An Efficient and Tasty Use of Atlantic Salmon Trimming: Microbiological and Chemical-Physical Evaluation of Salmon Frankfurters

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Abstract

The demand for easy-to serve fish based products, like frankfurters, is increasing, thanks to their convenience and quick preparation. In this study, cooked and cold-smoked salmon based frankfurters were evaluated for chemical-physical and microbiological characteristics during 90-days storage at 4°C. Every 15 days of storage, samples were submitted to complete microbiological analyses and to the determination of pH, Water Holding Capacity (WHC), colour parameters and texture (by Warner-Bratzler shear force test and folding test). The evaluation of lipid oxidation (by TBARS determination) was also performed. The results of microbiological analyses showed the absence of pathogens like *Listeria monocytogenes* and *Salmonella* spp., and the presence of very low total bacterial loads, with a gradual increase during the storage, exceeding 7 Log CFU/g after the 75th day. The identification of bacterial population by rRNA sequencing revealed the presence of a typical microflora, mainly composed by *Carnobacterium maltaromaticum* and *Brochothryx thermosphacta*. The physical-chemical properties, such as pH, WHC, colour, texture and lipid oxidation parameters were constant for all the period considered, at testing this product as a very stable easy-to prepare novel food.

Keywords: Frankfurters, Atlantic salmon, shelf-life, food microbiology.

Introduction

In the last years, with the increasing health concerns, the consumption of red meat is diminishing and seafood are becoming more popular: in fact, fish can be an optimal protein source substitute of pork, poultry and red meats. In particular, fish meat is also a good source of vitamins, minerals, carbohydrates and water-soluble components. Moreover, fatty fishes are an important source of omega-3 polyunsaturated fatty acids that are believed to reduce the risk of cardiovascular diseases (Wang *et al.* 2006). Fish consumption in Italy was approximately 19.8 kg *per capita* in 2012, 15.4% composed by fresh salmon (*Salmo salar*) (89.6%) and cold smoked salmon (10.4%), almost all farmed salmon (ISMEA, 2014). The introduction and development of novel fish food with the aim to reach young and health-conscious consumers, is now increasing: conventional fish products are evolving in new style packaging and formulation, matching also the changing lifestyle habits. The demand for ready-to-eat or easy-to serve products like frankfurters, also fish based, is increasing due to their convenience and quick preparation time but maintaining a high nutritional value. A large number of products both for export and internal markets based on lobsters, shrimp, squid, cuttlefish, bivalves and certain species of fishes, including salmon, have been evaluated and fish sausages are nowadays widely used all over the world. Several authors investigated the chemical and microbiological characteristics of several new fish based frankfurters or sausages composed of mullet (*Mugilcep halus*), North Pacific hake (*Merluccius productus*), channel catfish (*Ictalurus punctatus*), Indian sardine (*Sardinella longiceps*), Atlantic salmon (*Salmon salar*) and saithe (*Pollachiusvirens*), Cape hake (*Merlucciuscapensis*), rainbow trout (*Onchorynchus mykiss*), Nile tilapia (*Oreochromisniloticus*), common carp (*Cyprinuscarpio*), pink salmon (*Onchorhynchus gorbuscha*) and many others (Daley *et al.*, 1978; Medeiros *et al.*, 1986; Ravishankhar *et al.*, 1992; Nordvi *et al.*, 2007; Sini *et al.*, 2008; Dincer and Cakli, 2010; Klanikova *et al.*, 2013; Oliveira Filho *et al.*, 2012; Oliveira *et al.*, 2013). Recently, the inclusion in frankfurters and sausages, also fish based of many functional ingredients like chitosan (even in association with sodium lactate, carnitine and sulphite), fish oil and dietary fibre was also reported

