ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Optimization and Development of Functional Fiber Enriched Retort pouch processed Ready-To-Eat (RTE) Mixed Vegetable Curry by Response Surface Methodology

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Manuscript Info

Manuscript History:

Received: 15 March 2015
Final Accepted: 22 April 2015
Published Online: May 2015

Key words:

Response surface methodology (RSM), Mixed Vegetable curry, Retort processing, Beta-glucan, RTE

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Abstract

Beta glucan is one type of valuable dietary fiber present in cereal crops, especially in barley, oat, and some mushrooms. Thermal processing of Beta glucan enriched curry is perfrormed to extend the shelf-life with high quality and commercial sterility. The product was optimized using Response surface Methodology (RSM). In this study the experimental designs were performed to optimize the *β*-glucan enriched mixed vegetable curry, by varying the quantities of different variables like potato, capsicum and *β*-glucan, in order to obtain a response of maximum fibre content and overall acceptability. The predicted and the actual values obtained were almost similar. The fiber content of the curry was found to be dependent on the quantity of vegetables in combination with *β*-glucan content. The lethality of the *β*-glucan enriched mixed vegetable curry achieved the desired F_0 *value* 4.5 and found to be commercial sterility during entire storage with good sensory quality. The samples were analyzed for changes in proximate, sensory quality attributes, microflora and color. The product remained commercially sterile and confirmed the adequacy of the processing as well as its fitness for consumption.

INTRODUCTION

In recent years, there is an increase in consumer demand for the fiber-rich products due to their health concern. Dietary fiber (DF) plays an important role in decreasing the risks of many disorders such as constipation, diabetes, cardiovascular diseases (CVD), diverticulosis and obesity (Spiller, 2001). Presently fibre-rich products in the market are primarily those of natural origin (oat, peas, maize and grain bran) which may be added to food without modifications, as well as those that must be modified before they are added. *β*-glucan is the principal fiber present in barley and oat, although barley is an excellent source of *β*-glucan, yet on a worldwide basis, a limited amount of the barley is also used as a source of *β*-glucan in various foods for human consumption but the major quantities of barley are used for animal feed (FAO, 2001). Concentrated dietary fibre like *β*-glucan (oat and barley) obtained from cereal sources are used directly as meal supplements, as well as components of fibre-rich preparations. It is a principal fiber which reduce onset of colorectal cancer, reduction in glycemic index (Cavallero *et al*., 2002; Jenkins *et al.*, 2002; Granfeldt *et al.*, 2008), reduction in serum cholesterol levels, prevention of coronary heart disease, prevention of hepatic damage and promotion of the growth of beneficial gut microflora (Crittenden *et al*., 2002; Tungland, 2002). Apart from health and nutritional benefits (Malkki and Virtanen, 2001), β-glucan also has various suitable functional properties such as thickening, stabilizing, emulsification, and gelation. These properties determine the suitability of *β*-glucan to be incorporated in products like curries.

There is an increasing consumer demand for high quality convenient ready-to-eat food products and has led to an increase in the commercial production of ready-to-eat products (Kumar *et al.,* 2013). Ready-to-eat food is a convenience food which is commercially prepared for ease of consumption. These foods were initially consumed by military people, disaster victims, campers etc (Kanatt *et al*., 2005; Karadag & Gunes, 2008). Retort processing technology which has been widely familiar as one of the alternatives to metal cans for producing thermally processed shelf stable foods (Sabapathy *et al*., 2000). Retort processing has evolved significantly since its incorporation into Dept. of Defence (DOD) and NASA food systems. While the technology still relies on aggressive application and penetration of heat throughout foods, recent advancements in process engineering coupled with evolution of packaging technologies have allowed for an overall improvement of the technology (Goddard, 1994; Jun *et al*., 2006). The retort pouch has many advantages over canned and frozen food packages for both the customers as well as food manufactures. The advantages are pouch profile, storage and preparation efficiency, savings in transportation, package cost, improved flavour and savings of energy (Kumar *et al*., 2007).

Statistical design tools such as response surface methodology (RSM) are quite effective in optimizing the new product development or processing parameters. RSM uses a central composite rotatable design to fit a polynomial model by least-square technique. RSM helps to create a product using regression equations that describe inter-relations between input parameters and responses. The major constraint an optimizing procedure is that the desired degree of acceptance or health benefits must be achieved (Kathiravan *et al.,* 2013). Therefore, the main objective of this research work was optimization of vegetables and functional fiber (β-glucan) for the development of β-glucan enriched retort pouch processed ready-to-eat (RTE) mixed vegetable curry (MVC) by using Response Surface Methodology (RSM) with the aim of selecting the combination of vegetables with β-glucan to obtain ready-to-eat (RTE) mixed vegetable curry (MVC) with maximum fibre content as well as good sensory quality attributes.

Materials and Method

Samples

All the vegetables were procured from the Mysore local market. Vegetables were washed with tap water followed by sterile water and cut into pieces of approximately 1 inch size. The other ingredients, which were used for the preparation, were also procured from the Mysore local market. Beta glucan was commercially purchased from M/s. Impex Pvt (Ltd) Delhi.

