JEB
Website : www.jeb.co.in
E-mail : editor@jeb.co.in

Journal of Environmental Biology

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Determination and optimization of Vitamin B complex in xylanase enzyme treated polished rice by response surface methodology

Anjineyulu Kothakota*[1], J.P Pandey, A.H.Ahmad[2], Anil Kumar[3] and Wasif Ahmad[2]

[1]Department of Post Harvest Process & Food Engineering, College of Technology, G. B. Pant University of Agriculture and Technology, Pantnager-263 145, India

[2]Department of Veterinary Pharmacology & Toxicology, College of Veterinary Sciences, G. B. Pant University of Agriculture and Technology, Pantnager-263 145, India

[3]Department of Food Science & Technology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnager-263 145, India

Corresponding Author Email: **kothakotaanjanikumar23@gmail.Com**

Publication Info

Paper received:
20 November 2014

Revised received:
19 May 2015

Re-revised received:
04 June 2015

Accepted:
08 August 2015

Abstract

The present study provides information about the concentrations of Vitamin B (thiamine, riboflavin, pyridoxine and niacin) in polished brown rice treated with xylanase. Xylanase enzyme was produced from *Aspergillus awamori* MTCC 9166. Brown rice was treated with 60-100% enzyme (40ml of buffer -undiluted) for 30 to150 min (with variation of 30min) at 30 ºC to 50 ºC (with variation of 5 ºC) to attain a saturated moisture level of 35.5 $g100^{-1}g$ .The enzyme acted upon selective degradation (polishing time 10-50 sec) of bran layer facilitating retention of more vital nutrients along with the vitamins. Vitamin B content, detected through HPLC and optimized by response surface methodology (RSM) with central composite design (CCRD), demonstrated that selective degradation of bran layers for polished rice facilitated increase of thiamine (57%), riboflavin (48%), pyridoxine (90%) and niacin (55%) concentration in bio polished rice over normally milled rice.Enzyme treated bio-polished rice was considered to be better source of vitamin B complex than mechanically milled rice, hence more nutritionally efficacious.

Key words

Brown rice, HPLC, Response surface mathodology, Xylanase, Vitamin B complex

Introduction

The most recent European guidelines on "Prevention of cardiovascular disease in clinical practice in 2003" (De Backer *et al.,* 2003) emphasised on making healthy food choice as an integral part of total risk management. Everyone should receive professional advice on food and food choices to compose a diet associated with lowest risk of cardiovascular disease. A general recommendation is to encourage people to consume fruits and vegetables, whole grain cereals and bread, low fatty products and fish. Cereals are considered as an important source of nutrients, including vitamins B (De Backer *et al.,* 2003).

Among cereal rice forms staple food and is one of the cheapest source of energy and protein. Brown rice contain nutrients such as dietary fibres, essential amino acids, minerals, proteins, vitamin B and E and other non-nutrient essential photochemical concentrated in germinal and outer layers of starchy endosperm. These nutrients are removed as rice bran during whitening or milling operation. According to nutritionists (Lamberts *et al*., 2007), brown rice should be preferred because of its high nutritional value. Removal of bran layers during milling process improves appearance, cooking quality and palatability of rice, though major loss of nutrients and high percentage of brokenness results during mechanical milling (Das *et al.,* 2008). Therefore, producing

rice with minimum breakage, retaining maximum possible nutrients of brown rice and with preferable cooking attributes has been primary goal of rice processing industries. In order to retain loss of nutrients incurred during mechanical milling an alternative polishing method through enzymatic degradation of bran layers of brown rice was investigated (Lamberts *et al*., 2007; Cui *et al*., 2010 and Loveleen Kaur *et al*., 2011). In these studies attempts were made to use fungal enzyme xylanase (an endo nuclease), produced through solid-state fermentation by growing *Aspergillus awamori* (MTTCC 9166) on wheat bran (Das *et al.,* 2008; Loveleen Kaur *et al.,* 2011 and Yadav *et al.,* 2014) for hydrolysis of bran layer in order to retain Vitamin B (thiamine, riboflavin, pyridoxine and niacin) complex.

Different techniques were optimized to determine thiamine, riboflavin and pyridoxine in cereals and their derivatives. All of them relied on univariate approaches to obtain better resolution of Vitamin B complex. Fact that univariate methods are often time consuming, it would be advisable to use multivariate chemometric technique to optimize thiamine by HPLC. As reported previously, several studies have shown successful determination and optimization of riboflavin and thiamine by HPLC method using response surface methodology (Van de Velde *et al.,* 2012; Mirza and Tan 2001; Ferreira and Bruns, 2007). In lieu of above the aim of the present study was to optimize and enhance Vitamin B-complex in bio-polished rice through application of xylanase using response surface methodology.

