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Control of xerophilic mould in traditional Egyptian salted fish, "Molouha"

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Introduction

Abstract

Molouha is a traditional Egyptian salted fish prepared mainly from fresh water fish *Hydrocynous froskali* but also can be prepared from other fish types. Hence, xerophilic mould is predominant in this type of salted fish, this study was undertaken to investigate the efficiency of propionic acid to control the growth of xerophilic mould and mycotoxins production in Molouha. In this study, Molouha was prepared by wet salting method (ESS 2005) with the addition of propionic acid at concentration of 0.5, 1 and 2%. The results revealed that propionic acid treatment at 0.5% concentration reduced mould growth in the treated samples significantly ($P < 0.05$) and inhibited completely the pathogenic species from producing mycotoxins with better color, odor and appearance of the treated samples. While 1 and 2% propionic acid treatments led to complete inhibition of mould growth, but due to their sour taste it was not accepted by the panelist. This study suggests the using of propionic acid as an effective method in inhibiting mould growth and mycotoxins production in salted fish. Only 0.5% is needed for a significant inhibition of mould growth and mycotoxins production.

Introduction

Fish is considered one of the most important and widely accepted sources of protein. Salted fish "Molouha" is one of the most famous and traditional fish products in Egypt. The preservation effect of salt has been recognized and used to decrease water activity, to reduce microbial attack leading to increase in the shelf life time of the preserved fish (Aubourg and Ugliano, 2002).

Nevertheless, some organisms can survive, grow, multiply, and produce toxins under a diverse range of environmental conditions. In Molouha, xerophilic mould that is capable of growing at reduced water activity (APHA, 2001), can contaminate salted fish from different sources such as the raw fish itself (Bagy *et al.*, 1993) and the additives specially the salt (Delcourt *et al.*, 1994). *Aspergillus* species is the most predominant mould in the environment and can affect most kinds of food; it is capable of producing mycotoxins which present a great public health hazard associated with salted fish (Thom and Raper, 1945; Pitt and Hocking, 1985a).

Aflatoxins are produced in nature by *Aspergillus flavus* and *Aspergillus parasiticus*, the four major naturally produced aflatoxins are known as aflatoxin B1, B2, G1 and G2. Aflatoxins are both acutely and chronically toxic to animals, birds and Man, where they produce four distinct effects; acute liver damage, liver cirrhosis, induction of tumors and teratogenic effects (Stoloff, 1977). Ahmed *et al.* (2005) reported that xerophilic mould strains isolated from salted fish had the ability to produce mycotoxins, the most mycotoxin detected was stregmatocystin followed by B2 and G1.

Fish quality and preservative technique play an important role in the mycological quality of salted fish. Different means have been used to control fungal contamination and their formation of toxins. Organic acids and their salts are known to be efficient against microorganisms, particularly moulds (Bullerman 1985). These substances are generally used in conservation of food materials without leaving residues that may cause health hazards to the consumers (Oteng-Gyang 1984).

Propionic acid and propionate formulations such as calcium and sodium salts are highly effective mould inhibitors commonly used in the food industry especially in cakes, bakery products and cheese. They have been listed as preservatives which generally recognized as safe food additives (GRAS) (Kırbaşlar *et al*., 2006). They are permitted in foods primarily as mould inhibitors, they tend to be highly specific against mould with the inhibitory action is being primarily fungistatic rather than fungicidal (Jay, 1998). Rao and Valsen (1961) reported that spoilage by mould was delayed and overall shelf life is improved by dipping of salted fish in propionic acid.

Controlling xerophilic mould growth and

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mycotoxin production in salted fish has a great public health concern, therefore this study aimed to evaluate the efficacy of propionic acid on mould growth and mycotoxins production in salted fish (Molouha).

Materials and Methods

Sample collection

A total of six kg of sardine fish each about 20 cm in length and weighed 65 g were purchased from local fish markets in Ismailia City, Egypt. Fish were transferred to the Laboratory of Food Hygiene Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt, in an ice box with a suitable quantity of flaked ice.