(Roller *et al.*, 2002; Kook *et al.*, 2003; Lopez-Caballero *et al.*, 2005; Cardoso *et al.*, 2008; Josquin *et al.*, 2012). Considering hygienic aspects, it should be underlined that fish frankfurters are usually cooked and smoked during production. Spoilage and pathogenic microorganisms could stem from the raw fish, other ingredients, the environment, the equipment or through handlers during processing, affecting the final quality of the products. Moreover, the possible presence of thermoduric bacteria, organisms able to survive the industrial heat stresses even if not able to grow at high temperatures (Hull *et al.*, 1992), could determine health hazards and quality defects leading to the consequent reduction of the assigned shelf-life. These microorganisms are largely destroyed by pasteurization, but some cells can survive, resulting later in microbial spoilage. These microorganisms have their origin in the environment and are widely distributed: the main genera recognized as thermoduric are *Microbacterium*, *Micrococcus*, *Enterococcus*, some Lactobacilli and coryne form bacteria. Moreover, *Bacillus* and *Clostridium* species can be considered strictly thermoduric mesophiles, as they are spore formers; some of them are also able to replicate at 55°C. In particular, the spores of thermoduric bacilli may even survive to ultra-heat treatment (UHT) (Silva and Gibbs, 2010), preserving the stability of their proteins and enzymes and potentially producing off-flavours (Burgess *et al.*, 2010).

Objective of the current study was to determine the chemical-physical and microbiological characteristics of salmon based frankfurters during 90-days storage at 4°C, evaluating the possible presence of bacteria able to survive to the production process.

Materials and Methods

Fish Source, Ingredients and Chemical Composition

Samples of Atlantic salmon (*Salmo salar*) based frankfurters were obtained from a medium sized industry of Northern Italy. Briefly, the fresh salmon, on the second day from arrival was beheaded, filleted, trimmed, deboned and destined to other salmon processing. The meat trimmings and the meat left after deboning were promptly recovered, chopped and sieved in order to remove the bones. The meat mix was prepared adding other ingredients; ice, dietary fibre, salt, dextrose, antioxidant E301, natural flavours; afterwards the meat mix was stuffed into 17 mm water soaked collagen casings and cooked in order to achieve a core temperature of 75°C for 40 minutes. The products later were cold-smoked and peeled before vacuum packaging in 100-g packs of 4 frankfurters; the packs were finally pasteurized at 75°C for 5 minutes. The "use by" date was settled at 90 days at a temperature of 4°C.

After the production, the samples underwent to the following determinations: proximate composition (A.O.A.C 1990), water activity (RotronicHygromer Aw-DIO, Basserdorf, CH), and salt content as total chlorides (Pearson, 1973). Water Phase Salt content (WPS) was then calculated with the formula WPS = %salt (%salt + %moisture)$^{-1}$* 100 (Huss *et al.*, 1995). All the determinations were performed in sextuple.

Experimental Design

The salmon based frankfurter samples were stored at 4°C for the shelf-life evaluation. At settled sampling times (0, 15, 30, 45, 60, 75 and 90 days from the production) the samples were submitted to microbiological, chemical and physical analyses.

Microbiological Analyses and pH Determination

For microbial counts, 10 g of each sample were homogenized in 90 mL of a diluent solution (0.85% NaCl and 0.1% tryptone), and serial 10-fold dilutions were prepared. Total mesophilic count (TMC) was determined according to the ISO 4833:2003 method. Lactobacilli were enumerated according to ISO 15214:1998 method. The number of *Enterobacteriaceae* was determined by the ISO 21528-2:2004 method. *Escherichia coli* were enumerated according to ISO 16649-2:2001 method. Coagulase-positive Staphylococci were determined by ISO 6888-1:1999 method. The count of spores of sulphite-reducing *Clostridia*, was performed by ISO 15213:2003 method, after pasteurization of the dilutions. Detection and enumeration of *Listeria monocytogenes* were performed according to AFNOR methods (AFNOR BRD 07 ∕ 4–09 ∕ 98 and AFNOR BRD 07 ∕ 05–09 ∕ 01, respectively). Microbiological analyses were performed in triplicate. From the plates of the TVC at T90, a number of 5 colonies were selected on the base of the different morphology picked, and submitted to biochemical tests: isolates were tested for Gram reactions by KOH method and for standard cytochrome oxidase (Oxoid) and catalase reactions. In order to evaluate the thermal resistance, the isolates were streaked onto Plate Count agar (PCA; Oxoid, Basingstoke, UK), subsequently incubated at 55°C for 24 h. Isolates were then identified by rRNA sequencing (Eurofins, Germany) using the standard forward primersCC-CD as described by Rudi *et al.* (1997).

