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ORIGINAL ARTICLE

Effects of Garlic Extract and Lemon Juice on the Shelf Life of Fermented and Dried Fish Fillets (Lanhouin) during Storage

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Abstract

The effect of natural additives (lemon juice, garlic extract or their mixture) on the shelf life of Lanhouin fillets, a fermented fish, was investigated by monitoring the microbiological, physico-chemical and sensory changes in treated fillet samples and control. All analyzed samples showed a decreasing trend in water activity and total viable count during the storage period, while an increasing trend was recorded for the pH, total volatile nitrogen and biogenic amines at both 4 and 30°C storage conditions. The highest histamine content in the treated filet samples was 10.5 mg/kg while the maximal threshold suggested by European regulation for such fishery products is 20 mg/100g. The sensory evaluation revealed that the Lanhouin fillets treated with lemon juice, garlic extract or their mixture and stored at 4°C for 90 days exhibited a significantly higher visual appearance, odour and overall acceptability scores compared to those stored at 30°C. These ingredients which are readily available and affordable could be valorized in the production of Lanhouin as preservative agents in the cottage industry of West African countries.

Practical Application

Microbiological, physico-chemical and sensory changes are the main problems in fermented dried fish, especially during storage. The use of natural such as lemon juice, garlic extract or their mixture as natural additives could be an effective technique to improve the preservation of fermented dried fish.

Keywords: Lanhouin fillets, fermentation, histamine, garlic extract, lemon juice, storage.

1. Introduction

Salting and fermentation of fish are two main traditional preservation techniques used in many African countries to produce salted and fermented fish products with tender consistency and specific aroma (Anihouvi, Sakyi-Dawson, Ayenor, & Hounhouigan, 2009).

Preservative effect of salt has been associated to a decrease of water activity and less availability of free water for microbial spoilage (Anihouvi *et al.,* 2012; Kose and Hall 2011; Xu *et al.,* 2019; Zang *et al.,* 2020). However, bacteria activity depends on the processing and storage conditions of fish. Good storage conditions can stop or limit

microbial activity and spoilage. Storage conditions may also influence the sensory profile of the end-products. Therefore, storage conditions play an important role on the stability of fermented salted fish quality. To ensure the availability and accessibility to fishery products, different preservation techniques are commonly used (Rahman *et al.,* 2012; Thaker *et al.,* 2017). Nowadays, consumers require less preserved foods with maximum nutrient retention, without the addition of chemical preservatives. An increasing interest in the use of natural antimicrobials agents as food preservative has been reported by several authors (Boziaris *et al.,* 2011). Natural aromatic plants and spices have been widely used in many food products as flavouring, seasoning, antimicrobial and antioxidant agents (Akpo-Djènontin *et al.,* 2016). Spices and citrus fruit are also added to food such as fish and meat to delay the onset of rancidity and to reduce microbial proliferation (Kindossi *et al.,* 2012; Kumolu-Johnson et Ndimele 2011a; Saritha et Patterson 2012). The inhibitory effect of garlic on bacteria has been reported by Mah *et al.* (2009) for fermented fish preservation. Other studies on the preservation of fish products also showed that spices (garlic, ginger) and citrus fruit improve the stability of fish products during storage (Mah *et al.,* 2009). In Benin, Lanhouin, a fermented and dried fish is traditionally produced in cottage industries leading to unstable end-product with different quality (Anihouvi *et al.,* 2006; Kindossi *et al.,* 2016). To improve Lanhouin quality, ripening, fermentation and drying are the main steps of its production which need specific actions. The drying step was identified to be a critical point where specific actions should be applied. In Benin where plant materials such as garlic and lemon are readily available and affordable (Akpo-Djènontin *et al.*, 2016), their efficacy as antimicrobial agents in combination with ripening in brine could offer a safe approach to deal with major problems associated with the traditional processing and storage of Lanhouin.

Therefore, this study aims at assessing the physico-chemical, microbiological and sensory changes in Lanhouin fillets treated with garlic extract, lemon juice and their mixture stored at 4°C or at ambient temperature (30 ± 2°C).

2. Materials and Methods

2.1 Production and sampling of fermented and dried fillets

Fresh cassava fish (*Pseudotolithus* sp.) was bought at Cotonou seaport (Benin). The fish was transported in an ice box with dry ice to the Laboratory. The fish was washed, scaled, eviscerated, beheaded and washed twice before filleting. The fresh fillets (approximately 100 g per filet) were then ripened in brine (4% salt/mass of fillet) for 4 hours. The ripened fillets were salted (15 g of salt / 100 g of fillet), arranged in a perforated plastic bucket and fermented spontaneously during 4 days at ambient temperature (30 ± 2°C). The product obtained was called Lanhouin fillet.

2.2 Preparation of treatment solutions

Two preservative agents: lemon (*Citrus limon*) and garlic (*Allium sativum*) bought from the local market of Benin were used to treat the Lanhouin fillets.

2.3 Lemon juice solution (LJS)

The 8% lemon juice solution was prepared from 8 ml of pure lemon juice and 92 ml of salt solution (1 g of salt for 100 ml of water). The mixture was then filtered using a 0.63 µm diameter sieve.

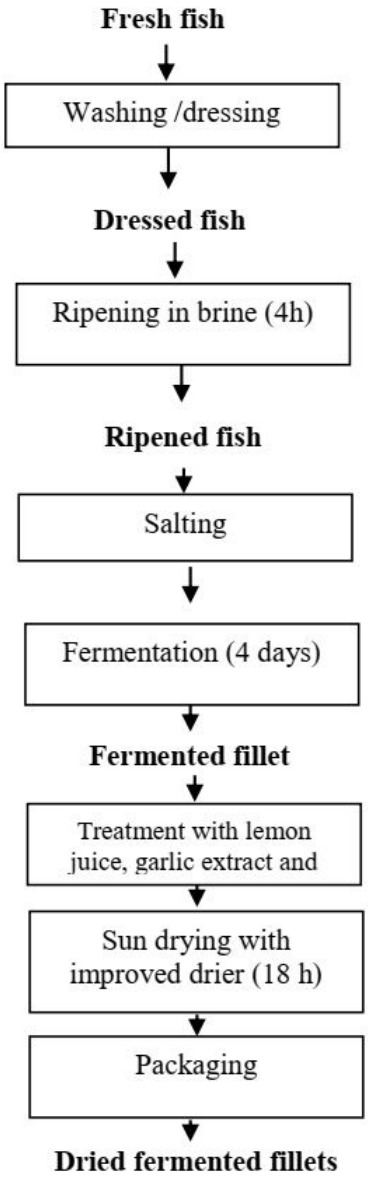

Figure 1: Process flow diagram for the production of Lanhouin fillets

2.4. Garlic extract solution (GES)

The garlic extract solution 8% was prepared by mixing 8 ml of garlic juice extracted from 150 g of garlic cloves in 92 ml of salt solution (1 g of salt for 100 ml of water). The mixture was then filtered using a sieve 0.63 µm diameter sieve.

