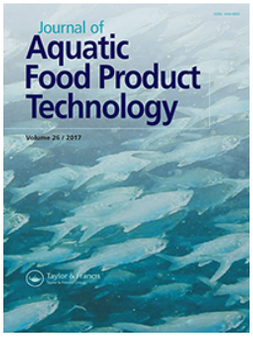

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Chilled Storage of *Pangasianodon hypophthalmus* Fillets Coated with Plant Oil Incorporated Alginate Gels: Effect of Clove Leaf, Clove Bud, Rosemary and Thyme Oils

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ABSTRACT

Essential oil incorporated alginate coating provides a novel way to improve the safety and shelf life of pangasius (*Pangasianodon hypophthalmus*) fillet. Oils from the leaves and buds of clove, flowering tops of rosemary, and dried seeds of thyme were incorporated separately in alginate coating. All the plant oils showed antibacterial activity, but the zone of inhibition was relatively larger for thyme oil. Alginate coating was performed using sodium alginate (1.5%), glycerol (10%), and calcium chloride (2%) and plant oil at 1% (v/v). The coated fillets were stored under chilled conditions and samples were analyzed for bacteriological, chemical, sensory, color, and texture parameters. Psychrotrophic counts crossed 7 log cfu/g by the 13th day and 15th day of chilled storage in control and plant oil treated fillets, respectively. The peroxide value of treated fillets was relatively low. Texture profile analysis indicated that plant oil incorporated alginate coating reduced the rate of loss of texture (softening) during chilled storage. Plant essential oil incorporated alginate gels were relatively better compared to control fillets in preserving pangasius fillet quality during chilled storage, and incorporation of thyme oil was relatively better compared to clove leaf oil, clove bud oil, and rosemary oils.

KEYWORDS

pangasius; alginate gel; clove leaf oil; clove bud oil; rosemary oil; thyme oil

Introduction

Coating foods with edible materials has been researched as an effective method to improve the food quality (Matuska et al., 2006). Many studies have shown that edible coatings made of proteins, polysaccharide, and oil-containing materials help to prolong the shelf life and preserve the quality of fish (Artharn et al., 2009; Fan et al., 2009). Alginate is a salt of alginic acid, a polymer of D-mannuronic acid and L-guluronic acid, and is isolated from brown algae (Lu et al., 2009). Coating fish, shrimp, scallop, and pork with sodium alginate showed that it can prolong their shelf life, reduce thawing loss, cooking loss, and weight loss, and maintain the functional properties during frozen storage (Wanstedt et al., 1981; Wang et al., 1994; Yu et al., 2008; Zeng and Xu, 1997). A few antimicrobial agents and antioxidants have been incorporated into edible coatings to suppress quality changes during storage (Chidanandaiah et al., 2009; Fan et al., 2008). Alginate-based edible coating containing vitamin C and tea polyphenols extended the shelf life of bream (Song et al., 2011). The coatings may serve as carriers for antimicrobial compounds and antioxidants in order to maintain high concentrations of preservatives on the surface of foods. Consumer preference for minimally processed food products poses challenges for ensuring food safety and has augmented the interest in non-toxic antimicrobial compounds to protect the food from contamination and the consumer against infection.

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Health consciousness is the rationale for the use of natural ingredients instead of synthetic preservatives in foods (Gennadios et al., 1997). Plant essential oils are volatile extracts that are commercially produced by steam distillation from flowers, buds, roots, bark, and leaves of aromatic and medicinal plants and have shown greater potential as antimicrobial agents against spoilage and pathogenic microorganisms (Van de Braak and Leijten, 1999; Jayasena and Jo, 2013). A number of plant essential oil components have been identified as effective antibacterials, e.g. carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde, and cinnamic acid, having minimum inhibitory concentrations (MICs) of 0.05 to 5 μl ml^{-1} *in vitro* but need a higher concentration to achieve the same effect in foods (Burt, 2004). Moreover, the positive influence of essential oils on the sensory attributes of foods makes them suitable candidates for incorporation in foods that are traditionally associated with herbs and spices (Mehlholm and Dalgaard, 2002; Harpaz et al., 2003; Burt, 2004). Plant essential oils are considered as Generally Recognized As Safe (GRAS). Sodium alginate (E 401), glycerol (E 422), and calcium chloride (E 509) are food additives generally permitted for use in foods. A combination of all these as essential oil incorporated alginate gel provides a novel way to improve the safety and shelf life of fish fillets.

Pangasianodon hypophthalmus is a fast growing fish species that is being widely farmed in India. Pangasius can easily be filleted due to the absence of intra-muscular pin bones. Pangasius meat has high nutritive qualities and excellent sensory properties making it a suitable candidate species for development of convenience products. The microbial activity is responsible for changes in flavor, odor, texture, and color that reflect the extent of post-harvest fish decomposition. Keeping the fish under chilled conditions (<4°C) from the time of harvest to cooking is generally the preferred practice for retarding spoilage of fish. Rao et al. (2013) reported that pangasius fillet could be stored for a period of 9 days under chilled conditions (<4°C). The present study intended to study the effect of essential oil incorporated alginate film coating on the microbiological and chemical quality and textural and color properties of pangasius fillet during chilled storage.

Materials and methods

P. hypophthalmus *fish fillets*

P. hypophthalmus (mean weight 1470 ± 330g) were procured from a wholesale fish market, Visakhapatnam, under fresh conditions and immediately brought to the laboratory under chilled conditions (<4°C). The fish were manually filleted on a stainless steel flat surface using sharp knives. The fish were beheaded, gutted, gilled, and finally washed thoroughly with 2ppm chlorinated water. The skin of the dressed fish was first removed by making a length-wise cut and pulling the skin manually. The exposed flesh of the fish was then cut parallel to the central bone frame, and hanging meat was trimmed off. Fish fillets (mean weight 243 ± 52g) (skinless, boneless, fish loin pieces) were maintained under chilled conditions (<4°C) and used for the experiment.

