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SANITARY EVALUATION OF SOME SALTED FISHES IN ALEXANDRIA

(With 5 Tables)

By

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التقييم الصحي لبعض الأسماك المملحة بالإسكندرية

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تمت الدراسة على ٦٠ عينة (٢٠ عينة من كل من الفسيخ والملوحة والسردين المملح) التي تم جمعها عشوائيا من أسواق الإسكندرية خلال الفترة من أغسطس ٢٠٠٥ وحتى مارس ٢٠٠٦. وقد فحصت هذه العينات للوقوف على مدى مطابقتها كيميائيا وبكتيريولوجيا للمواصفات القياسية المصرية (م ق م ١٧٢٥– ٣،٢،١ / ٢٠٠٥) للأسماك المملحة. ولقد أسفرت نتائج التحليل الكيميائي عن أن مستوى الهستامين في الأسماك المملحة تراوح بين ٩– ١٧٨مجم/١٠٠جم. كما أن تركيز الأس الهيدروجيني للعينات قد تراوح بين ٥,٩٢– ٧,٣ في عينات الفسيخ والملوحة والسردين المملح. ولقد أسفرت نتائج التحليل البكتيريولوجي عن أن متوسط عدد الميكروب العنقودي الذهبي ٢,٩ لو١٠ خلية /جم و ٢,٦٩ لو١٠ خلية /جم و ٢,٨٩ لو١٠ خلية /جم على التوالي. أما متوسط العدد الكلي للبكتيريا المختزلة للكبريت فقد كان ٣,٤٧ لو١٠ خلية /جم و ١,٦٨ لو١٠ خلية /جم و ٢,٨٦ لو١٠ خلية /جم على التوالي. ولقد أسفرت نتائج الدراسة الحالية عن أن ٥% من عينات الفسيخ فقط هي التي تجاوزت حدود المواصفات القياسية المصرية للنموات البكتيرية الحمراء المحبة للملح في الأسماك المملحة كما أن ١٠% من عينات الفسيخ أيضا كانت ايجابية للديكروب القولوني البرازي. أما بالنسبة للبكتيريا الممرضة مثل السالمونيللا والشيجلا والفبريو باراهيموليتكس والكولستريديم بوتيولينم فقد أسفرت النتائج عن خلو جميع العينات التي تم فحصها من هذه الميكروبات.

SUMMARY

A total of 60 Samples of salted fish [20 of each of Fesiekh (*Mugil cephalus*), Melouha (*Hydrocyons froskalii*) and sardine *(Sardina pilchardus)]* were collected at random from Alexandria markets through August, 2005 to March, 2006. The samples were examined chemically and bacteriologically. The achieved results of chemical analysis revealed that histamine level in the examined samples ranged from 9 to 178 mg/ 100 g. The salt content of the examined samples as expressed by the

water phase salt % was in a range of 3.2% to14.1%. On the other hand, the bacteriological analysis revealed that the mean value of *Staph. aureus* count in Fesiekh, Melouha and sardine samples was 2.9±0.03 log_{10} c.f.u/g, 2.69±0.1 c.f.u/g and 2.89±0.25 c.f.u/g, respectively. The mean value of Sulphite reducing anaerobic bacteria was 3.47± 0.60 log_{10} c.f.u/g, 1.68± 0.43 log_{10} c.f.u/g and 2.86± 0.09 log_{10} c.f.u/g, respectively. The red halophiles were detected only in 5% and *E. coli* in 10% of the examined Fesiekh samples. Sulphite reducing anaerobic bacteria were isolated in the rate of 85%, 70% & 55% and *Staphylococcus aureus* in the rate of 80%, 85% and 70% from Fesiekh, Melouha and salted sardine samples respectively. Whereas, *Vibrio parahaemolyticus*, *Clostridium botulinum*, *Salmonellae* and *Shigellae* were not detected through the current study.

Key words: *Salted fishes, evaluation, histamine, staph.aureus, E.coli, vibrio parahaemolytica, salmonella, C.botulinum.*

INTRODUCTION

The main objectives of salting fish are preservation and developing a desirable flavor. Preservation of fish by salting and fermentation could not be separated, since both were involved to a varying degree in most practical processes (Hobbs, 1987).

