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ORIGINAL ARTICLE

Effect of pasteurization of shell egg on its quality characteristics under ambient storage

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Abstract Three thermal processes viz. dry (55°C, 2 h), moist (57°C, 5 min) and microwave (power 9, 20 sec) were studied to determine their efficacy for the pasteurization of intact chicken eggs based on the extent of inactivation of artificially inoculated *Salmonella typhimurium* (ST) in the yolk of shell eggs and alteration in albumen protein solubility (APS). Moist heat treatment was superior to others as it brought about 2 log cfu/ml reduction of inoculated ST in much less time than dry heating but changes in APS were not significant. Subsequent quality evaluation of normal (uninoculated) eggs subjected to moist heat pasteurization during 15 days of ambient (35°C, 36% RH) (35 ± 0.5°C, 36 ± 2% RH)storage revealed no significant effect on percent loss in egg weight, albumen pH, viscosity of albumen and yolk and thiobarbituric acid values between pasteurized and unpasteurized eggs. Pasteurization had no adverse effect on foam volume and foam stability of albumen during storage in comparison to those of raw eggs. Naturally occurring aerobic mesophilic bacteria, coliforms, staphylococci, yeast and moulds on the egg shell surface and in egg contents got markedly reduced by pasteurization of shell eggs and their multiplication also retarded during storage. Both pasteurized and raw eggs remained fairly acceptable sensorily up to 10 days of storage at ambient conditions.

Keywords Shell egg · Pasteurization · *Salmonella typhimurium* · Inactivation

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Introduction

Microbial contamination of eggs, particularly with pathogenic bacteria is of increasing concern globally. As a result, the research on the shell egg pasteurization has drawn the attention of researchers in the recent past so as to provide wholesome table eggs to consumers (Hou et al. 1996, Stadelman et al. 1996, Hank et al. 2001). Although the major causes of spoilage of eggs are microbial contaminants and alteration in their chemical constituents during storage, incidence of contamination of eggs with pathogenic bacteria, especially *Salmonella* serotypes *S. typhimurium* (ST) and *S. enteritidis* (SE) has been of great concern from egg borne human salmonellosis viewpoint (Humphrey 1994, Henzler et al. 1998, Krishnamoorthy et al. 2003, Suresh et al. 2006, Messens et al. 2007). Attempts to eliminate these egg-borne pathogens from laying flocks to overcome vertical transmission have not been very successful. This has led to an alternative approach to explore the application of liquid egg pasteurization systems to intact shell eggs. Stadelman et al. (1996) subjected chicken eggs artificially inoculated with high levels of SE cells to thermal treatments to obtain yolk temperature of 55°C followed by holding at this temperature for varying times and found a drastic reduction of this pathogen without a significant change in egg albumen functional property. In a similar study carried out by Hank et al. (2001), no adverse effect was observed in the albumen quality between pasteurized (55°C, 3 h) and unpasteurized egg during 8 weeks of refrigeration (4°C) storage. In view of very limited information on shell egg pasteurization, and wide variation in the type and level of microbial contaminants (Bajaj et al. 2003, Reu et al. 2008) owing to variation in the egg production, handling and storage practices between developed and developing countries, attempts were made to optimize pasteurizing treatment for chicken shell eggs and evaluate its effect on their quality.

Materials and methods

Fresh chicken eggs were cleaned using 0.5% sodium carbonate solution (pH 11.8, 43 ± 0.5°C) followed by sanitizing in 100 ppm sodium hypochlorite solution prior to drying under fan. The cleaned eggs were candled to remove damaged eggs with hairline cracks.

