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The relationship between biogenic amines and the growth of spoilage related microorganisms in sliced cooked ham stored under different packaging

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ABSTRACT: Spoilage and pathogenic microorganisms are the most important factors affecting food safety and quality, and food packaging is the most important technical link to inhibit spoilage and pathogenic microorganisms in food transportation. The aim of this study was to investigate the development of biogenic amines (tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine) and spoilage-causing microorganisms in ham stored at 4 °C under different commodity packaging. The experimental packaging systems were Pack-1 (multilayer sheet + multilayer bag), Pack-2 (polycoupled sheet + metallized bag), and Pack-3 (polycoupled sheet + copper bag). The results showed that the Pack-2 has exceptionally high efficiency compared with the other two. The results of principal component analysis (PCA) applied to principal component 1 (PC1) was the most important variable in terms of differences among packing conditions, as it explained; 71.7%, 57.8%, and 83.5% of the total variability in Pack-1, Pack-2, and Pack-3, respectively. PC1 was positively associated with microbial analyses and protein content change (parts of biogenic amines content). PC1 differentiated the indicators from packaging conditions. PC1 was positively related to microbial analysis and protein change. Therefore, cadaverine, tryptamine and phenylethylamine could be used as the spoilage indicators of ham, of which the contents might reflect the spoilage degree.

KEYWORDS: ham, spoilage related microorganisms, biogenic amines, shelf life, packaging, principal component analysis

INTRODUCTION

Hams are generally cuts of pork that come from the hind leg. They can be cooked and served fresh, but most of them are cured in some way, i.e., dry cured and smoked or wet cured and then cooked or smoked [1]. Cooked ham is made from boned pork legs that are seasoned with a special flavoring mixture (salt, pepper, juniper, and laurel) and then steam-cooked at about 70 °C. The addition of nitrates and nitrites is allowed in cooked hams [2]. Ham fermentation depends on the environment and indigenous microorganisms to form a rich microbiota that is critical for flavor and aroma development. High microbial populations have been found on the surface of ham, which can influence the aging process. Low microbial counts inside ham are usually below 6 log colony forming units/gram (CFU/g). However, in exceptional cases, an abundant microbiota was found with total aerobic counts approaching 8 log CFU/g and *Lactobacillus* being the predominant microbial group [3]. The raw materials for cooked ham contain a variety of microorganisms, mainly from the genus *Lactobacillus*, *Pseudomonas* and *Brochothrix*. Nevertheless, cooking reduces the concentration and diversity of spoilage and pathogenic bacteria. Gram-positive, catalase-positive cocci are the most important microorganisms in dry-cured ham. Their proteolytic, lipolytic, catalase and nitrate reductase activities may contribute to the sensory characteristics of the product [3]. The production of cooked meat ham involves several steps that regulate the composition of microorganisms and their concentration, which affects the shelf life of the product. Studies have shown that *Leuconostoc* spp. is the most important genus in spoiled cooked ham [5]. The proportion and composition of the microbiota vary depending on processing, storage, and batch. However, due to cross-contamination during slicing, cooling and packaging, the likelihood of recontamination by microorganisms increases [6].

Although the curing process can extend the shelf life of foods, studies have shown that due to the microbial diversity of dry-cured meats, microbial hazards and potentially toxic metabolic compounds could eventually occur, with biogenic amines (BA) being particularly prominent [7]. BA is nitrogen compounds with low molecular weight generated mainly by microbial decarboxylation of amino acids. This enzymatic reaction can be transamination, reductive amination, degradation, and decarboxylation of certain precursor amino compounds [8]. BAs are used as shelf life marker of food spoilage, as high concentrations

of BAs are found when the hygienic quality of a product decreases [9]. The most relevant BAs that arise due to food spoilage are tryptamine (TRY), 2-phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), spermidine (SPD), and spermine (SPM). Due to the importance of harmful effects of BAs on food hygiene and health, it was concluded that individual BAs alone, or in combination, can be used as an important indicator for freshness, quality, and spoilage of food [10].

Fermented products usually contain higher concentrations of BAs because the fermentation process leads to increased free amino acid precursors of BAs [11]. TYR is the most representative amine in cured meat products. BAs are present in numerous dry-cured meats due to the growth of fermentative bacteria (such as *Lactobacillus*) and spoilage bacteria (such as *Enterobacteriaceae* and *Pseudomonas* spp.) [12, 13]. Among them, the most common are TYR, PUT, HIS, CAD, and SPD [14]. BAs were also detected in salami, adult bovine meat samples, dry sausages, onion sausage, smoked turkey fillets, and pepperoni sausage. The highest values of CAD, TYR and HIS in dry sausages were 790, 320 and 200 mg/kg, respectively. BAs were also detected in European dry ripened sausages made with horse, beef or turkey meat. The highest average total BAs content of 730 mg/kg was found in the turkey sausages; while the average contents in beef and horse sausages were 500, and 130 mg/kg, respectively [15]. The main bacterial groups that form BAs in meat fermented products are *Enterobacteriaceae* (i.e. *Escherichia*, *Citrobacter*, *Klebsiella*, *Proteus*, *Salmonella*, and *Shigella*), *Micrococcaceae* (*Staphylococcus* and *Micrococcus* genera), *Pseudomonas*, some strains belonging to *Bacillus* spp., as well as *Lactobacillus* [16]. BAs have been extensively studied, and their respective concentrations in food are an important area of research.

