Received: 6 May 2021

Revised: 28 September 2021

Accepted: 14 October 2021

Pre-treatment Effect on Physical and Microbial Parameters of Smoked Baltic Sprats

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Abstract. Smoked sprats are delicious and nutritionally valuable product. Besides protein, it contains high amount of unsaturated fatty acids and fat soluble vitamins. Nowadays, human interest for fish product consumption is increasing. It leads to the demand for increased shelf life of the smoked sprats. One way to prolong the shelf life is to use some pre-treatment prior to smoking. The aim of study was to analyse the effect of pre-treatment on smoked Baltic sprat quality and microbiological parameters. Various compositions of brine using salts (NaCl, CaCl₂) and acetic acid in different proportions were used for fresh sprat pre-treatment. Pre-treatment had the influence on physical parameters; pre-treated samples had higher salt and lower moisture content, and lower pH, which allowed prolonging the shelf life of smoked sprats. The highest pH was in the control sample 6.6, and it allowed growth of yeasts, moulds, *Pseudomonas* spp., and mesophilic and facultative anaerobic microorganisms. The results demonstrated that after two-week storage of the smoked sprats the growth of moulds (1×10^2 CFU g⁻¹) was observed only for smoked sprats without pre-treatment (control sample), indicating the positive effect of pre-treatment. *Salmonella* spp., *Clostridium perfringens*, *Listeria monocytogenes*, and *Escherichia coli* were not detected in any of the analysed samples. After pre-treatment in brine, containing combination of NaCl and CaCl₂, smoked fish had darker colour compared to control sample or sample with sodium chloride only. Any of applied pre-treatments, except brine containing NaCl, made firmer texture of the smoked fish.

Keywords: smoked sprats, microorganisms, physical parameters, texture, colour.

Introduction

Sprats (*Sprattus sprattus balticus*) are nutritionally valuable product, which is rich in proteins, contains unsaturated fats, fat soluble vitamins, and essential minerals (Babikova *et al.*, 2020). According to Central Statistical Bureau of Latvia the amount of sprats caught from 2019 till 2020 decreased from 38.7 till 28.9 thousand million tons in Latvia, but it is foreseen that fishing option for fisherman's will increase in 2021 for sprats by 6% (till 30 817 tons) (Muceniece, 2020). Fish were eaten already since ancient times, it was valuable and available product for everybody. Nowadays the demand for variety of fish products increases. However, it is hard to ensure the required amount and quality, because of fish spoilage, which starts soon after the catch. Therefore, it is necessary to refrigerate fish immediately after it is being caught to prevent microbial spoilage or ensure higher safety.

Smoking is one of the oldest methods, which has been used for fish processing to obtain delicious products. Traditional smoking is also used to preserve fish, when

smoking compounds such as phenols have a considerable importance for the preservation and sensory properties (flavour and colour) of the smoked products (Sérot *et al.*, 2004). There are identified at least 400 different compounds in wood smoke (acids, alcohols, esters, etc.), and each of them has a functional role in smoking process (Toledo, 2008). Typically, smoked and preserved fish products have very short shelf life, from hours till some days, that means it is hard to be stored longer or exported to other countries. Fresh smoked sprat shelf life can be a few days (72 h) till one week according to the manufacturer's internal regulations, but for sprats to be used in canned product 'Smoked sprats in oil', storage time should not exceed 16 h ("Requirements for Food Quality Schemes, Procedures for the Implementation, Operation, Monitoring, and Control Thereof," 2014). The use of pre-treatment allows to ensure microbiological safety of sprats for 2 weeks. Therefore, it is necessary to apply additional preservation for fish products to ensure safety and good quality of the products with sufficient shelf life.

