

# Report

## Sensory shelf life for protein products made from salmon backbones.

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# Report

## Sensory shelf life for protein products made from salmon backbones.

Effect of added antioxidants on the shelf life of taste neutral proteins

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**ABSTRACT**

The main goal of this project was to determine the sensory shelflife of proteins with neutral taste. Proteins were isolated from both the solid phase (sludge) from salmon oil production and from cooked salmon backbones. From both raw materials proteins were isolated after bone removal and subsequent removal of unwanted taste components by washing with warm water or warm water and ethanol. Effect of adding different antioxidants on the lipid oxidation in the protein products were studied. The best antioxidant were used in shelf life experiments where the lipid oxidation and sensory shelf-life of the products were studied.

The best antioxidants for the isolated protein products were BHT, propyl gallate and alfa-tocopherols. Methal chelators like EDTA and citrate had no effect. A commercial mixture of tocopherols where used for the shelf life study.

During the accelerated shelf life test (50 °C and 670 hours) no lipid oxidation was detected, neither by Schaal test nor by sensory analysis. This holds for all teted proteins powders with and without antioxidants. The shelf life dependence on temperature was estimated by comparison of shelf at both 50 °C and 70 °C through the use of the Arrhenius equation. This estimation indicates a shelf life of approximately 2 years at 20 °C for the isolated salmon proteins.

Two Norwegian companies have expressed interest in the results and wants to participate in developing this technology further towards industrial production.

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## 1 Aim of the project and background

The project "Utvikling av lukt-og smaksnøytrale marine proteiner" (SINTEF report nr A23364 September 2012) showed two possible sources and techniques for isolation of tasteless proteins from salmon backbone: a): the solid phase from salmon oil production based on salmon backbones and b): salmon backbones gently cooked before bone separation. The process where the salmon backbones were cooked before separation (the continuous cooker process) gave the highest product yield. Sensory analysis showed that freshly produced fish proteins from the salmon backbones by both techniques had taste intensity in the same range as soya isolates. A preliminary economic analysis shows that both processes have economical potential to succeed.

The stability and shelf-life of the product is a key factor for determining the potential market size and segment for the tasteless proteins from salmon backbone. Therefore the main goal of this project was to determine the sensory shelf life of the proteins. The specification set by the market is a shelf life of 2 years at room temperature.

Tasteless proteins isolated from salmon backbones by both techniques contained marine lipids. Shelf-life of product containing marine lipids is usually determined by the rate of lipid oxidation. This oxidation leads to the rancid taste development and is dependent on the pro-oxidants and antioxidants in the system. Therefore, the addition of the right antioxidants should help to prolong the shelf-life of the product.

This project had the following aims:

1. To screen and select possible antioxidant for the increase of the shelf-life of the product.
2. To perform an accelerated chemical shelf-life study in order to verify addition of antioxidant effect on the shelf-life of the protein product.
3. To perform accelerated shelf-life test to determine the sensory shelf-life of the product
4. Estimate activation energy for the shelf-life determination in order to be able to calculate the shelf-life changes at different temperatures

The project has been financed by The Norwegian Seafood Research Fund (FHF). The steering committee consisted of: Robert Wahren, Core Competance, Tore Remman, Nutrimar. Stein Ove Østvik acted as an observer from FHF.

## 2 Taste reduction in sea food products.

Production of protein products from fish can be divided into several product categories. In the fish meal category, the whole fish with exception of most of the lipids are dried. The resulting product has similar composition of water soluble compounds as the composition of the fish. In the hydrolysate category, the product also has a composition similar to fish, but some of the proteins from the fish are hydrolysed to free amino acids and oligomers of amino acids. These products contain less lipid (1 %) than the fish meal products (5-10 %), but still they contain all water soluble compounds found in the fish. A third product category is the surimi, where the insoluble muscle proteins (myofibrillar proteins) are separated and extensively washed with cold water and frozen after addition stabilizers. These products contain low fat and only the large proteins insoluble in water. Because of the gentle isolation procedure, these proteins have retained their functional properties, like the gelling property.

Fish is more perishable than most other food raw material. This is due to the chemical composition and the physical structure of seafood. Due to the lack of influence of gravity on fish in their natural habitat, their outer skin and the internal structures are weaker compared to land animals. These conditions reduce the ability to withstand the physical stress that is imposed upon fish during processing.

The weak structure of fish will make the way open for several bacterial infections during processing. These bacteria, when finding good growth conditions, reduce shelf life and give rise to off-flavour from the fish. The chemical composition of fish, also different from land animal, gives special growth conditions for bacteria. In cod-fish and pelagic species high amounts of trimethylamineoxide promotes bacterial growth. Fish has a low level of carbohydrates and a high level of proteins and amino acids. These nitrogen and sulphur containing amino acids are easily degraded by the bacteria due to the lack of carbohydrates. The amino acid degradation leads to production of off flavour, which is not found to the same degree in bacterial degradation of carbohydrates.

