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SafeSalt: Quality control of bacalao salt

Minh Van Nguyen
Sigurjón Arason
Hrönn Ólína Jörundsdóttir

Öryggi, umhverfi og erfðir

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Report summary

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Ágrip á íslensku:	Markmið verkefnisins var að þróa hraðvirka greiningaraðferð til að meta gæði salts sem notað er til saltfiskframleiðslu. Takmark verkefnisins var að lágmarka gulumyndun í saltfiski. Tilraunir með að nota þorskalýsi sem staðgönguefni til að meta þránun fitu af völdum málma sýndu lofandi niðurstöður og nauðsynlegt er að yfirfæra niðurstöður fyrir þorskalýsi yfir á þorskflök. Niðurstöður benda til þess að járn hafi meiri áhrif á þránun fitu en við kopar. Þránun mældist í fitu við allt að 5 ppm járnstyrk í salti. Nauðsynlegt er að skoða áhrif kopars og járns á oxun próteins í fiski.		
Lykilorð á íslensku:	*Saltfiskur, kopar, járn, gæðastaðall*		
Summary in English:	The objective of the project was to develope rapid test method to evaluate the quality of salt used in the production of heavily salted cod. This is done in order to reduce the risk of yellow discoloration in salted cod. Experiments where cod liver oil was used as surrogate material showed promising results and the next step is to extrapolate these results to cod filets. The results indicate that iron has stronger oxidazing effects on lipids compared to copper. Oxidation of lipids was detected at 5 ppm iron concentration in salt. Future research should aim at investigating the effects of copper and iron on protein oxidation in fish.		
English keywords:	*Salted cod, copper, iron, quality standard*		

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1. Introduction

Yellow discoloration in heavily salted and dried cod is a known problem in the production of heavily salted cod, often known as bacalao. This problem has occurred occasionally over the whole history of production of salted cod both in Iceland as well as from other countries. Until now, the salt used for brining and dry salting has been pointed out as source for this discoloration where recent studies indicate that there are several factors such as biological and environmental increasing the risk discoloration as well as processing technique and equipment. The mechanism for discoloration of salted cod appears to be more complicated than previously expected and yet not entirely understood.

Salted cod is produced in several steps. The first step is brine salting followed by the second step of dry salting for several days. In some cases if discoloration occurs, the discoloration appears during the dry salting period. In other cases, the discoloration does not appear until later, when the product has reached the marked. Main hypotheses have been up until now that copper or other oxidising agents in the presence of a catalyst, such as iron, oxidises unsaturated fatty acids, producing yellow discoloration, the influence of e.g. micro-organisms is not thoroughly understood (Ólafsson 1954; Lauritzsen, Martinsen et al. 1999). The long-chain highly unsaturated fatty acids eicosapentaenoic acid (EPA, 20:5ω) and docosahexaenoic acid (DHA, 22:6ω) are most prone for oxidation and constitute a major portion of the fatty acids present in cod (Khayat and Schwal 1983; Love 1988; Shahidi and Dunajski 1994).

Heavily salted fish are traditional products from the North-Atlantic fisheries and are regarded as ripened fish products in many countries. In Iceland, salted fish is one of the main fishery products and the traditional markets for Icelandic salted fish have been South Europe and Latin America. Nowadays, the United States is one of the growing markets. Salted fish products have in recent years constituted 15-20% of the total value of seafood exports from Iceland. However, yellow/brownish discoloration of the flesh threatens the quality of such skin-on salted products where Icelandic salted fish products have been categorized as high price-end products due to high quality and it is therefore of great importance to minimize the occurrence of discoloration as much as possible. The discoloration may cover the whole muscle surface or only sections of it or even penetrate into the fish muscle (Figure 1). As long as fifty years ago, studies on salted fish showed a positive correlation between the amount of copper and iron present in both the salt and fish muscle (Dyer 1949). Recent investigation indicates that manganese may cause certain discoloration (Arason, Jörundsdóttir et al. 2009). If occurs, this discolorations can appear late in the production process, even sometimes after the transport of the products to foreign markets.

There are several factors that can induce discoloration of salted cod. The aim of this project was to develop a rapid method for producers to determine the quality of salt for use in processing of salted cod as well as investigating further the impact of different metals on lipid oxidation. The project tested different methods for accelerating oxidation of lipids as described below.

Figure 1: The discoloration of salted cod products. Three pictures with typical copper discoloration and one with suspected manganese discoloration (lowest to the right).

2. Yellow discoloration of salted cod

2.1 Mechanism of yellow discoloration and discoloration experiments

Experiments have shown that copper in brine is quickly absorbed to the muscle tissue (Lauritzsen, Martinsen et al. 1999). It appeared, however, that the uptake of copper is more than merely an equilibrium process and a protein binding of positively charged copper is suggested to affect the uptake. Low post-mortem muscle pH seems to increase lipid oxidation (Lauritzsen, Martinsen et al. 1999). Reduced form of copper is particularly pro-oxidative (Kristin Lauritzsen 2004).

Discoloration of salted cod has been achieved experimentally with laboratory salt and added elements. An experiment produced yellow discoloration by adding $CuSO_4$ to NaCl containing low amounts of Ca (0.06%) and SO_4 (0.14%). The rate of discoloration increased if $CaCl_2$ (1%) was added, but no discoloration was achieved if the salt was sterilised and the fish washed in 0.5% $NaNO_2$ solution. High concentration of calcium increased the discoloration. The conclusion of this study was that calcium-requiring halophilic micro-organisms probably play a prominent role in the discoloration of salted cod (Ólafsson 1954).

Recent experiments indicate that low temperature seems to inhibit the discoloration (unpublished data).

2.2 Prevention of discoloration

Chelating agents have been used within the food industry to minimize the catalytic effect of transition metals in the oxidation process (Pokorný 1987; Løliger 1991) where the most important chelators are water soluble compounds such as citric acid, EDTA, phosphoric acid derivates and some amino acids (Hølmer 1995).

Polyphosphates reduces the discoloration probably through inhibiting the oxidation of unsaturated fatty acids (Kim, Hearnsberger et al. 1995; Goncalves and Ribeiro 2009). The mechanism by which phosphates prevent lipid oxidation appears to be related to their ability to sequester metal ions that are essential for prooxidation of lipid (Trout and Schmidt 1984; Masniyom, Benjakul et al. 2005; Cheng and Ockerman 2007). Some previous studies have dealt with the effects of phosphates on the quality of salted cod (Arnesen and Dagbjartsson 1973; Arnesen and Dagbjartsson 1974; Thorarinsdottir, Arason et al. 2001; Esaiassen, Nielsen et al. 2004; Esaiassen, Østli et al. 2005). The results obtained indicated that the use of phosphates during processing of salted cod may improve appearance and thereby consumers liking of the product.

