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Full Length Research Paper

Fundamental conditions required in extracting an alpha-amylase from *Cadaba farinosa* Forsk branches

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Crude extracts of *Cadaba farinosa,* Forsk plant are used for their starch-reducing power in most tropic countries to liquefy/sweeten starch gruels. The extraction process is a time consuming osmotic rehydrating process characterized by fluctuating extract yield and consequently, an unstable inherent starch-reducing power. In this study, dried tender branches were ground (Ø≤1 mm) and soaked for 50 min under agitation. The resulting mixture was clarified by centrifugation and tested for total proteins and α-amylase activity. Preliminary trials preceding extraction, generated process factors which were screened using the Plackett-Burman design. The most significant factors were modeled using Doehlert's design. Four of the eight factors, pH, concentration, velocity gradient of agitation and centrifugal force, were most relevant for extraction. Statistical analysis of the models of total proteins and total activity suggested a compromise zone where specific activity is always ≥ 2.1 Umg^{-1} under particular prevailing fundamental conditions. The complete extraction cycle of more than 6 h was now reduced to approximately 60 min. Investigating the marginal starch-reducing power of α-amylase from *Cadaba* seeds could reveal a better source.

Key words: *Cadaba farinosa* branches, extraction, fundamental factors, screening, cell-free extract, α-amylase.

INTRODUCTION

The downstream processing of α-amylases (Wolfgang and Sauter, 1995; Teotia et al., 2001; Nirmala and Muralikrishna, 2002; Febe et al., 2002; Ezeji and Bahl, 2006; Biazus et al., 2007; Tripathi et al., 2007; Tripathi et al., 2007; Wolfgang, 2007) from plant tissues starts with an appropriate preparatory step through an extraction-clarification phase for the turbid extract (Amersham, 1999; Clive, 2002; Deutscher, 2009). Extracts of *Cadaba farinosa* plant, geographically distributed in tropical and subtropical countries (ICRAF, 2010; Umesh et al., 2010) are being exploited in rendering starch gruels sweet. However, exploitation of their hydrolytic (proteolytic and starch - reducing) potentials are fastidious, time consuming and exposed to varied yields from various extraction methods (Ayo and Okaka, 1998; Gaffa and Ayo, 2001; Gaffa et al., 2002a; Gaffa et al., 2002b; Gaffa and Ayo, 2003; Glew et al., 2010). These variations prompted the need to investigate elementary conditions for the extraction of a highly active and stable alpha-amylase from *C. farinosa*. Therefore, the preservation and optimisation of the starch-reducing power expressed by the extracts have to be taken into consideration for a suitable extraction process.

In a number of extractions, we are neither really interested in studying the effects of factors nor the interactions between these effects. Instead, we are more concerned with wanting to know how one or several measured characteristics behave in a well defined experimental domain (Marczyk et al., 2005). Response surface methodology (RSM) was used (Mathieu and Phan-tan-luu, 1997; Marczyk et al., 2005; Goupy and Creighton, 2006) to elucidate the process.

The general objective of this work consisted in scrutinizing and identifying extraction conditions which could

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Table 1. Description of factors considered for screening in Plackett-Burman design.

Factor	Name	Abbreviation	Unit	Low level (-)	High level (+)
A	pH	pH	/	4.5	7.85
B	Buffer type	pKa	/	4.79	7.21
C	Centrifugal force	C_f	g	5000	7500
D	Extraction time	E_t	min	30	50
E	Velocity gradient of agitation	G	s^{-1}	214	1011
F	Centrifugation time	C_t	min	5	20
G	*Cadaba* concentration	C_c	w/v	0.14	0.2
H	Temperature	T	°C	30	45

enhance the starch-reducing power of extracts.

Preliminary studies were carried out to identify these extraction parameters which enhanced enzyme activity; the most relevant factors were selected based on the 20% rule of Wilfred Pareto and suitable extraction conditions established through modelling based on the percentage specific amylase activity.

MATERIALS AND METHODS

Tender branches of *C. farinosa* were obtained from farms in the neighbourhood of Bini-Dang, Ngaoundere. The chemicals used were, bovine serum albumin (BSA) from Amersham Pharmacia Biotech; 3,5 dinitrosalicyclic acid (DNS) and Sodium hydroxide (NaOH) were from Sigma Chemical Company; maltose, hydrated sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$), sodium potassium tartrate (Na_2KNO_4), soluble starch [$(C_{12}H_{24}O_{12})_n$] and hydrated copper sulphate ($CuSO_4.5H_2O$) were from Merck Eurolab GmbH; Folin and Ciocalteu reagent, and sodium carbonate (Na_2CO_3) were from Fluka Fleinchemikalien GmbH and were all of analytical grade.