Fish is highly perishable product, and it may have different microflora depending on the environmental factors and chemical composition of fish (Tahsin *et al.*, 2017). The microbiological spoilage is the main process which affects fresh fish quality and later also quality of the produced fish products. Microbial spoilage affects the sensorial properties, which initially cannot be seen. Total plate count (TPC), *Enterobacteriaceae* and *Escherichia coli* are often used as the indicators to assess the quality according to the legislation on safety of sea foods handling hygiene. TPC is used to assess the general microbiological quality of fish and can be a quality indicator for raw fish microbiological quality according to the EC Regulations 2073/2005; 852/2004; 853/2004; 1441/2007. Several pathogenic bacteria may either be present in the environment or contaminate fish during handling. *Salmonella* and *Listeria monocytogenes* are reported to cause foodborne infections in humans by the ingestion of raw or inadequately processed contaminated fish or fish products (Eizenberga *et al.*, 2015). Contamination by microorganisms can cause different human infections, which can even be lethal. One of the fish spoilers in cold storage process is *Pseudomonas* spp., which is harmful due to the enzyme produced toxic metabolites. Production of various alcohols, aldehydes and ketones (ethanol, 2-methylbutanal, 2-heptanone, 3-hydroxy-2-butanone or other) has been reported as a result of metabolic activity by microorganisms, such as *Pseudomonas* spp. or *Enterobacteriaceae* (Parlapani *et al.*, 2014). Typically for smoked fish the *Pseudomonas* spp. are not detected (Olayemi, Raji, & Adedayo, 2012). *Vibrio parahaemolyticus* is a halophilic bacterium usually is present in the marine environment (Wawire *et al.*, 2019). To decrease or stop microbiological spoilage in fresh fish, it is necessary to control other physical parameters, which can ensure stable quality during processing of fish such as pH, amount of added salt, moisture, and storage conditions (temperature and time). According to Yanar, Çelik, & Akamca (2006) the brine concentration, brine composition and time have influence on the fish product textural properties and microbiota. There is a trend to reduce amount of preservatives used in the production. However, before reducing the amount of preservatives, the fish processing industry needs to estimate survival and growth of microorganisms affecting the changes in product quality and safety.

The antimicrobial effect of organic acids depends on the nature of the acid, i.e. a degree of dissociation, nature of food and microorganisms. The use of pre-treatment has not only effect on microbiological properties, but it also affects the physical parameters. Preservation of fish inhibits bacterial growth in fish if acid is added in a brine. As a result, it reduces pH, and increases salt amount in fish flesh, it ensures longer product safety (Ayuba, Alhassan, & Jimmy, 2013) and makes also textural changes of the product. Acid and salt diffuse in the fish flesh; decrease water holding capacity bonds and makes firmer texture and has influence on water activity. Low water activity together with low pH reduce microbiological

growth (Kuda *et al.*, 2013). Sprats are fatty fish, fat content even in spawning season can be more than 10% (Timberg *et al.*, 2011), and as fatty fish it absorbs less salt than lean fish (Ruiz-Alonso *et al.*, 2021).

One of the problems to ensure stable quality of the final product is unstable quality of the fresh fish (nutritional elements protein, lipids, water) during the season. The changes are observed due to the variability in fish chemical composition. In the feeding season (July till October) sprats contain more lipids (about 15%), but less moisture (about 66%) compared to the spawning season (March till June) where lipid content is approximately 5%, but moisture till 77% (Usydus, Szlifder-Richert, & Adamczyk, 2012). Some studies show that using pre-treatment it is possible to improve quality of products (Sengun *et al.*, 2021) and fish, brining inhibits microorganism growth (Babikova *et al.*, 2020). Typically used materials, which have no impact on organoleptic attributes and are available to producers, are acids, salts, and other additives.

The aim of the study was to analyse the effect of pre-treatment on smoked Baltic sprat quality and microbiological parameters.

Materials and Methods

Fish. In the experiments, fresh Baltic Sea sprats (*Sprattus sprattus balticus*) caught in March, 2021 were used. Fish were transported to producing company within 48 hours, the temperature inside the fish was kept below 2 ± 2 °C. Fish was of the freshness category A and size category for sprats 1 (Council of the European Union, 1997). Fresh sprats were stored till processing (pre-treatment) in a container covered with ice (proportion fish to ice was 1:3) at 2 ± 2 °C in a refrigerated room not more than 48 hours to ensure fresh fish quality.

Pre-treatment materials. As the pre-treatment materials were used, food grade acetic acid CH_3COOH (E260), acetic acid amount 80%, (Safrāns, Latvia), NaCl (Mozirsol, Belarus), granulated CaCl_2 (E509, CaCl_2 content (97%), $110.99 \text{ g mol}^{-1}$) (Lachner, Czech Republic). The additives CH_3COOH and CaCl_2 are food additives and can be used in *quantum satis*.