Fish in its natural environment has its own micro-flora in the slime, on its body, in its gut and gills. These micro-organisms, as well as the enzymes in the tissues of the fish, bring about putrefactive changes in fish when it dies. Furthermore, the micro-organisms generally present in the salt used for salting also contribute to the degradative changes in the fish (Essuman, 1992). In case of poor hygiene, the contamination of salted fish may increase due to unsanitary procedures during processing and handling (Novotny *et al.*, 2004).

Salted fermented fishes, as Fesiekh, Melouha and salted sardine are among the popular fish products available in Alexandria. Such products could be consumed on a large scale during some occasions and feasts.

The first stage of the salting process is the addition of salt to the raw fish. Then the fish is stored in sealed barrels or containers at ambient temperatures. Fesiekh is a semi-putrid form of salted and dried Grey Mullet species (Mugil spp.), a saltwater fish. The traditional process of preparing it is to dry the fish in the sun for some days before being preserved in salt. Melouha is prepared from a freshwater fish.

The Egyptian Organization for Standardization and Quality Control, 2005 had laid down the chemical and bacteriological quality of salted fish (Fesiekh, Melouha and sardine) as the product must have salt concentration ≥ 6%, hydrogen ion concentration (pH value) between 6 and 6.5 and histamine content ≤ 20 mg/ 100 g. The product must be free from the pathogenic bacteria and its toxins, the red halophils, *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Clostridium botulinum* (*C. botulinum*) and *Escherichia coli* (*E. coli*). Also the counts of *Staphylococcus aureus* (*Staph. aureus*) and total sulfite reducing anaerobic bacteria must be ≤ 10^2 c.f.u/g (EOSQC, 2005).

In some instances, salted fishes in Egypt may be prepared under poor hygiene, which have been resulted in human illness and food poisoning outbreaks (Weber, *et al.*,1993), (Fawzi, 1999). So, the current study aimed at assessing the chemical and bacteriological quality of Fesiekh, Melouha and salted sardine from Alexandria markets in accordance to the Egyptian Organization for Standardization and Quality Control specifications (ESS 1725-1,2,3 /2005).

MATERIALS and METHODS

Sampling:

A total of 60 Samples of salted fish [20 of each of Fesiekh (*Mugil cephalus*), Melouha (*Hydrocyons froskalii)* and sardine *(Sardina pilchardus)]* were collected at random from Alexandria markets through August, 2005 to March, 2006.

The skin and bones of the fish was aseptically removed and the meat was comminuted using a sterile blender jar.

Chemical analysis:

Histamine level:

The histamine determination was done using liquid chromatography as described by Beljaars *et al.*, 1998.

Hydrogen ion concentration (pH):

20 g comminuted fish sample were blended with 40 ml distilled water (at room temperature) for 1 min. in a blender then the pH was measured using the pH meter method (Woyewoda *et al.*, 1986a).

Sodium chloride content:

It was determined as described in the CODEX STAN 167-1989, REV. 2-2005. In this procedure, the salt was extracted by water from the preweighed sample then the chloride concentration was determined by titration of an aliquot of the solution with a standardized silver nitrate solution and calculated as sodium chloride%.

Moisture content:

It was determined using the drying oven procedure (Woyewoda *et al.*, 1986b; Hilderbrand, 1992).

Water phase salt content:

Water phase salt (WPS) was Calculated as: % salt (%S) divided by % salt + % moisture (%M) multiplied by 100 (Hilderbrand, 1992).

$$WPS = \frac{\%S}{\%M + \%S} \times 100$$

Bacteriological analysis:

1. Decimal serial dilutions of samples were prepared and inoculated to Differential Reinforced Clostridial Medium (OXOID CM149) and to Baird Parker Agar (OXOID CM275) by pour plate method for sulphite reducing anaerobic bacteria and *staphylococcus aureus* count, respectively.
2. Red halophilic bacteria: were detected macroscopically as red slime on the surface of the salted fish, then microscopically as cocci and/or bacilli with various shapes or malformed cells (Karl, 2005).
3. Samples were examined also for the presence of *Salmonellae* and *Shigellae* (on XLD agar), *V. parahaemolyticus* (on TCBS agar), *C. botulinum* (on Reinforced Clostridial Medium) and *E. coli* (on Eosine methylene blue agar).
4. The isolated bacteria were identified and confirmed biochemically as described by Harrigan, 1998.