Experimental Designs

A face-centered central composite response surface analysis was used to determine the effect of potato, capsicum and functional fiber (β-glucan) on total fiber and sensory quality (OAA) of β-glucan enriched mixed vegetable curry. The selected responses were total fibre and sensory overall acceptability. The independent variables were potato (10-13 g), capsicum (9-13 g) and β-glucan (1-3 %). The levels for each independent parameter were presented in the Table 1. The experimental design was performed in one block of experiments. The order of assays within block was randomised and performed in triplicate. The results for the central composite rotatable designs (CCRD) were used to fit second-order polynomial equation. However, the regression analysis of the responses was conducted by fitting suitable models represented by (1) & (2).

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i \quad (1)$$

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i \neq j=1}^{n} \beta_{ii} x_i x_{ij} \quad (2)$$

where, β_0 was the value of the fitted response at the center point of the design, i.e., point (0,0,0) in case potato-capsicum-beta glucan; βi, βii and βij were the linear, quadratic and cross product (interaction effect) regression terms respectively and n denoted the number of independent variables (Kathiravan *et al.,* 2013).

Analysis of Variance (ANOVA) was performed to obtain the coefficients of the final equation for better accuracy. Design Expert 7.0.0 software (Stat Ease Inc., Minneapolis, MN) was used to generate models that fit the experimental data, draw the response surface plots and optimise the product. Three-dimensional surface plots were drawn to illustrate the interactive effects of two factors on the dependent variable, while keeping constant the other variables. The optimisation of product was done following the method of Kathiravan *et al.,* (2013). All the individual desirability functions obtained for each response were combined into an overall expression, which is defined as the geometrical mean of the individual functions. The higher the desirability value, the more adequate is the system. In the present study, desirability functions were developed in order to obtain optimized functional fibre (β-glucan) enriched mixed vegetable curry with the maximum sensory acceptability. All variables of the polynomial regression at a significance level of $p < 0.05$ were included in the model, and the coefficient of determination (R^2) was generated in order to assess the adequacy of the model.

Curry preparation

Mixed vegetable curry, a heterogeneous vegetarian product containing onion, garlic, ginger, spices and hydrogenated fat. The vegetables were blanched in boiling water bath. The 15 different combinations were prepared separately according to central composite rotatable designs (CCRD). Figure 1 describes all the different combinations of the mixed vegetable curry preparation and retort processed.

Packaging material

Pre-fabricated multilayer laminated retortable pouches consisting of 12 μm Polyester/12µm Aluminium foil/75 µm Cast-Polypropylene (PET/ Aluminium foil / C.PP) of dimension 15 cm × 20 cm were used to fill the product.

Filling and sealing

The optimized combination of mixed vegetable curry was prepared in bulk and 200 g of the product was filled manually and then, entrapped air in the head space was manually squeezed out before sealing the top of the pouch hermetically by an impulse heat sealer (Model: HP Impulse Sealer, M/S Sunray Industries Mysore, India). The filled and sealed pouches were loaded into Retort vessel.

Retort pouch processing of mixed vegetable curry

Retort pouch processing of mixed vegetable curry was carried out in a steam-air retort (M/s. Alpha Steritech, Bangalore, India) following the method of Kumar *et al.,* (2013). The retort was equipped with facility for using compressed air for over-riding pressure and a high-pressure water-circulating pump for pressurized cooling. The temperature of the product was continuously recorded during heat processing, through copper-constantan thermo couples, which were fixed at the geometric centres of the pouches and connected to a data logger (Model: E. Val flex, M/s. Ellab, Denmark).The pouches were placed at different locations in the retort. The temperature of the pouch and retort was calculated from the thermo-electro-motive force at regular intervals of 1 min. The F_0 *value* was calculated from the temperature and time history. The pouches were initially heated till there inside temperature reached 121°C. Subsequently, the pressure of the steam was raised in stages; from 5 lbs to 15 lbs. gauge pressure with the increase of temperature progressively. The processing was carried out to achieve a F_{18} *250 value* of 2.0 with maximum temperature of 118°C. After attaining the required F_0 *value***,** the product temperature was brought down to 50-55°C by pressurized cooling (compressed air and water) in 4-5 minutes. The cooled pouches were wiped dry and examined for any visual defects

Analysis of samples

Mixed vegetable curry with and without beta glucan was analysed for the changes in color, proximate composition [moisture, fat, protein, and carbohydrates (carbohydrates was calculated by difference)], microflora and sensory attributes according to the method described by Kumar *et al.,* (2013). Moisture and fat free samples were analyzed for their total dietary fiber (Insoluble and Soluble dietary fiber) contents by enzymatic and gravimetric method (Asp *et al.,* 1983). All the analysis was carried out in triplicate.

Result and Discussion

The vegetables and functional fibre content were optimized using Response Surface Methodology (RSM) and the responses were found to be fit with linear model. The *p-value* given in the parameters for each response were the model significance. The *p-value* indicates the p > F- values which should be less than 0.05 for model to be significant. The experimental design along with each experimental condition is shown in Table 2. The effect of changes in levels of selected variables on the response parameters has been represented in Figures 2-8

Experimental design for *β*-glucan enriched mixed vegetable curry

Table 2 represents the experimental design of *β*-glucan enriched mixed vegetable curry. The results show that the *β*-glucan variable had a significant effect, and was increasing by increase in fiber content in *β*-glucan enriched mixed vegetable curry. The fibres of potato and capsicum did not show much variation in fiber content of mixed vegetable curry.