2.3 Bacteria capable to induce discoloration in salted cod.

Jónsdóttir and Hannesson (1986) investigated the effects of bacteria able to induce yellow discoloration in salted and minced cod flesh (Jónsdóttir and Magnússon 1986). The bacteria strain was believed to be *Planococcus citreus* and believed to originate from the fish skin mucus. The optimum growth temperature for bacteria able to induce discoloration in salted cod was shown to be 30°C. The bacteria was able to grow in temperature above 5°C but no growth was detected <5°C. The increased salt concentration decreased the growth of bacteria and at 25% NaCl concentration in the minced fish flesh; the growth of bacteria was limited. The yellow discoloration caused by this bacteria usually appears early in the processing of salted cod. The yellow discoloration that appears after storing is not believed to be caused by these bacteria.

2.4 Salt standards

Icelandic salt quality standards are presented in Table 1.

Table 1: Icelandic salt quality standards

	Faulted	Good	Faulted	Unusable
Water		<3,5%	≥0,35%	
Ca	<0,055	0,05-0,205	0,20-0,35%	>0,35%
$CaSO_4$	<0,17%	0,17-0,70%	0,70-1,19%	>1,19%
Mg		<0,1%		≥0,5%
$MgSO_4$		<0,5%		≥0,5%
Fe		<20 mg/kg d.w.		≥20 mg/kg d.w.
Cu		<0,03 mg/kg d.w.	0,03-0,05 mg/kg d.w.	>0,05 mg/kg d.w.
Mn		<2 mg/kg d.w.		≥2 mg/kg d.w.

The CODEX salt quality standard is presented in Table 2.

Table 2: Maximum allowed concentration of contaminants according to CODEX standard for food grade salt

As	<0,5 mg/kg	Cd	<0,5 mg/kg
Cu	<2 mg/kg	Hg	<0,1 mg/kg
Pb	<2 mg/kg		

The standard presented in Table 1 is used by the Icelandic industry for quality assurance of salt. All salt that is imported and used for salted cod production, is analysed in order to minimize the risk of damage and reduced quality of salted cod due to metals or other quality factors of the salt.

3. Experimental design

3.1 Testing effect of metal concentration on fish homogenate

The first tests performed, a cod homogenate was used, adding salt with different metal concentration, both with and without the presence of iron. The salted fish homogenate was thoroughly mixed and stored on petri dishes with water runoff holes in order to allow the maximum contact with oxygen. Different methods of preparing the salt were tested, as well as different temperatures (4°C – room temperature). Tests were stored up to three weeks.

3.2 Testing effect of metal concentration on cod liver oil (Lýsi)

Commercial cod liver oil was used as a test matrix for inducing oxidation with metals. Cod liver oil was mixed with analytical salt at the ratio of 1:1 in round plastic containers. Metal ions were added with different concentrations and mixed thoroughly. The samples were stored at room temperature and the colour changes were measured by using a DFK 31BF03 CCD digital camera

3.3 Analytical parameters

The intensity of the fish homogenate colour was measured by using the Minolta CR-300 chromameter (Minolta Camera Co., Ltd; Osaka, Japan) in Lab* system (CIE, 1976) with CIE IlluminantC. The instrument records the L* (lightness), a* (redness) and b* (yellowness) values on CIELAB colour scale. The a* value describes the intensity in green colour (negative) and in red colour (positive). The b* value describes intensity in blue colour (negative) and in yellow colour (positive).

The colour of cod liver oil was measured by using machine vision. The images were taken in a light box with the dimensions 44.6 cm depth, 59.8 cm length and 67.0 cm height. Fluorescent bulbs were used and a polarized film covered the light. The samples were placed in the light box under a DFK 31BF03 CCD digital camera (The Imaging Source Europe GmbH). Images were then analysed using LensEye software (Engineering & Cyber Solutions, Gainesville, Fla, U.S.A.) by taking a contour plot that allows for colour parameters (L*, a* and b*) to be established.

4. Results and discussion

4.1 Effect of different metal concentrations on fish homogenate

As described in Section 3.1, effects of metals were first tested on fish homogenate. No noticeable change of colour or signs of lipid oxidation. Both visual changes of colour and chemical analyses were used to determine oxidation. The use of this experimental design was therefore discontinued. Figure 2 (a, b), Figure 3 (a, b), Figure 4 (a, b) show the changes in L*, a* and b* values, respectively, of fish homogenate that was mixed with different metal ions (iron-Fe^{2+},

copper-Cu^{2+}, manganese-Mn^{2+} and zinc-Zn^{2+}) and control during storage for 4, 11 and 18 days at room temperature.

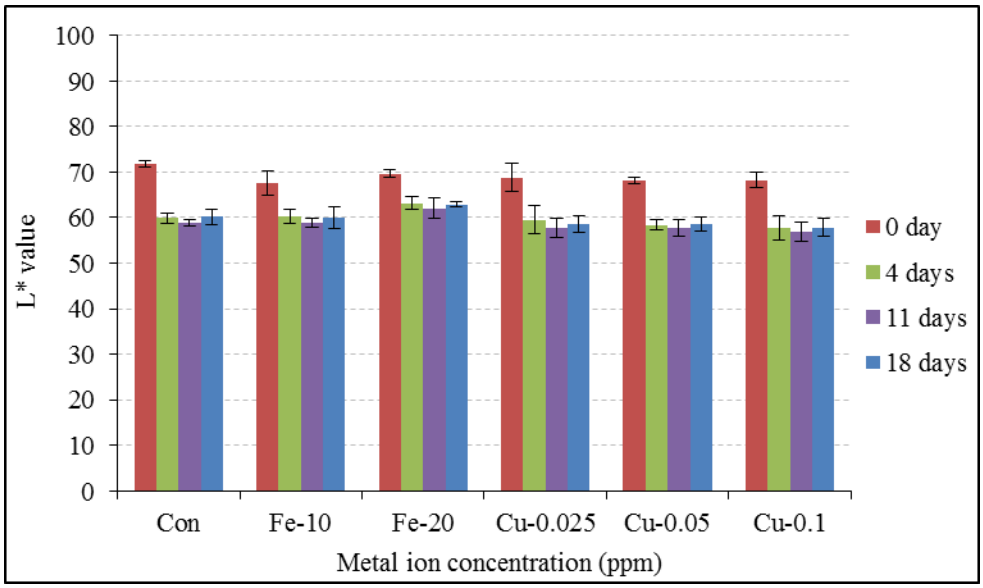


Figure 2a: Changes in L* values of fish homogenate mixed with different iron (Fe^{++}) and copper (Cu^{++}) concentrations during storage.