RESULTS

Table 1: Histamine level and hydrogen ion concentration (pH) of the examined salted fish samples.

Salted fish	Histamine level (mg/100g)					pH value				
	min	max	Mean ± SE	Samples exceeded ES limit*		min	max	Mean	Samples exceeded ES limit*	
				n	%				n	%
Fesiekh (*Mugil cephalus*) N=20	18	178	22.3 ± 2.0	6	30	6.30	7.30	6.90 ± 0.05	9	45
Melouha (*Hydrocyons froskalii*) N=20	13	65	19.1 ± 2.8	2	10	6.40	6.72	6.70 ± 0.01	2	10
Sardine (*Sardina pilchardus*) N=20	9	32	19.6 ± 1.7	3	15	5.92	6.84	6.40 ± 0.07	6	30

*ES limit = Egyptian standard limit for histamine is ≤ 20 for pH is 6

Table 2: Sodium chloride, Moisture and Water phase salt content of the examined salted fish samples.

Salted fish	% Sodium chloride			% Moisture			% Water phase salt				
	min	max	Mean	min	max	Mean	min	max	mean	Samples exceeded ES limit*	
										n	%
Fesiekh (*Mugil cephalus*) N=20	2.1	5.3	4.2	38	85	72	3.2	8.3	5.4	12	60
Melouha (*Hydrocyons froskalii*) N=20	4.5	8	9.5	30	64	51	4.2	14.1	13.7	1	5
Sardine (*Sardina pilchardus*) N=20	3.9	7.5	6.0	41	70	64	3.5	6.0	5.1	4	20

*Egyptian standard (ES) limit for Nacl in water phase is ≥6

Table 3: Counts of *Staphylococcus aureus* and sulphite reducing anaerobic bacteria in he examined salted fish samples.

Salted fish	*Staphylococcus aureus* (log_{10} c.f.u/g)			Sulphite reducing anaerobic bacteria (log_{10} c.f.u/g)		
	Min	max	Mean ± SE	Min	max	Mean ± SE
Fesiekh (*Mugil cephalus*) N=20	1.48	4.78	2.90 ± 0.03	1.95	6.69	3.47 ± 0.60
Melouha (*Hydrocyons froskalii*) N=20	1.60	4.20	2.69 ± 0.10	1.49	1.90	1.68 ± 0.43
Sardine (*Sardina pilchardus*) N=20	1.58	4.92	2.89 ± 0.25	1.55	4.99	2.86 ± 0.09

N=number of examined samples SE= standard error

Table 4: Frequency distribution of *Staphylococcus aureus* and sulphite reducing anaerobic bacteria counts in the examined salted fish samples

Salted fish	Bacteria	>10-10^2 c.f.u/g		>10^2-10^3 c.f.u/g		>10^3-10^4 c.f.u/g		>10^4-10^5 c.f.u/g		Saples exceeded ES limit*	
		n	%	n	%	n	%	n	%	n	%
Fesiekh (*Mugil cephalus*) N=20	*Staphylococcus aureus*	11	55	1	5	1	5	3	15	5	25
	sulphite reducing anaerobic bacteria	13	65	3	15	0	0	1	5	4	20
Melouha (*Hydrocyons froskalii*) N=20	*Staphylococcus aureus*	15	75	1	5	1	5	0	0	2	10
	sulphite reducing anaerobic bacteria	13	60	1	5	0	0	0	0	1	5
Sardine (*Sardina pilchardus*) N=20	*Staphylococcus aureus*	9	45	2	10	1	5	2	10	5	25
	sulphite reducing anaerobic bacteria	8	70	1	5	2	10	0	0	3	15

*Egyptian standard (ES) limit for of Staphylococcus aureus and sulphite reducing anaerobic bacteria is $\leq 10^2$ c.f.u/g

Table 5: Incidence of bacteria isolated from the examined salted fish samples.

