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Sardine Immersed Storage at 15°C in Brine Containing Sodium Benzoate

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Abstract

Sodium benzoate, used as a preservative in foods and generally recognized as safe (GRAS) when added within certain range, was tested in sardine preservation. Sardine were immersed in 4% brine and stored at 15°C. Previous immersion 0.3% sodium benzoate was added to the brine and the pH was adjusted to 5 with acetic acid.

According to the results in sensory evaluation and volatile base nitrogen (VB-N), treated samples were considered acceptable until the 6th day, whereas samples used as a control became spoiled on the second day. Microbial counts were not reliable indicators of changes in quality during storage.

Introduction

Benzoic acid, usually in the form of the sodium salt has long been used as an antimicrobial additive for foods. The sodium salt is preferred because of the low aqueous solubility of the free acid. In use the salt is converted to the acid, the active form¹⁾.

Sodium benzoate is generally considered to be most active against yeast and bacteria, and less active against molds²⁾. The majority of the reported studies are concerned more with the mode of action than with the type of microorganism against which it is effective²⁾.

There are many reports which depict the relationship of pH to inhibitory concentrations of sodium benzoate^{2,3)}. It has been also reported that sodium chloride has a considerable synergistic effect with sodium benzoate²⁾. Under regulations of the USA Food and Drug Administration (FDA), sodium benzoate and benzoic acid are generally recognized as safe²⁾ for use in foods. In some countries levels up to 0.2 and 0.3%^{1,3)} are permitted and are commonly used.

This preservative has especially wide applicability as an antimicrobial for foods and it is most suitable for foods and beverages which naturally are in the pH range below 4.0 or 4.5 or can be brought into that range by acid addition²⁾. Mixed to the food in small doses, is not deleterious to health¹⁾.

The inhibitory effect of sodium benzoate on some bacteria that cause foodborne illness or food spoilage has been studied by using different laboratory media as well as food products under various conditions¹⁻³⁾. For sodium benzoate to be effective the pH of the substrate must be low since the undissociated acid is believed to be

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responsible for its antimicrobial action. Currently, virtually no information is available on the effect of sodium benzoate in fish preservation.

On the other hand, previous works on fish immersed storage in 4% brine⁴⁾ and in 4% brine containing acetic acid⁵⁾ (0.05%) and stored at 15°C has been done with some promising results. However there is still a need to improve them. Therefore, continuing with this series of works, the effect of sodium benzoate was tested as another alternative for fish preservation under the conditions referred above, immersed storage in 4% brine held at 15°C. During storage, organoleptic changes in fish and changes in total aerobic counts, volatile base nitrogen (VB-N) and salt concentration of the fish and the brine of immersion were evaluated.

Materials and Methods

Raw material

Freshly caught sardine were purchased at Kami-iso in Hakodate Bay, were immediately placed in an ice box and transferred to the laboratory. Upon arrival they were removed from the ice box and rinsed with tap water. The fish were divided into lots of 2 kg, and used through the experiments. Each lot, destined for each day of sampling, was immersed in 2 l of sodium benzoate treated and untreated 4% brine (NaCl solution). This resulted in 2 lots for the control, untreated samples, and 4 lots for the treated samples.

The addition of sodium benzoate, to give a final concentration of 0.3% to the brine was done previous to fish immersion. Besides being certain of the better action of sodium benzoate at low pH^{1,3)}, pH of the brine was adjusted to 5 with acetic acid after the sodium benzoate addition.

Fish storage and sampling

Samples were kept at 15°C.

At predetermined times, two fish were randomly drawn from each lot for microbial counts, VB-N, and muscle salt uptake analyses. Parallel sampling was made on brine for microbial counts and changes in salt concentration.

Sensory evaluation

An overall score for quality was determined as stated in a previous work⁴⁾ and the quality score card presented in Table 1 was used.

Microbiological analyses

Total aerobic counts were determined in fish and in the brine of immersion using a standard pour plate technique and duplicate plates⁴⁾.

Chemical analyses

VB-N in fish muscle and in the brine was determined by the method of Conway⁶⁾.

The salt (NaCl) content of fish muscle and of the brine of immersion was determined by the method of Mohr⁷⁾.

Table 1. Quality score card for sardine immersed in brine.

Testing subject	Scores of alteration and description of features				
	A Completely fresh, highly acceptable	B Fresh and acceptable	C Fairly acceptable, borderline of acceptability	D Spoiled, unacceptable	E Completely spoiled, totally unacceptable
Overall appearance	Bright, metallic luster	Bright	Some loss of brightness	Bleaching	Bloom completely gone
Odor	Fresh	Very faint sour	Fishy, odor, slightly sour	Strong fishy or sour odor	Putrid
Texture	Firm and elastic	Slightly soft	Some loss of elasticity	Very soft	Totally soft and flabby
Slime	Clear, transparent	Becoming turbid	Opaque and milky	Sticky yellowish	Thick grayish or sticky red

Results and Discussion

Sensory evaluation

Changes in the overall appearance and acceptability scores of both fish immersed in treated 4% brine, and fish immersed in untreated 4% brine (control), are shown in Table 2.