Plant essential oils

Plant oils produced commercially (Kancor Ingredients Limited, Kerala, India) by the steam distillation process were tested for antibacterial activity. Oils from the leaves and buds of clove (*Eugenia caryophyllata*), flowering tops of rosemary (*Rosemarinus officianalis*), and dried seeds of thyme (*Thymus vulgaris*) were used in the study (Table 1).

Antibacterial activity of plant oils

The antibacterial activity of the plant essential oils was tested by the agar well test (Dorman and Deans, 2000) employing Mueller–Hinton (MH) agar and tested against *Salmonella typhimurium, Morganella morganii, Escherichia coli, Vibrio cholerae* O1, *V. cholerae* O139, *V. parahaemolyticus,*

Table 1. Details of plant essential oils used.

	Clove leaf oil	Clove bud oil	Rosemary oil	Thyme oil
Source of oil	Leaves	Dried unopened flower buds	Fresh flowering tops	Dried seeds
Extraction process	Steam distillation	Steam distillation	Steam distillation	Steam distillation
Specific gravity at 25°C	1.0436	1.0410	0.9140	0.8824
Refractive index at 20°C	1.5334	1.5304	1.4700	1.4890
Optical rotation	0°	0°	+2°	+1°

Staphylococcus aureus, and *Listeria monocytogenes*. MH agar supplemented with 1% salt was used for testing *V. parahaemolyticus*. Four wells (6 mm diameter) were made on pre-dried MH agar plates, and the well bottoms were sealed with sterile MH agar. Then, 100 µl of the bacterial culture was spread plated, and the inoculum was allowed to dry for 10–15 min. Twenty microliters of clove leaf oil, clove bud oil, rosemary oil, and thyme oil (undiluted) were dispensed into each well using a micropipette and incubated at 37°C for 16–18h. The zones of inhibition were measured and recorded for each essential oil against the specific bacterium.

Plant oil incorporated alginate coating of pangasius fillet

Four plant essential oils (clove leaf oil, clove bud oil, rosemary oil, and thyme oil) were used at the 1% v/v level. Pangasius fillets were divided into six batches: C1, C2-A, T1-CL, T2-CB, T3-RM, and T4-TY; where C1 and C2-A are control fillets and T1-CL, T2-CB, T3-RM, and T4-TY correspond to different treatments. Alginate coating was performed as per Song et al. (2011) using sodium alginate, glycerol, and calcium chloride. Sodium alginate solution (3% w/v) was prepared in distilled water and stirred at a controlled temperature of 80°C and cooled to ambient temperature and mixed with an equal quantity of chilled water containing 20% glycerol (v/v). Plant essential oil was added to a final concentration of 1% (v/v). Calcium chloride (2%) solution was prepared with chilled water.
The control and treated fillets were packed individually in pouches (12µ polyester laminated with 300 gauge low density polyethylene) and stored under chilled conditions by placing them in insulated boxes containing an adequate quantity of ice to maintain the temperature of chilled fillets at less than 4°C. After draining off the melted ice, re-icing was done every day to supplement the loss due to melting. The fillets were taken out at regular intervals on the first day and after 1 day, 3 days, 6 days, 9 days, 13 days, and 15 days of storage and analyzed for bacteriological, chemical, sensory, color, and texture parameters.

Chemical, bacteriological, and sensory analysis

Pangasius fillet samples were homogenized in Butterfield's phosphate-buffered water (1:10) in a Stomacher blender and analyzed for different microbiological parameters by pour plating. Aerobic plate count (APC) and psychrotrophic count was determined as per standard methods (APHA, 1976; BAM, 2011). Hydrogen sulfide (H_2S) producing bacteria were determined using peptone iron agar

C1	corresponds to control fillet (fillets were placed in chilled water for 1 min and drained for 1 min);
C2-A	corresponds to alginate-control (fillets were dipped in alginate solution for 30 sec, drained for 1 min followed by dipping in $CaCl_2$ solution and drained for 1 min);
T1-CL	fillets were dipped in alginate solution containing clove leaf oil (1%) for 30 sec, drained for 1 min followed by dipping in $CaCl_2$ solution and drained for 1 min;
T2-CB	fillets were dipped in alginate solution containing clove bud oil (1%) for 30 sec, drained for 1 min followed by dipping in $CaCl_2$ solution and drained for 1 min;
T3-RM	fillets were dipped in alginate solution containing rosemary oil (1%) for 30 sec, drained for 1 min followed by dipping in $CaCl_2$ solution and drained for 1 min;
T4-TY	fillets were dipped in alginate solution containing thyme oil (1%) for 30 sec, drained for 1 min followed by dipping in $CaCl_2$ solution and drained for 1 min.

(Gram et al., 1987). Moisture was determined as per standard methods (AOAC, 1990). Peroxide value (PV) was determined iodometrically (Method # 965.33 AOAC, 1990), and total volatile base nitrogen (TVBN) was determined by the Conway micro diffusion method (Conway, 1950).

Sensory analysis was performed on each sampling day. Uniform pieces from each fillet sample were cooked in boiling water for 5 min and scored by the panelists. The control and treated fillets were evaluated in terms of color, odor, flavor, and texture using a 9-point hedonic scale (1: dislike extremely to 9: like extremely) parameter wise, and the mean value of all the parameters was computed as overall acceptability (Amerine et al., 1965). A score of below 4 was considered as 'rejected'.

Color measurements and texture profile analysis

The L* (lightness) and a* (positive value-red; negative value-green) values of the fillets were measured using Hunter's colorimeter (ColorFlex EZ, Hunter Lab, Reston, VA, USA). Texture profile analysis (TPA) was performed at room temperature employing a food texture analyzer (Lloyd Instruments, AMETEK Ltd., Bognor Regis, UK) as per Anderson et al. (1994) equipped with a load cell of 50N. Texture measurements were performed on uniform raw fish fillet pieces of 2 cm^3, compressed twice by a cylindrical probe having a diameter of 50 mm and a test speed of 12 mm/min. From the force time curve, parameters such as hardness1 (N), hardness2 (N), cohesiveness, springiness (mm), springiness index, chewiness (Nmm), adhesiveness (kgf.mm), and stiffness (kgf/mm) were determined.

