Lipolytic and Proteolytic Activity of Yeasts Isolated from Fish

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Abstract:
This study was undertaken to determine lipolytic and proteolytic yeast count in 50 random samples of fish collected from different places at Sharkia governorate. Proteolytic activity was determined by using caseinate agar and lipolytic activity was determined by using plate count agar accompanied with tributyrin. The identification of the isolated yeasts was performed by a procedure which included two phases involved in the master key of simplified identification method (SIM). The mean values of total yeast count of fresh *Tilapia nilotica* and frozen fishes of *Pagellus acarne*, *Trachurus indicus*, *Lutjanus* and *Atherina*, were 3.76±0.18, 4.19±0.19, 2.49±0.30, 4.67±0.25 and 5.62±.016 $\log_{10}$cfu/g, respectively. The mean values of proteolytic yeast counts of fresh *Tilapia nilotica* and frozen fishes of *Pagellus acarne*, *Trachurus indicus*, *Lutjanus* and *Atherina*, were 3.73±0.31, 4.20±0.01, 2.51±0.34, 4.59±0.22 and 4.59±0.24 $\log_{10}$cfu/g, respectively. The mean values of lipolytic log yeast counts of fresh *Tilapia nilotica* and frozen fishes of *Pagellus acarne*, *Trachurus indicus*, *Lutjanus* and *Atherina*, were 4.44±0.13, 4.20±0.01, 2.96±0.21, 5.03±0.11 and 4.92±0.23 $\log_{10}$cfu/g, respectively. Four yeast genera could be identified including Candida, Cryptococcus, Trichosporon, Rhodotorula. The results determined that *C.glabrata* showed both proteolytic and lipolytic activities while *C.tropicalis* and *Rhodotorula glutinis* showed lipolytic activity and *Cryptococcus* and Trichosporon showed proteolytic activity. From the results of the present study, it can be concluded that yeast contamination of fish can lead to fish spoilage due to their lipolytic and proteolytic activities which characterized by off-odors, off-flavors and slime formation so consequently control of yeast growth in fish is necessary.

Keywords: yeast, fish, spoilage, lipolytic yeast, proteolytic yeast

Introduction:

In recent years, consumption of fish and fish products has greatly increased globally, consumers prefer fish because it is high in protein, vitamins (B complex), minerals including iron, calcium, and iodine ***(Stroud, 2001)***, as well as polyunsaturated fatty acids, which have beneficial effects against cardiovascular disease. On the other hand, sseafoods are very perishable products, and microbial load has strong influence on fish quality.

From the fishing to the consumption, fish are liable to contamination by different kinds of yeast which leads to spoilage of fish. Although, yeasts are smaller in number than bacteria, yeasts are widely distributed in seawater and non-saline water so numerous species of yeasts have been isolated from marine and fresh water fish ***(Deak and Beuchat, 1996)***, In a wider survey ***(kobatake et al.,1992)*** isolated yeast from seven kinds of fresh raw seafoods, the isolates comprised six genera, Candida, Cryptococcus, Debaryomyces, Rhodotorula, Sterigmatomyces and Trichosporon, All the isolates were psychrotrophic yeasts and showed varying degrees of lipolytic and proteolytic activities as *Candida lipolytica, Trichosporon pullulans,* and *Candida scottii*..

The degree of spoilage of yeasts isolated from protein-rich sea foods, can be evaluated according to the proteolytic activity of the isolated yeast. In light of this concern, the current study was carried out to investigate the level of yeast contamination in fish and to throw the light on the proteolytic and lipolytic activities of yeasts associated.

Material and Methods:

1. Sample collection:

A total of 50 samples of fresh and frozen fish (10 each from fresh *Tilapia Nilotica sp.,* frozen *Pagellus acarne sp.,* frozen *Trachurus indicus sp.,* frozen *Lutjanus sp.,* and frozen *Atherina sp*.) were collected from different places at Sharkia governorate. The collected samples were transferred to the laboratory without undue delay.