Fish sample preparation using pre-treatment. The brine solutions were prepared using different proportions of ingredients combined with cold water, no added ice (Table 1). The concentrations were selected according to the production experience and preliminary tests to find the best concentration of brine providing sufficiently firm fish texture. Six different pre-treatment methods were applied. Fish was soaked in proportion fish: brine (1:2) for 30 minutes periodically stirring, ensuring temperature not higher than 4 ± 2 °C, if necessary, chopped ice was added. Chopped ice can be used as an additional tool to ensure the required temperature. The sample without any pre-treatment was used as a control sample.

Table 1

Composition of the brine used for the pre-treatment of fresh sprats

Identification of samples	Ingredients per 1 kg of brine, g		
	NaCl	CaCl_2	CH_3COOH

Control	-	-	-
Na	29.13	-	-
3Na2Ca	28.64	16.71	-
2Na3Ca	16.70	28.64	-
Ace	-	-	9.90
NaAce	28.84	-	7.14
CaAce	-	28.84	7.14

After soaking in brine, fish was smoked in the hot smoking chamber (Rauch Spectrum, Germany) of the fish producing company using beech wood chips (with moisture below 15%). The main parameters for hot smoking (80 °C) – drying (35 minutes), cooking (10 minutes), steaming (2 minutes), adding smoke (10 minutes), and cooling (5 minutes). After smoking, fish was stored till at least 10 °C was reached and then placed in cutting machine to cut heads and tails (if necessary for packaging) and moved to the cooling chambers in plastic (HDPE) boxes for storage (2±2 °C). Each treatment was repeated in triplicate to make an average sample for analyses, the experiment was done in smoked fish producing company in Latvia. Samples were stored till analysing (next day after smoking, one week and two weeks) at 2±2 °C.

Determination of physical parameters. All smoked fish samples were analysed one day after smoking. The main control parameters were water activity (a_w) (method PN-ISO 21807:2005), pH (method PN-A-86782:1987), moisture content (method PN-A-86783:1962) and sodium chloride calculated from Na content ($\text{Na} \times 2.5$) according to the test

report, received from the external laboratory J. S. Hamilton (Poland). The samples were delivered to the laboratory in a cool box to ensure the same temperature as in the storage room (2±2 °C). Fresh smoked sprats were analysed for firmness using TA.HD.Plus texture analyser (Stable Microsystems, UK) equipped with Warner-Bratzler blade set with knife and colour in CIE L*a*b* system using Color Tec – PCM colour meter (Accuracy Microsensors Inc., USA) with software Color Tec – Color Soft QCW.

Determination of microbiological parameters. Microbiological parameters of sprats were analysed in the external laboratory J. S. Hamilton, one day after smoking and then after one and two-week storage to evaluate changes during storage. The samples were randomly selected from the plastic boxes stored in the cooling chamber (2±2 °C). The smoked sprats with different pre-treatments were analysed to check influence of pre-treatment method on the microbiological parameters indicated in Table 2.

Table 2

The methods used for the microbiological evaluation of smoked sprats

Microbiological parameters	Test method
Yeast	LVS ISO 21527-2:2008
Moulds	LVS ISO 21527-2:2008
Total plate count	LVS EN ISO 4833-1:2014
<i>Bacillus cereus</i>	LVS EN ISO 7932:2005
<i>Salmonella</i> spp.	LVS EN ISO 6579-1:2017
<i>Listeria monocytogenes</i>	LVS EN ISO 11290-2:2017
<i>Pseudomonas</i> spp.	PN-EN ISO 13720:2010
<i>Clostridium perfringens</i>	LVS EN ISO 7937:2005
β -glucuronidase positive <i>Escherichia coli</i>	LVS ISO 16649-2:2007
<i>Vibrio parahaemolyticus</i>	PN-EN ISO 21872-1:2017-10

Statistical analyses. Each sample was tested in triplicate, except colour and firmness, which were analysed in seven repetitions. The average values are reported. Averages and standard deviations were calculated using MS Office Excel 2016 (Microsoft, USA) software. Statistical analysis was performed using XLSTAT 2021 (Addinsoft Inc, USA). Comparisons were done by one-way ANOVA followed by t-test at the significance $p < 0.05$.