Materials and Methods

Enzyme preparation : Xylanase (Endoxylanase) enzyme was produced through solid-state fermentation by growing *Aspergillus awamori* (MTCC9166) on wheat bran (Kormelink, Searl-Van Leeuwen, Wood and Voragen, 1991). Xylanase activity on 4th day of incubation was 16 IUml^{-1}. Amylase activity in the same crude extract was 2.2 IUml^{-1}. The crude enzyme was centrifuged at 10,000 rpm and supernatant enzymes were concentrated through Ultra Filtration unit of 10kDa cut-off membrane. Endoxylanase activity was measured by modifying the method of Khanna and Gauri (1993), using 0.1 mol l^{-1} sodium acetate buffer, pH 5.0. For quantification of enzyme activity, 1% xylan solution, 0.5 ml, acetate buffer pH 6, was taken in test tubes and placed in water bath at 50^{0}C. Properly diluted enzyme solution (0.25 ml) was added to it and incubated for 10 min at 50^{0}C. The amount of reducing sugars released was determined using dinitro salicylic reagent. Enzyme activity was expressed in International Units (IU).

Enzymatic pre-treatment of brown rice : Brown rice (100 g) was soaked in 50 ml water for 24 hr. Water was changed at regular intervals for minimizing bacterial contamination. Rice was soaked for another 1 hr in 50 ml fresh water with 2.5 g of calcium carbonate at 60 ºC (10 ºC below gelatinization temperature of the rice) to saturate the grains to a 37-39% moisture content. Rice was washed in water to remove excess calcium carbonate and then with 150 ml of xylanase and cellulase enzyme solutions prepared from different concentration of 100% (undiluted), 90 % (90ml undiluted enzyme + 10 ml buffer), 80% (80 ml undiluted enzyme + 20 ml buffer), 70 % (70 ml undiluted enzyme + 30 ml buffer), 60 % (60ml undiluted enzyme + 40 ml buffer) with differentiation of 10 %, respectively at different temperatures (30 ºC, 35ºC, 40ºC, 45ºC and 50ºC) with differentiation of 5ºC for different time 30-150min (30, 60, 90, 120,150min). After treatment rice was washed to remove the enzymes. Superficial water was blotted off and then steamed in a preheated autoclave for 20 min under atmospheric pressure for complete gelatinization of rice.

Rice polishing and sample preparation : Pretreated samples were polished at different time (10, 20, 30, 40 and 50sec) by using abrasive type Satake laboratory polisher. Bran adhering to polished white rice was removed by sieving. After weighing, white rice and bran percentage was calculated. Whole kernels were separated from brokens by sizing of length. After polishing, xylanase treated rice was analyzed. Rice samples (10 g) were cooked in deionised water (20ml) for 20 -25 min, was proper cooking of sample to make a paste by piston mortar. The paste of cooked rice (1.5g) was dissolved in 5ml mobile phase in centrifugation tube and the mixture was homogenised in centrifuge at 6000 rpm for 15 to 20 min. Supernatant was taken and filtered through millipore membrane filter and was used for chromatographic separation (Das *et al.*, 2008).

Preparation of standard solutions : All the standard solutions were prepared following procedure of Das *et al.*, (2008).

Optimization procedure : A central composite rotatable design (CCRD) using four factors at five levels (coded levels -2, 0, and 2) was used for optimization of Vitamins B-complex: Vitamin B_1 (thiamine), Vitamin B_2 (riboflavin), Vitamin B_3 (nicotinamide) and Vitamin B6 (pyridoxine) determination in enzyme treated rice by HPLC. CCRD consisted of 30 experiments (chromatographic runs) under different conditions (Table 1).Five replicates in the central point were included in order to estimate the experimental error bran .Factors and levels were selected as reported previously (Das *et al.,* 2008 and Loveleen Kaur *et al.* 2011). Independent levels were coded for experimental design (Table 1). Experimental data were analyzed using design expert 8.0.6 statical software and second-order polynomial model predicted for optimization of dependent variables (Y).

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1.x_2 + \beta_{13} x_1.x_4 + \beta_{23} x_2.x_3 + \beta_{24} x_2 + x_4 + \beta_{34} x_3.x_4 + \varepsilon \quad (1)$$

Where, β_0 (constant), β_1, β_2, β_3, β_4 (coefficients for linear effects); β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34} (coefficients for interaction effects); β_{11}, β_{22}, β_{33}, β_{44} (coefficients for quadratic effects); and ε (random error).The response surfaces and contour plots for these models were plotted as a function of two variables, keeping the other variable at optimum level. Using the presented experimental design, each experiment was conducted by making duplicate injections of the extracted vitamin B complex Vitamin B_1 (thiamine), Vitamin B_2 (riboflavin), Vitamin B_3 (nicotinamide) and Vitamin B_6 (pyridoxine) (Nicolás michlig *et al.,* 2013).