However, there have been no data on the changes of BAs in different ham packages. In order to monitor and control the quality of ham, it is necessary to detect the quality changes and common indicators of ham in different packages, to obtain a theoretical basis for ham spoilage, and to provide technical support for the safety control and monitoring system of ham. In this study, the developments of BAs and spoilage-causing microorganisms in hams under different conditions were investigated by principal component analysis (PCA).

MATERIALS AND METHODS

Packaging materials

In the present study, we evaluated principally 5 different types of commercial packaging assembled in 3 ways for their ability to conserved cooked ham. Tested packages-multilayer sheet, multilayer bag, polycoupled sheet, and metallized bag were kindly provided by ESSEOQUATTRO s.p.a. industry (Carmignano di Brenta, Italy); and copper bag by Adercarta s.p.a. industry (Adro-Brescia, Italy).

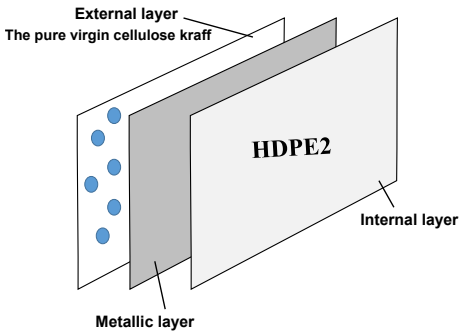

Fig. 1 Graphic structure of "multilayer" packaging.

"Multilayer" samples are protected by European Patent EP 1584464 A1. The base packaging was a sheet comprising a first layer formed by a virgin kraft of pure cellulose with very low weights and coupled to a second layer made of high density polyethylene (HDPE) with a third metallic layer (silver colour given by the presence of an aluminum powder equal to 0.005 g/m^2) fixed between them (Fig. 1). A multilayer bag is made from the multilayer sheet.

The polycoupled sheet is composed of 100% pure long-fibber cellulose coated with a treated film enabling it to preserve all the sensory qualities of food and to isolate the food completely from moisture, light, and ultraviolet (UV) rays.

The metallized bag is made of pure long-fibre kraft pulp to ensures excellent resistance to the weight of the food and is wet resistant to assist in proper food preservation.

The copper bag, an antibacterial freshness-preserving bag for fresh food industries, is composed of 99.05% Forest Stewardship Council (FSC) certified paper and 0.5% copper applied through sublimation and spraying. The copper bag is completed by an internal sheet that directly wraps the product, made in two versions: (1) recyclable and compostable, with internal biopolyethylene coating; (2) recyclable, with anti-grease treatment acting as an oxygen barrier. The 100% recyclable freshness bag, with a high light barrier, can reduce bacterial contamination by at least 99%.

Three types of packaging combinations:
Pack-1: multilayer sheet + multilayer bag,
Pack-2: polycoupled sheet + metallized bag,
Pack-3: polycoupled sheet + copper bag.

Sample collection

Sliced cooked ham (Prosciutto cotto, vacuum-sealed 150 g package) samples were supplied by a local supermarket in Camerino, Italy. Packages of the ham were opened. The sliced ham was further divided and

wrapped in previously prepared commercial multilayer sheet and polycoupled sheet for storage, with the ends of the packaging folded similarly to the wrapping method commonly used by consumers.

In Pack-1, the ham was wrapped inside the multilayer sheet and, thereafter, put inside the multilayer bag; then, the bag was sealed.

In Pack-2, the ham was wrapped inside the polycoupled sheet and, thereafter, put inside the metallized bag; then, the bag was sealed.

In Pack-3, the ham was wrapped inside the polycoupled sheet and, thereafter, put inside the copper bag; then, the bag was sealed.

Each Pack comprised around 3 slices (10–12 g/slice) of ham for further analyses at days 2, 5, and 7. All Packs were kept at 4 °C to simulate the consumer's storage conditions. Before doing the packaging combination, a part (around 40 g) of the ham was reserved and analysed on the same day to be used as the control at day 0 for each Pack sample. Three packages of individual Pack samples were used to perform the test on each of the designed analysis day. On the day of the analysis, each sample was divided into three portions: 20 g for microbiological analysis, 15 g for chemical analysis and 5 g for pH measurement. All analyses were performed in triplicate.

