Z Lebensm Unters Forsch (1992) 194:115–119

Zeitschrift für
Lebensmittel-
Untersuchung
und-Forschung

Original paper

Storage life of frozen salmonoids.

Effect of light and packaging conditions on carotenoid oxidation and lipid oxidation

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Received May 13, 1991

Die Haltbarkeit von Lachsforellen.
Einfluß von Licht und Verpackung auf die Carotenoid- und Fettoxydation

Zusammenfassung. Der kombinierte Effekt von Sauerstoffdurchgang durch Verpackungsmaterial und der Lichteinwirkung verschiedener spektraler Verteilung auf den oxidativen Abbau der Carotinoide und Fette in gefrorenen Lachsforellen wurde in einem Lagerungsversuch untersucht. Der Abbau des Carotenoids Astaxanthin wurde mit HPLC und *Tristimulus*-Calorimetrie beobachtet, und war empfindlich gegenüber UV-Licht und weniger empfindlich gegenüber der Sauerstoffdurchlässigkeit der Verpackung, was mit früheren Ergebnissen für Photooxidation der Carotinoide in Modellsystemen übereinstimmt. Dies widerspricht der Fettoxidation, die mehr von Sauerstoffzugang abhängig ist als von der Entwicklung des UV-Lichtes. Die Bildung von Peroxiden des Produktes kuliminierte nach 3monatiger Lagerung (bis 8,4 meq/kg Öl) und ging der Bildung von Thiobarbitursäure-aktiven Substanzen voraus. Das Produkt im Material mit hohem Sauerstoffdurchgang (60 cm^3 m^{-2} bar^{-1} pro 24 h), das bei normalen fluorescierendem Licht oder bei fluorescierendem Licht mit hohem UV-Anteil aufbewahrt wurde, erreichte einen TBS-Wert von ungefähr 5 µmol Malonaldehyd/kg Fleisch. Ein ranziger Geschmack wurde durch sensorische Bewertung nach 6monatiger Lagerung nicht erkannt, wohl aber ein bitterer Geschmack bei dem UV-Licht ausgesetzten Proben.

Summary. The combined effect of oxygen transmission of packaging material and of exposure to light with different spectral distribution on the oxidative degradation of carotenoids and lipids in frozen salmonoids has been characterized in a storage experiment with steaks of rainbow trout. The degradation of the carotenoid astaxanthin, as followed by HPLC analysis and tristimulus colorimetric measurement, was sensitive to the radiant flux density of UV light and less sensitive to the oxygen transmission rate of the packaging material, in agreement with previous findings for photooxidation of carotenoids in food model systems. This is in contrast to the lipid oxidation, which was found to be dependent on the accessability of oxygen rather than on exposure to UV light. Formation of peroxides in the product culminated after 3 months of storage (up to 8.4 mEq kg^{-1} oil), and preceded the formation of thiobarbituric-acid-reactive substances. The product in packaging material with a high oxygen transmission rate (60 cm^3 m^{-2} bar^{-1} per 24 h) reached a level of approximately 5 µmol malonaldhyde/kg^{-1} product, when stored exposed to standard fluorescent light or fluorescent light with a high UV component. Rancid taste was not detected by sensory evaluation for any of the products after 6 months of storage, whereas a bitter taste was noted for the product exposed to UV light.

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Introduction

Fish have, like other animals, no *de-novo* carotenoid synthesis, and the pigmentation of salmonoids by carotenoids depends solely on dietary sources [1]. Wild fish feed on crustaceans, and contain mainly the reddish carotenoid astaxanthin. Astaxanthin is in nature synthesized by phytoplankton, and is efficient as a scavenger of free radicals and as a quencher of singlet oxygen [2, 3]. Based on a growing body of evidence, astaxanthin has been identified as an important factor for the in vivo protection of the highly unsaturated lipids against oxidation, and the term "super vitamin E" has been coined [4]. In a previous study we found evidence for a similar protection of lipids in frozen salmonoid steaks during freezer storage, especially against light-induced oxidation of lipids during retail display [5].

