

Bacteriological Screening And Physiochemical Analysis Of Conventional And Organic Egg

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Abstract: Eggs are protein and nutrients packed nature's food. Egg is made up of mainly water which is 74 % of its mass and almost equal amount of protein and lipids. Chicken eggs are important as food to mankind since the beginning of time. A total of 50 eggs were purchased from different locations. These samples were examined for the sanitary quality using the total viable count and detection of the presence/absence of *Salmonella* and *Listeria* spp (pathogens) was done using standard microbiological methods. Total bacterial counts of Organic and Conventional chicken eggs shell and content ranged from log 4.8 for Retail egg shell (RES) to the highest log 7.6 for Cracked egg shell (CES) while the egg content ranged from log 2.12 for Organic egg content to the highest log 3.97 for Cracked egg content. *Listeria monocytogenes*, *Listeria innocua* and *Listeria florescedis* were isolated from shells of conventional and organic egg but none was isolated from the content. *Salmonella* spp were isolated from conventional and organic egg shell and organic egg content. Retail egg; Yolk pH values ranged 6.1 -6.8, Albumin pH ranged from 8.5 -9.4, Yolk and Albumin pH ranged from 7.1 -7.9 Market egg; Yolk pH values ranged 5.2 -6.8, Albumin pH ranged from 8.2 -9.7, Yolk and Albumin pH ranged from 6.9-7.9. Poultry farm egg; Yolk pH values ranged 5.4 - 8.1, Albumin pH ranged from 6.9 -7.5, Yolk and Albumin pH ranged from 7-7.7. Organic egg; Yolk pH values ranged 6.3 - 7.1, Albumin pH ranged from 5.9 -7.5, Yolk and Albumin pH ranged from 7.1 -7.8. Cracked egg; Yolk pH values ranged 6.2 - 6.9, Albumin pH ranged from 7.9 - 9.3, Yolk and Albumin pH ranged from 7 -9. It was deduced from this study that eggshells are prone to contamination than the egg content and this contamination can be attributed to farm environment and storage conditions.

I. INTRODUCTION

Eggs are regarded as protein and nutrients packed nature's food (Belitz *et al.*, 2009). Egg comprises of mainly water which is 74 % of its mass and almost the same amount of protein and lipids (Geister *et al.*, 2008). Chicken eggs are essential as food to mankind since the beginning of time. The chicken egg is a by thick calcareous and porous shell whitish to brownish in colour made up of inner membrane, albumen and yolk (De Reu, 2006). The inner part of the egg is the protein rich yolk which is covered by the egg white called albumen and membrane. The yolk contain layers of dark and light-colored material (Belitz *et al.*, 2008). The microbial quality of eggs became of public health significance as a result of egg-borne food poisoning by *Salmonella* Enteritidis (Humphrey 1994). The egg yolk, albumen, membrane / and shell can be contaminated by other bacteria of public health

significance (Bahrouz, 2005). Yolk provides an ideal environment for microbial proliferation (De Reu, 2006). Kingsbury (2006) stated that egg can become contamination during the laying process. Similar to De Reu (2006) bacterial contamination of egg may result from the infection of the laying egg which he called vertical transmission or from all surfaces, with which it makes contact with, this is called horizontal transmission. The intrinsic eggs defense against contamination by microorganisms is possible by the thick calcareous outer membrane (Jerzy and Dagmara, 2009). The albumen is composed of numerous egg white proteins that possess antimicrobial properties, especially the lysozyme, avidin, ovotransferrin and ovomucoid (De Reu, 2006). Ovomucoid possess the ability to stop bacterial from using the protein in albumen. The albumen pH can rise to over 9.0 and the viscosity, are not favourable to microbial growth (Mahdavi *et al.*, 2012). Mayes and Takeballi (1983) reported that

