Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

Melissa Moe Dahle

A comparison of atomized purified condensed smoke and traditionally wooden smoke in processing of cold smoked salmon (Salmo salar), in relation to quality, yield and food safety

Master's thesis in Biotechnology (MBIOT5)
Supervisor: Jørgen Lerfall
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Kunnskap for en bedre verden

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1 Abstract

Consumer demands are pushing the food industry to produce more healthy and less preserved products, which also is the case for cold smoked salmon (CSS). For CSS this involves reduced salt content, but changes in the smoking technology is also possible. One such technology of interest is purified condensed smoke (PCS). The use of PCS is regarded as more healthy due to lower content of polyaromatic hydrocarbones (PAH).

The effects of traditional wooden smoke and atomized PCS when producing CSS fillets regarding microbiological growth, processing yield, chemical and quality parameters, packing technologies and sensory attributes were studied through three experiments. The salt concentration (%) was held equal in fillets within each experiment (dry salted for 6 hours in Experiment 1 and 3, and 16.5 hours in Experiment 2). The smoking process ranged from 90-150 minutes (traditionally) and 3-5 cycles (PCS, 1 cycle consisted of 21 minutes smoking). Aerobic plate count (APC), lactic acid bacteria (LAB), pH, colorimetric properties, NaCl content, texture, ATP breakdown products, Napping®, processing yield and TPC were measured. In addition the effect of PCS and SGS treatment were studied on CSS inoculated with *L. innocua*.

Microbiological growth on APC had a tendency of being lower in PCS treated fillets. Lower microbial growth of PCS treated fillets is probably related to lower processing yield and pH. Texture (80 % force and breaking force) showed a tendency to be higher in fillets treated with PCS, probably caused by more extensive drying. Lightness (L^*), redness (a^*) and yellowness (b^*) were observed as lower in PCS fillets, although the assessors in sensory Napping® revealed no negative colour associations. No influential differences was seen in ATP breakdown products of traditionally or PCS smoked fillet. The effect of SGS treatment in PCS treated and traditionally smoked fillets showed reduced growth of APC and *Listeria* spp.

Sammendrag

Forbrukerkrav presser matindustrien til å produsere sunnere og mindre konserverte produkter, noe som også er tilfelle for kaldrøkt laks (CSS). For CSS innebærer dette redusering av saltinnhold, men endring i røyketeknologi er også mulig. En slik teknologi av interesse kan være renset kondensert røyk (PCS). Bruk av PCS anses for å være mer sunn på grunn av lavere innhold av polyaromatiske hydrokarboner (PAH).

Effektene av tradisjonell røyking og atomisert PCS ved produksjon av CSS-fileter ble studert gjennom tre eksperimenter, ved å fokusere på mikrobiologisk vekst, prosessutbytte, kjemiske og kvalitetsparametere, pakketeknologier, og sensoriske attributter. Saltkonsentrasjonen (%) ble holdt lik i fileter ved hvert eksperiment (tørrsaltet i 6 timer i eksperiment 1 og 3, og 16,5 timer i eksperiment 2). Røykeprosessen varierte fra 90-150 minutter (tradisjonelt) og 3-5 sykluser (PCS, en syklus besto av 21 minutter røyking). Aerobic plate count (APC), melkesyrebakterier (LAB), pH, fargeegenskaper, NaCl-innhold, tekstur, ATP-nedbrytningsprodukter, Napping®, prosessutbytte og TPC ble målt. I tillegg ble effekten av PCS og SGS-behandling studert på CSS inokulert med *L. innocua*.

Mikrobiologisk vekst på APC hadde en tendens til å være lavere i PCS-behandlet fileter. Lavere mikrobiell vekst av PCS-behandlede fileter er sannsynligvis relatert til lavere prosessutbytte og pH. Textur (80% kraft og bruddkraft) viser en tendens til å være høyere i fileter behandlet med PCS, sannsynligvis forårsaket av mer omfattende tørking. Lyshet (L^*), rødhet (a^*) og gulhet (b^*) ble observert som lavere i PCS-fileter, selv om vurderingene i sensorisk Napping® oppga ingen negative fargeassosiasjoner. Det ble ikke sett noen innflytelsesrike forskjeller i ATP-nedbrytningsprodukter av tradisjonell eller PCS-røkt filet. Effekten av SGS-behandling i PCS-behandlede og tradisjonelt røkt fileter viste redusert vekst av APC og *Listeria* spp.

Contents

2 Introduction

The interest of fresh and lightly preserved seafood is continuously growing with consumers that want less processed food [Kerry, 2012]. At the same time, these products are highly perishable and preservation methods are essential to assure safe products [Gram, 2009, Løvdal, 2015]. The combination of producing more lightly preserved seafood products and prevent spoilage is becoming more difficult [Özogul et al., 2010], and many different hurdles are being research at [Wiernasz et al., 2017, Porsby et al., 2008, Montiel et al., 2012]. Smoking is ascertained to be one of the oldest preservation methods [Doe, 1998], including in prevention of decomposition in seafood [Kerry, 2012]. Up to the late seventies products such as cold smoked salmon (CSS) was even considered as a high-valued product often sold in delicacy shops. Today CSS has lost some of its high prestige reputation [Røra et al., 2004], but it is still seen as a lightly preserved fish product of considerable economic importance world wide [Hansen et al., 1995].

In 2019 Fiskeri og Havbruksnæringens forskningsfond (FHF) declared NOK 1.5 million to provide knowledge as a basis for producing smoked salmon with a lower salt content than today's practice [Bævre-Jensen, 2020]. The background was to prevent lifestyle diseases such as high blood pressure and cardiovascular disease. With lower degrees of salting, other preservatives must be complemented to ensure safe products [Løvdal, 2015].

2.1 Cold smoked salmon (CSS)

Fish contains most of the minerals necessary for a nutritional diet, such as iodine, calcium and phosphorous [Burt, 1988]. Most importantly, salmon are known for its high content of long chain polyunsaturated fatty acids (PUFA), which is recognized as beneficial for human health [Kris-Etherton et al., 2002, Din et al., 2004, Rennie et al., 2003]. And, depending on the fish and other factors, fish is also a source for proteins [Burt, 1988]. All these beneficial nutritional factors makes fish a desired meal world wide.

The processing method of cold smoked salmon (CSS) consist of three fundamental stages; salting, dehydration and smoking [Doe, 1998, Özogul et al., 2010] (fig. 2.1). As mentioned, CSS is classified as a lightly preserved fish product. This implies that the product is only exposed to low concentrations of salt and smoke, in an absence of thermal treatment [Gram and Huss, 1996]. Furthermore, CSS is considered as Ready-To-Eat (RTE) product, which means that it should not require heating or any other processing upon eating. The combination of salting, dehydration and smoking in the production process, and the amount and time used, are a fine tuned balance in creating a safe CSS product that also have the right sensory attributes [Løvdal, 2015].

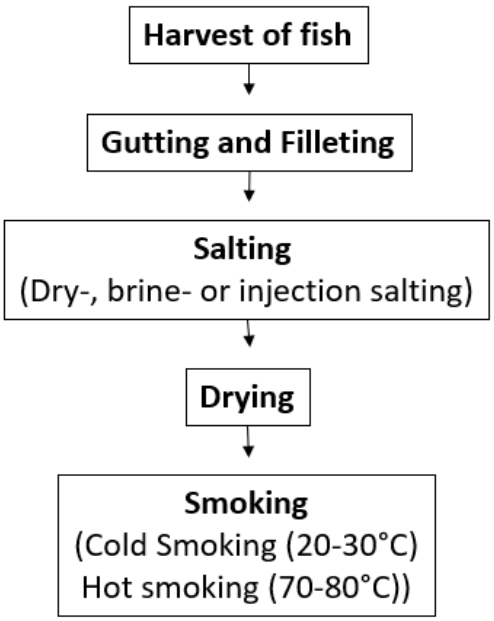

Figure 2.1: The main steps in a smoking process of salmon; harvesting, gutting and filleting, salting, drying and smoking. The time used in every step is a fine tuned balance to generate an excellent and valuable RTE product.

2.1.1 The effect of smoking

The most common practice for harvesting Atlantic salmon (*Salmo salar*) is by bleeding, gutting and icing the fish immidiately after harvesting. In this way the fish is prevented from bruising or mechanical gaping, which can be a result by handling the fish in-rigor [Skjervold et al., 2001, Birkeland et al., 2007].

Before the fish can be exposed to smoke it must be salted, either by injection, by dry salting or by brine salting [Adeyeye, 2019, Hansen et al., 1995, Birkeland and Bjerkeng, 2005], where the latter two being the most commonly used in salmon processing industry [Birkeland and Bjerkeng, 2005]. Until early 20th century, fish products were heavily salted. Modern refrigeration and packaging technologies have on the other hand reduced the need of severe curing methods [Doe, 1998, Burt, 1988, Horner, 1997]. The final salt concentration of smoked salmon is usually between 2.0-3.9% in the water phase [Hansen et al., 1998, Bannerman and Horne, 2001], thus categorizing smoked salmon as a "lightly preserved" product [Françoise, 2010], as earlier mentioned. Salt functions as a flavour enhancer [Terrell, 1983, Gallart-Jornet et al., 2007], increasing the perceptions of the fullness, thickness and sweetness of food. The uptake and distribution of salt in fish fillets depends on the salting methods used, the species, the thickness of the fillet [Gallart-Jornet et al., 2007], the fish:salt ratio and the flesh factors (composition of the muscle, rigor condition etc.) [Aursand et al., 2010]. When dry salt is used for salting, two simultaneous alterations occurs: the uptake of salt by the muscle and the loss of water [Horner, 1997, Rørå et al., 2004]. Furthermore, salt has a noticeable preservative effect by reducing the water activity and subsequenyly preventing growth of many spoilage microorganisms [Horner, 1997, Doe et al., 1983]. It also forms a more membranous surface which further inhibits microorganism growth [Cardinal et al., 2001].

Drying is usually performed after salting, to remove excess water from the flesh [Doe, 1998,

Burt, 1988]. Drying may involve addition of heat, removal of water, addition of bacteriostatic compounds and pH changes [Doe, 1998]. The water inside the pre-smoked product moves from within the product to the surface by a combination of molecular, vapor, and liquid diffusion [Maga, 2018]. At the same time solutes (salt and smoke components) can diffuse in the other direction [Doe, 1998]. Moisture is then continuously removed from the surface of the product by convection, where the rate of water evaporation is determined by air speed, temperature and humidity [Burt, 1988]. Thereby smoking, cooling, packaging and storage follows [Adeyeye, 2019].

Smoking is defined as the process where volatile compounds from thermal destruction of wood penetrates the surface of the product being smoked [Maga, 1987]. It represents a set of chemical, thermal, diffusive and biochemical processes that occur in a preliminary salted product. Taste, colour and aroma are positive developments from smoking [Burt, 1988, Maga, 1987, Guillén and Manzanos, 1996], including the preservative effect [Kristinsson et al., 2008]. The preservative effect is mainly due dehydration, and bactericidal and antioxidant properties from the smoke [Adeyeye, 2019]. The changes regarding colour, taste, aroma, texture and composition of the food depend on the composition of the smoke, smoke flavouring used, the composition of the fish itself, and the condition under which type of smoking is undertaken [Toth and Potthast, 1984]. In production of CCS, the absence of thermal treatment makes the parameters salting and smoking utmost important in order to minimize the risk of foodborne hazards and spoilage [Løvdal, 2015].

Effect of phenols
Numerous researchers have concluded that phenols are the main contributors to wood-smoke aroma, while others disagree - since there are other classes of compounds affecting typical smoke aroma properties. Phenols are aromatic hydrocarbons composed of benzene, with varying degree of hydroxy groups attached. Furthermore, phenolic compounds can also have other functional groups attached, such as aldehydes, acids, ketones and esters [Maga, 1987, Varlet et al., 2007]. Phenolic compounds such as syringaldehyde and coniferaldehyde get strongly retained by food and intensify the aroma of smoked fish. Their adhesion also prevents them from being lost over time by evaporation [Varlet et al., 2007]. Without considering wood type or combustion conditions, a relative high amount of phenols (10-60 times higher concentration than in vapor phase) can be produced in wood smoke. The type of smoke application utilized will although influence the phenolic concentration, and this must be taken into account since it will affect the smoked product flavor and shelf life [Maga, 2018].

Another important attribute from phenolic compounds is antimicrobial properties [Kristinsson et al., 2008]. Though, not all the phenolic compounds produced in wood smoke will end up in smoked food, and the concentration of phenols is said to be decreasing with storage. Another factor affecting the concentration of phenols in smoked products is their degree of penetration into food, leading to higher concentration of the outer parts. The concentration of phenols can also vary according to the fat content of the smoked product. In addition, solubility of most phenols will increase significantly with increasing acidity, thus at too low acidity some phenols can settle out during the prolonged storage of liquid smoke [Maga, 2018].

2.1.2 Smoking Technologies

Smoking is an important hurdle in the production of CSS [Doe, 1998, Burt, 1988]. The bactericidal properties of smoke is a result of the deposition of polyphenolic constituents on the product being smoked [Kristinsson et al., 2008]. Formaldehyde and acetic acid are the two main constituents found to show bacteriocidal effect, with preventing fungal growth and inhibit viral activities. Since the deposition of smoke mainly happens on the surface of the product, the

surface gets more effective against bacteria than the inner portion of the fish [Adeyeye, 2019].

Smoking can be performed cold (20-30 °C) [Mørkøre et al., 2001] and hot (70-80 °C). Cold smoking will not cook the flesh, coagulate the proteins, inactivate food spoilage enzymes or eliminate food pathogens. This means that storage at refrigerated temperatures is necessary to secure food safety [Adeyeye, 2019]. This process is designed to give the desired flavour to the product, rather than a significant degree of preservation [Burt, 1988, Guillén and Manzanos, 1996]. Therefore, in drying of CSS the process of salting will have a bigger impact in lowering the water content than the effect from increasing the temperature [Doe, 1998, Kristinsson et al., 2008].

Today, smoking happens mainly through the use of wood smouldering [Adeyeye, 2019]. Wood smoke consists of thousands individual components, where only 350 chemical compounds have been identified [Maga, 1987]. Composition of the compounds and their yield is dependent on temperature and oxygen concentration during thermal degradation of the wood, and to lesser extent the type of wood chosen [Doe, 1998]. The smoke contains many different compounds, mainly phenols, carbonyls, acids, furans, alcohols and polycyclic aromatic hydrocarbons (PAH) [Maga, 1987, Varlet et al., 2007]. Among those, PAH is known for its mutagenic and carcinogenic properties [Guillén and Manzanos, 1996, Pool and Lin, 1982]. This have resulted in technologies to eliminate PAH from the smoke aromas to avoid contamination of the food products [Pool and Lin, 1982]. PAH consist of multiple benzene rings fused together, and their presence in food has been of major concern. Smoked food products are though not the only source of PAH, they can also be found in other various sources, such as air pollution, incomplete combustion of carbon and hydrogen, petroleum and coal products [Maga, 2018].

Liquid smoke, also known as purified condensed smoke (PCS), is purified from PAH. PCS is often used in the production of meat [Maga, 2018]. Advantages of using PCS in products includes: (1) The flavour incorporated in the product can be performed uniformly throughout the product, instead of creating av gradient with lower intensity of smoking on the inside of the product, (2) there can be achieved closer control on the amount of smoke flavor transmitted to the product, (3) it can easily be used on a consumer level as well as in a commercial processing level [Maga, 2018], and (4) depending on the flavour and taste requested for, it has a chance to be the cheaper and healthier option to produce CSS [Singh et al., 2016]. The most important advantage of PCS is however, that in the production of PCS PAH are removed through heating, condensation and separation, before the smoke is filtrated [Singh et al., 2016, Guillén and Manzanos, 1996]. In the smoking process a liquid flow of atomized vapour is added in circulations to a closed chamber of compressed air. This creates a regenerated smoke, that can both be seen as more healthier and more environmental friendly [RedArrow, 2020].

Although condensed smoke can be created and utilized in various forms, the classical liquid smoke usually consists of smoke condensate dissolved in water, oil or smoke extracts in organic solvents [Maga, 2018]. The colour of freshly smoke condensed in water is usually bright yellow, but after being conducted on the product it darkens due to the formation of brown-colored condensation or polymerization products [Toth and Potthast, 1984, Maga, 2018], which can been seen as a negative effect [Valø et al., 2020]. The compounds usually consists of tarry products and PAH, but will settle out in time. The liquid can thereafter be decanted, which results in a liquid smoke low in PAH amounts. Smoke that on the other hand is condensed into water and contains solvents such as ethanol and glycerol does not precipitate tars and PAH. [Maga, 2018].

2.2 Sensory Properties

Smoking of meat and seafood often includes salting, curing and heating [Burt, 1988, Doe, 1998, Horner, 1997]. These processes cause desirable changes to proteins, where distinct ripening can occur. Smoking can also give changes in flavour and colour to the product [Guillén and Manzanos, 1996], but these changes depend on the degree of processing and the quality of the fish. In the beginning, methods of smoking were purely performed for pragmatic reasons - to avoid spoilage. Today, cold storage and distribution networks have been improved over the years, but the preference for taste and texture of smoked products are still being sought and demanded for [Doe, 1998].

2.2.1 Water Activity

Water activity is said to be a *measure of the water available in a system for biochemical and chemical reactions, and for microbial activity* [Wood, 1981]. It is defined as the vapour pressure of the product divided to the vapour pressure of pure water at the same temperature [Burt, 1988]. It should not be confused with moisture content, which is *a measure of the total amount of water in the food*. Some of this water is not available for microbial growth, since it is bound physically and chemically to proteins substrates or salts [Doe, 1998].Reduction of water activity is important to produce a product resistant to microbial activity [Burt, 1988, Horner, 1997]. The water activity is inversely related to the amount of smoke components and the content of salt in the water phase [Doe, 1998]. Important aims is though to avoid a product with exceeded desired levels of salt or a dry product with unpalatable appearance [Burt, 1988]. As mentioned previously, salting and drying of the product prior smoking decrease the water activity [Horner, 1997], and by such means contributes to preservation.

2.2.2 Colour and Appearance

Meat colour is the quality factor most used by consumers today, since this is the only sensory attribute that can be observed in the market [Kerry, 2012, Maga, 2018]. The pink coloration of salmonoid fish flesh is owed to carotenoid pigments, notably astaxanthin and canthaxantin, that fish accumulate through their diet. After smoking, the colour of the flesh can vary depending on (1) the pigments present, (2) quantity and composition of smoke deposits, (3) interactions between these and the flesh [Doe, 1998], temperature, moisture level and the method of smoke application [Maga, 2018].

In the concentration of reactants carbonyls are the reactant most influential to color formation [Toth and Potthast, 1984, Cardinal et al., 2004]. Research indicate a chemical reaction involving interactions between carbonyls in the smoke and amino acids on the food surface [Martinez et al., 2011]. Furthermore, some phenols are associated with the contribution of color formation in smoked foods. These phenols must have a high molecular weight, so as to have enough number of hydroxyl groups to cross-link proteins [Daun, 1972]. The number of such phenols in smoke are rather low, compared to the amount of carbonyls present. Simple phenols in smoke are on the other hand absorbed by lipids [Maga, 2018]. With temperature also influencing color formation, faster developing of colour will happen at increasing temperature of the product surface. This results in more surface color formation in hot smoking vs. cold smoking. It also appears that maximum colour formation on smoked products happens at surface moisture between 6 and 10%. This is due since if the surface of the smoke product is too moist, the reaction of colour formation will be too slow and in a dry smoke product on the other hand the carbonyls will not be absorbed [Maga, 2018].

Browning reactions are broadly classified as enzyme-catalyzed and nonenzymatic. They can

affect colour and appearance and may also influence the flavor, nutrition and possibly the safety of fish. Enzymatic browning is oxidation of phenols to *o*-quinones, where the product undergoes various cross-linking reactions leading to the formation of melanin. In non-enzymatic browning, the principal mechanisms in fishery products are Maillard browning [Ruiter, 1979] and protein-lipid browning. Maillard browning predominates at high water activities, and Protein-lipid browning at low water activities. Maillard browning requires amino-bearing compound, a reducing sugar and some water. The forming end products are melanin [Doe, 1998]. Oily fish tend to go browner because of the interactions between amino acids from proteins and carbonyl derivatives of lipids [Martinez et al., 2011], and is thus an example of protein-lipid browning. This reaction is more prominent in fatty fish than lean fish, and is influenced by factors affecting lipid oxidation [Doe, 1998].

Lipid oxidation is known for affecting odour and flavor, but can also affect colour. The red colour of fish muscle may decrease by autoxidation of the heme iron, and lipid oxidation lipids will lead to an increase in a more yellow hue [Kerry, 2012]. It has also been reported colour development from liquid smoke, which mainly depend on carbonyls, where volatile aldehydes give rise to orange in liquid-smoked fish [Varlet et al., 2007].

Images of food can be captured, and thus colours are determined using various system (e.g. L^*a^*b) to make correlations with food properties. L^*a^*b is an international standard for color measurements, adopted by the Commission Internationale d'Eclairage (CIE) in 1976 [Zhang et al., 1996, Pedreschi et al., 2006]. The L value represents lightness component on surface, and ranges from 0 to 100 (see fig. 2.2). The a and b values are chromatic components of redness to greenness and blueness to yellowness, that ranges from -120 to 120, respectively. The method gives uniformity in colour distribution and closeness to human perception [Hunt and Pointer, 2011, Jusoh et al., 2009, Pedreschi et al., 2006].

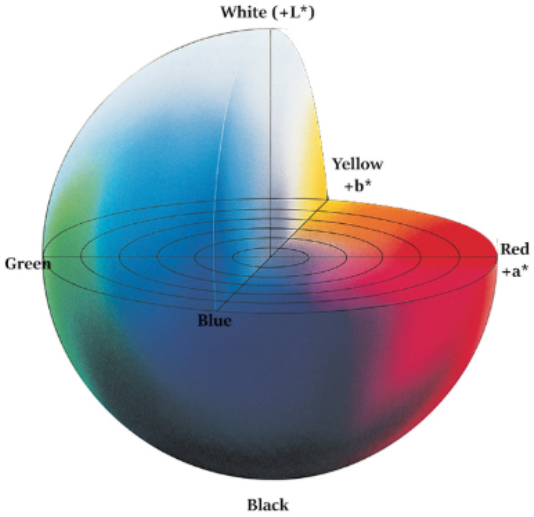

Figure 2.2: The CIE $L^*a^*b^*$ 1976 is an international standard used to measure colour. In the $L^*a^*b^*$ color space, L^* indicates lightness and a^* and b^* are chromaticity coordinates. a^* and b^* are color directions: +a* is the red axis, -a' is the green axis, +b* is the yellow axis and -b* is the blue axis [Konica Minolta Sensing Singapore Pte Ltd, 2020]

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2.2.3 Texture

The texture of seafood is affected by both autolytical changes that happens inside the fish, but also because of different conservation treatments performed on the product. Immediately after the fish is slaughtered an autolytic process starts, where degradation by endogenous fish enzymes happens, which affects texture[Kerry, 2012]. Protein changes during smoking can result in cross-linking of surface proteins. This can lead to firmer and more stable outer crust on the smoked product, which will be firmer than the softer inside portion of the product [Sigurgisladottir et al., 2000, Indrasena et al., 2000]. Tougher crust makes it hard for smoke constituents to penetrate toward the center of the product, which can result in uneven distribution of color and flavor [Maga, 2018].

A larger loss in weight during salting and drying/smoking decreases moisture content of the fish. Furthermore, a high salt content is avoided due to the trend of reducing dietary intake of sodium [Doe, 1998].

2.2.4 Water Holding Capacity (WHC)

Water loss can happen in chilled or frozen products in the form of desiccation, dehydration or evaporation. This can result in quality loss of the product [Kerry, 2012]. The ability of meat to retain its natural water content is called water-holding capacity (WHC) [Offer and Trinick, 1983].

Accordin to [Maga, 2018], Radetic et al found out that WHC was inversely related to the carbonyls and phenol concentration in smoked meat, and that pH and WHC was not significantly associated with each other. Areas with high concentration of carbonyls and phenols are therefore exposed to low degrees of WHC. Such areas is often the outer parts of the smoked product, where the carbonyls from the smoke usually attaches. A reason for this low degree of WHC can be that proteins on the outer parts of the smoked products usually will be connected to the water, but are now instead interacted with carbonyls and phenols [Maga, 2018]. In a texture speaking manner this will result in a dryer and tougher surface, compared to the interior of the product [Indrasena et al., 2000]. Based on this, the breaking force (the force to break through the surface of a smoked product) will be the most reliable measure to describe differences between smoked protocols used.

2.2.5 Lipid Oxidation and Rancidity

Fish lipids differs from other natural occurring fats in their chemical composition in that they (1) contain a greater proportion of highly unsaturated fatty acids, (2) they possess larger quantities of fatty acids with chain lengths longer than 18 carbons, (3) have polyunsaturates and (4) have a greater variety of lipid compounds. This high degree of unsaturated lipids makes fatty fish susceptible to the development of oxidative rancidity. In such a process, hydroperoxides are also formed and broken down to intermediatary products [Burt, 1988]. Such products are free fatty acids, diacylglycerides and monoacylglycerides, glycerol and nitrogen bases [Kerry, 2012]. These products are relatively unstable and can cause oxidation of pigments, loss of vitamins and the creation of rancid flavour [Burt, 1988]. Lipid oxidation is therefore recognized as one of the most important quality deterioration process, since both odour, flavour and nutritional value can be affected [Kerry, 2012].

The process of lipid oxidation can be caused by non-enzymatic processes such as autooxidation and photosensitized oxidation, but also by enzymes such as lipoxygenase [Kerry, 2012]. The role of water activity in lipid oxidation is controversial [Burt, 1988]. By reducing the water activity lipid oxidation will decrease because of the reduction in the mobility of the reactants. On the

other hand, at very low water activity lipid oxidation can be maximised, because the molecules are no longer "protected" by water and are now more accessible for oxidation [Damodaran and Parkin, 2017], and probably also because of the concentration of metal catalysts [Burt, 1988].

2.2.6 Taste and Aroma

The compounds responsible for taste of fresh fish include amino acids, peptides, organic acids, quarternary ammonium bases, and minerals. Many of these compounds are also contributing to taste in dried products, but to a large extent these compounds changes during the drying process [Doe, 1998]. A process of adhesion, condensation, diffusion and/or absorption between food and smoke occur [Toth and Potthast, 1984]. The aromatic flavour chemistry from smoking is quite complex, due to hundreds of new aromatic compounds can be formed. Some of the reactions contributing to changes in flavour in dried fishery products are browning reactions, lipid oxidation, action of endrogenous enzymes and microbial activity. From the smoke, the most important effect on flavor is exerted by polyphenols derived from the smoke, as earlier mentioned. Still, other volatile and water-soluble components can also contribute to the formation of the sensory profile of smoked fish. [Doe, 1998].

2.2.7 Breakdown Products of ATP

Post-mortem changes in fish muscle can shortly be summarized into: catching, rigor mortis, resolution of rigor mortis, autolysis and spoilage. The rate and extent of these reactions will among other things depend on the fish species, their health and the method of harvesting [Hong et al., 2017]. Fresh fish generally deteriorate by one of two mechanisms: bacterial spoilage or autolysis, where autolytic degradation results within the muscle from enzymatic changes [Surette et al., 1988].

During post-mortem storage of fish, the amount of ATP will decrease as a result of ceased ATP-production and because of the biochemical reactions that breaks down ATP (fig. 2.3). Inosine 5'-monophosphate (IMP) is one of the breakdown products, and is associated with a pleasant savory taste in seafood [Surette et al., 1988, Hong et al., 2017]. The enzymes responsible for the breakdown of ATP are normally inhibited from working. In a post-mortem fish on the other hand, the enzymes breaks down ATP rapidly. In the beginning, depletion of oxygen will force fish to produce ATP by the creatine phosphate (PCr) pathway and/or the anaerobic glycolysis pathway. These pathways do not produce ATP in the same extent, and in addition the storage of both PCr and glygocen will also deplete. At this time the breakdown of ATP will produce ADP, which is the substrate of the adenylayte kinase. Two ADP molecules can be converted to one ATP and one AMP. AMP can further be converted to IMP [Massa et al., 2005, Hong et al., 2017], which can be further degraded to inosine (ino), and later to hypoxanthine (Hx) [Saito and M., 1959].

The loss of IMP has been connected to the loss of freshness in some fish species ([Saito and M., 1959]). The rapid accumulation of Hx is apparently responsible for the offlavours and offodours sensed in perished seafood [Jones et al., 1964, Spinelli et al., 1964, Hong et al., 2017]. The amount of Hx and Ino produced can therefore be used as freshness indicators, but since many factors can affect their production [D'Amico and Di Natale, 2001] indexes with more than one compound from the ATP degradation products are often used [Nollet and Toldrá, 2009]. K value was originally defined by [Saito and M., 1959], as the ratio of Ino and Hx to the sum of ATP and its breakdown products expressed as percentage. However, [Karube et al., 1984] simplified the K-value by creating K_1-value, which excluded ATP, ADP and AMP. The reason was because several studies suggested that ATP, ADP and AMP dropped rapidly and IMP increased sharply after 24 h, and the amount of ATP, ADP and AMP would therefore not be

relevant [Hong et al., 2017]. The H value was considered as a better indicator for species having a high Ino accumulation, and is defined as the fraction of Hx to the sum of ATP degradation products in precentage (Hx/(Ino + IMP + Hx)*100 [Luong et al., 1992]. Regardless of the amount of indicators are used in indexes, some exceptions to the models can still be detected [Howgate, 2006].

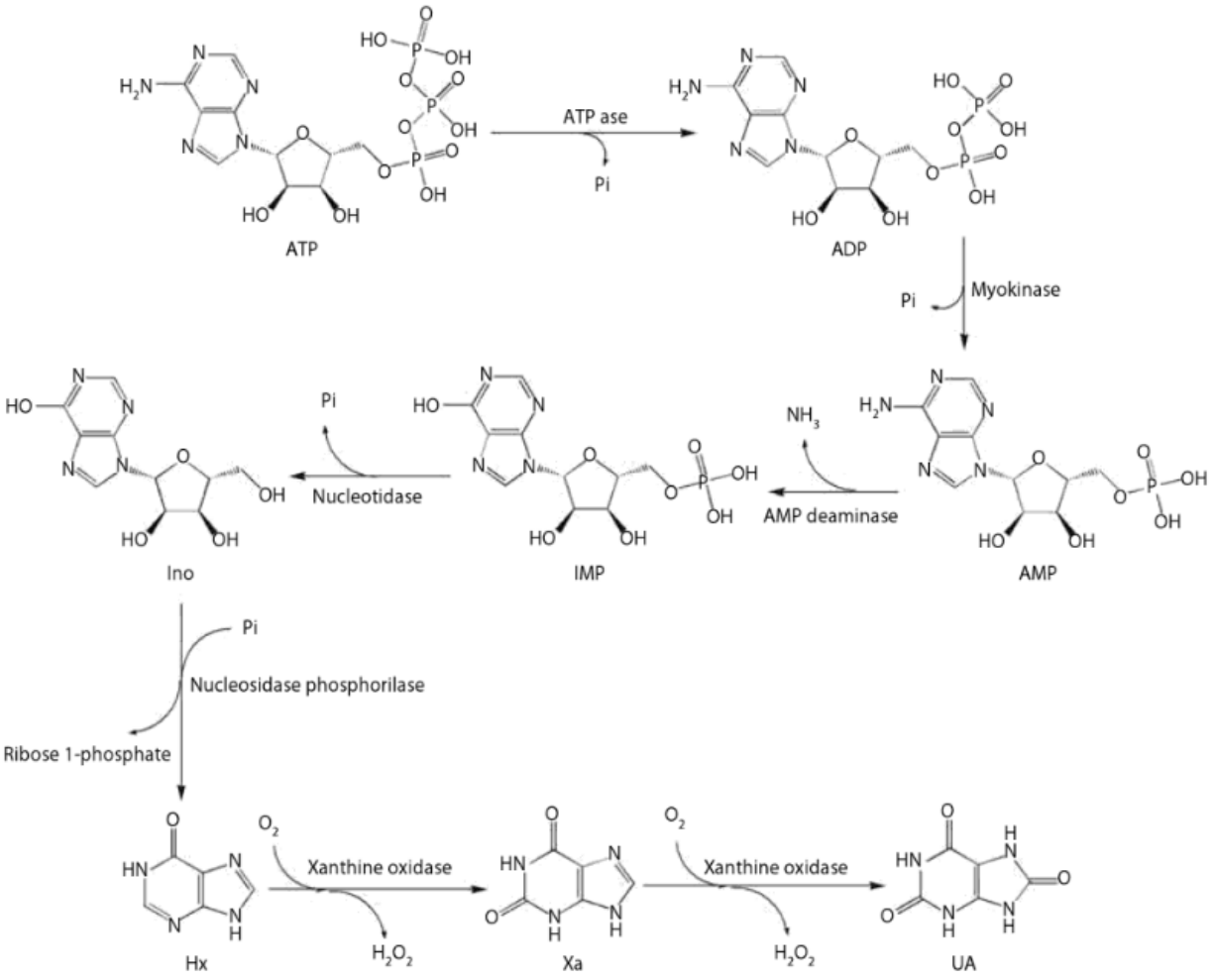


Figure 2.3: ATP degradation in postmortem fish muscle. A production of the breakdown products Ino and Hx are often connected to loss of freshness and development of perished seafood [Nollet and Toldrá, 2009].

