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Physical and chemical quality of sanitized commercial eggs experimentally contaminated with *Pseudomonas aeruginosa* and refrigerated during storage

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ABSTRACT - The objective of this study to verify the physicochemical quality of commercial washed and unwashed eggs, experimentally inoculated on the shell with *Pseudomonas aeruginosa* and stored at 5 and 25 °C for 30 days. A total of 384 eggs, classified as large, from light Dekalb White laying hens at 30 to 40 weeks of age, were used. The experimental design consisted of two blocks in a 2 × 2 × 2 factorial arrangement (contamination, washing, and refrigeration) with six replicates. The sanitization was performed by mechanical washing (hot water with chlorhexidine 20% and 8% active content). Eggs were contaminated by handling with 1.5×10^5 colony-forming units (cfu) of *Pseudomonas aeruginosa*/mL solution, and stored at 5 and 25 °C for 30 days. Each ten days, analyses of the eggs were carried out, for the assessment of physical (egg weight, specific gravity, shell thickness, yolk, albumen and shell percentage, Haugh unit, yolk and albumen rates) and chemical (albumen and yolk pH) characteristics. There were interactions between sanitization, storage temperature and contamination. The cooling process maintained the egg internal quality even when there was contamination on the shell by *Pseudomonas aeruginosa* inoculum. Cooling slows down the weight loss and promotes better internal physical and chemical quality of the eggs during the 30 days of storage regardless of the contamination and washing processes.

Key Words: bacterial contamination, cooling, physical characteristics

Introduction

Eggs are characterized as a type of food with elevated nutritional value because they contain a high biological value protein. However, for all the nutritional potential of the eggs to be absorbed by humans, they must be properly maintained during the period between oviposition and commercialization, which may take weeks (Pascoal et al., 2008).

Factors such as storage conditions, time and temperature, among others, influence the internal quality of eggs for consumption. The eggs maintained for long periods at elevated temperatures demonstrate acceleration of evaporation, albumen fluidizing, increase in the albumen and yolk pH values and, consequently, loss of protein (Solomon, 1991). Carvalho et al. (2007) mentioned that the longer this period is, the worse theinternal quality of eggs will be, and concluded that the storage period (without refrigeration) that does not compromise the internal quality is fifteen days in the maximum.

Research studies show that eggs refrigerated during storage have better physical and chemical quality (Carvalho et al., 2007; Stringhini et al., 2009). Although refrigeration is an important factor to maintain the quality of eggs, it is not an obligation in the stores.

The internal contamination of eggs may contribute to the reduction in their shelf life, which can be risky to the health of the consumer (Campopas, 2004). One of the bacteria with contaminating potential for eggs is the *Pseudomonas aeruginosa*, since it is able to cross the shell and the membranes of the egg, causing its degradation (De Reu et al., 2006). These bacteria are proteolytic and modify the sensory and physicochemical characteristics of the eggs by producing undesirable substances such as acid and hydrogen sulfide, ammonia, amines, indole and urea (Franco & Landgraf, 1992).

Andrade et al. (2004) found that approximately 16% of the eggs sold in the region of Goiânia were contaminated by *Pseudomonas* spp. These authors studied eggs produced in commercial farms as well as eggs of hens reared on ordinary farms. They concluded that eggs from farms in which no hygienic and sanitary aspects are observed and the storage is done in inappropriate places and for indefinite time are the most worrisome.

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The washing or non-washing of the eggs in the packaging process has been questioned by several researchers. Llobet et al. (1989) stated that the washing process results in better-looking eggs for sale and Laudanna (1995) mentions that the washing, when done well, reduces the concentration of microorganisms in the shell as well as the probability of contamination inside the egg. In Brazil, the Regulation of Industrial and Sanitary Inspection of Animal Products refers that eggs for industrialization should be washed, and the recommendation of chlorine use is at levels below 50 ppm (Aragon-Alegro et al., 2005).

On the other hand, conditions such as mishandling, improper packaging, egg contact with contaminated surfaces, and others, may favor their re-contamination. Stringhini et al. (2007) isolated *Pseudomonas* spp., *Enterobacter* spp. and *Escherichia coli* from hands, nasal cavity and oropharynx of the employees of the farm in the metropolitan region of Goiás.