“although the microflora found on the eggshell differ quantitatively and qualitatively indifferent geographical areas, the spoilage flora in eggs trends to be similar irrespectively of geographical area or husbandry methods, showing that the intrinsic defense mechanisms of the egg influence the selection of spoilage types. Probably because of their tolerance of dry conditions, the microflora of the eggshell is mainly Gram-positive bacteria which are gotten from dust, soil or faeces (Board and Tranter 1995).” Some common Gram-negative bacteria contaminants are members of the genera *Alcaligenes*, *Pseudomonas*, *Escherichia*, *Proteus* and *Aeromonas* (Mayes and Takeballi 1983; Board and Tranter 1995). *Salmonella* contamination of eggs shell has been severally reported in literatures (Gast and Beard, 1990; Musgrove *et al.*, 2005). Humphrey *et al.*, (1991b) reported *Salmonella* in egg content. Nitcheva *et al.* (1990) isolated *Listeria monocytogenes* from the eggshell. Moore and Madden (1993) isolated *Listeria spp* in raw egg. *Salmonella* contamination appears to be more in cracked eggs in a study conducted by Ernst *et al.* (1998). The quality of eggs is attributed to the characteristics that are appropriate from the point of view of consumers. Consumers checks for freshness, smell, shell colour and yolk colour. The quality of eggs is assessed by microbiological, hygienic, sensory and technological qualities as well.

Staphylococci are the most common bacteria contaminating eggshells. pathogens such as *Campylobacter jejuni*, *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica* and *Salmonella* serotypes had also been linked to eggs (Bahrouz, 2005; Howard *et al.*, 2005; Akbar and Anal, 2013). Others are species of *Aeromonas*, *Proteus*, *Pseudomonas*, *Citrobacter*, *Alcaligenes spp* and *Klebsiella* (Mayes and Takeballi 1983; Board and Tranter 1995; Musgrove *et al.*, 2004). The interest is in production systems (organic and conventional) and its effect on bacteriological quality of the eggs

II. MATERIALS AND METHODS

A total of 50 chicken eggs samples were randomly collected from retail shops, markets, poultry farm cracked egg and Organic egg in Port Harcourt, Rivers state Each sample was assessed for the total bacterial viable count by spread plate method. Also, detection of *Listeria* and *Salmonella* were performed.

SWAB SAMPLING OF EGGS

A sterile swab stick dipped in saline was used on the surface of each egg shell and swabs were then dipped into 10 mL of saline. The contents of the saline were mixed and inoculated into the different bacteriological media for detection of *Salmonella* and *Listeria* as described by Mahdavi, *et al.* (2012)

SAMPLING OF EGG CONTENTS

Egg sample was dipped in 90% ethanol for a few min after which the end of egg was flamed for some seconds. A

sterile scalpel blade was used to bore a hole on the shell through which the contents were transferred into a stomacher bag. The egg contents (yolk and albumen) were then blended for 30 s after which the mixture was used to inoculate appropriate bacteriological media. as described by Mahdavi, *et al.* (2015)

SALMONELLA DETECTION

The egg's content was mixed thoroughly and then 25 mL of egg contents or one ml of saline containing swabs were added to 225 and 25 mL of Buffered Peptone Water (BPW, Merck, Germany) respectively and incubated at 37°C overnight as pre-enrichment. One milliliter of the cultures were transferred to 9 mL of selenite cystine broth (Merck; Germany) and incubated at 37°C for 24 h for selective enrichment. The cultures were then streaked onto *Salmonella shigella* agar (Merck; Germany) agar and incubated at 37°C for 24–48 h. The plates were observed for typical *Salmonella*-like colonies. Each colony was isolated in a pure form by sub culturing for further studies and Biochemical identification

