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Research article

Evaluation of the microbial stability and shelf life of 50% NaCl-reduced traditional Greek pork meat product "Syglino of Monemvasia" stored under vacuum at different temperatures

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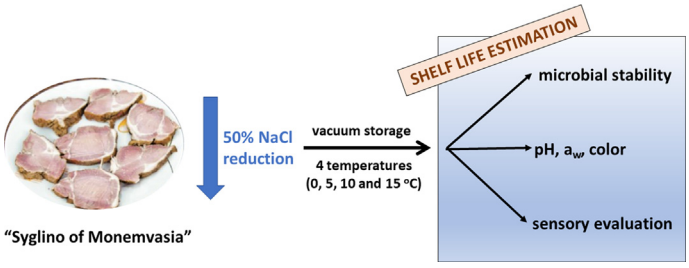
GRAPHICAL ABSTRACT



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ABSTRACT

Nowadays, consumers are increasingly concerned about nutrition and health issues. "Syglino of Monemvasia" is a traditional Greek cooked and smoked, sliced pork meat product. Although this is a nutritious food, its consumption should be done in moderation due to the pickling process of its preparation. This product was thus here optimized to contain half salt (NaCl) amount and its microbial stability and shelf life was then assessed in comparison to the already available commercial product. For this, the total viable counts (TVCs) and some critical specific spoilage associations were enumerated at each product type during vacuum incubation at four different temperatures (0, 5, 10, and 15 °C). The alterations in pH, a_w, color, and some other crucial sensory attributes of each product were also periodically monitored. The new low-salt product was found to remain microbiologically stable under refrigerated vacuum storage for approximately two weeks, being finally spoiled by *Brochothrix thermosphacta* grown above 10^7 CFU/g, ultimately resulting in the deterioration of taste, odor, and overall appearance of the product, and thus leading to its subsequent organoleptic rejection. Despite its limited shelf life, the 50% NaCl-reduced "Syglino" could be released in the local market provided that the cooling chain is maintained throughout its distribution (≤5 °C) and at the same time consumers are willing to accept its milder taste. Although salt replacers could have been used to improve its flavor and at the same time increase its shelf life, the

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product here developed without the use of such alternatives will hopefully contribute to the taste training of its consumers for less salt in their diet, keeping in parallel its clean label with no added preservatives and other food additives.

1. Introduction

Processed meat products have a history of thousands of years and were first created to cover the need to preserve meat when refrigeration was not a feasible option (Kerry and Kerry, 2000). Today, there are numerous such products available on the market throughout the world. These are in general divided in four categories depending on whether these are whole muscle or ground products, heat treated or not (Vandendriessche, 2008). Although some of them are shelf stable and can thus be stored at room temperature for months, most are preserved under low temperatures (i.e., refrigeration). Those products that do not receive thermal treatment are mainly preserved due to the reduction of their water activity (a_w) value (through salting, drying) combined with a pH decrease for those which are in parallel fermented (Talon and Leroy, 2014).

Salt (sodium chloride; NaCl) is the most frequently used additive in meat processing. This significantly contributes to the flavour of the final products, not only conferring the desired saltiness, but also acting as a flavour enhancer. In addition, salt results in a desirable gel texture upon cooking, by solubilizing and coagulating proteins, while it also facilitates emulsion formation through fat binding (Vidal et al., 2020). Finally, salt slows down the growth of (undesired) microorganisms, by binding water and as thus reducing a_w and extending products' shelf life. NaCl contains 39.3% sodium, which is an essential mineral for the human diet, necessary for the maintenance of plasma volume, acid–base equilibrium, transfer of nerve impulses and normal cell function (WHO, 2012). Processed foods in general, and in particular processed meats, consist important sources of sodium intake, with ready-to-eat (RTE) ones to normally contain about 1.5–2.5% salt (Inguglia et al., 2017; Ruusunen and Puolanne, 2005). However, the consumption of high doses of sodium i.e., more than 2 g per day for adults (equivalent to 5 g of salt per day) adversely affects health (WHO, 2012), since it is linked to hypertension. The latter may increase the risk of premature death from cardiovascular diseases (Rust and Ekmekcioglu, 2017).

Greece, like several other Mediterranean countries, has long history in the production of processed meats, with several of them being now produced in small industries or even in household still following the traditional way. One such product is the so-called "Syglino", a cooked and smoked, sliced pork processed meat mainly produced in the southernmost part of mainland Greece in the geographic region of Peloponnese, in the districts of Mani and Monemvasia. Nowadays, consumers are increasingly concerned about nutrition and health issues and therefore seek products that are not only safe and healthy, but also health-promoting (i.e., functional) and even helpful in combating degenerative diseases, such as cardiovascular ones and cancer (Gul et al., 2016). Although "Syglino" is a nutritious food, its consumption should be done in moderation due to the pickling process of its preparation. This is surely not recommended for people with hypertension and cardiovascular diseases. Undoubtedly the reduction of its high salt content (4.85% w/w) would make this product much healthier, but on the other hand would probably reduce its peculiar taste and shelf life, due to salt involvement in both those parameters.

