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Development of edible coating and its preservation effect on peeled and deveined brown shrimp (*Metapenaeus affinis*) during refrigerated storage

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Abstract
As consumer demand for additive-free seafood rises, so does the interest in natural preservatives for shrimp. This study investigated the effectiveness of edible coatings made from chitosan, gelatin, alginic acid, and their combinations with clove essential oil in maintaining the quality and extending the shelf life of brown shrimp (*Metapenaeus affinis*) during refrigerated storage (4±1 °C) for 15 days. All evaluated characteristics, including physical, biochemical, microbiological, and sensory aspects, showed significant improvement ($p \leq 0.05$) in coated shrimp compared to the control group. The coated samples exhibited superior quality throughout the storage period. Notably, coated shrimp had lower pH values than uncoated shrimp. Additionally, the treatment with 1% chitosan and clove essential oil solution demonstrated the most significant reduction ($p \leq 0.05$) in spoilage rate. Similar trends were observed in the biochemical analysis, with treated samples showing better results than the control. Sensory evaluations also revealed higher scores for coated shrimp samples. Overall, the study suggests that a chitosan coating combined with clove oil is the most effective treatment for preserving peeled and deveined shrimp, maintaining their quality attributes at an acceptable level during refrigerated storage for 15 days.

Keywords: Brown shrimp, chitosan, gelatin, alginic acid, clove essential oil

Introduction
Consumer demand for healthier food options, including fish and other seafood, has grown due to concerns about food quality. Shrimp, a vital commercial seafood worldwide, is particularly important in India. However, brown shrimp (*Metapenaeus affinis*) is highly perishable compared to other fresh products, necessitating frequent marketing as frozen or processed items. New preservation methods, such as active packaging with films or coatings, are needed to reduce microbial growth and control moisture, gas, and aroma migration (Hassan *et al*., 2018) [8]. As defined, edible coatings (EC) are thin layers of materials applied to food surfaces that are safe for consumption and considered part of the whole food product.
Edible coatings are small layers made of natural polymers that are applied to food surfaces using various methods including spraying, immersing and brushing (Dhall, 2013) [5], or using electrical deposition (Poverenov *et al*., 2014) [19]. There are many coating materials available but some edible biopolymer like Chitosan, Gelatin, Alginic acid with the mixture of essential oils like clove are effective. All of these act as antibacterial substance with approved GRAS (General recognition as safe) nature.
This study explores the use of natural biopolymers and essential oils for shrimp preservation. Chitosan, a polysaccharide derived from deacetylating chitin, the main component of crustacean shells, is one such biopolymer. Gelatin, another option, is obtained by partially degrading collagen found in animal bones, skins, and cartilages. Alginic acid, a naturally occurring hydrophilic polysaccharide, is extracted from brown seaweed. Finally, clove essential oil, extracted from clove seeds (Syzygium aromaticum), possesses antifungal, antioxidant, antibacterial, and insecticidal properties. Clove oil not only enhances flavor but also acts as an antimicrobial agent in food preservation. Studies by Hosseini *et al*. (2009) [9] have shown that clove oil, rich in eugenol, effectively inhibits the growth of pathogenic microorganisms.

This study aims to develop edible coatings using biocompatible materials combined with essential oils. These coatings target the extension of shelf life for peeled and deveined brown shrimp, while effectively maintaining their quality attributes at an acceptable level during refrigerated storage.

Materials and Methods
Materials
The study was undertaken in different phases as described below. An interval of 5 days was observed between different treatments for evaluation of quality changes during the study at refrigerated condition.

Preparation of 1% Chitosan Coating Solution
10 gm. of Chitosan powder mixed with 800 ml of 10% glacial acetic acid and let sit overnight. Then, filter the solutions with the help of Whatman filter paper no. 3 and make up 1000 ml solution with add distilled water. Then, add 0.75 ml/gm (Chitosan) glycerol into the solution as a plasticizer and stir for 10 minutes.