Salted fish / Bacteria	Fesiekh (*Mugil cephalus*) N=20		Melouha (*Hydrocyons froskalii*) N=20		Sardine (*Sardina pilchardus*) N=20	
	+ve	%	+ve	%	+ve	%
sulphite reducing anaerobic bacteria	17	85	14	70	11	55
Vibrio parahaemolyticus	0	0	0	0	0	0
Red halophilic bacteria	1	5	0	0	0	0
Clostridium botulinum	0	0	0	0	0	0
Staphylococcus aureus	16	80	17	85	14	70
Escherichia coli	2	10	0	0	0	0
Salmonellae	0	0	0	0	0	0
Shigellae	0	0	0	0	0	0

DISCUSSION

Chemical parameters:

Histamine level:

Histamine production in fish and other foods is by the decarboxylation of histidine through a reaction catalyzed by the enzyme histidine decarboxylase. It is reported, however that the decarboxylation reaction results largely from bacterial action These reports indicated that the fermentation of fish is a likely source of histamine (Ababouch *et al.*, 1991). In addition, various species of fish are known to have large amounts of free histidine in their muscle tissues as substrate for histidine decarboxylase.

Table (1) showed that histamine level in the examined salted fishes samples. The results showed that 30%, 10% and 15% of fesiekh, Melouha and sardine samples, respectively had exceeded the Egyptian standard limit of histamine in salted fish (≤ 20 mg/ 100g). Similar observation for sardine was obtained by Riad (1997), who reported that mean value of histamine level was 19.25 mg/100gm muscle with 45% of the examined samples exceeding the permissible limit. Lower findings (18.3 mg/100gm muscle), was stated by Samaha *et al.* (1997) who recorded that 10% of examined sardine samples exceeded the permissible limit. Moreover, Kassem (1996), and Fath El-Bab (2005) reported higher findings (44.95 and 30mg/100gm muscle, respectively).

In the present study, histamine levels were detected in the examined salted Melouha samples with a mean value 19.1mg/100gm muscle. The finding was lower than the data (44.31 mg/100gm muscle) of Kassem (1996) and Riad (1997), who noticed that 2.5% of *Hydrocyons froskalii* samples exceeded the permissible limit.

In respect of histamine level in Fessiekh, the results recorded in the present study (22.3 mg/100gm muscle) were lower than the finding (38.7 mg/100gm muscle) registered by Kassem (1996) and 35 mg/100gm muscle by Fath El-Bab (2005). Riad (1997), recorded that 42.5% of the samples exceeded the permissible limit.

Fish containing high levels of histamine results in histamine poisoning. This poisoning has historically been referred to as scombroid poisoning because of the frequent association of the illness with the consumption of spoiled scombroid fish (Taylor *et al.*, 1989). The US Food and Drug Administration (FDA), for instance, established a hazard action level (HAL) of 50 mg/100g in tuna products based on the investigation of previous histamine poisoning outbreaks and the defect

action level (DAL) of 20 mg/100g. Peconek *et al.*,1997, indicated that during storage of salted herring at ambient temperature the increase of histamine content in their flesh can occur. While, Fonberg-Broczek *et al.*, 2003, found that histamine content increased in low-salted sampled up to 35 g/kg during the period of storage. Taylor *et al.*, 1989 stated that Histamine formation in fish can be prevented by proper handling and refrigerated storage.

Hydrogen ion concentration (pH):

The pH value reflects the condition of the fish. In Table (1) the pH values of the examined salted fish samples were presented. the obtained results revealed that 45% of Fesiekh, 10% of Melouha and 30% of salted sardine samples seemed to exceed the Egyptian standard limits of pH values for salted fishes.

The pH values recorded in the current study were nearly in agreement wit that reported by Kassem, 1996 and El-Kewaiey, 2001 who observed a range of pH 5.9-7.2 and a mean value of 6.56. Also Fath El-Bab (2005), recorded a mean pH value of 7 and 6.9 in Fisiekh and salted sardine sample, respectively. The pH value between 5 and 6 was considered normal for fish, however, this value enhanced the histidine decarboxylase enzyme production which reached its maximum activity at pH 6.5 -6.5.

Salt content:

The Salt percentages of the examined samples were presented in Table (2) which revealed that Fesiekh, Melouha and sardine samples exhibited salt% range of 2.1%-5.3%, 4.5%-8% and 3.9%-7.5% respectively with means of 4.2%, 9.5% and 6% respectively.

