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EVALUATION OF CONTACT SURFACES CONTAMINATION IN SMOKED SALMON PLANT AND ITS EFFECT ON THE FINAL PRODUCT

(With 3 Tables)

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تقدير مدى تلوث الاسطح الملامسة للاسماك فى مصانع السالمون المدخن وتأثيرها على صلاحية المنتج النهائى

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من خلال هذه الدراسة فى أحد مصانع تدخين السلمون، حيث يطبق نظام الهاسب، تم تجميع عدد ٤٣٨ عينة من كل من العمال والأسطح الملامسة للغذاء والأسماك قبل وأثناء العمل من ثلاث مناطق هى منطقة التجهيز ومنطقة التصنيع ومنطقة التعبئة، حيث دلت النتائج أن جميع العينات قبل بدء العمل كانت سلبية سواء للعد أو العزل البكتيرى وأن منطقة التصنيع هى الأقل معنويا فى العد البكتيرى بالنسبة لباقى المناطق. أما بالنسبة للعمال فلم يتم عزل اى من الميكروبات سوى عترة واحدة من المكور العنقودى الذهبى (٨،٣٣%) فى منطقة التجهيز. أما الأسطح الملامسة للغذاء فقد تم عزل عترة واحدة بنسبة ٨,٣٣% من كل من الأيشريشيا القولونية والليستريا مونوسيتوجينز فى منطقة التجهيز. أما ميكروبات السالمونيلا فلم يمكن عزلها من أى من المسحات او الأسماك وكذلك لم يمكن عزل أى من ميكروبات التسمم الغذائى محل الدراسة من أى من المنتجات النهائية. وقد أوصت الدراسة بالتحقق من سلامة المواد الخام المستخدمة وزيادة عدد مرات النظافة والتطهير أثناء العمل للتقليل من خطر التلوث العرضى بالمصنع.

SUMMARY

A total number of 438 workers, surface swabs and fish samples were collected from cold-smoked salmon plant where HACCP system is in place. The samples were collected pre- and during work from three areas (preparing, processing and packaging) to be examined for the bacterial count and isolation of food poisoning microorganisms (*Salmonella, E. coli, S. aureus* and *L. monocytogenes*). All the preworking samples were negative for count and isolation. Meanwhile the production area was significantly ($P<0.05$) lower in count for both workers and contact

surfaces swabs than the other areas during work. For workers samples only *S. aureus* (1, 8.33%) was isolated from preparing area, while other microorganisms couldn't be isolated. For contact surfaces one strain (8.3%) of each of *E. coli* and *L. monocytogenes* were isolated from preparing area. Meanwhile, *E. coli* (1, 8.3%) and *L. monocytogenes* (2, 16.7%) were isolated from fish samples in the preparing area. Salmonella couldn't be isolated from any sample. Raw materials were the most probable cause of contamination. None of the investigated microorganisms was isolated from the finished products. For such plant, it's recommended to verify the quality of purchased raw material and to decrease the intervals of sanitization program to decrease the risk of cross contamination.

Key words: *Fish, Salmon, smoked fish, Salmonella, L.monocytogenes.*

INTRODUCTION

Smoking is among the oldest methods of food preservation. Smoked salmon as one of the ready-to-eat (RTE) seafood has been recorded as a vehicle for pathogenic microorganisms and food poisoning outbreaks occasionally occur. Improper processing and lack of suitable storage condition of these products is the main cause of food poisoning outbreaks (Gudbjornsdottir *et al.*, 2004; Eklund *et al.*, 2004).

Hazard analysis critical control point "HACCP" is becoming established and has been successful in the food industry, and improving the control of food production in catering establishments (Martinez-Tome *et al.*, 2000). The hygiene of the process and processing environment is a significant factor in the production of microbiologically safe and good quality products in the fish industry. Both quantity and the specific type of microbial flora are important factors for evaluating the hygiene of the processing environment (Miettinen *et al.*, 2001).

Although food poisoning microorganisms has been isolated from fresh frozen, and processed seafood, the production of RTE cold smoked salmon represents a particular concern, since the processing of these products does not include a thermal inactivation step (Ward, 2001). Meanwhile, salting represents the only weak bactericidal step (Neunlist *et al.*, 2005).

