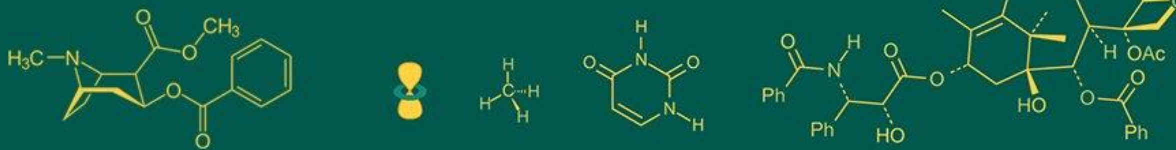

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Effect of prior plasma-activated water soaking and Carboxymethyl cellulose incorporated with clove essential oil on the shelf life of peeled shrimp (*Metapenaeus affinis*) during refrigerated storage

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Abstract
In the present research work, shrimp (*Metapenaeus affinis*) were treated with plasma-activated water (PAW) for 120 second, PAW for 120 second with clove essential oil (CEO) (1%), PAW for 120 second with carboxymethyl cellulose (CMC) (1%), and PAW for 120 second with clove essential oil (CEO) (1%) and carboxymethyl cellulose (CMC) (1%) respectively and stored under refrigerated condition (4±1 °C) for a total period of 25 days. Notable changes in chemical (pH, trimethylamine nitrogen (TMA-N), total volatile base nitrogen (TVB-N) and peroxide value (PV)), Microbiological (Total plate count and Total psychotropic counts), color (L*, a*, b*), and sensory parameters were recorded for the quality assessment. Based on our results, the pattern of increase in the Total plate count and Total psychrotrophic count in different groups were reported as the following order: PAW+CEO+CMC<PAW+CEO < PAW+CMC < control. Also, PAW for 120 second treatment along with the clove oil (1%) and carboxymethyl cellulose (1%) coating in peeled shrimp decreased the upward trend of pH, TVB-N, TMA-N and PV compared to the other treated group during storage at 4 °C. Besides, the textural and sensory properties of shrimp were significantly more acceptable compared to other treatments. Thus, PAW conjunction with CEO and CMC treatment was an effective hurdle technique for extending the shelf-life of shrimp upto 20 days in terms of biochemical, microbiological, color and sensory quality characteristics. This approach was practical and industrially applicable.

Keywords: Carboxymethyl cellulose, clove essential oil, coating, *Metapenaeus affinis*, plasma-activated water, refrigerated storage, shelf life

Introduction
Today, one of the most important problems of our world, which is in rapid change in social, economic, and cultural terms, is nutrition with adequate, balanced, and healthy foods. Nutritionists and health experts recommend to consume seafood more frequently since it is one of the primary sources of food, nutrition, livelihood, and money (Rahmaniya *et al.* 2018) [42]. Among seafood, edible crustaceans are prawn, shrimp, crab, and lobster which constitutes one of the important nutritious food sources for human being. Among them, one of the most demanded and traded seafood is shrimp (Tsironi *et al.* 2009) [54]. It contains a variety of nutrients, such as amino acids, peptides, calcium, vitamins, and other nutritionally useful substances (Zhang *et al.* 2015) [59]. In addition, is another benefit of shrimp meat that it has a relatively lower mercury content compared to other shellfish, including cephalopods (Bragagnolo *et al.* 2001) [11]. Shrimps also represent a healthy dietary choice because they are high in cholesterol and low in fat and calories (Ravichandran *et al.* 2009) [43]. They were rich in n-3 HUFA, known as omega-3 highly unsaturated fatty acids that have been associated with the prevention of cardiovascular disease. Furthermore, in the past three decades, health benefits from the consumption of omega-3-rich seafood have emerged as a significant and promising development in human nutrition and the prevention of diseases.
However, shrimp is a highly perishable product compared to other types of seafood, and once it is harvested, its quality rapidly degrades. For instance, Bak *et al.* (1999) [6] reported that shrimp have a lipid content of 1.2%, with highly unsaturated phospholipids constituting the majority of these lipids. Most of the lipids are found within the carapace shell.

Furthermore, shrimp exhibits susceptibility to both microbiological and chemical degradation throughout its storage period, primarily attributable to its high content of free amino acids and other soluble non-protein nitrogenous constituents (Mastromatteo *et al.* 2010) [34]. Consequently, the application of preservative treatments is an important aspect of increasing the shelf life of food and maintaining its quality and safety.

In response to a growing consumer preference for safer, more nutritious, and fresher food options, substantial research has been conducted in recent years on non-thermal food processing technologies. These methods include ultrasound, pulsed electric fields, cold plasma, and high hydrostatic pressure (Augusto 2020) [5]. Among them, the Plasma technology known as the fourth state of matter is a partially ionised gas comprised ions, electrons, ultraviolet photons, and neutral uncharged particles (such as molecules, atoms, and radicals) (Thirumdas *et al.* 2018) [52]. Atmospheric pressure plasma jet (APPJ) and dielectric barrier discharges (DBD) represent two common apparatuses employed in this technology, both of which are not widely used to treat large food surfaces and volumes due to limitations in their scalability. Additionally, the highly irregular surface structure of seafood products serves as several hiding places for microorganisms, increasing their resistance to cold plasma treatment. Plasma-activated water (PAW) has been developed as an alternative to overcome this limitation. Water that has undergone a plasma treatment is known as plasma-activated water (PAW). It is more eco-friendly than certain conventional chemical disinfectants, including those that contain chlorine compounds which produce byproducts that could be hazardous to the environment (Wu & Rioux 2010) [57]. Depending on storage conditions, PAW's bactericidal efficacy may last for a few days, providing the opportunity to scale up applications (Shen *et al.* 2016) [47]. However, one of the main disadvantages associated with cold plasma (CP) discharge or plasma-activated water (PAW) in fresh foods, with a particular focus on seafood products, is its tendency to accelerate the oxidation of lipids and proteins. This effect ultimately leads to a decrease in the overall acceptability of the final product (Olatunde *et al.* 2020) [40]. The use of antioxidants has been effective in preventing oxidation to overcoming this issue.

Since the beginning of history, essential oils have been used as flavour components in food. Essential oils (EOs) are secondary metabolites found in plants that have been demonstrated to have antioxidant and antibacterial properties (Burt 2004) [12]. For instance, Gómez-Estaca *et al.* (2010) [22] investigated the antimicrobial properties of various essential oils, they discovered that clove oil exhibited the most pronounced inhibitory effect against a variety of microorganisms. Ancient medicinal plant clove (*Syzygium aromaticum*), which has natural antimicrobial and antioxidant properties, contains a variety of active substances (eugenol, eugenyl acetate) which are useful for food packaging (Li *et al.* 2006) [31]. The active compound in clove essential oil (CEO), by interaction with the hydroxyl group, effectively inhibits the formation of amylase and protease enzymes in Gram-positive bacteria, along with enzyme activity in Gram-negative bacteria (Burt 2004) [12]. Essential oils have severe disadvantages, such as high volatility and low water solubility, despite their potential in the food industry. Essential oils have been combined with a variety of biopolymer-based coatings and films to overcome these limitations (Moradi *et al.* 2011) [36]. In the recent food processing industry, biodegradable, environmentally friendly edible films and coatings have recently gained a lot of attention. A variety of edible coatings are available, among which carboxymethyl cellulose (CMC), a linear anionic polysaccharide, water-soluble, and non-toxic derivative of cellulose, has several uses in the food and pharmaceutical industries (Tongdeesoontorn *et al.* 2011) [53]. CMC is produced as a cellulose derivative when cellulose reacts with sodium hydroxide and chloroacetic acid (Choulitoudi *et al.* 2017) [15]. Considering the aforementioned characteristics, CMC is an excellent substance as an alternative in the food industry. The quality and safety of shrimp were extensively investigated in relation to PAW treatment and PAW treatment with CEO-CMC coating. The findings of this study have great commercial value since they effectively enhance the safety profiles and comprehensive quality characteristics of refrigerated shrimp products.

Materials and Methods

Chemicals

Chemicals and reagents were obtained from Fisher Scientific and SRL Pvt. Ltd, India. Analytical or Guaranteed reagent grades were used for analysis, following their specifications and storage precautions.

