# FISCH ALS LEBENSMITTEL

# Relation between TMAOase activity and content of formaldehyde in fillet minces and bellyflap minces from gadoid fishes

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Minced fish is a significant component of a number of frozen fishery products like fish fingers, cakes and patties. Predominately minced fish is produced from gadoid species (Alaska pollack, cod, saithe, hake and others) possessing the enzyme trimethylamine oxide demethylase (TMAOase, E.C. 4.1.2.32) (Rehbein and Schreiber 1984).
TMAOase catalyses the degradation of trimethylamine oxide (TMAO) to formaldehyde (FA) and dimethylamine (DMA), preferentially during frozen storage of products (Hultin 1992). In most gadoid species light muscle contains only low activity of TMAOase, the activity of red muscle and bellyflaps being somewhat higher. In contrast, the TMAOase activity in blood, kidney and other tissues, residues of which may contaminate minced fish flesh, may be higher for several orders of magnitude (Rehbein and Schreiber 1984).

**Beziehung zwischen TMAOase-Aktivität und Formaldehydgehalt in Farcen aus Filet und Bauchlappen von Gadiden**

Zur Untersuchung des Zusammenhanges zwischen TMAOase-Aktivität und Formaldehydgehalt in tiefgekühltem Fischfleisch wurden Farcen (zerkleinertes Fischfleisch) aus Filet und Bauchlappen mehrerer Dorschfische hergestellt und bei -20 °C gelagert. Reine Filetfarce besaß nur sehr niedrige TMAOase-Aktivität und entwickelte relativ wenig FA. Im Gegensatz dazu war TMAOase-Aktivität in Bauchlappenfarce und Mischfarcen aus Filet und Bauchlappen deutlich meßbar. Dieses Probenmaterial wies überwiegend sehr hohe FA-Gehalte auf, die Werte bis zu 700 mg/kg erreichten. Eine strenge Korrelation zwischen der Höhe der TMAOase-Aktivität und dem Gehalt an freiem und gebundenem FA wurde nicht festgestellt, aber tendenziell stiegen die FA-Gehalte mit zunehmender TMAOase-Aktivität. Wird eine bestimmte Enzymaktivität im Produkt überschritten, ist mit hohen FA-Gehalten zu rechnen. Die Verarbeitung von Bauchlappen zu Erzeugnissen mit zerkleinertem Fischfleisch kann bei Gadiden nicht empfohlen werden, da mit erheblichen Gehalten an FA und DMA in den Erzeugnissen zu rechnen ist.

Addition of kidney has been frequently used to stimulate breakdown of TMAO in minced fish (Rehbein 1988, Chang and Regenstein 1997), but without determining the relation between the TMAOase activity of the final product and formation of FA or DMA. The work described here was performed to study the correlation between the TMAOase activity and FA content of minced fish flesh, the latter substance being of great importance for the quality of frozen-stored fish (Sotelo et al. 1995).

The composition of flesh separated from the bellyflaps has been reported to be similar to the composition of minced fillet (Thomson and Mackie 1982, McLay 1985). On the other hand, it has been found that minced bellyflaps from gadoid fish species produce high amounts of FA and DMA during frozen storage (Schubring 1995), indicating the presence of TMAOase in bellyflap mince.

## Materials and Methods

### *1. Preparation of samples*

Fish minces were prepared onboard the FRV "Walther Herwig III" using the Baader 694 bone separator equipped with drums containing holes of 3 or 5 mm in

