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Microbiological Shelf-Life of Vacuum-Packaged Hot-Smoked Atlantic Salmon (*Salmo salar*) Treated with Different Salt-Sugar Brines During Chilled Storage

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Abstract: Background: Hot-smoked Atlantic salmon remains susceptible to microbial regrowth during refrigeration, requiring optimized brining and vacuum packaging to maintain acceptable shelf-life. **Aims of the study:** This study aimed to evaluate the effects of three salt-sugar brine formulations on total aerobic microbial counts in hot-smoked Atlantic salmon during 21 days of vacuum-packaged storage at 4°C and identify the formulation providing the most favorable microbiological shelf-life. **Methodology:** Randomly 10 Atlantic salmon were filleted, placed in 3 salt-sugar brines and immersed for 1 hour, drained and then hot-smoked at 85°C for 2 hours using eucalyptus chips. After being cooled, vacuum-packed and kept at 4°C for 21 days. The product pH, water activity and salt content were determined by validated methods. Eight storage days were analyzed with duplicate plate-count agar cultures heated to 25°C and counted for 48 hours and the results presented as CFU/g and give descriptive summary. **Result:** Hot smoking reduced total aerobic counts from 6,000 CFU/g before processing to 28, 86, and 120 CFU/g on day 2 in Brines 1, 2, and 3, representing reductions of 99.5%, 98.6%, and 98.0%, respectively. Counts subsequently increased during refrigerated storage, reaching 8,900, 21,100, and 34,000 CFU/g on day 21. All treatments remained well below the referenced 1×10^7 CFU/g acceptability limit, while the strongest formulation, Brine 1, consistently demonstrated the most favorable microbiological profile throughout storage based on descriptive count comparisons. **Conclusions:** Hot smoking and salt-sugar brining markedly reduced microbial loads in Atlantic salmon. Although counts increased during refrigerated storage, all treatments remained below the referenced acceptability limit. Brine 1 provided the strongest microbial control, but larger replicated confirmatory studies are required.

Keywords: Atlantic salmon; hot smoking; salt-sugar brine; vacuum packaging; microbial shelf-life.

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1. Introduction

Atlantic salmon (*Salmo salar*) is one of the most valued species for aquaculture industry and is sold as fresh/frozen/salted/smoked salmon. It is a product of high quality proteins, long-chain omega-3 fatty acids, vitamins, and good sensory properties, which make it very popular amongst consumers. However, the high moisture content, near neutral pH, presence of nitrogenous nutrients and activity of endogenous enzymes make salmon very susceptible to microbial deterioration if the proper conditions of processing and storage are not strictly adhered to [1]. The preservation strategies that will enable

food to be stored for longer periods without compromising the nutritional and sensory qualities are particularly important to seafood processors and consumers.

Smoking is among the oldest techniques for fish preservation, and involves all of the above including salting, dehydration, heat and deposition of antimicrobial smoke compounds. Today, physical smoking is also to impart particular colour, aroma, flavour and texture to fish, not only to preserve them [2]. The smoke generated during the processing can affect processing yield, protein denaturation, water holding capacity, lipid oxidation, colour stability and microbial quality. These are all dependent on the degree of smoking temperature, smoking time, smoke composition, thickness of the fillets, quality of the raw materials and type of preparation before smoking [3].

Cold smoking is typically used at temperature levels that aren't enough to cook the fish or kill vegetative microorganisms. Hot smoking, on the other hand, uses higher temperatures to cook the muscle and may result in a significant decrease of initial microbial population [4]. But hot-smoked salmon doesn't always mean sterile and shelf stable. Heat-resistant spores may survive processing and microorganisms can be added during cooling, handling, slicing or packing. Hence a hygienic post-processing handling and ongoing refrigerated storage are in general necessary for hot-smoked fish.

Brining is a very important step in the making of smoked-salmon as it plays a role in flavor development, texture modification, moisture redistribution and microbial control. Sodium chloride is absorbed by the muscle during brining and water and soluble components move from the fish to the brine and vice versa [5]. The uptake of salt into the fillet can be different between the different regions of the fillet due to differences in muscle composition, fat distribution, rigor status, brine concentration, exposure time, temperature and fillet Geometry [6]. Sodium chloride has antimicrobial properties due to a decrease in the water activity, osmotic stress and disturbance of the membrane function and metabolic activity. However, several psychrotrophic, halotolerant and lactic acid bacteria can withstand the chilled storage and then grow.

