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Evaluation of chitosan treatment for lowering yolk cholesterol in chicken eggs#

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Abstract

Eggs are widely consumed as a nutritious food source, providing proteins, vitamins and minerals, yet their high yolk cholesterol content has raised concerns for human health. The present study explored the use of chitosan, a natural bioactive polymer, to reduce yolk cholesterol in chicken eggs while ensuring product acceptability. Fresh eggs were immersed in chitosan solutions at concentrations of 0.5% and 1% for different durations of 1, 2 and 3 hours to allow uniform coating across the shell surface. Sensory assessment was carried out to determine the most suitable formulation, and the treatment with 0.5% chitosan for 1 hour (T1) achieved the highest acceptability score, comparable to untreated eggs. This treatment was then subjected to cholesterol estimation using a colorimetric assay with a UV–VIS spectrophotometer. The analysis revealed a significant reduction (p < 0.05) in yolk cholesterol content of treated eggs compared with the control group. The findings indicate that chitosan treatment can effectively lower cholesterol in eggs through a simple surface-application process, offering a natural strategy to produce healthier egg products without compromising consumer preference.

Keywords: *Eggs, chitosan, cholesterol, reduction*

Eggs are recognised worldwide as a nutrient-dense food, rich in high-quality protein, vitamins and minerals essential for human health. India, being the second-largest producer of eggs globally. In recent years, egg production and consumption in the country have shown consistent growth, driven by factors such as rising incomes, urbanisation and greater awareness of the health benefits of eggs. As of 2023-2024, the per capita availability of eggs in India reached 103 eggs per year, reflecting their growing role in everyday diets (BAHS, 2023).

Eggs are highly nutritious but a major source of dietary cholesterol, raising concerns about cardiovascular health. Cholesterol, essential for hormone synthesis and cell membrane structure, is carried in the blood by LDL ("bad") and HDL ("good") lipoproteins. Excessive LDL intake can increase the risk of coronary heart disease, prompting interest in low-cholesterol food alternatives. Egg yolk is the primary source of cholesterol, with levels varying by species chicken eggs (7.65 mg/g), duck eggs (10.36 mg/g), and quail eggs (16.05 mg/g) (Aziz et al., 2012). Although chicken eggs have the lowest cholesterol, their widespread consumption makes it important to explore strategies to reduce cholesterol

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content without affecting nutritional quality, especially through post-laying interventions, which remain largely underexplored.

Chitosan, a biopolymer derived from crustacean shell chitin, has attracted attention for its functional properties, including antimicrobial activity, film formation and lipid-binding ability. Studies show that chitosan can reduce cholesterol absorption in the gastrointestinal tract. While its cholesterol-lowering effects are established in humans and animals, few studies have explored its direct application on eggs. Given the increasing demand for low-cholesterol foods, this study investigates the potential of post-laying chitosan treatment to reduce cholesterol in chicken eggs, offering a strategy to develop healthier poultry products and innovative functional foods that meet health-conscious consumer preferences.

Materials and methods

The study was conducted at the Meat Technology Unit, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. Fresh chicken eggs were obtained from the All India Coordinated Research Project (AICRP) on Poultry Breeding, Mannuthy, and carefully examined for cleanliness and shell integrity, with only those meeting quality standards selected for the study.

Preparation of chitosan solutions and egg treatments

Chitosan solutions were prepared at two concentrations, 0.5% and 1%, by dissolving 0.5 g and 1 g of chitosan powder, respectively, in 100 mL of 1% acetic acid. The mixtures were continuously stirred using a magnetic stirrer until completely dissolved and then filtered to remove any insoluble particles, yielding clear solutions.

Freshly collected chicken eggs were thoroughly cleaned using distilled water to remove surface dirt and impurities and then gently patted dry with clean tissue paper to eliminate excess moisture. Once dried, each egg was labelled according to its designated group. For the treatment groups, labels indicated the concentration of chitosan solution (0.5% or 1%) and the intended soaking duration (1, 2 or 3 hours). Eggs were then immersed in 0.5% and 1% chitosan solutions for the specified durations, with timers used to ensure precise soaking periods. Control eggs, which did not receive any chitosan treatment, were labelled as “control” and handled in the same manner, including washing, drying and arranging in clean, designated trays. After treatment, all eggs were rinsed with distilled water, air-dried at room temperature and stored in clean, labelled trays under controlled conditions until further sensory and cholesterol analyses.

