Evaluation of the alpha-amylase activity as an indicator of pasteurization efficiency and microbiological quality of liquid whole eggs

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ABSTRACT In order to evaluate the efficiency of the pasteurization process in liquid whole eggs, an UV/visible spectrophotometric method was developed and validated for the assessment of alpha-amylase activity. Samples were collected from 30 lots of raw eggs (n = 30) and divided into three groups: one was reserved for analysis of the raw eggs, the second group was pasteurized at 61.1°C for 3.5 minutes (n = 30), and the third group was pasteurized at 64.4°C for 2.5 minutes (n = 30). In addition to assessing alpha-amylase activity, the microbiological quality of the samples was also evaluated by counting total and thermotolerant coliforms, mesophilic aerobic microorganisms, *Staphylococcus* spp., and *Salmonella* spp. The validated spectrophotometric method demonstrated linearity, with a coefficient of determination (R^2) greater than 0.99, limits of detection (LOD) and quantification (LOQ) of 0.48 mg kg^{-1} and 1.16 mg kg^{-1}, respectively, and acceptable precision and accuracy with relative standard deviation (RSD) values of less than 10% and recovery rates between 98.81% and 105.40%. The results for alpha-amylase activity in the raw egg samples showed high enzyme activity due to near-complete hydrolysis of the starch, while in the eggs pasteurized at 61.1°C, partial inactivation of the enzyme was observed. In the samples of whole eggs pasteurized at 64.4°C, starch hydrolysis did not occur due to enzyme inactivation. The results of the microbiological analyses showed a decrease ($P < 0.0001$) in the counts for all the studied microorganisms and in the frequency of *Salmonella* spp. in the pasteurized egg samples according to the two binomials under investigation, compared to the raw egg samples, which showed high rates of contamination ($P < 0.0001$). After pasteurization, only one sample (3.33%) was positive for *Salmonella* spp., indicating failure in the pasteurization process, which was confirmed by the alpha-amylase test. It was concluded that the validated methodology for testing alpha-amylase activity is adequate for assessing the efficiency of the pasteurization process, and that the time-temperature binomial used in this study is suitable to produce pasteurized eggs with high microbiological quality.

Key words: liquid whole egg, pasteurization, alpha-amylase enzyme, microbiological quality

2017 Poultry Science 96:3375–3381
http://dx.doi.org/10.3382/ps/pex108

INTRODUCTION

Eggs are used in the food processing industry for various applications, such as emulsifying, foaming, gelling, and whipping. Because of their high nutrient content, care must be taken to prevent eggs from becoming a source of toxic infection and to ensure a high standard of quality when they reach consumers (Figueiredo et al., 2014).

Pasteurizing eggs has become an important alternative for increasing yields in egg-related industries and guaranteeing food safety. In addition to eliminating all the pathogenic microorganisms that may be present, pasteurization also greatly decreases microorganisms that cause deterioration, while increasing product shelf life. The principal objective of combining different binomials of time and temperature in egg pasteurization is to eliminate *Salmonella* spp., and may vary according to the legislation of each country. In the United States, for example, pasteurization of whole eggs is performed for 3.5 minutes at a temperature of 61.1°C, while in the European Community pasteurization is performed at a binomial of 64.4°C for 2.5 minutes (European Economic Community, 1989; USDA, 2013).

To ensure the safety of the final product, methods are needed to evaluate the efficiency of the pasteurization process in industry to prevent the release of lots which have not reached the appropriate combinations of temperature and time. Conducting microbiological tests on

Received January 12, 2017.
Accepted March 22, 2017.
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raw and pasteurized eggs and observing the elimination of pathogens and reduction in microbe counts after heat treatment is one way of evaluating the efficiency of the process. However, more time is required to obtain the results, which can be a problem for foods with a short shelf life.

As a result, the use of rapid methods to evaluate the efficacy of heat treatment is one alternative for ensuring product safety. Use of UV/visible spectrophotometry to assess the activity of the alpha-amylase enzyme present in raw eggs has been proposed by some authors to determine the efficiency of the egg pasteurization process (Brooks, 1962; Murthy, 1970; Codex, 1974). However, not only did the methodologies vary between the studies cited above, the results were not validated. Nonvalidated methods can generate unreliable results and are not officially recognized by international authorities and the scientific community (European Commission, 2002).

