PILOT STUDIES ON A NEW APPROACH TO PRODUCTION PROCESSES FOR COLD SMOKED SALMON

PILOTSTUDIER PÅ EN NY TILNÆRMING TIL PRODUKSJONSPROSESSEN FOR RØYKELAKS

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English abstract

Cold smoked salmon is a lightly salt cured, smoked product highly popular in northwestern Europe. Breaking with some of the principles and procedures of conventional production, a new methodology for producing cold smoked salmon was outlined, experimentally tested on a small scale, and evaluated by sensory means. By slicing salmon fillets thinly (3-4 mm) prior to brine salting, and by immersion in liquid smoke condensates for smoking, production times were reduced to minutes instead of days. It was not succeeded in producing a product of similar sensory preference to that of conventional smoked salmon – the most prominent barrier being pronounced off-flavours believed to originate from the liquid smoke condensates.

Norwegian abstract

Røykelaks er et lettsaltet, røkt produkt som er meget populært i Nord- og Vest-Europa. Ved å bryte med enkelte prinsipper og prosedyrer ved konvensjonell produksjon ble en ny produksjonsmetode skissert, testet eksperimentelt i liten skala og evaluert ved sensoriske vurderinger. Ved å skjære filéten i tynne skiver (3-4 mm tykke) før lakesalting, og ved å erstatte tradisjonell røyking med dypping i røykkondensat ble produksjonstiden redusert til minutter i stedet for dager. Det lykkedes ikke å produsere et produkt med tilfredsstillende sensorisk aksept. Den mest fremtredende årsaken til dette var usmaker antatt å stamme fra røykkondensatene.

Introduction

The background for this thesis was an invitation to participate (through the work on a master thesis) in a pilot development project with the ultimate goal of inventing new seafood production processes and food products. The pilot project was in its early stages at the beginning of – and during – the work on this master thesis, which is also reflected in the experimental work and approach of this thesis; in that it could not be planned in detail on beforehand, but rather consists of a sequence of smaller experiments planned and adjusted along the way.

The theme of this thesis was early decided to be 'extra low temperature salting and cold smoking of salmon and/or halibut'. It was later further decided to experiment with some twists on the production process; by thinly slicing the fillets before processing, and as well experiment with natural liquid smoke condensates as an alternative to conventional smoking.

The term 'extra low temperature' processing was in this context defined as keeping the temperature below 10 °C during production/processing – and ultimately as low as < 5 °C. The thought behind this was that it might retain freshness and texture of the fish better than the higher temperatures commonly used in the industry.

Slicing the fish fillets thinly (3-4 mm) *before* salting and smoking was thought to radically reduce production time by providing a larger surface area for uptake of salt and smoke, and a thinner flesh thickness for which salt would have to penetrate/travel to reach center and equilibrium.

Using natural liquid smoke condensate instead of conventional/traditional smoking was thought to have the advantage of being hugely time- and resource saving.

The main aim and focus of this thesis can be narrowed down as follows:

> Is it possible to produce a product largely similar to (and ultimately consumer preferable over) conventionally produced cold smoked salmon by using thinly sliced salmon fillets, extra cold production and processing temperatures, and substituting wood smoke with natural liquid smoke condensates?

The work of this master thesis should be regarded only as *initial* investigations and research, aiming to provide some orientation on the worthiness of industrially pursuing the previously mentioned production conditions and methods.

Sensory evaluation has been the main evaluation method in the experimental work because of its wide spectrum potential to detect and approximately measure different conditions, variations and changes.

Theory

Salt curing

Salting is an ancient conservation method used to preserve raw materials or simple food products. Salting is generally not sufficient as a single method to create a shelf stable product (Horner, 1992; Belitz et al., 2009), and is therefore most often combined with other preservation methods such as acidification/fermentation, air drying and smoking. One of the important functions of salting is to create an initial hurdle to bacterial growth during initiation of further preservation processing.

Sodium Chloride (NaCl) is the main content in salts used for curing. Impurities may be present in the form of sand and water, other salts and heavy metals. Bacterial contamination may also occur, particularly in sun-dried sea salt.

According to Horner (1997), salt diffusion through the fish flesh is by a dialysis mechanism. Salt migrates in the water phase, and will therefore diffuse and migrate slower in fatty muscle fibres, connective tissue and through skin, than in lean myofibrillar muscle. The rate of salt diffusion is a gradient between the outside salt concentration and the point in the fish furthest away from the salting medium. Diffusion will therefore be faster with stronger salt brine. The diffusion rate will slow down as salt concentrations moves towards osmotic equilibrium. Also, initial salt exchange at the fish surface will be much faster than diffusion deep in the muscle. Horner (1997) describes an example of cod dry-salting at 10 °C where 10 % salt concentration is reached 25 mm into the flesh in 24 h, but to reach the same concentration 50 mm into the flesh took 72 h.

Salt–protein interaction and water holding capacity of fish muscle

Salt affects muscle proteins tertiary structure in that salt concentrations up to a certain point opens up and swells the protein structure, giving room for more water. The main muscle proteins in fish (myosin and actin) have isoelectric points around pH 4.7-5.3 (Ranken et al., 1997), well below typical ulitmate pH of fresh fish muscle around pH 6-7. There will therefore be a surplus of positive charges in the proteins. The protein swelling by salting is in practice primarily due to Cl^- ions binding to the excess of positively charged sites on the proteins (Horner, 1997), as well as both Na^+ and Cl^- ions interfering with – and weakening – interaction between oppositely charged side chains in the protein. Mg^{2+} and Ca^{2+} ions from

impure salt bind more readily than Na^+ to the negatively charged sites on the proteins, and will thereby concentrate in the fish if present in the salt. Bound Mg^{2+} and Ca^{2+} may also form a barrier to the passage of Na^+ into the fish muscle, thereby slowing the salting rate (Horner, 1997).

The muscle water holding capacity is positively affected at approximately 1-9 % salt concentration (Horner, 1997), peaking around 5-6 % salt (Offer and Knight, as cited in Martínez-Alvarez et al., 2005). At 9-10 % salt content the protein denatures (Horner, 1997), collapsing the protein structure – the reason being too much of an interferrence with (breaking up of) ionic bindings and bridges holding up the swollen three dimensional structure, thereby causing water expulsion (drying) and textural changes (compacting/hardening by protein aggregation).

Some other factors influencing salt uptake in fish muscle

Temperature

Diffusion is the thermal motion of substances, and the activity increases with increasing temperature above absolute zero. As salt is distributed within the muscle by means of diffusion, temperature has a direct influence on the diffusion rate – being slower at lower temperatures.

Rigor status

Pre rigor processing is a relatively new production technique in salmon processing, particularly utilized to achieve fresh fillets of higher quality, e.g. on freshness, texture and colour (Skjervold et al., 2001).

Wang et al. (2000) found a considerably lower salt diffisivity when salting Atlantic salmon pre rigor compared with post rigor. They suggest this might be caused by ATP-driven ionic pumps maintaining concentration gradients across membranes pre rigor.

Freezing/thawing

Frozen and thawed salmon muscle has been found to have larger extracellular spaces and disrupted muscle cells (Sigurgisladottir et al., 2000). The degree of muscle damage is highly dependent on freezing method and frozen storage temperature and -time, as ice crystals form and develop differently dependent on conditions (Zhu et al., 2003; Alizadeh et al., 2007). Ranken et al. (1997) states very generally that freezing leads to a 30 % increase in salt

penetration of fish, whilst Sigurgisladottir et al. (2000) did not find significant differences in salt levels in the final product when treating both previously frozen and fresh salmon with the same salting and smoking regime.

Fish salting methods

The three primary salting methods in use are dry salting, brine immersion and brine injection. Alternative methods are mostly hybrids or enhanced versions of the three mentioned.

In dry salting, the fish is sprinkled (abundantly) with salt grains, often stacked in several layers in containers with holes in the bottom to let moisture run off as it is extracted from the raw material during the process. If instead the salt-sprinkled fish is put in containers with no run-off, the process would gradually transform into brine immersion as extracted moisture collects over time to create a brine covering the fish.

In brine immersion, the raw material is immersed in a salt-water solution with salt concentrations typically between 10 and 25 %. Contrary to dry salting, brine immersion often leads to a weight increase of the raw material as raised salt concentrations in the flesh encourages water uptake (up to a point). Gallart-Jornet et al. (2007b) reports a substantial weight gain of salmon fillets immersed in 4-18 % salt brine, and a slight weight loss in 25 % (saturated) brine.

In brine injection, a mechanic needle injector injects salt-water brine directly into the fish muscle. Brine injection is fast, but brings with it potential disatvantages such as introduction of microorganisms in the flesh, needle marks and internal tissue ruptures.

Farmed Atlantic salmon (*Salmo Salar*)

Farming of Atlantic salmon in Norway has seen a tremendous growth since the beginning in the early 1970's. According to Laksefakta (2013), Norwegian farmed salmon production was close to 1.2 million tons in 2012 – a new record high and more than a doubling of production since 2002 (Norwegian Directorate of Fisheries, 2012) – and about 60 % of the total global production that year. Over 96 % of this was exported, with several EU countries and Russia as main markets. With 74 % being exported fresh as whole fish, a potential for increased domestic earnings lies within increased value-adding by further processing prior to export.

