National Center of Radiation Research and Technology-Nasr City,
Cairo, Egypt.

EFFECT OF TRISODIUM PHOSPHATE AND LOW DOSE IRRADIATION TREATMENTS ON THE BACTERIOLOGICAL, CHEMICAL AND SENSORY STATUS OF FROZEN FISH FILLETS

(With 5 Tables)

By

WAFAA S. MOHAMED; ENSHRAH K.I. MIRA* and SUZAN M.A. ABOU-ZIED*

* Food Hygiene Department-Animal Health Research Institute-Dokki, Giza.

(Received at 17/12/2007)

تأثير المعاملات بفوسفات ثلاثى الصوديوم والتشعيع بجرعات منخفضة على الحالة البكتريولوجية والكيميائية والحسية لشرائح الأسماك المجمدة

وفاء سيد محمد ، إنشراح خليل إبراهيم ميره ، سوزان محمد أحمد أبو زيد

أجريت هذه الدراسة لبحث إمكانية استخدام أقل جرعة من أشعة جاما بالإضافة إلى تركيزات مختلفة من فوسفات ثلاثى الصوديوم لتخفيض المحتوى الميكروبى لشرائح السمك البلطى فور إعدادها وقبل تجميدها وعرضها بالأسواق. تم تقسيم شرائح السمك إلى أربع مجاميع حيث أعتبرت المجموعه الأولى ضابطة. تم غمر الجزء الثانى من العينات فى تركيزات مختلفة من فوسفات ثلاثى الصوديوم (١،٥،٣%) أما الجزء الثالث من العينات فتم تعريضه لجرعات مختلفة من الإشعاعات (١،٢،٣ كيلوجراى) وتم تعريض القسم الأخير لكلتا المعاملتين. وقد أسفرت نتائج فحص جميع العينات أن تعريضها لجرعة ١ كيلو جراى من أشعة جاما بعد غمرها فى محلول فوسفات ثلاثى الصوديوم تركيزه ٣% قد أدى إلى تخفيض العدد الكلى للبكتريا الملوثة للحم السمك. كما أدى تعريض العينات لجرعة ٢ كيلو جراى من أشعة جاما بعد غمرها فى محلول فوسفات ثلاثى الصوديوم تركيزه ٣% إلى القضاء تماما على بعض أنواع البكتريا وتخفيض مستوى حمض الثيوباربتيوريك والنيتروجين الكلى المتطاير وكذلك إعطاء أفضل دلالات من الجودة الحسية. لذا ننصح بتعريض شرائح السمك لجرعه ٢ كيلوجراى من أشعة جاما بعد غمرها فى محلول ٣% فوسفات ثلاثى الصوديوم لمدة عشر ثوانى للحصول على منتج خالى من الميكروبات ، صحى ومقبول اللون والملمس والرائحة.

SUMMARY

Development of a combination of gamma irradiation and trisodium phosphate (TSP) for preservation of fish fillets and improvement of its microbiological quality were studied. Irradiation doses of 1, 2 and

3 kGy were applied to the first group of the prepared samples; dipping in 1.5, 3 and 5% TSP concentrations was applied to the second group of samples, then a combination of irradiation and TSP treatments have been applied to the third group of the prepared samples. The results revealed that irradiation alone was effective in eliminating most of the detected organisms of importance in fish fillets gradually as the dose of irradiation increased. Immersion of fish fillets in TSP alone was not enough to reduce the microbial population while combination of 3% TSP and 1 kGy of irradiation resulted in great reduction in the counts of the contaminating microorganisms. However combination of 3% TSP and 2 kGy of irradiation reduced the aerobic plate count to less than 2 log cycles with the best sensory and chemical attributes.

Key words: *Fish, trisodium phosphate, irradiation.*

INTRODUCTION

After more than four decades of research and development; food irradiation has been demonstrated to be safe, effective and versatile as a mean of food preservation, decontamination or disinfection (Andrew *et al.*, 1998). Its various applications cover the destruction of parasites and pathogenic microorganisms in foods of animal and plant origin and decontamination of spices and food ingredients (Murano *et al.*, 1995). Such applications provide consumers with the increase in variety, volume and value of food (Hamza *et al.*, 1997).

Hackwood (1994) mentioned that in 1980, both the U.S. Food and Drug Administration and the World Health Organization of the United Nations accepted foods irradiated with an average dose as high as 10 KGy neither presenting any toxicological hazard nor introducing any special nutritional or microbiological problems and thus "safe for human consumption".

Fish fillets belongs to the semipershable food items because of its water activity, which favors the growth of spoilage organisms and the presence of enzymes that cause rapid spoilage and deterioration (Bari *et al.*, 2000). The quality of fresh water fish is a major concern to industry and consumers. Like marine fish; fresh water fish are extremely perishable food commodities. Deterioration of fish mainly occurs as a result of bacteriological activities leading to loss of quality and subsequent spoilage. Faulty rearing and harvesting practices can

result in contamination with food borne pathogenic bacteria (ICMSF, 1998).

