SHIPPING TRIAL OF IRRADIATED FROZEN SHRIMP FROM THAILAND TO THE NETHERLANDS

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บทคัดย่อ

ได้ทำการตรวจคุณภาพของกุ้งแช่แข็งไม่ฉายรังสีและฉายรังสีที่ทดลองส่งจากประเทศไทย ไปยังประเทศเนเธอร์แลนด์ กุ้งแช่แข็งทั้งชนิดมีเปลือกเด็ดหัว (Shell-on headless shrimp) และชนิดต้มปอกเปลือก (Precooked and peeled shrimp) ผ่านการฉายรังสี ด้วยปริมาณ 2 และ 3 กิโลเกรย์ ที่สำนักงานพลังงานปรมาณูเพื่อสันติ ประเทศไทย ส่งโดยทางเรือไปที่ International Facility for Food Irradiation Technology (IFFIT) ประเทศเนเธอร์แลนด์

ก่อนการขนส่งตรวจพบว่า กุ้งแช่แข็งทั้งสองชนิดมีคุณภาพดี รังสีปริมาณ 3 กิโลเกรย์ ทำให้เชื้อจุลินทรีย์ชนิดมีโซฟลาย (Mesophile) และไซโครโทรป (Psychrotrope) ลดลง 2-3 log cycles และไม่เปลี่ยนแปลงคุณภาพด้านประสาทสัมผัสอย่างมีนัยยะสำคัญ ในกุ้งฉายรังสีตรวจไม่พบเชื้อ Coliforms, Faecal coliforms, Escherichia coli, Salmonella, Vibrio parahaemolyticus และ Staphylococcus aureus ภายหลังการขนส่งซึ่งใช้เวลานาน 2 เดือน ตรวจไม่พบการเพิ่มปริมาณเชื้อจุลินทรีย์ในกุ้งแช่แข็งฉายรังสี การตรวจวิเคราะห์ Flora แสดงให้ทราบว่า รังสีไม่ก่อให้เกิดอันตรายจากการเปลี่ยนแปลง Microflora เชื้อจุลินทรีย์ที่รอดตายจากรังสีในกุ้งแช่แข็งทั้งสองชนิดประกอบด้วยเชื้อ Micrococcus spp. เป็นส่วนใหญ่ นอกจากนี้รังสีปริมาณ 3 กิโลเกรย์ ไม่เปลี่ยนแปลงคุณภาพด้านประสาทสัมผัสของกุ้งแช่แข็งภายในระยะเวลาการขนส่ง อาจสรุปได้ว่า การฉายรังสีสามารถปรับปรุงคุณภาพด้านแบคทีเรียของกุ้งแช่แข็งเพื่อการขนส่งไปประเทศเนเธอร์แลนด์ได้

ABSTRACT

Quality of non-irradiated and irradiated frozen shrimp after shipping trial from Thailand to the Netherlands were investigated. Commercial frozen shell on headless shrimp and precooked and peeled shrimp were irradiated with 0, 2 and 3 kGy at the Office of Atomic Energy for Peace (OAEP), Thailand, and transported by sea to the International Facility for Food Irradiation Technology (IFFIT), the Netherlands.

It was found that both types of frozen shrimp before shipment are of good quality. Radiation with 3 kGy resulted in 2-3 log cycles reduction of mesophilic and psychrotrophic colony counts and caused no significant change in sensory quality. The irradiated producted were found to be free from coliforms, faecal coliforms, Escherichia coli, Salmonella, Vibrio parahaemolyticus and Staphylococcus aureus. There was no increase in bacterial count in irradiated frozen shrimps after two months of transportation. The results of flora analysis show that radiation does not present any hazard resulting from a shift in the microflora. The surviving microflora in both types of irradiated frozen shrimp consisted mainly of Micrococcus spp.. Besides, irradiation with dose of 3 kGy did not affect the sensory quality of the frozen shrimp within duration of transportation. It can be concluded that irradiation improves the bacteriological quality of frozen shrimp for transportation to the Netherlands.