The main components of salmon meat are water, protein and fat. Norwegian producers of farmed Atlantic salmon (*Salmo Salaar*) cuts for the consumer market report nutrient contents

in the range of 13-16 % fat and 19-20 % proteins in their skin- and boneless salmon naturel products[1].

Lipid distribution within the 'white' muscle of Atlantic salmon has been found to vary greatly within the fillet. Katikou et al. (2001) found the belly part to contain the most fat (mean value 18.4 %), followed by the back area (mean value 12.24 %), whilst the middle section (longitudal midline) and the tail ranged from 2.37-6.08 %. An illustration to show the muscle sections is given in figure 1:

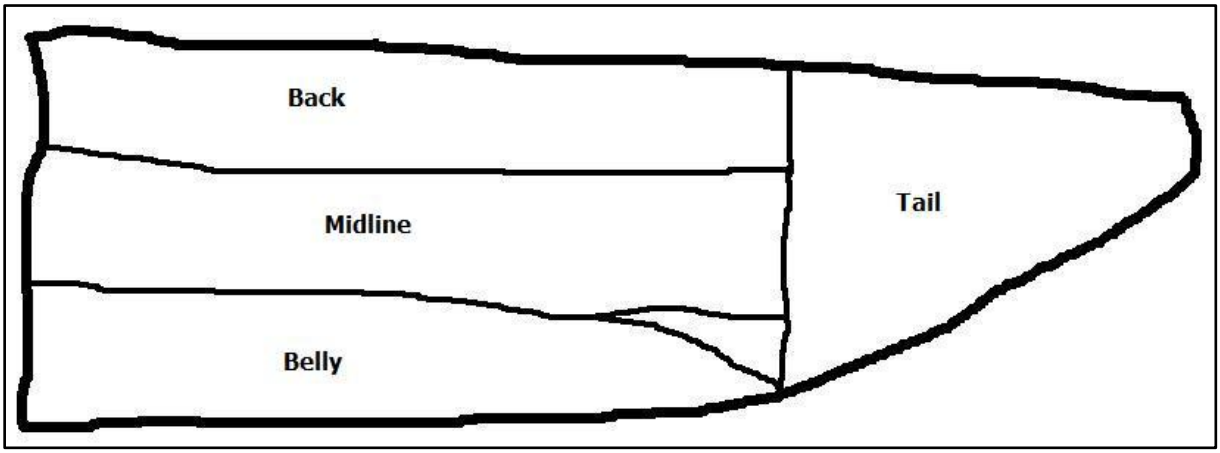

Figure 1. Salmon side fillet split in sections according to main trends in different fat content. The belly area had the highest fat content, followed by the back area, whilst midline- and tail area was found to have comparatively low amounts of fat (Katikou et al., 2001)

The research by Katikou et al. also confirmed a linear, inverted relationship between moisture and lipid content in the fillet.

Atlantic salmon muscle has characteristic red-orange coloured myofibrillar flesh intersectioned with white-gray stripes of connective tissue (figure 2). Connective tissue protein is usually 3-6 % of total muscle protein in boney fish (Ranken et al., 1997).

[1] http://www.salma.no/om-salma/salma-laks-n%C3%A6ringsinnhold
http://www.leroyseafood.com/Forbruker/Produkter/Produktdatabase/Laksefilet-Naturell/
http://www.leroyseafood.com/Forbruker/Produkter/Produktdatabase/Lakseloin-Finest/
http://www.seafraiche.no/no/nyheter/NYHET!+Sea+Fraiche%C2%AE+Lakseloin.9UFRrYXj.ips (24.04.2013)

Figure 2. Salmon slice showing muscle structure with separate pink/orange myofibrillar sections held together by thin layers of white/gray connective tissue

Cold smoked salmon production

Cold smoked salmon is a lightly preserved product with a salt content of 2.5-4.0 % (3.5-6.0 % in the water phase). Conventional production of smoked salmon can be broken down to the following main steps:

1. Leaving the fish to rest (2-4 days) until post rigor status is obtained
2. Filleting, leaving skin on
3. Salting (dry- or brine salting) – typically for 12-24 hours
4. Smoking at 20-30 °C, intersequenced with air drying steps – typically for at least 4-6 hours in total
5. Slicing
6. Packaging

The last use-by date is commonly 3-4 weeks from date of production when stored at 4 °C.

Smoking

Food smoking is an old practice anecdotally believed to have been discovered by chance thousands of years ago by humans hanging foods from the ceiling of the cave where they lived and made fires. Smoke would rise and concentrate in the ceiling, passing the hung foods on its way out, and thereby altering flavour and delaying spoilage.

Traditions of smoking (or indirectly smoke flavouring) foods have found extensive and diverse application around the world. Some examples showing the diversity are:

- Smoked whole meats, meat pieces and sausages. Examples include bacon, hams, legs and ribs of lamb, spareribs, hot dogs, beef jerky, dry cured hams and sausages etc.
- Fish and other seafood. Some popular examples are salmon, trout, whitefish, mackerel, eel, sardines, herring, oysters and scallops. The practice of smoking fish is found both in tropical and cold climates.
- Cheese, for example French goat cheese and Italian mozzarella.
- Smoke-dried peppers and chillies are staple spices in Spanish cooking, and heavily smoked jalapeños is a popular ingredient in Mexican and tex-mex cuisine.
- Tano-Debrah et al. (2007) describes smoke-flavoured water (produced by smoking the container before filling it with water) as a popular household produce, now seeing commercial production in Ghana.
- Smoked nuts (such as almonds and chestnuts) are popular snacks with a long history in many places of the world.
- Some types of tea include smoking as a step in the production, for example Lapsang Souchong from China.
- Wood barrels for wine and spirit ageing are charred on the inside to later release smoke-derived flavours to the drink.
- In Scotland malt is dried by smoking with peat for whisky production. Smoked malts are also used for some traditional beers, such as German *rauchbier*.

Definition

Food smoking is the condensation of smoke upon the raw materials or food´s surface by surrounding it with smoke over a period of time (normally from a few hours up to a few days – and even weeks).

The use of liquid smoke preparations challenge this definition as the smoke is condensed previous to application. The legal definition of food smoking varies between countries, where some requires products smoked with liquid smoke to be labeled as ‘smoked by liquid smoke’, ‘flavoured with smoke aroma’ or similar phrases.

Wood molecular composition

As smoke is created by pyrolytic degradation of wood, the wood’s chemical composition is essential as part of a thorough understanding of the different compounds present in wood smoke.

Besides water, the three major components of wood are the polymers cellulose, hemicellulose and lignin. They are found in different relative ratios between tree species. Also, the types and ratios of monomer building blocks in hemicellulose and lignin vary between species, as well as generally being particularly different in hardwood[2] and softwood[3]. It should also be noted that large variations exists between different parts, such as heartwood (core), sapwood and bark.

Wood is typically made up of 30-50 % cellulose, 25-35 % hemicellulose and 18-35 % lignin – all key elements to cell wall structure. Additional constituents are ash (0.2-0.8 %) and extractives (2-8 %). Lastly, wood also contains small amounts of proteins, pectin and starch.

Cellulose

Cellulose is a linear, unbranched homopolymer of β(1→4)-linked D-glucose (figure 3).

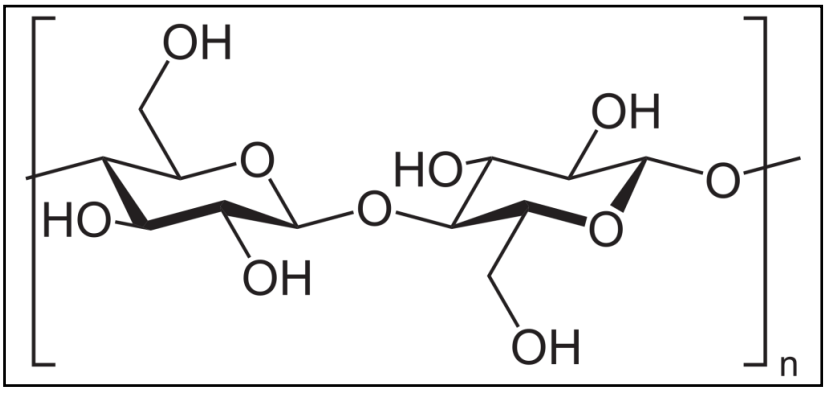

Figure 3. Cellulose molecular structure

[2] Angiosperm trees (mainly broad leaf trees)
[3] Gymnosperm trees (mainly conifers)

Polymer length is found to be from a few hundred to possibly up to 15 000 glucose units (Rowell et al., 2005). Abundant with hydroxyl groups, cellulose molecules form both inter- and intramolecular hydrogen bonds leading to the formation of microfibrils and further to crystalline cellulose fibers – giving wood its high tensile strength and stiffness. Pure cellulose is hydrophilic, but not water soluble.

