Original Research Article

Effects of oyster shell powder on the quality changes of Afitin,a traditional fermented condiment,during storage at 30ºC

ABSTRACT

Aims: Afitin is aperishable condiment due to its high water content.Various preservatives are used to extend its shelf life, and among them, salt (NaCl) has generated controversy due to potential health risks associated to exposure to elevated concentrations of salt.The present study aims at assessing the effect of oyster shell powder on the quality changes of Afitinduring storage at 30°C.

Methodology: Oyster shell powder was added to the condiment just before the natural fermentation at concentrations of 0.5%, 1% and 2%. At the end of the 24 h natural fermentation, the product was stored at 30°C. Afitin samples which did not receive the oyster shell powder served as controls. Samples were taken for microbiological, pH, water activity, and sensory analyses just before the fermentation, at the end of the fermentation, and at days 2 and 4 during storage.

Results: Oyster shell powder had a significant effect on the growth of microorganisms, on pH and water activity during the storage of Afitin. The average Total Viable Count (TVC)in the control samples was 8.9 Log CFU/g, whereas in the Afitin with 2% oyster shell powder, this load was 6.9 Log CFU/g after 2 days of storage. At the same time, the enterobacteria load in the control Afitin was 7.7 Log CFU/g, whereas in the Afitin with 2% oyster shell powder, it was below the detection limit. There was also a significant difference between the samples inoculated with 1% oyster shell powder and the control. From thesensory analysis, the control Afitin was rejected by the panellists after 48 h of storage, whereas the Afitin with 1% or 2% oyster shell powder was not rejected until the end of storage (4th day), indicating a notable prolongation of the product's shelf life by at least 100%.

Conclusion: The results show that oyster shell powder can potentially be used to improve the preservation of Afitin.

Keywords: Afitin, oyster shell powder, food preservation, microbiological quality, sensory quality

1. INTRODUCTION

Afitin is a condiment derived from alkaline fermentation of the African locust bean (*Parkia biglobosa*), produced in Benin by the *Fon* ethnic group[1]. This condiment has a high nutritional value namely in its high content in protein (35-41.8%), in lipid (29-36%) and zinc, copper, manganese, selenium, etc. [1,2].In Benin, this condiment is used by both rural and urban populations[3]. Although Afitin has several nutritional benefits, it is difficult to store it without additives for a long time.Allognissou (2014)[4] reported in a field study that Afitin cannot be preserved beyond 48 hours, due to its high moisture content, and is therefore a loss for women producers. In order to increase the shelf-life of Afitin, some producers use traditional preservation techniques, namely salt (NaCl)[5]. However, as recommended by the World Health Organization (WHO), consumersare seeking to reduce their salt (NaCl) intake, given that high sodium consumption may contribute to high blood pressure and an increase risk of heart disease and stroke. Elsewhere in the world, there is a growing promotion of oyster shell powder as a natural preservative of food products.. For example, Lu*et al*. (2022)[6] used oyster shell powder to extend the shelf life of white shrimps from 6 to 12 days at 4°C. Oyster shell powder not only preserves food better, but also improves its nutritional quality due to its high calcium content [6]. Benin, a country along the coast, boasts a notable abundance of oyster shells.While oyster shells are present in Benin, there is limited research on their application in food preservation. Therefore, it is advisable for Benin's food industry to investigate the potential of this product in preserving perishable local foods. The current study focuses on assessing the preservative efficacy of oyster shell powder on foods stored at room temperature (30°C), specifically examining the case of Afitin, a traditional condiment produced by alkaline fermentation of the African locust bean (*Parkia biglobosa*) in Benin. Specifically, it aims at(i) determining the effect of oyster shell powder on microbial growth inAfitin during storage at 30°C, (ii) evaluating the effect of oyster shell powder on the pH and water activity (aw) content of Afitin during storage at 30°C, and (iii) evaluatingthe effect of oyster shell powder on the sensory quality of Afitin during storage at 30°C.

2. MATERIALS AND METHODS

2.1. PRODUCTION OF OYSTER SHELL POWDER

The oyster shells used in this study were obtained from fishermen around the Lake Nokoué which is one of the most fishing areas in Benin (Dabadé et al., 2014) [9]. The oyster shells were soaked in water for a day andwashed with a brush to remove any foreign substances. The washed oyster shells were oven for 24 h at 105°C and then calcined in an oven at 900°C for 3 h as described by Lu *et al.* (2022)[6]. The oyster shell powder obtained was quickly cooled at -40°C for 1 h. After grinding and sieving, the powder was sterilised at 100°C for 30 min before use (Fig. 1).

