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The Effect of *Moringa oleifera* and *Gmelina aborea* Seed Oil Coatings on Internal Qualities and Shelf Life of Table Eggs

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Abstract

The objective of this study was to examine the effects of coatings made from *Moringa oleifera* (MOC) and *Gmelina aborea* (GAC) seed oils on the internal quality parameters of eggs maintained at room temperature (28–30°C) for four weeks. The assessed egg quality metrics comprised yolk pH, albumen pH, Haugh unit, yolk index, weight loss, and total bacterial count of egg contents. The impact of applying a coating on the weight reduction became evident after a week of storage, with notable disparities ($p < 0.05$) seen between eggs without a coating (1.46%) and eggs with a coating (0.27-0.31%). For Haugh unit, eggs coated with MOC consistently maintained a grade of "AA" (HU > 72) for the entire storage period. On the other hand, eggs coated with GAC and uncoated eggs showed a decrease in grade quality, transitioning from "AA" to "A" and "B" correspondingly. The eggs covered with MOC showed the highest yolk index of 0.41, followed by the eggs coated with GAC with a yolk index of 0.34. Both values were substantially different ($p < 0.05$) from the yolk index of uncoated eggs, which was 0.18. The data on egg microbiological quality indicated that the total viable count (TVC) in control eggs was higher and exhibited a statistically significant difference ($p < 0.05$) compared to that in coated eggs. This study emphasises a sustainable method for maintaining the internal quality and extending the shelf life of eggs stored at room temperature.

Keywords: egg internal quality, oil coatings, storage, microbial quality

Introduction

Eggs area notably rich sources of nutrients and bioactive components with multifaceted applications in the food industry (Lesnierowski and Stangierski, 2018). *In addition,* egg protein is used as a reference standard for assessing other foods (Eddin *et al*., 2019). Egg is rich in lysine, sulphur-containing amino acids, vitamins A, D, E, K and B, and essential minerals (Watson, 2008). In Nigeria, egg production has been rapidly expanding, and a yearly growth of 4.1% has been projected till 2030 (Heise *et al.*, 2017). Despite its nutritional and economic importance, eggs are highly perishable products and need to be transported from the farm to sale outlets under refrigerated conditions. In developing countries, the cost of refrigerated storage is way beyond the financial capabilities of most poultry farmers and retailers. Consequently, eggs are transported and marketed under ambient temperatures, thereby compromising the quality of the product. Immediately after laying, eggs undergo deterioration in their interior quality as a result of moisture loss and carbon dioxide escaping through the pores of the eggshell (Nongtaodum et al., 2013). Furthermore, they are exposed to contamination by microorganisms leading to loss of quality and subsequently spoilage (Xu *et al.*, 2017).

To overcome these drawbacks, the application of coating materials on eggshell designed to prolong its shelf life and retain the internal attribute have been investigated. These coating materials include polysaccharides (Bhale *et al*., 2003; Xie *et al*., 2002), proteins (Cho *et al*., 2002; Xie *et al*., 2002), oils (Jirangrat *et al*., 2010; Wardy *et al*., 2011), artificial polymers (Meyer and Spencer, 1973) and starch (Kim *et al*., 2007).

The earliest scientific report on the application of mineral oil as coating material to retain the shelf life of eggs was by Rosser (1942) and Romanoff and Yushok (1948) and the technique is currently being employed for preserving commercial table eggs (Stadelman *et al*., 2017). Similarly, coatings from different plant-based oils applied on eggshells have also been documented (Sabrani and Payne, 1978; Obanu and Mpieri, 1984; Wardy *et al*., 2010; Ryu *et al*., 2011; Wardy *et al.*, 2011; Nongtaodum *et al.*, 2013). More recently, the use of green coating made of Brazilian green propolis and rice protein concentrate was employed to extend the shelf life of eggs (da Silva Pires *et al.*, 2021). Similarly, the incorporation of beeswax and basil essential oil into chitosan based emulsion has been shown to be effective in the preservation of eggs (Sun *et al.*, 2021).

Moringa oleifera is considered as one of the most valuable trees in the world and while the benefits of different portions of the tree have been extensively investigated, published data on the application of *Moringa* seed oil in the food industry is scanty (Edeogu *et al*., 2020). Notwithstanding, a few studies have documented the antimicrobial and antioxidant activities of *Moringa oleifera* seed oil (Broin *et al*., 2002). *Gmelina aborea* is also another tree that has attracted considerable interest in the scientific community owing to the potential benefits ascribed to various parts of the tree. Oil extracted from the seeds is reported to possess antioxidant properties which could be exploited for other beneficial purposes. (Basumatary *et al*., 2012).