Chemicals and Reagents

All of the following chemicals: tryptamine hydrochloride (TRY, $C_{10}H_{12}N_2 \cdot HCl$, >99%, CAS No. 343-94-2), 2-phenylethylamine hydrochloride (PHE, $C_8H_{11}N \cdot HCl$, >98%, CAS No. 156-28-5), putrescine dihydrochloride (PUT, $C_4H_{12}N_2 \cdot 2\,HCl$, >98%, CAS No. 333-93-7), cadaverine dihydrochloride (CAD, $C_5H_{14}N_2 \cdot 2\,HCl$, >98%, CAS No. 1476-39-7), histamine dihydrochloride (HIS, $C_5H_9N_3 \cdot 2\,HCl$, >99%, CAS No. 56-92-8), tyramine hydrochloride (TYR, $C_8H_{11}NO \cdot HCl$, >98%, CAS No. 60-19-5), spermidine trihydrochloride (SPD, $C_7H_{17}N_3 \cdot 3\,HCl$, >98%, CAS No. 334-50-9), and spermine tetrahydrochloride (SPM, $C_{10}H_{26}N_4 \cdot 4\,HCl$, >98%, CAS No. 306-67-2) for standard solutions preparation; 1,7- diaminoheptane (98%, CAS No. 646-126 19-5) for the internal standard; and trichloroacetic acid (TCA, ⩾99.0%, CAS No. 76-03-9), acetone (⩾99.5% CAS No. 67-64-1), hydrochloric acid (HCl, 37%, CAS No. 7647-01-0), sodium hydroxide anhydrous (NaOH, ⩾98%, CAS No. 1310-73-2), acetonitrile (CH_3CN, HPLC gradient grade, ⩾99.9%, CAS No. 75-05-8), sodium carbonate anhydrous (Na_2CO_3, ⩾99.5%, CAS No. 497-19-8), dansyl chloride ($C_{12}H_{12}ClNO_2S$, 98% CAS No. 605-65-2), and methanol (CH_3OH, HPLC gradient grade, ⩾99.9%, CAS No. 67-56-1) for extraction and derivatization were from Sigma-Aldrich (Milano, Italy).

Stock solutions of BAs were prepared by dissolving 10 mg of each compound in 10 ml HCl 0.1 M (Merck-Darmstadt, Germany) and stored in glass stopper bottles at 4 °C. Standard working solutions of various concentrations were prepared daily from different aliquots of the stock solutions and appropriately diluted with deionized water (resistivity < 8 MΩ×cm) from the Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). The derivatization solution was prepared using dansyl chloride in acetone (10%).

Analyses of BAs

Chemical methods were based on monitoring molecules affecting the food and determining the degree of food deterioration. The BA analytical methods were based on previously published methods with slight modifications [17] and validated according to the criteria of European Regulations for quantitative methods of confirmation (EC 2002/657). Each slice of ham was cut into thin strips and blended. Then, 5 g of the sample was extracted in a centrifuge tube with 5% TCA by Ultra-Turrax S 18N-10G homogenizer (IKA-Werke Gmbh & Co., Staufen, Germany). To 1 ml of isolated supernatant, 0.2 ml of 10 mg/l 1,7-diaminoheptane solution (as internal standard), 0.3 ml of Na_2CO_3 saturated solution, and 50 µl of 2N NaOH were added. For derivatization, 2 ml of dansyl chloride solution was used, and the sample was placed at 45 °C for 45 min. Then, excess dansyl chloride was eliminated by adding 100 µl of 28% NH_4OH. SPE STRATA X 33 µ Cartridges, 200 mg/6 ml (Phenomenex, Bologna, Italy) were conditioned with 5 ml of CH_3CN followed by 5 ml of Milli-Q water. Samples were purified by the cartridge and eluted with 4 ml CH_3CN. Samples were stored at 4 °C and filtered on a 0.45 µm PTFE filter (Supelco Bellefonte, Pennsylvania, USA) prior to analysis. HPLC/DAD studies were performed using a Hewlett Packard (Palo Alto, CA, USA) HP-1090 Series II, made of an autosampler, a binary solvent pump, and a diode-array detector (DAD). BAs separation was achieved using the Gemini C18 analysis column (250×4.6 mm I.D., particle size 4 µm) from Phenomenex (Torrance, CA, USA). The column temperature was kept constant at 25 °C. The mobile phases analyzed by HPLC were Milli-Q water (A) and CH_3OH/CH_3CN 70:30 v/v solution (B) at a flow rate of 0.5 ml/min. The gradient program was: 0 min 60% B, 10 min 70% B, 20 min 90% B, 26 min 100% B, 29 min 100% B, and 32 min 60% B up to 40 min. The injection volume was 20 µl. The HPLC system was coupled to DAD, and the peak response was measured at 254 nm.

