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PREVENTION OF QUALITY LOSS AND MELANOSIS OF WHITE SHRIMP (*PENAEUS* SPP.), DURING COLD STORAGE *BY* MANGO SEED KERNEL EXTRACT

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Abstract

Background and Objective: Shrimps are highly perishable with a limited shelf-life, mainly associated with melanosis (discoloration), as well as chemical and microbial deteriorations. This study aimed to testify ethanolic mango seed kernel extract (MSKE) activity on the melanosis formation and quality of white shrimp (*Penaeus* spp.) during cold storage. Shrimp samples underwent immersion in different treatments (control group (C), sodium metabisulphite solution group (SMS; $Na_2S_2O_5$; 1.25%) and MSKE group (1 and 2%). Methodologies: Shrimp samples were packaged in polyethylene bags and stored inside the refrigerator (4 ± 1°C) to be periodically examined for their sensory quality, physicochemical parameters and bacteriological status. Results indicated that the melanosis formation was significantly delayed and sensory quality was significantly improved in shrimp treated with MSKE, compared with SMS and the control shrimp. The increase in the bacteria amounts (TVC, PTC and EBC), pH, total volatile basic nitrogen (TVBN) and thiobarbutric acid reactive substances (TBARS) were significantly decreased in shrimp treated with MSKE. The melanosis score, chemical indices and microbiological status of shrimp immersed in 2% MSKE was less than that treated by 1% MSKE. Conclusion: Based on chemical indices, microbial analyses and sensorial changes the shelf-lives of white shrimp was longest for 2% MSKE- (10 days), followed by SMS- (9 days), 1% MSKE- (8 days) and Control samples (6 days). These results suggested that 2% MSKE could be used as an effective natural alternative to synthetic antimelanosic agents to inhibit post-mortem melanosis, improve the quality of shrimp during cold storage.

Key words: MSKE, SMS, Shrimp blackspot (Melanosis), Shelf-life, Sensory acceptance. Chemical indices, Microbial quality.

Introduction

Marine white shrimp (*Penaeus* spp.), has been of increasing demand worldwide (Sae-leaw *et al*., 2017). Due to the high market and nutritional value, shrimp is a very important fisher resource all over the world. However, shrimp is highly perishable with a limited shelf-life, mainly caused by melanosis (discoloration), chemical and microbiological deteriorations (Gonçalves *et al.,* 2015). Melanosis or the formation of black spots in crustaceans, such as shrimp and crabs during postmortem storage, severely damage the market value and usually caused economical loss of this seafood (Yuan *et al.,* 2016).

Melanosis is caused by the action of polyphenoloxidase (PPO), also known as phenoloxidase, tyrosinase and cathecoloxidase (Zamorano *et al*., 2009), which oxidizes phenols to quinone. Quinone subsequently undergoes polymerization, giving rise to high molecular weight black pigment (Benjakul *et al.,* 2005). To retard melanosis, icing or refrigeration has been traditionally implemented. However, during iced or refrigerated storage, melanosis still takes place since PPO still remains active under these conditions (Nirmal and Benjakul, 2010).

To overcome melanosis in crustaceans and ensure perishables have a longer shelf-life antimelanosic agents such as 4-hexyl-1, 3- benzenediol (4-hexylresorcinol), sulphite-based compounds and phosphates have been intensively studied and proved to be effective to inhibit melanosis (Martinez-Alverez *et al*., 2008 and Thepnuan *et al*., 2008). These compounds interfere with the polymerization of quinones and do not allow the formation

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of dark pigments on shrimp (Rotllant *et al.,* 1983).. However, the use of synthetic compounds to inhibit melanosis in seafood is limited due to increasing regulatory attention and food safety concerns (Gomez-Guillen *et al*., 2005).

Natural products, especially natural antioxidants and antimicrobial agents, have been intensively examined as safe alternatives to synthetic compound. Recently, Grape seed extract is used to prevent melanosis in deep-water rose (Sun *et al.,* 2014). Rosemary extracts was used to retard quality deterioration in deep-water pink (Cadun *et al.,* 2008). In addition, green tea extract (GTE) showed the inhibitory activity towards melanosis of Pacific white shrimp during iced storage (Carrizo *et al*., 2014). Catechin in combination with ascorbic acid exhibited an inhibitory activity towards PPO of Pacific white shrimp (Yuan *et al.,* 2016).

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world. Mango seed kernels (MSK) are rich sources of phenolic compounds and flavonoids such as gallic acid, ellagic, pyrogallol, chlorogenic, catachin, mangiferin, protocatechuic, cinnamic, catechol. It also contained myricetin caffeine, coumaric, sinapic acid, ferulic acid, salicylic, kaempferol, quercetin and tannin, which showed antioxidant and chelating activity (Namngam *et al.,* 2018 and Abdel-Aty *et al.,* 2018). MSKE showed good antibacterial activity against pathogenic bacteria (Ahmed, 2015 and Raju *et al.,* 2019). MSKE is a suitable by-product that could represent a valuable input into functional foods production.

