Degradation of histamine in tuna soup by diamine oxidase (DAO)

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Abstract

Histamine is a biogenic amine, which can cause food poisoning when present at high concentrations (>500 ppm). In situations where the formation of histamine in food cannot be prevented through traditional methods such as refrigeration, diamine oxidase (DAO) enzyme may be a suitable method to reduce histamine concentration to safe levels. The aim of this work was to apply the enzyme to cooked tuna soup, which is one of the manufacturing steps of Rihaakuru, which often contains high levels of histamine. The DAO activity in tuna soup containing 500 ppm of histamine, at various pH values (5-7) and salt concentrations (1-5%) were examined. A central composite design (CCD) was used which contained a total of fifteen experiments. Histamine was completely degraded (L = 0) at pH 7 and 6.5, and at salt level of 2 and 3%. The rate of histamine reduction was optimum (r > 5) at pH 7 and salt level 3%. To obtain complete histamine degradation and optimum rate of degradation simultaneously, salt 3% and pH 7 was suggested.
Keywords: diamine oxidase, enzyme, histamine, tuna soup, rihaakuru, biogenic amines, maldives, fish paste, HPLC, histamine degradation.

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1 Introduction

Histamine forms in a variety of foods such as wine, sauerkraut, cheese, fish and fish products, and fermented meats. Scombroid poisoning is a fish poisoning caused mainly by eating scombroid fish such as tuna with elevated histamine [1, 2].

Rihaakuru, a traditional dish of the Maldives that has become a delicacy, is a cooked fish paste. The raw material is tuna including skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*), big eye (*Thunnus obesus*), frigate (*Auxis thazard*) and little tunny (*Euthynnus affinis*) [3]. Temperature abused tuna has a high concentration of histamine [2], and Rihaakuru made with temperature abused fish can have a histamine concentration at levels which are hazardous to health (>500 ppm) (Authors unpublished data).

Histamine poisoning symptoms are similar to allergic type reactions and include facial flushing, itching, hypotension, diarrhea and nausea [1]. These symptoms can be reduced by preventing histamine formation or by degrading histamine found in foods. Histamine can be degraded by bacteria or enzymes [4]. Bacteria that have been reported as histamine degraders are: *Micrococcus varians* [5], *Natrinema gari* [6], *Brevibacterium linen* [7], *Vergibacillus sp* SK33 [8], *Lactobacillus curvatus*, *L. sakei* [9], and *Staphylococcus xylosus* [10]. Similarly *Arthrobacter crystallopoietes* KAIT-B-007 is a potential histamine degrader as the bacterium possess diamine oxidase (DAO) that degrades histamine [11]. However, for food such as Rihaakuru, which is not a fermented product, the use of bacteria may not be an effective solution to the problem, as their action will change the nature of the product. The bacteria reported as histamine degraders do not to reduce histamine completely [4–10].

The enzyme DAO has been shown to degrade histamine in a fish silage by 99% in a model system (phosphate buffer, pH 7.0), and by 86% during the early stages of fermentation of fish silage [9]. The optimum activity of the enzyme has been reported to be pH 7 and 37°C [12].

Response surface methodology (RSM) is a technique for optimizing processes. The performance measure is the response and the input variables are independent variables. The data can be illustrated either as a response surface: showing the relationship between the response and two independent variables, where a corresponding value exists for each independent variable; or as a contour diagram – mainly a two-dimensional graph showing contours of response for independent variables on axis systems while the remaining variables are held constant [13]. The present work employs a RSM technique based on a five-level, two-variable central composite design (CCD) with one centre point, to optimize the degradation of histamine in tuna soup, using DAO.

2 Materials and methods

2.1 Materials

Chilled yellowfin tuna (*Thunnus albacares*) loins (4.84 kg) were purchased from Ocean Fisheries (Palmerston North, New Zealand) and transferred to the Food

Technology Pilot Plant freezer (-18°C) of Massey University until processed. Histamine dihydrochloride was obtained from Merck Limited (Auckland, New Zealand). DAO (EC 1.4.3.6, 0.18 units/mg) from porcine liver was obtained from Sigma-Adrich (St. Louis, USA). Other chemicals for this work were obtained from Sigma-Aldrich (St. Louis, USA).