Statistical analysis

Mean and standard deviation was calculated, and the data was analyzed employing IBM SPSS software version 20 (SPSS Inc., Chicago, IL, USA) by one-way analysis of variance (ANOVA) using Post-hoc Tukey HSD to test the difference of means at 0.05 level of significance.

Results and discussion

Antibacterial activity of plant oils

All the plant oils showed antibacterial activity against the Gram negative bacteria (*Salmonella Typhimurium, Morganella morganii, Escherichia coli, Vibrio cholerae* O1, *V. cholerae* O139, *V. parahaemolyticus)* and Gram positive bacteria *(Staphylococcus aureus* and *Listeria monocytogenes)*, but the zone of inhibition was relatively larger for thyme oil (34.4 ± 8.4 mm) compared to rosemary (23.4 ± 12 mm), clove bud (23 ± 6.7), and clove leaf (22.4 ± 7.3mm) essential oils (Table 2). *V. cholerae-O1* (30.5 ± 5.3), *V. choleare-O139 (*31.5 ± 6.8), and *L. monocytogenes* (33.3 ± 8.3) were found to be relatively more sensitive to plant essential oils. The gram positive bacteria, *S. aureus* and *L. monocytogenes*, were relatively more sensitive to rosemary and thyme oils. Thyme oil yielded relatively higher inhibition zones against both gram positive (40 mm) and gram negative bacteria (32.5mm).

Essential oils such as oregano, thyme, cinnamon, and clove have shown antibacterial activity (Dorman and Deans, 2000; Friedman et al., 2002; Burt, 2004). The antibacterial activity of essential oils was attributed to saponins, flavonoids, carvacrol, thymol, eugenol, terpenes, etc. Moreover, the hydrophobicity of plant essential oils enables them to partition in the lipids of the cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents (Burt, 2004). Generally, Gram-negative bacteria are more resistant to the plant-origin antimicrobials, because of the lipopolysaccharide outer membrane that restricts diffusion of hydrophobic compounds. The major components of clove oil are eugenol 75–85% and eugenyl acetate 8–15% (Bauer et al., 2001); major components of rosemary oil are α-pinene (2–25%), bornyl acetate 0–17%, and camphor (2–

Table 2. Antibacterial effect of plant essential oils.

	Clove leaf oil	Clove bud oil	Rosemary oil	Thyme oil	Plant essential oils (mean ± SD)
	Zone of inhibition (mm)				
Salmonella typhimurium, ATCC 51812	14.3 ± 0.6^{a}	15.3 ± 0.6^{ac}	9.00 ± 1.7^{b}	17.33 ± 0.6^{c}	13.8 ± 3.4
Escherichia coli	18.3 ± 0.6^{a}	20.00 ± 1.00^{a}	20.00 ± 1.00^{a}	33.3 ± 2.3^{b}	22.8 ± 6.9
Morganella morganii, ATCC 25829	18.00 ± 1.00^{a}	15.33 ± 0.6^{b}	10.00 ± 1.00^{c}	27.00 ± 1.00^{d}	17.5 ± 7.1
Vibrio cholerae O1, MTCC 3904	28.3 ± 1.5^{ab}	30.00 ± 1.7^{b}	26.00 ± 1.7^{a}	38.3 ± 0.6^{c}	30.5 ± 5.3
V. cholerae O139, MTCC 3906	34.3 ± 0.6^{b}	26.3 ± 1.5^{a}	26.7 ± 1.5^{a}	39.7 ± 0.6^{c}	31.5 ± 6.8
V. parahaemolyticus, ATCC 17802	28.7 ± 2.1^{b}	30.7 ± 1.2^{b}	16.3 ± 0.6^{a}	40.00 ± 0.00 ^{c}	28.8 ± 9.8
Staphylococcus aureus, ATCC 11632	15.00 ± 1.00^{a}	17.7 ± 1.5^{b}	40.00 ± 0.00^{c}	40.00 ± 0.00^{c}	28.3 ± 13.6
Listeria monocytogenes, ATCC 13932	23.3 ± 0.6^{a}	30.3 ± 0.6^{b}	40.00 ± 0.00^{c}	40.00 ± 0.00^{c}	33.3 ± 8.3
Gram –ve bacteria (Mean)	23.5	22.7	17.8	32.5	
Gram +ve bacteria (Mean)	19	24	40	40	

Results are Mean ± SD of triplicate determinations.
Values within the row with different superscript letters are significantly different ($p < 0.05$).

14%) (Pintore et al., 2002; Daferera et al., 2003); and major components of thyme oil are thymol 10–64%, carvacrol 2–11%, γ-terpinene, and p-cymene (Lens-Lisbonne et al., 1987; McGimpsey et al., 1994; Cosentino et al., 1999; Marino et al., 1999; Daferera et al., 2000; Juliano et al., 2000). Carvacrol and thymol are able to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP (Burt, 2004).

Effect of plant essential oil on alginate gel formation

The solution containing sodium alginate (1.5%) and glycerol (10%) formed a gel instantaneously when calcium chloride (2%) was added. Incorporation of plant essential oil up to 1.3% v/v (maximum concentration tested) in the final volume of alginate-glycerol solution did not affect the gel formation ability. Chilled water was used to make up a final volume of essential oil incorporated alginate solution and calcium chloride solutions. Gel formation was evident even at low temperature (<4°C), suggesting the suitably for application on fillets under chilled conditions.