1. INTRODUCTION

Shrimp is an important fishery export product of Thailand. Annual catches of shrimp is over one hundred thousand metric tons and this amont tends to increase every year [1]. Most of shrimp are exported as a frozen product. The problems of Thai export frozen shrimp are quality which sometime do not meet the requirement of bacteriological standard of importing countries. The presence of Salmonella and Arizona contamination in shrimp results in detention and rejection. To eliminate Salmonella and decrease the number of spoilage organism in seafood, gamma radiation is considered useful as a method for decontamination. Laboratory research have shown that a radiation dose of 2-4 kGy can be relied upon to eliminate Salmonella to a final low level that is acceptable in the sense of risk analysis[2].

These studies were organized upon the request by the Office of Atomic Energy for Peace (OAEP), Thailand, to the International Facility for Food Irradiation Technology (IFFIT), the Netherlands. To study a shipping trial of irradiated frozen shrimp from Thailand to the Netherlands, the quality of the samples was evaluated upon arrival at destination in order to compare with that evaluated before shipment. The quality evaluatiors were carried out as follows:

1.1 Determination of bacteriological quality of non-irradiated and irradiated frozen shrimp.

1.2 Determination of the effect of radiation on the microbial association of frozen shrimp.

1.3 Determination of sensory quality of irradiated frozen shrimp.

2. PROCEDURE

2.1 Samples

Frozen shell-on headless shrimp and precooked and peeled shrimps packed in plastic bag (1 kg/bag) 48 kgs were purchased from commercial frozen sea food company. Both types of shrimp were irradiated by Co-60 radiation source at doses of 0, 2 and 3 kGy at the OAEP , Thailand. The samples were kept at -18°C and despatched by sea to the Netherlands. Before despatching, the samples were taken for microbiological analysis and sensory evaluation. It took two months for shipment from Thailand to the Netherlands. Upon arrival at Rotterdam harbour, the samples were placed in ice box containing dry ice (CO_2) and transported to Research Institute ITAL, Wageningen.

2.2 Microbiological investigations

Frozen samples were thawed at 4°C for 20 hours before microbiological investigations commenced. The following media and incubation conditions were used.

2.2.1 Aerobic mesophilic and psychrotrophic colony count: spread plates on tryptone soya broth plus 3 g/l yeast extract and 12 g/l agar (TSBYA) incubated for 3 days at 30°C and 10 days at 7°C (3).

2.2.2 Aerobic mesophilic and psychrotrophic Gram negative rods: spread plates on TSBYA with 2 ug/ml crystal violet incubated for 3 days at 30°C and 10 days at 7°C (3).

2.2.3 Aerobic mesophilic and psychrotrophic Gram positive organisms: spread plates on TSBYA with 0.25% phenyl ethanol incubated for 3 days at 30°C and 10 days at 7°C (3).

2.2.4 Enterobacteriaceae, coliforms, faecal coliforms, E. coli, Lancefield group D streptococci, V.parahaemolyticus and Staph. aureus: the technique of liquid medium repair (LMR) for resuscitation [4] and the most probable number (MPN) were used.

2.2.5 Salmonella: determinded by the Presence-Absence test [5].

2.2.6 Lactobacillus: spread plates on Rogosa agar incubated in an anaerobic jar for 3 days at 30°C.

confirmation of the characteristic colonies obtained on selective isolation media was carried out by tests as described by Mossel et al [6].

2.3 Microbial community structure determination

The microbial community structure of non-irradiated and irradiated frozen shrimp were carried out by the method of so-called flora analysis. The key used for bacterial identification is present in table 2.3.1 [7].

Table 2.3.1

Preliminary grouping at genus level of bacteria of significance for foods and water