Results and Discussion

Pre-treatment effect on physical parameters of smoked sprats

Microbiological changes - spoilage, starts from the internal organs and gills, skin, and gut soon after fish have been caught. It is important to cool quickly to stop spoilage and to process fish in the fastest way to ensure the quality of finished products without any changes (Svanevik & Lunestad, 2011). Further the pre-treatment was applied with the aim to improve physical and microbiological parameters of the smoked sprats. In the sprat, smoking was used at least at 80 °C temperature; therefore, a lot of changes occurred in fish – changes of textural, flavour, and microbiological parameters. Because the product was cooked, proteins were denatured, and it decreased moisture content. Thus, it is possible to prolong the shelf life of smoked sprats and maintain the quality for longer time.

The pH of fresh fish is 7, which after smoking decreased till 6.6 in the sample without pre-treatment (Table 3). The

smoke led to a drop in pH, compared with the raw material values possibly due to the greater ionic strength of the internal solution in fish muscle cells associated with salt uptake (Rizo *et al.*, 2016). Pre-treatment reduced pH of smoked sprats depending on the brine composition. The lowest pH was 5.6 ± 0.2 in sprats treated with calcium chloride and acetic acid (CaAce) or 5.8 ± 0.2 when treated with brine containing the combination of sodium chloride and calcium chloride (2Na3Ca). The most similar pH to control sample was in fish treated with sodium chloride only (6.4 ± 0.2), which increased the amount of salt in fish. Typically during storage the pH increases, and it reflects the production of alkaline bacteria metabolites which starts to spoil the fish and starts increasing total volatile basic nitrogen (Abbas *et al.*, 2008). Rizo *et al.* (2016) compared smoked cod with fresh cod, the smoking reduced moisture, increased the NaCl concentration and lowered the a_w values probably due to NaCl uptake and dehydration. Analysing differences in water activity and moisture content among experimental samples after various pre-treatment methods no differences between average values was found, but significant differences were established for pH and sodium chloride content.

Brining reduced pH, water activity and increased salt content in flesh, the moisture decrease is affected by salt action and reduced water absorption availability. Acetic acid with or without salt can also inhibit microbiological growth and ensure longer shelf life of product (Kim & Lee, 2021). According to Abbas *et al.* (2009), water activity with moisture content has nonlinear relationship.

Table 3

Physical parameters of smoked sprats

ID of samples	pH	Water activity	Moisture, %	Sodium chloride, %
Control	6.6 ± 0.2 a	0.951 ± 0.028 a	67.5 ± 3.4 a	0.35 ± 0.07 c
Na	6.4 ± 0.2 ab	0.948 ± 0.028 a	66.7 ± 3.3 a	0.70 ± 0.13 ab
3Na2Ca	6.0 ± 0.2 bc	0.937 ± 0.028 a	62.5 ± 3.1 a	0.90 ± 0.17 a
2Na3Ca	5.8 ± 0.2 c	0.949 ± 0.028 a	61.5 ± 3.1 a	0.62 ± 0.12 ab
Ace	6.1 ± 0.2 bc	0.950 ± 0.028 a	65.7 ± 3.3 a	0.32 ± 0.06 c
NaAce	6.0 ± 0.2 bc	0.948 ± 0.028 a	62.9 ± 3.1 a	0.85 ± 0.16 ab
CaAce	5.6 ± 0.2 d	0.946 ± 0.028 a	61.7 ± 3.1 a	0.40 ± 0.08 bc

Average value ($n = 3$) $\pm$ SD. Means with the same letter in a column are not significantly different at $p < 0.05$ (t-test).