Hemicellulose

Hemicellulose is a group of heteropolymers including xylan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan. Their backbones are in most cases β(1→4)-linked and equatorially configured, and may consist of a single type or multiple different sugar monomers. Hemicelluloses are of amorphous character with little tensile strength, and are easily hydrolyzed.

It should be noted that there is some debate over which polysaccharides that should be included in the hemicellulose definition. Scheller and Ulskov (2010) includes only xyloglucan, xylans, mannans and gulucomannans, as well as the β(1→3,1→4)-glucans in their definition, excluding a list of other polymers (regarded by some as hemicelluloses) on the basis that these do not share the β(1→4)-linked structure, and as well may be part of pectin molecules.

Unlike cellulose, hemicellulose polymer backbones are often substituted with short side branches of 1-3 monomer units each. Degree of polymerization is limited to be mostly within 100-250 monomer units. Both backbone and side chains *may* consist of multiple different monomers – both pentoses and hexoses – as well as some actylated forms, such as glucuronic- and galacturonic acid (Sjöström, 1993). Common sugar monomers present in hemicelluloses are the hexoses glucose, mannose, galactose and rhamnose, and the pentoses xylose and arabinose. Hardwoods and softwoods differ in that hardwood primary hemicelluloses are glucuronoxylans, whilst softwood primary hemicelluloses are (galacto) glucomannans and arabinoxylans (Sjöström, 1993). According to Bungay (1981), the different types and proportions of hemicelluloses in woods lead to different contents and ratios of constituent monomers in a general manner as follows (in decreasing abundance):

- **Hardwood**

  Xylose → mannose → glucose → galactose → minor amounts of arabinose and rhamnose
- **Softwood**

  Mannose →xylose → glucose → galactose → arabinose

Lignin

Lignins are irregular, branched phenolic (aromatic alcohol) polymers of low solubility in most solvents. There are three different hydroxy- and methoxy-substituted phenylpropane monomer units found to be the main building blocks of lignins such as *p*-coumaryl alcohol in grasses and annuals, coniferyl alcohol in softwoods and hardwoods, and complementary sinapyl alcohol in hardwoods (Sjöström, 1993) (figure 4).

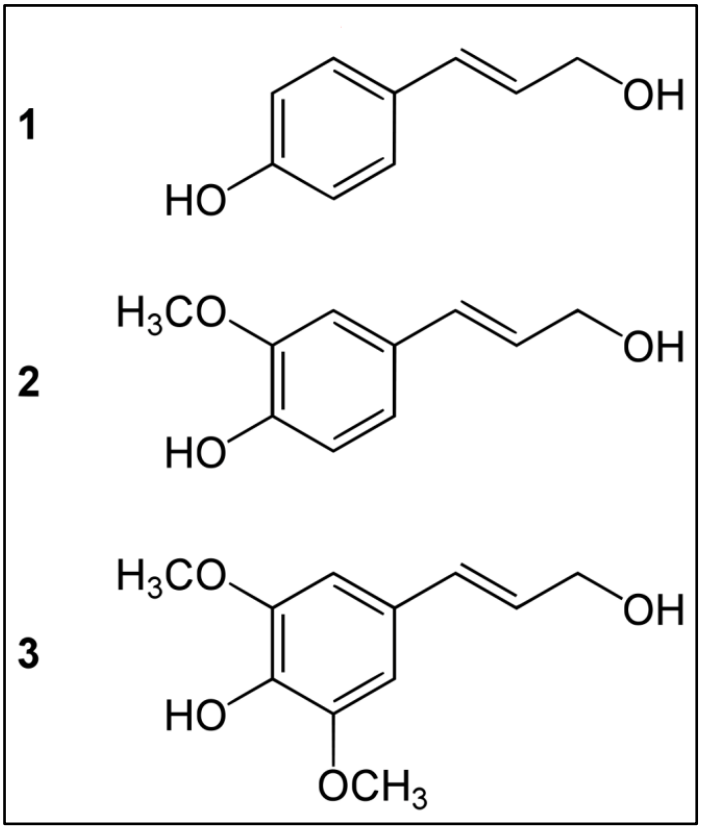

Figure 4. Lignin main constitutive monomers; *p*-coumaryl alcohol (1), coniferyl alcohol (2) and sinapyl alcohol (3)

Figure 5 shows a hypothetical illustration of how the phenylpropane monomers may be bound to make up the branched polymer lignin. The bindings are mainly C-C and C-O-C (ether bonds) – but somewhat randomly placed – making up a highly irregular polymer structure:

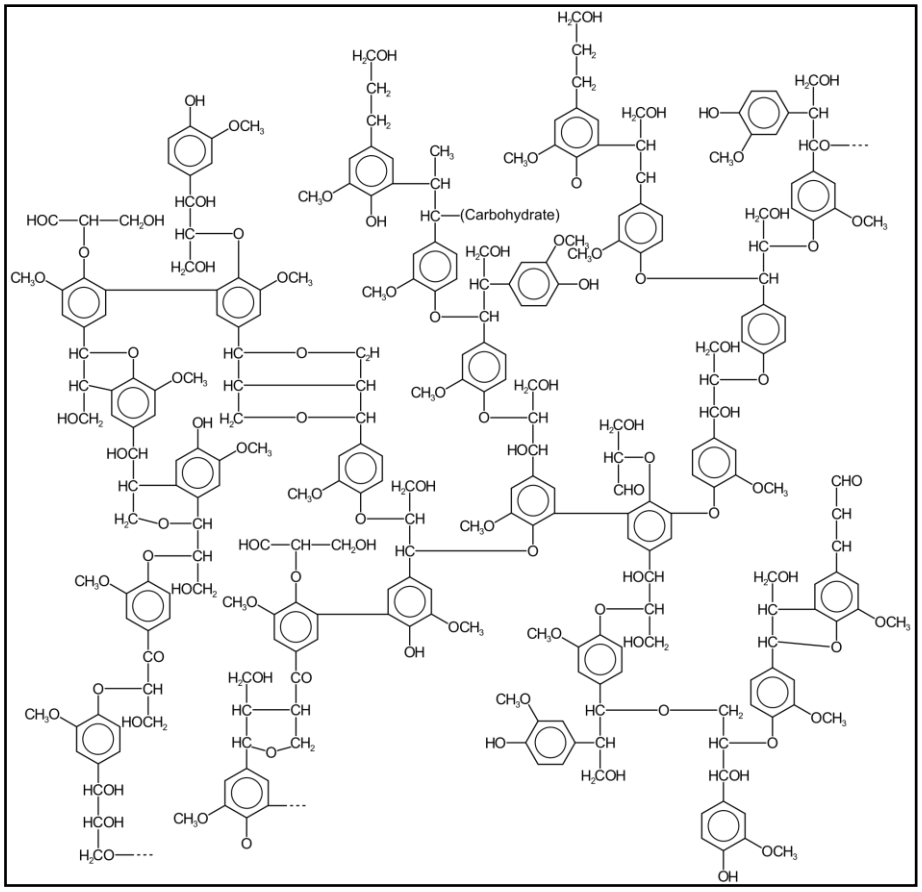

Figure 5. Hypothetic illustration of lignin partial molecular structure in softwood

The lignin content is considerably higher in softwoods than in hardwoods, in general 25-35 % and 18-25 % respectively (Rowell et al., 2005).

Extractives

Extractives are a varied group of organic wood chemicals that can be extracted using solvents. Many hundreds have been identified. Fats and fatty acids, resin acids, phenols, terpenes and waxes are some of the major extractives found. Select extractives are by large responsible for the wood colour and fresh wood smell, as well as aspects of wood durability. As a general rule, softwood contains substantially more extractives than hardwood. (Rowell, 2005)

Wood pyrolysis = smoke generation

The initial major occurrence during heating – and a prerequisite for pyrolytic degradation of wood – is the vapourization and escape of free- and loosely bound moisture, with a peak around 100-120 °C.

Degradation temperatures

Hemicellulose components start to degrade at about 225 °C, and will be completely decomposed at around 325 °C. Cellulose is more stable to thermal degradation, but will rapidly decompose at a narrow temperature range around 370 °C. In general, it can be said that most carbohydrate polymers will degrade between 300-375 °C (Rowell and LeVan-Green, 2005). Lignins are regarded much more stable to thermal degradation than the carbohydrate polymers. Although they begin to degrade at around 200 °C, it takes up to 500 °C to decompose most – and even further temperature increase to complete decomposition (Brebu and Vasile, 2009).

Note on (secondary) pyrolysis products and pathways

Due to the chemical complexity and diversity of woods, the exact mechanisms and pathways (as well as the thermal kinetics) in wood pyrolysis are largely unknown. Wood degradation partly leads to a multitude of unstable compounds that may react, combine and rearrange into a vast array of new, secondary compounds. It is also the impression of the author that the majority of advanced research on pyrolysis mechanisms is not directly related to food smoking, but rather for other industrial-chemical- and energy-utilizing purposes, as well as for environmental pollution issues.