Preparation of 1% Gelatin Coating Solution
10gm of Gelatin powder dissolved with 1000 ml distilled water by stirring on a magnetic stirrer at 35 °C for 15 minutes. Then, cool down the solution at 7 °C for 15 minutes and allow the solution to swell. Again, warm the solution at 55 °C for 30 minutes in a hot air oven.

Preparation of 1% Alginic acid coating solution
10gm of Alginic acid powder dissolved with 1000 ml distilled water by stirring on a magnetic stirrer at 35 °C for 15 minutes. Then, cool down the solution at 7 °C for 15 minutes and allow the solution to swell. Again, warm the solution at 55 °C for 30 minutes in a hot air oven.

Preparation of 1% Clove Essential Oil Solution
2 ml of tween 80 was added in 10 ml of clove oil as an emulsifier for complete dispersion of oil into the above biopolymers coating solutions.

Methods
The fresh Brown shrimp (*Metapenaeus affinis*) was brought from the Veraval fish landing centre. Then, the shrimp was dressed (peeled and deveined) and washed for further process. After that, dressed shrimp were divided into 4 groups, as T_1 (C), T_2, T_3 and T_4. T_1 (C) considered a control treatment considering no treatment was given.

In T_2 treatment, the shrimp were treated with 1% Chitosan with 1% Clove essential oil coating solution. For that 1% Chitosan coating solution was mixed with 1% clove essential oil solution. Both solutions were mixed with the help of a centrifuge for 5 minutes at 9000 rpm. Then after, the shrimp were dipped for 10 mins in a solution. Then allow standing for 2 minutes to drain. After that, put it in a hot air oven for 15 minutes at 35 °C for proper coating. Treated shrimp were packed in air packaging of an LDPE pouch and stored at refrigerated (4±1 °C) storage. Similarly, In T_3 treatment, shrimp were treated with 1% Gelatin with 1% Clove essential oil coating solution, for that first of all prepare the emulsion. For that, 1% Gelatin coating solution was mixed with 1% clove essential oil solution. Whereas, In T_4 treatment, shrimp were treated with 1% Alginic acid with 1% Clove essential oil coating solution, for that first of all prepare the emulsion. For that, 1% Alginic acid coating solution was mixed with 1% clove essential oil solution. All samples were packed in air packaging of an LDPE pouch and stored at refrigerated (4±1 °C) storage for further storage study.

Determination of Colour
The colour of shrimp samples was quantitatively assessed using a colour reader (CR-10, Konica Minolta Sensing, Inc., Japan). Measurements were conducted individually for each replication of the treatment. It shows L, a- and b+.

Where,
[L*]: Lightness
[a-]: Redness
[b+]: Yellowness

Texture Profile Analysis (TPA)
Texture profile analysis (TPA) is a technique used to evaluate the textural properties of food during consumption. It considers the interplay of mechanical characteristics (like firmness and elasticity), geometrical aspects (shape and size), fat and moisture content, and how these elements are perceived throughout chewing. TPA involves compressing a food sample with a flat probe, mimicking the multiple bites and chews humans experience. For shrimp, textural properties are also influenced by the internal structure, storage conditions, and even the specific species (Huriaux *et al*., 1999; Ladrat *et al*., 2003; Kim *et al*., 2005) [10, 13, 12].
During TPA, the force required for the initial bite (First compression) and any subsequent chews (Second compression) is measured in relation to how much the shrimp sample deforms. For this study, we'll specifically focus on hardness, which is determined by the peak positive force measured during the first compression.

Determination of pH
Measuring pH using pH meter. First take 5gm sample of dry fish and crush it in mortar pestle. Then add 50 ml of distilled water and mix with crushed sample. Calibrate pH meter with 4, 10 and 7 pH buffer solution. After that wash with distilled water and measure the pH of sample.

