Physicochemical characterisation of restructured Fenalår and safety implications of salt and nitrite reduction

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Abstract. – There is a new trend to produce dry-cured ham from lamb in shorter times by boning the ham before salting to later obtain restructured hams that are easier to dry and slice. However, little information about the physicochemical characteristics of Norwegian Fenalårs during the process or the safety implications of their elaboration procedures is reported in the literature. The aim of this study was to characterize the colour, texture and physicochemical properties of restructured Fenalårs when using Standard Salting (SS), Salt Reduced (SR) and a Non-Nitrite Salt Reduced (NNSR) treatments. Microbiological safety implications of the elaboration process when using the different salting treatments were also assessed using predictive microbiology. To do so, sixty Fenalårs were elaborated using a Standard Salting (SS), a Salt reduced (SR) and a Non-Nitrite Salt Reduced (NNSR) treatments. Physicochemical characterization (instrumental colour and texture and Zinc Protoporphyrin content) was performed at the end of the process using thirty Fenalårs. The rest of the Fenalårs were used to characterize the product through the elaboration process (pH and a_w) for the evaluation of microbiological hazards when using the different salting treatments using predictive microbiology. Results showed a significant increase in softness when reducing salt content and a decrease of redness when no nitrite was used, attributed to the formation of ZnPP content instead of nitrosylmyoglobin. In terms of risk assessment, the decrease of a_w through the elaboration process reduced the growth capacity of all the microorganisms evaluated. However, microbiological safety implications in salt reduced Fenalårs are important, especially when no nitrite was added, because the considerable increase of growth potential of *L. monocytogenes*. The increase of growth potential of proteolytic *C. botulinum* is very little and no relevant effect of nitrite on growth potential of *S. aureus* was observed.
Predictive microbiology and optimization of the process to enhance ZnPP formation can help to ensure safety and quality of salt reduced restructured Fenalårs without additives.

Key words – Dry-cured ham, lamb, colour, texture, ZnPP, nitrite elimination, salt reduction, predictive microbiology.

Highlights

– There is a new trend to reduce salt content and additives in Fenalår.
– Salt content reduction increases softness and microbial hazards.
– No addition of nitrite decreases redness and increases microbial hazards.
– Process optimization can help to improve non-nitrite salt reduced Fenalår quality.
– Predictive microbiology can help to ensure safety of elaboration process.

1. Introduction

Fenalår is a traditional Norwegian dry-cured product prepared from lamb or mutton leg. Fenalår fra Norge ("Fenalår from Norway") became a legal Protected Geographical Indication (PGI) in Norway in October 2012 (Håseth, Thorkelsson, Puolanne & Sidhu, 2014), and a PGI in Europe in 2017 (Regulation (EU) No. 1752/2017). According to the traditional elaboration process, the leg is pile salted with the bone inside. However, there is a new trend to remove the bone before salting to later obtain a restructured Fenalår from different meat pieces which can be elaborated in shorter times and easily sliced and sold as a ready-to-eat product. This restructured Fenalår could be as appreciated by consumers as PGI Fenalår. Although long-dry aged products are well appreciated by consumers (Villalobos-Delgado et al., 2014), other authors found that some consumers preferred dry-cured ham from sheep with short maturation times and cheaper prices (De Andrade *et al*., 2017).

Restructured elaboration procedures have been previously used to elaborate dry-cured products (Fulladosa, Serra, Gou & Arnau, 2009; Romero de Ávila, Hoz, Ordóñez & Cambero, 2014). Production of restructured Fenalår is also of interest to the Norwegian meat industry, but the safety implications and the quality of the final product need to be evaluated. Besides, the tendency to reduce salt content is also becoming more important for Fenalår, which has an above average salt content for dry-cured products, in order to comply with consumers demands and European nutritional recommendations (European Commission, 2020). However, salt content reduction in dry-cured meat products is not straightforward since it can lead to an increase in microbiological safety issues (Inguglia, Zhang, Tiwari, Kerry & Burgess, 2017; Taormina, 2010) and cause quality defects in the final product (Costa-Corredor, Serra, Arnau & Gou, 2009). Reduction or elimination of curing additives to comply with clean label requirements is also emerging. However, nitrite is not only used to achieve the typical cured colour in the final product (Cassens, Greaser, Ito & Lee, 1979; Honikel, 2008), but also for food safety purposes, i.e. to inhibit the growth of *Clostridium botulinum* (Honikel, 2008). Although dry-cured ham production without curing agents is feasible (Iacumin *et al*., 2019; Parolari, Aguzzoni & Toscani, 2016), it must be done with some caution (Buchanan & Phillips, 1990) especially when using restructured hams and salt reduced treatments in which microbiological contamination is more prone to occur (Fulladosa, Sala, Gou, Garriga & Arnau, 2012).