These characteristics, theoretically, make MSKE a very good alternative to sodium metabisulphite ($Na_2S_2O_5$). However, to our knowledge, there is no literature reporting the influence of MSKE on shelf life and quality of white shrimp. The aims of this study were to study the effect of sodium metabisulfite (SMS; 1.25%) and MSKE at different concentrations (1 and 2%) on the melanosis formation, sensory quality (appearance and odor), physiochemical properties (pH, total volatile basic nitrogen content (TVBN), thiobarbituric acid reactive substances (TBARS) content and microbial status (Total viable count (TVC), Psychrotrophic count (PTC) and Enterobacteriaceae (EBC) count of white shrimp (*Penaeus spp.*), during cold storage. Melanosis formation and sensory scores were evaluated at Z, 2, 4, 6, 8, 9 and 10 days, while the chemical indices and microbial status were examined after Z, 3, 6, 8, 9 and 10 days of cold storage, Proximate composition of MSK and raw shrimp were also investigated.

Materials and Methods

Chemicals and Reagents

Plate count agar (PCA), violet red bile glucose agar (VRBGA) and peptone water were purchased from Oxoid (Hampshire, UK). Methyl red, magnesium oxide, 2-thiobarbituric acid, bromocresol green, sodium metabisulfite (SMS), BHT and TCA were from Sigma-Aldrich (Germany). All other solvents and chemicals used were of analytical grade or the highest grade available.

Mango seed kernel source

A ripe mango seed as by-products (waste) was collected after mango pulp processing of Zebdia variety (*Mangifera indica* L.), during the summer season of 2018, from local fruit processing units (Farghly), Giza, Egypt. The kernels were removed manually from the seeds for further extraction.

Mango seed kernel extract preparation

Mango seed kernels after removal from the seeds were cleaned from extraneous matter and properly washed then dried in hot air-oven for 24 h at 40°C. The dried kernels were milled with grinder into a powdery form and kept in a closed dark glass bottle and stored at 4°C until further analysis.

According to the extraction method of El Anany, (2015), one hundred gram of MSK powder were extracted overnight with 1000 ml of 80% ethanol and methanol solutions in a shaking incubator (100 rpm) at room temperature. Then the extracts were centrifuged at 3500 rpm for 15 min. The supernatants were filtered through a Whatman No.1 filter paper, then extract solutions were concentrated to dryness in a rotary evaporator (Eyela, Rikakikai, Tokyo, Japan), at 40°C and complete the drying of extract in oven overnight at 40°C to form powder, which was stored at -20°C until further use. The extraction yield of MSK samples was calculated and reported as a percentage (g d wt. extract/100 g d wt. sample).

Shrimp source

Live marine whit shrimp (*Penaeus* spp.), with the size amounting to 55-60 shrimps/kg were purchased directly from fishing boats in Abi-kier, Alexandria, Egypt in December, 2019. The freshly caught shrimp were kept in ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, N R C, Egypt within 3 h after collection. Upon arrival, heads on, shells-on whole shrimp were used to represent the most problematic product form. Shrimp samples were divided into 4 groups each 2kg, washed in cold water and stored in ice until treatment (less than 1 h).

Shrimp Treatments

Whole shrimps were immersed in solution containing MSKE at various concentrations (1 and 2%, w/v) using a shrimp/solution ratio of 1:2 (w/v) at 4±1°C for 30 min. Another portion of shrimps were soaked in 1.25% sodium metabisulfite (SMS) at a ratio of 1:2 (w/v) for one min at 4±1°C. Shrimps without any MSKE or metabisulfite treatment were used as the control. Shrimp samples were drained on a screen for 3 min. Each group was subdivided into samples (15 shrimps), all packaged in polyethylene bags and stored at 4±1°C during experiment. Shrimp samples were monitored at 0, 2, 4, 6, 8, 9 and 10 days of cold storage for melanosis, chemical indices, microbial load and sensory properties.

Chemical Assessments

Chemical analyses were made on finely ground shrimp meat samples and MSKE. Analyses were conducted in triplicate. Proximate composition in terms of moisture, ash, lipid and total nitrogen of shrimp meat and MSKE were determined according to the methods described in the AOAC (1995). For pH determination 10 g of shrimp meat samples were homogenized in 90 mL distilled water for 1 min in a warring blender, and the pH value of the slurry was measured at room temperature using pH meter (JENWAY, 3510; UK). The total volatile basic nitrogen (TVB-N) expressed as mg TVB-N per 100 g shrimp meat and a thiobarbituric acid reactive substance (TBARS) as mg of malondialdehyde (MDA)/ kg shrimp meat were determined according to Pearson (1991).

HPLC analysis of phenolic compounds

The high performance liquid chromatography (HPLC) analysis was carried out for MSKE according to Kim *et al.,* (2006). The separation and determination were performed on Agilent 1260 series -C18 column (4.6 mm × 250 mm i.d., 5 µm). The column was eluted by water (solvent A) and 0.02% tri-floro-acetic acid in acetonitrile (solvent B) at a flow rate of 1 ml/min. The obtained peaks were monitored simultaneously at 280 nm. Commercial phenolic compounds were used as standards.

Melanosis assessment

Melanosis assessment of white shrimp was conducted through visual inspection at day 0, 2, 4, 6, 8, 9 and 10 day of cold storage, by ten panelists using ten-point scoring according to the method of Montero *et al*., (2001). Panelists were asked to give the melanosis score (0 to 10) for shrimp, where 0 = Absent; 2 = Slight (up to 20% of shrimps' surface affected); 4 = Moderate (20% to 40% of shrimps' surface affected); 6 = Notable (40% to 60% of shrimps' surface affected); 8 = Severe (60% to 80% of shrimps' surface affected); 10 = Extremely heavy (80% to 100% of shrimps' surface affected).

