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Optimization of ingredients for the development of wheatgrass based therapeutical juice using response surface methodology (RSM)

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Abstract
The aim of this study was to optimize the ingredients by response surface methodology (RSM) for the development of wheatgrass-orange blended therapeutical juice. Wheatgrass juice, orange juice and pectin were taken as independent variables in the range of 30-60%, 35-65% and 0.1-0.3, respectively and effect of these variables were studied on product responses (pH, TSS, sensory score, vitamin C and chlorophyll content). The storage study of optimized juice was studied at ambient and refrigerated conditions. In this investigation increase in concentration of wheatgrass juice resulted in juice with increased chlorophyll content, vitamin C and decreased TSS and sensory score while increase in concentration of orange juice resulted in juice with increased pH, TSS, sensory score and vitamin C and decreased chlorophyll content. Increasing level of pectin resulted exponential increase in TSS. 30% wheatgrass juice, 65% orange juice and 0.3% pectin were obtained as optimized results.

Keywords: Orange juice, wheatgrass (*Triticum aestivum*), RSM, shelf life study, therapeutical juice

Introduction

Fruit and vegetable juices have become a part of our diet due to enriched minerals, vitamins, low energy value and unique taste (Cassano *et al*., 2003) [4] but nowadays cereal grasses are also used as a health drink and are becoming popular due to an important sources of antioxidants, bioactive compounds, chlorophylls, amino acids, vitamins and enzymes (Mujoriya 2011) [18].

Wheatgrass is most popular among all grasses. It is basically extract from the cotyledons of the common wheat plant (*Triticum aestivum)* belonging to family Graminea. Nowadays wheatgrass juice is used as therapeutical food supplement because it improves body immunity and protects from various health problems. Wheatgrass juice is known as wonder juice as it has several therapeutical properties. It lowers the blood pressure, and effective against diabetes. It also purifies and cleanses the body, aids weight loss and promotes healthy skin. It is having antioxidant property and fight against free radicals that are responsible for damaging the cells (Das *et al*, 2012; Padalia *et al*. 2010; Siener *et al*. 2006, Kulkarni *et al*. 2006; Borek 2002) [6, 20, 25, 15, 3].

Orange juice is the excellent source of vitamin C, which is helpful in boosting the immune system. Natural antioxidants hesperidin is found in orange juice that helps in balancing of blood pressure. Orange juice decreases low density lipoprotein (bad cholesterol) in the body. Due to several medicinal properties it is used as a health drinks.

Zingiber officinale commonly known as ginger, belongs to the family Zingiberaceae (Sasi *et al*. 2013) [23]. Ginger juice has medicinal potential against lot of diseases (Grontved *et al*. 1988; Langner *et al*. 1998) [11, 13]. Number of literature provides the scientific evidence of potential health benefits of ginger. These literatures suggest that ginger is helpful against nausea (Ernst and Pittler, 2006; Langner *et al*. 1998) [13], inflammation (Grzanna *et al*., 2005) [10], hypertension (Afzal *et al*. 2001). It is also suggested that ginger lowers blood cholesterol and blood glucose levels (Ghayur *et al*. 2005) [9]. Preliminary evidence demonstrates the role of ginger in cancer prevention (Shukla and Singh, 2007) [24]. Thomson *et al*. 2002, suggested that ginger could be used as an cholesterol-lowering, antithrombotic and anti-inflammatory agent.

The ingredients were optimized with the help of response surface methodology (RSM) to obtain juice with desired sensorial (flavor and aroma) as well as nutritional and therapeutical properties. Storage study of optimized blended juice was also carried out at ambient and refrigerated condition to determine the consequence on physico-chemical properties of wheatgrass-orange blended therapeutical juice.

Materials and Methods

The study was carried out in the School of Vocational Studies and Applied Sciences (Department of Food Technology), Gautam Budhha University, Greater Noida, Uttar Pradesh, India. Wheat *(Triticum aestivum*) grains, orange *(Citrus sinensis),* lemon *(Citrus limon),* ginger *(Zingiber officinale) and* sugar were procured from local market Greater Noida, Uttar Pradesh, India. Pectin was purchased by Himedia laboratories Pvt. Ltd., Mumbai.