The objectives of this study were to develop and validate a spectrophotometric method to verify the efficiency of pasteurization of whole liquid eggs and to assess the impact of the variables of time and temperature of pasteurization (61.1°C for 3.5 minutes and 64.4°C for 2.5 minutes) on alpha-amylase enzyme activity in whole eggs and on microbiological quality.

MATERIALS AND METHODS

Solutions, Reagents, and Instrumental Parameters

The reagents used to assess alpha-amylase were soluble starch, trichloroacetic acid (**TCA**), resublimated iodine, and potassium iodide, which were obtained from Vetec (Sigma-Aldrich, St. Louis, MO). All reagents used were of pure grade for analysis (p.a.).

A 1% starch solution (pH 7.3) was prepared by dissolving 0.5 g of soluble starch in 50 mL of 0.02 M phosphate-saline buffer. This solution was heated to 90°C ± 2°C for the starch to fully solubilize.

A 15% TCA solution (v/v) and a stock solution of iodine and potassium iodide (0.1 M) were also prepared.

To prepare the iodine and potassium iodide solution for immediate use, 0.25 g of potassium iodide was added to 1 mL of the iodine and potassium iodide stock solution, which was completed to 100 mL with distilled water, resulting in a 0.001 M solution.

Validation Procedures

Samples of whole liquid egg pasteurized at 60°C for 3.5 minutes, 62°C for 3.5 minutes, and 64.4°C for 2.5 minutes (time-temperature condition to inactivate alpha-amylase) were used to evaluate the following performance parameters: linearity, matrix effect, precision, accuracy, limit of detection (**LOD**), limit of quantification (**LOQ**), and robustness.

To assess linearity, the samples were spiked with the 1% starch solution at concentrations of 0.5, 1.0, 2.0, 4.0, 5.0, and 6.0 mg kg^{-1} and the analysis was repeated seven times. After the analysis, a plot was created relating the absorbance obtained by the formation of the starch-iodine complex to concentrations, and by linear regression the curve equations were determined and the determination coefficients (R^2) (European Commission, 2002; INMETRO, 2010).

To evaluate the matrix effect, two calibration curves were developed: one with fortified matrix extracts and another without the matrix (using only standard starch solution). The samples were spiked with a 1% starch solution at concentrations of 0.5, 1.0, 2.0, 4.0, 5.0, 6.0 mg kg^{-1}. The curves were prepared and evaluated using F test and Student t test at 95% significance for evaluation of variance and means between slopes of the calibration curves (European Commission, 2002).

The precision of the analyses was assessed by determining the relative standard deviation (**RSD**) in terms of repeatability and intra-laboratory reproducibility, using the results obtained by successive analyses of the same sample over short time intervals and carried out under the same conditions and different conditions (equipment and analysts), respectively. The pasteurized egg matrices were fortified with the standard solution at three levels of concentration: low (1.0 mg kg^{-1}), medium (2.0 mg kg^{-1}), and high (6.0 mg kg^{-1}), considering the linear interval of the method with six replications (European Commission, 2002).

Accuracy was assessed via recovery tests using analyses with the linear range of the method in three concentrations: low (1.0 mg kg^{-1}), medium (2.0 mg kg^{-1}), and high (6.0 mg kg^{-1}), with six replications. The recovery obtained for each concentration was calculated using the equation $R = [(C1/C2)] \times 100$, which considers the concentration of starch obtained in the spiked sample (C1) and the concentration of starch added to the spiked sample (C2) (European Commission, 2002).

The LOD and LOQ were calculated from equations that consider the parameters of the analytical curve, $LOD = [(3 \times \sigma) / S]$ and $LOQ = [(10 \times \sigma) / S]$, using the standard deviation (σ) of the response and the slope of the analytical curve (S) (ICH, 2005).

Robustness was evaluated by the fractional factorial design proposed by Youden and Steiner (1975). Three analytical parameters, which were considered critical factors in the analysis, were selected, and small variations were induced in the nominal values of the method (Table 1). Then, six determinations were performed using blank samples spiked with starch solution at 3.0 mg kg^{-1} to determine the influence of each parameter and the different combinations in the final result. The influence of each parameter was evaluated by the Student t test and the effect of the combination of the parameters on method robustness was determined by the F test. The additional criteria used to determine the acceptability of the robustness was the

Table 1. Analytical parameters and variations for evaluation of robustness.