Sensory evaluation of raw shrimp

Appearance and odor attributes of raw shrimp were evaluated by modified acceptance test with 10 non-trained panel members of the laboratory staff. Five shrimp from each group were taken at regular intervals, and immediately packed in small white foam plates, then labeled and served to the panelists at room temperature in random order for evaluation their appearance (the first impression when looks the product), odor (the intensity of shrimp odor), using 9-points hedonic scales. The 9-points hedonic scales were 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely. A score less than 5 indicate that the shrimp is rejected (Mexis *et al.,* 2009).

Microbiological evaluation

Total viable count (TVC), Psychrotrophic count (PTC) and Enterobacteriace count (EBC) were determined following procedures recommended by APHA (2001), using plate count agar (PCA) and violet red bile agar (VRBA). After specific incubation periods plates showing 25-250 colonies were counted. The number of colonies were multiplied by the reciprocal of the respective dilution and expressed as log10cfu g^{-1}.

Statistical analysis

Results were expressed as means and standard deviation (M±SD) from triplicate determinations. Analysis of variance (ANOVA) was performed to compare the effect of MSKE or SMS dipping treatments on shrimp meat quality. Significant differences were defined as $P<0.05$; according to Rao and Blane (1985).

Results and Discussion

Proximate composition of white shrimp and MSKE

The proximate composition of raw fresh white marine shrimp is presented in table 1. These values are within the normal limits for the species and are in agreement with those found in the shellfish literature for white shrimp by Dincer and Aydin (2014), who reported that fresh white shrimp contained 77.47, 18.4, 1.30, 1.86 and 0.96% moisture, protein, fat, ash and carbohydrates contents (on fresh weight basis), respectively.

Table 1 also reveals that shrimp meat is characterized by a high protein and ash contents and very low fat content. These results confirmed the findings obtained by Boonsumrej *et al.,* (2007). However, slight differences may be due to the differences in the catching season,

Table 1: Proximate composition of MSKE and white shrimp (on fresh wt, basis).

Constituents	Shrimp	MSKE
Moisture%	76.05± 0.54[a]	7.65± 0.25[b]
Protein%	19.35± 0.32[a]	8.50± 0.23[b]
Fat%	1.43± 0.21[b]	11.14± 0.18[a]
Ash%	1.87± 0.14[b]	2.35± 0.12[a]
Total carbohydrates%	1.30± 0.17[b]	70.36± 0.16[a]
Extract yield % gm/100gm)	----	16.00± 0.57

All values reflect the mean and standard deviation are mean of triplicate determinations.

[There is no significant difference (P>0.05) between the values having the same superscripts in the same column. Total Carbohydrates = 100 - (Moisture + Protein + Intramuscular-fat + Ash].

geographical location, species and shrimp size variation (Rodde *et al.,* 2008).

The results of table 1 also cleared the proximate composition of MSKE which was in agreement with those found early by Muta *et al.,* (2017), who reported that MSKE contained 7.94% moisture, 9.20% protein, 10.92% fat and 2.44% ash. These results also confirmed the findings obtained by Gumte *et al.,* (2018), who found 7.79, 9.36, 9.59 and 1.31%, for the same mentioned proximate constituents, respectively.

Ethanolic extraction yields of MSK is given in table 1. After extraction, MSK provided higher yield (16.0± 0.57%). The variation in the yields of plant organs might be ascribed to the different availability of extractable components, resulting from the different chemical composition of plants. Similar results were achieved for MSK through the researches of (Arogba, 2015; Namngam *et al*., 2018 and Melo *et al*., 2019).

HPLC analysis of MSK methanolic extract

High performance liquid chromatography (HPLC) was used to identify and quantified the phenolic compounds that were present in the studied mango seed kernel methanolic extract and the results are illustrated in Fig. 1. From which it is apparent that, the components assayed for mango seed kernel methanolic extract (according to their retention times), were as follows: 3. 897- chlorogenic (14.64%), 5.630- methyl gallate (29.00%), 6.464- syringic acid (5.35%), 7.267 - rutin (12.81%),8.141- ellagic (5.61%), 9.797- vanillic 12.56%), 10.010- ferulic (8.58%) and 10.334- naringenin (11.45%) were positively identified in the present study by HPLC analytical system.

The HPLC chromatogram Fig. 1 also reveal that the dominant phenolic compound was methyl gallate (29.00%), while the peak produced for syringic acid (5.35%) was low which indicated that it was found in a small quantities. Such results are in close agreement with those reported by other authors authors (El-Kady *et al.,* 2016 and Abdel-Aty *et al.,* 2018).