Considering that *Pseudomonas* spp. is a microorganism with a great potential for deterioration of eggs besides being an opportunistic pathogen to humans, and that the egg may be contaminated after the washing by contact and mishandling of the employees in the egg room (Stringhini et al., 2009) or during the transportation and commercialization, the question is raised: does the sanitizer act as a protection against bacterial contamination after the washing of the egg?

The objective of this study was to verify if the sanitization, the experimental contamination (in the shell with *Pseudomonas aeruginosa)* and the storage temperature affect the internal quality of eggs stored for 30 days.

Material and Methods

The experiment was conducted at the Bacteriology Laboratory Department of Veterinary Medicine, Federal University of Goiás. A total of 384 eggs, without cracks, classified as large, from light Dekalb White laying hens at 30 to 40 weeks of age, were used. The experiment was set in random blocks at two different times. In the first stage, 192 eggs were used, and in the second stage, another 192 eggs as well, in a 2 × 2 × 2 factorial arrangement (sanitization × inoculation × refrigeration). Eight treatments were used: inoculated and refrigerated sanitized eggs; inoculated and non-refrigerated sanitized eggs; non-inoculated and non-refrigerated eggs; non-inoculated and refrigerated sanitized eggs; inoculated and refrigerated non-sanitized eggs; non-inoculated and refrigerated non-sanitized eggs; non-inoculated and non-refrigerated non-sanitized eggs; and inoculated and non-refrigerated non-sanitized eggs; with six repetitions each, in which the egg was the experimental unit.

Eggs from all groups were collected on the same day from a commercial farm. The group of non-sanitized eggs was directly collected in the laying house and the sanitized group was collected in the egg room, where they were submitted to a washing process with a sanitizer according to the commercialization practice, i.e., mechanically washing with water 10 °C above the room temperature containing 20% of chlorhexidine and 8% of active content.

After being collected, the eggs were subjected to UV light in an aseptic chamber for 24 hours, to reduce the initial bacterial load of the shell.

The solution for the contamination of the eggs was prepared using *Pseudomonas aeruginosa* ATCC 9027 at a concentration of 1.5 × 105 cfu/mL of a 0.85% sterile saline solution (Stringhini et al., 2009). To obtain the inoculums, the bacterial strain was peaked in cetrimide agar and incubated at 37 °C for 48 hours. Afterwards, the cells were suspended in a 0.85% buffered saline solution and maintained at 4 °C. The 1.5 × 105 cfu/mL concentration of sterile saline solution at 0.85% was adjusted with the Mc Farland nephelometric scale. The concentrations were confirmed by the plating of the serial decimal dilutions on cetrimide agar with a posterior incubation at 37 °C and enumeration of the colony-forming units (cfu) of *Pseudomonas aeruginosa*.

The contamination of the eggs from the contaminated group was performed in laboratory with the aid of a sterile tuberculin syringe. An aliquot of 0.1 mL of a 0.85% buffered saline solution containing the concentration of 1.5 × 105 cfu/mL of *Pseudomonas Aeruginosa* was deposited in the hands of the inoculators, who were wearing gloves, which were discarded after the inoculation of each unit. Each egg was maintained for a period of approximately 20 seconds in the contaminated hands, and their surface was completely moistened by the solution. Immediately after the inoculation, the eggs were taken for storage in a refrigerator (5 °C) or BOD chamber (25 °C), where the temperature was recorded once a day.

The analyses of physical (egg weight, specific gravity, percentage of yolk, albumen, and shell, Haugh unit, rate of yolk and albumen (Carbó, 1987) and chemical characteristics (pH of albumen and yolk) were performed every 10 days during 30 days.

The data was subjected to a variance analysis. Polynomial regression was also applied to the physical variables concerning the time of egg storage. The software used was SAS (Statistical Analysis System, version 8.0).

Results and Discussion

The results of the quality of eggs from the groups sanitized or not-sanitized at the beginning of the experiment (unpublished data) were consistent with those observed by Carvalho et al. (2007) with fresh eggs. The average of all analyzed eggs at the initial time for specific gravity was 1,085 and 70 Haugh units, indicating a good quality for shell and albumen. The Haugh unit value equal to or greater than 72 indicates an excellent quality, as long as the other factors are normal (Silversides et al., 1993). These results were similar to those found by Leandro et al. (2005) and Xavier et al. (2008), when they analyzed the external and internal quality of fresh eggs collected directly from the farm.