Group	Gram stain	Morphology	Biochemistry	Type genera
1	+	rods, no spores	catalase +	Corynebacterium/Brevibacterium/ Arthrobacter/Cellulomonas/ Curtobacterium/Microbacterium[2] Brachothrix[3]
2	+	ditto	catalase -	Lactobacillus
3	+	rods, with spores	catalase +	Bacillus
4	+	ditto	catalase -	
			glucose type[4]: 5-1	Clostridium
5	+	coccus	catalase +	
			glucose type 1-2	Micrococcus
			glucose type 3	Staphylococcus[5]
6	+	ditto	catalase -	
			glucose type 3	Streptococcus
			glucose type 4	Leuconostoc
7	-	rods[6], no spores	oxidase -	
			glucose type 1-2	
			motile	Xanthomonas
			non-motile	Acinetobacter
			glucose type 3-4	
			lactose +	
			citrate -	Escherichia[7]
			citrate +	
			MR +	Citrobacter/Kluyvera[8]
			MR -	
			motile	Enterobacter/Erwinia[9]
			non-motile	Klebsiella[1]
			lactose -	
			βGal -	
			motile	
			urea -	Salmonella
			urea +	Proteus
			non-motile	Shigella
			βGal +	
			H_2S +	Citrobacter[10.1]
			H_2S -	
			LDC -	Yersinia
			LDC +	
			arabinose +	Hafnia
			arabinose -	Serratia[11]
8	-	rods[4], no spores	oxidase +	
			glucose type 1	
			motile	Alcaligenes/Comamonas[12]
			non-motile	Moraxella/Flavobacter[1]
			glucose type 2	Pseudomonas[13]
			glucose type 3	Beneckea/Vibrio[12]
			glucose type 4	Aeromonas[14]
9	-	ditto	oxidize ethanol to acetic acid at pH 4.5	Acetobacter/Gluconobacter[12]

Table 2.3.1 (Continued)

LEGEND:

1 Reaction for approximately 90% of the most frequently encountered strains.

2 Mesophilic; D_w = 2 min.

3 Rods changing to cocci in old cultures; psychrotrophic D_{50} approx. 2 min.

4 Glucose type 1 = no attack; 2 = oxidative dissimilation; 3 = fermentative anaerogenic dissimilation; 4 = aerogenic fermentative dissimilation; 5 = strict anaerobic dissimilation.

5 Staph. aureus; mode of attack on mannitol type 3, i.e. fermentative but anaerogenic in over 90% of strains.

6 Gram negative cocci do exist and can be of great significance as causes of infective disease; however, they do not play an important role in the microbial ecology of foods and water.

7 E. coli: Aerogenic fermentation of lactose and formation of indole at 44±0.1°C in over 90% strains.

8 H_2S negative.

9 Enterobacter invariably aerogenic, Erwinia predominantly anaerogenic.

10 Formerly: the "Bethesda-Ballerup" group.

11 DNAse positive.

12 Respectively peritrichous and polar flagella.

13 Glucose type 1 types of Pseudomonas also exist.

14 Anaerogenic Aeromonas types are encountered occasionally; hence Aeromonas is ultimately to be distinguished from Vibrio based on its insentivity to pteridine compound 0/129.

2.4 Sensory evaluation

Samples were thawed at $4^{o}C$ overnight in a refrigerator before cooking and serving. Precooked and peeled shrimp were served to panelist without cooking again. Shell-on headless shrimp were cooked by dipping in 2% boiling brine solution for two minutes. Sensory panel evaluation of shrimp was carried out by the procedure as described by Larmond [8]. Twelve panelists were requested to taste and judge the shrimp for colour, odour, flavor, texture and general impression. The rating of shrimp desirabilities was given in numberical value ranging from the highest affirmative value "9" to the lowest affirmative value "1". The results were analysed by analysis of variance.

3. RESULTS AND DISCUSSION

3.1 Microbiological quality of frozen shrimp

The microbiological investigations of non-irradiated and irradiated samples of both types of shrimp before shipment were shown in tables 3.1.1 and 3.1.2. It was found that both types of frozen shrimp are of good quality. The initial mesophilic colony counts of frozen shell-on headless shrimp and precooked and peeled shrimp were approximately 5.1 and 5.0 (log cfu/g). The number cfu/g of Enterobacteriaceae, the most probable number of V. parahaemolyticus and Staph. aureus were low. Salmonella was not detected in both types of frozen samples. Radiation with 3 kGy resulted in 2-3 log cycles reduction of total mesophilic and psychrotrophic counts. Besides, the irradiated frozen shrimp were found to be free of coliforms, faecal coliforms, E.coli, Salmonella, V. parahaemolyticus and Staph. aureus.