Artificial inoculation: *ST* (E 2391) having resistance against nalidixic acid (NA) was procured from National Salmonella Centre, IVRI, Izatnagar. Hektoen enteric agar media containing NA (30 mg/l) was used to culture the resistant isolate to inhibit the growth of other bacteria. The broader end of clean intact shell eggs was gently perforated using 18-G needle. An inoculum of ST containing 10^7 cells in 100 µl was injected into the centre of yolk of each egg through the perforation using 25G needle. After inoculation, the holes were sealed with paraffin and the eggs were subjected to three pasteurizing treatments viz. dry heat (hot air oven) at 55°C for 2 h, moist heat (circulating water bath) at 57°C for 15 min and radiant energy (microwave oven) at power 9 for 20 sec. The yolk core temperature was measured by inserting a portable digital probe thermometer (Model ST-9269 Hwa Tai Tech. Co., Ltd, Taiwan) in the centre of the yolk through the perforation made into the broad end of thermally treated eggs. The heat treated and unheated (control) eggs were broken aseptically, yolk was separated from albumen, cultured, incubated (37°C, 18–24 h) and dark green colonies with light green colloidal zone having bull's eye appearance were counted and the counts were expressed as log cfu/g yolk.

Storage quality evaluation: Based on the magnitude of destruction of inoculated ST and egg albumen protein solubility assay as an indicator of its denaturation, moist heat (water bath) pasteurizing treatment was selected for keeping quality study. Both unpasteurized (control) and circulating water bath (57°C, 15 min) pasteurized eggs were stored for 15 days at ambient conditions (35 ± 0.5°C, 36 ± 2% RH) and evaluated at 5 days intervals for physico-chemical, microbiological and sensory quality.

Analysis: The loss in egg weight during storage was expressed as % of initial weight. Egg albumen pH was determined as per AOAC (1995). The distillation procedure of Tarladgis et al. (1960) was followed for measuring thiobarbituric acid (TBA) value in egg yolk whereas albumen protein solubility was measured as per Morr et al. (1985). Interior quality of eggs viz. Haugh unit (Haugh 1937), albumen index (Heiman and Carver 1936), yolk index (Funk 1948) and functional property viz. foam volume and foam stability (Baldwin 1977) were measured. Albumen and yolk Viscosity was determined directly, using a Brookfield Viscometer (Model DV-II PRO Viscometer USA). Aerobic plate count, coliforms, staphylococci, yeast and moulds counts were determined as per APHA (2001). All these measurements were done in triplicates, except egg weight loss and interior egg quality for which 10 eggs were utilized per treatment. Sensory evaluation for appearance, texture, flavour and overall acceptability was performed using 7-point Hedonic scale ranging from 7 (like very much) to 1 (dislike very much) by seven in-house semi-trained panelists. The data were analyzed statistically (Snedecor and Cochran 1994).

Results and discussion

ST inactivation: Application of dry heat (55°C, 2h) and moist heat (57°C, 15 min) brought about reduction ($p < 0.05$) in ST counts by 2.1 log and 2.0 log cfu/ml yolk, respectively while the same in eggs subjected to microwave heating (power 9, 15 sec) was found to be merely 1.2 log cfu/ml yolk (Table 1). Such variability observed in the reduction of *ST* counts might be due to variable yolk core temperature attained with different thermal treatments. Inoculated ST was not destroyed completely in any of these heat pasteurization treatments possibly due to non-attainment of critical temperature of 55 to 56°C necessary for maximum inactivation of salmonellae without denaturation of egg white proteins (Stadelman et al. 1996). Heat resistance of salmonellae is pH dependent and is maximum in the pH range of 5 to 6. This could be one of the reasons for heat resistance exhibited by ST in the yolk (pH 6.0–6.1) of fresh eggs as also reported by Cunningham (1977). In an earlier study, immersion of eggs in water for 30 min at 57°C also did not cause complete destruction of ST and prolongation of holding time at this temperature resulted in the coagulation of egg albumen proteins (Van Lith et al. 1995). However, in present study, water bath heating appeared more practicable than hot air oven as the former process required much less time due to faster heat transfer than the latter while both these pasteurization treatments exerted almost similar ST inactivation (Table 1).