Two aspects of carotenoid pigmentation clearly need further consideration in relation to the growing importance of aquaculture. In relation to the farming of salmonoids, it is important to develop feed with bioavailable and stable carotenoids, and great research effort is de-

voted to this aspect of aquaculture [6]. As for the processing, packaging and storage of the pigmented fish, detailed knowledge of the stability of the carotenoids in relation to exposure to oxygen and light, contact with blood pigments, enzymatic activity and other external and intrinsic factors are required. As part of our previous research we have developed a routine method for the quantitative determination of astaxanthin and canthaxanthin, the two carotenoids in use as feed additives, to be used for quality control of salmonoids during storage [7]. Moreover, since light in combination with oxygen seems to be the most important single factor in the degradation of carotenoids and in the initiation of lipid oxidation during freezer storage of salmonoids [5], we have investigated the role of oxygen pressure and of the spectral distribution of light on the oxidative stability of several carotenoids [8, 9]. From the results of these studies using model systems, it is predicted that the amount of residual oxygen in packs of frozen salmonoids is less important for oxidative stability than is the exposure of the product to light with a strong ultraviolet (UV) component. We have undertaken a practical test of this prediction in a large-scale storage experiment with steaks of rainbow trout. The quality of the frozen product was followed during 6 months of storage using several analytical methods, the results of which are reported here together with the results of a final sensory evaluation.

Materials and methods

Product and packaging. Rainbow trout (*Salmo gairdneri*) raised in net pens on astaxanthin-containing feed were obtained from a Danish fish farm. The fish (average mass 1.5 kg; total lipid 7.7% and dry matter 29.0%) were kept on ice during transportation. The head and tail of the fish were removed prior to sectioning into steaks (approx 2 cm thick). The steaks were vacuum-packed with two steaks in each pack, using two different transparent packaging materials: (I) A polyamide/polyethylene film with an oxygen barrier in the polyamide layer [Riloten 40/70 ×, from Otto Nielsen (Lyngby, Denmark), oxygen transmission rate (OTR) of 2 $cm^3 \cdot m^{-2} \cdot bar^{-1}$ per 24 h]. The water vapour transmission rate (WVTR) was measured on a Mocon infrared diffusometer (Modern Controls, Minneapolis, Minnesota, USA), and was 1.2 $g \cdot m^{-2}$ per 24 h. (II) A polyamide/polyethylene film (Amilon 15/60 from Otto Nielsen) with an OTR of 60 $cm^3 \cdot m^{-2} \cdot bar^{-1}$ per 24 h, and a WVTR of 0.5 $g \cdot m^{-2}$ per 24 h. The peaks were individually frozen in a blast freezer at an air temperature of about −30° C.

Storage. The packs were placed in the upper section of an illuminated freezer cabinet (gondola with forced air circulation). The surface temperature of the products was −18° C rising to −12° C during daily defrosting, as monitored by continuous registration. A total of 78 packs were used for the storage experiment. One third of the packs were covered with black plastic to protect the steaks from light.

Light sources. The freezer cabinet was divided into two sections. One section was illuminated with standard fluorescent tubes (Philips, Copenhagen, Denmark; TLD 36W/92) with a radiant flux density of 7.7 $W \cdot m^{-2}$ as measured by a Gossen Mavolux (FRG) digital photometer, and an UV component (300–400 nm) of 0.025 $W \cdot m^{-2}$ as measured by a Topcon (Tokyo, Japan) UV photometer. The other section was illuminated with tubes emitting a high amount of UV light (Philips TL 20W/09N) with a radiant flux tensity of 6.7 $W \cdot m^{-2}$ measured as the sum of visible (5.4 $W \cdot m^{-2}$) and UV (1.3 $W \cdot m^{-2}$) light. One third of the packs were placed under the light with high UV radiant flux.

