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Prediction of the amount and rate of histamine degradation by diamine oxidase (DAO)

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ABSTRACT

Histamine is a biogenic amine that forms in a variety of foods and can cause food poisoning at high concentrations (>500 ppm). In situations where the formation of histamine in food cannot be prevented through refrigeration, diamine oxidase (DAO) enzyme may be used to degrade histamine to safe levels. The aims of this work were to apply DAO in model (buffer) and real (cooked tuna soup used in the manufacture of a fish paste product, Rihaakuru) systems, in order to obtain predictions for the rates and amounts of histamine degradation. The two systems were set up with a constant concentration of histamine (500 mg/L) and the DAO enzyme (2534 units/L) at a temperature of 37 °C, agitation at 100 rpm and an incubation time of 10 h with variable pH (5–7) and salt concentrations (1–5%). A total of 15 experiments were designed for each system using central composite design (CCD). The data from these experiments were fitted into regression models; initially the data were used to generate an exponential decline model and then the data from this were fitted into a secondary response surface model (RSM) to predict the rate and amount of histamine degradation by DAO. The model system results indicated that DAO activity was not significantly affected by salt ($p > 0.05$), and that activity reached a maximum within the pH range of 6–6.5 with an optimum at pH 6.3. However, the results obtained with the tuna soup model showed that the optimum oxidation of histamine using DAO occurred between pH 6–7 and salt 1–3%. This study defined the conditions for the use of DAO to degrade 500 mg/L of histamine in tuna soup used to manufacture Rihaakuru. The models generated could also be used to predict the rate and amount of histamine degradation in other foods that have similar characteristics to tuna soup.

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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. Histamine at high concentration (>500 ppm) can cause histamine poisoning. Elevated levels of histamine in food result from mishandling, in particular from temperature abuse and a lack of good manufacturing practise. For example, histamine concentrations increased to unsafe levels when tuna was exposed to high temperatures due to the growth of bacteria containing the histidine decarboxylyase that converts histidine into histamine (Arnold, Price, & Brown, 1980). Any food that contains free histidine is susceptible to the production of histamine, providing conditions for bacterial growth are favourable (Shalaby, 1996).

Histamine poisoning symptoms are similar to allergy reactions, including facial flushing, itching, hypotension, diarrhoea and nausea (Arnold & Brown, 1978). Healthy people have an effective histamine detoxification system in the body so, unless an excess amount of histamine is taken, the system can degrade histamine efficiently into aldehydes, ammonia and hydrogen peroxide (Koutsoumanis, Tassou, & Nychas, 2010). For individuals on medication such as that used for depression, drugs can inhibit oxidase enzymes such as mono-, di- and polyamine oxidases in the detoxification system (EFSA Panel on Biological Hazards (BIOHAZ), 2011; Koutsoumanis et al., 2010).

Histamine poisoning can be prevented by preventing histamine formation or by degrading histamine in foods. Histamine formation is most commonly prevented through the control of temperature. Cold storage of food in combination with other methods such as packaging, preservatives and irradiation have been shown to reduce histamine production in food (Naila, Flint, Fletcher, Bremer, & Meerdink, 2010). However, control of the cold chain alone or combined with other methods may not be sufficient to control histamine, as evidenced by the fact that bacteria were found to produce histamine at low temperatures (<4 °C) (Dalgaard, Madsen,

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Samieian, & Emborg, 2006). Therefore, alternative methods to reduce histamine in food need to be explored.

Histamine can be degraded by bacteria or enzymes (Naila et al., 2010). Bacteria that have been reported as histamine degraders are: *Micrococcus varians* (Leuschner, Heidel, & Hammes, 1998), *Natrinema gari* (Tapingkae, Tanasupawat, Parkin, Benjakul, & Visessanguan, 2010), *Brevibacterium linens* (Leuschner & Hammes, 1998a), *Vergibacillus* sp. SK33 (Yongsawatdigul, Rodtong, & Raksakulthai, 2007), *Lactobacillus curvatus* and *L. sakei* (Dapkevicius, Nout, Rombouts, Houben, & Wymenga, 2000), and *Staphylococcus xylosus* (Mah & Hwang, 2009). Similarly *Arthrobacter crystallopoietes* KAIT-B-007 is a potential histamine degrader through the activity of diamine oxidase (DAO) that degrades histamine (Sekiguchi, Makita, Yamamura, & Matsumoto, 2004). These bacteria can be added to fermented foods, contributing to the flavor as well as the safety of the final product. However, for food such as Rihaakuru, which is not a fermented product, the use of bacteria may not be a practical solution to the problem as they are likely to change the nature of the product. In addition, the bacteria reported as histamine degraders only reduce histamine, but do not eliminate it completely (Dapkevicius et al., 2000; Leuschner & Hammes, 1998a; Leuschner & Hammes, 1998b; Naila et al., 2010; Tapingkae et al., 2010; Yongsawatdigul et al., 2007).