In our study, smoked fish had high moisture content – up to 67% for control sample (Table 3), but it may vary by smoking technologies, fish type and size. Salt and acids has the effect on proteins, it results in the destruction of proteins in brine while salt enters the muscle tissue (Martinez *et al.*, 2012). The highest salt content among the studied samples was 0.90 ± 0.17 in sample 3Na2Ca. The use of acids or salts decrease smoked fish moisture content, because the additives

diffuse in fish tissues, decrease juiciness, acetic acid degrades the connective tissue and its membranes, decreasing the water holding capacity with protein denaturation. The sample with the highest salt content 0.90% had the lowest water activity 0.937, while for the control sample with the lowest salt content, water activity was the highest (0.951 ± 0.028), which is in agreement with the study of Ruiz-Alonso *et al.* (2021), who reported that increasing the salt amount makes

lower water activity. Moisture decrease is similar using salt or acid because it starts enzymatic process and microbiological changes. Salt amount has the impact on bacteria growth. The addition of NaCl at a concentration of about 5.5-6.5% w/w, decreases the water activity (a_w) (Timberg, 2012).

Smoking of fish is one of the conventional procedures for reducing water activity (a_w) which expresses the moisture requirement of microorganisms. This is an effective tool in fish preservation as the nature and extent of fish spoilage depends on water activity, an important constituent of biological systems. Bjørnevik *et al.* (2018) analysed hot smoked fish and revealed that water activity must be less than 0.85 to make products stable at room temperature for a longer time. In fresh fish, water activity is close to 1, but for fresh sprats 0.97, and during processing it is typically decreased. The water activity could vary even if the moisture content does not change significantly it can be accelerated by up-take of moisture from the environment by the product, thus changing the water activity of the product during storage before equilibrium is reached (Wawire *et al.*, 2019). Water activity indicates possibility of microbiological activity. During storage it typically increases because these are good conditions (temperature, moisture, oxygen availability, time of storage, acidity and food nutrition's for future development) for microorganism growth. The highest water activity (0.951 ± 0.028) was observed in control sample without pre-treatment, but the lowest – using 3Na2Ca (0.937 ± 0.028); however, differences were not significant at $p < 0.05$. It shows that using pre-treatment it is possible to reduce a_w value, which is important for the microbiological activity reduction. Timberg (2012) found out when a_w decreases to 0.96, some Gram-negative bacteria are inhibited (e. g., *Pseudomonas* spp.) but some other may grow, while (Abbas *et al.*, 2009) reported, that water activity at least 0.91 is required for microorganism growth, and below 0.90 a lot of

bacteria cannot grow, only *Staphylococcus aureus* can grow till 0.85. Preservation was achieved by reducing the marinated product's pH value and water activity and increasing the salt content thereby inhibiting all microbial growth across all curing stages. Loss of moisture from the fish flesh caused by acetic acid decreased the total water content of sprat fillets. Babikova *et al.* (2020) also reported that water activity of marinated anchovies (*Engraulis anchoita*) was reduced from 0.99 to 0.94.

The smoked fish colour (Table 4) depends on smoking technique and also on pH, and salt content. There exists very strong linear correlation between pH and moisture ($r = 0.93$) increasing moisture the pH increases in linear proportion. Chan *et al.* (2020) finalised, higher pH results in darker colour (lower L^* value), but in the current research, there was no correlation between these parameters. Darker sprats were the ones, which were treated using 3Na2Ca and 2Na3Ca with pH 6 and 5.8, respectively, were increases pH, the same was observed by, no browning was observed when different salt at pH 4.5 was used. The control sample which had the highest pH 6.6 had an average L^* value but 3Na2Ca and 2Na3Ca had similar results for samples L^* value. Redness (a^*) had a weak negative correlation with water activity ($r = -0.55$), lower water activity increased redness of fish, for other colour parameters no correlations with physical parameters were established. In the production process (temperature above 70 °C) of smoking Maillard reaction also occurs, which affects smoked fish quality, changes organoleptic parameters, colour intensity and also protein functionality (heat stability and solubility of protein, textural properties). Metal ions have the ability to inhibit or promote production of Maillard reaction browning products. Ca^{+} ions and Na^{+} together with Cl slow down browning (Kwak & Lim, 2004). Higher salt concentrations slow down browning reactions (Yamaguchi *et al.*, 2009).

