Research Article

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Quality changes due to refrigerated storage in a traditional dry-cured pork belly salted with glasswort or KCl as partial substitutes for NaCl

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Abstract

BACKGROUND: Glasswort represents a novel alternative to KCl for replacing sodium in meat products. To evaluate the effects of Na reduction on the quality changes of a traditional dry cured belly due to storage, fresh bellies were dry-salted with 2% NaCl (BCON), with 2% of a mixture containing 50% NaCl and 50% KCl (BKCl) or with 1% of a mixture of 90% NaCl and 10% powdered glasswort (BGW), dry-cured, sliced, vacuum packaged and stored under refrigeration for 60 days.

RESULTS: The BKCl and BGW bellies were lower in sodium by one-third to one-half compared to BCON (with 1.6 g Na/100 g). Neither BKCl, nor BGW significantly differed from BCON in free fatty acids (FFA) before and after storage, whereas BGW showed almost twice as much 2-methylbutanal content as BCON. All bellies showed microbiological stability during storage. *Micrococcaceae* was the most abundant microbial group with values of 10^5 to 10^6 colony-forming units g^{-1}. The BGW presented higher *Micrococcaceae* counts (approximately one log unit) but lower microbial biodiversity than BCON.

CONCLUSION: The two alternative dry salting methods reduced the sodium content in bellies, at the same time as ensuring chemical and microbiological stability during refrigerated vacuum storage.

Keywords: traditional products; bacon; sodium-reduced; *Salicornia*; vacuum packaging

INTRODUCTION

Salting and drying are age-old ways of preserving meat that are still used. Traditional dry-cured bellies or traditional bacons are heat-treated but not fully cooked (e.g. 55 °C core temperature), normally smoked, dried meat products made from cured boneless pork bellies.[1] This product differs from industrial bacon, which is mostly injected with brine, pasteurized and scarcely or not dried.[2]

A major issue regarding the healthiness of dry-cured meat products is their high sodium content, which depends on the salting and drying conditions and intensity. Efforts to reduce NaCl should focus on ensuring the technological and sensory characteristics and maintaining shelf life.[3,4] A well-known strategy adopted to produce reduced sodium meat products has been the use of non-sodium salts, with KCl, $CaCl_2$ and $MgCl_2$ being the most commonly used. However, their use is limited because, above a certain amount, they can negatively affect the meat product quality.[4,5] A more recent strategy is the use of natural flavour enhancers, such as yeast extracts, herbs, spices and seaweeds.[6-8] They have the advantage of providing nutrients and bioactive compounds to meat products but can also have negative effects (e.g. on flavour or colour) depending on the ingredient and the amount used. The flavour enhancers include glasswort (*Salicornia herbacea*), which is an edible halophyte herb with a characteristic salty taste that contains micronutrients and antioxidant and antimicrobial phytochemicals.[9,10] Glasswort has been suggested to be a promising

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ingredient for sodium reduction in meat products[11-14]; however, its potential in this regard has not been thoroughly investigated.

In a previous study,[11] we investigated the use of KCl or glasswort as partial NaCl substitutes on the making process of a dry-cured belly and did not observe any negative effect on its quality characteristics during processing, nor on its sensory quality. The present study aimed to investigate how the use of those Na substitutes could affect the chemical and microbial composition of bellies and its stability during refrigerated vacuum storage.

MATERIALS AND METHODS

Production of pork bellies

Twelve fresh pancetta (weighing 1.3 ± 0.2 kg) from twelve Bísaro pigs reared in a mixed production system and weighing 135 kg at slaughter were used. The bellies were randomly assigned to the three different dry-salting procedures (four bellies per salt mixture): 4% NaCl (control; BCON), 4% of a mixture of NaCl and KCl (50% each; BKCl) and 2% of a mixture of NaCl and powdered glasswort (Salivitae Lda., Algarve, Portugal; 90% and 10%, respectively; BGW). The bellies were processed at the Polytechnic Institute of Bragança, Portugal, as described by Ferreira *et al*.[11] Briefly, after being manually rubbed with the salt mixtures, the bellies were kept under refrigeration for 48 h, then washed with tap water, hung to dry for 60 h under refrigeration, and heated twice for 1 h at 90 °C and 70–80% relative humidity (RH) with a resting period between heating of 48 h under refrigeration. Afterwards, the bellies were kept for 1 day at 10 °C and 30–35% RH, then cut into 1-cm slices (12–14 slices per belly), which were vacuum packed and stored at 4 °C for a maximum of 60 days. pH, water activity (a_w), proximate composition and mineral content of were determined the day of storage. Free fatty acid (FFA) and headspace-volatile compound (HVC) contents were assessed at the beginning and end of storage (days 1 and 60) and, for the microbial composition, an additional measurement point was included in the middle of storage (i.e. storage days 1, 30 and 60) to obtain a more complete pattern of change.