2. **Preparation of samples** ***(APHA, 2002):***

Twenty-five grams of muscle samples were aseptically homogenized with 225 ml of sterile peptone water 0.1% in stomacher (model 400, Seward Medical Londen, UK) at medium speed for 2 minutes (10^{-1} dilutions). A single tenfold dilution series in peptone water 0.1% were prepared up to 10^{6}.

3. **Enumeration of yeast count** ***(Deak and beuchat, 1996):***

Duplicate 0.1 ml samples of homogenate, as well as duplicate 0.1 ml from each of the previously prepared dilutions were spread plated on tryptone glucose yeast extract agar supplemented with chloramphenicol. The plates were incubated in an upright position,

plates showing from 10 to 100 colony forming units (CFU) were counted and recorded as total yeast count.

4. **Enumeration of proteolytic yeast count** ***(Lee and Kraft, 1984)***

Duplicate 0.1 ml from each of the previously prepared dilutions was spread plated on standard method caseinate agar supplemented with chloramphenicol. The inoculated plates were incubated at 25 ^{o}C for 3-5 days. The proteolytic activity was manifested by a clear hallo zone around the colonies.

5. **Enumeration of lipolytic yeast count** ***(Smith and Alford, 1984)***

Duplicate 0.1 ml samples of homogenate, from each of the previously prepared dilutions were spread plated on tributyrin agar supplemented with chloramphenicol. The inoculated plates were incubated at 25 ^{o}C for 3-5 days. The lipolytic activity was manifested by a clear hallo zone around the colonies**.**

6. Identification of the isolated yeasts:

Yeast isolates were identified according to the simplified identification method (SIM) which is confined to the most frequent foodborne yeasts ***(Deak and Beuchat, 1996),*** the identification was performed by procedure which included two phases. First phase, a primary characterization of isolates was carried out using six tests involved in the master key of SIM. These are the urease reaction, growth in the presence of 0.1% cycloheximide, and assimilation of nitrate, mannitol, celloboise and erythritol. Also, all isolates were examined microscopically for colony morphology. Then yeast isolates were divided into groups depending on the results of these tests. In the second phase of simplified identification method, selected tests according to each group were performed to approve and confirm identification. These are the Urease test, Growth with 0.01% cycloheximide, Assimilation of nitrate erythritol, mannitol, and cellobiose. In addition, all isolates were microscopically and for colony morphology on tryptone glucose yeast extract agar plates and for determine mode of reproduction by using rice agar medium. Based on the results of these tests, isolates were separated into groups and selected tests according to each group, were used in the second phase of SIM to reach and confirm identification. These tests included Growth without vitamins, Growth at 37°C, Fermentation of glucose, Production of starch like compound, Assimilation of inositol, galactose, maltose, melibiose, raffinose, 2-ketogluconate, 1-arabinose, methyleglucoside, ethylamine, cadaverine, ethylamine and lysine ***(Deak and Beuchat, 1996)***. These tests were performed according to the standard procedures described by ***Yarrow (1998).***

Results and Discussion:

1-Total Yeast counts in fish:

The results recorded in table (1) revealed that, the mean log $_{10}$values of total yeast counts were 3.76±0.18, 4.19±0.19, 2.49±0.30,4.67±0.25 and 5.62±0.16 in fresh *Tilapia nilotica* and frozen fishes of *Pagellus acarne, Trachurus indicus, Lutjanus* sp. and *Atherina sp.,* respectively. These results were nearly similar to those obtained by ***Ibrahim (2000)*** who reported the mean value of total yeast count/g were $7.1\times10^3\pm$ 3.6 $\times10^3$ in fresh *Tilapia nilotica*.

On the other hand, lower results were reported by ***El-Sayed (1990)*** who reported the mean value of total yeast count as 4.8×10^3 in *pagell acarne* species. Higher counts were detected in *trachurus fish* by ***El-Atabany et al. (1992).***

We can conclude that, due to their widespread distribution, yeast contamination of fish was high. The high yeast count was expected due to poor handling and the absence of adequate hygienic measures used during the fishing, transportation, handling, preservation, storage, and marketing of fish.