Single substance pyrolysis analyses on wood extracts and purified fractions (e.g. cellulose) are highly useful for identifying decomposition and secondary mechanisms, but their results and findings are not always so easily applicable to accurately predict the chemical outcome from whole wood pyrolysis, as there often is multiple competing mechanisms that will experience a shift in the competitive factors during the more complex conditions in pyrolysis of whole wood. Extracting and preparing many of the single wood substances for single substance pyrolysis analyses have also been shown in many cases to be difficult – often leading to changes in the substance, such as depolymerization – further deviating single-substance experimental findings from the actual conditions of whole wood pyrolysis.

Another consideration with regards to pyrolysis end products is that inorganic salts, alkali metals such as potassium, and alkaline earth metals such as magnesium and calcium – all which are naturally present to various degrees in wood – have been shown to act as reaction catalysts with the potential to drastically change the different chemical substance outcomes and yields/ratios in the smoke (Müller-Hagedorn et al., 2003; Wang et al., 2007; Aho et al., 2013).

The chemical products of wood pyrolysis

Pyrolysis of wood produces permanent gases, condensable volatiles and water (aqueous phase), tar (oily phase) and char residue (porous carbon-dominated skeletal solids).

Wood smoke has been defined physicochemically as;

> *"…an emulsion of droplets in a continuous phase of air and vapours stabilized by electrostatic charges on the droplets […] (where) a dynamic equilibrium exists between droplet and vapour phase of the smoke…"* (Horner, 1997)

The *dynamic* equilibrium is suggested to constantly change with tempererature, food products' (and surroundings) uptake of smoke, air to smoke ratio and smoke-air velocity. It is also suggested that the suspended droplets acts as a feeder of volatile substances to the vapour phase (Tilgner et al., as cited in Horner, 1997).

Permanent gases

Commonly produced permanent gases by pyrolysis of lignocelluloic biomass such as wood are carbon monoxide (CO), carbon dioxide (CO_2), hydrogen (H_2), methane (CH_4) and small amounts of ethene (C_2H_4) and ethane (C_2H_6) (Yang et al., 2007). CO is produced by decarbonylation (RCHO → RH + CO), and CO_2 by decarboxylation (RCOOH → RH + CO_2).

Water

Wood contains water in three forms:

- **Free water**
  Extracellular liquid moisture is found in cell voids, lumens and small pores – only held by capillary forces. A loss of this water will not affect wood volume.
- **Bound water**
  Chemically bound water located within the cell walls and held by hydrogen bonds, particularly to the many hydroxyl (OH) groups of cellulose, hemicellulose and lignin.
- **Water of constitution**
  Water of constitution is potential water incorporated in the molecular structures. The water can only be released by breaking up the molecule and thereby changing its structure, such as during decomposition by pyrolysis.

Water present in the wood will absorb latent heat of vaporization, and therefore act as an initial barrier and later retarding factor for the necessary temperature increase to conduct the pyrolysis degradation process. The more water in the wood, the more energy is required to reach and maintain an effective pyrolysis zone – and the more temperature increase is subdued.

Aqueous phase

As an example of smoke chemical complexity, Guillén and Manzanos (2002) detected over 200 different substances just in the aqueous phase (oily phase was filtered out) of condensed oak smoke produced with a maximum pyrolysis temperature of 557 °C. The main chemical groups they detected were aldehydes, ketones, diketones, furan and pyran derivatives, alcohols, esters, acids, phenols, guaiacol and derivatives, syringol and derivatives, lignin dimers, pyrochatecol derivatives and related, alkyl aryl esters, carbohydrate derivatives and nitrogenated compounds.

Oily phase (tar)

The oily/tar phase overlaps somewhat with the aqueous phase (as some compounds may have both hydrophilic and lipophilic properties), but also consist of heavier, non-volatile molecules, such as polycyclic aromatic hydrocarbons (PAH). Fast-pyrolysis produces an oily phase that is a pourable and pumpable liquid at ambient temperatures and of great chemical complexity, including phenolic compounds (sometimes referred to as ‘pyrolytic lignin’), carboxylic acids, aldehydes, sugars, ketones, alcohols and solids (Mohan et al., 2008). Slower pyrolysis produces a thicker tar of larger molecules due to molecular fusions in the prolonged time spent in the pyrolysis ‘hot-zone’.

PAHs have been reported to form in the wide temperature range from 300 °C up to 1000 °C during pyrolysis of biomass. McGrath et al. (2003) reports pyrolytic formation of 2-4 ring PAHs from 400 °C, and detectable amounts of 5-ring PAHs from 500 °C from pure cellulose pyrolysis. Another report (McGrath et al, 2001) also showed trends of increased total yields of PAHs with increased temperature – with a correlating shift with higher temperature from smaller to larger molecular size PAHs in the total PAH mixture. PAHs are also formed from polyphenolic (lignin) compounds (Sharma and Hajaligol, 2003).

Some general trends observed with increasing pyrolysis temperature is;

- Tar yield increase
- PAH yield and -molecular sizes increase
- Phenol and aroma-substance yield increase
- Char yield decrease

Factors that makes up a smokes' unique chemical characteristics are the chemical composition of the wood used (varying greatly with types of wood), and the degree of decomposition due to pyrolysis techniques and conditions such as temperature, oxygen availability, humidity, smoke velocity, inorganic catalysts etc.

Some smoke compounds affecting food product qualities

Carboxylic acids and phenolic substances play inhibitory roles against bacterias (Varlet et al. 2010). Particularly effective phenolic compounds found in smoke are isoeugenol, 4-methylguaiacol and guaiacol, having a stronger effect on *Listeria*, *Staphylococci* and *Bacillus* than on *E. coli* and *Lactobacilli* (Red Arrow, 2010). Other bactericides from smoke are formaldehyde and alcohols.

Phenolic compounds have antioxidant properties, generally stronger the higher boiling point of the particular compound. The polyhydroxyphenolic compounds are the strongest antioxidants among the phenolic compounds, but also the monohydroxyphenolic compounds such as 4-methylguaiacol, 4-vinylguaiacol and 4-propenylsyringol show good antioxidant properties (Varlet et al., 2010). These therefore aid to prevent lipid rancidification in the smoked product.

Phenolic compounds are also credited for most of the organoleptic properties (smoke flavour and aroma) from smoke (Maga, as cited in Red Arrow, 2010). Relatively recent research indicates that the role of carbonyls (as a complement to phenols) to smoke flavour may have been previously underestimated (Kostyra and Baryłko-Pikielna, 2006). In addition, smoke contains many other compounds found to be organoleptically active, but their roles in contributing to smoke flavour seem largely unknown.

As smoke is highly complex, one could expect interplaying effects of several chemical substances – with synergetic or antagonist effects on different properties such as microbial safety, chemical safety, flavour and appearance.

Smoke-derived harmful compounds – PAHs

Polycyclic aromatic hydrocarbons (PAHs) make a chemical group of over 100 identified substances. PAHs are of mostly lipophilic nature, and therefore found in higher quantities in surface-fatty smoked foods.

PAHs have long been seen as the key harmful compounds in wood smoke – proposing a significant health risk as mutagens and carcinogens – with benzo[a]pyrene (BaP) (figure 6) as the main PAH in focus for decades for its frequent appearance in foodstuffs, and its well documented carcinogenicity (Wester et al., 2012).

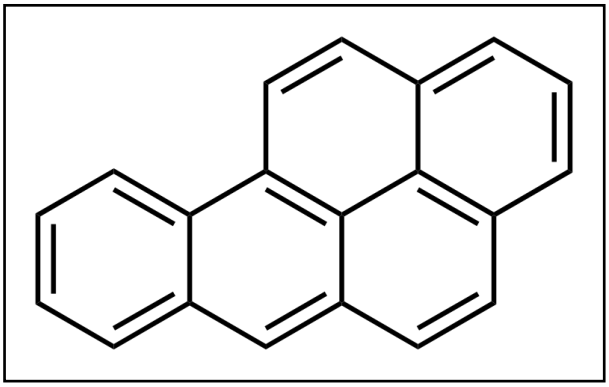

Figure 6. Skeletal chemical structure of benzo[a]pyrene

PAHs are secondary products from high temperature pyrolysis, mainly above 500 °C. They are considered environmental pollutants – their sources including vehicle exhaust, waste handling-, electric power- and industrial plants, residential wood burning, forest fires, volcano eruptions and cigarette smoke – and are found in many parts of the environment; in air, water and soil. Background levels of representative PAHs have been found to be 8-16 times higher in urban- than in rural areas (ATSDR, 1995). As examples of the widespread PAH pollution Barcan et al. (2000) reported 6-40 times higher than background levels of BaP in the topsoil layer corresponding to the take-off path from a nearby airport. Wretling et al. (2010) reports BaP levels exceeding EU regulations in Swedish commercially available handcraft “sauna” smoked fish. Essumang et al. (2012) reports levels of BaP in a large selection of Ghanaian traditionally smoked sardines a manyfold higher than EU maximum allowed levels. Norwegian fjord side aluminium works have been found responsible for PAH concentrations 1000 times higher than background concentrations in mussels within 1-2 km, and elevated concentrations traced 35-40 km in fjord bottom sediments (Naes et al., 1995). Deposition of

PAHs in soil and deep water sediments is particularly unfortunate, as PAHs are generally degradable by exposure to UV-light (Miller and Olejnik, 2001).