The microbiological quality of frozen shrimp carried out in the Netherlands was shown in tables 3.1.3 and 3.1.4. There were no change in total mesophilic and psychrotrophic counts after two months of shipment. Besides, amount of Enterobacteriaceae, coliforms, faecal coliforms, E. coli, V.parahaemolyticus and Staph. aureus determined by MPN determination seems to be decreased and lactobacillus counts of both types of shrimp were also reduced. However, there was an increase in psychrotrophic Gram netative rod shape bacterial count of non-irradiated frozen shell-on headless shrimp, but no increase in that of frozen precooked and peeled shrimp. A reason may be that frozen precooked and peeled shrimps was treated by heat and damaged cells could not be recovered in freezing condition. Irradiation of both types of frozen shrimp at dose of 3 kGy reduced mesophilic and

psychrotrophic counts by 2 log cycles. The irradiated products were also found to be free of coliforms, faecal coliforms, E. coli, Salmonella, Lancefield group D streptococci, V. parahaemolyticus and Staphylococcus aureus. No increase in bacterial count was found in irradiated shrimp after two months of transportation to the Netherlands.

3.2 Microbial community structure of frozen shrimp

Tables3.2.1 and 3.2.2 show the microbial association of shell-on headless shrimp and precooked and peeled shrimp. In non-irradiated shell-on headless shrimp, the coryneform group was predominant in the mesophilic flora and Micrococcus spp. in the psychrotrophic flora. In non-irradiated precooked and peeled shrimps, the coryneform was also the most prevalent organism amongst the mesophilic flora and yeast in the psychrotrophic flora. Irradiation of both types of frozen shrimps with 3 kGy resulted in a characteristic change in the composition of the bacterial flora, resulting in a predominance of Micrococcus spp.. Besides, the percentage of Gram positive cocci in total microflora increased with increasing radiation doses and that at the same time the Gram netative rods decreased. This phenomena has also been noticed in irradiated frozen chicken by Mulder (9).

3.3 Sensory evaluation

Mean flavor panel scores for all sensory factor of both types of non-irradiated and irradiated frozen shrimp which evaluated in Thailand were shown in tables 3.3.1 and 3.3.2. Irradiation with dose of 2-3 kGy did not affect the sensory quality of the frozen shrimp. There was no significant difference in mean scores for colour, odour, flavor and texture with respect to dosage used in both types of frozen shrimp.

Sensory quality of frozen shrimp carried out in the Netherlands was shown in tables 3.3.3 and 3.3.4. No significant change in sensory scores was observed in irradiated frozen shell-on headless shrimp. Only in irradiated frozen precooked and peeled shrimp was the significant change in colour observed. However, the such change was still within acceptable range on a nine point desirability scale. Therefore, irradiated frozen shrimp did not lose its sensory quality within duration of inter-country transportation.

4. CONCLUSIONS

Commercial frozen shell-on headless shrimp and precooked and peeled shrimp were irradiated with 0, 2 and 3 kGy gamma ray at the OAEP, Thailand and transported by sea to the IFFIT, the Netherlands. The microbiological and organoleptic quality of products were evaluated before shipping trial in order to compare with that evaluated at the destination in the Netherlands.

Both types of frozen shrimp used in this experiment are of good quality. Irradiation dose of 3 kGy reduced the mesophilic and psychrotrophic colony counts by 2-3 log cycles and, in addition, was adequate for eliminating coliforms, faecal coliforms, E.coli, V.parahaemolyticus and Staph. aureus. No significant change in sensory scroes for colour, odour, flavor and texture was observed resulting from irradiation application. Quality evaluation at destination showed that transportation condition (kept at $-18^{o}C$) did not affect the bacteriological and organoleptic quality of non-irradiated and irradiated frozen shrimp within trial duration (2 months) of transportation. Therefore, the quality control of original product is essential for the best quality at destination. It can then be concluded that for the transportation to the Netherlands the frozen shrimp, good manufacturing practices and supplemented with terminal processing by gamma radiation have been effective and shown potential use of irradiation as practical decontamination.

5. ACKNOWLEDGEMENTS

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Table 3.1.1 Microbiological investigation of non-irradiated and irradiated frozen shell-on headless shrimp before transportation