2-Proteolytic yeast counts in fish:

The results recorded in table (2) showed that the mean log $_{10}$ values of proteolytic yeast counts were 3.73±0.31, 4.20±0.01, 2.51±0.34, 4.59±0.22 and 4.59±0.24 in fresh *Tilapia nilotica* and frozen fishes of *Pagellus acarne, Trachurus indicus, Lutjanus* sp. and *Atherina* sp., respectively.

3-Lipolytic yeast counts in fish:

The results recorded in table (3) showed that the mean log $_{10}$values of lipolytic yeast counts were 4.44±0.13, 4.20±0.01, 2.96±0.21, 5.03±0.11 and 4.92±0.23, in fresh *Tilapia nilotica* and frozen fishes of *Pagellus acarne, Trachurus indicus, Lutjanus* and *Atherina* samples, respectively.

Relatively, we can't compare our results because there isn't much information about the occurrence and importance of proteolytic and lipolytic yeast in fish.

Because food microbiologists have not thought this information with any consistency, the true occurrence of proteolytic and lipolytic yeast in fish is unclear.

4-Distribution of yeast species in fish:

The results obtained in table (4) showed that of the 47 strains of yeasts isolated from different fish samples, these isolates were identified to 4 genera including Candida, Cryptococcus, Trichosporon and Rhodotorula.

The frequency and percentage of species in the isolates from fresh *Tilapia nilotica*, there were 20 isolates identified to *C.krusei* 1 (5%), *C.glabrata* 4 (20%), *Cryptococcus spp.* 3 (15%), *Trichosporon spp.* 5 (25%) and *Rhodotorula glutinis* 4 (20%), *Rhodotorula mucilaginosa* 3 (15%).

Nearly similar results were reported by ***Ibrahim (2000)*** who could isolate *Candida spp*. (43.3%), *Cryptococcus spp*. (16.7%), *Rhodotorula spp*. (40%) and

Trichosporon spp. (3.3) from fresh *Tilapia nilotica.*

Also, nearly similar results were reported by ***Samy et al, (2014)*** who found that *Candida spp.* were (43.3%) that higher than *Rhodotorula spp.* (33.3%) from *Tilapia nilotica*.

The frequency and percentage of species in the isolates from frozen *Pagellus acarne*, there were 9 isolates identified to *Cryptococcus spp*. 2 (22.2%), *Trichosporon spp*. 2 (22.2%), *Rhodotorula glutinis* 3 (33.4 %) and *Rhodotorula mucilaginosa* 2 (22.2%).

The frequency and percentage of species in the isolates from frozen *Trachurus indicus*, there were 5 isolates identified to *C.glabrata* 3 (60%) and *Trichosporon spp.* 2 (40 %).

The frequency and percentage of species in the isolates from frozen *Lutjanus sp.*, there were 5 isolates identified to *C.glabrata* 2 (40%), *Trichosporon spp.* 1 (20%) and *Rhodotorula glutinis* 2 (40%).

The frequency and percentage of species in the isolates from frozen *Atherina sp*., there were 8 isolates identified to *C.tropicalis* 2 (25%), *C.glabrata* 3 (37.5%), *Cryptococcus* 1 (12.5%), *Trichosporon* 1 (12.5%) and *Rhodotorula mucilaginosa* 1 (12.5%).

In general, these results agree with ***Michiko Kobatake et al. (1988) and kutty and Philip (2008)*** who reported that the yeast genera isolated with high frequency of occurrence were *Candida, Cryptococcus, Debaryomyces, Rhodotorula, Torulopsis, Trichosporon,* and others, which are present in various kinds of seafoods.

5-proteolytic and lipolytic activity of isolated yeast species:

The results determined that *C.glabrata* showed both proteolytic and lipolytic activities while *C.tropicalis* and *Rhodotorula glutinis* showed lipolytic activity and *Cryptococcus spp.* and *Trichosporon spp.* showed protoelytic activity.