Mixed vegetable curry with 10g of potato, 13g of capsicum and 1 % of *β*-glucan revealed 9.2812 % of fiber this indicated the reduction in fiber was due to decrease in *β*-glucan content. The same was observed when 11.50g of potato, 11g of capsicum and 0.32 % of *β*-glucan fiber content revealed to be 3.216 % which was also due to decrease in concentration of *β*-glucan. The product with variables of 11.5g of potato, 11g of capsicum and 2 % of *β*-glucan exhibited the fiber content as 11.00 %, which was slightly increased as compared to product containing 1 % of *β*-glucan The product with 11.5g of potato, 11g of capsicum and 3.68 % of β-glucan high exhibited high fiber as 16.218 % but was not acceptable due to inappropriate concentration of potato, capsicum and *β*-glucan. The maximum fiber content 15.39% was achieved when mixed vegetable curry contained 13g of potato, 13g of capsicum and 3% of *β*-glucan. ANOVA was made for the experiments and presented in the Table 3. The *β*-glucan concentration of the mixed vegetable curry was found to be significant role in the modification of the fibre content and the model found to be linear models described with an of accuracy $R^2 = 0.9032$. The 3D plot of figure 2, 3 and 4 represented the relative effect of variables (potato and capsicum), (*β*-glucan and potato) and (*β*-glucan and capsicum) on fibre content. Figure 2 a 3D plot depicting that the effect of potato and capsicum on fiber content of mixed vegetable curry and found to be not having much influence compared to *β*-glucan combination with vegetables. Figure 3 3D plot depicts the effect of potato and *β*-glucan on fiber content. The fiber content was increasing linearly with increasing β-glucan content. Figure 4 3D plots depicting the relative effect of capsicum and *β*-glucan content showed that almost the similar trend as the effect of potato and *β*-glucan on fiber content.

Over all acceptability (OAA) was the most important sensory quality criteria for over all acceptability of any RTE food products, it was also taken as a response for the *β*-glucan enriched mixed vegetable curry. Table 2 represents the experimental design for over all acceptability (OAA). The combination of the vegetables and functional fibre increased the *β*-glucan content of the product. Over all acceptability (OAA) of mixed vegetable curry with 10g of potato, 13g of capsicum and 1% of *β*-glucan was found to be 6.80. The product with 11.5g of potato, 11g of capsicum and 2% of *β*-glucan had OAA as 7.30. The mixed vegetable curry with 13g of potato, 13g of peas and 3% of *β*-glucan had OAA as 8.00, it was due to the combination of the potato, capsicum and *β*-glucan. The increase in *β*-glucan content increased the thickness of the curry and found to be good. The relative effect of variables (Potato and capsicum), (*β* glucan and potato) and (*β* glucan and capsicum) on overall acceptability was represented in 3D plot of figure 5, 6 & 7 respectively. Figure 5 3D plots depicting that the effect of potato and capsicum on OAA content of mixed vegetable curry has not much influence compared to *β*-glucan. Figure 6 & 7 3D plots depicting the effect of potato, capsicum and *β*-glucan content on OAA content. The increase in *β*-glucan content in mixed vegetable curry enhanced the texture of the curry and led to an increase in the Overall acceptability (OAA) of the mixed vegetable product. The multiple regressions were made to fit the model for β-glucan enriched mixed vegetable curry. Multiple regression equations (in terms of coded factors) was obtained for response of fiber content has been represented as follows:

*Total Dietary Fibre Y = +11.42 + 2.426E-003 * A +5.411E-003 *B +3.32 *C*

*OAA Y = +7.20 + 0.017 * A -0.12 *B + 0.51* C*

The synergy between factors of potato, capsicum and *β*-glucan content was significantly ($p < 0.05$) affecting the fibre content of the *β*-glucan enriched mixed vegetable curry.

Optimization of vegetables (potato and capsicum) and *β*-glucan content in *β*-glucan enriched mixed vegetable curry

The 20 experimental designs were made to optimize the *β*-glucan enriched mixed vegetable curry (Table 2). Mixed vegetable curry was prepared by conducting 15 different experiments in which five experiments were repeated. The 15 combination of fiber enriched curry were chosen by varying the quantities of different variables like potato, capsicum and *β*-glucan. The experiments were optimized with maximum fibre content and overall acceptability. The predicted values of potato, capsicum, *β*-glucan content, fibre content and over all acceptability were presented in the Table 5. The predicted combination were performed and analysed for actual value. The actual value of fibre content and overall acceptability almost found to be near of predicted value (Table 4). This optimized combination of vegetables and *β*-glucan content of mixed vegetable curry was prepared and retort processed in bulk. The retort pouch processed ready-to-eat (RTE) mixed vegetable curry was analysed for changes in colour, sterility, sensory attribute, proximate composition and total dietary fiber.