Organisms	log_{10}cfu/g		
	0kGy	2kGy	3kGy
Mesophilic bacteria	5.1	3.1	3.6
Psychrotrophic bacteria	4.1	3.3	<2.8
Mesophilic Gram negative rod-shape bacteria	4.4	<2.8	<2.8
Psychrotrophic Gram negative rod-shape bacteria	<2.8	<2.8	<2.8
Enterobacteriaceae	<2.8	<2.8	<2.8
Coliforms	1.0[a]	<-0.5[a]	<-0.5[a]
Faecal coliforms	0.6[a]	<-0.5[a]	<-0.5[a]
Escherichia coli	-0.4[a]	<-0.5[a]	<-0.5[a]
Salmonella	absent*	absent*	absent*
Vibrio parahaemolyticus	0.7[a]	<-0.5[a]	<-0.5[a]
Staphylococcus aureus	-0.3[a]	<-0.5[a]	<-0.5[a]
Lactobacillus	4.8	<2.8	<2.8

a = MPN determination

* = absent in 25g of sample

Table 3.1.2 Microbiological investigation of non-irradiated and irradiated frozen precooked and peeled shrimp before transportation

Organisms	$\log_{10}$ cfu/g		
	0kGy	2kGy	3kGy
Mesophilic bacteria	5.0	2.9	<2.8
Psychrotrophic bacteria	4.9	3.0	<2.8
Mesophilic Gram negative rod-shape bacteria	4.8	<2.8	<2.8
Psychrotrophic Gram negative rod-shape bacteria	<2.8	<2.8	<2.8
Enterobacteriaceae	<2.8	<2.8	<2.8
Coliforms	0.6[a]	<-0.5[a]	<-0.5[a]
Faecal coliforms	0.6[a]	<-0.5[a]	<-0.5[a]
Escherichia coli	-0.2[a]	<-0.5[a]	<-0.5[a]
Salmonella	absent*	absent*	absent*
Vibrio parahaemolyticus	<-0.5[a]	<-0.5[a]	<-0.5[a]
Staphylococcus aureus	0.3[a]	<-0.5[a]	<-0.5[a]
Lactobacillus	4.6	<2.8	<2.8

a = MPN determination

* = absent in 25g of sample

Table 3.1.3 Microbiological investigation of non-irradiated and irradiated frozen shell-on headless shrimp at destination

Organisms	$\log_{10}$cfu/g		
	0kgy	2kGy	3kGy
Mesophilic bacteria	5.2	3.3	3.2
Psychrotrophic bacteria	4.3	3.0	<2.8
Mesophilic Gram negative rod-shape bacteria	3.9	<2.8	<2.8
Psychrotrophic Gram negative rod-shape bacteria	3.3	<2.8	<2.8
Mesophilic Gram positive bacteria	4.8	<2.8	<2.8
Psychrotrophic Gram positive bacteria	3.6	<2.8	<2.8
Enterobacteriaceae	-0.4[a]	-0.4[a]	-0.4[a]
Coliforms	<-0.5[a]	-0.4[a]	<-0.5[a]
Faecal coliforms	<-0.5[a]	<-0.5[a]	<-0.5[a]
Escherichia coli	<-0.5[a]	<-0.5[a]	<-0.5[a]
Salmonella	absent*	absent*	absent*
Lancefield group D streptococci	1.6[a]	<-0.5[a]	<-0.5[a]
Vibrio parahaemolyticus	<-0.5[a]	<-0.5[a]	<-0.5[a]
Staphylococcus aureus	<-0.5[a]	<-0.5[a]	<-0.5[a]
Lactobacillus	2.9	<2.8	<2.8

a = MPN determination

* = absent in 25g of sample

Table 3.1.4 Microbiological investigation of non-irradiated and irradiated frozen precooked and peeled shrimp at destination

Organisms	$\log_{10}$cfu/g		
	0kGy	2kGy	3kGy
Mesophilic bacteria	5.5	<2.8	3.1
Psychrotrophic bacteria	4.5	<2.8	<2.8
Mesophilic Gram negative rod-shape bacteria	4.5	<2.8	<2.8
Psychrotrophic Gram negative rod-shape bacteria	<2.8	<2.8	<2.8
Mesophilic Gram positive bacteria	5.7	<2.8	<2.8
Psychrotrophic Gram positive bacteria	3.7	<2.8	<2.8
Enterobacteriaceae	-0.1[a]	<-0.5[a]	<-0.5[a]
Coliforms	-0.4[a]	<-0.5[a]	<-0.5[a]
Faecal coliforms	<-0.5[a]	<-0.5[a]	<-0.5[a]
Escherichia coli	<-0.5[a]	<-0.5[a]	<-0.5[a]
Salmonella	absent*	absent*	absent*
Lancefield group D streptococci	1.3[a]	<-0.5[a]	<-0.5[a]
Vibrio parahaemolyticus	<-0.5[a]	<-0.5[a]	<-0.5[a]
Staphylococcus aureus	<-0.5[a]	<-0.5[a]	<-0.5[a]
Lactobacillus	2.9	<2.8	<2.8

a = MPN determination

* = absent in <5g of sample

Table 3.2.1 Microbial community structure of non-irradiated and irradiated frozen shell-on headless shrimp at destination (in % of total colony count)*

