	4.9±0.002	19.96±0.1640
3	27±0.5	210.9±9.553	4.22±0.005	0.128±0.005	95±0.000	0.5±0.047	17.0±0.006	12.3±0.004	4.7±0.004	19.40±0.2501
4	28±1.0	151.3±8.987	4.21±0.005	0.185±0.005	139±1.414	0.66±0.032	15.0±0.004	12.6±0.005	2.4±0.004	18.13±0.3578
5	26±0.5	175.6±4.092	4.00±0.008	0.148±0.003	67.5±2.121	0.33±0.016	12.5±0.003	7.8±0.001	4.7±0.005	17.43±0.2376
6	24±0.0	156.8±0	4.07±0.008	0.153±0	69.5±2.121	0.33±0.016	10.0±0.008	7.5±0.007	2.5±0.015	19.83±0.1528
7	29±0.5	193.3±7.252	4.12±0.009	0.150±0.003	171±7.071	0.5±0.047	10.6±0.006	5.5±0.006	5.1±0.014	18.66±0.2082
8	27±0.0	214.2±10.172	4.19±0.022	0.126±0.006	111±5.656	0.66±0.023	9.3±0.010	4.3±0.011	5.0±0.003	17.83±0.2819
9	28±0.0	152.1±2.694	4.24±0.005	0.184±0.003	97±2.828	0.33±0.025	9.0±0.004	4.0±0.003	5.0±0.069	15.66±0.1546
10	36±0.5	191.4±5.652	4.15±0.000	0.188±0.003	197±1.414	0.33±0.025	10.0±0.006	5.0±0.006	5.0±0.004	17.50±0.1394
11	28±0.5	222.2±5.434	4.11±0.009	0.126±0.003	178.5±2.12	0.66±0.032	11.8±0.003	6.8±0.002	5.0±0.001	18.03±0.0596
12	30±0.5	250±11.193	4.29±0.005	0.120±0.003	118±18.384	0.83±0.198	9.3±0.007	4.3±0.004	5.0±0.091	18.06±0.2364
13	26±0.5	185.7±3.551	4.18±0.000	0.140±0	103.5±14.85	0.5±0.045	12.3±0.017	9.5±0.012	2.8±0.029	18.53±0.1116
14	26±0.0	191.1±4.397	4.16±0.009	0.136±0.003	190.5±0.71	0.33±0.040	11.8±0.006	6.8±0.001	5.0±0.006	18.26±0.1095
15	20.25±0.5	192.8±6.6105	3.98±0.010	0.105±0.006	75.5±2.121	0.5±0.021	13.7±0.011	8.25±0.003	6.45±0.014	18.53±0.1657
16	20.5±0.6	191.5±8.7164	3.99±0.005	0.107±0.003	81.5±7.778	0.33±0.016	17.2±0.006	6.77±0.002	11.8±0.008	18.40±0.1626
17	20.25±0.5	192.8±10.258	3.98±0.010	0.105±0.003	74.5±0.707	0.5±0.017	14.2±0.008	7.6±0.002	7.0±0.006	17.16±0.155
18	20.25±0.5	187.5±6.061	3.99±0.009	0.108±0.005	87±1.414	0.5±0.019	16.7±0.009	6.47±0.077	10.3±0.01	17.56±0.3220
19	20.25±0.5	194.7±8.691	3.99±0.005	0.104±0.003	86.5±6.364	0.5±0.01	14.4±0.015	7.47±0.002	7.0±0.0145	18.16±0.2614
20	20.25±0.5	194.7±8.691	3.99±0.005	0.104±0.003	89.5±2.121	0.5±0.025	16.9±0.012	7.25±0.006	9.7±0.017	17.76±0.2256

