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Dimethylamine and formaldehyde in cooked squid (*Illex argentinus*) muscle extract and mantle

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Trimethylamine oxide (TMAO) in the muscle of squid may be reduced by endogenous and bacterial enzymes to trimethylamine and demethylated in enzymatic or chemical reactions to dimethylamine (DMA) and formaldehyde (FA). The rate of TMAO degradation in heated Baltic cod and in squid extract and flesh, as well as the effect of FA generated in the tissues on the texture of cooked squid were investigated. The content of DMA in uncooked frozen stored mantle of 51 *Illex argentinus*, from three different shipments, was 88–452 μmoles/100 g meat. Boiling for 45 min resulted in a large accumulation of DMA and a lesser increase in free FA in squid, but only negligible changes in cod. The decomposition of TMAO could not have been caused by enzymatic reactions. In a series of experiments with squid of different lots no consistent correlations were found between the shear force required to cut the cooked squid mantle and the contents of free FA. The rate of increase in free FA in the heated samples of the water-soluble fraction of squid flesh did not correspond to that of DMA.

INTRODUCTION

Trimethylamine oxide (TMAO) has important functions in the living organism and an impact on the sensory properties of fish and squid muscle during storage and processing. It may be reduced enzymatically to trimethylamine (TMA), which is co-responsible for the fishy smell of the produce, or demethylated to dimethylamine (DMA) and formaldehyde (FA) (Tokunaga, 1964). In fish flesh, demethylation of TMAO may be either catalyzed by the TMAO demethylase, or by cations or other compounds present in the muscle tissues (Spinelli & Koury, 1979, 1981; Sikorski & Kostuch, 1982; Hultin, 1992). The direction and rate of degradation of TMAO in the fish flesh is affected by the state of the tissues, contents of required cofactors, and the temperature. In *Sebastes mentella* 15 min after death, the contents of TMAO, total volatile bases, TMA, DMA, and NH_3 are about 125, 15, 1·8, 0·2, and 10·6 mg/100 g, respectively (Oehlenschläger, 1989). In some frozen stored fillets of *Macrourus holotrachys* a very high TMAO demethylase activity, as well as correspondingly large amounts of FA were found in white and red muscles (Rehbein, 1991).

The sensory quality of fish of several species may deteriorate during frozen storage due to the development of undesirable texture, accompanied by loss in functional properties of the muscle proteins. The extent of freeze-denaturation in fish during storage is affected by the contents and distribution of fat in the tissues, as well as by the rate of accumulation of FA, different amino acids, and the products of nucleotide catabolism (Sikorski *et al.*, 1976; Matsumoto, 1979; Shenouda, 1980; Jiang & Lee, 1985; Jiang *et al.* 1987*a,b*; Sikorski & Kołakowska, 1993). The interaction of FA with reactive protein groups leads to the formation of stable protein aggregates. According to Ang and Hultin (1989), FA could possibly induce toughening in frozen stored fish, not only by direct cross-linking of the proteins, but also, by causing denaturation of the proteins by binding to their side-chain groups, it could lead to increased formation of aggregates, buttressed by non-covalent forces.

DMA and FA, accompanied by large amounts of TMA, can be generated (especially at cooking temperature) by nonenzymatic reactions, catalyzed by a low molecular weight, thermostable fraction of the squid flesh extract (Nitisewojo & Hultin, 1986; Hultin, 1992). Decomposition of TMAO to DMA and FA has been reported also in cooked flesh of *Illex argentinus* (Synowiecki & Sikorski, 1988). It is interesting to investigate whether any toughening, corresponding to the generation of DMA and FA, could be found in cooked fish and squid flesh.

MATERIALS AND METHODS

Fresh Baltic cod from the market, about 35 cm in length, was refrigerated in ice for not longer than 3

days after catching. The pH in the flesh was 6·55–6·65. The squid, *Illex argentinus*, 21–24 cm in mantle length, was purchased from the deep-sea fishing enterprise 'Dalmor'. Detailed information regarding the time elapsing between the catch and further treatment was not available. The squid was frozen on board in 10-kg blocks and stored in polyethylene sheets and cartons 7–12 months at −20°C. In the laboratory, after thawing in air at room temperature to about −3 to 0°C, the squid was gutted, skinned and refrozen, to be stored at −20°C until required for use. Samples of minced mantle tissue were homogenized with water (1 : 3) for 5 s at 7000 rpm plus 30 s at 12 000 rpm and centrifuged for 30 min at 4500 × *g*. The supernatant was heated on a water bath at 30–100°C for 15, 30, 45 and 60 min. In samples cooled to room temperature, DMA was determined according to Dyer and Mounsey (1945) and free FA according to Nash (1953).

DMA and FA were also determined in uncooked squid mantle and in Baltic cod flesh, as well as in mantle samples and cod fillets, about 2·5 × 2·5 cm, boiled 45 min in water (1 : 3). The shear force required for cutting the cooked mantle was determined using a previously described procedure (Kołodziejska *et al.*, 1992).

RESULTS AND DISCUSSION

The average content of DMA in uncooked frozen stored mantle of *Illex argentinus*, determined in 51 squids from 3 different shipments, was 250 μmole/100 g, within the range 88–452 μmole/100 g (Table 1). That was about five times higher than the amount reported by Nitisewojo and Hultin (1986) for *Illex illecebrosus* and by Synowiecki and Sikorski (1988) for one lot of squid *Illex argentinus*. This difference may stem from the species characteristics or treatment after catching. The content of DMA in fresh Baltic cod meat was, generally, at least one order of magnitude lower than in frozen *Illex argentinus* (Table 1).