Table 1 Effect of shell egg pasteurization on inactivation of inoculated *Salmonella typhimurium* in egg yolk and changes in albumen protein solubility

	Yolk core temp, °C	*S. typhimurium* count, log cfu/ml	Albumen protein solubility, %
Untreated (Control)	-	5.9 ± 0.09^{a}	87.5 ± 2.49
Microwave oven, * 20 sec	50	4.6 ± 0.59^{ab}	82.4 ± 1.36
Water bath, 57°C, 15 min	53	3.9 ± 0.50^{b}	84.6 ± 1.29
Hot air oven, 55°C, 2 h	54	3.8 ± 0.86^{b}	83.3 ± 0.93

*Power (9) Means ± SE with different superscripts in a column differ significantly ($p < 0.05$)

Since the incidence of naturally contaminated eggs with ST or SE has been reported in the low range of 0.6 to 2.9% of eggs produced (Humphrey et al. 1991, Yadav et al. 2008) with less than 100 cfu of SE per egg (Humphrey et al. 1989), it might be possible to eliminate these organisms from eggs with the level of destruction achieved in the present investigation.

Protein solubility: There were no significant differences in the solubility of egg white proteins (82.4–84.6%) among the thermal treatments and between thermally treated and unheated (87.5%) control groups (Table 1). This suggested that very little denaturation of egg albumen proteins occurred during the pasteurization process. This is in conformity with the report of Hank et al. (2001) who pasteurized eggs in a hot air oven at 55°C for 3 h.

Physico-chemical quality: A progressive increase in percent egg weight loss occurred with storage time regardless of treatments (Table 2). However, thermally treated eggs exhibited relatively more weight loss (14.2%) than untreated control group (12.8%) at 15th day of ambient storage. This could be attributable to the partial removal of cuticle during moist heat treatment, allowing more water vapour to escape from the egg contents.

Irrespective of treatments, the rate of increase in the pH value of egg albumen, arising from the loss of CO_2, was much faster during first 5 days than during subsequent storage till 10th day but thereafter it showed a slight decrease on 15th day of storage (Table 2). Relatively higher pH of thermally treated eggs than that of untreated control group might be due to more loss of CO_2 through shell pores following partial removal of cuticle during moist heat treatment.

Thermal treatment increased the viscosity of albumen from 81.7 cp in raw eggs to 100.7 cp in pasteurized eggs initially (Table 2), indicating partial protein denaturation. This is in conformity with the reports on egg white heated to 56–57°C by Cunningham (1977) and Jeantet et al. (1999) but contrary to the findings of Hou et al. (1996) who found a decrease in the viscosity of egg white of eggs subjected to moist heat treatment (57°C; 30 min) and those of Hank et al. (2001) who reported no appreciable change in the viscosity of albumen of eggs pasteurized in hot air oven for 3 h at 55°C. In the present investigation, the overall treatment effect was, however, not statistically significant. A drastic fall in the viscosity of egg albumen in both control and thermally treated groups during subsequent storage could be accounted for by the faster rate of liquefaction of thick

Table 2 Effect of moist heat (57°C, 15 min) pasteurization on changes in physico-chemical and functional properties of shell eggs under ambient (35°C, 36% RH) storage