Dry-cured belly quality traits

Two slices from each belly were randomly sampled on day one after packaging. Then, skin and the ends of the slices were removed. The slices were cut into 1-cm wide rectangular prisms and the prisms were mixed. Approximately half of the prisms were homogenized with a domestic food processor and used for the analysis of a_w, pH and whole belly composition. The other half of the prisms were trimmed into separate lean and fat, and the total weight and the separated fat weight were recorded to calculate separate fat percentage. The a_w was determined in duplicate at 25 °C using a CX-2 hygrometer (Decagon Devices Inc., Pullman, WA, USA). The pH was measured in duplicate in a homogenate of 2 g of sample and 8 mL of deionized water using a pHmeter (Model 507; Crison, Barcelona, Spain). Moisture, fat, protein and ash contents were analyzed following the AOAC Official Methods nos. 950.46, 991.36, 981.10 and 920.153, respectively.[15] Mineral contents (Na, K, P, Fe, Ca, Mg, Zn, Cu and Mn in the bellies; and the same plus B, Cd, Co, Cr, Ni and Se in glasswort) were determined in duplicate after a concentrated HNO_3 digestion of samples by inductively coupled plasma-optical emission spectrometry.[16]

Changes in free fatty acids and volatile compounds

One slice of each belly was sampled on the day one after packaging and other after 60 days of storage, the skin was removed and the slices divided into two similar portions. One was used for FFA and the other for HVC analyses. For FFA analysis, the slice portion was homogenized with a food processor and, in duplicate, lipids were extracted using the method of Folch *et al*.[17] The FFA fraction was separated by solid-phase extraction (SPE) following the procedure described by Pinkart *et al*.[18] For this, 50 ± 5 mg of extracted lipids were dissolved in 2 mL of hexane containing butylated hydroxytoluene (0.2 g kg^{-1}) and then 50 µL of a pentadecanoic acid (internal standard) solution in hexane was added. The mixture was placed into an aminopropyl silica minicolumns (DSC-NH_2; 500 mg; Supelco, Bellefonte, PA, USA) previously activated with 5 mL of hexane. Neutral lipids were removed with 7 mL of chloroform and FFA were eluted with 5 mL of a mixture of diethyl ether and glacial acetic acid (98:2). The fraction containing the FFA was evaporated to dryness under vacuum and the FFA were methylated with boron trifluoride (10%) in methanol for 20 min at 50 °C and the FFA methyl esters were extracted with 2 mL of hexane. The hexane phase was dried with Na_2SO_4 and injected in a gas chromatograph coupled with a mass spectrometer using a with a HP-88 column (100 m × 0.25 mm × 0.20 mm film thickness) (Agilent Technologies, Santa Clara, CA, USA) as described in Martin *et al*.[19]

For the HVC analyses, the correspondent half-slices were cooked in a convection oven in the fan-forced and upper and lower heating mode at 180 °C for 14 min. After cooking, fat excess was disposed, and the samples were homogenized with a food processor. A sample (3 ± 0.1 g) was placed into a 20-mL screw cap vial and the volatile compounds were analyzed in duplicate by gas chromatography–mass spectrometry using a solid-phase micro-extraction (SPME) method as described by Carballo *et al*.[20] with modifications. Briefly, the extraction of volatiles was carried out with a CTC Pal automated system (Agilent Technologies) equipped with an automatic SPME injection device at 200 °C using 75 µm carboxen/polydimethylsiloxane 1-cm-coated fused silica fibres. Vials with samples were incubated for 20 min at 45 °C and then exposed to the fibre for 40 min. The fibre was then moved to the injector port at 220 °C where adsorbed compounds were desorbed for 14 min. Separation conditions and identification procedure was the same as described in Carballo *et al*.[20] The concentrations of the identified HVC were expressed as area units × 10^8.

Microbiological changes

One slice of each belly was sampled on storage days 1, 30 and 60. In total, 25 ± 0.1 g of each slice was homogenized with 225 mL of 0.1 g L^{-1} peptone water for 2 min using a Stomacher-400 circulator (Seward, Worthing, UK). Serial decimal dilutions were prepared, and 1-mL aliquots of the appropriate dilutions were cultured in duplicate on the corresponding media (Oxoid Ltd, Basingstoke, UK). The media used and incubation conditions for the microbial groups determined were: aerobic mesophilic bacteria (AMB), plate count agar (PCA) at 35 °C for 48 h; lactic acid bacteria (LAB), De Man-Rogosa-Sharpe agar (MRS) with double agar layer at 30 °C for 72 h; *Micrococcaceae*, mannitol salt agar (MSA) at 35 °C for 48 h; for *Enterobacteriaceae*, violet red bile glucose agar with double agar layer at 35 °C for 24 h; for yeast, oxytetracycline glucose yeast extract agar (Oxoid) at 22 °C for 5 days.

From the growth in the MRS plates, 12 colonies were randomly picked for belly (four bellies), treatment (three treatments) and

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Table 1. Values of pH, a_W, separable fat and proximate and mineral composition in the dry-cured bellies

Characteristic	BCON	BKCl	BGW	SEM	*P*-value
pH	5.90	5.95	5.89	0.087	0.89
a_W	0.870	0.877	0.914	0.0152	0.144
Separable fat (%)	63.9	62.0	58.3	4.53	0.69
Proximate composition (%)					
Moisture	22.86	21.10	27.00	3.018	0.40
Fat	56.13	58.93	53.31	4.184	0.65
Protein	11.84	10.59	11.77	1.142	0.69
Ash	5.15	4.41	3.79	0.378	0.080
Macromineral (g/100 g)					
Na	1.611 a	0.752 b	1.072 b	0.1174	0.002
K	0.245 b	1.072 a	0.222 b	0.0827	< 0.001
P	0.148	0.122	0.125	0.0131	0.35
Mg	0.084	0.047	0.057	0.0119	0.129
Ca	0.071	0.023	0.016	0.0198	0.16
Trace mineral (mg/100 g)					
Zn	9.61 a	4.48 b	5.16 b	0.870	0.005
Fe	8.06	3.58	3.45	1.573	0.094
Cu	0.38 a	0.24 b	0.26 b	0.025	0.007
Mn	0.30	0.16	0.11	0.086	0.29

BCON, salted with 4 g of NaCl/100 g of fresh belly (control); BKCl, salted with 4 g of a mixture of NaCl and KCl (50% each)/100 g of fresh belly; BGW, salted with 2 g of a mixture of NaCl and powdered glasswort (90% and 10%, respectively)/100 g of fresh belly. Means in a row showing any common lowercase letter are significantly different ($P < 0.05$; Tukey's test).