Colour Parameters

Colour parameters were determined on 3-mm horizontal sections of frankfurters using a Minolta Chromameter CR-200 (Minolta, Osaka, Japan) working at CIEL ab system. The L*, a* and b* values, which describe the intensity of whiteness/brightness, red colour and yellowness respectively, were taken at six locations on the cut

surface immediately after opening the pack. Chroma was calculated as $\sqrt{(a^2+b^2)}$, the hue angle (h) was calculated as h = arctan (b*/a*), where h = 0 for red hue and h = 90 for yellowish hue. Total colour differences (ΔE*) between treated and control samples were calculated as: $\sqrt{(L1^*-L2^*)^2 + (a1^*-a2^*)^2 + (b1^*-b2^*)^2}$. A ΔE* more than 2.3 means a variation hardly perceptible to the human eye, while ΔE* more than 3.0 a variation well perceptible to the human eye.

Texture Analysis

The shear force was determined using a 5542 Instron Instrument, equipped with a Warner Bratzler blade. The texture of frankfurter slices was characterized for hardness by measuring the maximum force for a complete cut. Measurements were performed at 4°C on sections of raw and cooked frankfurter (boiled for 2 minutes). Six measurements for each sample were performed and mean values with standard deviation were recorded.

The folding test was performed according with the procedures described by Lanier (1992). Samples were cut into 3mm thick sections. The slides were held between the thumb and the forefinger folded to observe the way they broke. The scale used was as follows: 1: breaks by finger pressure; 2: cracks immediately when folded in half; 3: cracks gradually when folded in a half; 4: no cracks showing after folding in a half; 5: no cracks showing after folding twice.

Lipid Oxidation

Thiobarbituric acidreactive substances (TBARS) were determined in triplicate to evaluate the oxidation stability during storage in duplicate according to Ke *et al*. (1984).

pH Value

At the same sampling times, pH was measured by a pH meter (Amel Instruments, Milan, I): three independent measurements were performed on each sample.

Statistical Analysis

Data from physical-chemical analyses performed during the different sampling times were submitted to one-way ANOVA using PRISM graph pad 6. The threshold for statistically significant differences was settled at $P < 0.05$.

Results and Discussion

The results obtained are reported in the following sections.

Chemical Composition

Moisture content of Atlantic salmon based frankfurters was 52.0±0.5%; this value was lower than what found by Oliveira *et al*. (2013) in hot smoked pink salmon sausage supplemented with fish oil, coconut oil and wholegrain oats (63%) and was not within the range of 60-74% observed in many fish sausages (Rahaman *et al*., 2007, Oksuz *et al*., 2008, Sini *et al*., 2008, Al-Bulushi*et al*. 2013). Protein content was 13.9±0.2%, lowerthanin sausages made by pink salmon (22.4%) (Oliveira *et al*., 2013), crimson snapper (19.7%) (Al-Bulushi *et al*., 2013), rohu (18%) (Sini *et al*. 2008), African Catfish and Nile puffer (18.1 and 19.6% respectively) (Ahmed and Elhaj 2011) and in fermented saithe and salmon sausages (18.0-21.0%) (Nordvi *et al*., 2007). A high lipid content (29.6±1.0%) was detected in the product: although our samples did not contain animal fat sources as described in previous studies (Dincer and Cakli, 2010), the values observed were strongly higher than what found in fish-sausages without land-based animal fat additions (between 5 and 7%) (Nordvi *et al*., 2007; Sini *et al*., 2008; Olivera Filho *et al*., 2012; Al-Bulushi *et al*., 2013), probably due to the fatty level of the raw material used for the production of the frankfurters (trimmings comprehensive of the belly flaps). Ash content was attested at 2.6%, according with Oliveira *et al*. (2013) who found a content of 2.7% in hot smoked pink salmon sausages. Salt content was1.9±0.0%, slightly higher than the 1.5% suggested by Ruusunen *et al*. (2003) for the production of low salt -phosphate-free frankfurters. Apart from the tendency to limit the salt content, reducing the development of hypertension, saltcon tributes to enhance the perception of the typical flavour of cooked sausages (Desmond, 2006). In any case the salt content alone is not sufficient to inhibit the bacterial replication in these products.