Preparation of orange, lemon, ginger and wheatgrass juice

Fresh and mature orange, lemon and ginger fruits were used for extraction of juice. Peeled orange fruits were crushed in philips juicer & mixer (HL1631) for extraction of juice. Ginger were sliced and crushed with philips juicer & mixer (HL1631) for the extraction of juice. Lemons were cut lengthwise and squeezed with hand operated squeezer for the extraction of juice. The juice was filtered throughly and packed in pre sterilized airtight bottles.

Wheatgrass juice was extracted from young wheatgrass leaves through Philips juicer & mixer (HL1631) and obtained clear juice by passing through muslin cloth. After the extraction of all juices, the blend of juices was prepared and ingredients compositions of the blended juice were optimized by response surface methodology. The method used for the preparation of wheatgrass-orange blended juice is shown in figure 2.

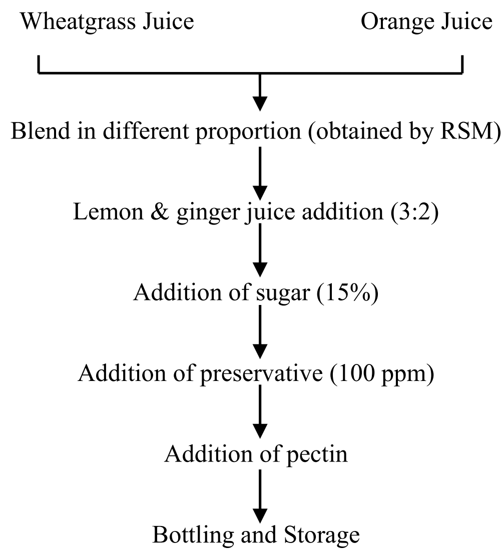

Fig 1: Preparation of wheatgrass-orange blended juice

Chemical analysis of blended juice

Blended juice was analyzed for total soluble solids (by ATAGO refractometer), total acidity (Ranganna 1994) [21], vitamin C by direct colorimetric method (Ranganna 1994) [21], total sugar, reducing sugar and by non reducing sugars by Lane and Eynon's method (Ranganna 1994) [21] and Chlorophyll content was determined by spectrophotometric method (Ranganna 1994) [21].

Experimental design for optimization of wheatgrass-orange blended juice

A central composite rotatable experimental design (CCRD) of response surface methodology was adopted to obtain best possible combinations of experiments (Kumar *et al*. 2014) [16]. For the same, three variables (five levels of each variable), wheatgrass juice (A; 30-60%), orange juice (B; 35-65) and pectin (C; 0.1-0.3) were taken. All the experimental trials were conducted in triplicate.

Table 1: Experimental (CCRD) design of blended juice and results of different responses

Run	Wheatgrass Juice %	Orange Juice %	Pectin%	pH	TSS	Sensory Score	Vitamin C	Chlorophyll
1	45.00	50.00	0.20	4.11	18	5.56	0.382	0.0351
2	60.00	65.00	0.10	4.29	17	5.4	0.143	0.033
3	60.00	35.00	0.30	4.18	18	5	0.122	0.0408
4	60.00	35.00	0.10	4.12	16	5	0.125	0.04006
5	19.77	50.00	0.20	4.14	17	7.3	0.775	0.0152
6	45.00	50.00	0.20	4.11	18	5.5	0.488	0.029
7	30.00	65.00	0.30	4.35	20	7	0.718	0.0302
8	45.00	75.23	0.20	4.35	19	6.5	0.778	0.0301
9	70.23	50.00	0.20	4.15	16	5.8	0.22	0.0456
10	45.00	50.00	0.20	4.22	16	6	0.503	0.0322
11	30.00	35.00	0.30	4.09	19	5.6	0.136	0.032
12	30.00	65.00	0.10	4.21	18	7.1	0.725	0.0241
13	30.00	35.00	0.10	4.03	17	5.6	0.138	0.028
14	60.00	65.00	0.30	4.51	18	5.4	0.14	0.0334
15	45.00	50.00	0.20	4.06	17	5.45	0.607	0.0325
16	45.00	50.00	0.20	4.05	17	6	0.406	0.0262
17	45.00	24.77	0.20	3.84	16	5.5	0.125	0.0427
18	45.00	50.00	0.37	4.1	20	5.5	0.398	0.0312
19	45.00	50.00	0.20	4.02	17	6.3	0.461	0.0309
20	45.00	50.00	0.03	4.07	13	5.3	0.412	0.0327