As far as it concerns shelf life, which surely is a significant economic factor affecting the product's trade and sales, besides its potential safety impact (Corradini, 2018), studies should be executed to evaluate the influence of salt reduction on the microbial stability of that specific product. This is because the intrinsic parameters of each food product (e.g., pH, a_w, composition, texture, redox potential), together with some other implicit factors (e.g., initial microbial ecology and subsequent microbial interactions), as well as the prevailing extrinsic factors (e.g., storage temperature, packaging atmosphere), may all together interactively influence microbial growth and behavior, and as thus the progression of the spoilage process (Gram et al., 2002; Huis in 't Veld, 1996; McMeekin and Ross, 1996).

Considering all the above, the main aim of the current study was to evaluate the microbial stability and shelf life of "Syglino of Monemvasia" produced by a small local company following the traditional way, either with the normal salt (NaCl) amount (during the pickling process) or half of it, incubated under vacuum at four different temperatures (0, 5, 10 and 15 °C). The pH, a_w and color of each product were in parallel determined through instrument measurements during each incubation treatment, whereas at the same time those products stored under refrigeration (i.e., 0 and 5 °C) were also organoleptically evaluated to estimate their odor, color, texture, taste, and overall appearance throughout their expected shelf life.

2. Materials and methods

2.1. Preparation of "Syglino" samples and experimental design

Two samples of "Syglino of Monemvasia" were manufactured by a local meat processing company based in Monemvasia (Peloponnese, Greece), one following the commercial traditional recipe with normal salt amount (SA), as control (i.e., 4.85% w/w NaCl), and another with half salt amount compared to that of the commercial recipe (SH). Only the pork shoulder was used to produce those samples. After all the surface fat was removed, it was further cut into strips 50 cm long and 5–8 cm thick. These were placed in specially shaped containers and were immersed in brine of either 17% or 8.5% w/v of salt concentration in which they remained for 7 days at 0 °C (pickling). After pickling, each sample was rinsed with clean water and allowed to dry for 20 h in cold rooms at 0 °C. Then, it was smoked in traditional built wood ovens, exclusively with natural olive wood smoke, up to 72 °C in the core of the product and after a second rinsing with clean water, each sample was boiled at 100 °C for 2 h in water containing wine, orange peel and a mixture of spices (laurel, allspice, cedar). Each sample was finally cooled to 0 °C and further chopped into slices (100 g each one and with 1 cm thick). All slices were then individually packed under vacuum, with the addition of extra virgin olive, in transparent vacuum bags of polyamides - polyethylene (VACUPACK, Oreokastro, Thessaloniki, Greece) and transported to the Laboratory of Food Quality Control and Hygiene (LFQCH) of the Agricultural University of Athens (Athens, Greece) under refrigerated conditions at 0–4 °C, within 3 h. Following samples receipt, their initial microbial load was immediately determined, together with their pH, a_w and color, as well as their main sensory attributes (see next sections below). These were subsequently separated and incubated at four different temperatures, namely at 0, 5, 10 and 15 °C. The manufacturing of the two "Syglino of Monemvasia" samples (SA, SH) was performed twice in different time periods (different batches), while two replicate samples were analyzed each time.

2.2. Microbiological analyses

At each time interval, 10 g of each sample (SA, SH) were aseptically weighted, diluted 1:10 in 90 mL of quarter-strength Ringer's solution (Lab M, Heywood, Lancashire, UK) and homogenized in a stomacher (Stomacher® 400 Circulator, Seward, UK) for 1 min. The homogenate was then serially decimal diluted in quarter-strength Ringer's solution and plated (0.1 mL) on selective agar media (all purchased from LabM unless otherwise stated) to enumerate the following microbial groups: (1)