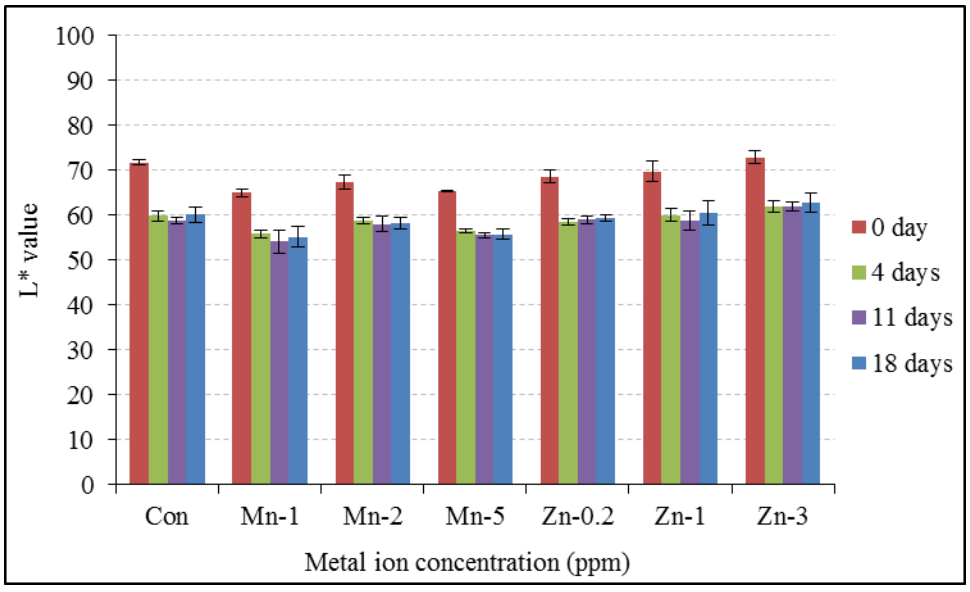


Figure 2b: Changes in L* values of fish homogenate mixed with different manganese (Mn^{++}) and zinc (Zn^{++}) concentrations during storage.

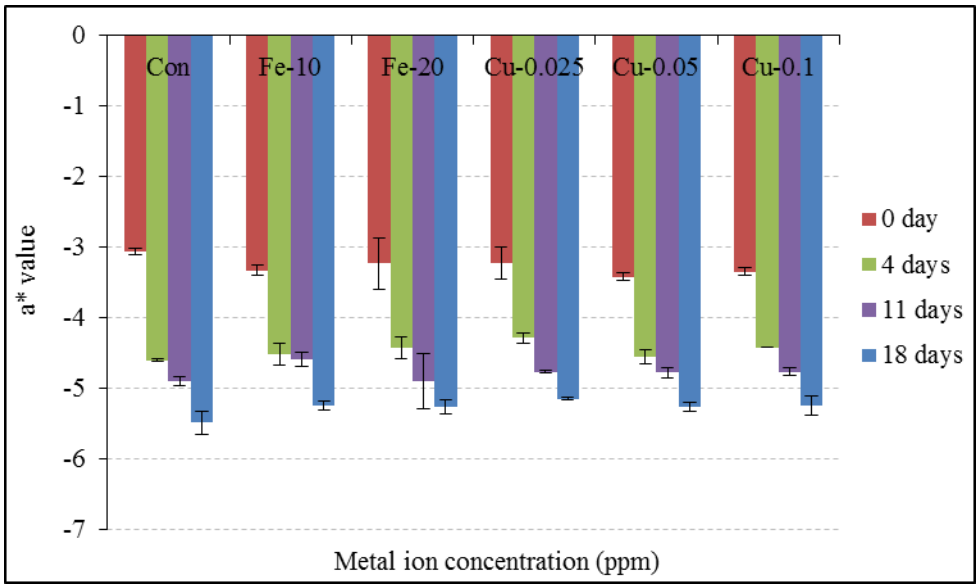


Figure 3a: Changes in a* values of fish homogenate mixed with different iron (Fe^{++}) and copper (Cu^{++}) concentrations during storage.

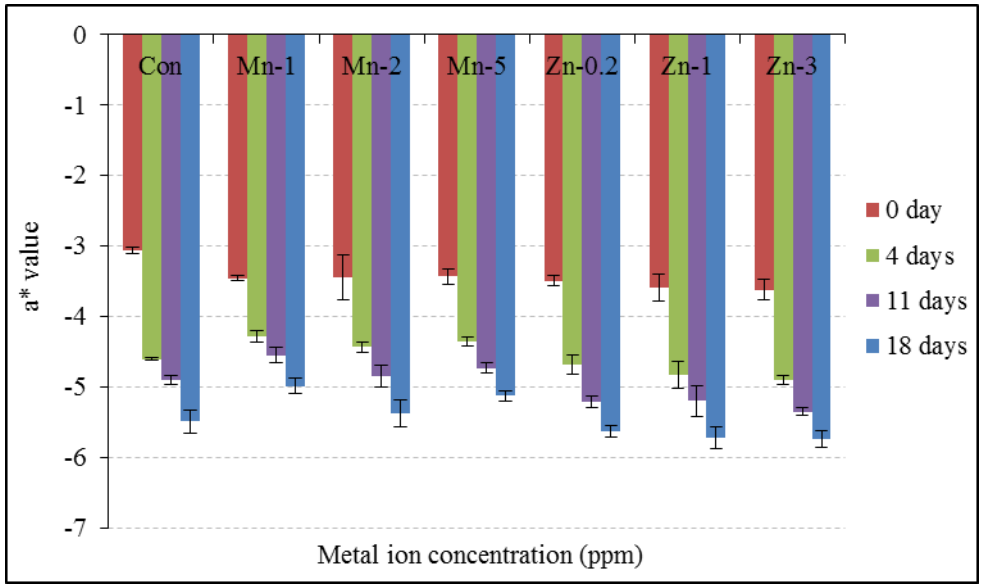


Figure 3b: Changes in a* values of fish homogenate mixed with different manganese (Mn^{++}) and zinc (Zn^{++}) concentrations during storage.