Determination of Moisture
Moisture content is one of the most commonly measured properties of food material. Seafood being a highly perishable commodity contains more than 65% moisture in general. Shrimp as it directly related to spoilage and affected the quality during processing (AOAC, 2006) [3].

$$\text{Moisture (\%)} = \frac{\text{Weight before drying (W1)} - \text{Weight after drying (W2)}}{\text{Weight of before drying (W1)}} \times 100$$

Determination of Ash
The principle of ash is thus to burn the organic matter and to determine which remain (AOAC 2006) [3].
Weigh an empty crucible (W1) and record the weight. Weigh exactly 5 grams of shrimp sample (W) and transfer it to the crucible. Heat the sample on a hot plate until smoking stops and the shrimp becomes completely charred. Caution: Never leave a hot plate unattended. Carefully place the crucible containing the charred sample (W) inside a muffle furnace. Heat the furnace to 550 °C and maintain that temperature for 3 to 4 hours. Once the furnace cools down

completely, retrieve the crucible. The ash remaining should be clean and white in appearance (W2).

$$\text{Ash (\%)} = \frac{(W2 - W1)}{W} \times 100$$

Sensory Analysis: Nine trained panelists evaluated the sensory quality of the shrimp samples using a 9-point hedonic scale (Peryam & Pilgrim, 1957) [18]. They assessed color, appearance, odor, texture, and overall acceptability following standard procedures.

Results and Discussion

Colour

Lightness [L*]

Table 1: Changes in Lightness [L*] value of Brown shrimp during refrigerated storage

Storage Period in Days	T_1(C)	T_2	T_3	T_4
0	53.03 ± 1.68^{b}	61.50 ± 3.40^{a}	54.00 ± 1.01^{ab}	56.30 ± 0.52^{ab}
5	53.03 ± 0.20^{b}	58.30 ± 1.57^{a}	53.86 ± 0.60^{b}	52.03 ± 0.55^{b}
10	53.40 ± 1.83^{b}	57.13 ± 1.89^{a}	49.93 ± 1.61^{bc}	44.10 ± 1.17^{c}
15	48.80 ± 1.56^{a}	48.80 ± 1.90^{a}	47.50 ± 1.96^{a}	43.96 ± 0.50^{b}

*Values are in mean ± SD, n=3. ^{a,b,c} Value with different superscripts in a column for each parameter differ significantly ($p<0.05$).

The lightness of the sample was observed to be differ significantly ($p<0.05$) from control and treated samples. In control and treated samples decreases with the time. Lightness value in control sample were found in the ranges of 53.03 to 48.80, which exhibit not much deviation however, it decreases with respect to storage period. Shrimp coated with chitosan and clove essential oil (T_2) found significantly decreasing trend in lightness value from 61.5 to 48.8. In the same way treatment combination of T_3 and T_4 observed decreasing in lightness from 54 to 47.5 and 56.3 to 43.96. It is may be due to application of the coatings on shrimp has make a slight darkish layer. Similar results for chitosan coating also observed in previous studies (Kaixi Zheng and Bo Li, 2023) [23]. It is also similar for chitosan & gelatin in Redfish (Huynh Thi Ai Van, 2019) [22].

Redness [a-]

Table 2: Changes in Redness [a-] value of Brown shrimp during refrigerated storage

Storage Period in Days	T_1(C)	T_2	T_3	T_4
0	-6.93 ± 0.66^{b}	-6.70 ± 0.26^{b}	-5.80 ± 1.47^{c}	-8.23 ± 0.28^{a}
5	-7.56 ± 1.95^{a}	-7.03 ± 0.90^{a}	-6.30 ± 0.69^{b}	-6.06 ± 0.65^{b}
10	-7.63 ± 0.83^{a}	-7.66 ± 1.46^{a}	-7.43 ± 0.77^{a}	-5.50 ± 0.50^{b}
15	-9.46 ± 0.05^{a}	-8.63 ± 0.90^{a}	-9.26 ± 0.32^{a}	-3.96 ± 0.75^{c}

*Values are in mean ± SD, n=3. ^{a,b,c} Value with different superscripts in a column for each parameter differ significantly ($p<0.05$).

Redness was more or less found similar in control sample (T_1), T_2 and T_3 treated samples however it observed to be significantly differ with T_4 treatment. In T_1 it increases from -6.93 to -9.46. This similar pattern also follows in T_2 and T_3 ranges from -6.7 to -8.63 and -5.8 to -9.26. But in T_4 it decreases from -8.23 to -3.96. It indicates that in first three treatments the spoilage takes place as redness increase but in first three treatments the control sample has higher redness means higher spoilage than T_2 and T_3. On the other hand, T_4 has initially higher redness and then it decreases as it is due to Alginic acid powder has red colour that increase reddening effect at initially after coating treatments.

