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Development of a hot water reconstitutable appetizer soup mix from *Coleus aromaticus* using response surface methodology

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Abstract

Indian borage/ Karpurvalli (*Coleus aromaticus*) is an herb with aromatic leaves with ajwain like flavour known for stimulating properties. The paper deals with the development of a shelf stable ready-to-reconstitute appetizer soup mix, convenient to the consumer. The central composite design with two ingredient variables i.e. karpurvalli leaves powder & green gram flour resulted in 13 experimental combinations with the help of Design expert® statistical software. All these combinations for the appetizer were processed by dehydration and proportionate dry blending using pre-processed ingredients and evaluated for quality parameters. The optimized product had the proximate composition of 19.1% protein, 6.3% fat, 8.3% ash, 5.2% crude fibre and 54.4% carbohydrate (by difference). The appetizer soup mix packed in metalized polyester pouches had a shelf life of 6 months at 28 ± 5°C as well as 37°C storage. The RTR (Ready-to-reconstitute) appetizer based on *Coleus aromaticus* was developed with excellent sensory properties and shelf stability.

Introduction

Indian borage/karpurvalli (*Coleus amboinicus* /aromaticus), a flavouring herb having ajwain (*Trachyspermum ammi*) like flavour due to similar chemical components. The herb is a perennial succulent shrub with fleshy and highly aromatic pubescent leaves. The plant is distributed throughout India, cultivated in the gardens, and known as Karpurvalli, Omavalli, Sugandhavalkam, Patta-ajwain, and Patharchur in several Indian languages (Shastri, 1962). In India, traditionally, the leaves of the plant are used for cold, cough, and fever as well as against skin irritations. Damanik *et al.* (2001) reported that the herb Bangun-bangun leaves (*Coleus amboinicus* Lour) are traditionally consumed by Batakneese women in North Sumatra Island Indonesia whilst nursing. Their group discussion study on Batakneese people revealed the belief that the consumption of these leaves can stimulate the production of the breast milk whilst nursing and these leaves may be consumed at any time and as much as possible (in the form of soup), without known adverse effects. Most participants found that consuming these leaves helped control postpartum bleeding and 'acted as a uterine cleansing agent'.

Several other properties such as antibacterial property (Subhas Chandrappa *et al.*, 2010), antimicrobial activity against the microbes of reproductive tract of women (Pritima and Pandian, 2007) and free radical scavenging components from herb extracts (Satish Rao *et al.*, 2006; Kumaran and Karunakaran, 2006, 2007; Rasineni *et al.*, 2008) have been reported. Karpurvalli leaves contain 74.41 mg/100 g ether volatiles while in stalk and stem it was 14.385 and 9.475 mg/100 g, respectively. Further, the principal compound carvacrol in leaves analyzed by HPLC was 22.8 µg/100 g (Wadikar, 2012). A ready-to-drink beverage prepared from the karpurvalli leaves was found to cause reduction in leptin levels in experimental animals and sustained consumption resulted in increased food intake and weight gain thereby indicating appetizing effect (Wadikar and Premavalli, 2011). The beverage was also found to cause reduction in leptin levels in human volunteers and resulted in improved appetite rating post consumption (Wadikar, 2012). The product can be recommended for anybody having appetite/ digestion problem and may help in weight gain in humans. There are several soup mixes available commercially; however karpurvalli herb has not been explored for soup mix preparation (Premavalli and Wadikar, 2011).

In view of this, the development of a convenience soup mix was attempted using RSM (response surface methodology). The use of RSM helps in unbiased development of the product and better optimization of the ingredients for the desired responses. This approach has been used by several researches (Wadikar *et al.*, 2008, 2011; Pandey *et al.*, 2009) in

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recent decade for product/process development.

Materials and Methods

Materials

Karpurvalli (*Coleus aromaticus*) leaves were obtained from the laboratory's garden-plot specifically cultivated. Green gram (*Vigna radiata*) dhal, corn flour, milk powder (Nestle/Amul brand), Common salt, ajwain (*Trachispermum ammi*) seeds, black pepper (*Piper nigrum*) powder (NKCA Pharmacy Ltd, Mysore), hydrogenated vegetable fat (Vanaspati brand) were procured from the local market.