Table 5: Regression Coefficients for Estimated Responses

COEFFICIENT	Brix (%)	pH (%)	Acidity (%)	Viscosity	Sedimentation (%)	Total Sugar (g)	Reducing Sugar (g)	Non-reducing Sugar(g)	Total Mean Sensory Score
β_0	20.106*	3.989*	0.11*	83.901	0.473*	15.955	15.955	8.711*	17.897
β_1	0.692	-0.008	2.91E-03	10.411	0.023	-0.009	-0.00866	-0.359	0.318
β_2	1.125	0.048*	1.53E-03	6.389	0.094*	-0.205	-0.20535	-0.007	-0.157
β_3	0.146	-0.029**	2.00E-05	10.348	-0.021	-1.350	-1.3503*	0.278	-0.233
β_{12}	0.25	0.01	4.13E-03	-0.75	0.04	-0.6	-0.6	0.0125	-0.683**
β_{13}	-0.5	0.03	-8.88E-03	-11.25	0	-0.725	-0.725	0.0375	0.233
β_{23}	0	0.015	-0.01**	12.625	0	-0.825	-0.825**	0.7375	0.083
β_{11}	3.547*	0.061*	0.027*	13.1298	-0.059*	-1.798**	-1.798	-1.327**	0.241
β_{22}	2.486*	0.063*	4.44E-03	13.572	0.0875*	-1.427**	-1.427	-1.327**	0.278
β_{33}	1.426	0.052*	9.75E-03**	13.1298	-0.029	-0.897	-0.897	-1.7155*	0.401
R^2	0.645	0.798	0.8104	- 0.1898	0.706	0.360	0.465	0.521	0.300
Model p value	0.0008	0.0002	< 0.0001	0.416	0.002	0.033	0.266	0.0034	0.139
Adequate precision	6.184	8.156	9.564	2.218	10.989	4.633	7.320	4.818	5.076
cv%	10.161	1.123	8.9	40.614	16.688	18.203	24.111	29.598	4.435
Lack of fit P value	0.1272				0.3136	0.127	0.0014	0.951	0.088

Table 6: Regression Equation for Estimated Responses

Responses	Regression equation
TSS	20.10642+0.692278*A+1.124973*B+0.146447*C+0.25*AB-0.5*AC+0*BC+3.547118*AA+2.486458*BB+1.425797*CC
pH	22.14309-1.68418*A-0.66085*B-8.9524*C+0.005*AB+0.6*AC+0.15*BC+0.060876*AA+0.015661*BB+20.81486*CC
Acidity	0.11+2.91E-03*A+1.53E-03*B+2.00E-05*C+4.13E-03*AB-8.88E-03*AC-0.01*BC+0.027*AA+4.44E-03*BB+9.75E-03*CC
Viscosity	83.90145+10.41084*A+6.388846*B+10.34762*C-0.75*AB-11.25*AC+12.625*BC+13.1298*AA+13.57175*BB+13.1298*CC
Sedimentation	0.473061+0.023431*A+0.094158*B-0.02093*C+0.04*AB+0*AC+0*BC-0.0592*AA+0.087525*BB-0.02915*CC
Total Sugar	15.95508-0.00866*A-0.20535*B-1.3503*C-0.6*AB-0.725*AC-0.825*BC-1.79846*AA-1.42723*BB-0.8969*CC
Reducing sugar	7.152883+0.350139*A-0.19803*B-1.62855*C-0.6125*AB-0.7625*AC-1.5625*BC-0.44059*AA-0.06936*BB+0.849879*CC
Non reducing sugar	8.710711-0.35879*A-0.00732*B+0.278244*C+0.0125*AB+0.0375*AC+0.7375*BC-1.32663*AA-1.32663*BB-1.71554*CC
Total mean sensory score	17.89714+0.318518*A-0.15699*B-0.23298*C-0.68333*AB+0.233333*AC+0.0833333*BC-0.24073*AA+0.277818*BB+0.401562*CC

A-Pulp; B-Sugarcane jaggery; C-Carboxymethyl Cellulose (CMC)

Fig. 1: 3D Surface Graph on Interaction of Independent Variables on Total Soluble Solids