Yellowness [b+]

Table 3: Changes in Yellowness [b+] value of Brown shrimp during refrigerated storage

Storage Period in Days	T_1(C)	T_2	T_3	T_4
0	8.86 ± 0.25^{b}	11.13 ± 1.35^{a}	11.7 ± 2.25^{a}	12.46 ± 1.81^{a}
5	8.76 ± 0.75^{b}	9.90 ± 0.95^{ab}	8.50 ± 1.57^{b}	11.060 ± 1.50^{a}
10	7.66 ± 0.35^{b}	9.26 ± 1.25^{a}	7.30 ± 0.81^{b}	9.23 ± 0.40^{a}
15	7.16 ± 0.90^{b}	8.13 ± 0.55^{a}	6.26 ± 0.37^{c}	8.06 ± 0.23^{a}

*Values are in mean ± SD, n=3. ^{a,b,c} Value with different superscripts in a column for each parameter differ significantly ($p<0.05$).

The yellowness of the shrimp samples differed significantly ($p<0.05$) between the control and treated groups. Interestingly, all samples exhibited a decreasing yellowness trend throughout the storage period. The control group showed a yellowness range of 8.86 to 7.16. Initially, the treated samples (T_2, T_3, and T_4) had higher yellowness values likely due to the added biopolymers. However, their yellowness also decreased during storage, ranging from 11.13 to 8.13 (T_2), 11.7 to 6.26 (T_3), and 12.46 to 8.06 (T_4), respectively. These findings suggest that regardless of the treatment, storage time plays a role in reducing the yellowness of shrimp, possibly due to natural discoloration or drying processes.

Hardness (Peak positive force)

Table 4: Changes in Hardness (gm/f) value of Brown shrimp during refrigerated storage

Storage Period in Days	T_1(C)	T_2	T_3	T_4
0	1829.65±1330.66^{a}	1610.04±1561.94^{b}	2053.45±1531.50^{ab}	2603.99±811.10^{bc}
5	2332.10±281.54^{a}	4163.91±1431.33^{c}	3743.80±1603.69^{b}	3075.40±2435.64^{ab}
10	3123.65±1341.58^{a}	4723.13±397.71^{b}	4445.34±2269.86^{ab}	3107.42±3522.13^{a}
15	7730.81±2441.33^{c}	6686.23±2226.88^{bc}	5107.49±2735.94^{b}	3921.48±945.64^{a}

*Values are in mean ± SD, n=3. ^{a,b,c} Value with different superscripts in a column for each parameter differ significantly (p<0.05).

Hardness significantly differed (p<0.05) between control and treated shrimp samples (T_2, T_3, and T_4) throughout the storage period. Interestingly, hardness increased for all groups over time. The control group exhibited a hardness range of 1829.65 to 7730.81, signifying a progressive increase. Similarly, treated samples showed increasing hardness, with ranges of 1610.04 to 6686.23 (T_2), 2053.45 to 5107.49 (T_3), and 2603.99 to 3921.48 (T_4). These findings align with Bindu *et al*. (2013) [4], who suggested that increased hardness might be due to protein denaturation leading to water loss and aggregation. However, despite the hardness increase, the coated shrimp generally remained more elastic and less rigid compared to the control. This indicates that the coating improved the overall rheological properties of the shrimp, potentially enhancing texture. Similar positive effects of chitosan coatings on fish texture have been reported by Mohan *et al*. (2012) [16].

pH

Table 5: Changes in pH value of Brown shrimp during refrigerated storage

Storage Period in Days	T_1(C)	T_2	T_3	T_4
0	7.03±0.15^{b}	6.23±0.05^{a}	6.96±0.05^{a}	7.20±0.10^{b}
5	7.10±0.10^{b}	6.26±0.05^{a}	6.93±0.05^{a}	6.86±0.05^{a}
10	7.70±0.10^{b}	6.20±0.10^{a}	6.60±0.10^{a}	6.63±0.05^{a}
15	7.70±0.10^{b}	6.06±0.05^{a}	6.60±0.10^{a}	6.53±0.11^{a}

*Values are in mean ± SD, n=3. ^{a,b,c} Value with different superscripts in a column for each parameter differ significantly (p<0.05).