Pseudomonas spp. on Cephaloridine, Fucidin and Cetrimide (CFC) agar incubated at 25 °C for 48 h, (2) yeasts and moulds on Rose Bengal Chloramphenicol (RBC) agar incubated at 25 °C for 48–96 h, (3) *B. thermosphacta* on Streptomycin Thallous Acetate Actidione (STAA) agar (Oxoid, Thermo Fisher Specialty Diagnostics Ltd, Hampshire, UK) incubated at 25 °C for 48 h. In addition, 1 mL of each dilution was pour-plated in: (4) Plate Count Agar (PCA), incubated at 30 °C for 48–72 h to enumerate total viable counts (TVCs), (5) de Man, Rogosa and Sharpe (MRS) agar, incubated at 30 °C for 48–72 h to enumerate lactic acid bacteria (LAB), and (6) Violet Red Bile Glucose Agar (VRBGA), incubated at 37 °C for 24–48 h to enumerate bacteria belonging to the Enterobacteriaceae family. All results were expressed as Colony Forming Units (CFU) per gram of meat product. Microbial counts (CFU/g) were always converted to logarithms (Log_{10} CFU/g) before the calculation of means and standard errors. In parallel to all those microbiological analyses and besides to the enumeration of Enterobacteriaceae (as a general food safety index against enteric pathogens), to better assure the safety of the samples at the two lower incubation temperatures (used for the organoleptic analyses), the presence of *Listeria monocytogenes* was excluded by plate counting on Palcam Agar incubated at 30 °C for 48 h (detection limit: 100 CFU/g).

2.3. Physicochemical analyses (pH, a_w and color measurements)

At each sampling point and for each tested temperature, the pH, a_w, and color of each sample (SA, SH) were determined. The pH value was recorded using a digital benchtop pH meter (pH 526 WTW, MultiCal) by immersing its electrode in the first decimal dilution previously prepared for the microbiological analyses. The pH meter was always calibrated before measurements, at ambient temperature, using pH buffers 4.01, 7.00 and 10.00 (Mettler Toledo, Columbus, Ohio, USA). a_w was determined on meat product slices using Hydrolab C1 water activity meter (Rotronic AG, Bassersdorf, Switzerland). Regarding the color, this was evaluated in ten different points on the surface of each replicate slice using the Konica Minolta CR-200 portable chroma meter (Konica Minolta Sensing, Tokyo, Japan), by determining the three L^*, a^*, and b^* parameters of the CIELAB color space as indicators of lightness, redness, and yellowness, respectively (Hernández Salueña et al., 2019; Kapetanakou et al., 2014, 2020). The colorimeter was initially calibrated as described in the user's manual using a white standard plate ($L = 100$).

2.4. Sensory evaluation

Each product stored under refrigeration (i.e., 0 and 5 °C) was organoleptically evaluated throughout its incubation (for up to 48 and 37 days, respectively), by a small panel of five assessors, which all belonged to the staff of the LFQCH and were familiar with meat spoilage sensorial characteristics. Informed consent was obtained from all assessors for taking part in the sensory evaluation. Each assessor was asked to evaluate the (1) odor, (2) color, (3) texture, (4) taste, and (5) overall appearance of each sample, without knowing its identity, using a standardized rating scale ranging from 1 (worst) to 3 (best), with 0.5 intervals between units (supplemented material). For any value equal or lower than 2 in any of the sensory attributes evaluated, the product was organoleptically rejected, while assessors were also aware of this rejection threshold. To carry out sensory analysis, at each sampling day and for each incubation temperature two replicate samples were periodically withdrawn from storage, left at room temperature for 30 min and sensory assessment was then carried out in artificial light and ambient temperature, while water and unsalted crackers were provided to the assessors between samples for palate cleansing (Pavli et al., 2020).

2.5. Statistical analysis

Factorial (three-way) analyses of variance (ANOVA) were applied on all the plate count and color measurements data to evaluate the effects of salt reduction, incubation day and temperature (used as the categorical predictors – independent factors), and their possible interactions on the tested dependent variables (i.e., microbial populations, and L^*, a^*, and b^* parameters of the CIELAB color space, respectively). Tukey's multiple range Post-hoc honestly significant difference (HSD) tests were then used for the discrimination of the means. With respect to the sensory evaluation data, non-parametric Friedman tests were applied on each sensory attribute (i.e., odor, color, texture, taste, and overall appearance) to check for any significant differences in panelists' responses between the different samples (i.e., SA/SH incubated at either 0 or 5 °C). All statistical analyses were done using the STATISTICA® software (StatSoft Inc.; Tulsa, OK 74104, USA), while all differences are reported at a significance level of 0.05.

3. Results and discussion

In this work, microbial growth was determined in two samples of "Syglino of Monemvasia", produced either with the normal salt (NaCl) amount (SA) or half of it (SH), and incubated under vacuum at four different temperatures (0, 5, 10 and 15 °C), for various time periods (Table 1). This was done by recording at regular time intervals the development of TVCs and in parallel targeting to the main spoilage specific associations previously described for those type of cooked meat products, i.e., *B. thermosphacta*, LAB, Enterobacteriaceae, *Pseudomonas* spp., yeasts and moulds (Borch et al., 1996; Nychas et al., 2008). It should be noted that those storage temperatures were selected given that "Syglino of Monemvasia" is a RTE product that is suggested to be stored at refrigeration temperatures till consumption (a shelf life of 3 months at 4 °C is indicated on the label by the company), and to include in parallel some temperature abuse conditions (10 and 15 °C). In our experiment, the total incubation period varied per sample (Table 1) and more specifically this decreased as temperature increased and was always shorter

Table 1. a_w ranges (mean values ± standard deviations), total incubation periods (d), and final pH values for the two samples of "Syglino of Monemvasia", produced either with the normal salt (NaCl) amount (SA) or half of it (SH), and incubated under vacuum at four different temperatures (0, 5, 10 and 15 °C). The estimated commercial shelf life, i.e., sampling day where the TVCs reached 10^7 CFU/g (microbial acceptability threshold) for each sample and temperature is also indicated.