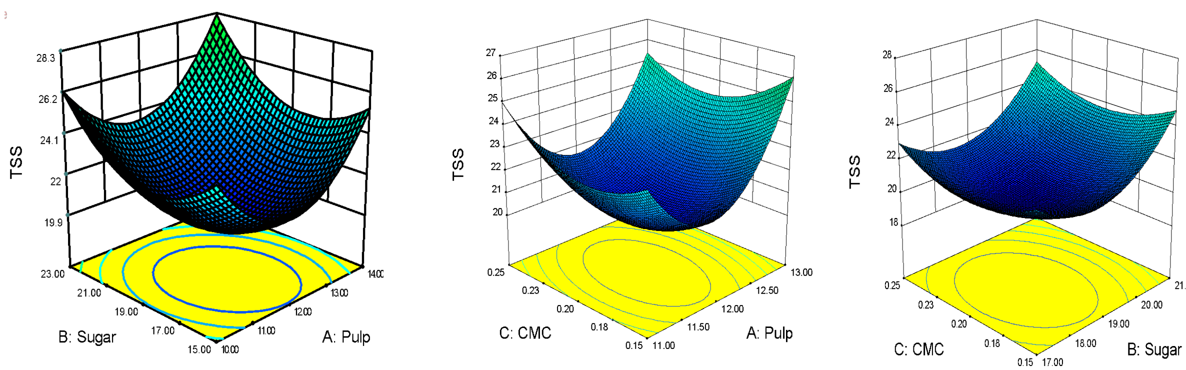


Fig. 2: 3D Surface Graph on Interaction of Independent Variables on pH

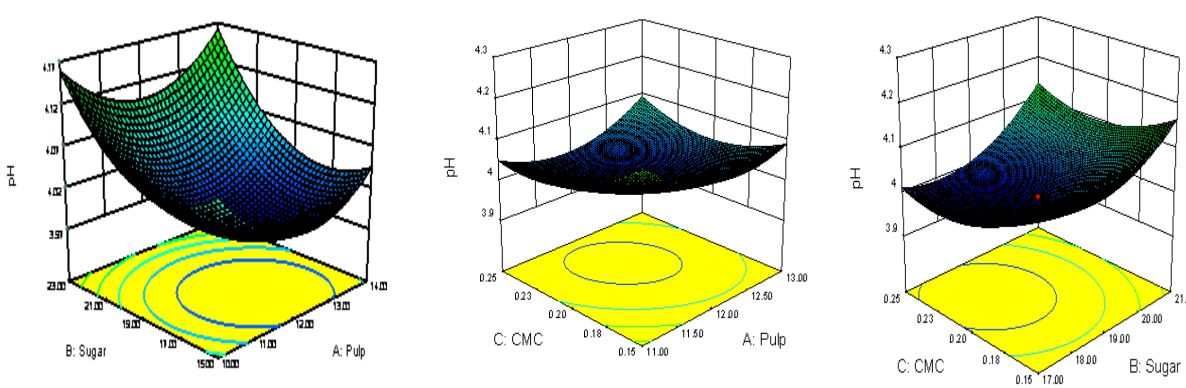


Fig. 3: 3D Surface Graph on Interaction of Independent Variables on Acidity