The microbiological safety implications of lowering the amount of salt and nitrite in restructured Fenalår have not been studied before. *Listeria monocytogenes* and *Salmonella* spp. are often involved in alerts of the Rapid Alert System for Food and Feed (RASFF) concerning dry-cured meat products. The current legislation establishes microbiological criteria for both hazards in ready-to-eat meats (European Commission, 2005). In addition, *E. coli* and *Salmonella* have been described as causative agents of outbreaks associated with the consumption of salted dry-cured meat products (Holck, Axelsson, McLeod, Rode & Heir, 2017; Omer *et al*., 2018). *Staphylococcus aureus* is also a pathogen of concern due to its ubiquitous and versatile character and its role in outbreaks linked to the consumption of dry-cured hams (Rajkovic, 2012; Portocarrero, Newman & Mikel, 2002). Besides, nitrite removal from meat product formulations could increase the probability of the growth of *C. botulinum* in these products (Taormina, 2010).

Predictive microbiology models have been applied by food safety authorities in order to evaluate the microbiological safety consequences of changes in food processing and preservation (Messens *et al.*, 2018), including salt reduction in Spanish cured meat products (AESAN, 2011). Predictive models are mathematical tools to estimate microbial behaviour in foods, i.e. growth, transfer, survival or inactivation, as affected by a series of intrinsic and environmental factors, such as pH, a_W, nitrite and temperature, without the requirement of time-consuming challenge-tests (Perez-Rodriguez & Valero, 2013). Robust predictive models accounting for the most relevant factors governing microbial behaviour in dry-cured products have been described and can be applied to evaluate safety implications (Zurera-Cosano *et al*., 2011).

The effects of salt reduction or the microbiological safety implications of nitrite elimination have been reported in pork cured meat products (Higuero, Moreno, Lavado, Vidal-Aragón & Cava, 2020), though no systematic studies for different salt levels in Fenalår can be found in the literature. Similarly, several types of pork based dry-cured meat products, such as PDO Parma ham (Laureati *et al*., 2014), Bayonne ham (Monin *et al*., 1997) or TEG Serrano ham (Guàrdia, Aguiar, Claret, Arnau & Guerrero, 2010) have been extensively characterized. Although some studies dealing with dry-cured products from sheep (De Andrade *et al*., 2017; Teixeira, Fernandes, Pereira, Manuel & Rodrigues, 2017) and goat (Ivanovic, Nesic, Pisinov & Pavlovic, 2016) from different countries have been found in the literature, little information regarding the textural and physicochemical characteristics of bone-in or restructured Norwegian Fenalår is reported.

Thus, the aim of our study was to characterize the colour, texture and physicochemical properties of restructured Fenalårs when using Standard Salting (SS), Salt Reduced (SR) and Non-Nitrite Salt Reduced (NNSR) treatments. Microbiological safety implications of the elaboration procedure when using the different salting treatments were also assessed using predictive microbiology.

2. Material and methods

2.1 Raw material selection and elaboration process of restructured Fenalår

Sixty Norwegian White lambs fed with coastal grazing were obtained. The animals were 4 months old and from the same production system. All the animals were slaughtered in Førde commercial slaughterhouse over the period of 1 day. After carcass dissection, legs were vacuum packed and stored at -20 ºC for 7 days. Frozen legs were thawed at 15 ºC (room temperature) for 24h. The ultimate pH in *Semimembranosus* muscle was 5.63±0.04 (pH meter, Mettler Toledo AG 8603 Schwerzenbach, Switzerland). The legs (n=120) were boned, and the connective tissue and subcutaneous fat removed. For restructured Fenalår production, 2 legs were netted (double rubber 110 net, ScotNet, Scotland) and manually rubbed in fine salt. The netted green hams were vacuum packed in shrink bags (polyamide/EVO/polyethylene; oxygen permeability of 12cc/m^2/24h at 23 ºC, 0% RH and 1 atm, and a water permeability of 8 g/m^2/24h at 38 ºC, 90% RH and 1 atm; Bemis® Company Inc. USA) together with the corresponding amount of salt inside the plastic bags according to the salting treatments described below.

Three different salting treatments were used: Standard Salting (SS) using 4.8g of salt/100g raw meat and 144 ppm nitrite (n=24); Salt Reduced (SR) using 3.9g of salt/100g raw meat and 144 ppm nitrite (8% reduction) (n=18); and Non-Nitrite Salt Reduced (NNSR) using only 3.9g of salt/100g raw meat (n=18). The salting step occurred in the cold room at 2-4 ºC for 42 days. After the salting period, Fenalårs were treated with a 4% potassium-sorbate solution for 1.5h at 13 ºC to avoid mould growth and dried at 18 ºC with an RH of 60% for 48h. Later, Fenalårs were dried at 13 ºC and an RH of 74 % for an additional 10 days. Then, Fenalårs were smoked with friction-smoke from beech wood for 6 hours before being returned to the chamber at 13 ºC for 13 days. Seven days later, the Fenalårs were pressed for 48 hours. Once this was finished, Fenalårs were hung up again at 13 ºC with a relative humidity of 74% until achieving a weight loss of 36% (16 days). A schematic representation of the restructured Fenalår elaboration process is shown in Figure 1. During the process, weight loss and temperature were determined twice per week.