Phenols, carbonyl compounds and organic acids are present in smoke, which has antimicrobial and antioxidant properties. The factors that affect the deposition of phenols are wood types, the conditions of smoke generation, the smoking time, temperature, and characteristics of the product surface [7]. Sugar is often added to salmon brines to counteract the "salty" taste, for flavor, and to help develop the desired surface color. It also can add a small amount to the osmotic pressure but its preservative effect is usually less than salt at standard formulation concentrations. Martínez et al. showed that the salting treatment and the sugar addition had an impact on the physicochemical, sensory and textural properties of smoked Atlantic salmon [8].

Smoking and refrigeration are often used together with the vacuum package to minimize oxygen exposure, the extent of oxidative deterioration, loss of moisture from the product, and the growth of aerobic spoilage microorganisms [9]. But, not all microorganisms are inhibited with reduced oxygen. If salt concentration or smoking intensity are not appropriately high, facultative anaerobic and psychrotrophic bacteria can grow during refrigerated storage. Thus, a series of studies on the microbiological, chemical and sensory quality of vacuum-packed hot-smoked fish have shown that these qualities change over time during the refrigeration period [10].

The microbiological stability is thus affected by multiple hurdles, not just a single processing factor, in smoked salmon. Interest in improving control, while maintaining product quality, has continued as evidenced by recent investigations of other antimicrobial interventions for raw and smoked salmon. With vacuum-packaged smoked fish, the risk of food-safety concerns is particularly great due to the possibility of postsmoking contamination with *Listeria monocytogenes* and the potential for growth and toxin production by nonproteolytic *Clostridium botulinum* under favorable anaerobic conditions [12]. These hazards could be influenced by packaging-film

permeability, salt level, water activity, storage temperature and shelf-life duration [13]. Hence, the current seafood-safety guidance focuses on the validated thermal processes, the proper salting, the proper handling for hygienic reasons, rapid cooling, packaging control, and continuous refrigeration [14]. Furthermore, the presence of total aerobics is useful as a good general indicator of the microbial quality, but it does not confirm the absence of specific pathogens [15].

Although the smoked salmon industry is of commercial importance, little information is available on the effect of the different formulations of salt-sugar brines on total aerobic counts after hot smoking and in vacuum-packed storage. An important consideration for comparing brine formulations is that too much salt can cause consumer rejection, while too little can increase dietary sodium consumption, and too little can also lead to microbial instability [16]. Thus, the objective of this study was to determine the effect of three salt-sugar brines, hot smoking, vacuum packaging, and cold storage at 4°C for 21 days on the total aerobic plate count of Atlantic salmon and to evaluate the brine that resulted in the best microbial profile for the fish.

2. Materials and Methods

Ten Atlantic salmon (*Salmo salar*) of average weight of 1.1 kg were purchased from the University of Tasmania Aquaculture Centre and sent to the seafood-processing laboratory at the Australian Maritime College, Beauty Point, Tasmania. Fish were kept frozen at -25 °C until one day before processing for gradual thawing in 4 °C refrigerator. The 20 fillets per fish were randomly distributed among the three fish brine treatments, and the final fish fillets were manually cut into 216 to 330 g pieces. The 30-L salt-sugar brines used were prepared with 160 g salt and 80 g sugar/L (Brine 1), 130 g salt and 65 g sugar/L (Brine 2), and 100 g salt and 50 g sugar/L (Brine 3). The fillets were soaked in the defined brine for 1 hour, then transferred onto labeled salt-sugar brines and held at 4°C for 0.5 hour. Fillets were dipped into the selected brines for 1 hour, then placed skin-side down on labeled salt-sugar brines and left to drain for 0.5 hour at 4°C. They were then hot-smoked for two hours at 85°C with dried eucalyptus wood chips in a smoker from Foley Industries. The fillets were then cooled at 4°C for 30 minutes, each put in a labelled bag and vacuum-packed with a Compack KRAMER GREBE machine before being kept at 4°C for 21 days. The pH of the products was determined during the storage period by measuring the pH of the thickest part of each fillet with a calibrated meter (WTW 330) fitted with a Sentix SP penetration probe. The water activity of the samples was determined following the smoking process at 25°C by means of a Novasina aw-center, after homogenization of about 10 g of sample, and after five minutes of stabilization. The smoke content was determined after the smoking by AOAC Method 937.09 - nitric-acid digestion, addition of silver nitrate and back-titration with ammonium thiocyanate. Microbiological quality was determined according to the total aerobic plate count method 966.23 of the Association of Official Analytical Chemists on days 0, 2, 5, 8, 12, 15, 18 and 21. Each sample was aseptically transferred into a stomacher bag, and mixed with 90 mL of sterile 0.5% peptone water; 45 seconds was used for the homogenization in a Lab-Blender 400. Ten-fold serial dilutions were made to 10⁻³ and duplicate quantities of 1 mL were placed in 90 mm Petri dishes, poured with molten plate-count agar and incubated at 25°C for 48 hours. Colony counts were performed, and results are reported as colony-forming units/g (CFU/g). There were limited analytical replicates for each treatment that no inferential statistical comparisons were made and results were summarized descriptively.