Incubation temperature (°C)	Sample	a_w [†]	Total incubation period (d)	Final pH	Commercial shelf life (d)
0	SA	$0.924^{a} \pm 0.010$	106	6.29 ± 0.11	after 106
	SH	$0.944^{b,c,d} \pm 0.008$	48	6.28 ± 0.23	28
5	SA	$0.926^{a,d} \pm 0.011$	59	6.20 ± 0.10	37
	SH	$0.951^{c} \pm 0.014$	37	5.91 ± 0.06	11
10	SA	$0.930^{a,b,d} \pm 0.006$	48	6.08 ± 0.01	28
	SH	$0.945^{b,c} \pm 0.015$	11	6.19 ± 0.24	5
15	SA	$0.926^{a} \pm 0.007$	37	5.97 ± 0.30	14
	SH	$0.945^{b,c} \pm 0.010$	11	6.36 ± 0.11	4

[†] Mean values sharing at least one common letter are not significantly different ($P > 0.05$; Tukey's HSD test).

for the samples containing the half salt amount (SH). This is because both temperature increase and salt reduction resulted, as expected (Fougy et al., 2016), to higher microbial growth rates and as thus to shorter periods for TVCs to reach the stationary phase and cause obvious spoilage-related sensory characteristics.

The generated growth curves for the microbial groups here studied are shown in Figure 1. It is observed that for all samples and independently of incubation temperature, *B. thermosphacta* was the specific spoilage organism (SSO), that is the microorganism that outcompete all others and reached first a high population density (i.e., $\geq 10^6$ CFU/g), being probably the main responsible for the spoilage of those products. *B. thermosphacta* is a facultative anaerobic Gram-positive psychrotrophic bacterium, closely related to the pathogenic species of *L. monocytogenes* (both belonging to the Listeriaceae family), is frequently implicated in the spoilage of refrigerated, both raw and processed (e.g., cooked, RTE), meat and seafood products incubated under vacuum or modified atmosphere packaging (MAP) (Papadopoulou et al., 2012; Vasilopoulos et al., 2015). This is due to its widespread occurrence throughout the food chain, its capacity to endure high-salt and low-pH conditions, together with its ability to grow at refrigeration temperatures, producing organoleptically unpleasant metabolites associated with off-odors, depending on the food matrix (Illikoud et al., 2019; Stanborough et al., 2017).

Besides *B. thermosphacta*, LAB was the microbial group that grow in all products here tested also probably contributing to their spoilage (Figure 1). Indeed, these have been previously identified as the major spoiling microorganisms of vacuum-packed meat and poultry, being most of them aerotolerant, fermenting sugars to lactic acid and leading to pH decrease, also producing slime, CO_2, and off-flavors (Borch et al., 1996; Nychas et al., 2008). Typical LAB spoilers usually belong to the genera *Lactobacillus*, *Leuconostoc*, *Enterococcus*, *Pediococcus* and *Carnobacterium*. In general, LAB grow slowly at refrigeration temperatures, being commonly outcompeted by pseudomonads, under aerobic conditions (Wickramasinghe et al., 2019). In addition, considering that these are normally present in low numbers, in the initial microflora of fresh meat, LAB rarely spoil fresh proteinaceous foods. However, the heat tolerance of some of them, combined with their psychrotrophic nature, results in their survival in heat-treated products, such as the samples here analyzed, subsequently exhibiting advantageous growth under oxygen-restrained conditions, such as vacuum or MAP, which prevent the growth of the aerobic spoilage microflora, such as the heat-sensitive pseudomonas (Borch et al., 1996; Nychas et al., 2008). However, it is worth to be noted that the samples that had been produced with the half salt amount (SH) and later incubated under vacuum, still showed a considerable growth of *Pseudomonas* spp. (Figures 1, S1 and S2). This is possible due to the possible failure of the commercial packaging here applied to fully ensure strict anaerobic conditions. On the other hand, the increased salt amount being present in the samples produced following the commercial traditional recipe, and as thus the reduction in a_w that this had provoked (Table 1), seems to drastically limit the growth of those Gram-negative bacteria (Figures 1, S1 and S2), which are generally known to be quite less resistant to drying and osmotic stresses compared to the Gram-positive species, such as LAB (Burgess et al., 2016).