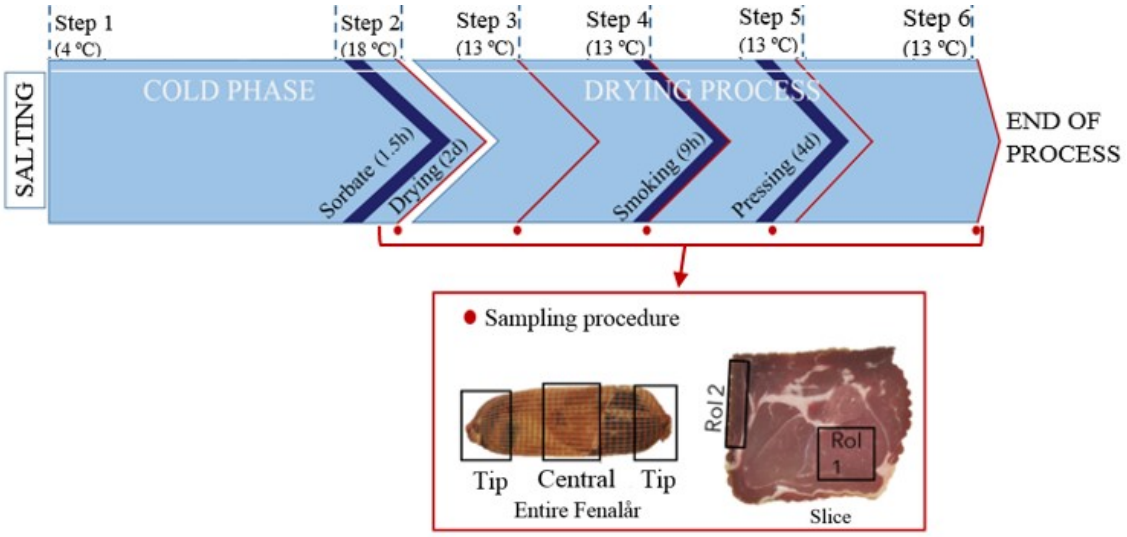

Figure 1. Schematic representation of restructured Fenalår elaboration process and sampling procedure performed at each step: Step 1 (42 days), Step 2 (2 days); Step 3 (10 days), Step 4 (13 days), Step 5 (7 days) and Step 6 (16 days).

2.2 Sampling procedure

Thirty restructured Fenalårs elaborated using SS (n=10), SR (n=10) and NNSR (n=10) salting treatments were sampled at the end of the process (when a weight loss of 36% was reached) (Figure 1). A 5cm thick slice was sampled in the central part of the ham and used for instrumental colour determinations. The remaining part of the Fenalår was used for instrumental texture analysis and the determination of salt, water and Zn-protoporphyrin (ZnPP) contents.

To study the microbiological hazards of the restructured Fenalår elaboration procedure, the rest of the Fenalårs (n=30) were sampled at different steps of the manufacturing process (after 44, 54, 67, 74 and 90 days of processing). At each step of the process, a total of 6 Fenalår from different salting treatments (SS, SR and NNSR) (see Table 2) were sampled in two different areas (the central and the tip area), obtaining 10cm thick slices in which two or three regions of interest (RoI), depending on the Fenalår, were selected (Figure 1). The a_w and pH were measured in all the ROIs (n=166).

2.3 Instrumental colour

A Minolta Spectrophotometer CM 700d colorimeter (Konica Minolta Optics, Inc. Japan) with 2° standard observer and D65 illuminant was used to measure colour in the CIE–LAB space (Commission Internationale de l'Eclairage, 1976): lightness (L*), redness (a*) and yellowness (b*) on the 5cm thick slice. Colour was measured in triplicate.

2.4 Instrumental texture

Three or four 2.0cm thick slices were obtained from which a minimum of five parallelepipeds were cut with the exact same dimensions (2cm x 2cm x 1.5cm) from the different muscles available. The identification of the muscle was not possible since it is a

restructured product. The pieces were wrapped in plastic foil to avoid drying and kept at 4 ºC ± 2 °C for 24h for temperature stabilization. A Stress Relaxation test was performed because it allows detection of defective textures (Morales et al., 2007). A Texture Analyser TA – HD plus 6014 (Stable Micro Systems Ltd, Surrey, England) provided with 30 kg load cell and a 60mm diameter compression plate (SMS P/45) was used. Samples were compressed to 25% of their original height, at a crosshead speed of 5mm/s and at a temperature of 4 ºC ± 2 °C. The force decay or relaxation versus time $Y_{(t)}$ was recorded obtaining a deformation curve and it was calculated as follows:

$$Y_{(t)} = \frac{F_0 - F_{(t)}}{F_0}$$

where F_0 (kg) is the initial force and $F_{(t)}$ is the force recorded after t seconds of relaxation. The force decay at 2s (Y_2) and 90s (Y_{90}) were calculated.

2.5 Physicochemical analysis

The a_w was measured with an AquaLab™ instrument (AquaLab Series 3, Decagon devices Inc. Pullman, Washington 99163, USA). Chloride content was determined according to (ISO 1841-2, 1996) using a potentiometric corning 926 chloride Analyzer. Moisture content was determined by drying at 103 °C ± 2 °C until a constant weight was reached AOAC (1990). All measurements were done in triplicate.