In addition, specific indexes were determined as freshness markers: Biogenic Amine Index (BAI), Chemical Quality Index (CQI), the total of the monitored (Total BAs). These indexes were obtained according to the following formula:

BAI = putrescine + cadaverine + histamine + tyramine

CQI = (putrescine + cadaverine + histamine)/(spermine + spermidine + 1)

Total BAs = putrescine + cadaverine + histamine + tyramine + spermine + spermidine + phenylethylamine.

The CQI was proposed to evaluate the quality of fish and seafood [18]. The levels of TYR and CAD were proposed to control poultry spoilage and beef quality during storage [19]. The BAI was created by Veciana [20] to improve the CQI. Therefore, it was suggested that cooked meat products should be classified into four levels. The Total BAs was used to have more sample visualized on the BAs evolution in different type of samples.

Microbiological analysis

Microbiological analysis is an important tool for assessing the level of food safety and hygiene. In this study, reliable microbiological parameters were taken into account to define the hygienic conditions of cooked ham products (in different types of packaging) found at the time of their deposition in the studio, and to monitor them during their shelf-life period for 7 days.

A 10 g ham sample from individual Packs was homogenized in 90 ml of peptone solution (0.1%) in a Stomacher-Easy MIX (AES Lactobacillusoratory, Bruz, France). A series of ten-fold dilutions (10^{-2} to 10^{-10}) was prepared and a given amount of each dilution was spread on several specific media: plate count agar (PCA, Oxoid Ltd., Basinstoke, UK), violet red bile glucose agar (VRBGA, Oxoid Ltd.), agar base with selective supplements CFC (PAB, Oxoid Ltd.) and streptomycin thallous acetate actidione agar (STAA, Oxoid Ltd.), tryptose sulphite cycloserine agar (TSC, Oxoid Ltd.) supplemented with streptomycin sulphate and thallous acetate for counting of mesophilics bacteria, *Enterobacteriaceae*, *Pseudomonas* spp. and *Brochotrix thermosphacta*, and *Clostridium perfringens*, respectively. The bacterial counts were performed after 24–48 h of aerobic incubation at 25 °C both for *Pseudomonas* spp. and *B. thermosphacta*; and after 24–48 h of aerobic incubation at 37 °C both for mesophilic bacteria and *Enterobacteriaceae*. For *C. perfringens*, the sample was incubated under anaerobic condition and counted. Gram stain, morphological,and biochemical analyses were performed on selected colonies isolated from each sample in order to confirm the strain identity.

pH measurement

A 5 g ham sample was chopped and subsequently transferred into a sterile stomacher bag. The pH of each sample at every time points were measured by an electronic pH meter (Mettler Toledo, Columbus, UK) equipped with a probe for solids.

Statistical analysis

Analyses were performed in triplicate, and data were expressed as mean ± standard deviation (SD). The relative standard deviation (%RSD = 100×SD/mean) was calculated to control the precision of the results obtained. *t*-test Student was applied to assess whether the results differences between the compared packaging were statistically significant. Probability level ($p < 0.05$) was considered statistically significant. Every measurement was repeated at least 3 times. Data were processed by analysis of variance (ANOVA). PCA was also applied to the data of indicators in each packaging. All statistical procedures were computed using SPS 25 and Origin 2021.

RESULTS AND DISCUSSION

Analysis of BAs

The HPLC-DAD chromatogram of 25 mg/l BAs mixture and 1,7-diaminoheptane, used as internal standard, is shown in Fig. S1. The respective values of the correlation coefficients R^2 of the analyzed molecules are reported, all the coefficients confirm the linearity of the method ($R^2 \geqslant 0.994$). Calculate the calibration curve for each BA was using the response factor (ratio of the BA peak area to the inner standard peak area). Under these HPLC conditions, each BA and internal standard was clearly resolved, indicating that the method can be used for the quantitative determination of BAs in food samples.

The aim of the present study was to evaluate and compare the efficiency of 3 types of packing materials (Pack 1, 2 and 3) in the preservation of cooked ham by monitoring BAs. Cooked ham's BA levels changed after seven days of storage in Packs 1, 2, and 3 (Fig. 2). Looking at the global BA values, the efficiency of package 2 tends to be higher than that of Pack 1 and Pack 3 in T5. However, the differences are not statistically significant (Fig. 2A). In fact, the BAI and CQI are lower in the samples of cooked ham stored in Pack 2. These differences are significant at T5 and T7 (Fig. 2B,C).

pH measurement

The pH values of all ham samples decreased steadily during the storage period, with around 0.5 in samples stored in Pack 2 and 3 While, around 0.75 in Pack 1 stored ham (Fig. 3).