Table 3: Anova for different responses of wheatgrass-orange blended juice

	Coefficient estimate					F Value					Prob > F				
	pH	TSS	Sensory Score	Vitamin C	Chlorophyll	pH	TSS	Sensory Score	Vitamin C	Chlorophyll	pH	TSS	Sensory Score	Vitamin C	Chlorophyll
Model	4.09	17.3	5.81	0.48	0.031	3.98	3.16	9.15	8.14	5.52	0.0210	0.0436	0.0009	0.0015	0.0067
A	0.032	-0.49	-0.51	-0.16	6.157E-003	1.60	2.59	39.01	25.23	35.67	0.2348	0.1386	< 0.0001	0.0005	0.0001
B	0.13	0.59	0.39	0.17	-3.028E-003	27.07	3.76	22.91	29.77	8.63	0.0004	0.0814	0.0007	0.0003	0.0149
C	0.039	1.37	0.017	-2.822E-003	6.383E-004	2.36	20.45	0.044	8.339E-003	0.38	0.1557	0.0011	0.8377	0.9290	0.5496
A^2	0.042	-0.012	0.22	-0.028	-2.778E-004	2.85	1.541E-003	7.24	0.84	0.077	0.1221	0.9695	0.0227	0.3815	0.7876
B^2	0.024	0.34	0.021	-0.044	1.844E-003	0.94	1.34	0.070	2.12	3.37	0.3543	0.2748	0.7968	0.1761	0.0961
C^2	0.020	-0.012	-0.19	-0.060	2.702E-004	0.69	1.541E-003	5.68	4.01	0.073	0.4272	0.9695	0.0385	0.0731	0.7932
AB	7.500E-003	-0.12	-0.26	-0.14	-1.095E-003	0.051	0.099	5.96	12.30	0.66	0.8251	0.7595	0.0348	0.0057	0.4352
AC	0.010	-0.12	0.012	3.750E-004	-1.120E-003	0.092	0.099	0.014	8.623E-005	0.69	0.7685	0.7595	0.9098	0.9928	0.4251
BC	0.030	-0.12	-0.013	-6.250E-004	2.200E-004	0.82	0.099	0.014	2.395E-004	0.027	0.3855	0.7595	0.9098	0.9880	0.8735

Table 3: Anova results for different responses

Response	Source	Sum of Square	F Value	P Value
pH	Lack of Fit	0.62	2.50	0.1683
TSS	Lack of Fit	*9.79*	3.45	0.0999
Sensory score	Lack of Fit	0.33	0.54	0.7408
Vitamin C	Lack of Fit	0.098	3.08	0.1213
Chlorophyll Content	Lack of Fit	9.759E-005	2.05	0.2244

Table 4: Statistical results of different responses

Parameters	Responses				
	pH	TSS	Sensory Score	Vitamin C	Chlorophyll
Std. Dev.	0.094	1.12	0.30	0.11	3.810E-003
Mean	4.15	17.35	5.84	0.39	0.032
C.V.	2.25	6.48	5.21	29.28	11.81
PRESS	0.51	78.45	3.33	0.79	8.110E-004
R-Squared	0.7819	0.7400	0.8917	0.8799	0.8324
Adj R-Squared	0.5856	0.5061	0.7942	0.7719	0.6816
Pred R- Squared	-0.2717	-0.6159	0.6104	0.2733	0.0635
Adeq Precision	7.405	6.598	9.840	9.228	8.894

Results and Discussion

Analysis of data

Analysis of experimental data was performed to observe the effect of independent variables on measured responses. The second order polynomial equation was used to examine the statistical significance of the model as given in equation 1. The responses (TSS, pH, sensory score, vitamin C and chlorophyll content) for different experimental conditions were performed and fitted in following equation as given below:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \varepsilon \quad (1)$$

Where,

Y = response variable

β_0 = constant

$\beta_1, \beta_2, \beta_3$ = linear effects of regression coefficient

$\beta_{11}, \beta_{22}, \beta_{33}$ = interaction effects of regression coefficient

$\beta_{12}, \beta_{13}, \beta_{23}$ = quadratic effects of regression coefficient

ε = random errors

x_1, x_2, x_3 = independent variables

Design expert software was used for analysis of experimental data. The adequacy of quadratic models for all responses was on the basis of R^2, F-value and p-value at 5 % level of significance.