	Parameter	Nominal condition[1]	Variation[2]
A/a	Temperature of water bath	44°C (A)	40°C (a)
B/b	pH of starch solution	7.3 (B)	6.6 (b)
C/c	Wavelength	585 nm (C)	595 nm (c)

[1]Capital letters (A, B, and C) denote the nominal values of the method.
[2]The corresponding lower case letters (a, b, and c) denote the alternative values.

comparison of standard deviation of intra-laboratory reproducibility (s_{repro}) with a standard deviation of the difference of factors (s_{factor}), with $s_{factor} > s_{repro}$ implying unacceptable robustness.

Assessment of the Efficiency of the Variables Time and Temperature in Pasteurizing Eggs

Sampling of Whole Liquid Eggs The samples of whole eggs were taken from 30 different production batches obtained from an egg producer which is under official inspection. Sample eggs were collected from each production batch prior to pasteurization (raw eggs). After collection, the lots of raw eggs were divided into two groups, one of which was pasteurized in a plate pasteurizer at 61.1°C for 3.5 minutes (n = 30) and the other pasteurized at 64.4°C for 2.5 minutes in a tubular device (n = 30). The pasteurizers have three distinct sections, namely: regeneration, heating and cooling. The cold raw product (0 to 7°C) is preheated at the regeneration section before being heated up to the pasteurization temperature, at the heating section. In the plate pasteurizer (Inoxil, Guarulhos, São Paulo, Brazil), this heating section consists of plate heat exchangers, where the whole egg reaches the temperature of 61.1°C for 3.5 minutes, while in the tubular pasteurizer (Actini, Evans-les-Bains, France) the pasteurization temperature reaches 64,4°C and is holding for 2.5 minutes in tubular heaters. At the regeneration section, the hot pasteurized product is used to preheat the incoming raw eggs before being cooled to a storage temperature (0 to 4°C) at the cooling section. After collection, the samples were kept refrigerated until analysis.

Analysis of Enzymatic Activity Using UV/visible Spectrophotometry

To evaluate the activity of alpha-amylase in the eggs, 0.5 g ± 0.0005 g of each sample, to which 250 μL of 1% starch solution was added, was weighed in duplicate. After this step, the samples were incubated in a water bath at 44°C for 30 minutes and immediately placed in an ice bath at an average temperature of −1°C for 2 minutes. Next, 5 mL of TCA 15% (v/v), 9 mL of boiling distilled water (90°C) and 9 mL of cold distilled water (5°C) were added to inactivate the enzyme. The resulting solution was decanted for 15 minutes and filtered in qualitative filter paper (J.Prolab, São José dos Pinhais, Brazil). Subsequently, 4 mL of the filtrate were transferred to a test tube and 2 mL of the solution with added iodine and potassium iodide were added. The final starch concentration in the samples of pasteurized whole liquid egg was obtained by linear regression based on the absorbency readings conducted in a UV/visible spectrophotometer (Biospectro, Curitiba, Brazil) at a wavelength of 585 nm using the line equation obtained in the linearity assessment. Next, alpha-amylase activity was measured by assessing the percentage of hydrolyzed starch through the equation: $[(C_0\text{-}C_f)/C_0]^*100$, which considers the initial concentration (C_0) and the final concentration of starch in the samples (C_f).

Microbiological Assays of the Samples of Whole Eggs

To analyze aerobic mesophilic bacteria in the samples of raw egg and whole liquid eggs, which were pasteurized at 61.1°C for 3.5 minutes and 64.4°C for 2.5 minutes, Petrifilm Aerobic Count Plates (3 M, St. Paul, MN) were used (Curiale et al., 1990) in dilutions of 10^{-1} to 10^{-4}. Populations of total and thermotolerant coliform bacteria were determined using Petrifilm *E. coli*/Coliform Count Plates (3M, St. Paul, MN) in plates with dilutions between 10^{-1} and 10^{-3}, in accordance with Curiale et al. (1991). *Staphylococcus* spp. were counted according to Bennet and Lancette (2001) in dilutions between 10^{0} and 10^{-3}. For *Salmonella* spp., the immunoenzymatic method (Elisa linked fluorescent assay, or **ELFA**) was used (Curiale et al., 1997) in a Vidas30 device (bioMeriéux, Hazelwood, MI).