Raw material preparation

Fresh tender branches of *C. farinosa* were separated from the extremely tough parts of the entire branch and then, chopped with a knife into chips which were dried at 45°C for 5 days using a convective dryer (CKA 2000 AUF). These dried chips were ground into coarse particle sizes using a rotating blade mill (Fryma maschines AG, ML-150: CH-4310 Rheinfelden-Switzerland) and further reduced using a laboratory hammer mill with sieve (Polymix, PX-MFC 90D: VWR International S.A.S Cedex-France) into fine particle sizes (Ø≤1 mm). The fine powder was stored in plastic paper wrapped with aluminium foil.

Enzyme extraction process

A portion (50 g) of powder was weighed using an electronic balance (Denver instrument, APX-3202, max 3200, d = 0.01 g) and completed to 250 ml with 0.2 M phosphate buffer at pH 6.15 using the Consort C863: multi parameter analyzer made in Belgium. The resulting slurry (0.2 w/v) contained in a 600 ml beaker ($Ø_{inner}$ = 8.0 cm; $Ø_{outer}$ = 8.5 cm; H = 14.8 cm) inside a water bath (Memmert, F-Nr 760) at 40°C was mechanically agitated (Prolabo, 22J) at a velocity gradient; velocity gradient (G) was measured as the square root of the mechanical power (Pm) using the Aoip tachymeter (FN5601, France) and the Voltcraft plus (Energy monitor: Anschluss 3000W, 13A max) compared to the product of the dynamic viscosity (µ) measured using the Haake falling ball viscosimeter (D5677, Germany) and the volume of solution (V) $G=\sqrt{Pm/\mu V}$ of 365 s^{-1} controlled using a rheostat (Rototransfo, 90NC: 210, Dereix S.A Paris) for 50 min. The solution was then clarified by centrifugation (Heraeus-Kendro Lab products, Biofuge primo R: D-37520, Fab n°: 284678, Germany) at 4875 *g* for 20 min to separate the supernatant (cell-free extract) from pellets (exhausted cell wall materials). The cell-free extract was then analyzed for total protein (Lowry et al., 1951) and total α-amylase activity (Fischer and Stein, 1969) spectrophotometrically using the Rayleigh vis-723N.

Design of experiments

RSM was used in the design of experiments, based on exploratory works and factor screening process. Exploratory work consisted in evaluating the moisture content, using the oven (Heraeus-kendro laboratory products, D-63450 type: T6, fabrication n° 20001046, Germany) method described by AOAC (1990); pH and temperature range for activity; enzyme dilution curve and buffer type.

Factors (Table 1) emanating from preliminary trials were screened using the method described by Plackett and Burman (1946). The design matrix used a factorial plan of 2^8*3/64 composed of 12 randomly ordered experiments carried out in a single block, considered linear without interactions. Significant factors identified by Plackett-Burman design were modeled using Doehlert's design (Mathieu and Phan-tan-luu, 1997) at three levels. Enzyme kinetics was equally studied, to elucidate the relationship between optimal yields and time. *Cadaba* concentration was varied against time for all other factors constant and the time it required enzymes to convert substrate into products was assessed under optimal conditions of pH and temperature.

For all experiments, total proteins (Lowry et al., 1951) and total α-amylase activity (Fischer and Stein, 1969) were determined as response to the system from three trials. Statgraphics (Windows version 5.0 software, Inc.) and Sigmaplot (Windows version 11.0 Build 11.0.0.77 Copyright ©2008 systat software, Inc.) were used in analysing all data.

Statistical analysis

The '*f* 'ratio was used to reject the null hypothesis at the $P \leq 0.05$ probability. The validity of each mathematical model was verified after carrying out trial tests of points other than experimental points. Their R^2 and the absolute analysis of deviation from mean (AADM, where, yi,exp and yi,fitted are respectively the experimental and fitted values and P the number of experiments carried out. ($-1 \leq AADM \leq 1$)) were used in evaluating the degree to which the

Table 2. Results for total proteins, total amylase activity and specific amylase activity of factors in the Plackett-Burman design.