Effect of independent variables on pH

pH value of 20 experiments of blended juice were measured in the range of 3.88 to 4.51 (Table 1). The analysis of variance for pH is shown in (Table 2). The model F-value for pH is significant (3.98) whereas the Lack of Fit F-value (2.50) is not significant (Table 3). The coefficient of determination (R^2 = 0.7819) indicates about the fitness of model and 78.89 % of variability of the response. Adeq. Precision is 7.405 which indicate an adequate signal (Table 4). On the basis of above statistical parameters, the model was obtained and selected for representing the variation of pH (Table 5).

Figure 2 clearly indicates the effect of concentrations of both juices (wheatgrass juice and orange juice) on pH. pH increases with increasing concentrations of orange juice. Wheatgrass juice also had slight positive effect on pH of the blended juice. The reason might be the higher pH of orange juice than wheatgrass juice.

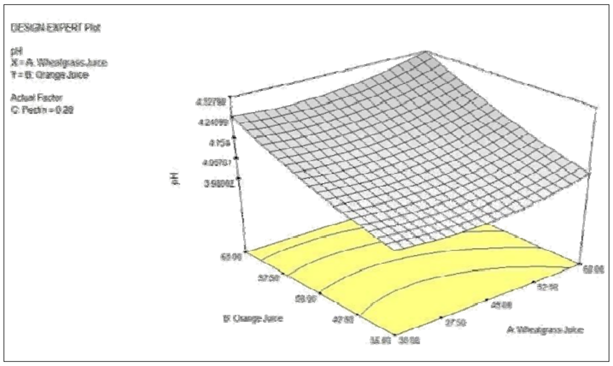

Fig 2: 3-D graph showing the effect of wheatgrass and orange juice on pH

Effect of independent variables on TSS

Total soluble solids of blended juice were measured in the range from 13 to 20 °Brix (Table 1). The analysis of variance for pH is shown in (Table 2). The model F-value for pH is significant (3.16) whereas the Lack of Fit F-value (3.45) is not significant (Table 3). There is only a 4.36 % chance that a Model F-Value this large could occur due to noise. The coefficient of determination ($R^2 = 0.7400$) indicates about the fitness of model and 74.00 % of variability of the response. Adeq. Precision is 6.598 which indicate an adequate signal (Table 4). A ratio greater than 4 is desirable and hence this model can be used to navigate the design space. On the basis of above statistical parameters the model was obtained and selected for representing the variation of TSS (Table 5).

The variation of total soluble solid with different variables of blended juice was shown in surface plot (fig. 3, 4 & 5). Figure 3 clearly indicates the effect of concentrations of both juices (wheatgrass juice and orange juice) on TSS. TSS slightly increases with increasing amount of orange juice while increasing amount of wheatgrass juice decreases the total soluble solid of blended juice. Figure 4 show that both wheatgrass juice and pectin affect the TSS. With the increase in the concentration of wheatgrass juice, TSS slightly decreases while TSS exponentially increases with increasing level of pectin in the blended juice. Figure 5 expresses that increase in the concentration of orange juice caused increase in the TSS while increase in the concentration of pectin gives the exponential increase in blended juice. It was found that pectin had more impact on TSS than orange juice. Similar result has been reported by various researchers (Neidhart *et al*. 2002; Singh and Singh 2012) [19, 26].