2.2 Preparation of tuna soup

Frozen yellowfin tuna was thawed overnight at 4°C, cut into approximately 8 cm cubes and washed with potable water. The tuna cubes were added to boiling salted (1%) water and boiled for 45 min while continuously removing the scum. The cooked tuna cubes were removed, the soup was filtered using cheese cloth and sterilized by UHT treatment (143°C for 10 s). The UHT treated soup was transferred aseptically into glass bottles and kept at 4°C until use.

2.3 DAO experiment on tuna soup

The DAO experiment was based on the method of Dapkevicius *et al.* [9]. Tuna soup (60 ml) was transferred into 100 ml glass bottles and the salt level adjusted to 3% (sodium chloride) and mixed for 30 s before 30 ml was added to two 50 ml glass bottles. Histamine dihydrochloride (500 ppm) was dissolved into one bottle of soup and DAO was dissolved (0.5631 g/60ml; or 2,534, units/L) into the other.

The soup bottles with histamine and DAO were incubated at 37°C for about 15 minutes, to bring the solution temperature to 37°C. To 21 number of centrifuge tubes was added soup containing DAO (1 ml) soup containing histamine (1 ml). The 0 h samples were immediately mixed using a vortex mixer, and boiled in a boiling water bath for 30 min, to inactivate the enzyme. The remaining samples were divided as per sampling time into plastic beakers with the sampling time labeled. The beakers were incubated at 37°C in a thermostat controlled shaker incubator at 100 rpm (Model Amper Chart Multitron II, Infors HT, Total Lab Systems Ltd, Christchurch, New Zealand). Sampling was carried out after 0, 0.5, 1, 3, 5 and 10 h with the enzyme being inactivated as described above. Control samples contained 2 ml of soup containing histamine. After inactivation all samples were stored at -80°C until analysis.

2.4 Histamine analysis

Samples stored at -80°C were thawed at 4°C overnight. Histamine analysis followed the methods of Hwang *et al.* [14], and Kung *et al.* [15], with a few modifications. A series of histamine standard solutions (0-500 ppm) were prepared to obtain a standard curve. Histamine standard solutions (2 ml) and each sample were combined with 1 ml of sodium hydroxide (2 M) and 10 µl of benzoyl chloride. The solution was mixed using a flask shaker at maximum speed (Griffin flask shaker, Kumar Group, Delhi, India) for 30 s and placed in a 30°C incubator for 40 min, for benzoylation. Benzoylation was stopped by adding 2 ml of saturated sodium chloride and for histamine extraction 3 ml

diethyl ether was added, and extracted in the flask shaker at maximum speed for 5 min. The extracted samples were centrifuged (4400 rpm for 15 min) (Eppendorf Centrifuge 5702, Bio-Strategy Ltd, Auckland, New Zealand), and 2 ml of the upper organic layer was transferred into 15 ml centrifuge tubes and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1.5 ml of methanol (HPLC grade) by mixing for 30 s, in the flask shaker, at maximum speed, and then centrifuged for 5 min at 4400 rpm. The dissolved residue was filtered through a nylon membrane filter (0.2 um, Startorium Stedim Biotech, Germany) into HPLC vials.

Histamine was analyzed by high performance liquid chromatography that consisted of the UV/VIS detector (UVD340U), thermostatted column compartment TCC-100, ASI-100 automated sample injector, P680 HPLC pump (Dionex, Dionex Pty Ltd ,New Zealand) and a LiChrospher 100 RP-18 reversed-phase column (5 um, 125x 4.6 mm, Merck, Ltd., New Zealand). The gradient elution program was set at a flow rate of 0.8 ml/min and the column oven temperature was set at 25°C, throughout the analysis. The gradient program started at 50:50 (methanol/water) for 0.5 min, linearly increasing to 85:15 for the next 6.5 min, held constant at 85:15 for 5 min and decreased back to 50:50 over the next 2 min. The volume injected was 20 µl and monitored at a 254 nm. Each sample was run for 28 min.

2.5 Statistical analysis

A standard 2-factor (salt and pH) 15-run central composite design (CCD) was employed to determine the optimum conditions, for DAO degradation of histamine in tuna soup (table 1).