Treatment of fish with propionic acid

Upon arrival, the fish were washed, drained and divided into four groups (1.5 kg each). The first group was selected as control (no acid treatment) and was salted by immersing the fish in a solution containing 150 g pure salt, 65 g sugar and 10 g spices (ESS, 2005). The second, third and fourth groups were salted in the same manner as above but treated with addition of 0.5%, 1% and 2% (v/v) propionic acid respectively. Brining processes were completed after 15 days at room temperature in a completely airtight container. After completing the brining process, samples were removed from the brining solution and left to drain on a sterile stainless steel wire mesh for 15 min. Each fish from each group were packaged in sterile polyethylene bags, labeled and stored at 4^{o}C for two months. At 15 days predetermined time intervals, four randomly chosen packages were taken from each group to be analyzed for mycological quality.

Sample preparation

The samples were prepared according to the method recommended by (APHA, 2001).

Determination of total xerophilic mould count:

The total xerophilic mould count was determined according to (Raper and Thom, 1949), and (Raper and Fennell, 1977) by using Czapex Dox agar and Malt Extract Agar with 10% NaCl media (Klare, 1970).

Identification of the isolated xerophilic mould:

The mould colonies were subjected to morphological and microscopical examinations, and when necessary slide culture technique was carried out according to (Arx, 1976). *Penicillium* species were identified according to (Raper and Thom, 1949); (Pitt, 1988) and (Samson, 1979). *Aspergillus* species were identified according to Raper and Fennell (1967) while other mould genera were identified according to (Arx, 1976).

Screening of mould toxigenicity

The qualitative toxicity screening for the identified mould species was carried out according to the technique described by (Hara *et al.*, 1974).

Sensory evaluation

The sensory quality of treated salted fish was evaluated by the method of (Kim *et al.*, 1995). Ten evaluators scored the samples for color, odor, taste, and overall acceptance by a five-point scoring method. The scoring method was: 5, like extremely; 4, like moderately; 3, neither like nor dislike; 2, dislike moderately; and 1, dislike extremely. The evaluations were conducted at storage periods of 0, 15, 30, 45 and 60 days.

Statistical analysis

The obtained results were evaluated statistically using SPSS software (Version 16).

Results and Discussion

Xerophilic mould is predominant in salted fish and is thought to play a role in food spoilage, mycotoxins production which can cause a great public health hazards as a result of salted fish consumption (Jonsyn and Lahal, 1992).

Effect of propionic acid on xerophilic moulds

Table one reveals that the addition of 0.5% propionic acid to salted fish significantly reduced the population of xerophilic mould, estimated by 2 logs whereas, treatment with 1% and 2% propionic acid cause complete inhibition of mould growth. (Rao *et al*., 1958) recorded reductions in mould population in fish dipped in various concentrations of propionic acid. Moreover, (Rao and Valsen, 1961) reported that spoilage by mould was delayed and overall shelf-life time is improved by dipping salted fish in propionic acid.

Table 1. Effect of propionic acid on xerophilic moulds in salted fish samples during 60 days in the study groups

	Storage time (days)/Mould count per g				
Propionic acid	0	15	30	45	60
Control(0%)	5 x 10^2c	8x 10^2b	5x 10^3b	8x 10^3b	5x 10^4b
0.5%	2x 10b	4x10a	5x 10a	4x 10^2a	8x 10^2a
1%	ND	ND	ND	ND	ND
2%	ND	ND	ND	ND	ND

Within the same storage time mean values in the same column that are not followed by the same letter are significantly different (P = 0.05).
ND =Not detected.

Table 2. Frequency distribution of identified xerophilic mould species in control and treated groups

Xerophilic mould	Control		Treated	
	F	%	F	%
A. amstelodami Thom&Chruch	10	10	8	14
A. chevalieri Thom&Chruch	5	5	10	18
A. nidulans Wint	4	4	12	21
A.niger Tieghem	7	7	ND	0
A. terrious var. aureus Thom and Raper	5	5	ND	0
P. chrysogenum Thom	6	6	5	9
P. verrecosum var. verrecosum Dierckx	9	9	ND	0
P. verrecosum var. cyclopium Dierckx	22	22	ND	0
P. restricum Gilman & Abbott	7	7	7	12
Alternaria Nees	8	8	8	14
Cladosporum herbarum Link.	17	17	7	12
Total	100	100	57	100

ND = Not detected.