To diversify and expand the functional properties of the oils from *Moringa* and *Gmelina*, their use as coating agents in extending the shelf life of egg was investigated. Consequently, this research reports, for the first time, the effect of coating with the oils of *Moringa oleifera* and *Gmenlina aborea* seeds on the interior quality of freshly-laid table eggs.

Materials and Methods

Preparation of coating materials

The oils from the seeds of *Moringa oleifera* and *Gmenlina aborea* were extracted using the soxhlet extraction technique; 150g of ground seeds was placed into a cellulose paper cone and extracted using petroleum ether (b.p 40–60 °C) in a Soxhlet extractor for 8 hours (AOCS, 1993).

Experimental eggs

One hundred and eight (108) freshly-laid eggs were used for the experiment. These eggs were purchased from a reputable commercial farm located at Asaba, Delta State, Nigeria. The eggs, on arrival, were initially inspected for flaws (cracks, fracture, and surface hygiene) and were kept in cold storage (4°C) prior to coating the following day. To prevent water condensation on egg surfaces, which could obstruct the coating process, eggs were made to acclimatise at room temperature (28 – 30°C) for two hours (Jirangrat *et al*., 2010).

Experimental design and egg treatment

Thirty-six (36) eggs were randomly assigned for each treatment; uncoated eggs (control), *Moringa oleifera*-coated (MOC) eggs and *Gmelina aborea*-coated (GAC) eggs. The coating ingredients were applied on the whole surface of each egg using a brush and allowed to dry overnight at room temperature to remove excess oil (Ezazi *et al*., 2021). The eggs were then placed in cardboard egg racks, with the small end facing down and stored at room temperature (28 ± 2ºC) for 28 days (Kim *et al.*, 2009). Nine (9) eggs per treatment were taken at one week (wk) interval for determination of weight loss, Haugh unit, yolk index, yolk pH and albumen pH. At the end of the storage period (28 days), the final batch of eggs from each treatment group was analysed for bacterial count in egg contents.

Determination of % weight loss in eggs

The % weight loss in uncoated, MOC and GAC eggs were calculated by deducting the final weight from the initial weight of egg, dividing by the initial weight and expressing it as a percentage (Jirangrat *et al*., 2010). The weight measurement was done at a specific time, and values recorded to within±0.001g employing a digital electronic balance (model Metler Toledo Pb153 GmbH, Greifensee, Switzerland).

Determination of Haugh unit and yolk index

Albumen and yolk heights were evaluated using a tripod micrometer (Mitutoyo, 0.01 mm, Japan), while albumin and yolk width were measured using a digital caliper (TMX PD - 150, China). Haugh unit was determined from the equation below (Lee *et al*., 1996).

$$HU = 100 \log [(H - 1.7 \times W^{0.37} + 7.57)]$$

Where, H is the thick albumin height measured in millimetres and W is the weight of the whole egg (g).Yolk index was determined as yolk height/yolk width.

Determination of albumen and yolk pH

Following the determination of Haugh unit and yolk index, the yolk and albumen were separated from each other. The contents of the albumen and yolk were mixed thoroughly and the pH measured using a pH meter (IQ150, IQ Scientific Instruments, San Diego, CA, USA). Before taking pH readings, the instrument was calibrated with buffer solutions of pH 4.0, 7.0 and 9.0.

Microbiological analysis of eggs

The internal content of the control (uncoated eggs), MOC and GAC eggs were analysed for bacterial pathogens (*Staphylococcus aureus*, *Proteus spp*, *E. coli*, *Streptococcus spp*) and the total viable bacterial counts after storage for 4 weeks at room temperature (28 ± 2ºC) using the method of Salihu *et al*. (2015). Before culturing of the content of eggs, ethanol solution (70%) was applied to the surface of each of the eggs as antiseptic. A sterile spatula was used to create an opening into the egg and the content of yolk and albumen were thoroughly homogenized in a stomacher (Bag mixer 400P). Nutrient agar and MacConkey agar media were utilized for isolation of bacterial pathogens. 1 mL of egg content was inoculated onto the above-mentioned culture media and after incubation, standard microbiological techniques including cellular morphology and staining, among others, were used for identification of isolated organisms. The colonies counted were expressed as log colony-forming units (CFU)/ml of egg contents.