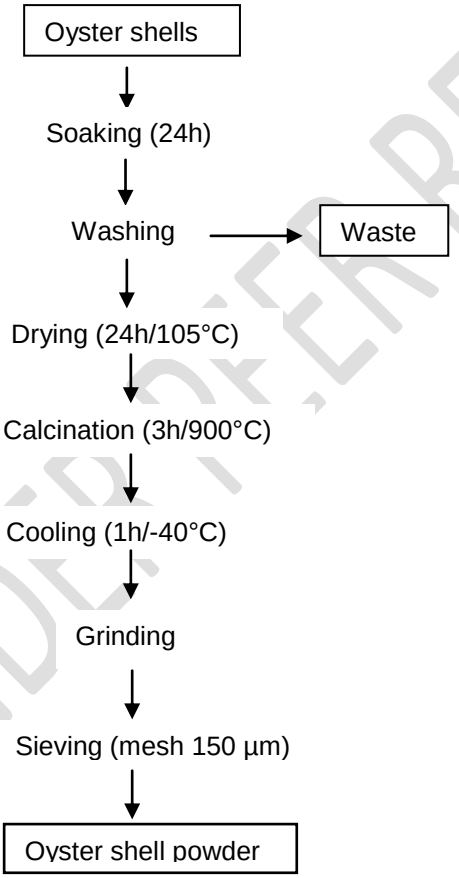


Fig. 1.Oyster shell powder production process [6]

2.2. PRODUCTION OF AFITIN

Afitin was produced by a professional womanusing the traditional technology described previouslyby Hounhouingan *et al.* (2001)[7]. Figure 2 shows the raw material (African locust beans) used to produce Afitin. However, just before the fermenation step, the cooked beans were divided into four batches of 1 kg each. To three baches; oyster shell powderwas addedat concentrations of 0.5%, 1% and 2%, respectively as reported previously by Kim *et al.*

Fig. 2. A: African locust pulp B: African locust beans C: Cooked African locust beans

(2007) [8]. The last bach of cooked beanswithout oyster shell powder was used as a control.

2.3. SAMPLING PREPARATION AND STORAGE CONDITIONS

Just after adding the oyster shell powder, samples from each batch were taken for laboratory analysis. This sampling point was considered as time zero (t_0). At the end of the fermentation (24 h later), samples were also taken from the four batches for laboratory analysis. The remaining Afitin samples (80 g) were aseptically packed in small glass containers, which were pre-sterilized at 180°C for 1 hour. They were then stored at 30°C and samples were taken for laboratory analysis at days 2 and 4 during storage.Two trials were conducted during this study.

2.4. MICROBIOLOGICAL ANALYSES

From each sample, 10 g was transferred aseptically to a stomacher bag and diluted 10 times in physiological saline peptone solution (0.85% NaCl, 0.1% peptone). The mixture was homogenized for 60 s using a stomacher (Seward Laboratory Stomacher 400, England) to get the first dilution from which successive decimal dilutions were prepared, as described by[10]. Total Viable Count (TVC) was enumerated on one layered plate of Plate Count Agar (PCA) medium and incubated at 30 °C for 72 hours. Enterobacteriawere enumerated on double-layered plates of violet-red bile glucose (VRBG) medium and incubated at 37 °C for 24 hours. For the double-layered plates, 1 mL of the appropriate dilution was inoculated into a Petri dish, then approximately 15 mL of the molten (45 °C) medium was poured into the Petri dish. After setting, the Petri dish was overlaid with approximately 10 mL of the same molten medium. All microbiological media used were from OXOID

2.5. PHYSICO-CHEMICAL ANALYSES

The pH was determined using 20 mL of distilled water and added to 10 g of ground inoculated Afitin. The mixture was homogenized and the pH was measured in duplicate using a pH meter (InoLab 7110, Germany). Water activity (aw) was measured at 26 °C using a water activity meter (Hygrolab, Rotronic AG, Switzerland) as describe by Houngbédji et al., (2020)[11].