Vitamin standards (thiamine hydrochloride, riboflavin, nicotinic acid, and pyridoxine- hydrochloride) and methanol (HPLC grade) for chromatographic analysis were obtained from Sigma-Aldrich (rudrapur, pantnager, India). Glacial acetic acid, calcium carbonate and hexane sulphonic acid sodium salt (analytical grade) were obtained from Sigma-Aldrich (rudrapur, pantnager, India). Other reagents used were of highest purity available. Aqueous solutions were prepared with deionized water. HPLC analysis of the samples was performed on Agilent 1100 HPLC system (Kyoto, Japan). Sample injections of 20µl were operated from Agilent 1100 auto sampler. Chromatograms were obtained from Nucleosil RP C18 HD 100–5µm 250 mm×4.6 mm (Macherey-Nagel part number: 721850.46, Kyoto, Japan) separations with a flow rate of 1 ml min^{-1} at 77 bar and 27ºC. The mobile phase (buffer / methanol 70:30, 5mm sodium salt of hexane sulphonicacid as buffer) was run under isocratic conditions. Awash-step with 100% mobile phase was used after each sample. Analysis was carried out in isocratic mode at a flow rate of 1ml min^{-1}, with column effluent being monitored at 254 nm wavelength. Data acquisition was done with Varian Star Software.

Results and Discussion

In recent years, chemometric tools have been frequently applied for optimization of different analytical methods considering their advantages such as reduction in number of experiments resulting in lower reagent consumption and less laboratory work (Van de Velde *et al.,* 2012; Mirza and Tan 2001). A five level central composite rotatable factorial design was used for optimization and estimation of vitamin B complexes: vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (nicotinamide) and vitamin B6 (pyridoxine) in enzymatic treated rice. This kind of response surface methodology (RSM) uses second-order polynomial models to describe response in the experimental design. The experimental data is shown in Table 2 and Table 5. From ANOVA results (Table 4), flow rate through lineal term and organic modifier proportion through quadratic term were factors that showed significant effects ($p \leq 0.05$).

Regression coefficients significant at 95% level, were selected for developing the models representing final equations in terms of coded factors. The resulting polynomial, after removing non-significant terms and all model term values, was calculated and is presented in Table 3. Regression analyses showed that thiamine was significantly ($p<.05$) affected by quadratic (x_1^2, x_2^2 and x_4^2), and interaction (x_1x_3, x_1x_4 and x_2x_3) terms only. Riboflavin and pyridoxine were affected by linear term (x_4), interaction (x_2x_4) term and quadratic (x_1^2, x_2^2, x_3^2 and x_4^2) terms. Linear term (x_1), interaction (x_3x_4) term and quadratic terms (x_1^2, x_2^2, x_3^2 and x_4^2) influenced niacin, respectively.

The goodness-of-fit of the models by evaluated correlation coefficient R^2 were 0.8652(Y_1), 0.8600(Y_2), 0.8618(Y_3) and 0.8618(Y_4), R^2 adj. were 0.8394(Y_1), 0.8079(Y_2), 0.7979 (Y_3) and 0.7461(Y_4), and the Fisher F-test values were 6.89(Y_1), 6.08(Y_2), 6.88 (Y_3) and 6.13 (Y_4), The derived P values of results were 0.0003(Y_1), 0.0003(Y_2), 0.0004 (Y_3) and 0.0001(Y_4), respectively. These all were significant at 5% level as observed in Table 4 and 5. However, the lack of fit was not significant for all Y_1, Y_2, Y_3 and Y_4 ($P>0.05$), while the F-values were significant for all Y_1, Y_2, Y_3 and Y_4 as they had lower P vales ($P<0.0005$).

It is evident from Eq. (2) that Y_1 depends on three factors. The negative coefficient of linear term (x_2 x_3 and x_4), quadratic terms (x_1^2, x_2^2, x_3^2 and x_4^2), and interaction terms (x_3x_4) decreased Y_1, whereas positive linear terms (x_1) and interaction terms (x_1x_2, x_1x_3 x_1x_4 and x_2x_3) increased Y_1. It was significant at p P>0.0005(0.0003)

Table 1 : Coded and actual values of independent variables for enzymatic treatment

Independent	Code	Levels in coded form				
Variables		-2	-1	0	+1	+2
Enzyme concentration (%)	X_1	100	90	80	70	60
Treatment time (min)	X_2	30	60	90	120	150
Polishing time (min)	X_3	10	20	30	40	50
Treatment temperature (ºC)	X_4	35	40	45	50	55

Table 2 : Experimental conditions and response values obtained for each of the 30 test points of the central composite rotatable design