2.3 Microbiological Aspects

2.3.1 Storage Life

According to Brown et al. [2011], *Shelf life has been defined as the period of time during which the food product will remain safe, be certain to retain desired sensory, chemical, physical and microbiological characteristics and comply with any label declaration of nutritional data.* Storage, or shelf life, is considered as the most important functional property. Products stated as spoiled has no longer those sensory properties acceptable by consumers or a nutritional value. The shelf life can be expressed as the number of days after processing, until the product quality is reduced. The shelf life of smoked fish is related to the initial bacterial contamination of the raw material, the decrease of water activity during processing, the effect of inactivating putrefactive microflora during heat treatment, the amount of smoke components that penetrate

the product, temperature during processing, air humidity and oxygen access during storage [Doe, 1998]. The shelf life of smoked salmon is often set to 3-4 weeks, though it may be up to 6 weeks [Rørvik et al., 1991].

Shelf life is controlled by three factors: product characteristics, environment exposing the product during distribution and storage, and properties of storage. The first factor is also known as an intrinsic factor, as it includes physical, chemical, biochemical and microbiological nature of the product - and also processing parameters. The second factor is on the other hand better known as an extrinsic factor, and includes light, temperature, consumer handling and humidity [Robertson, 2009].

Spoilage of food begins as soon as the fish dies, usually by mechanisms due to endogenous enzyme action (autolysis), nucleotide breakdown, bacterial actions and lipid oxidation [Burt, 1988], as previously mentioned. Differences regarding chemical biochemical, microbiological differences and innate composition gives unique spoilage patterns in different categories of seafood products. Depth of water at which the fish live is one example of a factor that will impact the microflora present in the seafood. [Kerry, 2012, Gram and Huss, 1996].

While the fish remains dead, enzymes (especially enzymes stored in the gut) are still active and will effectively break down fish tissue. The viscera of fish are a source to proteolytic enzymes, which are effective in breaking down fish flesh. These enzymes can leak out from the digestive tract and therefore initiate a faster breakdown of the fish flesh. [Gildberg, 1982] found out that these proteases are more effective at acid pH than alkaline pH. Furthermore, it is acknowledged that seafood favors bacterial growth, in such a degree that seafood will undergo autolysis more rapidly than terrestrial animals. This is due to low glycogen levels (the pH will not decline to such a low level as it does in terrestrial animals). Another reason is that the enzymes mentioned above are usually contained inside compartmentalized membranes, but with rough handling during processing the membranes are easily ruptured and enzymes will easily be spread throughout the seafood. Two other reasons that also contribute to the fast autolysis of seafood is the high moisture content and the high content of non-protein nitrogen [Gram and Huss, 1996].

In the first phase of deterioration muscle glycogen is degraded (following a fall in pH because of an increased level of lactate). The level of Adenosine triphosphate (ATP) in the muscle decreases, which causes a stiff muscle (rigor mortis) [Kerry, 2012, Hong et al., 2017].

Organisms from the environment can easily invade the fish during processing and storage through body openings and the skin. The bacterial count in food is known to be related to the temperature history of the food. A reduction in the temperature is said to be the most effective method to curtail bacterial and enzymatic spoilage [Burt, 1988]. Still, the effect on spoilage through these biochemical changes will not be proceeded until a certain level of specific spoilage organisms (SSOs) is reached. This level will usually not take place until a minimum of 6 days of storage in ice. After this time enzymes secreted by these microorganisms will also contribute to further deterioration, and therefore softening of the product [Kerry, 2012].

Usually, fresh food will deteriorate through autolysis and lipid oxidation, as earlier mentioned. Bacterial degradation is therefore not the main cause of degradation of seafood, but usually contributes with acceleration of degradation. In addition, many bacteria are found associated with degraded seafood - but in contrast only 10% of the bacterial flora is said to be responsible for producing sulfide spoilage odors. According to [Gram and Huss, 1996], it is degradation of Inosine monophosphate (IMP) that is responsible for fresh fish flavor [Surette et al., 1988]. IMP can further be broken down to hypoxanthine, inosine and xanthine, which also are contributors to unpleasant and rancid taste [Kerry, 2012, Surette et al., 1988], as previously mentioned.

As mentioned above, fish contain a high amount of non-protein nitrogen (free amino acids, TMAO). This nitrogen can serve as a nutrient source for bacteria, where TMAO gets reduced to trimethylamine (TMA) [Surette et al., 1988]. This compound is in limited quantities termed as a malodorous compound. TMAO is not usually present in freshwater species, but are more present in seawater species. [Kerry, 2012].

During the spoilage of muscle food stored under aerobic conditions strict aerobic Gram-negative organisms will dominate. On the other hand if storage of packing mechanisms involves the use of CO_2, the growth of normal spoilage flora will be inhibited (*Pseudomonas* spp., *Shewanella putrefaciens*). Although the understanding of how CO_2 affects the bacteria's individual gene expression is incomplete, the bacterias that gets affected is nevertheless documented [Kerry, 2012]. Examples of CO_2-tolerant microorganismst (mainly Gram-positive organisms) that will mainly dominate spoilage microflora are still *Lactobacillus* spp., *Photobacterium phosphoreum* and *Brochothrix thermospacta*. Although many food pathogens gets affected and inhibited by CO_2, *Listeria monocytogenes* and *Clostridium botulinum* are examples of two pathogens less affected [Dalgaard et al., 1997].

2.3.2 Lactic Acid Bacteria (LAB)

Lactic Acid Bacteria (LAB) constitute a group of gram-positive bacteria, which are connected through morphological, metabolically, and physiological characteristics. A general description of LAB include nonsporing, nonrespiring cocci or rods, facultative anerobic bacteria that produce lactic acid as their main end product during fermentation of carbohydrates [Axelsson et al., 2004]. Biopreservation refers to the use of natural microflora and/or their antibacterial products to extend storage life and enhance safety of foods [Stiles, 1996]. The LAB term is mainly associated with bacteria involved in food and feed fermentation, and the classification of LAB into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, and the ability to grow at different salt- and pH-concentrations [Axelsson et al., 2004]. LAB seem generally to be the dominant flora of the vacuum-packed smoked fish at the end of the storage period [Tomé et al., 2006, Huss et al., 1995b]. Vacuum packaging is a factor promoting LAB development, since LAB are facultative anaerobic bacteria [Leroi et al., 1998]. The role of LAB in CSS, on the other hand, is not clear. They may be found in elevated numbers before products get spoiled, and in vacuum-packed meat authors designate LAB as the main spoilage organisms [Huss et al., 1995b]. Still, some of LAB display a capacity to inhibit spoilage as well as several strains of pathogenic micro-organisms, e.g. *Listeria monocytogenes* [Tomé et al., 2006]. Inhibition is said to be caused by production of natural preservatives such as organic aciss, hydrogen peroxide and diacetyl, and antimicrobials such as bacteriocins, as well as competition for specific nutrients [Nilsson et al., 1999].

2.3.3 *Listeria monocytogenes*

Listeria monocytogenes is a well known non-sporeforming pathogen that can give rise to the disease listeriose [Løvdal, 2015]. It is a Gram(+), non-spore-forming rod bacteria with peritrichious flagella, that is halotolerant and facultative anaerob [Kerry, 2012]. The bacteria has therefore shown to survive salty conditions made from salting and brining [Espe et al., 2004], and can also grow well under both aerobically and anaerobically (vacuum packed) conditions at refrigerated temperature [Løvdal, 2015]. Because of its high adapted ability to grow in such challenging environments, *L. monocytogenes* has been an alarming threat to many RTE products, and therefore also CSS [Buchanan, 2004]. In fact, vacuum-packed CSS has been reported to show one of the highest prevalences of *L. monocytogenes* [Espe et al., 2004]. The

bacterium is especially a threat to people with low immune defence and pregnant women. A development of listeriosis among these can cause dangerous outcomes, and RTE products earlier tested exposed to *L. monocytogenes* is therefore recommended to avoid. A typical HACCP plan to assure destruction of the bacteria is usually included in US processing. [Kerry, 2012]. Smoked fish support growth of *L. monocytogenes* at temperatures above 0 ° C, even at 10% NaCl concentration and at a water activity 0,92. In the range of 2-25 ° C the bacteria shows an increased tolerance for NaCl [Doe, 1998].

Listeria is said to be sensitive to smoke components, and especially phenols [Doe, 1998, PAYNE et al., 1989, Vaquero et al., 2007]. Indeed, it has been identified that post-processing contamination of *Listeria* in smoked products is the main hazard in many foods, especially by slicing. *Listeria* may also be introduced to smoked products prior smoking, and therefore survive the steps of processing [Walsh and Kerry, 2012]. The growth rate of bacteria in CSS is depended on the condition and the initial number of the bacteria population, but most mainly on the temperature and water activity in the product. In addition, the growth of the bacteria can be affected by other microorganisms [Doe, 1998].

2.4 Packing Technologies

The goal of food packaging is to contain food in a cost-effective manner that satisfies industry requirements and consumer desires, maintain food safety and minimizes environmental impact. Today consumers demand minimal processed food, with fewer additives and preservatives [Sonneveld, 2000, Suppakul et al., 2003]. The main purposes for food packaging include containment of the product, protection against deteriorated effects, information to the consumer and in some cases ease use and convenience. Demands for both safety, convenience, quality and shelf life has led to improvements in packaging technologies, involving materials and incorporation of active materials [Robertson, 2016].

As earlier mentioned, there are several factors affecting seafood quality, including freshness and bacterial counts, colour, odour, flavour, texture and moisture content. To ensure a good cost-effective and responsible preservation of the product external conditions such as temperature and hygiene are two crucial factors. Nevertheless, packaging is also one of the most significant factors in reaching the same goal [Robertson, 2009]. New advances within packaging technologies are being produced and researched on every day, e.g. the use of additives to prolong shelf life [Kerry et al., 2006] (active packing) or the use of monitoring on products to give information about the quality condition during storage [Hogan and Kerry, 2008, Sivertsvik, 2003]. However, there are other barriers than just science that are holding these technologies back, such as difficulties having these new technologies accepted by regulatory bodies, concerns from producers that are afraid of their consumers reaction to chilled products with a long shelf life and the cost of implementing new technologies [Fletcher, 2012].

Today, overwrap packing is the dominant form of fresh meat retail packaging, while vacuum (VP) and modified atmosphere packaging (MAP) are more used for processed meat and for specialized applications. Both consumer demands for quality and flexibility, in addition to process economy and efficiency are important in choosing the right packaging system [Kerry, 2012].

MAP can be divided in two different forms; vacuum and MAP [Narasimha Rao and Sachindra, 2002, Sivertsvik et al., 2002b]. Vacuum stands for the packaging technology where air is removed by evacuation before the package is sealed. MAP includes packing with mixtures or combinations of gases before sealing the product [Kerry et al., 2006, Sivertsvik et al., 2002b]. Both of these technologies have seen to inhibit protein [Shin et al., 2003] and collagen degra-

dation [Masniyom et al., 2005], and thereby protect product firmness and texture. Although MAP both stands for the overall package technology for both vacuum and MAP, the word MAP usually refers to the latter technology [Kerry, 2012, Sivertsvik et al., 2002b].

Both vacuum and MAP have advantages and disadvantages, and which packaging technology to be used is often dependent on the product being stored. Even though USA have concerns over botulism in vacuum packed smoked fish [Fletcher, 2012], vacuum is commonly used for CSS [Dondero et al., 2004]. Still, MAP has also shown successfully results for storage of smoked fish [Cakli et al., 2006, Sivertsvik et al., 2002b].

2.4.1 Vacuum

Vacuum is a well accepted packaging technology today, as previously mentioned. The principle with vacuum is to evacuate air and thereafter seal the package. This packaging method prevent moisture loss and exclude O_2. Benefits associated with the use of vacuum is limiting the package volume and the prevention of oxidative spoilage - especially with the use of package material with high O_2 barriers. Disadvantages with the use of vacuum is the development of O_2-containing atmosphere with gases permeating through the product if the package inhabit vacuities [Robertson, 2016], in addition to the growth of anaerobic microorganisms such as *Clostridium botulinum* [Rizo et al., 2013].

2.4.2 Modified Atmosphere Packing (MAP)

Since the discovery of extended shelf-life of muscle food during storage in CO_2 rich atmosphere in 1930, many meat products have been stored using MAP. Nevertheless, MAP is today becoming the leading mild preservation method [Rotabakk and Sivertsvik, 2012]. MAP is a method for extending shelf life and at the same time ensure high quality of different food products, by storing the product in another atmosphere than ambient air [Narasimha Rao and Sachindra, 2002, Sivertsvik et al., 2002b].

Usually a mix of different gases is used (nitrogen, carbon dioxide and oxygen)[Sivertsvik et al., 2002b]. Some oxygen is sometimes added for its inhibition of biochemical changes (such as drip loss), but this is usually not done on products containing high amount of lipids [Rotabakk and Sivertsvik, 2012]. In addition, MAP contain frequently some oxygen, which act as a mechanism to prevent the growth of *C. botulinum* [Rizo et al., 2013]. An amount of oxygen above 10% is seen to inhibit the reduction of TMAO to TMA (known as the main spoilage compound) in cod fillets. On the other hand, oxygen is also a promoter of several types of deteriorate reactions in foods, like lipid oxidation and pigment oxidation. These reactions can be partly controlled by containing oxygen at a low concentration [Walsh and Kerry, 2012]. Also, CO_2 is added for its bacteriostatic effect [Rotabakk and Sivertsvik, 2012], to inhibit common aerobic spoilage bacteria, such as *Pseudomonas* species. Although, for retail packages of seafood, too high concentration of CO_2 can lead to packaging collapse and excessive drip loss [Sivertsvik et al., 2002b]. Nitrogen and argon are usually also included in MAP for their feature of being inert. [Rotabakk and Sivertsvik, 2012].

After their introduction into the product package the concentration of the different gases will inevitably change as they respond to the product. Since CO_2 is relatively soluble gas, it will dissolve into the liquid phase (water and the lipid liquid fraction) of the product [Abel et al., 2018]. This will result in a volume contraction if flexible packages are used, especially in the contraction that are most flexible - the top web. This is called packaging collapse and can be reduced by lowering the partial pressure to carbon dioxide, so that less soluble gases (N_2 and O_2) can increase in their partial pressure. Further on, oxygen will usually be consumed

by bacterial respiration and oxidative processes. The removal of oxygen will contribute to extending shelf life. Nitrogen is therefore added to prevent packaging collapse or to reduce the proportions of other gases. It does not support the growth of aerobic bacteria, nor repress the growth of anaerobic bacteria. [Rotabakk and Sivertsvik, 2012].

It has been showed that fish fillets stored using MAP typically show lower bacterial numbers compared with counterparts stored in air, and also MAP was shown to inhibit the breakdown of ATP derivatives [Rotabakk and Sivertsvik, 2012]. In fact, it is reported that the use of MAP can increase shelf life by 50-100%, where the limiting factor is growth of specific spoilage bacteria - where CO_2 has limited or no inhibitory effect. [Rotabakk and Sivertsvik, 2012, Sivertsvik et al., 2002b]. Also, MAP is seen to inhibit the growth of *Listeria* in trout, but not the prevention of growing [Yilmaz et al., 2009]. Although, only the highest quality fish and seafood products should be used to benefit from the extended shelf-life advantages of MAP. The achievable shelf life will depend on fat content, initial microbial amount, gas mixture, temperature of storage and the species [Sivertsvik et al., 2002b].

The biggest advantage of MAP is, as mentioned above, the extended shelf life [Sivertsvik et al., 2002b]. However, it has also other advantage effects. With the use of MAP costs will be reduced, as longer distribution distances are achievable and therefore fewer deliveries are required. This together allows a more centralized packaging and a higher portion control. In addition, MAP eases separation between sliced products, compared to vacuum, and can therefore be perceived by a cleared sight and with an all-around visibility. Last, but not least, the use of MAP are also decreasing the use of chemical preservatives, it prevents drip loss from the package and renders the product odourless and convenient [Sivertsvik et al., 2002b].

One of the disadvantages of MAP is that the packages contain often twice as much of gas compared with product, which can result in bulky packages. Furthermore, because of the isolating effect of the gas, the package will be difficult to chill. MAP was also shown to give a higher drip loss compared to air. When using MAP packaging there is also need for temperature control and special equipment and training of staff. Even though the products can be delivered by fewer transport deliveries the MAP packages have an increased packaging volume, which may raise the transport cost [Sivertsvik et al., 2002b]. Nevertheless, the benefits of using MAP is lost once the package is opened, different gas formulations are needed for each product type and special equipment and training is needed [Sivertsvik, 2000].

2.4.3 Soluble Gas Stabilisation (SGS)

There are at least two approaches to create a modified atmosphere, where one option is to generate CO_2 or remove O_2, or dissolve CO_2 into the product prior packing [Sivertsvik, 2000]. This way - increasing the effect of the packaging process is the use of soluble gas stabilisation (SGS). This method dissolves carbon dioxide into the product prior packaging under elevated pressures, and have a potential to prevent packaging collapse and still give a good quality and long shelf life. [Sivertsvik, 2000]. The method can be used as a pre-treatment in both packaging technologies such as vacuum and MAP [Fletcher, 2012]. SGS has been shown to be an especially good pre-treatment for products undergoing MAP-packaging. When CO_2 gets injected into the product package prior package closing the gas can dissolve into the product, which can result in a reduced packaging size and elevated transport efficiency. Especially for Atlantic salmon *Salmo Salar*, SGS has shown to be very successful with promising results. [Sivertsvik, 2000, 2003]. SGS has also shown to successfully extended shelf life when followed by vacuum packing [Mendes and Gonçalves, 2008].

Usually, the effect on spoilage bacteria from dissolving CO_2 into the product is directly associ-

ated to the partial pressure and the amount of dissolved CO_2 in the product containing bacteria [Devlieghere et al., 1998]. The amount of dissolved CO_2 entering the product is depending on temperature, pressure, packaging material and ratio between gas and product [Rotabakk and Sivertsvik, 2012]. The effect CO_2 has on bacteria is complex, but four activity mechanisms has been identified [Daniels et al., 1985, Dixon and Kell, 1989, Farber, 1991, Parkin et al., 1982]. These includes alteration of cell membrane function in effecting the bacteria's uptake of nutrients, direct inhibition or decreasing the activity of enzymes, penetration of the membranes leading to intracellular pH changes and the direct changes in physicochemical properties to the proteins.

By comparing SGS with the use of MAP (where the gas mix contain carbon dioxide), the CO_2 concentrations near the targeted equilibrium are reached before the product is packed. This will result in preventing packaging collapse, which normally is a disadvantage in the use of MAP since carbon dioxide usually dissolve into the product post packaging. [Fletcher, 2012].

Compared with any other atmospheric constituents CO_2 is many times more soluble in water [Rotabakk and Sivertsvik, 2012] and liquid phase of lipids [Abel et al., 2018]. How much CO_2 that gets dissolved into the product and how much that is surrounding the product is defined by Henry's law [Rischbieter et al., 1996]. This law states that the solubility of gas in a product is proportional to the partial pressure of the gas above the product. Henry's constant for solubility of carbon dioxide in water is dependent on temperature, where it decreases at higher temperatures. Further on, solubility of carbon dioxide seems to be similar in salmon fat as in water, as mentioned. The solubility is also connected to the partial pressure of carbon dioxide, where increased partial pressure gives an increased dissolving of carbon dioxide. Degree of filling (DF) is the amount of carbon dioxide that is available to dissolve into the given product, and is dependent on the partial pressure of CO_2. It has been showed that DF is important regarding the shelf life of MAP products [Sivertsvik et al., 2002b, Rotabakk and Sivertsvik, 2012].

Although, there are some disadvantages using VP and MAP with that high levels of CO_2 can result in liquid loss from raw seafood [Fletcher, 2012]. In addition, high carbon dioxide concentrations in the product is seen to result in a carbonated mouthfeel [Fletcher et al., 2005]. Also, as mentioned earlier, *L. monocytogenes* demonstrates the ability to grow in concentrations of carbon dioxide, which makes it very important to investigate the combination of different hurdles to decrease its growth [Rode et al., 2015].

2.5 Sensory Analysis

The main criticisms on product quality of smoked salmon often refers to appearance, texture related to fat content, concentration of salt and taste [Vergara et al., 2001]. Although, the only information available for consumers, and therefore constitute a big impact on the sales of the product, is appearance, brand name, processing references, shelf life and price [Cardinal et al., 2004]. In addition, the visual appearance of food is well known to influence flavour recognition [Teerling, 1992]. Developing a smoked salmon product with good sensory attributes is therefore essential.

Sensory science is a range of methods where human panelist or assessors uses their senses of sight, smell, taste, touch and hearing to measure the sensory attributes, characteristics and acceptability of food products. It is applicable to a variety of areas such as product development, quality control and process development [Watts et al., 1989]. Three types of methodology are commonly used, each with a different goal and each using different kind of panel [Watts et al., 1989, Lawless and Heymann, 2010]. Table 2.1 summarizes the three main types of methods

[Lawless and Heymann, 2010]. Descriptive tests are known to use high specialization of descriptive panels, which allows very detailed, robust and consistent, reproducible results, thus stable in time and within a certain sensory space [Moussaoui and Varela, 2010]. The most common descriptive method, Qualitative Descriptive Analysis (QDA^{TM}) [Stone et al., 2008], is a profiling test where a sensory panel chooses important descriptive attributes and scale the intensity of each attribute. Although, disadvantages often related with descriptive tests are that the sensory assessors need excessive training, which can be cost, time and resource consuming [Varela and Ares, 2012].

Table 2.1: Classification of test methods in sensory evaluation

Class	Question of interest	Assessor characteristics
Discrimination	Are products perceptibly different in any way	Screened for sensory acuity, oriented to test method, sometimes trained
Descriptive	How do products differ in specific sensory characteristics	Screened for sensory acuity and motivation, trained or highly trained
Affective	How well are products liked or which products are preferred	Screened for products, untrained

Recently, more rapid descriptive methods have been developed to improve utilization in the food industry [dos Santos Navarro et al., 2012], regarding reducing expensive costs of training assessors. Rapid Sensory Profiling Techniques are methods used to capture sensory perception, and some of them can be used as rapid and cheap alternatives to conventional descriptive methods [Delarue et al., 2014]. These methods are considered as cheaper, faster and less demanding according to performance and sensory panels [Varela and Ares, 2012]. There are multiple methods constituting this category. Two of the most frequently used rapid methods are Check-all-that-apply (CATA) [Adams et al., 2007] and projective mapping (PM) or Napping® [Pagès, 2005, Risvik et al., 1994].

2.5.1 Napping®

General Description

Napping® [Pagès, 2005], is a Rapid sensory profiling technique used in sensory analysis. The method is based on two-dimensional categorizing of related samples in a distinct way so that differences and similarities can be registered [Lê et al., 2015, Waldenstrøm, 2015]. In the performance of the analysis all the samples get randomly presented, and each assessor positions the samples on a bi-dimensional space (tablecloth, i.e. "nappe" in fresh), see fig. A.1 in Appendix A.1. Samples gets arranged on the tablecloth according to the differences and similarities between them - more similar samples would give closer placement [Lê et al., 2015, Perrin et al., 2008]. The dimensions of the space, its width (x-axis) and height (y-axis), are of utmost importance [Lê et al., 2015] (shown in fig. 2.4a).

The person executing the Napping® can decide which groups and criteria the samples should be analysed for [Valentin et al., 2012, Waldenstrøm, 2015]. In addition, the method can be performed on both appearance, smell, flavour and texture [Waldenstrøm, 2015], where the focus is on differences/similarities between the samples [Varela and Ares, 2012]. After the samples have been grouped according to the given criteria Ultra Flash Profiling (UFP) can be used. UFP is

based on that the assessors select terms to describe the samples or the distinct categories after grouping the samples [Waldenstrøm, 2015, Santos et al., 2013, Pagès, 2005, Perrin et al., 2008]. Napping® in combination with UFP can therefore gather product descriptions directly from assessors with their own vocabulary. These methods could therefore be viewed as interesting alternatives to profiling and the free descriptions assigned by the assessors could be more easily perceived [Perrin and Pagès, 2009].

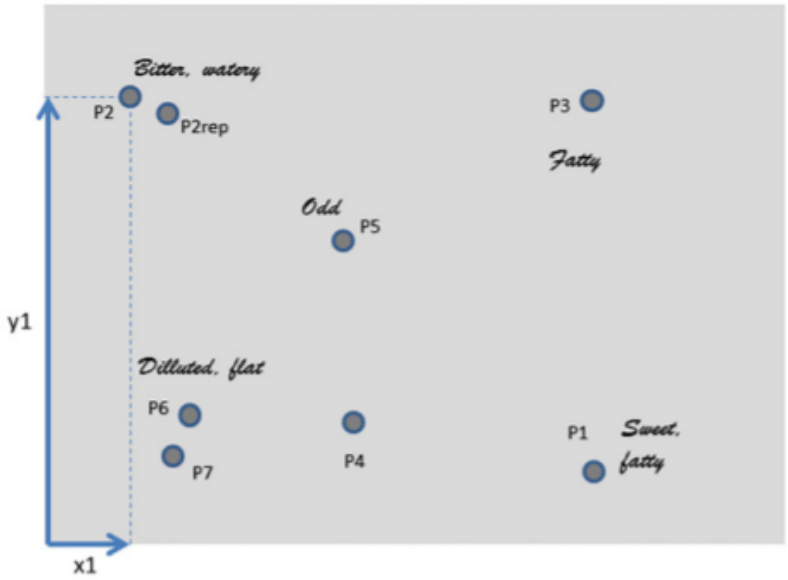
Data Analyses of Napping®

The results from Napping® are usually graphically displayed using some kind of Multivariate analyses. Multivariate analyses are used to present complex datasets with more than one variable [Lê et al., 2015], and can therefore be used to graphical display the results of Napping®. The analyses summarize complex data matrices in a two-dimensional plot by using correlations between variables [Næs et al., 2011]. Software such as XLSTAT can be used to select components in the dataset having the biggest variance. Then, multivariate analyses such as *Principal Component Analysis* (PCA) and *Multiple Factor Analysis* (MFA) can be used to analyse and present the data [Waldenstrøm, 2015, Pagès, 2005].

A PCA-plot explains the variance between samples in percentage, and present how much of the variance that can be explained by the Principal Component or the axis in the plot [Waldenstrøm, 2015]. MFA is based on PCA if the variables are quantitative and can be used in processing data received from Napping®. MFA takes into account for a group structure on the variables. It analyses several groups of variables defined for the same set of observations, and later expresses the relationships existing between the groups of variables. In that sense MFA can be seen as a weighted PCA [Lê et al., 2015, Pagès, 2005]. The main difference between PCA and MFA, is that MFA takes into account individual differences rather than averaging the data [Nestrud and Lawless, 2008]. In addition, when UFP is combined with Napping® the qualitative data with terms describing the groups are analysed as another data table, which give supplementary variables [Pagès, 2005, Varela and Ares, 2012, Perrin et al., 2008]. MFA works with multi-table data where the type of the variables can vary from one dataset to the other, but the variables should be of the same type within a given dataset [Chavent et al., 2014].

The dataset collected from Napping® can be organized as shown in fig. 2.4. The coordinates of the location assessor places the samples are measured for each assessor (fig. 2.4). The first set of results (Table 1 in fig. 2.4) will be the x and y coordinates (assessor 1: x1, x2), as every assessor have placed each sample (P1, P2, etc.) If UFP has been used, the number of times each term has been used for each sample (e.g. in fig. 2.4, how many times "sweet" has been mentioned for sample 1), are counted across consumers. The second set of results (Table 2 in fig. 2.4) will therefore be the amount of time the terms are mentioned for each sample. All the data will then be structured in a table, where rows presents the samples, and columns presents the coordinates (x,y) and the mentioned terms (fig. 2.4b) [Varela and Ares, 2012]. MFA will give a graphical display (biplots), where samples near each other were perceived as similar by the whole panel of assessors [Varela and Ares, 2012].

(a)



(b)

Sample	Table 1								Table 2				
	x1	y1	x2	y2	...	...	xn	yn	bitter	watery	sweet	...	fatty
P1													
P2	4.2	15.5	8.4	11			5.9	20.3	21	15	3		0
P2rep													
P3													
...													
P7													

Figure 2.4: a) An illustration of how to collect data of a Napping® test with 7 samples and one duplictae (P2rep). b) The table structure of the data matrix. The rows presents the 7 different samples and P2rep, and the columns presents the coordinates (assessor 1: x1, y1) as every assessor have placed each sample (P1, P2, etc.), and the term frequency for each sample (Table 2; bitter, watery etc.) [Varela and Ares, 2012]

.

Data Presentation

By analysing the data from Napping® with MFA the information on how the assessors used the axes (x,y) of the bi-dimensional space will be retained [Dehlholm et al., 2012]. The aim of the analysis is to represent the original data by a set of new (orthogonal) variables called principal components (PCs) - often called dimensions. The PCs are new variables that are constructed as linear combinations of the initial variables, that maximize the variance in the data. In this way the number of random variables will be reduced by obtaining PCs [Stanimirova et al., 2005]. The dimensions are ordered so that the first one has maximized the total variance. Studies [González-Mohíno et al., 2019, Pickup et al., 2018, Reinbach et al., 2014, Oliver et al.,

2018, Giacalone et al., 2013] where Napping® has been performed shows that the first two dimensions is often used if they explain over 50% of the total variance in the dataset.

Figure 2.5 and fig. 2.6 shows the product maps obtained from Napping®. The first two dimensions (F1 and F2) accounted for 62,98% of the explained variance (42,64% and 20,34%, respectively). The data from Napping® got analysed using MFA. As mentioned before, closer placed samples were perceived as more similar [Pagès, 2005], such as brandy samples B1R and B1. The placement of the brandies (B1-5, B1R) in the Observation plot corresponds to the placement of the different terms in the Variables plot. This means that the brandy samples B4 and B5 were associated with darker colours such as "Dark gold" and "Deep golden colour", compared to brandy sample B3, which was associated with "Light colour". B4 and B5 were also associated with rich aromas and flavours such as chocolate, coffee and apricots [Louw et al., 2013].