2.6. SENSORY ANALYSIS

The overall acceptance of the samples based on their odor, taste, color, and texture was assessed using a scale with three categories: 1 = sample with good quality, 2 = sample with marginal quality, but still acceptable, and 3 = spoiled samples [12–14]by 10 panelists experienced in Afitin evaluation. Sensory rejection time was defined as the moment when 50 % of the panelists evaluated samples to be in category 3[12–14].

2.7. STATISTICAL ANALYSIS

The means and standard deviations of the values of microbial loads; pH and water activity of the different samples, were carried out using Excel 2016 software. Significant differences between the means were estimated at the 5% threshold using the Tukey test and Statistica software.

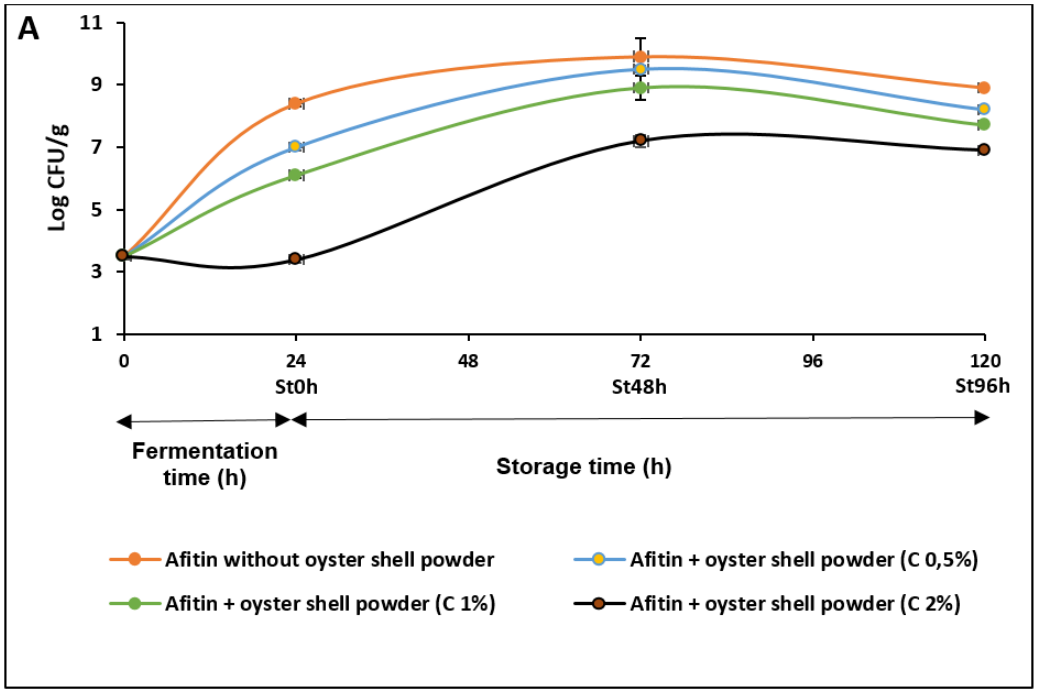
3. RESULTS AND DISCUSSION

3.1. EFFECT OF OYSTER SHELL POWDER ON THE MICROBIAL LOAD OF AFITIN

It was observed that the initial total viable counts (TVC) of the samples was on average 3.5 Log (CFU/g) and increased significantly during the 24 hours of fermentation as well as during the first 2 days of storage (Fig. 3A).The initial TVC obtained are similar to those reported by Azokpota *et al.* (2006) [1]. The lowest loads of TVC(3.5 ± 0.1 Log CFU/g)were obtained for samples analysed at the initial time and the highest load was generally obtained at day 2 of storage: 9.9 ± 0.6 Log CFU/g for the control; 9.5 ± 0.1 Log CFU/g for the sample inoculated with 0.5% of oyster shell powder; 8.0 ± 0.4 Log (CFU/g) for the sample inoculated with 1% of oyster shell powder and 7.2 ± 0.2 Log (CFU/g) for the sample inoculated with 2%of oyster shell powder.Statistical analysis showed that during storage, there was a significant ($P = .05$) increase of TVC concentrations. Likewise, there are significant differences between the samples. In addition, TVC concentrationsobtained for the control samples during storage were much higher than those obtained for the samples inoculated with oyster shell powder. It should therefore be noted that the oyster shell powders tested in this study had an inhibitory effect on Total Viable Count contained in cooked *Parkia biglobosa*beans.