Nearly similar results were reported by ***Michiko kobatake and Hiroshi Kurata (1983) and Ismail et al. (2000),*** they reported that proteolytic and/or lipolytic yeast species were widely distributed among the genera *Candida, Cryptococcus, Rhodotorula and Trichosporon.*

This study indicates that yeasts especially *C.glabrata, Trichosporon* and *Rhodotorula* play an important role in spoilage of fish. The ability of these and other yeasts to breakdown fish constituents, making nutrients more available for yeast growth which can enhance the rate of spoilage and shorten the shelf life.

It's also reasonable to conclude that yeasts, even in small populations, contribute significantly to lipolytic and proteolytic alterations in food. This fact highlights the significant function of yeast in the spoilage of fish, which results in undesirable

changes such as off odours, off tastes, and slime formation. As a result, yeast growth in fish must be controlled.

Table 1: *Statistical values of total yeast count (log_{10} cfu/g) on fresh and F: frequency, %: percentage*

product	Minimum	Maximum	Mean±SE
Fresh *Tilapia nilotica*	3.23	5.02	3.76±0.18
Frozen *Pagellus acarne*	3.60	4.98	4.19±0.19
Frozen *Trachurus indicus*	<1	3.53	2.49±0.30
Frozen *Lutjanus sp*.	3.30	5.41	4.67±0.25
Frozen *Atherina sp.*	5.06	6.19	5.62±0.16

Table 2: *Statistical values of proteolytic yeast counts (log_{10} cfu/g) on fresh and frozen fishes: - (No=10 of each)*

Product	Minimum	Maximum	Mean ±SE
Fresh *Tilapia nilotica*	<1	4.34	3.73±0.31
Frozen *Pagellus acarne*	4.16	4.26	4.20±0.01
Frozen *Trachurus indicus*	<1	3.95	2.51±0.34
Frozen *Lutjanus sp*.	3.45	5.62	4.59±0.22
Frozen *Atherina sp*.	3.72	5.95	4.59±0.24

Table 3: *Statistical values of lipolytic yeast counts (log_{10} cfu/g) on fresh and frozen fishes: - (No=10 of each)*

Product	Minimum	Maximum	Mean ± SE
Fresh *Tilapia nilotica*	3.89	4.92	4.44±0.13
Frozen *Pagellus acarne*	4.16	4.26	4.20±0.01
Frozen *Trachurus indicus*	2	3.95	2.96±0.21
Frozen *Lutjanus sp.*	4.46	5.81	5.03±0.11
Frozen *Atherina sp.*	4.04	5.72	4.92±0.23

Table 4: *Frequency and percentage of yeast species isolated from the examined samples fresh and frozen fish:*
F: frequency, %: percentage

samples / Yeast species	Tilapia nilotica		Pagellus acarne		Trachurus indicus		Lutjanus sp.		Atherina sp.	
	F	%	F	%	F	%	F	%	F	%
C.tropicalis	-	-	-	-	-	-	-	-	2	25%
C.krusei	1	5%	-	-	-	-	-	-	-	-
C.glabrata	4	20%	-	-	3	60%	2	40%	3	37.5%
Cryptococcus spp.	3	15%	2	22.2%	-	-	-	-	1	12.5%
Trichosporon spp.	5	25%	2	22.2%	2	40%	1	20%	1	12.5%
Rhodotorula glutinis	4	20%	3	33.4%	-	-	2	40%	-	-
Rhodotorula mucilaginosa	3	15%	2	22.2%	-	-	-	-	1	12.5%
total	20	100	9	100	5	100	5	100	8	100

Table 5: *Yeast species showing lipolytic and proteolytic activity:*

Yeast species	Lipolytic activity	Proteolytic activity
C.glabrata	+ve	+ve
C.tropicalis	+ve	-ve
Trichosporon spp	-ve	+ve
Cryptococcus spp	-ve	+ve
Rhodotorula glutinis	+ve	-ve