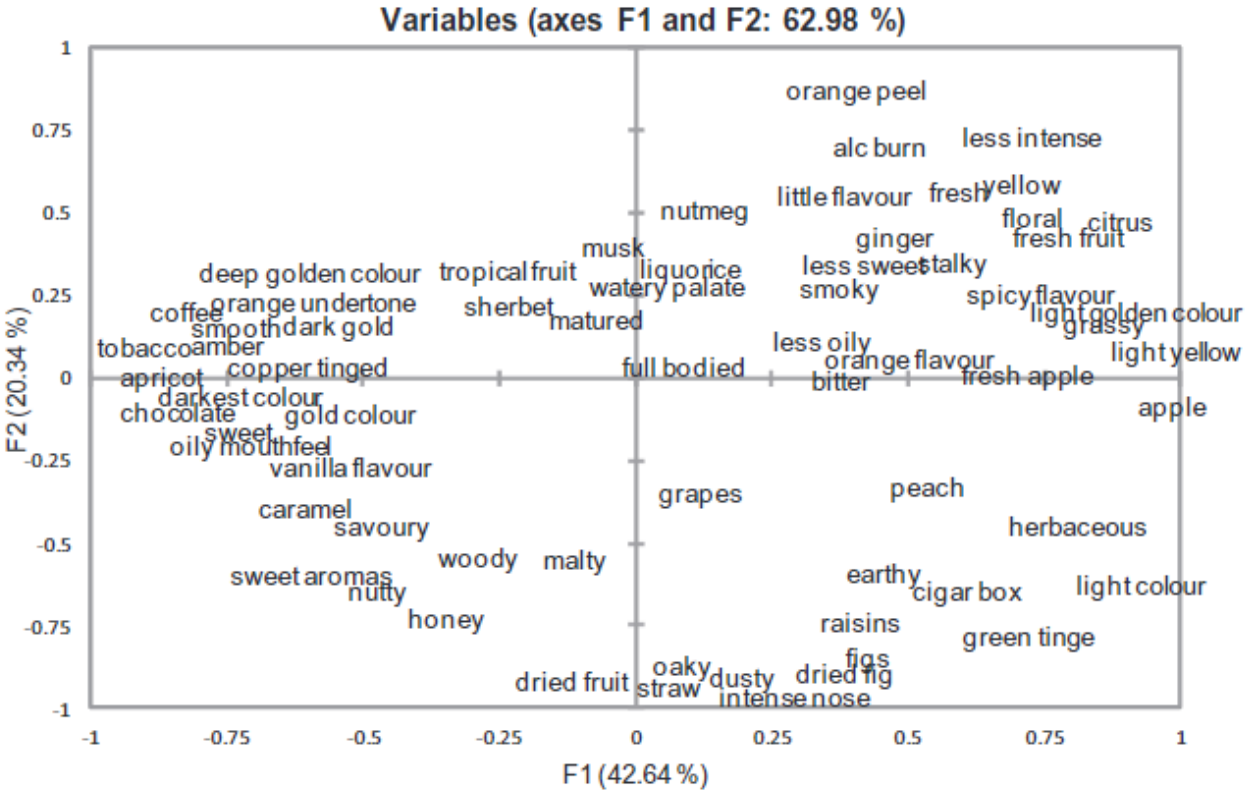


Figure 2.5: Variable plot obtained from Napping® of six brandies, B1-B5, with two blind replicates (B1 and B1R) [Louw et al., 2013].

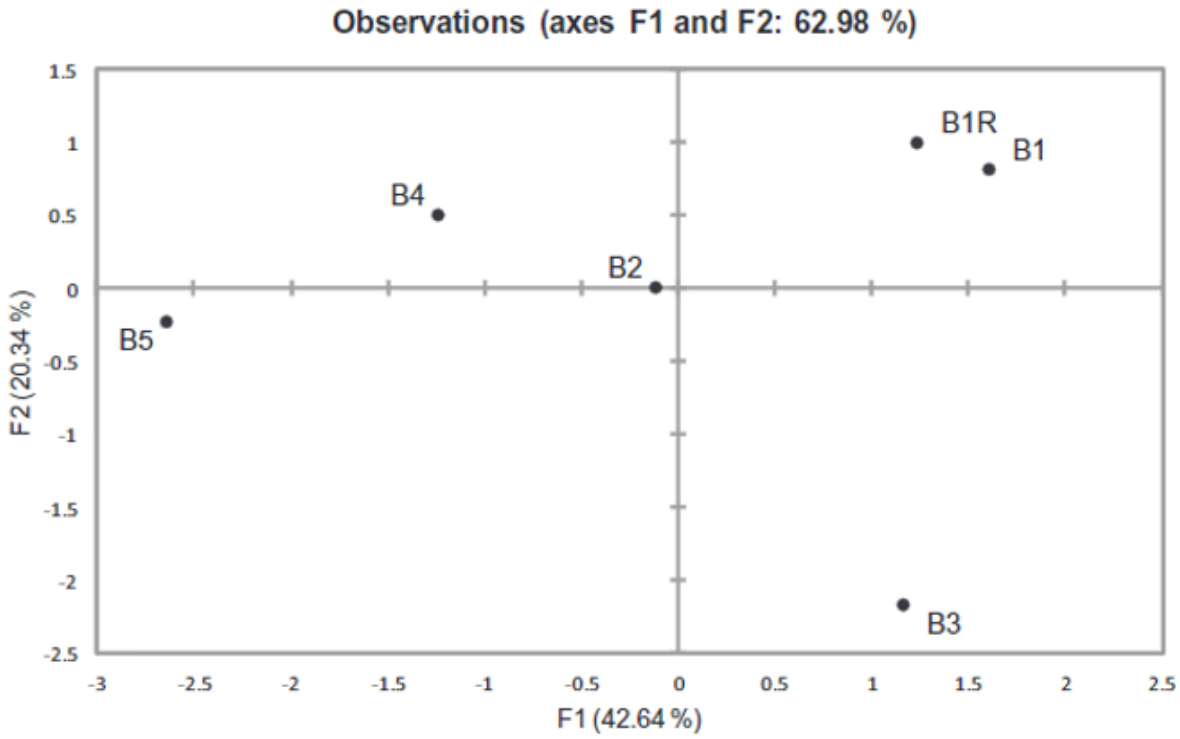


Figure 2.6: Observation plot obtained from Napping® of six brandies, B1-B5, and one replicate (B1R) [Louw et al., 2013].

Sensory Panels

Different types of sensory tests require a suitable degree of selection and training of assessors in a sensory panel. Assessors can be trained to a different extent of degree, and the three main groups of sensory panels are often said to be 1) Highly trained panel, 2) Laboratory panel and 3) Consumer panel [Powers, 1985, Lawless and Heymann, 2010]. For highly trained panels the attention is focused on judging the quality of the product [Lawless and Heymann, 2010], often using standards set by the food industry. The assessors are trained in distinguishing differences between product variations, and do not determine consumer preferences. A trained panel is successful when the panelists can determine the differences, reproduce their same results, and be consistent with the rest of the trained panel. A semitrained panel is a type of trained panel where the assessors are trained in sensory recognition and characterization, but not in the specific category of products or in scaling [Varela and Ares, 2012]. Laboratory panels are on the other hand usually small groups working at a company's laboratory, developing new products and determines how small changes may affect the quality of a product. Larger panels are more usual in consumer panels, and can be used by companies that wants to examine their products outside the laboratory. Such tests can be performed in grocery stores and shopping malls, to explore consumers accept [Lawless and Heymann, 2010].

Many of the sensory analyses used today demand the use of a trained panel, which can be both time and cost consuming. Napping® on the other hand can be conducted by all kinds of panels [Varela and Ares, 2014], and the method can therefore be a more rapid and cheaper sensory method - as mentioned. Although, profiling with a trained sensory panel will sometimes give information that the rapid methods cannot provide, and a trained sensory panel will also provide more precise product descriptions than a consumer panel [Waldenstrøm, 2015].

3 Aims of the Project

The aim of the present project was to study processing of CSS by using atomized PCS and to optimize processing protocols related to yield, quality and food safety. To compare PCS protocols, traditionally produced CSS was used. To reach the main objective, three different experiments was

- Experiment 1: The aim of the first experiment was to compare CCS fillets smoked by traditionally wood and by the use of atomized PCS (VTABB), by looking at microbiological, chemical and physiochemical parameters, quality parameters and processing yield.
- Experiment 2: The aim of the second experiment was to study three different PCS, by using 3-5 cycles with atomization. The quality of CSS fillets were evaluated by physiochemical, chemial and microbial parameters, and by sensory (Napping®)
- Experiment 3: The aim of Experiment 3 was to compare CCS treated with PCS against tradtionally CCS with focus on food safety, where we performed a challenge study with *L innocua*. Microbiological and chemical parameters were measured, to evaluate the growth of APC and *L. innocua*, with or without use of SGS treatment.

Chemical analyses consisted of measurements (depending on the experiment) such as pH, salt content (%), ATP breakdown products and total phenolic content. Whereas physiochemical parameters such as texture and colorimetric properties were also measured, dependent on the experiment.

4 Materials and Methods

In total, three different experiments were conducted. The goal of Experiment 1 was to study lightly salted Atlantic CSS produced with three different levels of smoke intensity of both Traditionally smoking by wood fire and by the use of atomized PCS. Both microbiological testes (APC and LAB), a sensory selection and chemical testes (pH, colorimetric properties, texture, breakdown products of ATP, TPC, salt content) were performed.

In Experiment 2, three different atomized PCS with three different levels of smoke intensity were used in sensory napping, together with one type of Traditionally Atlantic CSS. The goal was to study similarities and differences between CSS smoked by PCS and by traditionally smoking. Microbiological (APC and LAB), sensory and chemical analyses (pH, colorimetric properties, texture) were performed.

The purpose of Experiment 3 was to compare the growth of *L. innocua* on Atlantic CSS smoked by Traditionally Wood and atomized PCS, packed in vacuum with and without the use of SGS. The goal was to investigate if the use of atomized PCS and CO_2 (individually or used together) could decrease the growth of inoculated *L. innocua* to a lower amount than the amount found in Traditionally CSS packed in vacuum. Microbiological (APC and BLA), and chemical analyses (pH, colorimetric properties, texture, degradation products of ATP, TPC, salt content) were performed.

Material and methods is separated into two parts. The first part gives a general description of the three experiments, regarding experimental design and the sampling day for analyses (chemical, microbiological, sensory). The second part concerns the experimental descriptions of the processing technology performed throughout all the experiments.

4.0.1 Experiment 1

Comparison of Traditionally Wood smoked salmon and PCS smoked salmon

Twelve Atlantic salmon were filleted one day post slaughtering. Microbiological analyses of the raw material were performed on Iron Agar (IA) and de Man, Rogosa and Sharp Agar (MRS), with 3 dilutions (explained under "2.1.4 Microbiological Analyses").

After 2 days post slaughtering the fish was weighed before dry salted (6 hours). Post salting, the fillets were stored for three days before they were weighed once again, and dried (2.5 hours). After drying, the left fillets were smoked traditionally (explained under "2.8 Smoking"). Four fillets were taken out after 90 minutes, 120 minutes and 150 minutes, respectively. After smoking the fillets were cooled down, weighed and packed with plastic foil, and stored at 4°C. The right fillets were smoked by using atomized PCS (SmokEz VTABB, 9–12% acetic acid, pH = 2.0–3.2, Red Arrow, USA) the same day, after being dried for 20 minutes. Then 4 fillets were taken out after 3, 4 and 5 cycles, respectively (procedure explained under "2.8 Smoking"). See 4.1 for a flow sheet of the experimental design. After smoking the fillets were cooled down, weighed, and packed in plastic foil before stored at 4°C.

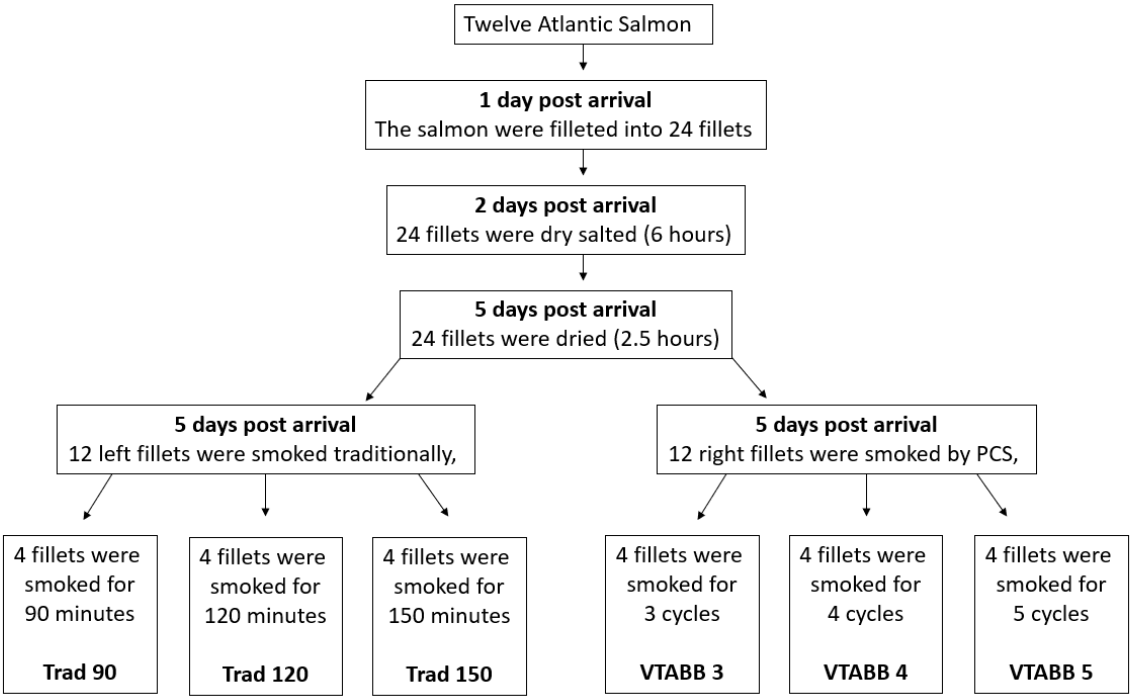


Figure 4.1: A flow sheet representing the experimental design for Experiment 1, including dry salting, drying and smoking (traditionally and by PCS using VTABB). Six different CSS were produced: Trad 90, Trad 120, Trad 150, VTABB 3, VTABB 4 and VTABB 5. Microbiological and chemical testes are not included.

Colorimetric properties were measured two days post processing, and the fillets were weighed and sliced into appropriate samples (fig. 4.2), before sealed in vacuum - each sample individually.

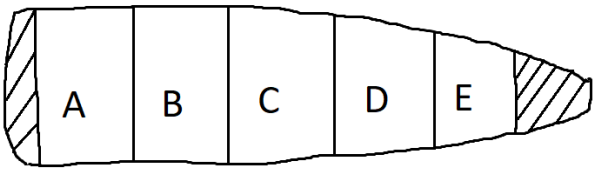


Figure 4.2: An illustration of how a CCS fillet were sliced into five approximately same samples (A-E) in experiment 1, after being smoked by either traditionally wood or by atomized PCS.

A sensory Selection was performed 21 days post processing on the six CSS: Traditionally smoked (90, 120 and 150 minutes) and PCS smoked salmon (3, 4 and 5 cycles). The goal was to determine which CSS that were most related and comparable to traditionally CSS.

The analyses performed in experiment 1 and sampling day for the analyses (microbiological, sensory and chemical) of CSS is given in table 4.1. On analysing day microbiological analyses (Aerobic Plate Count (APC) and Lactic Acid Bacteria (LAB)) were performed before pH, texture and colour. Measuring of salt content, breakdown products of ATP and total phenolic content (TPC) were all done on frozen (-40°C) CSS. The sampling day for analysing frozen CSS represents the number of days post processing the CSS were frozen down at.

Table 4.1: The analyses (microbiological, sensory and chemical) and the sampling day used in experiment 1. The day of sampling represents the number of days stored at 4 °C before analyses were conducted. Salt, Total Phenolic Content and ATP breakdown products analysis were performed on frozen fish samples, frozen down at indicated sampling day. Microbiological analyses (APC and LAB) were always performed before analysis of texture, pH and colour.

Analysis	**Day of sampling**
Total phenolic content	2
Salt Content	7
Texture	7 + 21
Sensory analysis	21
ATP breakdown products	2 + 35
Process yield	Before salting + 2
Aerobic Plate Count	2 + 7 + 14 + 21 + 28 + 35
Lactic Acid Bacteria count	2 + 7 + 14 + 21 + 28 + 35
Colorimetric Properties	2 + 7 + 14 + 21 + 28 + 35
pH	2 + 7 + 14 + 21 + 28 + 35

4.0.2 Experiment 2

Sensory Selection of CSS smoked by three different PCS with comparison of traditionally CSS

16 Atlantic salmon were manually filleted one day post slaughtering. Microbiological analyses (APC and LAB) of the raw material were performed with 3 dilutions.

The fillets were weighed before and after dry salting (16.5 hours). The right and left fillets were dry salted 1 and 2 days post mortem, respectively. The salting time was extended to 16,5 hours, since this experiment focused on sensory attributes. After the salt was washed off, the fillets were dried for 2,5 hours. The left and right fillets were weighed 2 and 3 days post mortem, respectively. The right fillets were smoked by using PCS, and the left fillets by traditional wood smoke (see fig. 4.3)

16 right fillets were smoked by using three different PCS (SmokEz VTABB, 9–12% acetic acid, pH = 2.0–3.2, SmokEz Enviro 24 (R24), 9.5-11.0 % acetic acid, pH = 2.2-3.5 and JJT01-160-C (30765475), a smoke type mixed based on sensory specifications provided by the institute, all collected from RedArrow, USA), with three different intensities. Fillets smoked with Enviro and JJT01 were processed by using 3, 4 and 5 cycles, whereas those smoked with VTABB were only smoked with 4 and 5 cycles. Two right fillets were used in every cycle, giving 2 * 8 cycles = 16 fillets of PCS smoked CSS.

16 left fillets were smoked by traditionally wood smoke (120+30 minutes) four days post slaughtering.

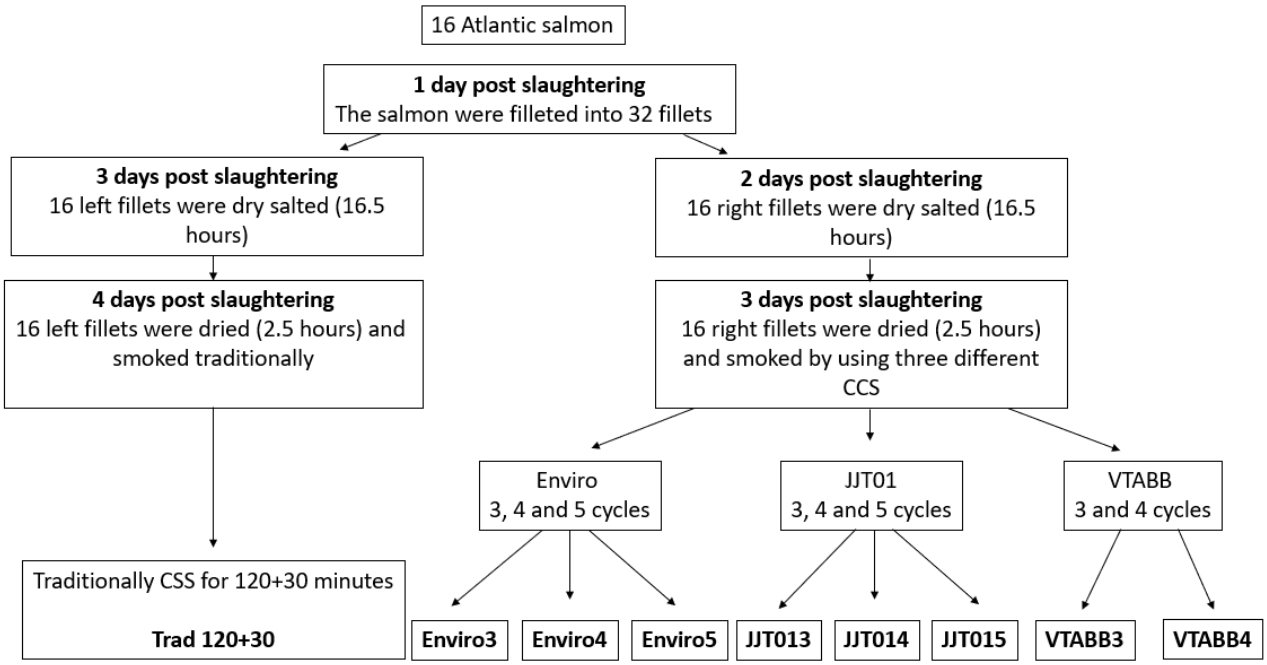


Figure 4.3: A flow sheet representing the experimental design for Experiment 2, including dry salting, drying and smoking (traditionally and by PCS using VTABB, Enviro and JJT01). Nine different CSS were produced: Trad 120+30, Enviro3, Enviro4, Enviro5, JJT013, JJT014, JJT015, VTABB3 and VTABB4. Microbiological and chemical testes are not included.

Weight was measured 2 days post processing on all CSS, before the CSS were sliced into appropriate samples (fig. 4.4) and vacuum sealed. The smallest samples (labelled A-E) were

used for microbiological analyses and pH measurements, and the biggest sample (F) was used for sensory and textural ansalyses.

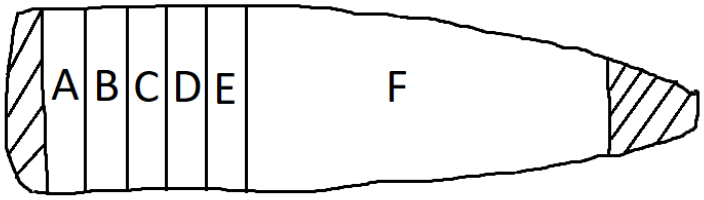


Figure 4.4: An illustration of how the CSS got sliced into six samples (A-F) in experiment 2, after being smoked by either Traditionally wood smoke or by PCS. The samples named A-E were used for microbiological analyses and pH measurements, and the sample named F were used for sensory and texture analyses.

After four days post smoke production, a Sensory Selection was performed on the 8 different products of PCS smoked salmon: Enviro smoked with 3, 4 and 5 cycles, VTABB smoked with 3 and 4 cycles and JJTO1 smoked with 3, 4 and 5 cycles. The goal was to determine which of the PCS smoked samples that were most related and comparable to the traditionally smoked salmon.

Napping® was performed 36 days post processing, on three different CSS smoked by PCS against its corresponding fillet smoked traditionally, see table 4.2. The goal was to find the PCS that were most similar to traditionally smoking, and study which terms and characteristics that were associated with the different CSS,. REFV and REFJ stands for the traditionally smoked filet corresponding VTABB and JJT01, respectively. A traditionally smoked corresponding filet for Enviro was not used, since this sample was considered unpleasant at the time of analysis.

Table 4.2: List of samples used in Napping® for Experiment 2. The right fillet of each fish (individual) was processed using three atomized purified condensed smoke (PCS) flavours (VTABB, JJT01 and ENVIRO). The numbers following the PCS label refers to the number of cycles the PCS fillets were smoked. All the left fillets were smoked traditionally (120+30 min).

PCS smoking (right filet)	**Traditionally smoking (left filet)**
VTABB 4	REFV
JJT01 4	REFJ
Enviro 3	-

The analyses performed in experiment 2 and sampling day for the analyses (microbiological, sensory and chemical) of the CSS is given in table 4.3. On analysing day microbiological analyses (APC and LAB) were performed before pH and texture. Weight measuring was performed before and after microbiological analyses.

Table 4.3: The parameters and their day of sampling used in the comparison of traditional wood smoke and PCS in experiment 2. The sampling day represents the number of storage days post the smoking day of PCS fillets. The sensory, texture and colour analyses were performed on the same fillet, and the pH measurements and microbiological testes were performed on another fillet.

Parameter	**Day of sampling**
Texture	27
Sensory analysis	4 + 35
Process yield	Before salting + 2* + 3*
Aerobic Plate Count	4 + 25 + 39
Lactic Acid Bacteria count	4 + 25 + 39
pH	4 + 25 + 39

2* were performed on PCS smoked fillets
3* were performed on traditionally smoked fillets

4.0.3 Experiment 3

A comparison of a selected PCS smoking and traditionally smoking protocol, regarding both the growth of *L. innocua* and packaging technology

5 fish of Atlantic salmon were filleted manually 4 days post slaughtering. All the fillets were dry salted (6 hours), before the salt were washed off and the fillets were stored wrapped in plastic at 4°C.

All the fillets were dried (2.5 hours) 5 days post slaughtering. The five left fillets got smoked by using traditionally wood smoke (150 minutes), and the right fillets were smoked by using PCS (SmokEz VTAAB, red arrow, USA) with four cycles (see 4.5).

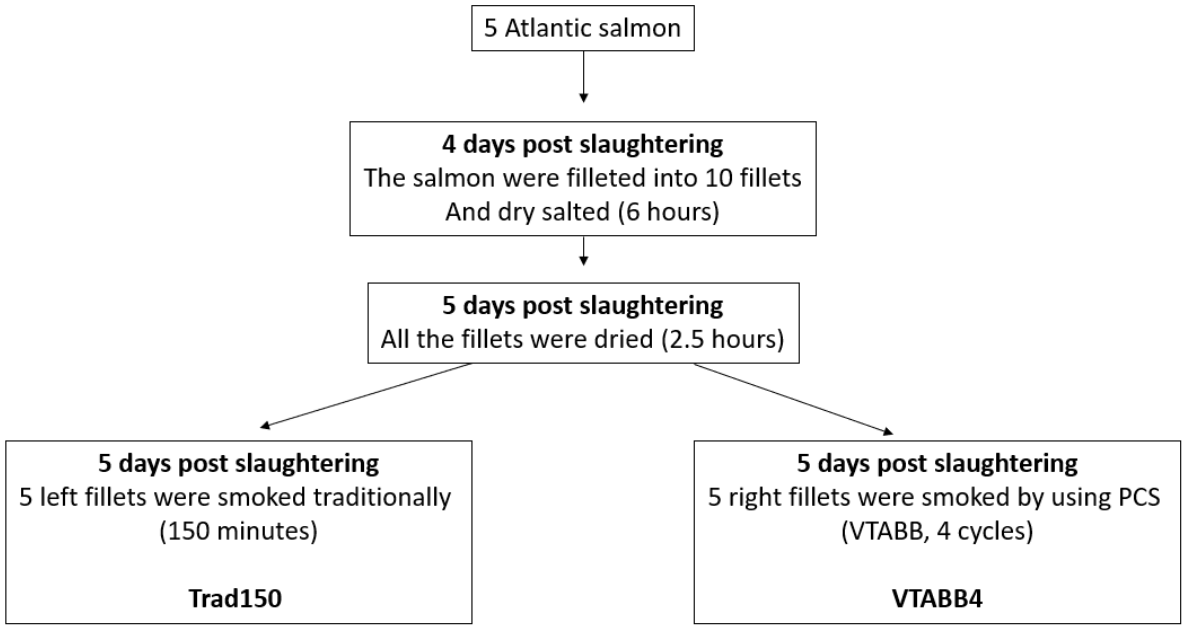


Figure 4.5: A flow sheet illustrating the experimental design for Experiment 3. Two different CSS were produced; Trad150 and VTABB4, by using traditionally wood smoke and PCS, respectively.

After smoking, pH were measured in all the fillets in the area of the caudal peduncle, just beneath the surface. All the CSS were later stored cold (4°C) in plastic foil until next day. Eight different processing groups were then made from the fillets (table 4.4).

Table 4.4: The 8 different groups of CSS produced in experiment 3. A-D include CSS smoked traditionally, where A and B were inoculated with *L. innocua*, packed in vacuum with or without SGS treatment. The groups E-H represents CSS with the same processing properties, only these samples were smoked by PCS (VTABB).

Label	Smoking method	Inoculated (Yes/No)	Packing method	Name shortening
A	Traditionally	Yes	Vacuum	Trad-V-Ino
B	Traditionally	Yes	SGS + vacuum	Trad-S-Ino
C	Traditionally	No	Vacuum	Trad-V-Nat
D	Traditionally	No	SGS + vacuum	Trad-S-Nat
E	PCS	Yes	Vacuum	VTABB-V-Ino
F	PCS	Yes	SGS + vacuum	VTABB-S-Ino
G	PCS	No	Vacuum	VTABB-V-Nat
H	PCS	No	SGS + vacuum	VTABB-S-Nat

177 samples on 10 g were cut from the 8 different CSS fillets smoked by PCS and traditionally wood smoke. All the samples included the smoked crust of the fillet. A fleet sheet illustrating the creation of the 8 different groups is shown in fig. 4.6.

For the groups not inoculated (C, D, G, H), here called "natural", the samples were packed in pairs; one sample for microbiology analyses and one sample for chemical analyses. For the inoculated groups (A, B, E, F), the samples were inoculated with *L. innocua* with a final concentration of 10^3 CFU/g, and packed individually. The inoculum was spread out on 10 g samples using a sterile hockey-stick cell spreaders.

Half of the samples (B, D, F, H) were pre-treated with CO_2 for 8 hours (SGS-treatment) before vacuum packed. The other half (A, C, E, G) were directly vacuum packed. All the samples were stored cold (4°C) upon analysing day.

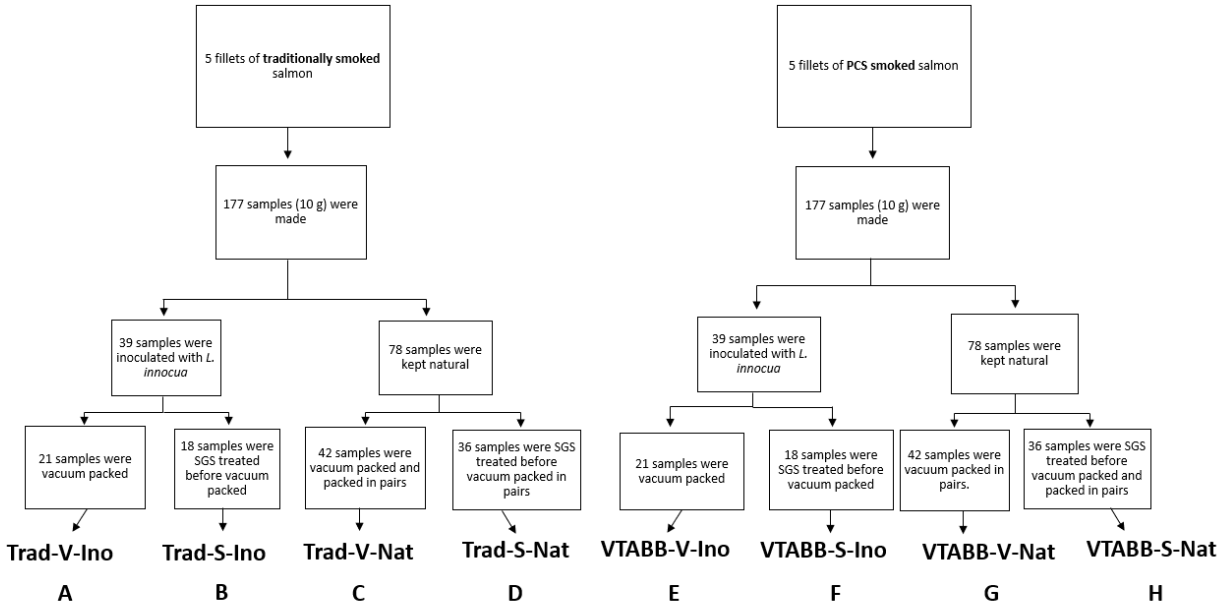


Figure 4.6: A flow sheet illustrating the eight different groups of CSS produced in experiment 3, including smoking protocol (PCS or traditionally wood smoke) and packing technology (vacuum with or without SGS treatment). A, B, E and F represents the samples inoculated with *L. innocua*. C, D, G and H represents the samples not inoculated, which were packed in pairs in one vacuum bag. Both label (A-H) and name shortening are included.