Microbiological Analyses

The microbiological evaluation of salmon based frankfurter showed (Figure 1), till 30 days after the production loads of total viable count (TVC) below the detection limit of 2 Log CFU/g: from that moment on a gradual increase of TVC was observed reaching after 90 days of storage the concentration of 7.1 Log CFU/g. Considering that the product was maintained in the optimal storage conditions, the threshold limit of 6 Log CFU/g, often used in food industries to indicate the end of shelf-life of a fish product (Olafsdottir *et al*., 2005), was got over only at the last sampling time (90 days). In some other studies, different limits are indicated, mainly linked to organoleptic characteristics: a total viable count above 7-8 Log CFU/g is often associated to sensory rejection. Moreover, von Holy *et al*. (1991) and Dykes *et al*. (1996), suggested a threshold value of
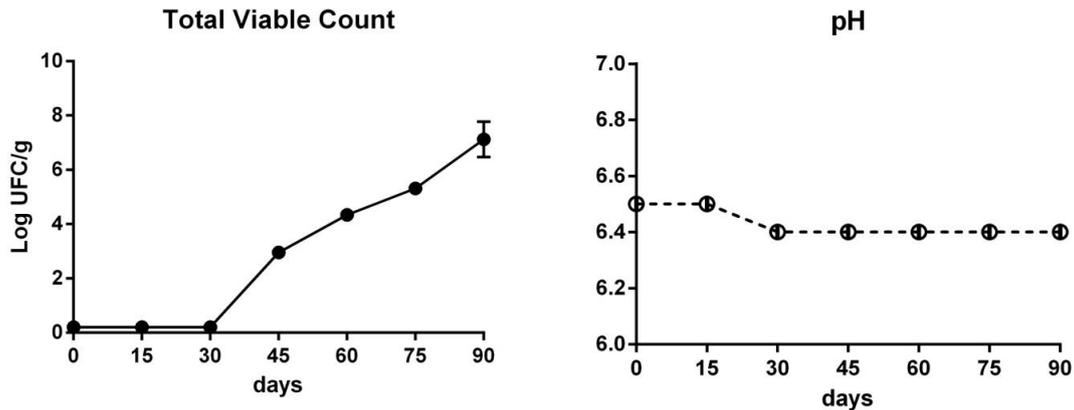
Figure 1. Total Viable Count and pH in Salmon-based frankfurters during the period considered.

6.7 Log CFU/g, based on TVC and LAB counts, to indicate imminent expiry of microbiological shelf life of vacuum-packaged Vienna sausages. In any case, these threshold limits are strictly linked to several factors related to the product characteristics, the industrial plant environment and to the different analytical methods (Espe *et al*., 2004).Based on the microbiological results, the shelf-life of the studied products should be limited to 75 days under refrigerated conditions.

The other parameters evaluated (Lactic Acid Bacteria, *Enterobacteriaceae*, *Escherichia coli*, Coagulase-positive Staphylococci and sulphite-reducing Clostridia) were, for all the period considered, under the limit of detection (2 Log CFU/g).