The pH of shrimp samples differed significantly (p<0.05) between the control and treated groups throughout storage. Notably, the control group exhibited a continuous increase in pH, ranging from 7.03 to 7.70. This rise is likely associated with the production of ammonia and amines, breakdown products of proteins by microbial enzymes, as reported by Triki *et al*. (2018) [21] for other refrigerated meats (Kamkar *et al*., 2021; Zhang *et al*., 2020) [11, 24]. Conversely, the treated samples (T_2, T_3, and T_4) displayed a decreasing pH trend during storage. For example, the chitosan-clove essential oil treatment (T_2) showed a decrease from 6.23 to 6.02. Similar decreasing trends were observed in T_3 (6.96 to 6.60) and T_4 (7.20 to 6.53). These findings align with Kaixi Zheng and Bo Li (2023) [23], suggesting chitosan coatings may help maintain lower pH levels.

Moisture

Table 6: Changes in Moisture (%) value of Brown shrimp during refrigerated storage

Storage Period in Days	T_1(C)	T_2	T_3	T_4
0	79.00±0.01^{c}	78.07±0.07^{b}	81.17±0.07^{a}	88.06±0.02^{a}
5	79.03±0.02^{b}	77.73±0.05^{c}	78.61±0.02^{b}	80.50±0.08^{a}
10	77.45±0.01^{b}	77.06±0.07^{b}	78.55±0.09^{a}	78.86±0.09^{a}
15	76.63±0.03^{b}	75.35±0.16^{c}	78.98±0.05^{a}	78.53±0.05^{a}

*Values are in mean ± SD, n=3. ^{a,b,c} Value with different superscripts in a column for each parameter differ significantly (p<0.05).

Moisture content, a crucial factor influencing microbial growth, decreased in all shrimp samples (Control and treated) throughout storage. The control group showed a slight decrease, ranging from 79% to 76%. Treated samples exhibited a similar trend: T_2 (78% to 75%), T_3 (81.17% to 78.98%), and T_4 (88% to 78%). Notably, T_4, containing alginic acid known for its high water holding capacity, displayed a statistically significant difference (p<0.05) compared to other treatments. The observed decrease in moisture content is likely due to a combination of factors. Protein binding water within the shrimp may undergo denaturation, or pressure on connective tissues might alter internal forces, leading to water loss. Farajzadesh *et al*. (2016) suggested similar mechanisms for chitosan-gelatin coatings, where the coating acts as a barrier to water loss and reduces exudates.

Ash

Table 7: Changes in Ash (%) value of Brown shrimp during refrigerated storage

Storage Period in Days	T_1(C)	T_2	T_3	T_4
0	0.76±0.02^{a}	0.73±0.02^{a}	0.72±0.02^{a}	0.89±0.01^{a}
5	0.87±0.05^{a}	0.86±0.04^{a}	0.84±0.01^{a}	1.08±0.05^{a}
10	1.07±0.01^{a}	1.02±0.02^{a}	1.09±0.01^{a}	1.26±0.04^{a}
15	1.20±0.01^{a}	1.34±0.01^{a}	1.23±0.02^{a}	1.54±0.01^{a}

*Values are in mean ± SD, n=3. ^{a,b,c} Value with different superscripts in a column for each parameter differ significantly (p<0.05).

There is not any significance difference in ash content of control and treated sample. In general, there was increment in the ash content in relevance to the storage period. In control sample it ranges from 0.76 to 1.2%, 0.73 to 1.3% for T_2, 0.72 to 1.2% for T_3 and 0.89 to 1.5% for T_4 respectively. Shrimp coated with alginic acid has higher ash content then other. Increment is understandable that decrement in moisture content inversely increase the fat content and that increase the ash content.