The analyses performed in experiment 3 and sampling day for the analyses (microbiological and chemical) of the CSS is given in table 4.5, where the sampling days represents days of cold storage (4°C) post processing. The pH measurements, colour, salt and phenol analysis were only performed on the samples not inoculated with *L. innocua* (labelled C, D, G and H). On analysing day microbiological analyses (APC and LAB) were performed before pH, texture and colorimetric measuring.

Table 4.5: List of parameters and days post processing of CSS the analyses were conducted in experiment 3 The pH measurement, texture, salt and TPC were only performed on sample pieces not inoculated (C, D, G and H) with *L. innocua*.

Parameter	**sampling day**
Total Plate Count	1 + 3* + 6 + 10* + 13 + 20 + 27 + 34 + 41 + 48
Listeria	1 + 3* + 6 + 10* + 13 + 20 + 27 + 34 + 41 + 48
pH	1 + 6 + 13 + 20 + 27 + 34 + 41 + 48
Salt	6 + 27 + 41
Colour	6 + 20 + 27 + 41
ATP Breakdown Products	6
Total Phenolic Content (TPC)	1*

1* only performed on group C and G
3* and 10* only performed on A, B, E and F

4.1 Processing Technology

4.1.1 Delivery of the fish

Head on gutted (h.o.g) Atlantic Salmon with an average weight of 4.5 kilograms were purchased from different producers depending on the experiment (see Appendix A.2, table A.1). The fish were brought to NTNU, Trondheim, Norway. All the fish were covered with ice in polystyrene boxes, and filleted manually after different amount of time after arrival, depending on the experiment. The colour of the fillets was measured in Experiment 1 and 2, before any processing. Microbiological samples (LAB, APC and H_2S) were taken from the raw material at every delivery of new fish (in total 3 deliveries). Here, 1 sample from 3 different salmon fillets were examined, since it was anticipated same microbiological flora on the fish delivered from the same production site with the same supply (the procedure is explained under "2.1.4 Microbiological sampling"). The salmon were manually filleted into left and right fillets and each individual where labeled from 1-X (X = total amount of fish purchased) and R and L for right and left fillet, respectively. All the fillets in Experiment 1 and 2 were weighed after filleting and stored at 4°C, each fillet wrapped individually in plastic foil upon the day of salting.

4.1.2 Salting

Salting were performed by dry salting in all of the experiments, where an approximately 1 cm thick layer of NaCl (GC Rieber, Food Grade, Fint Raffinert Salt, Lot# 24072019) where applied on the flesh side of the fillets. The salting time was dependent on the experiment. During salting, the fish where stored on grids at 4°C, with no packaging material. At the end of salting time, the salt was washed off using running cold water, and the fillets where weighed and wrapped individually in plastic foil and stored cold (4°C) upon smoking day.

4.1.3 Smoking

The fillets were placed on grids and dried for 2.5 hours at 25 °C. Depending on what smoking protocol performed first (traditional or PCS smoking), the left and right fillets got separated and either packed in plastic foil for temporary storage at 4°C or placed on grids for smoking. In all the experiments the right fillets were always smoked by using PCS, and the left fillets were smoked by using traditionally wood smoke. Both of the smoking procedures were performed by cold smoking protocols.

Traditionally
Smoking by traditionally wood smoke was performed using wooden chips in a smoking cabinet (Kerres smoke-air® show smoker CS700 EL MAXI 1001 smoking cabinet, Germany) at 22 °C. Grids loaded with five or six fillets were placed in the oven. Wood chips (RÄUCHERGOLD, HBK 750-2000, Delikatess-Räucherspäne, Beechwood Chips) was in advanced moisturized for 12 hours (2 kg wood chips with 1 L water). Depending on the smoking time for each experiment, the fillets were removed from the oven at the end of smoking time and weighed. For storage the fillets were packed individually in plastic foil and stored at 4°C for two days.

Purified Condensed Smoke
The grids loaded with five or six right fillets were placed inside the smoking cabinet (Kerres smoke-air® show smoker CS700 EL MAXI 1001 smoking cabinet, Germany), and first dried for 20 minutes. The salmon fillets were then smoked using atomized vapour (approximately 130-140 mL/cycle). One cycle consisted of 3 rounds of *Air circulation* and 1 round of *drying*. Each round of the maturing process included 15 seconds of atomizing PCS in the drying cabinet, and seven minutes of air circulation in the closed chamber. After three rounds of atomized vapour and air circulation, the fillets got dried for 20 minutes. The number of cycles performed

on the salmon fillets and the type of PCS used were dependent on the experiment. After the smoking the fillets were weighed and wrapped individually in plastic foil and stored at 4°C for two days.

After smoking
After two days of storage post processing of the CSS fillets the colour were measured, and the fillets were weighed. Then the fillet got separated into different samples depending on the experiment. Each sample was then vacuum-packed (Webomatic packaging system, Webomatic®, Germany) individually, and stored at 4°C upon sampling day. The processing yield was calculated using weight of raw material and fillet weight post salting (see Appendix A.5.1).

4.1.4 Microbiological Analyses

A 10 g sample of fish muscle was aseptically transferred to an individually sterile stomacher bag, together with 90 g of sterile Peptone Water (8.5 g/L NaCl and 1 g/L neutralised bacteriological peptone [Oxoid, LP0034]). The sample was homogenized vigorously for 60 seconds in a Stomacher 400 Lab Blender (Masticator Homogenizator, Basic/Panoramic, IUL Instruments, Spain). Appropriate serial dilutions, depending on the experiments, were made in sterile peptone water and spread on their respective agar plates. Aerobic plate count (APC) and H_2S-producing bacteria were quantified as total and black colonies, respectively, on Iron Agar (Lyngby) Without L-Cystein (IA; Oxoid, CM0964), supplemented with 0.04% L-cysteine (Sigma-Aldrich, Oslo, Norway, Lot STBH2586) and incubated at 22.0°C for 72 hours ± 6 hours, according to NMKL [2006]. Lactic Acid Bacteria (LAB) were quantified on M.R.S Agar (de Man, Rogosa, Sharpe [Oxoid, CM0361]), without any supplement, and incubated at 25°C for 5 days, according to NMKL [2007b]. Inoculated *L. inncoua* was quantified on Brilliance^{TM} Listeria Agar Base (BLA, Oxoid, CM1080), supplemented with Brilliance^{TM} Listeria Selective Supplement (Oxoid, SR0227E), according to NMKL [2007a]. The plates were incubated for 24 hours at 37°C. All sampling was done in accordance with NMKL [2010] and manufactures recommendations.

Execution day for the analyses of APC, H_2-producing bacteria, Listeria and LAB and which analysis performed were dependent on the experiment. Example of molecular growth calculations are given in Appendix A.5.6.

4.1.5 Colorimetric and Reflective Determination

Two different methods were used to measure the colorimetric properties of raw and smoked Atlantic salmon.

The surface colour of the muscle was measured instrumentally (CIE 1976 L*a*b*) in Experiment 1 and 2, and assessed by a digital photo imaging colour-measuring system (DigiEye 2.8 full system, VeriVide Ltd, UK). The samples were placed one-by-one in a standardized lightbox (daylight, 6400 K) and photographed using a digital camera (Nikon D80, 35 mm lens, Nikon Corp., Japan). The pictures were analysed and color quantified by DigiPix software (VeriVide Ltd., UK). Depending on the experiment, measuring of colour was performed at different samples and time.

In Experiment 3 the reflective properties were performed with a VideometerLab system (Videometer A/S, Hoersholm, Denmark). This multispectral imaging measured the light reflected from a vertical cut of the cold smoked sample. The system is based on a high-intensity integrating sphere illumination featuring light emitting diodes (LED) together with a high-resolution monochrome grayscale camera (Dissing et al., 2011). The data acquisition was done by imaging the muscle at 18 different wavelengths ranging from 405 to 970 nm. The data collected from

the image at each wavelength was an average of all pixels recorded in the area of interest of each sample.

4.1.6 pH Determination

A multi-meter (Testo 206-pH1, USA) was used to measure the pH of both the raw material and CSS. The analysis was performed by pressing the multi-meter 2 cm inside the muscle right beneath the surface. The pH was taken at each sampling day, where the sampling day was dependent on the experiment.

4.1.7 Degradation products for ATP

Frozen (-40°C) samples of smoked salmon got rasped up using a kitchen rasp. 1.2-1.6 g of rasped fish was added a centrifuge tube with 7.5 mL TCA (7%, Sigma Aldrich, Lot STBJ3637, CAS 76039), and homogenized for 2 minutes using a Ultra Turrax (T25 digital ULTRA-TURRAX®).

After homogenization, 3.25 mL of KOH (1.0 M, VWR International, Cas# 1310-58-3) was added, before centrifuging for 15 minutes at 1800 rpm (Hettich® ROTINA 420/420R centrifuge). The supernatant was transferred to plast tubes (15 mL), and stored at -40°C upon analyzing day.

At analyzing day the samples were defrosted, and 1 mL was filtrated using a syringe and a syringe filter (25 mm w/0.45 μm Polyethersulfone membrane, VWR International), before the solution were transferred into a HPLC bottle. Samples were analysed by using reverse phase high performance liquid chromatography (HPLC, Agilent 1290 Infinity II LC system), connected to a diode array UV-VIS detector (Agilent 1260 Infinity Diode Array and Multiple Wavelength Detector). The ATP breakdown products were separated by using a Infinitylab Poroshell 120 column EC-C18, 3.0 x 100mm 2.7-Micron (Agilent, USA), with a UHPLC guard column InfinityLab Poroshell 120 EC-C18, 3.0 x 5mm 2.7 Micron (Agilent, USA). The eluent was 3.5% acetonitrile in H_2O (Cas # 75-05-8), 0.25 M KH_2PO_4 (Cas# 7778-77-0) and 0.0023 M $[CH_3(CH_2)_3]_4N(HSO_4)$ (Cas# 32503-27-8). The flow was a gradient with 0.2 mL/min from 0-2 minutes, 0.8 mL/min from 2-9 minutes and 0.2 mL/min from 9-10 minutes. The detection wavelength was set to 210 and 260 nm. Standard curves of the ATP breakdown products and calculations are shown in A.5.3 and A.5.4, respectively.

4.1.8 Texture Determination

Instrumental textural analyses were performed (duplicate measurements) using a Texture Analyser TA-XT plus (Stable Micro Systems Ltd, England) equipped with a 25 kg load cell and a flat-ended cylindricala plunger (d = 20 mm, type P/1SP). Force-time graphs were documented and analysed by Texture Exponent light software for Windows (Version 4.12, SMS). Breaking force was measured as the force (N) recorded when breakage of the sample surface was observed. The resistance force (F80) was measured as the force (N) needed to press the plunger down 80% of the fillet thickness, and are used to describe sample firmness. The measurement was performed with a constant speed of 2 mm/s close to the lateral line.

4.1.9 Total Phenolic Content [TPC]

Total Phenolic Content were performed based on the guidelines given by "HiST Matteknologisk utdanning" named "Total Fenol" under the subsection "Kjøtt". Frozen smoked salmon got rasped up using a kitchen rasp. Rasped salmon (5 g) were mixed with methanol:water (100:80, 20 mL) to remove lipids, using a Ultra Turrax (T25 digital ULTRA-TURRAX®) at 13,000 rpm

(40 seconds). The supernatant got filtered using a filter paper (Schleicher Schuell, Rundfilter 598/1, 70 mm). Distilled water (5.0 mL) and supernatant liquid (1.0 mL) were oxidized with Folin-Ciocalteu (1.0 mL, VWR International, France, Lot 19J174020) for 8 minutes. The solution got neutralized with sodium carbonate (7%, 10 mL, VWR International, Lot# 17F154110, CAS# 497-19-8), before dissolved with distilled water to a final volume of 100 mL. The absorbance was measured at 750 nm (Shimadzu UV Spectrophotometer, UV-1800, Japan), with distilled water as a blank test, after 2 hours of incubation of the test solution. Example of TPC calculations are given in Appendix A.5.2.

4.1.10 Salt content analysis

1.5-2.0 g frozen rasped salmon was mixed with destilled water (40 mL) for 40 seconds at 13.000 rpm by using a Ultra Turrax (T25 digital ULTRA-TURRAX®). Hot distilled water (60°C, 60 mL) was added the mix, together with 1 mL HNO_3 (1 M, VWR International, Lot# 15L070509). The salt content was measured using TitroLine 7000 with Titration Agent 0.1 M silver nitrate solution ($AgNO_3$, VWR International, Lot# 18E254120 CAS# 7761-88-8), with the program *TitriSoft 3.15*. Two parallels were conducted from each sample. The program measured %chloride content of the sample, and example of calculations is shown in Appendix A.5.5.

4.1.11 Inoculum Preparations of *L. innocua*

Stock cultures of *L. innocua* (CCUG 15531T) were stored at -80°C. Strains were streaked onto a BHI agar plate, and incubated at 37°C. 100 mL of BHI (Oxoid) was inoculated with a single colony of *L. innocua*, and incubated at 37°C with shaking (230 rpm) for 24 hours. 1 mL inoculum were transferred to a new BHI (100 mL), and incubated with shaking (230 rpm) at 8°C for four days. The inoculum was diluted with BHI to a OD_{600}-value of 0.1 (which express $8.7*10^7$ cells/mL). The solution was diluted with BHI, to a final concentration of $8.7 * 10^5$ CFU/mL. 0.1 mL of this inoculum was spread out on a 10 g sample of smoked salmon, ending up with a final concentration of 10^3 CFU/g salmon.

4.1.12 Headspace gas analysis and volume

The headspace gas composition ($\%O_2$ and $\%CO_2$) was measured using an oxygen and carbon dioxide analyser (Checkmate 9900analyzer, PBI-Dansensor, Ringsted, Denmark) as described by [Abel et al., 2018]. The gas compositions were measured in empty trays after packaging and 6 days post packing, and in the trays containing CO_2-treated CSS after packing.

The headspace gas volume (mL) was measured at day 6 post packing by using a texture analyser (StableMicro System Ltd, TA-XT plus, Godalming, UK) as described by [Abel et al., 2018].

4.1.13 Sensory Analyses

Sensory selection were performed in Experiment 1 and 2, whereas Napping® was only performed in Experiment 2. The sensory selection was conducted by a small group (4 and 5 assessors), all members of NTNUs semi-trained panel. The Napping® was conducted by all of the assessors in NTNUs semi-trained panel (10 assessors).

Sensory Selection
The sensory panel only consisted of 4 and 5 participants in Experiment 1 and 2, respectively. The selection was based perception of characteristics such as appearance, texture, smoking character and salt taste.

Napping®
Vacuum packed smoked salmon was held at room temperature two hours before being cut into slices (3 mm wide). Two slices were served each assessor in plastic containers (65 mm in diameter, Veriplast Holland BV), in room temperature. The napping were performed at NTNU, Kalvskinnet at "Sensorikklaben", a lab designed according to the ISO standard "General guidance for the design of test room" ISO8589:2007, in white tasting booths (with controlled air conditioning and lightning). All samples were served simultaneously in a randomized order, coded with 3-digit codes. Room tempered water and neutral crispy bread (Korni Flatbrød) were supplied. Before the analysis, a plenary discussion controlled by the panel leader was conducted to prepare a list of terms, see Appendix A.4, fig. A.2. The Napping® with UFP allowed the assessors to select terms from the list of terms.

The data were captured using the software EyeQuestion (Logic8 BV) (fig. A.1, Appendix A.1). All statistical analyses were performed using the XLSTAT software (Addinsoft, Paris, France). The X and Y coordinates and the frequency mention of terms were analysed by MFA.

4.1.14 Statistics

One-Way ANOVA analysis with Tukey Pairwise Comparison test was achieved for calculations comparing the experimental groups, by using IBM SPSS (Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) Significance level (α) was set to 5%. Paired-Samples T-test was used when comparing parameters of the two smoking protocols (traditionally smoking and PCS atomized smoking), with a confidence interval of 95%. All results are given as means ± standard deviation (SD) if not stated otherwise. To meet the requirements of equal variance and normal distribution, all statistical analyses of microbial growth were done on $\log_{10}$-transformed data.

5 Results

Three Experiments (1, 2 and 3) were performed by smoking Atlantic salmon with purified condensed smoke (PCS) and traditionally wooden smoke, respectively.

In the first experiment three intensities of PCS, and traditionally wooden smoke were conducted to produce CSS. Microbiological, sensory and chemical analyses were performed to compare the different smoking protocols.

The second Experiment was mainly based on comparing three different types of PCS, with different level of smoke intensity. Microbiological and chemical analyses were performed, and also sensory selection and Napping®.

The third experiment was conducted to study the effect of the most promising PCS (decided from microbiology analyses and Napping® in Experiment 2) compared to traditionally wooden smoke, to inactivate or inhibit the growth of *L. innocua*. Both microbiological and chemical analyses were conducted.

5.1 Experiment 1: Comparison of Traditionally Wooden smoked salmon and PCS smoked salmon

5.1.1 Processing Parameters

Processing Yield

In the present experiment a difference in smoking protocol ($p < 0.005$) was observed with traditionally smoked groups having a higher content (93.32 ± 0.90 %) than VTABB atomized groups (92.66 ± 0.75 %). However, smoke intensity did not affect processing yield ($P > 0.227$), indicating similar water holding capacity and water content of the products (fig. 5.1). However, Trad 90 and Trad 120 showed a tendency of a higher total yield with averages of 93.66 ± 0.51% and $\pm$ 1.02%, respectively, and VTABB 5 with a lower at 92.33 ± 0.23%, but none of these values were significant.

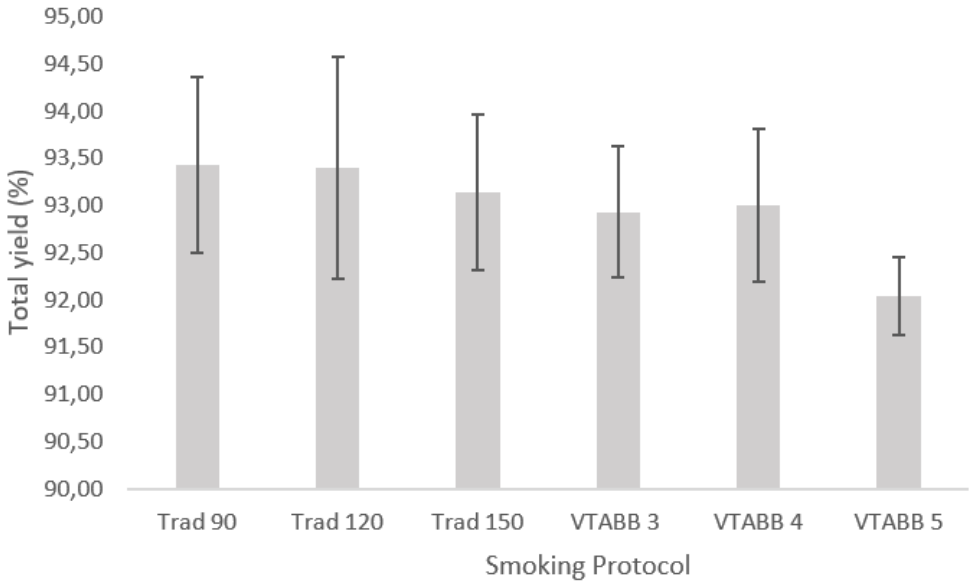


Figure 5.1: The processing yield (%) (mean ± SD) of smoked salmon produced by traditionally wooden smoke (Trad) and atomization of purified condensed smoke (VTABB), from Experiment 1 (n = 4). The total yield was measured using weight before salting and two days post processing. The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. The results showed an average of higher processing yield of traditionally smoked groups than VTABB atomized groups ($p < 0.005$).

5.1.2 Chemical Parameters

pH

Based on the experimental design a main effect of smoking protocol was observed on the fillet pH. By comparing the initial and final pH of the smoking protocols, VTABB atomized groups had significant lower pH than the traditionally smoked groups ($p < 0.001$). Average initial pH were 6.29 ± 0.10 and 5.94 ± 0.09, and mean final pH were 6.45 ± 0.02 and 6.24 ± 0.12, for traditionally smoked and PCS atomized groups, respectively.

By comparing the initial pH of experimental groups, the traditionally smoked groups had significantly higher pH than the VTABB atomized groups ($p < 0.029$), whereas Trad 150 and PCS 3 ($p > 0.063$) only showed a tendency.

Overall, the pH regarding all the experimental groups seemed to increase during storage. However, only Trad 90, VTABB 3 and VTABB 4 had significant difference between first and final storage day ($p < 0.026$, $p < 0.044$ and $p < 0.023$, respectively). VTABB 5 had a significantly lower pH than the other groups from day 14 and throughout the storage period ($p < 0.040$). VTABB 3 and 4 seemed to be lower than the traditionally smoked groups throughout storage too, although this was only sporadically significant. At the initial and final day VTABB 4 seems to have lower pH than VTABB 3, although this was not significant ($p > 0.867$). The pH between the traditionally smoked groups, were on the other hand, more sporadically, and was not observed to be different from each other at any measuring throughout storage.

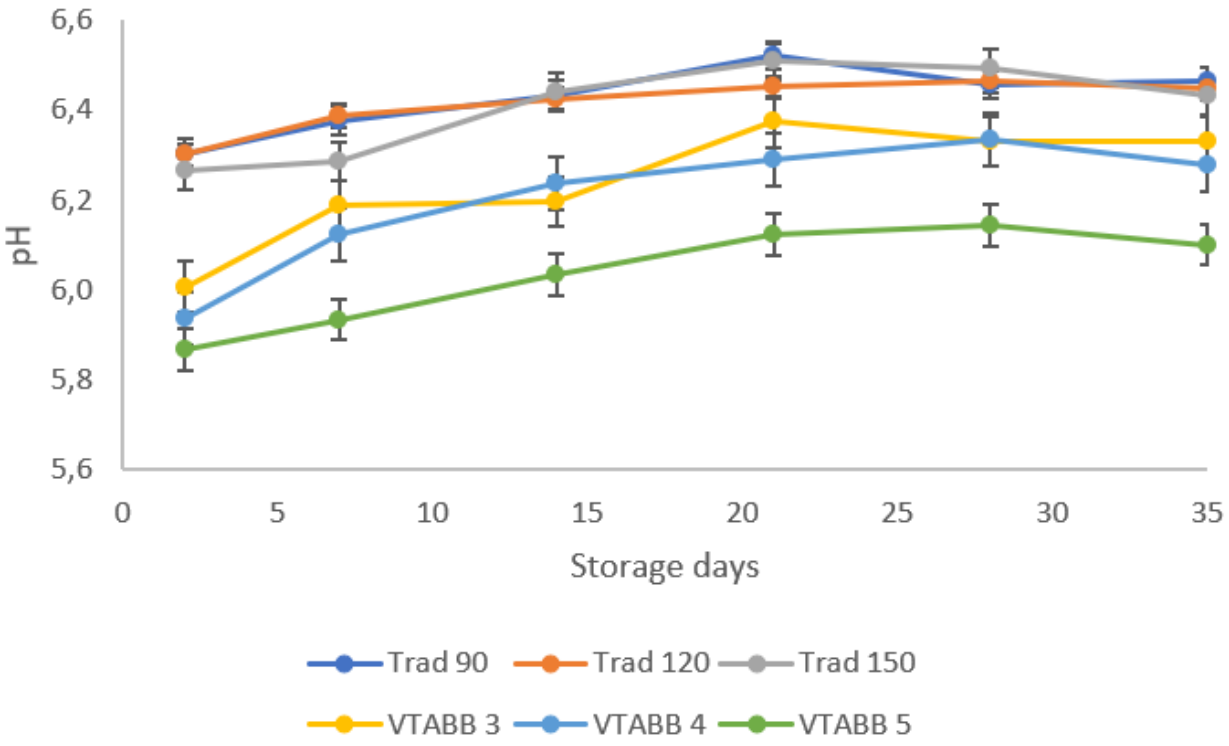


Figure 5.2: pH measured throughout storage of cold smoked salmon produced in Experiment 1 (n = 3). The groups labelled "Trad" are smoked by using traditionally wooden smoke, and the groups labelled "VTABB" by atomization of purified condensed smoke (VTABB). The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. A significant difference ($p < 0.001$) was observed between the final mean pH of the smoking protocols, where traditionally smoked groups had a mean of 6.45 $\pm$ 0.02 and VTABB atomized groups 6.24 $\pm$ 0.1)

Total Phenolic Content (TPC)

A comparison of the two smoking protocols revealed a higher ($p < 0.001$) TPC of VTABB atomized groups (0.41 $\pm$ 0.037 mg/100 g raw material) than traditionally smoked groups (0.37 $\pm$ 0.026 mg/100 g raw material).

Trad 90 was observed to have a significant lower TPC than VTABB 4 and VTABB 5 ($p < 0.001$), with a content of 0.35 $\pm$ 0.02 mg/100 g raw material. Trad 150 was also observed to have a lower TPC than VTABB 4 ($p < 0.021$), where VTABB 4 had a TPC at 0.43 $\pm$ 0.02 mg/100 g raw material. The traditionally smoked groups were not observed to be significant different in TPC from each other. However, VTABB 3 was observed to have a significant lower TPC than VTABB 4 and VTABB 5 ($p < 0.011$ and $p < 0.031$, respectively).

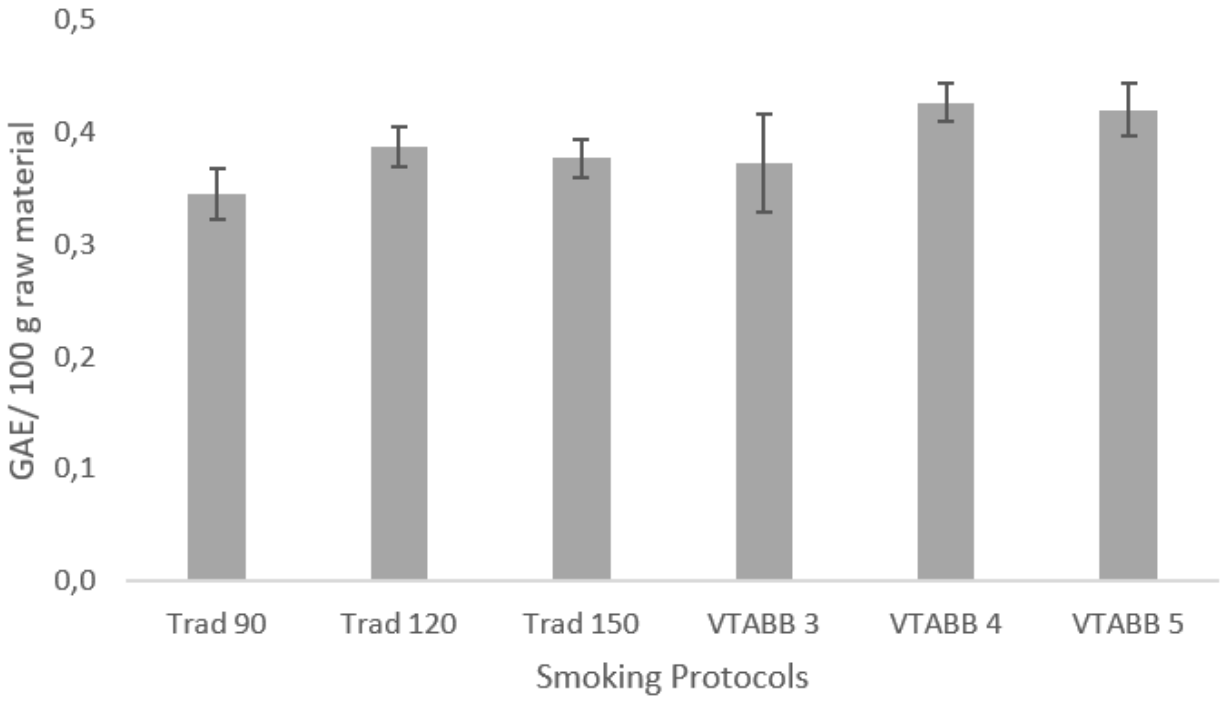


Figure 5.3: The total phenolic content measured of smoked salmon produced by traditionally wooden smoke (Trad) and atomization of purified condensed smoke (VTABB), from Experiment 1 (n = 3). The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. VTABB atomized groups were measured to have a higher content than traditionally smoked groups ($p < 0.001$).

ATP Breakdown Products

Breakdown products from ATP were measured after 2 and 35 days storage (fig. 5.4). After 2 days storage, 3 peaks were detected in the chromatogram, see table A.2. They were most likely the ATP breakdown products hypoxanthine (Hx), inosine monophosphate (IMP) and inosine (Ino) (see Appendix A.3, table A.3). The content of the Hx, IMP and Ino, including K_1-value, H-value and Hx/Ino ratio can be seen in table 5.1.

Based on the experimental design, a main effect of the smoking protocols was observed after 2 days storage regarding the mean μmol/g of Hx ($p > 0.002$) (fig. 5.4A), where the traditionally smoked groups had a higher content. Including that Trad 150 was observed to have significant higher amount of Hx than VTABB 4 ($p < 0.009$). After 35 days storage the amount of Hx had significantly increased in all the experimental groups ($p < 0.001$). Although, no main effect of the smoking protocols was observed ($p > 0.133$). On the other hand, VTABB 3 was observed to have significant higher amount of Hx compared to Trad 120, Trad 150 and VTABB 5 ($p < 0.007$), with a mean of 1.87 ± 0.15 μmol/g.

Average μmol/g of Ino in traditionally smoked and VTABB atomized groups were found to be 4.18 ± 0.17 and 3.63 ± 0.23, respectively ($p > 0.008$), 2 days post processing. Trad 150 was observed to have a higher content of Ino than both VTABB 3 and VTABB 4 ($p < 0.017$ and $p < 0.035$, respectively), with a mean of 4.55 ± 0.57 μmol/g. After 35 days storage the amount of Ino had raised higher in all the experimental groups ($p < 0.001$). The results also indicate a difference in the content of Ino regarding smoking protocols, where VTABB atomized groups had a higher content ($p < 0.001$). Trad 120 and Trad 150 were both observed to have significant lower Ino values than VTABB 4 and VTABB 5 ($p < 0.046$), with means of 6.62 ± 0.68 μmol/g and 6.49 ± 0.75 μmol/g, respectively.

The amount of IMP (fig. 5.4C) observed after 2 days storage was significant higher than Hx ($p < 0.001$) in all groups, where traditionally and VTABB atomized groups showed an average concentration of μmol/g of 6.61 $\pm$ 0.47 and 6.32 $\pm$ 0.4 µmol/g, respectively ($p > 0.106$). However, the results do not indicate a difference in IMP content between the smoking protocols ($p > 0.662$). Nonetheless, a significant difference was observed between VTABB 5 and VTABB 4 ($p < 0.002$), with 4.78 $\pm$ 0.82 and 8.13 $\pm$ 1.22 μmol/g, respectively. There was not observed any amount of IMP after 35 storage days in any of the experimental groups.

The K_1-value was only valuable 2 days post processing, since IMP was not detected at day 35, and therefore gave a 1.00 value. The H-value was reduced regarding all the experimental groups from 2 to 35 days storage. The Hx/Ino ratio increased from day 2 to 35 post processing. No significant differences regarding K_1-value and Hx/Ino ratio were observed between the smoking protocols ($p < 0.423$).