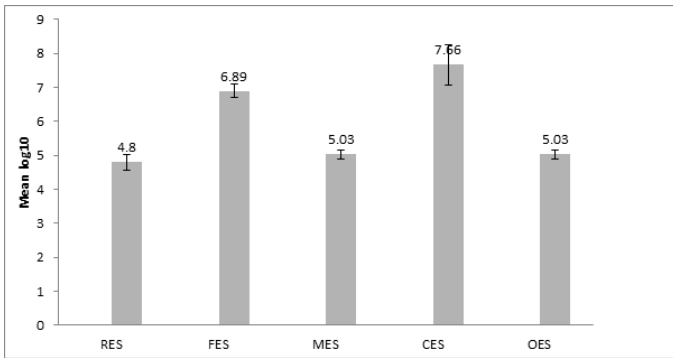
LISTERIA DETECTION

Samples were analyzed for the presence of *Listeria spp.* using selective enrichment and isolation protocol. Egg content was mixed thoroughly and then 25 mL of egg contents or one ml of saline containing swabs were added to 225 ml of half fraser broth and incubated at 37°C overnight as pre enrichment. Then, 1 mL of the cultures were transferred to 9 mL of (Fraser broth) and incubated at 37°C for 24–48 h for selective enrichment. The cultures were then streaked onto PALCAM (Merck; Germany) agar and supplemented with PALCAM Selective Supplement (HC784958 Merck; Germany) and incubated at 37°C for 24–48 h. The plates were observed for typical *Listeria*-like colonies. After incubation at 37°C for 48 h, five characteristic colonies is selected from the PALCAM agar plates and streaked onto tryptone soya yeast glucose agar (TSAYE) plates for purification. Isolates will be tested for catalase, Gram reaction, motility test, carbohydrate utilization.

TOTAL VIABLE COUNT OF THE EGG SAMPLES

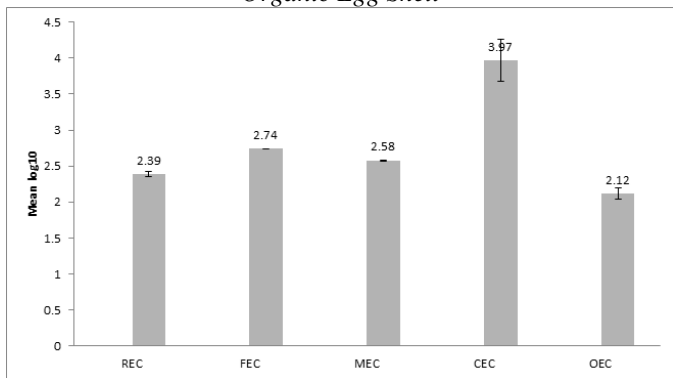
For the total bacterial count, a swab method was applied following (Loongyai *et al.*, 2010). For the enumeration of bacteria in egg contents the method of De Reu (2006) was applied. The isolates were further confirmed to genus and species level with the help of biochemical test and were identified according to Bergeys manual of determinative bacteriology.

III. RESULTS



LEGEND:RES-Retailer egg shell, FES-farm egg shell, MES-Market egg shell, CES-cracked egg shell, OES-organic egg shell.EACH ERROR BAR REP MEAN ± STD DEV

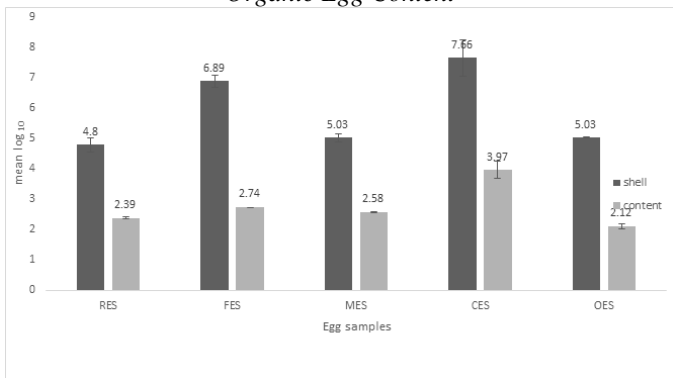
Figure 1: Total Bacterial Counts Of Conventional And Organic Egg Shell



LEGEND ;RES-Retailer egg content, FES-farm egg content, MES-Market egg content, CES-cracked egg content, OES-organic egg content

EACH ERROR BAR REP MEAN ± STD DEV

Figure 2: Total Bacterial Counts Of Conventional And Organic Egg Content



LEGEND;RE-Retailer egg ,FE-Farm egg ,ME-Market egg CE-Cracked egg ,OES-Organic egg Content- Yolk + Albumin

EACH ERROR BAR REP MEAN ± STD DE

Figure 3: Total Bacterial Counts Of Conventional And Organic Egg Shell And Contents