If the proliferation of bacteria is hampered in seafood, other off flavours could be produced from the oxidation of lipids. The off-flavours produced are dependent upon the chemical structure of the lipids in the fish. Because of the high number of unsaturated chemical structures in the marine lipids compared to lipids from land animal, these marine lipids will oxidize faster and also produce oxidation compounds that have a lower threshold level. The results are development of rancid off-flavour at a speed and intensity higher than what is experienced from land animal. To combat development of off-flavour from marine fish, both bacterial growth and lipid oxidation has to be prevented.

In fish meal the origin of flavour is both related to the conditions of the raw material and process conditions. To be able to produce fish meal with neutral flavour, the fish has to be fresh and especially the drying process has to be gentle. As the fishmeal is a dried product, bacterial growth is generally not a problem in the dry product. Fish meal contains viscera, and the flavour of the viscera makes the removal of flavour complicated.

The development of flavour upon storage of fish meal is caused by oxidation of the remaining fish oil and phospholipids in the meal. This can to some extent be prevented by use of strong synthetic antioxidants like etoxyquine, but synthetic anti-oxidants are not generally allowed in food for human consumption. Other ways of preventing lipid oxidation is the addition of ascorbate and vegetable oil, preventing iron associated oxidation (Doisaki et al. 2007). Other ways of deodorizing fish meal is by extraction with ethanol and methanol (Meade and Miller 1971) and hexane (Oterhals, Å, 2012).

The production of surimi is based on extensive washing of minced fish muscle with cold water and was developed for Alaskan Pollock. This washing removes taste compounds and fat. (Ohsima et al., 1993). Due to the solubility of sarcoplasmic proteins, these proteins are washed out together with other water soluble compounds in the fish muscle. This loss of dry matter and protein results in a process with low yield. Surimi

production has also been tried from Norwegian fish species (Langmyhr, E. and Sørensen, N. K., 1987) and (Langmyhr, E., et al., 1990).

The surimi product has neutral taste and is used for production of fish and crab substitutes like crab sticks. The production of fish protein hydrolysates leads to formation of bitter peptides. Selection of the hydrolytic enzyme and process conditions has been shown to influence the produced bitter taste (Slizyte, R., et al. 2011). Production based on fish viscera give additional bitter taste due to bitter compounds from the gall salts (Dauksas, E., et al., 2004). Several means have been tried to reduce the bitter taste in hydrolysates, both by selective absorption of taste compounds by ion exchange materials (Dauksas, E. et al. 2004), beta-cyclodextrin and active carbon (Hu, W. et al. 2007), but these methods result in a product loss of 10 -20 %. Also microorganisms has been tried, both lactic acid bacteria and yeast (Jin, J. and Zhou, J., 2007). In Chinese publications a mixed process with both chemical adsorption and microbial degradation is recommended.

### 3 Materials and methods

#### 3.1 Materials

Antioxidants: TOCOVET® M-70 Oil (mixed tocopherols 70% in oil) - Fenchem Biotek Ltd, alfa tocopherol (>97%) – Fluka, rosmarin extract – (multiple component mixture) Fenchem Biotek Ltd; Butylated hydroxytoluen (BHT) (>99%) – Sigma Aldrich; propyl gallate (>98%) – Fluka; Ethylenediaminetetraacetic acid (EDTA, >99.5%) – Fluka, citrate (>99.8) – Merck.

Salmon backbones from the filleting line in (SalMar ASA, Kverva-Norway) were used in the tests.

#### 3.2 Methods

Isolation of proteins was performed according to the SINTEF report nr A23364 September 2012. Two sources/techniques were used: the *solid phase from salmon oil production based on salmon back bones* and *salmon backbones* gently cooked before bone separation. The de-fatting process using hot water and ethanol was performed as described in the SINTEF report nr A23364 September 2012. Therefore, four different proteins were isolated:

**CSW**- proteins from cooked salmon backbones before bone separation and using hot-water defatting process

**CSWE**- proteins from cooked salmon backbones before bone separation and using hot-water followed and ethanol defatting processes

**SPW** - proteins from solid phase from salmon oil production and using hot-water defatting process

**SPWE** - proteins from solid phase from salmon oil production and using hot-water followed and ethanol defatting processes.

Chemical composition of the isolated proteins is given in Table 1.

Table 1. Chemical composition of the proteins.