Preparation of plasma activated water

Plasma-activated water (PAW) preparation was carried out using a dielectric barrier discharge (DBD) pencil plasma jet (PPJ) device, specifically designed and developed by the Atmospheric Plasma Division at the Institute for Plasma Research (IPR) located at Gandhinagar, Gujarat, India. The experimental setup of the Dielectric Barrier Discharge Plasma Jet (DBD-PPJ) involves a coaxial assembly of cylindrical electrodes, encompassing both a grounded electrode and a power electrode. These electrodes are spatially isolated by a tube made up of quartz serving as a dielectric barrier. The working gas for PAW production was air. A high-voltage, high-frequency alternating current (AC) power supply that has a 10 kVA power rating, adjustable voltage within the range of 0 to 10 kV, variable current spanning 0 to 999 mA, and adjustable frequency range of 0 to 40 kHz used to power the DBD-PPJ setup. A 600 ml glass beaker was kept which contains 200 ml of distilled water. The vertical distance between the surface of the water and the lower extremity of the DBD-PPJ setup is kept fixed at 10 mm. To facilitate controlled airflow from the top into the DBD-PPJ setup, an air pump is employed. The airflow rate is consistently regulated at 10 L/min, a value constantly monitored through the utilization of an air rotameter. A magnetic stirrer is used to stir the water, thereby enhancing the solubility of plasma-derived reactive species in the medium.

The pH, EC (Electrical Conductivity), ORP (Oxidation-Reduction Potential), and TDS (Total Dissolved Solids) of PAW were assessed using precise measuring instruments. These physicochemical parameters were monitored using a pH meter (Hanna Instruments), EC meter (Contech CC-01), ORP meter (Contech COR-01), and TDS meter (HM digital), all with least counts of 0.1, 0.1 μS/cm, 0.1 mV, and 1ppm, respectively. Reactive oxygen and nitrogen species (such as nitrate, nitrite, and hydrogen peroxide) in PAW

were preliminary measured using nitrite and hydrogen peroxide test strips, along with a colorimeter nitrate detection kit. For the determination of dissolved ozone concentration in PAW indigo colorimetric method is used (APHA 2005) [4]. Physicochemical properties and RONS present in PAW are shown in Table 1.

Table 1: Physicochemical properties and RONS present in PAW

Sr. No.	Property	Concentration
1	pH	3.70±0.12^a
2	ORP (Oxidation-reduction potential) (mV)	730.00±0.14^b
3	EC (Electrical conductivity) (µS/cm)	27.50±0.09^b
4	NO_3^- (g/L)	1.20±0.01^b
5	NO_2^- (mg/L)	3.40±0.06^b
6	H_2O_2 (mg/L)	22.20±0.11^b
7	Dissolve O_3 (mg/L)	1.80±0.02^b

*Values are the mean of triplicate measurements ± standard deviation (SD); values with different lowercase letters in the same column indicated a significant difference at $p<0.05$.

Preparation of coating solution

Commercial carboxymethyl cellulose CMC (High Viscosity 1100-1900 CPS) with an average molecular weight of 90 kDa and Clove essential oil (Extrapure, 80%) was obtained from the Sisco Research Laboratories (SRL) Pvt. Ltd, Maharashtra, India. The carboxymethylcellulose (CMC) and clove oil coating were prepared by the method following Rezaei *et al.* (2021) [44] with some modifications. For the preparation of 1% CMC solution, 10 gm CMC powder was mixed with 1 liter distilled water while agitating the mixed solution with a magnetic stirrer at 80 °C for about 45 min to achieve a clear solution. For the preparation of 1% clove oil, 10 ml clove oil was mixed with tween-80 as a surfactant and 96% ethanol as a co-surfactant. The mixture was then kept under a closed bottle for 1 h at 86 °C. Then distilled water was added up to a volume reached 1 liter. To produce the CMC gel incorporated with clove oil, the clove oil was combined with CMC gel at a specific weight-to-volume ratio (W/V) and subsequently subjected to homogenization at a speed of 20,000 rpm for 5 minutes.

Soaking, coating and storage procedure

Fresh shrimp (*Metapenaeus affinis*) with an average weight and length of about 17-18 gm and 15– 16 cm were purchased from Veraval fish landing center (Longitude 20° 54.259 and Latitude 70° 22.439') and transported under iced condition in Styrofoam box with storage temperature range between 0-2 °C to Department of fish processing technology laboratory, College of Fisheries Science, K.U., Veraval. The raw material was thoroughly washed with chilled water to remove the dirt, slime, and other foreign particle. The length and weight of the shrimp sample were taken and later stored according to the size of the material and afterward beheading, peeling, and deveining (PD) of shrimp was done for further analysis.

For the experiment, shrimps were randomly divided into five groups, including control (without any treatment), PAW group (PAW treatment for 120 seconds), PAW+CEO group (PAW treatment for 120 seconds with dipping in CEO solution), and PAW+CMC group (PAW treatment for 120 seconds with dipping in CMC solution) PAW+CEO+CMC group (PAW treatment for 120 seconds with dipping in CEO and CMC solution). Each experimental group was divided into three subgroups, each containing 50 shrimps. The shrimps were dipped in the solution for 15 minutes (with the ratio of 1:1 (w/v) at 4°C) except for the control group. After dipping, shrimp were allowed to drain for 15 minutes before being packaged in polyethylene bags and stored in refrigerated temperature at 4°C ± 1 for a duration of 25 days. Sampling was carried out every 5 days of storage for further analysis.

Proximate composition of shrimp

On the first day of the experiment, the moisture, protein, lipid, and ash contents of the shrimp were assessed following the procedures determined by AOAC (2006) [3]. The values were expressed as %.

Shrimp quality evaluation

Biochemical quality evaluation

pH determination

Determination of pH in the shrimp sample was determined by the pH meter method described by AOAC (2006) [3]. A 10 g of shrimp meat was homogenized in 20 ml of distilled water and the mixture was filtered. The pH of the filtrate was measured using a digital pH meter (U TECH INSTRUMENTS - 733060) at ambient temperature.

Total volatile base nitrogen (TVB-N) analysis

The determination of total volatile bases in the sample was conducted using the total volatile base nitrogen (TVB-N) determination method as given by Beatty and Gibbons (1937) [7]. In this procedure, 1 ml of standard N/100 sulfuric acid was placed within the inner chamber of the micro diffusion unit. Simultaneously, in the outer chamber, 1 ml of trichloroacetic acid (TCA) extract was introduced, followed by the addition of 1 ml of saturated potassium carbonate. The micro diffusion unit was then sealed with a glass lid and left undisturbed overnight. The unreacted acid remaining in the inner chamber was subsequently titrated against a standard N/100 sodium hydroxide solution with Tashiro's indicator. Similarly, a blank test was also conducted without a sample. The TVB-N content was then calculated and expressed in milligrams per 100 grams of the sample.

Trimethylamine nitrogen (TMA-N) analysis

The determination of trimethylamine in the sample was conducted using the trimethylamine nitrogen (TMA-N) determination method as given by Beatty and Gibbons (1937) [7]. In this procedure, 1 ml of standard N/100 sulfuric acid was placed within the inner chamber of the micro diffusion unit. Simultaneously, in the outer chamber, 1 ml of TCA extract was introduced, followed by 0.5 ml of neutralized formaldehyde and 1 ml of saturated potassium carbonate. The micro diffusion unit was then sealed with a glass lid and left undisturbed overnight. The unreacted acid remaining in the inner chamber was subsequently titrated against a standard N/100 sodium hydroxide solution with Tashiro's indicator. Similarly, a blank test was also conducted without a sample. The TMA-N content was then calculated and expressed in milligrams per 100 grams of the sample.

Peroxide value (PV) analysis

The peroxide value (PV) of the shrimp sample was determined using an iodometric method for lipid extraction in accordance with Jacobs (1958) [27]. Initially, 10 grams of the shrimp sample were weighed and thoroughly ground with 15 grams of anhydrous sodium sulfate. The resulting homogenate was transferred into a 100 ml stoppered flask,

and 30-50 ml of chloroform was added. This mixture was then placed in a dark environment for approximately 15-20 minutes, with intermittent shaking. Subsequently, vacuum filtration was employed to separate and filter the homogenate. The filtrate, constituting the chloroform extract, was then transferred into a stoppered container for further analysis.