Run	Experimental matrix								Response						Response	
	Factor								Total activity			Total protein			Specific activity	
	A	B	C	D	E	F	G	H	A_{obs} (U)	A_{fit} (U)	*R*	P_{obs} (mg)	P_{fit} (mg)	*R*	SA_{Calcul} (Umg^{-1})	SA_{theo} (Umg^{-1})
1	-	-	+	+	+	-	+	+	28.34	32.37	-4.04	15.00	15.85	-0.85	1.89	2.04
2	-	+	+	+	-	+	+	-	49.08	48.99	0.09	20.31	20.60	-0.29	2.42	2.38
3	+	+	-	+	-	-	-	+	22.47	26.42	-3.95	20.01	20.53	-0.52	1.12	1.29
4	+	-	+	-	-	-	+	+	51.14	47.10	4.04	17.67	16.82	0.85	2.89	2.80
5	-	-	-	-	-	-	-	-	11.07	11.12	-0.04	14.35	14.99	-0.64	0.77	0.74
6	+	+	+	-	+	+	-	+	11.72	11.75	-0.03	18.98	19.63	-0.65	0.62	0.60
7	-	-	-	+	+	+	-	+	8.07	4.04	4.04	17.95	17.10	0.85	0.45	0.24
8	+	+	-	+	+	-	+	-	40.85	36.92	3.93	22.81	22.30	0.51	1.79	1.66
9	-	+	+	-	+	-	-	-	4.11	4.10	0.02	17.18	16.55	0.63	0.24	0.25
10	+	-	+	+	-	+	-	-	24.54	24.62	-0.07	16.27	15.97	0.30	1.51	1.54
11	-	+	-	-	-	+	+	+	43.07	43.14	-0.07	23,00	22.70	0.30	1.87	1.90
12	+	-	-	-	+	+	+	-	25.32	29.27	-3.95	19.32	19.83	-0.51	1.31	1.48

A_{obs} and A_{fit} = observed and fitted activity; P_{obs} and P_{fit} = observed and fitted amount of protein; **R** = residual values; **SaCal** and **SaTheo** = calculated and theoretical specific activity. **A** = ph; **b**=buffer; **c** = centrifugal force; **d** = extraction time; **e** = velocity gradient of agitation; **f** = centrifugal time; **g** = *Cadaba* conc and **h** = temperature.

$$AADM = \frac{\left[\sum_{i=1}^{p}\left(\frac{y_{i,exp}-y_{i,fitted}}{y_{i,exp}}\right)\right]}{p} \quad (1)$$

models were representative. This is calculated as follows;

RESULTS AND DISCUSSION

The moisture content of *C. farinosa* samples used was evaluated at 9.47 ± 0.47%. Umesh et al. (2010) reported 9.0% moisture content during their investigation of the pharmacognostic and phytochemical nature of the roots.

Exploratory results and factor screening

Results of the exploratory works suggested that, the effects of the factors: buffer pH, buffer pKa, extraction time, velocity gradient of agitation, concentration, temperature, centrifugal force and centrifugation time, had an influence on the extraction process. Plackett-Burman design with results expressed in units of specific amylase activity is shown in Table 2. Specific activity (Umg^{-1}) was the number of units of activity per milligram of total protein and a measure of the purity of an enzyme sample.

Screening revealed that, buffer type, concentration and centrifugal force were the most relevant factors for extracting proteins. The velocity gradient of agitation and concentration were determinant for preserving the activity function of the extracted proteins. These were 20% of the factors responsible for 80% of the process variations. However, pH, velocity gradient of agitation and centrifugal force made a negative contribution to the extraction process. The rate of agitation was proportional to the rate of denaturation of amylase activity of extracted proteins. Most denatured proteins were subsequently inactivated and became susceptible to separation proportionally to the applied centrifugal force with spent materials.

Modeling and statistical analysis of the extraction process

The following models where; Yi = response, X1 = Cadaba concentration (w/v); X2 = pH of buffer (pH); X3 = centrifugal force (g); X4 = velocity gradient of agitation (s-1) for total proteins and total amylase activity were obtained using Doehlert's design (Table 3).

Total proteins

$$Y_P = 86.39 + 163.42X_1 - 12.1X_2 - 0.002X_3 - 0.091X_4 + 0.716X_1X_2 - 0.001X_1X_3 + 0.042X_1X_4 + 0.0X_2X_3 + 0.011X_2X_4 + 0.0X_3X_4 - 49.941X_1^2 - 0.067X_2^2 + 0.0X_3^2 + 0.0X_4^2 \quad (2)$$

Total activity

$$Y_A = 129.931 - 72.012X_1 - 15.526X_2 - 0.028X_3 - 0.460X_4 - 23.889X_1X_2 + 0.028X_1X_3 + 0.554X_1X_4 + 0.001X_2X_3 + 0.058X_2X_4 - 0.0X_3X_4 + 172.914X_1^2 + 1.036X_2^2 + 0.0X_3^2 + 0.003X_4^2 \quad (3)$$

Table 3. Results for total proteins, total amylase activity and specific amylase activity for Doehlert's design.