Experiment Design

The assays were conducted in a randomized block design with the blocks represented by the different whole eggs used. Three products were evaluated: whole unpasteurized egg (raw egg), whole egg pasteurized at 61.1°C for 3.5 minutes, and whole egg pasteurized at 64.4°C for 2.5 minutes, with 30 repetitions each. To assess alpha-amylase activity and evaluate the counts for mesophilic aerobic bacteria, total and thermotolerant coliforms, and staphylococci bacteria, the data were transformed and the data which did not exhibit normal distribution and/or homoscedasticity were submitted to non-parametric statistical analysis. The results were compared using the Friedman test at a significance level of 0.01%. To assess the frequency of *Salmonella* spp., the data were distributed in a contingency table and interpreted by the chi-square test.

RESULTS AND DISCUSSION

Validation of the Spectrophotometric Method for Evaluating Activity of Alpha-amylase as an Indicator of the Efficiency of Egg Pasteurization

The analytical method used to study alpha-amylase activity to indicate that liquid whole eggs had been pasteurized was adequate for use only in eggs pasteurized at 64.4°C for 2.5 minutes. Partial inactivation of the enzyme, when time and temperature were less than 64.4°C or 2.5 minutes, affected the linearity of the method, and the samples of liquid eggs that were pasteurized at 60°C for 3.5 minutes and 62°C for 3.5 minutes could not be used to evaluate the other performance parameters required in the procedure to validate the method. Therefore, the validation procedures were performed using only pasteurized egg samples at 64.4°C for 2.5 minutes. Other authors have also observed that the use of alpha-amylase enzyme as an indicator of the efficiency of the pasteurization process is possible for pasteurized eggs at temperatures above 64.4°C for 2.5 minutes (Brooks, 1962; Shrimpton et al., 1962; Murthy, 1970). However, the validation of the methodology was not performed in these studies. According to EURACHEM (2014), one method should be validated when it is necessary to demonstrate that its performance characteristics are adequate for use for a particular purpose. It is stated in ISO/IEC 17025 (2005) that the laboratory shall validate: non-standard methods; laboratory-designed/developed methods; standard methods used outside their intended scope; amplifications and modifications of standard methods.

The equations of the curves and the coefficients of determination (R^2) and correlation, which were used to evaluate linearity in the range from 0.5 to 6.0 mg kg^{-1}, are presented in Table 2. The values obtained show that the model is adequate because the coefficient of determination is greater than 0.99, which is considered evidence of an excellent fit between the data and the regression line. The estimate of R^2 provides an evaluation of the quality of the curve obtained, because the closer its value is to 1.0, the lower the dispersion and uncertainty of the set of experimental points. According to the criteria of European and Brazilian legislation, values higher than 0.99 are recommended for linearity tests (European Commission, 2002; INMETRO, 2010).

Comparison of the slopes obtained from the linear regression curves of the spiked blank matrix and the analyte in solution showed that the matrix effect is significant (Figure 1). Therefore, when the starch is added to the pasteurized egg matrix, lower absorbance values for the starch-iodine complex are found in comparison with the absorbencies generated with the same concentrations of starch in the fortified blank matrix.

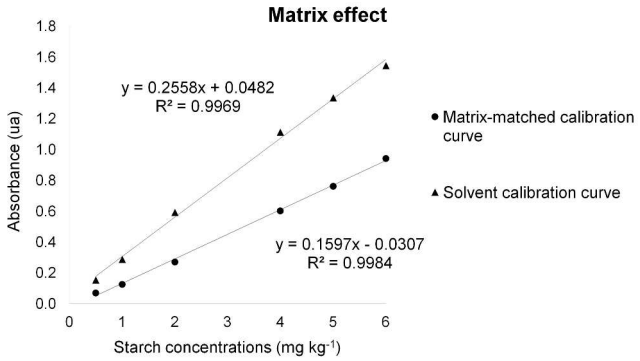

Figure 1. Linear regression equations used in the matrix effect evaluation for spiked blank matrix and analyte in solution (starch solution at concentrations of 0.5, 1.0, 2.0, 4.0, 5.0, and 6.0 mg kg^{-1}).

Table 3. Repeatability, reproducibility, and recovery values obtained from analyzing the samples pasteurized at 64.4°C for 2.5 minutes and fortified with three different concentrations of starch (1.0, 2.0, and 6.0 mg kg^{-1}).