Retort processing and microflora of beta glucan enriched mixed vegetable curry

The optimized *β*-glucan enriched product was prepared bulk and retort processed. The come up time for the product to reach 121°C was 10-12 min. After attaining 121°C the product was subjected to steam-air mixture (15 psi

+ 5psi) and the product temperature reached to 118°C in 25 min. The lethality of the product was achieved the desired F_0 *value* 4.5. Then it was brought to 40-45°C by pressurized water-cooling. The time-temperature and lethality history curve of the product is shown in Figure 8. The total plate count, coliforms and spores count were found to be 3.4×10^2, 0.5×10^1, nil respectively (data not shown). The microbiological analysis of retort pouch processed *β*-glucan enriched mixed vegetable curry showed that the product remained commercially sterile and confirmed the adequacy of the processing as well as its fitness for consumption.

Changes in CIE color values of mixed vegetable curry

The CIE Color (L*, a*, b*, dL*, da*, db* and dE*) values of mixed vegetable curry was measured by Hunter Lab Scan Spectrophotometric colorimeter. There was a major colour change in mixed vegetable curry due to addition of *β*-glucan. The control sample before processing had a redness (a*), yellowness (b*) and luminosity (L*) values as 14.15, 36.13, 33.04 and fiber enriched sample had values as 11.29, 38.88, 38.85, respectively. The total difference was found to be 7.04. The product after processing did not show much change in colour but there was change in total difference (dE*). Retort processed control (without beta glucan) sample had redness (a*), yellowness (b*) and luminosity (L*) values as 15, 41, 35.54 and fiber enriched sample had values as 13.11, 41.07 40.79, with the total difference of 5.58. It is also observed that the product after processing did not show much change in colour but there was change in total difference (Table 5).

Changes in proximate composition and sensory of mixed vegetable curry

The proximate composition of retort processed mixed vegetable curry was analysed. The product of mixed vegetable curry before and after retort processing was analyzed for its moisture, total fat, protein, total dietary fiber, carbohydrates. The values (in %) of control (without fibre enriched) and fiber enriched product was found to be 71.0.08 ± 0.5, 9.00 ± 0.04, 2 ± 0.08, 4.484 ± 0.0004, 13.016 ± 0.008 and 59.7 ± 0.08, 8.6 ± 0.08, 1.8 ± 0.04, 15.3946 ± 0.004, 14.5054 ± 0.0007 respectively. Retort processed control and fiber enriched mixed vegetable curry was found to be 70.52 ± 0.004%, 9.1 ± 0.04%, 2.01 ± 0.008%, 4.256 ± 0.0004%, 14.114 ± 0.% and 58.32 ± 0.008%, 8.5 ± 0.08%, 1.75 ± 0.009%, 15.123 ± 0.0004%, 16.307 ± 0.0004%. A significant change in the moisture content was found due to addition of *β*-glucan. Malkki and Virtanen (2001) also reported that *β*-glucan also has various suitable functional properties such as thickening, stabilizing, emulsification and gelation. The total fat, protein, total dietary fiber and carbohydrates did not show much variation on retort processing. Our results were in concurrence with other authors who studied the retort pouch processed ready-to-eat foods (Kumar et al., 2013) they also reported that, there was not much changes during retort pouch processing on proximate compositions.

The Sensory quality of control (without beta glucan) mixed vegetable curry using a 9-point hedonic scale score revealed that initially the product scored 8.5 ± 0.06 for color, 8.3 ± 0.08 for flavor, 8.2 ± 0.03 for texture, and 8.4 ± 0.03for taste 8.35 ± 0.07 for overall acceptability. Beta glucan enriched mixed vegetable curry sensory score was found to be 7.9 ± 0.08 for color, 8.2 ± 0.09 for flavour, 8.1 ± 0.04 for texture, 8.2 ± 0.03 for taste, 8.1 ± 0.08 for OAA. The OAA was decreased due to changes in color, thus clearly indicating the product. However, the samples of beta glucan enriched mixed vegetable curry was acceptable and the product remained in good.

Variables		Range of Levels			
		Low Actual	Low Coded	High Actual	High Coded
A	Potato (g)	10.00	-1.000	13.00	1.000
B	Capsicum (g)	9.00	-1.000	13.00	1.000
C	*β*-glucan (%)	1.00	-1.000	3.00	1.000

Table: 1 Experimental ranges and levels of independent variables used in RSM in terms of actual and coded factors for beta glucan enriched mixed vegetable curry

Run Order	**Factors**			**Responses**	
	Potato (g)	**Capsicum (g)**	**β-glucan (%)**	**Total Dietary Fibre (%)**	**Sensory (OAA)**
1	10.00	13.00	1.00	9.28	6.50
2	11.50	11.00	2.00	11.01	7.30
3	10.00	9.00	1.00	9.42	6.80
4	8.98	11.00	2.00	11.21	7.50
5	13.00	13.00	3.00	15.39	8.00
6	11.50	11.00	2.00	11.01	7.30
7	10.00	9.00	3.00	15.09	7.60
8	13.00	9.00	3.00	15.13	7.40
9	13.00	13.00	1.00	9.32	6.70
10	11.50	11.00	2.00	11.01	7.30
11	10.00	13.00	3.00	15.28	7.80
12	14.02	11.00	2.00	11.12	7.40
13	11.50	11.00	2.00	11.01	7.30
14	11.50	11.00	2.00	11.01	7.30
15	11.50	11.00	2.00	11.01	7.30
16	11.50	14.36	2.00	11.12	6.20
17	13.00	9.00	1.00	9.41	7.00
18	11.50	11.00	0.32	3.22	6.00
19	11.50	11.00	3.68	16.22	7.90
20	11.50	7.64	2.00	11.22	7.30