Effect of MSKE and SMS on melanosis formation

Melanosis scores for white shrimp with and without treatments of SMS, 1 and 2% MSKE during the

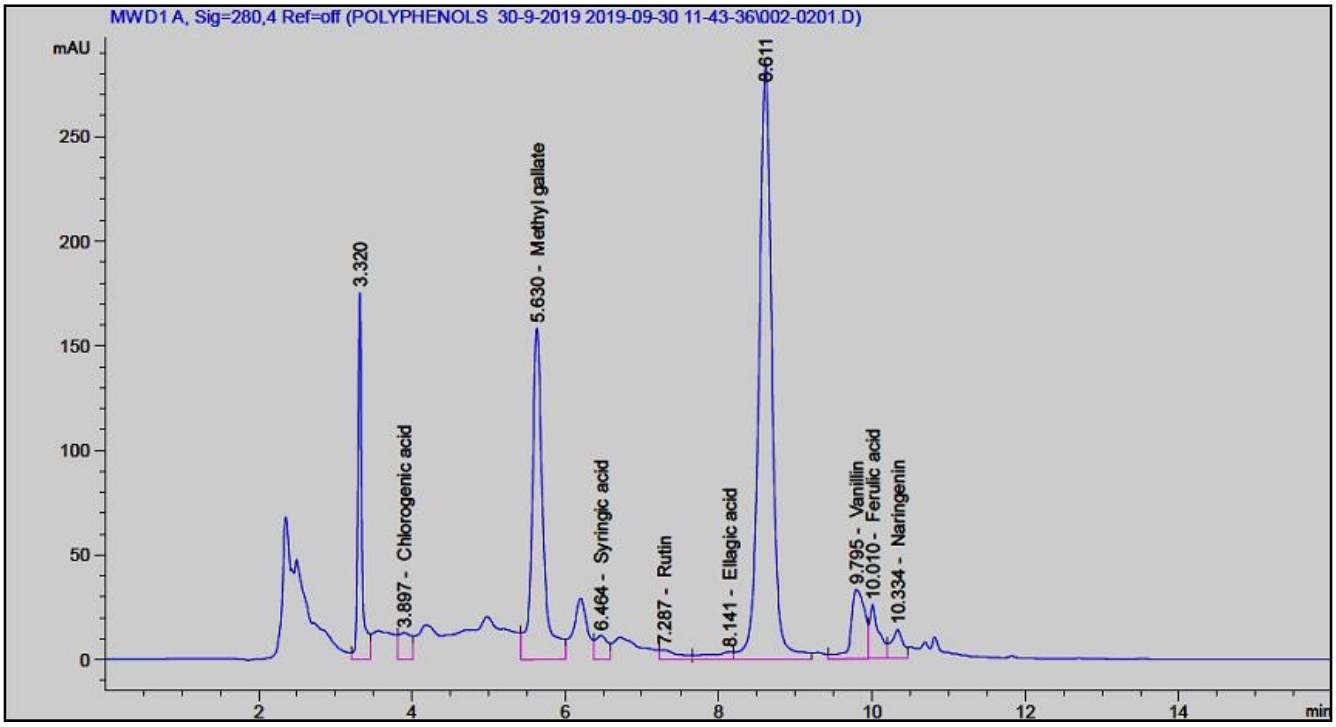

Fig. 1: HPLC chromatogram of MSKE (Retention time, Component identifications and Area%).

refrigerated storage are illustrated in Fig. 2. At day 0, all samples had no melanosis (score = 0). During 2 days of storage, treated samples showed no difference in melanosis scores ($P<0.05$). When the storage time increased, melanosis score in the shrimp samples increased ($P<0.05$), which is similar to previous studies (Sun *et al.,* 2014; Yuan *et al.,* 2016 and Sae-leaw *et al.,* 2017. Concerning melanosis scores, it is worth mentioning that a score of 4 or less indicated high quality product with minimal melanosis. A score between 4 and 10 was considered indicative of shrimp with measurable quality defects. A score of 8 or greater represented serve defects, approaching unacceptable (Sun *et al.,* 2014).

Melanosis score of shrimp was in descending order: control, 1% MSKE, SMS and 2% MSKE samples. Overall, MSKE was able to retard the melanosis in shrimps. Treatment of shrimps with 2% MSKE showed higher effectiveness in lowering melanosis, when compared with SMS treatment. During 8–10 days of storage, the lowest melanosis score was observed in the sample treated with 2% MSKE, followed by those treated with SMS and 1% MSKE, respectively while severe melanosis was found in the control Fig. 2. The melanogenesis was significantly inhibited and visual quality was significantly improved in white shrimp treated with MSKE in the present study. The retardation of melanosis formation in MSKE treated shrimp was related with high phenolic compounds, chelating activity as well as reducing power (El-Kady *et al.,* 2016 and Abdel-Aty *et al.,* 2018).

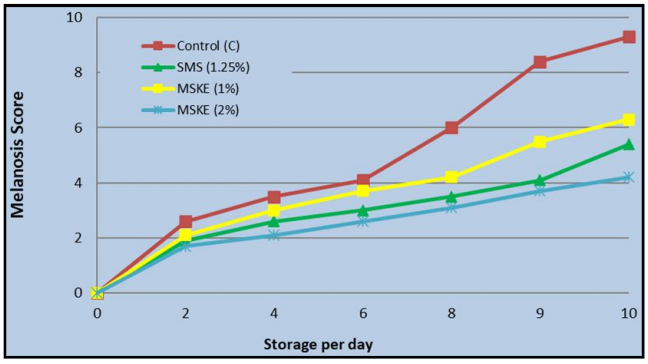

Fig. 2: Effect of MSKE and SMS on melanosis formation of white shrimp.