Figure 3B shows the evolution of the enterobacteria concentrations in the samples before fermentation, at the end of fermentation and during the storage. At the beginning of experiment;the enterobacteria load was below the detection limit for all samples.During the

fermentation step, the Enterobacteria load increased significantly. At the end of storage, the Enterobacteria load was 7.6 Log CFU/g for the control. Whereas. For Afitin with oyster shell powder the Enterobacteria concentrations increased to 2.4 Log CFU/g and 1.8 Log CFU/g at the beginning of storage (24 h after the beginning of the experiment)respectively for samples with 0.5% and 1% of oyster shell powder, before decreasing below the detection limitafter 48 h of Afitin storage. The enterobacteria load of the samples with 2% of oyster shell powder remained below the detection limit throughout the experiment.Statistical analysis also showed that during the fermentation, there was no significant difference between the Enterobacteria loads of the samples inoculated with oyster shell powder. However, the Enterobacteria loads of the control samples were significantly different from the inoculated samples (*P*=.05).Theseresults show that the oyster shell powder has inhibitory effects on the microorganisms during storage. Indeed, oyster shells are made up of approximately 95% calcium carbonate ($CaCO_3$) [6,15,16]. After calcination, calcium carbonate $CaCO_3$ is converted into calcium oxide (CaO) [16]. CaO is considered as an antimicrobial agent applicable in food and medicine fields due to its strong biocidal activity, various antimicrobial mechanisms and biocompatibility [15]. The presence of this compounds and the biochemical reactions it causes in an alkaline environment can strongly affect cellular integrity [16,18]. According to Oikawa *et al.* (2000)[19], oyster shell powder can inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Listeria, Salmonella*, *Cactus bacillus*, *Micrococcus luteus*, *Aspergillusniger* and *Penicilliumfuniculosum*. The inhibitory effect observed on Total Viable Count (TVC) and Enterobacteria in this study could be elucidated by all the above-mentionedfactors.