<i>Proteins</i>	composition, g/100g dry powder			
	protein	lipid	ash	water
SPW - proteins from solid phase from salmon oil production and using hot-water defatting process	68,2	16,9	16	2,1
SPWE - proteins from solid phase from salmon oil production and using hot-water followed and ethanol defatting processes.	75,3	6,3	15,4	6,7
CSW- proteins from cooked salmon backbones before bone separation and using hot-water defatting process	82,3	18,1	1,8	7,8
CSWE- proteins from cooked salmon backbones before bone separation and using hot-water followed and ethanol defatting processes	90,1	8,0	1	6,7

Isolated wet proteins were divided into two parts. One part was added a selected antioxidant (dissolved in ethanol) and both parts were freeze-dried. Therefore total 8 proteins: CSW, CSWE, SPW, SPWE and the same proteins bearing exogenous antioxidant: CSWA, CSWEA, SPWA, SPWEA were produced for the shelf-life test.

### 3.3 Chemical analyses

The moisture in the samples (raw material and the solid fractions) was determined gravimetrically by drying at 104°C for 24 h. Ash content was estimated by charring in a crucible at 550°C until the ash had a white appearance (AOAC, 1990). The measurements were performed in triplicate.

The analyses of total lipid content in raw material and sediments were performed according to the method of Bligh and Dyer (Bligh and Dyer, 1959). The total fat content was determined and expressed as gram lipid pr. gram sample material. The measurements were performed in duplicate.

Total nitrogen (N), determined by CHN-S/N elemental analyser 1106 (Costech Instruments ECS 4010 CHNSO Analyser) and crude protein was estimated by multiplying total N by a factor of 6.25. The measurements were performed in four parallels.

### 3.4 Screening of antioxidants

The activity of antioxidants was measured as the effectiveness to reduce dissolved oxygen (oxidation substrate) uptake in 10% of CSW and CSWE protein solutions in water. The uptake of dissolved oxygen by the 10% protein solution in a closed, stirred, water jacketed cell was used as a measure of lipid oxidation. The reduction of dissolved oxygen was measured continuously by a polarographic oxygen electrode (Hansatech Instrument Ltd., Norfolk, UK). After measuring oxygen uptake for 5-10 min 20µL of antioxidant solution was injected. Principle oxidation figure is given in the Figure 1. The anti-oxidative activity was expressed as inhibition of oxygen uptake rate (OUR) in %. Oxidation experiment was performed at 30°C.



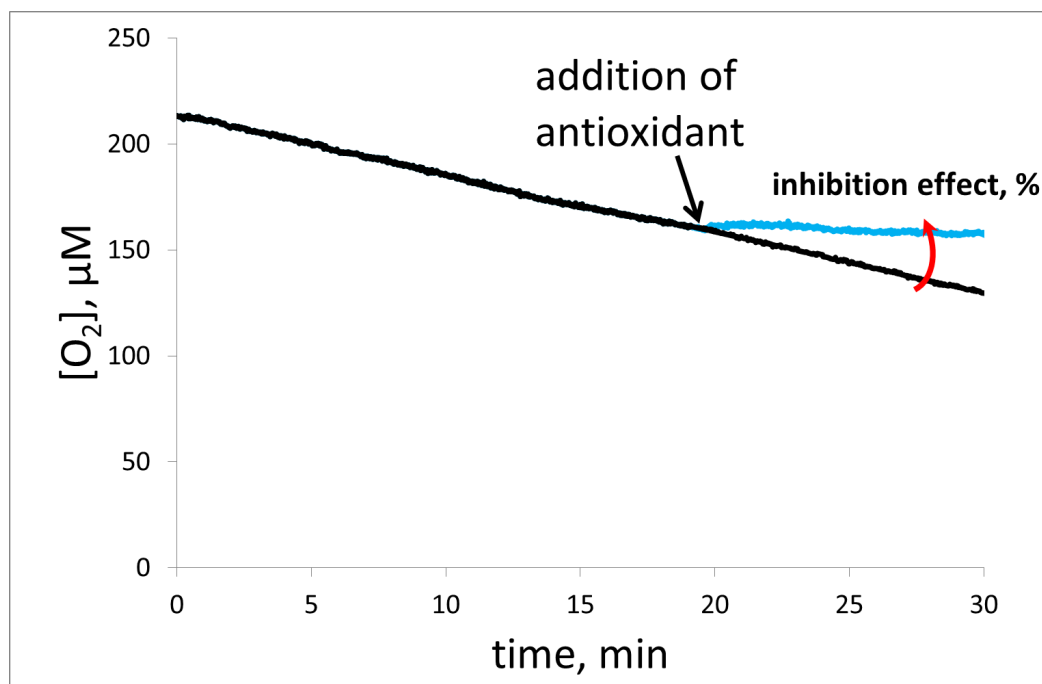


Figure 1. Principle scheme of lipid oxidation in the 10% protein solution when oxidation is followed by measuring dissolved oxygen concentration changes.