Independent variables				Dependent variables			
Enzyme concentration (%) X_1	Treatment time (min) X_2	Polishing time (min) X_3	Treatment temperature (°C) X_4	Thiamine mg $100g^{-1}$ Y1	Riboflavin mg $100g^{-1}$ Y2	Pyridoxine mg $100g^{-1}$ Y3	Niacin mg $100g^{-1}$ Y_4
70	60	20	35	0.164	0.025	1.441	1.668
90	60	20	35	0.146	0.027	1.575	1.723
70	120	20	35	0.186	0.035	1.521	1.745
90	120	20	35	0.155	0.036	1.634	1.822
70	60	40	35	0.172	0.037	1.549	1.787
90	60	40	35	0.162	0.036	1.449	1.891
70	120	40	35	0.155	0.031	1.519	1.786
90	120	40	35	0.167	0.041	1.692	1.744
70	60	20	45	0.178	0.042	1.621	1.841
90	60	20	45	0.182	0.037	1.701	1.892
70	120	20	45	0.147	0.039	1.452	1.744
90	120	20	45	0.149	0.036	1.761	1.799
70	60	40	45	0.152	0.042	1.672	1.567
90	60	40	45	0.177	0.037	1.587	1.663
70	120	40	45	0.145	0.033	1.598	1.592
90	120	40	45	0.167	0.039	1.457	1.713
60	90	30	40	0.144	0.038	1.591	1.643
100	90	30	40	0.156	0.036	1.611	1.753
80	30	30	40	0.179	0.034	1.629	1.789
80	150	30	40	0.185	0.041	1.567	1.873
80	90	10	40	0.154	0.044	1.586	1.783
80	90	50	40	0.141	0.041	1.632	1.815
80	90	30	30	0.167	0.034	1.499	1.779
80	90	30	50	0.146	0.039	1.677	1.719
80	90	30	40	0.198	0.045	1.442	1.952
80	90	30	40	0.188	0.045	1.353	1.892
80	90	30	40	0.198	0.045	1.353	1.892
80	90	30	40	0.188	0.049	1.353	1.952
80	90	30	40	0.188	0.045	1.353	1.952
80	90	30	40	0.188	0.045	1.393	1.952

Selected thiamine values of the products are reported in Fig. 1a–b as a function of independent variables. The response surface graphs obtained from this model showed that highest retention of thiamine was obtained at 85–90% enzyme concentration,40-45 ºC temperature, 20-28sec polishing time and 100-108 min treatment time due to the fact that enzyme at optimum level highly act on brown layer even after cooking. Brown rice contain xylan which is covalently bonded to lignin and non-covalently bonded to cellulose that maintains cellulose integrity *in situ* and control the overall speed of xylolytic hydrolysis reaction, exhibiting a crucial effect on polymer's enzymatic degradation (Beg *et al.,* 2001), This crucial polymer enzyme effect causes less loss of water soluble fractions during cooking and soaking of enzyme treated rice, because all the nutrients moves to endosperm during treatment. The present observations is in conformation with the previous report of (Hegde *et al.,* 2006; Uffen, 1997).

The model equation predicting this response is given in Eq. (3). Comparing the magnitude of coefficients, it is evident from Eq. (3) that treatment temperature had a strong effect on Y_2 as it had largest coefficient followed by enzyme concentration, polishing time and treatment time. The positive linear coefficients and (x_1x_2 and x_1x_3) interaction terms contributed to increase of Y_2, whereas remaining negative interaction terms and all negative quadratic coefficients had a reverse trend on Y_2. However, all the quadratic coefficients considered were significant within the level of coefficient selected. It was significant at p P>0.0005(0.0003)

Riboflavin increased from 0.025 to 0.49 mg $100g^{-1}$ as a function of both enzymatic treatment time and temperature (Fig. 1 c and d). Also, during enzymatic treatment process, higher enzyme concentration and polishing time led to low retention of riboflavin in enzyme treated rice by HPLC.

Table 3 : Final equation in terms of coded factors

Response	Equation			
Y_1	$0.19+1.250E-003x_1-2.083E-003x_2-1.500E-003x_3-2.167E-003x_4-9.708E-003x_1^2$ $-1.708E-003x_2^2-0.010x_3^2-8.083E-003x_4^2+2.500E-004x_1x_2+5.750E-003x_1x_3$ $+6.250E-003x_1x_4+2.500E-004x_2x_3-6.250E-003x_2x_4-1.250E-003x_3x_4$			
	R^2adj.= 0.8394	(2)	R^2=0.8652	F=6.89
Y_2	$0.046+4.167E-005x_1+8.750E-004x_2+5.417E-004x_3+1.958E-003x_4-2.760E-003x_1^2-$ $2.635E-003x_2^2-2.635E-003x_3^2-2.885E-003x_4^2+1.438E-003x_1x_2+9.375E-004x_1x_3-$ $1.187E-003x_1x_4-1.437E-003x_2x_3-1.812E-003x_2x_4-1.562E-003x_3x_4$			
	R^2adj.= 0.8079	(3)	R^2=0.8600	F=6.09
Y_3	$1.37+0.022x_1-3.542E-003x_2-3.792E-003x_3+0.034x_4+0.053x_1^2+0.052x_2^2+0.055x_3^2+$ $0.050x_4^2+0.027x_1x_2-0.049x_1x_3-9.813E-003x_1x_4-1.312E-003x_2x_3-0.042x_2x_4-0.016x_3x_4$			
	R^2adj.= 0.7979	(4)	R^2=0.8618	F=6.88
Y_4	$1.93+0.031x_1-6.625E-003x_2-0.018x_3-0.020x_4-0.062x_1^2-0.029x_2^2-0.037x_3^2-0.049x_4^2-$ $5.937E-003x_1x_2+2.563E-003x_1x_3+8.063E-003x_1x_4-3.687E-003x_2x_3-8.937E-003x_2x_4-$ $0.062x_3x_4$			
	R^2adj.= 0.7461	(5)	R^2=0.8612	F=6.13