ZnPP was quantitatively extracted in subdued light conditions with an ethyl acetate/acetic acid/dimethyl sulfoxide solvent mixture (10:2:1, v/v/v) in quadruplicate as described (Bou, Llauger, Arnau & Fulladosa, 2018). In brief, 2g of ground sample were weighed into 50ml capacity centrifuge tubes and homogenized using an UltraTurrax T25 model disperser (IKA Werke GmbH & Co. KG, Staufen, Germany) for one minute at 9000 rpm with 10ml of the solvent mixture while the tube was immersed in ice. The sample residues were re-extracted (few second burst) with the same volume of solvent mixture and added to the previous one. After extraction on ice for 20 minutes and centrifugation (1100g, 14 min, 4 °C), the supernatant was filtered through a filter paper (grade 1) and collected into a volumetric flask. The solvent extractions were performed until the final volume was attained (typically 20ml). Two hundred microliters of extracts were transferred to 96-microwell plates and sealed with polyolefin acrylate sealing tape. The samples were then incubated for two minutes at 30 °C and shaken for 30 seconds before measuring the fluorescence of ZnPP using a Thermo Fisher Scientific Varioskan microplate reader (Waltham, Massachusetts, USA) with the excitation at 416nm and the emission at 588nm. Ethyl acetate/acetic acid/dimethyl sulfoxide solvent mixture (10:2:1, v/v/v) was used as a blank. Each sample was analysed four times, and the excitation and emission spectra of the standards and samples were compared. ZnPP content was calculated using a calibration curve prepared with ZnPP standard solutions and expressed on the wet weight (ww) basis and dry matter (dm) basis (ZnPP content DM = ZnPP (mg)/(sample (kg) – water (kg))).

2.6 Assessment of food safety implications associated with different elaboration treatments

The behaviour of *Listeria monocytogenes, Salmonella, Staphylococcus aureus* and *Clostridium botulinum* non-proteolytic and proteolytic in restructured Fenalår elaborated with different salting treatments was assessed and compared by applying the predictive models available in ComBase Predictor (www.combase.cc). For simulations with the predictive models, worse case scenarios were set by using the highest values of pH, a_w and temperature recorded in each step of the restructured Fenalår elaboration process as model inputs (Table 2). Simulations using nitrite concentration of 72 ppm as the input value for the model were performed to consider the uneven diffusion of the added nitrite to the internal parts of Fenalår and the possible transformation to non-active substances such as nitrate (estimation 10-40%), bound to lipids (1-15%) or lost as gas (1-5%) (Cassens *et al*., 1979; Honikel, 2008). Kinetic parameters, i.e. growth rate ($1/h^{-1}$) resulting from the doubling time (h) provided by the predictive tool was estimated for each sampling step of the production process, i.e. after 42, 44, 54, 67, 74 and 90 days of processing. To assess the impact of the elaboration process of restructured Fenalår, the index time increase (t_{inc}) was used following the approach of the Spanish Agency for Food Safety (Zurera-Cosano *et al*., 2011). This index is defined as the time required by the microorganism to increase its concentration a given magnitude, e.g. 1 log unit. The impact of the salt and/or nitrite concentration was assessed in relative terms, e.g. the percentage of reduction of t_{inc} in the SR and NNSR products with respect to the SS. The antimicrobial effect of sorbate and the phenolic compounds present in the smoke was not evaluated due to the lack of predictive models including these factors. However, since sorbate and smoking were equally applied in all elaboration treatments, it was assumed that their influence on microbial behaviour was similar for SS, SR and NNSR products.

2.7 Statistical analysis

A one-way ANOVA was used to evaluate the effect of the salting treatment (SS, SR and NNSR) on chemical, instrumental colour and instrumental texture characteristics. Differences between mean values were tested by means of Tukey's test at $\alpha = 0.05$. All the analysis was performed using the statistical package XLSTAT v.2016.3. (Addinsoft SARL, Paris, France).

3. Results and discussion

3.1 Physicochemical characterization

Chemical characteristics of restructured Fenalårs are shown in Table 1. Mean salt content of SS Fenalårs was 5.8%, being similar to traditional bone-in Fenalår reported to be 5.42% by Petrova, Tolstorebrov, Mora, Toldrá & Eikevik (2016). "Fenalår fra Norge PGI", which comprises the varieties "Traditional" (minimum 30% desiccation and dry-curing period between three and six months) and "Matured" (minimum 35% desiccation and a dry-curing period between five and nine months) establish a maximum salt content lower than 9% and 7%, respectively (Regulation (EU) No. 1752/2017). However, salt content of Fenalår production can vary greatly, showing values from 5% to 10%, as reported by Håseth *et al*. (2014). Other lamb/sheep dry-cured products also show a large variation but lower salt contents (Stojković *et al*., 2015). Teixeira *et al*. (2017) reported a salt content of 3.8% in goat and 4.7% in sheep cured legs. Ivanovic *et al*. (2016) found values of 4.5% in goat smoked ham, whereas Paleari, Moretti, Beretta & Caprino (2006) reported a salt content of 3.53% in dry-cured lamb thighbone from Lamon and Bergamasca breeds. In comparison to pork dry-cured ham on the market, both SS and SR restructured Fenalårs showed similar salt content. A market study (http://www.innovacc.cat/wp-content/uploads/2017/05/Annex_9.2._Informe_final.pdf) showed salt content values of 5.26%, 4.81%, 5.32% and 5.40% for Parma PDO, Alto Addigio PGI, TGE Serrano and Culatello di Zibello PDO, respectively. Tomažin *et al*. (2020) studied Kraški pršut (Slovenian ham), reporting salt values between 3.61% and 5.68%.