The results of the variance analyses for the quality of the eggs after ten days of storage (Table 1) showed that there was no interaction between the factors of sanitization, contamination, and storage temperature, for the variables of egg weight, albumen rate, albumen and yolk percentage and the Haugh unit. The contaminated eggs showed lower albumen pH when compared with the non-contaminated eggs.

There was a ($P<0.05$) sanitization effect on the weight of the egg and albumen and yolk percentage, in which the sanitization reduced the egg weight and probably this was the reason for the increase in the albumen and yolk percentage. The higher weight loss of the sanitized eggs should be related to the increase in shell porosity, given the washing procedure, which caused a greater water loss by evaporation. Pinto & Silva (2009) observed weight loss of 1.04 g in eggs washed and stored at 8 °C for 14 days. According to these authors, the loss was due to the removal of the protection cuticle caused by the brushing during the washing of the eggs, which influenced the evaporation rate.

Regarding the refrigeration, the eggs kept at a 25 °C temperature showed a decrease of the physical quality (egg weight, Haugh unit, albumen rate and percentage) compared with those kept at 5 °C after ten days of storage. Carbó (1987) reported that the chemical and physical changes experienced by the eggs occur in the first days after being laid and the decline of quality is accelerated by higher storage temperatures. Similarly, Keener et al. (2006) demonstrated that eggs maintained for seven weeks at 5 °C had higher values of Haugh unit than the ones stored at 22 °C.

There was an interaction between the contamination × refrigeration factors for the yolk pH (Figure 1-A), showing that non-contaminated and refrigerated eggs showed better pH values. However, refrigeration did not improve the yolk pH values in eggs contaminated by *Pseudomonas aeruginosa.*

For the albumen pH (Figure 1B) there was an interaction effect between the sanitization × refrigeration groups. The results show that when the eggs were sanitized, the

Table 1 - Physicochemical quality of eggs contaminated or not by *Pseudomonas aeruginosa* and stored under different temperatures during 10 days

Experimental groups	Variables of physicochemical quality									
	Egg weight (g)	Specific gravity (g/cm^3)	Albumen rate	Albumen (%)	Albumen pH	Yolk rate	Yolk (%)	Yolk pH	Shell (%)	Haugh unit
Contamination (cont.)[1]										
No	57.6	1.065	0.03	52.3	8.5a	0.38	27.7	5.8	10.3	52.1
Yes	57.9	1.065	0.03	49.0	8.2b	0.37	26.3	6.2	10.8	49.2
Sanitization (san.)[2]										
No	59.0a	1.066	0.04	50.4b	8.4	0.38	26.7b	6.0	10.4	50.2
Yes	56.5b	1.064	0.03	50.9a	8.3	0.36	27.4a	6.0	10.7	51.1
Temperature (temp.)										
25 °C	56.7b	1.062	0.01b	48.2b	8.6	0.30	27.1	6.1	10.6	34.8b
5 °C	58.8a	1.068	0.05[a]	53.1a	8.1	0.44	26.9	5.8	10.6	66.4a
P value										
Contamination	0.67	0.39	0.83	0.35	0.01	0.10	0.50	0.01	0.20	0.44
Sanitization	0.0001	0.20	0.08	0.0004	0.81	0.003	0.02	0.86	0.38	0.80
Temperature	0.0007	0.0001	0.0001	0.0001	0.0001	0.0001	0.68	0.07	0.93	0.0001
Cont. × san.	0.93	0.01	0.90	0.97	0.04	0.25	0.07	0.95	0.001	0.35
Cont. × temp.	0.76	0.13	0.31	0.23	0.44	0.21	0.31	0.0001	0.77	0.20
San. × temp.	0.21	0.05	0.95	0.56	0.04	0.002	0.52	0.79	0.24	0.75
Cont. × san. × temp.	0.94	0.28	0.76	0.84	0.74	0.02	0.74	0.78	0.04	0.33
Coefficient of variation (%)	0.05	0.003	0.25	0.09	0.05	0.05	0.08	0.13	0.15	0.36

Averages followed by equal letters in the same column do not differ by the F test (0.05).
[1] Experimental contamination with *Pseudomonas aeruginosa* on the egg shells.
[2] Mechanical sanitization using chlorhexidine in the eggs washing water.