Changes in bacterial counts during chilled storage of pangasius fillets coated with plant oil incorporated alginate gels

The APC of plant oil (1% v/v) treated fillets (T1-CL, T2-CB, T3-RM, T4-TY) was lower than the control fillets (C1 and C2-A) but the APC values were relatively lower in thyme oil treated fillets (Figure 1a). The initial reduction in APC of C1, C2-A, and T1-CL fillets was observed up to the end of the 3rd day of chilled storage, and thereafter the APC values showed a steady increasing trend. T2-CB and T4-TY showed a steady increasing trend in APC only after the end of the 6th day of chilled storage. APC of the T3-RM decreased until the 9th day of chilled storage, but the APC values were higher than those of T4-TY trial. The antibacterial activity was relatively higher for thyme oil. The APC on the 13th day was in the order C1> C2-A> T3-RM> T1-CL> T2-CB> T4-TY, with values of 5.08, 4.9, 4.8, 4.4, 4.2, and 4.0 log cfu/g, respectively. However, both control and treated samples had APC values of less than 5 x 10^{5} cfu/g, the maximum acceptable limit (FSSAI, 2011), even at the end of the 15th day of chilled storage. Analysis of the samples for bacteria capable of multiplication at lower temperature (<4°C) might provide better inference of the quality of chill stored products. Psychrotrophic counts showed an initial reduction up to the first day for C1, C2-A, and T2-CB and

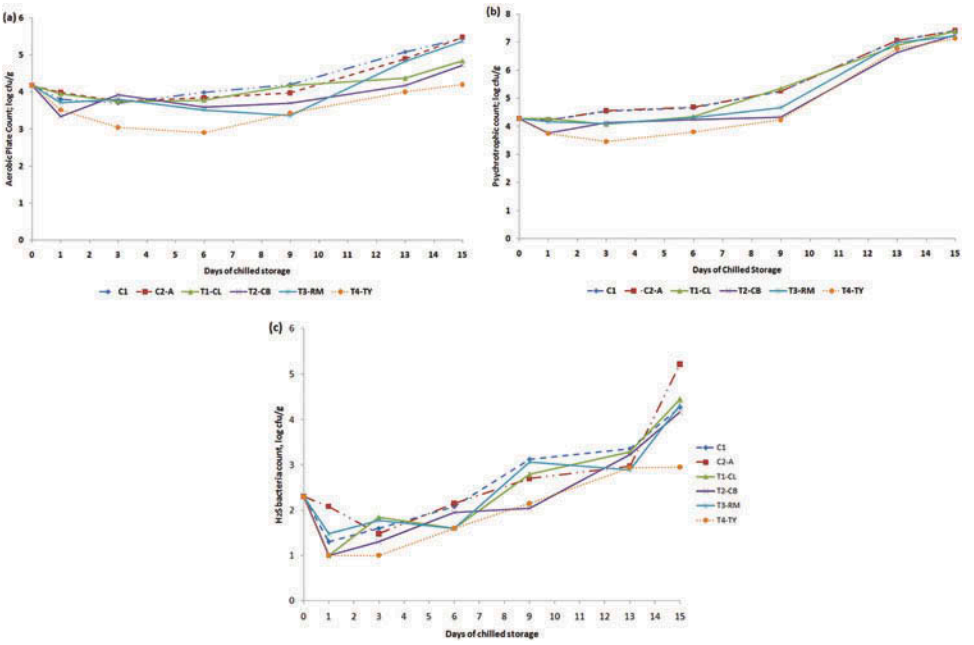

Figure 1. Changes in the bacterial counts of pangasius fillets coated with plant oil incorporated alginate gels. (a) Changes in aerobic plate counts. (b) Changes in the psychrotrophic counts. (c) Changes in H_2S producing bacteria counts.

up to third day for T1-CL, T3-RM, and T4-TY, followed by steady increase and crossed 7 log cfu/g by the 13th day in control fillet samples (C1 and C2-A); whereas the plant oil treated T1-CL, T2-CB, T3-RM, and T4-TY fillets crossed 7 log cfu/g (ICMSF, 1986) on the 15th day of chilled storage (Figure 1b), indicating that the shelf life was 9 days for control fillets and 13 days for the essential oil incorporated alginate coated fillets. Hydrogen sulfide producing bacteria tolerate low temperatures and are extremely important in spoilage of proteinaceous foods during chilled storage. H_2S producing bacteria showed an increasing trend in all samples during chilled storage, but the increase was slower in thyme oil (T4-TY) treated fillets (Figure 1c). The reduction in APC of T1-CL, T2-CB, T3-RM, and T4-TY may be attributed mainly to the antimicrobial activity of clove oil, rosemary oil, and thyme oil. The antibacterial activity of essential oil is due to saponins, flavonoids, carvacrol, thymol, eugenol, terpenes, etc. (Burt, 2004). Moreover, the results indicate that physical structure of the coating has not limited the antibacterial activity of the essential oil. Initial decrease in APC during the first 3 days of chilled storage of gutted and whole pacu has been reported (Murthy et al., 2015). Non-sterile foods that rely on refrigeration for shelf life are subject to spoilage by psychrotrophic bacteria, and the presence of large numbers of psychrotrophic bacteria in refrigerated seafood may reflect growth of the initial population during storage (Gilliland et al., 1976). Psychrotrophs can grow at 0 to 7°C even though they have optima between 20 and 30°C (Prescott et al., 2005). Song et al. (2011) observed that APC of bream fillets during refrigerated storage increased after 4 days and exceeded the maximum acceptable level of 7.0 log CFU/g (ICMSF, 1986) after 15 days of refrigerated storage, whereas the APC of vitamin C and tea polyphenol incorporated alginate coated bream fillet reached 5.54 and 5.63 log CFU/g, respectively, on day 21. The increase in total viable count and total psychrotrophic count during chilled storage of bighead carp fillets coated with sodium alginate-horsemint essential oil coating was slower than the uncoated control fillets (Heyadri et al., 2015). Similarly, Ozyurt et al. (2015) also observed that bacteria grew more quickly in uncoated fillets than in fillets with protein-based coating.