Within this process, 5 ml of the chloroform extract was transferred into a 100 ml stoppered flask, and 25 ml of a solvent consisting of 2 volumes of glacial acetic acid and one volume of chloroform was added. Following this, 1 ml of a potassium iodide solution was introduced and allowed to react for 1 minute. To this mixture, 35 ml of distilled water and 1 ml of a starch solution were added. The liberated iodine was subsequently titrated against a 0.02N standard sodium thiosulfate solution and expressed as milliequivalents of peroxide per kilogram of lipid.

Microbial quality evaluation

Preparation of sample

For the preparation sample, 10 grams of shrimp sample were aseptically placed in a sterile sample dish and subsequently transferred to a sterile plastic bag. The shrimp sample was homogenized by adding 90 ml of sterile phosphate buffer or normal saline solution. The homogenized sample was then transferred to a stomacher blender and subjected to a tenfold dilution (i.e., 10^{-1}). For subsequent dilutions, 1 ml of the 10^{-1} dilution was mixed with 9 ml of the appropriate medium for plating, resulting in dilutions of 10^{-2}, 10^{-3}, and 10^{-4}.

The determination of total plate count and total psychrophilic bacterial count was conducted following the procedure described by AOAC [3]. Plate count agar (PCA) was utilized as the medium for enumerating both total plate count and total psychrotrophic count. For this purpose, 1 ml from the respective dilutions was aseptically transferred into separate serial petri dishes in duplicate. Subsequently, approximately 18-20 ml of sterile Plate Count Agar (PCA) was evenly spread over the surface of each petri dish, thoroughly mixed, and allowed to solidify. The incubation of the petri dishes was carried out at a temperature of 37 ± 1 °C for a duration of 48 hours for the determination of total plate count and at 7 ± 1 °C for 5 days for the determination of total psychrotrophic count. The results were then reported as logarithmic values expressed as log10 colony-forming units per gram (CFU/g).

Physical quality evaluation

Drip Loss

Drip loss was calculated according to the methodology described by Santos and Regenstein (1990) [46]. Initial weight of all shrimp pouch and trays were recorded. he final weights of these pouches and trays were recorded after thoroughly draining all water from the pouches at regular interval of sampling (every 5 days).

Instrumental color analysis

The color of shrimp samples was quantitatively assessed using a color reader (CR-10, Konica Minolta Sensing, Inc., Japan). Measurements were conducted individually for each replication of the treatment. The color values corresponding to L*, a*, and b* were recorded. The L* value represents the lightness of the sample, with 100 indicating white and 0 indicating black. The a* value indicates the chromaticity, where positive values indicate redness and negative values indicate greenness. The b* value also represents chromaticity, with positive values indicating yellowness and negative values indicating blueness) (Papadakis *et al.* 2000) [41]. These measurements provided objective data regarding the color characteristics of the shrimp samples.

Texture Analysis

Texture profile analysis was performed using the TA. XAPC1i Texture Analyzer (Stable Microsystems, UK) with P/75 cylindrical Perspex probe (Diameter:75mm) used to compress the sample 50% of the original thickness to stimulate the chewing process. A trigger force of 50g was applied at a constant speed of 1.0 mm/s. The result of force-time curves for TPA was defined by Bourne (2002) [10], which includes hardness, cohesiveness, chewiness and springiness.

Sensory Analysis

At specified time intervals, including 0, 5, 10, 15, 20, and 25 days of refrigerated storage, both treated and untreated shrimp samples were placed onto stainless-steel trays and covered with a layer of aluminum foil. A sensory evaluation was conducted by a panel consisting of 10 untrained panelists aged between 23 and 30 years, employing a 9-point hedonic scale where 9 represented "extremely desirable," 7 represented "moderately desirable," 5 represented "neither desirable nor undesirable," 3 represented "moderately undesirable," and 1 represented "extremely undesirable" (Meilgaard *et al.* 1999) [35]. The sensory parameters evaluated included color, odor, texture, and overall acceptability. Shelf life criteria assumed that rejection would occur when the sensory scores declined below 5. Color, odor, texture and overall acceptability were analyzed as sensory parameters.

Statistical analysis

Statistical analysis was based on triplicate analysis for each sample at each specific storage time. ANOVA (Analysis of variance) statistical technique was used to find out the significant difference in samples between the treatments as per the standard statistical methods. (Snedecor & Cochran, 2014) [50]. The analysis of variance (ANOVA) was carried out based on the experimental data using IBM BASIC windows release 1.13 to know the significant difference between different treatment combinations and to find the best treatment combination.

Results and Discussion

Proximate composition of shrimp

At first day of experiment, shrimp had the moisture content was about 78.03±0.06%, protein content was 18.17±0.15%, fat content was 1.42±0.03% and ash content was 1.09±0.01% (mean ± SD). The result revealed that major component is moisture followed by protein and crude fat. A slight variation observed in present study may be due to different in size, sex, weight and season. Both intrinsic and extrinsic factors can affect proximate composition of shrimp.

Biochemical quality changes

Changes in pH during refrigerated storage

The pH level is often used as a means of assessing the freshness of seafood due to its susceptibility to alterations caused by microbial or enzymatic actions (Varlik *et al.*

2000) [56]. The initial pH of all treatments ranged between 6.35±0.06[b] and 7.15±0.06[a] (Fig. 1). During refrigerated storage, autolytic processes generate a significant amount of alkaline substances, and their accumulation contributes to an increase in the pH levels of shrimp (Nirmal & Benjakul 2009) [37]. Here, the highest pH was obtained in the control than the other treatment, resulting in a final pH of 7.97±0.10[c] (p<0.05). This proves the findings of Shiekh and Benjakul (2020) [48], the control group exhibited the most pronounced pH increase in *Litopenaeus vannamei* for all the days of sampling. This result was also consistent with the results of Yan *et al.* (2020) [58], where it was observed that after two days of storage, the pH level of the prawns in the control group increased at the maximum rate, while the pH level of prawns treated with electrolyzed water pH increase comparatively slower rate. Similar results were found by Jung *et al.* (2017) [29], where meat batter exposed to cold plasma (CP) discharge for 25 and 30 minutes exhibited a lower pH than the untreated control. When the pH was above 7.6, shrimp were not accepted (Nirmal *et al.* 2011) [38]. This value exceeded the level for the control at day 10 of the storage (Fig. 1). However, the lower pH rate obtained in shrimp treated with PAW in conjunction with CEO and CMC compared to other treatments might have been due to the presence of antimicrobial constituents, such as eugenol derived from CEO, the oxygen barrier effect provided by the CMC coating, and the potential influence of reactive oxygen species (ROS) in PAW. These combined mechanisms may contribute to pH reduction by inactivating microorganisms capable of producing either acidic or alkaline metabolites, as well as aerobic bacteria (Li *et al.* 2006; Varela *et al.* 2011) [31,55]. Rezaei *et al.* (2021) [44] demonstrated that a combination of clove essential oil (CEO) and carboxymethyl cellulose (CMC) coating resulted in a reduced rate of pH increase during refrigerated storage as compared to the untreated samples.

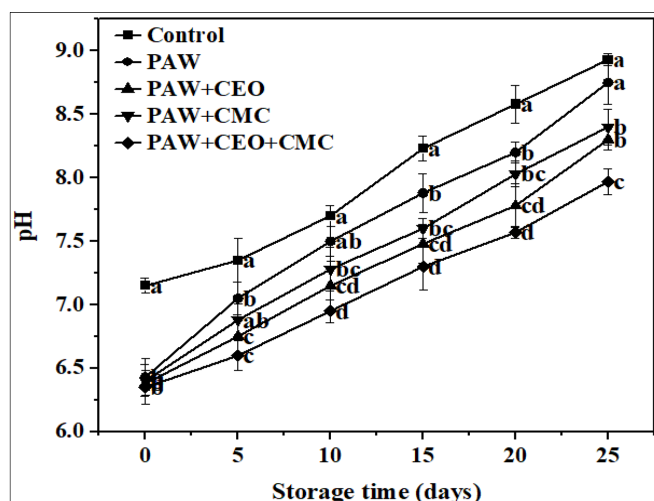

Fig 1: Changes in pH of shrimp (*Metapenaeus affinis*) during refrigerated storage Lines represent the mean of triplicate measurements ± standard deviation (SD); Different lowercase letters on the lines within the same sample indicate significant differences (p<0.05).

