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CONTRACT WORK/ PROJECT REPORT

Accessibility: Open	**Date: 04.10.2006**
Title: Final report for project "Level of residual nitrite by smoking of smoked salmon"	**Contract Work No:** O3036
Purpose of the contract work/ project: To find the contribution from wood smoke to the level of residual nitrite in smoked salmon	
Client: Fiskeri- og Havbruksnæringens Landsforening	**Client's reference:** Henrik Stenwig
Contract work/ project leader and co-workers: Oddvin Sørheim, Tom Chr. Johannessen og Karin Solgaard	**Signature:**
Director: Ragni Ofstad	**Signature:**

ABSTRACT

Conclusions

Based on data from Norwegian producers of smoked salmon, a small pilot study was conducted with dry salting and brine injection, followed by smoking and 45 days chilled storage. Analysis of residual nitrite after processing, revealed traces of nitrite (up to 2.3 mg/kg) in dry salted and smoked salmon. After storage, low levels (2.1 – 2.6 mg/kg) of nitrite were found in the products, either because of formation of nitrite during storage or analytical error. Four samples of commercial Norwegian smoked salmon contained no residual nitrite (< 1 ppm). A literature review showed few pertinent studies, except on the effect of nitrite on pathogenic bacteria in smoked salmon.

Recommendations

Due to the limited number of samples in this pilot study, we will recommend a broader study to determine the effect of various smoking conditions on formation of nitrite in smoked salmon.

INTRODUCTION

Nitrite ($NaNO_2$) is not permitted as an additive to smoked salmon in Norway. In the fall 2005, smoked salmon from several Norwegian processors was analysed to contain high levels of residual nitrite, due to use of curing salt (sodium chloride - NaCl with $NaNO_2$). In other samples, low levels of nitrite of appr. 5 mg/kg were also measured, in which the processors claimed not to have added nitrite. In the latter cases, it was considered that residual nitrite could be formed from other sources in the process with special attention to smoking. Based on results from processing of meat products, it is known that nitrite can be formed by exposure to nitrous gases in wood smoke. The knowledge of the effects and fate of nitrite in smoked salmon and other processed fish seems very limited.

The project was initiated by Fiskeri- og Havbruksnæringens Landsforening (FHL) and Norske Sjømatbedrifters Landsforening (NSL), and funded by Fiskeri- og havbruksnæringens forskingsfornd (FHF). The project was mainly performed at Matforsk AS – Norwegian Food Research Institute from June to September 2006, and was separated in four sub-tasks:

A. Information about typical conditions for production of Norwegian smoked salmon
B. Pilot study on smoking of salmon
C. Test of a few commercial Norwegian smoked salmon samples for residual nitrite
D. A literature search for relevant research reports.

THE PROJECT

A. Data on processing of Norwegian salmon

Information about the processing of Norwegian salmon was obtained from five companies by interviews by NSL. A standardised form, developed by Matforsk and NSL, was used for the interviews. The data were the basis for planning of the following pilot study (B).

A summary of the collected data:
Raw materials: farmed salmon weighing 3 – 6 kg (whole fish), with variation in classification (Superior to Ordinary)
Salting method: dry salting with NaCl of fine crystals, sometimes sugar is added
Salting time: 6 – 48 hours, large variation, but usually less than 24 hours
Salt concentration in the final products: 1.5 – 4 %
Drying prior to smoking: usually 3 – 8 hours at 10 – 30 °C, or over night at 3 °C
Smoking: by using cut beech chips in smoke generators, at smoking temperatures of 16 - 28 °C, for 4 – 17 hours
Holding time: the smoked salmon is usually chilled over night before packaging
Packaging: in vacuum
Microbiological shelf life: 21 – 45 days.

B - Pilot test on smoking

Materials and methods

The experiment consisted of 10 series with two randomly selected salmon fillets with skin per serie, a total of 20 fillets. The fillets weighed 0.884 to 1.267 kg. Four series were produced with NaCl dry salting (no nitrite) with variable smoking times. Two series were produced with NaCl brine injection (no nitrite) with two smoking times. After producing the six non-nitrite series, two series were produced with brine injection with nitrite, but without smoking. Fillets and samples containing added nitrite were kept strictly separate from the non-nitrite samples. One serie of dry salted (NaCl) without smoking and one serie of raw unprocessed fish were used for nitrite analysis as well.