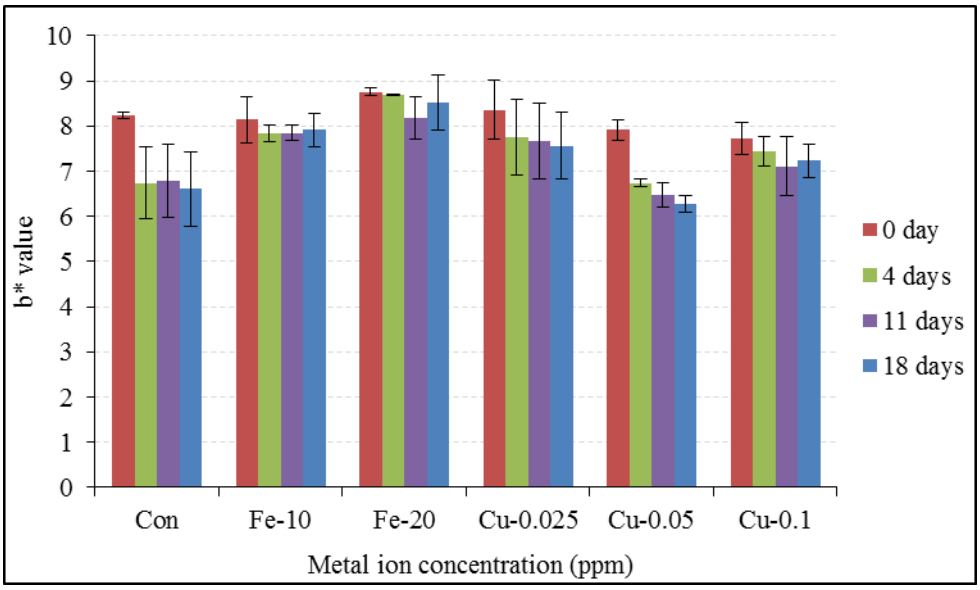


Figure 4a: Changes in b* values of fish homogenate mixed with different iron (Fe^{++}) and copper (Cu^{++}) concentrations during storage.

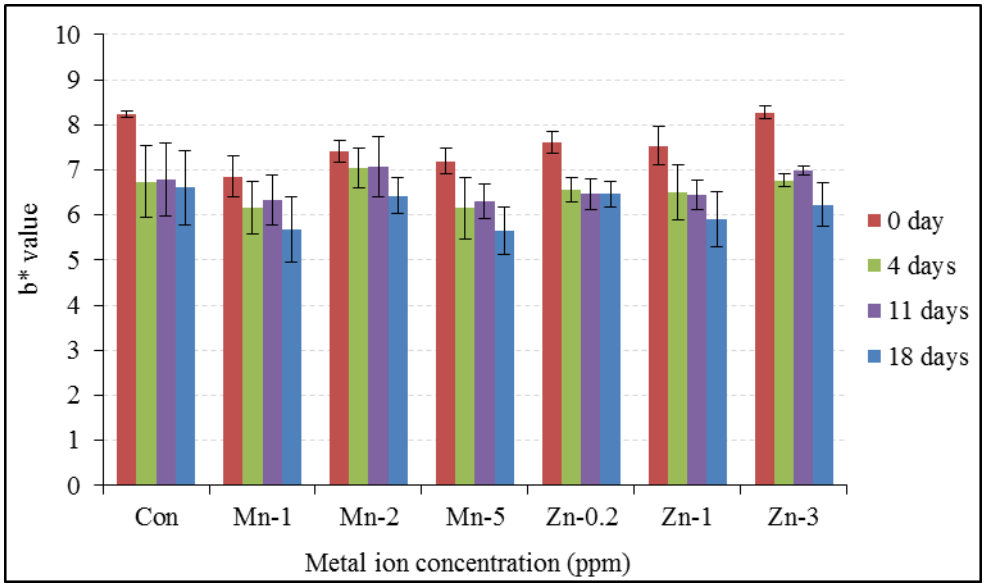


Figure 4b: Changes in b* values of fish homogenate mixed with different manganese (Mn^{++}) and zinc (Zn^{++}) concentrations during storage.

Figure 3a and 3b show that there are changes in a* value for fish homogenate for different metals but the same changes are seen for the control sample. The changes are therefore due to storage, but not to metal induced oxidation.

4.2 Effect of different metal concentrations on cod liver oil

4.2.1 Effect of different copper concentrations

Cod liver oil was used as a surrogate material for investigating effects of metal on lipid oxidation. Copper was mixed in different concentrations and stored at room temperature for two weeks and colour change was measured during the experiment. No visual changes (L* value, Figure 5) was seen in the colour of cod liver oil with different copper concentrations. Limited changes were in the a* and b* values (Figure 6 and 7).

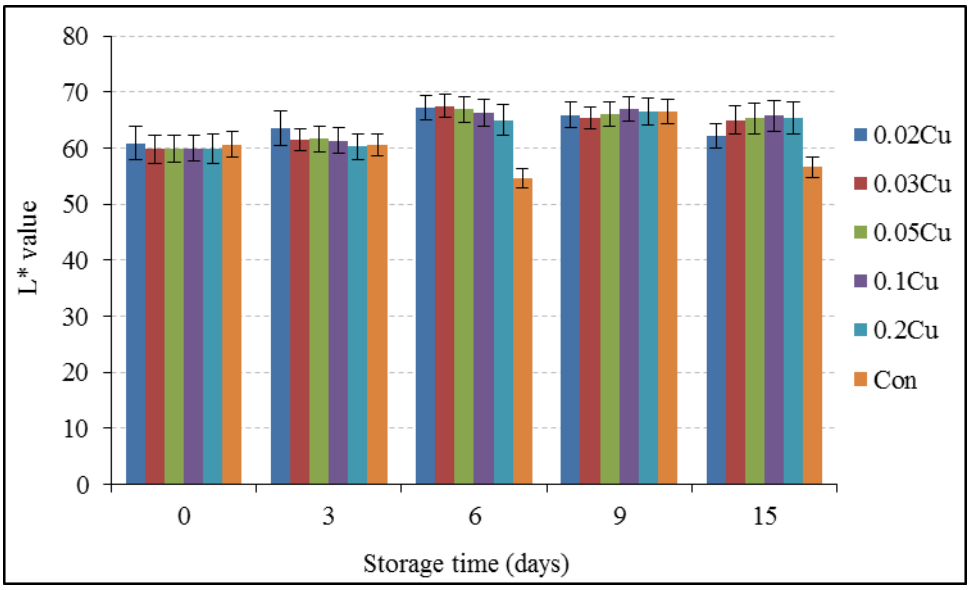


Figure 5: L* value for cod liver oil mixed with different concentrations of copper (Cu^{2+}).