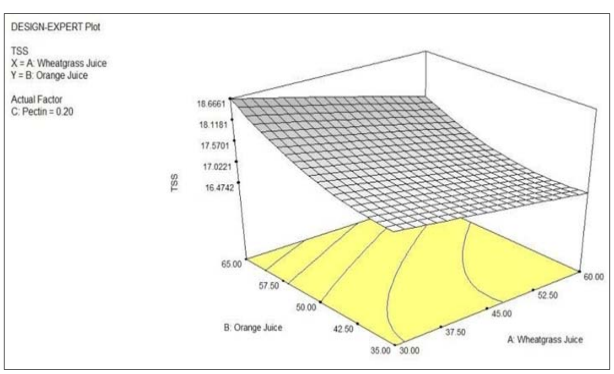

Fig 3: 3-D graph showing the effect of wheatgrass and orange juice on TSS

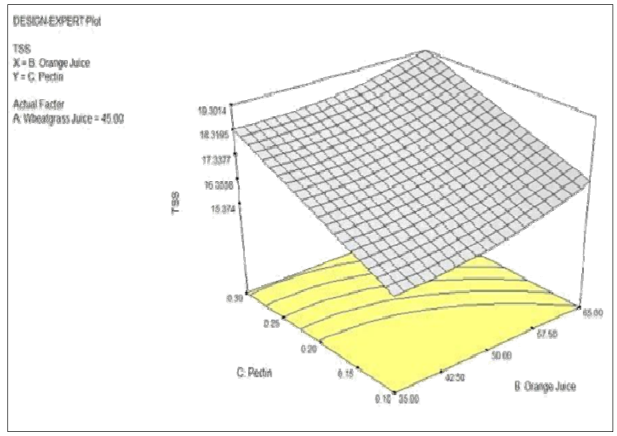

Fig 4: 3-D graph showing the effect of wheatgrass juice and pectin on TSS

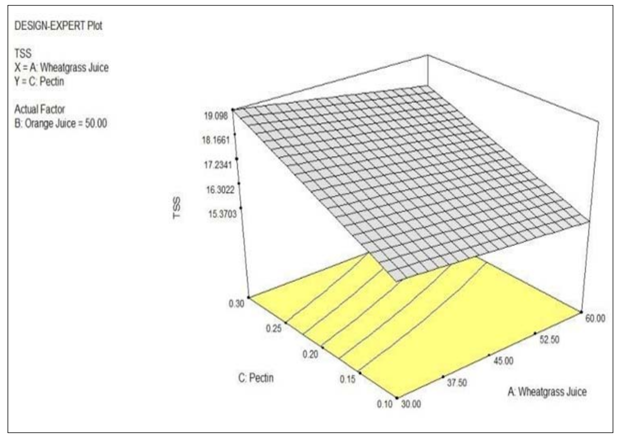

Fig 5: 3-D graph showing the effect of orange juice and pectin on TSS

Effect of independent variables on product Sensory Score

Sensory score has significant value as well as nutritional factors for the consideration of product quality. So it is very important to evaluate the effect of variables on product sensory score. The sensory score were found in the range of 5 to 7.3 (Table 1). The coefficient of model and other statistical attributes of sensory score are shown in Table 2. In regression model, the Model F-value of 9.15 is significant. There is only a 0.09% chance that a Model F-value this large could occur due to noise, whereas the Lack of Fit F-value (0.54) indicates the significance of the model (Table 3). The coefficient of determination ($R^2 = 0.8917$) indicates that 89.17% of variability of the response could be explained by the model. Adeq. precision was 9.840 which indicate an adequate signal (Table 4). Considering all the above criteria, the model obtained is given in Table 5.

The variation in sensory score of beverage with different ingredients was shown in 3D surface plot (Figure 6). It was found in the study that sensory score decreases as the level of wheatgrass juice increases in blended juice. This might be due to poor taste and low shelf life of wheatgrass juice. It was observed that orange juice had positive effect in the sensory score. Similar result of better sensory score with wheatgrass juice was obtained (Singhal *et al.* 2012) [27]

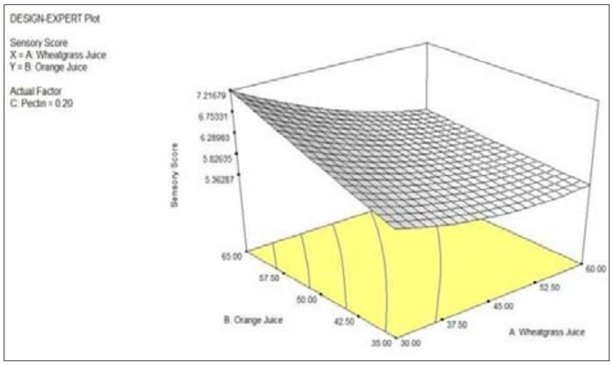

Fig 6: 3-D graph showing the effect of wheatgrass & orange juice on sensory Score