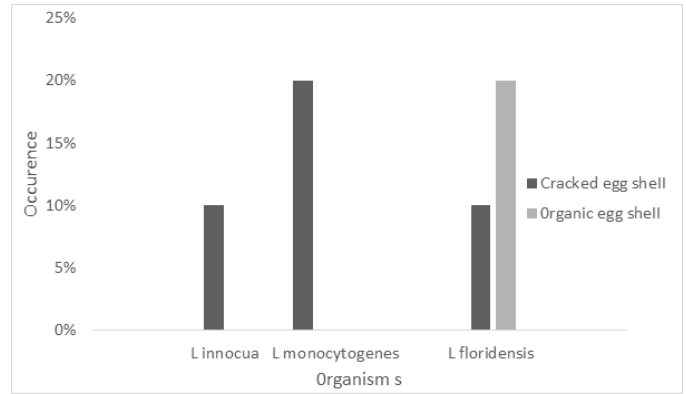
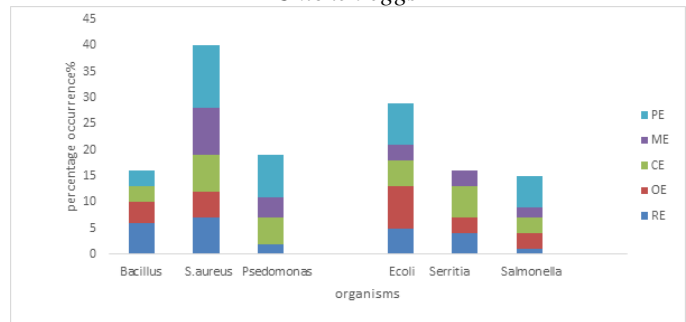
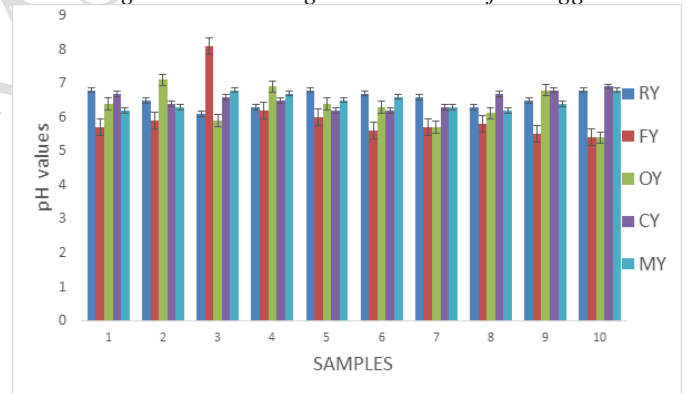


Figure 4: Percentage Occurrence of Listeria spp from Chicken eggs



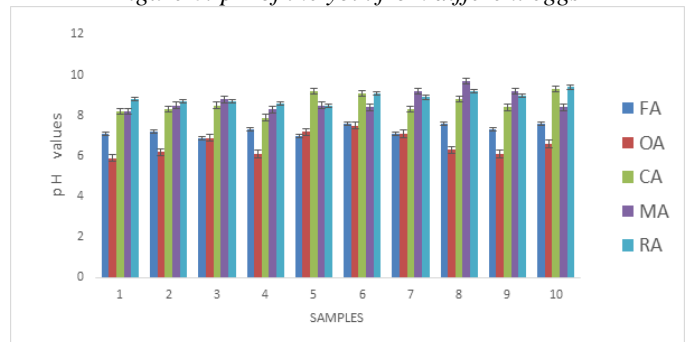
LEGEND;PE –Poultry Egg,ME- Market Egg, CE- Cracked Egg,OE-Organic Egg, RE –Retail Egg

Figure 5: Other Organisms isolated from eggs



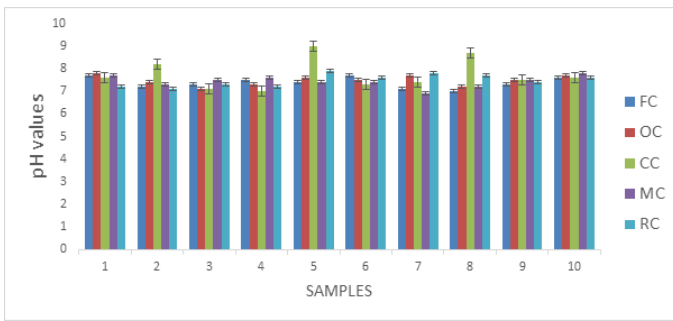
LEGEND: RY; Retailer egg yolk, FY; Farm egg yolk, OY, Organic egg yolk, CY; Cracked egg yolk, MY; Market egg yolk