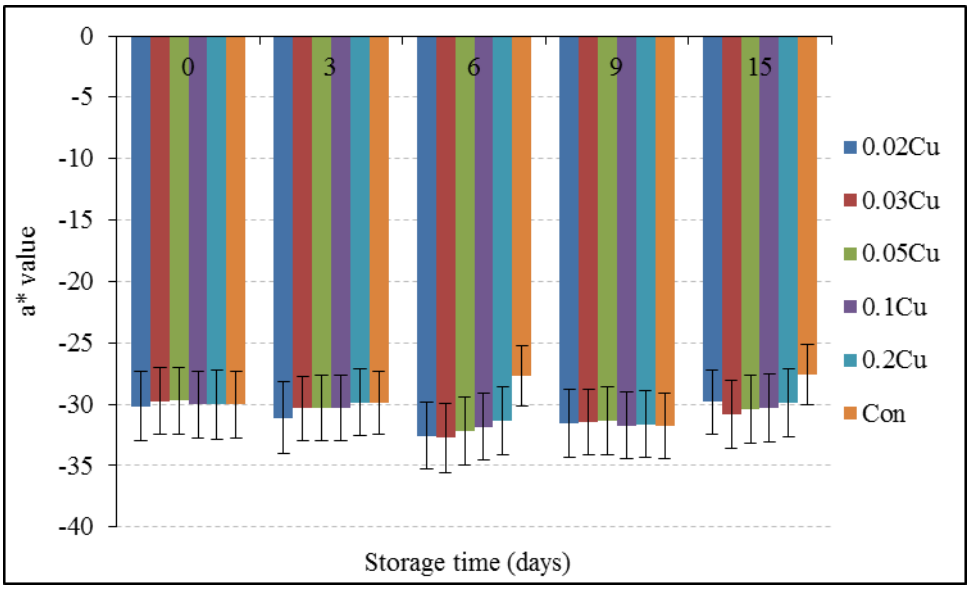


Figure 6: a* value for cod liver oil mixed with different concentration of copper (Cu^{2+}).

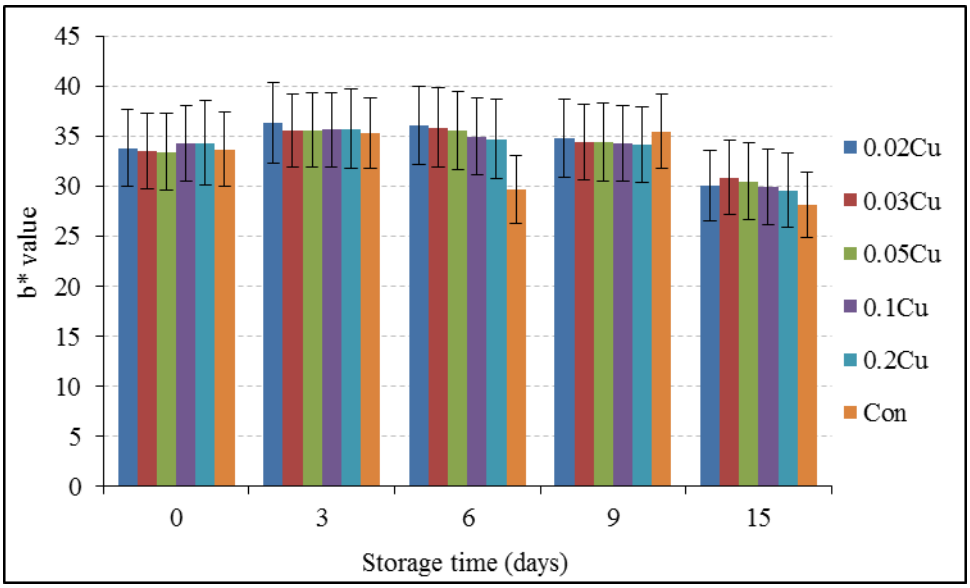
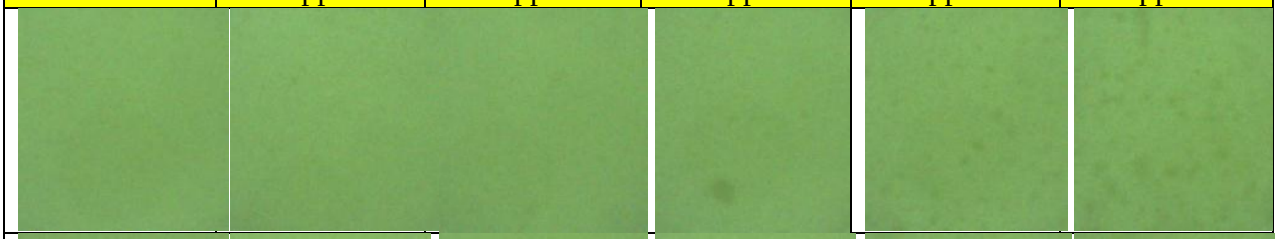
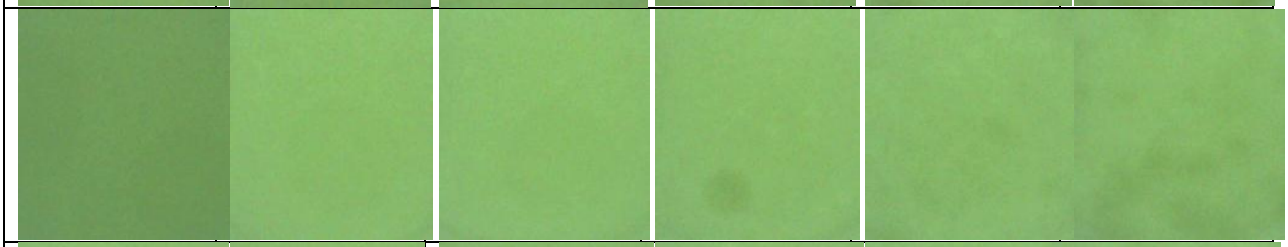
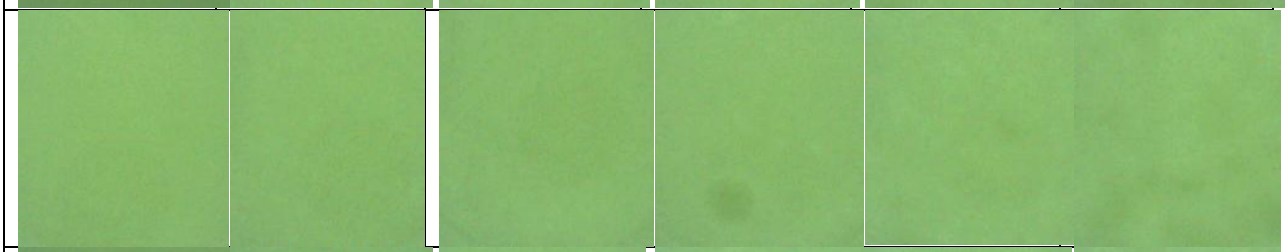
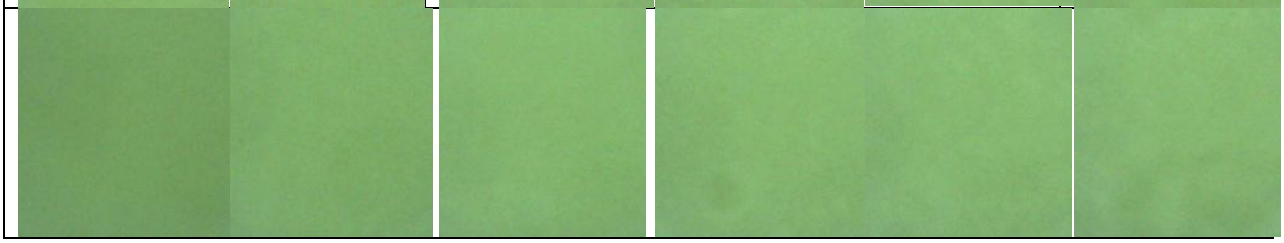