The application of diamine oxidase (DAO, EC 1.4.3.6) or bacteria containing this enzyme are emerging approaches to degrade histamine in food (Dapkevicius et al., 2000; Mondovi, Rotilio, Costa, & Agrõ, 1971; Naila et al., 2010). DAO has been isolated from many sources such as the organs of pigs (liver, kidney), human placenta and blood plasma, and from micro-organisms including *Microbacterium lacticum* (Voigt & Eitenmiller, 1978) and *Arthrobacter crystallopoietes* KAIT-B-007 (Sekiguchi et al., 2004). Voigt and Eitenmiller (1978) found that cheese had both DAO and amino acid decarboxylase activity. Low amine content was found in cheese which had more DAO activity and a high amine concentration was found in cheese that had more amino acid decarboxylase activity suggesting that amine concentration in foods is dependent on the ratio of amine producing and degrading enzymes. Leuschner and Hammes (1998b) reported tyramine degradation in fermented sausages inoculated with one bacterial strain containing tyramine decarboxylase activity and another containing tyramine oxidising activity. In the presence of tyramine oxidising bacteria, the total amount of tyramine formed was reduced to 40% of the concentration found in sausage inoculated with the tyramine decarboxylating bacteria. DAO from porcine liver has been shown to lower histamine accumulation in ensiled fish slurry (animal feed) during the early stages of fermentation when the product had a high pH (initial pH 6.4) and a high oxygen concentration (Dapkevicius et al., 2000). The research by Dapkevicius et al. (2000) focused on degrading histamine in animal feed as histamine can cause liver damage to animals eating feed with elevated histamine concentrations (Křížek, 1991). Since the potential for DAO to degrade histamine has been shown in animal feed, its application in other foods needs to be investigated. It has previously been reported that DAO activity is optimum at pH 7 and 37 °C, in the presence of oxygen (Beutling, 1992). Enzyme activity is likely to be affected by the various conditions found in different environments or foods. Many

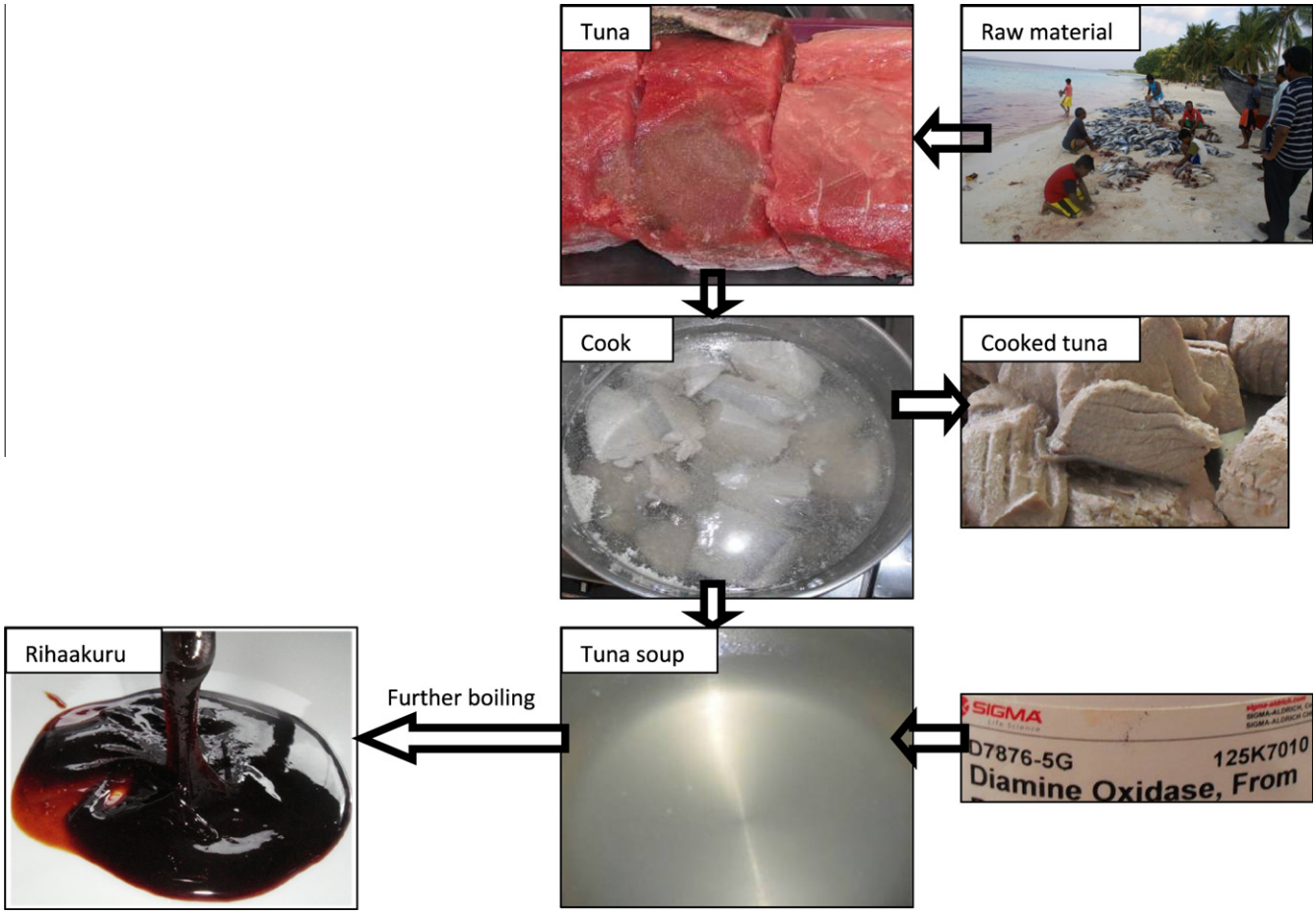

Fig. 1. Rihaakuru manufacturing steps, including the tuna soup step where DAO can be added after which the soup is further boiled until it becomes Rihaakuru. The boiling will kill all the pathogenic microorganisms that may have grown during DAO treatment.

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