Chemicals

Sodium bicarbonate ($NaHCO_3$), Magnesium oxide (MgO) and Potassium meta-bi-sulphite (KMS) were procured from M/s. s d fine Chemicals Ltd, Mumbai and M/s. Hi-media Laboratories Ltd, Mumbai, and used for blanching of karpurvalli leaves.

All the chemicals and reagents used for analysis were of AR grade and procured from M/s. s d fine Chemicals Ltd, Mumbai and M/s. Hi-media Laboratories Ltd, Mumbai and M/s. Merck specialties Pvt. Ltd, Mumbai.

Raw material processing

The leaves were carefully plucked leaving aside the over-mature leaves and subjected to standardized hot water blanching at 90°C for 30 seconds followed by cold water dipping. The blanching liquor contained Potassium Meta-bi-Sulfate (KMS), Sodium bicarbonate ($NaHCO_3$) and Magnesium oxide (MgO) (Wadikar, 2012). The leaves were further dehydrated at a constant temperature of 60°C for about six hours in cross air flow drier (M/s. Everflow Tray Drier, Chennai, India). The dried leaves were finely powdered and sieved through 60 mesh sieve (M/s. Jayant scientific, Mumbai).

Green gram dhal was cleaned and roasted in an open pan to a pale brown colour to get a pleasant flavour. It was allowed to cool and ground finely in a mill. Then it was sieved through 60 mesh sieve.

Experimental design

A statistical software Design expert® version 8.0 from Statease Inc; USA, was used to construct as well as to analyze the design. Dehydrated Karpurvalli leaves (DKL) powder and Green gram dhal flour (GGDF) were taken as independent variable with sensory score, Total carotenoids and acidity as the responses. The central composite rotatable design (CCRD) based on two independent variables i.e. GGDF and DKL resulted in 13 experimental combinations. The design consists of 4 factorial points, 4 axial points and five centre point replications (Myers and Montgomery, 2002). The center points were selected with ingredients at levels expected to yield satisfactory experimental results. The α-values in the design outside the ranges were selected for rotatability of the design (Thompson 1975). The independent variables with their coded and actual values are given in Table 1. The regression analysis of the responses was conducted by fitting suitable models represented by equations 1 and 2.

Table 1. Experimental ranges and levels of independent variables used in RSM in terms of actual and coded values for Karpurvalli soup mix

Variables	Range of levels					
	Actual	Coded	Actual	Coded	Actual	Coded
A: GGDF	8	-1	10	0	12	+1
B: DKL	1	-1	1.25	0	1.5	+1

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i X_i \quad \ldots 1$$

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i \neq j=1}^{n} \beta_{ij} X_i X_{ij} \quad \ldots 2$$

where,

β_o was the value of the fitted response at the center point of the design, i.e., point (0, 0, 0); β_i, β_{ii} and β_{ij} were the linear, quadratic and cross product (interaction effect) regression terms respectively and 'n' denoted the number of independent variables.

Product preparation

The ingredients green gram dhal flour, karpurvalli powder, starch powder (corn flour), milk powder, vegetable fat, salt, and spices were weighed as per the design and mixed. Melted vegetable fat was poured in the above mixture and mixed uniformly in the mix. The soup was prepared by adding water to the mix in the ratio 6:1 and simmering it for two minutes. These soup preparations were evaluated for the sensory responses.

To establish the shelf life, the karpurvalli soup mix prepared with optimized ingredient levels was packed in metalized polyester (78 µm thickness) pouches and stored at ambient conditions (18-33°C) and 37°C.

Analytical evaluation

The acidity was determined by titrometry. Proximate analysis of the product was carried out by standard AOAC procedures (AOAC, 1975, 1984). Changes in the thio-barbituric acid (TBA) value during storage were estimated by steam distillation method as described by Tarladgis *et al.* (1960).