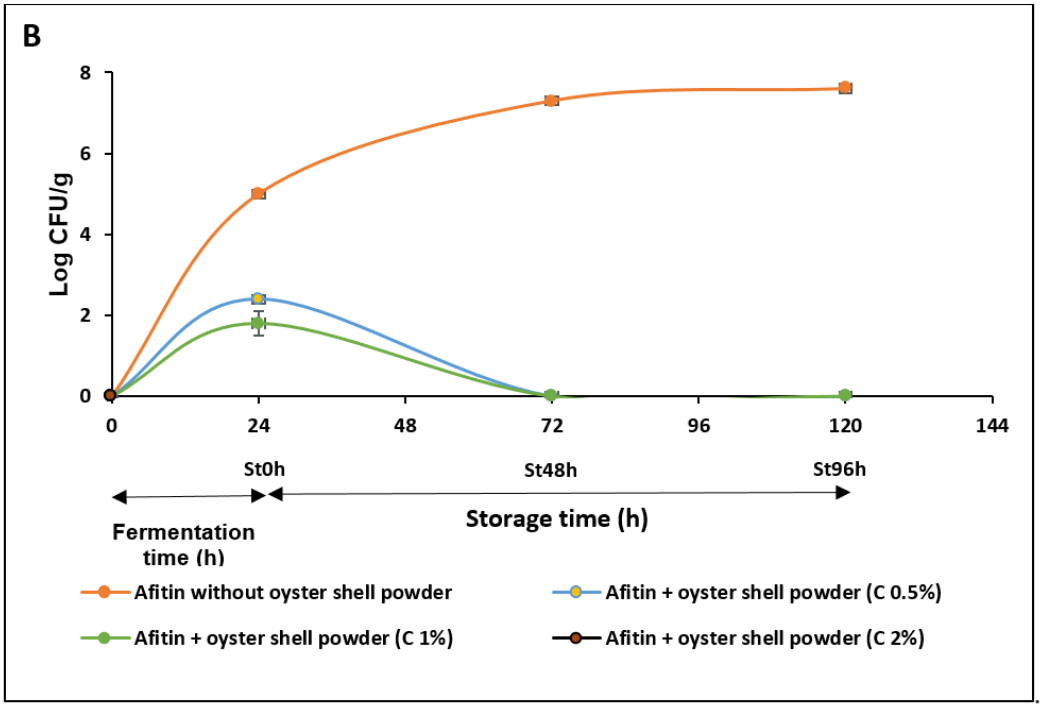
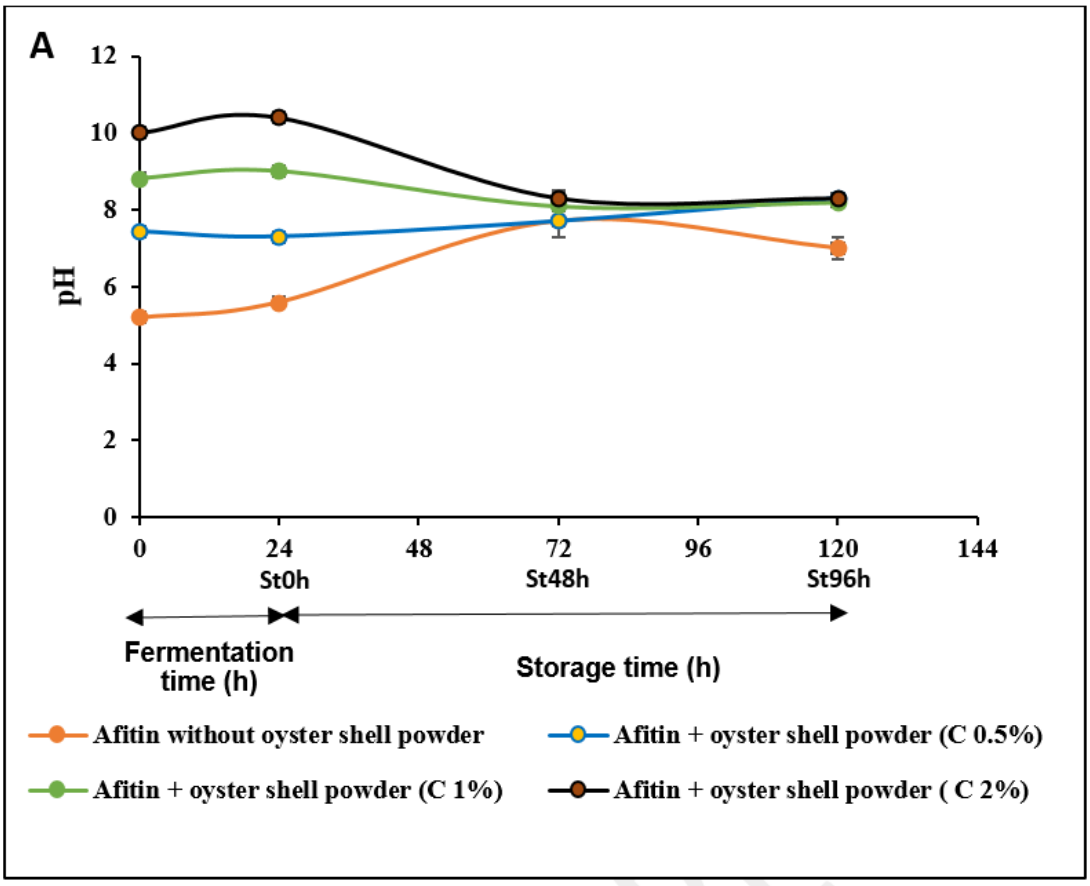


Fig. 3. Evolution of TVC (A) and Enterobacteria (B) in the samples. (St = Storage time)