2.5. Mixed solution of lemon juice and garlic extract (LJGS)

The mixed solution of lemon juice and garlic extracts was prepared from equal quantities of lemon juice solution and garlic extract solution.

2.6. Treatment of Lanhouin fillets samples before storage

At the end of fermentation and before drying, the fermented fish fillets were rinsed with tap water and divided into four batches each containing, approximately 40 fermented fish fillets. Three batches of fermented fish filets were respectively immersed into 80 ml of lemon juice, garlic extract solution and their mixture for 5 min. The fourth batch was only in salt solution and served as control.

2.7. Drying of treated and untreated fermented fish fillet samples

All the fermented fish fillet samples were dried using a solar drier of GERES/GRET for 18 hours according to processors' habits as reported by Kindossi *et al.* (2012). After drying, the treated fermented fish fillet samples from each solution and control were packed in plastic bag (Type Walovac 90 B), divided into two portions and stored in the refrigerator at 4°C and at ambient temperature (30 ± 2°C) for 90 days.

2.8. Sampling

Samples of Lanhouin fillets were collected at 0, 60 and 90 days of storage for the microbiological, physico-chemical and sensory analyses.

2.9. Microbiological analysis

Ten (10) g of each sample was introduced aseptically in a sterile stomacher bag and 90 ml of sterile diluent containing 0.1% peptone (Oxoid L37, Basingstoke, Hampshire, England)

and 0.8% sodium chloride (NaCl) (Merck KGaA, Germany) with pH adjusted to 7.2. The mixture was then homogenized for two min using a Stomacher (Lab-Blender, Model 80, Seward Medical, and London, UK) (ISO-6887-1, 1999). One ml of suspension was serially diluted and used for microbial counts according to ISO norms. Total viable counts (TVC) of *Enterobacteriaceae* and *Staphylococcus aureus* were enumerated using Plate Count Agar (PCA, Oxoid CM0325, Basingstoke, Hampshire, England) (ISO-4833, 2003), Violet Red Bile Glucose Agar (VRBG, Oxoid, CM0485, Basingstoke, Hampshire, England) (ISO-21528, 2004) and Baird Parker agar base (Oxoid CM0275, Basingstoke, Hampshire, England) supplemented with egg yolk tellurite emulsion (SR54, Basingstoke, Hampshire, England) (ISO-6888, 1999) respectively.

2.10. Physico-chemical analysis

All the Lanhouin fillet samples were firstly ground using porcelain pestle and mortar before physico-chemical analysis. The pH of samples was measured using a pH meter (Hanna Instrument HI 9318) according to the reference method (ISO-2917, 1999). Moisture content was determined by drying 5 g of ground sample at 103 ± 2 °C till constant weight using an oven (Heraeus T5042, Geprufte Sicherheit) according to the Association of Official Analytical Chemists (AOAC) method 950.46 (AOAC, 1995). Water activity (a_w) was measured according to the reference method (ISO-21807, 2005), using a thermo-hygrometer recorder C056696 (Rotronic-Hygrolab 2, 8303 Bassersdorf). Total volatile nitrogen (TVN) was estimated according to the method recommended by the European Commission (EC, 2005). In fact, 10 g of ground Lanhouin was homogenized with 90 ml perchloric acid 6% (SIGMA) solution for 2 min. After filtering, 50 ml of extract solution was alkalinized with 20% solution hydroxide, and submitted to steam distillation. The steam distiller (Büchi distillation Unit K-350, Switzerland) was set to produce approximately 100 ml of distillate in 10 min. the volatile base components absorbed by 3% of boric acid solution in a beaker were determined by titration using hydrochloric acid solution (0.01 mol).

Biogenic amines (histamine, cadaverine, putrescine and spermidine) were analyzed according to a modified method developed by Mah *et al.* (2002). 50 mg of Lanhouin sample was homogenized and extracted with 3 ml of 0.4 M perchloric acid solution following homogenization and centrifugation at 3000 x g. 250 µl of the sample extract-was mixed with 50 µl of 2 M sodium hydroxide and 75 µl saturated sodium bicarbonate (Na_2CO_3). Derivatisation step was performed with 500 µl of dansyl chloride incubated at 60°C for 45 min in the dark. After this period, 25µl of ammonium hydroxide (25%) was added to remove the excess of dansyl chloride and the mixture was left to stand in the dark for 30 min at ambient temperature. After this second period, 375 µl of acetonitrile was added and the mixture was filtered using a 0.45 µm pore-size filter (Millipore Co., Bedford, MA). 10 µl of the mixture was injected on HPLC (Knauer system, Germany) equipped with a-Rheodyne 7125 injector, an on-line solvent degasser with LPG Smartline manager 5050 (ADA110606103, Knauer, Germany), Smartline RI Detector 2300 (n°110542, Knauer, Germany) and Spectra system UV2000, a Knauer-system controller-Smartline, a pump 1000 (n°111235, Knauer, Germany) as described by Kindossi *et al.* (2016).

2.11. Sensory evaluation of Lanhouin fillet samples

Lanhouin fillet samples kept for different storage periods (0, 60 and 90 days) were evaluated for visual appearance (texture, colour), odour and overall acceptability using a 9 points verbal hedonic box scale which varied from 'extremely dislike' to 'extremely like'(Kindossi *et al.*, 2013). Twenty one naive people were chosen to form the panel.