The target optimizations were rate (r = exponential rate constants for the decline in % over time), and asymptote (L = ultimate limits to the decline in %) of the reaction. Initially exponential models were fitted over time to each of the 15 runs, from which the parameters a (histamine amplitude in %,), L, and r, were determined, using nonlinear regression analysis. The exponential model was developed using eqn (1);

$$Y = L + a*e^{(-r*t)} \quad (1)$$

where L = ultimate limits to the decline in %, a = initial histamine amplitude in %, r = exponential rate constants for the decline in % over time, and t = time. The L value was constrained; $L \geq 0$.

Then RSM models were developed with salt (y) and pH (x) as independent variables, and L and r the dependent variables using regression.

Table 1: Central composite design (2factors, pH, salt).

No. of exp.	pH	Salt
1	5.5	2
2	6.5	2
3	5.5	4
4	6.5	4
5	5.5	2
6	6.5	2
7	5.5	4
8	6.5	4
9	6	3
10	5	3
11	7	3
12	6	1
13	6	5
14	6	3
15	6	3

Analysis of variance (ANOVA) was used to test the significance of the regression models and their coefficients. The data analysis was performed using Minitab statistical software (Version 15, Minitab Private Ltd, Sydney NSW, Australia). A backward elimination method was utilized for the RSM fits. Significance was accepted for $P<0.05$. Contour diagrams were plotted for L and r with the factors of pH and salt.

3 Results and discussion

3.1 DAO ability to degrade histamine in tuna soup

The exponential models fitted over time to each of the 15 runs, goodness of fit (R^2) were between 0.8-0.998 indicating good fits for observed data. The regression models for observed, and predicted fitted values for L and r are presented in table 2.

The developed RSM models with salt (y) and pH (x) as independent variables, and L and r the dependent variables, yielded eqns (2) and (3) (table 3):

$$L = 72.12 - 12.58x + 1.08y^2 \quad (2)$$

$$r = 7.14 - 2.28x + 0.20x^2 + 0.41y + 0.07y^2 \quad (3)$$

where L = ultimate limits to the decline in %, x = pH, y = salt in %, R = exponential rate constants for the decline in % over time.

Table 2: Comparison of the observed rate (r) and percentage (L) of histamine degraded by the DAO enzyme with prediction using regression models for L and r.

x	x2	y	y2	xy	r	r-fit	L	L-fit
5.5	30.25	2	4	11	0.21	0.20	0	7.27
6.5	42.25	2	4	13	0.40	0.36	0	-5.31
5.5	30.25	4	16	22	0.12	0.21	19.52	20.28
6.5	42.25	4	16	26	0.19	0.37	0	7.70
5.5	30.25	2	4	11	0.16	0.20	0	7.27
6.5	42.25	2	4	13	0.47	0.36	0	-5.31
5.5	30.25	4	16	22	0.12	0.21	3.85	20.28
6.5	42.25	4	16	26	0.42	0.367	0	7.70
6	36	3	9	18	0.20	0.17	13.49	6.40
5	25	3	9	15	0.28	0.21	38.63	18.98
7	49	3	9	21	0.46	0.53	0	-6.18
6	36	1	1	6	0.34	0.44	0	-2.27
6	36	5	25	30	0.55	0.45	37.91	23.75
6	36	3	9	18	0.15	0.17	0	6.40
6	36	3	9	18	0.16	0.17	0	6.40

Table 3: Model coefficients.

Dependent variable		t	Sig	coefficients
r	constant	2.59	0.03	7.14
	pH (x)	-2.43	0.04	-2.28
	salt (y)	-3.22	0.01	-0.41
	pH x pH (x^2)	2.45	0.04	0.20
	Salt x Salt (y^2)	3.29	0.01	0.07
L	constant	2.27	0.043	72.12
	pH (x)	-2.41	0.033	-12.58
	Salt x Salt (Y^2)	2.54	0.026	1.08

The coefficient of determination (R) of the dependent variables, L and r, was 0.71 and 0.83, respectively (table 4), which indicates that the model is suitable to represent the real relationships among the selected parameter ranges for pH and salt. Moreover, the correlation between salt and pH on the activity of DAO enzyme was strong and the prediction based on the regression line was good.

The ANOVA results showed that the model for dependent variable L, was a significantly good as $F(2, 12) = 6.11$ at $p = 0.02$ was significant ($P<0.05$). Similarly, the model for dependent variable r, was significantly good as $F(4, 10) = 5.43$ at $p = 0.01$ was significant ($P<0.05$) (table 5).