Effect of independent variables on vitamin C content

Ascorbic acid content was found in the range of 0.122 to 0.778 mg/ml (Table 1). The analysis of variance for ascorbic acid content is shown in (Table 2). The coefficient of model and other statistical attributes of vitamin C were shown in Table 3. Regression model fitted to experimental results for vitamin C shows that Model F-value of 8.14 is significant (values of Prob > F less than 0.0500 indicate model terms are significant). There is only a 0.15% chance that a Model F-Value this large could occur due to noise, whereas the Lack of Fit F-value of 3.08 implies the Lack of Fit is not significant. There is a 12.13 % chance that a Lack of Fit F-value this large could occur due to noise. The coefficient of determination (R^2=0.8799) indicates that 87.99 % of variability of the response could be explained by the model. 9.228 values of Adeq Precision indicate an adequate signal (Table 4). The model can be used to navigate the design space as the ratio is greater than 4. The model obtained for representing the variation of vitamin C is given in is given in Table 5.

The variation of sensory score of beverage with different ingredient was shown in 3D surface plot (Figure 7). It was found in the study that increases in the concentrations of wheatgrass juice slightly increases the vitamin C and orange juice caused liner increase in vitamin C concentration. Similar finding of vitamin C content has been reported by USDA food database. It appeared that orange juice has more vitamin C content (50 mg/100gm) than wheatgrass juice i.e. 1 mg/28.35g.

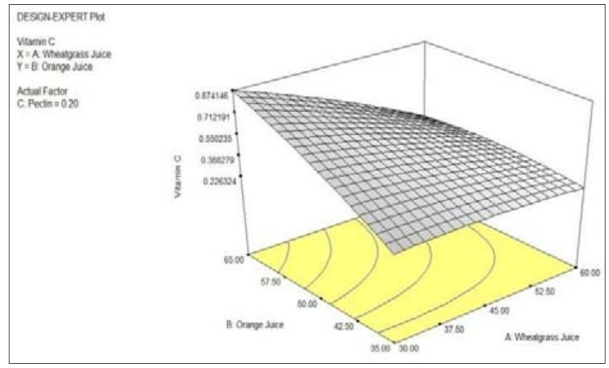

Fig 7. 3-D graph showing the effect of wheatgrass & orange juice

Effect of independent variables on chlorophyll content

Chlorophyll is desirable due to its therapeutic activity. The chlorophyll content of the blend beverage varied from 0.0152 to 0.0456 (Table 1). The Model F-value of 5.52 was found significant, whereas the Lack of Fit F-value of 2.05 connotes the Lack of Fit is not significant. The fit of model was also expressed by the coefficient of determination R^2 = 0.8324, which shows that 83.24 % of variability of the response could be explained by the model. The Adj R^2 was 0.6816. An adequate signal obtained as the value of Adeq. Precision was 8.894 (Table 4). The model obtained for representing the variation of chlorophyll is given in Table 5.

Figure 8 shows the positive and elevated effect of wheatgrass juice and negative effect of orange juice on total chlorophyll content. Chlorophyll content was found more in wheatgrass rich blend (Table 1), It is due to the presence of high chlorophyll content (70 %) in wheatgrass juice Chauhan 2014; Rana *et al*. 2011; Padalia *et al*. 2010) [20, 22, 20].

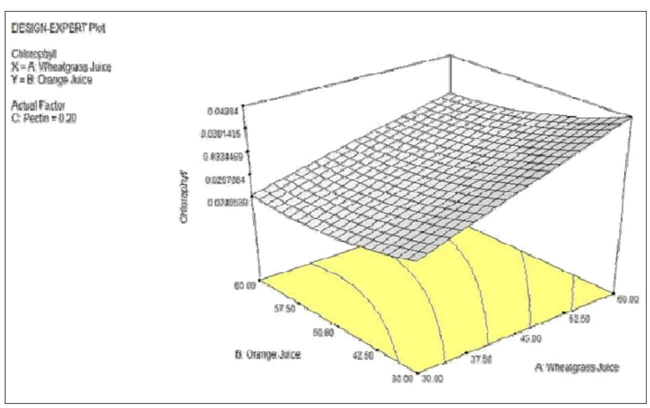

Fig 8: 3-D: graph showing the effect of wheatgrass & orange juice on chlorophyll content