Y_1=Thiamine, Y_2=Riboflavin, Y_3=Pyridoxine, Y_3=niacin; R^2-coefficient of determination; Adj R^2=adjusted; R^2Pred = predicted

Table 4 : Analysis of variance (ANOVA) for second-order polynomial model fitted to response surface

Response	Source	df[a]	SS	MS	F-value	P
Y_1	Model	14	7.883E-003	5.631E-004	6.88	0.0003*
	Lack-of- fit	10	1.095E-003	1.095E-004	4.10	0.0662**
	Pure error	5	1.333E-004	2.667E-005		
	Residual	15	1.228E-003	8.187E-005		
	Total	29	9.111E-003			
Y_2	Model	14	8.068E-004	5.999E-005	6.84	0.0003*
	Lack-of- fit	10	1.211E-004	1.102E-005	2.58	0.1532**
	Pure error	5	2.133E-005	4.267E-006		
	Residual	15	1.316E-004	8.772E-006		
	Total	29	9.492E-004			
Y_3	Model	14	8.399E-004	0.024	6.68	0.0004*
	Lack-of- fit	10	1.102E-004	4.705E-003	3.49	0.0903**
	Pure error	5	6.748E-003	1.350E-003		
	Residual	15	1.316E-004	3.586E-003		
	Total	29	9.715E-004			
Y_4	Model	14	0.29	0.021	7.94	0.0001*
	Lack-of- fit	10	0.035	3.473E-003	3.62	0.0843**
	Pure error	5	4.800E-003	9.600E-004		
	Residual	15	0.040	2.635E-003		
	Total	29	0.33			

Y_1=Thiamine, Y_2=Riboflavin, Y_3=Pyridoxine, Y_3=niacin; respectively.*Significant at Pd"0.0005; ** non significant Pe"0.5; SS: sum of square; MS: mean of square.[a] Degrees of freedom.

Quantitative estimation of major B-vitamins in cooked rice through HPLC

Vitamin mg/100g in rice	Brown rice	Optimized enzyme treated rice	Polished rice
Thiamine	0.179041	0.160246	0.069021
Pyridoxine	1.592312	1.58592	0.144032
Niacin	1.834119	1.809691	0.824134
Riboflavin	0.041011	0.038727	d"0.02

Generally, enzyme concentration and polishing time increased riboflavin retention up to 80-85% and 20-25 sec thereafter decreased retention due to swollen (temporarily producing long uncontrolled fibres compounds by xylanase) action of xylanase with bran layer. This action causes uncontrolled hydrolytic reaction between enzyme and bran layer (xylan, lignin and cellulose) making fours structure in bran layer leading to loss of water soluble fractions