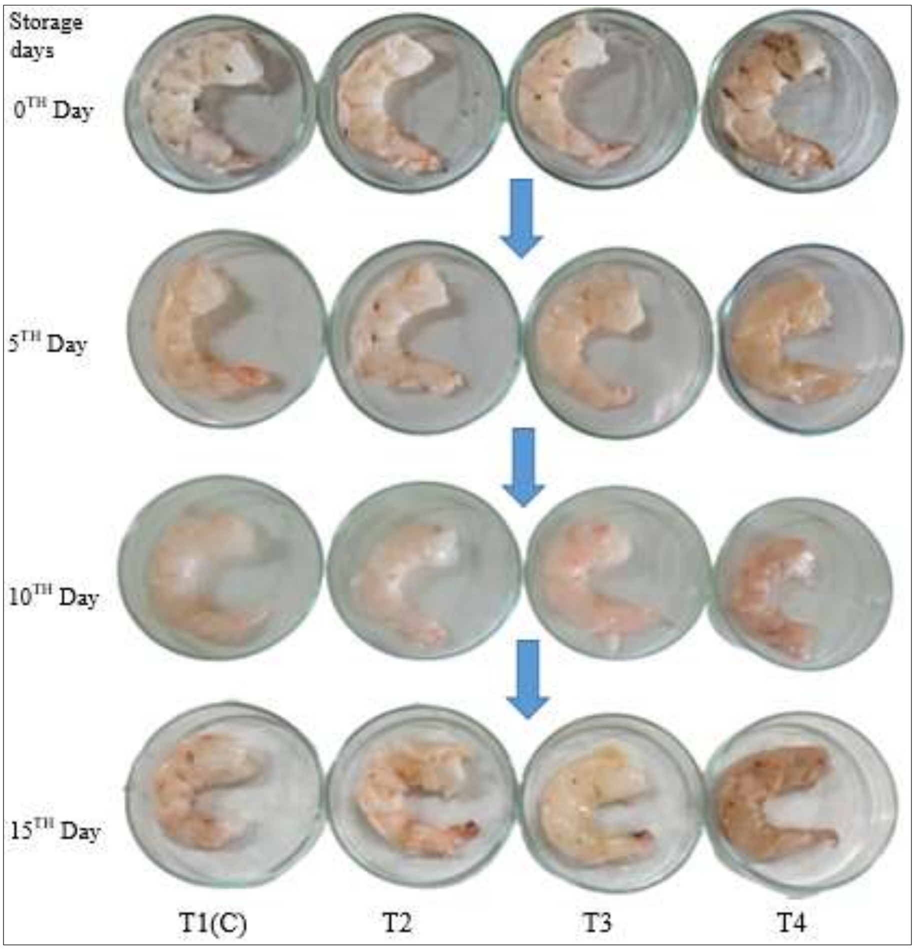

Fig 1: Changes in colour of sample during refrigerated storage

Sensory Analysis
Colour

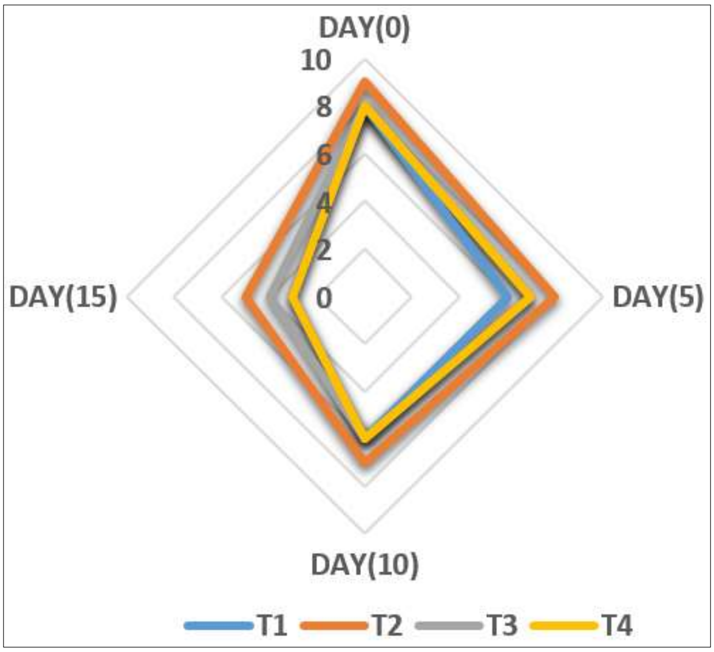

Fig 2: Changes in colour score for Brown shrimp during refrigerated storage

As shown in Figure 2, color changes were evident in the shrimp samples during storage. Sensory evaluation results revealed significantly lower scores (Indicating poorer color perception) for the control group compared to the treated samples (Chitosan and other treatments) throughout the storage period. Notably, the control group's color only remained acceptable for the first 10 days. These findings suggest that the coatings, particularly the chitosan coating, effectively helped maintain better shrimp color over time.

Appearance

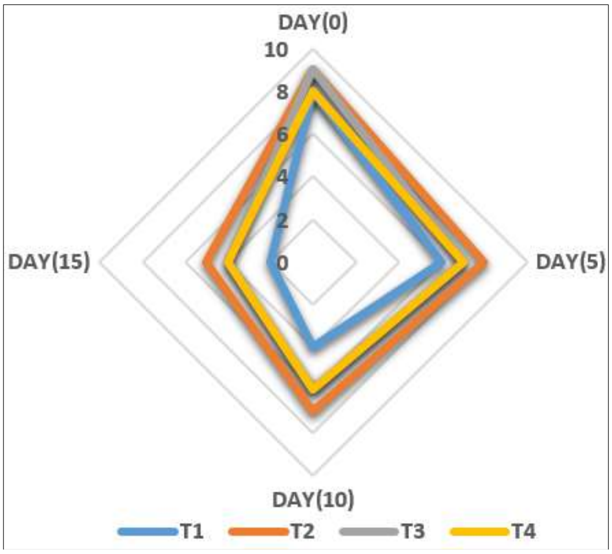

Fig 3: Changes in Appearance score for Brown shrimp during refrigerated storage