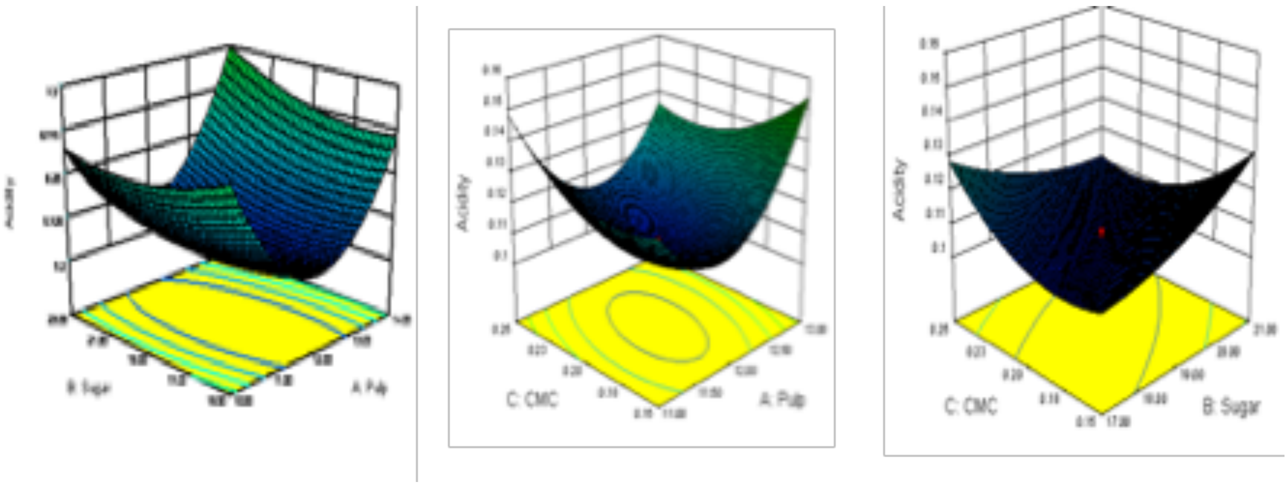


Fig. 4: 3D Surface Graph on Interaction of Independent Variables on Viscosity

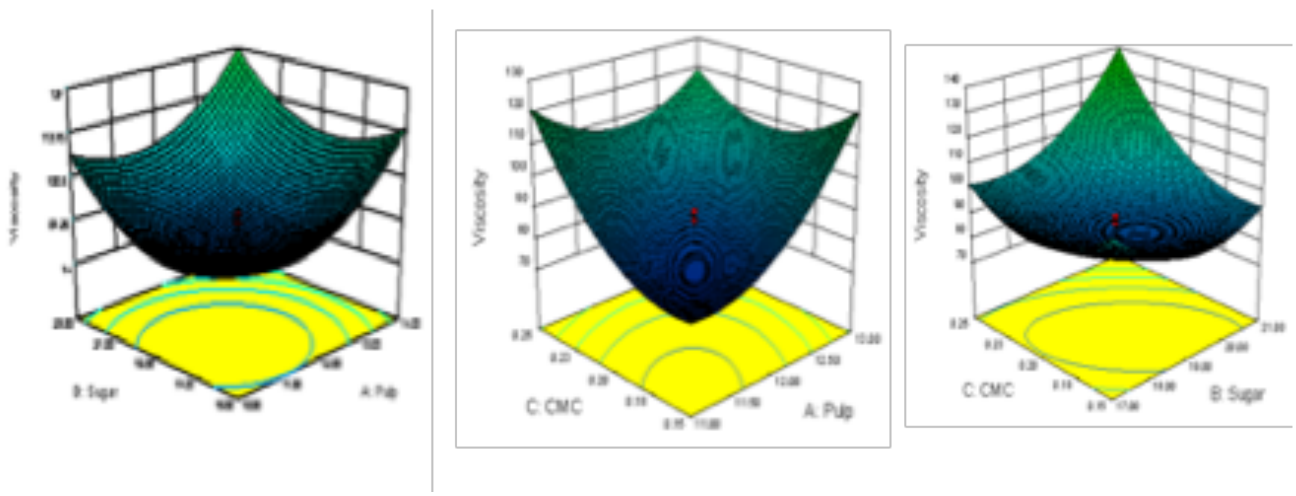


Fig 5: 3D Surface Graph on Interaction of Independent Variables on Sedimentation