Colour measurement. The surface colour of the frozen steaks were measured through the packaging material using a tristimulus colorimeter (Hunterlab D-25 equipped with a D-25 M sensory head, diameter 2.5 cm). The instrument was calibrated against a white standard with $L=90.7$ $a=-0.9$ and $b=-0.1$. The reported colour measurements are mean values of four individual measurements on different sections of each of the packs.

Analysis of pigment content. The concentration of carotenoids was determined by the HPLC method previously described [7]. Each analysis required 30 g minced flesh, and a whole steak was minced prior to extraction and isolation of pigment.

Assessment of lipid oxidation. The peroxide value (PV) was used to characterize the initial stage of lipidoxidation. Ten grams of the product surface (an approx. 0.5-cm deep layer) was extracted according to Bligh-Dyer [10], and the extract was used for a combined determination of PV and oil content. Half of the extract was allowed to oxidize Fe^{2+} to Fe^{3+}, followed by complex binding of Fe^{3+} to SCN^- and spectrophotometric detection at 505 nm, according to the method of Stine et al. [11]. The values were expressed as meq peroxide kg^{-1} oil. The oil content was determined gravimetrically on the other half of the extract. Thiobarbituric-acid (TBA) reactive substances were measured by the extraction-method of Vyncke [12]. The samples (10 g) were taken from the product surface (an approx. 0.5 cm deep layer), and values were expressed as μmol malonaldehyde/kg flesh.

Sensory evaluation. At the end of the storage experiment the taste of cooked samples were evaluated by a six-member trained sensory panel using a ±5 hedonic scale. The steaks were heated in boil-in bags for 8 min in a water-bath at 80° C.

Results

The storage experiment with steaks of farmed rainbow trout was designed in order to evaluate the combined effect of OTR of packaging material and of exposure to light on oxidative degradation of carotenoids and lipids during freezer storage. Two different packaging materials were combined with three different illumination conditions, in effect yielding six different set of storage conditions, as outlined in Table 1. Oxidation of carotenoids

Table 1. The radiant flux density on the product surface for the six combinations of oxygen transmission rate of packaging materials and illumination conditions used in the storage experiment with frozen steaks of rainbow trout

Packaging material	Protected against light		Standard fluorescent tubes		Tubes with strong UV-component	
	VIS[a]	UV[b]	VIS[a]	UV[b]	VIS[a]	UV[b]
I: Polyamide/polyethylene with OTR[c]=2, WVTR[d]=1.2	0.0	0.0	7.7	0.025	5.4	1.3
II: Polyamide/polyethylene with OTR[c]=60, WVTR[d]=0.5	0.0	0.0	7.7	0.025	5.4	1.3

[a] VIS: visible light, radiant flux density in $W \cdot m^{-2}$ for $\lambda \geqq 400$ nm
[b] UV: ultraviolet light, radiant flux density in $W \cdot m^{-2}$ for $300 \leqq \lambda \leqq 400$ nm
[c] Oxygen transmission rate in $cm^3 \cdot m^{-2} \cdot bar^{-1}$ per 24 h
[d] Water vapour transmission rate in $g \cdot m^{-2}$ per 24 h

Table 2. Astaxanthin concentration in steaks of rainbow trout ($mg \cdot kg^{-1}$ flesh)

Packaging material[a]	Illumination[b]	Prior to storage[c]	After 6 months of storage[d]
I	Dark	8.5±0.4	10.2±0.2
	Standard tubes	8.5±0.4	8.2±0.2
	UV	8.5±0.4	6.2±0.2
II	Dark	8.5±0.4	8.8±0.5
	Standard tubes	8.5±0.4	8.6±0.2
	UV	8.5±0.4	7.1±0.1

[a] See Table 1 for OTR and WVTR
[b] See Table 1 for radiant flux density
[c] Mean of four determinations
[d] Mean of two determinations

was followed by colour measurement of the product surface and by HPLC analysis of carotenoid content in the flesh. The early stages of lipid oxidation were detected as peroxides whereas secondary oxidation products were determined as TBA-reactive substances. The quality of the product stored under the six different sets of storage conditions was followed for 6 months and a sensory evaluation concluded the storage experiment.