Microbiological analysis

The aim of the present study was to evaluate and compare the efficacy of 3 types of packaging materials on the preservation of cooked ham using microbiological parameters. Microbial strains included total aerobic mesophiles, *Escherichia coli* (β-glucoronidase positive), *Enterobacteriaceae*, *Staphylococcus* spp. (coagulase positive), *Bacillus cereus* (presumed), and *C. perfringens* (a sulfite-reducing anaerobic bacterium). The counts of

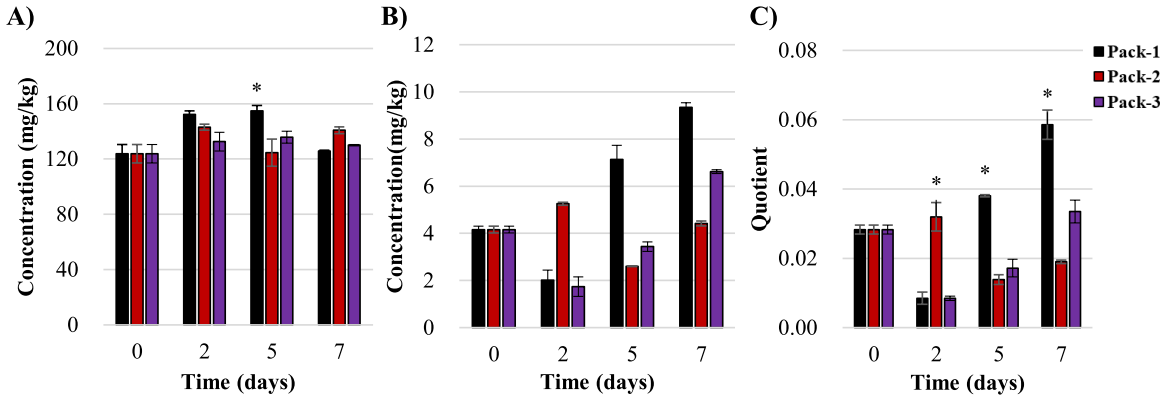

Fig. 2 Changes of BAs of cooked ham inside Pack-1, Pack-2 and Pack-3 during 7 days of storage. A), Total BA;, B), BAI; C), CQI. * Statistically significant different ($p < 0.05$, Student's t-test).

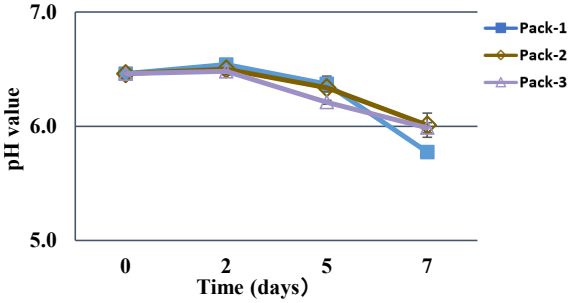

Fig. 3 Changes of pH value of cooked ham inside Pack-1, Pack-2 and Pack-3 during 7 days of storage.

C. perfringens and *E. coli* were under detection limits in ham during the whole study period [21].

For total aerobic mesophiles, Pack-2 maintained its value below 6 Log CFU/g for 5 days, with a slight increase after day 7. In contrast, the sample from Pack-3 contained mesophiles below 6 Log CFU/g for up to 2 days (Fig. 4A). With respect to *Enterobactriaceae*, all samples showed an increase after day 2 of storage, and the number was maintained untilday day 5, followed by a further increase after day 7. Pack-2 had a relatively lower number of *Enterobactriaceae* than the other two (Fig. 4B). Pack-2 and Pack-3 were able to maintain relatively low levels of *Staphylococcus* spp. at day 2 of storage (Fig. 4C). Fig. 4D shows that all samples showed a similar growth trend over time, while Pack-2 showed a better limitation of number compared with Pack-1 and Pack-3. Hence, Pack-2 showed a better preservation of the microbiological quality of the cooked ham by limiting the numbers of total aerobic mesophiles, *B. cereus* and *Staphylococcus* spp. until day 7 of storage compared with the other two (significant differences were observed mainly after days 2, 5 and 7). In addition, all 3 types of packaging were efficient in limiting the numbers of *Enterobacteriaceae* up to 5 days. In conclusion, Pack-2 was the best packaging for preservation of microbiological quality of the cooked ham, maintaining values compatible with the acceptance criteria established in the guidelines for microbiological analysis of foods.