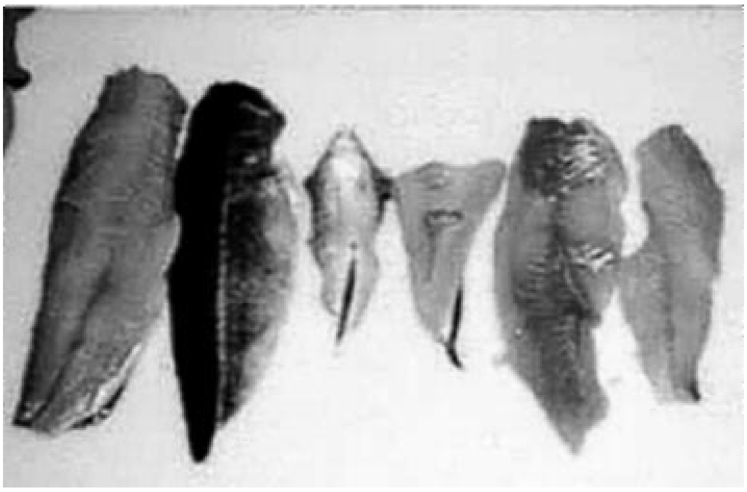
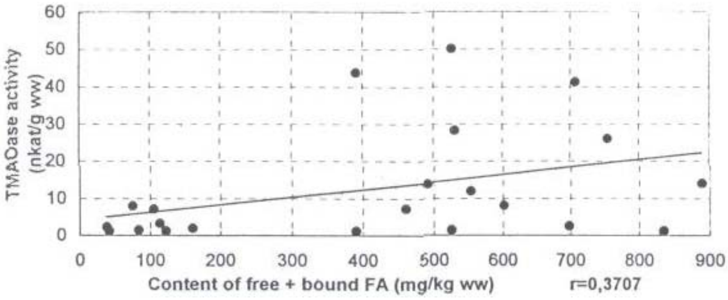


Fig. 2: Relationship between TMAOase activity and formaldehyde content in different types of gadoid minces

Fig. 1: Raw material for preparing fish minces (from left: skin-on fillet, bellyflaps, skinned fillet)




diameter. The minces were packed in plastic bags, and frozen-stored at -20 °C.

Bellyflaps were recovered from fish directly after the catch (Figure 1). The fish was filleted, the bellyflaps were cut off the fillets, skinned and frozen-stored at -25 °C. Part of the bellyflaps were frozen after removal of the peritoneum.

### *2. Chemical analysis*

1. Free FA was measured after extraction with perchloric acid, and free plus bound FA was determined after steam destillation of acidified samples (Rehbein 1987).

2. TMAOase was extracted with Triton X-100/phosphate buffer, and its activity was measured using a two-step test system (Rehbein et al. 1996). In the first step, catalysed by TMAOase, TMAO is split into FA and DMA, and in the second reaction released FA is determined by means of formaldehyde dehydrogenase measuring the formation of NADH (reduced nicotinamide adenine dinucleotide) at 340 nm. Often measurement of TMAOase activity of bellyflaps or fillet was hampered by turbidity of the extracts. Therefore, not only blanks without extract were run; in addition, extinction was read after 10 and 20 min of reaction time, and the difference of theses values was used to calculate the TMAOase activity.

3. The protein concentration of extracts was determined by means of the Coomassie dye-binding assay (Bio-Rad Protein Assay) using bovine serum albumine as protein standard.

## Results and Discussion

### *Formaldehyde content of fish minces*

In the different types of frozen-stored minces prepared from fillets and bellyflaps of cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and saithe (*Pollachius virens*), variable amounts of free plus bound FA were detected (Table 1). Bellyflap minces of all the three gadoid species contained up to 700 mg/kg of free plus bound FA, whereas low values (40-160 mg/kg) were found in the case of minced fillet. The values obtained for minced hadddock were surprisingly high, as it has been reported by Gill et al. (1979) that haddock does not accumulate formaldehyde in frozen-stored fillets. However, Gill et al. had used fillet instead of mince, and the fish was held 2-3 days in ice before filleted and frozen-stored. This period may be sufficient to destroy some of the TMAOase activity.

In mixtures of minced fillet and bellyflaps formation of FA was stimulated by addition of bellyflaps. When stored for 11 months, minces of high FA content showed reduced amount of extractable protein (Table 1 and 2), indicating protein denaturation by FA during storage. In general TMAOase activities reflected FA contents (Table 2). High activities were measured in bellyflaps (in whole as well as in minced tissue), whereas the activity of fillets was near or below detection limit. However, a significant linear correlation between TMAOase activity and FA content could not be established (Figure 2). This might be explained by several reasons:

(i) Denaturation of the enzyme during frozen storage.
(ii) Depletion of physiological, but yet unknown cofactors.
(iii) Exhaustion of the subtrate TMAO; the concentration of TMAO in white muscle of haddock, saithe and cod ranges from 20 to 60 mmol/kg wet weight (Rehbein 1988), enabeling the formation of 600 to 1800 mg FA/kg wet weight.
(iv) Reaction of FA with proteins to compounds resistant to acid destillation.