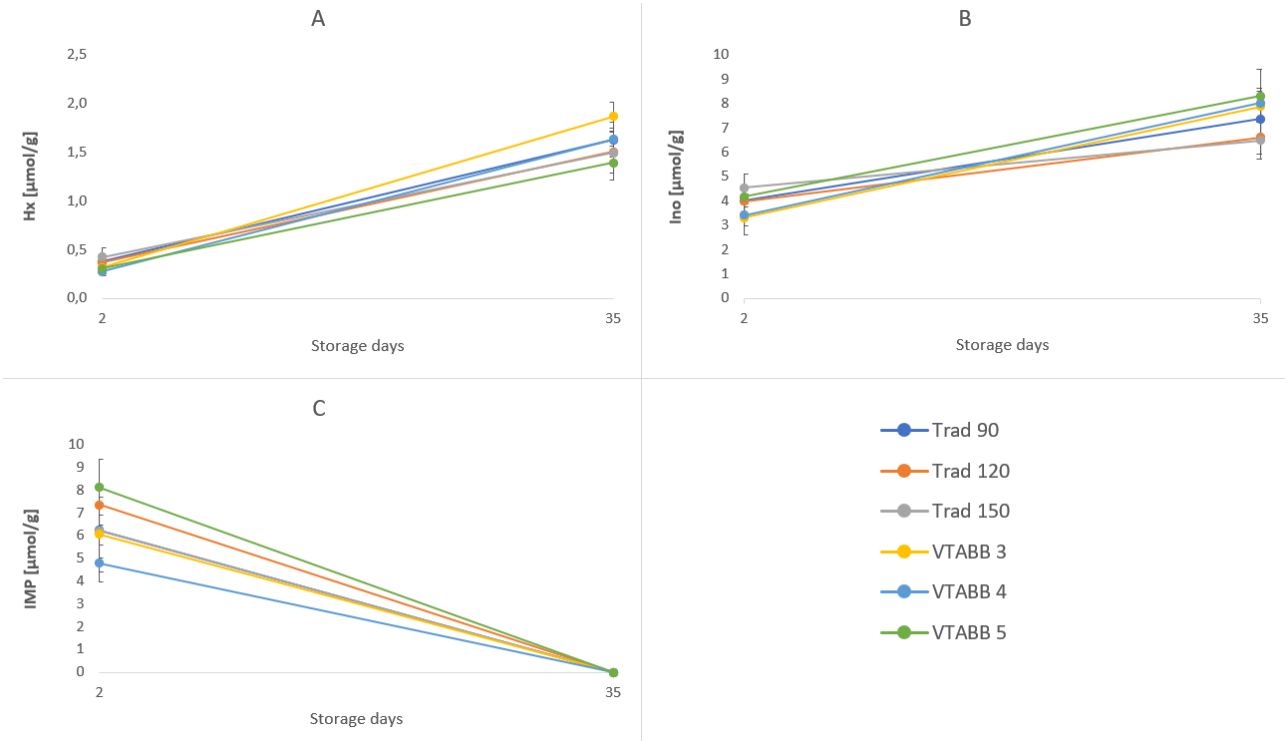


Figure 5.4: ATP breakdown products measured 2 and 35 days post processing of cold smoked salmon produced in Experiment 1 (n = 3). Hypoxanthine (Hx), inosine (Ino) and inosine monophosphate (IMP) are represented in figure A, B and C, respectively. The groups labelled "Trad" are smoked by using traditionally wooden smoke, and the groups labelled "VTABB" by atomization of purified condensed smoke (VTABB). The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. The content of Hx and Ino were observed to be significant higher 35 days post processing than after 2 days ($p < 0.001$). No amount of IMP was observed at day 35.

Table 5.1: The amount of the ATP breakdown products hypoxanthine (Hx), inosine monophosphate (IMP) and inosine (Ino) in μmol/g, including measured H-value (Hx/(Hx+IMP+Ino)), K_1-value ((Hx+Ino)/(Hx+Ino+IMP)) and Hx/Ino ratio.

Experimental group	**Hx (μmol/g)**	**Ino (μmol/g)**	**IMP (μmol/g)**	**K_1-value**	**H-value**	**Hx/ino ratio**
Day 2 post processing						
Trad 90	0.38 ± 0.07	6.24 ± 1.21	4.00 ± 0.27	0.41	3.55	0.09
Trad 120	0.37 ± 0.07	7.35 ± 0.87	4.00 ± 0.55	0.37	3.14	0.09
Trad 150	0.42 ± 0.10	6.23 ± 1.80	4.55 ± 0.57	0.44	3.77	0.09
VTABB 3	0.32 ± 0.06	6.05 ± 1.63	3.31 ± 0.72	0.37	3.26	0.10
VTABB 4	0.28 ± 0.05	4.78 ± 0.82	3.42 ± 0.45	0.44	3.26	0.08
VTABB 5	0.31 ± 0.03	8.13 ± 1.22	4.17 ± 0.92	0.36	2.44	0.07
Day 35 post processing						
Trad 90	1.63 ± 0.12	7.37 ± 0.90	-	1.00	0.18	0.22
Trad 120	1.51 ± 0.13	6.62 ± 0.68	-	1.00	0.19	0.23
Trad 150	1.50 ± 0.21	6.49 ± 0.75	-	1.00	0.19	0.23
VTABB 3	1.87 ± 0.15	7.89 ± 0.63	-	1.00	0.19	0.24
VTABB 4	1.63 ± 0.18	8.03 ± 0.58	-	1.00	0.17	0.20
VTABB 5	1.39 ± 0.18	8.32 ± 1.08	-	1.00	0.14	0.17

Salt Content

There was not observed any differences in comparison of smoking protocols ($p > 0.881$), nor the experimental groups ($p > 0.993$), regarding the salt content (%) 7 days post processing (fig. 5.5). The mean NaCl % for the traditionally smoked and VTABB atomized groups were 1.90 ± 0.06% and 1.92 ± 0.07%, respectively.

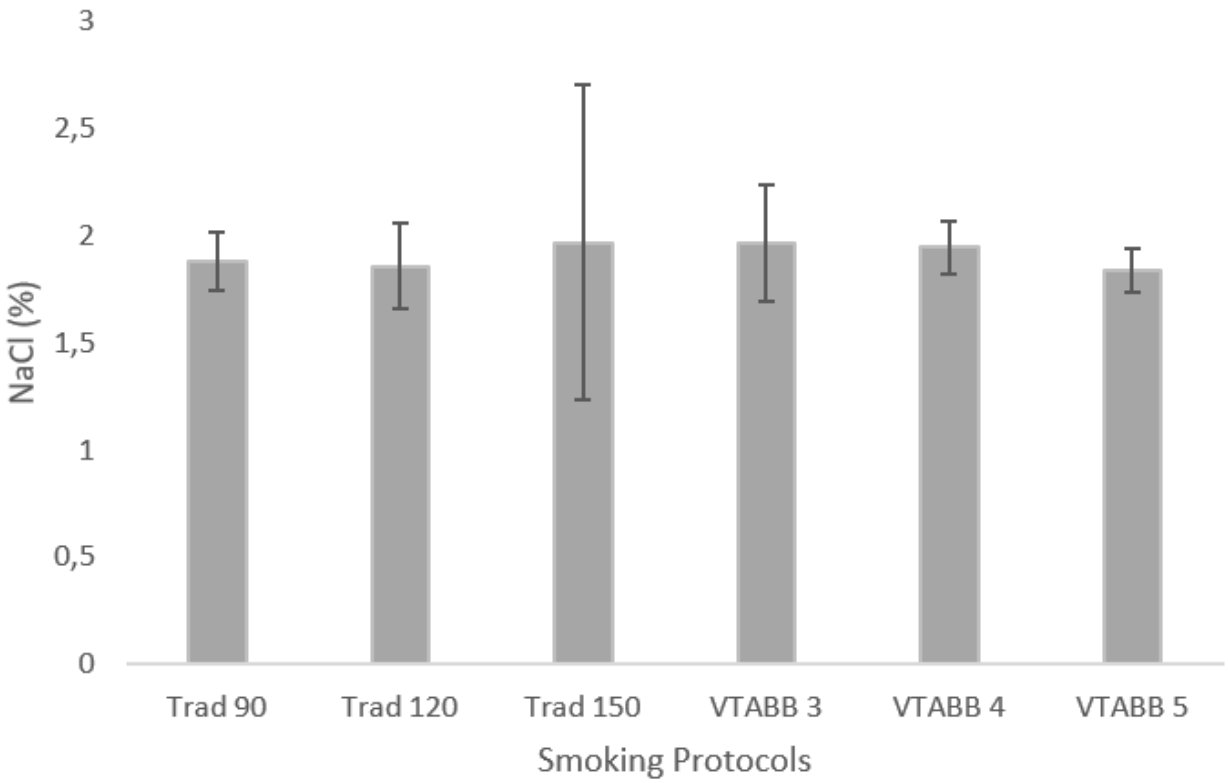


Figure 5.5: The salt content (% NaCl) measured 7 days post processing in Experiment 1 (n = 3). The groups labelled "Trad" are smoked by using traditionally wooden smoke, and the groups labelled "VTABB" by atomization of purified condensed smoke (VTABB). The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. The results showed no significant differences between the groups, nor the smoking protocols.

5.1.3 Microbiological Parameters

Aerobic Plate Count (APC)
After 21 days storage Trad 150 was observed to have a growth in APC. The growth was held stable throughout day 35, with a $\log_{10}$(CFU/g) of approximately 2.86 $\pm$ 2.82.

The results from aerobic plate count (APC) indicated no detection of growth regarding the groups Trad 120, VTABB 3 and VTABB 5. The groups Trad 90 and VTABB 4 were on the contrary detected to have a sporadically growth at day 28, but these levels were under the

Lactic Acid Bacteria (LAB)
The results of lactic acid bacteria (LAB) indicated no detection of growth regarding all the experimental groups until 21 days storage. After 21 days, Trad 150 was measured to have a $\log_{10}$(CFU/g) of 2.87 $\pm$ 2.87. After 28 days storage the level of LAB regarding Trad 90 and VTABB 4 had a sporadically growth, but with levels under the qualifying limit. The final storage day (day 35 post processing) had no detection of growth regarding LAB.

5.1.4 Other Quality Parameters

Texture
By comparing the smoking protocols the results of F80 (N) indicate a firmer texture of the VTABB atomized groups, 7 and 21 days post processing ($p < 0.013$ and $p < 0.031$, respectively). Average F80 values of traditionally smoked and VTABB atomized groups after 7 days storage

were 28.65 ± 2.04 N and 39.03 ± 5.58 N. After 21 days storage the average values were 31.93 ± 4.27 N (traditionally smoked) and 41.77 ± 7.67 N (VTABB atomized).

There were also no significant differences observed in F80 (N) regarding the experimental groups of CSS in Experiment 1, 7 days post processing (fig. 5.6). After 21 days storage VTABB 5 had a significant higher F80 compared to Trad 120 ($p < 0.015$) and Trad 150 ($p < 0.028$), respectively. A comparison among the experimental groups shows no difference in F80 when comparing day 7 and 21 post processing ($p > 0.258$).

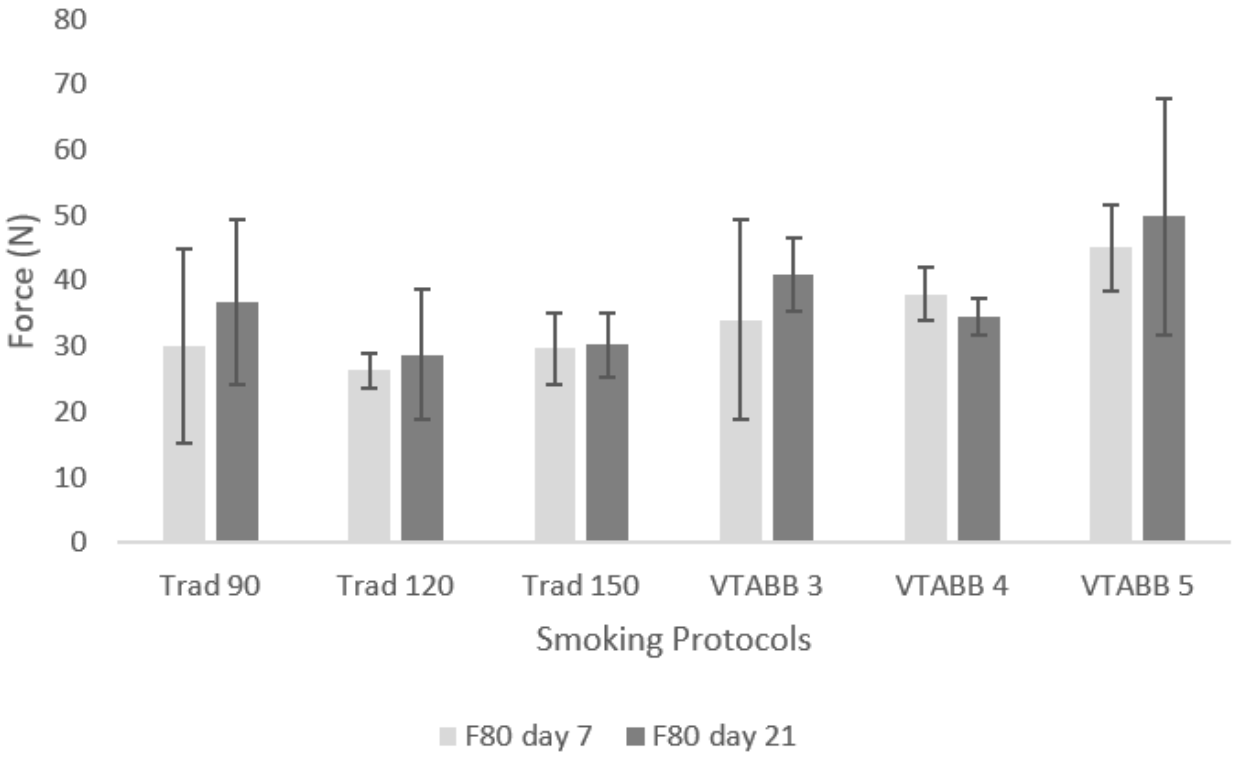


Figure 5.6: The resistance force (F80) measured 7 and 21 days post processing of cold smoked salmon produced in Experiment 1 (n = 3). The groups labelled "Trad" are smoked by using traditionally wooden smoke, and the groups labelled "VTABB" by atomization of purified condensed smoke (VTABB). The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. The results of F80 (N) 7 and 21 days post processing indicate a firmer texture of the VTABB atomized groups than the traditionally smoked groups ($p < 0.031$).

The breaking force (N) regarding the smoking protocols showed a significant higher toughness among the VTABB atomized groups at day 7 ($p < 0.012$) fig. 5.7. However, no significant differences were detected after 21 days storage between the smoking protocols ($p > 0.125$). fig. 5.7.

When comparing each group, VTABB 5 was observed to have significant higher breaking force than Trad 120 ($p < 0.036$) 7 days post processing, with values of 46.46 ± 10.97 N and 32.03 ± 1.92 N, respectively. There was not observed any significant differences between the groups regarding breaking force (N) 21 days post processing. When comparing day 7 and 21 for each group, Trad 120 was detected to have less toughness in breaking force at day 27 ($p < 0.026$).

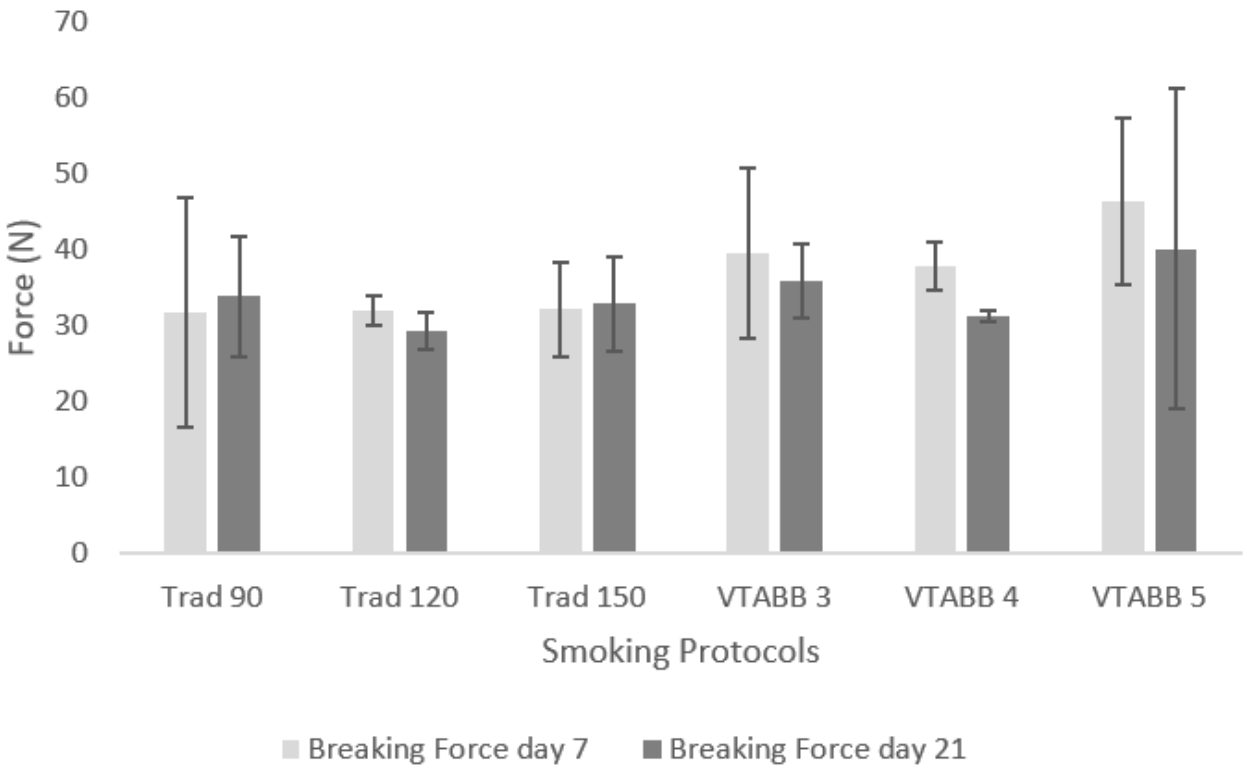


Figure 5.7: The Breaking force (N) measured 7 and 21 days post processing of cold smoked salmon produced in Experiment 1 (n = 3). The groups labelled "Trad" are smoked by using traditionally wooden smoke, and the groups labelled "VTABB" by atomization of purified condensed smoke (VTABB). The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. The breaking force (N) among the smoking protocols showed a significant higher toughness of the VTABB atomized groups at day 6 ($p < 0.012$), but not 21 days post processing ($p > 0.125$).

Colorimetric Properties

The fillet lightness (L^*), redness (a^*) and yellowness (b^*) were measured for the experimental groups of CSS produced in Experiment 1, see fig. 5.8, 5.9 and 5.10, respectively.

By comparing the lightness between the raw material and last storage day among each group, all the VTABB atomized groups and Trad 150 had significance lower lightness ($p < 0.038$). The change in redness, on the other hand, was only observed in Trad 120 ($p < 0.022$) and VTABB 3 ($p < 0.050$), and with a tendency in VTABB 5 ($p > 0.054$).

Throughout storage the results indicate an average higher lightness among the traditionally smoked groups than the VTABB atomized groups ($p < 0.008$). After 2 and 14 days storage fillet lightness (L^*) was observed to be significant higher for all the traditionally smoked groups of CSS, compared to VTABB 4 and VTABB 5 ($p < 0.026$). However, there was not observed any significant differences 7 days post processing. At 14 days post processing the mean L^* value of the traditionally smoked and VTABB CSS were 58.80 ± 0.35 and 56.11 ± 0.42, respectively. At the final storage day (35 days post processing), Trad 90 was observed to have a significantly higher value of L^* than VTABB 4 ($p < 0.036$) and VTABB 5 ($p < 0.011$).

Throughout the whole storage experiment there was not observed any significant differences regarding the mean of the two smoking protocols ($p > 0.290$).

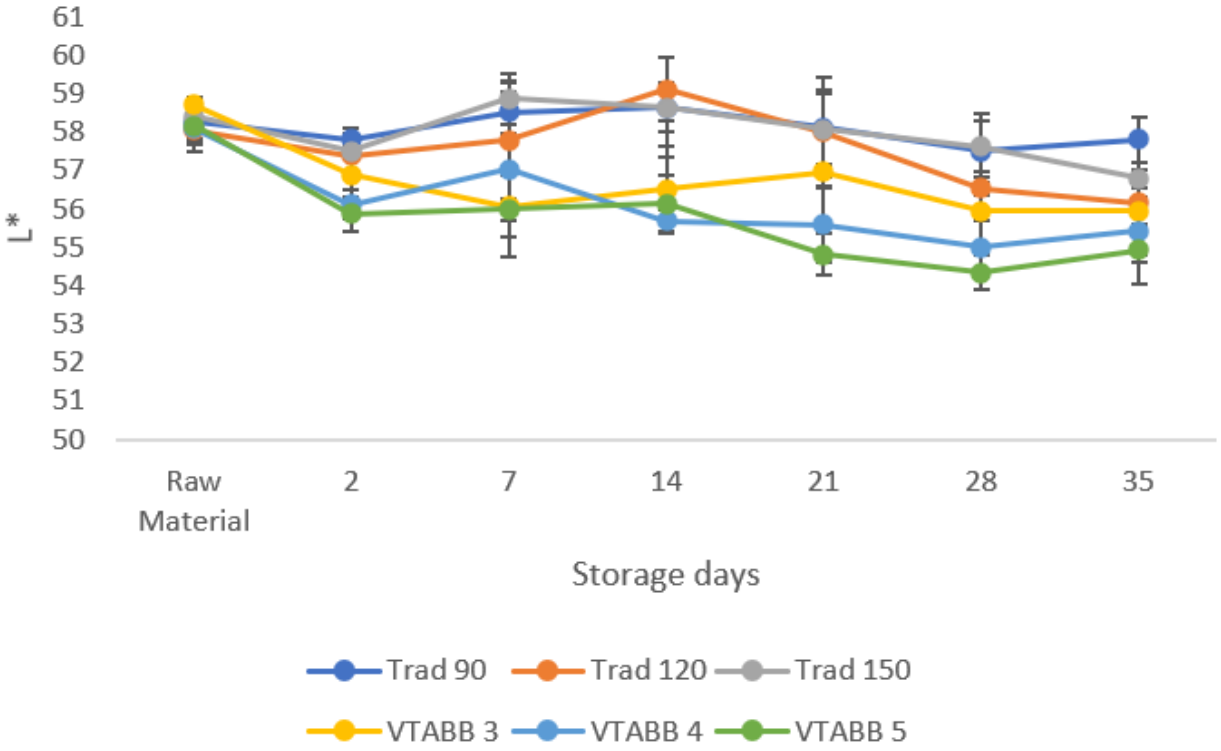


Figure 5.8: Fillet lightness (L*) measured in the raw material (n = 4), and throughout storage of cold smoked salmon produced in Experiment 1 (n = 3). The groups labelled "Trad" are smoked by using traditionally wooden smoke, and the groups labelled "VTABB" by atomization of purified condensed smoke (VTABB). The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. Traditionally smoked groups had an average higher lightness throughout the storage period than VTABB atomized groups ($p < 0.008$). At the final storage day Trad 90 was observed to have a higher level of L^* than VTABB 4 and VTABB 5 ($p < 0.036$).

The traditionally smoked groups had a significantly higher redness than VTABB atomized groups at day 7 and throughout day 28 post processing ($p < 0.024$). After 14 days storage Trad 90 and Trad 120 had the highest value of a^*, with a mean of 16.53 ± 0.42, when compared to VTABB 4 and VTABB 5 ($p < 0.014$). In addition, Trad 90 was observed to have a significant higher redness than Trad 150 and VTABB 3 ($p < 0.028$).

At the final storage day, Trad 90 was observed to have a significant higher fillet redness than VTABB 5 ($p < 0.013$), with values of 13.43 ± 0.33 and 8.05 ± 1.44, respectively.

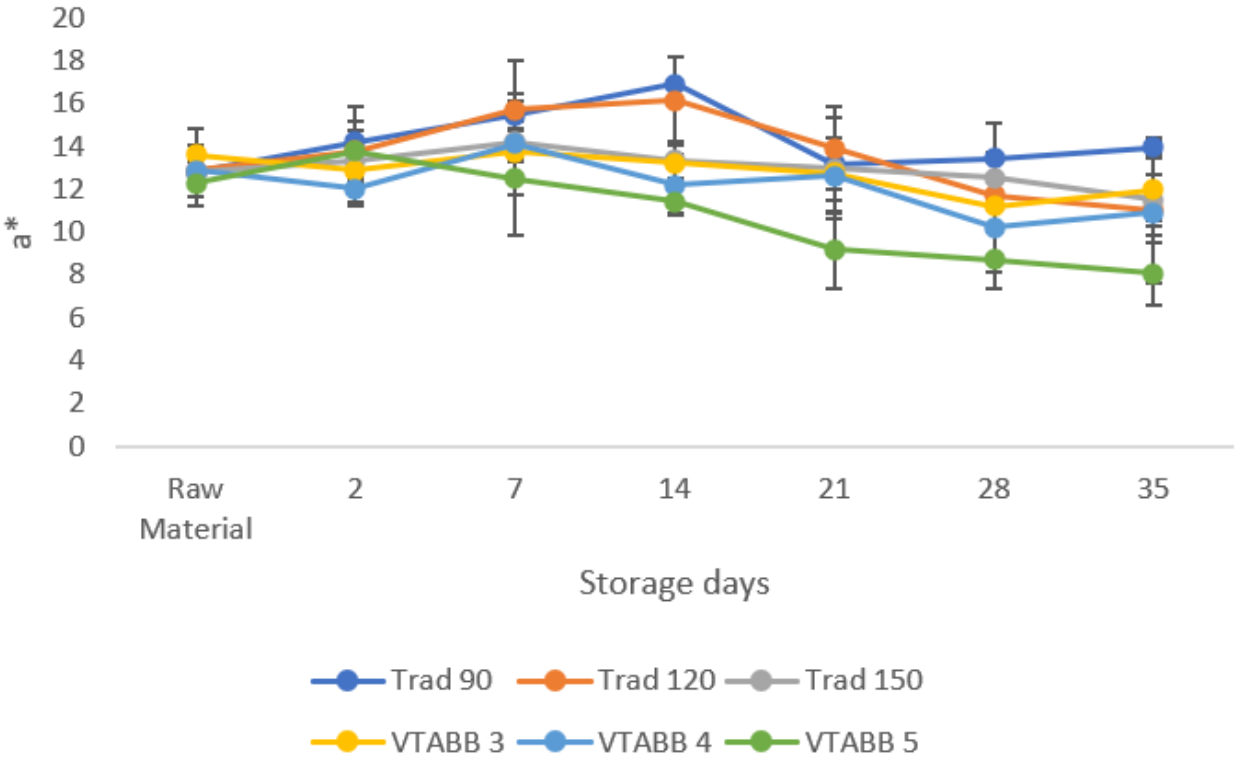


Figure 5.9: Fillet redness (a*) measured in the raw material (n = 4), and throughout storage of cold smoked salmon produced in Experiment 1 (n = 3). The groups labelled "Trad" are smoked by using traditionally wooden smoke, and the groups labelled "VTABB" by atomization of purified condensed smoke (VTABB). The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. Traditionally smoked groups had a higher redness than the VTABB atomized groups from day 7-28 post processing ($p < 0.024$). There was not observed any significant differences in fillet redness (a^*) between first and final storage day for each group. At the final storage day Trad 90 was observed to have a higher value of a^* than VTABB 5 ($p < 0.011$).

VTABB atomized groups were detected significant lower in yellowness than the traditionally smoked groups when comparing the smoking protocols from day 7 and throughout day 28 post processing ($p < 0.046$). A significant difference in fillet yellowness (b^*) was observed at day 14 post processing, where Trad 90 and Trad 120 were observed to have a significant higher b^* value than the VTABB atomized groups ($p < 0.030$). In addition, Trad 90 was observed to be significant higher in fillet yellowness than Trad 150 ($p < 0.045$), and Trad 150 was significant higher in fillet yellowness than VTABB 5 ($p < 0.016$). To sum up, all the traditionally smoked groups were significant higher in fillet yellowness than VTABB 5 after 14 days storage.

At the final storage day, Trad 90 and VTABB 3 were found to have significant higher values for b^* than VTABB 5 ($p < 0.005$ and $p < 0.047$, respectively). At this day, Trad 90 had a b^* value of 11.34 $\pm$ 1.53 and VTABB 5 had a b^* value of -4.18 $\pm$ 3.07.

By comparing the yellowness of raw material and final storage of each group, VTABB 5 had a significantly lower yellowness ($p < 0.022$). Although, Trad 150, VTABB 3 and VTABB 4 showed similar tendencies ($p > 0.051$, $p > 0.065$ and $p > 0.068$, respectively).

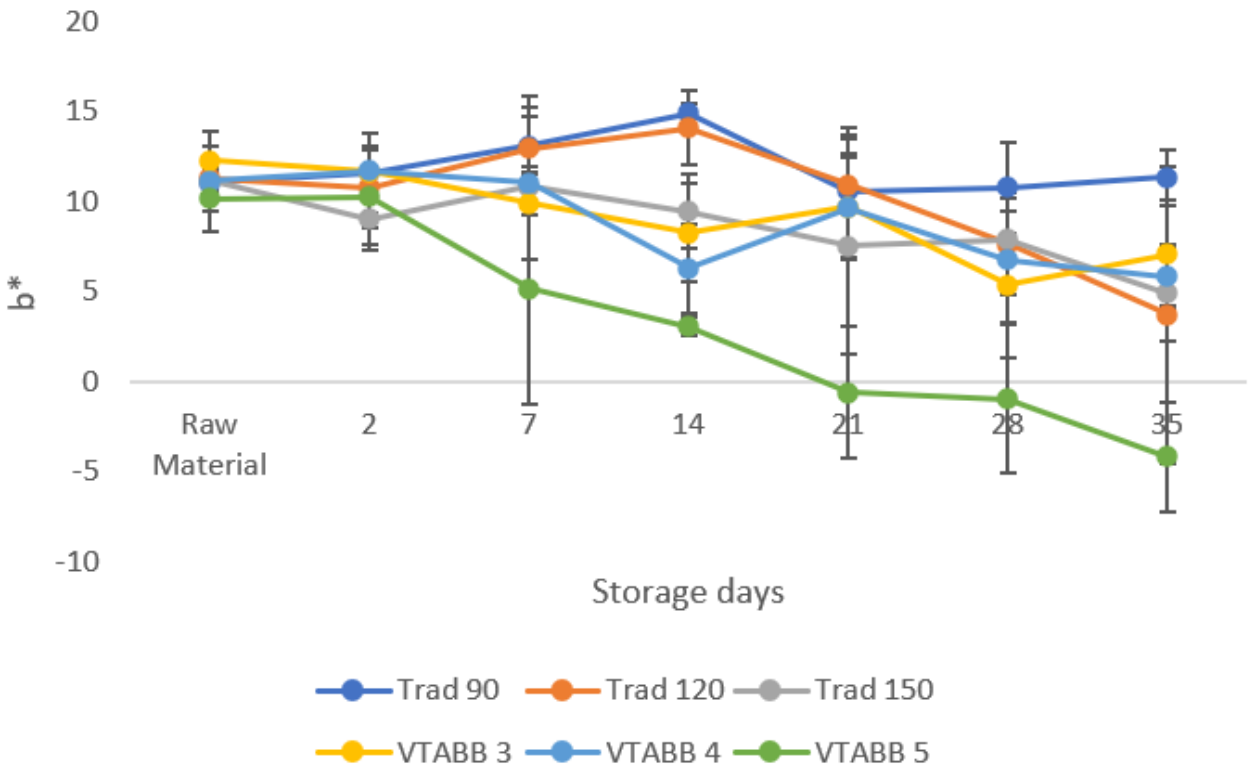


Figure 5.10: Fillet yellowness (b*) measured in the raw material (n = 4), and throughout storage of cold smoked salmon produced in Experiment 1 (n = 3). The groups labelled "Trad" are smoked by using traditionally wooden smoke, and the groups labelled "VTABB" by atomization of purified condensed smoke (VTABB). The numbers following "Trad" (90, 120, 150) represents the smoking time (min), and the numbers following "VTABB" (3, 4, 5) represents the level of smoke intensity. From day 7 and throughout day 28 post processing VTABB atomized groups were detected a lower yellowness than traditionally smoked groups ($p < 0.046$). VTABB 5 was observed to have a lower $b*$ value when comparing first and last storage day ($p < 0.022$). At the final storage day Trad 90 and VTABB 3 were observed to have a higher level of $b*$ than VTABB 5 ($p < 0.005$ and $p < 0.047$, respectively).