Methods used for evaluation of fish quality can be grouped into two categories: those that determine sensory quality and those that use physical, chemical, and/ or bacteriological principles. Microbiological criteria that exist for raw fishery products include plate counts for total bacterial count, fecal coliforms count (most probable number), and *Staphylococcus aureus* count (Gonzalez-Rodriguez *et al.*, 2001).

Polyphosphates are organic additives widely used to increase water holding capacity and reduce cooking losses in the manufacturing of meats and meat products (Sheard *et al.*, 1999 and Erasmus *et al.*, 2003). Trisodium polyphosphate (TSP) has been used in the United States since 1992 when it was granted by the FDA (Coppen *et al.*, 1998). The extreme alkaline pH (10-13) of TSP is detrimental to microorganisms since they are not able to adjust and carry out cellular functions. TSP poses a problem with its high pH and phosphate removal making disposal difficult and expensive (Fabrizio *et al.*, 2002).

This study was undertaken to determine the efficacy of TSP dipping and/or low doses of irradiation in reducing pathogenic and spoilage populations of bacteria in filleted fish as it is exposed to contamination from many sources such as the water where it lives, contamination during gutting, deboning, handling, washing, bad storage and packaging. Further investigations will be needed for evaluation of the status of treated fish during storage.

MATERIALS and METHODS

Sampling:

Fresh Tilapia fishes were purchased from Giza markets with average 500 grams weight, washed, degutted and filleted then sent to the laboratory in an ice box. The prepared fish fillets were classified into four groups:

1- The first group of samples was left as a control.
2- Dipping in TSP: the second group of samples was dipped in 1.5, 3 and 5% solutions of trisodium phosphate (TSP) for 10 seconds. Afterwards; the samples were allowed to drain excess solution before being placed in sterile polyethylene bags.
3- Irradiation: the third group of samples was irradiated at a specific irradiation dose (1, 2, 3 kGy)

4- The fourth group of samples was subjected to both dipping and irradiation treatments with the fore mentioned concentrations of TSP and doses of irradiation.

Each fish sample was packed in a polyethylene bag that was sealed under aseptic conditions and marked with the specific dose of irradiation and the specific TSP dilution. The samples were frozen at -18°C for 7 days to assimilate the commercial product.

Irradiation of samples:

Frozen fish fillets samples were exposed to gamma irradiation at doses of 1, 2 and 3 kGy using the Russian (CM-20) Gamma cell located at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The source was giving a dose rate of irradiation of about 6 kGy / hour at the time of experiments.

The frozen samples were subjected to the bacteriological, chemical and sensory examinations before and after treatments.

1- Bacteriological analysis:

Samples were subjected to bacteriological examinations for evaluation of the reduction in bacterial counts immediately after treatments. 10 grams of fish fillets were homogenized aseptically with 90 ml of sterile 0.1% peptone water and serial dilution was conducted to determine the aerobic plate count (APC), Most Probable Number (MPN) of Coliforms and *Staphylococcus aureus* count according to the methods recommended by APHA (1992).

2- Chemical analysis:

a- The pH of a homogenate consisting of 5 g of fish fillet samples in 50 ml of distilled water was measured with pH meter (Digital, Jen co 609) as described by Carballo *et al.* (1995).

b- Total volatile nitrogen content (TVN) was determined according to the method of Conway, as recommended by FAO, (1980).

c- The value of thiobarbituric acid (TBA) was determined according to Tarladgis *et al.* (1960) and Pikul *et al.* (1983).

Sensory evaluation:

TSP immersed fish fillet samples, irradiated samples, samples that received both treatments and control samples were sensorily evaluated for their color odor and texture by 6-person trained panelists (Torres, 2001). A 5- point hedonic scale was used to score the various attributes and a score of 2.5 was considered as rejected samples which were the quality criterion used for both control and treated samples.

RESULTS

Table 1: Log mean viable counts of the microbial flora of irradiated fish fillets.

Irradiation dose /kGy	APC	Total psychrotrophic count	Coliform count	*Staph. count*
0 (control)	6.40	7.30	2.68	2.38
1	3.30	2.3	2.53	<2
2	2.60	2.07	<3	<2
3	2.10	2.01	<3	<2

Table 2: Log mean viable counts of the microbial flora of TSP immersed fish fillets.

TSP dilutions	APC	Total psychrotrophic count	Coliform count	*Staph. count*
Control	6.4	7.30	2.68	2.38
1.5%	6.3	6.47	2.66	2.38
3%	6.1	5.47	2.60	2.30
5%	5.4	4.23	2.30	2.01

Table 3: Log mean viable counts of the microbial flora of fish fillets (TSP immersed and irradiated).