Phenolic compounds and plant extracts have been shown to inhibit PPO and reduce melanosis in crustaceans. Nirmal and Benjakul (2010), reported that Pacific white shrimps treated with 2% ferulic acid had the lower melanosis score after 10 days of iced storage. Pacific white shrimp treated with 0.5% ethanolic green tea extract with prior chlorophyll removal possessed the lower melanosis, compared with the control and showed similar score to those treated with SMS 0.5 or 0.75% EGCG showed the higher efficiency in retardation of melanosis in Pacific white shrimp, compared to SMS and 0.25% EGCG. The use of 1% CE for soaking whole shrimp for 30 min was able to retard the melanosis in shrimp during the refrigerated storage (Sae-leaw, and Benjakul, 2019). As a consequence, SMS could be replaced by MSKE to provide SMS-free shrimp.

Sensory evaluation:

Appearance and odor of a product can be the criteria for rejecting of any kind of food if they differed significantly from what is expected by the consumers (Chouliara *et al.*, 2007).. The sensory characteristics, appearance and odor for the samples of white shrimp during cold storage are shown in Fig. 3. It was found that, the control and

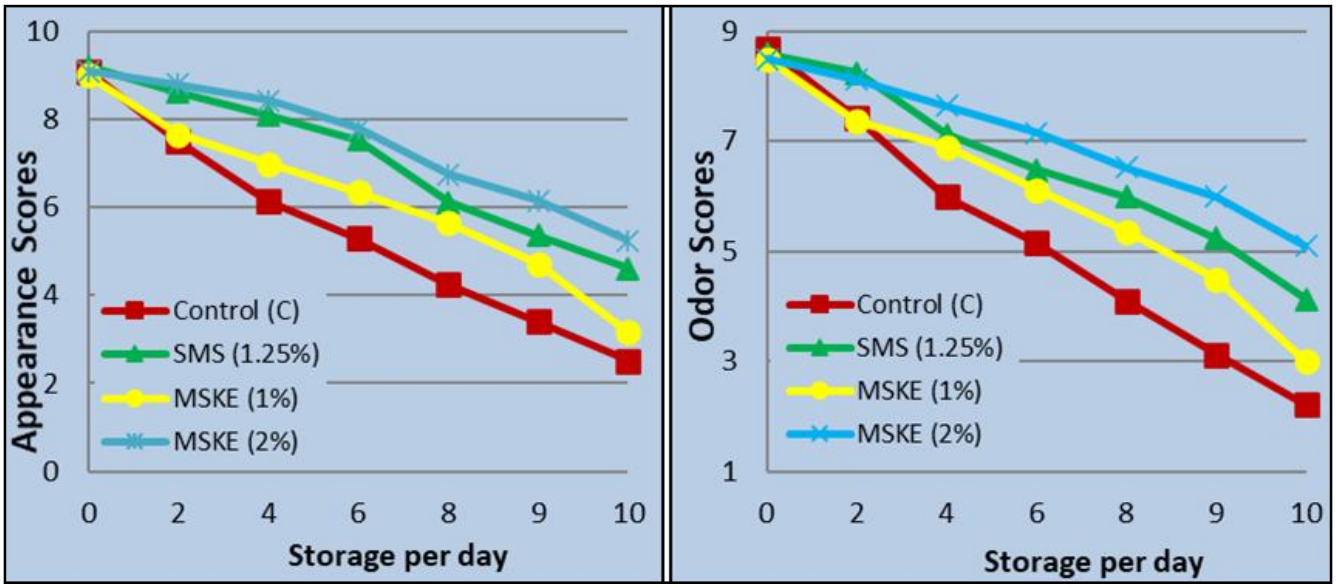

Fig. 3: Appearance and odor scores of white shrimp during cold storage.

treated shrimp samples showed excellent odor and appearance scores by panelists at zero day of evaluation, off-odor and appearance were noticed after the day 6, 8, 9 and 10 days of storage in control, 1% MSKE, SMS and 2% MSKE treated groups, respectively. In general the decrease in sensory scores was highest in the control group for all the sampling days and was significantly inhibited after treatment with SMS and MSKE for 10 days.

For the control shrimp samples, in the first two days, there were no changes for odor attribute, being fresh to sea odor. After six days, the odor of meat showed a progressive decrease, which reached from ammonia odor to the maximum score putrid. Appearance attribute, being excellent to great at the first two days of refrigerated storage, after six days, the appearance of shrimp showed a marked decrease, which reached from good appearance to the maximum score inacceptable Fig. 3. The higher scores for appearance and odor were found in shrimps treated with MSKE, SMS compared to the control ($P < 0.05$). The results were coincidental with the lower melanosis in MSKE and SMS treated samples Fig. 2. This result was also confirmed by subsequent microbiological analysis table 6. The result suggested that treatment of white shrimps with MSKE or SMS could improve the sensory properties. A similar tendency is visible in the research of (Sun *et al.,* 2014; Yuan *et al.,* 2016 and Sae-leaw and Benjakul, 2019).

Quality Indices Alterations

pH changes:

Changes in pH values in shrimp samples during cold storage are depicted in table 2, from which it is clear that the initial pH value of untreated samples (control) and treated shrimp with SMS, 1% MSKE and 2% MSKE was 7.24, 7.12, 7.18 and 7.15, respectively at zero day of refrigeration storage. The pH value of all shrimp samples slightly decreased during the first 3 days of storage, by more time of refrigeration storage pH values increased in different degrees within untreated and treated shrimp samples, with the control samples always being the highest. Similar trends of pH changes have been observed by other authors (Sun *et al.,* 2014; Yuan *et al.,* 2016 and Khodanazary, 2019).