Table 4

Colour and texture of smoked fish after pre-treatment and smoking

ID of samples	Colour			Texture	
	L^*	a^*	b^*	Work of shear, $N\ mm^{-1}\ s$	Cutting strength, $N\ mm^{-1}$
Control	$57.28 \pm 6.7\ a$	$-5.14 \pm 6.2\ a$	$15.54 \pm 9.0\ c$	$1.27 \pm 0.28\ b$	$0.54 \pm 0.12\ c$
Na	$63.65 \pm 11.8\ a$	$-13.84 \pm 13.5\ b$	$32.42 \pm 12.8\ ab$	$0.94 \pm 0.21\ d$	$0.37 \pm 0.08\ d$
3Na2Ca	$45.58 \pm 8.9\ b$	$-1.04 \pm 12.1\ a$	$26.20 \pm 15.6\ abc$	$2.38 \pm 0.92\ abc$	$1.05 \pm 0.49\ abc$
2Na3Ca	$47.59 \pm 13.9\ b$	$-6.9 \pm 9.9\ ab$	$16.88 \pm 12.2\ bc$	$1.42 \pm 0.25\ bc$	$0.62 \pm 0.13\ ac$
Ace	$52.66 \pm 13.0\ ab$	$-6.71 \pm 5.9\ b$	$26.56 \pm 5.1\ ab$	$1.75 \pm 0.30\ abc$	$0.71 \pm 0.14\ abc$
NaAce	$62.89 \pm 10.2\ a$	$-8.32 \pm 10.4\ b$	$17.19 \pm 13.0\ abc$	$1.76 \pm 0.54\ abc$	$0.71 \pm 0.24\ abc$
CaAce	$61.19 \pm 10.5\ a$	$-7.19 \pm 16.2\ b$	$26.38 \pm 17.2\ abc$	$1.73 \pm 0.26\ abc$	$0.82 \pm 0.12\ abc$

Average value ($n = 7$) $\pm$ SD. Samples with the same letter have no significant difference in a column at $p < 0.05$ according to t-test.

Smoked sprats texture was affected by pH and moisture content. The decreased moisture content and lower pH resulted in a firmer texture. Work of shear differed between $1.27 \pm 0.28 \text{ N mm}^{-1} \text{ s}$ (control) and $1.76 \pm 0.54 \text{ N mm}^{-1} \text{ s}$, when brine contained acetic acid. The use of acids in the pre-treatment increases the ionic strength (Çağlak, 2015) and decreases pH values, which makes firmer texture. In the sample with the highest salt content Ruiz-Alonso *et al.* (2021) obtained similar results for work of shear fillets with brining at 7% and 10% salt were firmer than 5% treated fillet samples because of salt penetration in fish flesh. The combined use of salt and acetic acid increased work of shear more than any separate pre-treatment agent. Increased sodium chloride amount in sample 3Na2Ca made texture firmer ($2.38 \pm 0.92 \text{ N mm}^{-1} \text{ s}$) than the texture of control sample without pre-treatment ($1.27 \pm 0.25 \text{ N mm}^{-1} \text{ s}$). Salting makes internal protein changes which results in modification in fish texture and water holding capacity (Martinez *et al.*, 2012). Acids also made texture firmer for smoked pre-treated fish because of acid penetration in fish flesh. There exists a moderate correlation between water activity and texture that shows the increased water activity decreasing work of shear ($r = -0.75$) and cutting strength ($r = -0.78$), it may also have an influence on microbiological parameters.

Pre-treatment effect on microbiological parameters of smoked sprats

Fish contains a lot of bacteria, but during processing some of them are inactivated, but some only start their activity. Shelf life of fresh or smoked sprats typically is some days, but

using the pre-treatment it is possible to improve these microbiological parameters without significant sensory changes. Huong (2014) also researched that smoking inhibits the formation of toxins in products and reduce the growth of microorganism, due to lower water activity, or pre-treatment (salting, acid use) which creates a physical surface barrier for microbiological growth. The spoilage microflora of smoked fish can be impacted from smoking parameters or pre-treatment method, but also time and temperature of storage, packaging material type are important.