Sensory evaluation

Sensory evaluation was conducted to assess the organoleptic acceptability of the treated eggs. Eggs from each group were scrambled without oil, salt or seasoning to maintain consistency. Samples were coded with randomized three-digit numbers to prevent bias and evaluated by a trained panel of 5–10 members under controlled conditions. Panelists rated appearance, colour, taste, off-odour, undesirable taste, aftertaste and overall acceptability using an eight-point hedonic scale (Ranganna, 2000). Scores were recorded for all attributes and the treatment with the highest acceptability score was selected for cholesterol analysis. Cholesterol content was determined using a colorimetric method with a UV–VIS spectrophotometer.

Preparation of yolk filtrate for cholesterol estimation

The egg yolk was carefully separated from the albumin, transferred to a clean glass container, and homogenized thoroughly. A 0.5 g portion of the homogenized yolk was weighed and mixed with 7.5 mL of chloroform–methanol solution (2:1, v/v), followed by manual shaking twelve times to ensure complete mixing. Distilled water (2.5 mL) was then added, and the mixture was shaken again twelve times. The sample was centrifuged at 2500 rpm for 10 minutes, and the upper aqueous methanol layer was removed. The remaining chloroform layer was evaporated on a water bath at 90°C. After evaporation, 4 mL of glacial acetic acid was added, and the mixture was centrifuged at 5000 rpm for 10 minutes. A 0.1 mL aliquot of the resulting solution was mixed with 6.9 mL of ferric chloride–acetic acid reagent, incubated for 15 minutes to allow protein flocculation, and centrifuged at 3000 rpm for 10 minutes. The clear supernatant was then analysed spectrophotometrically at 500 nm using a double-beam UV–VIS spectrophotometer following the colorimetric method of Rudel and Morris (1973) and the Zak method (Herdyastuti & Daniar 2019). Reagents were added according to the specifications provided in the experimental design table 1.

Table 1. Reagent composition for cholesterol estimation

	BLANK	STANDARD	TEST
Supernatant	-	-	5mL
Standard (0.1ml std+6.9ml ferric chloride-acetic acid reagent.	-	5mL	-
Ferric chloride-acetic acid Reagent	5mL	-	-
Sulfuric acid	3mL	3mL	3mL
Mix well and kept for 30 min, measure OD at 500 nm			

Table 2. Median sensory scores of control and chitosan-treated egg samples for various organoleptic attributes

Sensory attributes	C	T_1	T_2	T_3	T_4	T_5	T_6	p value
Appearance and colour	7(1.00) ^{a}	7(0.25) ^{ab}	7(0.37) ^{ab}	7(1.00) ^{b}	7(1.00) ^{b}	7(1.00) ^{ab}	7(1.00) ^{b}	0.000**
Egg taste	7(0.87) ^{a}	7(0.69) ^{a}	7(1.00) ^{ab}	6(1.00) ^{bc}	6.5(1.00) ^{abc}	6(1.37) ^{cd}	5.25(2.00) ^{d}	0.000**
Off odour	7(1.00) ^{a}	7(0.50) ^{a}	7(0.18) ^{ab}	7(0.87) ^{b}	7(0.87) ^{ab}	7(1.00) ^{b}	7(1.00) ^{b}	0.000**
Undesirable taste	7(1.00) ^{a}	7(1.00) ^{ab}	7(0.50) ^{ab}	6.5(1.00) ^{bc}	7(1.25) ^{bc}	6(1.00) ^{cd}	5.5(1.93) ^{d}	0.000**
Undesirable aftertaste	7(0.87) ^{a}	7(0.88) ^{a}	7(1.00) ^{ab}	6(1.00) ^{bcd}	7(1.00) ^{ab}	6(1.00) ^{cd}	5(2.37) ^{d}	0.000**
Overall acceptability	7(0.50) ^{a}	7(0.94) ^{ab}	7(0.50) ^{abc}	6.5(1.00) ^{cde}	7(1.00) ^{bcd}	6(1.37) ^{de}	5.5(1.37) ^{e}	0.000**