The correlation of indicators of the ham in different packaging

PCA allows a better overview of the relationship between variables. The results of PCA applied to the mean values of the parameters of cooked ham were summarized in Fig. 5–Fig. 7. For cooked ham in Pack-1, PCA showed that about 88.7% of variability was explained by two first principal components, while two principal components in Pack-2 and Pack-3 explained about 85.0% and 90.8% of variability, respectively. Principal component 1 (PC1) was the most important variable in terms of differences among packing conditions, as it explained 71.7%, 57.8%, and 83.5% of the total variability in Pack-1, Pack-2, and Pack-3, respectively. PC1 was positively associated with microbial analyses and protein content changes (parts of BAs content). Histamine cannot be detected in cooked ham.

In Fig. 5, all indicators were on the positive side of PC1 except for putrescine and spermidine; but the levels of *Enterobacteriaceae*, *B. cereus*, and tyramine were significantly lower than the others. Total aerobic mesophiles, *Staphylococcus* spp., cadaverine and tryptamine were not only together, but also had high values on the positive side of PC1. The indicators of cooked ham in Pack-1 were related to total aerobic mesophiles, *Staphylococcus* spp., cadaverine, tryptamine, phenylethylamine, and spermine; other BAs could not reflect shelf life. The values of spermidine and spermine were on the negative side of PC1 (Fig. 6), while the others were not only together but also had high values on the positive side of PC1. The indicators of cooked ham in Pack-2 were related to BAs,

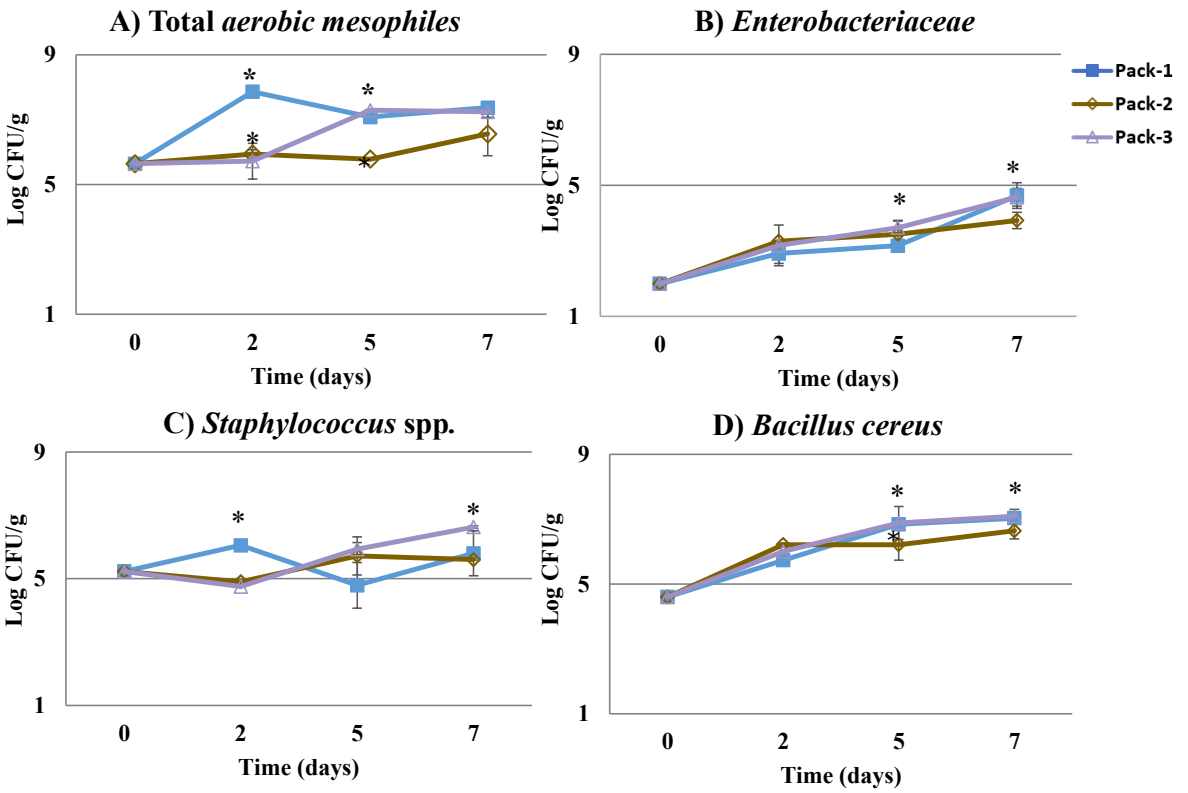

Fig. 4 Changes of microorganism of cooked ham inside Pack-1, Pack-2 and Pack-3 during 7 days of storage. A), Total aerobic mesophiles; B), *Enterobacteriaceae*; C), *Staphylococcus* spp.; D), *Bacillus cereus*. * Statistically significant different ($p < 0.05$, Student's t-test).