Figure 7: b* value for cod liver oil mixed with different concentration of copper (Cu^{2+}).

Visual changes of the cod liver oil with different copper concentrations and different storage time are shown in Table 3.

Table 3: Pictures of cod liver oil with different concentrations of copper (Cu^{2+}) during storage.

	Control	0.02 ppm Cu	0.03 ppm Cu	0.05 ppm Cu	0.1 ppm Cu	0.2 ppm Cu
0 day						
3 days						
6 days						
9 days						
15 days						

4.2.2 Effect of different iron concentrations

Prooxidant effects of iron were tested using cod liver oil as surrogate matrix. Colour changes were seen after 3 days, where the sample darkened and became browner. As seen in 8, the colour change increased with time and also with concentration of iron. Limited change was seen for the two weakest concentrations, 1 and 5 ppm iron. Figure 9 shows changes in a* value in the cod liver oil samples where changes minimal for the two weakest concentrations, 1 and 5 ppm iron. When the iron concentration is increased, the colour changes increased as well. Limited changes were detected in the b* value (Figure 10).

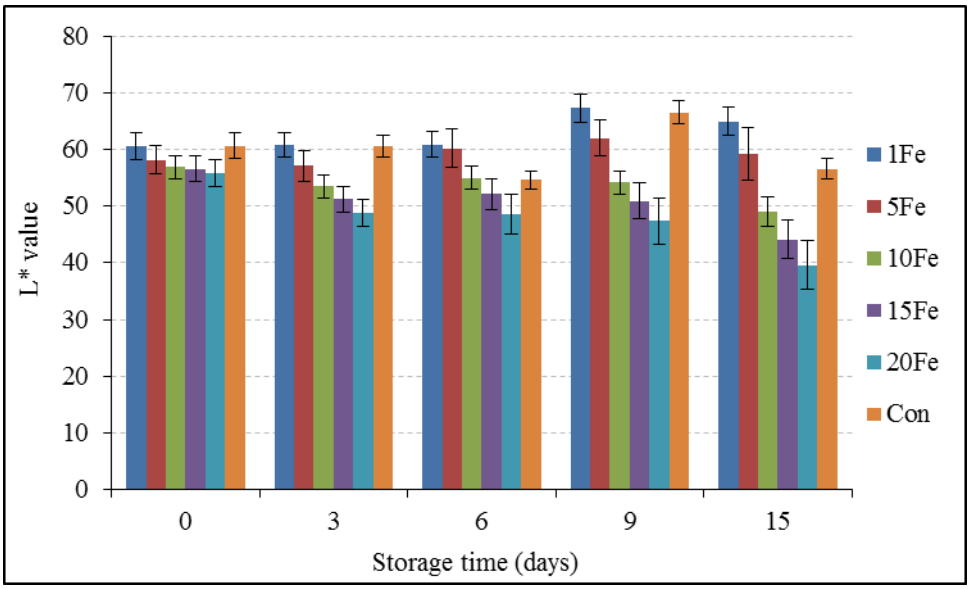


Figure 8: L* value for cod liver oil mixed with different concentrations of iron (Fe^{2+}).

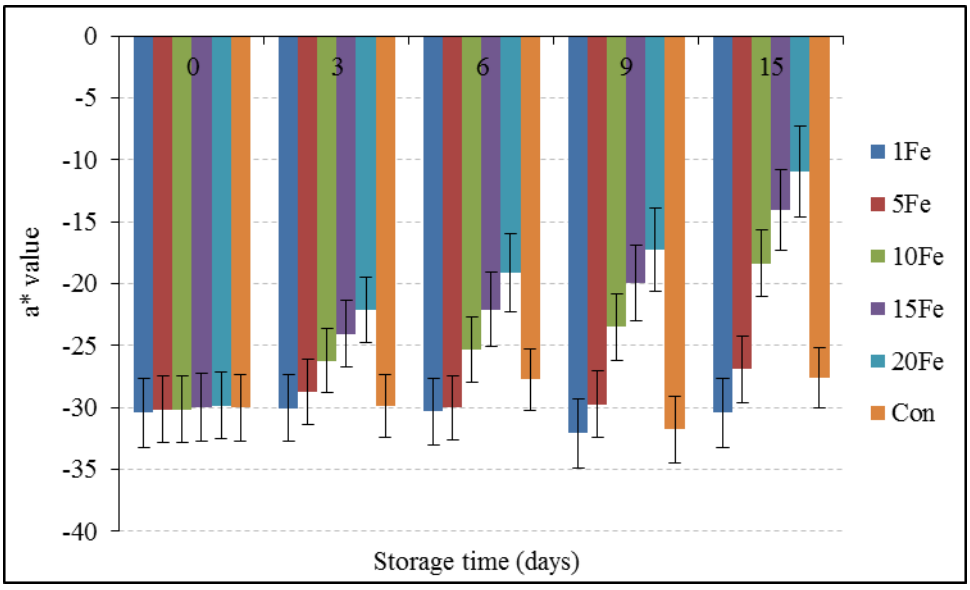


Figure 9: a* value for cod liver oil mixed with different concentration of iron (Fe^{2+}).