Table 2. Main free fatty acids (mg/100 g of fat) in the dry-cured bellies on days 1 and 60 of storage

	BCON		BKCl		BGW			*P*-value		
Fatty acid	1	60	1	60	1	60	SEM	Treatment (T)	Storage (S)	T × S
C14:0	28.0	50.4	27.1	48.3	32.0	55.1	3.31	0.64	< 0.001	0.99
C16:0	214.5	369.8	248.6	383.8	283.1	446.6	24.43	0.34	0.003	0.96
t-C16:1*n*-7	5.2	10.6	5.9	10.4	6.3	12.3	0.77	0.64	< 0.001	0.91
C16:1*n*-7	47.0	102.9	56.1	99.4	58.5	99.6	6.77	0.96	0.002	0.87
C18:0	99.2 b	161.5 ab	117.6 ab	163.4 ab	125.3 ab	210.8 a	11.11	0.20	0.002	0.67
C18:1*n*-9	605.8	1095.8	757.7	1120.9	803.7	1290.8	71.03	0.38	0.002	0.90
C18:1*n*-7	110.2	207.0	141.8	214.6	150.5	238.6	13.54	0.47	0.003	0.93
C18:2*n*-6	206.0 b	369.4 ab	247.6 ab	452.3 ab	278.6 ab	499.9 a	32.21	0.26	0.001	0.89
C18:3*n*-3	9.2 a	17.4 ab	10.3 ab	20.7 ab	12.4 ab	23.8 b	1.63	0.36	0.003	0.88
C20:1*n*-9	16.2	31.4	20.0	32.0	21.2	35.4	2.06	0.67	0.005	0.96
C20:2*n*-6	7.3 b	12.8 ab	9.5 ab	17.1 ab	10.7 ab	19.8 a	1.32	0.100	0.002	0.79
C20:4*n*-6	20.9	31.0	23.6	44.3	37.0	49.5	3.31	0.25	0.18	0.89
Total	1394 b	2497 ab	1695 ab	2644 ab	1844 ab	3024 a	171.34	0.33	0.001	0.95

BCON, salted with 4 g of NaCl/100 g of fresh belly (control); BKCl, salted with 4 g of a mixture of NaCl and KCl (50% each)/100 g of fresh belly; BGW, salted with 2 g of a mixture of NaCl and powdered glasswort (90% and 10%, respectively)/100 g of fresh belly. Means in a row showing any common lowercase letter are significantly different ($P < 0.05$; Tukey's test).

sampling day (3 days), reaching a total of 432 colonies. Isolates were grown in tryptone soy broth (TSB) (Bacto, Mt Printchard, NSW, Australia) with 5 g L^{-1} of yeast extract (YE) (Difco, Leeuwarden, The Netherlands) (TSB-YE) at 37 °C for 24 h. Next, 1 mL of aliquot was centrifuged (50 000 × *g* for 3 min) in Eppendorf tubes and the pellets were resuspended in 1 mL of a mixture of TSB-YE glycerol 0.5 L L^{-1} and frozen at −40 °C until further analysis. Isolates were recovered in TSB-YE at 37 °C for 24 h and then a bacterial loop was subcultured in MRS agar. One colony from each recovered isolate was prepared and identified by the matrix-assisted laser desorption/ionization-time of flight technique.[18] Identification, at genus or species level, were considered if scores were above 1.7 and 2.0, respectively (Compass Data Analysis software package; Bruker Daltonics, Bremen, Germany).

Statistical analysis

The statistical analysis was performed using the SPSS, version 24 (IBM Corp., Armonk, NY, USA). Data for a_w, pH, belly proximate

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composition and mineral content were analyzed by one-way analysis of variance (ANOVA), with salting treatment as the fixed factor. Free fatty acids, HVC and microbial group counts were analyzed by two-way ANOVA, with salting treatment and storage times and their interaction as fixed factors. When the fixed factors showed significant difference ($P < 0.05$) a Tukey's post-hoc test was carried out.

RESULTS AND DISCUSSION

Proximate and mineral composition

The values of pH, a_W, proximate composition and mineral contents in the dry-cured bellies used in the present study are shown in Table 1. No differences were found for pH, a_W and proximate composition between treatments. However, the ash content in the BGW showed a trend towards significance ($P < 0.1$) compared to BCON, suggesting that the amount of salt entering the BGW during the salting phase was higher than in the BCON. Also, the mean a_w value of BGW (as opposed to both BCON and BKCl) was above 0.89, which is considered a critical point for dry-cured meat products to reach storage stability at room temperature and to develop a stable curing colour.[21] The macrominerals contents differed ($P < 0.01$) for Na and K, as expected. The belly BKCl contained approximately half the Na content and four times more potassium than BCON, whereas, on comparing BCON with BGW, in the latter, there was a Na reduction of approximately one-third. Trace mineral results indicated significantly higher amounts of Zn and Cu ($P < 0.01$) and a near to significant ($P < 0.1$) higher amount of Fe in the BCON than in the other bellies. A higher presence of these minerals in the NaCl than in the KCl used for salting would explain the differences between BCON and BKCl, and the differences between BCON and BGW could be attributed to the higher proportion of NaCl used in the former. The Na, K, P, Mg and Ca contents of glasswort (mean ± SD), expressed as g Kg^{-1}, were 178.0 ± 13.8, 13.3 ± 1.5, 2.5 ± 0.3, 6.1 ± 0.5 and 17.2 ± 1.33, respectively; and those for Zn, Fe, Cu, Mn, B and Se, expressed as mg kg^{-1}, were 20,2 ± 0.44, 8.2 ± 0.4, 23.0 ± 2.1, 31,0 ± 7.8 and 16.2 ± 0.4. At the levels used, salting with glasswort, despite its high mineral content, did not increase BGW mineral levels compared to CON or BKCl.