### 3.5 Accelerated storage tests - Schaals test

The principle of Schaals test is that when the fat oxidises it binds oxygen and the weight increases. Protein powders (10g) were placed in a glass Petri dish (d=7cm). The dish with protein powder was placed in a cabinet with the appropriate temperature with free access to air. The weight of the powders was checked after two hours in order to get the dried powder weight – zero weight. Later the weighing was performed once or twice during the day. (Pokorný, J. et al. 1995).

### 3.6 Sensory analysis

The aim of the analysis was to evaluate the changes of the taste intensity of the produced protein powders during accelerated storage test compared with reference powders. The two reference samples were used. One reference sample (ref A) was assigned the taste intensity 1 and the second (ref B) was with the taste intensity 5.

- Reference A– 1 % protein solution made from protein powder after ethanol wash from the solid phase from salmon oil production. This reference sample taste intensity was evaluated as "1". This reference represents the best powders that have been produced.
- Reference B – 2% protein solution. Protein powders with neutral taste from cooked salmon backbones were oxidised by incubation at 70°C till they bound 30 mg oxygen per 5 g protein powder. This reference sample taste intensity was evaluated as "5". The taste of reference B was slight rancid.

Aliquots of the reference solutions were kept at minus 80° C and thawed immediate before use.

Semi-trained sensory evaluators (six to eight persons, both sexes represented, age around 35 years) were asked to evaluate the taste intensity of six coded protein solutions (1% concentration) according to the reference samples using the scale from 1 (Reference A) to 5 (Reference B).

## 4 Results

### 4.1 Screening of antioxidants

The activity of tested antioxidants to inhibit oxidation in 10% protein solution is given in Figure 2. The antioxidants that work as metal chelators (EDTA and citrate) had no effect on the dissolved oxygen uptake by the taste-reduced proteins. Therefore, iron mediated oxidation is not active in the proteins powders. However, anti-oxidants that work as radical scavengers (butylated hydroxytoluene, propyl gallate, tocopherols, rosmarine extract) showed inhibition of dissolved oxygen uptake, and indicate reduced oxidation. Because tocopherol is a natural antioxidant and showed good activity in preventing oxidation of the lipids in the taste reduced proteins, this antioxidant was chosen for the shelf life experiments. Tocovet is a commercial antioxidant that contains a 70% mixture of tocopherols. Therefore, 1500 ppm of this antioxidant should show equal activity as 1000ppm of alfa-tocopherol. Tocovet (1500 ppm based on the dry weight basis) was added before freeze-drying to the isolated proteins CSW, CSWE, SPW and SPWE to be used in the shelf life experiment.