Statistical analysis

The mean ± standard deviation based on nine determinations (eggs) for each treatment were used for the analysis. Results on % weight loss, Haugh unit, Yolk index, and albumen and yolk pH were analysed using the Statistical Analysis System (SAS) version 9.4 software and significant differences among uncoated, MOC and GAC eggs were evaluated with the least significant difference (LSD) test ($p \leq 0.05$).

Results and Discussion

Percentage weight loss

A precise method for directly measuring the quality and shelf life of egg during storage is the weight loss (da Silva Pires *et al.*, 2020). The data on % weight loss during storage of eggs (Table 1) revealed that after the first week, there was a significant difference ($p<0.05$) between control (1.46%) and coated eggs (0.27- 0.31%).

Table 1: Weight loss (%) in control (uncoated) and coated eggs with seed oil from *Gmelina aborea* (GAC) and *Moringa oleifera* (MOC) stored for 4 weeks at room temperature (28-30 ºC).

	Weight loss %			
Coating Material	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	$1.46±0.6^{aC}$	$3.12±1.2^{aB}$	$7.07±2.3^{aA}$	$8.17±1.8^{aA}$
GAC eggs	$0.27±0.9^{bB}$	$0.96±0.3^{bB}$	$1.45±1.7^{bB}$	$4.50±1.7^{bA}$
MOC eggs	$0.31±0.4^{bB}$	$0.83±0.2^{bB}$	$1.06±0.9^{bB}$	$3.80±0.8^{bA}$

The values represent the mean ±SD of nine replicates per treatment (n=9).
$^{A-C}$ Means with different uppercase superscript on the same row are significantly different p<0.05.
$^{a-c}$Means with different lowercase superscript on the same column are significantly different p<0.05.

Weight loss in eggs increased as storage advanced and at the end of week 4, weight loss in uncoated eggs was 8.17%, while weight losses in GAC and MOC eggs were 4.5 and 3.8% respectively. Several studies have shown the impact of coating on weight loss during egg storage. Based on a previous study, Pires *et al*. (2019) showed that eggs that were left uncoated lost weight (5.43%), while eggs coated with rice protein alone lost weight (4.23%), and eggs coated with rice protein and tea tree (4.10%), co-paba (3.90%), and thymol (4.08%) lost weight after 6 weeks. A study also found that eggs coated with soy protein isolate alone and montmorillonite at different percentages (0.2%, 0.5%, 0.8%) resulted in weight losses of 10.45, 7.13, 8.56, and 8.91%, respectively, while those uncoated had a loss of 14.19 percent after 6 weeks of storage. (Xu *et al*., 2017). Egg moisture loss during storage depends on environmental factors (temperature, relative humidity, air flow), and as time passes, these effects become more pronounced, especially during storage under ambient conditions (Feddern *et al.*, 2017). Our findings from this study showed that *Moringa* and *Gmelina* oils coatings were able to block pores of the eggs, thereby reducing the evaporation of moisture and gas.

***Haugh Unit* (HU)**

The Haugh unit is the most used index for assessing albumen quality, and it is based on albumen height and egg weight (Yücee, and Caner, 2015). According to the USDA (2020), the grading of eggs based on HU is as follows: HU ≥ 72 (AA); HU from 71 to 60 (A); HU from 59 to 31 (B) and class C, when HU score is< 31.

Table 2: Haugh unit in control (uncoated) and coated eggs with seed oil from *Gmelina aborea* (GAC) and *Moringa oleifera* (MOC) stored for 4 weeks at room temperature (28-30ºC).

	Haugh Unit				
Coating Material	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	78.1±4.68^{aA}	74.11±3.07^{aAB}	70.77±5.63^{aBC}	68.66±5.12^{abC}	60.22±5.52^{cD}
GAC eggs	78.1±4.68^{aA}	76.44±5.5.83^{aAB}	72.66±3.87^{aB}	69.22±5.65^{bC}	66.35±4.25^{bC}
MOC eggs	78.1±4.68^{aA}	76.58±4.83^{aAB}	76.22±4.22^{aAB}	75.00±2.87^{aAB}	72.77±1.06^{aB}

The values represent the mean ±SD of nine replicates per treatment (n=9).
$^{A-C}$ Means with different uppercase superscript on the same row are significantly different p<0.05.
$^{a-c}$ Means with different lowercase superscript on the same column are significantly different p<0.05.