Table 3. Headspace volatile compounds content (area units x 10^{-8}) in the dry-cured bellies on days 1 and 60 of storage

	BCON		BKCl		BGW			*P*-value		
Compound	1	60	1	60	1	60	SEM	Treatment (T)	Storage (S)	T × S
Aldehydes										
Ethanal	0.32	0.35	0.25	0.33	0.33	0.25	0.017	0.83	0.90	0.63
Propanal, 2-methyl-	3.73	3.48	4.06	3.70	5.12	4.78	0.269	0.35	0.69	1.00
Butanal, 3-methyl-	11.00	13.11	12.74	13.73	15.95	16.80	0.875	0.082	0.40	0.93
Butanal, 2-methyl-	14.79 b	16.89 b	21.49 ab	19.61 ab	26.53 a	28.71 a	2.213	0.008	0.77	0.78
Pentanal	8.53	6.75	8.71	8.23	11.40	6.05	0.759	0.93	0.33	0.72
Hexanal	20.17	13.30	12.52	12.11	19.19	6.10	2.110	0.53	0.070	0.36
Heptanal	1.79	1.47	1.19	1.13	2.15	1.30	0.162	0.149	0.106	0.41
Octanal	1.35	1.41	1.21	0.98	1.01	0.61	0.120	0.093	0.35	0.64
Nonanal	ND	ND	ND	ND	0.29	0.10	0.035	–	0.33	–
Hydrocarbons										
Pentane (+ acetone)	17.54	18.38	23.18	22.42	22.65	9.22	2.166	0.131	0.116	0.088
Hexane	1.66	1.80	1.69	1.80	1.614	1.05	0.115	0.49	0.75	0.60
Heptane	9.93	13.63	14.61	11.95	16.66	8.43	1.244	0.92	0.43	0.29
Octane	8.67	11.21	11.59	8.07	13.85	4.91	1.287	0.98	0.23	0.24
Heptane, 2,2,4,6,6-pentamethyl-	1.82 b	2.46 ab	1.27 b	2.47 ab	2.37 ab	4.65 a	0.469	0.016	0.006	0.33
Decane	ND	ND	0.10	0.58	0.78	0.83	0.166	0.38	0.61	0.68
Ketones										
2,3-Butanedione	0.11	0.22	0.33	0.21	0.09	0.13	0.036	0.38	0.91	0.57
2-Butanona	0.40	0.21	0.29	0.17	0.40	0.19	0.043	0.87	0.19	0.94
2-Heptanone	0.50	0.37	0.22	0.09	0.53	0.10	0.080	0.53	0.26	0.77
Octanedione (+ furan, 2-pentyl)	1.33	2.41	1.09	1.36	0.52	0.17	0.318	0.076	0.52	0.53
Others										
Metanethiol	0.54	0.51	0.69	0.57	0.68	0.38	0.047	0.72	0.24	0.65
Disulfide, dimethyl	2.99	0.37	0.49	0.28	0.47	0.53	0.429	0.42	0.30	0.40
Cyclopentane, butyl-	0.60	0.82	0.74	0.72	0.44	0.09	0.109	0.21	0.83	0.62
Total	107.77	109.15	118.35	110.49	142.93	95.27	6.534	0.84	0.23	0.36

BCON, salted with 4 g of NaCl/100 g of fresh belly (control); BKCl, salted with 4 g of a mixture of NaCl and KCl (50% each)/100 g of fresh belly; BGW, salted with 2 g of a mixture of NaCl and powdered glasswort (90% and 10%, respectively)/100 g of fresh belly. ND, non-detected. Means in a row showing any common lowercase letter are significantly different ($P < 0.05$; Tukey test).

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Free fatty acids and volatile compounds

An a_w value in dry-cured meat products close to 0.90 does not inhibit enzymatic activity even at low temperatures.[21] Accordingly, a significant increase in total FFA was observed in the bellies as a result of lipolytic activity during storage ($P < 0.01$) (Table 2). The increase in individual FFA was significant for all but the longest and more polyunsaturated fatty acid C20:4*n*-6. The lack of increase in this FA may be due to oxidation (i.e. its instability to oxidation reactions).[22,23] The NaCl reduction approaches did not significantly affect FFA levels either at the beginning or at the end of storage compared to control. Thus, the lower salt content in BGW compared to BCON was not sufficient to increase lipolysis.