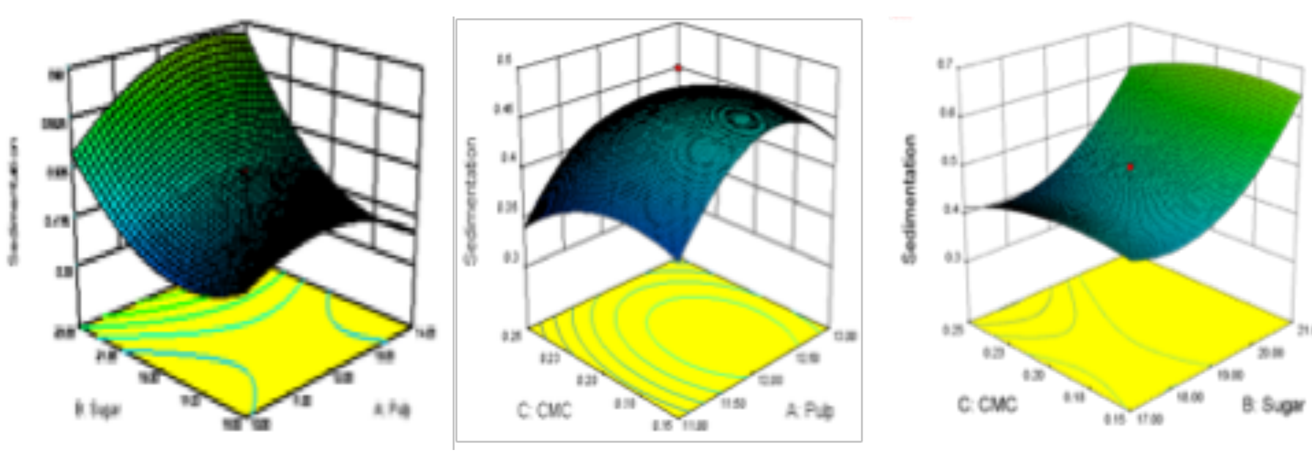


Fig. 6: 3D Surface Graph on Interaction of Independent Variables on Total Sugar

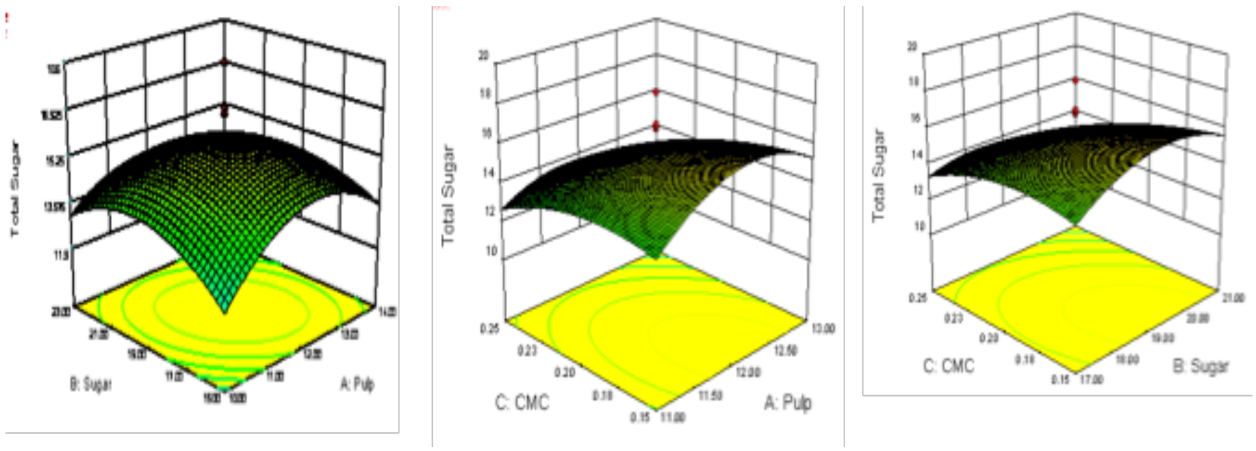


Fig 7: 3D Surface Graph on Interaction of Independent Variables on Reducing sugar