Changes in total volatile basic nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N) during refrigerated storage

TVB-N is regarded as one of the most widely used indices for assessing the overall freshness and quality of seafood. The term includes the measurement of ammonia (produced by amino acid deamination and nucleotide breakdown), trimethylamine (produced by deteriorating bacteria), dimethylamine (produced by autolytic enzymes during storage), and various other volatile basic nitrogenous compounds associated with seafood spoilage (Gram & Huss 1996) [23]. At day 0, the TVB-N level ranged from 10.05±0.04[a] to 10.21±0.18[a] mg N/100 g in all samples, indicating good shrimp quality (Fig. 2). After 25 days of refrigerated storage, the TVB-N values of all samples increased. Similar increasing trends were also observed by Fatemeh *et al.* (2016) [20], where the control group shrimp had an initial TVB-N value of 10.48 mg N/100g, and it gradually increased throughout the storage period. In the control sample, a higher rate of TVB-N accumulation was observed as compared to PAW, PAW+CMC, PAW+CEO, and PAW+CEO+CMC treated group (p<0.05). Most previous investigations established a TVB-N value of 30 mg/100 g as the limit of acceptability, which means that a TVB-N of above 30 mg/100 g is regarded as the indicator of poor quality for consumption the shrimp products (Altissimi *et al.* 2018) [2]. The TVB-N content exceeded the level at day 10 for control, PAW treated group was acceptable up to day 10, the PAW+CMC and PAW+CEO treated group was up to day 15, and the PAW+CMC+CEO treated group was acceptable up to day 20 (p<0.05). The decrease in TVB-N content observed in the PAW+CEO+CMC combined treatment, in contrast to other treatments, can be attributed mainly due to the combined antimicrobial effects of plasma-activated water (PAW) and the natural antimicrobial compounds found in clove essential oil (CEO) and carboxymethyl cellulose (CMC). This combination effectively prevented bacterial growth and deamination of non-protein nitrogenous compounds, or both, which ultimately contributed to the lower TVB-N content observed (Behbahani *et al.* 2019; Dashipour *et al.* 2014; Zhao *et al.* 2020) [8,17,60] (Fig. 2). This is an agreement with finding of Chaijan *et al.* (2022) [13] demonstrated that in comparison compared to individual treatments involving PAW soaking (PS) and coating with a mixture of whey protein isolate (WPI) and crude ginger extract (CGE), the combined treatment of PS and WPI-CGE coating led to a decrease the rate of accumulation of total volatile compounds in fish steak during refrigerated storage. Furthermore, Furthermore, Olatunde *et al.* (2020) [40] investigated the most pronounced reduction of total volatile compound formation in sea bass slices when a combined treatment of cold plasma (CP) and liposomal-encapsulated ethanolic coconut husk extract (LE-ECHE) was applied, as opposed to fish treated exclusively with CP or LE-ECHE alone.

Trimethylamine (TMA) is one of the most well-known compounds to evaluate the freshness and degree of spoilage in seafood. It will produce a typically fishy odor due to the presence of Trimethylamine (TMA) which is predominantly produced through the reduction of trimethylamine oxide (TMAO), a process primarily mediated by enzyme activity of certain bacteria, although there is also a potential contribution from endogenous enzymes. Fig. 3 shows the TMA-N changes of treated and untreated shrimp throughout refrigerated storage. In general, the levels of TMA-N content in all samples increased as the refrigerated storage increased (p<0.05). However, it's noticed that the rate of this increase was significantly more pronounced in the control sample (Fig. 3). At day 15, TMA-N of the control and PAW

treated sample was 17.82±0.11[a] mg N/100 g and 15.68±0.13[b] mg N/100 g. Samples treated by PAW+CEO and PAW+CMC had TMA-N of 15.58±0.16[d], and 18.34±0.11[c] mg N/100 g, respectively at 20 days storage period. Nevertheless, TMA-N in samples treated by PAW+CEO+CMC was 13.58±0.41[e] mg N/100 g at the same period. These values were higher than the acceptable limit of 10-15 mg N/100 g for good quality seafood at day 15 for the control and PAW treated group, while PAW+CEO+CMC combined treatment acceptable up to 20 days which was showing lowered TMA-N formation during the refrigerated stored shrimp as compared to other treatment (Connell 1995) [16]. The decrease in TMA-N content can be attributed to the lower microbial load in the PAW+CEO+CMC treated sample, particularly the H2S-producing bacteria capable of converting trimethylamine oxide (TMAO) into trimethylamine (TMA) (Huss 1988) [25]. The present observation was in line with the result obtained by Singh and Benjakul (2020) [49], as they found that the rate of trimethylamine (TMA) production was notably higher in the control sample, sea bass slices treated with squid pen chitooligosaccharide, and fish treated with high voltage cold atmospheric plasma (HV-CAP), as compared to the sample subjected to the combined treatment of chitooligosaccharide and HV-CAP during refrigerated storage.

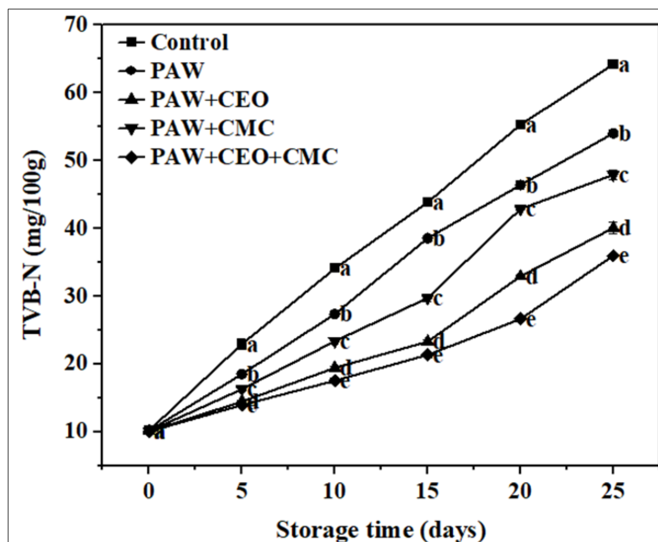

Fig 2: Changes in TVB-N of shrimp (*Metapenaeus affinis*) during refrigerated storage. Lines represent the mean of triplicate measurements ± standard deviation (SD); Different lowercase letters on the lines within the same sample indicate significant differences ($p<0.05$).

Changes peroxide Value (PV) during refrigerated storage

The peroxide value (PV) is employed as an indicator to express the oxidative states of lipid-containing food products. It measures the initial phase of oxidative rancidity. The initiation of oxidation is characterized by the generation of free radicals from fatty acids through the homolytic disruption of a hydrogen atom situated adjacent to a double bond which results in the formation of hydroperoxides (Nirmal and Benjakul 2009) [37]. The initial PV was between 1.51±0.02[a] meq O_2/kg and 1.58±0.07[a] meq O_2/kg which was consistent with the 1.6 ± 0.3 meq O_2/kg by Okpala *et al.* (2014) [39]. Fig. 4 shows an increasing trend in peroxide value (PV) with progression in storage for all samples ($p<0.05$). Here, the highest peroxide value was obtained in the control and the lowest peroxide value was obtained in the PAW+CEO+CMC combined treatment. Also, the values of these samples were lower than 10 meq O_2/ kg of lipid, regarded generally as the acceptable level up to day 20 (Jeon *et al.* 2002) [28]. PAW could inhibit the microbial growth, which retarded the lipid oxidation of shrimps. Nevertheless, the presence of oxidizing agents in PAW could potentially have adverse effects on shrimp lipid oxidation. However, the findings of this study suggest that the impact of oxidizing agents in PAW on shrimp lipid oxidation was minimal and can be ignored. Furthermore, the synergistic effect of carboxymethyl cellulose (CMC) acting as an oxygen barrier and the antioxidant properties of clove essential oil (CEO), influenced by its polyphenol content, effectively retards the process of lipid oxidation (Suppakul *et al.* 2003; Varela *et al.* 2011) [51, 55]. Similar results were also found by Shiekh and Benjakul (2020) [48], who demonstrated that chamuang leaf extract followed by high voltage cold atmospheric plasma (HV-CAP) combined treatment prevented lipid oxidation in *Litopenaeus vannamei* during storage as compared to HV-CAP or chamuang leaf extract (CLE) treatments alone. Singh and Benjakul (2020) [49] also found lower hydroperoxide production in sea bass slices when subjected to a combined treatment involving HV-CAP and chitooligosaccharide, in contrast to fish treated individually with HV-CAP or CLE, during the refrigerated storage. Therefore, PAW treatment followed by CEO and CMC prevented lipid oxidation in *Metapenaeus affinis* during refrigerated storage.