Treatment	APC	Total psychrotrophic count	Coliform count	*Staph. count*
Initial count	6.4	7.3	2.68	2.38
1 kGy+TSP				
1.5%	3.2	2.1	2.4	<2
3%	2.2	<2	<3	<2
5%	2.1	<2	<3	<2
2 kGy+TSP				
1.5%	2.1	2.05	<3	<2
3%	<2	<2	<3	<2
5%	<2	<2	<3	<2
3 kGy+TSP				
1.5%	2.06	<2	<3	<2
3%	2.03	<2	<3	<2
5%	<2	<2	<3	<2

Table 4: The effect of irradiation and TSP concentrations on the chemical properties of fish fillets.

Treatment	pH	TVBN	TBA
Control	6.61	20.4	0.25
1 kGy+TSP			
1.5%	6.6	15.9	0.18
3%	6.9	15.6	0.18
5%	7.1	15.3	0.18
2 kGy+TSP			
1.5%	6.8	15.6	0.16
3%	6.9	15.5	0.16
5%	7.0	15.1	0.15
3 kGy+TSP			
1.5%	6.15	14.1	0.11
3%	6.90	14.0	0.11
5%	7.0	14.0	0.11

Table 5: The effect of irradiation and TSP concentrations on the sensory attributes of fish fillets.

	colour	odour	Texture
Control	4.5	4.8	4.4
Tsp			
1.5%	4.5	4.6	4.4
3%	4.6	4.6	4.7
5%	4.6	4.6	4.8
Irradiation			
1	4.5	4.5	4.4
2	4.5	4.4	4.4
3	4.3	4.2	4.3
1 kGy+TSP			
1.5%	4.8	4.5	4.6
3%	4.6	4.5	4.5
5%	4.6	4.6	4.4
2 kGy+TSP			
1.5%	4.5	4.5	4.6
3%	4.6	4.5	4.7
5%	4.6	4.5	4.7
3 kGy+TSP			
1.5%	4.3	4.3	4.4
3%	4.4	4.3	4.5
5%	4.5	4.4	4.5

DISSCUSION

Microbiological aspects:

1- The effect of irradiation on log means viable counts of the microbial flora of irradiated fish fillets:

The initial bacterial loads of fish fillets were recorded in Table (1), where the mean log cfu value of Aerobic plate counts, psychrotrophic counts, coliform counts and *Staph. aureus* counts were 6.4, 7.30, 2.68 and 2.38 respectively. The obtained results were similar to that recorded by Russell (1998); Myer and Etzel (2000); Kim *et al.* (2000). These results were higher than the results achieved by Reddy *et al.* (1997); Bala *et al.* (2000); ICMSF (1986) who stated that at an APC of 10^7cfu/gm or more, fish should be considered unacceptable and rejected. Romos and Lyon (2000) declared that the flesh of live fish is bacteriologically sterile, however, the fillets are usually contaminated during processing (deheading, skinning, evisceration and filleting) operations. Other factors that affect the bacterial load of the fillets are storage time, temperature and their combined action which significantly alter the bacterial growth and consequently the overall sensory attributes.

The effect of irradiation as log viable counts of all the inspected microorganisms showed gradual reduction as the irradiation dose increased. These results agreed with Badr (2004) and Lacroix *et al.* (2004). It was observed that the applied doses of 1, 2 and 3 kGys reduced log the aerobic plate counts (APC) by 3.1, 3.8 and 4.3 log cycles respectively. Fish fillets treated with the same doses of irradiation recorded a reduction in the viable counts of psychrotrophic bacteria by 5.00, 5.23 and more than 5 log cycles respectively. A dose of irradiation of 2 kGy was sufficient to decrease the viable counts of the detected coliforms in fish fillets to less than 2 log cycles; while a dose of 1 kGy was enough to eliminate *Staph. aureus* from the examined fish fillets. Nearly similar results were recorded by Bari *et al.* (2000) while Torres *et al.* (2001) reprted that a radiation dose of 1 kGy applied to Saural fillets decreased the initial microbial population by 2 log cycles; higher results were noticed by Tabiri *et al.* (2003) and Badr *et al.* (2005).

2- The effect of TSP on log mean viable counts of the microbial flora of TSP immersed fish fillets:

Results recorded in Table (2) showed the effect of trisodium phosphate (TSP) treatment on the bacterial load. Concerning the mean

APC of treated fish fillets, results indicated that there is no significant reduction of log APC of samples treated with 1.5, 0.3 and 5% as APC after treatment recorded 0.1, 0.3 and 2 reduction rate respectively. Nearly similar results were recorded by Marshall and Jindal (1997); Ahmed (2006) and who recorded that microbial count reduction achieved by TSP may be due to the higher pH of these treatment solutions. Dipping fish fillets in 1.5 and 3% TSP solution reduced log psychrotrophic count by 0.83 and 1.83 cfu/g respectively while, samples treated with 5% TSP solution recorded reduction by log 2.07 compaired with untreated samples. Nearly similar results were recorded by Zhuang *et al.* (1996) and Ahmed (2006).

