Evaluation of the Direct Thiobarbituric Acid Extraction Method for Determining Oxidative Rancidity in Mackerel (Scomber scombrus L.)*

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A comparison was made between the thiobarbituric acid (TBA) values determined by the direct extraction method with trichloroacetic acid and those assessed by the distillation method. When applied to mackerel kept in frozen condition the TBA-values obtained with both methods showed a similar evolution in course of time. A regression analysis carried out on 50 samples taken at random confirmed these findings. The correlation coefficients for white and red muscles were 0.901 and 0.886 respectively.

The thiobarbituric acid (TBA) test is now widely used for measuring oxidative rancidity in fat-containing foods. In a previous paper, the possibilities of the direct determination of the TBA-values in trichloroacetic acid extracts instead of distillates of fish were evaluated and showed to be promising. In the same period, V. Witte et al. also reported favourable results with a direct extraction method for pork and beef, using a mixture of trichloroacetic acid and phosphoric acid as extracting solution. These authors obtained a recovery of 94% of malonaldehyde and a correlation coefficient of 0.845 between TBA-values as determined by the extraction method relative to these determined by the distillation method. Their data compared favourably with our results of 95.3% (teleost fish) and 0.854 respectively.

The direct extraction procedure is now being further investigated in our laboratory on different fish species. This paper reports results obtained with mackerel (Scomber scombrus L.). Two experiments were carried out.

In a first experiment, the possibilities to assess rancidity with the extraction method as compared with the distillation method were evaluated. For this purpose, a batch of quick-frozen mackerel was analysed four times during a 28 weeks storage period at two different temperatures (−28°C and −18°C). TBA-values of white and red muscles were determined separately.

In a second experiment, the relationship between TBA-values determined by both methods was studied on fifty samples of fish or frozen mackerel taken at random.

Experimental

Fish

First experiment: mackerel (Scomber scombrus L.) of ca. 600–700 g caught in October 1972 in the Southern North Sea and kept for about five days in ice on the fishing boat were quick-frozen in a plate freezer and double-glazed. Half of the batch was kept at −28°C, the other half being stored at −18°C. The average fat content of the white and red muscles was 16.8 and 23.1% respectively.

Second experiment: fresh or frozen mackerel of varying fat content and degree of rancidity were used.

Sampling for analysis

The frozen fish was thawed in a circulated water bath kept at 20°C. The fish were filleted and the white and red muscles of one fillet were carefully separated. The flesh of five fillets from different fish was put together, minced and thoroughly mixed.

TBA tests

Distillation procedure: according to B. Tarladgis et al. but using Antanacopoulos' still; 5 g of minced fish together with 5 ml of a 0.5% solution of propyl gallate and EDTA were distilled for 10 min. giving 100 ml of distillate.

Extraction procedure: 20 g of fish was homogenized with 100 ml of 7.5% trichloroacetic acid solution containing 0.1% of both propyl gallate and EDTA for one minute in a Waring blender and filtered.

TBA reaction: 5 ml of 0.02 M 2-thiobarbituric acid (British Drug Houses) in distilled water was added to 5 ml of distillate or filtrate in test tubes with screw caps; they were placed for 40 min. in a boiling water bath. Absorbance was read at 538 nm after cooling in tap water; a Hitachi-Perkin-Elmer model 139 spectrophotometer was employed.

Results were expressed in mg malonaldehyde per kg. For the final calculations, the recoveries of 66% and 95% for the distillation and extraction procedures respectively were taken into account.

Statistical analyses

Linear regression and its significance were calculated as outlined by G. Snedecor and W. Cochran.

Results and discussion

Storage experiment

The evolution of TBA-values obtained with both methods is shown in the figure. The scale of the ordinate

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of the distillation graph was doubled to stress the similarity of both TBA patterns when absolute values were not taken into account (see further).