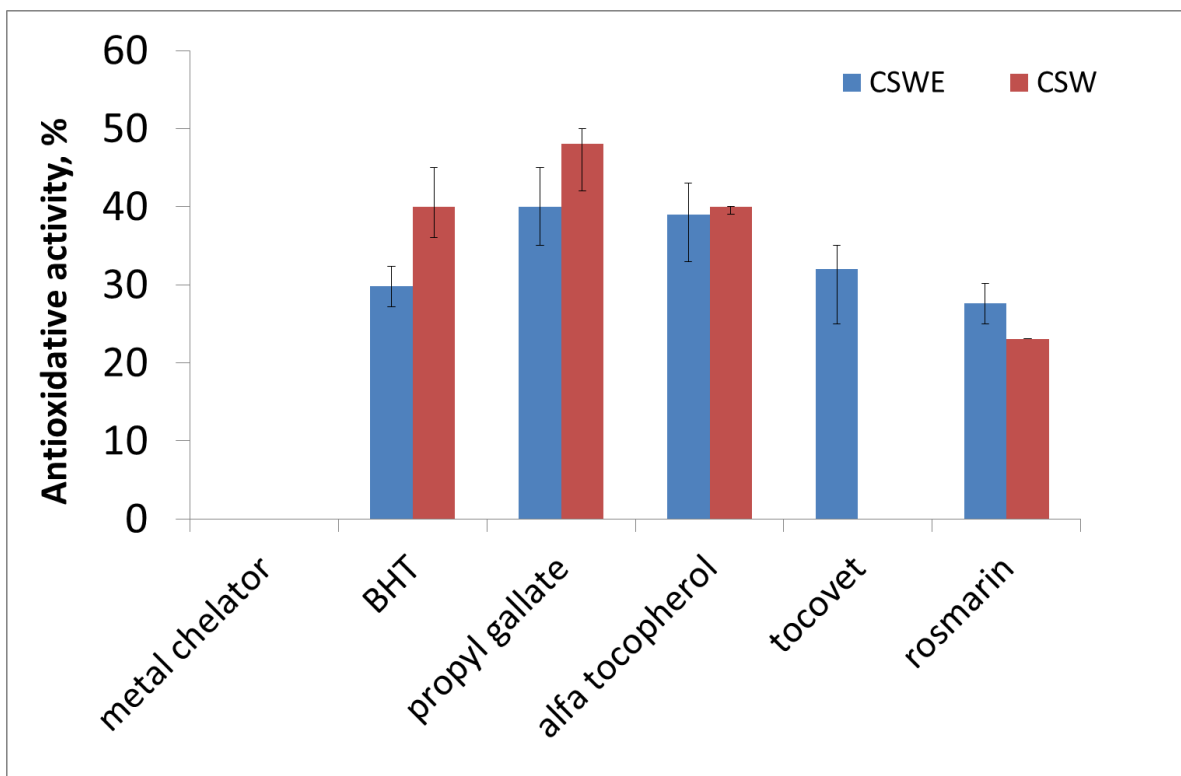


Figure 2: Activity of antioxidants to inhibit oxidation in 10% protein solution of taste reduced proteins. Proteins that were tested: CSWE – proteins from salmon backbones, gently cooked and defatted using hot-water followed by ethanol extraction. CSW - proteins from salmon backbones gently cooked and defatted by hot-water. Antioxidants tested: metal chelator EDTA 1000 ppm and citrate 1000 ppm, radical scavengers like BHT – butylated hydroxytoluene 100 ppm, propyl gallate – 100 ppm, tocopherol – 1000 ppm, Tocovet – commercial tocopherol mixture (70% tocopherol)– 1500ppm, rosmarin extract – 1000 ppm. Values are given as a middle value with the bars that represent highest and lowest values of the measurements

## 4.2 Shelf-life by accelerated storage tests - Schaals test

When the taste reduced proteins were stored at 70°C, the weight increase after approx storage hours was obtained (Figure 3).

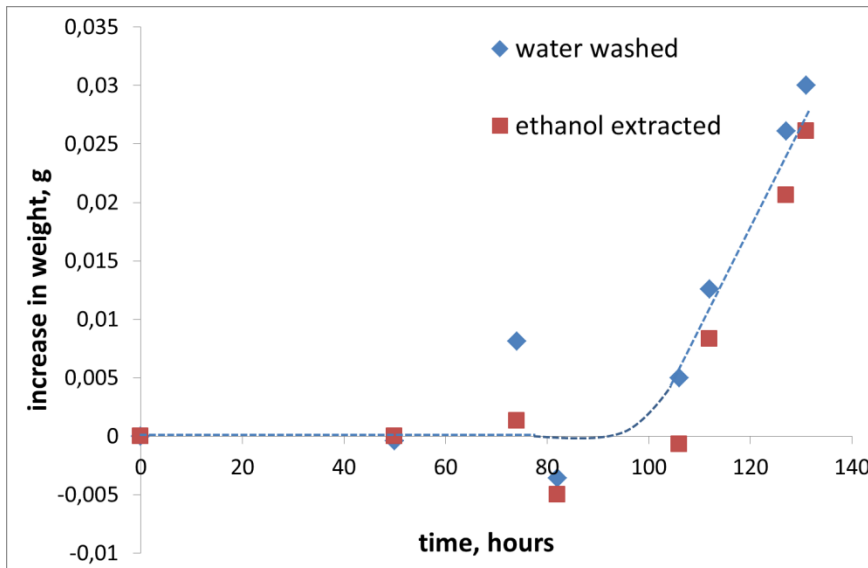


Figure 3. Weight increase for protein powders (5 g) stored at 70 °C with free access to air. Blue diamonds: only water washed, red squares water washed and ethanol extracted. (Mozuraityte, R. et al. 2012).

Freshly made CSW, CSWE, SPW, SPWE and the same proteins powders bearing 1500 ppm antioxidant tocovet: CSWA, CSWEA, SPWA, SPWEA were used for the shelf-life determination using storage temperature at 50°C. After an initial weight equilibration up to 200 h, the weight of most of the protein powders remained fairly stable. The expected increase in weight for these products to indicate lipid oxidation was close to 0,07g after reaching weight equilibrium. The the weight changes in the powders stored at 50°C did not show the expected weight increase, and no clear point of increase of weight were observed as seen in Figure 4A and Figure 4B.

