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Experimental and modelling studies provide kinetic insights on bio-transformation of locust bean to condiment

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Abstract

Influence of temperature (370C, 480C) and particle size (whole, powder) in relation to time (1- 96 h) of incubation on bio-conversion of locust bean to condiment was evaluated using three bio-conversion indices namely; free amino acid (FAA), total phenolic content (TPC) and relative reducing power (RRP). In addition, data obtained was subsequently subjected to three model conformation analysis with view to gain kinetic insight on bio- transformation of locust bean to condiment. Experimental result showed that a period of 96 h was adequate to convert any of the samples to condiment. Sample subjected to high temperature and large surface area were characterised with high FAA and attainment of FAA bio-conversion equilibrium at short incubation duration in comparison to samples characterised with small surface area subjected to low temperature incubation condition. A result of kinetic model analysis showed the process of bio-conversion of locust bean to condiment can best be described by first and pseudo-first order reaction for all the markers evaluated for the bio-conversion of locust bean to condiment. The result of this study provides insight to mechanism of bio-conversion of locust bean to condiment which should find application in designing of an improved process for the bio-conversion of locust bean to condiment.

Introduction

Palatability of food is important in nutrition for some reasons notably, selection, intake, absorption and digestion of food. Although, all the five senses are involve in the determination of food palatability, with taste playing a major role (Yamaguchi and Ninomiya, 2000). In terms of source, tastant can be synthetic or natural in origin. Preference for tastant of natural origin to tastant of synthetic origin is most importantly due to safety concern on tastant of synthetic origin.

In Africa, there many condiments used to enhance palatability of foods, most importantly fermented oil seeds such as locust bean, cotton, melon, and soybean (Odunfa, 1981; Odunfa 1985). The potential commercial opportunity that awaits the production of locust bean condiment necessitate studies to gain understanding of the conversion of locust bean to condiment with view to develop production process that obviate the laborious process associated with the traditional process as well as facilitate the preparation of a product not associated with the short coming of product derived from the traditional method. In a bid to gain scientific comprehension of bioconversion of locust bean to condiment a series of investigation (Daramola *et al*., 2009; Daramola and Osanyinlusi, 2013) had been initiated. In this paper, experimental studies was carried out with view to provide kinetic insight on bio-conversion of locust bean too condiment.

Materials and Methods

Processing/fermentation of locust bean to condiment

Two broad stages are involved in the preparation of locust bean to condiment, namely, 1. cooking stage and 2. fermentation stage.

Preparation of substrate for the process fermentation

The procedure for the preparation of substrate for fermentation was carried out essentially according to the procedure described by Kok *et al.* (1986). The raw beans were soaked overnight in water and the adhering pulpy material was removed manually. The resultant material referred to as ”whole beans “ were boiled in water using pressure pot for 6 h to loosen leathery testa. The testa and endosperm were then separated manually by rubbing the beans between palms of the hand under running water to obtain “dehulled beans”. The dehulled beans were subsequently cooked for another 30mins with view to soften-cooked the beans. Adequacy of cooking was determined by the development of characteristic

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brown colour from the reaction of protein and sugar.

Bio-conversion of cooked locust bean to condiment

Beans were divided into 4 principal groups to facilitate investigation on fermentation/incubation temperature of 37^0C and 480C in relation to two particle size (whole and powder). Fermentation of a typical (sample) experimental set-up of a designated substrate particle size was carried out at a particular temperature using laboratory incubator as the fermentor. Substrate was placed in a pyrex- plastic container lined with solid foam to absorb traces of moisture. Samples were taken from fermentor from a period of 1h to 96 h at 24 h intervals. Recovered samples were dried at 50^0C, milled and packaged for bio-conversion marker analysis.

Kinetic modelling

Three models namely; first order, pseudo first order, and second order were used to investigate the bioconversion mechanism of locust beans to condiment under the experimental conditions employed in this study.