Analytical data on similar products are not available in literature; studies conducted on fish frankfurters or other fish sausages gave variable results principally due to the different processing. Nordvi *et al*. (2007)found in uncooked fermented and dried salmon and saithe sausages, a TVC around 4.6 Log CFU/g 4 days after the production that substantially did not show any decrease in the final dried product (4.2 Log CFU/g CFU/g) after 30 days. In the same study a load of LAB around 8 Log CFU/g was also detected: this could be explained by the inoculation of *L. sakei*as starter culture and consequently extend the shelf-life. Oliveira *et al*. (2013) found loads of TVC (both mesophilic as psychrotrophic) around 4.1 Log CFU/g in fish sausages from pink salmon cooked (60°C for 60 minutes), while the bacterial count in the hot smoked (core temperature of 71°C) product was less than 2 Log CFU/g, in close agreement with the results obtained by Oliveira Filho *et al*. (2012) who found the same result in aerobic psychrotrophic bacteria in Nile Tilapia based sausages. Oksuz *et al*. (2008) found in uncooked African catfish sausages (prepared with 84.6% of minced catfish meat and 15.5% of other ingredients like garlic, salt, red and black peppers, cinnamon, spices, clove, cumin, ginger, coconut spice, olive oil and salicylic acid) a starting TVC around 2.5 Log CFU/g; this load decreased during the storage of 70 days at 4°C till 1.7 Log CFU/g, due probably to the decrease in moisture content (from 72.0-74.4% at T0 till 45.0% after 70 days at 4°C). As well, *Enterobacteriaceae* and *Staphylococcus aureus,* reduced their loads respectively from 2.5 Log CFU/g to 0.2 Log CFU/g and from 1 Log CFU/g to an undetectable level during the storage period.

Moreover, the presence of *Listeria monocytogenes,* was researched in our samples, as it represents one of the most widespread pathogens in light-preserved fish products and also it has been already recognized as responsible for outbreak in post-processed contaminated frankfurters. In particular, a multistate outbreak of listeriosis took place in the USA, affecting at least 50 people in 11 States (CDC 1999) with six deaths after the consumption of prepacked hot-dogs (opened and previously none opened). In the studied salmon frankfurters this pathogenwas always absent in 25 g in all the samples analysed, confirming the safety of these products.

Randomly, from the bacteria grown onto the PCA plates, a number of 5 colonies were isolated andwere submitted to identification. These isolates resulted to be Gram positive, catalase and oxidase negative and able to survive at 55°C; four colonies were identified by DNA sequencing as *Carnobacterium maltaromaticum* and one as *Brochothrix thermosphacta*: these microorganisms were already recognized as major spoilage microbiota especially in frozen and thawed products under vacuum or modified atmosphere packaging (Laursen *et al*. 2005). Even Rudi *et al*. (1997) reported *Carnobacterium maltaromaticum, Carnobacterium divergens* and *Brochothrix thermosphacta* as the dominant flora in salmon fillets packed in an atmosphere consisting of 60% CO_2 and 40% N_2. None of the isolates belongs to the bacterial groups known as strictly thermoduric. The thermal treatment performed during the production resulted to be not enough to obtain a complete microbial inactivation even if the residual microflora is not of major concern.

Physical-Chemical Analyses

Colour Analysis

In Table 1 are presented the results obtained from colour analysis. The L*, a* and b* values during the storage period did not show a clear trend. A slight decrease in L values was observed after 75 days (P<0.05), associated to a gradual small increase of the red index (P<0.01). Moreover, in this study, the hue value, a form of data reduction involving both a* and b*, did not point out any significant difference in the whole period. Chroma, also termed saturation index, used as an indicator of the loss of colour saturation, was quite stable and did not show important differences between the times considered. ΔE (calculated between two consecutive times or between T0 and the other times) was always below the value of 2.3, considered as a threshold limit above which the consumer can perceive a change in the colour product. The highest ΔE was detected between T60 and T75, suggesting a more pronounced modification in this storage period, even if not manifest.