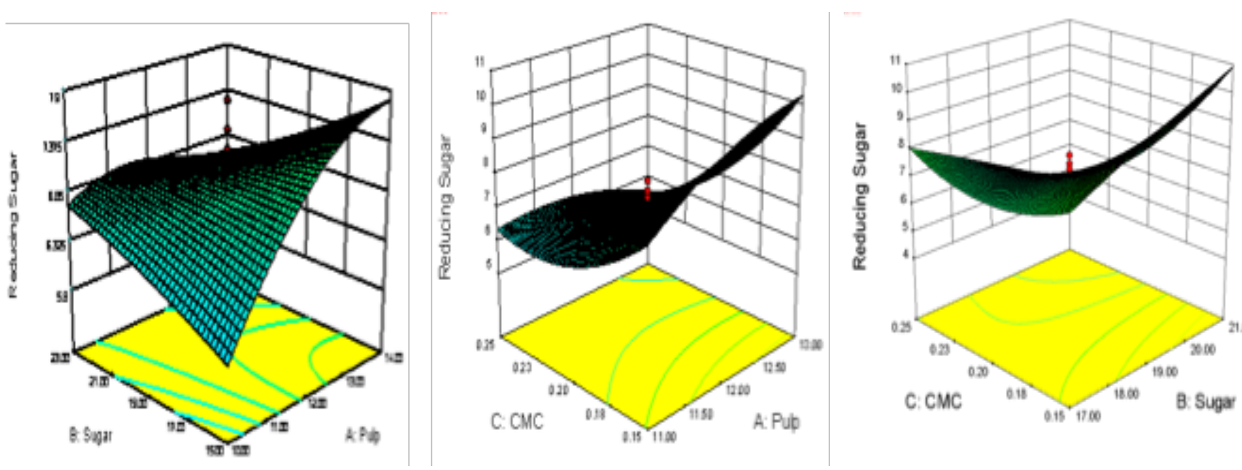


Fig 8: 3D Surface Graph on Interaction of Independent Variables on Non reducing sugar