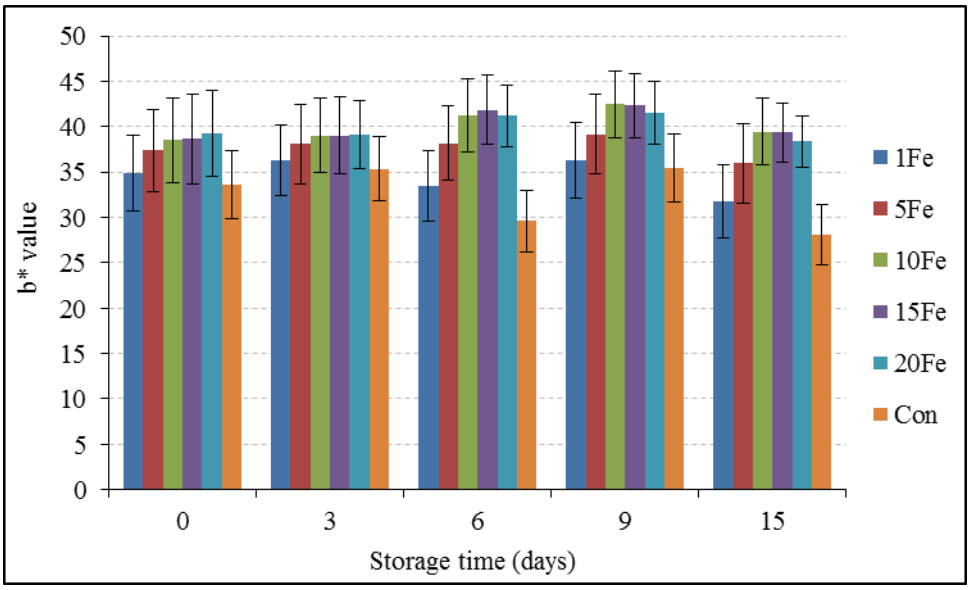


Figure 10: b* value for cod liver oil mixed with different concentration of iron (Fe^{2+}).

Table 4 and 5 show visual changes in cod liver oil with increased concentration of Fe^{2+} and Fe^{3+}, respectively, during storage time. It seems that solid particles are formed in the mixture as well as the mixture darkens.

Table 4: Pictures of cod liver oil with different concentrations of Fe^{2+}.

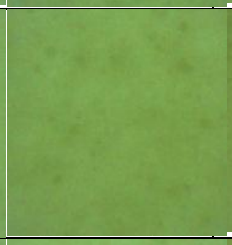
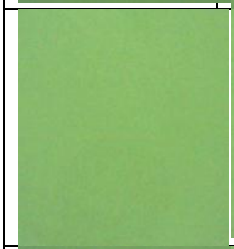
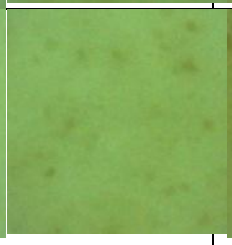
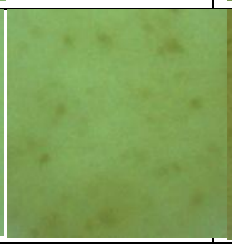
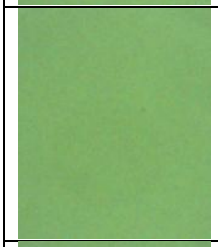
	Control	1 ppm Fe	5 ppm Fe	10 ppm Fe	15 ppm Fe	20 ppm Fe
0 day						
3 days						
6 days						
9 days						
15 days						

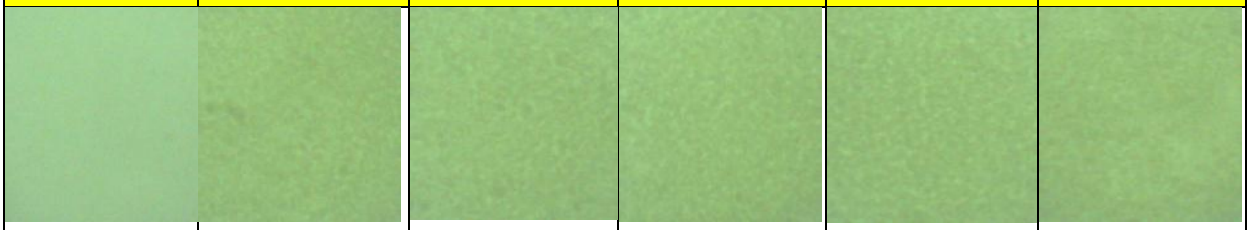
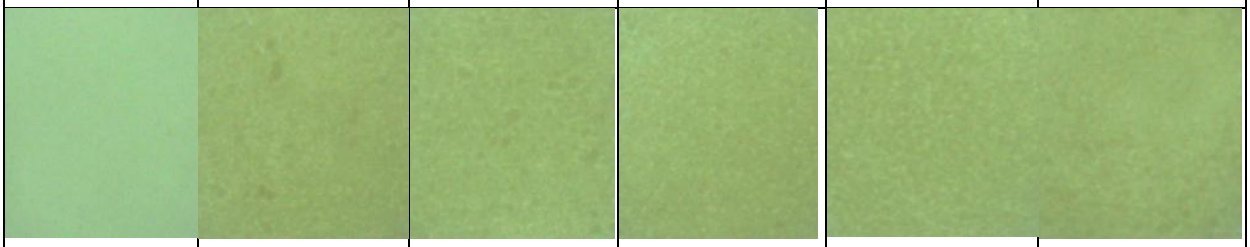
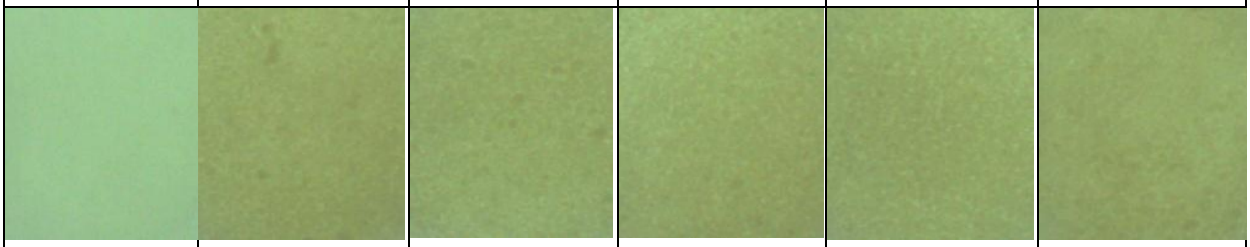
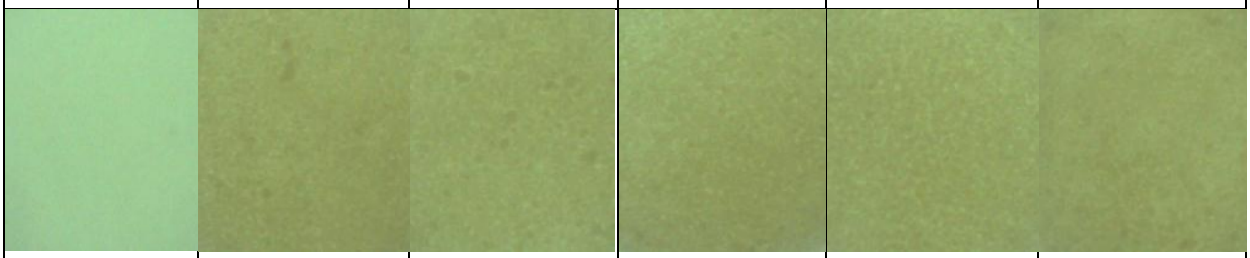
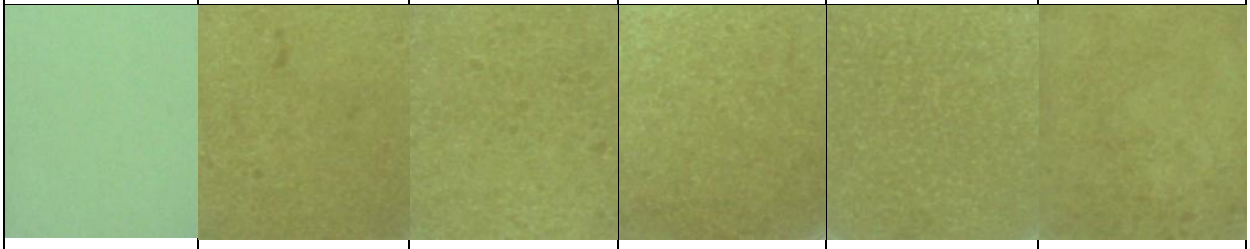
Table 5: Pictures of cod liver oil with different concentrations of Fe^{3+}.