Sensory Selection

A sensory selection was performed on the experimental groups of CSS in Experiment 1, 21 days post processing.

The experimental groups VTABB 3, Trad 90 and Trad 120 were perceived as being too raw, and to have too little smoke characteristics, regarding both taste and odour. VTABB 5 was noted as having a hard surface, compared with the traditionally smoked groups. All the groups atomized with VTABB were considered as having a degree of acidic taste, which grew with increased level of smoke intensity. The experimental groups flavoured with VTABB were perceived as darker in its colour and with a more yellow-brown colour, compared with the groups of traditionally CCS. The colour intensity was considered as highest for VTABB 5.

Both the traditionally smoked and VTABB atomized groups were perceived as having a soft texture, independent of smoking degree. Although, by comparison of the two smoking protocols, the VTABB flavoured groups were perceived as having the firmest texture.

After the sensory selection it was decided to change the processing parameters, by excluding the groups VTABB 3 and VTABB 5, and Trad 90 and Trad 120. It was also decided to increase the salting time, to achieve a firmer texture and a less raw consistency before Napping® in Experiment 2.

5.2 Experiment 2: Sensory Selection of CSS smoked by three different PCS with comparison of traditionally CSS

5.2.1 Processing Parameters

Processing Yield

By comparing processing yield between the smoking protocols, PCS atomized groups had a significantly lower yield compared to Trad 120+30 ($p < 0.001$), with means of 89.15 ± 0.68 % and 90.44 ± 0.60 % (fig. 5.11). However, VTABB4 was observed to be significantly lower than Trad 120+30 ($p < 0.012$), with 88.52 ± 0,63% and 90.44 ± 0.60%, respectively. There was also a significant difference in processing yield between JJT01 5 at 88.72 ± 0.70% and Trad 120+30 ($p < 0.032$).

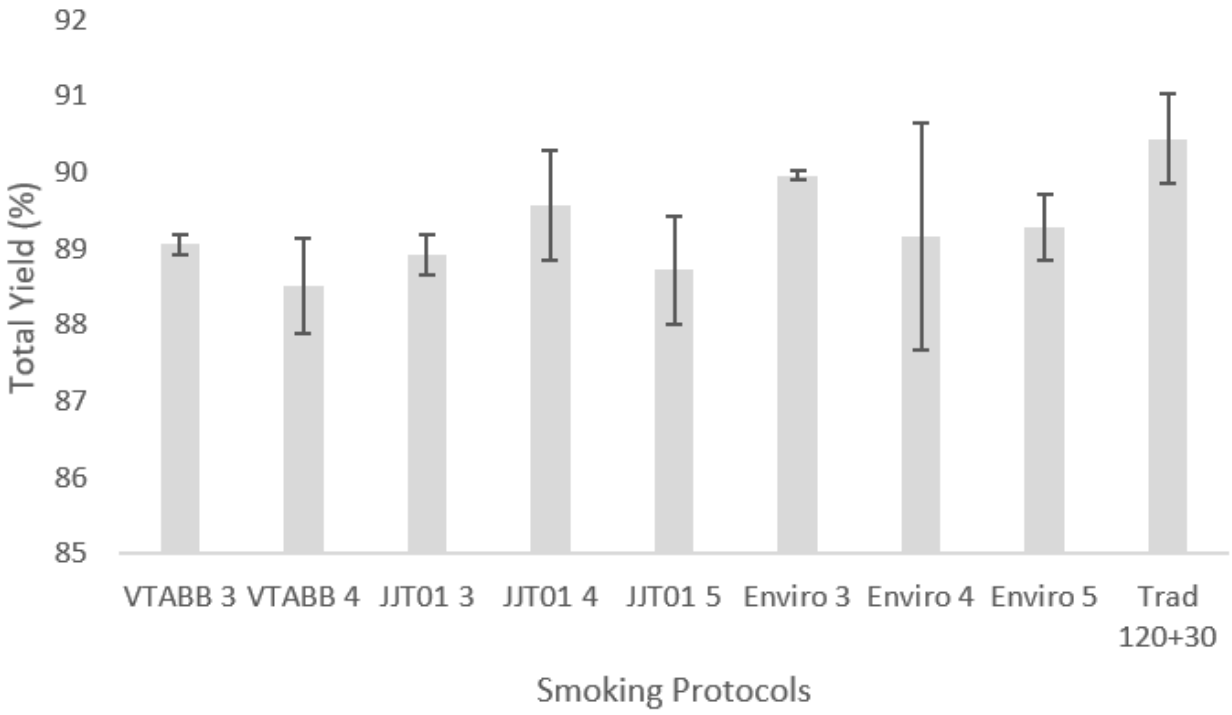


Figure 5.11: Processing yield (%) (mean ± SD) of smoked salmon produced by traditionally wooden smoke (Trad, n = 16) and atomization of purified condensed smoke (VTABB, JJT01, Enviro, n = 2), from Experiment 2. Calculations were based on weight before salting and two days post processing. The group "Trad" was smoked in 120+30 minutes. The numbers following the others groups represents the level of smoke intensity. PCS atomized groups had a lower processing yield than Trad 120+30 ($p < 0.001$). A Significant difference in the processing yield was found between VTABB 4 and Trad 120+30 ($p < 0.012$), and JJT01 5 and Trad 120+30 ($p < 0.032$).

5.2.2 Chemical Parameters

pH

PCS atomized groups had an average lower initial pH (5.81 ± 0.04) than Trad 120+30 (6.28 ± 0.03), with $p < 0.004$ (fig. 5.12). Although, Trad 120+30 had a significant higher pH at day 4 and day 25 post processing ($p < 0.002$) than all the PCS atomized groups. No significant differences were found between the experimental groups at the final measurement. By comparing the initial and final pH for each group, there was a significant difference regarding VTABB 4, JJT01 4 and Enviro 3 ($p < 0.006$, $p < 0.001$ and $p < 0.032$, respectively).

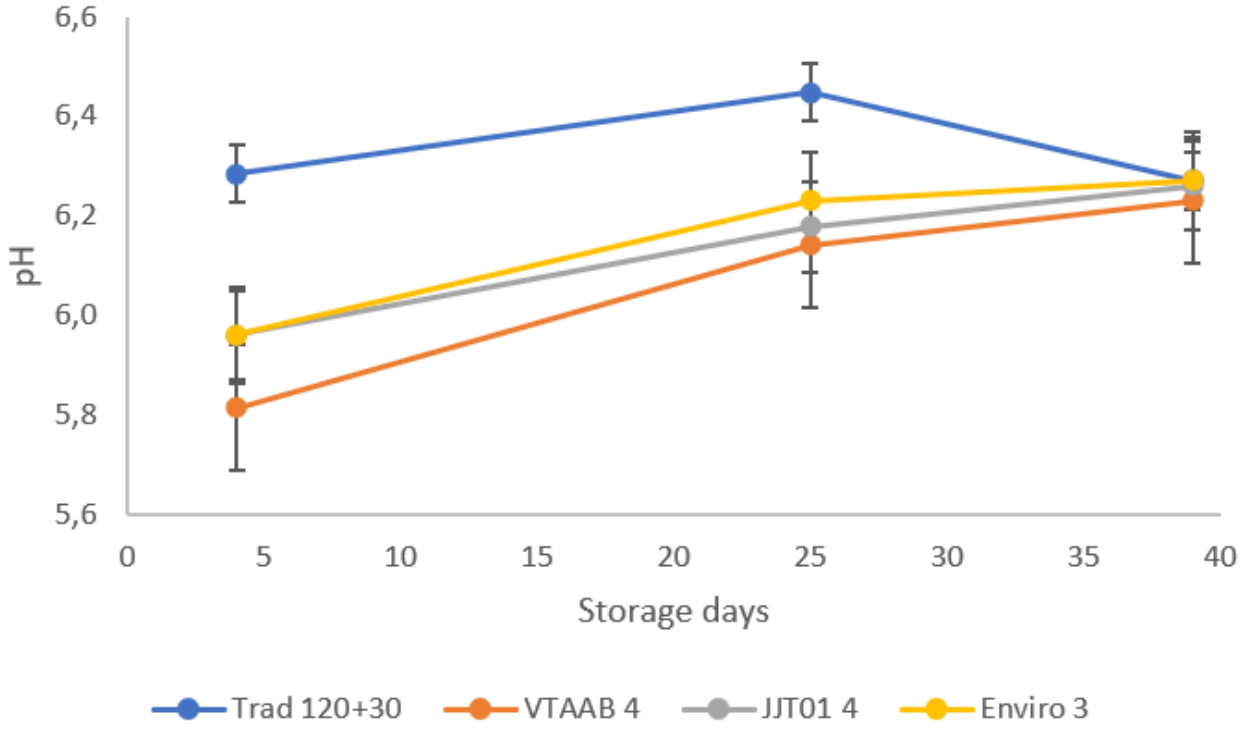


Figure 5.12: pH measured throughout storage of cold smoked salmon produced in Experiment 2 (n = 3), by traditionally wooden smoke (Trad) and atomization of purified condensed smoke (VTABB, JJT01, Enviro) The group "Trad" was smoked in 120+30 minutes. The numbers following the others groups represents the level of smoke intensity. At day 2 and 25 post processing, Trad 120+30 was observed to have a significant higher pH than the groups smoked by purified condensed smoke ($p < 0.002$). The mean pH ± SD for Trad 120+30 and the groups smoked by purified condensed smoke were found to be 6.28 ± 0.02 and 5.91 ± 0.09, respectively.

5.2.3 Microbiological Parameters

Aerobic Plate Count (APC)

Measured APC 4 days post processing did not show detection of growth in any of the experimental groups (fig. 5.20). Trad 120+30 showed a high level of APC at day 25 and 39 (with $\log_{10}$(CFU/g) of 3.00 ± 3.24 and 3.15 ± 3.38, respectively), although this level was not observed to be significant higher than the other groups ($p > 0.528$). Regarding JJT01 and VTABB 4 the level of APC grew at day 25 and 39, respectively, but this growth was not significant compared with the content of Enviro 3. Enviro 3 had no detection of growth throughout the storage period.

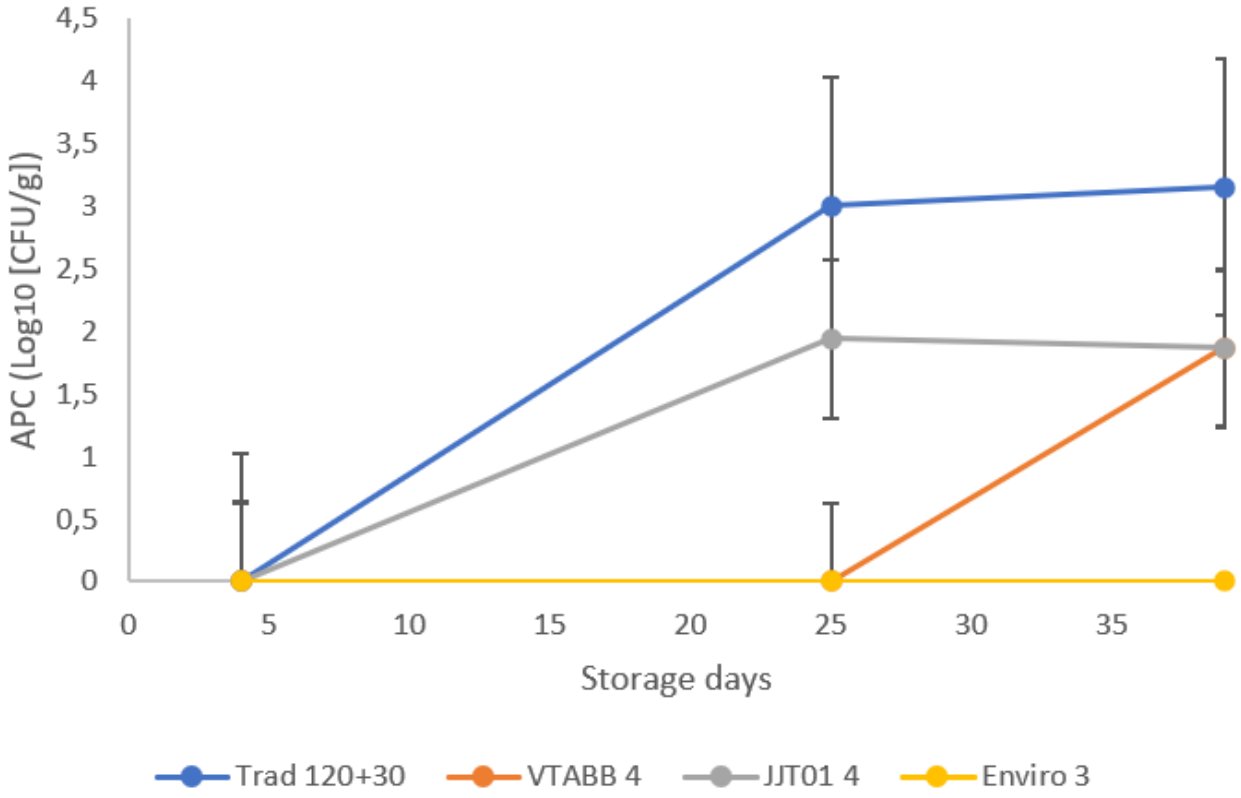


Figure 5.13: A semi-logarithmic plot of aerobic plate count (APC) measured throughout storage of cold smoked salmon produced in Experiment 2 (n = 3). The groups were produced by traditionally wooden smoke (Trad) and atomization of purified condensed smoke (VTABB, JJT01, Enviro). The group "Trad" was smoked in 120+30 minutes. The numbers following the other groups represents the level of smoke intensity. Although the level of APC grew for Trad 120+30, JJT01 and VTABB 4, this growth was not observed to be significant compared with Enviro 3, nor each other.

The growth of LAB in Experiment 2 was also measured, but none of the four experimental groups of CSS had any growth throughout the storage period.

5.2.4 Other Quality Parameters

Texture

F80 (N) and breaking force (N) of the experimental groups produced in Experiment 2 were measured 27 days post processing (see fig. 5.14 and fig. 5.15, respectively). There was not observed any significant differences between the experimental groups nor the smoking protocols regarding F80 (N). The PCS atomized groups had a firmer average breaking force than Trad 120+30 ($p < 0.015$), with values of 52.59 ± 4.57 N and 48.34 ± 0.13 N, respectively. However, no differences were measured between the experiment groups.

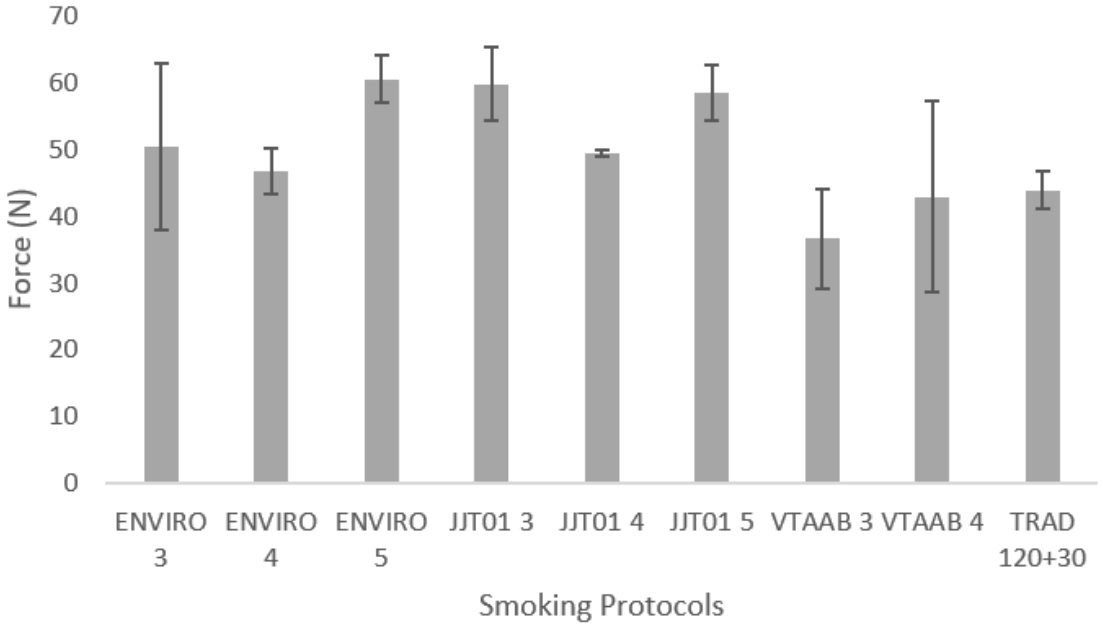


Figure 5.14: Resistance force (F80) measured 27 days post processing of cold smoked salmon produced in Experiment 2 (n = 1), by traditionally wooden smoke (Trad) and atomization of purified condensed smoke (VTABB, JJT01, Enviro). The group labelled "Trad" was smoked in 120+30 minutes. The numbers following the others groups represents the level of smoke intensity. The PCS atomized groups were in average recorded as firmer in texture than Trad 120+30 ($p < 0.015$).

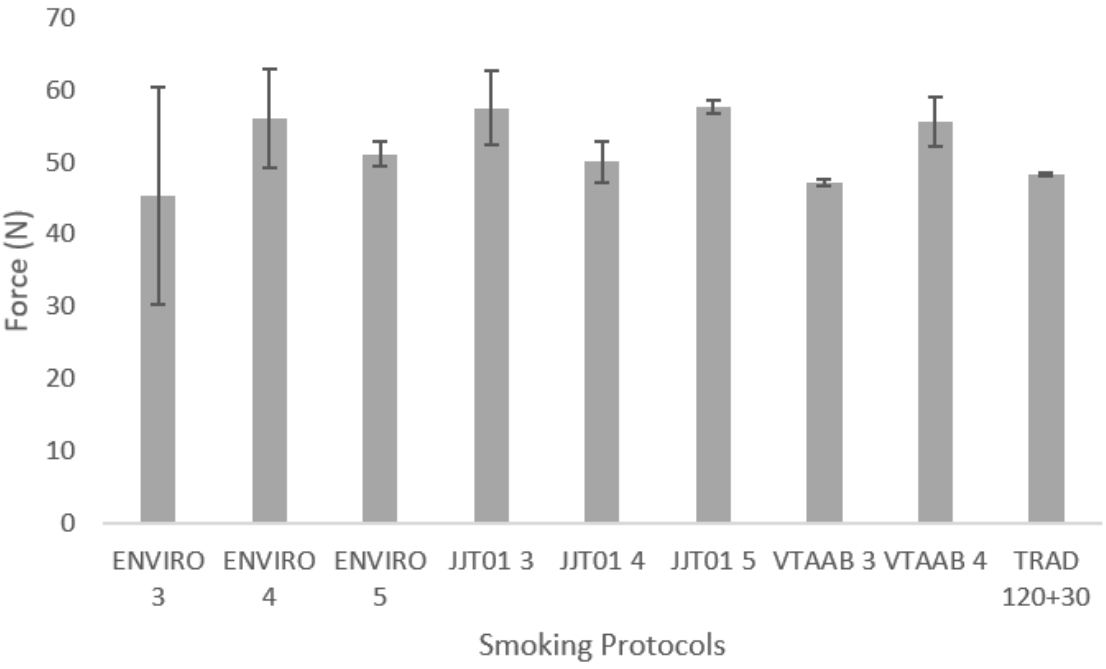


Figure 5.15: Breaking force (N) measured 27 days post processing of cold smoked salmon produced in Experiment 2 (n = 1), by traditionally wooden smoke (Trad) and atomization of purified condensed smoke (VTABB, JJT01, Enviro). The group labelled "Trad" was smoked in 120+30 minutes. The numbers following the others groups represents the level of smoke intensity. There was not observed any significant differences between the different experimental groups.

Sensory Selection
A sensory selection of the eight experimental groups of CSS smoked by using atomized PCS was performed. VTABB 3 and JJT01 3 were considered as "too lightly", regarding smoke intensity. On the other hand, JJT01 5, Enviro 4 and Enviro 5 were perceived as "too strong" in smoke intensity. Based on this VTABB 4, JJT01 4 and Enviro 3 were considered as good candidates for Napping®.

Napping®
Figure 5.16 shows the product map obtained from Napping®. The first two dimensions accounted for 70,15% of the explained variance (43,19% and 26,95%, respectively). Only terms mentioned 6 times or more totally are included in the variables plot. fig. A.2 displays terms and frequency cited by the assessors for defining the similarities or dissimilarities between the five evaluated groups of CSS.

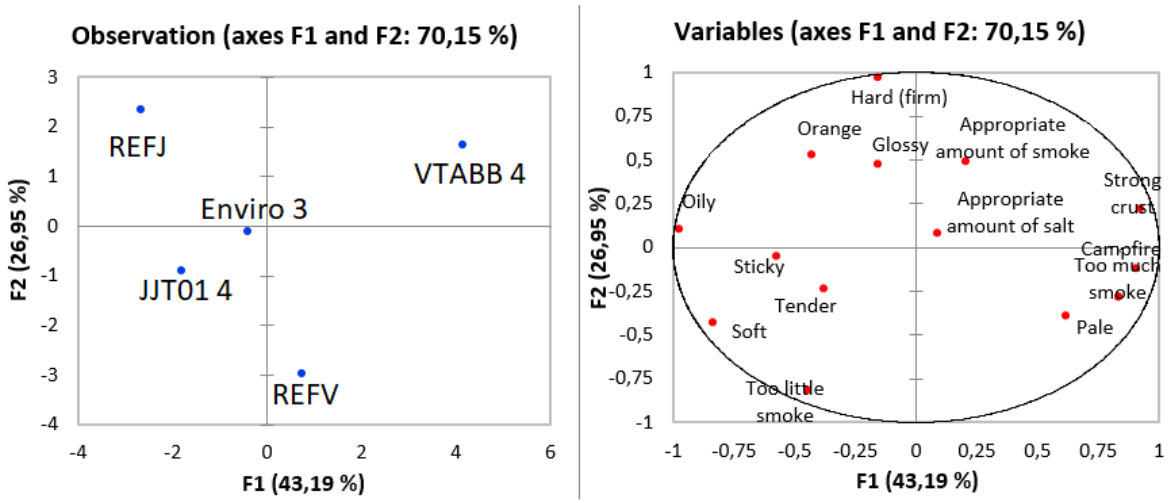


Figure 5.16: Observation and variables plots obtained from Napping® with UFP by 10 semi-trained assessors. VTABB 4, JJT01 4 and Enviro 3 represents groups smoked by purified condensed smoke, by using either 3 or 4 levels of smoke intensity. The reference groups, REFV and REFJ, are the corresponding cold smoked fillets smoked traditionally (150 minutes). Only terms mentioned 6 times or more totally are shown.

By looking at observatin in fig. 5.16 VTABB 4 differ from Enviro 3 and JJT01, and is described with terms like "Appropriate amount of smoke" and "strong crust". On the other hand, Enviro 3 and JJT01 are described as "Sticky" and "soft". REFJ and REFV are placed far apart from each other and therefore are perceived as different, where REFJ are described as "orange" and REFV as "too little smoke".

By looking at fig. A.3 in the Appendix A.4 and "Squared cosines of the observations" it seems like VTABB 4, REFV and REFJ are explained well through the two first dimensions (F1 and F2). Some of the variance in the data set can also be explained by dimension three and four (F3 and F4), where Enviro 3 and JJT01 4 seems to be best explained, respectively.

Since the PCA plot in fig. 5.16 only are represented through dimension 1 and 2, it seems like Enviro 3 and JJT01 4 are not well explained in this plot. For Enviro 3, 0.98 of the

variance is explained mainly through dimension 3, where terms like "glossy", "orange", "red", "appropriate amount of smoke", "appropriate amount of salt", "too much salt" and "metallic" are best explained (see A.4 in the Appendix A.4). Dimension 4 gets high values for the terms "sticky", "tender", "too little salt", "insipid" and "off-taste", and these terms are therefore anticipated to be important in the description of JJT01 4.

5.3 Experiment 3: A comparison of a selected PCS smoking and traditionally smoking protocol, regarding both the growth of *L. innocua* and packaging technology

5.3.1 Chemical Parameters

pH

A comparison between the two smoking protocols showed significant higher average pH of the traditionally smoked groups than the VTABB atomized groups throughout the storage period ($p < 0.048$), except day 34 post processing ($p < 0.098$), see fig. 5.17.

The average initial pH ± SD of the traditionally smoked and VTABB atomized groups were 6.07 ± 0.05 and 6.08 ± 0.1, respectively. However, after one day storage the pH dropped significantly in the VTABB atomized groups ($p < 0.001$), to 5.42 ± 0.1.

There was not a significant change in pH among the groups from initial to final storage day ($p > 0.301$). Although, at the final storage day, Trad-V-Nat had a significant higher pH at 6.15 ± 0.07 than VTABB-V-Nat ($p < 0.025$) and VTABB-S-Nat ($p < 0.045$).

When comparing the pH of groups smoked by the same protocol, with and without SGS treatment, a difference was measured day 20 and 27 post processing. Trad-V-Nat was measured higher than Trad-S-Nat after 27 days of storage. However, after 20 days VTABB-V-Nat was measured lower than VTABB-S-Nat.

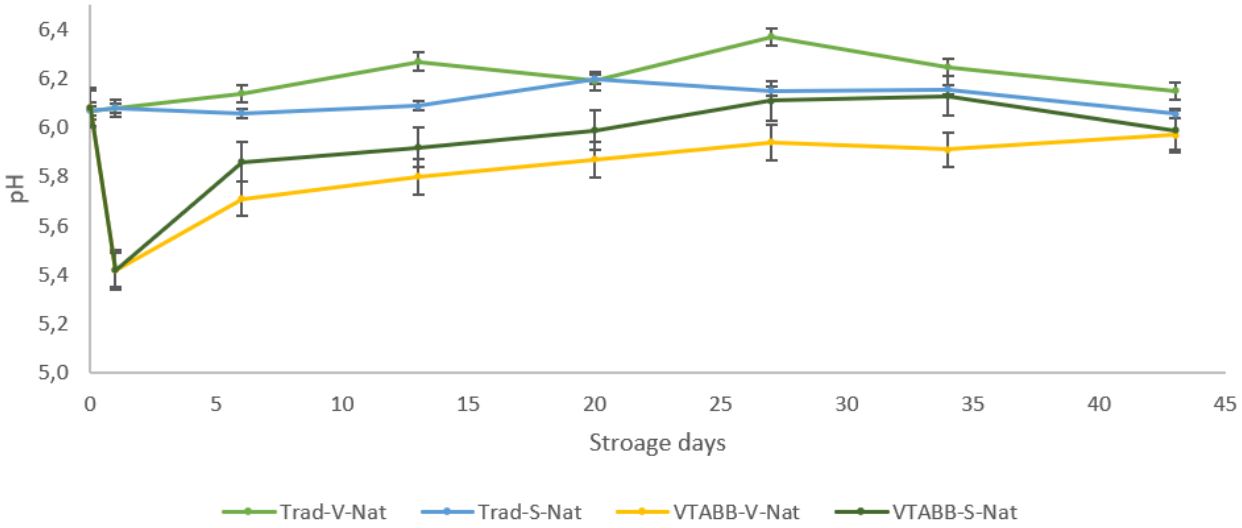


Figure 5.17: pH measured throughout the storage of cold smoked salmon produced in Experiment 3 (n = 3). The initial pH (n = 5) was measured immediately post processing. The groups labelled "Trad" were smoked traditionally for 150 minutes, and were packed with ("S") or without the use of SGS ("V") in vacuum. The VTABB atomized groups ("VTABB") were smoked by using atomized purified condensed smoke with a smoke intensity of 4 cycles, and packed under the same conditions as the traditionally smoked groups. The term "Nat" stands for groups not inoculated with *L. innocua*. No significant difference was observed regarding the initial pH, however Trad-V-Nat had a significant higher pH at the final storage day than VTABB-V-Nat ($p < 0.025$) and VTABB-S-Nat ($p < 0.045$).

Total Phenolic Content (TPC)

The TPC of two experimental groups from Experiment 3, Trad-V-Nat and VTABB-V-Nat, were measured to be 0.53 ± 0.02 and 0.62 ± 0.03 mg/100 g raw material ($p > 0.001$).

ATP Breakdown Products

Measuring of ATP breakdown products in Experiment 3 (fig. 5.18) revealed three peaks which indicated most likely the products Hx, In and IMP (see Appendix A.3, table A.3).

The average Hx content of VTABB atomized groups (1.16 ± 0.36 μmol/g) were measured to be higher than the traditionally smoked groups (0.77 ± 0.32 μmol/g), with $p < 0.008$. By comparing the experimental groups, VTABB-V-Nat was observed to have a significant higher content than Trad-S-Nat ($p < 0.025$), with means of 0.72 ± 0.34 and 1.32 ± 0.44, respectively. When comparing the content of Hx and In, all the groups were observed to have a significantly higher amount of In ($p < 0.035$). By comparing the average In content of the smoking protocols, VTABB atomized groups had a higher content (4.99 ± 1.59 μmol/g) than traditionally smoked groups (3.35 ± 0.44 μmol/g)), with $p < 0.004$. Trad-V-Nat was recorded to have higher amount of In than VTABB-V-Nat ($p < 0.033$), with means of 3.21 ± 0.33 and 5.30 ± 2.13 μmol/g, respectively.

Regarding IMP, VTABB atomized groups had a higher amount than the traditionally smoked groups ($p < 0.001$), with averages of 3.45 ± 1.16 μmol/g and 1.62 ± 0.43 μmol/g, respectively. Trad-V-Nat and Trad-S-Nat were both observed to have a significantly lower amount than VTABB-S-Nat ($p < 0.001$), with means of 1.56 ± 0.50 and 1.68 ± 0.35 μmol/g, respectively. VTABB-S-Nat had a content of 3.87 ± 1.13 μmol/g.