1. Dry NaCl – smoking for 1 hour
2. Dry NaCl – smoking for 2 hours
3. Dry NaCl – smoking for 3 hours
4. Dry NaCl – smoking for 5 hours
5. Brine injection NaCl – smoking for 2 hours
6. Brine injection NaCl – smoking for 5 hours
7. Brine injection with NaCl + $NaNO_2$ – no smoking
8. Brine injection with NaCl + $NaNO_2$ – no smoking
9. Dry salt NaCl – no smoking
10. Raw fish unprocessed.

Dry salting was performed by adding salt in excess at the inner and skin sides of the fillets. The salting time was 12 hours, followed by removal of the salt by using water, and finally a holding and equilibration period over night. The temperature during salting and holding was 3 °C. The salt content of the dry salted smoked fillets was analysed (chloride titration).

Brine injection was performed with a Sühner WS 80F machine (Sühner, Bremgarten, Switzerland) equipped with 2 mm thick needles in 10 mm distance. The brine contained 20 % NaCl. Two types of brine were used: one with only NaCl, and one with NaCl (1/2) and NaCl with 0.6 % $NaNO_2$ (1/2). The fillets were injected one or two times to obtain a target of appr. 15 % weight increase, followed by salt equilibration over night at 3 °C. The weight of the fillets was recorded after injection and prior to possible smoking.

Smoking was performed in a Doleschal UM SC 2000 smoking house (Doleschal, Steyr, Austria). Smoke was produced in a smoke generator supplied with cut beech (*Fagus sylvatica*) of type Raucher Gold KL/2/16 (J. Rettenmeier & Söhne, Rosenberg, Germany). The smoking program started with 30 min. of drying, followed by smoke production for 10 min., smoke circulation for 20 min. and exchange with fresh air for 5 min. The three latter steps were repeated throughout the whole smoking program. The temperature in the smoking house was 28 +/- 2 °C.

After smoking, the fillets were chilled at 3 °C over night. The next day, the fillets were weighed and samples of >50 g were collected for nitrite analysis. The smoked and unsmoked products were then vacuum packaged in polyamide bags (oxygen transmission rate 30 ml/m^2/ 24h/ atm at 23 °C and 0 % relative humidity), and stored in darkness at 1 °C for 45 days. The samples were photographed after storage. The second nitrite analysis was performed at the

end of storage. The samples were collected from the same fillets as for the first nitrite analysis, and in positions next to the first samples. Due to a mistake by the laboratorium, the samples for the second analysis were frozen for an additional 10 days before analysis.

The nitrite samples were submitted to Analytica AB (Oslo). At the same day the samples were received, they were sent by chilled air freight to GBA Gesellschaft für Bioanalytik (Hamburg, Germany). The nitrite analysis was based on extraction with water, protein precipitation with Carrez reagents, and photometric determination (Shimadzu UV-160-A spectrophotometer) of nitrite after derivatisation with sulfanilamide and naphthyl-ethylendiammonium dichloride. The detection limit is appr. 0.5 – 1 mg/kg. The uncertainty of the measurements is appr. 15 % at low nitrite values, and 5 – 10 % at high nitrite values.

Visual colour of raw, unprocessed salmon fillets and smoked salmon fillets stored for 45 days was evaluated by a three-member trained panel. The Roche colour scale was used as a reference for evaluation of the fresh salmon.

Results and discussion

In evaluating the results of the pilot test, it is important to keep in mind that the number of samples is very limited, and that the results have a high degree of uncertainty.

Table 1.
Yield and colour of salmon

Serie	% yield after salting	% yield before storage	Visual colour of raw fish*	Visual colour before storage
1 – Dry salt, 1 h smoke	---	91	Red/Yellow 30	Yellow/Brown
2 – Dry salt , 2 h smoke	---	93	R/Y 30	Y/B
3 - Dry salt, 3 h smoke	---	89	R/Y 27	Y/B
4 – Dry salt , 5 h smoke	---	89	R/Y 28	Y/B
5 – Brine salt, 2 h smoke	111	107	R/Y 28	Y/B
6 – Brine salt, 5 h smoke	115	107	R/Y 29	Y/B
7 – Brine salt + nitrite, no smoke	118	117	R/Y 28	Bright red
8 – Brine salt + nitrite, no smoke	112	111	Y 26	Bright red
9 – Dry salt, no smoke	---	93	R/Y 30	Red/Yellow
10 – Raw unprosessed fish	---	---	R/Y 31	---