	Control	1 ppm Fe	5 ppm Fe	10 ppm Fe	15 ppm Fe
0 day					
2 days					
5 days					
6 days					

4.3 Effect of mixture of copper and iron

Combined oxidizing effects of iron and copper were investigated. Iron was added in the same concentration (20 ppm) where the concentration of copper ranged between 0.02 and 0.1 ppm. The results are shown in Table 6.

Table 6: Pictures of cod liver oil with different concentrations of iron (Fe^{2+}) and copper (Cu^{2+}) (ppm).

	Control	0.02 Cu + 20 Fe	0.03Cu + 20 Fe	0.05 Cu + 20 Fe	0.1 Cu + 20 Fe	20 Fe
0 day						
1 day						
2 days						
3 days						
4 days						
7 days						

All samples show visual changes in colour, the cod liver oil darkens. Still, there seems to be no correlation between dark colour and increased copper concentration. The same colour change was seen with only iron and no copper. The colour change is therefore probably due to iron being present in the mixture instead of copper. A study by Thanonkaew *et al.* (2006) where oxidating effects of Fe and Cu on cuttlefish (*Sepia pharaonis*) are investigated, shows that Fe induces lipid oxidation but Cu protein oxidation. The protein oxidation reported by Thanonkaew et al. (2006) is likely oxidation of phospholipids in proteins. The content of phospholipids in cod liver oil is probably not high enough for the test setup in the present study to show any copper induced oxidation.

4.4 Effects of manganese and zinc

The effects of manganese (Mn^{2+}) and zinc (Zn^{2+}) were also investigated, using cod liver oil as surrogate material. No colour change related to oxidizing effects was seen for either manganese or zinc. The changes in colour of cod liver oil during storage as influenced by different concentrations of manganese and zinc are depicted in Table 7 and 8, respectively.

Table 7. Picture of cod liver oil with different concentrations of manganese (Mn^{2+}).

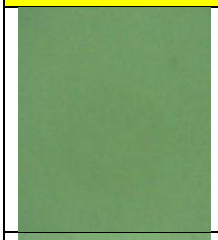
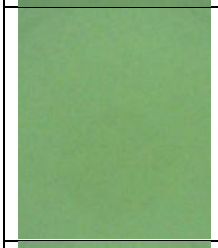
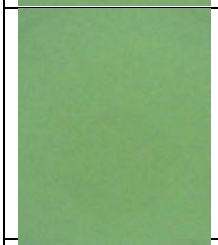
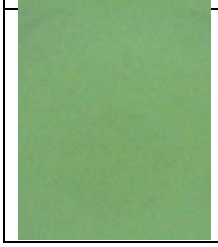
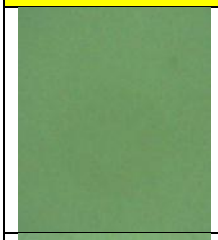
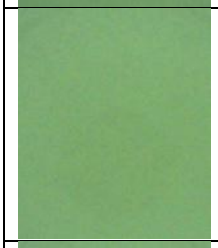
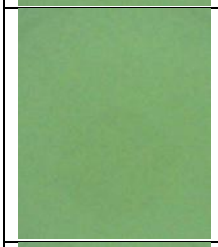
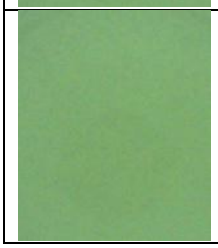
	Control	1 ppm Mn	3 ppm Mn	5 ppm Mn	7 ppm Mn
0 day					
2 days					
3 days					
13 days					

Table 8. Picture of cod liver oil with different concentrations of zinc (Zn^{2+}).

	Control	0.2 ppm Zn	1 ppm Zn	3 ppm Zn	5 ppm Zn
0 day					
2 days					
3 days					
13 days					

5. Conclusions

Of the four inorganic trace elements tested, iron (including Fe^{2+} and Fe^{3+}) showed to increase lipid oxidation, visualised as colour change in cod liver oil. Further, the lipid oxidation seems to be linearly correlated to increased iron concentration. Copper (Cu^{2+}), manganese (Mn^{2+}) and zinc (Zn^{2+}) did not show the same lipid oxidizing effects.

Using cod liver oil as surrogate test material for cod fillets showed promising results. This material is easy to work with and could be developed further into a test kit for salted cod producers and salt importers. Some modifications are necessary, e.g. to include protein fraction. Next step would be to extrapolate the results from the cod liver oil to cod fillets.

The study indicates that iron causes oxidative effects in cod liver oil and effects were detected in a concentration of 5 ppm Fe in salt on cod liver oil. These results have to be extrapolated to fillets before any recommendations can be given.

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