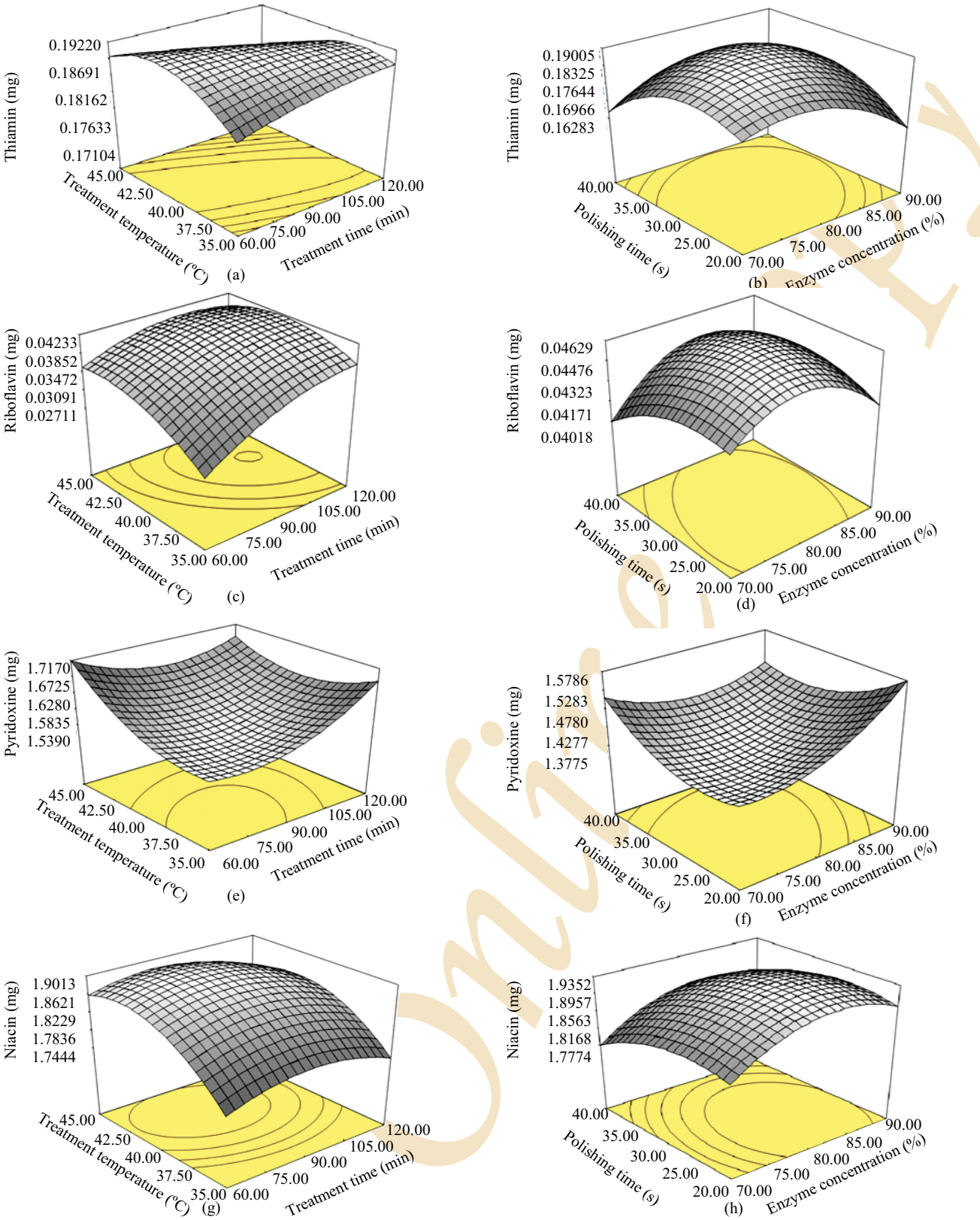

Fig. 1 : Effect of enzymatic treatment parameters on thiamine, riboflavin, pyridoxine and niacin concentration: (a), (c), (e) and (g) treatment temperature vs. Treatment time (enzyme concentration: 90% and polishing time: 20 sec); (b), (d), (f) and (h) polishing time vs. enzyme concentration (treatment temperature: 400C and Treatment time: 90 min

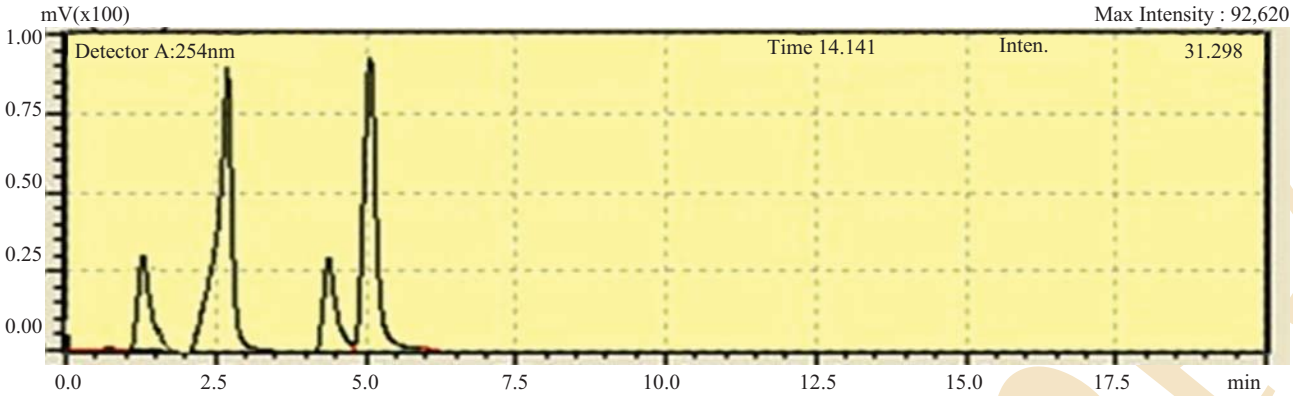

Fig. 2 : Typical chromatograms view of retention for B complex vitamins (thiamine at1.5min, riboflavin at 2.5 min, and pyridoxine at 4.4min and niacin at 5.5min) in optimized enzyme treated rice samples

(Riboflavin) during cooking and soaking process (Saloheimo *et al*., 2002). However during treatment condition of time and temperature of enzyme treated rice increased, leads to more retention of riboflavin occurred due to inactive formation of xylane fragments (it causes a partial damage and loose structure on xylane layer) because this inactivation causes development of hard xylolytic layer outside the endosperm and aleuronic layer in brown rice which makes more stagnant of water soluble nutrients in endosperm and aleuronic layer during soaking and cooking and reduces the loss of nutrients in enzyme treated rice during polishing (Wong, 2006).