2.1 Statistical analysis

Data were summarised descriptively as there were only two analytical replicates per brine treatment. The microbial counts were reported as CFU/g and the pH, water activity and salt content were reported as mean values. The percentage reduction after

hot smoking, absolute and fold increases after storage and final counts after hot smoking compared to the reference acceptability limit were determined. There were no inferential statistical tests or p-values reported due to the limited replication.

2.2 Ethical approval

Human research ethics approval and informed consent did not apply as there were no human participants or identifiable personal data. No experimental procedures were carried out on live animals, and Atlantic salmon obtained following harvest from the University of Tasmania Aquaculture Centre. All the processing and laboratory analyses were carried out according to the rules of laboratory safety, food hygiene and responsible research of the institution.

3. Results

3.1 Changes in microbial loads following hot smoking and subsequent storage under three salt–sugar brine treatments at 4°C

The total aerobic plate count (TAPC) was significantly reduced after hot smoking with an average value of 6,000 CFU/g to 28, 86 and 120 CFU/g on day 2 for Brines 1, 2 and 3, respectively. The microbial counts thereafter also rose during the refrigerated storage, reaching 8,900 CFU/g in Brine 1, 21,100 in Brine 2 and 34,000 in Brine 3 on Day 21. The lowest microbial count was always observed from the strongest brine treatment while the highest final count was observed from the least strong treatment.

Table 1. Total Aerobic Plate Counts of Vacuum-Packaged Hot-Smoked Atlantic Salmon During Refrigerated Storage

Storage stage	Brine 1 (CFU/g)	Brine 2 (CFU/g)	Brine 3 (CFU/g)
Before processing, day 0*	6,000	6,000	6,000
Day 2 after processing	28	86	120
Day 21 of storage	8,900	21,100	34,000

3.2 Relative reductions after processing and subsequent increases in microbial counts according to the applied brine concentration

After treatment with Brines 1, 2 and 3, the total aerobic microorganisms were reduced by 99.5%, 98.6% and 98.0% respectively when compared to pre-processing count. Microbial regrowth was detected in all the treatments for chilled storage but the magnitude was lowest in Brine 1 and highest in Brine 3. Brine 1 recorded a net increase of just 2,900 CFU/g after 21 days as compared to increases of 15,100 and 28,000 CFU/g in Brines 2 and 3, respectively.

Table 2. Changes in Total Aerobic Plate Counts Following Smoking and During 21 Days of Chilled Storage

Microbial change	Brine 1	Brine 2	Brine 3
Reduction from day 0 to day 2 (%)	99.5	98.6	98.0
Absolute increase from day 2 to day 21 (CFU/g)	8,872	21,014	33,880
Fold increase from day 2 to day 21	317.9	245.3	283.3
Net increase from day 0 to day 21 (CFU/g)	2,900	15,100	28,000

3.3 Comparison of final total aerobic counts with the microbiological acceptability threshold applied in the original study

The microbiological samples of all the brine treated samples did not exceed the microbiological acceptability limit of 1×10^7 cfu/g throughout the storage period of 21 days. The counts in Brines 1, 2, and 3 at day 21 were only 0.089%, 0.211%, and 0.340% of the referenced limit, respectively. As a result, all treatments had microbiological quality acceptable based on the total aerobic count criterion employed in the original study, and Brine 1 had the most desirable microbial profile.