2.12. Statistical analysis

The Statistical software (version 7.1, StatSoft France, 2006) was used to analyze the data and significance was accepted at probability $p < 0.05$ with two-way analysis of variance (ANOVA). Least Significant Difference of Fisher post-hoc test was used for means structuration.

3. Results and Discussion

3.1 Effect of preservative agents on quality changes of Lanhouin fillet samples during storage

Figure 2 shows the changes in total viable counts (TVC) of control and Lanhouin fillet samples treated with lemon juice (LJS), garlic extract (GES) and their mixture (LJGS), and stored at 30 ± 2°C and at 4°C. All samples showed a decreasing trend in TVC with storage period. The decrease in TVC can be due to the decrease in water activity and this together with the accumulated salt in the flesh, led to the suppression of bacteria growth during storage period. TVC of Lanhouin fillet samples treated with LJS was lower than that of Lanhouin fillet samples treated with GES. The TVC of Lanhouin fillet samples treated with the mixture of lemon juice and garlic extract (LJGS) was lower than those of Lanhouin fillet treated with each solution.

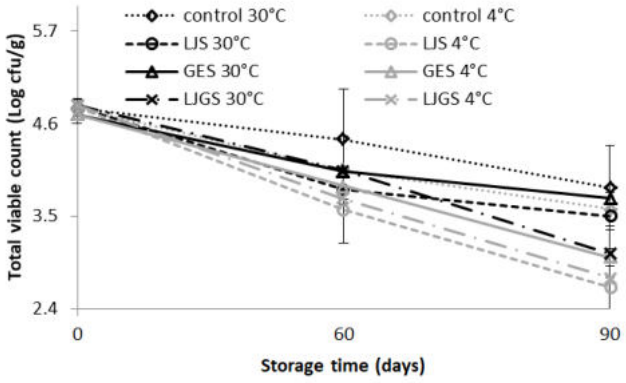

Figure 2: Changes in total viable count of Lanhouin fillets during storage. Control 30°C, control 4°C: untreated Lanhouin samples storage at 30°C and 4°C respectively LJS 30°C, LJS 4°C: Lanhouin treated with lemon juice solution and stored at 30°C and 4°C GES 30°C, GES 4°C: Lanhouin treated with garlic extract solution and stored at 30°C and 4°C LJGS 30°C, LJGS 4°C: Lanhouin treated with mixture of lemon juice and garlic extract solution, and stored at 30°C and 4°C

TVC of treated Lanhouin fillet samples were significantly ($p < 0.05$) lower than that of the control. Lanhouin fillet samples treated with mixed solutions exhibited synergistic effect with a high reduction of TVC. This can be attributed to their bactericidal effect. However, the Lanhouin fillet samples treated with lemon juice (LJS), garlic extract (GES) and their mixture (LJGS), and stored at 4°C presented the lowest TVC loads followed by samples stored at 30°C. TVC of treated Lanhouin fillet samples were significantly lower ($p < 0.05$) than that of the control after 60 days storage. This difference can be attributed to the action of lemon juice and garlic extract used to treat the Lanhouin fillet samples. These results confirm the antimicrobial activity of garlic extract and lemon juice against microbial growth as reported by Mah *et al.* (2009) and Prasongwatana *et al.* (2013). Similar results were reported on chicken sausage treated with different concentrations of garlic paste and stored at 3-4 °C for 21 days (Sallam *et al.*, 2004),

and on smoked catfish treated with different concentrations of fresh ginger and stored at 25-30 °C for 21 days (Kumolu-Johnson et Ndimele, 2011). Likewise, *Staphylococcus aureus* was detected in all the samples (<1-3.6 Log cfu/g), while *Enterobacteriaceae* was lower than 1 Log cfu/g for all Lanhouin fillet samples during the entire storage period (**Table 1**). The changes in. *aureus* were observed for control samples at ambient temperature. Keeping Lanhouin samples at ambient temperature without lemon juice and garlic extract is favourable for microbial growth which increases more with storage time, than at 4 °C. The European Commission (EC/n°2073, 2005) limits the level of *S. aureus* in fish products which is between 2 and 3 Log cfu/g. So, after 90 days, Lanhouin samples stored at ambient temperature without lemon juice and garlic extract were not in agreement with this regulation.

The a_w values of Lanhouin fillet samples stored at 4 °C decreased with storage time (**Figure 3**). The decrease in a_w during storage can be attributed to the loss of water-holding capacity of the protein found in Lanhouin fillet samples (Kilinc *et al*, 2006). The a_w of treated Lanhouin fillet samples stored at 4 °C was significantly ($p < 0.05$) lower than those of treated Lanhouin samples stored at 30 °C. This might be due to the fact that treated Lanhouin samples stored at 30 °C regained moisture. In addition, the a_w of treated Lanhouin fillet samples was significantly ($p < 0.05$) lower than that of the control under all storage conditions. This may be attributed to the effect of lemon juice and garlic extract used. The pH values of stored Lanhouin fillets were statistically different ($p < 0.05$) and increased gradually with storage period (**Figure 4**). The Lanhouin fillet samples treated with lemon juice (6.63) and the mixture of LJGS (6.62), and

Table 1 : Changes in *Enterobacteriaceae and Staphylococcus aureus* of Lanhouin fillets kept at variable storage conditions

Parameters	Storage condition	Storage period	Lanhouin fillets			
			Control	Treated with lemon juice	Treated with garlic extract	Treated with mixed lemon juice and garlic extract
Enterobacteriaceae	30°C	First day	<1	<1	<1	<1
		60 days	<1	<1	<1	<1
		90 days	<1	<1	<1	<1
	4°C	60 days	<1	<1	<1	<1
		90 days	<1	<1	<1	<1
Staphylococcus aureus	30°C	First day	1.7 ± 1.0	<1	<1	<1
		60 days	1.7 ± 1.0	<1	<1	<1
		90 days	2.2 ± 1.4	<1	<1	<1
	4°C	60 days	<1	<1	<1	<1
		90 days	<1	<1	<1	<1

stored at 4°C had the lower pH values because of the presence of citric acid contained in the lemon juice. In this research, lemon juice, garlic extract and their mixture did not have any significant ($p > 0.05$) effect on the pH changes of Lanhouin fillets as compared to the control. Moreover, no significant ($p > 0.05$) differences were recorded between the three treatments, same for the storage conditions.