Table 2: Design of experiment of β-glucan enriched mixed vegetable curry

Term Model	Response	
	Fibre Linear	OAA Linear
F Value	49.74	13.62
P > *F*	< 0.0001 Significant	0.0001 Significant
Mean	11.42	7.20
Standard deviation	1.00	0.30
C V %	8.79	4.23
R squared	0.9032	0.7186
Adjusted *R* Squared	0.8850	0.6658
Predicted *R* Squared	0.8262	0.4929
Adequate Precision	24.861	12.654

Table: 3 ANOVA and model statistics for β-glucan enriched mixed vegetable curry

Values	**Variables**			**Response**	
	Potato (g)	Capsicum (g)	β-glucan (%)	Fiber (%)	Sensory (OAA)
Predicted	13.0	13.0	3.0	14.751	7.60
Actual	13.0	13.0	3.0	15.382	7.9

Table 4: Predicted responses vs. actual response for β-glucan enriched mixed vegetable curry

Parameters/ Samples	Before Retort processing		After Retort processing	
	Control	β-glucan enriched	Control	β-glucan enriched
L*	33.04	38.85	35.54	40.79
a*	14.15	11.29	15.00	13.11
b*	36.13	38.88	41.00	41.07
dL*	-	5.81	-	5.25
da*	-	-2.87	-	-1.9
db*	-	2.75	-	0.08
dE*	-	7.04	-	5.58

Table 5: Changes in CIE Colour values of β-glucan enriched mixed vegetable curry

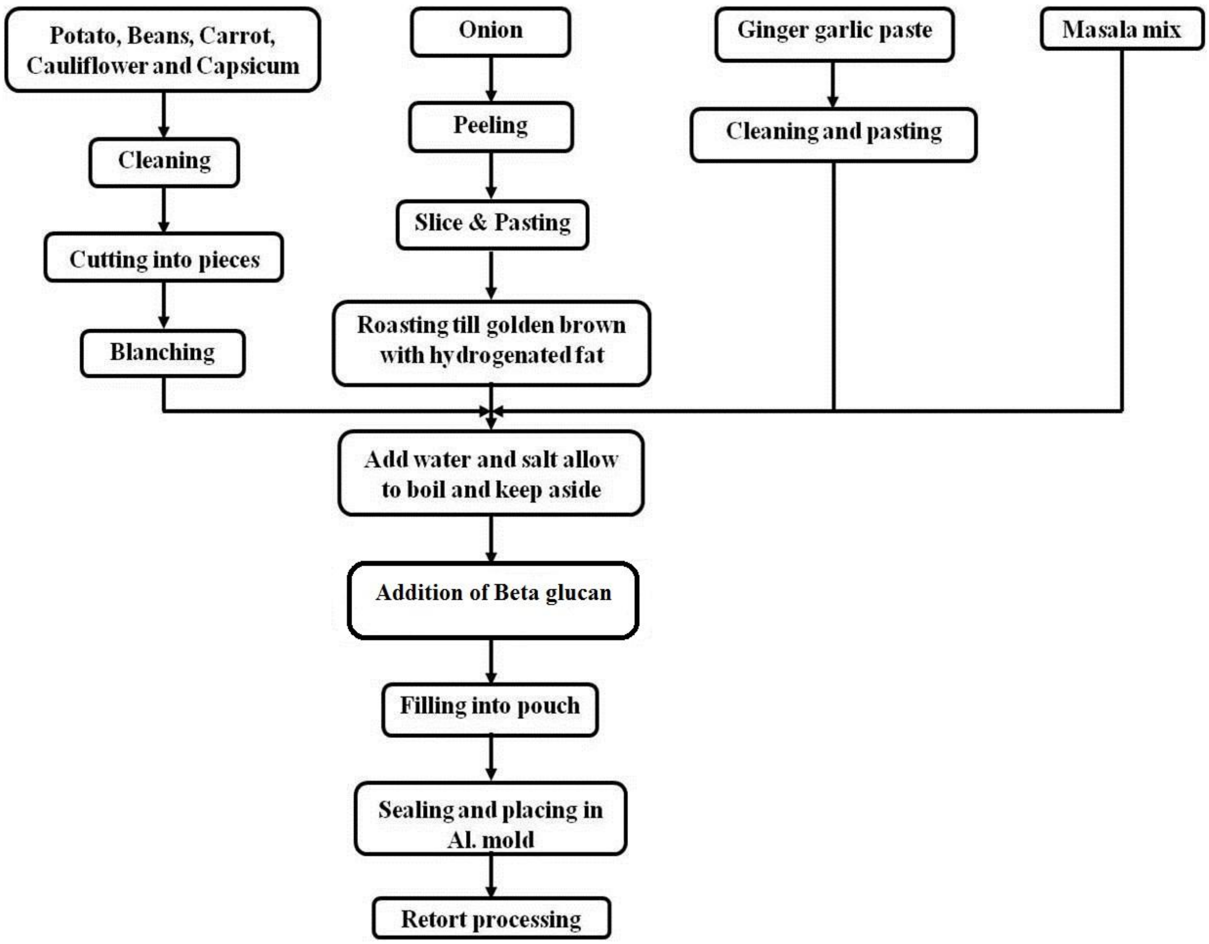

Figure 1: Flow chart for preparation and processing of mixed vegetable curry.