Run	Experimental matrix				Response						Response	
	Factor				Total activity			Total protein			Specific activity	
	X_1	X_2	X_3	X_4	A_{obs} (U)	A_{fit} (U)	*R*	P_{obs} (mg)	P_{fit} (mg)	*R*	SA_{obs} (Umg^{-1})	SA_{fit} (Umg^{-1})
1	0.2	6.15	4875	289	66.61	72.53	-5.92	43.70	43.32	0.38	1.52	1.67
2	0.18	6.15	4875	289	73.61	67.69	5.92	44.89	45.28	-0.39	1.64	1.50
3	0.195	6.3	4875	289	77.22	72.83	4.39	46.46	47.11	-0.65	1.66	1.55
4	0.185	6.0	4875	289	40.28	44.67	-4.40	47.65	47.00	0.65	0.85	0.95
5	0.195	6.0	4875	289	106.39	100.85	5.54	44.97	44.94	0.03	2.37	2.24
6	0.185	6.3	4875	289	118.61	124.16	-5.55	46.99	47.02	-0.03	2.52	2.64
7	0.195	6.19	5000	289	135.00	133.29	1.71	48.35	48.70	-0.35	2.79	2.74
8	0.185	6.11	4750	289	129.44	131.14	-1.70	51.19	50.83	0.36	2.54	2.59
9	0.195	6.11	4750	289	75.28	73.70	1.58	49.81	50.29	-0.48	1.51	1.47
10	0.19	6.24	4750	289	80.28	76.83	3.44	47.14	46.35	0.79	1.70	1.66
11	0.185	6.19	5000	289	89.44	91.00	-1.56	50.59	50.12	0.48	1.77	1.82
12	0.19	6.06	5000	289	70.83	74.25	-3.42	43.46	44.25	-0.79	1.63	1.68
13	0.195	6.19	4900	365	103.89	103.72	0.17	53.08	52.36	0.72	1.96	1.98
14	0.185	6.11	4849	215	128.06	128.23	-0.17	49.30	50.02	-0.72	2.59	2.56
15	0.195	6.11	4849	215	91.39	92.93	-1.54	47.81	47.85	-0.04	1.91	1.94
16	0.19	6.24	4849	215	78.39	80.72	-2.33	45.22	45.27	-0.05	1.73	1.78
17	0.19	6.15	4951	215	120.22	116.18	4.04	49.08	48.27	0.81	2.45	2.41
18	0.185	6.19	4900	265	85.56	84.02	1.54	52.19	52.15	0.04	1.64	1.61
19	0.19	6.06	4900	365	54.28	51.95	2.33	47.73	47.68	0.05	1.14	1.09
20	0.19	6.15	4798	365	88.67	92.71	-4.04	51.68	52.49	-0.81	1.72	1.77
21	0.19	6.15	4875	289	43.33	44.18	-0.84	47.57	49.29	-1.72	0.91	0.90
22	0.19	6.15	4875	289	41.39	44.18	-2.79	51.27	49.29	1.98	0.81	0.90
23	0.19	6.15	4875	289	44.72	44.18	0.55	48.49	49.29	-0.81	0.92	0.90
24	0.19	6.15	4875	289	47.22	44.18	3.05	49.84	49.29	0.55	0.95	0.90

[1] A_{obs} and $\mathbf{A_{fit}}$ = observed and fitted activity; $\mathbf{P_{obs}}$ and $\mathbf{P_{fit}}$ = observed and fitted amount of protein; **R** = residual values; $\mathbf{Sa_{Cal}}$ and $\mathbf{Sa_{Theo}}$ = calculated and theoretical specific activity. X_1 = Concentration; X^2 = pH; X_3 = centrifugal force; X_4 = velocity gradient of agitation.

Table 4. Contribution of factors to the models of total proteins and total amylase activity in the extraction process, respectively.

Variable	X_1	X_2	X_3	X_4	X_1X_2	X_1X_3	X_1X_4	X_2X_3	X_2X_4	X_3X_4	X_{12}	X_{22}	X_{32}	X_{42}
Y_P (%)	23.8	1.7	0.0	0.13	1.05	0.0	0.06	0.0	0.02	0.0	72.9	0.1	0.0	0.0
Y_A (%)	25.1	5.4	0.01	0.2	8.34	0.01	0.19	0.0	0.02	0.0	60.4	0.4	0.0	0.0

The percentage contribution of factors (Table 4) to the models suggests that X_1 accounted for more than 85% in both single and quadratic effect combined to the entire process implying that, extracting more proteins with amylase activity from *C. farinosa* required a high concentration relatively to the contributions of other factors. X_1X_2 accounted for 6 and 8%, respectively to Y_P and Y_A. All other coefficients made just minute contributions to the models. The velocity gradient for extraction and the centrifugal force are factors whose contributions were small but significant enough to produce a negative influence on the overall process. The contour plot representation of their concerted effects shows a shaded region which is a compromise zone (Figure 1) where the average minimum specific activity was ≥ 2.1 Umg^{-1}. Extraction parameters like concentration, pH, centrifugal force and velocity gradient of agitation, were operated for values within the ranges of: 0.194 to 0.200g/ml, 6.0 to 6.05, 4920 to 5000 g and 220 to 260 s^{-1} respectively.