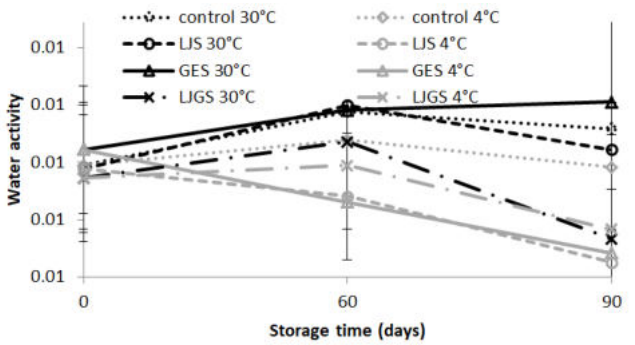

Figure 3: Changes in water activity of Lanhouin fillets during storage. Control 30°C, control 4°C: untreated Lanhouin samples storage at 30°C and 4°C respectively LJS 30°C, LJS 4°C: Lanhouin treated with lemon juice solution, and stored at 30°C and 4°C GES 30°C, GES 4°C: Lanhouin treated with garlic extract solution, and stored at 30°C and 4°C LJGS 30°C, LJGS 4°C: Lanhouin treated with mixture of lemon juice and garlic extract solution, and stored at 30°C and 4°C

Figure 5 shows the changes in TVN of control, Lanhouin treated with lemon juice and garlic extract and their mixture. TVN in control samples reached 129.7 and 115.6 mg N/100 g after 90 days of storage at 30°C and 4 °C respectively. The treated Lanhouin fillet samples stored at 30°C and the control stored at 4°C showed an increasing trend in TVN with storage period. However, the TVN values of treated Lanhouin fillet samples stored at 4°C were almost stable with small fluctuations during the storage period. The preservative agents used had significant effect ($p < 0.05$) on the TVN contents of treated Lanhouin fillet samples. TVN production in fish flesh is mainly associated with bacterial decomposition (Özyurt *et al.*, 2009).

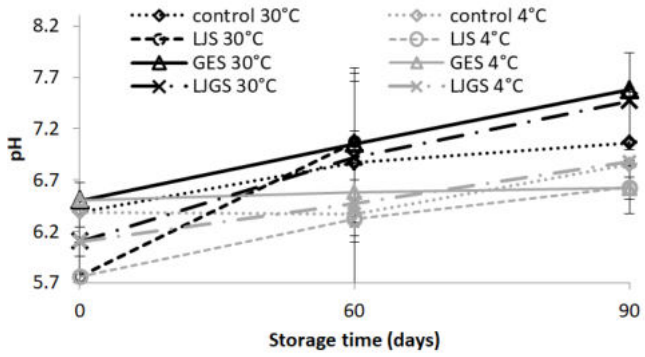

Figure 4: Changes in pH of Lanhouin fillets during storage period. Control 30°C, control 4°C: untreated Lanhouin samples storage at 30°C and 4°C respectively LJS 30°C, LJS 4°C: Lanhouin treated with lemon juice solution, and stored at 30°C and 4°C GES 30°C, GES 4°C: Lanhouin treated with garlic extract solution, and stored at 30°C and 4°C LJGS 30°C, LJGS 4°C: Lanhouin treated with mixture of lemon juice and garlic extract solution, and stored at 30°C and 4°C

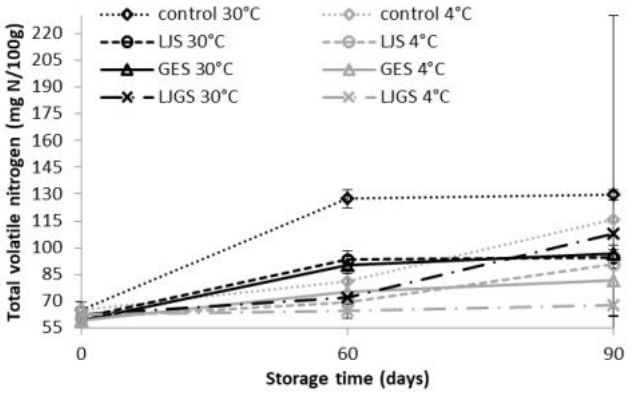

Figure 5: Changes in total volatile nitrogen of Lanhouin fillets during storage period. Control 30°C, control 4°C: untreated Lanhouin samples storage at 30°C and 4°C respectively LJS 30°C, LJS 4°C: Lanhouin treated with lemon juice solution, and stored at 30°C and 4°C GES 30°C, GES 4°C: Lanhouin treated with garlic extract solution, and stored at 30°C and 4°C LJGS 30°C, LJGS 4°C: Lanhouin treated with mixture of lemon juice and garlic extract solution, and stored at 30°C and 4°C