Table 4: Model summary.

Dependent variable	R	R Square	Adjusted R Square	Std. Error of the Estimate
L	0.71[a]	0.50	0.42	10.46
r	0.83[b]	0.69	0.56	0.10

[a]Predictors (Constant), Y^2, X(pH)
[b]Predictors (Constant), X(pH), Y^2, Y(salt), X^2

Table 5: ANOVA.

Dependent variables		Sum of Squares	df	Mean Square	F	Sig.
L	Regression	1336.65	2	668.32	6.11	0.015[a]
	Residual	1313.54	12	109.46		
	Total	2650.19	14			
r	Regression	0.20	4	0.05	5.43	0.014[b]
	Residual	0.09	10	0.009		
	Total	0.29	14			

[a]Predictors: (Constant), Y^2, XpH
[b]Predictors: (Constant), XpH, Y^2, Ysalt, X^2

The contour diagram for dependent variable r-fit against salt (y-axis) and pH (x-axis) of DAO activity in tuna soup is shown in fig. 1.

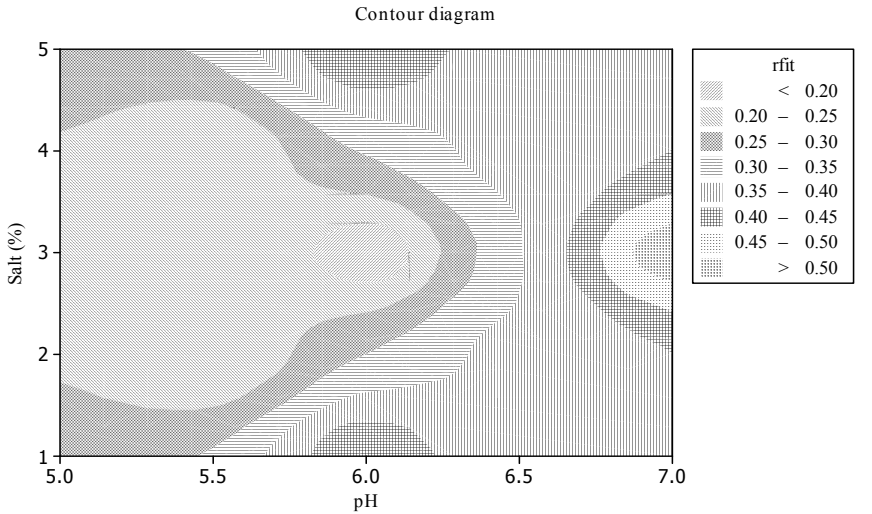

Figure 1: Interaction between pH and salt concentration (%) on rate (r-fit) of histamine degradation by DAO in tuna soup.

Of the contour diagram areas, rate of histamine degradation, by DAO, was weakest between pH 5 to approximately 6.35 and salt range of about 1.85 to 4.2 % (R<0.2 and between 0.2-0.25), where pH 6 and salt 3% being the area the rate of the reaction was slowest. The acceptable area in the contour diagram may be considered at R ≥ 0.3 which is approximately between pH 6.35 – 7, and salt 1-5%. Similarly, the R value is greater than 0.3 at pH between 5-7 and salt approximately below 1.5%, and pH between 5-7 and salt above 4.5%. Of the experimented regions, pH approximately between 6.8 and 7, and salt approximately between 2.8 to 3.2%, provide the maximum region for r value > 0.5, where pH 7 and salt 3% being the optimum. Although the maximum rate of histamine degradation was obtained at pH 7 and salt 3%, the average natural condition of the soup fall between pH 5.5 and 6.5 and salt between 1 and 3%, even then, the acceptable region of pH and salt conditions is covered, as the DAO activity is at the acceptable range. The r-fit model has shown that the rate of the enzyme activity reached maximum up to 0.5 in tuna soup, within the experimental region; may be due to the interaction of enzyme with the tuna soup matrix, thus the rate of the reaction could not elevate further.

A contour diagram of dependent variable L-fit against salt (y-axis) and pH (x-axis) of histamine degradation by DAO in tuna soup is shown in fig. 2.