Texture Analysis

The results obtained from shear force test (Table 2) showed the absence of a clear trend during the period considered: the values measured ranged, both in cooked and in raw samples, from 6.91 to 11.50 Newton) at the settled sampling times. Generally, our values were comparable with those obtained by Dincer and Cakli (2010) who found values in frozen

rainbow trout frankfurters between 5.16 and 12.38 N, with a clear trend of decrease during the storage considered. In the same study, higher values (16.59-29.89 N) were detected in the product obtained from fresh product. Shear force is an important property for the consumer, because determine the texture acceptability of a product (7). According with Dingstad *et al*. (2005), the cut-off point of 47.3 N and above results in consumer acceptability less than 60% as expected in all the stages of the study our samples resulted to be desirable.

The folding test, a simple and fast method, is often used to measure the quality of gel springiness in frankfurters. As shown in Table 2, the folding test scores of salmon frankfurters were stably 5 at all the times considered, indicating a good elasticity of the product.

Lipid Oxidation

According to Ke and Linke (1982) and Ke *et al*. (1984), in all the sampling times considered, TBARs values of salmon based frankfurters remained below the threshold limit of 8μmol/kg that indicates the absence of rancidity developed in the products. These data suggested that there were not evident oxidative problems in the samples analysed, due also to the vacuum packaging (Table 2).

pH

Slightly acid pH values were detected in all the samples (values ranging from 6.38 and 6.47), without

Table 1. Values of L*, a*, b*, Hue angle, Chroma and ΔE values measured during the sampling time

Time	L*	a*	b*	Hue Angle	Chroma	ΔE between 2 consecutive times	ΔE between T0 and the other times
T0	80.60±0.88	5.36±0.12	20.76±0.25	75.51	21.44	-	-
T15	80.55±0.41	5.40±0.12	20.50±0.12	75.25	21.20	0.26	0.26
T30	81.16±0.17	5.54±0.12	20.36±0.08	74.77	21.10	0.64	0.71
T45	81.00±0.29	5.39±0.10	20.19±0.09	75.06	20.89	0.28	0.70
T60	80.44±0.35	5.74±0.17	20.62±0.25	74.44	21.41	0.79	0.43
T75	78.65±0.49	6.18±0.09	21.42±0.11	73.89	22.29	2.01	2.22
T90	79.63±0.21	6.50±0.05	21.22±0.17	72.97	22.19	1.05	1.56

Values of L*, a* and b* are expressed as mean ± standard deviation.

Table 2. Shear force of cooked and uncooked products, textural properties and production of TBARs (thiobarbituric acids) in salmon based frankfurters during the storage period

Time	Shear force uncooked (N)	Shear force cooked (N)	TBARs μmol/Kg	Folding test
T0	10.55±0.53	12.96±0.68	4.35	5
T15	6.91±0.38	8.04±0.30	5.36	5
T30	11.38±0.55	11.18±0.60	6.34	5
T45	9.75±0.98	11.50±0.31	4.61	5
T60	11.70±0.55	11.89±0.77	6.05	5
T75	11.58±0.57	10.49±0.73	5.19	5
T90	9.69±0.91	11.25±0.46	6.53	5

modifications for all the period considered.

Conclusions

Salmon based frankfurters were proved to be microbiologically safe: the production process allowed obtaining the absence of pathogens and only low concentrations of very typical microbiota were found till the 75th day; afterwards the loads overcame the limit of 6 Log CFU/g. The physical-chemical properties, like colour and texture parameters results, were constant for all the period considered and no evident lipid oxidation was observed in the sampling sessions, attesting this product as a very stable easy-to prepare novel food. This suggests that the shelf-life of the product is strictly limited by the microbial growth.

Acknowledgements

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