Comparative studies suggest an increasing loss in Y_A (Figures 2a and 2b) corresponds to a simultaneous increase loss in Y_P for increasing values of X_1 up to a break-even point. Any further increase in X_1 instead leads to an increase in Y_A for decreasing Y_P (Figure 3). Both models

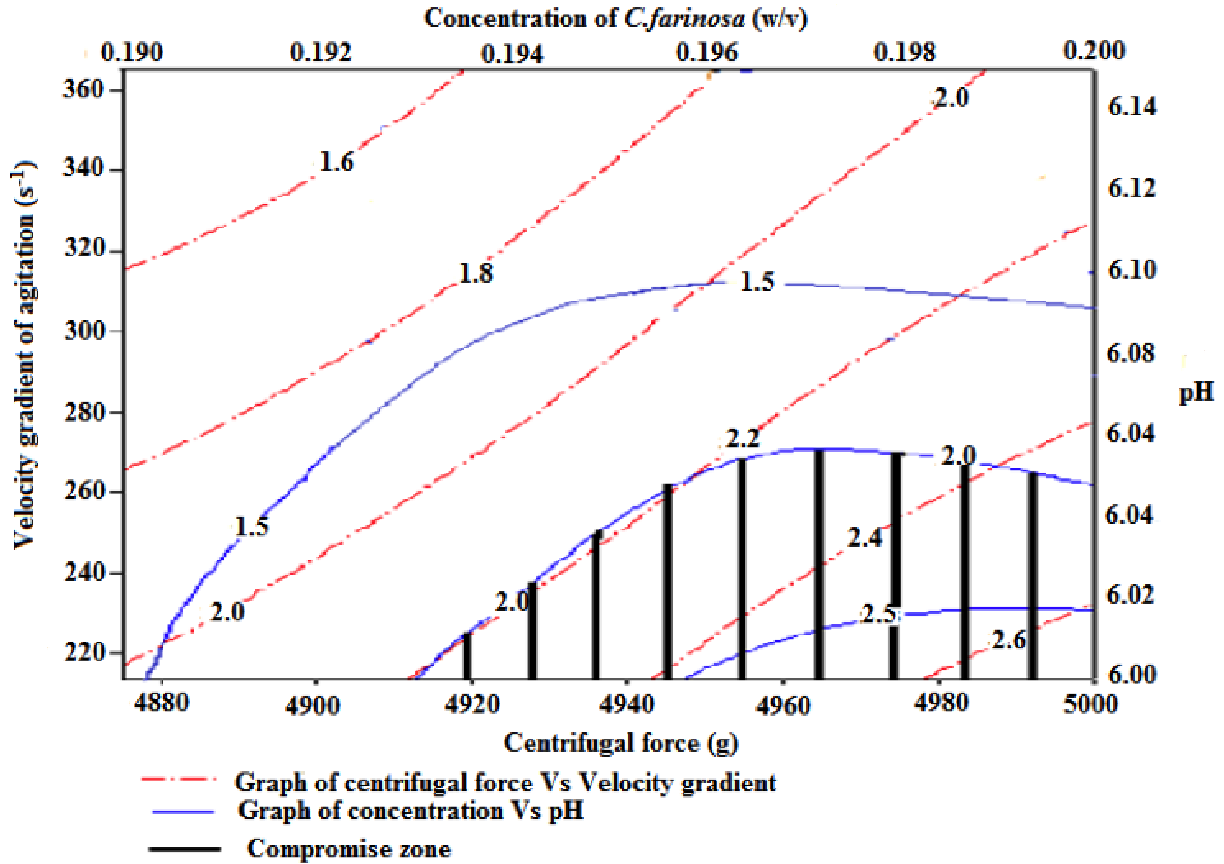

Figure 1. Variation of specific amylase activity as a function of pH of the buffer solution, concentration, velocity gradient of agitation and centrifugal force for separation. A specific amylase activity of ≥ 2.5 Umg^{-1} corresponds to boundary conditions of 6.0 ≤ pH ≤ 6.02; 0.196 ≤ concentration ≤ 0.2; 4950 ≤ g ≤ 5000; 215 ≤ s^{-1} ≤ 230.