Figure 6: pH of the yolk from different eggs



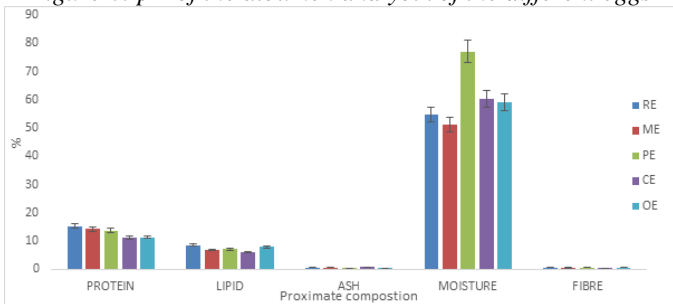
LEGEND: RA; Retailer egg albumen, FA; Farm egg albumen, OA; Organic egg albumen, CA; Cracked egg albumen, MA; Market egg albumen

Figure 7: pH of the albumen from the different eggs



LEGEND: RC; Retailer egg content, FC; Farm egg content, OC; Organic egg content, CC; cracked egg content, MC; market egg content.

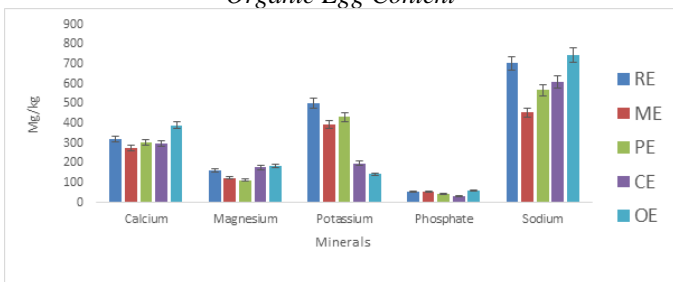
Figure 8: pH of the albumen and yolk of the different eggs



LEGEND; RE-Retailer egg, MA-Market egg, PE-Poultry egg, CE-Cracked egg, OE-Organic egg

EACH ERROR BAR REP MEAN \pm STD DEV

Figure 9: Proximate Composition Of Conventional And Organic Egg Content



Legend; RE: Retailer egg, ME: Market egg, PE; Poultry egg, CE: Cracked egg, OE: Organic egg

EACH ERROR BAR REP MEAN \pm STD DEV

Figure 10: Mineral contents of conventional and organic eggs

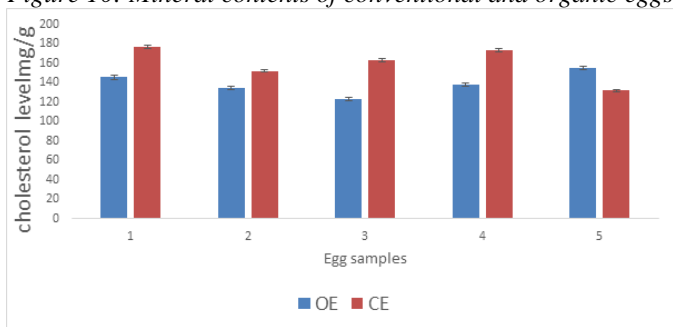


Figure 11: Cholesterol level in Conventional egg and organic eggs

Samples/Metals	zinc	iron	copper	lead	nickel	chromium	cadium
Conventional egg	118	48	-	-	0.03	-	0.04
Organic egg	75	67	0.01	-	-	-	0.01

Table 1: Heavy metal level in the egg samples

IV. DISCUSSION

BACTERIOLOGICAL ANALYSIS OF CONVENTIONAL AND ORGANIC EGG

From the analysis of aerobic count of conventional egg which comprise of retailed eggs, market eggs, farm eggs and cracked eggs were evaluated. Cracked egg shell had the highest count of log cfu/g 7.66, while the retail egg shell had the least count of 4.80. Organic egg shell had a log cfu/g of 5.03 which is lower than cracked egg shell. This quite similar to the studies of (Arathy *et al.*, 2009; Siriporn *et al.*, 2015). Egg shells are already infected when passing through the vent, contamination occurs within a short time after the egg is laid due to contact with dirty surfaces including faecal matter and this could increase the presence of organisms on the egg surface as a result lead to danger of egg surface and egg content infection (Siriporn *et al.*, 2015; Arathy *et al.*, 2009). Conventional egg content ranged from log cfu/ml 2.39 to 3.97, while organic egg content had a log 2.12 cfu/ml. In their study of table egg content in Ghana, Ansah *et al.* (2009) reported 7.26, 6.54, 7.18 and 6.9 for different cities. The finding of Abdul *et al.*, (2012) in Saudi Arabia was close in range to this study (3.02 log₁₀ cfu/mL).