Table 5: The quadratic model equations for pH, TSS, sensory score, vitamin C and chlorophyll content

Responses	Quadratic model equations
pH	$4.09 + 0.032\ A + 0.13\ B + 0.039\ C + 0.042\ A^2 + 0.024\ B^2 + 0.020\ C^2 + 7.5\times10^{-3}\ AB + 0.010\ AC + 0.030\ BC$
TSS	$17.13 - 0.49\ A + 0.59\ B + 1.37\ C - 0.012\ A^2 + 0.34\ B^2 - 0.012\ C^2 – 0.12\ AB - 0.12AC - 0.12\ BC$
Sensory score	$5.81 - 0.51\ A + 0.39\ B + 0.017\ C + 0.22\ A^2 + 0.021\ B^2 - 0.19\ C^2 - 0.26\ AB + 0.012\ AC - 0.013\ BC$
Vitamin C	$0.48 - 0.16\ A + 0.17\ B - 2.822\times10^{-3}\ C – 0.028\ A^2 - 0.044\ B^2 - 0.060\ C^2 - 0.14\ AB + 3.75\times10^{-4}\ AC – 6.250\times10^{-4}\ BC$
Chlorophyll	$0.31 + 6.157 \times 10^{-3}\ A – 3.028 \times 10^{-3}\ B + 6.383 \times 10^{-4}\ C - 2.778E-004\ A^2 + 1.844 \times 10^{-3}\ B^2 + 2.702 \times 10^{-4}\ C^2 - 1.095\ 10^{-3} - 1.120\ 10^{-3} + 2.200 \times 10^{-4}$

Optimization of ingredients and verification of results

For the development of therapeutical blended juice, wheatgrass juice, orange juice and pectin were optimized in the level of 30%, 65 % and 0.3 %, respectively by design expert software. The values of predicted responses were 4.32, 19.99°Brix, 7.02, 0.8126 mg/ml and 0.0266 gm/ lit for pH, TSS, sensory score, vitamin C and chlorophyll content, respectively and the real values of experimental sample under the optimum ingredients condition resulted 4.21, 18° Brix, 7.18, 0.725 mg/ml and 0.0241 gm/lit for pH, TSS, sensory score, vitamin C and chlorophyll content, respectively. No significant differences were found in the results of predicted responses and the values of experimental samples. Therefore the model validates the experimental and predicted values of the responses.

Table 6: Comparison of predicted and actual values of different responses

Responses	Predicted Value	Actual Value
pH	4.32	4.21
TSS	19.99	18
Sensory Score	7.02	7.18
Vitamin C	0.8126	0.725
Chlorophyll	0.0266	0.0242

Storage study of wheatgrass-orange blended juice at ambient (25°C) and refrigerated condition (4°C)

Optimized blended juice was carried out for storage study at ambient (25°C) and refrigerated condition (4°C) for 0-30 days. The significant changes in pH, acidity, TSS, reducing sugar, non reducing sugar, total sugar, vitamin C and chlorophyll content during storage have been observed and shown in Table 7, 8, 9 and 10.

During storage at ambient temperature, the pH values decreased ceaselessly and observed in the ranged from 4.21 to 3.27. The decrease in pH was due to increase in titrable acidity. Slight changes take place in TSS during ambient storage condition (Table 7). The TSS increased in wheatgrass-orange blended juice with passage of storage time at ambient condition, which might be due to hydrolysis of polysaccharides into simpler carbohydrates (Jan and Masih, 2012) [12]. Similar results were also reported by Bhardwaj and Nandal 2014 and Jan and Masih 2012 [12]. A substantial change was seen in vitamin C and chlorophyll content during storage at ambient temperature. Vitamin C decreased from 0.725 mg/ml to 0.25 mg/ml because it is an unstable compound, prone to oxidation, breaks down easily and demeans steadily during storage. (Fennema 1977) [8]. The similar results were also reported by in therapeutical blended juice, Ullah *et al*. 2015 [29] in blended RTS. A significant change was also seen in chlorophyll content. Initially Chlorophyll content was 0.0241 and after 30 days period of storage, decreased up to 0.00407 gm/lit. Drastic loss of Chlorophyll occurred. It is due to chlorophyllase enzyme which functions at high temperatures. Reducing sugar, non reducing sugar & total sugar content was not significantly changed during ambient storage condition. (Table 8)