ATSDR (1995) states that consumption of food is one of the primary sources for PAH exposure for humans.

Until recently, BaP has been used as a single marker for PAH contamination in food, with set rules for maximum levels in the EU. EC R1881/2006 sets maximum levels of BaP in foodstuffs to 1.0 µg/kg wet weight for a range of foods intended for infants and young children, 2.0 µg/kg wet weight for oils and fats, and for meat and fish muscle meat, 5 µg/kg wet weight for non-smoked crustaceans and for smoked fish and meat, and finally 10 µg/kg wet weight for bivalve mollusks (clams etc.). Some exceptions and specifications are not mentioned here.

The EC R835/2011 follow-up to EC R1881/2006 finds that BaP is insufficient as a marker for PAHs in food, and suggests a system of PAHs – the so called *PAH4* – consisting of BaP, benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR). EC R835/2011 introduces maximum levels for *PAH4* in addition to BaP, as well as a stepwise lowering of maximum values for both in many of the food categories towards year 2015. EU current and planned maximum PAH levels with regards to seafood and smoked seafood products are shown in table 1.

Table 1. Recent and near future EU regulations regarding maximum levels of PAHs in seafoods for human consumption. (EC R835/2011)

Foodstuffs		**Maximum levels (µg/kg)**	
		Benzo[a]pyrene	**PAH4**
6.1.5	Muscle meat of smoked fish and smoked fishery products, excluding fishery products listed in points 6.1.6 and 6.1.7. The maximum level for smoked crustaceans applies to muscle meat from appendages and abdomen. In case of smoked crabs and crab-like crustaceans (*Brachyura* and *Anomura*) it applies to muscle meat from appendages.	5,0 until 31.08.2014 2,0 as from 01.09.2014	30,0 as from 01.09.2012 until 31.08.2014 12,0 as from 01.09.2014
6.1.6	Smoked sprats and canned smoked sprats (*sprattus sprattus*); bivalve molluscs (fresh, chilled or frozen); heat treated meat and heat treated meat products sold to the final consumer.	5,0	30,0
6.1.7	Bivalve molluscs (smoked)	6,0	35,0

The stepwise introduction of reduced maximum values is meant to enable smoked food producers time to adjust their equipment and production processes.

Cold- and hot smoking

A useful categorization of smoked foods is whether they have been cold- or hot smoked, which is determined by the smoke temperature at the point where it surrounds the raw material:

- **Hot smoking**
  Hot smoking involves smoke temperatures at 65-95 °C. Proteins in the raw material will therefore largely denature – as well as fats melt and drip off – completely changing the foods texture and to some extent nutrient content and -availability. Due to the high temperatures involved, inhibitory chemical substances from the smoke and the surface drying as a result of the smoking process, most microorganisms are inhibited or eliminated.
- **Cold smoking**
  Cold smoking usually involves smoke temperatures at around 18-37 °C. This leads to none or very little protein denaturation due to temperature. Some fat melting (and dripping) may occur – particularly in fish, where fats are highly unsaturated and therefore of low melting point. Although the temperature is ideal for the growth of many bacteria, the smoking process (including raw material surface drying and smoke-derived gases and compounds) inhibits bacterial activity and survival to some extent.

The smoking temperatures are simply controlled by passively or actively chilling the smoke upon introduction to the food smoke chamber – in practice most often by adjusting the distance from- and the size of the smoke source.

Direct and indirect smoking

Direct smoking is defined as having the smoke source inside the smoking chamber/house, whereas indirect smoking is defined as having the smoke source/generator separate from the smoking chamber.

Traditional smoking

Traditional smoking is typically a direct, batch smoking procedure where the food material is hung high in the smoking house/chamber and a wood fire lit on the floor. By closing the door, oxygen supply will be limited and thereby encouraging pyrolysis and smoke generation.

Traditional smoking is a particular concern for its directness between the smoke source and the food material, long-time smoking (often for weeks) and the lack of control over important smoking parameters such as pyrolysis temperature and air/smoke humidity. Research have shown a general trend of much higher levels of PAHs in traditionally smoked fish compared to smoking with modern equipment, often manyfold exceeding EU maximum levels for BaP – and large variations between batches (Wretling et al., 2010; Essumang et al., 2012).

Modern industrial smoking methods

Modern smoking equipment consists of stainless steel product chambers connected to an external electric or mechanical smoke generator. Most systems are semi- or fully automated, computer controlled and multistage programmable – serving both ease of use and control of important processes such as drying and smoking cycles, and control of smoking parameters such as pyrolysis temperature, air and smoke flow/circulation, humidity and smoke temperature. Other in-built, common features of modern smoking systems are electrostatic filtering of heavier molecules (including PAHs) and particles from the smoke, automatic cleaning and sanitation, optional product heat treatment/cooking and rapid product cooling at the end of smoking.

Various smoke generating systems have been developed, the most common in large, industrial smoking systems being:

- **Electric heating element**
  Smoke is being created by the feeding of wood dust on an electric heating element. Smoking control parameters are heat plate temperature, air supply and wood moisture content.
- **Friction**
  Smoke is generated through friction by wood logs pressing against a rotating disc. Can be hermetically sealed and flameless (Red Arrow, 2010).
- **Super heated steam**
  Super heated steam (400 °C) is passed through wood chips, generating a mild, gentle

smoke. A fire- and explosion safety advantage in that there is no glow or open flame in the generator.

- **Liquid smoke systems**

  Liquid smoke systems are differing in that they utilize pre-manufactured condensed smoke concentrates instead of wood. The liquid smoke concentrate is *atomized* to smoke by compressed air through calibrated nozzles in the smoking chamber. With this technique there will be no gaseous phase, only small droplets similar in size to that of conventional smoke (15-20 μm).

Liquid smoke

Liquid smoke condensate is wood smoke that is condensed and captured in cool water (or organic solvents). This crude liquid condensate may be further processed and refined into a diverse range of smoke flavour products (Varlet et al., 2010). From information provided by Red Arrow (2010) – a world leading producer of smoke condensates for food application – a flowchart outlining the primary production process can be made as shown in figure 7.

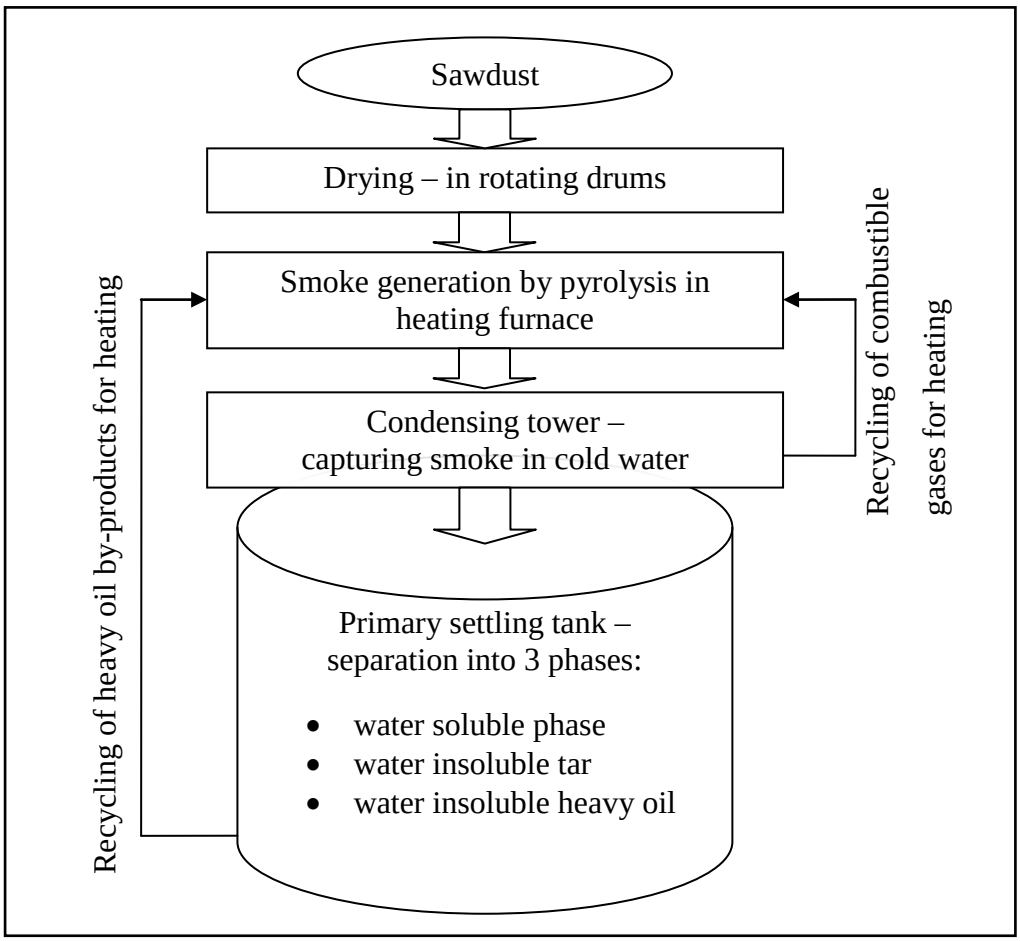

Figure 7. Flowchart outlining Red Arrow's natural smoke condensate production

The water soluble phase and the water insoluble tar phase are drained off separately, filtered and processed into aqueous- and oily smoke flavourings respectively. From these, further smoke flavouring products can be made, such as smoke powders and –salts, smoke concentrates, browning products and smoke distillates. Due to the low pH of the aqueous smoke, partly neutralized or buffered smoke flavours are also made for uses where the acids would have had an undesired effect on the product, for example by denaturation of proteins. Liquid smoke is widely used in the food industry; 75 % of smoked foods are treated with liquid smoke in the US, and 20-30 % in Europe (Varlet et al., 2010).