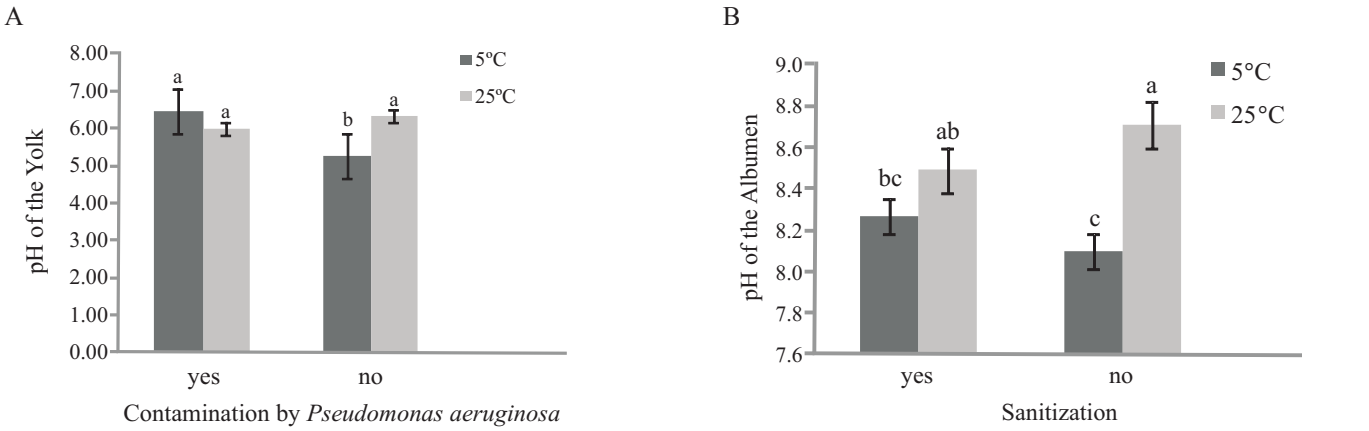

Figure 1 - Values of the yolk pH (A) and of the albumen pH (B) of eggs stored for 10 days.

refrigeration did not affect them, but when the eggs were not sanitized, refrigeration significantly improved the quality of the albumen.

For the albumen pH (Figure 1B) there was an interaction effect between the sanitization × refrigeration groups, and the unwashed and refrigerated groups showed the best results of albumen pH. Probably, there was no removal of the cuticle in the unwashed eggs and the gas exchange with the environment was lower. Cardoso et al. (2001) reported that the disadvantage of washing the eggs is the removal of the protective cuticle of the shell pores, which may increase the loss of water and CO_2.

On the other hand, the yolk rate variable was influenced by sanitization, contamination and refrigeration (Figure 2), in which the eggs refrigerated for 10 days during the storage showed better yolk rate, regardless of sanitation and contamination. These results are similar to the ones shown in the study of Hara-Kudo et al. (2001). These authors observed that the water penetration from the albumen to the yolk, with consequent weakness of the vitelline membrane, is delayed when the eggs are kept refrigerated. The non-refrigerated eggs showed higher yolk rate when not contaminated and not sanitized compared with the eggs from all other groups.

The refrigeration preserved the yolk rate, which is a physical quality of the eggs even when contaminated with *Pseudomonas aeruginosa*, and this was observed for the yolk pH variable, in which refrigerated and contaminated eggs were worse than the refrigerated and not contaminated ones.

Concerning the period of 20 days of storage (Table 2), there was no interaction (P>0.05) between the contamination, sanitization and refrigeration factors for egg weight, specific gravity, albumen and yolk rate, percentage of shell, albumen and yolk, and for albumen and yolk pH. There was interaction (P<0.05) between the three studied groups (sanitization × contamination × refrigeration) for albumen rate, yolk and albumen pH, and for the Haugh unit variables.

In relation to egg weight, a significant effect could be seen only regarding the sanitization. Sanitized eggs were lighter after a period of 20 days of storage. There was a temperature effect on yolk rate, albumen and yolk percentage, and the best results were found in eggs stored in refrigerated rooms, which corroborates the experiments of Barros et al. (2001), Hara-Kudo et al. (2001) and Moura et al. (2008).