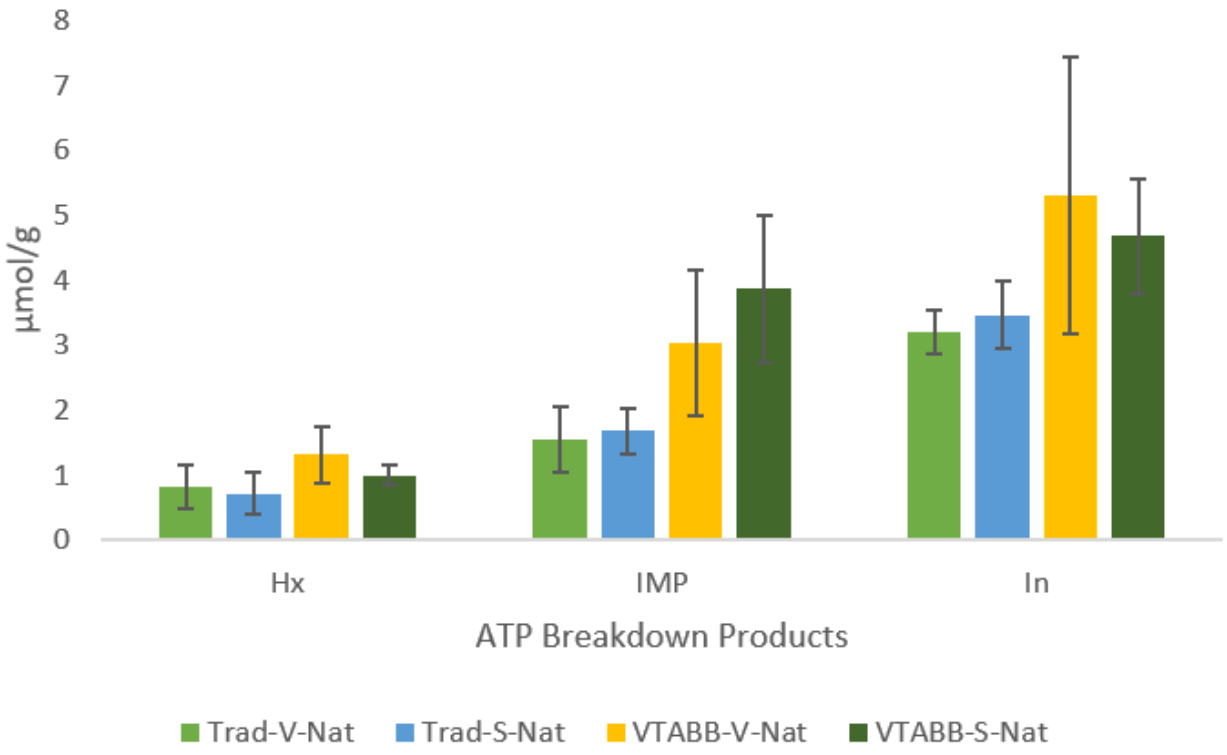


Figure 5.18: The ATP breakdown products (Hx, In, IMP) measured in the experimental groups of cold smoked salmon (n = 3), produced in Experiment 3. The groups had been stored for 6 days. Hx stands for hypoxanthine, In stands for inosine and IMP for inosine monophosphate. The groups labelled "Trad" were smoked traditionally for 150 minutes, and were packed with ("S") or without the use of SGS ("V") in vacuum. The VTABB atomized groups ("VTABB") were smoked by using purified condensed smoke, with a smoke intensity of 4 cycles, and packed under the same conditions as the traditionally smoked groups. The term "Nat" stands for groups of cold smoked salmon not inoculated with *L. innocua*. VTABB atomized groups had an average higher content of all breakdown products than the traditionally smoked groups ($p < 0.008$). The content of Hx was observed to be significant lower than the content of In regarding all the groups ($p < 0.001$).

Salt Content

The salt content (%) was measured in the experimental groups from Experiment 3, performed 6, 27 and 41 days post processing (fig. 5.19). 6 days post processing Trad-S-Nat was observed to have a higher content of NaCl than VTABB-V-Nat ($p < 0.044$), with $2.64 \pm 0.16\%$ and $2.01 \pm 0.31\%$. The average content of NaCl of the traditionally smoked groups and VTABB atomized groups 6 days post processing were $2.57 \pm 0.09\%$ and $2.11 \pm 0.14\%$ ($p < 0.022$). No significant differences were observed regarding any of the groups nor the smoking protocols 27 and 43 days post processing. The average content of NaCl of the traditionally smoked groups and VTABB atomized groups 27 days post processing were $2.76 \pm 0.09\%$ and $2.69 \pm 0.16\%$, respectively ($p > 0.756$).

By comparing the salt content 6 and 27 days post processing VTABB-V-Nat was observed to have a higher content 27 days post processing ($p < 0.026$). However, after 43 days the NaCl content was reduce in both the groups ($p < 0.005$). The other groups had no significant change in NaCl content between day 6 and 27, and day 27 and 43.

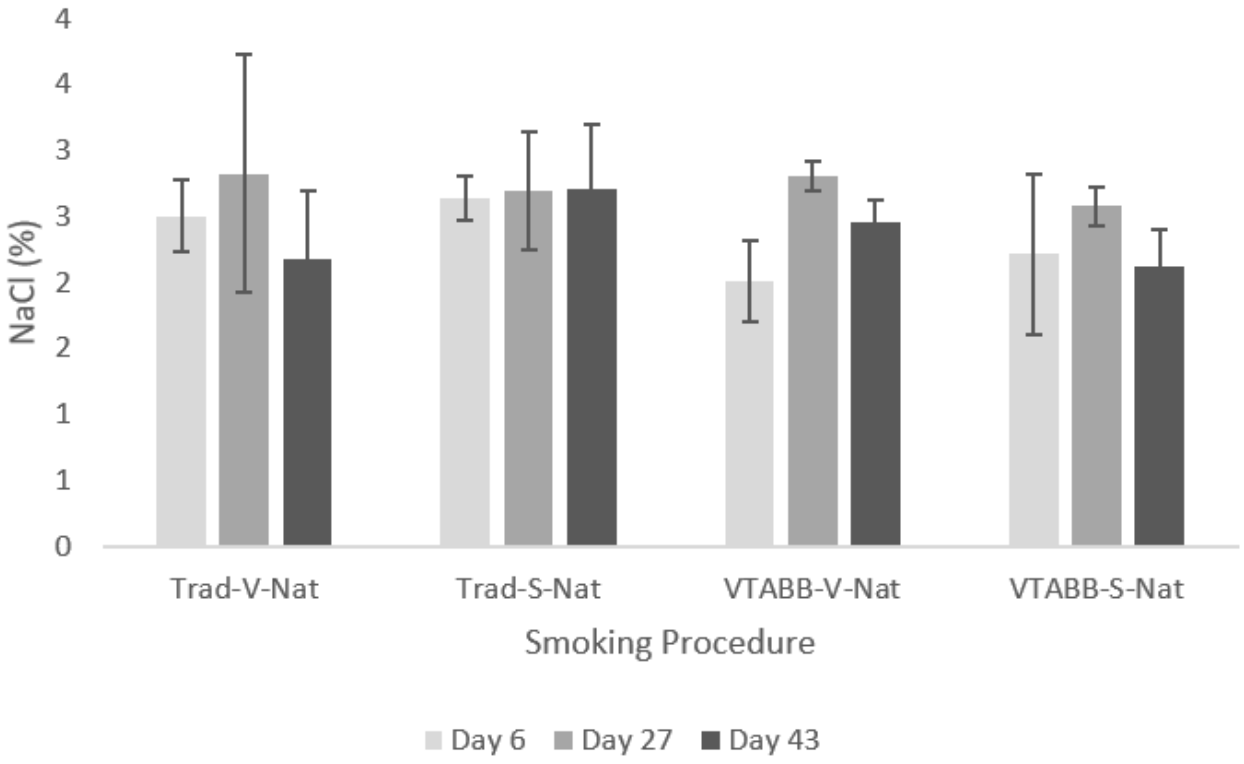


Figure 5.19: The salt content (% NaCl) measured in the experimental groups of cold smoked salmon, produced in Experiment 3. The analysis was performed on groups stored for 6 and 27 days post processing. The groups labelled "Trad" were smoked for 150 minutes, and were packed with ("S") or without the use of SGS ("V") in vacuum. The VTABB atomized groups ("VTABB") were smoked by using purified condensed smoke with a smoke intensity of 4 cycles, and packed under the same conditions as the traditionally smoked groups. The term "Nat" stands for groups of cold smoked salmon not inoculated with *L. innocua*. Trad-S-Nat was observed to have significantly higher NaCl% than VTABB-V-Nat, with mean values of $2.64 \pm 0.16\%$ and $2.01 \pm 0.31\%$.

5.3.2 Microbiological Parameters

Aerobic Plate Count (APC)

The results of APC in Experiment 3 indicate a higher growth in Trad-V-Ino, although this was only recorded as significant 26 and 43 days post processing ($p < 0.008$) (fig. 5.20). At the final storage day the $\log_{10}$(CFU/g) of Trad-V-Ino was 4.00 ± 3.55. Trad-S-Ino was also observed to have a significant higher level of APC at the final storage day than VTABB-V-Ino ($p < 0.005$) and VTABB-S-Ino ($p < 0.021$), with a $\log_{10}$(CFU/g) of 3.63 ± 2.67.

The two groups VTABB-V-Ino and VTABB-S-Ino CSS did not have a significant different APC level from each other at any measuring point throughout the whole storage experiment.

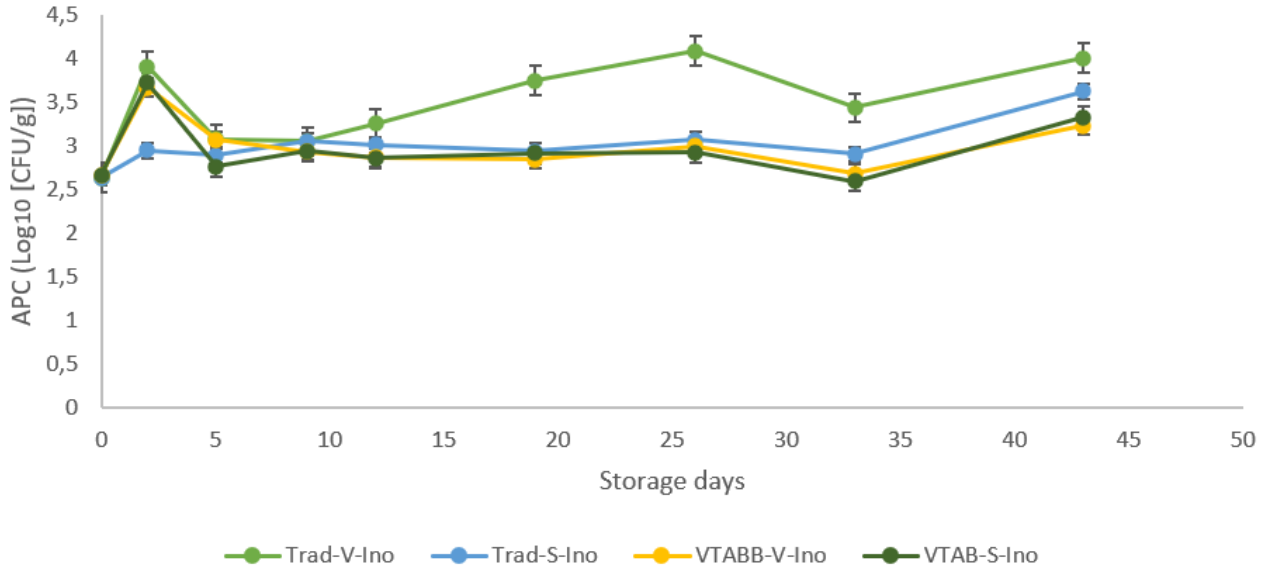


Figure 5.20: A semi-logarithmic plot illustrating the level of aerobic plate count throughout the storage of cold smoked salmon produced in Experiment 3 (n = 3). The groups labelled "Trad" were smoked traditionally for 150 minutes, and were packed with ("S") or without the use of SGS ("V") in vacuum. The VTABB atomized groups ("VTABB") were smoked by using purified condensed smoke, with a smoke intensity of 4 cycles, and packed under the same conditions as the traditionally smoked groups. The term "Ino" stands for groups inoculated with *L. innocua*. The groups were stored for a total of 43 days. Trad-V-Ino had a significant higher APC day 26 and 43 post processing (p<0,008), with a $\log_{10}$(CFU/g) value of 4.00 ± 3.55.

***Listeria* spp.**

By measuring the growth of *Listeria* spp. in Experiment 3 (fig. 5.21), Trad-V-Ino had a significant higher amount at the final storage day (3.99 ± 0.04 ($\log_{10}$(CFU/g)) than initially (3.16 ± 0.07 $\log_{10}$(CFU/g)) with $p < 0.004$. In addition, Trad-V-Ino was observed with a significantly higher growth of Listeria 19 and 43 days post processing ($p < 0.017$).

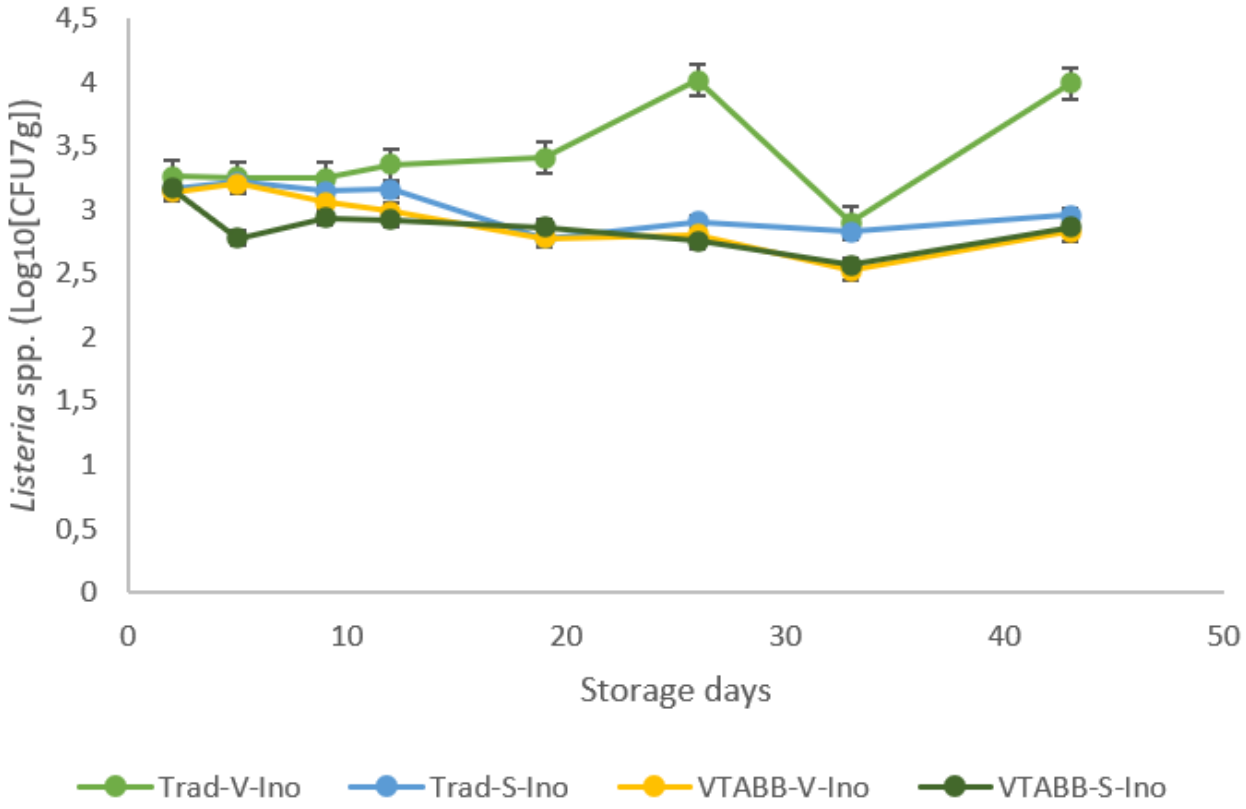


Figure 5.21: A semi-logarithmic plot illustrating the growth of *Listeria* spp. throughout the storage of cold smoked salmon produced in Experiment 3 (n = 3). The groups labelled "Trad" were smoked traditionally for 150 minutes, and were packed with ("S") or without the use of SGS ("V") in vacuum. The VTABB atomized groups ("VTABB") were smoked by using purified condensed smoke, with a smoke intensity of 4 cycles, and packed under the same conditions as the traditionally smoked groups. The term "Ino" stands for groups inoculated with *L. innocua*. The groups were stored for a total of 43 days. Trad-V-Ino had a significant higher growth of *Listeria* spp. than the other groups at day 19 and 43 ($p < 0.017$).

6 Discussion

Experiment 1: Comparison of Traditionally Wooden smoked salmon and PCS smoked salmon

Processing yield affected by smoking protocol was lowest among fillets smoked with VTABB. This was also shown by [Valø et al., 2020], which used the same type of PCS. A lower yield regarding these groups was expected, considering longer drying time of PCS treated groups [Gómez-Guillén et al., 2000, Indrasena et al., 2000]. The water activity was not measured, however it is assumed that prolonged drying and smoking gave higher drip loss and subsequently lower yields, due to evaporation of water from the product [Doe, 1998, Maga, 1987]. The results from [Indrasena et al., 2000, Valø et al., 2020] also support these findings. One of the factors affecting the migration mechanism in flesh is lipid content [Wang et al., 2000], where diffusion of water in flesh of lean fish is faster than in fat fish, and subsequently leads to faster dehydration in lean fish [Løvdal, 2015]. However, the lipid content was not measured, and since the salmon collected in each experiment was from the same production site, the lipid content was assumed to be the same in the fillets.

A firmer texture regarding F80 was recorded after 7 and 21 days storage in the VTABB atomized groups, which can also be seen as a consequence to more excessive drying [Indrasena et al., 2000, Rørå et al., 2004, 1998]. However, by studying the breaking force no difference regarding smoking protocols was observed 21 days post processing, even though VTABB atomized groups were measured having a higher breaking force 7 days post processing. These results reflects [Hultmann et al., 2004, Valø et al., 2020], which reported no significant linear dependence between textural properties and storage time. Although, a tendency of lower breaking force can be seen in VTABB 3, 4 and 5 when comparing each group individually at day 7 and 27. This could be caused by changes in myofiber to-myofiber and myofiber-to-myocommata attachments [Birkeland et al., 2004, Taylor et al., 2002], giving tender texture to the VTABB treated fillets. Traditionally smoked groups had on the other hand more fluctuations between sampling days regarding F80 and breaking force after 7 and 27 days, and signs of texture softness can therefore not be stated.

Higher TPC was observed in fillets processed by using atomized VTABB, where VTABB 4 and VTABB 5 showed a tendency of higher TPC, and Trad 90 lower. Higher TPC was also observed in work by [Valø et al., 2020], of VTABB treated groups. A dryer product will have higher concentration of lipids (due to loss of water) [Indrasena et al., 2000, Cardinal et al., 2001], which phenolic substances can attach by hydrophobic interactions [Maga, 2018, Martinez et al., 2007]. VTABB groups are already stated to have lower processing yield, and subsequently higher concentration of lipids and phenols [Indrasena et al., 2000]. A correlation between processing yield and TPC can also be argued for Trad 90, with having a tendency of high processing yield and low TPC. However, average TPC in VTABB treated filets (0.41 $\pm$ 0.037 mg/100 g sample) and traditionally smoked fillets (0.37 $\pm$ 0.026 mg/100 g sample) were still slightly lower than the French standard NF V45-065, which according to [Løvdal, 2015] is corresponding to 0.6 mg/100 g sample. These values of TPC are also lower than those observed by [Valø et al., 2020], but this is probably due to longer smoking regarding both the smoking protocols in [Valø et al., 2020]. This assumption is strengthened by [Birkeland et al., 2004, Cardinal et al., 2004], which showed that duration of the smoking process seem to affect the accumulation of TPC in smoked fillets.

Trad 90 and Trad 120 were expected to have higher bacteria growth, due to low amount of processing through smoking [Løvdal, 2015]. However, Trad 150 was the only group that showed a stable growth of APC from day 21 and throughout day 35, although the number was considered as being low (2.86 $\pm$ 2.82 $\log_{10}$(CFU/g)) compared to levels observed by [Valø

et al., 2020]. pH and salt content have showed to influence the growth of bacteria [Løvdal, 2015]. However, a difference in pH and salt content was not observed between Trad 150 and the other groups. A contamination of the samples before or during microbiological analysis can therefore be the explanation of growth.

A contamination of the CSS during microbiological analysis can be discussed. Two out of three parallels were detected having APC colonies 21 days post processing. However, microbiological analyses 28 and 35 days post processing only showed 1 parallel of Trad 150 with detected APC growth. A contamination of the CSS sample before microbiological analyses can therefore not be excluded. Although, no growth of LAB were detected 28 and 35 days post processing of Trad 150, but one parallel was detected after 21 days of storage. This can imply that non-LAB were detected after 21, 28 and 35 days of storage, on one parallel in Trad 150, and therefore not detected on MRS. Another cause can be that the parallels on each day were contaminated by bacteria with no ability of growing on MRS. A consideration regarding the processing yield and microbiology growth can be discussed since there was no differences in yield between the traditionally smoked groups. This can indicate that 30-60 minutes longer smoking time do not have a significant effect regarding yield and water content (without that being measured), and therefore does not give a more cured product with limitation of bacteria growth. The Trad 150 raw fillets was also generally bigger in size (kg), which also can cause higher processing yield compared to smaller sizes of fillets [Rørå et al., 1998].

A sporadically microbiological growth was also detected in Trad 90 and VTABB 4 day 28 days after storage in one parallel regarding APC and LAB. Detection of growth after 35 days of storage was on the other hand not detected. Since only one parallel showed growth of APC and LAB, and no growth was detected 35 days post processing, a contamination might have occurred before plating out on IA and MRS. The pH in groups processed with VTABB had a tendency to be lower than those smoked traditionally, which was also the case in PCS treated fillets of [Valø et al., 2020]. A lower pH in VTABB processed groups was expected, since VTABB consists of organic acids (9.0-12.0% acetic acid), which can deposit on the surface of fillets [Hassan, 1988]. A low pH can prevent growth of bacteria [Russell and Dombrowski, 1980, Rhee et al., 2011, Theron and Lues, 2007] and high content of phenols is known to have antimicrobial potential [Gimenez and Dalgaard, 2004, Løvdal, 2015, Tocmo et al., 2014, Thurette et al., 1998]. In this study where the salt content is held low, the microbiological stability and food safety need to be enhanced by other barriers, which subsequently can be higher content of phenols and/or low pH, as stated by [Valø et al., 2020]. However, [Lebois et al., 2004] observed that phenol concentration in liquid smoke had no effect on L. monocytogenes in a BHI broth, and [Leroi and Joffraud, 2000] stated that APC and LAB were mainly inhibited by salt concentration and to a lesser degree phenols. However, since differences in bacteria growth between traditionally smoked and VTABB processed groups was not ascertained, the effect of pH and TPC can not be confirmed.

The values of colorimetric properties ($L^*a^*b^*$) were reduced during storage in VTABB 5, where especially yellowness was recorded to be severe low 35 days post processing. A stronger yellowness was expected of the VTABB atomized groups, since VTABB has a yellow-brown colour which will deposit on the CSS fillet. This was also seen in fillets smoked by VTABB in [Valø et al., 2020]. According to [Cardinal et al., 2004] pasty fillets are often pink in colour, whereas firmer ones are more orange/brownish. This corresponds to the results of VTABB treated fillets, which were also seen having lower processing yield than traditionally smoked fillets. Comparing the raw material and CSS at last storage day, decreased fillet lightness was detected in Trad 150 and all the VTABB processed fillets. Lower lightness was furthermore seen in smoked fillets of [Birkeland et al., 2004, Cardinal et al., 2001, Mørkøre et al., 2001].

The results also reflects observations from [Valø et al., 2020, Lerfall et al., 2016], where the latter stated that extensive dehydration gave a more intense dark colouring due to slightly increased carotenoid concentration. A tendency of lower yellowness was observed in salmon smoked with VTABB and Trad 150 too, which is the contrary of the results of [Cardinal et al., 2001]. However, as reported by [Birkeland et al., 2004, Mørkøre et al., 2001] initial fillet fat content affects the yellowness of dry-salted smoked fillets. But, since the lipid content was not measured, this can not be stated as the reason of lower yellowness. Although, VTABB treated fillets in [Valø et al., 2020], was also measured having a lower yellowness as an effect of storage. Therefore, the reduced yellowness/increase in blueness are most likely due to the choice of PCS. The consequence of lower $L^*a^*b^*$ values in VTABB treated fillets giving a possible undesirable colour effect will be discussed in Experiment 2, relative to results given by Napping® In addition, this was stated in the sensory selection, where VTABB 5 was recorded as "darker". A solution can therefore be less intense PCS treatment, as stated by [Valø et al., 2020].

The content of IMP in all of the experimental groups was reduced when measuring 35 days post processing. This was anticipated, since IMP will break down during storage of seafood to Ino and Hx [Murata and Sakaguchi, 1986, Huss et al., 1995a, Sallam, 2007] After 35 days of storage the amount of Hx was not different between smoking protocols. Hx is often associated with perished seafood and has therefore been used as freshness indicator in fish [Jones et al., 1964], [Spinelli et al., 1964]. The conversion of Ino to Hx is mostly performed by bacteria belonging to the deterioration flora [Hong et al., 2017]. And, since no significant difference in microbiological growth was observed between smoking protocols throughout storage, a similar content of Hx was expected. A similar Hx content in the smoking protocols could therefore not designate a better smoking protocol against perversity. Furthermore, there was not observed any differences in H-value nor Hx/ino ratio between the smoking protocols 35 days post processing. Although, VTABB fillets had a tendency to have lower Hx/Ino ratios, thus having less produced Hx in comparison to the content of Ino. However, VTABB atomized smoke can not be designated as being more resistant against Hx production than traditionally smoked, or vice versa.

Experiment 2: Sensory Selection of CSS smoked by three different PCS with comparison of traditionally CSS

The results from Experiment 2 indicated lower processing yield of fillets smoked with atomized PCS compared to those smoked traditionally (Trad 120+30). As this was the case in Experiment 1 as well, the extra drying performed to remove condensed vapour on the surface (caused by atomized PCS) is probably the reason for lower yield [Gómez-Guillén et al., 2000, Indrasena et al., 2000]. There was a tendency of lower processing yield among groups with higher smoke intensity, which also strengthens this assumption.

A lower initial pH was measured in the fillets atomized with PCS than traditionally smoked fillets (Trad 120+30), which was also seen in PCS treated fillets in [Valø et al., 2020]. This is previously mentioned when discussing the pH from Experiment 1. However, at the final day the pH considering all the groups were equivalent, which was also the case in Experiment 1. Previous work have also reported an increase in pH during storage [González-Fandos et al., 2005, Martinez et al., 2010, Hultmann et al., 2004, Sallam, 2007, Swastawati et al., 2012], and [Olsen et al., 2006] stated production of basic nitrogen as an explanation.

A tendency of higher APC growth in salmon fillets smoked traditionally (Trad 120+30) was observed after 25 days storage, but without a significant difference. Growth was also detected in JJT01 4, but these values were also relatively low (2.0-3.0 $\log_{10}$(CFU/g)), compared traditionally smoked fillets reported by [Valø et al., 2020]. The APC growth in Trad 120+30 and JJT01 4 were stable through day 39, including VTABB 4 with sporadically measured growth this day. The APC growth regarding all the groups were detected in only one parallel, both on day 25 and 39 post processing. As a result, a difference in growth can therefore not be stated. Detection of growth on MRS was not detected, and one can therefore assume no growth of LAB throughout the storage period.

Regarding texture, the groups were measured as similar in F80, but PCS atomized groups were firmer in breaking force. This can be seen in context with longer drying performed for PCS atomized groups, which would create a firmer crust [Indrasena et al., 2000, Valø et al., 2020]. As explained by [Valø et al., 2020], extended drying when smoking by using PCS is important, due to avoid soft fillets generating from condensates in the drying cabinet. In the sensory selections performed in Experiment 1 and 2, the lightest smoked fillets (both PCS treated and traditionally smoked) were stated as being "too raw" and having "soft texture". This could be a consequence from less salting and smoking performed on the fillets, giving higher water content and less dried product [Indrasena et al., 2000, Birkeland et al., 2004]. As a result, firmer texture given by PCS treatment could be a positive effect in lightly treated CSS.

The results collected from Napping® showed that the judges found JJT01 4 and Enviro 3 more similar with each other, than with VTABB 4. However, corresponding fillets smoked traditionally (REFJ and REFV), were placed far apart from each other - even though these fillets were smoked by the same protocol and method. This far placement of identical groups can question the judges ability to distinguished the CSS fillets [Varela and Ares, 2012]. Although, since the F2 axis "only" explains 26.95 % of the variance, a similar distance between groups in the F1 axis would have a bigger impact, because of higher explanation of variance and therefore represent further difference between groups. The variables plot implied VTABB 4 to have "appropriate amount of smoke", "appropriate amount of salt" and a "strong crust". The strong crust can be seen in context with the breaking force, which had a tendency to be higher than the other smoked fillets examined by Napping®. The two other groups processed with PCS were described with terms more unfavourable, such as "sticky", "soft" and "oily", in addition to "metallic" and "insipid". As a conclusion, it seemed like VTABB 4 was rated best among groups processed with PCS, but as previously mentioned, skepticism regarding the

judges must be taken in consideration. The final colorimetric properties of VTABB 4 fillets in Experiment 1 was observed lowered than the raw material. [Valø et al., 2020] stated that smoking and drying time should be shortened for the PCS protocol to achieve a better CSS result. However, negative terms regarding the colour of VTABB 4 was not mentioned by the assessors in Napping®. It can therefore be assumed that even though VTABB 4 was still observed having low $L^*a^*b^*$ values, this did not notably affect the sensory apperance.

The goal of Experiment 2 was to find the most promising PCS out of three different PCS used. Based on the results, none of the PCS were outstanding regarding processing yield, pH, microbiology, texture nor Napping®. Since the institute have done earlier research on VTABB [Valø et al., 2020] and Napping® showed generally more acceptance regarding this PCS protocol, VTABB was chosen to do further research on in Experiment 3.

Experiment 3: A comparison of a selected PCS smoking and traditionally smoking protocol, regarding both the growth of *L. innocua* and packaging technology
The results from Experiment 3 indicated higher pH among the traditionally smoke protocol day 1 and throughout day 27 post processing. Although, there was not measured difference among the groups immediately after smoking, and after 34 days storage. Higher pH in traditionally smoked fillets is seen in Experiment 1 and 2, including [Valø et al., 2020], and is probably caused by high concentration of acetic acid and the deposition of organic acids on the fillet surface [Hassan, 1988]. In Experiment 1 and 2, the initial pH have been taken 2 and 4 days post processing, respectively. On these days, the traditionally smoked and VTABB atomized groups were significant different in pH, where traditionally smoked groups measured highest. An assumption in higher pH immediately after smoking by PCS compared with 2 days later is presented in [Valø et al., 2020]. Here, it is believed that the product surface will first be acidified, following by a diffusion of organic acids from the fillet surface to the sample core over time. This would explain the high initial pH values and the drop in pH after 1 day in the VTABB processed fillets. Since pH was not measured right after smoking in the first two experiments, this hypothesis is only an assumption.

It was presumed that SGS treated groups would have a lower pH, because of the solubility of CO_2 in the samples [Sivertsvik et al., 2002a]. However, this was only the case for the traditionally smoked groups after 27 days storage, but not for the VTABB atomized groups. In traditionally smoked fillets, there was a tendency of lower pH in fillets treated with SGS (Trad-S-Nat). However, in VTABB processed salmon, fillets treated with SGS (VTABB-S-Nat) had a tendency of higher pH throughout storage. Although, this was only significant 20 days post processing. Why the pH was lower for VTABB fillets not treated with SGS is not clear. By the end of the storage period, the pH of all the groups seemed to be at approximately same level, like seen in Experiment 1 and 2 [Hultmann et al., 2004, Sallam, 2007]. As mentioned before, the raise in pH regarding the VTABB treated fillets is probably due to production of basic nitrogen [Olsen et al., 2006].