A general decrease in HU was seen during egg storage (Table 2), with control eggs experiencing the largest reduction. In week 3, MOC eggs had the highest HU (75.0) and differed significantly (p<0.05) from uncoated eggs (68.6). MOC eggs maintained grade "AA" quality throughout the storage period, but GAC and uncoated eggs reduced from "AA" to "A" and "AA" to "B", respectively.

A previous study found that the grade of uncoated eggs stored at 25°C fell from "AA" to "B" and "C" after 1 and 3 weeks, respectively, whereas emulsion-coated eggs maintained "A"-grade quality after 4 weeks (Torrico *et al*., 2011). In addition, Ryu *et al*. (2011) found that the grade of eggs stored at 25ºC fell from "AA" to "B" after 3 weeks while eggs coated with different oils decreased from "AA" to "A" after 5 weeks of storage.

During storage of eggs, there is a reduction in HU due to the liquefaction of the dense albumen, the liquid albumen portion increasing while the dense portion decreases *(*Vlčková *et al*., 2019). The release of water by hydrolysis and the subsequent degradation of amino acids also leads to a loss of velocity, and fluidisation of the dense albumen (Moreng and Avens, 1990). One theory put forward for the reduction in HU during storage is the collapse of the ovomucin-lysozyme complex, the reduction in the carbohydrate content of ovomucin, and the increase in pH (Chen *et al*., 2005; Yuceer and Caner, 2014). The data on HU from this experiment demonstrates that coatings from *Moringa oleifera* and *Gmelina aborea* seed oils were able to maintain the albumen quality of eggs as their grade did not fall below ''A'' after 4 weeks of storage.

Yolk Index

Egg freshness can also be evaluated by the yolk index (YI), which is calculated by comparing yolk height to width (Stadelman *et al*., 2017). The YI of a freshly laid egg is about 0.45 and as the egg ages, the YI decreases; therefore, a higher YI is synonymous with better yolk quality (Yuceer and Caner, 2014). The reduction in YI during storage of eggs is caused by a weakening of the vitelline membrane, allowing water to diffuse out of the albumen as it loses its structure causing yolk liquefaction. (Obanu and Mpieri, 1984). YI changes during storage (Table 3) revealed that no significant differences (P > 0.05) existed between the stored eggs irrespective of treatment. After 3 weeks of storage, YI of uncoated eggs declined by 46.94%, while that of GACs and MOCs decreased by 28.57% and 14.28% respectively. At the end of storage, MOC eggs had the highest YI (0.41), followed by GAC (0.34), and uncoated eggs (0.18).The results in this study are also in agreement with findings from another research (Vandyousefi and Bhargava, 2017), which established that coating eggs with cinnamon oil - chitosan emulsions at varying ratios resulted in lower reductions (19.56-43.47%) in YI compared to uncoated eggs (47.82%). Following similar trends, YI in uncoated and washed eggs lowered to below 0.3 after 12 and 17 days of storage at 30°C, respectively, but YI in mineral-coated eggs remained above 0.3 after 30 days of storage (Park *et al*., 2003). Almeida *et al*. (2016) also showed that eggs (clean or uncleaned) coated with milk protein had higher YI after 42 days of storage compared to uncoated eggs. Differences in YI between studies can be attributed to coating material, initial egg characteristic (size and quality) and storage conditions (Mueller, 1958).

Table 3: Yolk index in control (uncoated) and coated eggs with seed oil from *Gmelina aborea*(GAC) and *Moringa oleifera* (MOC) stored for 4 weeks at room temperature (28-30ºC)

	Yolk Index				
Coating Material	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	$0.49±0.04^{aA}$	$0.41±0.08^{aB}$	$0.38±0.09^{bCD}$	$0.26±0.09^{bCD}$	$0.18±0.07^{bD}$
GAC eggs	$0.49±0.04^{aA}$	$0.43±0.05^{aAB}$	$0.35±0.07^{abB}$	$0.35±0.07^{abB}$	$0.34±0.06^{aB}$
MOC eggs	$0.49±0.04^{aA}$	$^{0}.43±0.08^{aA}$	$0.42±0.09^{aA}$	$0.42±0.09^{aA}$	$0.41±0.07^{aA}$

The values represent the mean ±SD of nine replicates per treatment (n=9).
$^{A-C}$ Means with different uppercase superscript on the same row are significantly different p<0.05.
$^{a-c}$ Means with different lowercase superscript on the same column are significantly different p<0.05.