The results of the physical and chemical quality analysis of eggs after a period of 30 days of storage (Table 3) showed that the egg weight, yolk rate and pH were affected by the temperature. It could be observed that the pH of non-refrigerated eggs was higher (P<0.05) than the pH of the ones kept at 5 ºC. These results corroborate the findings of Oliveira & Silva (2000) and Hara-Kudo et al. (2001), who mentioned that storage at lower temperatures maintains the chemical characteristics of the eggs. According to Mateos & Coren (1991), the decrease in the internal egg quality

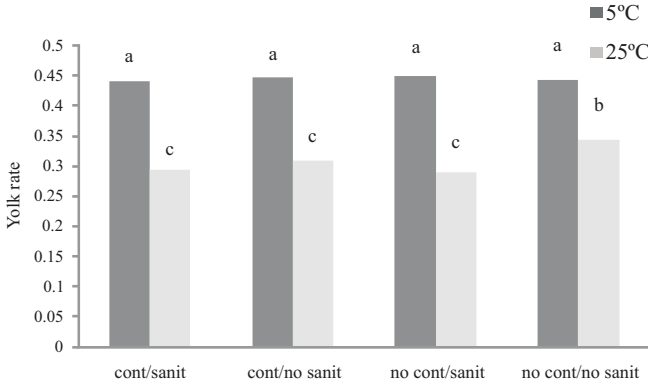

Figure 2 - Values of the yolk rate of eggs stored for 10 days.

is associated with loss of carbon dioxide and is directly proportional to storage temperature.

An effect of the storage temperature on the eggs weight stored for a period of 30 days was not observed. Similarly, the inoculation of the bacteria *Pseudomonas aeruginosa* did not influence the egg weight, the percentage of the albumen pH and yolk and albumen and the pH of the albumen yolk rate (P<0.05). However, Pinto & Silva (2009) found an increase in the pH of yolk and albumen in eggs contaminated with *Salmonella Enteritidis* and *Escherichia coli*, regardless of its storage conditions. The differences in results may be related to the type of pathogen and the methodology used in this experiment.

Table 2 - Physicochemical quality of eggs contaminated or not by *Pseudomonas aeruginosa* and stored under different temperatures for 20 days

Experimental groups	Variables of physicochemical quality							
	Egg weight (g)	Albumen rate	Albumen (%)	Albumen pH	Yolk rate	Yolk (%)	Yolk pH	Haugh unit
Contamination (cont.)[1]								
No	55.7	0.22	50.3	8.0	0.32	29.6	6.2	32.8
Yes	56.7	0.26	49.6	8.2	0.32	29.8	6.3	38.6
Sanitization (san.)[2]								
No	57.2a	0.24	50.3	8.2	0.32	29.3	6.3	39.0
Yes	55.2b	0.24	49.5	8.1	0.32	30.1	6.2	32.4
Temperature (temp.)								
25 °C	55.6	0.06	47.2b	8.2	0.23b	30.6a	6.1	19.6
5 °C	56.8	0.42	52.6a	8.0	0.41a	28.8b	6.4	51.8
P value								
Contamination	0.20	0.12	0.40	0.41	0.69	0.75	0.60	0.06
Sanitization	0.01	0.80	0.34	0.64	0.72	0.10	0.85	0.03
Temperature	0.16	0.001	0.001	0.16	0.001	0.0008	0.01	0.001
Cont. × san.	0.42	0.03	0.37	0.05	0.45	0.48	0.31	0.72
Cont. × temp.	0.72	0.06	0.07	0.50	0.66	0.94	0.001	0.44
San. × temp.	0.83	0.96	0.12	0.06	0.64	0.85	0.19	0.12
Cont. × san. × temp.	0.86	0.01	0.77	0.05	0.23	0.34	0.01	0.0003
Coefficient of variation (%)	0.07	0.5	0.07	0.78	0.17	0.08	0.11	0.42

Averages followed by equal letters in the same column do not differ by the F test (0.05).
[1] Experimental contamination with *Pseudomonas aeruginosa* on the eggs shells.
[2] Mechanical sanitization using chlorhexidine in the eggs washing water.

