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 highly perishable 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 TVCconcentration in the control was 8.9 Log CFU/g, whereas in the afitin with 2% oyster shell powder, this load was 6.9 Log CFU/g after 3 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 a sensory 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).

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 the fermentation of *Parkia biglobosa* seeds, 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 the use of salt [5]. However, as recommended by the World Health Organization (WHO), populations are seeking to reduce their salt intake, given that high sodium consumption contributes to high blood pressure and an increased risk of heart disease and stroke. Elsewhere in worldwide, a natural preservative product is increasingly use, which is oyster shell powder, used for storing various types of food. 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 is a coastal country where oyster shells are found in significant quantities. Although oyster shells are available in Benin, few studies have been carried out on the use of this product for food preservation. Therefore, it would be better for Benin's food industry to study the ability of this product to preserve perishable local foods. The present work aimed to evaluate the preservative effect of oyster shell powder on foods stored at room temperature (30°C): case of Afitin - a condiment from *Parkia biglobosa* seeds - produced in Benin. Specifically, it aims to (i) determine the effect of oyster shell powder against the microbial growth into afitin stored at room temperature (30°C), (ii) evaluate the effect of oyster shell powder on the pH of afitin stored at room temperature (30°C) and (iii) evaluate the effect of oyster shell powder on the organoleptic quality of afitin stored at room temperature (30°C).

2. MATERIAL AND METHODS

2.1. EQUIPMENT

The materials used in this study include :

- African locust beans from Houèto, a village in the municipality of Abomey-Calavi in southern Benin;
- oyster shells from the fishermen of Sainte-Cécile Gbèdjromédé in Cotonou, Benin;
- afitin production equipments:containers, gas stove, ash or fine sand, etc.
- laboratory equipment (pH meter, smoke hood, Stomacher, petri dishes, PCA and VRBG culture media) required for laboratory analyses.

2.2. PRODUCTION OF COOKED *PARKIA BIGLOBOSA*SEEDS

The production ofcooked*Parkia biglobosa*seedswasdone by a producerat Houèto (a village in the Abomey-Calavi commune of southern Benin) using the technology described previouslyby Hounhouingan *et al.* (2001)[7]. The cooked seeds (afitin) obtained were divided into four batches of 1 kg each in hermetically sealed sterile Stomachers bags. The batches were placed in a cooler filled with ice.They were then taken to the laboratory for sampling.

2.3. PRODUCTION OF OYSTER SHELL POWDER

The oyster shells used in this study were obtained from fishermen at Sainte-Cécile (Cotonou, southern Benin). The oyster shells were cleaned with a brush and soaked in water for a day to remove any foreign substances. The washed oyster shells were oven for 24 h at 105°C and then calcined in an amorphous furnace at 900°C for 12 h as described by Lu*et al.* (2022)[6]. The oyster shell powder obtained was quickly cooled in a biobank at -40°C for 1 h. After grinding and sieving, the powder was sterilised at 100°C for 30 min before use (Fig 1).

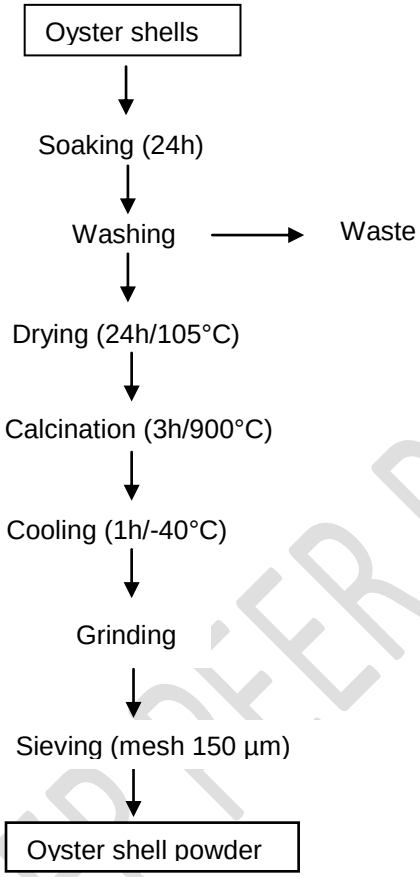


Fig. 1.Oyster shell powder production process [6]