	Starch concentration (mg kg^{-1})			
Validation parameter	1.0	2.0	6.0	Mean
Repeatability (RSD)[1]	5.02%	8.41%	7.51%	6.98%
Reproducibility (RSD)[1]	8.66%	8.43%	7.16%	8.08%
Recovery (%)[2]	100.10%	105.40%	98.81%	101.44%

[1]RSD: relative standard deviation used to evaluate the precision of the method.

[2]Recovery of additions of low (1.0 mg kg^{-1}), medium (2.0 mg kg^{-1}), and high (6.0 mg kg^{-1}) starch concentrations obtained in assess of the accuracy of the method.

The accuracy of the validated method was adequate, with mean recovery values from 98.81% to 105.4% (Table 3). The accuracy was within the range recommended by the European Commission (2002) and the Codex Alimentarius (2009), which determined a recovery percentage from 70 to 110% and from 80 to 110%, respectively, for analyte concentrations higher than 1 mg kg^{-1} (European Commision, 2002; Codex Alimentarius, 2009).

The RSD for the results obtained in the repeatability tests ranged from 5.02% to 8.41%, while the RSD in the reproducibility tests ranged from 7.16% to 8.66%. These values are within the range established by the European Commission (2002), which indicates a maximum RSD of 16% for assessment of reproducibility and 10% for evaluation of repeatability (Table 3).

The results of the study of method robustness showed that change in wavelength was the only significant

Table 2. Linearity of the spectrophotometric method used to study alpha-amylase activity obtained using starch concentrations from 0.5 to 6.0 mg kg^{-1}.

Regression equation	Coefficient of determination (R^2)	Coefficient of correlation (r)
y = 0.1597x - 0.0307	0.9984	0.9992

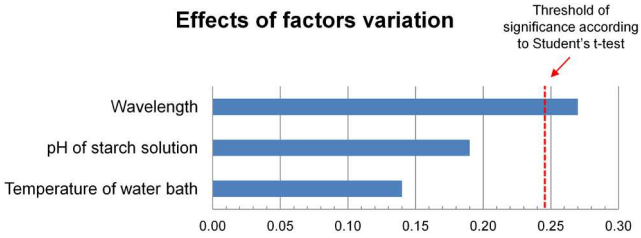

Figure 2. Effect of variation of each factor studied to determine the robustness of the method.

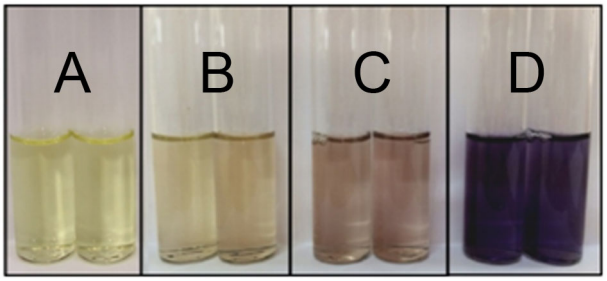

Figure 3. Colors obtained by adding iodine solution to water (**A**) and adding the starch and iodine solution to raw egg (**B**), to egg pasteurized at 61.1°C for 3.5 minutes (**C**), and egg pasteurized at 64.4°C for 2.5 minutes (**D**).

factor ($P < 0.05$) in evaluating the effects of variation of each factor in isolation (Figure 2). However, altering this factor did not significantly affect the robustness of the method, as the value of the standard deviation of the effects (0.37) was similar to the standard deviation of the intra-laboratory reproducibility of the method at a concentration of 3.0 mg kg^{-1} (0.24) by the F test ($P > 0.05$).

Evaluating alpha-amylase activity by quantifying starch hydrolysis can also be used as a quick test in industrial platforms, because it provides a visual assessment of pasteurization efficiency at different temperatures. The colorations observed differ significantly according to the combination of temperature and time to which the eggs are subjected. Samples from raw eggs are yellow, indicating that nearly all of the starch added to the matrix was hydrolyzed and that there was no formation of the starch-iodine complex. The eggs pasteurized at 61.1°C for 3.5 minutes were purplish-pink, indicating partial hydrolysis of starch, while the samples of eggs subjected to temperatures above the point of enzymatic inactivation (64.4°C for 2.5 minutes) were blue-violet, demonstrating the formation of the starch-iodine complex (Figure 3).