Most of the HVC detected in the dry-cured bellies (Table 3) have been previously described in other processed pork bellies,[24-26] and presumably originated from lipid degradation, such as straight-chain aldehydes, 2-pentyfuran, 2-heptanone, octanedione and hydrocarbons, and from amino acid Strecker degradation, such as branched-chain aldehydes.[22,27]

The presence of 2-methylbutanal and the sum of branched-chain aldehydes were affected by salting treatment, being higher in BGW than in BCON both at the beginning and at the end of storage. The lower amount of salt used for salting BGW compared to that used for BCON might have facilitated the amino acids Strecker degradation during heating and drying. Accordingly, Wang *et al.*[28] reported a higher content of branched-chain aldehydes in low-salt fermented sausages than in conventional fermented sausages. High levels of branched-chain aldehydes could affect the flavour of the BGW more intense compared to BCON due to their low odour thresholds.[24] Moreover, the flavour of BGW could be affected any distinctive and unique volatile compound provided by the glasswort. Their concentration, however, would have been below the detection limit of the analytical method because any HVC detected in the BGW was different from those detected in the other two treatments, and none of the volatile compounds reported for glasswort leaves in previous studies[9] were detected in the BGW. In partial agreement, in our previous study,[11] we found that the use of a same amount of glasswort in dry-cured bellies resulted higher flavour intensity before cooking than in control bellies; however, no differences were found in flavour between glasswort and control bellies after cooking.

The levels of 2,2,4,6,6-pentamethylheptane were also higher in BGW than in BCON, and increased with storage. Accordingly, 2,2,4,6,6-pentamethylheptane has been related to storage time.[29] This compound can presumably originate from lipid degradation[30] or migration from the packaging material.[31] Its role in belly flavour cannot be established because there are no data in the literature on odour thresholds for this compound.

Microbiology

The levels of AMB (PCA counts) were similar to those of *Micrococaceae* (MSA counts), which was by far the most abundant group, followed by those of LAB and yeast (MRS and oxytetracycline glucose yeast extract counts). The microbial load in dry-cured bellies or brine-injected bacon could be the result of colonization of bacteria from the belly surface, growth of colonizing bacteria during salting, drying or cooling, and incomplete thermal inactivation of microorganisms during heating.[32,33] The AMB concentration found for this type of meat products in other studies have shown a variability. Gong *et al.*[32] found similar values to those in the present study in the muscle portion of a Sichuan-style Chinese bacon; meanwhile, lower values (1.5–3.3 log colony-forming units g^{-1}) were reported for several varieties of Chinese bacon.[34,35] The microbial composition in the dry-cured bellies (Table 4) partially agrees with that reported by Egan and Shay[36] on traditional Australian bacon, consisting of a mixture of micrococci, coagulase-negative staphylococci, lactobacilli and some Gram-negative rods. By contrast, Wang *et al.*[35] found, in a variety of Chinese traditional bacons with a_W in the range 0.8–0.9, that the dominant microbial species was halotolerant proteobacteria from genus *Salinivibrio* and *Vibrio*, as well as *Staphylococcus*.

Storage did not change the microbial concentration of the microbial groups except for an approximately 0.5 log colony-forming unit g^{-1} increment of *Micrococcaceae* during the first month of storage, which was significant when the counts of all the treatments were considered together ($P < 0.05$; not shown). This microbial stability, which is in line with the stability observed for the HVC, can be attributed to the refrigeration temperature and low a_w. By contrast, studies on industrial bacon reported a steady growth of LAB during refrigerated vacuum storage that eventually spoil the bacon.[37,38] Presumably, these bacons were salted by brine injection and were not dried or were slightly dried, and so their a_w would be not lower than 0.95 because the growth of LAB decreases dramatically as a_w decreases from this value.[39]

Treatment affected the levels of *Micrococaceae*, LAB and yeast in the dry-cured bellies (Table 4). *Micrococaceae* levels were higher in BGW than in BCON ($P < 0.05$) on day 1 of storage (and considering all 3 days of storage together; not shown), the highest LAB levels were found in BGW, and the lowest yeast counts were found in BCON. Differences in *Micrococaceae* and LAB levels could be attributed to increased growth during belly processing due to the lower amount of NaCl absorbed in BGW salting compared

Table 4. Concentration (Log colony-forming units g^{-1}) of relevant microbial groups in the dry-cured bellies on days 1, 30 and 60 of storage

	BCON			BKCl			BGW				*P*-value		
Media	1	30	60	1	30	60	1	30	60	SEM	Treatment (T)	Storage (S)	T × S
PCA	4.49	5.45	5.42	5.71	5.47	5.26	5.48	6.03	5.92	0.129	0.20	0.20	0.22
MSA	4.47 b	5.42 ab	5.09 ab	5.53 ab	5.94 a	5.20 ab	5.88 a	6.25 a	5.73 ab	0.120	0.001	0.036	0.54
MRS	2.03 ab	1.40 b	1.53 b	3.42 ab	1.87 ab	1.88 ab	3.96 a	3.32 ab	3.78 a	0.254	0.002	0.115	0.74
OGYE	2.49 abc	2.07 c	1.86 c	3.74 ab	3.65 ab	2.36 bc	3.60 ab	3.58 ab	3.86 a	0.183	0.001	0.28	0.35

BCON, salted with 4 g of NaCl/100 g of fresh belly (control); BKCl, salted with 4 g of a mixture of NaCl and KCl (50% each)/100 g of fresh belly; BGW, salted with 2 g of a mixture of NaCl and powdered glasswort (90% and 10%, respectively)/100 g of fresh belly. PCA, plate count agar; MRS, De Man-Rogosa-Sharpe; MSA, mannitol salt agar; OGYE, oxytetracycline glucose yeast extract agar. Means in a row showing any common lowercase letter are significantly different ($P < 0.05$; Tukey's test).