Another one microbial group that exhibited adequate growth in the samples here analyzed, especially in those produced with the normal salt amount (SA) and incubated at the two higher temperatures (i.e., 10 and 15 °C), was yeasts and moulds (Figure 1). These two fungal groups are known to be xerophilic and acid resistant, frequently thus implicated in the spoilage of low moisture and/or acidic foods (Hernández et al., 2018; Rico-Munoz et al., 2019). Considering that the selective medium here used for their isolation/enumeration (i.e., RBC) does not easily discriminate between those two fungal groups, the numbers reported in the graphs correspond to both. However, these should normally in the vast majority consist of yeasts, given that the growth of most of the moulds should be drastically restricted under the vacuum incubation (Rico-Munoz et al., 2019). On the contrary, yeasts are facultative anaerobic and can hence continue growing under anaerobic conditions, although to a lesser extent compared to other psychrotrophic meatborne bacteria (e.g., *B. thermosphacta*) (Hernández et al., 2018; Nielsen et al., 2008). Important yeast isolates of cooked meat products belong to the species *Candida sake*, *Candida zeylanoides*, *Cryptococcus carnescens*, *Cryptococcus victoriae*, *Debaryomyces hansenii*, *Kluyveromyces marxianus*, and *Yarrowia lipolytica* (Vasilopoulos et al., 2015).

It should be emphasized that salt reduction significantly affected ($P < 0.001$) the growth of all the examined microbial groups, except xerophilic yeasts and moulds, as well as osmotolerant LAB (Burgess et al., 2016), in those products that were stored under refrigeration (0 and 5 °C) (Table S1). Hence, microbial populations (Log_{10} CFU/g) were found in general to be significantly higher in those products containing half salt amount (Figure S1), accelerating their spoilage, and decreasing their shelf life (Fougy et al., 2016). On the other hand, when products were stored under temperature abuse conditions (10 and 15 °C), salt reduction significantly increased ($P < 0.001$) the growth rates of only *Pseudomonas* spp. and *B. thermosphacta* (Table S2). It thus seems that upon storage temperature is increased, the inhibitory effects of salt (i.e., decrease of a_w) on overall microbial growth are somehow limited. For instance, the populations recorded for TVCs do not seem to significantly differ

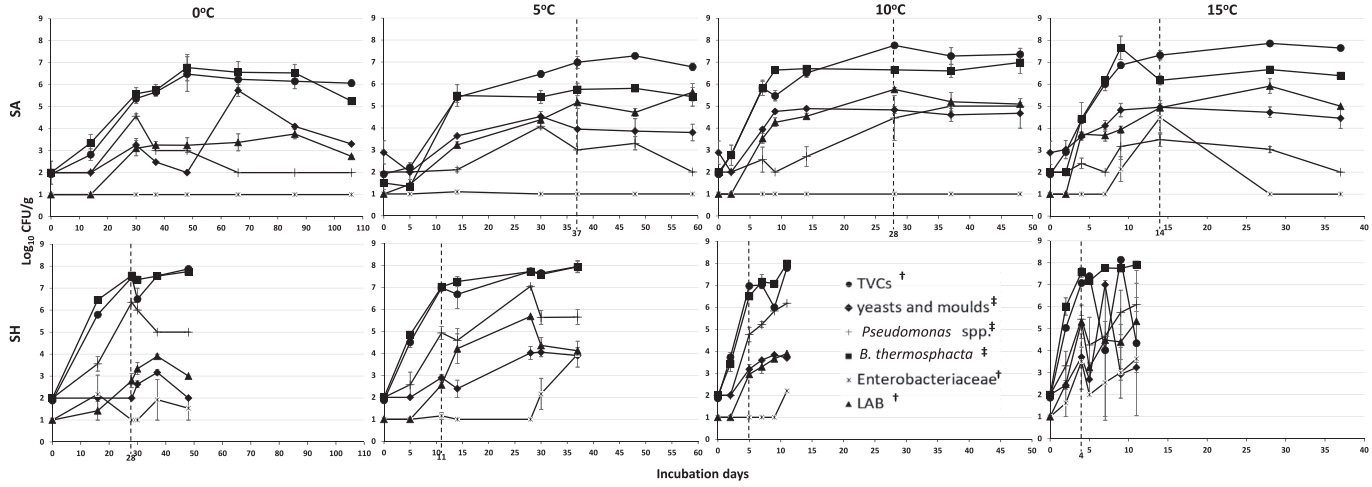

Figure 1. Growth curves of the examined microbial groups of the two samples of "Syglino of Monemvasia", produced either with the normal salt (NaCl) amount (SA) or half of it (SH), and incubated under vacuum at four different temperatures (0, 5, 10 and 15 °C). The points at each sampling point represent mean values ±standard error ($n = 4$). Vertical dotted lines indicate sampling day where the TVCs reached 10^7 CFU/g (microbial acceptability threshold). † detection limit: 10 CFU/g. ‡ detection limit: 100 CFU/g.

between the two types of products (SA, SH) stored at either 10 or 15 °C the first 9 days of incubation (Figure S2). Nevertheless, at the same time *B. thermospacta* numbers surpassed the critical limit of 10^7 CFU/g, much earlier in those products containing the half salt amount (SH) possibly resulting in their quick spoilage.