Total plate count for fresh fish is allowed to be not more than $1 \times 10^5 \text{ CFU g}^{-1}$, but in smoked or smoked brined fish it should not exceed $1 \times 10^4 \text{ CFU g}^{-1}$ ("Технический регламент евразийского экономического союза 'о безопасности рыбы и рыбной продукции' (ТР ЕАЭС 040/2016) (Technical Regulation No. 040/2016 On safety of fish and fish products," 2016), pathogens *Pseudomonas* spp. (for fish typically including *Pseudomonas aeruginosa*, *Pseudomonas anguilliseptica* "red spot disease") or moulds are not allowed in smoked fish, yeasts in smoked fish are allowed up to $1 \times 10^3 \text{ CFU g}^{-1}$, but need to follow Good Hygiene and Manufacturing practice program, because yeasts indicate the efficiency of cleaning and disinfection. Typically, yeasts are present in fresh fish, but during processing it can be additionally contaminated from processing environment and equipment (Bagge-Ravn *et al.*, 2003). On the 1st day after smoking the lowest TPC was determined in the sample pre-treated with combination of sodium chloride and acetic acid. Sengun *et al.* (2021) reported that the acid has an inhibiting role for microbial growth. The lowest result for TPC after smoking and in storage was obtained using any of pre-treatment methods even on the 2nd week of storage only in the control sample rapid growth till $3.3 \times 10^8 \text{ CFU g}^{-1}$ was observed (Table 5).

Microbiological parameters during storage of smoked sprats

Table 5

ID of samples	Total plate count, CFU g ⁻¹			<i>Pseudomonas</i> spp., CFU g ⁻¹		Yeasts, CFU g ⁻¹			Moulds, CFU g ⁻¹		
	1 st day	1 st week	2 nd week	1 st day	1 st week	1 st day	1 st week	2 nd week	1 st day	1 st week	2 nd week
Control	4.2×10^2	2.3×10^7	3.3×10^8	<10	7.0×10^8	<10	3.9×10^5	1.2×10^6	<10	<10	1.0×10^2
Na	5.6×10^2	3.5×10^5	9.5×10^6	<10	2.9×10^3	<10	1.1×10^3	4.2×10^5	<10	<10	<10
3Na2Ca	3×10^2	2.6×10^3	7.2×10^5	<10	1.0×10^3	<10	1.5×10^2	9.5×10^4	<10	<10	<10
2Na3Ca	4.5×10^2	9.6×10^3	3.4×10^4	<10	<10	<10	5.8×10^3	3.4×10^4	<10	<10	<10
Ace	7.0×10^1	5.2×10^2	1.2×10^5	<10	<10	<10	3.7×10^2	8.3×10^4	<10	<10	<10
NaAce	6.0×10^1	2.6×10^4	2.1×10^6	<10	<10	<10	2.5×10^6	1.7×10^6	<10	<10	<10
CaAce	8.0×10^1	6.4×10^2	4.4×10^5	<10	<10	<10	7.5×10^2	2.5×10^5	<10	<10	<10

Some moulds and yeasts are salt tolerant, but using pre-treatment (salts, acids) it is possible to decrease mould activity (Musa *et al.*, 2018). Without pre-treatment yeasts started growing after the 1st week 3.9×10^5 CFU g⁻¹ while in the samples Ace or Na lowest growth 10^2 CFU g⁻¹. Moulds need water activity around 0.85, but yeasts can grow at higher water activity (Tournas *et al.*, 2001) and experiment showed similar results, because yeasts started growing after the 1st week of storage and moulds after the 2nd week of storage in the control sample because it had higher pH. At the beginning, yeasts and moulds were below <10 CFU g⁻¹, but after one week the number of yeast colonies started to increase fast. Moulds stayed stable after the 1st week. In the sample 3Na2Ca, the yeast count was the lowest (1.5×10^2 CFU g⁻¹), but in the sample NaAce the highest 2.5×10^6 CFU g⁻¹ and in 2nd week the highest were also NaAce 1.7×10^6 CFU g⁻¹). Using pre-treatment, it is possible to inhibit mould growth, because only in control sample after two-week storage moulds (1.0×10^2 CFU g⁻¹) were detected. Moulds are not allowed in processed fish, moulds metabolite products are mycotoxins, which are carcinogenic (Ayuba, Alhassan, & Jimmy (2013)). Thus, using any of the selected pre-treatments, it is possible to reduce mould activity.