At refrigerated condition, the pH values of blended juice during storage decreased continuously and observed in the ranged of 4.31 to 3.72. This is due to increased acidity. There was not significantly change in TSS content during storage (Table 9). Similar results have been reported for TSS of wheatgrass based blended juice. A minor change was seen in vitamin C content. In fresh juice vitamin C was 0.725 mg/ml and changed up to 0.650 mg/ml during storage period of 30 days. A substantial change was found seen in total chlorophyll content. In fresh juice, chlorophyll content was 0.0241 and decreased up to 0.00809 gm/lit. Negligible change in reducing sugar, non reducing sugar & total sugar was observed during refrigerated storage condition (Table 10). Similar findings of losses in vitamin C and chlorophyll content during storage of wheatgrass blended juice at refrigerated storage condition were observed.

Table 7: Physico-chemical characteristics of wheatgrass-orange blended juice during storage at ambient condition

Tests/Days	0 day	6th Day	12th Day	18th Day	24th	30th Day
pH	4.21	4.00	3.65	3.46	3.36	3.27
TSS(°Brix)	18	19	19	19	19	19
Acidity (%)	0.896	1.024	1.28	1.40	1.53	1.40

Table 8: Physico-chemical characteristics of wheatgrass-orange blended juice during storage at ambient condition

Tests/Days	0 Day	15th Day	30th Day
Reducing Sugar (%)	0.725	0.675	0.25
Total Sugar (%)	1.26	1.13	1.16
Non reducing Sugar (%)	5.92	5.36	5.23
Vitamin C (mg/ml)	4.72	4.28	3.86
Chlorophyll (gm/lit)	0.0241	------	0.00407

Table 9: Physico-chemical characteristics of wheatgrass-orange blended juice during storage at refrigerated condition

Tests/Days	0 day	6th Day	12th Day	18th Day	24th	30th Day
pH	4.31	4.24	4.28	4.25	4.17	3.72
TSS (°Brix)	18	18	19	19	19	19
Acidity (%)	0.896	0.896	0.896	0.896	1.024	1.28

Table 10: Physico-chemical characteristics of wheatgrass-orange blended juice during storage at refrigerated condition

Tests/Days	0 Day	15th Day	30th Day
Vitamin C (mg/ml)	0.725	0.675	0.65
Reducing Sugar (%)	1.34	1.22	1.16
Total Sugar (%)	5.92	5.36	5.21
Non reducing Sugar (&)	4.64	4.20	3.84
Chlorophyll (gm/lit)	0.0241	------	0.00809

Conclusion

This study was carried out to optimize the ingredients composition through response surface methodology for the development of therapeutical juice. Effect of temperature and other storage conditions were also studied to observe the final product quality.

For the development of therapeutical beverage, experimental design with wheatgrass juice (30-60%), orange juice (35-65%) and pectin (0.1-0.3%), as independent variables produced 20 different combinations that were studied using response surface methodology to investigate the effect of these variables on product responses (pH, TSS, sensory score, vitamin C and chlorophyll content). Multiple regression equations were obtained to describe the effect of each variable on product responses. A central composite design (CCRD) for three variables at five levels was used for experimental combinations and optimization of ingredients. The effects of independent variables on response parameter were analyzed through design expert software. The second order polynomial response models were fitted for each response variables and ANOVA tests were performed for each response. Optimum values for wheatgrass juice, orange juice and pectin were found to be 30, 65 and 0.3 %, respectively. Loss of vitamin C in wheatgrass-orange blended juice was seen 65% in ambient condition and 10.34% in refrigerated condition. Loss of chlorophyll was seen 83.11% in ambient condition and 66% in refrigerated condition. Thus it was found that wheatgrass-orange blended juice stored in refrigerated condition had better result and was more palatable and nutritionally adequate.

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