2.4. SAMPLING PREPARATION AND STORAGE CONDITIONS

Samples of afitin (80 g) were taken aseptically in small boxes. The latter were pre-sterilized at 121°C for 1 hour. Oyster shell powder was added to these samples at concentrations of 0.5%, 1% and 2% as reported previously by Kim *et al.* (2007)[8], then stored at room temperature (30°C). Cooked seeds that had not received oyster shell powder were used as a control. Samples were taken at different times: before the fermentation (0h), at the end of the fermentation (24h), at days 2 and 4 during storage.Two trials were conducted during this study. All microbiological media and chemicals used were from OXOID and SIGMA, respectively.

2.5. MICROBIOLOGICAL ANALYSES

From each samples, 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[9]. 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.

2.6. 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)[10].

2.7. 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 [11–13]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.

2.8. 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.

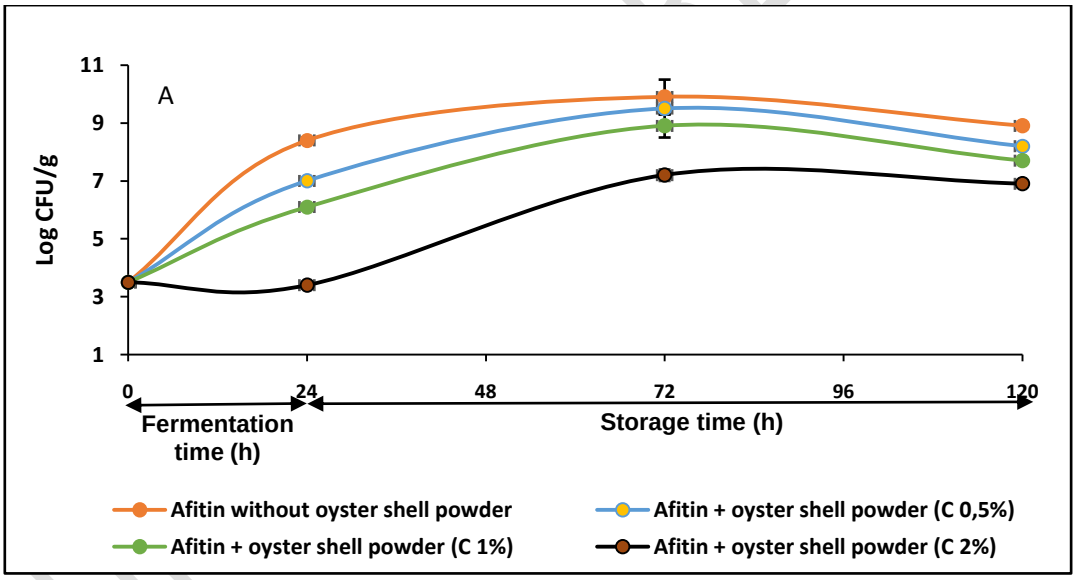
3. RESULTS AND DISCUSSION

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

It was observed that the initial TVC loads 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.The initial TVC loads obtained are similar to those reported by Azokpota *et al.* (2006) [1]. The lowest loads of TVC were obtained for samples analysed at the initial time (3.5 ± 0.1 Log CFU/g) 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 (TVC) contained in cooked *Parkia biglobosa* seeds.