Sensory evaluation

All the combinations of the Karpurvalli soup mix were evaluated for their colour, aroma, taste, texture and overall acceptability (OAA) on 9-point hedonic scale by semi-trained panel of 15 members during product development as well as storage study. The 9-point Hedonic scale grading was as follows: 9 = Excellent, 8 = Very good, 7 = Good, 6 = Good above fair, 5 = Fair, 4 = Fair above poor, 3 = Poor, 2 = Very poor, 1 = extremely poor. The statistical analysis for significance was carried out using IBM® SPSS® 19 trial version software.

Results and Discussion

Development of Karpurvalli soup mix

The leaves dehydrated with the optimized process and then powdered were used for the product development. The Karpurvalli soup mix was developed using a CCRD based on two independent variables i.e. green gram dhal flour (GGDF) and dehydrated karpurvalli leaves (DKL) leading to 13 experimental combinations. The design consists of 4 factorial points, 4 axial points and five centre point replications (Myers and Montgomery, 2002). Wadikar *et al.* (2008) reported a chakota soup mix based on dehydrated leaves of *Artiplex hortensis* wherein the chlorophyll content was taken as a variable during product development. However, in the present context carotenoids content was chosen as a variable due to their therapeutic value. Kumar and Vallikannan (2010) also reported on carotenoids content of the herb.

The ranges of the independent variable are represented in Table 1 while the total 13 combinations along with observed responses of OAA, Total carotenoids and acidity are given in Table 2. The dry ingredients were properly mixed for uniformity of dispersion as per the experimental combinations and soup was prepared by adding water to the mix in the ratio 6:1 and simmering it for two minutes. These soup preparations were evaluated for the responses. Both OAA and total carotenoids were fit with the quadratic models and the P<F values lower than 0.05 showed that the models were significantly fit (Table 3). However, the acidity response could not be fit with any model so was not used for further navigation. The deviation from the centre point in independent variables resulted in variation in responses. The perturbation and 3D graphs (Figure 1) shows that OAA was equally affected by levels of GGDF and DKL with GGDF showing slightly more influence.

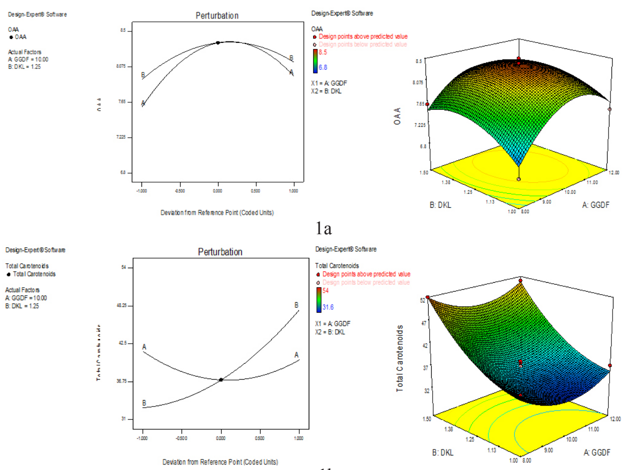
Table 2. Experimental design for Karpurvalli soup mix

Run Order	Green Gram Dhal flour (GGDF),g	Dehydrated Karpurvalli leaves(DKL), g	Overall Acceptability Score	Total Carotenoids, µg	Acidity, %
1	10.00	1.25	8.2	36.8	0.44
2	10.00	0.90	7.0	31.6	0.432
3	10.00	1.60	7.6	54.0	0.45
4	7.17	1.25	7.0	44.8	0.456
5	12.83	1.25	7.4	42.7	0.448
6	8.00	1.00	6.8	38.2	0.44
7	10.00	1.25	8.4	37.0	0.44
8	12.00	1.50	7.8	51.0	0.42
9	10.00	1.25	8.3	35.7	0.436
10	10.00	1.25	8.5	37.5	0.443
11	10.00	1.25	8.4	38.0	0.44
12	12.00	1.00	7.5	37.0	0.41
13	8.00	1.50	7.6	52.0	0.416