Table 3 - Physicochemical quality of eggs contaminated or not by *Pseudomonas aeruginosa* and stored under different temperatures for 30 days

Experimental groups	Variables of physicochemical quality							
	Egg weith (g)	Albumen rate	Albumen (%)	Albumen pH	Yolk rate	Yolk (%)	Yolk pH	Haugh unit
Contamination (cont.)[1]								
No	54.5	0.31	30.8	8.1	0.26	18.7	6.3	40.2
Yes	55.6	0.37	30.1	8.0	0.25	22.3	6.2	38.8
Sanitization (san.)[2]								
No	56.3a	0.36	30.5	8.1	0.27	19.0	6.1	42.4a
Yes	53.7b	0.32	30.4	8.0	0.24	22.0	6.4	36.7b
Temperature (temp.)								
25 °C	53.6b	0.17	30.6	7.9	0.12b	24.2	6.6a	12.1
5 °C	56.4a	0.52	30.3	8.2	0.40[a]	16.8	5.9b	67.0
P value								
Contamination	0.12	0.006	0.61	0.34	0.56	0.03	0.36	0.48
Sanitization	0.0003	0.02	0.91	0.62	0.06	0.06	0.11	0.005
Temperature	0.0001	0.0001	0.84	0.06	0.0001	0.001	0.0001	0.001
Cont. × san.	0.41	0.12	0.30	0.63	0.32	0.69	0.19	0.32
Cont. × temp.	0.96	0.006	0.55	0.51	0.98	0.03	0.87	0.009
San. × temp.	0.24	0.02	0.01	0.05	0.13	0.22	0.65	0.44
Cont. × san. × temp.	0.70	0.12	0.64	0.87	0.93	0.38	0.93	0.22
Coefficient of variation (%)	0.06	0.26	0.21	0.09	0.29	0.37	0.1	0.24

Averages followed by equal letters in the same column do not differ by the F test (0.05).
[1] Experimental contamination with *Pseudomonas aeruginosa* on the eggs shells.
[2] Mechanical sanitization using chlorhexidine in the eggs washing water.

The highest albumen rate in non-sanitized eggs was observed when eggs were refrigerated (Figure 3). Similarly, the albumen pH was better in the group refrigerated and non-sanitized.The results of albumen pH suggest that the sanitization may have caused mechanical removal of the cuticle that protects the pores in the shell, which facilitates the penetration of microorganisms through the shell and loss of humidity and CO_2. According to Mateos & Coren (1991), carbonic acid is one of the components of the buffer system in the albumen which dissociates itself, forming water and carbon dioxide. This gas diffuses through the pores of the shell, being released to the environment with consequent pH increase and change in the taste of the egg.

The fact that the sanitized and refrigerated egg presented a worse result of albumen rate in relation to the non-sanitized and refrigerated egg may be due to a microbial contamination before the egg washing. Laudanna (1995) and Campopas (2004) report that inappropriate conditions of temperature, pH, and filthy washing water may increase the bacterial load of the egg, which reduces its quality. Gama et al. (2008), when evaluating the bacterial quality of 272 water samples of the classification room of eggs from commercial farms, found that 7.35% of the samples were contaminated with total coliforms and 5.88% with fecal coliforms. The increase in the fluid albumen and the alterations in the anti-bacterial defenses promote the bacterial growth and the deterioration of the internal quality of eggs (Carbó, 1987).

An interaction between the contamination × temperature factors occurred for albumen rate, yolk percentage and Haugh unit (HU). Concerning the HU (Figure 4-A) it could be observed that contaminated and not-refrigerated eggs presented lower values; however, when the eggs were refrigerated, they did not show contamination effect. Therefore, the refrigeration proved to be efficient in maintaining the albumen quality of the eggs, even when they were contaminated by *Pseudomonas aeroginosa*.