In addition to atomization, there are two common ways to apply liquid smoke:

- **Showering or drenching**
  The product is showered with water-diluted aqueous liquid smoke, or the product is dipped in the solution. The technique is highly efficient in applying smoke flavour. Depending on the liquid smoke concentration, showering or drenching takes from a few seconds to a few minutes, and is therefore well suited for continuous food production.
- **Injection**
  Diluted aqueous liquid smoke is applied to the product through injection needles penetrating the product. This technique is also suited for continuous production, and can as well be combined with brine salting into one production step. Injection leaves smoke flavour throughout the product more so than topical application, but may also introduce bacteria into the product.

In addition to the above mentioned methods, smoke condensate derived flavourings are used in several ways. Smoke oils are suitable for incorporation in fatty emulsions, marinades and sauces, smoke powders in dehydrated mixes such as powdered soups or spice mixes, and smoke flavour impregnated films and casings can be used to completely eliminate smoke application steps in the food manufacturing.

Materials and methods

Experiments were mainly conducted in the kitchen facilities of the dairy pilot plant of The Norwegian University of Life Sciences during spring 2013.

Materials

Equipment

- Weighing scale
- Filleting knife
- Chefs knife
- Knife sharpener
- Steel forks and spoons
- Cutting board
- Absorptive kitchen paper
- 3 x 2L Polypropylene (PP-05) plastic containers, food grade (Plast Team AS)
- Vacuum sealing machine
- Clear plastic bags for vacuum sealing
- Petri dishes
- Plastic cups – 250 ml
- Plastic film
- Thermometer

Food materials

- Fresh, farmed halibut (approx. 8-9 kg whole weight)
  (Bremnes Seashore AS)
- Pre rigor filleted deep frozen salmon back- and belly loin fillets
  (SALMA®. Salmon Brands AS)
- Salt, fine, refined, NaCl > 99.8 %, anti caking agent E535 max 0,001 % (NorSal)
- Sugar – beet root sucrose (Nordic Sugar AS)

- Tapwater[4]
- MilliQ, distilled and filtered water

Smoke flavourings

- Unlabeled water soluble liquid smoke condensate (obtained from the meat pilot plant at Nofima, Ås)
- PA24 water soluble liquid smoke condensate (Red Arrow LLC)
- SMOKEZ 5111water soluble liquid smoke condensate (Red Arrow LLC)
- SMOKEZ OIL H SF oil soluble natural liquid smoke flavouring (Red Arrow LLC)

Cooling/storage facilities

- Cold storage room, air circulated, 4 °C
- Freezing room, –40 °C

Calculations and statistical evaluations

- Calculator (Casio fx-9750G)
- Microsoft Excel 2007 on Windows Vista Home Premium Service Pack 2 OS.

Methods

In addition to several smaller tests not mentioned in this thesis, three main experiments were conducted (in chronological order):

1. Halibut: Different salting methods before equal treatment with liquid smoke

This first experiment was conducted as a starting point to get a feel for the salting process and the use- and effects of liquid smoke.

Pieces of fresh skin- and boneless halibut fillet (average 215g ±39g each) were salted in three different brine concentrations (12 %, 20 % and 25 % salt (by weight) in water), or dry salted. For brine salting, 3 parts brine were used per 1 part fish – calculated to match exact fillet weight. 0.5 parts salt was used per 1 part fish for dry salting. Ingredients and fish were put in

[4] Surface water treated at water plant with $Al_2(SO_4)_3$, sand filtered, pH adjusted with NaOH, and disinfected with sodium hypochlorite (Oppegård kommune, 2012). The authors' attempts to find up to date public information on water quality and ion content failed, but the water is known for low content of salts – probably < 50 ppm in total

individual plastic bags and carefully shaken until all salt were dissolved, and until the fillet were completely covered in salt in the case of dry salting. Most air was removed before the bags were sealed in the vacuum machine without applying vacuum, and then left for 50 hours at 4 °C. Next the fillets were briefly rinsed in tap water, immersed for 30 seconds in a 50/50 smoke condensate/water-solution (unlabeled smoke), drained for 10 seconds, packed and sealed in new individual plastic bags and left for approximately 3 days at 4 °C before freezing at –40 °C for storage until sensory evaluation. Individual fillet weights were registered fresh, after salting and before freezing (3 days after smoke flavouring).

For sensory evaluation, the fish were thawed in bags in cold tapwater, one edge cut off about one third into the fillet and discarded before slicing by hand at a slight angle to create cross-sectional slices approximately 5 mm thick. Individual slices were put in three digits randomly marked petri dishes. Six panelists, all involved in the development project, were used for evaluation. The panelists were spread around a table, but instructed not to communicate during the evaluation. Each had a blank A4 white paper sheet in front of them to place the petri dish on when evaluating. Samples were firstly evaluated one by one in an evaluation form asking to write comments in the three categories appearance, smell/aroma, and taste/mouthfeel. In the end the panelists were asked to a forced ranking of the four different samples from least to most salt intensity.

Brief summary of the process:

1. Halibut filleting, de-skinning and cutting into portions
2. Four different salt treatments for 50 hours at 4 °C; brine salting in 12 %, 20 % and 25 % salt brines + dry salting
3. Equal treatment by immersion in liquid smoke-in-water solution for 30 seconds.
4. Storage for 3 days at 4 °C, then freeze storage at –40 °C
5. Sensory evaluation

Fillet weight changes in percent in relation to original fillet weight were calculated by the following formula;

$$\left(\frac{W_t \times 100}{W_o}\right) - 100 = weight\ change\ in\ percent$$

where W_o represents original fillet weight, and W_t fillet weight at measuring point in salt/smoke treatment.

Relative weight changes during the 3 days after smoke treatment were calculated by;

$$\left(\frac{RFW_{smoke} \times 100}{RFW_{salt}}\right) - 100 = relative\ weight\ change\ in\ percent$$

where RFW_{salt} represents relative fillet weight in percent after salting, and RFW_{smoke} relative fillet weight (compared to original fillet weight) in percent 3 days after smoking.

Binominal distribution tables (Sensorisk studiegruppe [Sensory study group], 2009) were used to compute statistical significance of results from the forced ranking of samples for salt intensity.

2. Salmon: First brine salting and liquid smoke treatment of thin slices

The main approach used in this experiment was to put what is the last step in conventional smoked salmon production first; namely slicing the fish fillet *before* salting and smoking, instead of after. Additional important factors was to keep the temperature much lower than conventional processing, and as well using liquid smoke condensate to ease and speed up the process. The main aim in this experiment was to establish some salting time limits with regards to what salting times would be appropriate.

Factory frozen, pre rigor filleted skin- and boneless salmon fillets were used as the base raw material. Two 'back loin' fillets, about 500g in total, were thawed overnight at 4 °C. Two different salting brines were used – 12 % and 25 % salt. To round off the flavour, as is common in the industry, 1 part sugar was added per 3 parts of salt, thereby adding 4 % sugar in the 12 % brine, and 8,33 % sugar in the 25 % brine (table 2). Ingredients were added to plastic containers, lid-sealed and shaken to dissolve before being placed at 4 °C for temperature stabilization until the next day. The 25 % brine was completely saturated, as a very small (negligible) amount of salt never dissolved.

Table 2. Brine recipes

	12 % salt brine	**25 % salt brine**
Salt	180g	375g
Sugar	60g	125g
MilliQ Water	1260g	1000g
Total	1500g	1500g

The thawed salmon fillets were carefully hand sliced at an angle of about 45°, to produce 32 slices of each fillet – a total of 64 slices of approximately 3-4 mm in thickness. The narrowing end of the fillets was discarded, as slices of these would have been substantially smaller in overall size (figure 8).

Figure 8. Partly sliced salmon 'back loin' fillet. The left- and rightmost pieces were discarded. The middle piece shows the slicing angle on its left side

32 slices were immersed in each of the two brines, which were carefully stirred throughout the salting to ensure complete brine coverage of each individual slice. Four slices were taken out at intervals of 1, 3, 5, 7, 10, 15, 20 and 30 minutes of immersion, immediately dipped in water to remove excess brine, put on kitchen paper to remove excess water and finally put in plastic cups covered with plastic film and placed at 4 °C for resting. Temperatures of the brines were registered at the end of the salting.