The moisture percentages of the examined samples were also found in Table (2) which showed that Fesiekh, Melouha and sardine samples revealed moisture percentage range of 38%-35%, 30%-64% and 41%-70% with mean value of 72%, 51% and 64% respectively.

The salt content of the examined samples as expressed by the water phase salt % was illustrated in Table (2). Water phase salt means the percent salt (sodium chloride) in the finished product. The results of salt content in the current study revealed that 60%, 5% and 20% of Fesiekh, Melouha and sardine samples respectively, exceeded the Egyptian standard limits of salt content for salted fishes.

Salt preserves fish by the removal of water from the flesh to a level that impedes microbial growth and enzymatic activities. The reduced use of salt results in uncontrolled fermentation. Under such conditions, the fish muscle becomes ideal for the growth of pathogenic

organisms and the product may decay within a short period. In situations where brine is reused a number of times, the chemical composition of the salt solution is altered. Significant amounts of organic material are introduced and the bacterial load of the brine becomes extremely high especially the red halophiles. Common defect of salted fermented fishery products called pink is the result of spoilage by red halophilic bacteria which grows in brine solutions at temperatures ranging from 15° to 55°C. (ICMSF, 1980). The moisture content of fermented fish varies from about 12 %in smoked dried anchovies to 65 % in wet salted cured fish. Products with high moisture content tend to deteriorate faster than drier products especially if the salt level is low. (FDA, 2001a).

A few pathogenic organisms can proliferate at salt concentrations higher than 10 %. However, it is known that many of these organisms survive in saturated salt solutions. For instance, salmonella can survive in 10 % salt solutions for one to three months. *Escherichia coli* and *Staphylococcus aureus* can survive for many weeks in salted fish (ICMSF, 1980).

In a study on fesiekh processing, Abdalla (1989) reported that the pH of the product ranges from 6.4 to 6.9 and the salt level is 6 to 7%. These are favourable conditions for the growth of *C. botulinum* and other proteolytic bacteria. This could possibly be the reason for fatalities involving the consumption of fessiekh in Egypt where the raw product is a delicacy among some people.

Bateriological parameters

***Staphylococcus aureus* count:**

Results presented in Table (3) revealed that the mean counts of *Staph. aureus* in Fesiekh, Melouha and sardine samples were 4.78, 4.2 and 1.58, respectively. Whereas, the results presented in Table (4) showed that 25%, 10% and 25% of the examined Fesiekh, Melouha and sardine samples respectively had counts higher than 10^2 c.f.u/g (2 $\log_{10}$ c.f.u/g) which exceeded the Egyptian standard limits of *Staph. aureus* count in salted fishes.

Nearly similar results were recorded by Kassem (1996) who isolated *Staph. aureus* from salted fishes in Cairo and Giza in a range of <2-5 $\log_{10}$ c.f.u/g and found that the mean was 3.69 $\log_{10}$ c.f.u/g. She attributed the higher counts of *Staph. aureus* in Fesiekh to the excessive contamination to which mullets were subjected during the swelling stage. Higher results were obtained by El-Kewaiey, (2001) who reported a count ranged from 2.6 to 5.83 $\log_{10}$ c.f.u/g and a mean value of 4.75 $\log_{10}$ c.f.u/g. He reported also that only 12% of samples satisfied the

Egyptian standards,1985. In addition, Basti *et al.*, (2004), in Iran, stated that *Staph. aureus* more than 3 $\log_{10}$ c.f.u/g were found in 10% of the examined salted fish. Whereas, Sen &Temelli (2003) examined marinated anchovy in Turkey and found that the mean count of *Staph. aureus* was 2 $\log_{10}$ c.f.u/g. Results of El-Tahan *et al.*, (1998) showed that *Staphylococcus aureus* counts were higher than the acceptable limit.

Sulphite reducing anaerobic bacteria (Clostridia) count:

From Table (3) the range and mean counts of Sulphite reducing anaerobic bacteria with 3.47, 1.6 and 2.86 2 $\log_{10}$ c.f.u/g in the examined samples, respectively. The rate of the samples exceeded the Egyptian standard limits of Sulphite reducing anaerobic bacteria count was 20%, 5% and 15% of the examined Fesiekh, Melouha and sardine samples respectively (Table 4). However, Sen &Temelli (2003), recorded no sulphite reducing anaerobic bacteria in samples of marinated anchovy in Turkey.