Organisms	30°C			7°C		
	0kGy	2kGy	3kGy	0kGy	2kGy	3kGy
Gram negative rod						
Acinetobacter	-	-	-	-	11	-
Alcaligenes/Comamonas	-	-	3	-	-	-
Moraxella/Flavobacter	-	-	6	-	-	-
Xanthomonas	-	-	-	3	6	-
Gram positive cocci						
Aerococcus	-	-	-	3	-	-
Micrococcus	19	88	78	52	61	89
Staphylococcus	7	7	-	3	22	11
Streptococcus	13	-	-	22	-	-
Gram positive rod						
Corynebacterium	58	5	-	10	-	-
Lactobacillus	-	-	-	5	-	-
Bacillus	3	-	10	5	-	-
Yeasts	-	-	3	2	-	-

* Limit of significance 2-5%

Table 3.2.2 Microbial community structure of non-irradiated and irradiated frozen precooked and peeled shrimp at destination (in % of total colony count)*

Organisms	30°C			7°C	
	0kGy	2kGy	3kGy	0kGy	2kGy
Gram negative cocci	-	2	-	-	-
Gram negative rod					
Moraxella/Flavobacter	1	-	-	2	-
Gram positive cocci					
Micrococcus	3	28	77	10	86
Staphylococcus	1	9	-	2	-
Streptococcus	-	-	-	20	-
Gram positive rod					
Corynebacterium	89	20	23	3	14
Yeasts	6	41	-	63	-

* Limit of significance 2-5%

Table 3.3.1 Mean[1] flavor panel scores for non-irradiated and irradiated frozen shell-on headless shrimp before transportation

Sensory factor	Radiation dose (kGy)			F-value
	0	2	3	
Colour	7.58	7.17	7.33	1.061[2]
Odour	7.00	6.67	7.00	1.913[2]
Flavor	6.67	6.08	6.25	1.092[2]
Texture	7.00	6.67	7.00	1.257[2]

[1] n = 12 judgements

[2] non significant difference $p \geq 0.05$

Table 3.3.2 Mean[1] flavor panel scores for non-irradiated and irradiated frozen precooked and peeled shrimp before transportation

Sensory factor	Radiation dose (kGy)			F-value
	0	2	3	
Colour	7.67	7.50	7.25	1.208[2]
Odour	6.08	5.67	6.00	0.951[2]
Flavor	5.00	4.92	5.00	0.027[2]
Texture	6.12	6.08	5.92	0.299[2]

[1] n = 12 judgements

[2] non significant difference $p \geq 0.05$

Table 3.3.3 Mean[1] flavor panel scores of non-irradiated and irradiated frozen shell-on headless shrimp at destination

Sensory factor	Radiation dose (kGy)			F-value
	0	2	3	
Colour	6.91	6.83	6.96	0.28[2]
Odour	6.29	5.88	6.08	0.69[2]
Flavor	7.00	6.67	6.75	0.75[2]
Texture	7.08	7.08	7.00	0.33[2]
General impression	7.17	6.75	6.75	2.92[2]

[1] n = 12 judgements

[2] non-significant difference $p \geq 0.05$

Table 3.3.4 Mean[1] flavor panel scores of non-irradiated and irradiated frozen precooked and peeled shrimp at destination

Sensory factor	Radiation dose (kGy)			F-value
	0	2	3	
Colour	7.42	7.13[ab]	6.79[b]	11.38[3]
Odour	6.75	6.29	6.29	0.45[2]
Flavor	6.71	6.46	6.13	0.97[2]
Texture	6.75	6.67	6.83	2.73[2]
General impression	7.08	6.54	6.46	2.16[2]

[1] n = 12 judgements

[2] non-significant difference $p \geq 0.05$

[3] significant difference $p \leq 0.05$

Mean scores with the same exponent letter did not differ significantly

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