Treatment	Storage days				Overall mean
	0	5	10	15	
			Weight loss,%		
C	-	3.6 ± 0.27	7.3 ± 0.54	12.8 ± 0.91	5.9 ± 0.80
P	-	4.1 ± 0.24	8.2 ± 0.50	14.2 ± 0.85	6.6 ± 0.87
			Albumen pH		
C	7.9 ± 0.02	8.9 ± 0.07	9.3 ± 0.01	9.2 ± 0.02	8.8 ± 0.16
P	7.9 ± 0.02	9.0 ± 0.00	9.4 ± 0.06	9.3 ± 0.02	8.9 ± 0.18
			Viscosity of albumen, cp		
C	81.7 ± 20.45	15.1 ± 2.45	10.8 ± 0.51	0	26.9 ± 10.63
P	100.7 ± 0.63	13.5 ± 1.24	24.7 ± 1.48	0	34.7 ± 11.85
			Viscosity of yolk, cp		
C	410.7 ± 23.59	326.1 ± 62.03	229.7 ± 29.04	150.8 ± 10.24	279.3 ± 33.42
P	434.3 ± 20.00	258.5 ± 67.15	185.4 ± 6.15	224.4 ± 46.25	275.6 ± 33.58
			TBA value, mg MA/kg yolk		
C	0.2 ± 0.02	0.2 ± 0.02	3.2 ± 0.09	3.4 ± 0.01	1.7 ± 0.32
P	0.2 ± 0.02	0.3 ± 0.03	3.3 ± 0.03	3.9 ± 0.07	1.9 ± 0.36
			Foam volume, ml/25 ml albumen		
C	26.0 ± 1.15	25.0 ± 2.88	23.3 ± 2.02	21.3 ± 0.66	23.9 ± 0.98^a
P	41.3 ± 1.58	27.0 ± 5.77	24.7 ± 2.02	24.0 ± 3.00	29.2 ± 2.30^b
			Foam stability, ml/25 ml albumen		
C	24.2 ± 1.20	19.3 ± 2.66	20.0 ± 1.73	18.0 ± 1.00	20.4 ± 1.04^a
P	38.7 ± 0.88	21.0 ± 1.52	20.7 ± 1.76	19.0 ± 1.35	24.8 ± 2.31^b

C=Control, P= Pasteurized Overall means ± SE with different superscripts in a column differ significantly ($p<0.05$)

albumen and a decline observed in the viscosity of egg yolk during storage might be due to diffusion of water from albumen to yolk via vitelline membrane.

TBA value showed an increasing trend during storage regardless of treatment (Table 2). However, the rate of increase in TBA value was much faster after 5 days of storage

Table 3 Effect of moist heat (57°C, 15 min) pasteurization on the interior quality of eggs under ambient (35°C, 36% RH) storage

Treatment	Storage days				Overall mean
	0	5	10	15	
			Haugh unit		
C	71.8 ± 2.12	62.4 ± 3.88	30.3 ± 4.26	ND	41.1 ± 28.18[a]
P	71.0 ± 11.50	53.8 ± 18.15	51.2 ± 7.80	43.5 ± 7.64	54.9 ± 15.40[b]
			Albumen index		
C	0.067 ± 0.003	0.053 ± 0.055	0.008 ± 0.003	ND	0.032 ± 0.030
P	0.070 ± 0.006	0.039 ± 0.006	0.033 ± 0.004	0.007 ± 0.003	0.037 ± 0.004
			Yolk index		
C	0.34 ± 0.01	0.21 ± 0.01	0.14 ± 0.00	0.04 ± 0.01	0.19 ± 0.02
P	0.33 ± 0.01	0.20 ± 0.01	0.16 ± 0.01	0.07 ± 0.02	0.19 ± 0.02

C, P: As in table 2 ; ND= Not done Overall means ± SE with different superscripts in a column differ significantly ($p < 0.05$)

Table 4 Effect of moist heat (57°C, 15 min) pasteurization on changes in the microbial quality of shell eggs under ambient (35°C, 36% RH) storage