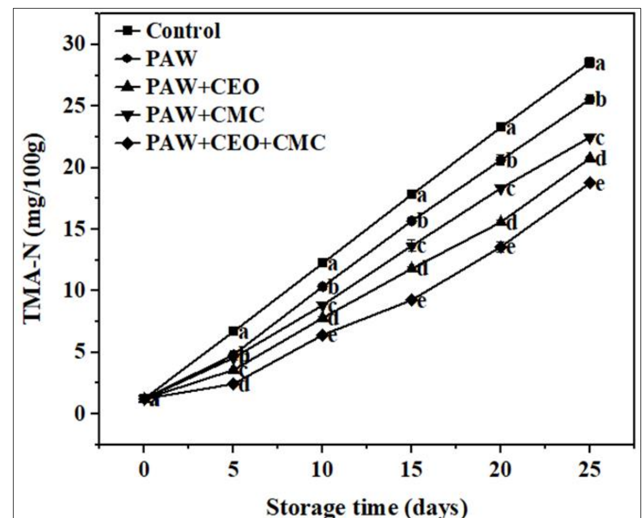

Fig 3: Changes in TMA-N of shrimp (*Metapenaeus affinis*) during refrigerated storage. Lines represent the mean of triplicate measurements ± standard deviation (SD); Different lowercase letters on the lines within the same sample indicate significant differences ($p<0.05$)

Microbiological quality changes

Seafood products act as a reservoir for bacterial proliferation, which although not posing a significant health hazard to consumers, frequently leads to premature degradation (characterized by sensory defects) even before the expiry date. Additionally, the microbiological risk associated with seafood products was predominantly reflected in their ability to cause foodborne poisoning. Contaminations from spoilage microorganisms represent a prominent factor contributing to the deterioration of shrimp, thereby resulting in the degradation of shrimp quality (Liao

et al. 2018) [32]. In fish and fishery products total bacterial count is used as an index of spoilage.

As shown in Fig. 5 and 6, increasing refrigerated storage periods resulted in an increase in Total plate count (TPC) and psychrotrophic bacterial count (PBC) in all samples (p<0.05). Initially, the total plate count (TPC) in all the samples ranged from 3.42±0.13[b] to 4.11±0.13[a] log CFU/g, which indicated that the shrimp samples were fresh. The control group exhibited a more pronounced growth in total plate count (TPC), reaching the permissible limit of 6 log CFU/g by Day 10 (FSSAI 2017) [21]. Over the 25-day storage period, all treatment groups reported varying rates of increase in TPC (p<0.05). On Day 20, the TPC for PAW+CEO and PAW+CMC exceeded the acceptable limit, whereas the TPC for the PAW+CEO+CMC group remained below the acceptable limit during the 20-day refrigerated storage period (Fig. 5). Combined impact of PAW followed by CEO and CMC coating in *M. affinis* was more effective in lowering the microbial growth, compared to others. This can potentially be attributed to the plasma-activated water (PAW) bactericidal properties, which involve the reactive species (such as nitrate, nitrite, and H_2O_2) reaction and significant acidification. As a result, oxidative stress is generated within the cell membrane, causing the deterioration of cell membrane integrity via the formation of pores on the cell surface and the occurrence of physical damage to the cell membrane (Zhao *et al.* 2020) [60]. Additionally, the presence of phenolic compounds in CEO, such as eugenol, has been associated with its antibacterial properties. Furthermore, the combined application of an edible coating containing CEO and PAW helped to prevent the penetration of oxygen into the interior of the shrimp, ultimately leading to a reduction in microbial growth. This is in agreement with the finding of Chaijan *et al.* (2022) [13] demonstrated that in comparison to the PS and WPI-CGE coating treatments alone, the PS + WPI-CGE coating combined treatment resulted in inhibiting the growth of microorganisms in fish steak during cold storage.

The majority of microbes that cause fish and shellfish spoilage are Gram-negative psychrotrophic bacteria, and these organisms are sometimes responsible for digestive disorders (Nirmal & Benjakul 2011) [38]. Low psychrotrophic bacteria were found at the 0 days but as storage time increased their count also increased (p<0.05) (Fig. 6). The initial value of the Total psychrotrophic count of all samples ranged from 2.93±0.21[a] log CFU/g to 3.41±0.20[a] log CFU/g. The control showed a higher Total psychrotrophic count as compared to other treatments at the end of the storage period (Fig. 6). Similar result was also obtained by De Souza Silva *et al.* (2019) [18], where psychrotrophic bacterial count of cold plasma (CP) treated *Litopenaeus vannamei* was lower than that of control. The acceptable limit of total psychrotrophic count for consumption of fishery products has been estimated as 7 log CFU/g (ICMSF 1986) [26]. At Day 20, only the samples subjected to the PAW+CEO+CMC treatment exhibited PBC of less than 7.0 log CFU/g. In contrast, samples treated with PAW, PAW+CEO, and PAW+CMC exceeded the threshold of 7.0 log CFU/g by Day 20. These results indicate that shrimp treated with a combination of PAW, CEO, and CMC coating exhibited more effective inhibition of psychrotrophic microbial growth during refrigerated storage. This was in line with the finding of Shiekh and Benjakul (2020) [48], who reported that in comparison to the high voltage cold atmospheric plasma (HV-CAP) and chamuang leaf extract treatments alone, the CLE followed by HV-CAP combined treatment prevents the psychrotrophic bacterial count in *Litopenaeus vannamei* during refrigerated storage. The findings indicate that treating shrimp with PAW, especially when combined with CEO and CMC, can effectively delay the increase of both total TPC and PBC, thereby extending the shelf life of shrimp during refrigerated storage.

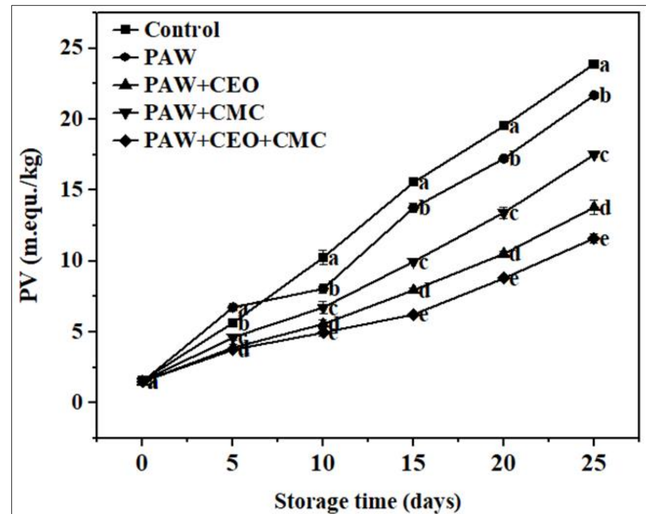

Fig 4: Changes in PV of shrimp (*Metapenaeus affinis*) during refrigerated storage. Lines represent the mean of triplicate measurements ± standard deviation (SD); Different lowercase letters on the lines within the same sample indicate significant differences (p<0.05).

Physical quality changes

Changes in Textural Properties during refrigerated storage

Generating and interpreting texture profile information, with instrument or sensory means, it's called texture profile analysis. It is an important sensory property in seafood products for its acceptability. Variation in textural properties of seafood also depends on the microstructure of the product, the storage condition (frozen or chilled storage), and the species (Huriaux *et al.* 1999; Kim *et al.* 2005; Ladrat *et al.* 2003) [24, 19, 30]. Four textural parameters of both treated and untreated samples were assessed through the texture profile analysis (TPA) test, and the outcomes of this evaluation are presented in Table 2.