Carotenoid concentration

The major carotenoid of the rainbow trout was identified as astaxanthin, in an initial concentration of 8.5 ± 0.4 $mg \cdot kg^{-1}$ flesh, which is a relatively high concentration for salmonoids [5, 7]. Canthaxanthin was present in trace amounts (approx. 0.2 $mg \cdot kg^{-1}$). After 6 months of storage, a moderate decrease in astaxanthin concentration (Table 2) was detected for the product exposed to the light with a strong UV component. It should, however, be noted that the carotenoid concentration was determined in homogenized flesh from the steaks and not in the surface layer.

Colour measurement

The high astaxanthin content in the flesh gave the product a red to dark orange appearance. The colour was quantitatively characterized by tristimulus colorimetric parameters, which prior to storage had the value Hunter $L = 40.6 \pm 2.7$, Hunter $a = 26.3 \pm 1.7$, and Hunter $b = 15.8 \pm 1.1$ (mean values for 78 packs, each measured four times). The Hunter b parameter (yellowness) changed only marginally during storage. Hunter L (the lightness parameter) increased, and Hunter a (the redness parameter) decreased for certain storage conditions. The increase in the Hunter L parameter was most significant for the packaging material with high WVTR (I, see Table 1) and is most likely due to dehydration of the product surface and changes in the reflectance properties of ice crystals. In total, this dehydration appears to be of minor importance and localized in the product surface, as evidenced by a constant oil content of the product (determined regularly during storage). The Hunter a parameter has been shown to give a high correlation with subjective

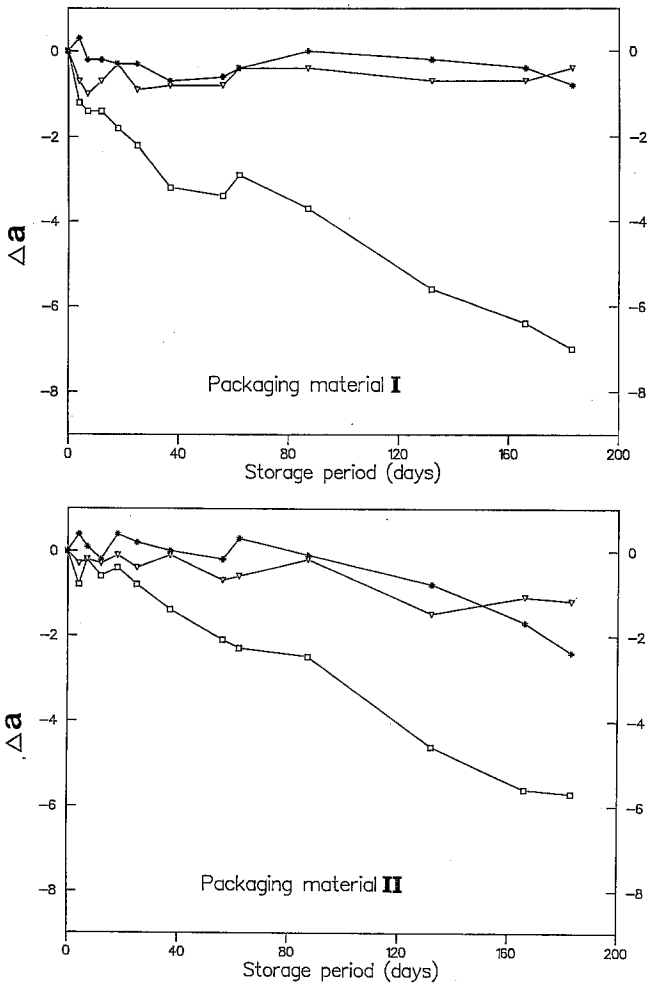

Fig. 1. Colour stability, measured as changes in the redness parameter Hunter a, of steaks of rainbow trout during freezer storage. Six different storage conditions are compared (two packaging materials and three conditions of illumination, see Table 1). (–□–) UV-light; (–▽–) light; (–#–) dark