Treatment	Storage days				Overall mean
	0	5	10	15	
Egg shell surface, log cfu/cm^2					
			Aerobic plate count		
C	2.9 ± 0.01	2.0 ± 0.07	2.2 ± 0.02	3.9 ± 0.08	2.8 ± 0.12[a]
P	0.2 ± 0.23	1.2 ± 0.28	2.2 ± 0.10	2.9 ± 0.39	1.6 ± 0.33[b]
			Coliforms		
C	1.1 ± 0.22	1.4 ± 0.14	1.7 ± 0.05	1.7 ± 0.43	1.5 ± 0.13[a]
P	0.4 ± 0.23	0.7 ± 0.33	1.2 ± 0.26	1.3 ± 0.16	0.9 ± 0.15[b]
			Staphylococcus		
C	0.7 ± 0.03	1.7 ± 0.05	1.8 ± 0.01	1.8 ± 0.00	1.5 ± 0.13[a]
P	0.2 ± 0.23	0.6 ± 0.59	0.8 ± 0.51	1.4 ± 0.37	0.8 ± 0.23[b]
			Yeast and molds		
C	1.4 ± 0.08	1.6 ± 0.16	1.8 ± 0.08	1.9 ± 0.06	1.7 ± 0.07[a]
P	0.9 ± 0.17	0.9 ± 0.17	1.0 ± 0.52	1.7 ± 0.01	1.2 ± 0.18[b]
Egg content, log cfu/ml					
			Aerobic plate count		
Control	3.4 ± 0.07	4.2 ± 0.15	4.2 ± 0.15	4.2 ± 0.51	4.0 ± 0.16[a]
Pasteurized	0	0.8 ± 0.39	1.6 ± 0.83	3.7 ± 0.12	1.5 ± 0.46[b]
			Coliforms		
C	1.5 ± 0.26	1.9 ± 0.96	2.5 ± 0.18	2.7 ± 0.02	2.2 ± 0.26[a]
P	0.2 ± 0.23	0.8 ± 0.10	0.9 ± 0.47	1.3 ± 0.13	0.8 ± 0.16[b]
			Staphylococcus		
C	1.3 ± 0.02	1.4 ± 0.01	1.5 ± 0.01	1.6 ± 0.05	1.4 ± 0.03[a]
P	0	0.2 ± 0.23	0.5 ± 0.49	1.4 ± 0.36	0.5 ± 0.20[b]
			Yeast and molds		
C	1.0 ± 0.49	1.1 ± 0.20	1.2 ± 0.02	1.4 ± 0.07	1.2 ± 0.12
P	0.7 ± 0.37	0.9 ± 0.14	1.5 ± 0.10	1.7 ± 0.29	1.2 ± 0.16

C, P: As in table 2 Overall means ± SE with different superscripts in a column differ significantly ($p<0.05$)

in both raw and pasteurized eggs. In general, yolk lipids in intact shell eggs are known to exhibit little autoxidation due to exclusion of proxidants like light and oxygen by the shell and shell membranes, phosvitin as iron-chelator, tocopherol as free-radical terminator and outer protein layer of yolk lipoproteins acting as a barrier between the lipid molecules and oxidative factors (Cotterill et al. 1977, Pike and Peng 1985). However, thermal treatment and/or storage at elevated ambient temperature (35ºC) might be accountable for increased lipid oxidation, particularly during later part of storage.

Functional properties: The functional quality measured in terms of foam volume and stability of the albumen foam revealed that the pasteurized eggs maintained better functionality of albumen than untreated control group throughout the storage (Table 2). An initial increase observed in the volume of foam and its stability following mild thermal treatment could be due to the partial unfolding of albumen proteins and resultant increase in their surface hydrophobicity (Hou et al. 1996).

Interior egg quality: Haugh unit (HU), albumen and yolk indices of both raw and pasteurized eggs progressively declined with storage time (Table 3). The HU of raw eggs registered a decrease from 71.8 on day zero to 30.3 on the 10th day at ambient storage. This was consistent with the observation of Kumar et al. (1969). Corresponding values for pasteurized eggs were 71.0 and 51.2. The overall treatment means for HU were statistically significant ($p < 0.05$). However, no significant differences were noted between treatments in respect of albumen and yolk indices, indicating little adverse effect of mild moist heat treatment on these interior quality attributes of egg up to 10 days of ambient storage. Albumen quality of untreated eggs could not be measured on 15th day due to complete liquefaction of thick albumen into thin albumen.