Changes in chemical parameters during chilled storage of pangasius fillets coated with plant oil incorporated alginate gels

The peroxide value of control fillet (C1) crossed 10 meq/kg fat by the 13th day and increased to 15 meq/kg fat by the 15th day of chilled storage (Figure 2a). The alginate coated control fillets (C2-A) had a PV of 6.95 meq/kg fat at the end of the 15th day. Thyme oil (4.5 meq/kg fat), clove bud oil (5.18 meq/kg fat), and clove leaf oil (5.2 meq/kg fat) treated pangasius fillets showed relatively lower PV values at the end of 15 days of chilled storage. The PV values were within acceptable limits (Connell, 1975) for essential oil incorporated alginate fillets even at the end of 15 days of chilled storage. Coating of pangasius fillet with only alginate gel (C2-A) resulted in relatively reduced lipid oxidation, but essential oil incorporation into the alginate gel provided further protection from lipid oxidation due to well-established antioxidant properties in the essential oil. The results show that the hydroperoxides formed in control fish fillet might have been subsequently oxidized to secondary compounds at a faster rate compared to plant oil incorporated alginate coated fillets. Alginate coating might have acted as a barrier preventing the exposure of the fillet surface to air, and the plant oil might have imparted antioxidation properties. TVBN values did not show any particular trend, but all the values were less than 35 mg/100 g (EC, 1995) at the end of the 15th day of chilled storage (Figure 2b). Moisture content of pangasius fillets increased during chilled storage. The moisture values ranged between 76% and 80%. Immediately after treatment, it was observed that the moisture content of alginate coated pangasius fillets (76.35 to 78.43%) was higher than uncoated fillets (76.03%). Song et al. (2011) observed that moisture in uncoated bream fish (gutted) evaporated much more rapidly compared to vitamin C and tea polyphenol incorporated alginate coated bream fish.

Results of sensory evaluation of essential oil treated fillets showed that among the treated samples, rosemary oil treated fillets rated higher for sensory evaluation, followed by thyme oil treated fillets samples. The overall acceptability of C1 sample rated lowest during sampling days. The control fillets C1 and C2-A crossed the acceptable limit on day 13 of storage. Scores of all the treated samples reached below 4 on day 15 of storage (Figure 3). Off-odors, discoloration, and texture softening limited the overall acceptability of pangasius fillets during chilled storage. The relatively better sensory quality of essential oil treated fillets is attributed to relatively lower APC, psychrotrophic bacteria, H_2S producing bacteria, and lower lipid oxidation in the treated fillets compared to the control fillets. Sensory analysis correlated well with microbiological analysis of coated and uncoated fish fillets (Andevari and Rezai, 2011; Ozyurt et al., 2015). Strong essential oil odor was noticed in rosemary and clove leaf oil treated fillets. In general, the sensory acceptability of the samples over the entire storage period followed the order T3-RM>T4-TY>T1-CL>T2-CB>C2-A>C1.

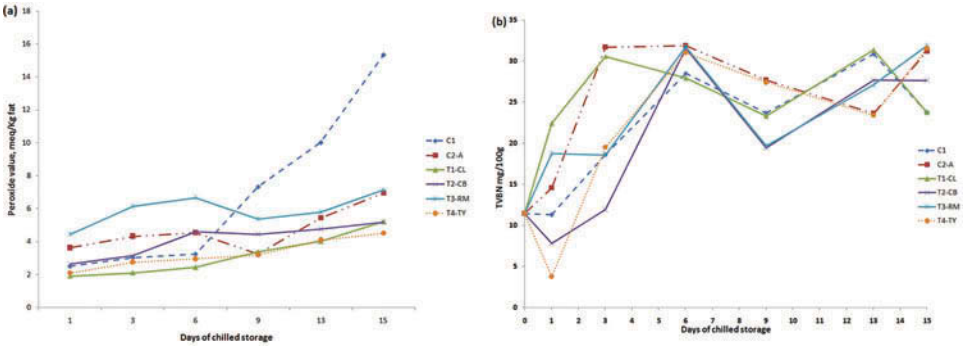

Figure 2. Changes in chemical parameters of pangasius fillets coated with plant oil incorporated alginate gels. (a) Peroxide value. (b) Total volatile base nitrogen.

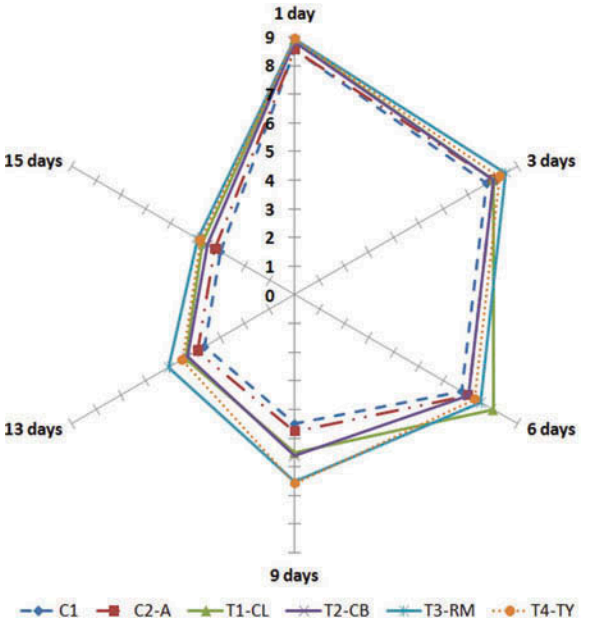

Figure 3. Changes in the sensory quality of pangasius fillets coated with plant oil incorporated alginate gels.

Changes in the color and texture parameters during chilled storage of pangasius fillets coated with plant oil incorporated alginate gels

Lightness values increased during chilled storage (from 51 to 62), but there was no appreciable difference between control and treated fillets (Figure 4a). The a* values decreased during chilled storage from 16 to 5.6, but there was no appreciable difference between control and treated fillets (Figure 4b). A similar increase in L* value with increase in chilled storage period of seabass fillets (Cheret et al., 2005), meager fillets (Hernandez et al., 2009; Genc et al., 2013), and rainbow trout fillets (Ozyurt et al., 2015) has been reported. Fish fillet color has been associated with the heme based pigment, physical structure of the muscle, and the amount of unbound water, which influences light scattering. Jouki et al. (2014) reported an increase in L* value during chilled storage (4°C) of rainbow trout fillets coated with quince seed mucilage films containing thyme and oregano oils.