There is a paucity of the literature on propionic acid treatment effect on moulds in salted fish; therefore, the results of this study were compared to (Dixit and Singh, 2011) who reported that propionic acid inhibited the mould growth and aflatoxin production but in fenugreek seeds. They screened some organic acids to inhibit mould count and aflatoxin production , and reported that the maximum inhibition of mould count was noted at 0.4% concentration of Propionic acid, this concentration completely inhibit production of aflatoxin B2 and G1.

Table two reveals that *Penicillium* and *Aspergillus* species were predominant in control and treated samples representing 44 and 31% isolates and 21 and 30% respectively. Other mould species were detected in control and treated samples with different percentages, however *A. niger*, *A. terrious* var. *aureus*, *P. verrecosum* var. *verrecosum* and *P. verrecosum* var. *cyclopium* could not be detected in treated samples.

The obtained results agree with those reported by (Atapattu and Samarajeewa, 1990; Diagada and Adebajo, 1994; Sallenave-Namont *et al*., 2000), who isolated xerophilic mould from salted and dried fish products and reported that the *Aspergillus* and *Penicillium* were the predominant mould species.

Mycotoxins screening

Toxic mould metabolites, mycotoxins, are a broad spectrum of biologically active substances that occur as a result of growth of moulds on various types of feed and foods. Aflatoxins are the most significant mycotoxins and pose a quadruple threat to human and animal as they produce four distinct effects such as acute liver damage, liver cirrhosis, induction of tumors and teratogenic effect (wild, 2007). The Toxigenicity screening of isolated and identified xerophilic mould from salted fish samples was reported in table three. The results show that

Table 3. Mycotoxins production of the identified xerophilic mould in propionic acid treated and control salted fish

Mould Species	No.	Treated		Control		Mycotoxin
		No.	%	No.	%	
A. amstelodam iThom & Chruch	10	ND	ND	8	80	Stregmatocystin
A. chevalieri Thom & Chruch	5	ND	ND	4	80	Stregmatocystin
A. nidulans Wint	4	ND	ND	4	100	AFB2
P. verrecosum var. verrecosum Dierckx	22	ND	ND	8	36.4	AFG1

ND = Not detected.

Table 4. Sensory evaluation of control and treated salted fish

		Concentration of Propionic acid			
Storage time/d	parameter	0	0.5%	1%	2%
0	Color	3.40a	3.60a	3.82a	4.01b
	Odor	3.60b	3.91c	3.93c	3.88c
	Taste	4.00b	3.53c	3.30c	3.00a
	O..A	4.00ab	4.00a	4.01a	4.20b
15	Color	3.30a	3.55b	3.70b	3.80c
	Odor	3.32b	3.88c	3.91c	3.77b
	Taste	3.89b	3.43[d]	3.00c	2.00a
	O. A	3.66a	3.80[a]	3.85b	3.90b
30	color	3.00a	3.50b	3.55bc	3.60c
	odor	2.95a	3.70b	3.82c	3.85d
	Taste	3.20a	3.10c	2.60b	2.00d
	O.A.	3.20a	3.70b	3.77bd	3.80d
45	Color	2.90a	3.10b	3.40c	3.50c
	Odor	2.70a	3.00ab	3.20b	3.33b
	Taste	3.15a	3.00c	2.20b	2.00b
	O.A.	2.20a	2.89b	3.00c	3.00c
60	Color	2.50a	2.81b	2.98b	2.99b
	Odor	2.30a	2.30a	2.40b	2.65b
	Taste	2.00a	2.22ab	2.56b	2.00d
	O.A.	2.40a	2.50b	2.77b	2.89c

O.A. overall acceptance

24 (24%) xerophilic mould strains had the ability to produce mycotoxin in control samples only and four genera including *A. amesteldomi*, *A. chevalier*, *A. nidulans* and *P. vericosum* var *vericosum* produced predominately Stregmatocystin followed by aflatoxin G1 and aflatoxin B2.