Table 1: Mean ± standard deviation of chemical, instrumental colour and instrumental texture parameters of restructured Fenalår at the end of the process using different salting treatments.

	Salting treatments				
	SS	**SR**	**NNSR**	**RMSE**	**p-value**
n	10	10	10		
Weight loss (%)	36.4±0.9	36.7±0.49	36.5±0.32	0.43	0.543
Physicochemical parameters					
NaCl (%)	5.8±0.4^{a}	4.9±0.2^{b}	4.8±0.3^{b}	0.30	0.001
Moisture (%)	56.8±0.5	56.6±1.6	57.9±3.6	2.38	0.661
pH	5.6±0.0	5.7±0.0	5.6±0.0	0.03	0.179
a_w	0.909±0.007	0.917±0.001	0.918±0.001	0.005	0.066
ZnPP (mg/kg)ww[1]	0.03±0.09^{b}	0.18±0.15^{b}	10.11±2.9^{a}	1.752	< 0.0001
ZnPP (mg/kg) dw[2]	0.07±0.16^{b}	0.42±0.36^{b}	23.70±4.88^{a}	2.915	< 0.0001
Instrumental colour parameters					
L*	34.9±1.9	33.6±1.0	35.4±2.0	1.88	0.345
a*	12.2±1.1^{a}	12.3±1.29^{a}	6.9±1.3^{b}	1.42	0.000
b*	4.7±0.8	4.8±0.6	4.2±1.5	1.11	0.675
Instrumental texture parameters					
F_0 (kg)	1.73±0.43^{a}	1.18±0.32^{b}	1.09±0.43^{b}	0.391	0.005
Y_2	0.398±0.009^{b}	0.426±0.023^{a}	0.434±0.027^{a}	0.0220	0.005
Y_{90}	0.708±0.014^{b}	0.735±0.021^{a}	0.744±0.026^{a}	0.0210	0.004

^{ab}Means within rows with a different letter are significantly different ($p \leq 0.05$). SS: Standard Salting; SR: Salt Reduced; NNSR: Non-Nitrite Salt Reduced. RMSE: root mean square error of the linear model. [1]Expressed on a wet weight basis; [2]Expressed on a dry weight basis.

In this study, SR treatment produced a significant decrease of salt content in the final product ($p \leq 0.05$). However, the achieved reduction was only ~15% and therefore the product could not be labelled as a salt reduced product (Official Journal of the European Communities C 371 01.12.1998). Further reduction of the salt in restructured Fenalår production would require additional investigation of product safety. All the salting treatments, SS and SR/NNSR restructured Fenalårs showed a_w lower than 0.90 in the final product, according to the general rule of Fenalår fra Norge stablished in the Regulation (EU) No. 1752/2017. However, variation of pH, a_w and temperature during the elaboration process should be evaluated to study the microbiological safety implications of the salt reduced treatments. Figure 2 shows fluctuations of pH and a_w during the restructured Fenalår elaboration process.