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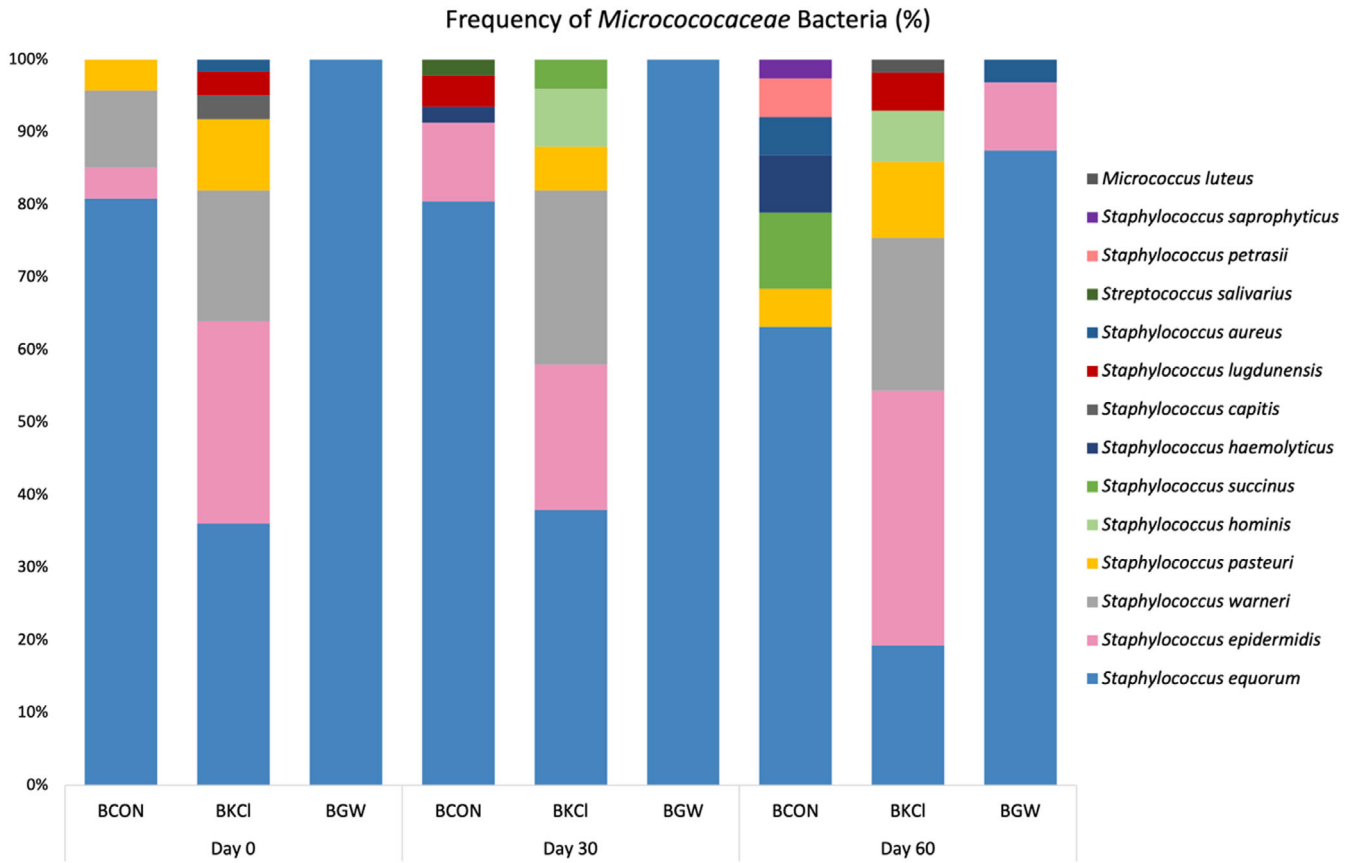

Figure 1. Frequency of *Micrococcaceae* bacteria isolated from dry-cured pork bellies. BCON, salted with 4 g of NaCl/100 g of fresh belly (control); BKCl, salted with 4 g of a mixture of NaCl and KCl (50% each)/100 g of fresh belly; BGW, salted with 2 g of a mixture of NaCl and powdered glasswort (90% and 10%, respectively)/100 g of fresh belly.

Table 5. Occurrence of the most abundant species isolated from the mannitol salt agar plates (into brackets is the percentage of total isolates)

Species	BCON (n = 136)	BKCl (n = 185)	BGW (n = 108)
Micrococacceae			
Staphylococcus equorum	99 (73)	52 (28)	100 (93)
Staphylococcus epidermidis	7 (5)	47 (25)	3
Staphylococcus warneri	5 (4)	35 (19)	0
Staphylococcus pasteuri	4	15 (8)	0
Staphylococcus hominis	0	8 (4)	0
Subtotal	131 (96)	168 (91)	104 (96)
Others			
Subtotal	5 (4)	17 (9)	4 (4)

BCON, salted with 4 g of NaCl/100 g of fresh belly (control); BKCl, salted with 4 g of a mixture of NaCl and KCl (50% each)/100 g of fresh belly; BGW, salted with 2 g of a mixture of NaCl and powdered glasswort (90% and 10%, respectively)/100 g of fresh belly.

to BCON. The higher levels of *Micrococcaceae* and LAB could be responsible for the also higher levels of methylated aldehydes in BGW (Table 3) because the metabolism of these micro-organisms is involved in their production.[40]

Identification results (Fig. 1 and Table 5) showed that, as expected, most of the isolates from MSA plate were assigned to the family *Micrococcaceae*, and, within this family, *Staphylococcus* species such as *Staphylococcus equorum*, *Staphylococcus epidermidis* and *Staphylococcus warneri*. As for the type of salting, BKCl treatment showed the highest microbial diversity and BGW the lowest (i.e. in BGW bellies *S. equorum* showed an occurrence of more than 90%). The low variability might suggest a possible intraspecies selective antimicrobial effect of glasswort against *Staphylococcus* spp., or the reason for the lower biodiversity is

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the lower total salt content. Diversity within treatments was not noticeably affected by storage time except for an apparently higher variety of *Micrococacceae* species in BCON on day 60 day of storage than on days 0 and 30.