colour score for the redness of raw salmon [13, 14]. Hunter a thus provides an objective measure of redness of raw salmon flesh, and was also previously used to follow colour fading of salmonoids during storage [5]. The changes in Hunter a values ($\Delta a_t = a_t - a_{t=0}$) during storage are shown in Fig. 1 for the six different storage conditions. The product exposed to high intensities of UV-light shows a significant decrease in redness and this colour fading progressed from the start of the storage period or with only a short induction period. The light from standard fluorescent tubes had no effect on colour fading during storage relative to dark storage. The OTR of the packaging material is seen to be less critical. However, after 2 months of storage, colour fading was initiated for the product packed in packaging material II with the highest oxygen transmission rate.

Lipid oxidation

The primary stage of oxidation, the formation of peroxides, were detected by reduction of the peroxides by

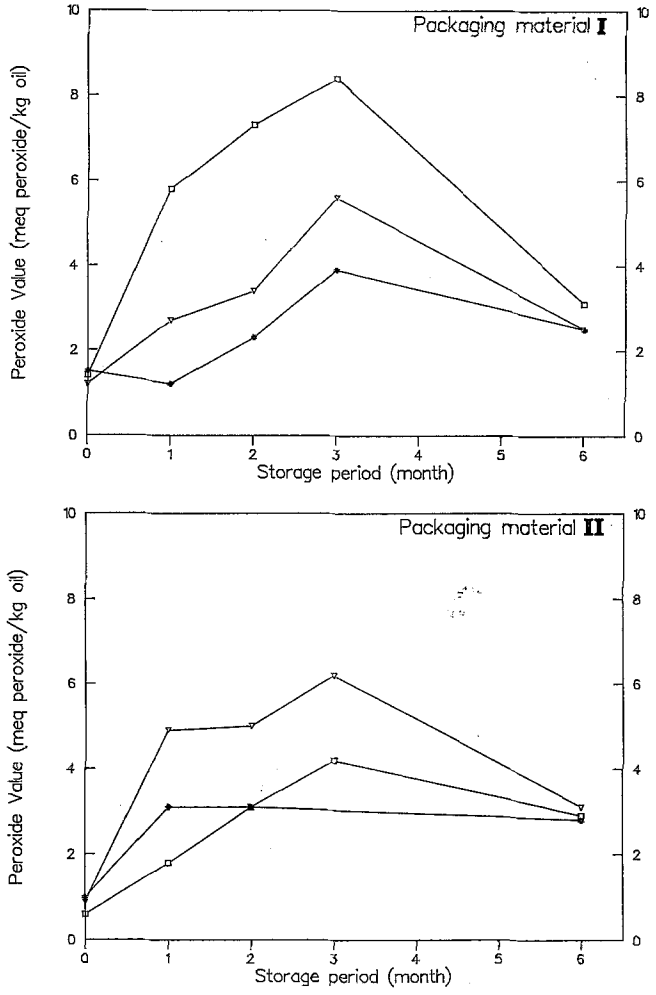

Fig. 2. Progression of primary oxidation determined as peroxide value in the surface layer of steaks of rainbow trout (mEq · kg^{-1} oil) during freezer storage. Six different storage conditions are compared (two packaging materials and three conditions of illumination, see Table 1). For symbols see legend of Fig. 1

iron(II). The peroxide value increased during the initial period of storage and reached a maximum value after approximately 3 months. The onset of peroxidation was only delayed for the product packed in the plastic material with low oxygen permeability and kept in the dark. As may be seen from Fig. 2, a decline in PV was observed after 3 months of storage indicating that the degradation of peroxides was faster than the formation, and that further oxidative degradation processes were becoming dominant. The product packed in the plastic material with high oxygen transmission and exposed to light with a strong UV component showed an unexpectedly low PV level. This observation could indicate cleavage of the peroxides by UV light, as an initiator of further oxidative degradation. The level of secondary oxidation products rose faster for the product in the packaging material with high oxygen transmission and exposed to light, supporting this suggestion. The secondary oxidation products derived from the peroxides were measured as TBA-reactive substances, and the change in TBA-values during storage are shown in Table 3. The OTR is highly important for the development of secondary oxidation products, and this effect is clearly accentuated by light, whereas the spectral distribution is apparently of minor importance.