3.2. EFFECT OF OYSTER SHELL POWDER ON pH AND WATER ACTIVITY OF AFITIN

The pH of the samples analysed ranged from 5.2 ± 0.0 to 10.4 ± 0.1 (Fig. 4A). The highest pH values were obtained for samples inoculated with oyster shell powder. The statistical analysis carried out showed that there was a significant difference between all the pH values of the inoculated samples (0,5%, 1% and 2%) compared with the pH values of the control. In fact, Oyster shell powder is essentially constitute of calcium oxide (CaO) which is a basic compound [17] and should therefore increase the pH of the samples. Only the samples inoculated with 0.5% of oyster shell powder, did not show a significant difference with the control at 48h of storage (72h after the beginning of the experiment). This can be explained by the low level of powder inoculated this sample. For each type of Afitin (inoculated or not), there is no significant difference between the pH values obtained at 0h comparated to 24h (the end of fermentation) and between the pH values obtained at 48h comparated to 96h of storage. The initial pH of *Parkia biglobosa*beans was 5.2±0.0, but rose rapidly during fermentation and storage for the control group, reaching 7±0.3 around day 3 to day 4 of the experiment. These data are similar to those observed by Koné *et al.* (2023)[20] on*Parkia biglobosa* beans during *soumbala*(also derived from alkaline fermentation of the African locust beans (*Parkia biglobosa*)) production, where pH values was 5.5 on day 1, rising to 7.9 on day 3. The increase in pH observed during fermentation of *Parkia biglobosa*beans could be explained by the activity of *Bacilus*, the predominant flora[20]. These microorganisms degrade bean proteins, leading to the release of peptide amino acids and abundant ammonia production from amino acid deamination [21]. The results obtained also corroborate those of several authors who have observed an increase in pH during bean fermentation [22,23]. The initial pH of the treated groups with 1% and 2% oyster shell powder, was around 8.8±0.0 and 10±0.0, respectively, reaching an optimum after 24h (after the beginning of the experiment) at 9.0± 0.1 (inoculated with 1% oyster shell powder) and 10.4±0.1 (inoculated with 2% oyster shell powder) before dropping to 8.2±0.0 (inoculated with 1% oyster shell powder) and 8.3±0.1 (inoculated with 2% oyster shell powder) at 96 h of storage. On the other hand, control samples and samples inoculated with 0.5% oyster shell powder, maintained virtually increasing trend.

Figure 4B shows changes in water activity contents of the samples between 0 h and 120 h during fermentation (24 hours) and storage (96 hours) at room temperature (30°C). The water activity of the samples analyzed varied between 0.952±0.0and 0.974±0.0. The highest values are obtained of samples inoculated with 1% and 2% oyster shell powder. Statistical analysis shows that at the end of fermentation, the water activity levels of samples inoculated with 1% and 2% oyster shell powder were significantly different from those of the control sample and those of samples inoculated with 0.5% oyster shell powder. The difference of water activity value between control and samples could be due to the ability of powder components such as CaO to chelate the water molecular getting from the biochemical reactions in the samples during storage as previously reported by Lu et al. (2022)[6]. On the other hand, after 120h of the experiment, there was no significant difference between samples in terms of water activity. During storage, control samples and those inoculated with 0.5% oyster shell powder remained almost identical in terms of water activity at the 5% threshold.