Table 3. ANOVA and Model statistics of the Karpurvalli soup mix

Sl No	Term	Response		
		OAA Score	Total Carotenoids	Acidity
1	Model	Quadratic	Quadratic	Mean
2	F-value	26.23	101.51	--
3	P>F	0.0002	<0.0001	--
4	Mean	7.27	41.25	0.44
5	S.D.*	0.16	1.08	0.014
6	CV%	2.24	2.62	3.10
7	R squared	0.9493	0.9864	--
8	Adjusted R squared	0.9131	0.9767	--
9	Predicted R squared	0.7184	0.9307	--
10	Adequate precision	12.859	28.624	--

*Standard deviation



1a

1b

Figure 1. Perturbation and 3-D graph for OAA and Total Carotenoids of Karpurvalli soup mix

The total carotenoids were mainly dependent on the level of DKL (Figure 1b). The multiple regression equations generated for both the responses were:

Overall acceptability of Karpurvalli soup =

$$+7.86+0.18^{*} A+0.16^{*} B - 0.12^{*} A^{*} B - 0.57^{*} A^{2} - 0.39^{*} B^{2}$$

Total Carotenoids of Karpurvalli soup mix =

$$+37.00 - 0.65^{*} A+7.43^{*} B + 0.050^{*} A^{*} B + 3.69^{*} A^{2} + 3.22^{*} B^{2}$$

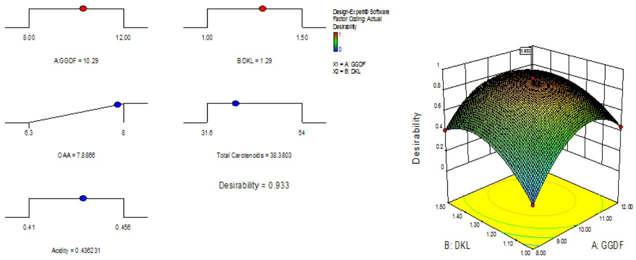


Figure 2. Optimised levels of variables and their desirability plot for Karpurvalli soup mix

Table 4. Predicted Vs Actual response values for the optimized composition of Karpurvalli soup mix

Response	PRV	ARV
Overall Acceptability Score	7.83	7.78
Acidity, %	0.43	0.42
Total Carotenoids, µg/g	38.38	38.25

PRV: predicted response value, ARV: actual response value

Table 5. Changes during storage of Karpurvalli soup mix (#n =10)

Storage period, months	Storage Temperature, °C	Overall Acceptability Score#	Acidity, %	TBA* value
0	-	7.78±0.4^{a}	0.42	0.20
2	18-33	7.71±0.4^{a}	0.456	0.23
	37	7.65±0.2^{a}	0.47	0.31
4	18-33	7.30±0.3^{a}	0.49	0.28
	37	7.30±0.8^{a}	0.523	0.36
6	18-33	7.02±0.4^{b}	0.53	0.34
	37	6.85±0.1^{b}	0.55	0.42
8	18-33	6.20±0.5^{b}	0.56	0.40
	37	6.00±0.4^{c}	0.60	0.46

*Thiobarbituric acid value (mg malonaldehyde/ kg sample) (n = 2)
#values with different superscripts within the column are significantly different from each other (p ≤ 0.05)

Since the mean model was suitable for acidity which indicates that the average of observations in the experimental design is a better predictor or a representative value of the response. The numerical optimization was achieved with maximizing the OAA score response and with constraint of total carotenoids to be in the experimental range. The optimized ingredient levels with the predicted responses and desirability plot is represented in Figure 2. The optimized karpurvalli soup mix was prepared by mixing the dry ingredients roasted green gram dhal flour, dehydrated karpurvalli leaves, milk powder, corn flour, ajwain powder, black pepper powder, salt and hydrogenated vegetable fat. This mix was reconstituted and evaluated for the responses. The predicted and actual response values (Table 4) were in close concurrence with each other.