CONCLUSIONS

The alternative dry salting methods used in the present study reduced the sodium content in a traditional dry-cured belly at the same time as ensuring microbiological stability during refrigerated vacuum storage. Salting with NaCl-glasswort mixture resulted in a belly richer in microorganisms and selected aromatic compounds typical for dry-cured meat products. Meanwhile, salting with the KCl and NaCl mixture did not significantly affect those quality traits compared to conventional dry-salting. Despite the higher microbial counts, NaCl-glasswort dry-cured belly had a lower biodiversity of *Micrococococaceae*, suggesting a possible selective action of this salting procedure on specific species. A consumer sensory analysis is recommended for future studies to evaluate the acceptability of the NaCl-replaced dry-cured bellies.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1 Laranjo M, Elias M, Patarata L and Fraqueza MJ, Entremeada and paia de toucinho, in *Production of Traditional Mediterranean Meat Products. Methods and Protocols in Food Science*, ed. by Lorenzo JM *et al*. Springer Nature, Berlin, pp. 169–176 (2022).
2 Bohrer BM, in *Bacon: The Processing, Shelf Life, and Macronutrient Composition of Bacon Manufactured from Pork Bellies, in Pork*, ed. by Munekata ES, Pateiro M, Franco D and Lorenzo JM. CRC Press, Boca Raton, pp. 363–389 (2021).
3 Xiao-Hui G, Jing W, Ye-Ling Z, Ying Z, Qiu-Jin Z, Ling-Gao L *et al*., Mediated curing strategy: an overview of salt reduction for dry-cured meat products. *Food Rev Intl* **39**:1–16 (2022).
4 Vidal VAS, Paglarini CS, Lorenzo JM, Munekata PES and Pollonio MAR, Salted meat products: nutritional characteristics, processing and strategies for sodium reduction. *Food Rev Intl* **39**:1–20 (2021).
5 Pateiro M, Munekata PES, Cittadini A, Dominguez R and Lorenzo JM, Metallic-based salt substitutes to reduce sodium content in meat products. *Curr Opin Food Sci* **38**:21–31 (2021).
6 Jia S, Shen H, Wang D, Liu S, Ding Y and Zhou X, Novel NaCl reduction technologies for dry-cured meat products and their mechanisms: a comprehensive review. *Food Chem* **431**:137142 (2024).
7 Kim TK, Yong HI, Jung S, Kim HW and Choi YS, Technologies for the production of meat products with a low sodium chloride content and improved quality characteristics—a review. *Foods* **10**:957 (2021).
8 Gullón P, Astray G, Gullón B, Franco D, Campagnol PCB and Lorenzo JM, Inclusion of seaweeds as healthy approach to formulate new low-salt meat products. *Curr Opin Food Sci* **40**:20–25 (2021).
9 Rahmani R, El Arbi K, Sassi Aydi S, Hzami A, Tlahig S, Najar R *et al*., Biochemical composition and biological activities of *Salicornia europaea L*. from southern Tunisia. *J Food Meas Charact* **16**:4833–4846 (2022).
10 Ekanayake S, Egodawatta C, Attanayake RN and Perera D, From salt pan to saucepan: *Salicornia*, a halophytic vegetable with an array of potential health benefits. *Food Front* **4**:641–676 (2023).
11 Ferreira I, Leite A, Vasconcelos L, Rodrigues S, Mateo J, Munekata P *et al*., Sodium reduction in traditional dry-cured pork belly using glasswort powder (*Salicornia herbacea*) as a partial NaCl replacer. *Foods* **11**:3816 (2022).
12 Kim HW, Hwang KE, Song DH, Kim YJ, Ham YK, Yeo IJ *et al*., Effects of red and green glassworts (*Salicornia herbacea L*.) on physicochemical and textural properties of reduced-salt cooked sausages. *Korean J Food Sci Anim Resour* **34**:378–386 (2014).
13 Kim HW, Hwang KE, Song DH, Kim YJ, Lim YB, Ham YK *et al*., Effect of glasswort (*Salicornia herbacea L*.) on the texture of frankfurters. *Meat Sci* **97**:513–517 (2014).
14 SeoNg PN, Seo HW, CHo SH, Kim YS, KaNg SM, Kim JH *et al*., Potential use of glasswort powder as a salt replacer for production of healthier dry-cured ham products. *Czech J Food Sci* **35**:149–159 (2017).
15 AOAC, Official method 950.46 moisture in meat; Official method 991.36 fat (crude) in meat and meat products; Official method 981.10 crude protein in meat; Official method 920.153 ash in meat, in *Meat and Meat Products*, 16th edn, ed. by Cunniff P. Official methods of analysis of the AOAC International, Gaithersburg, MD, pp. 1–15 (1999).
16 Osorio MT, Zumalacárregui JM, Bermejo B, Lozano A, Figueira AC and Mateo J, Effect of ewe's milk versus milk-replacer rearing on mineral composition of suckling lamb meat and liver. *Small Ruminant Res* **68**: 296–302 (2007).
17 Folch J, Lees M and Stanley GH, A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**:497–509 (1957).
18 Pinkart HC, Devereux R and Chapman PJ, Rapid separation of microbial lipids using solid phase extraction columns. *J Microbiol Methods* **34**: 9–15 (1998).
19 Martín A, Giráldez FJ, Mateo J, Caro I and Andrés S, Dietary administration of l-carnitine during the fattening period of early feed restricted lambs modifies lipid metabolism and meat quality. *Meat Sci* **198**: 109111 (2023).
20 Carballo DE, Andrés S, Giráldez FJ, Khanjari A, Caro I, Llamazares D *et al*., The effects of storage and hop extract on aroma and flavour compounds in Balkan-style sausages packed under a CO2-containing anaerobic atmosphere. *Heliyon* **6**:e05251 (2020).
21 Tapia MS, Alzamora SM and Chirife J, Effects of water activity (aw) on microbial stability as a hurdle in food preservation, in *Water Activity in Foods, Fundamentals and Applications*, ed. by Barbosa-Cánovas GV, Fontana AJ Jr, Schmidt SJ and Labuza TP. Wiley-Blackwell, Hoboken NJ, pp. 239–271 (2008).
22 Flores M, Understanding the implications of current health trends on the aroma of wet and dry cured meat products. *Meat Sci* **144**:53–61 (2018).
23 Domínguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W and Lorenzo JM, A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants* **8**:429 (2019).
24 Aheto JH, Huang X, Tian X, Lv R, Dai C, Bonah E *et al*., Evaluation of lipid oxidation and volatile compounds of traditional dry-cured pork belly: the hyperspectral imaging and multi-gas-sensory approaches. *J Food Process Eng* **43**:e13092 (2020).
25 Poligne I, Collignan A and Trystram G, Effects of salting, drying, cooking, and smoking operations on volatile compound formation and color patterns in pork. *J Food Sci* **67**:2976–2986 (2002).
26 Yang Y, Zhang X, Wang Y, Pan D, Sun Y and Cao J, Study on the volatile compounds generated from lipid oxidation of Chinese bacon (unsmoked) during processing. *Eur J Lipid Sci Technol* **119**:1600512 (2017).
27 Shahidi F and Oh WY, Lipid-derived flavor and off-flavor of traditional and functional foods: an overview. *J Food Bioact* **10**:20–31 (2020).
28 Wang J, Aziz T, Bai R, Zhang X, Shahzad M, Sameeh MY *et al*., Dynamic change of bacterial diversity, metabolic pathways, and flavor during ripening of the Chinese fermented sausage. *Front Microbiol* **13**: 990606 (2022).
29 Santos MD, Matos G, Casal S, Delgadillo I and Saraiva JA, Quality evolution of raw meat under hyperbaric storage – fatty acids, volatile organic compounds and lipid oxidation profiles. *Food Biosci* **42**: 101108 (2021).