It should be noted that the regulation on microbiological criteria for foods applied in Europe (Commission Regulation [EC] No 1441/2007) mainly concern pathogenic (and not spoilage) microorganisms, to assure safety of foods for the consumers. For instance, in RTE foods able to support the growth of *L. monocytogenes*, such as the two products here analyzed, those regulations require that the population of that pathogenic bacterium will not surpass 100 CFU/g during the shelf life. Both products (i.e., produced with the normal salt amount and half of it) complied with this criterion (data not shown). On the other hand, foods are spoiled by spoilage microorganisms, such as *B. thermosphacta*, Enterobacteriaceae, and *Pseudomonas* spp., reaching the spoilage detection level (microbial acceptability threshold) (Huis in 't Veld, 1996). For many types of perishable food products, including pork meat products, this latter is generally considered as 10^7 CFU/g (Chai et al., 2017; Corradini, 2018; Tang et al., 2013), while this level is also defined in the legislation of some countries (Greece including) as the maximum allowed number of aerobic microorganisms in preserved meats. The commercial shelf life estimated microbiologically in our study complied to this general recommendation.

The initial pH of the samples produced with the normal salt amount (SA) was 6.35 ± 0.04, while a pH value of 6.18 ± 0.02 was recorded for those samples produced with the half salt amount (SH), confirming for all their non-acidic nature (pH ≥ 4.6). Those pH values remained almost constant till the end of incubation at all temperatures here tested (Table 1). Thus, it seems that the metabolic activity of the microorganisms that grow in those products either does not result in the important production of pH-altering compounds or there was a steady balance between the production of acidic and alkaline compounds. Indeed, *B. thermosphacta* commonly does not show as high acidification potential as classical LAB. Nonetheless, a possible alternative explanation be also associated with the counteracting effect of parallel growth of pseudomonads or even yeasts and moulds, e.g., due to film permeability incapable of maintaining vacuum for long. This in turn, may have caused a (slight to significant) pH rise, i.e., buffering the pH reduction due to growth of LAB and *B. thermophacta* and concomitant production of basic metabolites associated with proteolytic activity, including total volatile basic nitrogen (TVBN), etc., or consumption of organic acids (Dainty, 1996; Skandamis and Nychas, 2002). Considering a_w, there was always a difference of 0.02–0.03 between the two product types (Table 1), with the one produced with the half salt amount (SH) presenting the higher value, something that is explained by the well-known water binding activity of salt (NaCl). This small difference in a_w values was again maintained almost stable till the end of incubation at all temperatures here tested (data not shown).

The color of meat and other food products is surely an important sensory attribute that affects the desire of consumers at purchase (Hung and Verbeke, 2018). The variation of the three-color parameters *L**, *a**, and *b**, at the two samples of "Syglino of Monemvasia", during their vacuum incubation at the four different temperatures is presented in Figure 2. In general, these parameters did not differ between the two samples at time 0. However, under the refrigerated storage (i.e., 0 and 5 °C), the samples that contained the half salt amount (SH) tent to show an increase in lightness (*L** parameter) as incubation progressed, accompanied with a parallel decrease in their redness (*a** parameter). This was more evident at 5 °C, where for all sampling days (i.e., 5, 14, 30 and 37), significant differences in those two parameters were observed in the low-salt samples with respect to time 0. With respect to the two higher storage temperatures (i.e., 10 and 15 °C), some significant variations in these parameters were also observed in both samples, depending on the day of incubation, which again generally resulted in the increase of lightness combined with a decrease of both redness and yellowness (*b** parameter). Previous studies with meat products have shown similar variations of meat color as spoilage progressed (Kapetanakou et al., 2014).