Both the coating treatments and storage duration significantly impacted the shrimp's appearance. While all samples initially possessed acceptable sensory characteristics, these scores declined as storage time increased.

Odour

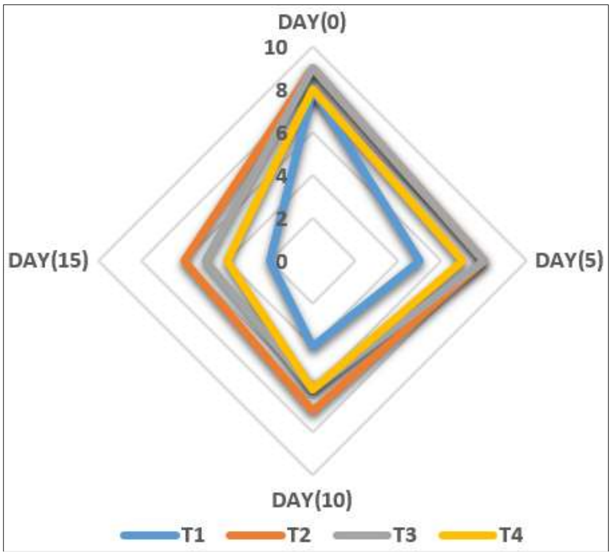

Fig 4: Changes in Odour score for Brown shrimp during refrigerated storage

Odor analysis (Figure 4) revealed significant effects ($p < 0.05$) of both the treatments and storage time. Initially, all shrimp samples, control and treated, had high odor scores. However, odor scores decreased for all samples as storage time increased. After 15 days, the control group exhibited a very poor odor compared to all treated samples. Interestingly, the chitosan coating with clove oil treatment maintained the highest odor score at the 15^{th} day, suggesting its effectiveness in preserving odor quality.