First order kinetic model:

An integrated form of First order equation yields

$$-\ln\left(1-\frac{X_A}{X_{AE}}\right) = K_1\left(1+\frac{1}{K}\right)t \quad \text{.....(1)}$$

Thus equation (1) can be re-written as:

$$\ln [1- U_t] = -K_r t \quad \text{.....(2)}$$

where: K_r = rate constant, U_t = fractional attainment of equilibrum

$$Ut = \frac{X_A}{X_{AE}} \quad \text{.....(3)}$$

Therefore, a plot of –ln [1- U_t] versus time t will generate a straight line. The slope of the straight line gives K_r = rate of (reaction) conversion of locust bean to condiment.

Pseudo first order model

The pseudo first order model can be represented as:

$$\frac{d q_t}{d_t} = K_s (q_e - q_t) \quad \text{.....(4)}$$

Equation (4) can be integrated for the following boundary conditions to obtain equation (5) thus; t=0, t=t, $q_t = q_t$

$$-\log (q_e - qt) = \log q_e + \frac{K}{2.303} t \quad \text{.....(5)}$$

A plot of –log (q_e-q_t) versus t will give a straight line with the slope given a conversion rate constant K/2.303.

Pseudo second order model:

The pseudo second order model reaction can be expressed as equation (6)

$$\frac{d q_t}{dt} = K_s (q_e - q_t)^2 \quad \text{.....(6)}$$

On integration of equation (6) for the following boundary conditions: t = 0, q_t = 0, t=t, $q_t = q_t$ and on rearrangement gives equation (7)

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t \quad \text{..... (7)}$$

where K_2 is the rate constant for the pseudo second order model.

Determination of free amino acids

Free amino acids in the locust bean condiment samples were extracted with 80% ethanol (v/v) in accordance with the method of Odibo *et al.* (1990). The free amino acids in the ethanolic extract were estimated using the ninhydrin colorimetric method (Rosen, 1957) using glycine as standard.

Evaluation of total phenolic content

Total phenolic content in the locust bean condiment samples were evaluated according to the method described by Taga *et al*. (1984). Briefly: A 100 µL of Folin-Ciocalteau reagent (2N wrt acid Fluka Chemic AG-Ch-9470 BUCHS) was added to each sample (20 µL) and well mixed after addition of 1.58 mL of water. After 30 seconds, 300 µL of 2% sodium carbonate solution was added and the sample tubes were left at room temperature for 2h. The absorbance (A) of the developed blue colour was measured at 750nm using Unicam Helios & UV/ VIS/Spectrophotmeter. A plot of A750nm against corresponding concentration was used to calculate phenolic content (g/g ascorbic acid equivalent).

Determination of relative reducing power

Relative reducing power in the locust bean condiment samples were determined in accordance with the method of Oyaizu (1986). Simply, each sample (1mg/mL) in ethanol (2.5 mL) was mixed with sodium phosphate buffer (pH 6.6). the buffered sample was mixed with conditioning reagents (1%K_3-Fe-CN_6, 10% TCA, 0.1% $FeCl_3$) centrifuged, diluted using distilled water and absorbance was measured at 700 nm. Higher absorbance indicates a higher reducing power. A plot of A_{750nm} against

Table 1. Hydrolysate indices profile during biotransformation of locust bean to condiment

Temp	Particle size	Day 0	Day 1	Day 2	Day 3	Day 4
			[1]Free amino acids			
37°C	Whole	0.0002	0.02	0.026	0.030	0.034
	Milled	0.0002	0.016	0.034	0.036	0.028
48°C	Whole	0.0002	0.006	0.020	0.044	0.032
	Milled	0.0002	0.012	0.016	0.028	0.022
			[2]Total phenolic content			
37°C	Whole	1.906×10^{-3}	4.813×10^{-3}	8.594×10^{-3}	8.594×10^{-3}	7.813×10^{-3}
	Milled	1.906×10^{-3}	5.813×10^{-3}	8.594×10^{-3}	7.031×10^{-3}	4.688×10^{-3}
48°C	Whole	1.906×10^{-3}	4.25×10^{-3}	6.563×10^{-3}	9.375×10^{-3}	8.594×10^{-3}
	Milled	1.906×10^{-3}	4.00×10^{-3}	9.469×10^{-3}	7.813×10^{-3}	7.031×10^{-3}
			[3]Relative reducing power			
37°C	Whole	1.039×10^{-3}	2.009×10^{-3}	5.357×10^{-3}	5.002×10^{-3}	4.911×10^{-3}
	Milled	1.039×10^{-3}	3.23×10^{-3}	5.134×10^{-3}	5.580×10^{-3}	5.357×10^{-3}
48°C	Whole	1.039×10^{-3}	2.679×10^{-3}	4.241×10^{-3}	5.357×10^{-3}	5.357×10^{-3}
	Milled	1.039×10^{-3}	4.018×10^{-3}	4.687×10^{-3}	4.911×10^{-3}	4.911×10^{-3}