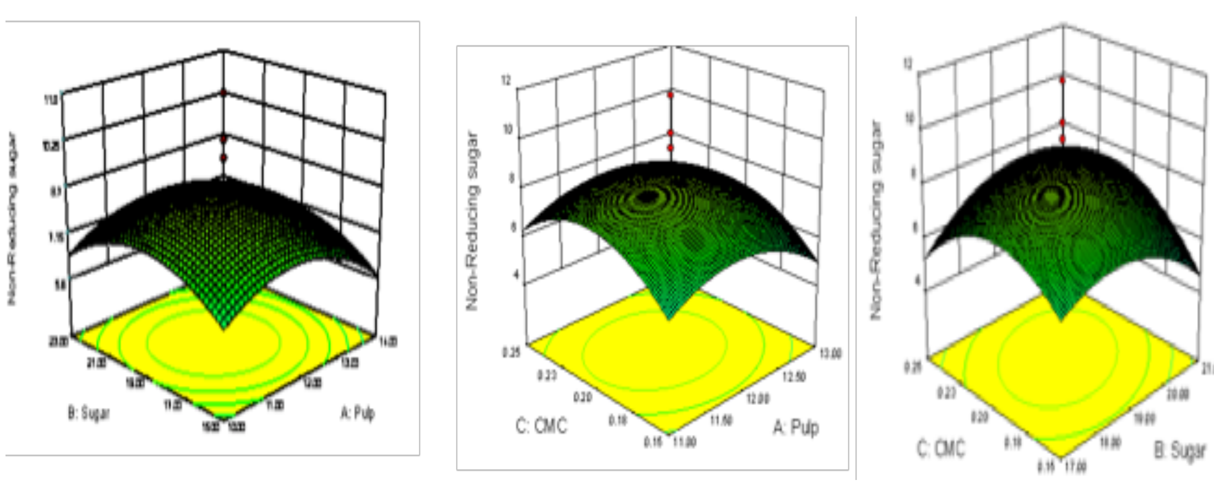
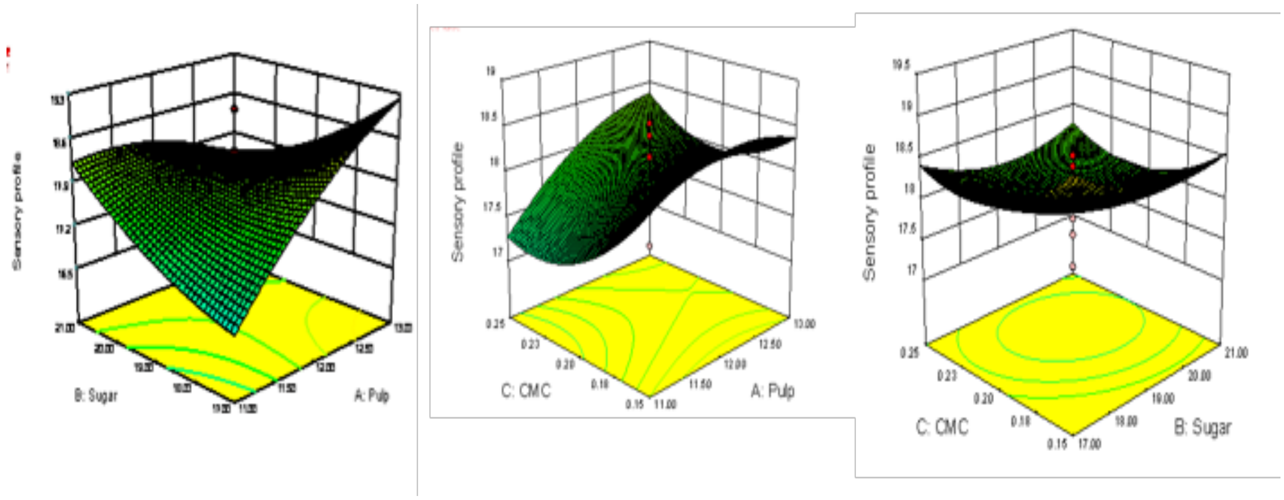


Fig. 9: 3D Surface Graph on Interaction of Independent Variables on Overall Acceptability Sensory Score



The sugarcane jaggery and CMC showed a negative significant interactive effect on acidity and reducing sugar at $p<0.05$.

Response Surface Model Evaluation

The coefficient of determination (R^2), model p value, lack of fit p value, CV% and adequate precision value of the regression model using CCRD for each response are presented in Table 5. The adjusted R^2 above 0.8 for pH (0.798) and acidity (0.8104) showed good fit of the model with experimental data, while R^2 value between 0.5 and 0.8 for TSS (0.645), sedimentation level (0.706), non –reducing sugar (0.521) and reducing sugar (0.465) indicating fair fit of the model with the experimental data.

The CV% value less than 10% for pH (1.123), acidity (8.9%) and overall sensory score (4.435) showed that the experiments conducted were precise and reliable. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The adequate precision value greater than 4 for TSS (6.184), pH (8.156), acidity (9.564), sedimentation level (10.989), total sugar (4.6.330), reducing sugar (7.320), non-reducing sugar (4.818) and overall sensory score (5.076) indicated adequate signal for better prediction and optimization. The prediction of viscosity by numerical optimization was inadequate.

The significance of the model indicating fit of the proposed second order quadratic polynomial equation model for proper prediction of data was significant at $p<0.05$ for total sugar and at $p<0.01$ for TSS, pH, acidity, sedimentation value and non –reducing sugar. But the proposed second order quadratic polynomial model was not fit for the prediction of viscosity, reducing sugar, and overall sensory score. The suggested fitted model was mean, 2F1and 2F1for viscosity, reducing sugar and overall sensory score respectively. The insignificant lack of fit p value for all determined responses except for reducing sugar and viscosity predicted that the proposed quadratic model was probably appropriate and adequate for prediction / optimization.