However, considering the results obtained from both the validation of the spectrophotometric method as well as through visual evaluation, pasteurization using the binomial 64.4°C for 2.5 minutes was seen to be more suitable for egg pasteurization than that of 61.1°C for 3.5 minutes, which is recommended by the Food and Drug Administration (USDA, 2013) because it permits the use of a method which is fast and reliable in verifying the efficiency of the process. The absence of alpha-amylase activity was crucial for the validation procedures and for distinguishing a stronger color differentiation between the samples of raw egg and pasteurized eggs. Furthermore, some changes were made in the methods described by Brooks (1962), Shrimpton et al. (1962) and Murthy (1970) to enable the quantitative assessment of starch hydrolysis and the visual evaluation of results. These changes were made in the volume of the starch solution added to the egg sample, in the volume of boiling water and cold water added during the extraction process, which was standardized to 9 mL of boiling distilled water and 9 mL of cold distilled water, and in the final ratio between the extract volume and the volume of iodine solution (4 mL of extract and 2 mL of iodine solution).

Assessment of the Efficiency of the Variables Time and Temperature in Pasteurizing Eggs

Assessment of enzyme activity using UV/visible spectrophotometry in the raw egg showed that alpha-amylase was present and active in all samples due to the high percentages of hydrolyzed starch found (Table 4). In these samples, high counts of mesophilic aerobic microorganisms, total coliforms, and thermotolerant bacteria, as well as *Staphylococcus* spp. were also found (Table 4). Furthermore, 23 of the 30 samples (76.67%) tested positive for *Salmonella* spp. (Table 5).

The European Union does not require testing for *Staphylococccus* spp. in pasteurized eggs, only testing

Table 4. Results (log CFU mL^{-1}) of the counts for mesophilic aerobic bacteria, coliforms at 35°C, coliforms at 45°C, *Staphylococcus* spp., and percentage of hydrolyzed starch in samples of raw egg, egg pasteurized at 61.1°C for 3.5 minutes, and egg pasteurized at 64.4°C for 2.5 minutes.

	Raw egg			Egg pasteurized at 61.1°C			Egg pasteurized at 64.4°C		
Parameter	Min.[1]	Max.[2]	Median	Min.[1]	Max.[2]	Median	Min.[1]	Max.[2]	Median
Mesophilic microorganism	3.00	>6.18	4.81[a]	<1.00	3.60	2.00[b]	<1.00	3.70	1.30[b]
Total coliforms	2.26	>5.18	4.11[a]	<1.00	<1.00	<1.00[b]	<1.00	<1.00	<1.00[b]
Thermotolerant coliforms	<1.00	>5.18	2.70[a]	<1.00	<1.00	<1.00[b]	<1.00	<1.00	<1.00[b]
Staphylococcus spp.	1.30	5.30	4.04[a]	0.00	1.74	0.81[b]	0.00	2.15	2.08[b]
Hydrolyzed starch (%)	57.12	85.05	73.15[a]	0	75.53	14.04[b]	0	0	0[c]

[1]Min. = minimum values.
[2]Max. = maximum values.
[a–c]Means followed by different letters on the same line differ among themselves according to the Friedman test ($P < 0.0001$).

Table 5. Results of the analyses of *Salmonella* spp. in samples of raw egg, egg pasteurized at 61.1°C for 3.5 minutes, and egg pasteurized at 64.4°C for 2.5 minutes.

Parameter	Raw egg				Egg pasteurized at 61.1°C				Egg pasteurized at 64.4°C			
	Positive		Negative		Positive		Negative		Positive		Negative	
Salmonella spp.	n[1]	%[2]	n[1]	%[2]	n[1]	%[2]	n[1]	%[2]	n[1]	%[2]	n[1]	%[2]
	23[a]	76.67	7	23.33	1[b]	3.33	29	96.67	0[c]	0	30	100

[1]Absolute frequency.
[2]Relative frequency.
[a–c]Frequency of positive samples followed by different letters on the same line differ among themselves by the chi-square test ($P < 0.0001$).

for S*taphylococcus aureus* (European Economic Community, 1989); nevertheless, testing for other microorganisms of this genus is important because, when present in high numbers, *Staphylococcus* spp. also produce thermostable enterotoxins (Cunha et al., 2006). Despite chemical and physical properties in the egg that prevent the multiplication of microorganisms, after laying, the eggs can be contaminated by microorganisms present in the environment or which comprise the microbiota in the avian intestinal tract, such as bacteria of the genus *Salmonella* (Quinn et al., 2005). The use of eggs that are dirty, cracked, or that have defects in the shell increases susceptibility to contamination during industrial processing, which may explain the high microbial counts found in the raw eggs.