Two persons (familiar with commercial smoked salmon) evaluated samples from all the different salting times for salt intensity by sensory means about 1 hour after the salting process was finished. The samples were tasted in the same order as they were produced; starting with the 12 % salt brine immersed slices, and the shortest immersion time first to minimize desensitisation to the salty taste. Raw, untreated fish was also tasted as a control, and to check for quality defects. Water was used to rinse the mouth between tastings when needed. The samplers discussed and took shared notes during the tasting, registering salt intensity in the order of not enough-, possibly too little-, probably right-, possibly too much- and definitely too much salt (in comparison to the salt levels of commercially produced smoked salmon). Some notes were also taken on texture, mouth feel and taste in samples of different salt treatments.

After sensory evaluation of salt intensity, some of the remaining samples that were not found to be definitely too salty were used to do a brief taste test of newly acquired liquid smoke products:

Two solutions of different concentration were made with the PA24 water soluble liquid smoke condensate; 25 % (50g) and 10 % (20g) smoke condensate in water – topped up with water (150g and 180g respectively) to make 200g of solution each. A 10 % solution of the SMOKEZ OIL H SF smoke oil were also made by mixing 10g with 90g of refined rapeseed oil (figure 9).

Figure 9. Liquid smoke dilutions: 10 % oil soluble (left), 10 % water soluble (middle) and 25 % water soluble (right)

Three slices that were previously salted for 1 minute in the 25 % salt brine were put in the 25 % water soluble smoke condensate solution, and one taken out after 5, 30 and 60 seconds of immersion. The slices were immediately dipped in pure water to remove excess liquid smoke and then put on kitchen paper to remove excess water. The procedure was repeated with the fish salted for 1 minute in 12 % salt brine with the 10 % water soluble smoke condensate solution. Also, three slices of the fish salted for 3 minutes in 12 % salt brine were immersed in the 10 % smoke oil dilution and taken out one each at the same time intervals (5, 30 and 60 seconds), and directly put on kitchen paper to drain off excess oil.

The smoke flavoured salmon slices were then tasted and discussed by the same two persons evaluating the salting, and joint agreed notes were taken on flavour and textural changes.

Brief summary of the process:

1. Slicing salmon fillet to slices of approximately 3-4 mm in thickness

2. Immersion of salmon slices in 12 % and 25 % salt brine for different times (1, 3, 5, 7, 10, 15, 20 and 30 minutes)
3. Sensory evaluation of salt intensity in brined salmon slices
4. Test-immersion of salted salmon slices in water- and oil soluble natural smoke condensates
5. Sensory descriptive evaluation of salted and smoke flavoured salmon slices

3. Salmon: Second brine salting and liquid smoke treatment of thin slices – new liquid smoke flavour recommended for fish, adjusted process, and an additional side-experiment

After reviewing the results and experiences from the previous experiment, an adjusted experiment was conducted, mainly narrowing in on salting times and changing the smoke condensate to one particularly recommended for fish products:

An 18 % salt brine with a 1:3-relation of added sugar to salt were made by mixing 360g salt, 120g sugar and 1520g tap water to a total of 2000g brine. The brine was placed for a few hours at 4 °C to stabilize temperature. Two frozen salmon loins were thawed in cold tap water, then the thickest part of the fillets were sliced by hand at an angle to make 29 slices of 3-4 mm in thickness.

1000g of the brine were used to immerse salmon slices for 30, 60, 90, 120 and 180 seconds – five slices for each salting time, a total of 25 slices. The slices were immediately dipped in pure water to remove excess brine and then put on kitchen paper to remove excess water.

A 10 % and a 33 % liquid smoke condensate solution were made by mixing 20g and 66g SMOKEZ 5111 with 180g and 134g tap water respectively. Four slices from each salting time treatment were subsequently immersed in the 10 % liquid smoke solution. Two were removed after 30 seconds and the other two at 60 seconds. The slices were immediately dipped in tap water and put on kitchen paper to drain off excess water. The remaining one slice from each salting time treatment were immersed in the 33 % liquid smoke solution for 5 seconds, dipped in water and put on kitchen paper.

As there were 4 slices left, a tiny experiment were put together by diluting remaining salt brine with 10 % SMOKEZ 5111 liquid smoke condensate, making up a brine of 16.2 % salt, 5.4 % sugar, 10 % smoke condensate and 68.4 % water. 2 slices were immersed for 5 seconds and the remaining two slices for 30 seconds. One slice from each salting time treatment was

dipped in water to remove excess smoking brine, while the other was not. All slices were drained on kitchen paper.

Each group of two- or single slices was vacuum sealed individually and stored at 4 °C for sensory evaluation the next day.

The differently treated salmon slices (a total of 19) were evauated individually by two persons. Samples were coded in letters and numbers. The samples were evaluated on hedonic scales of 1-9 points for salt intensity, smoke flavour intensity, smoke aroma liking and overall liking (total impression). Some notes were also taken on texture, mouthfeel and flavour. Drinking water and spitting buckets were available throughout the tasting.

Brief summary of the process:

1. Slicing of salmon fillets to slices of approximately 3-4 mm in thickness
2. Immersion of salmon slices in 18 % salt brine for different times (30, 60, 90, 120 and 180 seconds)
3. Immersion of salmon slices in 10 % and 33 % water soluble liquid smoke condensate solutions for different times (5, 30 and 60 seconds)
4. Immersion of salmon slices in combined salt and smoke condensate brine for different times (5 and 30 seconds) – with or without rinsing samples after treatment
5. Vacuum packing of samples and storage at 4 °C for one day
6. Sensory evaluation of all samples using hedonic 9 point scales and descriptive evaluation

Calculations on results (mean values, variance and standard deviations) were done by their respective in-built functions in Microsoft EXCEL.

Results

1. Halibut: Different salting methods before equal treatment with liquid smoke

Different salting methods – as well as treatment with a liquid smoke solution – *indicate* dramatic and rather systematic impact on fillet weight change. Immersion in the brine of lowest salt concentration gave the highest weight gain among the brined fillets, whereas dry salting led to a weightloss. Liquid smoke application and storage for three days led to weight losses (liquid losses) in all samples, with a concise trend in that the fillet that gained the most weight during salting also lost the most weight in the three days after smoke treatment, whereas the fillet that lost weight during salting (dry salted) lost the least weight in the days after smoke treatment (table 3, rightmost column). It must be stressed that these "results" can only be taken as mere suggestions or indications, without any statistical evidential backing – as the experiment was too simple to provide any of such.

Table 3. Weight changes in halibut fillets after various salting treatments and subsequent equal liquid smoke treatment

Salting method	Weight change compared to fresh fillet weight		Relative weight change during the 3 first days after smoke treatment (%)
	After salting for 50 hours (%)	3 days after liquid smoke treatment/ final weight change upon consumption (%)	
12 % brine	+ 20.7	+ 7.8	– 10.7
20 % brine	+ 9.4	+ 0.4	– 8.2
25 % brine	+ 0.8	– 6.3	– 7.0
Dry salting	– 15.2	– 17.7	– 2.9

With regards to sensory evaluation, the participants found the halibut salted in 12 % brine as the least salty, and the dry salted as the most salty out of the differently salted samples ($p < 0.05$). It can not be shown with statistical significance that the participants were able to separate the 20 %- and 25 % brine salted halibut with regards to perceived salt intensity.

The participants' free written descriptions of each sample revealed that all but the 12 % brine treated halibut were above pleasantly salty. Also, all participants reported smoke and smoke related aromas and flavours in all samples; descriptive words used being *distinct-*, *strong-*, *mild-* and *fresh smoke aroma/taste*, as well as *bonfire*. Two of the participants also

commented on observations of distinctly *yellowish* to *orange-like* coloured rinds/edges on all samples.

2. Salmon: First brine salting and liquid smoke treatment of thin slices

Sensory evaluation of salmon slices immersed for different lengths of time shows that an immersion time of ten minutes or more in 12 % salt brine resulted in a product described as *definitely too salty* compared to commercial smoked salmon. For 25 % salt brine the line was drawn at 5 minutes of immersion (table 4).

Notes on texture shows that the 12 % salt brine was gentle on the salmon, whereas the 25 % brine led to immediate and severe, negatively associated effects on texture from 5 minutes of immersion and outwards (table 4). Additionally it is worth mentioning that the slices immersed for 30 minutes in 25 % salt brine had developed a clearly rancid flavour.