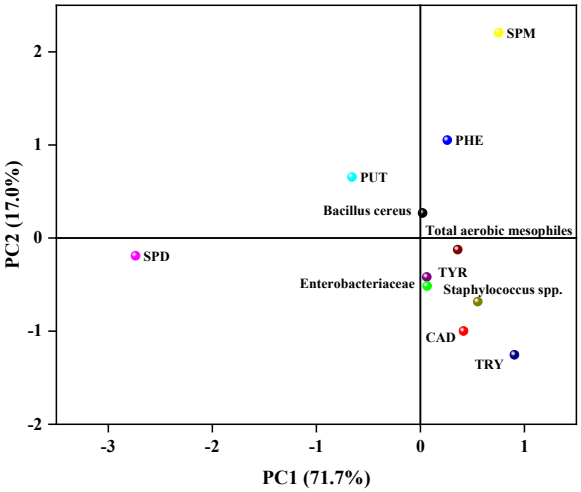

Fig. 5 Relationships among BAs and microbial counts properties of Pack-1 sample obtained by PCA.

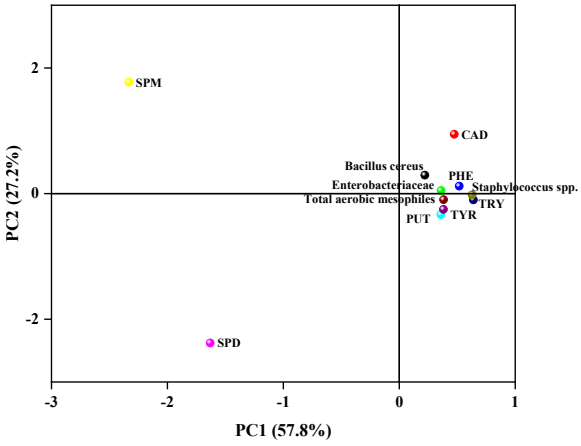

Fig. 6 Relationships among BAs and microbial counts properties of Pack-2 sample obtained by PCA.

and microbial analysis had high values on the positive side of PC1, except spermidine and spermine. In Fig. 7, all indicators were on the positive side of PC1, except spermidine. In particular, spermine, cadaverine and tryptamine had high values on the positive side of PC1. Tyramine, phenylethylamine, and microbial indicators had a lower value on the positive side of PC1. The indicators for the Pack-3 samples were cadaverine, tryptamine and spermine, which could contribute to the reduction of cooked ham shelf life, while other BAs

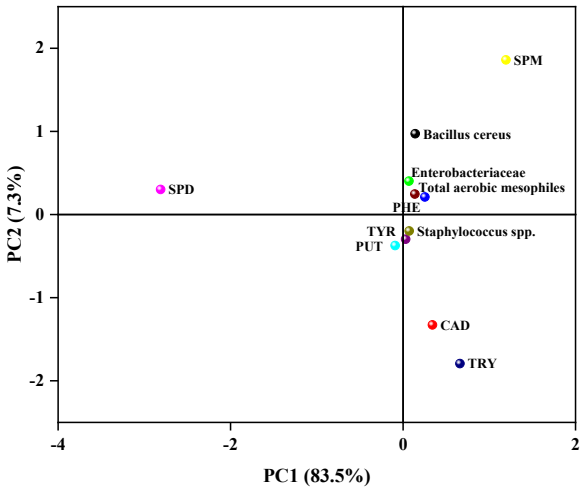

Fig. 7 Relationships among BAs and microbial counts properties of Pack-3 sample obtained by PCA.

and microbial indicators did not reflect the shelf life.

There were significant differences in BAs in the 3 types of packaging. Similar values were found by different authors. Under different packing conditions, packaging affected the formation of BAs during storage of sardines [22]. Cadaverine and tryptamine could be used as spoilage indicators for trout (Salmo trutta) in ice storage [23]. Galgano indicated that cadaverine was mainly associated with the number of *Enterobacteriaceae*; while *B. cereus* was strongly correlated with tyramine formation and, to a lesser extent, with putrescine, cadaverine, and histamine [24].

The changes in BAs, microbial content, and pH during ham preservation are very small compared with meat [25], likely due to the reduction in moisture and increased NaCl concentration in the ham. Studies have shown that NaCl concentration has a significant effect on endogenous microbiota (Lactic acid bacteria, *mesophilic aerobic* bacteria, *psychrotrophic* bacteria, *Staphylococcus* spp., and Enterobacteriaceae) and biological amine content (histamine, tyramine, putrescine, cadaveramine, and spermatine) in dry-cured meat. The higher NaCl cluster showed a lower content of BAs. A negative correlation between microbial count and BA content in the lower NaCl cluster suggested that the higher BA content in the lower NaCl cluster might be the result of a stress response mechanism. On the other hand, the salt concentration in the higher NaCl cluster had an inhibitory effect on the formation of BA except histamine. The collective results suggested a NaCl threshold that minimizes the formation of BAs in dry cured meats [26].