The sensory evaluation showed marked differences between the two samples. Benzoate treated fish remained in good condition up to the 4th day. They declined slightly up to the 6th day to be classified as acceptable or in the borderline of acceptability. This decline continued and the fish was valued "spoiled" on the 7th day of storage.

Samples used as control suffered an extremely fast decomposition. Although they were recognized as acceptable on the first day of sampling, they were found to be completely spoiled by the next day. In this case, the rate of change of the different parameters evaluated was very fast. The first day and average for all of them was "category C". The fish had a good appearance even though some fishy odor existed. In addition when pressed in the dorsal side the muscle was slightly soft and in most instances the slime was still clear. When evaluated the second day, all the parameters, without exception, had completely decayed making the fish unacceptable.

On the other hand changes for the treated samples were not the same. Appearance and odor were observed to change progressively while changes in texture and slime were more slowly until the 6th day. Then a sudden decline of all the parameters evaluated indicated the fish had spoiled. However when compared with the controls, it could be said that sodium benzoate extended the shelf life of the treated samples a great deal from 1 day (control) to 6 days (treated sample).

Microbial counts

The results of colony forming units (cfu) in the sardine and in the brine of immersion are presented in Fig 1. The initial bacterial load on sardine (surface and

Table 2. Overall quality scores and acceptability of sardine immersed in brine.

Testing subject	Before storage	Untreated		Treated			
		Days		Days			
		1	2	2	4	6	7
Overall appearance	A	B	D	A	B	C	D
Odor	A	C	D	A	B	C	D
Texture	A	C	D	A	A	B	D
Slime	A	B	D	A	A	C	D
Acceptability	A	C	D	A	B	C	D

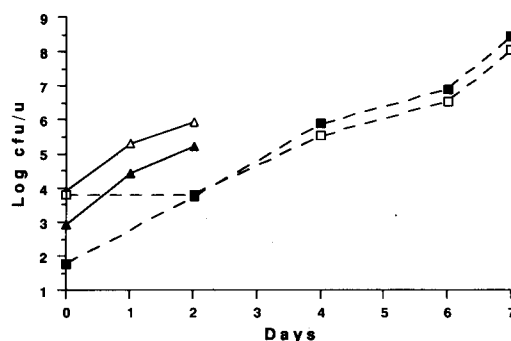


Fig. 1. Total colony forming units in fish (g) and in the brine (ml) of immersion during storage at 15°C.

(□---□) Fish in treated brine. (△—△) Fish in untreated brine.
 (■---■) Treated brine. (▲—▲) Untreated brine.

flesh) after thoroughly rinsing it with tap water, once it arrived to the laboratory, was almost 10^4 cfu g^{-1} . It has been reported^{8,9)} that the surface slime of fish is an excellent medium for bacterial growth and that rinsing the fish not only reduces bacterial counts but improves organoleptic quality.

Any increase in the total bacterial count of treated samples was observed until the second day. From the second day, it increased progressively from almost 10^4 cfu g^{-1} (initial load) to nearly 10^8 g^{-1} after 7 days of storage. Between the 6th and 7th day the rate of increase was observed to be a little faster than in previous days. The lag period observed for treated samples was not observed for untreated, in which growth started to occur immediately after immersion. On the second day, when samples were considered to be spoiled, according to results of VB-N and sensory evaluation, bacterial count was nearly 10^6 cfu g^{-1} .

On the other hand bacterial populations in the brine increased continuously after the sardine immersion. When benzoate was present, the initial numbers were 10 fold lower than the numbers of the control determined at the same time. However rates of growth were parallel for both samples.

Except for treated samples during the first two days, the pattern of microbial growth in the sardine flesh and the respective brine was similar in each case.

Results for fish immersed in the control brine showed no clear relation between the outcome of sensory tests and microbial counts. The fish were sensorily unacceptable before the increase in bacterial count was considered to have a level that is supposed to show spoilage¹⁰⁻¹². On the other side, microbial rate of growth in the brine was similar to that recorded in a previous work⁴) under the same conditions.

It is known that sodium benzoate retards trimethylamine formation in muscles of certain fish even though it does not retard bacterial growth¹⁴). Therefore, in this work, it could be explained the presence of high bacterial number, on the 6th day, in fish immersed in the treated brine and the lag phase observed between the first two days could be better attributed to the action of the acid⁵). Besides, these results also did not fit with sensory evaluation, since high bacterial counts were recorded before the appearance of any spoilage characteristic.