Values are expressed in median

*Median bearing different letters as superscript differ significantly at 0.05 level *p < 0.05; **p<0.001; ns – non- significant; C – Control, T1 – 0.5% for 1 hour, T2 - 0.5% for 2 hour, T 3 - 0.5% for 3 hour, T4 - 1% chitosan for 1 hour, T5 - 1% chitosan for 2 hour and T6 - 1% chitosan for 3 hour ; Scoring range – 1 - 8 Hedonic scale*

Results and discussion

Sensory evaluation

A sensory evaluation was conducted to assess the organoleptic acceptability of eggs treated with different concentrations of chitosan. Parameters such as appearance and colour, egg taste, off-odour, undesirable taste, undesirable aftertaste and overall acceptability were assessed across different treatments. The data were analysed using a non-parametric statistical test, and the median values are presented in table 2.

The sensory evaluation showed that all chitosan-treated eggs retained relatively high organoleptic scores, with T1 (0.5% chitosan for 1 hour) and T2 performing best across attributes such as appearance, colour, taste, off-odour, aftertaste and overall acceptability. T1 consistently achieved the highest overall acceptability score (median 7.00, IQR 0.94), comparable to the control group, and maintained favourable ratings for taste and minimal off-odour. In contrast, higher chitosan concentrations and longer soaking times, such as T5 (1% for 2 hours) and T6 (1% for 3 hours), scored lower for taste and aftertaste, indicating that excessive chitosan may adversely affect sensory properties. These results are consistent with Herdyastuti & Daniar (2019), who reported that moderate chitosan concentrations and optimal soaking durations improve cholesterol binding while preserving sensory qualities. Overall, treating eggs with 0.5% chitosan for 1 hour offers an effective compromise between reducing cholesterol and maintaining consumer acceptability, making it a suitable strategy for producing healthier eggs.

Cholesterol estimation in control and chitosan-coated eggs

Based on the results of the sensory evaluation, the chitosan treatment with the highest acceptability score (T_1) was selected for cholesterol analysis. This treatment, along with the control (untreated) egg, was evaluated to determine the effect of chitosan on yolk cholesterol content. The analysis was conducted using the colorimetric method with a UV-VIS spectrophotometer. The mean cholesterol values obtained for both control and treated eggs are presented in the table 3.

Table 3. Mean cholesterol (mg/g) of control and chitosan treated whole egg samples

Samples	Cholesterol (mg/g)	*P* value
Control	15.91±1.24^{a}	0.039*
Treatment	9.86±0.26^{b}	

*Means bearing different letters as superscript differ significantly at 0.05 level *p < 0.05;*

ns-non-significant 0.05 level; Treatment- 0.5%chitosan for 1 hour; The values are expressed as their Mean± Standard error

The cholesterol content of egg yolk was significantly affected by chitosan treatment. In the control group, yolk cholesterol was measured at 15.91 ± 1.24 mg/g, whereas eggs treated with chitosan showed a markedly reduced level of 9.86 ± 0.26 mg/g, representing an approximate 38% reduction. Statistical analysis confirmed that this decrease was significant (p < 0.05).

The observed reduction in yolk cholesterol can be attributed to the biochemical properties of chitosan, a naturally occurring cationic polysaccharide capable of binding negatively charged molecules, including dietary lipids, bile acids and cholesterol. This binding likely facilitates the sequestration and removal of cholesterol from the egg yolk. These findings are agreement with previous studies; Herdyastuti & Daniar (2019) they demonstrated that immersing quail eggs in chitin solution effectively lowered cholesterol levels, with smaller chitin particles showing enhanced efficacy due to increased binding surface area. Similarly, Sunarno et al. (2023) reported significant cholesterol reduction in duck eggs using nanochitosan as a feed additive, highlighting that dietary inclusion of chitosan can effectively decrease yolk cholesterol.

Conclusion

The present study demonstrates that post-laying application of chitosan on chicken eggs effectively reduces yolk cholesterol while maintaining desirable sensory qualities. Treatment with 0.5% chitosan for 1 hour was identified as the optimal condition, achieving approximately 38% reduction in cholesterol without compromising appearance, taste or overall acceptability. These findings highlight chitosan as a practical, natural and non-invasive agent for producing healthier eggs, offering a promising approach to meet the growing consumer demand for low-cholesterol functional foods. Further research may explore its potential application on a commercial scale and its long-term effects on egg quality and nutritional value.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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