Figure 2B 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 storage;the Enterobacteria load was below the detection limit for all samples.During the concentration, 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 after 24 h respectively for samples with 0.5% and 1% of oyster shell powder, before falling down below the detection limitafter 72 hours.The enterobacteria load of the samples with 2% of oyster shell powder remained below the detection limit throughout storage.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 ($CaCO_3$) [6,14,15]. 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 [14]. The presence of this compounds and the biochemical reactions it causes in an alkaline environment can strongly affect cellular integrity [15,17]. According to Oikawa *et al.* (2000)[18], oyster shell powder can inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, Listeria, Salmonella, *Cactus bacillus*, *Micrococcus luteus*, *Aspergillus niger* and *Penicillium funiculosum*. All this could explain the inhibitory effect that oyster shell powder had on TVC and Enterobacteria in this study.



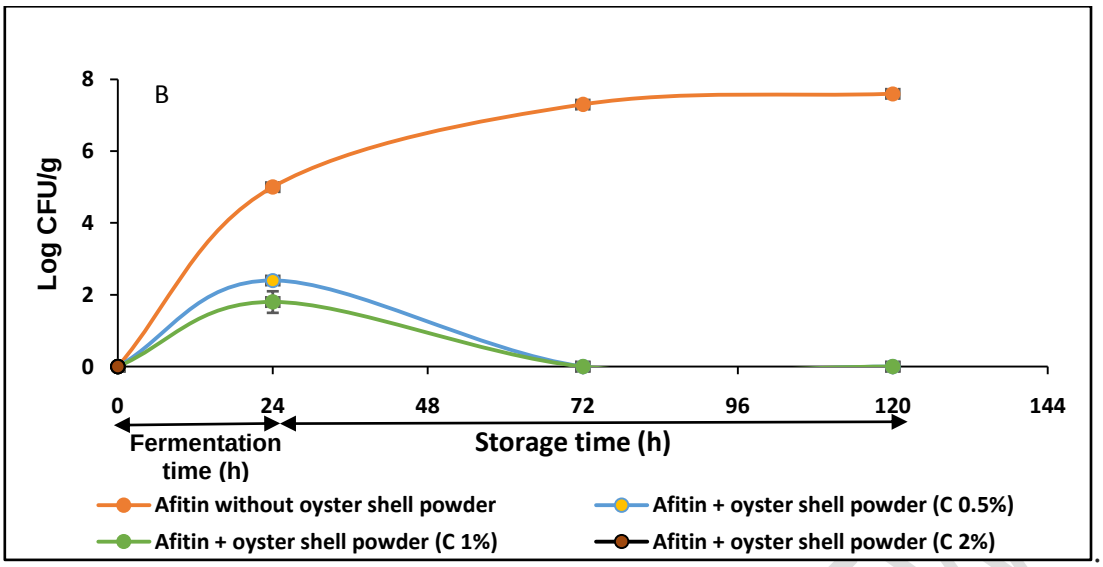


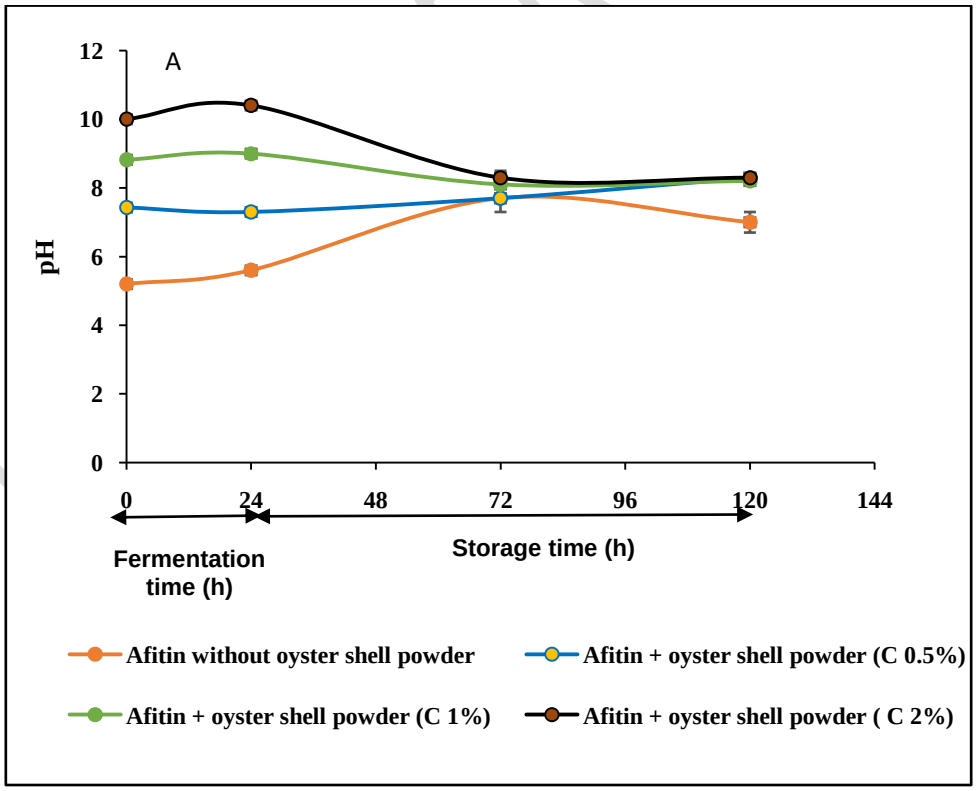
Fig. 2. Evolution of TVC(A) and Enterobacteria (B) in the samples

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 3A). 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 [16] 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 72h of conservation. This can be explained by the low level of powder inoculated at this level. For each type of afitin (inoculated or not), there is no significant difference between the pH values obtained at 0h comparated to 24h of storage and between the pH values obtained at 72h comparated to 120h of storage. The initial pH of *Parkia biglobosa* seeds 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 fermentation and storage. These data are similar to those observed by Koné *et al.