Numerical Optimization and Validation

Numerical multi-response optimization was adopted to determine the optimum level of each independent variable and the respective predicted level of responses as per the set goals with maximum desirability function is reported in Table 7. The optimum level of independent variables for RTS beverage preparation was in compliance with prescribed limit of response suggested by FPO and FSSAI, 2011 and as well as in comparable with commercial mango juice available in the market. As per the set goals for optimization, the independent variables were 12.65g% of wood apple pulp, 17.83g% of sugarcane jaggery and 0.25g% of CMC. The predicted levels of each response for optimized independent variable levels were validated through analysis of variance between predicted level and experimental data with Duncan's test as post – hoc comparison. The experimental viscosity of the RTS beverage was significantly higher than the predicted level, which could confirm the unfit of the proposed model for prediction of viscosity level.

Table 7: Optimum Level of Independent Variables and Determined and Predicted Response Values

S.No.	Responses	Predicted Value	Determined Value
Optimum level of independent variables: 12.65 g% wood apple pulp, 17.832 g% sugarcane jaggery and 0.25 g% CMC			
1	Total soluble solids	23.398	23±0.816
2	pH	4.03	4.08±0.005
3	Acidity	0.128	0.1216±0.005
4	Viscosity	106.19	638±10.630*
5	Sedimentation	0.37	0.35±0.005
6	Total sugar	12.813	11.9±0.006
7	Reducing sugar	7.156	7.3±0.008
8	Non reducing sugar	5.621	4.6±0.008*
9	Total mean sensory score	18.719	21.76±0.118*
10	Brix/acid ratio	181.944	189.14

*Significantly different at $p<0.01$

Robust Process Protocol for the Preparation of RTS Beverage from Wood Apple Pulp

The following flowchart depicts the robust process protocol for the preparation of RTS beverage from wood apple pulp with optimized level of ingredients along with quality assurance system and indication of CCP.

Production Cost of RTS Beverage from Wood Apple Pulp

The cost of production of 250ml of developed wood apple RTS beverage with optimum level of ingredients is tabulated as follows (Table 8).

Table: 8 Production of cost of Wood apple RTS/250ml.

Expenses / 250 ml	Cost of Production in Rs.
Personnel	2.00
Total(A)	**2.00**
Raw materials	
a) Wood apple pulp	5.0
b) Sugarcane Jaggery	5.50
c) CMC	0.25
Total(B)	**10.75**
Utilities	
a) Fuel	0.50
b) Water	0.50
c) Power	0.50
Total(C)	**1.50**
Contingency expenses	
a) Transport	0.50
b) Publicity, Postage, Telephone, Stationary	0.50
c) Packaging material cost	4.00
Total(D)	**5.00**
Depreciation on building (@5%)for 100L production/ day	0.86
Depreciation on machine (@1%)	0.69
Interest on capital investment (@12%)(Rs.3750000)	3.13
Total(E)	**4.68**
Total cost of production (A+B+C+D+E)	**16.09**
Net profit ratio@10%	1.61
Cost of developed production per kg	25.54
Valid cost of production per kg	**26.00**

The total cost of production of 250 ml of bottled RTS beverage like *Maa* drink was Rs.26.00/- which reflects the feasibility, affordability and viability of the preparation of RTS beverage from wood apple pulp by *Maa* drink manufacturing companies.

CONCLUSION

The wood apple fruit pulp based RTS beverage could be made by combining 12.65g% thermally processed wood apple pulp, 17.8g% sugarcane jaggery, and 0.25% carboxy methyl cellulose as a thickening agent, as per the safe standard limit of TSS, pulp%, and acidity prescribed by FSSAI, 2011. Thus, despite numerous limitations, Tamil Nādu has a great deal of potential for the production and marketing of underutilized wood apple fruit as well as for the use of another seasonal industrial source to support the mango pulp processing industry's continuous functional propagation.

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