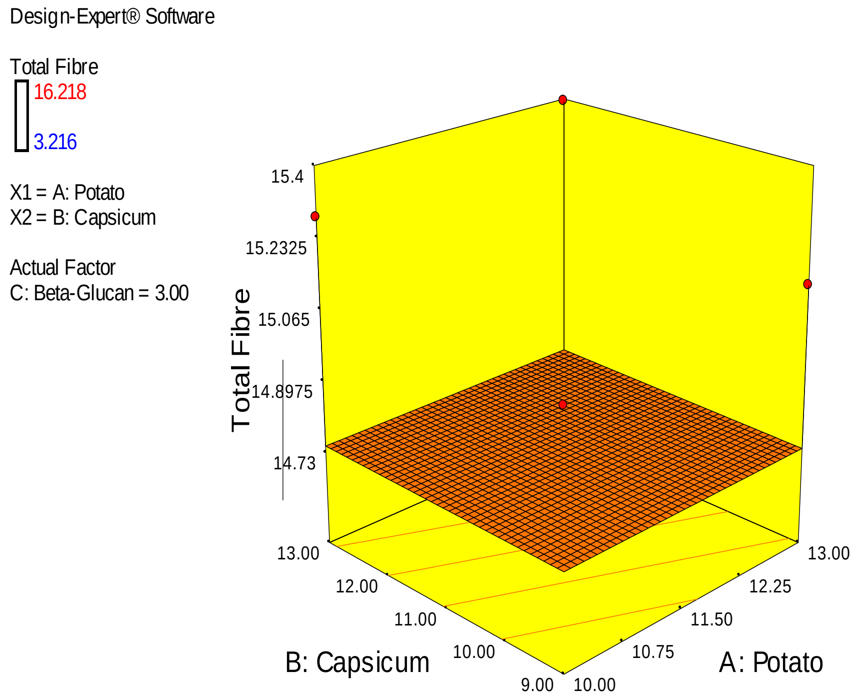

Figure 2: 3D plot depicting effect of Potato and Capsicum on total fibre content

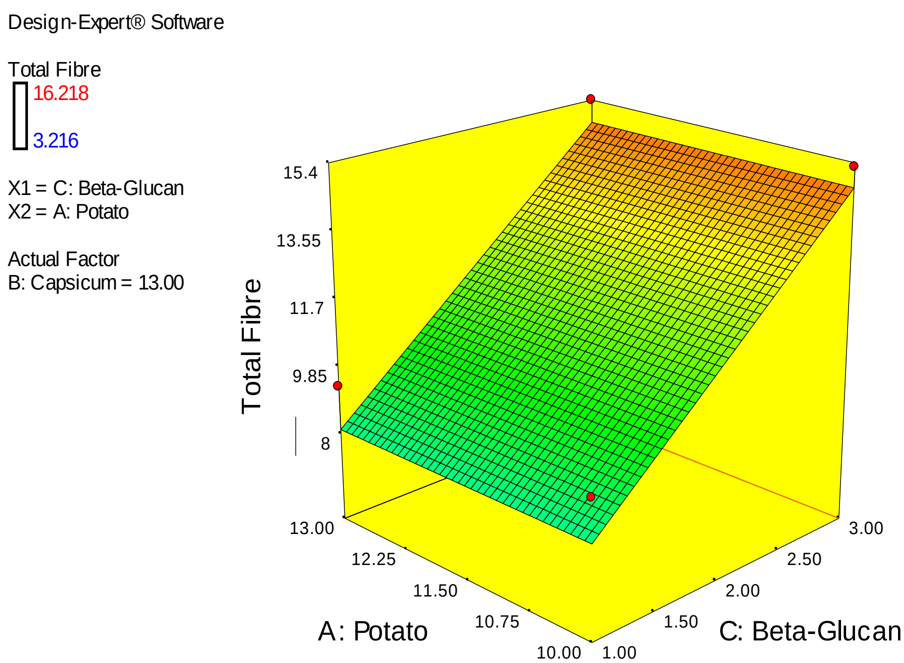

Figure 3: 3D plot depicting effect of Potato and β-glucan on total fibre content

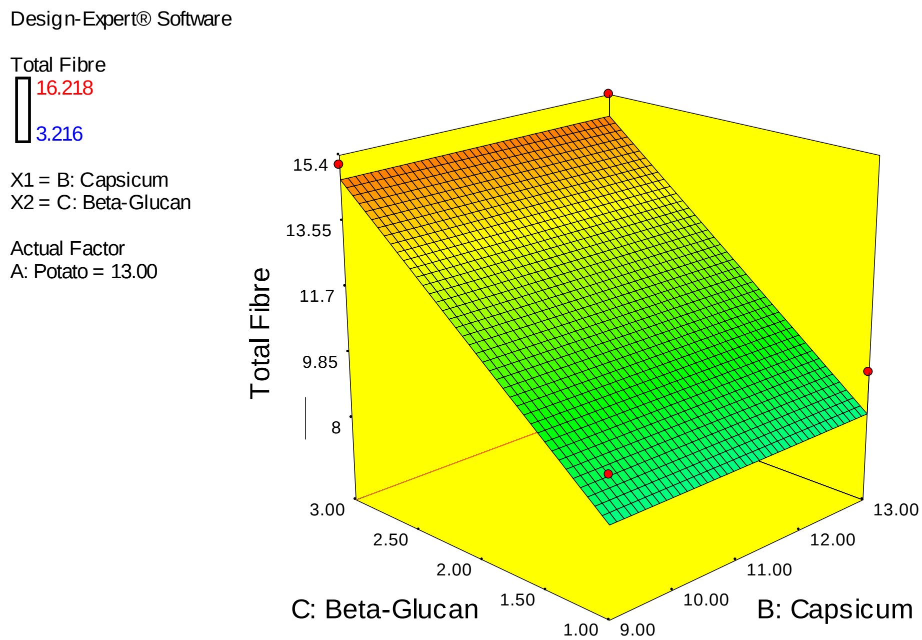

Figure 4: 3D plot depicting effect of Capsicum and Beta glucan on Total fibre content