L. monocytogenes can contaminate finished products through employee contact surfaces such as hand tools, gloves, aprons, and food contact surfaces (Tompkin *et al.*, 1999). FDA identified smoked fish in

the high risk category for causing listeriosis. *L. monocytogenes* can cause listeriosis in pregnant women, immuncompromized and eldery (Slutsker and Schuchat, 1999). There are several reports of listeriosis associated with the consumption of RTE foods (Anon, 2001). Some of these epidemics resulted in mortalities, as well as large scale recalls of implicated RTE foods (USDA, 2002). Listeriosis has a mortality rate of 20-30% and thus represents a serious public health concern (Schuchat, *et al.,* 1991). The prevalence of *Listeira* spp. in RTE foods have been variable ranging from 1.8% to 48% (Gibbons *et al.,* 2006).

Other enteric pathogens have been isolated form RTE foods including *E. coli* and *Salmonella* emphasizing the risk posed by consumption of these foods (Faustini *et al.,* 2003; Lee and Middleton, 2003). In many countries *S. aureus* is the second or third common cause of foodborne disease outbreaks behind *Salmonella* (Rosec *et al.,* 1997). *S. aureus* was the predominant microorganism found on hands of food preparators (Ryan *et al.,* 1996). Scott and Bloomfield (1990) identified the ability of *S. aureus* to cause cross-contamination for up to 24 h via the fingertips.

The aim of this study was to determine the level of food contact surface hygiene and occurrence of *Listeria* spp. *Salmonella* spp., *E. coli* and *S. aureus* and its effect on the final product in cold-smoked salmon factory where HACCP system is implemented.

MATERIALS and METHODS

In a cold-smoked salmon plant where HACCP system is in place, the processing steps were as follows; imported eviscerated whole frozen salmon fishes were left to thaw in a cold room (less than 10 °C), washed with water and acetic acid, deheaded, cut into two halves, deboned, salted then smoked (cold smoking), trimmed, sliced and finally vacuum packed.

Through six visits, a total number of 438 samples were collected as follows: 126 consisted of 36 workers and 90 food contact surfaces swabs were collected pre-work directly after cleaning and disinfection. Another 252 samples consisted of 72 workers, 180 food contact surface swabs and 60 food samples were collected during at least midway through a shift and towards the end of the working day from various processing stages. All swabbed surfaces were in direct contact with food. The plant was divided into three areas representing preparing area, processing area and packaging area. Each area was sampled for workers

(gloves and aprons), surfaces [utensils (tables, knives, containers, shovels and cutting boards), machines (skinning, trimming and slicing)] and fish (frozen, thawed, salted, smoked and final packed product). Swabs from workers' hands and 25 cm^2 from food contact surfaces using sterile swab moisten with neutralizing buffer and 25 grams of food were collected. All samples were kept in an icebox and transported without delay to the laboratory where they were analyzed for the following:

Bacteriological analysis:

- Preparation of samples

On arrival to the laboratory, samples were homogenized with buffer peptone (1:10). Ten-fold serial dilutions were prepared using buffer peptone for the following analysis:

1. Aerobic plate count: using pour plate technique onto plate count agar (Oxoid, CM0325) and incubation at 35 °C for 48 h according to APHA (1992).
2. Isolation of *Salmonella*: samples were pre-enriched on buffer peptone for 24 h at 37 °C, enriched on Rappaport Vassiliadis (Lab M 86) for 24 h at 41.5 °C and tetrathionate broth (Oxoid CM0029) at 37 °C for 24 h then plated on XLD (Oxoid CM0469) and Brilliant green agar at 37 °C for 24 h, according to APHA (1992).
3. Isolation of *E. coli*: using lauryl sulphate broth (Biolife) incubated at 35 °C for 24-48 h followed by inoculation of a loopful from positive tubes on *E. coli* broth (Britania) incubated at 45.5 °C for 24-48 h then plated on eosin methylene blue agar (Biolife) and incubated at 35 °C for 24-48 h according to APHA (1992).
4. Isolation of *S. aureus*: using selective enrichment procedure, by enrichment on brain heart infusion broth (Oxoid CM225) at 37 °C for 24 h, then streaking on Barid Parker agar (Biolife) at 35° C for 48 h according to APHA (1992).
5. Isolation of *L. monocytogenes*: using *Listeria* enrichment broth (Biolife) at 30 °C for 48h and plating on Oxford agar (Lab M 122) at 35 °C for 24-48 h according to FAO (1992)

The results were statistically analysed according to Selvin 1996), using SPSS for Windows version 10. "SPSS Inc. Headquarters, Chicago, Illinois USA".

