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RESEARCH PAPER

Development of Cucumber Fermented Beverage using Response Surface Methodology

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Abstract

Cucumber is valued for its nutritive and medicinal properties. The optimization of the fermentation process with reference to yeast concentration and the period of fermentation has been attempted by using Response Surface Methodology (RSM) for its value addition. The statistical design suggested 13 formulations, with the yeast concentration ranging from 0.29-1.70% and the fermentation process period varied from 0.17-5.82 days. The responses such as acidity, pH, Brix, ascorbic acid, and sensory quality attributes were studied. Overall the fermentation time and yeast concentration were optimized with 1.3% yeast concentration and 1 day fermentation time for preparation of fermented beverage. The optimized results were acidity (%) 0.25, pH 3.56, Brix° 22.7, Vitamin-C 5.28 mg/100ml. The product gave a score of sensory evaluation for more than 8 in various attributes.

Keywords: Cucumber, RSM, fermentation, yeast, sensory parameters, vitamin C

Fermentation is a method of preservation through formation of acidulants, alcohols, antibacterial compounds and improving digestibility of protein and carbohydrates, helps to reduce or eliminate antinutrients, natural toxicants, cooking time and fuel requirements thus, improving the nutritional value and flavour (Campbell, 1994).

Fermented foods and beverages enhance the pleasure of eating and their nutritional roles include indirect contributions through subjective enhancement of appetite and have several health benefits as it aids in digestion, promote healthy flora in digestive tract, produce beneficial enzymes and help to absorb vitamins and minerals more effectively from foods. Some fermented foods regulate the level of acidity in the digestive tract, also act as antioxidants and provide anti-carcinogenic effect. (Parvez *et al.*, 2006).

Yeast helps to synthesize vitamins and minerals in vegetable juices (Rakin *et al.*, 2005). Vegetables are strongly recommended in the human diet since they are rich in antioxidants, vitamins, dietary fibres and minerals. The major part of the vegetables consumed in the human diet are fresh, minimally processed, pasteurized or cooked by boiling in water or microwaving. The availability of vitamins and minerals of vegetables can be improved by fermentation process. Though, vegetable fermentation has received attention of researchers worldwide, the vegetable juices, beverages fermentation have seen a limited approach and the studies on nutrient profile are scanty.

Cucumber (*Cucumis sativus* L.) belongs to the Cucurbitaceae family, is a major vegetable crop consumed worldwide. It is the fourth most important vegetable crop after tomato, cabbage, and onion

(Tatlioglu, 1993). Although its calorie and nutritional value is very low, it is a primary source of vitamins and minerals in the human diet (Mah, 1989). It is widely used as a natural diuretic and thus, can serve as an active drug for secreting and promoting flow of urine. Due to its high content of potassium (50-80 mg/100g), cucumber is useful for in the management of both high and low blood pressures (Kashif *et al.,* 2008). Different varieties of fresh cucumbers, such as Holenarasipur, Dotted, Zucchini, Regular, English, Organic Zucchini, Organic regular are available in the market. *Pranic* Healing is an Ancient Science and Art of healing using *Prana* or life force (Sui 1992). It is receiving scientific attention in biology, physics, nano-technology and health, however, the art still eludes scientific definitions and measurability. The agronomic impact of *pranic* treatment is being investigated, reports suggest increase in agriculture yield, improve nutritional quality and shelf–life (Jois *et al.,* 2016a & b).

An earlier investigation on the nutritional, functional, quality parameters and shelf stability of different varieties of locally available cucumbers revealed that *pranic* treated samples showed better stability at room temperature and refrigerated storage (Keerthika *et al.,* 2016).

Hence, in the present study, Pranic treated cucumbers were utilised in the development of fermented beverage to investigate the effect of Pranic healing on different nutritional and sensory attributes of the beverage. The statistical design tool, Response Surface Methodology (RSM) was used for the optimization of Fermentation process with respect to time and starter culture.

MATERIALS AND METHODS

Materials

Good quality raw materials i.e. cucumber (*Cucumis sativus*), wet yeast (Saf yeast) and sugar were procured from local market. All the chemicals, standards, organic solvents and acids used were of analytical grade and procured from M/s. S D fine Chemicals Ltd, Mumbai, SRL, Acuro Organics Ltd, New Delhi.