Table 4. Descriptive sensory evaluation of salmon slices immersed for different lengths of time and in brines of different salt concentration

<table>
<tr><th rowspan="2">Brining time (minutes)</th><th colspan="2">12 % salt brine</th><th colspan="2">25 % salt brine</th></tr>
<tr><th>Salt level</th><th>Texture</th><th>Salt level</th><th>Texture</th></tr>
<tr><td>1</td><td rowspan="2">Probably right</td><td rowspan="6">No obvious surface protein denaturation</td><td>Probably right</td><td rowspan="2">No obvious surface protein denaturation</td></tr>
<tr><td>3</td><td>Possibly too salty</td></tr>
<tr><td>5</td><td rowspan="2">Possibly too salty</td><td rowspan="2">Def. too salty</td><td rowspan="4">Dry and miscoloured. Distinct protein denaturation worsening with increased salting time.</td></tr>
<tr><td>7</td></tr>
<tr><td>10</td><td>Def. too salty</td><td rowspan="3">Extremely salty</td></tr>
<tr><td>20</td><td rowspan="2">Very salty</td></tr>
<tr><td>30</td></tr>
</table>

Trends in the effects of different liquid smoke preparations on salted salmon slices were that all gave disturbing off-flavours (in addition to smoky flavours) – worst for the oil soluble liquid smoke. The strongest liquid smoke solution (25 % liquid smoke dilution in water) also led to distinct surface denaturation/drying on the salted salmon slices immersed for 30- and 60 seconds, but not for 5 seconds (table 5).

Table 5. Descriptive sensory evaluation of salted salmon slices immersed in different liquid smoke products and dilutions for 5, 30 and 60 seconds

Smoke flavour product and dilution	Smoke immersion time (seconds)		
	5	30	60
10 % PA24 in water	Edible. Unpleasant perfume/chemical after-taste		Too intense perfume/chemical flavour
25 % PA24 in water	Horrible, perfume/chemical flavour	Acid denaturation, dry surface Horrible, perfume/chemical flavour	
10 % SMOKEZ OIL H SF in rapeseed oil	Oily, soft surface and texture Horrible perfume/chemical flavour, worse than for the water soluble smoke		

3. Salmon: Second brine salting and liquid smoke treatment of thin slices – new liquid smoke flavour recommended for fish, adjusted process, and an additional side-experiment

In total, none of the 19 different samples received higher than 3 out of 9 points on a hedonic scale for *overall liking*. Combined mean value score for overall liking of *all* 19 samples as assessed individually by two persons was 2.08 with a variance of 0.62 and standard deviation of 0.78. Results by sensory evaluation on salting were inconclusive in if the samples became sufficiently salted, but showed trends in rising salt content with increased salting time.

Sensory descriptive notes and afterwards discussion revealed that the samples had a peculiar off-flavour similar to those in the first salmon experiment (perfume/chemical). It was also consequently noted a considerable unpleasant surface protein denaturation/drying on samples immersed for 5 seconds in 33 % liquid smoke solution as well as in the samples of the side-experiment of 5 and 30 seconds immersion of salmon slices in a combined salt (16.2 %) and liquid smoke (10 %) brine. These samples were also described as particularly *watery*.

Discussion

The base structure in experimental design of the conducted experiments are clearly too simple to produce any strong evidential findings based on statistical significance. This was also not the intention, as the experiments were merely intended as initial orientation on the approachability to largely "unknown terrain", both for the author, the others involved in the pilot project, and as well with regards to available scientific literature.

The author would also like to argue that in the early stages of new product development it may even be an advantage not to have a too strict and narrow experimental design as new discoveries are often (at least partly) unintentional and by accident. This angle of incidence has also been attemptively maintained in that open descriptive evaluations and discussions have been encouraged throughout evaluation of experimental samples.

Still, the possibly greatest advantage for result documentation and evaluation would have been to check the persons involved in sensory evaluation for sensibility- and ability to recognize as well as discriminate basic tastes (salt, sweet, bitter, sour and umami). Also, the participants should have been properly trained and coordinated to a common platform of understanding for hedonic scales, sensory concepts and descriptive language (Lawless & Heymann, 2010).

Another issue, which is highly probable to affect findings to some degree, particularly with the small sample production and without conducting repetitions in sensory evaluation, was the handslicing of salmon fillets by knife. This most certainly led to some unevenness in slice thickness, both within- and between slices, which could lead to deviances in salt uptake and impact of smoke flavour intensity.

Also, much processing was done in room temperature. This should not have affected salt uptake too much, as care was taken to properly cool brines and fish before processing, as well as considering the fast production times. Salt brine temperatures were measured to have risen from 4 °C at the beginning of salting to 11 ±1 °C at the end of the 30 minute salting runs in the first salmon salting experiment. Salmon slices left longer than the first 10 minutes in 12 % salt brine, and longer than the first 5 minutes in 25 % salt brine was regarded as 'definitely too salty' and therefore irrelevant. One can therefore roughly assume that the slices brined to a desired salt level were salted within the temperature range of 4 °C to maximum 8 °C.

The intention throughout the experimental work was to conduct a final and more thorough sensory evaluation comparing 'the new smoked salmon product' with conventional cold smoked salmon, but as the samples produced never reached satisfactory acceptability in the initial tests it was concluded that a comprehensive sensory evaluation would have been an unnecessary waste of resources at this stage.

The first experiment (on salting and liquid smoke treatment of halibut) functioned to demonstrate that liquid smoke condensate has the potential to give fish products similar flavours and aromas to that of conventional smoking. All participants in the sensory evaluation clearly recognized smoke flavours and -aromas in all the different samples, but an informal discussion held after the assessment revealed that at least some of the participants were not satisfied with the flavour from the liquid smoke. Birkeland and Skåra (2008) concluded that use of smoke condensate is suitable to produce cold smoked salmon with quite similar quality characteristics to conventionally smoked salmon, but stresses the importance of production procedures- and smoke condensate formula optimization. Varlet et al. (2010) emphasized the large variation- and potentials in tailoring smoke condensate formulas for specific needs, and pointed out the importance of finding 'the exact right' smoke condensate for the specific raw material and production process to succeed in producing appealing smoke flavoured seafood products. Finding the right smoke condensate may also be important for other properties such as textural and physicochemical, where for example Martinez et al. (2007) found largely different and contrasting influences between two different smoke condensates on salted salmon.

The halibut experiment also demonstrated expected but somehow dramatic changes in fillet weights during salting, depending on salting method and brine concentration. A surprise was that the fillet in 25 % salt brine held its weight (+ 0.8 %), as Gallart-Jornet et al. (2007a, 2007b) reported weight losses in the order of 5-10 % of fillet weight in salmon and cod under the same conditions (4 °C storage for 50 hours, 25 % salt brine, 3 parts brine to 1 part fish). These findings are beside the focus of this thesis, but demonstrate the influence of salting method on yield – which of course is of great economical interest to food producers.

The sliced salmon salt brining experiments showed that just a few minutes in salt brine would be sufficient to gain a similar salt content to that of conventionally smoked salmon. This is a dramatic saving in time spent salting, as conventional brine- or dry salting of whole salmon fillets typically take at least 12-24 hours. Finding a suitable liquid smoke with regards to

desired flavour profile did not succeed in these experiments, but if one were to find one, this would also have been considerably saving on production time, as immersion or drenching in liquid smoke condensates generally seldom exceeds three minutes in production of smoke flavoured foods. Conventional smoking on the other hand typically takes at least a few hours, and often longer.

A suggestion for further research would be to conduct physical salt analyses on salted salmon slices to better document the salting times needed – also in relation to different raw material qualities (fresh, frozen/thawed, rigor status, slice thickness etc), different low temperatures during salting, and the effects of different brine strengths.

With regards to the liquid smoke off-flavours found, it could be a step forward to put some work into identifying the specific compounds responsible, and if these can be removed from the smoke condensate or at least if the liquid smoke application method can be adjusted to not favour adhesion/uptake of these in the fish. The off-flavours was described as perfumy/chemical, which probably translates (at least partly) to the term *medicinal* (hospital, chemist's, laboratory) as used by Ojeda et al. (2002) to describe smoke flavourings. Findings by Ojeda et al. attribute this sensory quality to the substances guaiacol, d-camphor, m-cresol and o-cresol. In the experimental work for this thesis, temperatures were particularly low during processing. Also, a comprehensive muscle surface drying regime – as is a common production step in conventionally smoked salmon – was left out to prevent surface crusting of the salmon slices. This may have caused a different interaction between the salmon and certain specific substances from the smoke condensate – leading to a highly different flavour profile.

The sliced salmon brining technique demonstrated here could possibly open for new production techniques – in changing from the conventional batch production to a continuous production flow. It is possible that the sliced salmon liquid smoke treatment technique proves too difficult for producing a product similar to conventional smoked salmon, as it – and the results it produces – might deviate too much from consumer preferences. Smoking – also with liquid smoke – leads to a coloured surface/rind on the conventionally produced fillet product. This would obviously be undesired on the pre-production sliced salmon, as this would then consist of only the coloured and dry surface and none of the succulent meat, as is revealed when slicing a conventionally produced smoked salmon fillet.

The pre-production slicing technique could also (and possibly better) be utilized for faster production of smokeless salt/sugar cured fish products such as ‘gravlax’ or similar products, by introducing other non-smoke flavourings with the salt brine.

Conclusion

The main finding in this work is that in brine salting of salmon slices (< 5 mm), salting times are reduced to mere seconds or minutes – in comparison to hours or days for salting of whole fillets. It is still unclear if the extra low temperature processing conditions may have any particularly beneficial effects on product quality. The use of liquid smoke condensates for smoking was not successful in producing a tasty product due to prominent off-flavours from all liquid smoke products tested.

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