* (2023)[19] on of *Parkia biglobosa* seeds during Soumballa 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* seeds could be explained by the activity of *Bacilus*, the predominant flora[19]. These microorganisms degrade seed proteins, leading to the release of peptide amino acids and abundant ammonia production from amino acid deamination [20]. The results obtained also corroborate those of several authors who have observed an increase in pH during seed fermentation [21,22]. The initial mean 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 of storage at 9.0±0. 1 (inoculated with 1% oyster shell powder) and 10.4±0.1 (inoculated with 2% oyster shell powder) before dropping after

72h of storage to 8.2±0.0 (inoculated with 1% oyster shell powder) and 8.3±0.1 (inoculated with 2% oyster shell powder) at 120h of storage. On the other hand, control samples and samples inoculated with 0.5% oyster shell powder, maintained virtually increasing tedance. This shows that treatment superior or equal 1% oyster shell powder has a greater effect on *Parkia biglobosa* seeds during storage at 30°C, depending on seed pH.

Figure 3B shows the changes of water activity in the samples between 0 h and 120 h of storage 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 in the samples as previously reported by Lu et al. (2022)[6]. On the other hand, after 120h of fermentation and storage, 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. We can therefore conclude that treatment with 1% or more oyster shell powder has a greater effect on *Parkia biglobosa seeds* during storage at 30°C, depending on the water activity of the seeds.