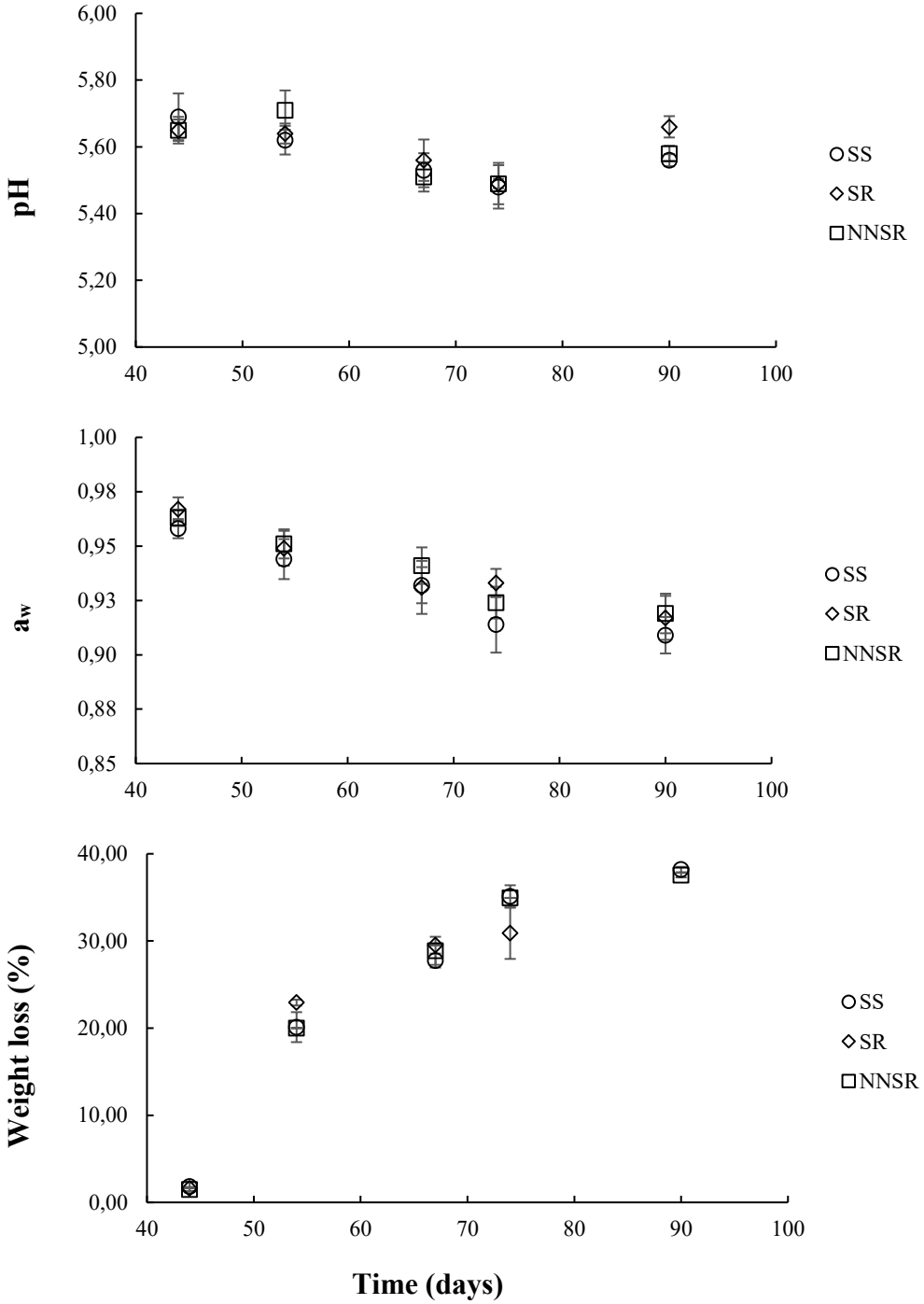

Figure 2. Evolution of pH, water activity (a_w) and weight loss through the restructured Fenalår elaboration process using different salting treatments. (○) SS: Standard Salting; (◊) SR: Salt Reduced; (□) NNSR: Non-Nitrite Salt Reduced.

3.2 Characterization of colour and texture

Colour measurements showed no significant changes between SS and SR treatments for any of the studied parameters (L*, a* and b* values). In contrast, no addition of nitrite (NNSR) produced a significant decrease of redness (a* values) ($p \leq 0.05$). In the case of NNSR, nitrosylmyoglobin is not formed because nitrite has been omitted in the elaboration process. The obtained red colour was in part attributed to ZnPP (Table 1), although other porphyrins such as metmyoglobin could also be present. Given that ZnPP is quite similar to NO-heme, the presence of oxidized forms of myoglobin may contribute to reduced redness values. Wakamatsu, Okui, Ikeda, Nishimura & Hattori (2004) proved in pork meat that when no nitrites are used, Zn-protoporphyrin (ZnPP) is formed instead of nitrosylmyoglobin, decreasing the redness intensity. To our knowledge, this is the first time that ZnPP formation is reported in lamb dry-cured ham.

Mean ZnPP content in the studied NNSR Fenalår was 23.70mg/kg on a dry weight basis (Table 1), whereas it was negligible when using nitrite (SS and SR) because of the inhibition that nitric oxide produces on ZnPP formation (Wakamatsu, Hayashi, Nishimura & Hattori, 2010). Variations found within Fenalårs from the same group (contents between 19 and 31mg/kg ZnPP dw) can be due to the effect of raw ham pH or salt content variations which have been reported to influence ZnPP formation (Bou, Llauger, Arnau, Olmos & Fulladosa, 2020; Wakamatsu *et al*., 2019). Higher concentrations of ZnPP were reported for non-nitrified Parma dry-cured ham (43.8 ±14mg/kg on a dw basis) (Bou *et al*., 2018) and also for Spanish dry-cured ham elaborated without additives (between 67±24 and 95±11mg/kg on a dw basis) (Bou *et al*., 2020). Wakamatsu, Odagiri, Nishimura, and Hattori (2009) reported ZnPP contents that ranged between 27 and 47mg/kg on a wet weight basis in three different muscles (*Semimembranosus*, *Semitendinosus* and *Biceps femoris*) of Parma ham. However, Ghadiri Khozroughi, Kroh, Schlüter & Rawel (2018) mentioned that red meat (lamb and beef) yielded up to four times higher ZnPP concentrations compared to porcine meat muscle, while there was almost no ZnPP quantified in poultry samples. De Maere *et al*. (2017) also reported differences of ZnPP formation in different *in vitro* meat sources, showing a higher ZnPP formation rate in lamb. However, in this study, this higher formation rate in lamb has not been observed. Lower ZnPP concentration found in lamb Fenalår could be related to the shorter maturation period (4 months) in comparison to the Parma and Spanish dry-cured hams (12-24 months), since processing time is a crucial factor for ZnPP formation (De Maere *et al*., 2017). These results suggest that redness of NNSR Fenalår could be improved by optimizing elaboration procedures, i.e. increasing ageing times and/or processing temperatures at the different steps of the process or by selecting raw material with characteristics that enhance formation of ZnPP. It must be remarked that elaboration with or without the bone can also produce important differences on a* values. Coll-Brasas, Kåsin *et al*. (2019) reported that traditional bone-in Fenalår with standard salting treatment had significantly ($p \leq 0.05$) lower redness values (a*=7.41) than restructured Fenalårs analysed in this study (a*=12.2). This fact could be explained by the easier diffusion of curing agents inside the product because of the higher nitrified surface of restructured Fenalårs.