Tab. 1: Content of free plus bound formaldehyde of minced fish stored at -20 °C for 8 (cod, haddock) or 11 month (saithe)

| Type of mince | | Formaldehyde content (mg/kg wet weight) Free FA | Free + bound FA |
|---|---|---|---|
| **Cod** | | | |
| Skinned fillet | | 22.8 | 114.5 |
| Skin-on fillet | | 25.5 | 161.2 |
| Bellyflaps | | 168.6 | 515.6 |
| **Haddock** | | | |
| Skinned fillet | | 7.6 | 38.5 |
| Skin-on fillet | | 10.1 | 83.9 |
| Bellyflaps | | 193.2 | 531.0 |
| **Saithe** | | | |
| Skinned fillet | | 6.5 | 41.9 |
| Skin-on fillet,SOF | | 16.9 | 123.1 |
| Bellyflaps, BF | | 244.1 | 601.9 |
| SOF/BF | 3/1[a] | 122.1 | 390.5 |
| SOF/BF | 1/1 | 164.1 | 525.6 |
| SOF/BF | 1/3 | 262.0 | 698.3 |

a: ratio of the mixture of SOF/BF

The minces described in Table 1 and 2 had been stored for 8 or 11 months when analysed for TMAOase activity and FA content. To get further insight into the relation between both parameters, another set of experiments was performed where free FA was measured directly after preparation of minces, and TMAOase activity and free plus bound FA were determined after about one month of frozen storage.

The results, as given in Table 3 and 4, demonstrate that in case of saithe, in contrast to cod and haddock, FA is formed very rapidly after mincing the bellyflaps, even before freezing. After a period of about one month, minced bellyflaps of saithe and haddock contained high amounts of free plus bound FA, whereas cod expressed much lower values. For each of the three species, the FA content of minced fillet was considerably lower than that of the minced bellyflaps.

In most experiments use of a drum with 3 mm holes instead of 5 mm holes resulted in lower content of extractable protein and higher amount of FA in frozen-stored products. The process of disruption and mixing is expected to be more extensive when tissues are passed through smaller holes.

The TMAOase activity of minced bellyflaps of the three species was similar, ranging from 7 to 14 nkat/g wet weight. In minced fillet, the enzyme activity was below the detection limit.

As found for the first series of experiments, FA formation was high, if a threshold (under these experimental conditions being in the range of 10 nkat/g ww) of TMAOase activity was exceeded. Again a direct correlation between both parameters could not be established.

Tab. 2: TMAOase activity of minced fish (cod, haddock, saithe) stored at -20 °C for 11 months

| Type of mince | | Extractable protein[a] (mg/ml) | TMAOase activity[b] (nkat/mg prot) | (nkat/g ww) |
|---|---|---|---|---|
| **Cod** | | | | |
| Skinned fillet | | 14.2 | 0.046 | 3.25 |
| Skin-on fillet | | 10.9 | 0.036 | 1.95 |
| Bellyflaps | | 9.5 | 3.18 | 151.3 |
| **Haddock** | | | | |
| Skinned fillet | | 13.0 | 0.036 | 2.34 |
| Skin-on fillet | | 11.6 | 0.026 | 1.53 |
| Bellyflaps | | 5.5 | 1.03 | 28.2 |
| **Saithe** | | | | |
| Skinned fillet | | 9.8 | -[c] | - |
| Skin-on fillet | | 10.0 | - | - |
| Bellyflaps | | 3.5 | 0.62 | 8.04 |
| SOF/BF | 3/1 | 5.9 | - | - |
| SOF/BF | 1/1 | 5.2 | 0.080 | 1.54 |
| SOF/BF | 1/3 | 3.8 | 0.18 | 2.56 |

a: Amount of protein in the extract used for determination of TMAOase activity. Minces were extracted with 4 volumes of 0.3 % (w/v) of Triton X-100 in 0.1 M sodium phosphate pH 7.0.

b: Enzyme activities are given in nkat/mg extractable protein and nkat/ gram wet weight.

c: below detection limit (E = 0.05).