Fig. 7 shows a range of hardness values observed among the various groups during the refrigerated storage. The hardness of all treatments decreased as the storage period increased (p<0.05), reflecting a degradation in the structural integrity of proteins. These results exhibited a strong correlation with the expressible drip as shown in Fig. 8, which could potentially be influenced by the enzymatic breakdown of muscle proteins by microorganisms (Chaijan *et al.* 2020) [14]. Within the control group, samples exhibited the most rapid decrease in hardness, followed by those in the PAW-treated group. Subsequently, the PAW+CMC and PAW+CEO groups demonstrated a more gradual reduction in hardness, while the PAW+CMC+CEO group displayed the slowest decline in this attribute. These observations indicate that the combined treatment can delay the degradation of myofibrillar proteins, a process influenced by intrinsic biological factors such as enzymes and

microorganisms. This may be due to less drip loss, and *antimicrobial* and antioxidant activities as mentioned above. A similar result was also obtained by Zhao *et al.* (2020) [61], who observed that textural parameters reduction was higher in the untreated sample compared to the PAW fresh beef at the end of the storage period. In addition, CMC fortified three different concentrations of CEO-coated shrimp also confirmed the current findings (Razaei *et al.* 2021) [44].

Table 2: Changes in Textural properties of shrimp (*Metapenaeus affinis*) during refrigerated storage.

Treatments	Storage Time (Days)					
	0	5	10	15	20	25
Hardness (kgf)						
Control	4.86±0.01^{a}	4.17±0.03^{e}	3.85±0.04^{e}	3.25±0.04^{e}	2.96±0.02^{d}	2.82±0.03^{d}
PAW	4.84±0.01^{a}	4.31±0.03^{d}	4.16±0.04^{d}	3.75±0.04^{d}	3.10±0.10^{d}	2.90±0.01^{d}
PAW+CEO	4.86±0.02^{a}	4.53±0.04^{b}	4.39±0.05^{b}	4.19±0.05^{b}	3.64±0.07^{b}	3.44±0.04^{b}
PAW+CMC	4.88±0.02^{a}	4.43±0.03^{c}	4.27±0.03^{c}	3.95±0.05^{c}	3.39±0.05^{c}	3.16±0.05^{c}
PAW+CEO+CMC	4.88±0.02^{a}	4.65±0.04^{a}	4.51±0.04^{a}	4.35±0.05^{a}	4.11±0.11^{a}	3.54±0.04^{a}
Chewiness (kgf.mm)						
Control	2.54±0.04^{a}	2.34±0.02^{b}	2.29±0.01^{c}	2.04±0.03^{d}	1.84±0.03^{d}	1.45±0.05^{d}
PAW	2.53±0.02^{a}	2.39±0.04^{ab}	2.33±0.05^{bc}	2.18±0.05^{c}	2.07±0.05^{c}	1.84±0.03^{c}
PAW+CEO	2.51±0.03^{a}	2.45±0.04^{a}	2.40±0.03^{ab}	2.34±0.01^{ab}	2.23±0.05^{ab}	2.14±0.04^{ab}
PAW+CMC	2.51±0.03^{a}	2.45±0.03^{a}	2.37±0.02^{ab}	2.26±0.04^{bc}	2.15±0.03^{bc}	2.05±0.03^{b}
PAW+CEO+CMC	2.52±0.01^{a}	2.46±0.03^{a}	2.42±0.03^{a}	2.37±0.01^{a}	2.29±0.03^{a}	2.21±0.02^{a}
Cohesiveness (N)						
Control	0.45±0.03^{a}	0.39±0.01^{b}	0.33±0.02^{c}	0.30±0.01^{c}	0.25±0.01^{c}	0.21±0.01^{c}
PAW	0.45±0.02^{a}	0.40±0.01^{ab}	0.36±0.01^{b}	0.32±0.02^{bc}	0.28±0.01^{c}	0.24±0.02^{c}
PAW+CEO	0.45±0.02^{a}	0.44±0.01^{a}	0.40±0.01^{a}	0.37±0.02^{a}	0.34±0.02^{ab}	0.31±0.01^{ab}
PAW+CMC	0.45±0.02^{a}	0.42±0.03^{ab}	0.39±0.01^{ab}	0.35±0.02^{ab}	0.32±0.01^{b}	0.29±0.01^{b}
PAW+CEO+CMC	0.46±0.02^{a}	0.43±0.01^{a}	0.42±0.01^{a}	0.39±0.02^{a}	0.36±0.02^{a}	0.33±0.02^{a}
Springiness (mm)						
Control	2.89±0.03^{a}	2.60±0.05^{b}	2.49±0.06^{c}	2.36±0.05^{d}	2.19±0.05^{d}	2.04±0.03^{c}
PAW	2.87±0.01^{a}	2.67±0.06^{ab}	2.56±0.03^{bc}	2.40±0.04^{cd}	2.33±0.03^{c}	2.21±0.05^{b}
PAW+CEO	2.87±0.06^{a}	2.74±0.03^{a}	2.66±0.03^{ab}	2.56±0.05^{ab}	2.44±0.02^{ab}	2.37±0.03^{a}
PAW+CMC	2.87±0.02^{a}	2.69±0.04^{ab}	2.60±0.05^{abc}	2.48±0.05^{bc}	2.39±0.05^{bc}	2.30±0.04^{ab}
PAW+CEO+CMC	2.87±0.03^{a}	2.78±0.05^{a}	2.70±0.06^{a}	2.61±0.05^{a}	2.53±0.04^{a}	2.41±0.05^{a}

*Values are the mean of triplicate measurements ± standard deviation (SD); values with different lowercase letters in the same column indicated a significant difference at $p<0.05$.

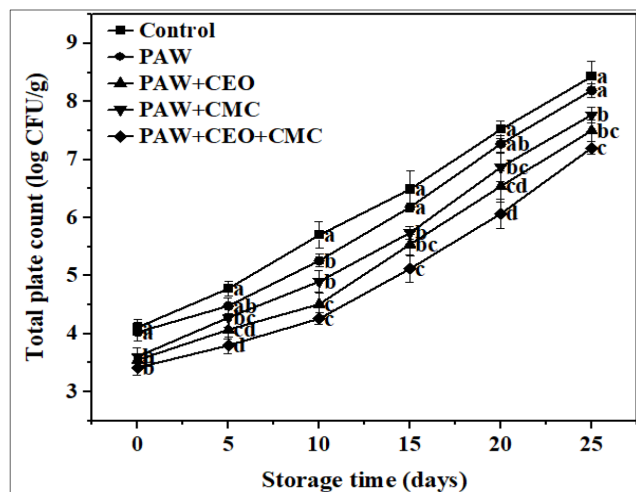

Fig 5: Changes in Total plate count of shrimp (*Metapenaeus affinis*) during refrigerated storage. Lines represent the mean of triplicate measurements ± standard deviation (SD); Different lowercase letters on the lines within the same sample indicate significant differences ($p<0.05$).

Changes in drip loss during refrigerated storage

Drip loss is the result of decreased weight in the final product due to the thawed muscle being unable to reabsorb all of the separated water that has been bound. This represents one of the physical characteristics that can impact the texture and sensory attributes of food products. The drip loss of the samples increased significantly during the time ($p<0.05$) (Fig. 8). The highest drip loss was observed in the control over a period followed by PAW, PAW+CMC, PAW+CEO, and PAW+CEO+CMC treated group during refrigerated storage ($p<0.05$). These findings could be related to the degradation of muscle protein structural integrity triggered by both microbial and endogenous proteases, as supported by the highest microbial growth observed in the control group (Fig. 8). Formation of drip loss brings down the loss of weight, nutrient and flavor components, causing the unpleasant appearance of seafood. CMC coatings efficiently protect against the dehydration of shrimp in refrigerated storage due to their protective barrier properties (Varela *et al.* 2011) [55]. In addition, carboxymethyl cellulose (CMC) demonstrates excellent moisture sorption capability, primarily attributed to the presence of hydroxyl and carboxyl functional groups (Akhtar *et al.* 2018; Rincón *et al.* 2019) [1, 45]. CMC forms a semi-permeable film that coats the surface, facilitating the retention of moisture. The formation of films composed of carboxymethyl cellulose (CMC), which tends to cluster water within its polymer matrix, might explain the decrease in drip loss observed in the CMC-treated samples. This effect could be counteracted and enhanced through the incorporation of hydrophobic essential oils into the carboxymethyl cellulose (CMC) film. Our results confirmed this and showed that shrimp treated with CMC+CEO coating in conjunction with PAW can retain more weight compared with other treatments. Therefore, the incorporation of CEO and CMC with PAW treatment optimally reduces drip loss of stored shrimp. Liu *et al.* (2020) [33] also demonstrated chitosan coating combined with clove oil could contributed retained more weight compared to the other sample throughout the storage period.