RESULTS

Table 1: Mean APCs and incidence of isolated microorganisms in different areas for workers samples

Area	Samples	No.		APC		Isolates No. (%)				
		B	D	B	D	B	*E. coli*	*L. monocytogenes*	*S. aureus*	*Salmonella*
Preparing area	Workers	12	24	-ve	$2.5X10^2$ a	-ve	-ve	-ve		-ve
	Gloves	6	12		$2.0X10^2$				-	
	Aprons	6	12		$3.0X10^2$				1(8.33%)	
Production area	Workers	12	24	-ve	8.0X10 A	-ve	-ve	-ve	-ve	-ve
	Gloves	6	12		5.0X10					
	Aprons	6	12		$1.1X10^2$					
Packing area	Workers	12	24	-ve	$1.0X10^2$ a	-ve	-ve	-ve	-ve	-ve
	Gloves	6	12		8.0X10					
	Aprons	6	12		$1.2X10^2$					

B: before work D: during work

There is significant difference between means containing the same capital and small letter

Table 2: Mean APCs and incidence of isolated microorganisms in different areas for surfaces samples

Area	Samples	No.		APC		Isolates No. (%)				
		B	D	B	D	B	*E. coli*	*L. monocytogenes*	*S. aureus*	*Salmonella*
Preparing area	Utensils	24	48	-ve	$6.0X10^2$ a	-ve			-ve	-ve
	Tables	6	12		$3.0X10^2$		-	-		
	Knives	6	12		$2.2X10^2$		-	-		
	Containers	6	12		$1.0X10^3$		-	1(8.3%)		
	Cutting-boards	6	12		$9.0X10^2$		1(8.3%)	-		
Production area	Utensils	24	48	-ve	$1.1X10^2$ A	-ve	-ve	-ve	-ve	-ve
	Tables	6	12		$1.0X10^2$					
	Shovels	6	12		2.0X10					
	Containers	6	12		$2.4X10^2$					
	Shelves	6	12		8.0X10					
Packing area	Surface	42	84	-ve	$3.9X10^2$ a	-ve	-ve	-ve	-ve	-ve
	Machines	18	36		$3.0X10^2$					
	Skinning	6	12		$2.0X10^2$					
	Trimming	6	12		$2.1X10^2$					
	Slicing	6	12		$5.0X10^2$					
	Utensils	24	48		$4.8X10^2$					
	Tables	6	12		$6.0X10^2$					
	Knives	6	12		$4.3X10^2$					
	Containers	6	12		$3.0X10^2$					
	Cutting-boards	6	12		$6.0X10^2$					

There is significant difference between means containing the same capital and small letter

Table 3: Mean APCs and incidence of isolated microorganisms in different areas for food samples

Area	Samples	No.	APC	Isolates No. (%)			
			D	*E. coli*	*L. monocytogenes*	*S. aureus*	*Salmonella*
Preparing area	Frozen	12	$5.4X10^2$ A	-	2 (16.7%)	-ve	-ve
	Thawed	12	$1.2X10^4$ a	1 (8.3%)	-		
Production area	Salted	12	$2.0X10^2$ A	-ve	-ve	-ve	-ve
	Smoked	12	$4.3X10^3$ a	-ve	-ve		
Packing area	Final	12	$6.2X10^3$ a	-ve	-ve	-ve	-ve

There is significant difference between means containing the same capital and small letter

DISCUSSION

The pre-work samples of the food contact surfaces and workers swabs for the 3 sampled areas were negative for aerobic plate count and isolation (Table 1, 2 and 3). This result indicates the efficiency of the processes of cleaning and disinfection SSOPs (sanitary standard operating procedures). On the other hand, the bacterial loads for workers, utensils and machines were significantly elevated during work in the different three areas.

This result is reasonable considering that HACCP system is established at the plant i.e. food hygiene training of the staff, physical separation of raw food materials and unpacked smoked salmon by means of separate areas, utensils and machines, the use of separate staff, control and monitoring of temperature. This result agrees with Gillespie *et al.*, (2000) who stated that significantly unsatisfactory or unacceptable samples were obtained from premises where no HACCP system was in place as compared with these which had an undocumented or documented hazard analysis system.

Results highlighted that raw salmon was the most probable cause of contamination of each of workers (hands and aprons), machines and utensils during work. This indication can be observed from the bacterial count of frozen and thawed salmon (Table, 3). The condition that emphasize the spread of bacteria in the working environment during preparing salmon fishes. This result agrees with Gorman *et al.* (2002) who observed that contaminated raw materials contributed to an increase in the contamination level of worker's hands, counter tops and draining

boards during preparation. Also held the opinion of Eklund *et al.* (1995) who stated that sanitation and cleanup procedures adequately eliminated *L. monocytogenes* from the processing line and equipment, but recontamination occurred soon after resumption of processing. The primary source of contamination proved to be the surface areas of frozen or fresh raw fish coming into the plant.