Red halophilic bacteria:

The red halophiles were isolated from the examined Fesiekh samples only in the incidence of 5% (Table, 5). The red halophilic bacteria causes the pink slime on the surface of salted fish which gradually spread leading to fish degradation due to the active proteolytic enzymes produced by the bacteria. These bacteria belong to two genera of bacteria namely Halobacterium and Holococcus. Halobacterium is rod-shaped requiring at least 10-15% salt concentration for growth whilst Halococcus can thrive at 5-10% salt content. Both genera are strictly aerobic growing optimally at 37^{0}C and produce red carotenoid pigments (Krieg & Holt, 1984). Gram & Huss (1995) stated that Fish products with high salt contents may spoil due to growth of halophilic bacteria.

Incidence of pathogenic bacteria:

The incidence of pathogenic bacteria in the examined samples was presented in Table (5). This table revealed that sulphite reducing anaerobic bacteria were isolated in the rate of 85%, 70% & 55% and *Staphylococcus aureus* in the rate of 80%, 85% and 70% from Fesiekh, Melouha and salted sardine samples, respectively. Results of *Staph. aureus* were higher than that obtained by Nassar & Ahmed, 1997 (15.5%) and slightly lower than that obtained by El--Kewaiey, 2001 (88%). Whereas, *Vibrio parahaemolyticus*, *Clostridium botulinum*, *Salmonellae* and *Shigellae* were not detected through the current study.

It should be assumed that *C. botulinum* will be present in any raw fishery product, particularly in the viscera. *C. botulinum* forms toxin more rapidly at higher temperatures than at lower temperatures. Failure to isolate *C. botulinum* through the current study, is not a proof that salted ungutted fishes processed in sealed containers at ambient temperature are free from such organism or its toxin in every instance. Telzak, *et al.* (1990), described an international outbreak of type E botulism due to uneviscerated salted fish in New York city and Israel, Whereas, Weber, *et al.* (1993) described another massive outbreak of type E botulism associated with traditional salted fish in Cairo.

In Iran, the study of Basti *et al.*, (2004) reported a high rate of *Vibrio parahaemolyticus* (50%) and a lower rate of *Staph. aureus* (10%). But *Salmonella* and coliforms were not detected. Also Results of El-Tahan *et al.*, (1998) showed that. All the samples under observation were free from Salmonella and Clostridia.

Rodriguez-Jerez *et al.* (1993) reported that the counts of the different bacterial groups decreased during the first two weeks of ripening, with the exception of the sulphite-reducers and vibrio, but later stabilized. The count of the sulfite-reducers remained unchanged during the whole ripening process. Vibrio were not detected in any of the samples studied.

In a study conducted by Nerquaye-Tetteh *et al.* (1978) to isolate various micro-organisms, no Salmonella spp. were isolated from samples of fermented fishery products obtained from the open markets in Ghana. The absence of Salmonella spp. from fermented fishery products could be attributed to the high salt level and low water activity of the products. These conditions do not favour the growth of salmonella. However, Arkoudelos *et al.* (2003) reported that *Salmonella enteritidis* and *Staph. aureus* survived for 60 and 90 days respectively during the ripening period of salted sardine.

From the obtained results of this study, we can notice that the sulphite reducing anaerobic bacteria and *Staph. aureus* were very important contaminants of salted fishes from Alexandria markets. The processing methods of salting fishes particularly, Fesiekh could create avenues for microbial infection and risks of food poisoning.

Therefore, it is recommended that salted fermented fishes stored under reduced oxygen at ambient temperature should be designed to consistently achieve a water phase salt level of at least 20% (based on the maximum water phase salt level for growth of *S. aureus*); a pH of 4.6 or below; or a water activity of 0.85 or below (based on the minimum

water activity for growth of *Staph. aureu*). Expert knowledge of pickling/brining/formulation processes is required to establish such a process. Such knowledge can be obtained by education or experience or both (FDA, 2001). As the spores of *Clostridium botulinum* are known to be present in the viscera of fish, any product that will be preserved by salting, drying, pickling, or fermentation must be eviscerated prior to processing. Without evisceration, toxin formation is possible during the process even with strict control of temperature. Evisceration must be thorough and performed to minimize contamination of the fish flesh.

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