INFLUENCE OF CHEMICAL INHIBITORS AND HIGH PRESSURE ON MELANOSIS OF PRAWNS (*Penaeus japonicus*)

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INTRODUCTION

The shelf life of prawns, shrimps and other fresh crustaceans is largely determined by two factors. One is the growth of large quantities of free amino acids and other substances during chilled storage – an ideal substrate for microbial growth – and the second is the appearance of melanosis.

Melanosis is a process that is triggered by a biochemical mechanism consisting of oxidation of phenols to quinones by means of an enzymatic complex known as polyphenoloxidase (PPO). This is followed by non-enzymatic polymerization of the quinones, giving rise to pigments of high molecular weight and very dark or black colouring. These same reactions are responsible for enzymatic browning in fruit and vegetables, on which there are numerous studies in the literature (Constantinides and Bedford, 1967; Mayer and Harel, 1979; Chen et al ,1991; Lee-Kim et al., 1997; Hernández and Cano, 1998; Duangmal and Owusu, 1999).

In crustaceans, PPO has various different locations. It is found on the exoskeleton, chiefly on the shell of the cephalothorax, uropods and on the pleuron in the region of the connection with the pleopods (Ogawa et al., 1984). It is also found on the haemolymph (Nagagawa and Nagayama, 1981). Because of the intense irrigation of the cephalothorax, this is where PPO is most commonly found. It remains active under refrigeration (with or without ice) and in thawed product. This pigmentation spreads rapidly and hence places a limit on the shelf life of crustaceans.

In order to prevent this phenomenon as far as possible, a variety of compounds have been used which act as inhibitors. The most widely used of these are sulphites and derivatives. These chemical substances interfere in the polymerization of the quinones, combining irreversibly with them and forming colourless compounds (Embs and Markakis, 1965). However, they are not invariably effective, as they do not prevent melanosis entirely. It has been necessary to search for alternatives that show effective inhibitory effects on melanosis but are devoid of health concerns to consumers. However, the number of chemicals that can actually be used in food

systems to inhibit melanosis is limited by off-flavours, off-odours, toxicity, and economic feasibility (Chen et al., 1991).

Processes like vacuum packing and pressurization could be very useful technologies in chilled storage of these products for total or partial elimination of these factors, which negatively affect storage of crustaceans.

The objective of this study was to partially characterize the PPO in prawn and to determine the effect exerted on the appearance of melanosis by certain kinds of processing – vacuum and high pressure – and the addition of certain compounds.

CHARACTERIZATION OF PPO

The species used was live farmed imperial tiger prawn (*Penaeus japonicus*) from Acuinova Andalucía, S.A., Ayamonte, (Huelva).

Firstly a study was carried out with enzymatic extracts obtained from different parts of the prawn (cephalothorax shell, uropods/telson, pleuron and muscle) to determine the zones in which the highest proportion of this activity is located. The cephalothorax shell exhibited much higher specific activity than the rest, followed by the caudal zone (uropods and telson), and lastly the pleuron and the muscle. The rest of the characterization was therefore carried out using the cephalothorax shell.

The temperature profile of PPO activation presented two plateaux between 25 °C and 40 °C in which activity increased by around 20 % per 10 °C increase in temperature. At temperatures over 55 °C there was loss of activity, which could have been due to thermal denaturation of the enzyme. Thermal stability declined noticeably when the enzymatic extract was subjected to temperatures over 35 °C. The profile of PPO activity versus different pH values had two very sharp peaks of high activity, one in the acid zone (pH 5) and the other in the basic zone (pH 8). Stability versus pH diminished completely at very acid pH levels, below pH 5. This pronounced enzymatic instability at acid pH levels suggests that the treatment of prawns with acid solutions would go a long way towards inhibiting melanosis; in contrast, at pH levels close to neutral (pH 7.5), more instability was observed. Similar effects have been found by other authors in a variety of crustaceans, although there are differences attributed to the intrinsic characteristics of the species.