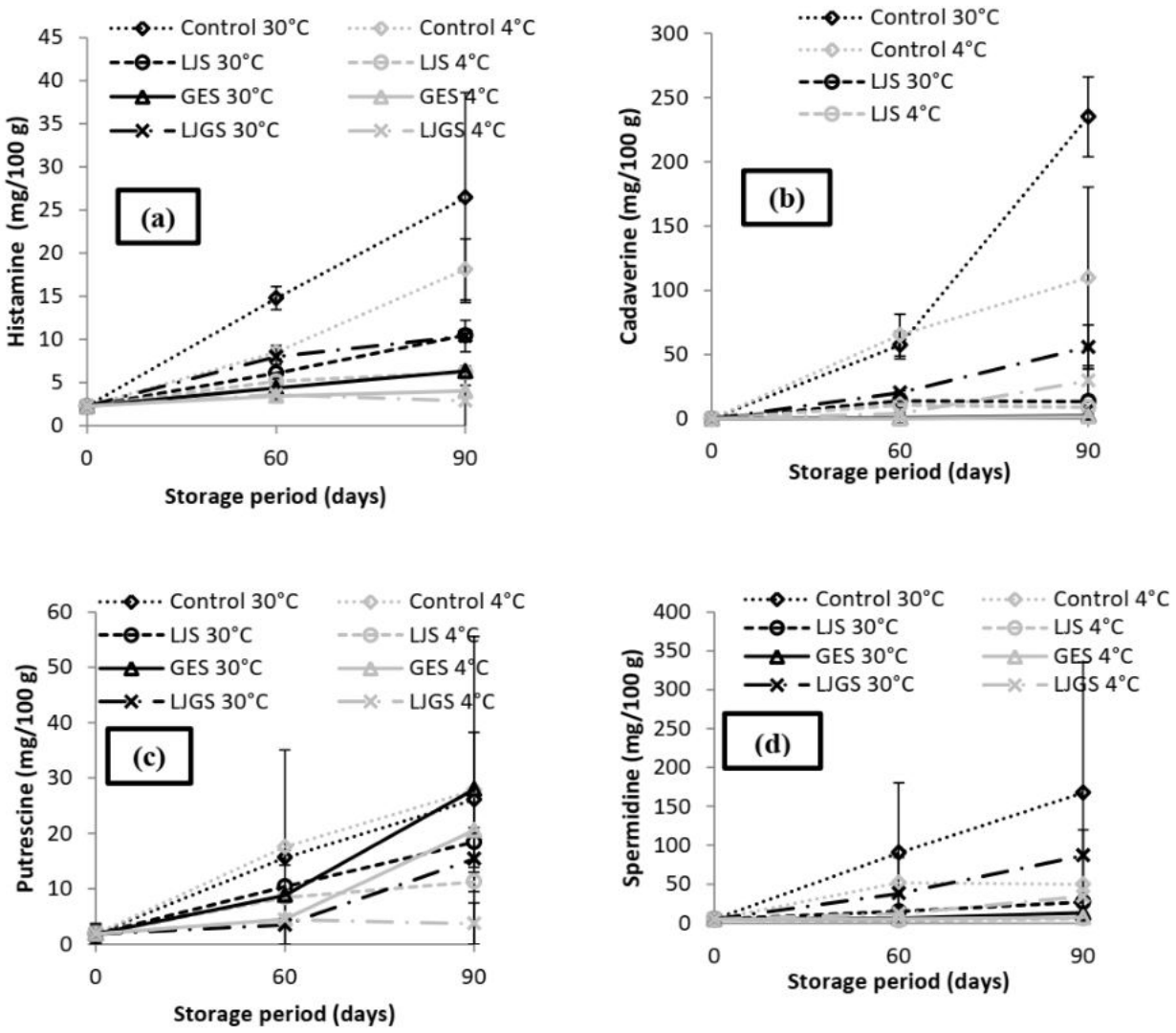

Figure 5: Changes in biogenic amines of Lanhouin fillets during storage period. Control 30°C, control 4°C: untreated Lanhouin samples storage at 30°C and 4°C respectively LJS 30°C, LJS 4°C: Lanhouin treated with lemon juice solution, and stored at 30°C and 4°C GES 30°C, GES 4°C: Lanhouin treated with garlic extract solution, and stored at 30°C and 4°C LJGS 30°C, LJGS 4°C: Lanhouin treated with mixture of lemon juice and garlic extract solution, and stored at 30°C and 4°C

Figure 6 shows the contents of biogenic amines in Lanhouin fillet samples treated with LJS, GES, and LJGS and untreated (control) during 90 days of storage at 4 and 30°C. All the biogenic amines increased progressively during storage. In the first storage period of 60 days, histamine contents increased from 2.24 to 5.13 mg/100 g (4°C) and from 2.24 to 6.10 mg/100 g (30°C) in Lanhouin fillet samples treated with LJS; from 2.10 to 3.44 mg/100 g (4°C) and from 2.10 to 4.38 mg/100 g (30°C) in Lanhouin fillet samples treated with GES and for Lanhouin fillet samples treated with LJGS (**Figure 6a**). When stored at 4°C for 90 days, the histamine contents of Lanhouin fillet samples treated with LJS, GES and LJGS increased from about 2.2 (Control, first day) to 6.18 mg/100 g (LJS), 4.02 mg/100 g (GES) and 3.36 mg/100 g (LJGS). When stored

at 30°C for 90 days, the histamine contents of Lanhouin fillet samples treated with LJS, GES and LJGS increased to 26.45 mg/100 g (Control), 10.48 mg/100 g (LJS), 6.30 mg/100 g (GES) and 10.38 mg/100 g (LJGS). However, the histamine content (26.45 mg/100g) in control stored at 30°C exceeded the maximal limit (20 mg/100g) set by European Union regulation for fishery products which have undergone enzyme maturation treatment in brine (EC/n°2073, 2005). All the treated Lanhouin fillet samples were safe during the 90 days of storage at 4°C and 30 °C. There was a significant ($p < 0.05$) difference between samples stored at 4°C and those stored at 30°C. This indicates that the storage temperature has a significant effect on histamine content of Lanhouin fillet samples, confirming the findings of Silva *et al.* (1998) and Naila *et al.* (2010). Also, the histamine contents in treated Lanhouin fillet samples were significantly ($p < 0.05$) lower than those in control after 60 days storage and under all storage conditions. The histamine content in Lanhouin fillet samples treated with GES and stored at 30°C for 60 days was significantly ($p < 0.05$) lower than that in Lanhouin fillet samples treated with LJGS. However, no significant difference ($p > 0.05$) was recorded between the histamine contents of Lanhouin fillet samples treated with LJS and GES; and Lanhouin fillet samples treated with LJS and LJGS. The histamine content in Lanhouin fillet samples treated with LJGS and stored at 4°C for 60 days was significantly ($p < 0.05$) lower than that in Lanhouin fillet samples treated with LJS. However, it was not significantly different ($p>0.05$) from that in Lanhouin fillet samples treated with GES. The greatest reduction effect on histamine was observed in samples treated with garlic extract and lemon juice. The histamine values were similar to 11.7 mg/100g reported by Mah *et al.* (2009) in Myeolchi-jeot, a Korean salted and fermented anchovy treated with garlic extract.