The results of the sensory evaluation of the two samples stored under refrigeration (i.e., 0 and 5 °C) are presented in Figure 3. In accordance with our previous relevant publication on pork meat

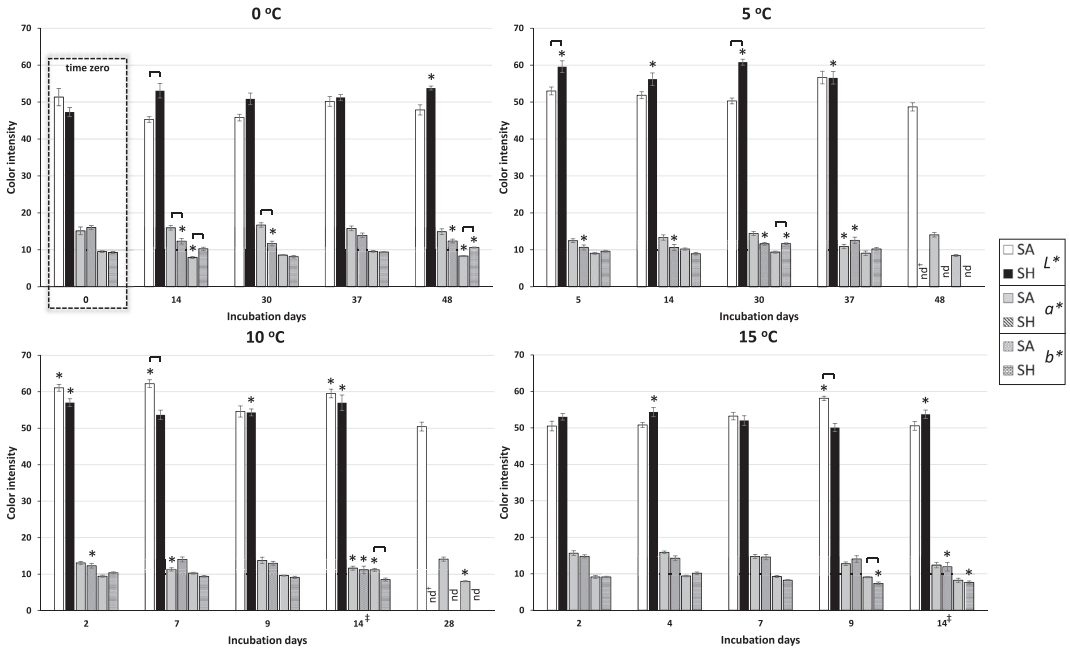

Figure 2. Values of the three parameters of the CIELAB color space (i.e., *L**, *a**, and *b**; as indicators of lightness, redness, and yellowness, respectively) for the two samples of "Syglino of Monemvasia", produced either with the normal salt (NaCl) amount (SA) or half of it (SH), and incubated under vacuum at four different temperatures (0, 5, 10 and 15 °C). Each bar represents mean value ±standard error ($n = 20$; i.e., 2 replicate pork meat samples x 10 surface points). Brackets (⌐¬) and asterisks (*) denote statistically significant differences ($P < 0.05$) between the two samples (SA, SH) and with respect to time 0, respectively. † not determined. ‡ The values presented for the SH samples correspond to the 11th incubation day.