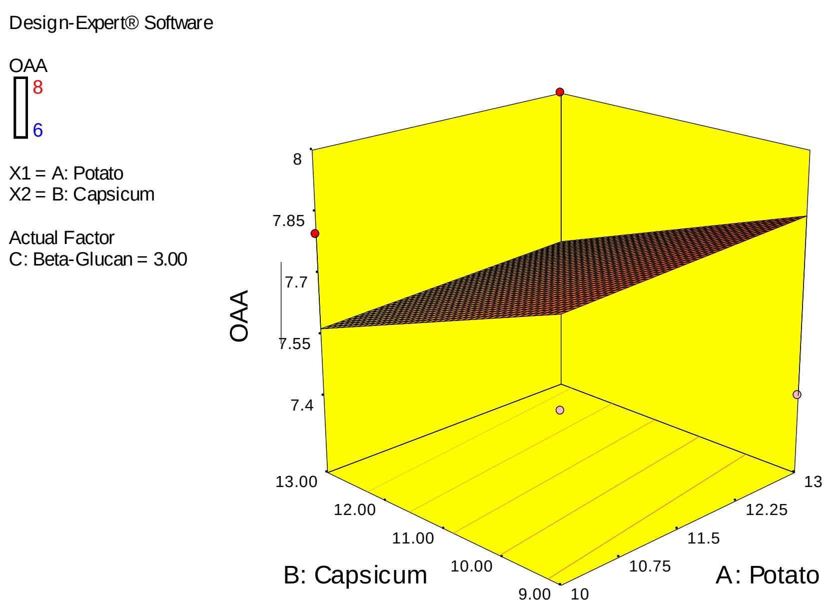

Figure 5: 3D plot depicting effect of Potato and Capsicum on OAA

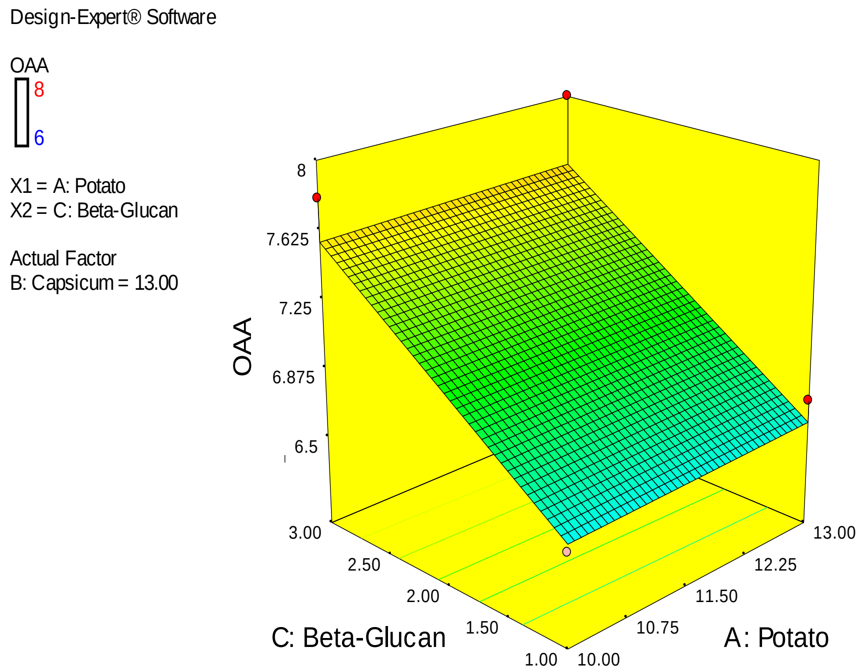

Figure 6: 3D plot depicting effect of Potato and Beta glucan on OAA

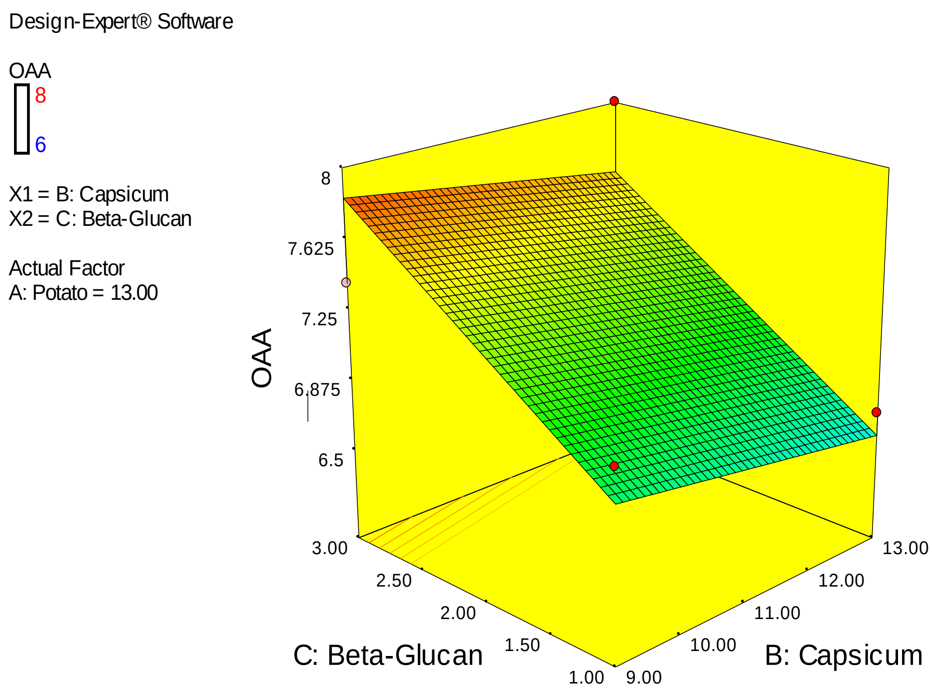

Figure 7: 3D plot depicting effect of Capsicum and Beta glucan on OAA

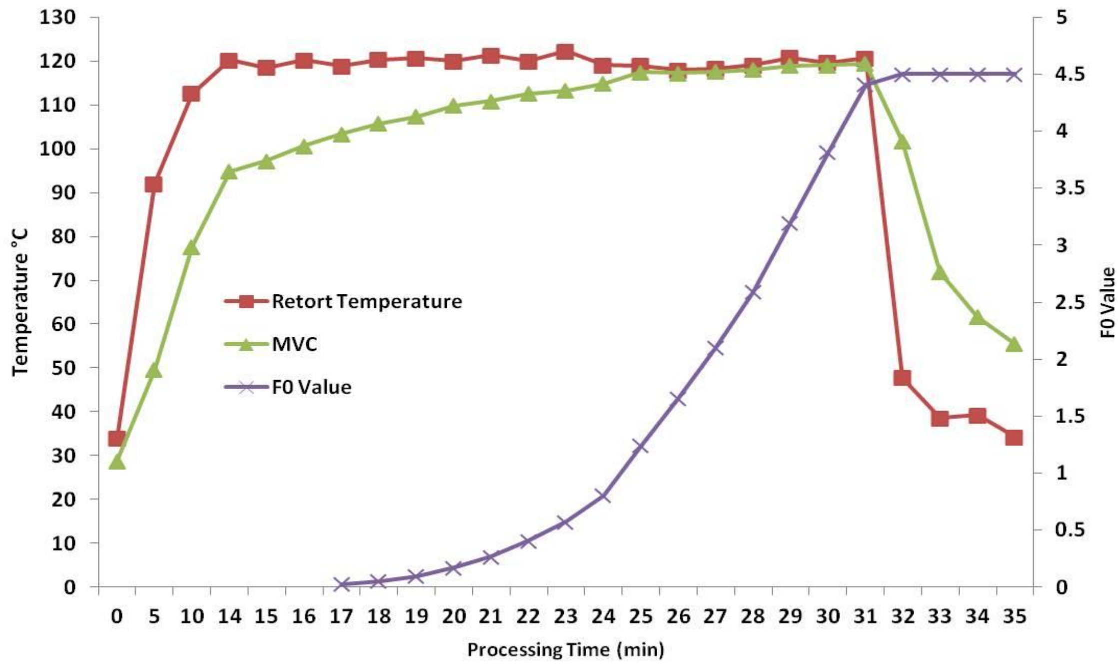

Figure 8: Time-temperature and lethality history curve of the *β*-glucan enriched mixed vegetable curry

Summary and conclusion

Beta glucan is one type of valuable dietary fiber present in cereal crops, especially in barley, oat, and some mushrooms. The present study describes the use of Response surface Methodology (RSM) was one of the best methods to optimize the new food products. In this study 20 experimental designs were made to optimize the *β*-glucan enriched mixed vegetable curry. Out of 20 experiments 5 experiments were repeated. The 15 combination of fiber enriched curry were chosen by varying the quantities of different variables like potato, capsicum and *β*-glucan. The experiments were optimized with maximum fibre content and overall acceptability. The predicted and actual values of potato, capsicum, *β*-glucan content, fibre content and over all acceptability were almost similar. The factors of the vegetable in combination with *β*-glucan content were significantly modifying the fibre content of the product. Thermal processing of foods is one of the most important preservation techniques in the food industry to extend the shelf-life of any foods with high quality and commercial sterility. The lethality of the *β*-glucan enriched mixed vegetable curry was achieved the desired F_0 *value* 4.5 and found to be commercial sterility during entire storage with good sensory quality.

Acknowledgment

The authors express deep gratitude to The Director, Defence Food Research Laboratory, Mysore, for his constant support and encouragement.

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