[1]g glycine equivalent/mL of sample extract, [2,3] g ascorbic acid activity equivalent /ml of sample extract, [1,2,3] average of two determinations

corresponding concentration was used to calculate phenolic content (g/g ascorbic acid equivalent).

Results and Discussion

Influence of temperature and particle size on conversion of locust bean to condiment

Influence of temperature and particle size was studied on the conversion of locust bean to condiment. This was informed by the fact that temperature and particle size (surface area) are two fundamental factors affecting hydrolysis rate which signifies progress in bioconversion of locust bean to condiment. Three indices of bio-conversion were evaluated namely; Free amino acid, Total phenolic content and Relative reducing power.

Free amino acid

Free amino acid (FAA) gives insight to locust bean hydrolysis and release of tastant components and precursors of amino acids origin (Ninomiya and Yamaguchi (2000). As shown in Table 1 there was an increase in the quantity of FAA (g glycine equivalent/mL of sample extract) to the maximum of 0.044 hydrolysed at 48^{0}C in comparison to 0.034 at temperature of 37^{0}C and equilibrium quantity was quickly attained at high temperature of 48^{0}C. This can be explained by the fundamental principle that reaction rate increase as temperature increases. In addition, it is important to state that temperature of 480C was neither too much for the micro-organisms involved in the bio-transformation nor the enzyme released by the micro-organisms.

Total phenolic content

Total Phenolic content (TPC) was assessed for two reasons namely; 1. As a contributor to the taste of the condiment. 2. As a contributor to antioxidant quality of the condiment. In an earlier study by Omafuvbe *et al* (2004), tocopherol and other phenolics were identified in locust bean condiment. Examination of the results in Table 1 showed that high values 9.469 of TPC (g ascorbic acid activity equivalent /ml of sample extract) was obtained for treatment at 48^{0}C in comparison to low 8.594 values of TPC for samples fermented at 37^{0}C. Also, it can be observed that it takes shorter duration at 48^{0}C to attained equilibrium TPC when compared to sample set-up at 37^{0}C.

Relative reducing power

Relative reducing power (RRP) was evaluated because it is antioxidant marker. Effect of temperature on RRP (g ascorbic acid activity equivalent /ml of sample extract) appeared not profound. This suggests that the antioxidative activity of locust bean during bio-conversion to condiment does not necessarily depend on only TPC but could be affected by other substances such as pro-oxidants, and minerals (Giese, 1996).

Influence of particle size

Considering the results on Table 1 with respect to particle size in a particular temperature, it is apparent that high amount (0.036) of FAA at three days was obtained for fixed particle size sample in comparison to FAA of 0.034 and duration of four days. However, a contrary result was obtained for different particle size at fixed temperature of 48^{0}C. This trend of the result was probably due to uncoordinated polymerisation and de-peptidation reactions. Similarly, high (data not shown) equilibrium TPC was attained at a shorter duration in a set-up with smaller particle

Table 2. Kinetic parameters of the hydrolysate indices during biotransformation of locust bean to condiment