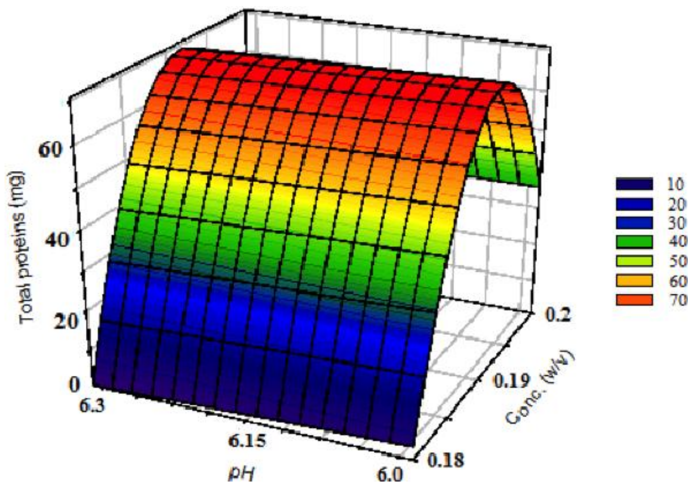

Figure 2a. Trends in total proteins as a function of pH versus concentration. Total proteins increases to about 68 mg at a pH of 6.0 and drops after a concentration of 0.195 g/mL to about 45 mg.

provide the evidence that, specific activity is more dependent on the pKa and pH, not concentration. They accounted for 3 Umg^{-1} (X_2) and 8 Umg^{-1} (X_1X_2).

The analysis of variance after 24 runs generated six significant effects for total protein and 14 significant effects for total activity, at the 95.0% confidence interval (P ≤ 0.05). The coefficient of determination indicated that the models (Table 5) as fitted could explain 98.1%, of the variability in specific amylase activity and when adjusted accounted for 97.8% of the overall phenomenon. The AADM was evaluated at 0.005. In principle, it is another form of Chi-squared which characterizes the degree of dispersion between points of the models. Student's distribution test was also used to verify the veracity of the null hypothesis between means of the experimental and fitted values.

It showed no significant difference for an average specific amylase activity of 2.37 ± 0.01 Umg^{-1} at the 95.0% confidence interval ($t_{exp'tal}$ = 0.012; t_{95} = 1.68). In partial conclusion, experiments were more exposed to systematic errors than chance errors.

Trends in total proteins and α-amylase activity

Increasing concentrations of *C. farinosa* were investigated as a function of time. General aspects of the profile in

Table 5. Analysis of variance for both models of total proteins and total amylase activity for the extraction process.

Source	Total protein					Total Amylase activity				
	Sum of squares	Df	Mean square	F-ratio	P-value	Sum of squares	Df	Mean square	F-ratio	P-value
X1	7.92	1	7.92	5.36	0.04	157.67	1	157.67	4.11	0.07
X2	5.68	1	5.68	3.85	0.08	1347.04	1	1347.04	35.08	0.00
X3	17.06	1	17.06	11.55	0.00	2967.05	1	2967.05	77.27	0.00
X4	12.47	1	12.47	8.44	0.01	286.28	1	286.28	7.46	0.02
X1X1	33.25	1	33.25	22.50	0.00	253,23	1	253,23	6.59	0.03
X1X2	1.15	1	1.15	0.78	0.39	950.86	1	950.86	24.76	0.00
X1X3	0.48	1	0.48	0.33	0.58	1131.11	1	1131.11	29.46	0.00
X1X4	1.01	1	1.01	0.69	0.42	150.51	1	150.51	3.92	0.08
X2X2	5.52	1	5.52	3.74	0.08	1687.67	1	1687.67	43.95	0.00
X2X3	36.69	1	36.69	24.82	0.00	593.84	1	593.84	15.47	0.00
X2X4	17.52	1	17.52	11.86	0.00	475.95	1	475.95	12.40	0.01
X3X3	0.41	1	0.41	0.28	0.61	2298.74	1	2298.74	59.86	0.00
X3X4	0.04	1	0.04	0.03	0.86	179.01	1	179.01	4.66	0.06
X4X4	4.60	1	4.60	3.12	0.11	2320	1	2320	60.44	0.00
Total error	13.30	9	1.47			345.59	9	38.39		
Total (corr.)	162.83	23				8101.16	23			
R2	91.83%					95.7%				
Adjusted	79.12%					89.1%				
AADM	0.0051					0.005				

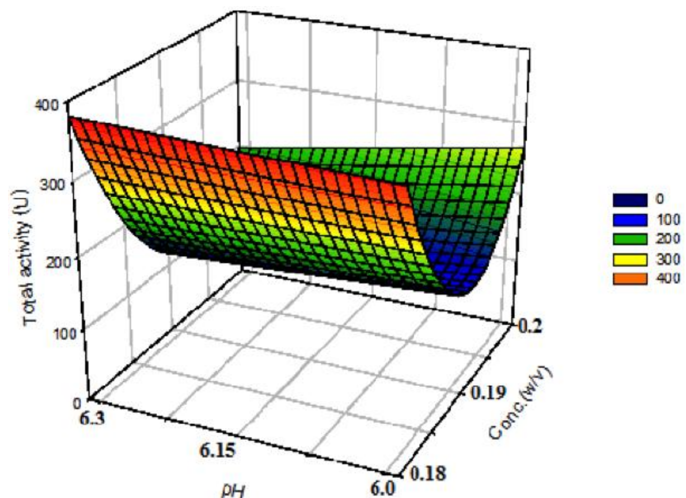

Figure 2b. Trends in total amylase activity as a function of pH versus concentration. Enzyme activity at pH 6.0 drops from 400 U to a minimum of about 150 U at a concentration of 0.19 g/ml.