The proximate composition of the optimized karpurvalli soup mix revealed the presence of 19.1% protein, 6.3% fat, 8.3% ash, 5.2% crude fibre and 54.4% carbohydrate (by difference). The soup mixes and concentrates available in Indian market and

Table 6. Changes in sensory parameters# during storage of Karpurvalli soup mix (n =10)

Storage period, months	Storage Temperature, °C	Colour	Aroma	Taste	Texture
0	-	7.8±0.3^{a}	7.70±0.6^{a}	7.65±0.3^{a}	8.00±0.1^{a}
2	18-33	7.50±0.1^{a}	7.38±0.6^{a}	7.63±0.4^{a}	7.81±0.2^{a}
	37	7.40±0.2^{a}	7.25±0.5^{a}	7.45±0.1^{a}	7.75±0.3^{a}
4	18-33	7.65±0.3^{a}	7.10±0.5^{a}	7.41±0.2^{a}	7.73±0.3^{a}
	37	6.70±0.3^{b}	6.90±0.2^{b}	7.24±0.3^{a}	7.52±0.4^{a}
6	18-33	6.60±0.1^{b}	6.70±0.4^{b}	7.11±0.6^{a}	7.38±0.4^{a}
	37	6.50±0.2^{b}	6.60±0.3^{b}	6.73±0.3^{b}	7.17±0.1^{b}
8	18-33	6.42±0.1^{b}	6.20±0.1^{b}	6.08±0.2^{b}	7.0±0.5^{a}
	37	6.36±0.2^{b}	5.85±0.7^{c}	5.83±0.6^{c}	6.80±0.7^{b}

#values with different superscripts within the column are significantly different from each other (p ≤ 0.05)

marketed by different manufacturers include several variants of vegetables soup, tomato soup, sweet corn soup, mushroom soup and some variants of chicken soup. The ingredient profile of most of these soup mixes/concentrates (Premavalli and Wadikar, 2011) reflects that the spices occupy the lower end indicating the low levels of spices. Cecil *et al.* (1999) have also reported that high carbohydrate and high fat soup consumption leads to appetite suppression and reduced meal intake. The Karpurvalli soup mix in the present context has been kept low in fat content and adequate enough for the shelf stability.

This optimized Karpurvalli soup mix with 6.4% of dehydrated leaves was prepared in bulk, packed in laminated pouches (78 µm thickness), and stored at RT and 37°C. The results of periodic evaluation (Table 5) revealed that the initial acidity of 0.42% of the product increased with storage up to 0.56% and 0.60% at the end of 8 months storage at RT and 37°C, respectively. The TBA value of 0.2 increased to 0.40 to 0.46 mg/kg after eight months of storage. The OAA score of 7.78 decreased to 6.85 after six month storage at 37°C. Further after eight months storage, the OAA scores were 6.15 and 6.00 for the samples stored at RT and 37°C, respectively. Table 6 represents the changes in other sensory parameters such as the colour, aroma and taste and the scores revealed that those were markedly affected after six months storage and rated low. The initial values of aroma and taste were 7.7 and 7.65 which were reduced to 6.6-6.7 and 6.73-7.11, respectively after six months but still the samples were acceptable. However, the scores further reduced after eight months to below 6 which was in the unacceptable range. Hence, considering the sensory properties of the product, the soup mix shelf life was established as six months at RT. A shelf life of six months for such soup mixes based on black pepper namely spiced drink mix, spiced tomato mix and chakota soup mix have been reported (Wadikar *et al*., 2008) in tri-laminated (Paper/Al Foil/Polythene) pouches. Singh *et al.* (1999) reported a shelf life of 8 months for mushroom-whey soup powder when packed in metalized polyester and stored at 30°C. They reported that the optical density (OD) for TBA values increased from 0.108 to 0.188 during storage of the soup powder samples at 30°C and from 0.108 to 0.286 at 45°C after 8 months of storage in metalized polyester. Another report (Bawa *et al*., 2006) on convenience mixes based on finger millet and spices too revealed the shelf life of 5-6 months. The reduced OAA scores and increased TBA and acidity values as a result of storage were reported as quality markers. However, the changes were not remarkable.

In conclusion, a ready-to-reconstitute and functional soup mix based on *Coleus aromaticus* was developed and optimised using response surface methodology and the product had a shelf life of six months at ambient conditions. The herb can be further explored for its other culinary and functional applications as appetising/digestive. The soup mix being a novel one holds good commercialisation potential.

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