The presence of relatively high TMAOase activity in bellyflaps of various gadoids raised the question of the origin of the enzyme. Bellyflaps consist of different tissues: (i) light muscle tissue is located in the centre; (ii) layers, being rich in connective tissue and lipids, are covering the central muscle tissue; (iii) on the side of the abdominal cavity bellyflaps are covered by a thin layer belonging to the peritoneum, most of which was removed before mincing (Figure 1).

Bellyflaps with or without peritoneum were assayed for TMAOase activity. Removal of the peritoneum decreased the TMAOase activity of the bellyflaps, as shown for cod

Tab. 3: Content of free and bound formaldehyde in minced bellyflaps (BF) and minced skin-on fillet (SOF); the diameter of the holes of the separator drum is given in brackets.

| Sample | Formaldehyde content (mg/kg wet weight) Free FA[a] | Free + Bound FA[b] |
|---|---|---|
| **Cod** | | |
| Minced (3 mm) BF | 3.92 | 75.66 |
| Minced (5 mm) BF | 3.95 | 105.64 |
| Minced (3 mm) SOF | 3.60 | 21.08 |
| Minced (5 mm) SOF | 1.07 | 44.96 |
| **Saithe** | | |
| Minced (3 mm) BF | 328.63 | 491.45 |
| Minced (5 mm) BF | 159.74 | 460.96 |
| Minced (3 mm) SOF | 28.85 | 94.34 |
| Minced (5 mm) SOF | 11.81 | 72.16 |
| **Haddock** | | |
| Minced (5 mm) BF | 4.02 | 552.81 |
| Minced (5 mm) SOF | 1.74 | 75.85 |

[a]: Measured directly after preparation of minces
[b]: Measured after one month of frozen storage

(Table 4) and also found in many cases for other gadoids (data not given here). In case of saithe the TMAOase activity of the bellflaps varied widely, between the detection limit and 85 nkat/g wet weight. This variation may be due to inhomogenities of the different preparations of the bellyflaps or contamination by body fluids during preparation of samples.

Tab. 4: TMAOase activity in bellyflaps, minced bellyflaps and minced fillet

| Sample | Extractable protein (mg/ml) | TMAOase activity (nkat/mg prot) | (nkat/g ww) |
|---|---|---|---|
| **Cod** | | | |
| Bellyflaps (BF), 1[b] | 14.3 | -[a] | - |
| Bellyflaps (BF), 2[c] | 17.3 | 0.092 | 7.95 |
| Minced (3 mm) BF | 7.4 | 0.22 | 7.95 |
| Minced (5 mm) BF | 12.0 | 0.12 | 7.20 |
| Minced (3 mm) SOF | 7.7 | - | - |
| Minced (5 mm) SOF | 10.4 | - | - |
| **Saithe** | | | |
| Bellyflaps (BF), 1[c] | 15.0 | - | - |
| Bellyflaps (BF), 2[c] | 14.4 | 0.29 | 20.99 |
| Bellyflaps (BF), 3[c] | 12.0 | 1.42 | 85.04 |
| Minced (3 mm) BF | 9.7 | 0.29 | 13.85 |
| Minced (5 mm) BF | 10.0 | 0.15 | 7.24 |
| Minced (3 mm) SOF | 8.6 | - | - |
| Minced (5 mm) SOF | 11.5 | - | - |
| **Haddock** | | | |
| Minced (5 mm) BF | 12.0 | 0.20 | 11.97 |
| Minced (5 mm) SOF | 9.6 | - | - |

[a]: below detection limit (E = 0.05)
[b]: bellypflaps without peritoneum
[c]: bellyflaps with peritoneum
SOF: skin-on fillets
The diameter of the holes of the separator drum is given in brackets

The FA content of fish minces reported here is in the range found for frozen fishery products from retail shops in Germany (Rehbein et al. 1995).

## Conclusions

1. In all different types of minces of gadoid fishes analysed in the present study formaldehyde was produced during frozen storage.

2. Considerable TMAOase activity was found in minces containing bellyflaps. In minced fillet the activity was very low, and often below the detection limit.

3. The relation between the amount of formaldehyde andTMAOase activity was such that a certain enzyme activity was sufficient for the production of high amounts of FA.

4. Use of bellyflaps from gadoid fish species for production of minced fish is not recommended, because considerable formation of FA (and DMA) in frozen products has to be expected, due to TMAOase activity of this tissue.

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