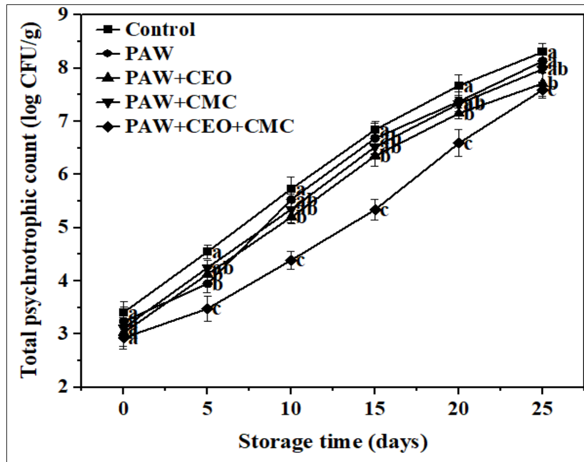

Fig 6: Changes in Total psychrotrophic count of shrimp (*Metapenaeus affinis*) during refrigerated storage. Lines represent the mean of triplicate measurements ± standard deviation (SD); Different lowercase letters on the lines within the same sample indicate significant differences (p<0.05).

Changes in color properties during refrigerated storage

In seafood products, color constitutes the most important visual attribute for consumers. The color assessment of treated and untreated shrimps during the refrigerated storage period is shown in Table 3. In terms of color property assessment, the enzyme polyphenol oxidase initiates a reaction with phenolic compounds, leading to the formation of insoluble black pigments known as melanosis, results in a reduction of the L* values. Notably, this reduction occurred at a higher rate within the control group as compared to the treated groups. The application of a coating to shrimp samples has the potential to restrict the availability of oxygen, thereby mitigating the processes of myoglobin and lipid oxidation.

The presence of phenolic compounds within clove essential oil (CEO) may additionally retard the oxidation of meat pigments and lipids, consequently leading to reduced color fading in shrimp. A similar result was obtained by Olatunde *et al.* (2020) [40], who observed that fish slices, whether they were pre-treated with ethanolic coconut husk extract or its liposomal form, demonstrated less color change compared to those that did not undergo this pre-treatment, regardless of exposure to cold plasma (CP). The changes in the 'a*'-value in shrimp showed a slightly increasing trend towards redness with the increasing refrigerated storage period (p<0.05). A similar increasing trend with time for the 'a*'-values was observed in white prawn by Bindu *et al.* (2013) [9], they attributed these color shifts to lipid oxidation resulting from the hydrolysis of carotenoids by endogenous proteases and their release from the protein matrix within the muscle. The redness value was higher in the control group compared to the treated groups. The changes in the 'b*'-value in shrimp showed a slightly increasing trend towards yellowness with the increasing refrigerated storage period (p<0.05). The yellowness value was higher in the control group compared to the treated groups. Bak *et al.* (1999) [6] observed a significant increase in the yellowness parameter of untreated shrimp during storage under refrigeration, resulting in a perceptible shift in color from a red to a more pronounced yellow appearance. Rezaei *et al.* (2021) [44] demonstrated that CMC combination with CEO had a lower color difference observed during refrigerated storage when compared to untreated samples. To recap, the combination of PAW treatment and the application of CMC coating incorporated with CEO could influence the color characteristics of the shrimp sample throughout the storage period.

Table 3: Changes in Color properties of shrimp (*Metapenaeus affinis*) during refrigerated storage.

Treatments	Storage Time (Days)					
	0	5	10	15	20	25
Lightness (L*)						
Control	55.10±0.10^{c}	52.47±0.50^{d}	49.57±0.21^{d}	47.90±0.10^{d}	45.77±0.35^{d}	44.37±0.32^{c}
PAW	55.57±0.06^{b}	53.67±0.21^{c}	51.33±0.35^{c}	49.43±0.51^{c}	47.17±0.60^{c}	45.30±0.30^{c}
PAW+CEO	55.73±0.15^{b}	54.87±0.15^{ab}	53.13±0.35^{ab}	50.70±0.30^{b}	49.10±0.66^{b}	47.97±0.15^{a}
PAW+CMC	55.77±0.15^{b}	54.20±0.53^{bc}	52.10±0.56^{bc}	50.17±0.12^{bc}	48.50±0.26^{b}	46.33±0.60^{b}
PAW+CEO+CMC	56.27±0.25^{a}	55.30±0.36^{a}	54.23±0.51^{a}	52.23±0.21^{a}	50.90±0.01^{a}	48.50±0.36^{a}
Redness-greenness (a*)						
Control	-10.47± 0.15^{ab}	-10.27±0.12^{b}	-9.10±0.10^{b}	-8.20±0.20^{b}	-7.60±0.26^{b}	-7.17±0.21^{b}
PAW	-10.53±0.21^{ab}	-10.10±0.20^{b}	-9.47±0.15^{ab}	-8.53±0.45^{ab}	-8.07±0.21^{ab}	-7.70±0.20^{b}
PAW+CEO	-10.47±0.12^{ab}	-10.40±0.10^{ab}	-9.70±0.30^{a}	-9.07±0.40^{a}	-8.40±0.40^{a}	-8.03±0.25^{ab}
PAW+CMC	-10.20±0.10^{b}	-10.33±0.06^{ab}	-9.57±0.12^{ab}	-8.70±0.17^{ab}	-8.27±0.12^{ab}	-7.87±0.06^{ab}
PAW+CEO+CMC	-10.87±0.15^{a}	-10.67±0.12^{a}	-9.80±0.20^{a}	-9.23±0.25^{a}	-8.63±0.21^{a}	-8.23±0.15^{a}
Yellowness-blueness (b*)						
Control	5.83±0.06^{a}	6.47±0.15^{a}	6.80±0.26^{a}	7.33±0.15^{a}	8.43±0.45^{a}	9.13±0.31^{a}
PAW	5.67±0.12^{ab}	6.07±0.06^{b}	6.60±0.10^{a}	7.07±0.40^{ab}	7.90±0.20^{ab}	8.53±0.25^{ab}
PAW+CEO	5.40±0.40^{b}	5.63±0.31^{c}	6.10±0.20^{bc}	6.63±0.15^{bc}	7.30±0.36^{bc}	7.90±0.46^{bc}
PAW+CMC	5.47±0.45^{b}	5.90±0.10^{bc}	6.47±0.15^{ab}	6.80±0.40^{abc}	7.57±0.35^{abc}	8.27±0.25^{b}
PAW+CEO+CMC	5.50±0.10^{b}	5.07±0.58^{d}	5.93±0.15^{c}	6.33±0.31^{c}	6.90±0.20^{c}	7.43±0.06^{c}

*Values are the mean of triplicate measurements ± standard deviation (SD); values with different lowercase letters in the same column indicated a significant difference at p<0.05.

Sensory quality changes

At the 0 day, sensory evaluations, including assessments of appearance, odor, texture, and overall acceptability, were conducted for the shrimp samples treated with PAW, both with and without the addition of CEO and CMC were shown in Table 4. The sensory scores decreased significantly as the storage period increased, with a slower decrease for the treated samples (p<0.05). Shrimp samples that received scores below 5 were considered unsatisfactory, characterized by the presence of undesirable attributes such as a rancid odor, absence of a shiny color, a soft and rigid texture, and a lack of overall acceptability. On the 10th day

of storage, the control became unacceptable, and evident off-odor was announced, while the PAW+CEO+CMC treated shrimp remained acceptable throughout the 20-day storage period. Shiekh and Benjakul (2020) [48] demonstrated that chamuang leaf extract followed by high voltage cold atmospheric plasma (HV-CAP) combined treatment prevented lipid oxidation lead to a decreased rate of off-odor and flavour in *Litopenaeus vannamei* during storage as compared to HV-CAP or chamuang leaf extract treatments alone. Based on the overall acceptability score, it can be concluded that the shelf life of refrigerated shrimp was 10 days for the control samples and extended to 20 days for those treated with PAW+CEO+CMC combined treatment. This can be attributes to the functional attributes of PAW/CMC/CEO, including their antioxidant, oxygen barrier, and antimicrobial properties. These outcomes find support in the results obtained from chemical quality analyses. The utilization of high-voltage atmospheric cold plasma (HVCAP) in conjunction with the incorporation of chitooligosaccharides derived from squid pen has demonstrated the ability to prolong the shelf life of sea bass slices as indicated by maintaining a likeness score above the acceptable limit (Singh & Benjakul 2020) [49]. Zouelm *et al.* (2019) [62] found that cold plasma (CP) treatment exhibited superior efficacy over sodium metabisulfite in terms of inactivating spoilage microorganisms in *Litopenaeus vannamei* and maintaining sensory quality. Therefore, the CEO and CMC in conjunction with PAW prior treatment were able to maintain the quality of shrimp and hence shelf-life extension was achieved.