30 Gorraiz C, Beriain MJ, Chasco J and Insausti K, Effect of aging time on volatile compounds, odor, and flavor of cooked beef from pirenaica and friesian bulls and heifers. *J Food Sci* **67**:916–922 (2002).
31 Rivas-Cañedo A, Fernández-García E and Nuñez M, Volatile compounds in fresh meats subjected to high pressure processing: effect of the packaging material. *Meat Sci* **81**:321–328 (2009).
32 Gong W, Zhu Y, Shi X, Zhang W and Wen P, Influence of tissue type on the bacterial diversity and community in Pork Bacon. *Front Microbiol* **12**:799332 (2021).
33 Taormina PJ and Bartholomew GW, Validation of bacon processing conditions to verify control of *Clostridium perfringens* and *Staphylococcus aureus*. *J Food Prot* **68**:1831–1839 (2005).
34 Li X, Xiong Q, Zhou H, Xu B and Sun Y, Analysis of microbial diversity and dynamics during bacon storage inoculated with potential spoilage bacteria by high-throughput sequencing. *Front Microbiol* **12**: 713513 (2021).
35 Wang Y, Wang Z, Han Q, Xie Y, Zhou H, Zhou K *et al.*, Comprehensive insights into the evolution of microbiological and metabolic characteristics of the fat portion during the processing of traditional Chinese bacon. *Food Res Int* **155**:110987 (2022).
36 Egan AF and Shay BJ, The microbiology of vacuum-packaged pork, in *Proceedings of the 30th European Meeting of Meat Research Workers*. Bristol, United Kingdom, pp. 215–216 (1984).
37 Aaslyng MD, Vestergaard C and Koch AG, The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Sci* **96**:47–55 (2014).
38 Li X, Li C, Ye H, Wang Z, Wu X, Han Y *et al.*, Changes in the microbial communities in vacuum-packaged smoked bacon during storage. *Food Microbiol* **77**:26–37 (2019).
39 Liu SQ, Asmundson RV, Gopal PK, Holland R and Crow VL, Influence of reduced water activity on lactose metabolism by *Lactococcus lactis* subsp. *cremoris* at different pH values. *Appl Environ Microbiol* **64**: 2111–2116 (1998).
40 Smit BA, Engels WJM and Smit G, Branched chain aldehydes: production and breakdown pathways and relevance for flavour in foods. *Appl Microbiol Biotechnol* **81**:987–999 (2008).

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