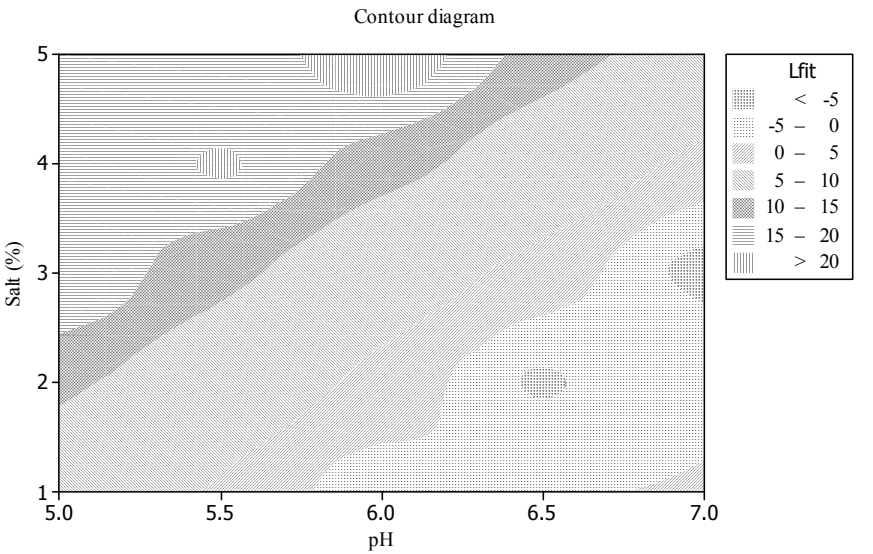

Figure 2: Interaction between pH and salt concentration (%) on total percent (L-fit) of histamine degradation by DAO in tuna soup.

The unacceptable regions, where histamine remained between 5 and > 20%, were pH between 5 to 6.7 and salt about 1.85 to 5%, where pH 6 and salt 5%, and pH 5.5 and salt 4% had L value greater than 20%. The acceptable region (L: 0-5%) in the contour diagram was like a valley that fall approximately

between pH 5.75 to 6.75 where salt concentration was about 1 to 1.3%; and pH between 6.3 to 7, where salt concentration was about 1 to 2%; likewise at pH 6.5 and salt 2.5%; and at pH 7 and salt about 3.5%. Within this acceptable region the maximum condition for complete histamine degradation was at pH 7 and salt 3% (L = 0). Based on the L-fit model, most histamine (80-100%) can be degraded by DAO within the ranges of pH and salt tested. However, since the natural condition of tuna soup is of pH between 5.5 and 6.5, and salt between 1 and 3%, the maximum condition may not be applied in practice, but the stated natural condition falls within the acceptable range, where most of the histamine can be degraded by 95- 100%.

The most effective way of degrading histamine from tuna soup may be by merging the optimum conditions that degrade all histamine and the optimum conditions that produce the highest rate of degradation, within the experimental region. When the contour diagrams of r-fit and L-fit response are overlapped, the common area where the values were at optimum for both is the area of interest. In this work the common point where the maximum condition for both r and L was at pH 7 and salt 3%. However, the natural conditions in tuna soup had a pH between 5.5 and 6.5, and salt concentration between 1 and 3%, thus to achieve maximum reaction, the pH of the soup need to be adjusted to 7. The most practical way to approach this is to find a common area from both RSM models of L and r where the natural conditions of tuna soup exist; that is pH 5.5 to 6.5, and salt concentration of 1 to 3%. The common optimum point within the experimental region is pH 6 and a salt concentration of 1%, where histamine can be degraded by 100% (L =0) and the rate of reaction by DAO enzyme is acceptable (r: 0.4-0.45). Once the histamine is degraded from the soup, the soup has to be further processed into Rihaakuru without any delay, as otherwise the bacteria may produce more histamine.

In conclusion, a method to degrade histamine formed in processed food has been reported. The regression model obtained, from the experimental data of histamine degradation in tuna soup using DAO, has a good correlation with that of observed and predicted variables. Under the natural pH (5.5-6.5) and salt concentration (1-3%) in tuna soup degradation of histamine occurred with the process being optimal at a pH value of 7 and salt concentration of 3%. The significance of this study is that high histamine levels in Rihaakuru can be controlled since the production of tuna soup is one of the manufacturing steps of Rihaakuru.

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