Albumen and Yolk pH

A freshly laid egg has an albumen pH that ranges from 7.6 to 8.5 (Waimaleongora-Ek *et al*., 2009). However during storage, the pH may increase to around 9.7, due to the breakdown of carbonic acid into water and carbon-dioxide (which escapes through the pores of the egg shell), causing the thinning of the thick albumen and subsequent increase in albumin pH (Figueiredo *et al*., 2013; Kemps *et al*., 2007). In both uncoated and coated eggs, the albumen pH increased during storage (Fig 1A), and after 4 weeks of storage, the albumen pH of uncoated, MOC, and GAC eggs had risen from 7.86 to 8.94 and 8.44, respectively. Furthermore, significant differences (p<0.05) between albumen pH of coated and uncoated eggs were observed after the storage period. In another study, the albumen pH in uncoated eggs increased from 8.71 to 9.42 after 5 weeks of storage, while eggs coated with mineral oil of different viscosities had values ranging from 8.64-9.37 (Waimaleongora-Ek *et al*., 2009).

For yolk pH, the value is normally around 6.0, with slight variation (6.4 to 6.9) occurring even after prolonged storage (Oliveira and Oliveira, 2013). In spite of not having a drastic change in yolk pH after 4 weeks (Fig.1B), eggs coated with Moringa and Gmelina oils had average values of 6.71 and 6.72 respectively, and were significantly lower ($p<0.05$) than those of uncoated eggs (6.76). Biladeau and Keener (2009) showed that yolk pH in uncoated eggs increased from 6.0-6.27 after 12 weeks storage, while pH of eggs coated with whey protein isolate, paraffin wax, soy protein isolate and mineral oil varied from 5.97-6.11. Another study found that after 5 weeks of storage, the pH in the yolk of uncoated and coated eggs (whey protein isolate, sodium montmorillonite nanoparticles, and sodium metabisulfite) varied from 6.0- 6.66 and 6.09- 6.57, respectively (de Araújo Soares *et al*., 2021). In another study, values of yolk pH in uncoated and coated eggs (whey protein isolate, sodium montmorillonite nanoparticles and sodium metabisulfite) ranged from 6.0- 6.66 and 6.09 - 6.57 respectively, following 5 weeks of storage.

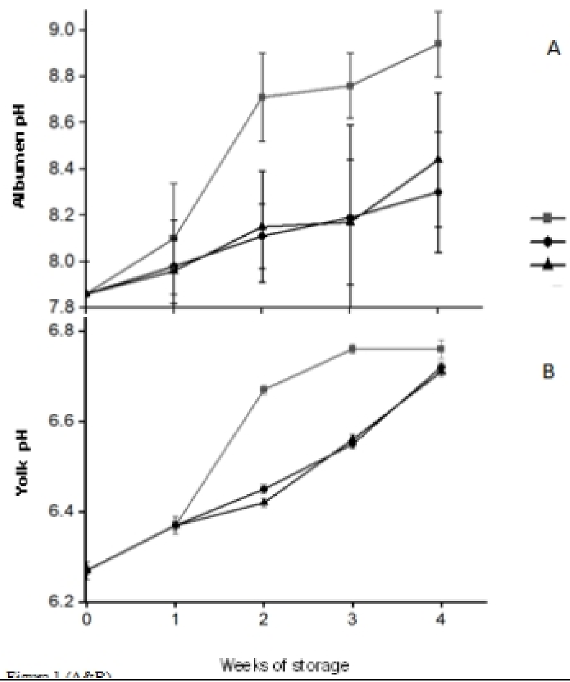

Figure 1: Changes in albumen and yolk pH in control (non-coated) eggs (■), *Gmelina aborea* coated (GAC) eggs (▲) and *Moringa olifera* coated (MOC) eggs (▲) respectively during 4 weeks of storage at room temperature (28 – 30°C). The values represent the mean ±SD of nine independent measurements for each treatment.