In the samples pasteurized at 61.1°C for 3.5 minutes, a lower percentage of hydrolyzed starch was found when compared to the samples of raw egg, indicating that enzymatic activity was present, but to a lesser degree. Total inactivation of alpha-amylase occurs when temperature and time variables are greater than or equal to 64.4°C and 2.5 minutes. In this treatment, a reduction was also seen in the counts for mesophilic aerobic bacteria, total and thermotolerant coliforms, and *Staphylococcus* spp. These results were expected, because one of the goals of pasteurization is to eliminate pathogenic microorganisms and reduce those that cause deterioration. However, *Salmonella* spp. was present in one of the 30 samples (3.33%). The presence of this microorganism in a pasteurized product may indicate error during thermal processing or re-contamination after the process. To assess whether this sample was affected by error during the pasteurization process, we calculated the confidence interval of the mean of the percentage values for hydrolyzed starch in the raw egg. This sample contained 75.53% hydrolyzed starch. This value is inserted into the confidence interval for the percentage of hydrolyzed starch for 99% of the raw egg samples, which ranges from 68.43% to 76.43%. This confirms that there was failure in the pasteurization process of this sample. Some serovars of *Salmonella* are sensitive to thermal treatments reaching temperatures greater than or equal to 58°C (van Asselt and Zwietering, 2006; Lianou and Koutsoumanis, 2013; Jakočiūnė et al. 2014). In addition, associating the result obtained from analysis of alpha-amylase test with the presence of *Salmonella* spp. in the sample, it can be argued that this sample did not reach the combination of temperature and time required for pasteurization.

In the samples of egg pasteurized at 64.4°C for 2.5 minutes, the starch was not hydrolyzed. Considering that the same samples were evaluated before and after the pasteurization process, the absence of hydrolyzed starch demonstrates that the enzyme was completely inactivated in this treatment (Table 4). The results of the microbiological analyses demonstrated a reduction in microbial counts, as well as total elimination of *Salmonella* spp. (Table 5). The counts for mesophilic aerobic bacteria observed in this treatment were similar to those obtained in the samples pasteurized at 61.1°C for 3.5 minutes, and the values found for both treatments were lower than the limits recommended by the European Union and the United States, which are 5.0 log CFU mL^{-1} and 4.0 log CFU mL^{-1}, respectively (European Economic Community, 1989; USDA, 2013). The counts for total and thermotolerant coliforms were also similar among the pasteurized egg treatments, thereby indicating good hygienic and sanitary quality in the industrial processes. International agencies recommend testing pasteurized eggs for microorganisms belonging to the *Enterobacteriaceae* family. In the United States (USDA, 2013), a limit of 1.0 log CFU mL^{-1} has been determined, while the European Union has established a limit of 2.0 log CFU mL^{-1} (European Economic Community, 1989).

Microbiological analysis can be used as a tool to assess the efficiency of the egg pasteurization process, because differences were found between the results for raw eggs and pasteurized eggs. However, the greater time needed to obtain the results and the required laboratory structure makes industrial application impractical. The methodology proposed to test alpha-amylase activity by quantifying starch hydrolysis using spectrophotometry was effective in differentiating the samples of raw and pasteurized eggs, and can be used as a routine methodology in industry because of its ease of implementation, low cost, and rapid results.

CONCLUSION

The UV/visible spectrophotometric method presents the performance characteristics required for validation and evaluation of alpha-amylase activity by quantifying

starch hydrolysis is adequate for determining the efficacy of pasteurization in whole liquid eggs. Furthermore, pasteurization using the time/ temperature binomials of 61.1°C for 3.5 minutes and 64.4°C for 2.5 minutes is effective in reducing microbe counts and eliminating pathogenic microorganisms.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of the Collegiate of Postgraduate Studies in Animal Science at the Escola de Veterinária at the *Universidade Federal de Minas Gerais* (EV/UFMG), the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and the *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG) for providing funds for publication and research. The authors also thank the *Instituto Mineiro de Agropecuária* (IMA) for assisting with the analyses.

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