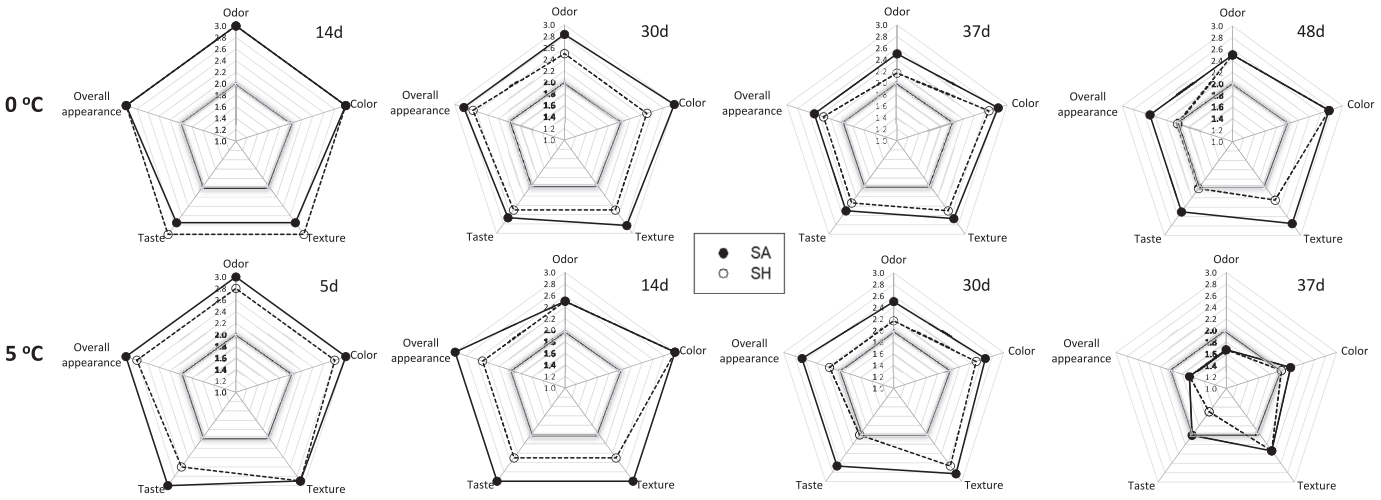

Figure 3. Sensory evaluation (odor, color, texture, taste, and overall appearance) of the two samples of "Syglino of Monemvasia", produced either with the normal salt (NaCl) amount (SA; •) or half of it (SH; ○), and incubated under vacuum at two different refrigeration temperatures (0 and 5 °C). For each sample and temperature, 4 different incubation days have been selected, ranging from 14 to 48 and from 5 to 37 days for 0 and 5 °C, respectively. In each graph, the inner pentagon with the shaded outline indicates the organoleptic rejection values (≤2.0).

spoilage (Kapetanakou et al., 2014) and since the panel was not trained, we decided to set a strict threshold for acceptance at the score of 2. Thus, samples receiving scores equal or lower than 2 were characterized as spoiled, indicating the end of product shelf life. It is observed that the commercial shelf life initially here estimated microbiologically (Table 1), that is the time needed for TVCs to reach 10^7 CFU/g, was most of the times shorter than the period estimated using the sensory evaluation (Figure 3). This means that assessors still considered as acceptable products where TVCs have surpassed 10^7 CFU/g, indicating that spoilage detection level concerning the total number of aerobic microorganisms should be either higher and/or spoilage appears later due to metabolic activities and interactions resulting in the production of spoilage-associated metabolites getting this way worse the sensory attributes of the product conceived by the consumer. Thus, for instance in the sample with the half salt amount (SH) incubated at 5 °C, TVCs surpassed 10^7 CFU/g by the 11^{th} incubation day (Figure 1), whereas at the same time this product was organoleptically rejected only after the 30^{th} incubation day (due to its unpleasant taste, receiving a score of 2.0 for this sensorial attribute; Figure 3). Similarly, in the same product incubated at 0 °C, TVCs surpassed the acceptability threshold by the 28^{th} incubation day (Figure 1), whereas its sensorial characteristics remained rather acceptable (i.e., >2.0) till the 48^{th} incubation day, where an unpleasant taste was again evident, together with an undesirable overall appearance (Figure 3). Friedman tests also confirmed that there were statistically significant differences ($P < 0.05$) in panelists' responses concerning both these two latter sensory attributes from the 37^{th} incubation day between the four different samples (i.e., SA/SH incubated at either 0 or 5 °C) (data not shown). Undoubtedly, this seeming disagreement that was here observed between the microbial and sensory rejection thresholds is surely not something peculiar considering that microbial growth limits with respect to shelf life estimation (i.e., numbers of microorganisms needed for product's deterioration) can greatly vary between 10^6 and 10^9 CFU/g, depending on the type of food, the actual intrinsic, extrinsic and implicit factors and the activity of SSOs (Kapetanakou et al., 2014). This is thus justified when someone considers the complex spoilage mechanisms occurring in foods of animal origin (and other food products as well which are spoiled due to microbial growth), being determined not only by the total number of microorganisms, but more importantly their types, interactions, and overall metabolic activities (Odeyemi et al., 2020).

4. Conclusions

A "Syglino of Monemvasia" cooked and smoked, sliced pork traditional meat product was here optimized to contain half salt amount compared to the already available commercial product. This remained microbiologically stable under refrigerated vacuum storage for approximately two weeks, being finally spoiled by *B. thermosphacta* grown above 10^7 CFU/g. Hence, the populations (Log_{10} CFU/g) recorded for this SSO were, in general and throughout shelf life, significantly higher in that product containing half salt amount, independently of storage temperature (0, 5, 10, and 15 °C), accelerating this way its spoilage and ultimately leading to its organoleptic rejection. Despite its limited shelf life, this product could be released in the local market provided that the cooling chain is maintained throughout its distribution (≤5 °C) and at the same time consumers are willing to accept its milder taste. Although salt replacers could have been used to improve its flavor and at the same time increase its shelf life, the product here developed without the use of such alternatives will hopefully contribute to the taste training of its consumers for less salt in their diet, keeping in parallel its clean label with no added preservatives and other food additives.

Declarations

Author contribution statement

Eleni Michelakou: Performed the experiments; Analyzed and interpreted the data.

Efstathios Giaouris: Analyzed and interpreted the data; Wrote the paper.

Dimitrios Doultsos: Performed the experiments.

Constantina Nasopoulou: Conceived and designed the experiments.

Panagiotis Skandamis: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare the following conflict of interests: Efstathios Giaouris; [is currently serving as associate editor in Heliyon Food Science and Nutrition], Constantina Nasopoulou; [is currently serving as advisory board member in the same journal].

Additional information

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