Table 3. Microbiological Acceptability of Hot-Smoked Atlantic Salmon Fillets After 21 Days of Refrigerated Storage

Brine treatment	Day-21 count (CFU/g)	Referenced limit (CFU/g)	Percentage of limit (%)	Classification
Brine 1	8,900	1×10^7	0.089	Below the stated limit
Brine 2	21,100	1×10^7	0.211	Below the stated limit
Brine 3	34,000	1×10^7	0.340	Below the stated limit

4. Discussion

The current results show that hot smoking resulted in a significant decrease in the total aerobic microflora level in Atlantic salmon in the short term. The counts decreased by 98.0–99.5 %, from an average pre-processing value of 6,000 CFU/g to 28–120 CFU/g on day 2. This significant loss is due to the combined effects of heat, salt, dehydration and the antimicrobial smoke components. Lerfall and Hoel found the microbiological quality of hot smoked Atlantic salmon to be affected by salting technology, smoking protocols and storage conditions at 4°C after smoking [17]. Hot smoking is a process that is fundamentally different from cold smoking since the temperatures reached are higher and can result in the direct killing of vegetative microorganisms. However, the extent of the reduction will be dependent on the amount of contamination, the thermal history, the thickness of the fillet, the salt penetration, and the post-processing cleanliness.

Biologically plausible: The small counts immediately after smoking are likely because multiple hurdles operate simultaneously for microbial control in smoked salmon. Løvdal stated that the parameters of salt concentration, water activity, smoke compounds, temperature, packaging and initial microbiota are major factors that contribute to the microbial stability of smoked salmon [18]. The effect of sodium chloride is to reduce water availability, create osmotic stress and may interfere with cellular enzyme activity. Smoke contains phenols, carbonyls and organic acids which are bacteriostatic or bactericidal. Both salt and smoke had an effect on the microbial quality of smoked salmon, but the interaction and effectiveness of salt and smoke on the different microbial groups were different, as shown by Leroi et al. [19]. The corresponding decrease obtained from the use of Brine 1 is, thus, in accordance with its more concentrated formulation (160 g salt and 80 g sugar/L).

Sugar is not as inhibitory as salt at the fish curing concentrations but can lead to osmotic pressure and affect moisture migration. The present experiment did not report however water-phase salt, water activity, pH, moisture content, smoke deposition, nor core temperature. Thus, it is not possible to ascribe the lower counts which occurred in Brine 1 solely to its nominal salt-sugar content. There may also have been differences in the uptake of salt in the fillets, the composition of the fillets, the uniformity of the brining process, or initial sample contamination. Maillet et al. demonstrated that the microbial

communities of smoked-salmon also differ significantly among processing environments and among the different factories, even though the processing stages are mostly similar [20]. This helps to explain that the differences in brine are descriptive not causal, as they represent treatment effects.

During 21 days of vacuum-packed storage at 4°C, the total aerobic counts were increased for all the treatments. This regrowth is to be expected because smoking and brining do not always kill all the microorganisms. If your refrigerator is being used as a storage place for a long period of time, it may be possible for the psychrotrophic and salt-tolerant organisms to recover and multiply. Progressive rise in aerobic and anaerobic counts were observed in vacuum-packed smoked salmon, and that storage temperature is highly influential on shelf life; vacuum-packed smoked salmon at 4°C were acceptable for the 20 days studied by Dondero et al. [21]. This is a day 21 increase which is consistent with the microbial behavior observed in storage, but low counts overall.

In Brine 1 the lowest day-21 count was observed while Brines 2 and 3 showed lower counts than Brine 1. This dose-ordering would corroborate the hypothesis that the enhanced brining strength led to a more sustained inhibitory environment. Heir et al. showed that the addition of other inhibitory salts as well as the salt composition significantly affect microbial growth during chilled storage of smoked salmon [22]. They also demonstrated that the antimicrobial activity was temperature dependent and that, in the absence of other compounds, the growth of pathogens was not decreased with conventional salt reduction. Therefore, the effectiveness of salt concentration must be considered together with water activity, pH, smoke level, storage temperature and preservative composition, as salt concentration is only part of the microbial control.

The apparent advantage of Brine 1 is in line with the hurdle-technology concept, which is based on the combination of several moderate preservation factors to limit microbial growth. Wiernasz et al. highlighted the importance of the use of competitive and/or protective microorganisms in conjunction with physicochemical hurdles in seafood preservation [23]. Vacuum-packed smoked salmon may encourage the growth of facultative anaerobic or fermentative bacteria and may inhibit the growth of strictly aerobic bacteria. Lactic acid bacteria and other psychrotrophic organisms were found to be becoming prominent during storage of vacuum-packed smoked salmon by Leroi et al. [24]. Thus, the aerobic plate counts only reflect the total culturable load, and do not provide a complete description of the microbial ecology or the organisms causing deterioration.