CONCLUSION

In our study, we mainly analyzed 3 types of packaging, assembled in 3 different ways for their ability to preserve cooked ham. The analytical methods based on extraction, derivatization, purification, and determination in HPLC-DAD allowed us to quantify the content of BAs in ham inside different types of packaging. The results showed that a chemical quality index of all the samples studied was lower than 3. The total content of BAs showed slight differences during shelf life. Chemical, microbiological, and pH analyzes confirmed the equivalence of the different packaging. It could be concluded that packaging provided a high level of food safety for consumer protection.

However, Pack-2 (polycoupled sheet + metallized bag) had an exceptionally high efficiency compared with Pack-1 (multilayer sheet + multilayer bag) and Pack-3 (polycoupled sheet + copper bag). The samples stored in the 3 types of packaging did not show a significant increase in BAs during the study. The packaging system could help distinguish the development and correlation of BAs and spoilage-related microorganisms in the product by PCA analysis. PC1 was positively related to microbial analysis and protein change (parts of BA content). There were significant differences in tyramine, putrescine and spermine levels among the 3 types of packaging. In conclusion, the results showed that PC1 could distinguish the indicators from the packaging conditions; cadaverine, tryptamine and phenylethylamine could be used as spoilage indicators for ham.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.044.

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Appendix A. Supplementary data

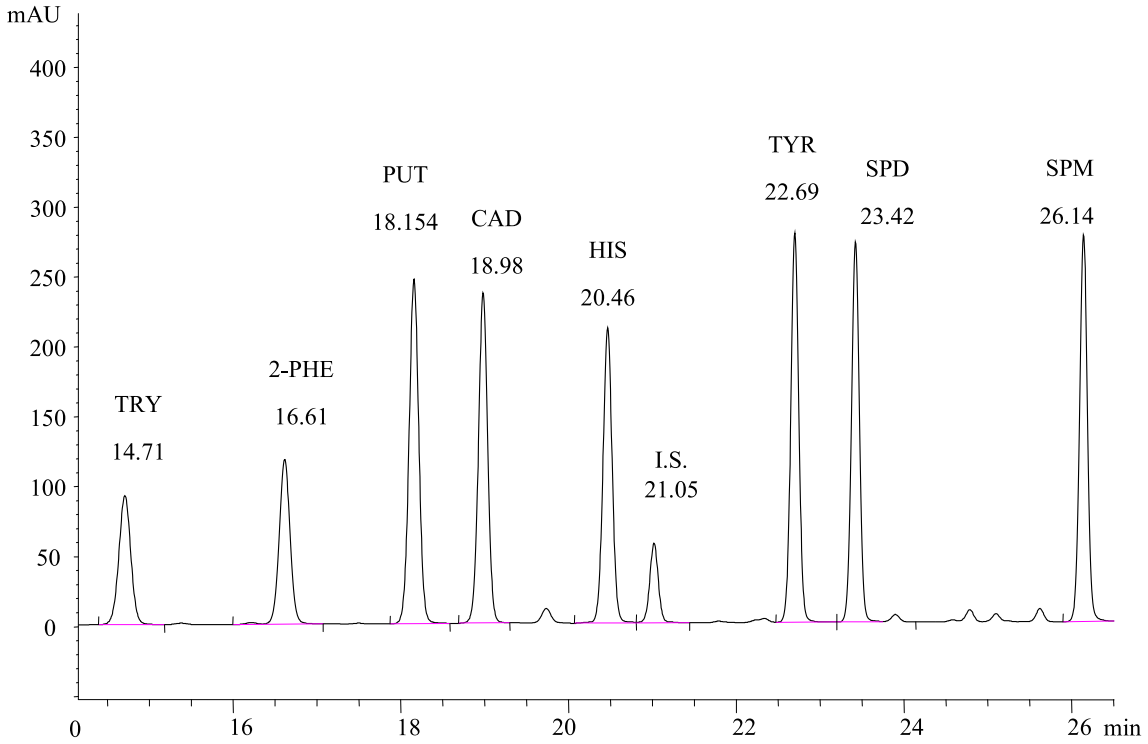

Fig. S1 HPLC-DAD chromatogram of a 25 mg/l standard solution of all analysed BAs and Internal Standard. Legend: Tryptamine (TRY), 2-Phenylethylamine (2-PHE), Putrescine (PUT), Cadaverine (CAD), Histamine (HIS), 1,7-Diaminoheptane Internal Standard (I.S.), Tyramine (TYR), Spermidine (SPD), Spermine (SPM).