![Evolution of TBA-values during storage of frozen mackerel](image)

Evolution of TBA-values during storage of frozen mackerel
a: dark muscles, \(-18^\circ C\); b: white muscles, \(-18^\circ C\); c: dark muscles, \(-28^\circ C\); d: white muscles, \(-28^\circ C\);

Although it is not always easy to assess accurately oxidative rancidity by organoleptic means owing to interferences by other compounds (e.g. free fatty acids) formed during storage, there was a fair agreement between the sensory assessment and TBA-values.

The difference between the red and white muscles on one hand and between the storage temperatures of \(-18^\circ C\) and \(-28^\circ C\) on the other hand was clearly shown. At \(-18^\circ C\), rancidity started after about 8 weeks of storage but was delayed for another 9 weeks at \(-28^\circ C\). The mackerel kept at \(-18^\circ C\) was considered to be no longer satisfactory after ca. 4 months (17 weeks), whereas the samples stored at \(-28^\circ C\), although slightly rancid, were still judged suitable for further processing (e.g. smoking) at the end of the experiment (28 weeks).

The TBA-values of red muscles were markedly higher than the corresponding values of white muscles, clearly reflecting the greater sensitivity of these muscles towards oxidation, a fact which is known since many years. The most striking chemical differences between both types of muscle for Scombridae are a higher content of lipids, cytochrome C, haemoglobin, myoglobin, pantothenic acid, riboflavin, cobalamin and thiamine in dark muscles (review by R. Love\(^8\)). The intimate contact between myoglobin and fat contributes both to rancidity and discoloration\(^5\). Myoglobin oxidizes to the brown ferric compound fourteen to sixteen times as rapidly as haemoglobin when exposed to atmospheric oxygen\(^6\).

There was practically no increase in TBA-values in the ordinary muscles stored at \(-28^\circ C\) and only a slight increase at \(-18^\circ C\), contrasting with the evolution of the corresponding values in dark muscles.

More experiments are necessary to investigate if threshold TBA-values could be established for mackerel (and other fish species). Nevertheless, the present work stressed the advantage of taking only the dark muscle and not a mixture of the whole fish (dark and white muscles) for the assessment of rancidity by the TBA-method.

**Relationship between distillation and extraction methods**

Since the apparent correspondence between the results obtained with both methods was excellent (figure), it was decided to study this relationship more closely with fifty other samples of mackerel taken at random.

The regression equation of the fifty TBA-values of white muscle of mackerel as determined by the distillation method relative to the TBA-values of the same samples determined by extraction was:

\[ Y = 0.184 + 0.411X \]

where \(Y\) is the TBA-value as determined by the extraction method and \(X\) the TBA-value determined by the distillation method.

For red muscles, the equation was:

\[ Y = 0.226 + 0.460X \]

The correlation coefficients were 0.901 and 0.886 for white and red muscles respectively and were highly significant. There was no significant difference between both coefficients. The same conclusion could be drawn for the regression coefficients (0.411 and 0.490) and the intercepts (0.184 and 0.224). The latter values on the other hand were significantly different from 0.

These results indicate that white and red muscles, which differ in composition and sensitivity towards oxidation, nevertheless gave the same relative TBA-values when the extraction or the distillation methods were used. Mean TBA-values determined by the distillation procedure however were approximately twice as large as those determined by the extraction method. Either the heat of distillation increased the quantities of aldehyde from lipid precursors or heat disrupted certain carbonyl amino acids, pyrimidine or protein\(^7\).

Finally, it should be stressed that the correlation coefficients obtained were not significantly different from the value of 0.824 obtained in earlier experiments with cod (Gadus morhua L.), redfish (Sebastes marinus L.), herring (Clupea harengus L.), plaice (Pleuronectes platessa L.) and spurdog (Squalus acanthias L.)\(^8\).

The regression equation (not quoted in the previous paper) was also very similar:

\[ Y = 0.205 + 0.452X \]

This would indicate an identical relationship between the TBA-values determined by both methods with all species of untreated fish. Further investigations with other fish species are necessary to confirm this hypothesis.

The conclusion of this work is that at least for mackerel the extraction method can be used instead of the distillation method. As outlined in the previous paper\(^1\) the direct extraction procedure has the advantage of speed and simplicity.

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