The TPC was measured for both smoking protocols not treated with SGS (Trad-V-Nat and VTABB-V-Nat), since it was anticipated that SGS did not affect TPC. The results indicated higher TPC in fillets atomized with VTABB, as observed in Experiment 1 and by [Valø et al., 2020], which is probably due to more intense drying, as mentioned earlier in this discussion [Birkeland et al., 2004, Indrasena et al., 2000]. Although, [Sérot et al., 2004] showed that the deposition rate of TPC was very rapid for PCS vaporisation, with an approximately linear increase between 16 and 32° C, whereas the increase of deposition was very gradual for the smouldering smoke production between 16 and 24° C. A temperature of 22° C was used in this Experiment, and rapid deposition rate with PCS treatment can therefore be a reason for higher TPC in VTABB treated fillets.

The level of IMP was found to be higher than the level of Ino and Hx in all the fillets, regardless of smoking protocol or SGS treatment [Hong et al., 2017]. This was expected since the analysis was performed only 6 days post processing, and it usually takes some time before IMP breaks down, as shown by [Murata and Sakaguchi, 1986]. However, the levels of IMP, Ino and Hx were higher in the fillets treated with atomized VTABB than the traditionally smoked groups. The reason is though not clear.

The content of NaCl seemed to be stable in fillets smoked traditionally (Trad-S-Nat and Trad-V-Nat) throughout the storage period. The NaCl content was also measured higher in traditionally smoked groups than VTABB atomized groups, as also shown in [Valø et al., 2020], after 6 and 27 days storage. Observed differences between smoking protocols might be a result of differences in the raw material characteristic affecting the salt uptake, e.g. contents of lipids

[Gallart-Jornet et al., 2007, Wang et al., 2000], or muscle temperature at point of salting [Telis et al., 2003], as explained by [Valø et al., 2020]. Although, since the salmon was obtained by the same production site, differences in lipid content are hardly plausible. However, the content varied in VTABB atomized fillets (VTABB-V-Nat and VTABB-S-Nat), by first increasing from day 6 to 27, and then reducing from day 27 to 43. Fluctuations in NaCl content between day 6 and 27 could be a result of uneven salt concentration in the fillets due to dry salting. [Rørå et al., 2004, Indrasena et al., 2000] have reported higher oscillation of salt content in fillets salted by dry salting than injection salting. Analysis with more parallels could have given a better representation of the salt content in each smoke processing, and therefore reduced the fluctuations in NaCl content. Even though fluctuations in salt content appeared, the final NaCl (%) were 2.76 ± 0.09% (traditionally smoked fillets) and 2.69 ± 0.16 % (VTABB atomized fillets). Therefore the salt content was in the ranged according to the French standard NF V45-065 (NaCl % between 2.5-3.5), but the CSS fillets of both smoking protocols were stilled regarded as a lightly preserved product when compared to [Leroi et al., 2001, Løvdal, 2015, Espe et al., 2004].

No growth of either APC or *Listeria* spp. was detected in the non-inoculated control groups (Natural). This indicates that even though the salmon had been salted only 6 hours, and smoked 150 minutes or atomized in 4 cycles, the bacteria prevalence was still low. [Valø et al., 2020] showed higher growth of APC in both VTABB processed and traditionally smoked fillets, although these fillets were only smoked for 3 h (compared to 6 h), but smoked for 180 minutes (compared to 150 minutes). This corresponds to the results observed by [Leroi et al., 2000], which stated that microbial growth was mainly inhibited by the salt concentration in meat, and to a lesser extent by phenol content - if smoking degree can be seen as the concentration of phenols. However, in inoculated samples, growth was detected as both APC and *Listeria* spp. This indicates that all growth detected on APC most likely originate from the inoculated *L. innocua*, which also was shown by [Al-Zeyara et al., 2011], who found APC and L. monocytogenes to be highly positively correlated.

Further on, traditionally smoked fillets not treated with SGS (Trad-V-Ino) was expected to have higher bacteria growth than the other groups, since Trad-V-Ino did not have the reduced pH from PCS nor the CO_2 [Valø et al., 2020, Sivertsvik et al., 2002a] to inhibit bacterial growth. The results indicate tendency of higher APC growth regarding Trad-V-Ino throughout storage, and this was measured to be significant day 26 and 43 post processing. Regarding *Listeria* spp. Trad-V-Ino showed a tendency of higher growth, until day 33 where there was a drop in growth. Reason for this drop is unclear, since the growth seems to increase 43 days post processing. One reason can be that the samples analyzed 33 days post processing were unintended treated with less *L. innocua* inokulum, or that these bacteria were more stressed than the other days and therefore did not grow as good. It is implausible to assume that there was a drop in *Listeria* spp. concentration, since the growth increased at day 43 again. Although, by studying the APC growth there is also a drop 33 days post processing here as well, and this regards all the groups. Another reason for the drop can be that another type of stomacher was used that exact day, which did not shatter the samples in the same degree. Since there was a drop in APC as well, and the drop was not so big in the other groups of *Listeria*, there is not assumed that there was something wrong with the agar used for *Listeria* or APC.

Regarding the APC growth in other smoked fillets, fillets smoked traditionally and SGS treated (Trad-S-Ino) were measured to be higher at the final storage day than fillets atomized with PCS. Therefore, it can seem like the effect of PCS on inhibit bacterial growth is larger than the effect of CO_2. However, this difference was not seen on *Listeria* spp. It seems like the content of APC and *Listeria* spp. were held constant throughout the storage period regarding

traditionally smoked fillets with SGS treatment (Trad-S-Ino) and fillets atomized with VTABB (VTABB-V-Ino and VTABB-S-Ino). Some fluctuations were seen in traditionally smoked fillets without SGS treatment (Trad-V-Ino). This means that inoculated *L. innocua* did not die during storage in Trad-S-Ino, VTABB-V-Ino and VTABB-S-Ino fillets, but neither did grow, as seen in [Milly et al., 2008]. Since this shows that *L. innocua* is not killed, growth can (according to [Løvdal, 2015]) resume after the package is opened, even if the salmon is stored at refrigerator temperature, and thus constitute a major food safety risk. An assumption of steady content of APC is that the cells were stressed, and therefore did not thrive and divide. Although, at the last APC measurements the growth had a tendency to increase in all the smoked fillets. This was not the case regarding the growth of *Listeria* spp., which got reduced in all the groups except Trad-V-Ino. A reason for this can be that other bacteria started to grow in the samples, and subsequently gave higher APC growth. However, since there was not detected any growth on the natural samples, this can only be seen as an assumption.

7 Conclusion

Based on the three experiments performed in this thesis it can be concluded that CSS fillets produced with atomized PCS gave lower yield, firmer texture and higher TPC compared to traditionally CSS. This indicates that PCS gave dryer products with increased phenolic content. Both the salt content (%) and TPC of all measured fillets had a trend to be slightly below the French Standard requirements of 2.5-3.5 % NaCl and 0.6 mg phenols/100 g, with measurements ranging from 1.88-2.82 % salt and 0.34-0.62 mg phenols/100 g sample. The microbial growth was moreover found low within both PCS and traditionally smoked CSS, and the growth of LAB was non-existing. However, after inoculating CSS fillets with *L. innocua* traditionally fillets were measured having higher APC and *Listeria* spp. growth. The effect of SGS treatment showed reduced growth of APC and *Listeria* spp. in both traditionally smoked and PCS treated fillets. pH was found to be lower in PCS fillets, as expected due to acetic acid from the PCS. The TPC was also observed lower in PCS fillets, and it was assumed to be a consequence of extensive drying which gave frequent hydrophobic interactions between lipids and phenolic substances. Colour is an essential appearance factor for the consumer. The salmon fillets treated with PCS were darkened, more red and yellowish, but the results from Napping® indicated no negative colour associations with PCS treated fillets. The overall texture was found to be significantly firmer for the PCS samples, although this was not found negative through sensory selection with 4 semi-trained assessors. This study shows that PCS can be a good alternative to traditional smoke when producing CSS with low salt content. The PCS did moreover seems to inhibit *Listeria innocua*, which indicate a potential to increase the food safety of CSS.

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A Appendix

A.1 Theory: Sensory - Napping®

An illustration of the setup arranged for performing Napping® is shown in fig. A.1. The first step is to place the different samples according to similarities and differences, where similar samples are placed close to each other. The second step is to describe the groups made by terms either choose in advance, or by the assessor.

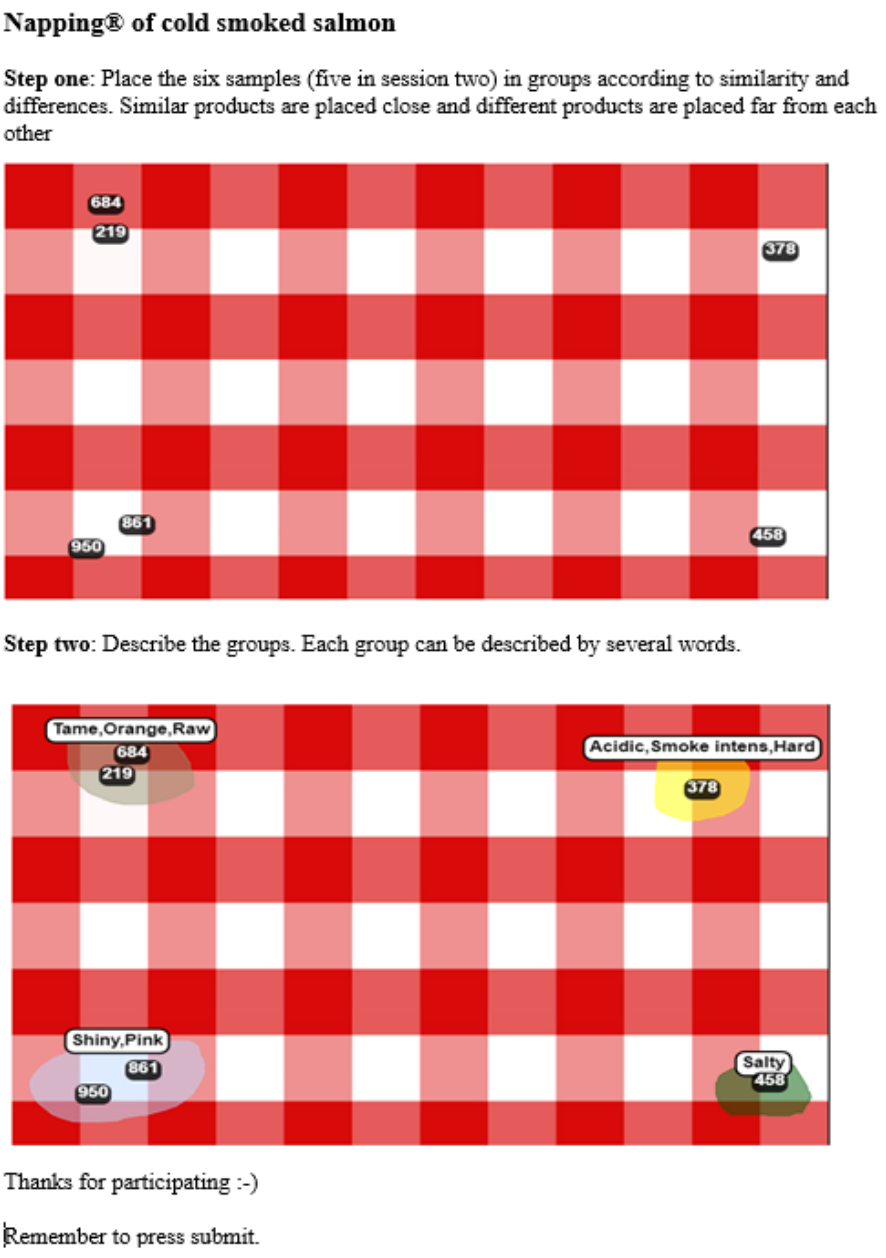


Figure A.1: Example of a competed ballot (Napping® with UFP in EyeQuestion). The assessors placed products with different sensory characteristics far from each other, and products with similar sensory attributes closer (Step one). A cited list of terms were later used to describe the different categories created (Step two).

A.2 Fish Supplying Farm and Producer

Information about the fish supplying farm, producer and harvesting date regarding all the three experiments are shown in table A.1.

Table A.1: The producers and supplying farms contributing with Atlantic salmon for every experiment. The experiment numbers reflects the experiments in the order they were performed, from first (1.) to last (4.)

Experiment number	Fish supplying farm	Producer	Harvest Date
1.	SalMar	SalMar	25.09.19
2.	Akvafuture AS	SalmoSea AS	09.12.19
3.	Akvafuture AS	SalmoSea AS	30.01.2020

A.3 Results - ATP Breakdown Products

The retention times (min) with each breakdown products of the standard mix (STDmix) and mean retention times (min) of the experimental groups from Experiment 1 and 3 is shown in table A.2 and table A.3, respectively.

Table A.2: The retention times (min) and breakdown products of a standard mix (STDmix) and mean retention times (min) of all the experimental groups (n = 6) observed at day 2 and day 35 post processing, from Experiment 1.

Peak #	**RetTime (min) STDmix**	**Breakdown Product**	**RetTime (min) Day 2**	**RetTime (min) Day 35**
1	2.76	Hypoxanthine	2.80	2.79
2	3.16	Inosine Monophosphate	3.32	-
3	3.66	Inosine	3.60	3.51

Table A.3: The retention times (min) and breakdown products of a standard mix (STDmix) and average retention times (min) of all the experimental groups (n = 3) from Experiment 3 , observed 6 days post processing.

Peak #	**RetTime (min) STDmix**	**Breakdown Product**	**RetTime (min) Day 6**
1	2.76	Hypoxanthine	2.72
2	3.16	Inosine Monophosphate	2.95
3	3.66	Inosine	3.35

A.4 Results - Napping®

The cited terms and their frequency from the Napping® performed in Experiment 2 is shown in fig. A.2. Only terms mentioned 6 times or more were included in the variables plot. The Eigenvalues and squared cosines of the observations is shown in fig. A.3, where it seems like JJT01 and Enviro (here labelled Enviro24) are best explained in dimension 4 and 3, respectively. The four different dimensions and squared cosines of the terms is shown in fig. A.4. The bold numbers highlights the terms which explains its dimension the most.

Figure A.2: Cited terms and frequency by 10 assessors doing Napping® with modified UFP on traditionally (REFV and REFJ) cold-smoked Atlantic salmon and three types (VTABB, JJTO1 and ENVIRO24) of smoke-flavored Atlantic salmon (PCS). The analysis was performed in Experiment 2.

Samples/ Terms	VTAB	REFV	JJTO1	REFJ	ENVIRO24	AVERAGE (per sample)	SUM
Glossy	3	2	2	4	0	2,2	11
Oily	1	2	4	4	3	2,8	14
Smooth	0	0	1	2	1	0,8	4
Dry	2	2	0	0	0	0,8	4
Grey	1	1	0	0	0	0,4	2
Pink	1	1	1	1	1	1	5
Orange	1	1	1	3	0	1,2	6
Red	0	0	0	1	2	0,6	3
Pale	3	4	1	1	0	1,8	9
Artificial	0	0	1	1	0	0,4	2
Perfumed	1	0	0	0	0	0,2	1
Stinging Odor	1	0	0	0	0	0,2	1
Rotten Odor	0	0	0	0	0	0	0
Rancid	0	0	0	1	0	0,2	1
Chemical	0	1	0	0	0	0,2	1
Sticky	0	0	3	1	3	1,4	7
Hard (firm)	2	0	1	3	1	1,4	7
Soft	0	3	4	3	4	2,8	14
Tender	2	3	2	3	3	2,6	13
Strong crust	4	2	0	1	1	1,6	8
Raw	0	1	2	0	1	0,8	4
Stingy	2	1	0	0	0	0,6	3
Tame	0	0	1	1	0	0,4	2
Appropriate amount of smoke	3	2	1	3	4	2,6	13
Too little smoke	0	3	3	1	1	1,6	8
Too much smoke	2	1	1	0	1	1	5
Appropriate amount of salt	3	3	2	3	5	3,2	16
Too little salt	0	1	0	1	0	0,4	2
Too much salt	1	0	1	1	2	1	5
Mild	0	0	2	1	2	1	5
Sweet	0	0	0	1	0	0,2	1
Acidic	3	0	0	1	1	1	5
Campfire	4	3	0	1	0	1,6	8
Metallic	1	0	0	0	0	0,2	1
Juicy	0	0	0	0	1	0,2	1
Insipid	0	0	1	0	0	0,2	1
Off-taste	0	1	0	1	0	0,4	2

Figure A.3: Eigenvalues and Squared cosines of the observations from Napping®. The eigenvalues show that 70,15% of the variance is explained in the first two dimensions. The squared cosines of the observations show in which of the dimensions the different samples of smoked salmon are best explained. VTABB and REFJ (the corresponding fillet to JJT01, smoked traditionally) seems to be best explained in dimension 1 (F1). Which terms in dimension 1 that explained these two smoke samples can be seen in fig. A.4.

Napping® with modified UFP

Eigenvalues

	F1	F2	F3	F4
Eigenvalue	5,71	3,56	2,20	1,74
Variability (%)	43,19	26,95	16,67	13,18
Cumulative %	43,19	70,15	86,82	100,00

Squared cosines of the observations:

	F1	F2	F3	F4
VTAB	**0,85**	0,13	0,01	0,01
REFV	0,05	**0,74**	0,05	0,16
JJTO1	0,34	0,08	0,07	**0,51**
REFJ	**0,48**	0,37	0,05	0,10
ENVIRO24	0,02	0,00	**0,98**	0,00

Figure A.4: Squared cosines of the terms used in Napping®. The bold numbers show in which dimension (F1, F2, F3, F4) the terms are best explained.

Statistics XLSTAT

Squared cosines of the variables/terms

	F1	F2	F3	F4
	F1	F2	F3	F4
Glossy	0,00	0,27	**0,68**	0,05
Oily	**0,95**	0,01	0,00	0,04
Smooth	**0,72**	0,27	0,01	0,00
Dry	**0,70**	0,08	0,12	0,09
Grey	**0,70**	0,08	0,12	0,09
Pink	**0,00**	0,00	0,00	0,00
Orange	0,18	0,27	**0,41**	0,14
Red	0,13	0,08	**0,72**	0,06
Pale	**0,39**	0,16	0,34	0,11
Artificial	**0,59**	0,10	0,21	0,10
Perfumed	**0,76**	0,19	0,03	0,03
Stinging Odour	**0,76**	0,19	0,03	0,03
Rotten Odour	**0,00**	0,00	0,00	0,00
Rancid	0,31	**0,39**	0,08	0,22
Chemical	0,02	**0,62**	0,07	0,28
Sticky	0,33	0,00	0,31	**0,36**
Hard (firm)	0,03	**0,93**	0,03	0,01
Soft	**0,70**	0,18	0,11	0,01
Tender	0,16	0,03	0,13	**0,69**
Strong crust	**0,86**	0,05	0,02	0,07
Raw	0,14	**0,48**	0,01	0,38
Stingy	**0,90**	0,00	0,09	0,01
Tame	**0,59**	0,10	0,21	0,10
Appropriate amount of smoke	0,04	0,23	**0,51**	0,22
Too little smoke	0,19	**0,68**	0,10	0,02
Too much smoke	**0,82**	0,01	0,01	0,16
Appropriate amount of salt	0,01	0,01	**0,86**	0,13
Too little salt	0,11	0,02	0,21	**0,66**
Too much salt	0,02	0,23	**0,64**	0,10
Mild	**0,45**	0,00	0,27	0,29
Sweet	0,31	**0,39**	0,08	0,22
Acidic	**0,52**	0,48	0,01	0,00
Campfire	**0,70**	0,00	0,18	0,12
Metallic	**0,76**	0,19	0,03	0,03
Juicy	0,01	0,00	**0,99**	0,00
Insipid	0,15	0,06	0,08	**0,72**
Off-taste	0,11	0,02	0,21	**0,66**

A.5 Example Calculations

A.5.1 Processing Yield

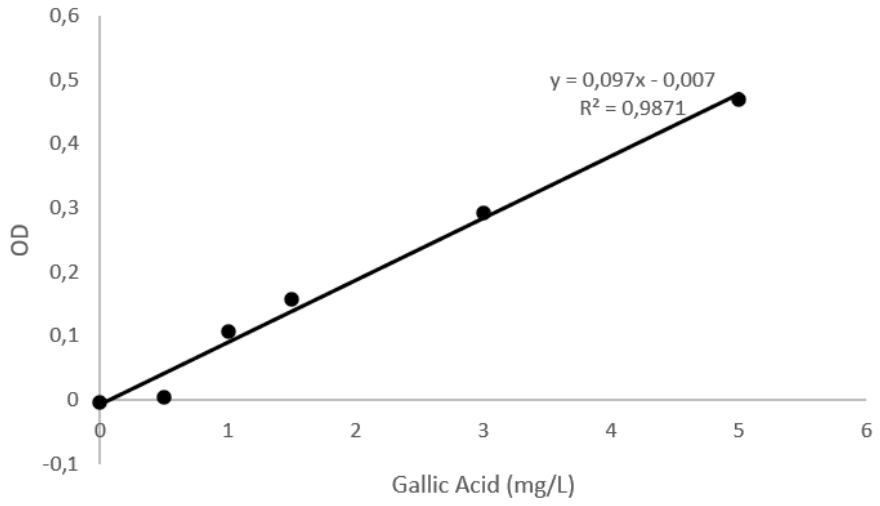
The processing yield (%) was calculating using the weight of the fillet before salting (Weight before salting), and 2 days post smoking (Weight 2 days post smoking). An example of a calculation from Experiment 1 is shown eq. (A.1).

$$ProcessingYield(\%) = \frac{Weight\ 2\ days\ post\ smoking\ (g)}{Weight\ before\ salting} * 100\% = \frac{1{,}3624}{1{,}4718} * 100\% = 92{,}8795\% \tag{A.1}$$

A.5.2 Total Phenolic Content

A standard curve was made by plotting the absorbance (750 nm) of gallic acid as a function of known concentration (mg/L), see fig. A.5. Linear regression gave a function of y = 0.097x - 0.007. The TPC was calculated using the slope (0.097) of the standard curve, and an example of TPC calculation of Trad 90 from Experiment 1 is shown in eq. (A.2).

Figure A.5: The standard curve used to calculate the total phenolic content, made by plotting the absorbance of gallic content (750 nm) as a function of concentration (mg/L). The trendline gave a function of y = 0.097x - 0.007, with a R^2 of 0.9871.



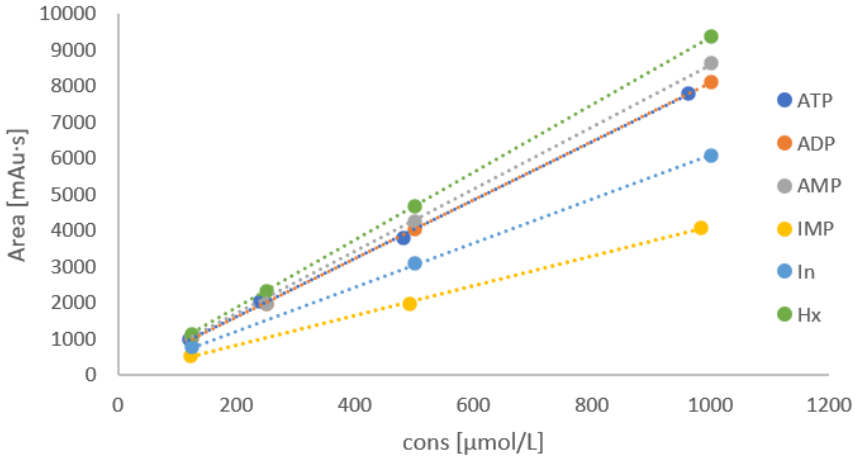
$$GAE/100\ g\ sample = \frac{a * Abs * \frac{b}{c}}{\frac{W}{d}} = \frac{0\ 097 * 0\ 084 * \frac{100}{1}}{\frac{5{,}0336}{0{,}020}} = 0{,}324\ GAE/100\ g\ sample \tag{A.2}$$

a = Slope from the equation in the standard curve
Abs = Absorbance measured at 750 nm
b = Size of the volumetric flask (100 mL)
c = Volume taken out of the extract (1 mL)
W = Weight of the sample (g)
d = Volume extraction (0.020 L)

A.5.3 ATP Breakdown Products

A standard mix (STDmix) containing ATP, ADP, AMP, IMP, Ino and Hx, in concentration of 1000, 500, 250 and 125 μmol/L were ran through the HPLC system first, at the same conditions as the samples explained in "2.1.7 Degradation products of ATP". A standard curve for each product was made as the area (mAu*s) of each peak as a function of the concentration (μmol/L) (see fig. A.6). The slope of the function was used to calculate the content of each breakdown product, as shown in eq. (A.3) where the Hx content of Trad 90 is calculated.

Figure A.6: The standard curves used to calculate the content of ATP breakdown products, with area (mAu*s) as a function of concentration (μmol/L). The function of IMP, Ino and Hx was y = 4.1x, y = 6.1x and y = 9.4x, respectively. ATP, ADP and AMP were not detected in the samples, therefore their function was not used.



$$\frac{Area\% * V}{a * W} = \frac{389\ 8633 * 0{,}0106}{9{,}4 * 1{,}2396} = 0{,}3443\ \mu mol/L \tag{A.3}$$

Area% = The area in % of the peak from the chromatogram
V = Volume solvent (L)
a = Slope of the standard curve
W = Weight of the sample

A.5.4 K_1- and H-value

The K_1- and H-value were calculated using eq. (A.4) and eq. (A.5), respectively. The examples shows calculation in Trad 90, after 2 days storage.

$$K_1 - value = \frac{Ino + Hx}{Ino + Hx + IMP} = \frac{4{,}0 + 0{,}38}{4{,}0 + 0{,}38 + 6{,}24} = 0{,}41 \mu mol/g \tag{A.4}$$

$$H - value = \frac{Hx}{Hx + Ino + IMP} = \frac{0{,}38}{0{,}38 + 4{,}0 + 6{,}24} = 3{,}55 \mu mol/g \tag{A.5}$$

A.5.5 Salt content %

The salt content % was measured using eq. (A.6), where the example shows the calculation of Trad 90.

$$\frac{(EQ1 - B) * T * M * F1}{W} = \frac{(5{,}99 - 0) * 0\ 1 * 58{,}443 * 0{,}1}{1{,}8635} = 1{,}88\% \tag{A.6}$$

EQ1 = Consumption at the equivalence point (mL)
B = 0 (Blank value)
T = 0.1 (Exact concentration of the titrant in mol/L)
M = 58.443 g/mol (Molecular weight of NaCl)
F1 = 0.1 (Conversion factor for %, 100/1000)
W = Weight of the sample (g)

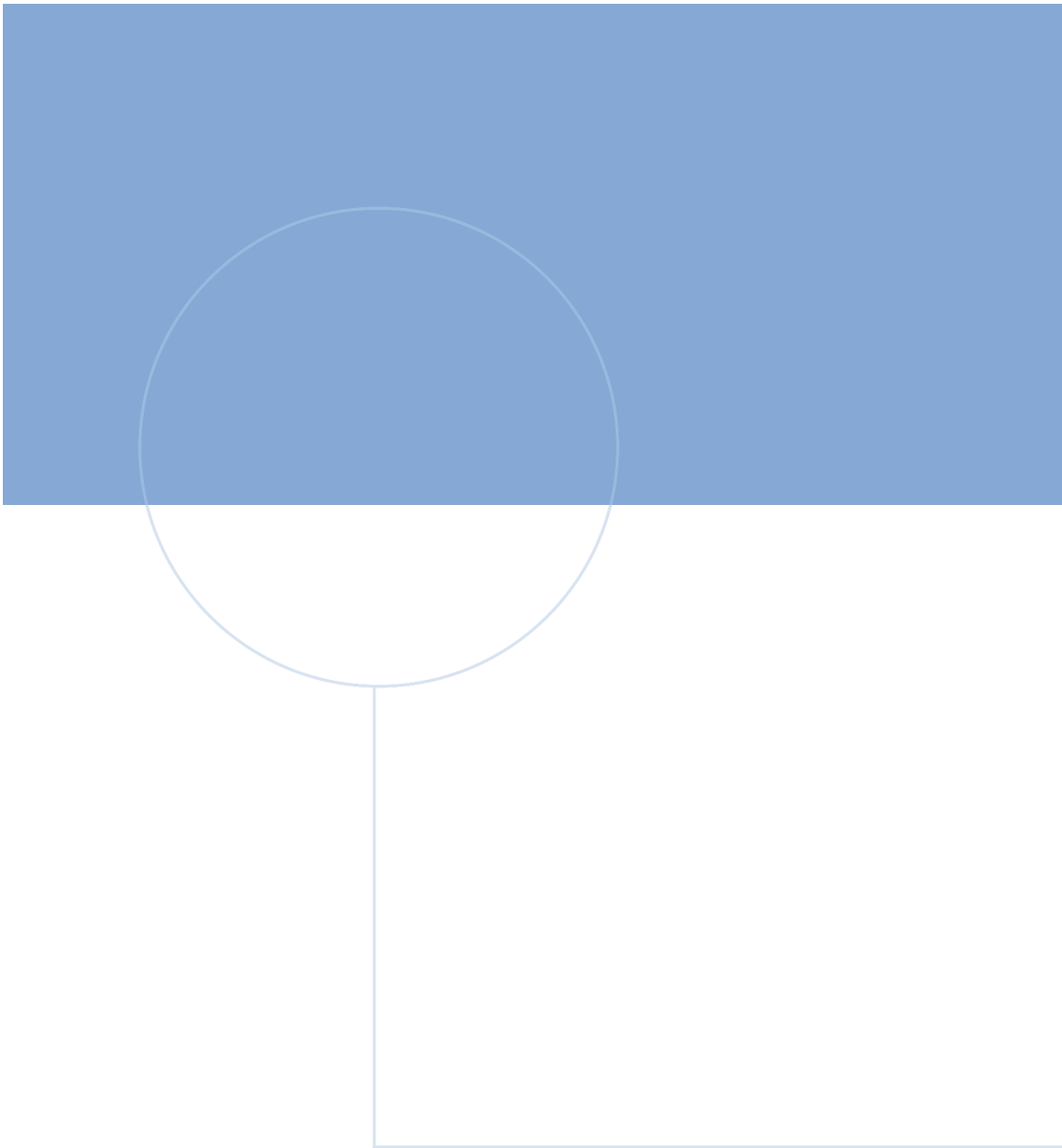
A.5.6 Molecular growth

CFU/g of colonies counted on petri dishes for both APC, LAB and *Listeria* spp. were calculated using eq. (A.7). For APC, 1 mL bacteria culture were plated on each petri dish, therefore a -1 (10^{-1}) diluted culture will be -1 diluted when plated on a petri dish. For LAB and *Listeria* spp. only 0.1 mL were plated on each petri dish, therefore a -1 diluted culture would give -2 (10^{-2}) dilution when plated on a petri dish, an so an.

$$\frac{C1 + C2 + C3}{D1 + D2 + D3} = \frac{54 + 20 + 0}{0{,}1 + 0{,}01 + 0} = 672{,}73\ CFU/g \tag{A.7}$$

C1 = Number of colonies
C2 = Number of colonies
C3 = Number of colonies
D1 = Dilution factor of petri dish C1
D2 = Dilution factor of petri dish C2
D3 = Dilution factor of petri dish C3

multiplied for each dilution made. For APC and LAB 1 mL and 0.1 mL (equals 0.1 and 0.01 g fish due to 1:10 dilution (fish:peptone water)) were plated on petri dishes, respectively. For a total 10 – 3-dilution for APC a multiplication of 10 3 give CFU/g fish. If 65 colonies were counted, CFU would be 65000 (Equation A.2). Due to additional dilution by using 0.1 mL for LAB, multiplying with 10 is needed. CFU = C · DF1 · DF2 = 65 · 103 = 65000CFU/g(A.2) Where: CFU = Colony Forming Units (CFU/g) C = Colonies on petri dish DF1 = Dilution factor made of peptone water DF2 = Dilution factor, 10, only for LAB



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Kunnskap for en bedre verden