Processing of the beverage

Cucumber was deskinned, removed the rind portion, and cut into pieces, blanched at 90°C for 3 mins and extracted the juice using a juice extractor. The extracted juice was processed further using sugar syrup. Yeast was added (0.29 – 1.70%) and fermented at room temperature for 0.17 to 5.82 days of period. After fermentation for varied periods, the respective samples were filtered in muslin cloth immediately and centrifuged to get a clear juice and pasteurized for 15 mins at 90 °C. The samples were analysed immediately after the completion of the fermentation process.

Experimental design

The percentage of yeast and time of fermentation was as per the runs obtained by design expert statistical software. A central composite rotatable design was used without blocking. The numbers of design points were obtained on basis of the number of independent variables decided. The design considered 4 factorial points, 4 axial points and 5 central points leading to 13 sets of experiments. (Devaki and Premavalli, 2012a, 2012b). Optimized ranges of the variables are shown in Table 1.

Table 1: Process variables their levels and experimental design

Process variables	-1.414 (α point)	-1 (factorial point)	0 (centre point)	+1 (factorial point)	+1.414 (α point)
Yeast (%)	0.29	0.5	1	1.5	1.70
Fermentation (Days)	0.17	1	3	5	5.82

X_1	X_2	Runs
± 1	± 1	4
± 1.414	0	2
0	± 1.414	2
0	0	5

Yeast concentration and fermentation period were selected as independent variables and acidity (%), pH, brix°, vitamin C (mg/100ml) and organoleptic

evaluation (score) – appearance, flavour, consistency, taste and overall acceptability were selected as the responses. Since overall acceptability is an important criteria for the product acceptance, it has been taken as one of the responses. As the process used was fermentation with yeast sources, the responses such as acidity, TSS and pH was considered for the study and the changes in the nutritional profile such as ascorbic acid were considered as responses. Each independent variable investigated in this experiment had five levels which were -1.4142,-1, 0, +1 and +1.4142. A total of 13 combinations (design points) were generated for the two independent variables and the alpha values in the design outside the ranges were selected for rotatability of the design. The centre point (the level combination in which the value of each coded variable was 0) was repeated five times for the two-variable design and was selected keeping ingredients at levels expected to yield satisfactory experimental results. The regression analysis of the responses was conducted by fitting suitable models represented by the following equation:

$$Y = \beta o + \sum_{i=1} \beta i Xi + \sum_{i=1}^{n} \beta ii Xi2 + \sum_{i \neq 1=1}^{n} \beta ij Xi Xij$$

where, βo was the value of the fitted response at the center point of the design, i.e., point (0, 0, 0); βi, βii and βij were the linear, quadratic and cross product (interaction effect) regression terms respectively and 'n' denoted the number of independent variables.

Analytical Evaluation

The fermented cucumber juice was analysed for acidity (Ranganna, 1986), Vitamin C (AOAC 1970), pH and °Brix (Hand Refractometer - Atago). The analysis for all the parameters was carried out in duplicate and the average value has been reported. The analysis for all the parameters was carried out in duplicate and the average value has been reported.

Sensory Evaluation

A semi trained panel evaluated the samples for colour, flavour, taste, consistency and over all acceptability using a nine point hedonic scale (Devaki and Premavalli, 2012a). Samples were randomly drawn for each experimental block, coded and served to the panelists.

RESULTS AND DISCUSSION

The experimental central composite rotatable design with the independent variables and responses is given in Table 2. Since over all acceptability is an important criterion for the product acceptance, it has been taken as one of the responses. Over the 13 combinations, acidity (%) ranged from 0.18 to 0.37, pH 3.38 to 3.68, Brix° 16.4 to 24.1, vitamin C (mg/100ml) 4.52 to 6.14 and sensory evaluation (score) – appearance 8.4 to 8.9, flavour 6.5 to 8.8, consistency 7.9 to 8.7, taste 6.5 to 8.8 and overall acceptability ranging from 6.9 to 8.73. The effects of the independent variables using yeast and fermentation time on the response at linear, quadratic and interactive levels are presented in Table 3. The sign and magnitude of the coefficients indicate the effect of the variable on the responses. Negative sign of a coefficient at linear level indicates decrease in response with an increase in level of the variable where as at interactive level; level of one variable could be increased while that of the other decreased to obtain the same response. All linear, quadratic and interactive effects were calculated for each model. Quadratic response surface models were selected for all the responses. The adequacy was calculated by F-ratio, mean, standard deviation, coefficient correlation and lack of fit test. R^2 value was more than 90% and lack of fit was highly non-significant. The regression coefficient, correlation coefficient for the responses were 0.99 for acidity, pH, Brix, appearance, and 0.90 for taste, consistency, respectively indicating that all the values were more than or equal to 90%. The R^2 value for vitamin C was 0.89, flavour 0.85 and OAA 0.84, but because of the subjective nature of the response the model is considered significant. The closer the value of R^2 to unity, the better the empirical models fits the actual data. On the other hand, the smaller the value of R^2 the less relevance the dependent variables in the model have in explaining the behavior of variations. (Mendenhall, 1975).