Texture has been attributed as one of the most important quality factors for acceptability of a product (Di Monaco et al., 2008) and is defined as an expression of structural, mechanical, and

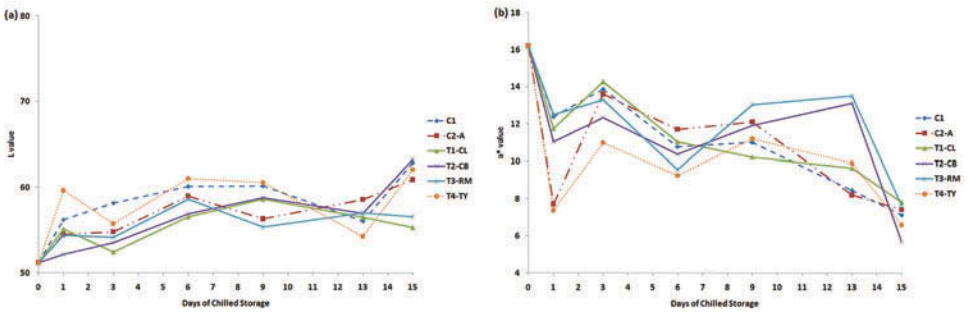

Figure 4. Changes in color parameters of pangasius fillets coated with plant oil incorporated alginate gels. (a) Changes in lightness value. (b) Changes in a* value (redness).

surface attributes detected through human senses (Szczesniak, 2002). Hardness, springiness, adhesion, and cohesion are the basic mechanical variables that characterize texture of food (Casas et al., 2006). The texture and structure of fish muscle are vital freshness quality attributes that largely depend on hardness, cohesiveness, springiness, chewiness, resilience, and adhesiveness, as well as the internal cross-linking of connective tissue and the detachment of fibers (Cheng et al., 2014). Structurally, fish muscle is comprised of alternating muscle sheets that are anchored by connective tissue. Collagen in the connective tissue plays an important role in maintaining fish fillet integrity, textural toughness, and muscle cohesiveness, but enzymes such as collagenases and proteases disrupt the structure of collagen and myofibrils (Suarez et al., 2005). Degradation of textural properties is attributed to weakening of connective tissue and loss of the Z-lines (Laksmanan and Piggott, 2003). The variation of textural properties of fish muscle depends on factors such as the structure of contractile protein, the framework of connective tissue, and lipid oxidation (Aussanasuwannakul et al., 2012). Texture loss due to enzymolysis is mainly attributed to autolytic and microbial changes (Ayala et al., 2010). Hardness 1 refers to the peak force during first compression. The texture profile analysis of pangasius fillet coated with essential oil incorporated alginate coating showed a decrease in hardness 1 (Figure 5), but the rate of decrease was lower in treated fillets (T1-CL, T2-CB, T3-RM, and T4-Ty) and alginate control (C2-A) compared to the control fillets (C). Similarly, alginate coating resulted in a relatively lower rate of decrease in springiness, springiness index, stiffness, and chewiness during chilled storage compared to the control fillets.

The TPA of fresh pangasius fillet showed hardness 1 of 36.67 ± 0.8 N, hardness 2 of 25.75 ± 3 N, springiness of 5.56 ± 0.7 mm, chewiness of 30.3 ± 12.5 Nmm, springiness index of 0.62 ± 0.2, cohesiveness of 0.15 ± 0.04, adhesive force of 0.47 ± 0.05 N, adhesiveness of 0.05 ± 0.05 kgf.mm, and stiffness of 0.89 ± 0.2 kgf/mm. On the 13^{th} day of chilled storage, the control fillets were organoleptically rejected (<4 score) and had higher psychrotrophic counts (>10^7 cfu/g), whereas the fillets that were given essential oil incorporated alginate coating were organoleptically acceptable and had relatively lower psychrotrophic counts. The TPA result at the end of 13 days of chilled storage showed that the hardness 1 of C1 (10.52 ± 4.02) was lower compared to C2-A (23.42 ± 6.0), T1-CL (24.98 ± 3.02), T2-CB (30.87 ± 8.80), T4-TY (30.92 ± 2.73), and T3-RM (34.33 ± 6.26). Stiffness was higher in essential oil incorporated alginate coated pangasius fillets (T1-CL: 2.63 ± 0.48; T2-CB: 2.57 ± 0.66; T4-TY: 2.2 ± 1.1; T3-RM: 2.06 ± 0.82) and alginate coated control (2.32 ± 0.81) fillets compared to non-coated control fillets (1.49 ± 0.31). Chewiness was higher in essential oil incorporated alginate coated pangasius fillets (T1-CL: 23.27 ± 2.69; T2-CB: 20.52 ± 4.06; T3-RM: 20.25 ± 4.36; T4-TY: 14.77 ± 3.36) and alginate coated control (14.08 ± 6.02) fillets compared to non-coated control fillets (5.25 ± 2.43). Springiness and springiness index of essential oil coated pangasius fillets (T1-Cl: 4.41 ± 1.10 and 0.75 ± 0.18; T2-CB: 3.47 ± 0.25 and 0.59 ± 0.04; T4-TY: 3.16 ± 0.28 and 0.54

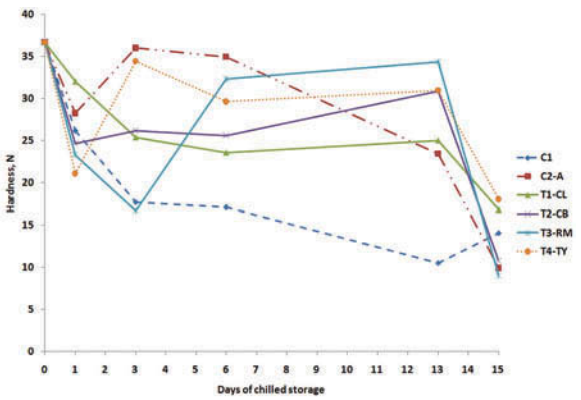

Figure 5. Changes in the hardness of pangasius fillets coated with plant oil incorporated alginate gels during chilled storage.