Boiling the squid flesh for 45 min resulted in a large accumulation of DMA and a lesser increase in FA (Table 1). In the experiments of Synowiecki and Sikorski (1988) the mantle flesh of *Illex argentinus* contained (after 45 min cooking) about 18 times more DMA and only twice as much free FA as the uncooked sample. In cod, no significant change in the contents of these compounds was found (Table 1). This result could not have been caused by a corresponding difference in the original contents of TMAO in the muscles of the investigated species. Boiling of the samples also precluded the involvement of enzymatic processes in the generation of DMA. Thus the results obtained in this experiment must have been caused by nonenzymatic factors present in the squid flesh participating in the reaction, which were lacking in the cod meat. According to Tokunaga, cited by Nitisewojo and Hultin (1986), in white fish flesh the rate of thermal decomposition of TMAO was generally lower than in red muscles of fish and in the flesh of squid and clams.

There was as yet no justification for checking the correlation between the shear force and the bound FA in the cooked mantle tissue. It is not known whether the thermal decomposition of TMAO in squid yields equimolar amounts of DMA and FA or whether the amount of bound FA may be computed as the difference between the concentration of DMA and free FA. However, if as in the case of cod, about 50% of the total FA in squid reacts with the tissue components, a correlation between the rheological properties of the cooked mantle and free FA could be expected.

The content of free FA in 51 cooked samples of squid mantle, determined in three series of experiments involving different lots of squid, was not consistently correlated to the shear force required to cut the tissue (Table 2), whereby the shear force changed only in a small range of values. The results presented in Table 2 may have been caused either by the biological differences in the squid of the investigated lots, by slightly different handling after catching, or by some unknown factors, affecting the interaction of FA with the squid constituents.

Heating of the water-soluble fraction of frozen stored *Illex argentinus* mantle meat above 50°C resulted in the formation of DMA, at a rate increasing with temperature (Fig. 1). After 60 min the content of DMA

Table 1. DMA and free FA in raw and cooked flesh of *Illex argentinus* and Baltic cod

Sample	Raw meat		Cooked[a] meat	
	FA	DMA (μmole/100 g)	FA	DMA
Squid[b]	23–92 40 ± 19	88–452 250 ± 146	83–240 160 ± 43	1025–2402 1490 ± 376
Cod[b]	9–14 11 ± 2	8–51 21 ± 19	5–12 8 ± 3	5–48 20 ± 19

[a] DMA and free FA were determined in the whole homogenized sample, containing both the flesh and the cooking liquor.
[b] Fifty-one squid and three cod from three different shipments.

Table 2. The correlation between the shear force and the contents[a] of free FA in cooked *Illex argentinus* mantle

Series[b]	Shear force (N)	Free FA (mg/100 g)	Correlation coefficient
I *n* = 15	2·4–3·7	4·1–7·8	0·72
II *n* = 18	3·5–5·5	2·5–5·6	0·73
III *n* = 18	2·8–7·0	2·9–5·6	−0·61

[a] Free FA was determined in whole homogenized sample, containing both the flesh and the cooking liquor.
[b] *n*—number of squid. All determinations were made in triplicate.

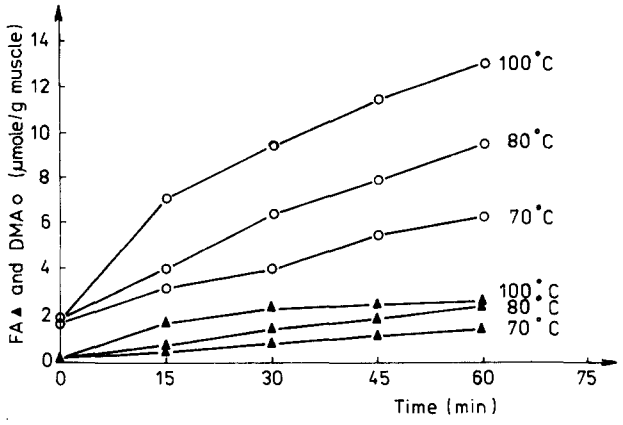

Fig. 1. The effect of time and temperature of heating of squid muscle extract on the accumulation of free FA (▲) and DMA (○). Means of 3 determinations, coefficient of variation ≤5%.

in samples heated at 100°C was about eight times higher than in uncooked squid mantle. The process must have been nonenzymatic, as there was no accumulation of DMA during 60 min heating up to 50°C. Nitisewojo and Hultin (1986) reported on the formation of DMA in extracts of *Illex illecebrosus* flesh already at room temperature, in the presence of Fe^{2+} and ascorbate, whereby only about 10–15% of the total amount of DMA was generated due to enzymatic demethylation of TMAO.

The rate of increase in free FA in the heated samples of the water-soluble fraction of squid flesh did not correspond to that of DMA (Fig. 1). This may have been caused either by nonequimolar generation of DMA and FA in nonenzymatic processes or by a high rate of interaction of FA with different components of the extract at the elevated temperature. According to Castell *et al.* (1974) the enzymatic demethylation leads to generation of equimolar amounts of DMA and FA, but in nonenzymatic reactions other compounds are also formed and the 1:1 relation of DMA and FA may not apply (Hultin, 1992). FA formed *in situ* from TMAO or added to the fish flesh may react with several components of the tissues (Castell *et al.*, 1974; Kostuch & Sikorski, 1977). In cod minces containing 10 mg added FA per 100 g, about 60% of the added FA was, at pH 6·7, irreversibly bound after 24 h at 4°C. At pH 4·7 and 8·7 the amounts of bound FA were 15 and 30%, respectively (Kostuch & Sikorski, 1977).

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