However, the decrease of pH indicates that some fermentation occurs during storage. The last pH values increase might have been due to the liberation of ammonia compounds as a result of enzyme activity or the proteolytic microbial flora present in the raw shrimp (López-Caballero *et al.,* 2007 and Sriket *et al.,* 2007). However, the rise of the pH of shrimp was significantly inhibited by different concentration of MSKE treatment in the present study, similar with the effect of green tea extract, pomegranate peel extract and cinnamaldehyde (Gomez-Guillen *et al*., 2005; Nirmal and Benjakul, 2010 and Sun *et al.,* 2014). Generally, a pH value of 7.8 is reported to be a critical value in determining the acceptability of shrimp (Cobb *et al.,* 1976 and Sundararajan, 2010). Considering these studies, control, 1% MSKE, SMS and 2%MSKE treated shrimp in this study had an acceptable and good quality with regard to pH index to 6, 8, 9 and 10 days at refrigerated storage.

Total volatile basic nitrogen (TVB-N) changes

Changes in TVB-N values are presented in table 2 The increase in TVB-N is related to the activity of

Table 2: Quality indices changes of raw white shrimp during cold storage for 10 days.

Treatment/Day		0	3	6	8	9	10
Control (C)	pH Value	7.24 ± 0.18^{a}	6.98 ± 0.10^{a}	7.42 ± 0.14^{a}	7.92 ± 0.10^{a}	8.16 ± 0.11^{a}	8.32 ± 0.16^{a}
SMS(1.25%)		7.12 ± 0.14^{a}	6.79 ± 0.17^{a}	7.14 ± 0.13^{c}	7.34 ± 0.12^{c}	7.68 ± 0.19^{c}	7.96 ± 0.12^{c}
MSKE (1%)		7.18 ± 0.21^{a}	6.90 ± 0.23^{a}	7.32 ± 0.18^{b}	7.76 ± 0.15^{b}	7.92 ± 0.28^{b}	8.12 ± 0.14^{b}
MSKE (2%)		7.15 ± 0.10^{a}	6.86 ± 0.28^{a}	7.08 ± 0.15^{d}	7.17 ± 0.24^{d}	7.42 ± 0.13^{d}	7.71 ± 0.10^{d}
Control (C)	TVB-N	16.40 ± 0.18^{a}	21.32 ± 0.13^{a}	30.20 ± 0.17^{a}	38.00 ± 0.24^{a}	46.90 ± 0.11^{a}	52.70 ± 0.17^{a}
SMS(1.25%)		13.70 ± 0.12^{c}	15.85 ± 0.16^{c}	21.80 ± 0.13^{c}	27.65 ± 0.18^{c}	30.10 ± 0.10^{c}	34.20 ± 0.12^{c}
MSKE (1%)		14.15 ± 0.21^{b}	19.14 ± 0.13^{b}	25.50 ± 0.11^{b} 25 ± 0.11^{b}	29.34 ± 0.21^{b}	36.78 ± 0.08^{b}	42.85 ± 0.14^{b}
MSKE (2%)		12.65 ± 0.14^{d}	16.50 ± 0.17^{d}	19.56 ± 0.10^{d}	24.30 ± 0.15^{d}	27.85 ± 0.19^{d}	30.00 ± 0.23^{d}
Control (C)	TBARS	1.04 ± 0.17^{a}	1.36 ± 0.12^{a}	1.78 ± 0.16^{a}	2.36 ± 0.10^{a}	3.00 ± 0.17^{a}	3.76 ± 0.14^{a}
SMS(1.25%)		0.90 ± 0.14^{a}	1.15 ± 0.10^{b}	1.45 ± 0.11^{c}	1.87 ± 0.12^{c}	2.03 ± 0.14^{c}	2.57 ± 0.12^{c}
MSKE (1%)		0.98 ± 0.15^{a}	1.23 ± 0.13^{b}	1.66 ± 0.14^{b}	2.08 ± 0.18^{b}	2.85 ± 0.08^{b}	3.25 ± 0.10^{b}
MSKE (2%)		0.94 ± 0.21^{a}	1.14 ± 0.11^{b}	1.27 ± 0.10^{d}	1.62 ± 0.13^{d}	1.84 ± 0.12^{d}	2.08 ± 0.11^{d}

All values reflect the mean and standard deviation (SD), are mean of triplicate determinations. Mean values in the same column bearing the same superscript do not differ significantly ($P<0.05$). Total volatile basic nitrogen (TVBN, as mg N/100g shrimp). Thiobarbituric acid reactive substances (TBARS, as mgMDA/kg shrimp). – SMS: Sodium metabisulphite, MSKE: Mango seed kernel extract (1, 2%).

spoilage bacteria and endogenous enzymes, which impart the characteristic unpleasant "fishy" odor (Castro *et al.,* 2012). As shown in table 5, the TVB-N content was initially higher in control shrimp as compared to treated shrimp, because of the effect of SMS and MSKE. During cold storage TVB-N increased steadily with a higher rate ($P<0.05$) in control samples than in treated samples for all the sampling days table 5. These results confirmed the findings obtained by (Sun *et al.,* 2014; Yuan *et al.,* 2016 and Khodanazary, 2019).

As can be noticed from the same results of table 2, samples pre-treated with SMS and MSKE exhibit less protein deterioration as evident from lower TVBN contents than control shrimp throughout the ability of SMS and MSKE to reduce microbial load, thereby decreasing protein breakdown and hence lower values of TVBN were formed (Rajkowski and Summers, 2012).