The effect of independent variables on pyridoxine is shown in Table 4. Eq. 4 shows that positive effect of linear terms such as x_1 and x_4, all quadratic terms and interaction terms of x_1x_2 on pyridoxine led to increased pyridoxine concentration. Negative effect of x_2, x_3 linear terms, x_1x_2, x_1x_4, x_2x_3, x_2x_4 qudratic terms and x_3x_4 interaction terms on pyridoxine decresed concentration of pyridoxine, which was significant at P>0.0005(0.0004) lavel.

Fig. 1 e and f shows selected response surface for pyridoxine against independent variables. Pyridoxine concentration of all the enzymes in treated rice ranged from 1.353 to 1.701 mg100g^{-1}. During enzymatic treatment process, retention of pyridoxine concentration increased by increasing all the parameters (enzyme concentration, treatment temperature, treatment time and polishing time). Among them enzyme concentration, treatment time and treatment temperature were significantly affected, but for polishing time it showed reverse trend. Generally retention of pyridoxine concentration increased by decreasing polishing time, thereafter increased due to bonding nature of xylan, lignin and cellulose in bran layer . Xylan, covalently bonded to lignin and non-covalently bonded to cellulose, helps in maintaining integrity of bran layer which is otherwise affected during removal of bran layer during polishing time (Das *et al*., 2007). Nicolas Michlig *et al.,* (2013) demonstrated that increasing all the parameters (enzyme concentration, treatment temperature, treatment time and polishing time) of enzyme treatment process increased retention of pyridoxine concentration due to significant quadratic coefficients.

Niacin retention concentration values in response surface experiments varied widely between 1.567 and 1.952 mg100g^{-1}.Also, during enzymatic treatment process retention of niacin concentration increased initially in enzyme treated rice by increasing all the dependent variables such as enzyme concentration, treatment temperature, treatment time and polishing time, Thereafter decreased significantly due to the affect of all quadratic coefficients. Among all dependent variables, enzyme concentration and treatment temperature were affected significantly.

High value of niacin retention concentration was obtained at 85–90% concentration, 40^0-45^{0}C temperature, 20-28% polishing time and 100-108 min treatment time as observed in Fig.1.This was due to affect of parallel cellulose and xylonaose chains interact with bran layer through hydrogen bonds and vander Waals forces, producing microfibriles. They cause the water soluble nutrients to move to inner part of endosperm, resulting in less loss nutrients during polishing (Glazer & Nikaido, 2007; Somerville *et al*., 2004).

The predicted response values were compared with the experimental values by plotting the experimental values (thiamine, riboflavin, pyridoxine and niacin) against those calculated from the predicted models. The plotted data indicated a good fit for responses (thiamine, riboflavin, pyridoxine and niacin) with regression coefficient R^2 of 0.8652, 0.8601, 0.8618, and 0.8612, respectively. The result indicated that there was good agreement between the experimental and theoretical values for thiamine, riboflavin, pyridoxine and niacin as predicted by the models.

The present study concluded that selective bio polishing of rice helped in retaining the major vitamin B like

Thiamine (57%), Riboflavin (48%), pyridoxine (90%) and Niacin (55%) in enzyme treated brown rice due to the less loss of water soluble nutrient (vitamin B complex) fractions during soaking and cooking. This conclusions shows that the enzyme treated brown rice more nutritious compared to mechanically milled rice.

Acknowledgment

The financial support received from the Technical Education Improvement Programme-II, College of Technology, G. B. Pant University of Agriculture and Technology is highly acknowledged.

References

Beg, Q.K., M. Kapoor, L. Mahajan and G.S. Hoondal: Microbial xylanases and their industrial applications: a review. *App. Microbiol. Biotechnol.*, **56**, 326–338 (2001)

Cui, L., Z. Pan, T. Yue, G.G. Atungulu and J. Berrios: Effect of ultrasonic treatment of brown rice at different temperatures on cooking properties andquality. *Cereal Chem.,* **87**, 403–408 (2010).

Das, M., Banerjee, R., & Bal, S: Enzymatic polishing of rice, A new processing technology. *LWT –Food Science and Technology* . **41,** 2092-2096 (2007).

Das, M., R. Banerjee and S. Bal: Multivariable parameter optimization for endoglucanase production by *Trichoderma reesei* Rut C30 from *Ocimum gratissimum* seed. *LWT-J. Food Sci. Technol*., **41**, 2079-2084 (2008).

De Backer, G., E. Ambrosioni, K. Borch-Johnsen, C. Brotons, R. Cifkova and J. Dallongeville: European guidelines on cardiovascular disease prevention in clinical practice. *Euro. Heart J.*, **24**, 1601–1610 (2003).