On the other hand, propionic acid 0.5% completely inhibited the growth of pathogenic mould species which produce mycotoxin before, in the treated salted fish samples. Due to lack of literature concerning the effect of propionic acid on mycotoxin production in salted fish we cannot compare our results to any published data. However, Larous *et al*., (2007) reported that propionic acid 0.15% inhibited the growth of *P. expansum* and production of mycotoxin in apple fruits while (Dixit and singh, 2011) reported that 0.4% propionic acid inhibit mould growth and aflatoxin production in fenugreek seeds.

It is clear from the obtained results that propionic acid 0.5% has an inhibitory effect on mould growth and consequently to mycotoxin elaboration during storage of salted fish. Therefore, the addition of propionic acid 0.5% can be recommended to be used during salted fish processing as a preservative. Moreover, propionic acid is affordable, eco-friendly and has no residual toxicity.

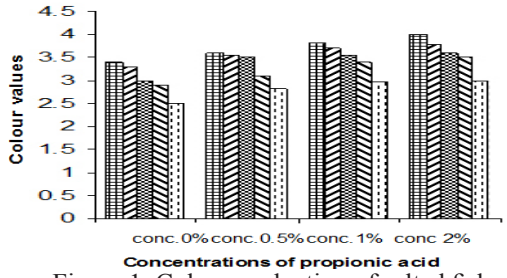


Figure 1. Colour evaluation of salted fish

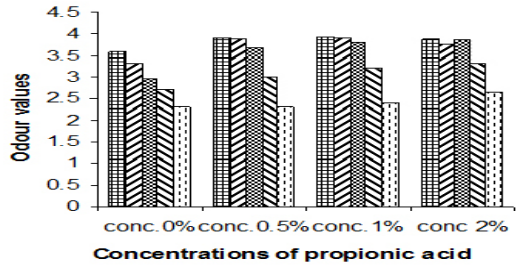


Figure 2. Odour evaluation of salted fish

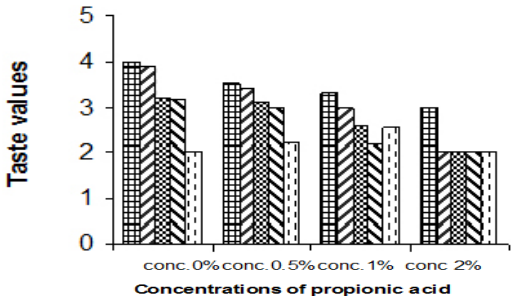


Figure 3. Taste evaluation of salted fish

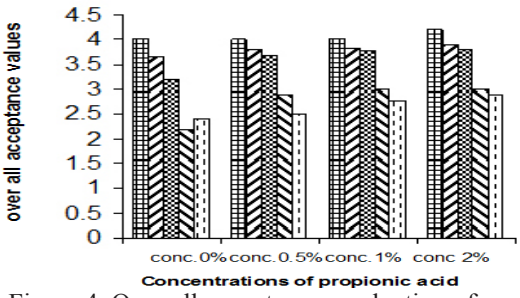
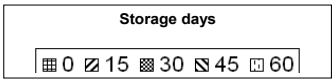


Figure 4. Over all acceptance evaluation of salted fish



Sensory evaluation

The organoleptic evaluation of food products to any processing technology is very important in determining the consumer acceptability. Table four shows the sensory evaluation of treated and control samples. Treated samples with 0.5%, 1% and 2% showed high levels of the acceptability in color, odor and overall acceptance, while treated samples with 1% and 2% propionic acid showed the lowest acceptability as the samples had a sour taste.

The achieved results as shown in figures 1, 2, 3 and 4 reveal that there were a significant difference ($P < 0.05$) in sensory parameters between control and treated groups. The color, odor, taste and overall acceptance parameters of treated groups were significantly changed upon increasing storage period.

There were no significant differences in preference of color, odor, taste and overall acceptance between 1% and 2% propionic acid.

Conclusion

This study highlights the possibility of using propionic acid as an effective method in inhibiting mould growth and mycotoxins production in salted fish. Due to its high efficacy, relative good palatability at lower inclusion rates and low cost, propionic acid may reasonably be considered as one of the most economical organic acids for field applications. Only 0.5% is needed for a significant inhibition of mould growth and mycotoxins production in salted fish.

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