In terms of texture, salt reduction decreased F_0 and increased force decay at 2 (Y_2) and 90 seconds (Y_{90}) (Table 2). These results are in agreement with other studies carried out in pork dry-cured ham. Ruiz-Ramírez, Arnau, Serra & Gou (2005) found that dry-cured ham muscles from pork with lower NaCl content showed lower hardness, cohesiveness and springiness. Benedini *et al*. (2012) found an increase of Y_2 and Y_{90} in low salt class dry-cured ham. Morales, Serra, Guerrero & Gou (2007) also found that BF muscles from pork dry-cured ham with levels of NaCl lower than 2% were more prone to show soft textures. Optimization of the Fenalår elaboration process and corrective actions using emerging technologies (Coll-Brasas, Arnau *et al*., 2019; Contreras *et al*., 2020) could help to yield SR and NNSR Fenalårs with similar textural characteristics to SS Fenalårs.

Table 2: Results of the simulations applying predictive models in order to evaluate the implications of different Fenalår salting treatments on the behaviour of *Listeria monocytogenes*.

Step of the process	Temperature (°C)*	Duration (days)	Type	Maximum a_w	Maximum pH	Growth rate (h^{-1})	Doubling time (h)	Time for 1 log increase (d)	Reduction in comparison to SS (%)
Cold phase	4	42	SS	0.959	5.70	0.004	71.25	10.4	0
			SR	0.969	5.67	0.005	54.98	8.3	20
			NNSR	0.965	5.66	0.007	44.19	6.0	43
Drying	18	2	SS	0.957	5.69	0.042	7.22	1.0	0
			SR	0.964	5.64	0.046	6.57	0.9	9
			NNSR	0.962	5.63	0.061	4.90	0.7	31
Storage	13	10	SS	0.956	5.68	0.020	14.77	2.1	0
			SR	0.960	5.68	0.023	13.24	1.8	13
			NNSR	0.962	5.82	0.035	8.52	1.2	43
Smoking and storage	13	13	SS	0.943	5.62	0.013	23.49	3.2	0
			SR	0.943	5.61	0.013	43.49	3.2	0
			NNSR	0.951	5.57	0.023	13.27	1.8	43
Pressing and storage	13	7	SS	0.932	5.63	0.009	32.70	4.6	0
			SR	0.943	5.63	0.013	23.49	3.2	31
			NNSR	0.933	5.50	0.012	24.56	3.5	25
Final process	13	16	SS	0.918	5.61	0.000	0.000	-	-
			SR	0.927	5.69	0.009	34.80	4.6	-
			NNSR	0.928	5.63	0.011	26.53	3.8	-

SS: Standard Salting; SR: Salt Reduced; NNSR: Non-Nitrite Salt Reduced.

*Temperature of the air of the drying room where the hams were stored. It is assumed as a worst-case overestimate (in general representative of the surface of the product) as a lower temperature could be expected in the product in most of the steps.

3.3 Microbial safety implications associated with the different restructured Fenalår salting treatments

The results of the simulations using predictive models indicated that microbial behaviour in restructured Fenalår varies according to the microorganism and the physicochemical characteristics of the products, which are dependent on the salting treatments used (SS, SR and NNSR) and the temperatures at which each manufacturing step is carried out.

During the cold phase, most of the microbiological hazards evaluated would not be able to grow in Fenalår, either because the temperature was below the minimum growth temperature, i.e. *Salmonella*, *S. aureus, E. coli* and proteolytic *C. botulinum*, and/or due to the presence of nitrites (e.g. non-proteolytic *C. botulinum*). At this step, among the microorganisms assessed only *L. monocytogenes* would be able to grow in Fenalår in agreement with its psychrotrophic nature. The t_{inc} of *L. monocytogenes* in SR and NNSR would be affected in comparison with the value of this index in SS (Table 2). The t_{inc} of *L. monocytogenes* in NNSR products would be 43% lower in comparison with the SS product with 72 ppm of active nitrite. This could be attributed to the fact that salt reduction would yield products with physicochemical characteristics that are slightly different in comparison with the SS product, e.g. a higher maximum a_w (Table 2). These differences determine changes in the *L. monocytogenes* growth rate. It was previously reported that under certain circumstances, such as refrigerated storage, nitrite is effective to control *L. monocytogenes* (EFSA, 2003; Tompkin, 2005). The reduction of added nitrite from 144 ppm to 0 ppm in the present study results in products with more favourable conditions for *L. monocytogenes* growth.