Microbial quality: The initial aerobic plate counts (APC) on the shell surface (2.9 log cfu/cm^2) and in egg contents (3.4 log cfu/ml) of raw egg were lower (Table 4) than those reported by Panda and Panda (1973) but higher than the findings of Anand et al. (1993). Pasteurization of shell eggs caused a marked reduction in APC, coliforms, staphylococci, yeast and moulds on the shell surface initially (Table 4). Although these microbial counts evinced a gradual increase with storage time, the counts remained significantly ($p < 0.05$) less in thermally treated than in control group. More or less similar trends of multiplication of these microflora in egg contents were observed during storage, with the exception that pasteurization treatment reduced APC and staphylococci to an undetectable level initially which tended to show a modest growth viz. 3.7 log cfu /ml and 1.4 log cfu /ml on 15th day of storage respectively. Low counts of APC, yeast and moulds in stored egg products subjected to more severe thermal treatment have also been reported (Modi et al. 2008).

Sensory quality: The appearance and texture scores of hard-cooked eggs of both control and treated groups showed an improvement on 5th day of storage (Table 5) possibly due to rise in albumen pH (Table 2) and associated improvement in the peeling characteristics of eggs (Stadelman and Rhorer 1984). Subsequent storage, in general, evinced a decline in these and other sensory attributes like flavour and overall acceptability owing to flattened appearance of broad end of cooked eggs, alteration in flavour arising from increased lipid oxidation (Table 2) and somewhat rubbery texture of egg albumen. However, both raw and pasteurized eggs remained sensorily acceptable up to 10 days of ambient storage.

Conclusion

Moist heat (57°C, 15 min) pasteurization of intact chicken eggs in a circulating water bath appeared suitable as it gave

Table 5 Effect of moist heat (57°C, 15 min) pasteurization on the sensory quality of shell eggs under ambient (35°C, 36 % RH) storage

Treatment	Storage days				Overall mean
	0	5	10	15	
Appearance					
C	3.1 ± 0.45	5.4 ± 0.20	5.9 ± 0.26	3.3 ± 0.18	4.4 ± 0.27^a
P	5.1 ± 0.34	6.1 ± 0.14	4.7 ± 0.18	3.4 ± 0.20	4.8 ± 0.21^b
Texture					
C	4.1 ± 0.40	5.7 ± 0.18	5.3 ± 0.28	3.8 ± 0.40	4.8 ± 0.21
P	5.3 ± 0.18	6.0 ± 0.00	4.8 ± 0.34	4.0 ± 0.30	5.0 ± 0.18
Flavour					
C	5.6 ± 0.36	5.6 ± 0.20	5.3 ± 0.35	2.3 ± 0.18	4.7 ± 0.30^a
P	5.7 ± 0.28	6.1 ± 0.14	5.4 ± 0.29	3.7 ± 0.18	5.3 ± 0.20^b
Overall acceptability					
C	5.0 ± 0.30	5.4 ± 0.20	6.0 ± 0.21	3.1 ± 0.14	4.9 ± 0.23^a
P	6.1 ± 0.14	6.3 ± 0.18	5.6 ± 0.20	4.0 ± 0.00	5.5 ± 0.18^b

C, P: As in table 2 Overall means ± SE with different superscripts differ significantly ($p < 0.05$)

about 2 log cycle reduction of *ST* artificially inoculated in the yolk of shell eggs in a much shorter time than dry heat treatment without adverse effect on albumen protein solubility. This pasteurization process produced little or no adverse effect on the physico-chemical, interior and functional properties of eggs and retarded multiplication of naturally occurring microflora in eggs during 15 days of storage at ambient conditions (35 ± 0.5°C, 36 ± 2% RH). Both pasteurized and unpasteurized eggs remained sensorily acceptable up to 10 days of storage. Further research is needed to explore combination of dry or moist heat with microwave heating regimen for quicker and more uniform heat distribution in the yolk of shell eggs.

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