An increase in cadaverine level for 90 days storage was also recorded (**Figure 6b**). There was a significant ($p < 0.05$) difference between cadaverine content of samples stored at 4°C and cadaverine of samples stored at 30°C. The cadaverine contents of treated Lanhouin fillet samples were significantly lower than those of the control and under all the storage conditions. However, no significant ($p > 0.05$) difference in the cadaverine content was observed between treated Lanhouin fillet samples. Garlic extract and lemon significantly delayed the increase of cadaverine content in Lanhouin fillet samples.

An increase in putrescine level during 90 days storage was also recorded (**Figure 6c**). During the first 60 days, the putrescine level increased significantly ($p < 0.05$) in the control sample stored at 4°C and 30°C, and in Lanhouin fillets treated with LJS, GES and LJGS respectively and stored at 4 and 30°C respectively. After 90 days storage, the putrescine was still increasing in the control, in Lanhouin fillets treated with LJS, GES and LJGS and stored at 4°C and 30°C respectively. No significant difference in putrescine content was recorded between samples stored at 4°C and those stored at 30°C. This indicates that the temperature had no significant ($p > 0.05$) impact on the putrescine content of Lanhouin fillet samples during the storage periods. The putrescine contents of treated Lanhouin fillet samples were significantly lower than that of the control under different storage conditions. The putrescine content in Lanhouin fillets treated with LJGS was significantly ($p < 0.05$) lower than those in Lanhouin fillet samples treated with GES or with LJS, showing the effectiveness of the LJGS on

the reduction of putrescine compared to LJS or GES.

An increase in spermidine level during the 90 days storage period was also recorded (Figure 6d). During the first 60 days, the spermidine level increased significantly ($p < 0.05$) in the control (4°C and 30°C), in Lanhouin fillets treated with LJS, GES, LJGS and stored at 4°C and 30°C respectively. There was no significant ($p > 0.05$) difference in the spermidine content of samples stored at 4 and 30°C respectively. This indicates that the temperature had no significant ($p > 0.05$) effect on the spermidine content of Lanhouin fillet samples during storage period. At 30°C, the spermidine contents of treated Lanhouin fillet samples were significantly lower than those of the control. Garlic extract and lemon juice presented significant ($p < 0.05$) reduction effect on the cadaverine content of Lanhouin fillet samples stored at 30°C. However, no significant ($p > 0.05$) difference in spermidine content was recorded between treated Lanhouin fillet samples. Additionally, at 4°C, no difference in spermidine content was recorded between all Lanhouin fillet samples. The low storage temperature (4°C) had no effect on the evolution of spermidine in Lanhouin fillet samples treated with garlic extract or lemon juice. These low levels of biogenic amine especially those of histamine in treated Lanhouin samples are in agreement with the acceptable level (EC/n°2073, 2005).

3.2. Sensory evaluation of Lanhouin fillet samples after storage period

Table 2 shows the average scores for the appreciation of the visual appearance (texture, colour), the odour and the overall acceptability of Lanhouin fillets according to the treatment and storage period. For the visual appearance (texture, colour), the control and treated Lanhouin fillet samples received score values varying from 7.0 to 7.5. The treated Lanhouin fillet samples treated with lemon juice exhibited the lowest score of 7.0 while the highest score was attributed to the Lanhouin fillet samples treated with mixed lemon juice and garlic extract. However the recorded scores for appearance and for the control (7.3) were not significantly different ($p > 0.05$) from those of the treated Lanhouin fillet samples (7.0 - 7.5) at the 60^{th} storage day.

The visual appearance scores of control samples stored at 30°C for 60 (6.5) and 90 days (6.2) were significantly ($p < 0.05$) higher than those of treated Lanhouin fillet samples (score < 5) stored under similar conditions. However, the visual appearance of the control and Lanhouin fillet samples treated with garlic extract and stored at 4 °C for 60 and 90 days were significantly ($p < 0.05$) higher than those of Lanhouin fillet samples treated with lemon juice and the mixture of LJGS, and stored in the same conditions. The visual appearance scores of treated Lanhouin fillet samples were significantly ($p < 0.05$) affected at 30°C than 4°C. It appears that for the control samples, the longer the storage period, the lesser the visual appearance was appreciated by the consumers. Same goes for all other storage methods. The control and treated Lanhouin fillet samples stored at 4°C presented an acceptable visual appearance score (5). Garlic extract preserved the appearance of Lanhouin fillet samples during storage at 4°C.

The score for odour of Lanhouin fillet samples is presented in **Table 2**. The odour of fermented fish mainly originates from the breakdown of protein and lipid through the action of microbial and endogenous enzymes (Anihouvi *et al.*, 2009; Zang *et al.*, 2020). Also the development of

odour is affected by the product formulation particularly spices, preservatives, processing conditions (Kaban et Kaya, 2009).

Table 1: Sensory evaluation scores of Lanhouin fillets kept at variable storage conditions