* Roche colour scale for fresh salmon

Yield and colour of the salmon samples are shown in Table 1. The salmon fillets were salted and smoked according to information from commercial processors in Norway (see part A). Dry salting of salmon is the most common method in Norway, but brine injection was included in the test for better possibility of controlling the concentrations of salt and nitrite in the final products. The measured concentration of NaCl in the dry salted and smoked samples no. 1 – 4 was 2.5 – 3 % (results not shown), and the final yield was appr. 90 % (Table 1). The calculated concentration of NaCl in the brine injected samples was 2.2 – 3.6 %. The yield of the brine injected samples with NaCl only was 107 %, and the texture was softer than the dry salted samples. The brine injected samples with salt and nitrite were not smoked, and therefore had a higher final yield than those samples that were smoked.

The colour of the raw, unprocessed salmon fillets varied quite much from scores of 26 to 31 on the Roche colour scale (Table 1). Figure 1 demonstrates the colour differences between the samples after 45 days storage. The unsmoked samples with injected salt and nitrite had an untypical bright red colour, which was easily differentiated from samples with only NaCl. Increasing smoking times increased the thickness of a yellow-brownish outer layer of the smoked samples.

Figure 1
Photo of salmon samples stored for 45 days at 1 °C. From left to right:

- no. 1 – dry salt, 1 h smoke
- no. 3 – dry salt, 2 h smoke
- no. 5 – dry salt, 3 h smoke
- no. 7 – dry salt, 5 h smoke
- no. 9 – dry salt, no smoke
- no. 10 – brine injection with salt, 2 h smoke
- no. 12 – brine injection with salt, 5 h smoke
- no. 15 – brine injection with salt and nitrite, no smoke
- no. 17 – brine injection with salt and nitrite, no smoke.

The analyses of residual nitrite are presented in Table 2. Two days after processing, levels of 1.3 – 2.3 mg nitrite/kg were found in dry salted and smoked salmon. Increasing smoking time did not appear to result in systematic increase in levels of nitrite. In samples with brine injection of salt and smoking no. 5 and 6 and the raw sample no. 10, no nitrite was found. The dry salted sample without smoking no. 9, contained 1.1 mg nitrite/kg. According to calculations, samples with brine injection of salt and nitrite should contain maximum 108 and 72 mg nitrite/kg for samples no. 7 and 8, respectively. Levels of appr. 70 % of these values were recovered in the initial nitrite analysis, due to expected losses of nitrite during the processing, mainly by chemical break-down.

At 45 days storage, samples no. 1 – 6 and 9 all seem to have a background level of appr. 2.0 – 2.5 mg/kg nitrite (Table 2). If this small increase in nitrite level from day 2 to 45 is caused by reactions in the products or by analytical errors, is not known. Levels of nitrite in samples no.

7 and 8 were considerably reduced during storage to 4 – 9 mg nitrite/kg. The relative reduction in nitrite concentration during storage was greater in sample no. 7 than 8.

Table 2.
Level of residual nitrite ($NaNO_2$) in salmon fillets.

Serie	Nitrite (mg/kg) before storage	Nitrite (mg/kg) after 45 days storage
1 – Dry salt, 1 h smoke	1.3	2.3
2 – Dry salt, 2 h smoke	2.3	2.6
3 – Dry salt, 3 h smoke	1.8	2.3
4 – Dry salt, 5 h smoke	1.5	2.3
5 – Brine salt, 2 h smoke	< 1.0	2.5
6 – Brine salt, 5 h smoke	< 1.0	2.1
7 – Brine salt + nitrite, no smoke	75.0	3.6
8 – Brine salt + nitrite, no smoke	49.2	8.9
9 – Dry salt, no smoke	1.1	2.4
10 – Raw, unprocessed fish	<1.0	---

C - Residual nitrite in commercial smoked salmon

Four samples of commercial smoked salmon from three Norwegian processors were analysed for residual nitrite. None of the samples contained nitrite (< 1 mg/kg). The age of the samples at the time of analysis was not known.

D - Literature review

A literature search was conducted to collect pertinent reports on nitrite in smoked salmon. Only a few relevant reports were found, probably due to the fact that nitrite is not used or permitted for smoked salmon in large parts of the world. No reports on undesirable nitrite formation in uncured, smoked salmon were found, neither any reports on colour effects of nitrite in smoked salmon.