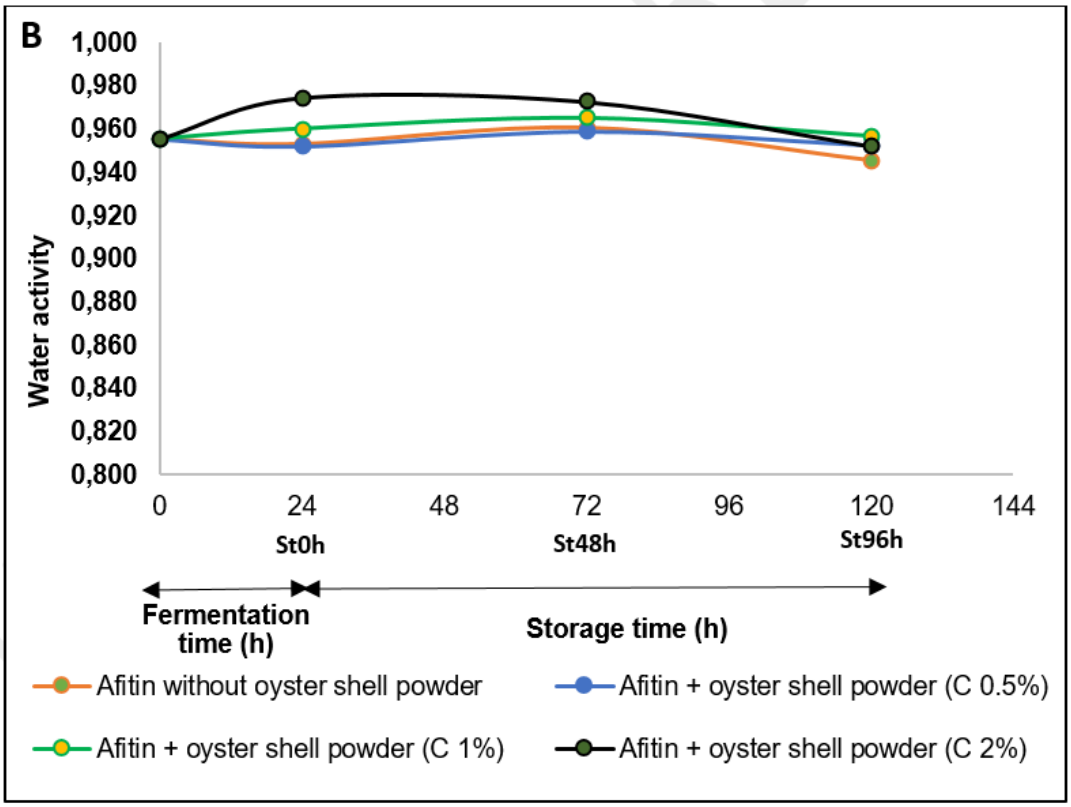


Fig. 4. Changes in pH (A) and water activity (B) in the samples. (St = Storage time)

3.3. EFFECT OF TREATMENT WITH OYSTER SHELL POWDER ON THE OVERALL QUALITY OF AFITIN

The results of the Afitin sensory evaluation are presented in Table 1. Afitin samples were deemed to be good quality by all panelists at the beginning of storage time. As expected, the percentage of the panelists evaluating the Afitin samples to be marginal or defective quality increased with storage time. The quality of the Afitin was judged defective when there was off-odor production and/or noticeable changes in Afitin texture. The sensory rejection time defined as the time when at least 50 % of the panelists evaluate Afitin to be spoiled was 48 hours storage time for the Afitin without oyster shall powder (Afitin control) and for the Afitin with 0.5 % oyster shall powder. While there was no rejection time for the Afitin samples with 1% and 2% oyster shall powder until the end of the storage. Similar to our findings, Allognissou (2014) [4] reported in a field study that Afitin cannot be preserved beyond 48 hours, due to its high moisture content. Thus, the treatments of Afitin with at least 1 % oyster shell powder could prolong the product's shelf-life by at least 100% (from 48 h to 96 h).

Table 1. Overall assessment (in % of panelists) of treatment with oyster shell powder on the sensory quality of Afitin during storage at 30 °C

	Overall quality assessment criteria	Fermentation and Storage time (hours) 0h	24h	72h	120h
Afitin without oyster shell powder		**0h**	**St0h**	**St48h**	**St96h**
	1 = Good quality product;	100	90	0	0
	2= Slightly poor quality, but still acceptable;	0	10	50	0
	3 = Poor quality, unacceptable product	0	0	50	100
	Overall quality assessment criteria	**Fermentation and Storage time (hours) 0h**	**24h**	**72h**	**120h**
Afitin + oyster shell powder(0.5%)		**0h**	**St0h**	**St48h**	**St96h**
	1 = Good quality product;	100	100	0	0
	2= Marginal quality, but still acceptable;	0	0	50	33.3
	3 = Poor quality, unacceptable product	0	0	50	67.7
	Overall quality assessment criteria	**Fermentation and Storage time (hours) 0h**	**24h**	**72h**	**120h**
Afitin + oyster shell powder (1%)		**0h**	**St0h**	**St48h**	**St96h**
	1 = Good quality product;	100	100	33.3	50
	2= Marginal quality, but still acceptable;	0	0	67.7	50
	3 = Poor quality, unacceptable product	0	0	0	0
	Overall quality assessment criteria	**Fermentation and Storage time (hours) 0h**	**24h**	**72h**	**120h**
Afitin + oyster shell powder (2%)		**0h**	**St0h**	**St48h**	**St96h**
	1 = Good quality product;	100	100	67.7	33.3
	2= Marginal quality, but still acceptable;	100	0	33.3	67.7
	3 = Poor quality, unacceptable product	67.7	0	0	0

St = Storage time

4. CONCLUSION

The present study evaluated the effect of oyster shell powder on the preservation of Afitin. The microbiological analyses carried out for this purpose showed that oyster shell powder had a significant effect at 5% threshold on the Total Viable Count (TVC) and on enterobacteria concentration in Afitinduring storage at 30°C. The analysis of the pH and water activity of the samples revealed a significant effect of oyster shell powder. The sensory evaluation revealed that the control sample and the 0.5% sample were rejected after 48 h by the panelists, while the samples inoculated with 1% and 2% were not rejected until the end of storage (4 days).The study shows that the use of oyster shell powder at a concentration of 1% or higher can prolong the shell-life of Afitin by at least 100%. This study presents Afitin processors,

traders, and consumers with an alternative preservation method, offering a means for a better quality management of the product.

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