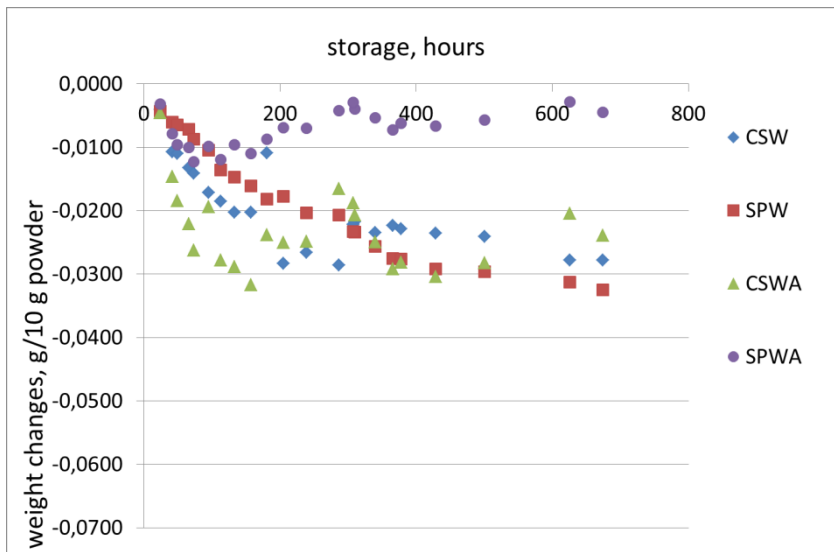


Figure 4A. The changes in weight of the protein powders (10 g) defatted by hot-water and stored at 50°C. CSW- proteins from cooked salmon backbones before bone separation and using hot-water defatting process  
 SPW - proteins from solid phase from salmon oil production and using hot-water defatting process  
 CSWA and SPWA are the same proteins as above added 1500 ppm Tocovet.

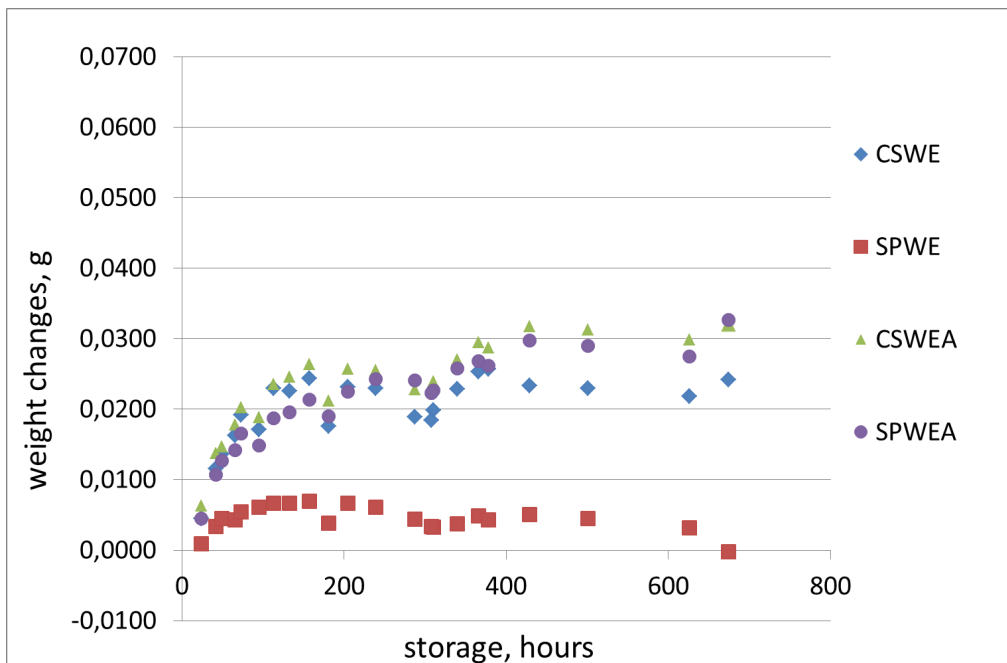


Figure 4B. The changes in weight of the proteins defatted by ethanol extraction and stored at 50°C. CSWE- proteins from cooked salmon backbones before bone separation and using hot-water followed and ethanol defatting processes  
 SPWE - proteins from solid phase from salmon oil production and using hot-water followed and ethanol defatting processes.  
 CSWEA, and SPWEA are the same proteins as above added 1500 ppm Tocovet.

### 4.3 Shelf-life by sensory test

Sensory shelf-life tests were performed on the same powders that were used in the Schaals test at 50°C. The taste intensity of the powders is given in Figure 5. During the 670 hours of storage 8 sensory tests were performed. Judges could not detect significant sensory changes during these 670 hours storage at 50°C. These results show that no lipid oxidation is detectable by sensory analysis during 670 h at 50°C, neither for the taste reduced proteins nor for taste reduced proteins added anti-oxidant (Tocovet, 1500 ppm). This is a remarkable result, pointing to a stable product that has a long shelf life at room temperature.

