MICROBIAL LOAD, CHOLESTEROL LEVELS AND SENSORY QUALITY OF HARD-BOILED COMMERCIAL EGGS

JOSEPH J.K[1*], ADENIYI J.O[1] AND ABU O.A[2]

[1]Animal Products and Processing Unit, Department of Animal Production, University of Ilorin, [2]Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

* Author for correspondence

Microbial flora and load, cholesterol levels and sensory quality of attributes of hard-boiled commercial eggs being hawked along Lagos-Ilorin Road were monitored in this study. The bacteria isolated from the eggs include; *Achromobacter sp. and Staphylococcous aureus*. While the eggs sampled from Ibadan, Ogbomoso and Ilorin had lower microbial load (20 – 33 X 10^4 CFU/g). On the average a 56g egg was found to contain 235mg cholesterol. The results of sensory quality evaluation showed that the panelists that rated the eggs for appearance of albumen, and yolk, juiciness, flavour, firmness and overall acceptability preferred and rated higher eggs obtained from Ibadan, Ogbomoso and Ilorin when compared with eggs obtained from Lagos and Oyo states.

The implications of these findings from the standpoint of food poisoning and public health were discussed.

INTRODUCTION

Eggs are rich sources of high quality protein, unsaturated fatty acids (mainly oleic), iron, phosphorus, trace minerals, vitamins A E and K and the B- vitamins including B12. It provides a unique, well- balanced source of nutrients for persons of all ages (young children, teenagers and old people) Their high nutrient content, low calorie value and ease of digestibility also makes egg valuable in many therapeutic diets for adults (Stadelman and Cotterill, 1977). It has also been reported that the availability, modest cost, ease of preparation, popular taste appeal and low caloric value give eggs a deserved place in geriatric diets (Stadelman and Cotterill, 1977).

A considerable amount of eggs are consumed boiled in Nigeria, probably because of the ease of preparation. Unpeeled hardboiled eggs are sold in small retail outfits, along the major roads and street corners. The usual practice is to package three or four eggs, depending on the egg size in a film bag (Joseph and Ndikintum, 1999). Many travelers see such hard-boiled eggs as convenient food, which go well with bread and "pure" water while in the vehicle on a journey. A close observation of the way such eggs are hawked revealed a potential for health hazard to the consumers, especially from the standpoint of microbial load and

cholesterol intake. This is because most of the products are not freshly boiled eggs and consumers usually take three to four of such eggs on a trip.

A lot of works has been done and reported on the effects of egg lipids on serum lipids in man, although results reported are often contradictory (Stadelman and Cotterill, 1977). Cholesterol has been implicated in atherosclerotic heart diseases because blood (serum) cholesterol and fatty acids were found in thickened inner walls of arteries of people with atherosclerotic heart diseases (Stadelman and Cotterill, 1977).

Lowering the total fat content of the diet and raising the ratio of polyunsaturated to saturated fatty acids have been reported to decrease serum cholesterol (Stadelman and Cotterill, 1977).

Several factors influence directly or indirectly the concentration of nutrients and contaminants in eggs. These include age, breed and strain of hens differnces in eggs produced by individual hens, rations and environmental temperatures for hens, egg storage conditions and length of storage time, preparation and cooking methods (Stadelman and Cotterill, 1977, Awosanya, *et al*, 1998; Ayorinde, *et al.*, 19990. This work was therefore designed to

investigate the microbial load, cholesterol levels and sensory quality of hard-boiled commercial egs being sold along Lagos-Ilorin road with a view to ascertaining their safety from public health point of view.

MATERIALS AND METHODS

Eggs used for this study were purchased from vendors randomly selected at each point, which included Lagos tollgate, Ojoo motor park at Ibadan, Petrol station at Oyo, general hospital at Ogbomoso and Sawmill garage at Ilorin. The experiments were carried out once a month for three consecutive months (i.e. September, October and November 1998).

Microbial Examination

Microbial isolations, identifications and total viable bacterial counts were performed on macerated egg content in sterile distilled water. Isolations, identifications and total bacterial counts were performed on representative samples from each point's samples using the standard plate count method on suitable serial dilutions. (1mL of appropriate dilution plated in duplicate). Nutrient agar (0X01D) plates were incubated at 37°C for 24h. Microbial isolates were identified according to Collins and Lyne (1970) and Buchanan and Gibbon (1974).