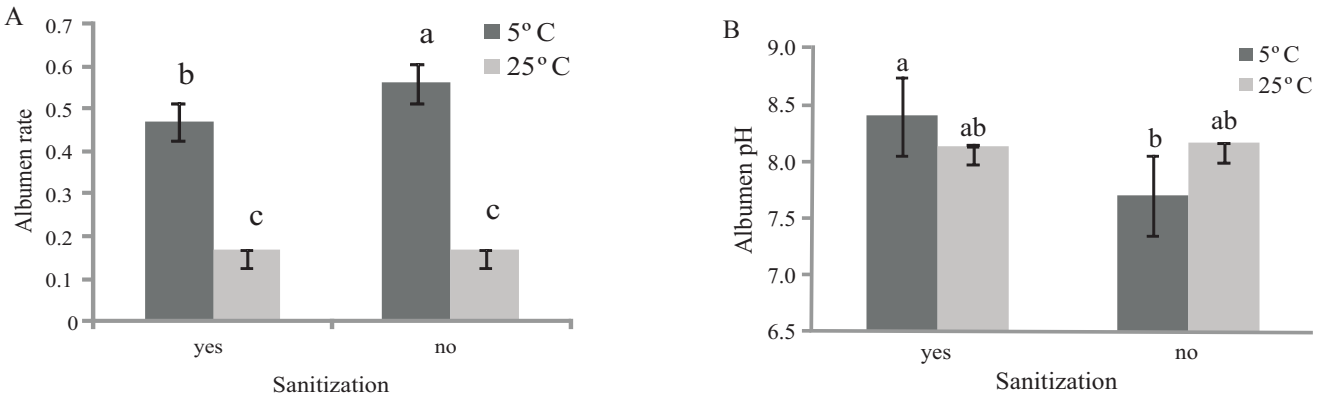

Figure 3 - Interaction between the mechanized sanitization groups using chlorexidine in the water of washing eggs with different temperatures of storage for the yolk rate (A) and the albumen pH (B).

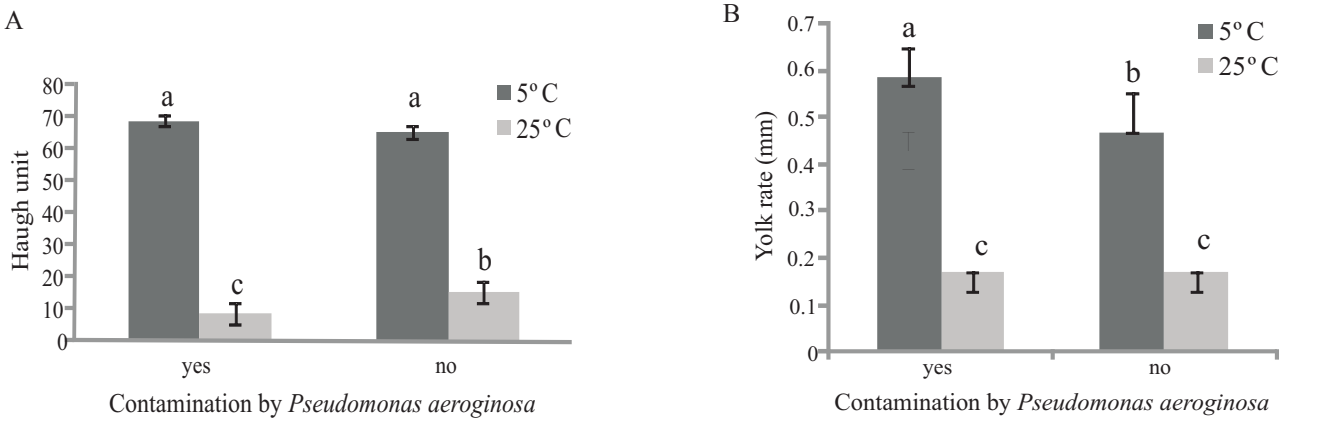

Figure 4 - Values of Haugh unit (A) and yolk rate (B), for the interaction between the groups of storage temperature and experimental contamination of eggs with *Pseudomonas aeroginosa.*

Mendes (2010) reported that shell and content of eggs contaminated with *Pseudomonas aeruginosa* and maintained under refrigeration presented lower counts of *Pseudomonas aeruginosa* than the contaminated eggs kept at a 25 ºC. Psychotropic bacteria, such as *Pseudomonas aeruginosa*, when maintained at low temperatures, have slower reactions caused by the generation of energetic activity and lower biochemical changes during the bacterial growth phase (Cousin et al., 1992).

One can observe that the storage time (P<0.05) affected all variables, except for yolk rate and percentage (Table 4).