± 0.49) and T3-RM (3.38 ± 0.27 and 0.57 ± 0.05) were higher compared to alginate control C2-A (3.05 ± 0.58 and 0.52 ± 0.11) and control fillets (3.02 ± 0.67 and 0.52 ± 0.12). Similar observations of texture softening during chilled storage of fish have been reported (Jouki et al., 2014; Ozyurt et al., 2015; Viji et al., 2015). Storage conditions that minimize bacterial growth and slow the lipid oxidation can slow down spoilage. Chilled storage of fish slows but does not stop the decrease in texture (Taylor, 2006; Roy et al., 2012). Correlations between microbiological attributes and texture of both the essential oil treated and control pangasius fillets was found to be negative, ranging between −0.702 and −0.558 for psychrotrophic counts and the respective hardness values; correlations ranged between −0.524 and −0.305 for APC and hardness. Similar observations were reported by Genc et al. (2013). Microbial proteases of psychrotrophic proteolytic bacteria might have been the potential source of progressive protein degradation observed during chilled storage of fillets (Benjakul et al., 1997; Liu et al., 2010). The antibacterial activity of the plant essential oils and the alginate coating acting as physical barrier might be the reason for the delay in loss of texture of the treated pangasius fish fillets.

Conclusion

The results indicate that plant oil incorporated alginate gels were relatively better as reflected by low APC, low psychrotrophic counts, low PV, better sensory characteristics, and reduced rate of loss of texture compared to control fillets in preserving pangasius fillet quality during chilled storage. The control fillets were organoleptically rejected (< 4 score) and had higher psychrotrophic counts (> 10^7 cfu/g) on the 13^{th} day of chilled storage, indicating a shelf life of 9 days; whereas the fillets that were given essential oil incorporated alginate coating became organoleptically unacceptable and had psychrotrophic counts of more than 10^7 cfu/g on the 15^{th} day, indicating a shelf life of 13 days during chilled storage. The incorporation of thyme oil was found to be better compared to clove leaf oil, clove bud oil, and rosemary oil.

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References

AOAC. 1990. Official Methods of Analysis. 15^{th} edn. Association of Official Analytical Chemists, Washington, DC, USA.

Amerine M. A., Pongborn R. H., and Roescler, E. B. 1965. Principles of sensory evaluation of food. New York, NY: Academic, p. 602.

Anderson, U. B., Stomsnes, A. N., Thomassen M. S., and Steinsholt, K. 1994. Fillet gaping in farmed Atlantic salmon. J. Agric. Sci. 8: 165–179.

Andevari, G. T., and Rezai, M. 2011. Effects of gelatin coating incorporated with cinnamon oil on the quality of fresh rainbow trout in cold storage. Int. J. Food Sci. Technol. 46: 2305–2311.

APHA. 1976. Compendium of methods for the microbiological examination of foods. In: Speck, M. L. (Eds.). Washington, DC: American Public Health Association, p. 702.

Artharn, A., Prodpran, T., and Benjakul, S. 2009. Round scad protein-based film: Storage stability and its effectiveness for shelf-life extension of dried fish powder. Food Sci. Technol. 42: 1238–1244.

Aussanasuwannakul, A., Slider, S. D., Salem, M., Yao, J., and Kenney, P. 2012. Comparison of variable-blade to Allo-Kramer shear method in assessing rainbow trout (*Oncorhynchus mykiss*) fillet firmness. J. Food Sci. 77: S335–S341.

Ayala, M. D., Abdel, I., Santaella, M., Martınez, C., Periago, M. J., Gil, F., Blanco, A., and Albors, O. L. 2010. Muscle tissue structural changes and texture development in sea bream (*Sparus aurata*) during postmortem storage. LWT-Food Sci. Technol. 43: 465–75.

BAM. 2011. Bacteriological Analytical Manual (BAM), U.S. Food and Drug Administration, http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods.htm (Accessed 29 November 2013).