Microbiological Analysis

At the time of laying, most eggs have minimal or no contamination, and the subsequent deterioration and spoilage occasioned by microorganisms is dependent on the environmental conditions before and during storage (Gentry and Quarles, 1972). It is estimated that the eggshell contains up to 17,000 pores that serve as route for pathogens and other microorganisms to gain entrance into the internal contents of eggs (Jin *et al.*, 2013). The data on microbial analysis of uncoated and coated eggs (Figure 2) showed that the average total viable count (TVC) of egg contents were less than the maximum accepted 6.0 log10CFU/mL, recommended by the

International Commission on the Microbiological Specification for Food (Roberts and Tompkin, 1996). Furthermore, the data showed that contents of individual microbes (*Staphylococcus aureus, Proteus* spp*., E. coli*, *Streptococcus* spp.) and TVC in uncoated eggs were higher and significantly different (p<0.05) from MOC and GAC eggs. Several studies have demonstrated the ability of coating materials to slow down microbial invasion into the egg contents (Morsy *et al*., 2015; Xu *et al.*, 2017; Yüceer and Caner, 2020). There is evidence to suggest that *Moringa oleifera* seed oil extract inhibits the growth of bacteria and fungi, due to the presence of phytochemicals in the seeds that exert both antimicrobial and antioxidant effects (Dinesha *et al*., 2018). Similarly, the seed of Gmelina arborea is reported to be rich in phytochemicals (tannins, alkaloids, flavonoids, saponins and phenols) with preservative properties (Ogutuga *et al*., 2020). Comparing the impact of both coating materials showed that Moringa coating was more potent in preserving egg internal qualities than Gmelina and this may be due to the higher phenolic content of the former as reported in the literature (Jahan *et al.*, 2018; Shoeb *et al*., 2014).

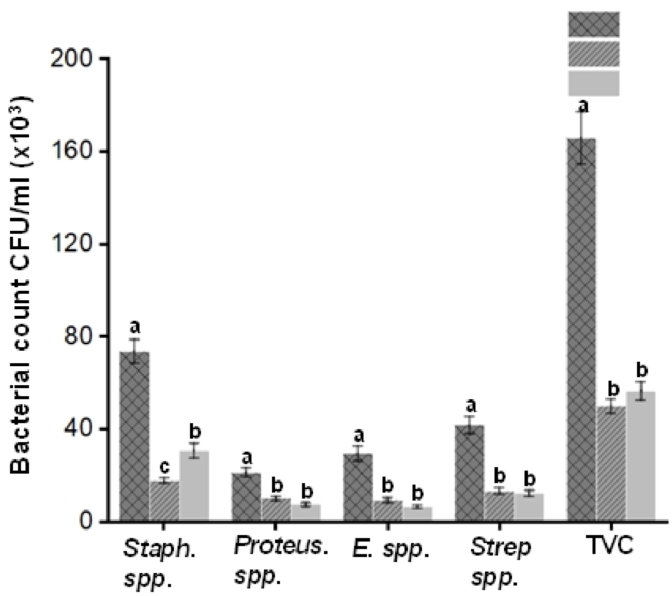

Figure 2: Bacterial counts (CFU/ml) in control (non-coated) eggs ( ), *Gmelina aborea* coated (GAC) eggs ( ) and *Moringa olifera* coated (MOC) eggs ( ) after 4 weeks of storage at room temperature (28 – 30°C). The values of each bar represent the mean ±SD of nine independent measurements and values with different letters are significantly different (p ≤ 0.05).

Key: CFU/ml = colony forming unit per ml.*Staph spp*: *Staphylococcus spp*: *E. spp*: *Escherichia spp*: *Strep spp*: *Streptococcus spp*: TVC: Total viable count.

Conclusion

The application of oil coating from seeds of *Moringa oleifera* and *Gmelina aborea*was was able to maintain the internal qualities of eggs for up to 4 weeks compared to uncoated eggs. Eggs coated with *Moringa oleifera* seed oil performed better by having the lowest % weight loss and highest scores for Haugh unit (AA) and Yolk index (0.41). In addition, the ability of the coating materials to seal off egg pores was reflected in the total viable counts (TVC) which was lower in coated compared to uncoated eggs. The application of the oils from these underutilised plant sources represents a promising green technology for preservation of table eggs, and future studies can evaluate their effectiveness in conjunction with other coating materials.

Furthermore, the use of oil coating materials from *Moringa* and *Gmelina* seeds in developing countries is recommended to reduce the burden cost of refrigeration and sustain the internal quality of eggs during transportation and storage.

Declaration

Conflict of interest: The authors have no conflict of interest.

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