Generally, it is worth mentioning that a level of 30 mg N/100g flesh for TVBN is usually regarded as the limit beyond which seafood will develop an objectionable odor/taste (Cobb *et al.,* 1976 and ES, (5021/2017). Consequently, control samples remained acceptable with regard to TVB-N index for six days in comparison to 8, 9 and 10 days at 4±1°C for 1% MSKE, SMS and 2% MSKE treated shrimp, respectively. The above results agree with those of previous studies decreased (Nirmal and Benjakul, 2010 and Sun *et al.,* 2014), which reported that the TVB-N values of white shrimp treated with ferulic acid, cinnamaldehyde and grape seed extract were significantly decreased.

TBARS Changes

Display of data demonstrated in table 2, it is obvious that at the beginning of the storage period TBARS values of control, 1% MSKE, SMS and 2% MSKE treated shrimp were determined as 1.04, 0.98, 0.90 and 0.94 mg MDA/kg flesh; respectively. The outcome was in agreement with the results for white shrimp (Ali, 2011 and Sun *et al.,* 2014). As can be seen from the results of the present study table 2 there is a trend towards an increase in TBARS values during the storage period, with the control always being the highest followed by 1% MSKE, SMS and 2% MSKE, respectively. Thus, the ability of MSKE in prevention of lipid oxidation was dependent upon the concentration. The rise of TBARS content may be due to the partial dehydration of seafood and interacting lipids with air oxygen (Kilincceker *et al.,* 2009). These results confirmed the findings of other researchers (Sun *et al.,* 2014; Yuan *et al.,* 2016; Khodanazary, 2019 and Sae-leaw and Benjakul, 2019).

Phenolic compounds in MSKE more likely acted as antioxidants, which could provide electron or H atom to radical (Nilsuwan *et al.,* 2018). Additionally, the retardation of lipid oxidation of MSKE treated shrimps might be attributed to the metal chelation of MSKE. In general, the compounds with metal chelating property, known as the secondary antioxidant, could help retard the initiation stage of oxidation (Gordon, 2001). As a result, lipid oxidation in shrimp meat could be impeded and rancidity could be prevented. In the present study, the lowered lipid oxidation of shrimps treated with SMS and MSKE solution was in accordance with the lowered microbial growth table 3. Concerning TBARS of shrimp samples, it is worth mentioning that, seafood products of good quality will have TBARS values less than 2 mg MDA/kg, while consumption limits should be less than 5 mg MDA/Kg flesh (Goulas and Kontominas, 2007 and Moini *et al.,* 2009 and ES, (5021/2017)). In this study, TBARS value of control, 1%MSKE, SMS and 2% MSKE treated shrimp were had acceptable TBARS limit after 6, 8, 9 and 10 days of refrigerated storage.

Microbiological evaluation

The total viable count (TVC), Psychrotrophilic (PTC) count and Enterobacteriaceae count (EBC) of control and treated white shrimp during 10 days of refrigerated storage are shown in table 3. The lower initial value of TVC and PTC showing a correct handling was performed after catching and that products were microbiologically of high quality. The Gram-negative Psychrotrophic bacteria are the major group of microorganism responsible for spoilage of chilled stored seafood (Gram and Huss, 1996). In general, TVC and PTC bacteria counts increased continuously as the storage time increased ($P < 0.05$). In addition, TVC and PTC were the highest in the control group for all the sampling days, followed by 1% MSKE, SMS and 2% MSKE treated shrimp, respectively. MSKE is considered rich source of polyphenolic compounds Fig. 1 that show antioxidant and antimicrobial effects (Mutua *et al*., 2016). These results confirmed the findings reported by other authors (Gomez-Guillen *et al*., 2005; Sun *et al.,* 2014; Rahimabadi *et al.,* 2016; Nilsuwan and Prodpran, 2018 and Khodanazary, 2019).

The statistical analysis of TVC and PTC revealed significant difference ($P>0.05$) between examining shrimp samples at zero day of refrigeration storage, while the increase in the storage time produce significant proliferations in TVC and PTC ($P<0.05$), whatever the treatment conditions. The use of MSKE effectively lowered deteriorative alteration caused by psychrophilic microorganisms. However, TVC and PTC reached and exceeded a value of 6 log cfu/g, considered as the upper

Table 3: TVC, PTC and EBC (as logCFU/g) of white shrimp during cold storage for 10 days.