Table 2: Experimental design for cucumber fermented beverage

Run	Variable levels (uncoded)		Responses (per 100ml)								
	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9
	Yeast	Fermentation	Acidity	pH	Brix	Vitamin C	Appearance	Flavour	Consistency	Taste	OAA*
	(%)	(Days)	(%)		°	(mg/100ml)	(Score)	(Score)	(Score)	(Score)	(Score)
1	1	3	0.3	3.48	20	4.52	8.8±0.42	8.3±0.48	8.0±0.47	8.0±0	8.1±0.31
2	1.71	3	0.25	3.5	22.6	5.65	8.7±0.42	6.9±0.87	8.2±0.91	6.9±0.73	6.9±0.73
3	0.5	5	0.29	3.38	20	4.52	8.5±0.52	7.9±0.69	8.3±0.48	8.0±0.63	8.1±0.63
4	1	3	0.3	3.48	20	4.52	8.8±0.42	8.3±0.48	8.1±0.56	8.2±0.42	8.1±0.31
5	1	3	0.29	3.47	20	4.52	8.8±0.42	7.9±0.87	8.2±0.63	7.7±0.48	7.8±0.63
6	1	0.17	0.23	3.67	23.2	6.14	8.9±0.31	8.8±0.63	8.7±0.48	8.9±0.31	9.0±0
7	1	5.82	0.37	3.39	16.4	4.52	8.4±0.51	8.6±0.42	7.9±0.31	8.7±0.42	8.7±0.42
8	1.5	5	0.32	3.49	18.7	5.65	8.5±0.52	7.6±0.31	8.0±0	7.6±0.42	7.5±0.31
9	1	3	0.3	3.48	20	4.52	8.8±0.42	7.5±0.87	8.0±0	7.5±0.48	7.0±0
10	1.5	1	0.24	3.54	23.7	5.06	8.8±0.42	8.0±0	8.6±0.51	8.0±0	8.1±0.56
11	0.29	3	0.18	3.52	24.1	4.52	8.6±0.69	6.5±1.26	8.2±0.78	6.5±1.17	6.6±1.07
12	0.5	1	0.18	3.68	24	5.8	8.8±0.42	7.5±0.70	8.3±0.67	7.6±0.69	7.5±0.52
13	1	3	0.3	3.48	20	4.52	8.8±0.42	7.4±1.07	8.0±0	7.4±0.51	7.4±0.78

* Over all acceptability (OAA) scored on nine point hedonic scale

Effect of variables on quality parameters:

Acidity, pH and Brix were considered as quality parameters for the independent variables. The acidity (% citric acid) ranged from 0.18 to 0.37%, pH from 3.38 to 3.68 and Brix° ranged from 16.4 to 24.1. Table 3 reveals that at linear level, yeast and fermentation time had a significant ($p<0.0001$) positive effect on acidity, and a negative significant effect on pH and Brix ($p<0.0001$). At quadratic level yeast had a negative significant effect ($p<0.0001$) on acidity and positive and significant effect ($p<0.0001$) on pH and Brix and fermentation time had a positive significant level at $p<0.001$ for pH and at negative effect for acidity and Brix. Yeast concentration and fermentation time had positive significant effect ($p<0.0001$) on pH but negative effect on acidity and Brix at interactive level.

Considering the statistical analysis for optimization of ingredients of cucumber fermented beverage the model obtained from the regression can be written as follows for quality parameters.

$$\text{Acidity} = 0.298 + 0.023\,X_1 + 0.048\,X_2 - 0.041\,X_1^2 + 0.001\,X_2^2 - 0.0075\,X_1X_2 \qquad R^2 = 0.99$$

$$\text{pH} = 3.478 - 0.007\,X_1 - 0.093\,X_2 + 0.016\,X_1^2 + 0.027\,X_2^2 + 0.063\,X_1X_2 \qquad R^2 = 0.99$$

$$\text{Brix} = 20 - 0.465\,X_1 - 2.327\,X_2 + 1.68\,X_1^2 - 0.093\,X_2^2 - 0.25\,X_1X_2 \qquad R^2 = 0.99$$

Table 3: Coefficient of second order polynomial regression models of cucumber fermented beverage with wet yeast

Coefficient	Acidity	pH	Brix	Vitamin C	Appearance	Flavour	Consistency	Taste	OAA*
	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9
β_0	0.298	3.478	20	4.52	8.8	7.88	8.06	7.76	7.68
β_1	0.023[a]	-0.007[d]	-0.465[a]	0.245[d]	0.025[b]	0.089	0.001	0.069	0.056
β_2	0.048[a]	-0.093[a]	-2.327[a]	-0.373[c]	-0.171[a]	-0.009	-0.216[c]	-0.006	-0.031
β_{11}	-0.041[a]	0.016[a]	1.68[a]	0.295[d]	-0.056[a]	-0.573[d]	0.083	-0.512[d]	-0.459
β_{22}	0.001	0.027[b]	-0.093	0.418[b]	-0.076[a]	0.441[d]	0.133[d]	0.553[d]	0.599[a]
β_{12}	-0.0075[b]	0.063[a]	-0.25[a]	0.468[d]	-0.015	-0.215	-0.15[d]	-0.203	-0.293[c]
R^2 %	99.73	99.61	99.87	88.98	99.61	85.14	89.51	90.14	83.63
p%	<0.0001	<0.0001	<0.0001	<0.01	<0.0001	<0.05	<0.05	<0.05	<0.05
Mean	0.273	3.50	20.97	4.95	8.72	7.79	8.19	7.79	7.77
SD	0.004	0.007	0.108	0.269	0.013	0.332	0.102	0.273	0.371
F-value	510.63	359.73	1110.8	11.31	357.50	8.023	11.947	12.798	7.149

Values with different superscripts are significant at the level, a: p>0.0001, b: p<0.001, c: p<0.01, d: p<0.05

* Over all acceptability (OAA)

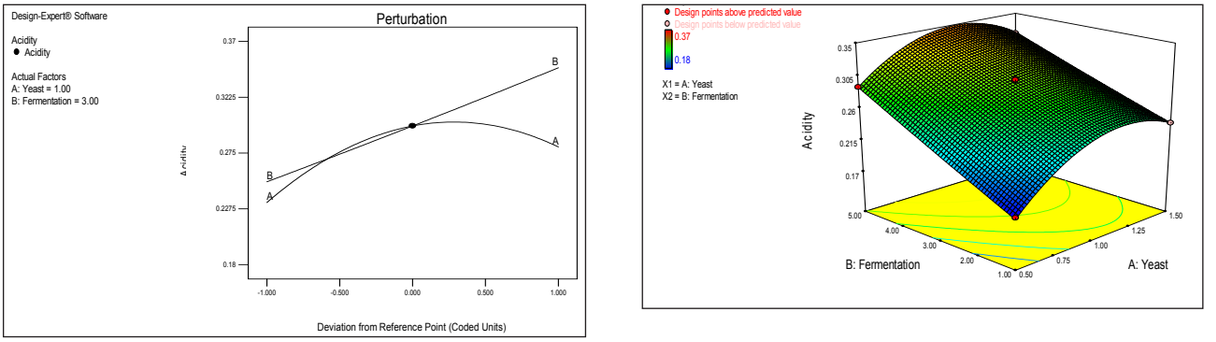
The surface plots for acidity, pH and Brix in relation to yeast and fermentation time are shown in Fig. 1a to 1c respectively. As yeast concentration increased, Brix decreased and as fermentation time increased there was increase in acidity and as time decreased there was decrease in pH and Brix of beverage.

Effect of variables on nutritional parameter

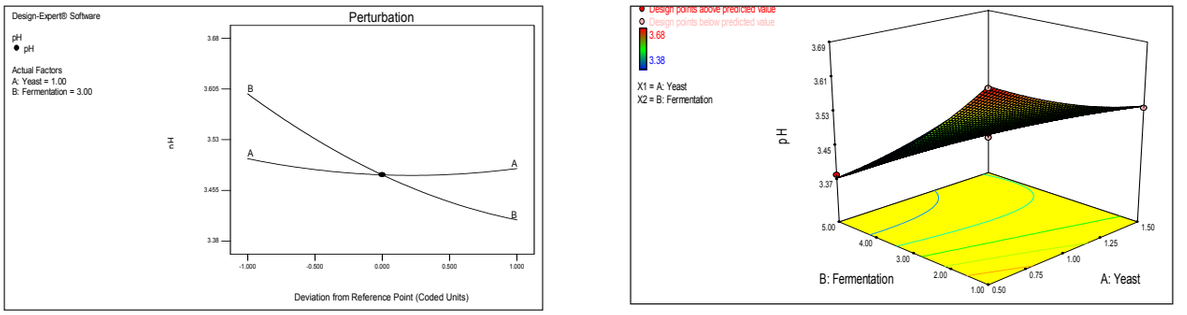
Nutritional parameters refer to as the minor nutrients such as vitamins are of great importance in the human diet. It is known that, the improvement of nutritional characteristics is seen during fermentation. In the present investigation, vitamin C has been studied and the concentration has been found to be 4.52 to 6.14 mg of per 100 ml fermented beverage. Fermented vegetable juices such as sauerkraut, red-beet, carrot, celery, tomato and hot pepper is reported to contain high amounts of protein, vitamins such as vitamin C, β-carotene, thiamine, riboflavin and minerals (Afanaseva *et al.,* 1992). Use of brewer's yeast in fermentation of vegetable juices is reported to have beneficial effects (Rakin *et al.,* 2005). Fermented carrot juice had higher calcium, phosphorus, iron and β-carotene, while fermented beetroot juice had higher contents of betanin and vitamin C. The fermented juices, a mixture of beetroot and carrot juices with brewer's yeast autolysate, represents the product with optimum proportions of pigments, vitamins and minerals. Considering the statistical analysis for optimization of ingredients of cucumber fermented beverage the model obtained from the regression can be written as follows for nutritional parameter.

$$\text{Vitamin C} = 4.52 + 0.245\ X_1 - 0.373\ X_2 + 0.295\ X_1^2 + 0.418\ X_2^2 + 468\ X_1 X_2 \qquad R^2 = 0.89$$

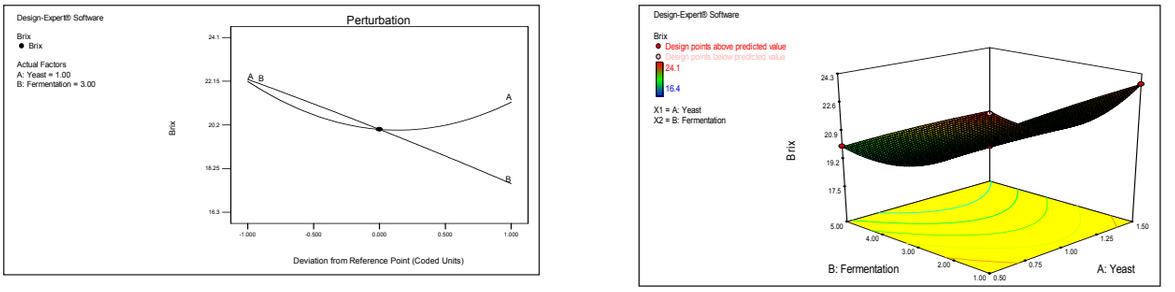
From the above equation, it can be concluded that vitamin C had significant negative effect on linear level for fermentation time (p<0.01) and positive effect for yeast (p<0.05). Yeast concentration and fermentation time had positive effect at quadratic level and interactive level. It is clear from Fig. 2, as there was increase in yeast concentration, there was increase in vitamin C. Abbas (2006) has reported that among the vitamins and other enzyme cofactors that are accumulated and/or synthesized by yeast are thiamine, nicotinic acid, pyridoxine and pantothenic acid. In the present study, with the increase in fermentation time there was decrease in vitamin,



1a:Acidity



1b: pH



1c:Brix

Fig. 1: Perturbation and 3D graph for quality parameters; 1a: acidity, 1b: pH and 1c: brix of cucumber fermented beverage

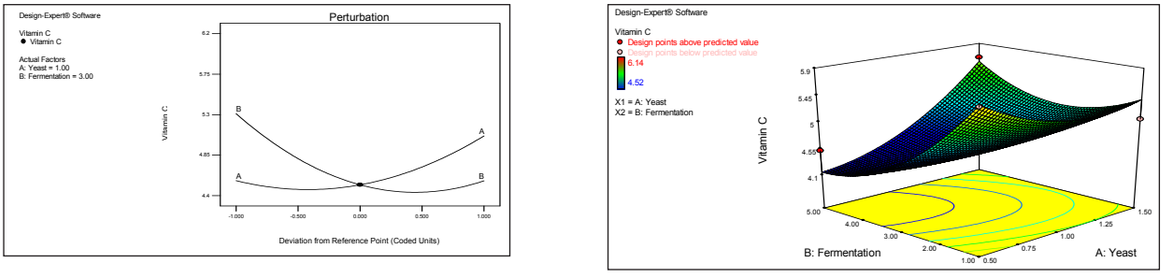


Fig. 2: Perturbation and 3D graph for nutritional parameters; vitamin C of cucumber fermented beverage

therefore the fermentation time has great influence on vitamin profile.

Effect of variables on sensory parameters

Sensory evaluation such as, appearance, flavour, consistency, taste and overall acceptability were selected as sensory parameters. The scores of sensory parameters ranged from 8.4 to 8.9 for appearance, 6.5 to 8.8 flavour, 7.9 to 8.7 consistency 7, 6.5 to 8.8 taste and 6.9 to 8.73 for overall acceptability on nine point hedonic scale. In the present study, optimization of the cucumber beverage showed that at linear level on all the sensory parameters such as appearance, flavour, consistency, taste and overall acceptability, yeast had a positive effect and fermentation time had a negative effect. At quadratic level, yeast effect on appearance, flavour, taste and overall acceptability were negative and fermentation time effect on flavour, consistency, taste and overall acceptability were positive. Lopez *et al.* (2008) also showed a positive effect of fermentation process by confirming that yeast are present throughout the fermentative process and it is generally accepted that they can produce compounds with important organoleptic attributes determining the quality and flavour of the final product. Considering the statistical analysis for optimization of ingredients of cucumber fermented beverage the model obtained from the regression can be written as follows for quality parameters.

$$\text{Appeanence} = 8.8 + 0.025\,X_1 - 0.171\,X_2 - 0.056\,X_1^2 - 0.076\,X_2^2 - 0.015\,X_1X_2 \qquad R^2 = 0.99$$

$$\text{Flavour} = 7.88 + 0.089\,X_1 - 0.009\,X_2 - 0.573\,X_1^2 + 0.441\,X_2^2 - 0.215\,X_1X_2 \qquad R^2 = 0.85$$

$$\text{Consistency} = 8.06 + 0.001\,X_1 - 0.216\,X_2 + 0.083\,X_1^2 + 0.133\,X_2^2 - 0.15\,X_1X_2 \qquad R^2 = 0.90$$

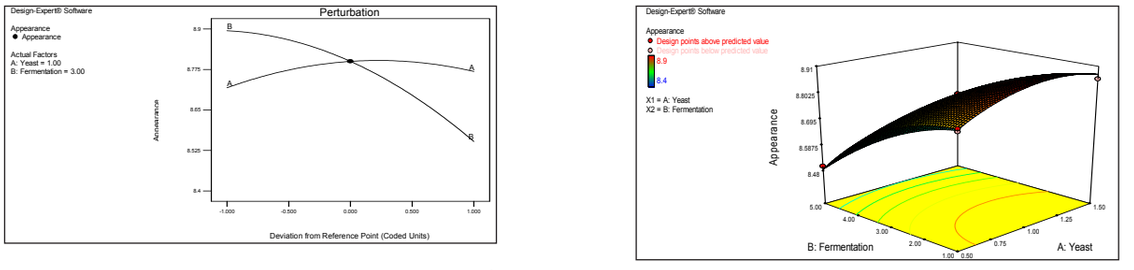
$$\text{Taste} = 7.76 + 0.069\,X_1 - 0.006\,X_2 - 0.512\,X_1^2 + 0.553\,X_2^2 - 0.203\,X_1X_2 \qquad R^2 = 0.90$$

$$\text{Over all acceptability} = 7.68 + 0.056\,X_1 - 0.031\,X_2 - 0.459\,X_1^2 + 0.599\,X_2^2 - 0.293\,X_1X_2 \qquad R^2 = 0.84$$

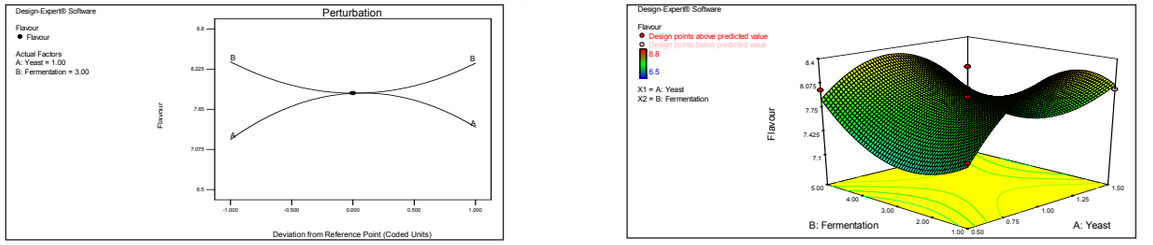
Fig. 3a to 3e represents the change in sensory parameters of fermented beverage with respect to yeast concentration and fermentation time. It is clear from Fig. 3a to 3e that fermentation time influenced all sensory parameters. Querol and Fleet (2006) also reported that in wines, among the 1,000 volatile compounds identified, more than 400 are produced by yeasts. Esters produced by yeasts can contribute to both aroma and flavour and several factors can contribute to aroma production by yeasts.

Optimization of independent variables

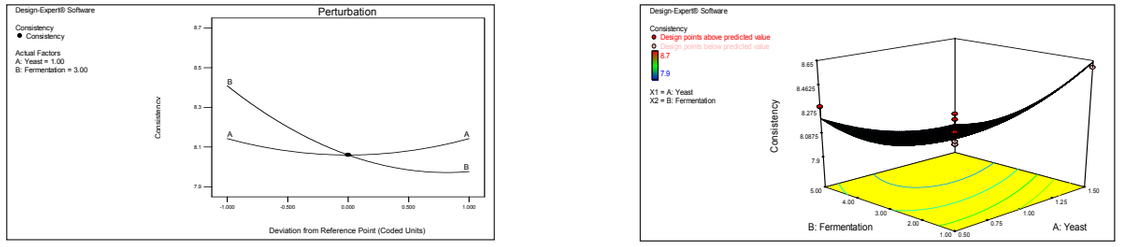
Numerical optimization of independent variables, yeast and fermentation time was achieved using design expert software. The predicted and actual values of the response are given in Table 4. The aim of the experiment was to increase over all acceptability score. The optimized solution was 1.3% yeast and 1 day fermentation time with best fit desirability of 0.74 (Fig. 4). The optimized results were acidity (%) 0.25, pH 3.56, Brix° 22.7, vitamin C 5.28 mg/100ml and organoleptic evaluation (score) – appearance 8.9, flavour 8.3, consistency 8.5, taste 8.3 and overall acceptability 8.4. The predicted response value as against actual value for responses as shown in Table 4 were in concurrence with each other, hence the fitted models are suitable for predicting the responses. RSM showed that quadratic response surface models were fitted. F values were significant in all selected responses and a high R^2 value of >90% showed fitness of the polynomial regression models for describing the effect of variables.



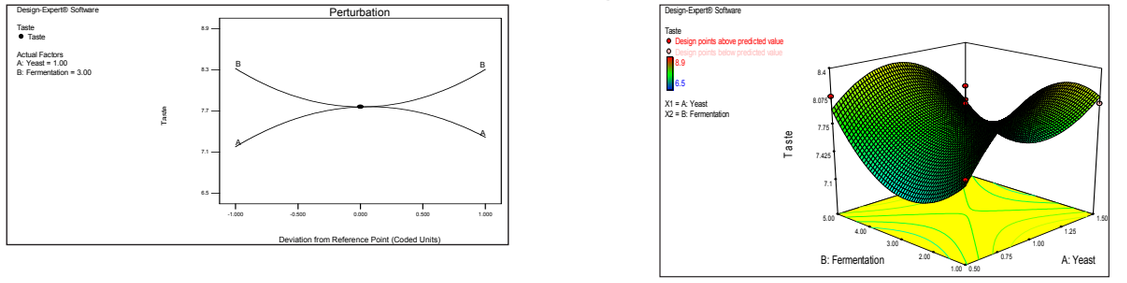
3a: Appearance



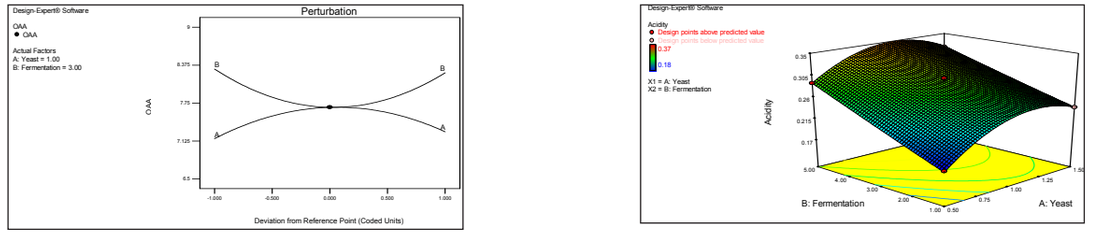
3b: Flavour



3c: Consistency



3d: Taste



3e: Overall acceptability

Fig. 3: Perturbation and 3D graph for quality parameters; 3a: appearance, 3b: flavour, 3c: consistency, 3d: taste and 3e: overall acceptability of cucumber fermented beverage

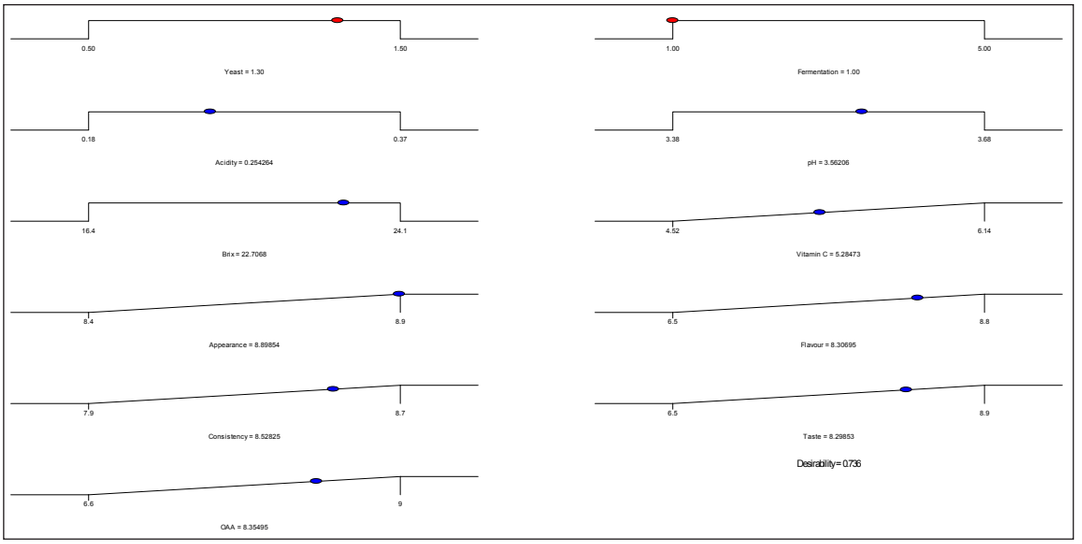


Fig. 4: Optimised levels of variable and their desirability plot for cucumber fermented beverage

Table 4: Predicted and actual response values

Responses (per 100ml)	Predicted	Actual (n=3)
Acidity (%)	0.25	0.24±0
pH	3.56	3.5±0
Brix °	22.7	22.6±0
Vitamin C (mg)	5.28	5.3± 0
Appearance (Score)	8.9	8.7±0.309
Flavour (Score)	8.3	8.3±0.429
Consistency (Score)	8.5	8.5±0.216
Taste (Score)	8.3	8.3±0.412
OAA*	8.4	8.4±0.209

Note: *Over all acceptability (OAA) scored on nine point hedonic scale

CONCLUSION

The results of the study indicated that the effect of yeast concentration and fermentation time were significant to all the selected responses and RSM could be useful in optimizing the yeast concentration and fermentation time with maximum retention of vitamin and sensory scores. Overall, the fermentation time and yeast concentration is optimized with 1.3% yeast concentration and 1 day fermentation time. From the observations, it can be concluded that fermentation helped to improve the functional and nutritional components of the fermented beverage when compared to the raw vegetable.

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