Table 4: Changes in Sensory properties of shrimp (*Metapenaeus affinis*) during refrigerated storage.

Treatments	Storage Time (Days)					
	0	5	10	15	20	25
Appearance						
Control	8.20±0.29^{a}	7.10±0.57^{c}	4.70±0.95^{c}	3.70±0.67^{c}	2.40±0.70^{d}	2.00±0.00^{c}
PAW	8.40±0.52^{a}	7.50±0.53^{bc}	6.20±0.42^{b}	4.40±0.97^{c}	2.60±0.70^{d}	2.30±0.67^{c}
PAW+CEO	8.80±0.12^{a}	8.10±0.32^{ab}	7.00±0.00^{ab}	6.50±0.53^{a}	5.10±0.57^{b}	3.30±0.48^{b}
PAW+CMC	8.60±0.20^{a}	7.80±0.79^{abc}	6.50±0.71^{b}	5.60±0.70^{b}	4.20±0.63^{c}	2.40±0.84^{c}
PAW+CEO+CMC	8.90±0.10^{a}	8.30±0.48^{a}	7.40±0.84^{a}	7.10±0.57^{a}	6.00±0.00^{a}	4.70±0.67^{a}
Odor						
Control	8.40±0.32^{a}	7.00±0.00^{b}	4.60±0.70^{d}	4.10±0.32^{d}	2.50±0.85^{d}	1.90±0.74^{c}
PAW	8.50±0.15^{a}	7.20±0.79^{b}	5.40±0.52^{cd}	4.60±0.84^{cd}	3.40±0.70^{c}	2.70±0.95^{bc}
PAW+CEO	8.30±0.48^{a}	7.70±0.82^{ab}	6.50±0.53^{ab}	5.40±0.70^{b}	5.10±0.57^{ab}	3.50±0.71^{ab}
PAW+CMC	8.50±0.43^{a}	7.40±0.84^{ab}	6.10±0.57^{bc}	5.00±0.00^{bc}	4.30±0.48^{b}	3.10±0.32^{ab}
PAW+CEO+CMC	8.60±0.30^{a}	8.10±0.32^{a}	7.30±0.82^{a}	6.40±0.52^{a}	5.80±0.79^{a}	3.70±0.48^{a}
Texture						
Control	8.50±0.33^{a}	6.90±0.57^{b}	4.70±0.48^{c}	3.90±0.32^{d}	2.30±0.82^{c}	1.70±0.67^{c}
PAW	8.50±0.21^{a}	7.30±0.82^{ab}	5.30±0.48^{c}	4.40±0.84^{cd}	3.30±0.67^{b}	2.50±0.71^{b}
PAW+CEO	8.60±0.20^{a}	7.60±0.97^{ab}	6.50±0.71^{b}	5.80±0.79^{ab}	5.20±0.42^{a}	3.40±0.52^{a}
PAW+CMC	8.60±0.12^{a}	7.40±0.70^{ab}	6.10±0.74^{b}	5.10±0.32^{bc}	3.80±0.79^{b}	2.60±0.52^{b}
PAW+CEO+CMC	8.70±0.27^{a}	8.20±0.42^{a}	7.40±0.52^{a}	6.40±0.52^{a}	5.70±0.48^{a}	4.10±0.57^{a}
Overall acceptability						
Control	8.60±0.12^{a}	7.30±0.82^{b}	4.80±0.63^{c}	4.10±0.74^{b}	3.40±0.84^{d}	2.50±0.71^{c}
PAW	8.60±0.20^{a}	7.70±0.67^{ab}	6.40±0.97^{b}	4.40±0.84^{b}	3.90±0.74^{cd}	2.90±0.32^{c}
PAW+CEO	8.80±0.12^{a}	7.70±0.82^{ab}	7.20±0.63^{ab}	6.30±0.48^{a}	5.00±0.00^{ab}	3.80±0.79^{ab}
PAW+CMC	8.80±0.12^{a}	7.50±0.53^{ab}	6.70±0.67^{b}	5.70±0.48^{a}	4.60±0.52^{bc}	3.20±0.63^{bc}
PAW+CEO+CMC	8.90±0.10^{a}	8.20±0.42^{a}	7.60±0.52^{a}	6.50±0.71^{a}	5.60±0.70^{a}	4.20±0.63^{a}

*Values are the mean of triplicate measurements ± standard deviation (SD); values with different lowercase letters in the same column indicated a significant difference at p<0.05.

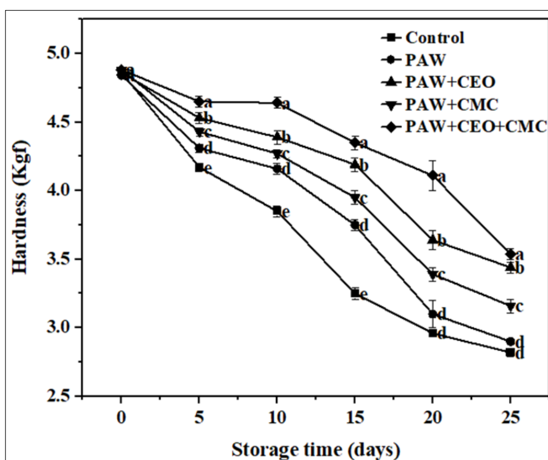

Fig 7: Changes in Textural properties of shrimp (*Metapenaeus affinis*) during refrigerated storage. Lines represent the mean of triplicate measurements ± standard deviation (SD); Different lowercase letters on the lines within the same sample indicate significant differences (p<0.05).

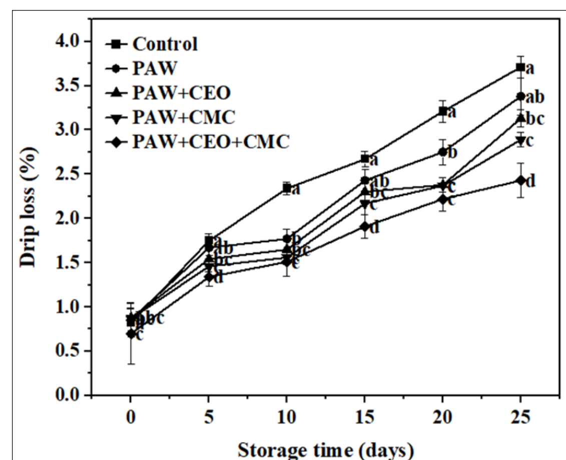

Fig 8: Changes in Drip loss of shrimp (*Metapenaeus affinis*) during refrigerated storage. Lines represent the mean of triplicate measurements ± standard deviation (SD); Different lowercase letters on the lines within the same sample indicate significant differences (p<0.05).

Conclusion
The treatment of PAW+CEO+CMC was has proven to be an efficient approach for increased shelf life of the refrigerated peeled shrimp. This study showed very significant importance by extending the shelf life of PAW treated shrimp for 10 days around compared to simple refrigerated shrimp. Furthermore, the shelf life of PAW+CEO+CMC combined treatment was extended to 20 days (based on pH, TMA-N, TVB-N, TPC, and PBC), whereas the PAW+CMC and PAW+CEO had shelf-life of 15 days. Slower color changes and texture softening in shrimp sample were also observed in PAW+CEO+CMC versus the control. Highest sensory score was attained in shrimp with PAW, 1% CEO and 1% CMC treatment compared to other treatment. Therefore, PAW treatment combined with these coatings offers a promising alternative for reducing the dependency on conventional chemical additives and synthetic materials in food formulation. This approach has the potential to enhance food quality and extend the shelf life of seafood products.

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Statements and Declarations
Competing Interest
The authors have no conflict of interest to declare.

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Availability of data and materials
Date available on request

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