Sensory attributors	Storage condition	Storage period	Lanhouin fillets			
			Control	Treated with lemon juice	Treated with garlic extract	Treated with mixed lemon juice and garlic extract
visual appearance (texture, color)		First day	7.3 ± 0.3^{a1}	7.0 ± 0.0^{a1}	7.1 ± 0.7^{a1}	7.5 ± 0.3^{a1}
	$30°C^{e}$	60 days	6.5 ± 0.3^{b2}	3.6 ± 0.1^{c2}	4.1 ± 0.4^{a2}	4.6 ± 0.4^{a2}
		90 days	6.2 ± 0.3^{b2}	3.5 ± 0.7^{a2}	4.5 ± 2.1^{ab2}	4.1 ± 0.1^{a2}
	$4°C^{f}$	60 days	6.3 ± 0.3^{a2}	4.5 ± 0.7^{b2}	6.2 ± 0.3^{a1}	5.4 ± 0.2^{b2}
		90 days	5.9 ± 0.5^{a2}	5.0 ± 1.4^{a2}	7.5 ± 0.7^{b1}	5.2 ± 0.6^{a2}
Odor		First day	5.2 ± 0.6^{a1}	6.5 ± 2.1^{ab12}	7.0 ± 1.4^{ab1}	7.1 ± 0.3^{b1}
	$30°C^{e}$	60 days	3.5 ± 0.3^{a2}	6.0 ± 1.4^{b1}	6.6 ± 0.6^{b1}	6.4 ± 0.4^{b1}
		90 days	2.8 ± 0.8^{a2}	4.5 ± 0.7^{b2}	5.0 ± 1.4^{b1}	5.5 ± 0.2^{b2}
	$4°C^{f}$	60 days	4.7 ± 0.1^{a1}	5.7 ± 1.0^{ab1}	6.5 ± 0.7^{b1}	6.9 ± 0.2^{b1}
		90 days	3.9 ± 0.1^{a2}	5.5 ± 0.7^{b1}	6.0 ± 0.0^{b1}	6.0 ± 0.1^{b2}
Overall acceptability		First day	5.3 ± 0.4^{a1}	6.0 ± 1.4^{ab12}	6.5 ± 0.7^{b1}	6.5 ± 0.2^{b1}
	$30°C^{e}$	60 days	4.0 ± 0.4^{a2}	5.0 ± 1.4^{ab1}	6.0 ± 1.4^{b12}	5.2 ± 0.1^{b2}
		90 days	3.0 ± 0.3^{a3}	4.5 ± 0.7^{b1}	5.0 ± 0.0^{c2}	4.8 ± 0.1^{c3}
	$4°C^{f}$	60 days	4.4 ± 0.2^{a2}	7.0 ± 0.0^{b1}	6.5 ± 0.7^{b1}	6.0 ± 0.2^{b2}
		90 days	4.2 ± 0.6^{ab2}	5.0 ± 0.0^{c2}	5.0 ± 0.0^{b2}	6.0 ± 0.1^{b2}

^{abc}: Means with different letters in the same row are significantly different ($p < 0.05$); ^{1,2,3}: Means with different numbers in the same column with the storage condition are significantly different ($p < 0.05$); ^{ef}: storage conditions with different letters according to each column and each sensory attributors are significantly different effect ($p < 0.05$)

In this work, the odour score of the control was lower than that of treated Lanhouin fillet samples. It was not significantly different ($p > 0.05$) from those treated with LJS and GES but significantly different with the samples treated with the mixture of both solutions. It appears that, the longer the on-going storage period, the lesser the odour is appreciated by the consumers. The odour scores of control samples were significantly lower ($p < 0.05$) than those of treated Lanhouin fillet samples stored under similar conditions.

The odour of all treated Lanhouin fillet samples was acceptable (score ≥ 5) after 60 and 90 days of storage and for all storage conditions. The odour was more appreciated when the Lanhouin fillet samples were kept at 4°C. The odour of Lanhouin fillet samples stored was significantly ($p < 0.05$) and negatively correlated with TVC ($r = -0.57$) and TVN ($r = -0.81$).

These correlations indicate that microorganisms and their metabolites affected the odour scores of Lanhouin fillet samples during storage and under different conditions. These correlations are in line with those found in the literature which reported that microorganisms and the total volatile nitrogen were associated with changes in odour of fermented seafood products (Jaffrès *et al.*, 2011). For the overall acceptability, the average scores of treated Lanhouin fillet samples stored at 30°C and 4°C for 60 and 90 days were significantly higher ($p < 0.05$) than those of the control samples kept under similar conditions. It appears that, the longer the storage period, the lesser the overall acceptability of control is desired; while for the treated Lanhouin fillet samples kept at 4°C, the overall acceptability scores remained almost constant during the entire storage period.

Conclusions

The use of garlic extract and lemon juice was found to significantly improve the shelf life of Lanhouin fillets under the tested storage conditions, especially regarding their microbial and physicochemical parameters. The histamine contents recorded after application of garlic extract and lemon juices individually or in combination during the 90 days of storage at both 4 and 30°C are very low (around 10 mg/kg) to involve any public health issue. In addition, these treatments conferred acceptable scores to visual appearance and odour of Lanhouin fillets when stored at 4°C for 90 days or 30°C for 60 days. These ingredients which are readily available and affordable could be valorized in the production of Lanhouin in the cottage industries of West African countries.

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Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

References

Akpo-Djènontin, D.O.O., Anihouvi, V.B., Vissoh, V.P., Gbaguidi, F. & Soumanou, M. (2016). Processing, storage methods and quality attributes of spices and aromatic herbs in the local merchandising chain in Benin. *African Journal of Agricultural Research*, *11*(37), 3537–3547. https://doi.org/10.5897/AJAR2016.11262

Anihouvi, V.B., Ayernor, G.S., Hounhouigan, J.D. & Sakyi-Dawson, E. (2006). Quality characteristics of lanhouin : A traditionally processed fermented fish product in the Republic of Benin. *African Journal of Food Agriculture Nutrition and Development*, *6*(1), 1–15.

Anihouvi, V.B., Kindossi, J.M. & Hounhouigan, J.D. (2012). Processing and Quality Characteristics of some major Fermented Fish Products from Africa: A Critical Review. *International Research Journal of Biological Sciences*, *1*(7), 72–84.

Anihouvi, V.B., Sakyi-Dawson, E., Ayenor, G.S. & Hounhouigan, J.D. (2009). Biochemical Changes and Aroma Development during the Spontaneous Fermentation of Cassava Fish into Lanhouin and their Influence on Product Acceptability. *Journal of Aquatic Food Product Technology*, *18*, 370–384. https://doi.org/10.1080/10498850903224919

AOAC. (1995). *Official Methods of Analysis of the Association of Official Analytical Chemists*. Washington, USA: AOAC.

Boziaris, I.S., Proestos, C., Kapsokefalou, M. & Komaitis, M. (2011). Antimicrobial Effect of Filipendula ulmaria Plant Extract Against Selected Foodborne Pathogenic and Spoilage Bacteria in Laboratory Media, Fish Flesh and Fish Roe Product. *Food Technology and Biotechnology*, *49*(2), 263–270.

European Commssion. EC/n°2073. (2005). Regulation n°2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, *L 338*, 1–26.