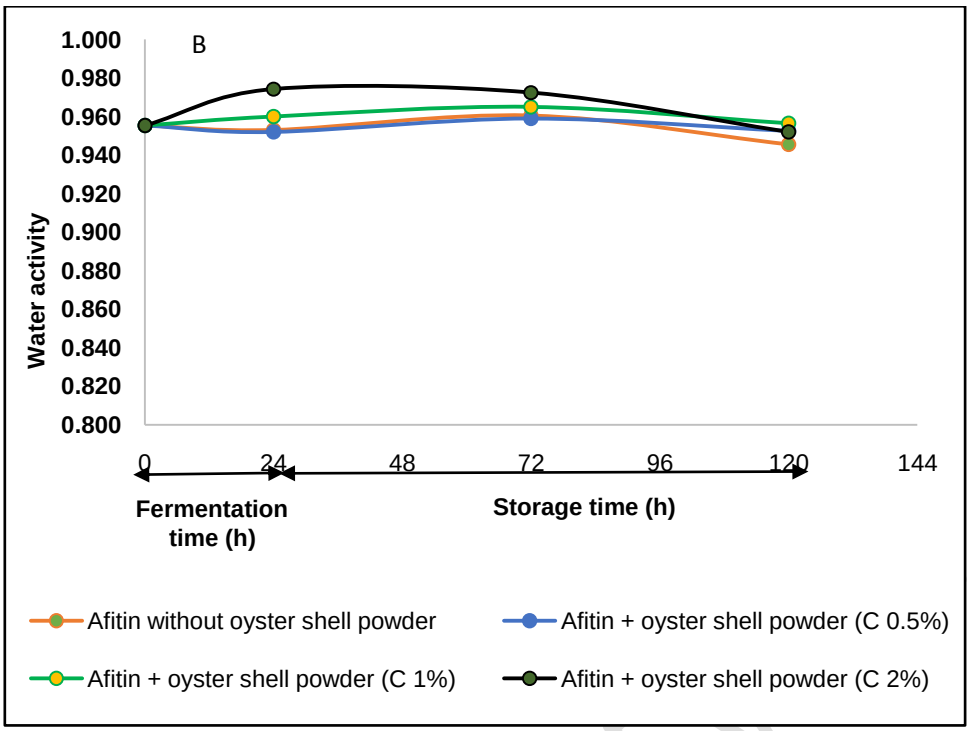


Fig. 3. Changes in pH (A) and water activity (B) in the samples

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

Table 1 summarises the evaluation of the impact of treatment with oyster shell powder on the sensory quality of afitin when stored at room temperature (30°C). It shows the percentage of panellists who agree on the same overall assessment of afitin without oyster shell powder and with oyster shell powder at each sampling time. The sensory rejection time for afitin without oyster shell powder and with oyster shell powder was identified. For the batch of afitin without oyster shell powder and the batch treated with oyster shell powder at a concentration of 0.5%, the rejection time was t = 3 days. The batches of afitin treated with oyster shell powder at concentrations of 1% and 2% were not rejected during all the experimentation(5 days). Thus, treatments with concentrations of oyster shell powder greater than 1 % could extend the shelf life of afitinduring storage, depending on the organoleptic parameters.

Table 1. Overall assessment of treatment with oyster shell powder on the sensory quality of afitin during storage at 30 °C

Afitin without oyster shell powder	Overall quality assessment criteria	Fermentation and storage time (days)			
		0	1	3	5
	1 = Good quality product;	100	33.3	0	0

	2= Slightly poor quality, but still acceptable;	0	66.7	50	50
	3 = Poor quality, unacceptable product	0	0	50	50
Afitin + oyster shell powder(C 0.5%)	**Overall quality assessment criteria**	**Fermentation and storage time (days)**			
		0	**1**	**3**	**5**
	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	6	50	67.7
Afitin + oyster shell powder (C 1%)	**Overall quality assessment criteria**	**Fermentation and storage time (days)**			
		0	**1**	**3**	**5**
	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
Afitin + oyster shell powder (C 2%)	**Overall quality assessment criteria**	**Fermentation and storage time (days)**			
		0	**1**	**3**	**5**
	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

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 load of Total Viable Count (TVC) and enterobacteria contained in afitinover 120 h of preservation. 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 72 h by the panellists, while the samples inoculated with 1% and 2% were not rejected until the end of storage (5 days). The effect of the powder was therefore highly significant with 1% and 2% concentrations on the microbiological, physico-chemical and sensory parameters.

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