Lipids Evaluation:

Two gramme of yolk sample was extracted three times for three hours each time with cholesterol: methanol (2: 1 v/v). The extract was filtered and concentrated by evaporation to 50cm². The crude extract was purified by addition of 0.3 volume of 0.73% sodium chloride solution to the extract in chloroform: methanol. To remove any interphase impurities the extract was washed three times with a mixture of chloroform: methanol: 0.58% sodium chloride solution (3:48:47 v/v/v).

Quantitative Estimation of Individual Lipids

The individual lipids were identified by the use of thin layer chromatography as described by Amenta (1964).
Chloroform: methanol: water (65:25:4 v/v/v) solvent was used for the separation of phospholipids, while petroleum ether (b.p 40-60°C): diethylether: acetic acid (80:20:1 v/v/v) solvent was used for the separation of natural lipids. The plates were spotted with iodine vapour and the RF values of various spots in both the phospholipids and neutral lipid separation were calculated and compared with those of known lipid standards. The spots were scraped from TLC plates and eluted with

chloroform: methanol (2:1 v/v). The solvent was evaporated off and the amount of lipid quantitated by colorimetric method. This method depends on the reduction of acidified dichromate (0.5cm³ of dichromate reagent) by the lipid of the sample.

Sensory Evaluation:

After the screening of recruited individuals from within and outside the University community, a thirty-member taste panel was selected. The panelists were familiarized with the scoring procedure (a nine-point hedonic scale) during preliminary training sessions. Panelists were served a plate containing one-coded sliced samples of the boiled eggs obtained from the different sources.
The samples were served at room temperature and the panelists were provided with water for month rising after each test, conducted in a room with normal lighting (standard white fluorescent lighting). The panelists scored the samples for appearance, flavor, firmness, juiciness and overall acceptability. All data collected were analyzed using a multiple comparison difference analysis (Larmond, 1977).

RESULTS AND DISCUSSION

Microbial Examination:

The bacteria isolated from hard-boiled eggs obtained from the different locations along, Lagos-Ilorin road shown in Table 1. All the isolated and identified bacteria were found in the eggs from Lagos. Proteus vulgaris was not found in eggs obtained from Ibadan and Ilorin. Bacillus sp was absent in eggs obtained from Oyo and Ogbomoso. The variation in microbial flora could be attributed to the different level and source of pollutants after the egg was freshly boiled. All the identified bacteria have been grouped as present occasionally (proteus), present on most eggs but in small numbers (*Staphylococcus, Pseudomonas, Bacillus, Achromobacter, Escherichia* and *Aerobacter*) or always present in large numbers (Micrococcus) (Stadelman and Cotterill, 1977). Interestingly, *Salmonella*, one of the organisms of public health concern in eggs was not isolated from any of the samples.

Figure 1 shows the total viable bacteria counts of hard-boiled eggs obtained from the different locations. Eggs obtained from Lagos and Oyo were heavily loaded with microbes (Lagos (62-68X10⁴ CFU/g) and oyo (65-7-X10⁴ CFU/g), while those obtained from Ibadan (30-33X10⁴ CFU/g), Ogbomoso (21-29 X10 CFU/g)

and Ilorin (20-25X10⁴ CFU/g) were lighter in terms of microbial load. Although we are not aware of any recommended safe level of microbial load for eggs we assumed that the microbial load levels (20-70 X 10⁴ CFU/g) observed in this study are tolerable.

Lipid Evaluation

Results of qualitative and quantitative evaluation of lipids extracted from the yolk of hard-boiled eggs obtained from different locations are shown in table 2. Total lipids of the eggs ranged between 30.8 to 32.6g/ 100g of yolk. This means that an egg which between 5.24 – 5.54g will be lipids. The cholesterol contents of the samples were also found to be between 1.36 to 1.41g/100g of yolk. This converts to 231.2 to 239.7mg cholesterol per 17g yolk of a 56g weight egg. The results of cholesterol contents obtained in this study is similar to the 230mg cholesterol per 17g yolk reported earlier (Standelman and Cotterill, 1977). The eggs total lipids were also partitioned into 13.11 to 13.71g free fatty acids and 14.10 to 16.00g phosopholipids per 100g of egg yolk.

From the results in this study, a regular consumer of one egg (56g) per day will take on the average 235mg cholesterol. And since a daily intake of between 40 and 500mg cholesterol has been found to affect the patient's serum cholesterol level (Stadelman and Cotteril, 1977) potential consumers of such egg should be advised not to take more than one a day. Our observations revealed that since the hard-boiled eggs are retailed in threes for ₦20 or ₦25, the general tendency was that the consumers take the three eggs per trip. This habit should be discouraged.