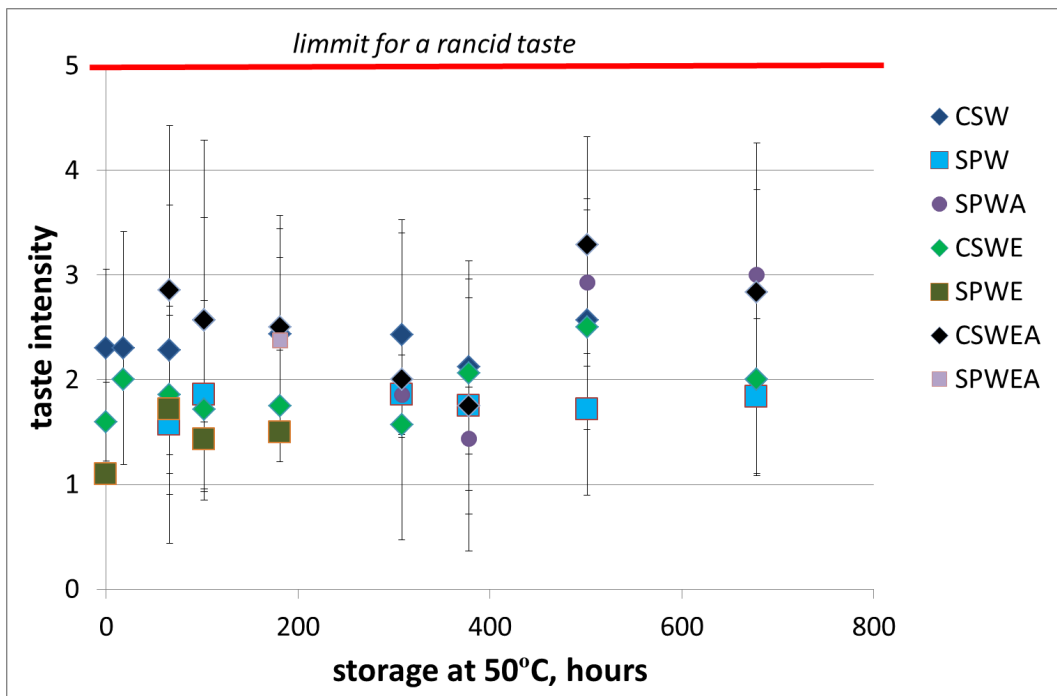


Figure 5. Sensory test of the protein powders stored at 50°C.

CSW- proteins from cooked salmon backbones before bone separation and using hot-water defatting process  
 CSWE- proteins from cooked salmon backbones before bone separation and using hot-water followed and ethanol defatting processes

SPW - proteins from solid phase from salmon oil production and using hot-water defatting process

SPWE - proteins from solid phase from salmon oil production and using hot-water followed and ethanol defatting processes.

CSWEA, SPWA and SPWEA are the same proteins as above added 1500 ppm Tocovet

However, taste panellists usually recognize fish proteins with added antioxidant (70% tocopherol mixture – Tocovet 70) bearing slightly higher intensity taste. The difference in taste intensity between protein with antioxidant (sample CSWEA) and the protein without added anti-oxidant (CSWE) was relatively stable (Figure 6), except for one sampling point at 370 h.

The results from the sensory analyses were in agreement with the Shaal-test where no weight increase due to lipid oxidation could be detected.