Similarly, during drying at 18 °C for 2 days, the salt reduction would result in products with a maximum a_w slightly higher in comparison with SS (Table 2). The t_{inc} for *L. monocytogenes* would be 31% and 9% lower in NNSR and SR products respectively in comparison with SS products, with 72 ppm of active nitrite. Regarding *Salmonella* and *E. coli*, assuming that the inhibitory mechanism of nitrites differs among different species and that it is not considered effective to control Gram-negative bacteria such as these pathogens (EFSA, 2017), the reduction of nitrite would not have a relevant effect on the t_{inc} for NNSR and SR products with respect to SS.

The t_{inc} of *S. aureus* would increase from 12h during the drying step at 18 °C to 30-63h during the subsequent manufacturing steps carried out at 13 °C in NNSR products, evidencing the positive correlation between temperature and growth rate. Research on the effects of nitrite removal on the behaviour of *S. aureus* in dry-cured products similar to restructured Fenalår are scarce, but research has demonstrated that nitrite failed to control *S. aureus* growth during the production process of a dry-sausage (Bang, Hanson & Drake, 2008; Gonzales-Barron *et al*., 2015). It could also grow in medium-salted bacon, independent of the concentration of nitrite or ascorbate (Crowther, Holbrook, Baird-Parker & Austin, 1976; Tompkin, 2005). Furthermore, the addition of increasing salt up to 3.64% (w/w) and nitrite at 154 ppm did not affect the growth capacity of *S. aureus* during drying of a pork sausage (Bang *et al*., 2008). Therefore, based on the available scientific information, the t_{inc} for *S. aureus* in SS, SR and NNSR products would be

equivalent, i.e. independent of the salt and nitrite concentrations used in the assessed products.

In general, the decrease of a_w during the steps at 13 °C would drastically reduce the growth capacity of all the microorganisms evaluated. Model simulations indicated that slight differences in t_{inc} of *L. monocytogenes* in SS and SR products would be marked, since the slight differences in the physicochemical parameters of SS and SR Fenalår do not determine changes in growth rate, except after the pressing and storage step (Table 2). *Clostridium botulinum* non-proteolytic would not be able to grow in products in any of the steps of the manufacturing production process, as the minimum a_w enabling its growth is 0.97 (ICMSF, 1996), while during drying at 18 °C, proteolytic *C. botulinum* would not reach 1 log increase in Fenalår (t_{inc}=14d) for longer than the actual duration of the drying step. Merialdi *et al.*, (2016) detected a slight growth of 2 – 2.5 Log units of proteolytic *C. botulinum* in 2 out of 9 samples after 7 days of drying of Parma ham (dry ham without nitrite and nitrate) at 20 ºC, though the toxin formation was not evidenced until 14 days.

The reduction of nitrite in the Fenalår elaboration is not a determining factor limiting the growth of *Salmonella, E. coli* and *S. aureus*, it increases very little the growth potential of proteolytic *C. botulinum* but considerable increases the growth potential of *L. monocytogenes*, consequently the implementation of complementary antimicrobial hurdles (including a decrease of temperature during the process) would be needed in order to assure the microbiological safety of nitrite-reduced products.

It is worth mentioning that the simulations with the ComBase model, which was developed in growth media, usually provide fail-safe predictions, overestimating the growth that would actually occur in real food matrices. Despite the limitations, the tool provides useful simulations to calculate the t_{inc} for comparing the relative impact of different scenarios (SS, SR and NNSR) of input data.

4. Conclusions

Salt reduced restructured Fenalår is challenging to produce since soft textures, important changes in colour and safety hazards during the elaboration process can occur, especially in non-nitrite salting treatments. The elaboration of Fenalår without nitrite must be cautious as it can increase the growth potential of *L. monocytogenes* and slightly that of proteolytic *C. botulinum*. However, predictive microbiology and the optimization of the process to enhance ZnPP formation can help to ensure the safety and quality of salt reduced restructured Fenalårs without additives.

5. Acknowledgements

This work was supported by the CERCA Programme/Generalitat de Catalunya,), Nortura SA (Norway) and Foods of Norway, Center for Research-based Innovation (Research Council of Norway; grant 237841). Elena Coll Brasas is the recipient of a doctoral fellowship awarded by INIA (Spanish Government, CPD2015–0054, FPI2015–0023). The authors would also acknowledge the contribution of Karoline Kåsin, Lene Ruud, Trond Livden and Anne Mette Grøtli.

CRediT authorship contribution statement

E. Coll-Brasas: Investigation, Formal analysis, Writing - original draft, Visualization. **A. Possas:** Data curation, Writing - original draft, Formal analysis. **P. Berg:** Supervision, Funding acquisition. **V. Grabež:** Project administration, Writing - review & editing, Resources. **B. Egelandsdal:** Project administration, Supervision, Writing - review & editing, Resources. **S. Bover-Cid:** Visualization, Supervision, Writing - review & editing, Data curation, Formal analysis. **E. Fulladosa:** Visualization, Supervision, Writing - review & editing, Formal analysis.

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