The microbiological parameters are the main factor that can have an impact on the smoked fish safety during its storage. Ayuba, Alhassan, & Jimmy (2013) investigated bacterial pathogens, which may be transferred from fish to human beings include: *Clostridium botulinum* type E (botulism), *Pseudomonas aeruginosa* (wound infections), and *Vibrio parahaemolyticus*. González-Rodríguez *et al.* (2002) also confirmed *Salmonella* spp. and *Listeria monocytogenes* are food poisoning pathogens. In the current study, neither in freshly smoked nor stored fish these bacteria were detected. If fish contain *Salmonella* spp. or *Listeria monocytogenes* or other pathogen spores, only the application of sterilization or high pressure can destroy them; in smoking process the spores preserve their activity (Evelyn & Silva, 2019). But if *Listeria monocytogenes*, *Salmonella* or *Clostridium botulinum* were in smoked sprats, there is a possibility to destroy them, because Mejlholm, Devitt, & Dalgaard (2012) reported *Listeria monocytogenes* and *Salmonella* were not able to grow at 7.7 °C in brined (together lactic acid 0.32%, acetic acid 0.28 % of organic acids in the water phase) and drained modified atmosphere packaged shrimp that resembled commercial products with either acetic and lactic acids or benzoic, citric acids. *Vibrio parahaemolyticus* were not detected in the smoked fish, but using brine (0.21% benzoic acid, 1.65% citric acid, 0.10% sorbic acid and salt 3.30% percentage of chemicals (w/v) (Mejlholm, Devitt, & Dalgaard (2012)) it is possible to inactivate also *V. parahaemolyticus* (at 2.2 ± 0.4 °C, pH 4.0 till pH 4.4). *V. parahaemolyticus* was reduced more than 3.0 log CFU g⁻¹ in mussels that were dipped in 2% (v/v) lactic acid brine for 15 min (Belleggia *et al.*, 2020). Using pre-treatment, it is possible to stop growth or inactivate *V. parahaemolyticus* pathogens. *Clostridium perfringens* was not detected in storage time of smoked sprats using pre-treatment or without treatment; the same was observed for *Escherichia coli* (hygiene conditions) and *Bacillus cereus*, which was lower than 10 CFU g⁻¹. Smoked sprats during storage did not contain any of these pathogens which means that no effect of the pre-treatment on pathogen

growth can be observed according to this research.

Pseudomonas one day after smoking were less than 10 CFU g⁻¹ (Abd El-Aziz, 2015) for all tested samples, but during storage the amount started to increase in the samples without pre-treatment; after the 1st week it started to increase in the control sample as it had the highest pH 6.6 and had the highest count (7.0×10^8 CFU g⁻¹) after the 1st week of storage. Duman *et al.* (2021) reported that *Pseudomonas* spp. stability and growth application vary with salt amount, temperature and pH. Using acetic acid in combination with salt (Ace, NaAce, CaAce) or only salt with CaCl₂ (2Na3Ca) it stays the same as one day after smoking <10 CFU g⁻¹. Wang *et al.* (2014) research on freshwater grass carp during storage for 12 days indicated the increased *Pseudomonas* spp. growth from 3% till 37%. Sterniša *et al.* (2020) found correlation with fish weight and *Pseudomonas* species (Duman *et al.*, 2021). Thus, the use of acetic acid or CaCl₂ in majority of cases allowed to slow down the growth of *Pseudomonas* spp., and the product is acceptable for use in further production after one week of storage.

Conclusions

The use of brine ensures migration of salt and acid evenly in fish tissues, and it results in lower moisture content and lower pH, which demonstrates the influence of pre-treatment on both physical and microbiological parameters. The pre-treatment can improve texture and colour of freshly smoked sprats. Textural parameters, such as cutting strength and work of shear are influenced by moisture content and pH. Colour is affected both by salt content and pH. The pre-treatment also ensures better microbiological parameters, which can ensure longer shelf life of smoked sprats, reducing the growth of TPC, moulds and yeasts. Pre-treated smoked sprats have higher salt content compared to fish without pre-treatment, and it ensures safer use of sprats in further processing, when different products from smoked fish (canned, pasteurized) are made. The pre-treatment in a brine, containing combination of sodium chloride and calcium chloride, improved textural parameters and ensured improvement of microbiological parameters of the smoked Baltic sprats.

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Acknowledgment

This work was supported by the programme “Strengthening research capacity in the Latvia University of Life Sciences and Technologies” project Z43 and fish producing company SIA “NS ESTATE”.