Texture

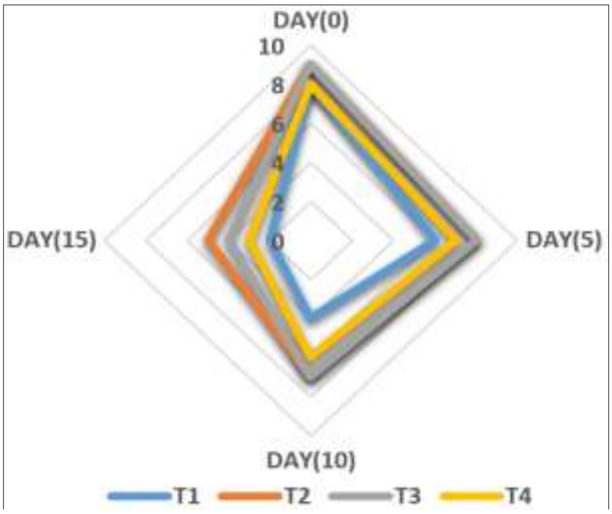

Fig 5: Changes in Texture score for Brown shrimp during refrigerated storage

Texture analysis (Figure 5) revealed significant effects ($p \leq 0.05$) of the treatments and storage time. Interestingly, the clove oil treatment initially had the highest mean texture value compared to the control and other treated samples. However, over storage, the texture scores for all samples, including the clove oil treatment, decreased consistently. By the end of storage, the control group had the lowest texture score, while the treatment combining chitosan and clove oil maintained a relatively high texture value. These findings suggest that while clove oil might offer some initial textural benefits, the combination of chitosan and clove oil might be more effective in preserving shrimp texture over extended storage.

Overall acceptability

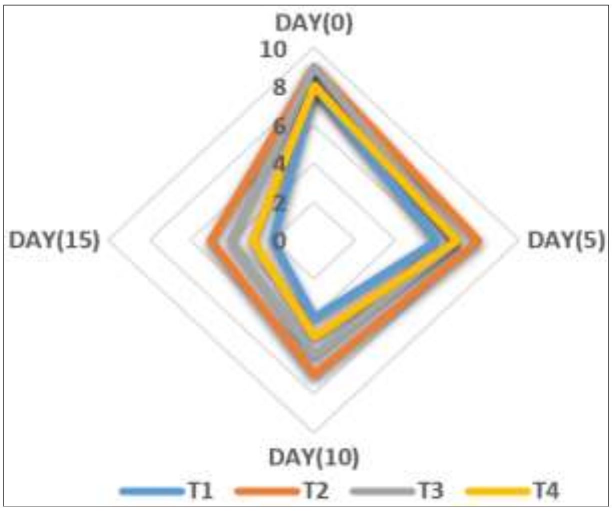

Fig 6: Changes in Overall acceptability score for Brown shrimp during refrigerated storage

Overall acceptability, a key indicator of consumer preference and safety for consumption (Ojagh *et al*., 2010) [17], declined rapidly in the control group as storage time increased (Figure 6). Conversely, all treatment groups exhibited improved acceptability scores, with the chitosan-coated shrimp maintaining the highest average score throughout storage. This aligns with findings by Li *et al*. (2012) [14] who observed a significant decrease in consumer acceptance of uncoated large yellow croaker after eight days, likely due to increased bacterial growth. Our results suggest that chitosan-based coatings, particularly those containing clove oil, effectively preserve sensory quality by potentially preventing oxidation. Chitosan's established antioxidant and chelating properties (Tayel, 2016) [20] might contribute to this preservation by inhibiting the activity of oxidation enzymes.

Conclusion

This study explored the application of edible coatings containing essential oils to preserve peeled and deveined brown shrimp (*Metapenaeus affinis*) during refrigerated storage. The results demonstrated that treatments combining chitosan, gelatin, alginic acid, and clove essential oil effectively extended the shelf life of the shrimp compared to the control group. Notably, shrimp coated with 1% chitosan blended with clove essential oil exhibited the best quality characteristics throughout refrigerated storage. These findings suggest the potential of such coatings for shrimp producers to deliver fresh, high-quality shrimp to consumers with an extended shelf life under refrigeration.

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