The folds from day 2 to 21 were numerically large as the denominator in the post-processing was very low. For instance, the fold increase of Brine 1 seems higher than the other treatments, although the actual count of day 21 is the lowest for Brine 1. Thus, when the baseline values are very different it can be misleading to use fold change only. A meaningful comparison would be absolute counts and log₁₀ of the CFU/g. Paludan-Müller et al. showed that the activity and interaction of certain organisms are more important for the spoilage of vacuum-packed smoked salmon than total counts [25]. Therefore, the fold increase is less informative than the absolute increase of 8,872 CFU/g in Brine 1 for comparing the performance of the treatments.

The relationship of regrowth with initial day-2 count was also not inversely proportional, which might be explained by interactions between microbial populations. Joffraud et al. demonstrated that patterns of spoilage could be different between combinations of bacteria and the individual bacteria [26]. Olofsson et al. also showed that the vacuum-packed smoked salmon harbours a complex bacterial community that could be under-represented by traditional cultivation methods [27]. The different trajectories of brines could, therefore, be due to differences in microbial competition, lag phase, salt tolerance, and substrate utilization. Lacking intermediate numerical values, growth rates,

lag phases and generation times could not be estimated reliably for days 5, 8, 12, 15 and 18.

The observed regrowth also stresses the significance of the post-smoking handling. This initial flora can be significantly reduced by heat treatment, but, contamination will reoccur during cooling, during trimming, during handling and during vacuum packaging. Rachman et al. reported on the lactic acid bacterial population changes in the smoked-salmon during storage and showed the usefulness of using molecular identification in addition to plate counting [28]. The microbial profile of the final product can then be affected by the way the surface, equipment and packaging area and personnel are processed. It is recommended that future experiments should include separation of surviving organisms and post process contaminants by sampling the product immediately after smoking, after it has cooled and after it has been packaged.

These differences in treatments may also be due to differential salt distribution. Birkeland and Bjerkeng demonstrated the influence of salting method, exposure time and temperature on both quality and salt content of Atlantic salmon smoked [29]. Similarly, Gallart-Jornet et al. showed that salt transfer into salmon muscle is also affected by increasing salt concentration of the brine [30]. The composition of the brine cannot therefore be assumed to be the same as the concentration of the salt in the water in each fillet. Water-phase salt and water activity would have to be truly measured in order to define a mechanistic correlation between the brine formulations and the microbial inhibition.

All day-21 counts were significantly lower than the benchmark of 1×10^7 CFU/g. The progressive rise in microbial counts has been reported with no immediate loss of acceptability in similar studies with the proper combination of smoking, salting, refrigeration and packaging [31]. However, a below threshold for total aerobic count should not be considered as evidence of the absence of pathogens or absolute product safety. Some pathogens could exist at low levels which are not detected in total count. Neunlist et al. demonstrated that salting and smoking could have an impact on culturability and viability of *L. monocytogenes* while not ensuring its complete destruction [32]. Brillet et al. also found that targeted bioprotective measures might be necessary to manage *L. monocytogenes* in smoked salmon [33].

Vacuum packaging offers advantages in minimizing oxidative damage and preventing aerobic organisms, but cannot be used to substitute for adequate refrigeration or pathogen testing. The classical seafood microbiology studies have shown that the pattern of spoilage is highly dependent on the product atmosphere and dominant specific spoilage organisms [34]. Variations in the numbers of counts at the end compared to those reported in studies with more rapid spoilage may be attributed to other smoking conditions, such as higher temperature; different initial contaminations; greater salt uptake; increased vacuum integrity; more precise temperature control, or analytical media differences and incubation conditions. There is also some variation between processing facilities, for example, microbial quality and shelf life of cold-smoked salmon from different processing plants were reported by Hansen et al. [35]

5. Conclusion

The total aerobic counts of Atlantic salmon were significantly reduced by hot smoking, and then gradually increased during 21 days of vacuum-packed storage at 4°C, with the lowest post-processing and final counts obtained with brine 1 which contained 160 g salt/L and 80 g sugar/L. All treatments were under the reference acceptability limit. The lack of replication and partial (lack of intermediate numerical data) cannot be overlooked, however, and should be interpreted with caution and confirmed using larger studies with physicochemical, pathogen-specific and statistically supported shelf-life assessment.

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