Egg shells were significantly high in aerobic counts than the content (p<0.05). In a heavily contaminated area the egg gets contaminated through the shell and bacteria are then transferred to the inside (Arathy *et al.*, 2009). Bacteria grow faster in the shell membrane (Rickeet *et al.*, 2001). This supports the elevated bacteria presence in the shell membrane; indicating poor hygienic condition in the locations. Comparing the counts of both organic and conventional egg with the ICMSF bacteria standard of 6.0 log₁₀ cfu/ml showed egg content and shell exceeded the standards/limits. On the other hand retailed egg and organic egg shell did not exceed the stated limit. Obi and Igbokwe 2007 and Abdul *et al.*, 2012 reported a comparable result. When comparing total aerobic counts of egg types, conventional egg had slightly higher aerobic count than organic egg. This is not in concord with the study of Yasser (2015) who found no significant difference in both eggs. Other researchers however reported high counts for conventional egg than organic egg (El-Kohyl *et al.*, 2014; Hafez *et al.*, 2013).

Microorganisms that were been isolated from egg shell and content include: *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter spp*, *Klebsilla spp*, *Proteus spp*, *Bacillus spp*, *Citrobacterspp*, *Shigella spp*, *Serritia spp*, *Salmonellaspp*, *Listeria spp*, *Pseudomonas spp*. The major contaminants were *Staphylococcus sp*, *Streptococcus spp*, *Bacillus sp* and *Micrococeus spp*. Rajmani and Verma (2011) and Arathy *et al.* (2009) both stated similar genus from eggs. Eggs laid and stored in a dirty environment do have high level of bacterial contamination than egg laid in a clean environment (Bruce & Drysdale, 1994; Stepien, 2010). *Listeria monocytogenes*, *Listeria innocua* and *Lisreria floresedsies* were isolated from shells of conventional and organic egg but none was isolated from the content. A similar report was presented by Jones *et al.* (2011), different from study conducted by (Mahdavi *et al.*, 2012). *Salmonella spp* were isolated from conventional and organic egg shell and organic egg content. Jones *et al.* (2012)

also isolated *Salmonella spp* from egg shell more in number than egg content.

PH VALUES OF CONVENTIONAL AND ORGANIC EGGS

There was a gradual increase in egg pH as observed in this study. The first of chemical changes that can occur in egg is pH changes (Rhim *et al.* 2004). The initial pH of yolk is slightly acidic (5.9- 6.2) and rises slightly during storage to about 6.8 (Scott and Silversides, 2001). Egg white is initially in the region of 7.6 and rises to 8.9-9.4 after storage due to CO₂ loss through the shell (Sams, 2001). The natural ratio of egg white to egg yolk in an egg is 2:1 and therefore when mixed together (albumen and yolk) has a pH range 7.2 to 7.9, this corroborates to this present findings. The higher pH values of eggs from retail store might be due to long storage period before marketing. Storage is critical to egg pH as fresh eggs often have lower pH than old ones (Li-Chan *et al.* 1995). Waimaleongora-Ek *et al.* (2009) likewise demonstrated that holding time before use affect egg pH.