The refrigeration delayed the weight loss of eggs and the ones refrigerated during the 30 days of storage (P>0.05) had lower loss regardless of the contamination. The sanitation increased the weight loss of theeggs; also, it was observed that the loss in the non-sanitized group was smaller. Weight loss was accelerated in the treatments where the eggs were washed and stored at 25 °C. The contaminated eggs had lower weight loss compared with the non-contaminated ones. This shows that the contamination did not affect the acceleration of weight loss of eggs.

Albumen rate values decreased linearly (P<0.05) with increasing storage period. The albumen lost the viscosity and after 30 days of storage, it was almost liquid and the yolk was breaking easily. These results can be explained by the mucin and lysozyme interaction, decreased solubility of proteins, loss of carbohydrate from the ovomucin molecules, loss of consistency due to the interaction of glucose with albumen proteins, loss of sialic acid associated with the egg proteins; and the reductive division of the disulfide with alkaline pH connections (Sgarbieri, 1996).

There was an interaction effect between contamination and storage temperature (P<0.05) for albumen rate. Considering the whole experimental period, it was observed that the eggs contaminated and stored in room temperature were more affected. Storage at high temperatures decreases the efficiency of the defense mechanisms of the eggs (Hara-Kudo et al., 2001). Mendes et al. (2010), studying the behavior of *Pseudomonas aeruginosa* in experimentally contaminated eggs observed that there was a greater multiplication of bacteria when eggs were stored at 25 ºC for 30 days, i.e., when they had more fluid albumen and low Haugh unit values.

A negative linear effect occurred for the Haugh unit, considering the entire storage period of the eggs; however, the r^2 values were below 0.2. The Haugh unit worsened during the storage time; nevertheless, the refrigerated eggs, when compared with the groups stored at a temperature of 25 ºC, presented better internal quality results throughout the 30 days storage. Baião & Cansado (2008) obtained a Haugh unit of 36.93 for eggs refrigerated and stored for 35 days. The decrease in the Haugh unit is due to the fluidization of the albumen, and occurs quickly in the first days after the egg-laying, whereas the increase in storage temperature accelerates the rate of chemical degradation reactions of the ovomucin present in the thick albumen (Austic & Nesheim, 1990). With the hydrolysis of amino acid chains, water is released, which increases the fluid albumen rate, resulting in loss of egg quality (Carbó, 1987).

Table 4 - Variables of the physicochemical quality of eggs submitted to contamination, sanitization and refrigeration, considering all the periods of storage

	Variables of physicochemical quality P value								
Experimental groups	Egg weight (g)	Albumen rate	Albumen (%)	Albumen pH	Yolk rate	Yolk (%)	Yolk pH	Shell (%)	Haugh unit
Contamination (cont.)[1]	0.12	0.006	0.61	0.34	0.56	0.03	0.36	0.52	0.48
Sanitization (san.)[2]	0.0003	0.02	0.91	0.62	0.06	0.06	0.11	0.02	0.005
Temperature (temp.)	0.0001	0.0001	0.84	0.06	0.0001	0.001	0.0001	0.95	0.001
Cont. × san.	0.41	0.12	0.30	0.63	0.32	0.69	0.19	0.95	0.32
Cont. × temp.	0.96	0.006	0.55	0.51	0.98	0.03	0.87	0.77	0.009
San. × temp.	0.24	0.02	0.01	0.05	0.13	0.22	0.65	0.95	0.44
Cont. × san. × temp.	0.70	0.12	0.64	0.87	0.93	0.38	0.93	0.09	0.22
Time	0.0001	0.01	0.0001	0.0001	0.68	0.09	0.0001	0.0001	0.0002
Coefficient of variation (%)	0.06	0.26	0.21	0.09	0.29	0.37	0.1	0.04	0.24

P values from the variance for the variables of the physicochemical quality of eggs.
[1] Experimental contamination with *Pseudomonas aeruginosa* on the eggs shells.
[2] Mechanical sanitization using chlorhexidine in the eggs washing water.

Conclusions

The contamination by *Pseudomonas aeruginosa* worsens the quality of the eggs, especially when they are sanitized and maintained at 25 ºC. Sanitization does not improve the quality of eggs stored without refrigeration (5 ºC). Refrigeration delays the loss of internal quality of eggs stored for up to 30 days. Eggs, especially the sanitized ones, must be stored at 5 ºC.

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