Treatment		First order			Pseudo first order			Second order		
Temp	Particle size	q_e	k_r	r	q_e	k_r	r	q_e	k_r	r
					Free amino acids					
37°C	Whole	0.034	0.0289	0.9861	0.034	0.0134	0.9944	0.034	22.54	-0.337
	Milled	0.036	0.0623	0.9698	0.036	0.02175	0.9405	0.036	36.94	-0.265
48°C	Whole	0.044	0.047	0.8970	0.044	0.0146	0.9256	0.044	12.50	-0.758
	Milled	0.028	0.069	0.9023	0.028	0.0191	0.9340	0.028	41.67	-0.081
					Total phenolic content					
37°C	Whole	0.034	0.0293	0.967	0.034	0.024	0.9582	0.034	101.33	0.9073
	Milled	0.036	0.0513	0.9896	0.036	0.028	0.9849	0.036	141.44	0.9461
48°C	Whole	0.044	0.0439	0.9313	0.044	0.0205	0.9264	0.044	75.71	0.9477
	Milled	0.028	0.0584	0.9192	0.028	0.0247	0.9214	0.028	114.70	0.9016
					Relative reducing power					
37°C	Whole	$5.359X10^{-3}$	0.0371	0.9061	0.034	0.05366	0.8986	0.034	170.16	0.9061
	Milled	$5.58X10^{-3}$	0.0465	0.9877	0.036	0.0353	0.8539	0.036	185.46	0.9854
48°C	Whole	$5.357X10^{-3}$	0.0333	0.9743	0.044	0.0219	0.9689	0.044	178.77	0.235
	Milled	$4.911X10^{-3}$	0.0442	0.9448	0.028	0.029	0.9136	0.028	192.41	0.999

size in comparison to set-up with bigger particle size substrate. However, increase in surface area does not lead to concomitant increase in RRP during conversion of locust bean to condiment. This result could be probably due to high vulnerability to oxidation associated with substrate of comparative high surface area (small particles sizes) thereby destroying antioxidant activity of active components of tests samples.

Kinetic conversion of locust beans to condiment

Study on kinetic conversion of locust beans to condiment was accomplished at two temperatures and substrate particles. Three hydrolysate indices namely FAA, TPC and RRP were evaluated and subsequently analysed for model insights. Results showed that an experimental duration of 96 h was sufficient for conversion of all the locust beans to condiment with respect to attainment of bean-condiment- conversion equilibrium. The kinetic of locust bean conversion to condiment is described using three kinetic models: the first order, pseudo first and pseudo second order models. The fitness of the models was ascertained using rate constant comparative analysis and correlation coefficients. The computed results from the experimental data are shown in Table 2. Examination of the result showed that hydrolysis rate (bio-transformation rate) for FAA generally increased with increase in temperature and increase in surface area (decrease in substrate particle size) for both first order model and pseudo first order model. The two models were found adequate. More importantly, a comparison between the correlation coefficients of the two latter models suggests that pseudo first order fits much more than first order model. However, correlation coefficient of the result (Table 2) of second order model appeared to be grossly inadequate for bio-conversion of locust beans to condiment, hence did not merit discussion.

Total phenolic content (TPC)

Both the particle size and temperature enhanced bio-conversion of locust bean to condiment as shown by high K_r = 0.0584-0.0513 for samples with high surface area in comparison to low K_r = 0 0293-0.0439 for samples with low surface area for first order model. Similar trends were obtained using pseudo first and second order model. The result of the calculated correlation coefficient showed that all the models described adequately the formation TPC during bio-conversion of locust bean to condiment with first and pseudo first order models being the best. Also, temperature exerted similar Kr and correlation coefficient trend, hence similar articulation for model adequacy as above.

Relative reducing power

Using hydrolysis rate and correlation coefficients, the influence of temperature and particle size on RRP result were similar to the above reported results. The order of fitness of models was: first > pseudo first > second order.

Conclusion

This study provides kinetics insight into biotransformation of locust beans to condiments. Analysis of indices of transformation into condiment such as FAA, total phenolic index and dietary antioxidant marker, RPP showed that all the values increased and attained maximum values within fermentation duration of 3 or 4 days. The values obtained filled well to pseudo first order and first order kinetic models used with correlation coefficient

range of 0.8539 to 0.9944 under the conditions employed in this study.

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