PROXIMATE AND CHOLESTEROL COMPOSITION OF EGG

Protein content was high in retailed egg can be attributed to their feed as suggested by Isahmus *et al.* (2015). Retailed egg and organic egg were relatively higher in lipid when compared with the study by Gordan 2002 and Emmual *et al.* (2011). Ash content represents the presence of appreciable amount of mineral in a given sample. Poultry farm egg had a high moisture content probably because they were freshly laid. Moisture content of eggs exceeded 15% making it suitable for microbial growth. The nutritional differences in egg can be as a result of difference in diet, age, sex and breed (Clum *et al.*, 1998), The differences in cholesterol content might be as a result of genetics, environment condition, rearing system, nutrition and disease (Fletcher, 2002). The conventional eggs had a significantly higher cholesterol when compared with Organic eggs (p<0.05). Ewonetu *et al.* (2015) also reported lower cholesterol from Organic chicken eggs relative to Conventional chicken eggs

MINERAL ANALYSIS OF CONVENTIONAL AND ORGANIC EGGS

The mineral contents of egg basically depend on the hen's feeding (Taber *et al.*, 2011). Calcium (391mg/kg), Magnesium (183mg/kg), Sodium 744 (mg/kg) and Phosphate (57mg.kg) were significantly high in organic egg when compared with conventional eggs. Calcium (298.7mg/kg), Magnesium (143 mg/kg), Sodium (583mg/kg), Phosphate (46mg/kg). This is in agreement with studies of Kiczorowska *et al* (2015) and Odoemena *et al.* (2006). Sahin *et al.*, 2003) observed a similar nutritional effect on egg originating from hen's kept in various production system. Taber *et al.* (2011) eggs from organic poultry were the richest in nutrient because their usual dietary ration is supplemented by whatever the pick as they roam freely

HEAVY METAL LEVEL IN ORGANIC AND CONVENTIONAL CHICKEN EGGS

Hen's egg mayt have high level of heavy metals that originates from food, feed and water which are influenced with the surrounding environment. The intake of heavy metal for consumption of 1 egg per day for Zinc is 1.50mg. Zinc level was found in both conventional egg (118mg/kg) and organic egg (78mg/kg) but higher in conventional egg.(p<0.05). This is similar to these studies (Azza *et al.*, 2011; Siddiqui *et al.*, 2011). Variation of zinc content in organic and conventional egg might come from dietary sources or contamination of the environment (Falchuck and Montorizi, 2001; Hashish *et al.*, 2012; Azzaet *al.*, 2012).

Iron content in organic egg (67mg/kg) was significantly higher than conventional egg (48mg/kg) (p<0.05) The intake of heavy metal for consumption of 1 egg per day for Iron is 2.99 mg. Other studies showed variation in eggs iron content (Siddiqui *et al.*, 2011; Hashish *et al.*, 2012; Abdul khaliq *et al.*, 2012). Copper was not detected in conventional egg but was detected in organic egg (0.11mg/kg). The intake of heavy metal for consumption of 1 egg per day for copper is 0.07mg. Scott *et al.*, 1982 showed copper concentration in white egg as 2.5 ppm and yet in another study concentration of copper in fresh egg was reported to be 1.7ppm (Hasetline *et al.*, 1978). Lead was not detected in organic and conventional egg, Contrary to our findings Hashish *et al.*, (2012); Abdulkhaliquet *al.*, (2012); Azzaet *al.*, (2011); Ulvozlu *et al.*, (2009) had Lead ranging from 0.31-12.1 mg/kg.

Nickel was not detected in organic egg but in conventional egg 0-0.03mg/kg. The intake of heavy metal for consumption of 1 egg per day for 0.02 mg/g., This is quite similar to the study by Ulvozlu *et al.*, 2011; Nisimankia *et al.*, 2009) in which Nickel was detected in table egg. The absence of nickel in organic eggs is similar to studies by Hashish *et al.* (2012) and Azzaet *al.* (2011). Chromium was not detected in organic and conventional egg. The intake of heavy metal for consumption of 1 egg per day for Chromium is 0.19 mg/kg. Similar to our findings chromium was not detected in organic and conventional egg as reported by Hashish *et al.*, (2012); Azzaet *al.* (2011); Abdulkhaliqu *et al.* (2012.) Contrary to this study, Siddiqui *et al.*, (2011); Ulvozlu *et al.* (2009), detected high Chromium in table egg. Cadmium level was slightly high in conventional egg than organic egg. The intake of heavy metal for consumption of 1 egg per day for Chromium 0.19ug. Limited data exist to show the exposure of humans to cadmium from egg consumption (Jeng and Yang 1995). However, hen's egg might contain elevated levels originating from food and wat.

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