Although results in changes in microflora are not presented here, (still under study) both results, for treated and untreated fish samples, could only be explained on the basis of changes in microflora. Even if it is true that the microbial count recorded in fish used as a control was low in comparison with results presented in other works^{14,15}), spoilage was obvious. Therefore it could be supposed that spoilage microorganisms rapidly displaced the initial flora, causing decomposition of the fish. For the contrary, it could be expected that the microflora of the fish treated sample was composed mostly of low activity spoilage bacteria or the spoilage bacterial activity inhibited by the action of the benzoate¹³). This could be at least, until the 6th day, after which rapid changes in it or inactivation of the benzoate by changes in the characteristics of the brine during fish immersion, were thought to be the reason for making the fish decay and become unacceptable.

VB-N

Changes in the total VB-N of the sardine flesh during immersion in treated and untreated brine, and of the brine themselves are presented in Fig 2. The VB-N values for treated fish increased imperceptibly from an initial 8 mg% to almost 28% on the 6th day of storage. At this time this tendency changed abruptly and a sudden increase showed the VB-N to reach nearly 60 mg%. This pattern in VB-N production carried on by bacterial activity^{10,16}) was very similar to that observed for

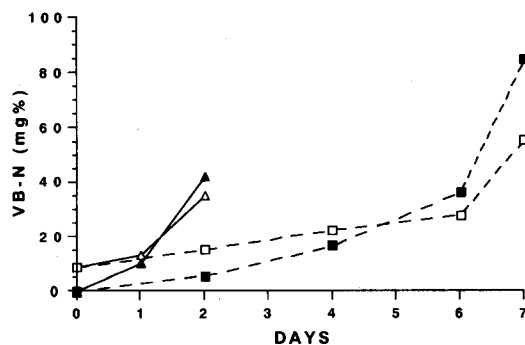


Fig. 2. VB-N changes in fish (g) and in the brine (ml) of immersion during storage at 15°C. Symbols are the same as in Fig. 1.

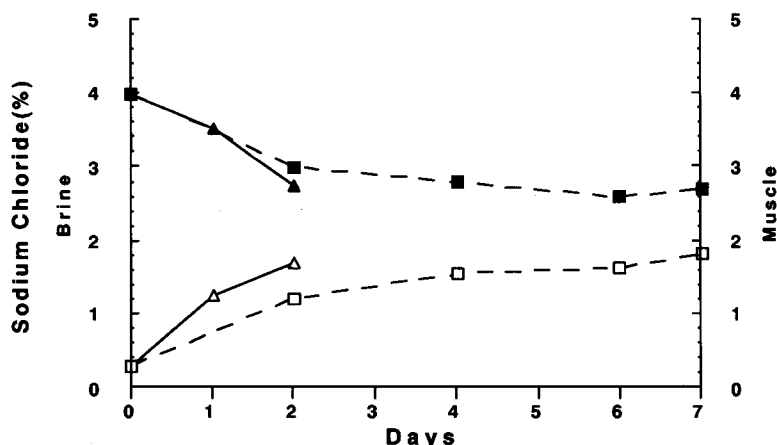


Fig. 3. Sodium chloride changes in fish and in the brine of immersion during storage at 15°C. Symbols are the same as in Fig. 1.

microbial growth.

According to this result and to that of sensory evaluation these samples were thought to be acceptable for consumption until the 6th day. However, they did not last longer and on the 7th day spoilage was evident.

The VB-N of fish used as control was almost the same to that of treated fish on the first day. From the first to the second day, this value increased very fast so that it reached nearly 40 mg%, which has been reported as a sign of spoilage¹⁶⁾. A clear coincidence between these results and the outcome of sensory test, that also showed this sample to be spoiled by the 2nd day, was observed during this work. These results also showed concordance with results obtained in a previous work⁴⁾.

The VB-N values for the treated and untreated brine were seen to change similarly to that of the corresponding sample of fish, with only small differences in the final value for the control and until the 6th day for the treated samples. At this time, the brine presented little higher ones. Here, it could be supposed that bacterial activity was responsible for this difference. It can be assumed that it is easy for the bacteria to use already broken down compounds coming out from the fish and becoming available in the brine in more quantities than those present in the fish itself. Therefore, it could be expected this difference to be larger as spoilage advances.

Salt content

A complementary study on salt penetration was done with the purpose to test Whether or not there is any effect of benzoate on salt penetration into fish muscle immersed in the treated brine. According to the results showed in Fig. 3, rate of increase and final salt concentration were recognized to be the same as that in results obtained in a previous work⁴⁾. Therefore, it could be said that there is no influence when using sodium benzoate on salt penetration.

Although further studies need to be done, especially those concerning changes in microflora. An important conclusion of this work may be the fact that 0.3%

sodium benzoate added to 4% brine when working together with the acetic acid, used to adjust the pH, could be a good alternative for sardine preservation when it is stored at 15°C. Results of this synergistic action²⁾ clearly showed that samples were acceptable up to the 6th day of storage.

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