Bauer, K., Garbe, D., and Surburg, H. 2001. Common Fragrance and Flavor Materials: Preparation, Properties and Uses. Weinheim, Germany: Wiley-VCH, p. 293.
Benjakul, S., Seymour, T. A., Morrissey, M. T., and An, H. 1997. Physicochemical changes in Pacific whiting muscle proteins during iced storage. J. Food Sci. 62: 729–733.
Burt, S. 2004 Essential oils: Their antibacterial properties and potential applications in foods—a review. Int. J. Food Microbiol. 94: 223–253.
Casas, C., Martinez, O., Guillen, M. D., Pin, C. and Salmeron, J. 2006. Textural properties of raw Atlantic salmon (*Salmo salar*) at three points along the fillet, determined by different methods. Food Control 17: 511–515.
Cheng, J. H., Sun, D. W., Han, Z., and Zeng, X. A. 2014. Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: A review. Compr. Rev. Food Sci. Food Saf. 13: 52–61.
Chidanandaiah, Keshri, R. C., and Sanyal, M. K. 2009. Effect of sodium alginate with preservatives on the quality of meat patties during refrigerated (4°C) storage. J. Muscle Foods 20: 275–292.
Cheret, R., Chapleau, N., Delbarre-Ladrat, C., Verrez-Bagnis, V., and de Lamballerie, M. 2005. Effects of high pressure on texture and microstructure of sea bass (*Dicentrarchus labrax* L.) fillets. J. Food Sci. 70: 477–483.
Connell, J. J. 1975. Control of Fish Quality. Surrey, England: Fishing News (Books) Ltd, p. 179.
Conway, F. 1950. Microdiffusion and Analysis and Volumetric Error, 3rd edn. London: Crosby Lockwood and Sons. Pp. 87–107.
Cosentino, S., Tuberoso, C. I. G., Pisano, B., Satta, M., Mascia, V., Arzedi, E. and Palmas, F., 1999. In vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. Lett. Appl. Microbiol. 29: 130–135.
Daferera, D. J., Ziogas, B. N., and Polissiou, M. G. 2000. GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. J. Agricult. Food Chem. 48: 2576–2581.
Daferera, D. J., Ziogas, B. N., and Polissiou, M. G. 2003. The effectiveness of plant essential oils in the growth of *Botrytis cinerea, Fusarium sp*. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Prot. 22: 39–44.
Di Monaco R., Cavella S. and Masi P. 2008. Predicting sensory cohesiveness, hardness and springiness of solid foods from instrumental measurements. J. Texture Stud. 39: 129–149.
Dorman, H. J. D., and Deans, S. G. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88: 308–316.
EC. 1995. Commission decision 95/149/EEC of 8th March 1995 relating to total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used. Official J. Eur. Communities, L. 97: 84–87.
Fan, W. J., Chi, Y. L., and Zhang, S. 2008. The use of a tea polyphenols dip to extend the shelf life of silver carp (*Hypophthalmicthys molitrix*) during storage in ice. Food Chem. 108: 148–153.
Fan, W. J., Sun, J. X., and Chen, Y. C. 2009. Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food Chem. 115: 66–70.
Friedman, M., Henika, P. R., and Mandrell, R. E. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes* and *Salmonella enterica*. J. Food Prot. 65: 1545–1560.
FSSAI. 2011. Food Safety and Standards Authority of India. Microbiological requirements for seafoods. FSS (Food product standards and food additives) regulation 2011, *Part II, The Gazette of India: Extraordinary part III*, Sec. 4, p. 449–529.
Genc, I. Y., Esteves, E., Anibal, J., and Diler, A. 2013. Effects of chilled storage on quality of vacuum packed meagre fillets. J. Food Eng. 115: 486–494.
Gennadios, A., Milford, A., and Hanna. 1997. Application of edible coatings on meats, poultry and seafood: A review. Lebensm. Wiss. U. Technol. 30: 337–350.
Gilliland, S. E., Michener, H. D., and Kraft, A. A. 1976. Psychrotrophic microorganisms. In: Compendium of methods for the microbiological examination of foods (Speck, M. L., Ed), American Public Health Association, Washington, pp. 173–178.
Gram, L., Trolle, G., and Huss, H. H. 1987. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. Int. J. Food Microbiol. 4: 65–72.
Harpaz, S., Glatman, L., Drabkin, V., and Gelman, A. 2003. Effects of herbal essential oils used to extend the shelf life of freshwater reared Asian sea bass fish (*Lates calcarifer*). J. Food Prot. 66: 410–417.
Hernandez, M. M., Lopez, M. B., Alvarez, A., Ferrandini, E., Garcia, B. G., and Garrido, M. D. 2009. Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. Food Chem. 114: 237–245.
Heydari, R., Bavandi, S., and Javadian, S. R. 2015. Effect of sodium alginate coating enriched with horsemint (*Mentha longifolia*) essential oil on the quality of bighead carp fillets during storage at 4°C. Food Sci. Nutr. 3: 188–194.
ICMSF. 1986. Microorganisms in foods. 2. Sampling for microbiological analysis. Available from (2nd ed.). Toronto: University of Toronto Press. http://www.fao.org/DOCREP/003/T1768E/T1768E04.htm
Jayasena, D. D., and Jo, C. 2013. Essential oils as potential antimicrobial agents in meat and meat products: A review. Trends Food. Sci. Technol. 34: 96–108.

Jouki, M., Mortazavi, S. A., Yazdi, T. F., Koocheki, A. and Khazaei, N. 2014. Use of quince seed mucilage edible films containing natural preservatives to enhance physico-chemical quality of rainbow trout fillets during cold storage. Food Sci. Human Wellness. 3: 65–72.

Juliano, C., Mattana, A., and Usai, M. 2000. Composition and in vitro antimicrobial activity of the essential oil of *Thymus herba-barona* Loisel growing wild in Sardinia. J. Essent. Oil Res. 12: 516–522.

Laksmanan, J. R., and Piggott, P. A. 2003. Potential application of high pressure for improvement in salmon quality. Trends Food. Sci. Technol. 14: 354–362.

Lens-lisbonne, C., Cremieux, A., Maillard, C., and Balansard, G. 1987. Methodes d'evaluation de l'activite´ antibacterienne des huiles essentielles: application aux essences de thym et de cannelle. J. Pharm. Belg. 42: 297–302.

Liu, S., Fan, W., Zhong, S., Ma, C., Li, P., Zhou, K., Peng, Z., and Zhu, M. 2010. Quality evaluation of tray-packed tilapia fillets stored at 0°C based on sensory, microbiological, biochemical and physical attributes. Afr. J. Biotechnol. 9: 692–701.

Lu, F., Liu, D. H., and Ye, X. Q. 2009. Alginate calcium coating incorporating nisin and EDTA maintains the quality of fresh northern snakehead (*Channa argus*) fillets stored at 4°C. J. Sci. Food Agric. 89: 848–854.

Marino, M., Bersani, C., and Comi, G. 1999. Antimicrobial activity of the essential oils of *Thymus vulgaris* L. measured using a bioimpedometric method. J. Food Prot. 62: 1017–1023.

Matuska, M., Lenart, A., and Lazarides, H. N. 2006. On the use of edible coatings to monitor osmotic dehydration kinetics for minimal solids uptake. J. Food Eng. 72: 85–91.

Mcgimpsey, J. A., Douglas, M. H., Van Klink, J. L., Beauregard, D. A., and Perry, N. B. 1994. Seasonal variation in essential oil yield and composition from naturalized *Thymus vulgaris* L. in New Zealand. Flavour Frag. J. 9: 347–352.

Mejlholm, O. and Dalgaard, P. 2002. Antimicrobial effect of essential oils on the seafood spoilage micro-organism *Photobacterium phosphoreum* in liquid media and fish products. