RESEARCH ARTICLE

APPLICATION OF RESPONSE SURFACE METHODOLOGY (RSM) IN OPTIMIZATION OF LACTIC ACID FERMENTATION OF CARROT: INFLUENCE OF ADDITION OF SALT, ADDITIVES AND GROWTH STIMULATORS

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ABSTRACT

Response surface methodology involving Central Composite Design (CCD) was applied to evaluate the effect of additives and growth stimulators with salt on natural lactic acid fermentation of carrot at room temperature. The variables investigated in this study were salt (2% - 3%), Lactose, $MgSO_4$ + $MnSO_4$ and Mustard (1%,1.5% and 2%) ; and various responses involved were per cent titrable acidity (Lactic Acid), pH, per cent salt and Lactic Acid Bacterial count (LAB count). The statistical analysis of the results showed that, only the treatments having powdered mustard had an appreciable effect on lactic acid fermentation. The best results were obtained in the treatment with 2.5 % salt, 1.5 % lactose, 1.5 % $MgSO_4$ + $MnSO_4$ and 2.0 % mustard. These optimized concentrations increased titrable acidity (1.69% as compared to the lowest value of acidity obtained i.e. 1.50%), lowered pH (2.47 as compared to the highest value of pH obtained i.e.3.30) and increased LAB count (9 X 10^8 cfu/ml as compared to the lowest value of count obtained i.e.6.13 X 10^8). The second-order polynomial regression model determined that the desirable characteristics would be obtained at these concentrations of additives. Hence, the values for different additives and growth stimulators optimized in this study could successfully be employed for the lactic acid fermentation of carrot.

INTRODUCTION

Among different vegetables produced and consumed in India, carrot (*Daucus carota*), occupies a significant place in Indian diet. Carrot belongs to the family Umbelliferae, is a native of Europe and is grown as a winter season root crop. These are highly regarded for their nutritional value because they are important source of provitamin A, thiamin, riboflavin, β-carotene and are consumed either raw or cooked (Burns 1997). In the production time there is glut of crop in the market leading to the postharvest losses. Lactic acid fermentation of vegetables has successfully used as one of the alternatives for preservation and to provide healthful product with diversified taste for consumers (Joshi and Sharma 2009; Joshi *et al.*, 2011). This vegetable in its natural state can be preserved for a very short period only and thus, their availability to the consumers remains seasonal. Among different methods, fermentation is one of the oldest methods of food preservation in the world (Joshi and Sharma 2009). The natural lactic fermentation a natural process is brought about by the lactic acid bacteria (LAB) present in the raw food or those derived from a starter culture. Also, its bio-preservation nature has

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gained increasing attention as natural means for enhancing the shelf-life and safety of food products (Ananou *et al.*, 2007; Joshi *et al.*, 2011). LAB are present on the raw vegetables as constituents of natural microflora and this can be employed for carrying out fermentation by adjusting the proper concentrations of salt, additives and growth stimulators (Daeschel *et al.*, 1987). Hence, at present fermentation of majority of vegetables is done by naturally occurring lactic acid microflora rather than by defined starter cultures (Pederson 1971). Lactic acid fermentation of vegetables has been successfully employed as for preservation and to provide healthful products to the consumers (Joshi and Sharma 2009). Recently, Lactic acid fermented vegetables have been employed to make various products viz., Ready- to-Serve drinks, sauces, appetizer and instant chutney mix powders with diversified taste and good acceptability (Joshi and Sharma 2010, Joshi *et al.*, 2011). Salt has historically been used for directing the fermentation of cucumber, radish and carrot (Fleming *et al.* 1978, Hudson and Buescher 1985, Fleming *et al.*, 1987). In LAF, salt withdraws water (plasmolysis) and nutrients from the vegetable tissue and the latter acts as the substrate for growth of lactic acid bacteria and thus, promoting anaerobiosis in the medium and inhibits the growth of unwanted microorganisms (Pederson 1971). Many ingredients apart from salt can be used in the preparation of lactic acid

fermented fruits and vegetables. These ingredients include, a source of nutrients such as sugars (Lactose), mineral salts (MgSO4 + MnSO4) and vitamins for fermentation causing microorganisms, help to restrict the growth of unwanted bacteria either through a regulatory effect on pH or by producing inhibitory substances and spices that have a final flavour determining role in fermented vegetables (Montet *et al.*, 1999). Mustard seeds in powdered form are also of great interest and has been used by various workers for the lactic acid fermentation of vegetables with varying concentrations (Anand and Das 1971; Sethi 1990). Mustard, like other condiments and spices not only imparts flavour and taste to the food products but also possesses preservative action (Anand and Johar 1957). Response surface methodology (RSM) is a useful approach to identify the optimum operating conditions of a given system. This method is employed to find out how a particular objective is affected by a given set of operating conditions of interest, and identify the optimum operating conditions (Box *et al.*, 1978; Giovanni 1983). The major advantage of RSM is to minimize the load of experiments as well as time (Tzeng *et al.*, 2009). Keeping all these advantages and uses of lactic acid fermentation of carrot into consideration, the present study was conducted to determine the effect of salt, additives and growth stimulators on natural lactic acid fermentation of carrot at room temperature, employing a Central Composite Design (CCD) and Response Surface Methodology (RSM) in order to optimize their concentrations for natural lactic acid fermentation. The results obtained are summarized in this paper.

MATERIALS AND METHODS

Raw materials

Carrot, mustard and salt used in the experiments were procured from the local market. The chemicals, *viz*. Lactose, Magnesium Sulphate (MgSO4), Manganese Sulphate (MnSO4) were of analytical grade and obtained from CDH ®.

Experimental design for optimization of lactic acid fermentation

Response surface methodology was applied to the experimental data using a commercial statistical package (Design expert, Trial version 8.0.5.2, State Ease Inc., Minneapolis, IN statistical software) for the generation of response surface plot and optimization of process variables. A factorial experiment was used to study the effects of various independent variables *viz*., salt (A), lactose (B), MgSO4 + MnSO4 (C) and mustard (D) on the response variables such as per cent titrable acidity (Y1), pH (Y2), per cent salt (Y3) and LAB count (Y4) of lactic acid fermentation of carrot. The averages from three replicated values of each run were taken as dependent variables or responses. For the statistical analysis, the numerical levels were standardized to -1, 0 and +1 (Table 1). The standard scores were fitted to a quadratic polynomial regression model for predicting individual Y responses by employing at least square technique (Wanasaundara and Shahidi 1966). The second order polynomial equation was fitted to the experimental data of each dependent variable as given. The model proposed to each response of Y was:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<j=1} \beta_{ij} X_i X_j \qquad \text{------------1}$$

Where βo, βi, βij are intercepts, quadratic regression coefficient terms Xi and Xj are independent variables. The model permitted evaluation of quadratic terms of the independent variables on the dependent variable. The response surface and contour plot were generated for different interactions of any two independent variables, where holding the value of third variables as constant at central level. The optimization of the process was aimed at finding the optimum values of independent variables.

Table 1. Process variables and their levels of experimental design

Symbols	Independent variable	Range and levels		
		-1	0	+1
A	Salt (%)	2	2.5	3
B	Lactose (%)	1	1.5	2
C	Magnesium sulphate + Manganese sulphate (%)	1	1.5	2
D	Mustard (%)	1	1.5	2

Statistical Analysis

Response Surface Methodology (RSM) was used to estimate the main effects of salt, lactose, MgSO4 + MnSO4, and mustard on lactic acid fermentation process. A central composite design was used with salt (2%, 2.5% and 3%), lactose (1%, 1.5% and 3%), MgSO4 + MnSO4 (1%, 1.5% and 3%) and mustard (1%, 1.5% and 3%) being the independent process variables (Table 1). For the generated 30 experiments, RSM was applied to the experimental data using design expert 8 (Design expert, Trial version 8.0.5.2, State Ease Inc., Minneapolis, IN statistical software). Data were statistically analyzed by using RSM (*F* test for analysis of variance ANOVA).

Lactic acid fermentation

Fermentation of carrot was performed similar to that practiced for sauerkraut fermentation and as done in our earlier studies (Joshi *et al.*, 2009). Optimally mature roots of carrot were washed, peeled and grated into shreds. The required concentration of salt (2%, 2.5% and 3%) along with additives and growth stimulators (1%, 1.5% and 3%) were added uniformly throughout the mass of shredded carrot. The shredded carrots were then packed tightly into the glass jars and then weighed down to expel the air in-between. During fermentation, the surface of carrot shreds was covered with a plastic bag. Sufficient water was added into the bag so that the carrot shreds slide down into the brine until the surface of the uppermost shreds was covered with the brine. The shreds were allowed to ferment at room temperature (25+2°C) in the laboratory. Appropriate parameters of Lactic Acid Fermentation viz. % titratable acidity, pH, % salt and LAB (Lactic acid bacteria) count were measured after the completion of Lactic Acid Fermentation (when no more acid was produced).

Analytical methods

The carrot shreds and brine were monitored for titratable acidity (%), pH and lactic acid bacterial (LAB) count during fermentation according to the standard methods. The pH was determined with digital pH meter (CRISON Instrument Ltd.

Table 2. Experimental design and results of the central composite design of 30 experiments for lactic acid fermentation of carrot

	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3	Response 4
Run	A	B	C	D	Y_1	Y_2	Y_3	Y_4
1	2.50	1.50	1.50	2.00	1.69	2.49	2.45	8.88
2	3.00	2.00	2.00	2.00	1.65	2.47	2.89	8
3	2.50	1.50	1.50	1.50	1.58	3	2.41	8
4	2.00	2.00	2.00	2.00	1.62	2.99	1.89	8.33
5	3.00	1.00	2.00	1.00	1.56	3.01	2.81	8
6	2.00	1.00	2.00	1.00	1.61	2.98	1.91	8.23
7	2.50	2.50	1.50	1.50	1.52	3.23	2.39	7.8
8	2.00	1.00	1.00	1.00	1.53	3.21	1.85	8
9	2.50	1.50	1.50	1.50	1.54	3.2	2.41	7.66
10	2.50	1.50	1.50	1.50	1.56	3.19	2.38	7.7
11	3.00	2.00	2.00	1.00	1.51	3.25	2.91	6.66
12	2.50	1.50	2.50	1.50	1.52	3.21	2.49	6.13
13	2.00	2.00	1.00	2.00	1.64	2.5	1.89	8.33
14	3.00	1.50	1.50	1.50	1.58	2.99	2.89	7.66
15	2.00	2.00	2.00	1.00	1.51	3.24	1.98	7
16	2.50	1.50	1.50	1.50	1.59	2.99	2.15	7.61
17	2.50	1.50	1.50	2.00	1.69	2.47	2.49	9
18	2.50	0.50	1.50	1.50	1.51	3.22	2.39	6.66
19	3.00	1.00	2.00	2.00	1.62	2.96	2.79	8.33
20	2.00	1.00	2.00	2.00	1.64	2.51	1.79	8.9
21	2.50	1.50	0.50	1.50	1.58	2.99	2.48	6.71
22	2.50	1.50	1.50	1.50	1.51	3.24	2.49	6.61
23	3.00	1.00	1.00	1.00	1.5	3.3	2.88	6.66
24	3.00	2.00	1.00	1.00	1.51	3.2	2.79	6.66
25	2.00	1.50	1.50	1.50	1.58	2.89	2.48	6.67
26	3.00	2.00	1.00	2.00	1.64	2.49	2.99	9
27	3.00	1.00	1.00	2.00	1.62	2.97	2.89	8.33
28	2.00	2.00	1.00	1.00	1.52	3.21	1.89	8.16
29	2.00	1.00	1.00	2.00	1.61	2.99	1.9	6.66
30	2.50	1.50	1.50	1.50	1.55	3.2	2.35	6.78

Spain) that was standardized with buffers of pH 4.0 and 7.0 before pH measurements. Titratable acidity and per cent salt estimation was measured bv the method of AOAC (1980) and Ranganna (2010), respectively.

LAB Count

Lactic Acid Bacterial (LAB) count of microorganisms was taken according to the method given by Harrigan and McCance (1996) using MRS agar media having composition (g/L): Peptic digest of animal tissue (10), yeast extract (5), beef extract (8), dextrose (20), polysorbate 80 (1), ammonium citrate (2), sodium acetate (5), magnesium sulphate (0.2), manganese sulphate (0.05), dipotassium phosphate (2) and agar (12). pH at 25oC of the medium was adjusted to 5.7+0.2. Incubation temperature for the cultures was 37+1oC.

RESULTS AND DISCUSSION

Experimental results of various responses

Table 2 reveals the experimental results of various responses (Y1, Y2, Y3 and Y4). It is discernible from the table that addition of the various additives and growth stimulators along with salt, especially mustard has resulted in imparting the desirable characteristics to the products (increased acidity, lowered pH, high LAB count). Mustard seeds contain allyl isothiocynate, a volatile aromatic compound with antibacterial and antifungal properties which may be responsible for the desired characters. Ground mustard seeds or oil have been used to increase lactic acid levels during fermentation of turnip (Anand and Das 1971). Among all the 30 runs employed, the range of per cent titrable acidity, pH, per cent salt and LAB count varied from 1.50% – 1.69%, 2.47 – 3.25, 1.79% - 2.99%

and $6.13 – 9 \times 10^8$, respectively. The highest per cent titrable acidity, lowest pH and highest LAB count was obtained with the Run 17 having salt- 2.5%, lactose- 1.5%, MgSO4 + MnSO4- 1.5% and mustard- 2 %. Addition of lactose at this concentration would have proved a potential energy substrate for lactic acid bacteria. However, the alternation (increase or decrease) in the concentration of the variables resulted in remarkable changes in the responses. For instance, at salt- 3%, lactose- 2%, MgSO4 + MnSO4- 1% and mustard- 1%, the per cent titrable acidity, pH, per cent salt and LAB count were recorded to be 1.51%, 3.2, 2.79% and 6.66×10^8, respectively which were less in magnitude as desirable. Hence, it can be inferred that their concentration at a moderate range is favourable for the lactic acid fermentation of carrot. This may be due to the favourable condition being generated inside the fermentation vessel by supporting the development of anaerobic conditions for the growth of LAB and hence, increasing the acidity and lowering the pH (Buckenhuskes *et al.,* 1988). Also, this concentration of salt (2.5 %) has been successfully used in the lactic acid fermentation of radish for shelf-stability and pickling (Joshi and Sharma 2009). It is also clear from the table that Y3 (per cent salt) remained more or less the same as it was added initially in the fermentation vessel indicating that salt was used for the stimulation of LAB and has not been used up.

Fitting Models

Experiments were performed according to the CCD experimental design given in Table 2 in order to search for the optimum combination of various parameters for the lactic acid fermentation of carrot. The experimental results along with the theoretically predicted values of all the responses are shown in Table 2. The responses of the CCD designs were fitted in the

mathematical expression of relationship in terms of coded factors with variables as shown below:

$$Y1 = 1.56 - 3.889 \times 10^{-3} A - 2.917 \times 10^{-3}B + 1.250 \times 10^{-3}C + 0.052D + 6.875 \times 10^{-3} AB - 6.250 \times 10^{-4}AC - 0.012BC + 0.013BD - 6.875 \times 10^{-3}CD - 8.000 \times 10^{-4}A2 - 0.013B2 - 4.475 \times 10^{-3}C2 + 0.038D2 \quad \text{------(1)}$$

$$Y2 = 3.07 + 6. \times 10^{-3}A - 0.023B - 8.333 \times 10^{-4} C - 0.24D - 0.067AB - 5.000 \times 10^{-3} AC - 0.014AD + 0.098BC - 0.086BD + 0.026CD + 0.014A2 + 0.048B2 + 0.017C2 - 0.21D2 \quad \text{….....(2)}$$

$$Y3 = 2.44 + 0.46A + 0.017B - 3.750 \times 10^{-3}C + 0.019D + 6.250 \times 10^{-4}AB - 0.012AC + 0.021AD + 0.021BC + 0.011BD - 0.032CD + 0.11A2 - 0.021B2 + 3.150 \times 10^{-3} C2 - 0.13D2 \quad \text{…….(3)}$$

$$Y4 = 7.40 + 1.111 \times 10^{-3}A + 0.096B + 0.062C + 0.47D - 1.875 \times 10^{-3}AB + 1.875 \times 10^{-3}AC + 0.37AD - 0.31BC + 0.30BD + 0.11CD - 0.26A2 - 0.045B2 - 0.25C2 + 1.04D2 \quad \text{…..(4)}$$

where Y1, Y2, Y3 and Y4 are per cent titrable acidity, pH , per cent salt and LAB count (cfu X 10^8) respectively, and A, B, C and D are the coded values of the test variables, salt, lactose, MgSO4 + MnSO4 and mustard respectively. The statistical

Table 3. Analysis of variance (ANOVA) for Response Surface Quadratic Model for titrable acidity during lactic acid fermentation of carrot

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	0.074	14	5.315E-003	6.79	0.0003*
A	2.722E-004	1	2.722E-004	0.35	0.5641
B	2.042E-004	1	2.042E-004	0.26	0.6169
C	3.750E-005	1	3.750E-005	0.048	0.8297
D	0.044	1	0.044	56.56	< 0.0001*
AB	7.563E-004	1	7.563E-004	0.97	0.3411
AC	6.250E-006	1	6.250E-006	7.989E-003	0.9300
AD	7.563E-004	1	7.563E-004	0.97	0.3411
BC	2.256E-003	1	2.256E-003	2.88	0.1101
BD	2.756E-003	1	2.756E-003	3.52	0.0801*
CD	7.562E-004	1	7.562E-004	0.97	0.3411
A^2	2.087E-006	1	2.087E-006	2.668E-003	0.9595
B^2	4.853E-003	1	4.853E-003	6.20	0.0250*
C^2	5.556E-004	1	5.556E-004	0.71	0.4126
D^2	4.628E-003	1	4.628E-003	5.92	0.0280*
Residual	0.012	15	7.823E-004		
Lack of Fit	6.785E-003	9	7.539E-004	0.91	0.5666[NS]
R-Squared	0.8638				
Adj R-Squared	0.7366				
Pred R-Squared	0.4015				
Adeq Precision	8.475				

(Df- Degree of freedom, NS- Not Significant, *- 5 % level of Significance)

Table 4. ANOVA for Response Surface Quadratic Model for pH during lactic acid fermentation of carrot

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	1.83	14	0.13	4.81	0.0023*
A	8.000E-004	1	8.000E-004	0.029	0.8661
B	0.013	1	0.013	0.48	0.4987
C	1.667E-005	1	1.667E-005	6.130E-004	0.9806
D	0.94	1	0.94	34.59	< 0.0001*
AB	0.073	1	0.073	2.68	0.1223
AC	4.000E-004	1	4.000E-004	0.015	0.9051
AD	3.025E-003	1	3.025E-003	0.11	0.7433
BC	0.15	1	0.15	5.59	0.0319*
BD	0.12	1	0.12	4.38	0.0538*
CD	0.011	1	0.011	0.41	0.5339
A^2	6.331E-004	1	6.331E-004	0.023	0.8808
B^2	0.065	1	0.065	2.39	0.1428
C^2	8.169E-003	1	8.169E-003	0.30	0.5917
D^2	0.14	1	0.14	5.21	0.0374*
Residual	0.41	15	0.027		
Lack of Fit	0.35	9	0.038	3.72	0.0618 [NS]
R-Squared	0.8179				
Adj R-Squared	0.6479				
Pred R-Squared	0.0698				
Adeq Precision	8.256				

(Df- Degree of freedom, NS- Not Significant, *- 5 % level of Significance)

Table 5. ANOVA for Response Surface Quadratic Model for per cent salt during lactic acid fermentation of carrot

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	3.90	14	0.28	11.86	< 0.0001*
A	3.79	1	3.79	161.27	< 0.0001*
B	7.004E-003	1	7.004E-003	0.30	0.5932
C	3.375E-004	1	3.375E-004	0.014	0.9062
D	5.720E-003	1	5.720E-003	0.24	0.6289
AB	6.250E-006	1	6.250E-006	2.659E-004	0.9872
AC	2.256E-003	1	2.256E-003	0.096	0.7610
AD	6.806E-003	1	6.806E-003	0.29	0.5984
BC	6.806E-003	1	6.806E-003	0.29	0.5984
BD	1.806E-003	1	1.806E-003	0.077	0.7854
CD	0.016	1	0.016	0.69	0.4186
A^2	0.036	1	0.036	1.54	0.2343
B^2	0.012	1	0.012	0.50	0.4899
C^2	2.753E-004	1	2.753E-004	0.012	0.9152
D^2	0.054	1	0.054	2.28	0.1514
Residual	0.35	15	0.024		
Lack of Fit	0.29	9	0.032	2.83	0.1089[NS]
R-Squared	0.9171				
Adj R-Squared	0.8398				
Pred R-Squared	0.7601				
Adeq Precision	10.489				

(Df- Degree of freedom, NS- Not Significant, *- 5 % level of Significance)

Table 6. ANOVA for Response Surface Quadratic Model for LAB count (cfu X 10^8) during lactic acid fermentation of carrot

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	17.69	14	1.26	3.79	0.0075
A	2.222E-005	1	2.222E-005	6.665E-005	0.9936
B	0.22	1	0.22	0.67	0.4269
C	0.093	1	0.093	0.28	0.6061
D	3.71	1	3.71	11.14	0.0045*
AB	5.625E-005	1	5.625E-005	1.687E-004	0.9898
AC	5.625E-005	1	5.625E-005	1.687E-004	0.9898
AD	2.14	1	2.14	6.42	0.0230*
BC	1.54	1	1.54	4.63	0.0481*
BD	1.47	1	1.47	4.41	0.0531*
CD	0.21	1	0.21	0.63	0.4405
A^2	0.22	1	0.22	0.66	0.4276
B^2	0.056	1	0.056	0.17	0.6880
C^2	1.70	1	1.70	5.09	0.0394*
D^2	3.54	1	3.54	10.62	0.0053*
Residual	5.00	15	0.33		
Lack of Fit	3.42	9	0.38	1.45	0.3365[NS]
R-Squared	0.7796				
Adj R-Squared	0.5738				
Pred R-Squared	-0.1384				
Adeq Precision	7.278				

(Df- Degree of freedom, NS- Not Significant, *- 5 % level of Significance)

significance of the model equations were evaluated by the F test for analysis of variance (ANOVA). The results of multiple linear regressions conducted for the second order response surface model are given in Table (3-6). The significance of each coefficient was determined by Student's t-test and p-values. Model F-values of 6.79, 4.81, 11.86 and 3.79 for Y1, Y2, Y3 and Y4 respectively, imply that the models were significant; there is only 0.03%, 0.23%, 0.01% and 0.75% chances respectively that a "Model F-Value" this large could occur due to noise. Table 3-6 reveal that for titrable acidity D, BD, B2 and D2 were significant model terms; for pH D, BC, BD and D2 were significant; for salt estimation A was significant model terms; whereas, in case of LAB count D, AD, BC, BD, C2 and D2 were significant model terms. The "Lack of Fit F-values" of 0.0067, 0.35, 0.29 and 3.42 for Y1,

Y2, Y3 and Y4 respectively, imply that the Lack of Fit is not significant relative to the pure error which indicates towards the fitness of models. The goodness of fit of the models were checked by the determination coefficient (R2). All the independent variables showed the values of R2 to be > 0.75 and "Adeq Precision" greater than 4 which indicated aptness of the model and adequate signal respectively. Hence, the models were used to navigate the design spaces (Box *et al.*, 1978)
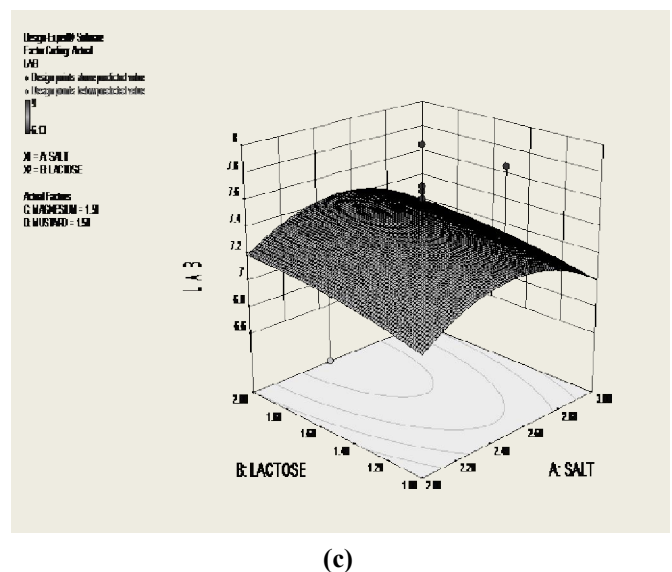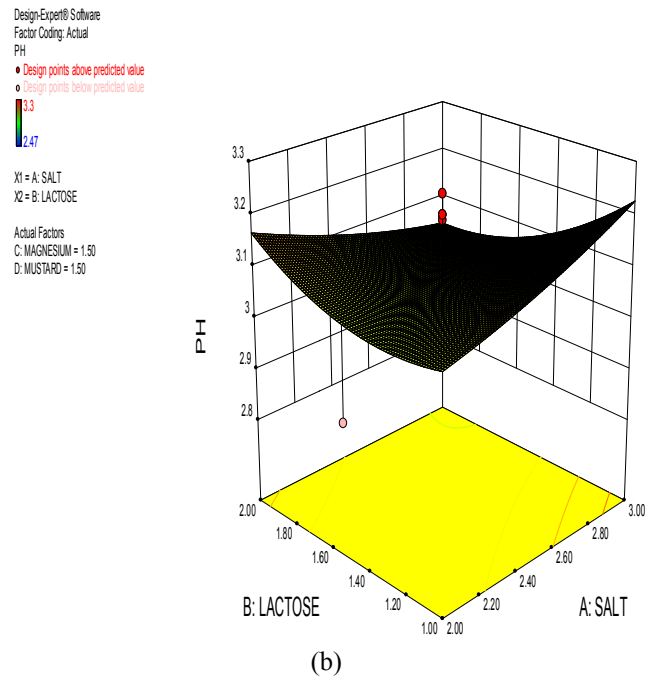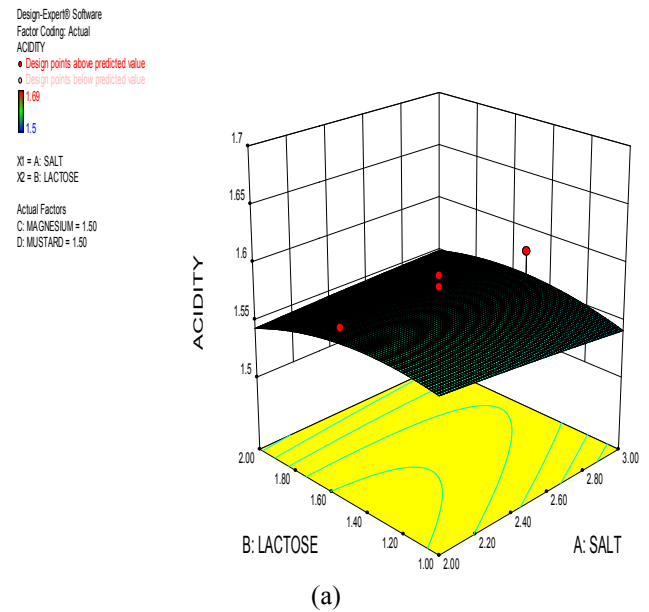
3D RESPONSE SURFACE GRAPHS

Response surface plots as a function of two factors at a time, maintaining all other factors at fixed levels are more helpful in understanding both the main and the interaction effects of these two factors. These plots can be easily obtained by

calculating from the model, the values taken by one factor where the second varies with constraint of a given Y value. The response surface curves were plotted to understand the interaction of the variables and to determine the optimum level of each variable for maximum response. The response surface curves for Y1, Y2, Y3 and Y4 are shown in Figure 1. The nature of the response surface curves shows the interaction between the variables. From figures it was observed that the elliptical nature of the contour in 3D-response surface graphs depict the mutual interactions of all the variables in providing significant effect on responses. In general all the additives contributed commutatively towards the fermentation process by increasing the acidity and LAB count; and lowering the pH. However, the analysis of the results and response graphs showed that, in the range studied, only the treatments having grounded mustard had an appreciable effect on lactic acid fermentation w.r.t these desirable characteristics. The response surface graph (Fig. 1a) depicts the main effect and interaction of lactose and salt on titrable acidity while keeping the concentration of mustard and MgSO4 + MnSO4 constant at 2.0%.

It is clear from the shape of the graph that with the increase in the concentration of additives and growth stimulators, there was increase in the titrable acidity. Maximum acidity was achieved at salt (2.5%), lactose (1.5%), mustard (2%) and MgSO4 + MnSO4 (1.5%). It's also clear from the graph that the titrable acidity decreased to 1.50 when lactose and salt were at 1 and 1 per cent, respectively. It is apparent that this concentration of lactose and salt was less and not optimum. This reduced acidity was obtained in our earlier work (Joshi *et al*., 2008). Fig. 1b depicts the interaction of various variables on pH in the form of 3D response graph. It can be inferred from the graph that ph was indirectly proportional to the concentration of variables used in the experiments. The lowest value of ph was desirable for the fermentation processs. The lowest ph was observed when fermentation was carried out at salt (2.5%), lactose (1.5%), mustard (2 %) and MgSO4 + MnSO4 (1.5%). Lowering of ph helps to prevent the growth of pathogenic and undesirable microorganisms which leads to biopreservation.

The interactions between lactose and salt at 1.5 % of mustard and MgSO4 + MnSO4 on per cent salt estimation is depicted in the form of 3D Response surface graph (Fig. 1c). The flattened and straight slope of response plots indicated that the estimation of salt content did not show any specific pattern or alterations and neither this was expected except that equilibrium salt contents are relevant for the LAB to grow and show the activities. The main function of salt is to stimulate the LAB and it can neither be excreted nor synthesized, therefore its concentration did not vary in the entire process. Similar but little elliptical graph was observed in case of LAB count as compared to the response graph of titrable acidity which also indicated that the variables under study have significant effect on LAB count (Fig. 1d). Maximum bacterial count was observed when the fermentation was carried out by using salt (2.5%), lactose (1.5%), mustard (2 %) and MgSO4 + MnSO4 (1.5%). However, close to this value of LAB count was observed at lactose, mustard, MgSO4 + MnSO4 (1-1.5 %) and salt (2-2.5%). Further, it is evident from the magnitude of P and F values that there is maximum positive contribution of
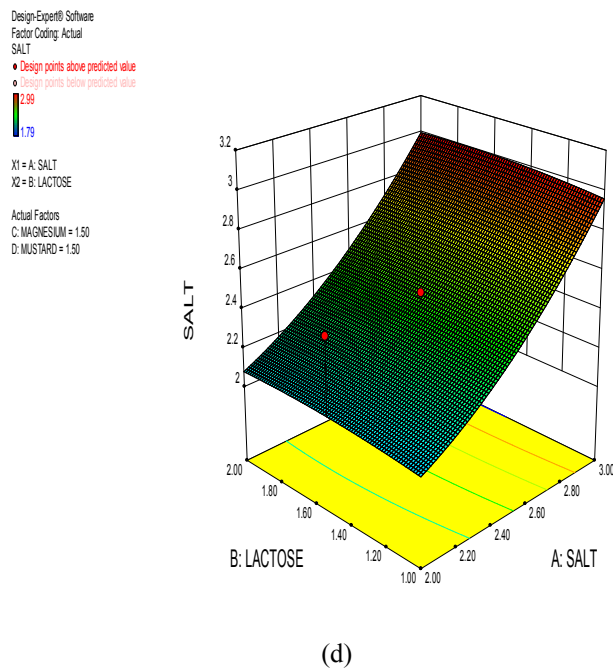

(a)

(b)

(c)

Design-Expert® Software
Factor Coding: Actual
SALT
Design points above predicted value
Design points below predicted value
2.99
1.79

X1 = A: SALT
X2 = B: LACTOSE

Actual Factors
C: MAGNESIUM = 1.50
D: MUSTARD = 1.50

(d)

Fig.1. 3D Response surface graph of carrot fermentation depicting the interactions between Lactose and Salt at 1.5 % Mustard and Mg SO$_4$ + MnSO$_4$ on Acidity (a), pH (b) Salt (c) and LAB count (d)

all the variables viz., salt , lactose , mustard and MgSO4 + MnSO4 on the various responses: titrable acidity, pH and LAB count during the lactic acid fermentation of carrot (Tables 3 – 6).

Optimum concentrations of additives and growth stimulators

Optimum concentrations of salt, additives and growth stimulators for lactic acid fermentation of carrot was determined to obtain the best results w.r.t the desirable characteristics i.e. increased titrable acidity, low pH and high LAB count for Lactic Acid Fermentation. Second order polynomial models obtained in this study were utilized for each response in order to determine the specified optimum concentrations of salt, additives and growth stimulators. The sequential quadratic programming using design expert 8.0.5.2 was used to solve the second degree polynomial regression equation. The optimum values obtained by substituting the respective coded values of variables are: salt – 2.5%, lactose- 1.5 %, MgSO4 + MnSO4 – 1.5 % and mustard 2 %. At this point, all the desirable characteristics were achieved to be the best in fermentation process as these optimized concentrations increased titrable acidity (1.69% as compared to the lowest value of acidity obtained i.e. 1.5%), lowered pH (2.47 as compared to the highest value of pH obtained i.e.3.30) and increased LAB count (9 X 10^8 cfu/ml as compared to that obtained with the lowest value of 6.13 X 10^8).

Conclusion

In this study, RSM was used to determine the optimum operating conditions that yield increased titrable acidity, low pH and high LAB count for Lactic Acid Fermentation of carrot. Analysis of variance has shown that the effects of all the process variables including salt, lactose, MgSO4 + MnSO4 and mustard were statistically significant. Second order polynomial models were obtained for predicting per cent titrable acidity, salt, pH and LAB count. Among 30 runs conducted, run 2 has got the optimum concentration of salt – 2.5%, lactose- 1.5 %, MgSO4 + MnSO4 – 1.5 % and mustard - 2 % for lactic acid fermentation of carrot.

REFERENCES

Anand JC, Das L .1971. Effects of condiments on lactic fermentation in sweet turnip pickle. *J. Food Sci. Technol.*, 8(3): 143.

Anand JC, Johar DS .1957. Effect of condiments on control of Aspergillus niger in mango pickle. *J. Sci. Indus. Res.*, 16A: 370.

Ananou S, Maqueda M, Martínez-Bueno M, Valdivia E .2007. Biopreservation, an ecological approach to improve the safety and shelf-life of foods Communicating Current Research and Educational Topics and Trends in Applied Microbiology

Aoac. 1980. Official methods of analysis, 13th edn, Association of Official Analytical Chemists, Washington DC

BOX GEP, Hunter WG, Hunter JS. 1978. Statistics for Experimenters, pp. 91-334, John Wiley & Sons. New York. NY.

Burns EE .1997. Carrots. In: Processing Vegetables Science and Technology. D.S. Smith, J.N. Cash, W.K. Nip, Y.H. Hue (eds.). Technomic Publishing Co., Ind. Lancaster, USA.

Daeschal MA, Andersson RE, Fleming HP .1987. Microbial ecology of fermenting plant materials. *FEMS Microbial Rev.*, 46: 357-367.

Fleming HP, Mcfeeters RF, Thompson RL .1987. Effects of sodium chloride concentration on fermness retention of cucumbers fermented and stored with calcium chloride. *J. Food Sci.*, 52: 653-657.

Fleming HP, Thompson RL, Bell TA, Hontz LH .1978. Controlled fermentation of sliced cucumbers. *J. Food Sci.*, 43: 888.

Giovanni WA. 1983. Response surface methodology and product optimization. *Food Technol*. 37, 41-45.

Harrigan WF, McCance EM .1966. (eds.) Laboratory Methods in Microbiology. 2nd edn. Academic Press, London.

Hudson JM, Buescher RW .1985. Pectic susbtances and firmness of cucumber pickles as influenced by CaCl2, NaCl and brine storage. *J. Food Biochem.*, 9: 211-215.

Joshi VK, Sharma Somesh. 2010. Preparation and evaluation of sauces from lactic acid fermented vegetables. *Journal of food science and technology*. 47(2). 214-218

Joshi VK, Somesh Sharma .2009. Lactic acid fermentation of radish for self stability and pickling. *Natural Product Radiance*. 8(1). 19-24

Joshi VK, Somesh Sharma, Neerja Rana .2011. Preparation and evaluation of appetizers from lactic acid fermented vegetables. *Journal of Hill Agriculture*. 2(1). 20-27

Joshi VK, Sharma S, Thakur NS. 2008. Effect of temperature, salt concentration and type of microorganism on lactic acid fermentation of carrot. *Acta Aliment* 37:205–219

Montet D, Loiseau G, Zakhia N, Mouquet C .1999. Fermented fruits and vegetables. In: Biotechnology: Food Fermentation. Vol. II. V.K. Joshi and Ashok Pandey (eds.). *Edu. Publ. Distri.*, New Delhi. pp. 951-969.

Pederson CS.1971. In: Microbiology of Food Fermentation. AVI Publishing Co. Inc., Westport, Connecticut, pp. 108-152.

Ranganna S .1986. Handbook of Analysis of Quality Control for Fruit and Vegetable Products. 2nd Edn. Tata McGraw Hill Publ. Co., New Delhi.

Sethi V. 1990. Lactic fermentation of black carrot juice for spiced beverage. *Indian Food Packer*, 44(3): 7-12.

Tzeng Daw-I, Chao-Ying Tai, Yi-Chen Chia, Yeu-Pyng Lin, Andi Shau-Mei Oi. 2009. Investigation of fermenting conditions for sugarcane (Saccharum Officinarum l.) Wine using response surface methodology. *Journal of food processing and preservation*. 33. 330-346

Wanasundara F, Shahidi. 1996. Optimization of hexa metaphosphate-Assisted Extraction of Flaxseed Proteins Using Response Surface Methodology. *Journal of Food Science,* Volume 61, Issue 3, pages 604-607.

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