On the other hand, coliform counts (MPN) in samples treated with 1.5, 3 and 5% TSP recorded limited reduction rate compared with non treated samples (0.02, 0.06 and 0.38 log.). Nearly similar results were recorded by Ahmed (2006) while, Delmore *et al.* (2000) and Ramirez *et al.* (2001) reported that TSP (10%) reduced total coliform counts and *E. coli* counts by 1.8 log. Comparing the initial *Staph. aureus* count of the treated and untreated samples with TSP revealed that there was a limited effect of TSP solution on the reduction of the organism count. These results are nearly similar to those reported by Torres *et al.* (2001).

3- The combined effect of TSP and irradiation on Log mean viable counts of the microbial flora of fish fillets:

The recorded results in Table (3) showed the effect of combination of TSP and low doses of irradiation on APC, psychrotrophic counts, coliforms and *Staph. aureus* counts of the treated fish fillets samples. Results revealed that the application of TSP immersion alone had a limited effect on the reduction of the counts of the detected microorganisms; a dose of irradiation of 2 kGy and TSP dilution of 3% was sufficient to reduce the total aerobic plate count of fish fillets to less than 2 log cycles while the log viable counts of psychrotrophic bacteria was less than 2 at a radiation dose of 1 kGy and 3% TSP dilution.

On the other hand the application of 1 kGy and TSP at 3% concentration reduced the log viable counts of coliforms to <3 while the log viable counts of *Staph aureus* was <2 at 1.5% at the same irradiation dose.

Chemical aspects:

The value of thiobarbituric acid (TBA) is used as an index for measuring oxidative rancidity. It is well known that gamma irradiation induces lipid oxidation through the release of free radicals, mainly hydroxyl radicals, upon radiolysis of fish water which enhances lipid oxidation (Rincon *et al.,* 1997). Results in Table (4) recorded the chemical criteria of control; TSP immersed and irradiated fish fillets. The results revealed that the mean pH value of the control samples was 6.61 which is within the value specified by ESS (No. 3494/ 2005) for fish meat. Nearly similar findings were obtained by El-Shater (1999) and Ahmed *et al.* (2006). While higher values were recorded by Zhuang *et al.* (1996) and Reddy *et al.* (1997). The pH of fish fillets dipped in TSP solution ranged from 6.9 to 7.4 according to the concentration of the solution. The pH of the irradiated samples ranged from 6.54 to 5.16 which is considered due to the dose of irradiation. While samples received both treatments recorded pH values of 6.15-7.1 which is in the range considered by the values of ESS (2005) for fish meat.

TVB-N ranged from 40.4 (control) to 17 mg/100gm (TSP, irradiated and combination treatment ssamples). Nearly similar results were recorded by Gopal *et al.* (1999), higher results were recorded by El-Shater, (1999) and Ahmed, (2006).

The ESS (2005) specified that the limit of acceptance for TVB-N for fish flesh is 30 mg/100g. From the achieved results, it was clear that treatment with TSP or/and irradiation improve the TVB-N values of fish fillets samples.

Concerning the TBA number Table (4) showed a value of 0.25 mg MD/Kg for control samples, while that of all treated samples with TSP or / and irradiated samples ranged from 0.20-.011 mg MD/Kg. It was clear that all the three treatments reduced the value of TBA of all samples compared with the control samples.

Undeland *et al.* (1999) emphasized the significant of rancidity as of a vital concern to fish industry, as the development of lipid oxidation often limits the possibilities for storage and processing of some fish species. ESS (2005) stated that the permissible limit for TBA number for chilled fish should be lower than 4.5 mg MD/Kg.

Sensory evaluation:

Table. (5) shows that immersing fish fillets to different concentrations of TSP had no noticeable effect on the odour of the samples while the texture and colour were slightly improved as recorded by the panelists and considered acceptable according to both limits

specified by ICMSF (1986) and ESS (2005), similar findings were recorded by Ahmed, (2006). While exposing the samples to low dose gamma irradiation alone reduced the colour and the odour but at a neglicable extent; the texture became friable as the dose of irradiation increased. The best sensory attributes with the least irradiation dose and the least TSP concentration reached at a dose of 1 kGy and 3% TSP.

In conclusion; the combination of 3% TSP with 1 and/or 2 kGy dose of irradiation was sufficient to eliminate all the spoilage microorganisms that contaminate fish fillets in this experiment without affecting the chemical attributes of the product and with the best sensory characters.

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