Treatment/Day		0	3	6	8	9	10
Control (C)	TVC	4.10±0.12^{a}	5.10±0.14^{a}	5.92±0.18^{a}	6.45±0.16^{a}	7.00±0.15^{a}	7.53±0.13^{a}
SMS(1.25%)		3.96±0.15^{a}	4.73±0.17^{c}	5.28±0.12^{c}	5.74±0.18^{c}	6.10±0.22^{c}	6.87±0.18^{c}
MSKE (1%)		4.00±0.17^{a}	4.98±0.12^{b}	5.54±0.14^{b}	6.12±0.13^{b}	6.57±0.14^{b}	7.25±0.21^{b}
MSKE (2%)		3.92±0.13^{a}	4.46±0.15^{d}	5.00±0.11^{d}	5.38±0.10^{d}	5.84±0.16^{d}	6.10±0.15^{d}
Control (C)	PTC	3.75±0.11^{a}	4.52±0.13^{a}	5.46±0.19^{a}	6.00±0.14^{a}	6.65±0.13^{a}	7.12±0.14^{a}
SMS(1.25%)		3.67±0.18^{a}	4.18±0.11^{c}	4.73±0.13^{c}	5.41±0.11^{c}	5.92±0.18^{c}	6.45±0.12^{c}
MSKE (1%)		3.71±0.21^{a}	4.40±0.14^{b}	5.15±0.10^{b}	5.70±0.17^{b}	6.28±0.12^{b}	6.73±0.10^{b}
MSKE (2%)		3.60±0.16^{a}	3.95±0.16^{d}	4.62±0.13^{d}	5.24±0.21^{d}	5.43±0.17^{d}	5.87±0.17^{d}
Control (C)	EBC	2.20±0.14^{a}	2.55±0.10^{a}	2.86±0.17^{a}	3.20±0.12^{a}	3.41±0.25^{a}	3.66±0.13^{a}
SMS(1.25%)		2.10±0.12^{a}	2.32±0.17^{c}	2.42±0.15^{c}	2.78±0.15^{c}	3.10±0,13^{c}	3.28±0.24^{c}
MSKE (1%)		2.15±0.19^{a}	2.46±0.13^{b}	2.67±0.12^{b}	3.00±0.23^{b}	3.32±0.10^{b}	3.44±0.16^{b}
MSKE (2%)		2.06±0.10^{a}	2.14±0.15^{d}	2.38±0.14^{d}	2.61±0.16^{d}	2.96±0.15^{d}	3.12±0.11^{d}

All values reflect the mean and standard deviation (SD), are mean of triplicate determinations. Mean values in the same column bearing the same superscript do not differ significantly ($P<0.05$). Total viable count (TVC), total Psychrotrophilic (TPC), Enterobacteriaceae count (EBC) – SMS: Sodium metabisulphite, MSKE: Mango seed kernel extract (1,2%).

microbiological limit for good quality shrimp, as defined by the Egyptian Standard, after the six day for the control samples, indicating a shelf life of about 6 days compared with 8, 9 and 10 days at 4±1°C for 1% MSKE, SMS and 2% MSKE treated shrimp, respectively. Similar trend of changes was reported (Okpala *et al.,* 2014; Sun *et al.,* 2014 and Gonçalves *et al.,* 2015).

Finally, Enterobacteriaceae (EBC) is considered as a hygiene indicator (Barbosa *et al.*, 2009), the results of shrimps treated with MSKE at different concentrations in comparison with the control (without treatments) and those treated with SMS are shown in Table 3. The initial Enterobacteriaceae bacterial count for fresh control, SMS, MSKE (1%), MSKE (2%), white shrimp was 2.20, 2.10, 2.15 and 2.06 log CFU/g. Compared to control, Enterobacteriaceae grew in SMS or MSKE treated samples at a slower rate and never exceeding 10^3 CFU/g. However, Enterobacteriaceae count was retarded with the treatment of MSKE. Inhibition was more pronounced with increasing concentrations ($P < 0.05$). These results are in accordance with those of (Gonçalves *et al.,* 2015 and Sae-leaw and Benjakul, 2019).

Phenolic compounds in MSKE might form complexes with proteins in the cell wall of microorganism, causing the leakage or disruption of cell wall. Furthermore, phenolics, might chelate some metal ions required for microbial growth (Gordon, 2001 and Nilsuwan and Prodpran, 2018). Therefore, microbial growth in shrimp could be retarded to some degree by the treatment with MSKE. The maximum limit for Enterobacteriaceae in foods including fishery products is 4 log CFU/g (Cobb *et al.,* 1976 and Gram and Huss, 1996). It was noted that shrimps treated with 1 and 2% MSKE and those treated with SMS had the Enterobacteriaceae counts below the maximum limit after 10 days of refrigerated storage.

Heleno *et al*., (2015) reported that phenolic acids such as protocatechuic, vanillic ferulic and caffeic acids could be used as antimicrobial agents because of the presence of carboxylic acid (COOH), two hydroxyl (OH) groups in *para* and *ortho* positions of the benzene ring and also a methoxyl (OCH3) group in the *meta* position.

Conclusions

It may be concluded that, mango seed kernel which represent about 9 per cent of total fruit weight and treated as processing waste, can be enhanced the chemical indices, microbial status and sensory scores of white shrimp (*Penaeus* spp.). Shrimps treated with 2% MSKE solution had the lower microbial growth, pH, TVB-N content, TBARS value and melanosis score throughout the refrigerated storage for 10 days. Furthermore, treatment of shrimp with MSKE could improve sensory properties of shrimp after refrigerated storage. This led to the extended shelf-life of shrimp by 2-4 days over that of control samples. Therefore, 2% MSKE could be used as an alternative melanosis inhibitor to sulfiting agents. Additionally, it acted as natural preservative for shrimp.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Significance statements

This study demonstrates the potential use of MSKE to improve sensory characteristics, microbial quality, retard lipid oxidation, maintain the quality indices and

prevent melanosis formation of raw white shrimp (*Penaeus* spp.) during cold storage at 4±1°C for 10 days. According to microbiological, organoleptic and chemical indices analyses it was also shown that the application of such natural components extended the shelf-life of treated shrimp by 2-4 days over that of control (6 days). Therefore, they could be a good replacement for the synthetic antimicrobials and antioxidants currently used by the fish industry.

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