Ferreira S.L.C. and R.E. Bruns: Determination of thiamine in wheat flours using a validated isocratic HPLC-Fluorescence method previously optimized by Box Behnken Design. *Anal. Chemical. Acta,* **597,** 179-184 (2007).

Glazer, A.N. and H. Nikaido: Biomass. In: Microbial Biotechnology: Fundamentals of Applied Microbiology. (Ed.: A.N. Glazer, H. Nikaido). Cambridge University Press, ISBN 978-0-521-84210-5, New York, **6**, pp. 430-455 (2007).

Guggisberg. D., M.C. Risse and R. Hadorn: Determination of Vitamin B_{12} in meat products by RP-HPLC after enrichment and purification on an immunoaffinity column. *Meat Sci.,* **90**, 279–283 (2012).

Kormelink, F.J.M., M.J.F. Searl-Van Leeuwen, T.M. Wood and A.G.J. Voragen: Uurification and characterization of a (1, 4)-b-D-arabinoxylan arabino furano hydrolase from *Aspergillus awamori*. *App. Microbiol. Biotechnol.*, **35**, 753-758 (1991).

Khanna, S. and Gauri: Regulation, purification, and properties of xylanase from *Cellulomonas fimi*. *Enz. Microb. Technol.*, **15**, 990-995 (1993).

Lamberts, L., E. De Bie, G.E. Vandeputte, W.S. Veraverbeke, V. Derycke and W. De Man: Effect of milling on colour and nutritional properties of rice. *Food Chem.,* **100**, 1496–1503 (2007).

Loveleen Kaur, S., M. Arora, V.K. Sehgal and S. Bhatia: The use of fungal enzymes viz protease, cellulase and xylanase for polishing rice. *Inter. J. Food Saf.*, **13**, 26-37 (2011).

Li, K., P. Azadi, R. Collins, J. Tolan, J.S. Kim and L. Eriksson Karl-Erik: Relationships between activities of xylanases and xylan structures. *Enz. Microb. Technol*., **27**, 89–94 (2000).

Mirza, T. and H.S.I. Tan: Determination of B_1, B_2, and B_6. in meat products by using HPLC previously optimized by central composite design. *J. Pharmacol. Bio Medi. Anal* ., **6**, 25-39 (2001).

Montgomery, D.C.: Design and Analysis of Experiments. New York "Wiley", 416-419. (2001).

Myers, R.H.: Response surface methodology. Ann Arbor, Edwards Brothers Inc (2001).

Michlig, N., F. Van de Velde, M.R. Freyre and C.M. del H. Bernardi: Determination of thiamine in wheat flours using a validated isocratic hplc-fluorescence method previously optimized by box–behnken design. *Food Anal. Meth*.,**10**, 13-9688-3(2013).

Reilly, P.J.: Xylanases structure and function. In: Trends in the biology of fermentation for fuels and chemicals (Ed.: A. Hollaender). Plenum, New York, pp 111–129(1981).

Saloheimo, M., I. Lund, J.M. Djahromi, B.S. Paulsen, J.K. Wold and Knutsen: Swollenin, a *Trichoderma reesei* protein with sequence similarity to the plant expansins, exhibits disruption activity on cellulosic materials. *Euro. J. Biochem.*, **269,** pp. 4202–4211, (2002).

Somerville, C., S. Bauer, G. Brininstool, M. Facette, T. Hamann, J. Milne, E. Osborne, A. Paredez, S. Person, T. Raab, S. Vormerk and H. Youngs: Toward a system approach to understanding plant cell wall. *Science*.**, 306**, 2206-2211 (2004).

Valss, F., M.T. Sancho, M.A. Fernandez-Muino and M.A. Checa: Determination of vitamin B_6 in cooked sausages. *J. Agri. Food Chem.,* **49**, 2001, pp. 38-41(2001).

Van de Velde .F , Pirovani .M.E, Cámara .M.S , and Güemes .D, del .H, Bernardi C.M: optimization of Thiamine and riboflavin in cereal foods by using response surface methodology. Food *Anal Meth* .,**5,** 1097 -1104 (2012).

Xueyan, T., D.A. Cronin and N.P. Brunton: Simplified approach to the determination of thiamine and riboflavin in meats using reverse phase HPLC. *J. Food Compos. Anal.,* **19**, 831–837 (2006).

Wong, K.K.Y., L.U.L. Tan and J.N. Saddler: Multiplicity of β-1, 4-xylanase in microorganisms, functions and applications. *Microbial Rev* ., **52**, 305–317 (2006).

Yadav, K.K., N. Garg, D. Kumar, S. Kumar, A. Singh and M. Kumar: Application of response surface methodology for optimization of polygalactuornase production by *Aspergillus niger. J. Enviro. Biol.,* **36**, 255-259 (2014)