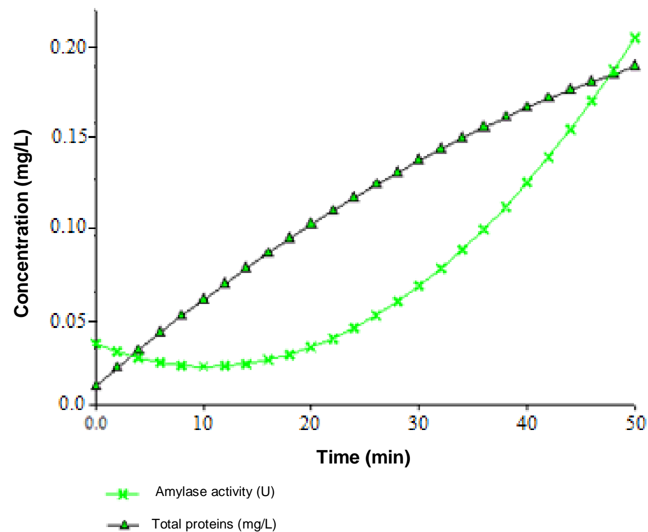

Figure 3. Trends in the evolution of total proteins and α-amylase activity based on the concentration of *C. farinosa* as a function of time. Total proteins and α-amylase activity both have a critical minimum concentration.

yields (Figure 3) showed a lag in effect of α-amylase activity for increasing concentrations of *C. farinosa*. Initially, some amounts of proteins were responsible for the observed activity but the presences of contaminants impeded functional proteins responsible for the activity.

This occurred within the first 15 min. After approximately 30 min, alpha-amylase activity grew exponential cause of increase protein concentration, less protein denaturation and less mobility of most contaminants. Then, at approximately 50 min of extraction, a break-even point was reached where more α-amylase activity could be accounted for by a small amount of total proteins.

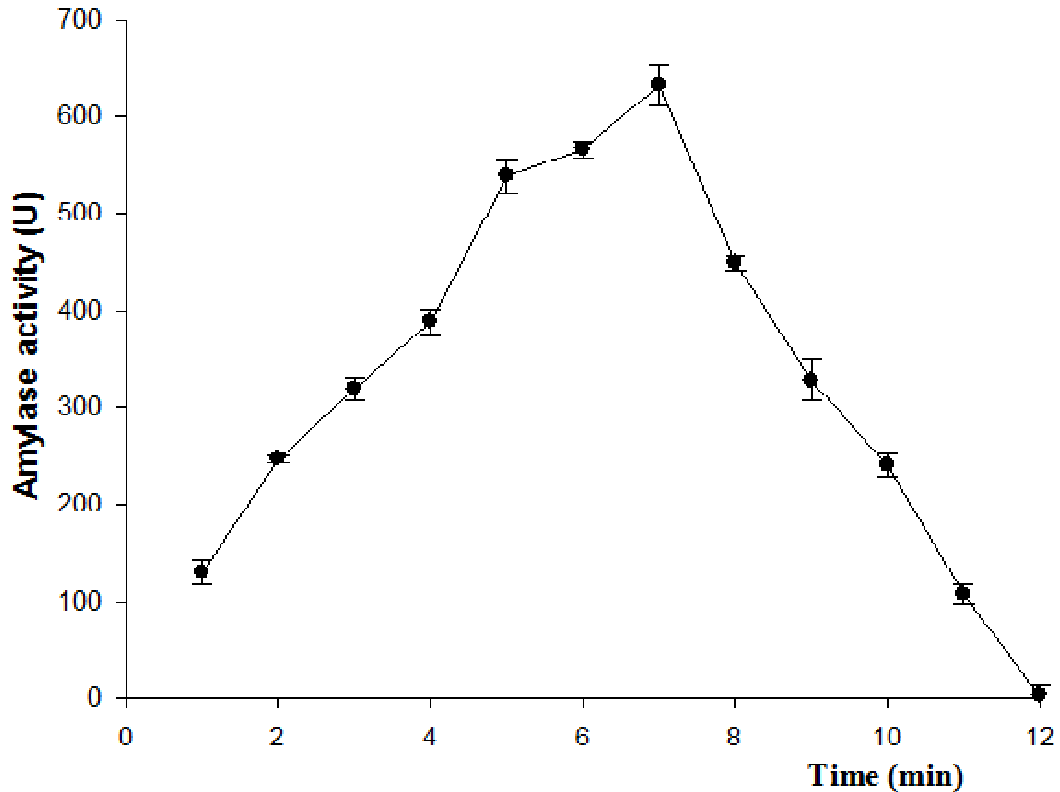

Figure 4a. Trend of enzyme activity as a function of time at pH 6.0 and 70°C. Maximum amylase activity of about 650 U was obtained after 7 min of reaction time.