Table 3. Progression of secondary oxidation determined as the thiobarbituric acid value in the surface layer of steaks of rainbow trout (μmol malonaldehyd · kg^{-1} flesh) during freezer storage

Packaging material[a]	Illumination[b]	Storage for (months)				
		1	2	3	4	6
I	Dark	1.7	2.1	2.1	2.1	1.7
	Standard tubes	0.5	1.8	1.9	1.7	1.7
	UV	0.4	2.1	1.1	3.4	1.4
II	Dark	2.6	3.0	4.7	4.4	2.9
	Standard tubes	1.2	7.1	3.9	6.0	5.5
	UV	1.2	5.6	4.1	5.6	4.4

[a] See Table 1 for OTR and WVTR
[b] See Table 1 for radiant flux density

Sensory evaluation

After 6 months of storage a sensory evaluation was performed for cooked samples. Taste, odour and appearance were evaluated separately, and the product exposed to light with a high UV component was given the lowest score in each category. Rancid taste was not detected for any of the samples, and the taste was still acceptable for all samples. A bitter taste was, however, noted for the steaks exposed to UV light during storage.

Discussion

The practical storage life of frozen fish products is often determined by the development of a rancid taste. Intrinsic factors such as enzymes and blood pigments are of importance for the initiation of lipid oxidation in fish products [15], together with the actual storage conditions [5, 16]. Carotenoids in salmonoids are associated with lipids and the oxidation of the two types of compounds are linked together; thus bleaching of salmonoids is coupled to development of rancidity [17]. In the present study, we have focused on the importance of oxygen accessibility and exposure to light for the initiation of oxidation of both carotenoids and lipids in frozen steaks of rainbow trout during storage.

The availability of oxygen for the product was kept at two levels regulated by the OTR of the plastic films, both of which are currently in use for food packaging. The product was stored in the dark or exposed to light, and for the illuminated samples, two different types of fluorescent tubes were used, having comparable total radiant flux density but differing in spectral distribution. The fluorescent tubes with the strong UV component are not in common use in the retail trade, and the storage conditions obtained with these fluorescent tubes should accordingly be considered as an accelerated storage test (Table 1).

The carotenoid concentration in the steaks was constant during the storage period as found by chemical analysis, except for the product exposed to light with the strong UV component. The decrease in the surface redness of the product was likewise most significant for the product exposed to UV light. These observations can be rationalized on the basis of wavelength dependence for the quantum yield for photooxidation of carotenoids. For each of the four carotenoids lutein, β-carotene, astaxanthin and canthaxanthin, the efficiency with which monochromatic light promotes oxidation increases with decreasing wavelength according to [8, 9]:

$$\Phi^{irr} = A \cdot \exp(-a\lambda_{irr}). \quad (1)$$

An exponential increase with decreasing wavelength has been confirmed for several solvents including carotenoids solubilized in water [9], and the present findings confirm that the results obtained in the previously developed model systems can be applied, at least qualitatively, to carotenoid-pigmented foods. Moreover, the photodegradation of the surface colour is hardly dependent on the accessibility of oxygen (Fig. 1), an observation that relates to the dependence of the photooxidation quantum yield for carotenoids on the oxygen partial pressure:

$$\Phi^{irr} = k_{car} \cdot \sqrt{P(O_2)}. \quad (2)$$

Equation (2) has been demonstrated for solubilized β-carotene and lutein serving as food models [8]. Compared to a linear dependence, the square root dependence on oxygen pressure decreases the improvement to be expected for carotenoid stability as a result of diminishing the residual oxygen in the product. Similar arguments apply to the OTR of the packaging material (for the OTR of materials II and I, compare the ratio 60/2 with the ratio $\sqrt{60}/\sqrt{2}$). These results on the influence of the spectral distribution of light and of the OTR of the packaging materal obtained in chemical model experiments and confirmed during practical storage should be considered when designing packaging material for carotinoid-pigmented foods. It is also of interest to compare these recommendations for packaging of salmonoids with the optimal packaging conditions found for cured meat products [18]. For sliced ham, the spectral distribution of light reaching the product surface is of little importance, whereas the level of residual oxygen is critical for oxidation of the pigment nitrosylmyoglobin.

The OTR of the packaging film was found to be important for the development of secondary oxidation products from the lipids in rainbow trout steaks during freezer storage (Table 3). Exposure to light likewise promoted lipid oxidation, and was of special importance in the product packed in the film with low OTR. However, the influence of the spectral distribution of the light was less significant for the progression of lipid oxidation. Based on a comparison with other foods such as pork patties [19], an increase in secondary lipid oxidation due to exposure to UV light could be expected. This was not seen for steaks of rainbow trout, despite the fact that the formation and degradation of peroxides was influenced by exposure to UV light (Fig. 2). A possible explanation is that the radicals formed by degradation of the primary oxidation products (i.e. the peroxides) were effectively scavenged by astaxanthin in its role as "super vitamin E". We shall, however, refrain from further speculation until current work on light-induced oxidation processes in homogenized flesh from salmonoids can be presented.

Acknowledgements. The present work was supported by grant 16-4185/4361 H from the Danish Technical Research Council. The authors wish to thank Otto Nielsen Inc., Lyngby/Denmark, for providing packaging material, and for help with the packaging of the products. Further we wish to thank the Research Laboratory, Danish Ministry of Fisheries especially Lisbeth B. Knudsen for advice on performing the PV- and TBA-methods on fish products.

References

1. Simpson KL (1982) In: Martin RE (ed) Chemistry and biochemistry of marine food products, chap 10. AVI, Westport
2. Burton GW, Ingold KU (1984) Science 224:569
3. Di Mascio P, Kaiser S, Sies H (1989) Arch Biochem Biophys 274:532
4. Miki W (1991) Pure Appl Chem 63:141
5. Andersen HJ, Bertelsen G, Christophersen AG, Ohlen A, Skibsted LH (1990) Z Lebensm Unters Forsch 191:119
6. Torrissen OJ (1985) Aquaculture 46:133
7. Christophersen AG, Knuthsen P, Skibsted LH (1989) Z Lebensm Unters Forsch 188:413
8. Jørgensen K, Skibsted LH (1990) Z Lebensm Unters Forsch 190:306
9. Christophersen AG, Huang J, Jørgensen K, Skibsted LH (1991) Z Lebensm Unters Forsch 192:433
10. Bligh EG, Dyer WJ (1959) Can J Biochem Physiol 37:911
11. Stine CM, Harland HA, Coulter ST, Jenness R (1954) J Dairy Sci 37:202
12. Vyncke W (1975) Fette, Seifen, Anstrichm 77:239
13. Skrede G, Storebakken T (1986) J Food Sci 51:804
14. Little AC, Martinsen C, Sceurman L (1979) Color Res Appl 4:92
15. Fischer J, Deng JC (1977) J Food Sci 42:610
16. Chen HM, Weyers SP, Hardy RW, Biede SL (1984) J Food Sci 49:1337
17. Stone RA, Kinsella JE (1989) J Agric Food Chem 37:866
18. Andersen HJ, Bertelsen G, Boegh-Soerensen L, Shek CK, Skibsted LH (1988) Meat Sci 22:283
19. Andersen HJ, Skibsted LH (1991) J Food Sci (in press)