Results of bacterial load of food contact surfaces (workers and surfaces) and fish samples showed weak but significant ($P<0.05$) decrease in the production area than preparing and packing areas (Table 1, 2 and 3). This decrease can be attributed to the washing effect of thawed fishes (raw material) with acetic acid beside the effect of salting. This result agrees with that recorded by (Neunlist *et al.*, 2005).

Prior to food preparation, all workers' hands, machines and utensils were free of all the tested microorganisms. However, after preparation and processing *L. monocytogenes* was isolated from the containers in preparation area (1 (8.3%) (Table, 2). The pathogen could also be isolated from 2 (16.7%) of frozen fishes (Table, 3). This agrees to some extent with Cabedo *et al.* (2008) who isolated *L. monocytogenes* from 20% of frozen fishes.

The ability of foodborne microorganisms as *Salmonella* and *Listeria* spp. to become disseminated from naturally contaminated foods to various hands and food contact surfaces is well known (Gorman *et al.*, 2002). Throughout the present study we observed that in spite of the studied plant applied restrict sanitary programs according to HACCP system *L. monocytogenes* could be isolated from utensils. The same findings were achieved by several studies which have shown that *L. monocytogenes* is capable of contaminating food processing machines with bacterial attachment and biofilm formation representing a source of potential contamination of food products (Autio *et al.* 1999). Once attached to a surface, *L. monocytogenes* appear to be more difficult to be removed (Autio *et al.*, 1999 and Beresford *et al.*, 2001).

Although *L. monocytogenes* was isolated from the preparation area and frozen fishes it couldn't be isolated from other surfaces or finished products. This could be attributed to the effective SSOPs and the combined effect of processing steps (washing with acetic acid, salting and smoking). This correspond with the opinion of Neunlist *et al.* (2005) who stated that the combination of steps of cold-smoking significantly lowered *L. monocytogenes* by 1.6 log cfu/g. Also Gonzalez *et al.* (2002) couldn't isolate the organism from any of the samples of

smoked salmon. On the other hand, Cabedo *et al.* (2008) isolated *L. monocytogenes* from 7.9% of smoked salmon.

It was observed through this study that *S. aureus* was the only isolated pathogen from one worker's apron (8.33%) after preparation of raw fishes. In this concern, Gorman *et al.* (2002) achieved the same result and claimed that *S. aureus* was the predominant microorganism found on hands of food preparators following food preparation. Food preparator hands were recorded as the main factor contributing up to 39% of domestic food poisoning outbreaks (Ryan *et al.*, 1996). Scott and Bloomfield (1990) identified the ability of the *S. aureus* to cause cross contamination for up to 24 hours via fingers' tips.

E. coli could be isolated from 1 (8.3%) of the cutting boards (preparation area) and from 1 (8.3%) of thawed fishes. It seems that the cleaning process eliminated the pathogen so it couldn't be isolated from any of the finished products. This result agrees with Gonzalez *et al.* (2002) who couldn't isolate *E. coli* from the smoked salmon samples. In this concern, Gorman *et al.* (2002) reported that from 7 isolates of *E. coli* during the preparation of food, four of which were found to cross contaminate one or more of the premises surfaces such as counter top and draining boards.

Although *Salmonella* has been found to survive on dry surfaces for long periods of time (Humphrey *et al.*, 1994) it couldn't be detected in the examined raw food premises surfaces or final products. In the same way, Gonzalez *et al.* (2002) couldn't isolate *Salmonella* from the smoked salmon samples. However, Gorman *et al.* (2002) reported that a small number of *Salmonella* infected food (8%) had the ability to cause 100% cross contamination with other sites in the preparing premises including the counter top.

It was obvious in the present study that all the final products were negative for all the tested pathogens although the raw food materials and some of the processing facilities were contaminated. The result which indicates the proper and efficiency of the production processes. At the same time, such a plant may need to decrease the intervals between cleaning and sanitization item to increase its ability to eradicate pathogens such as *L. monocytogenes*.

Overall, it was undoubtful that the contaminated raw food material is the real cause of disseminating microorganisms and contamination of various facilities (equipments and utensils) and workers' hands. Therefore, it is recommended to verify the quality of purchased raw material and to increase frequency of cleaning and

disinfecting of the premises and the hygienic practice of the workers to decrease the risk of cross contamination.

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