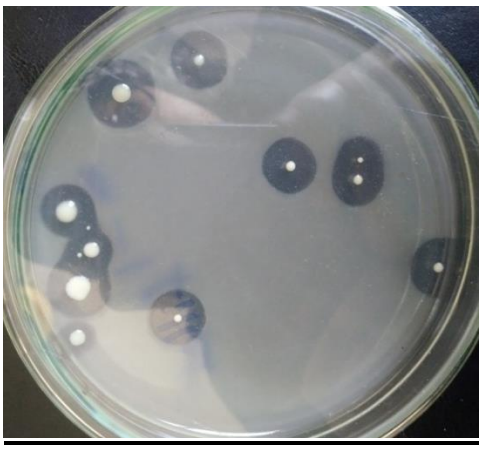

Figure 1: Different yeast colonies showing lipolytic activity on tributyrine agar media.

Figure 2: Different yeast colonies showing lipolytic activity on tributyrin agar media.

Figure 3: Different yeast colonies showing proteolytic activity on caseinate agar media.

Conclusion: From the results of the present study it could be concluded that there is high level of contamination by yeast in fish samples collected from different places in Sharkia. Moreover, yeast contamination in fish can lead to spoilage because of their lipolytic and protoelytic activities**.**

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الملخص العربي
الخمائر المحللة للدهون والبروتينات المعزولة من الأسماك

أُجريت هذه الدراسه لمعرفة عدد الخمائر المحللة للدهون والبروتينات في خمسين عينة من عينات الأسماك المختلفه التي تم تجميعها من أسواق محافظه الشرقية, في تلك الدراسة تم استخدام نوعين من المنبتات (standard method caseinate agar) لمعرفة الخمائر المحللة للبروتينات و(tributyrin agar)لمعرفة الخمائر المحللة للدهون, كان متوسط لوج العدد الكلي للخمائر في عينات لحوم أسماك البلطي الطازجة و أسماك كلا من المرجان و الباغة و الشخرم والباساريا المجمدة هو 3,76±0,18, 4,19± 0,19 , 2,49±0,30 , 4,67±0,25 و 5,62±0,16 لكل جرام على التوالي. وكان متوسط لوج عدد الخمائر المحللة للبروتينات في عينات لحوم أسماك البلطي الطازجة و أسماك كلا من المرجان و الباغة و الشخرم والباساريا المجمدة هو3,73±0,31, 4,20±0,01, 2,51±0,34, 4,59±0,22 و4,59±0,24 لكل جرام على التوالي. وكان متوسط لوج عدد الخمائر المحللة للدهون في عينات لحوم أسماك البلطي الطازجة و أسماك كلا من المرجان و الباغة و الشخرم والباساريا المجمدة هو 4,44±0,13 , 4,20±0,01 , 2,96±0,21, 5,03±0,11 و 4,92±0,23 لكل جرام على التوالي. تم عزل وتصنيف اربعة انواع من الخمائر تشمل كلا من : الكانديدا و كريبتوكوكاس و تريكوسبورون و الرودوتورولا, وتبين من النتائج أن كانديدا جلابراتا لها القدرة على تحليل الدهون والبروتينات و أن كلا من كانديدا تروبيكالس و رودوتورولا جلوتينيس لهم القدرة على تحليل الدهون وأن كلا من كريبتوكوكاس و تريكوسبورون لهم القدره على تحليل البروتينات.

أكدت نتائج هذه الدراسة أن الخمائر لها دور هام في فساد لحوم الأسماك المختلفة وذلك نظرا لقدرتها على تحليل الدهون والبروتينات مما قد يؤدي الى تكون مادة لزجة على سطح لحوم الأسماك أو تواجد طعم ورائحة غير مقبولين للمستهلك ولذلك يجب السيطرة على نموهم حتى تكون المنتجات صالحة للإستهلاك الآدمي وذات جودة جيدة.