In meat products, nitrite is known as an antimicrobial agent, colorant and antioxidant (Cornforth, 1996, Møller & Skibsted, 2002). In fish products, microbiological studies have shown that nitrite affected *Clostridium botulinum* by substantially reducing the salt level required to prevent toxin production in smoked salmon (Pelroy et al., 1982). Nitrite also inhibited growth of *Listeria monocytogenes* in smoked salmon (Pelroy et al., 1994). In the same study, the growth of naturally occurring aerobic microorganisms was inhibited as well, but to a lesser extent than *L. moncytogenes*.

The reduction of levels of nitrite in smoked salmon during storage was depending much on temperature (Pelroy et al., 1982). At 3.3 °C, initial levels of 150 – 200 mg nitrite /kg did not change during 22 days of storage. In contrast, at 25 °C, nitrite levels were substantially reduced to below 10 mg nitrite/kg at 10 days and later storage.

A requirement for coloration of salmon with nitrite is that sufficient heme, either in the forms of hemoglobin or myoglobin, is present in the salmon muscle. No scientific reports on the heme content of farmed salmon was found, but a survey showed that wild salmon contained appr. 25 % of the heme iron of beef, which should indicate a heme content of 0.1 - 0.2 %, similar to chicken (University of Wisconsin, 2004)

The development of undesirable pink/red colour in meat products has been investigated. Application of aquous solutions with nitrite to surfaces of pork muscles yielded a red colour with as low as 1 – 2 mg added nitrite/kg (MacDougall & Hetherington, 1992), demonstrating that very low levels of nitrite contamination can affect colour. In a study of gas oven burners, nitrogen dioxide (NO_2) was found to be the main contributor to formation of nitrite and an undesirable red colour in meat surfaces, due to its high water solubility (Cornforth et al., 1998).

References

Cornforth, D. (1996). Role of nitic oxide in treatment of foods. In Nitric oxide: principles and actions. Ed. Lancaster, J.Jr. Academic Press Inc., San Diego, USA, 259 – 287.

Cornforth, D.P., Rabovitser, J.K., Ahuja, S., Wagner, J.C., Hanson, R., Cummings, B. & Chudnovsky, Y. (1998). Carbon monoxide, nitric oxide, and nitrogen dioxide levels in gas ovens related to surface pinking of cooked beef and turkey. J. Agric. Food Chem., 46, 255 – 261.

MacDougall, D.B. & Hetherington, M.J. (1992). The minimum quantity of nitrite required to stain sliced and homogenised pork. Meat Sci. 31, 201 – 210.

Møller, J.K.S. & Skibsted, L.H. (2002). Nitric oxide and myoglobin. Chem. Rev. 102, 1167 – 1178.

Pelroy, G.A., Eklund, M.W., Paranjpye, R.N., Suzuki, E.M. & Peterson, M.E. (1982). Inhibition of *Clostridium botulinum* types A and E toxin formation by sodium nitrite and sodium chloride in hot-processed (smoked) salmon. J. Food Protect. 45, 833 – 841.

Pelroy, G.A., Peterson, M.E., Paranjpye, R., Almond, J. & Eklund, M.E. (1994). Inhibition of *Listeria monocytogenes* in cold-processed (smoked) salmon by sodium nitrite and packaging method. J. Food Protect. 57, 114 –119.

University of Wisconsin (2004). UW heath – Online health fact. At http://www.uhs.wisc.edu/docs/uwhealth_iron_182.pdf.

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CONCLUSIONS

Based on data from Norwegian producers of smoked salmon, a small pilot study was conducted with dry salting and brine injection, followed by smoking and 45 days chilled storage. Analysis of residual nitrite after processing, revealed traces of nitrite (up to 2.3 mg/kg) in dry salted and smoked salmon. After storage, low levels (2.1 – 2.6 mg/kg) of nitrite were found in the products, either because of formation of nitrite during storage or analytical error. Four samples of commercial Norwegian smoked salmon contained no residual nitrite (< 1 ppm). A literature review showed few pertinent studies, except a few reports on the effect of nitrite on pathogenic bacteria in smoked salmon.

RECOMMENDATIONS

The pilot study was performed with a limited number of samples and did not fully reflect the variation in raw materials, processing equipment and processing conditions that can be found in commercial production of smoked salmon in Norway. We will recommend:

- a broader and more thorough study to determine the effect of smoking on the formation of residual nitrite in smoked salmon
- visits to producers of smoked salmon, where elevated levels of residual nitrite have been determined, to look for sources of nitrite
- evaluation of the suitablity of analytical methods for nitrite and their level of error at concentrations near zero nitrite.