Sensory Evaluation

Eggs obtained from Ibadan, Ogbomoso and Ilorin were rated high by the sensory panelists in terms of appearance of yolk and albumen, juiciness flavour, firmness and overall acceptability (Table 3), while eggs ontained from Lagos and Oyo were rated low for all the parameters.

These results appear to confirm the result on microbial load because eggs from which high microbial load (Lagos and Oyo eggs) were recorded were rated low in terms of sensory quality. This may be attributable to the breakdown of proteins, organic and other compounds by proteolytic organisms (Lepedes, 1977; Jay, 1978).

TABLE 1:
Bacteria Isolated From Hard-Boiled Eggs Obtained from Different Lcations along Lagos-Ilorin Road.

Microorganisms	LOCATIONS				
	Lagos	Ibadan	Oyo	Ogbomoso	Ilorin
Bacillus cereus	+	+	–	–	+
Bacillus megaterium	+	+	–	–	+
Eschrichia coli	+	+	+	+	+
Aerobacter sp	+	+	+	+	+
Micrococcus sp	+	+	+	+	+
Proteus vulgaris	+	–	+	+	–
Pseudomonas sp.	+	+	+	+	+
Staphylococcus aureus	+	+	+	+	+
Achromobacter sp.	+	+	+	+	+

+ = Present   - = Absent.

TABLE 2:
Quantitative Evaluation of Lipids Extracted from Hard-Boiled Eggs Purchased at Different Locations along Lagos-Ilorin Road.

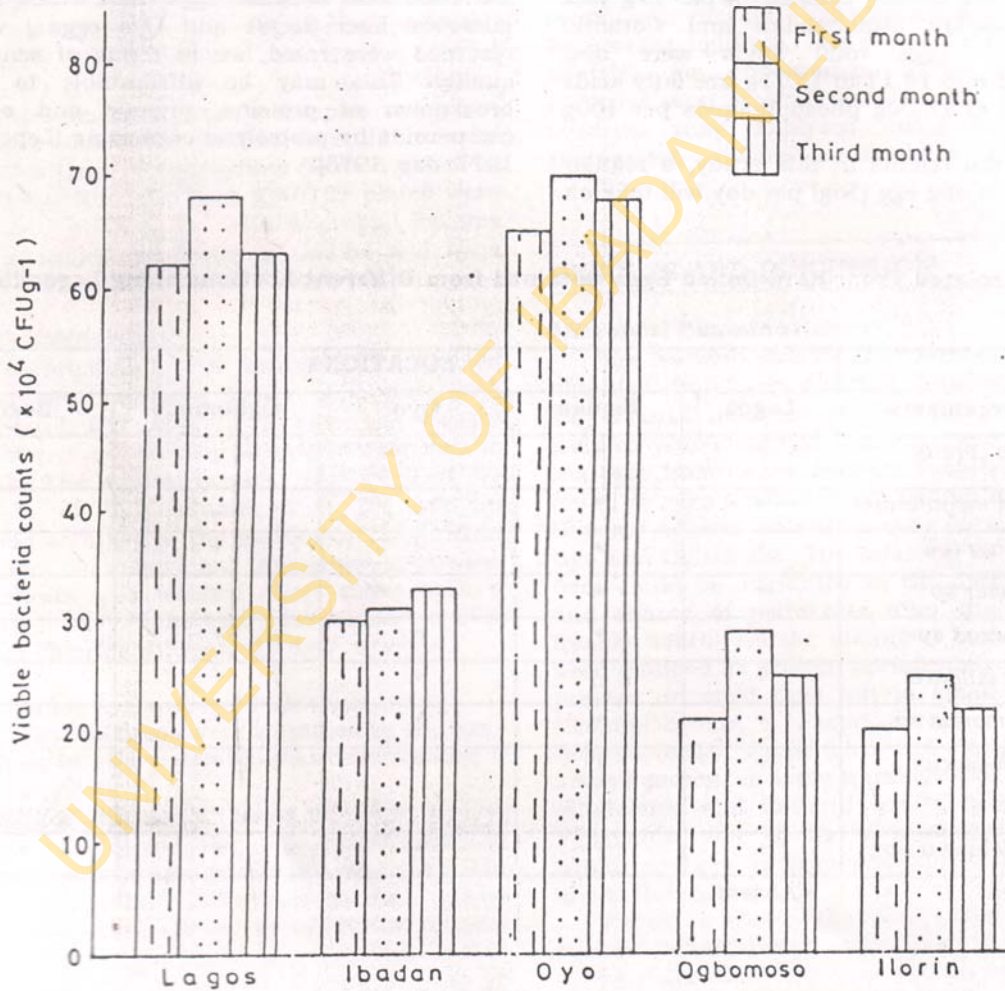| LIPIDS	LOCATIONS				
	Lagos	Ibadan	Oyo	Ogbomoso	Ilorin.
Total Lipid (of 100g yolk)	31.00	30.80	32.60	30.96	31.40
Cholesterol	1.36	1.38	1.37	1.41	1.39
Cholesterol ester	1.58	1.67	1,53	1.46	1.11
Free fatty acids	13.16	13.56	13.71	13.11	13.27
phospholipids	14.90	14.10	16.00	14.30	15.60