Practically, at a baseline concentration of about 0.08 mg/L corresponding to an extraction time of 23 min, the amount of extracted proteins produced a proportionate effect of α-amylase activity, if and only if a compromise was established. When the concentration falls, very few proteins account for more activity in competition with contaminants. Increasing concentrations showed that, more of the proteins with the active function were being extracted at the expense of contaminants.

Optimum α-amylase activity

Based on preliminary studies, the evolution of amylase activity at optimum pH (6.0) and temperature (70°C), showed a general rise from 129.6 U after 1 min of catalysis to 632.6 U corresponding to a 5-fold increase in activity after 7 min (Figure 4a) suggesting that, the enzyme catalytic rate was exactly 5 times faster at 7 min than at 1 min; every 1 min of enzyme stability corresponded to an average catalytic factor of 0.7. Extrapolation of the degree of bonding existing between enzyme and substrate concentration showed a strong correlation over a reaction period of 7 min (Figure 4b).

Loss in residua activity after 7 min of reaction must have partly being due to a progressive reduction in substrate-enzyme protection as substrates were converted to products (Wolfgang, 2007). A significant loss in the amount of Ca^{2+} ions responsible for activity and stability of the metalloenzymes (Fisher and Stein, 1969) was another possibility. Studies by Glew et al. (2010) testify the presences of significant amounts of Ca^{2+} ions which are likely to be responsible for their stability at high temperatures. Therefore, in partial conclusion, a pH of about 6.0 and temperatures not exceeding 70°C were suitable for maximum enzyme activity. Addition of Ca^{2+} ions to the extraction buffer could be a remedy to heat denaturation of the active sites of the enzymes.

Conclusion

Cell-free extracts of *C. farinosa* expressed the value of a potential substitute to the commercial brands. The very long durations required for extracting α-amylases from *C. farinosa,* (more than 6 h) were now reduced to just an hour. Approximately, 60 min was required for extraction of α-amylase. Few proteins accounted for most of the expressed α-amylase activity though influenced by contaminants. Thus, concentrating the bulk proteins to remove contaminants and obtained proteins with enhanced

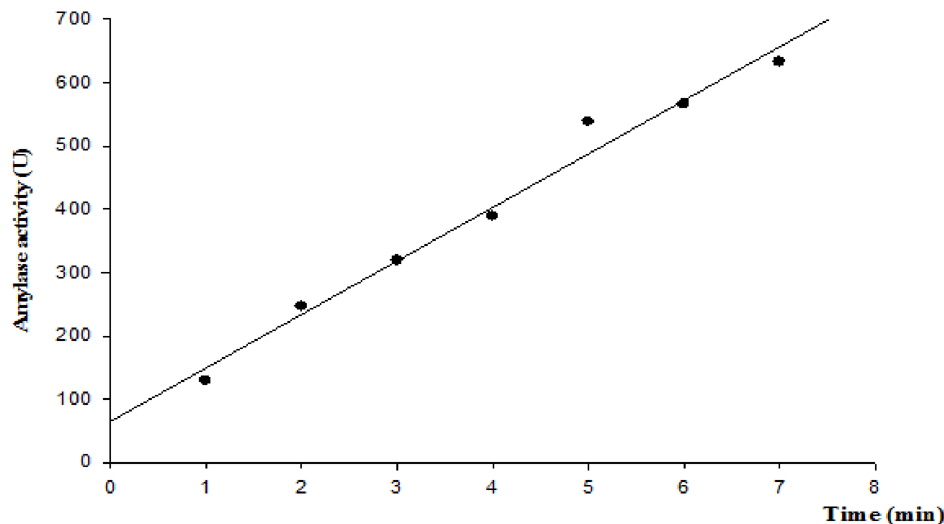

Figure 4b. Correlation between enzyme activity and the 7 min reaction duration at pH 6.0 and temperature, 70°C. The regression equation y = 0.17x + 79 was obtained with an R^2 = 0.99 whose significance was the existence of a first order kinetics for the enzyme catalyzed reaction.

starch-reducing properties is desirable.

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