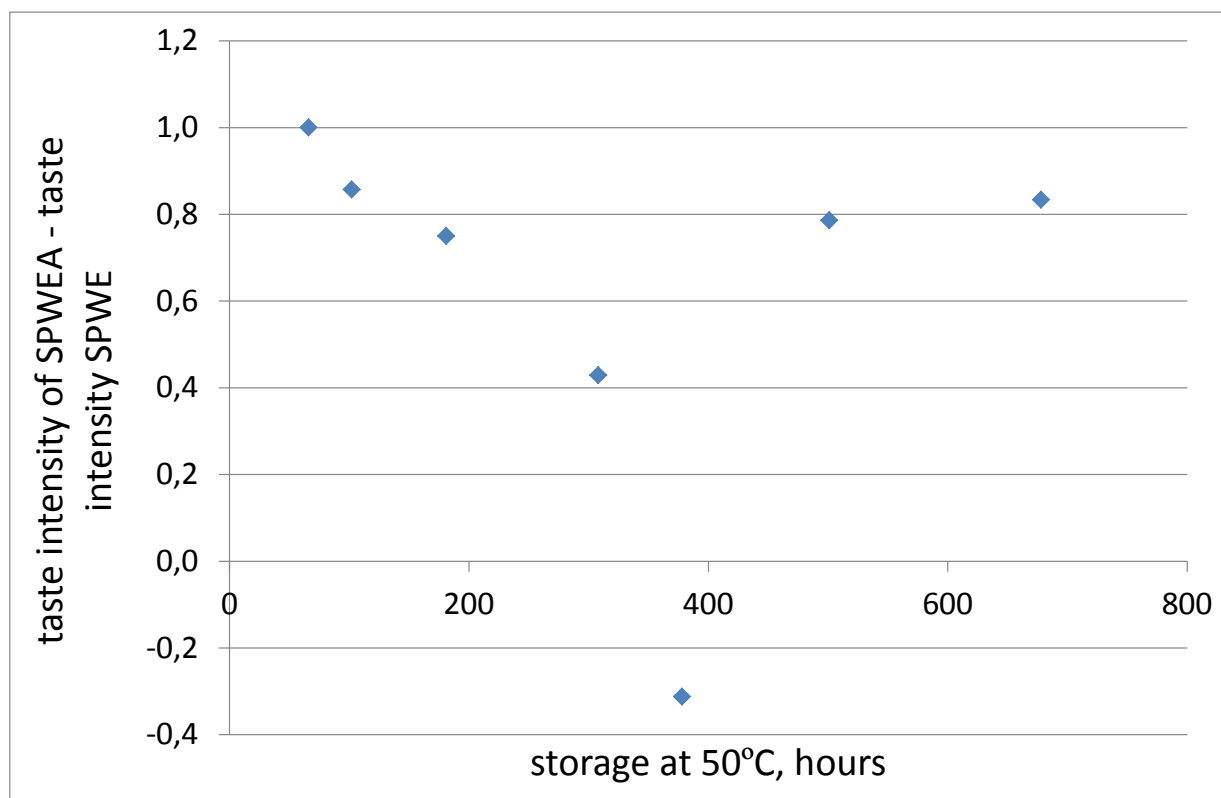


Figure 6. The difference in taste intensity between protein with antioxidant (SPWEA sample) and without antioxidant (SPWE) during storage test. SPWEA was added 1500 Tocovet.

## 5 Calculation of shelf life.

In an earlier report (SINTEF report A23364) (Figure 3) the stability of taste reduced proteins, without added antioxidant, were found to be 100 hours at 70°C, before lipid oxidation could be measured. At 50°C the stability of taste reduced proteins was longer than 700 hours. For biological processes a rule of thumb is that the rate doubles per 10°C increase in temperature. Extrapolation of data from 70°C to 20°C after this rule of thumb, gives a shelf life of 133 days, or 4,4 months. Extrapolation of data from the 50°C experiment to 20°C give a shelf life of more than 226 days or 7,4 months. To be able to extrapolate data in such a case where the rule of thumb does not fit, the activation energy has to be calculated for the rate limiting reaction determining the shelf life. The activation energy is calculated according to Arrhenius equation ( $k = A \cdot e^{E/RT}$ , where  $k$  is the rate constant (1/shelf life),  $A$ : the pre exponential factor,  $E$ : activation energy,  $R$ : the gas constant,  $T$ : absolute temperature) to 89 kJ/(gmol\* K). Based on the two data points the extrapolated shelf life at 20°C can be estimated to be more than 2 years. An acceptable shelf life for commercial application is according to industry 2 years (Wahren, R. 2012).

## 6 Minor survey of industrial interest in marine proteins with neutral taste.

Five companies were briefly presented for the technology for the production of marine proteins with neutral taste. Four companies currently producing protein and protein hydrolysate from reset raw materials from salmon were contacted. Of these four, two wanted the research to be continued. The aim should be to establish an industrial process based on this new technology. Both companies expressed interest in

participating in continuing projects. One company said it needed more time to take a decision, and the last company would focus only on hydrolysates. The fifth company producing from silage was not interested.

Three possible customers of taste neutral marine proteins have been approached and they all have shown great interest in testing the new product.

## 7 Conclusions

To reduce development of off flavour in dried products from marine fish, lipid oxidation has to be prevented. Isolation of proteins from salmon was performed according the SINTEF proposed techniques for production fish proteins with neutral taste. The compounds responsible for taste were washed out by hot water and ethanol. Two raw materials were used: the solid phase from salmon oil production based on salmon backbones and salmon backbones gently cooked before bone separation.

Study of lipid oxidation showed that iron mediated oxidation is not active in these fish proteins. Antioxidants, known as lipid radical scavengers, were effective to reduce oxidation in the proteins. Sensory shelf life of the isolated proteins with and without antioxidants was identified to be longer than 670 hours at 50°C. The sensory analyses were in agreement with the Schaal test where no lipid oxidation could be detected. Based on extrapolation, the shelf life at 20°C can be estimated to be more than 2 years.

Two companies producing salmon proteins and salmon proteins hydrolysates have expressed interest in developing the technology further.

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