EFFECT OF INHIBITORS

The following inhibitors were assayed: bisulphite, kojic acid, 4-hexylresorcinol, sodium benzoate, ascorbic acid and citric acid.

EFFECT OF DIFFERENT CONCENTRATIONS OF INHIBITORS ON THE ACTIVITY OF THE ENZYMATIC EXTRACT AT PH 8

Metabisulphite produced 30 % inhibition at concentrations of 10 µg/ml; inhibition was total at higher concenrations, between 80–100 µg/ml. In the case of kojic acid, higher concentrations than those of sulphite were required to attain similar levels of enzymatic inhibition, so that 150-200 µg/ml was needed to achieve 80 % versus 40 % µg/ml of sulphite. On the other hand kojic acid lacks the toxic drawbacks of sulphite. 4-hexylresorcinol produced 40 % inhibition at concentrations of 0.15 g/l, reaching 80 % inhibition with 1 g/l. Sodium benzoate was more effective than hexylresorcinol, producing 70 % inhibition at concentrations of 0.1 g/l. With ascorbic acid and citric acid no inhibition was detected on measurement at pH 8. However, at lower pH levels originated by the acid itself (3.1 and 2.8 respectively), inhibition was total. These results suggest that their effect was due to instability produced in the enzyme by the acid pH.

In real systems, however, melanosis as manifested by the appearance of black spots in prawns kept in chilled storage without additives was very rapid, and a score of 4 on the scale described by Otwell and Marshall (1986) was reached in four days. A score of 4 or more is considered indicative of product degradation, although Otwell et al. (1992) do not consider it unacceptable until a score of 8 is reached.

The two acids assayed, ascorbic and citric, did not slow down the progress of melanosis despite the fact that in a model system the enzyme was inhibited in an acidic medium. In a real system (live prawn), therefore, it could be useful to increase the concentration of the acid and the exposure time to facilitate its action. Sodium benzoate was somewhat more effective, producing slightly lower scores. At the experimental concentrations, kojic acid showed a clear inhibiting effect on melanosis. The prawns treated with kojic acid presented scarcely any black spots until the fifth day of storage and maintained a score of 4 for at least four days more. 4-hexylresorcinol was the most effective inhibitor at the experimental concentrations, keeping the prawns in quite acceptable conditions for at least a week. The effect of 4-hexylresorcinol was also studied in combination with ascorbic acid and citric acid. In both cases there was a similar mechanism that enhanced the effect exerted by each one separately, and the lots treated in this way scored lowest throughout the storage period. This suggests that each of these substances could suppress the pigment formation mechanism at different stages or else favour the action of 4-hexylresorcinol.

EFFECT OF HIGH PRESSURE

As regards the effect of pressurizing the enzyme extracts in basic conditions (pH 8), activity was highest at atmospheric pressure (0.1 MPa) and tended to decrease with gradual raising of the pressure applied: slightly between 100-200 MPa and

more strongly between 300 – 400 MPa.

However, when assays were performed on whole prawn there was no visually appreciable decrease of melanosis. We have found no references in the literature to the effect of high pressure on the PPO of crustaceans. But there have been studies in various vegetable products, where the behaviour was quite different. When an enzyme is unprotected – as in the case of purées and juices – 300 MPa is sufficient to produce inhibition. However, in order to produce inhibition in whole fruits, much higher pressures are required. Such pressures could not be used with crustaceans while retaining their raw appearance.

SUMMARY

Briefly, then, it is necessary to study concentrations and treatment times with inhibitors in real systems to standardize the most suitable conditions for each species in question, including the combination of antioxidants and PPO inhibitors. The combined action of 4-hexylresorcinol and citric or ascorbic acid is of particular interest as this reduces the appearance of melanosis and the microbial load.

High pressure treatment in a model system inhibits the appearance of melanosis, but in a real system (live prawn) it tends to activate it.

The combination of vacuum and high pressure could be especially useful for prolonging the shelf life of commercially valuable crustaceans since this favours inhibition of PPO and microbial growth.

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