European Commission. EC. (2005). Commission Regulation No 2074/2005, annex II, section II, chapter III. Determination of the concentration of TVBN in fish and fishery products. *Official Journal of the European Union, L338, 27-59.*

ISO-21528. (2004). Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of Enterobacteriaceae – Part 2:Colony-count method *(ISO 21528-2)* (1st ed., pp. 1–10; ISO-Microbiology, Ed.). 1st ed., pp. 1–10.

ISO-21807. (2005). Microbiology of Food and animal feeding stuffs Determination of water activity (ISO, Ed.). International Organization for Standardization.

ISO-2917. (1999). Meat and meat products Measurement of pH (reference method) (2nd ed.; ISO, Ed.). 2nd ed. International Organisation for Standardisation.

ISO-4833. (2003). Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of microorganisms Colony-count technique at 30 °C *(ISO 4833)* (3rd ed., pp. 1–9; ISO-Microbiology, Ed.). 3rd ed., pp. 1–9. Retrieved from http://www.iso.org

ISO-6887-1. (1999). Microbiology of food and animal feeding stuffs Preparation of test samples, initial suspension and decimal dilutions for microbiological examination Part 1: General rules for the preparation of the initial suspension and decimal dilutions (p. 5; ISO-Microbiology, Ed.). p. 5.

ISO-6888. (1999). Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) Part 1: Technique using Baird-Parker agar (ISO 6888-1) (1st ed., pp. 1–11; ISO-Microbiology, Ed.). 1st ed., pp. 1–11.

Jaffrès, E., Lalanne, V., Macé, S., Cornet, J., Cardinal, M., Sérot, T. & Joffraud, J.J. (2011). Sensory characteristics of spoilage and volatile compounds associated with bacteria isolated from cooked and peeled tropical shrimps using SPME-GC-MS analysis. *International Journal of Food Microbiology*, *147*(3), 195–202. https://doi.org/http://dx.doi.org/10.1016/j.ijfoodmicro.2011.04.008

Kaban, G. & Kaya, M. (2009). Effects of Lactobacillus plantarum and Staphylococcus xylosus on the Quality Characteristics of Dry Fermented Sausage "Sucuk". *Journal of Food Science*, *74*(1), 58-63. https://doi.org/10.1111/j.1750-3841.2008.01014.x

Kilinc, B., Cakli, S., Tolasa, S., & Dincer, T. (2006). Chemical, microbiological and sensory changes associated with fish sauce processing. *European Food Research and Technology*, *222*(5-6), 604-613.

Kindossi, J.M, Anihouvi, V.B., Vieira-Dalodé, G., Akissoé, N.H., Jacobs, A., Dlamini, N. & Hounhouigan, D.J. (2012). Production, consumption, and quality attributes of Lanhouin, a fish-based condiment from West Africa. *Food Chain*, *2*(1), 117–130. https://doi.org/10.3362/2046-1887.2012.009

Kindossi, J.M, Akpo-Djenontin, O.O.D., Anihouvi, V.B., Akissoé, N.H., Anne-Laure, D., Vieira Dalodé, G. & Hounhouigan, J.D. (2013). Sensory Evaluation and Consumer Acceptability of an

African Fish Based Flavouring Agent and Taste Enhancer. *Indian Journal of Applied Research*, *3*(8), 317–321. https://doi.org/10.15373/2249555X

Kindossi, J.M., Anihouvi, V.B., Akpo-Djenontin, O.O.D., Vieira-Dalodé, G., Hounsou, M., Akissoé, N. H. & Hounhouigan, J.D. (2016). Microbial population and physico-chemical composition of an African Fish based flavouring agent and taste enhancer. *African Journal of Food Science*, *10*(10), 227–237. https://doi.org/10.5897/ajfs2016.1443

Kumolu-Johnson, C.A. & Ndimele, P.E. (2011). A Review on Post-Harvest Losses in Artisanal Fisheries of Some African Countries. *Journal of Fisheries and Aquatic Science*, *6*, 365–378. https://doi.org/10.3923/jfas.2011.365.378

Mah, J.H., Han, H.K., Oh, Y.J., Kim, M.G. & Hwang, H.J. (2002). Biogenic amines in Jeotkals, Korean salted and fermented fish products. *Food Chemistry*, *79*, 239–243.

Mah, J.H., Kim, Y.J. & Hwang, H.J. (2009). Inhibitory effects of garlic and other spices on biogenic amine production in Myeolchi-jeot, Korean salted and fermented anchovy product. *Food Control*, *20*, 449–454. https://doi.org/10.1016/j.foodcont.2008.07.006

Naila, A., Flint, S., Fletcher, G., Bremer, P. & Meerdink, G. (2010). Control of Biogenic Amines in Food-Existing and Emerging Approaches. *Journal of Food Science*, *75*(7), R139–R150. https://doi.org/10.1111/j.1750-3841.2010.01774.x

Özyurt, G., Kuley, E., Özkütük, S. & Özogul, F. (2009). Sensory, microbiological and chemical assessment of the freshness of red mullet (Mullus barbatus) and goldband goatfish (Upeneus moluccensis) during storage in ice. *Food Chemistry*, *114*, 505–510. https://doi.org/10.1016/j.foodchem.2008.09.078

Rahman, M.M., Nasrun, M., Hossain, M.Y. & Aa`zamuddin, M. (2012). A Comparison between Ice and Salt Storages on Bacteriological Quality of Asian Seabass (Lates calcarifer). *Pakistan Journal of Biological Sciences*, *15*, 583–588. https://doi.org/10.3923/pjbs.2012.583.588

Sallam, K.I., Ishioroshi, M. & Samejima, K. (2004). Antioxidant and antimicrobial effects of garlic in chicken sausage. *Lebenson Wiss Technology*, *37*(8), 849–855.

Silva, C.C.G., Da Ponte, D.J.B. & Dapkevicius, M. (1998). Storage temperature effect on histamine formation in Big Eye Tuna and Shkipjack. *Journal of Food Science*, *63*, 644–647.

Zang, J., Xu, Y., Xia, W. & Regenstein, J.M. (2020). Quality, functionality, and microbiology of fermented fish: a review. *Critical Reviews in Food Science and Nutrition*, *60*(7), 1228–1242. https://doi.org/10.1080/10408398.2019.1565491

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