Fig. 1.
Total viable bacteria counts of hard-boiled eggs from different locations

TABLE 3:
SENSORY QUALITY RATINGS OF HARD-BOILED EGGS PURCHASED AT DIFFERENT LOCATIONS ALONG LAGOS-ILORIN ROAD.

Locations	SENSORY QUALITY SCORES					
	Appearance		Juiciness	Flavour	Firmness	Overall acceptability
	Yolk	Albumen				
Lagos	5.07[a]	6.13[a]	5.50[a]	5.50[a]	5.73[a]	5.17[a]
Ibadan	6.27[c]	6.47[ab]	6.13[b]	6.20b	5.90[ab]	6.25[b]
Oyo	5.87[b]	6.13[a]	5.58[a]	5.98[ab]	5.67[a]	5.33[a]
Ogbomoso	6.47[c]	6.90[bc]	6.33[b]	6.31[b]	5.87[a]	6.40[b]
Ilorin	6.10[bc]	7.00[c]	6.20[b]	6.56[b]	6.30[b]	6.83[b]

Different subscripts on means within a column indicate significant difference (P<0.05). Higher values indicate greater preference.

In conclusion, it appears that most of the hard-boiled eggs hawk in Nigeria are safe in terms of microbial and cholesterol load. But those obtained from Lagos and Oyo were suspect because of the higher microbial load $(62 - 7- \times 10^4$ CFU/g) and lower sensory quality of the egg samples obtained from the two locations. An acceptable shelf life should be determined for hard-boiled eggs after which the vendors should not be able to hawk such eggs. The current practice in which consumers purchase hard-boiled eggs without quality assurance is risky and prone to food poisoning.

REFERENCES

AMENTA, J.S. (1964): A rapid chemical method for quantification of lipids separated by thin-layer chromatography. J. Lipid Research 5: 270 – 272.

AWOSANYA, B: JOSEPH, J.K AND OLAOSEBIKAN, O.D. (1998): The effect of age of bird on shell quality and component yield of eggs. Nig. J. Anim. Prod. 25:68-70.

AYORINDE, K.L.: JOSEPH, J.K, ADEWALE, O.E. AND AYANDIBU, I.J. (1999): Growth laying performance and egg quality traits of NAPRI commercial layers" on deep litter and in cages. Trop. J. Anim. Sci. 1(1): 147-155.

BUCHANAN, R.E. AND GIBBONS, N.E. (1974): Bergeys Manual of determinative Bacteriology, 8th edn. Williams and Wilkings Co. Baltimore, MD.

COLLINS, C.H. AND LYNE, P.M. (1970): Microbiological Methods, 3rd edn, Butterworth, London.]

JAY, J.M (1978): Modern Food Microbiology 2nd edn. D. Van Nostrand Company, New York.

JOSEPH, J.K AND NDIKINTUM, F.N. (1999): Peelability and shelf life of hard-boiled shell egg. Appl. Trop. Agric (In press).

LARMOND, E. (1977): Laboratory Methods for Sensory Evaluation of Food. Publication No. 1637. Canada department of Agriculture, Ottawa.

LEPEDES, D.N. (1977): McGraw-Hill Encyclopedia of Food Agriculture and Nutrition. McGraw-Hill Book Company, New York.

STADELMAN, W.J. AND COTTERILL, O.J.(1977): Egg Science and Technology 2nd edn. Avi. Publ. Inc. Westport, Connecticult.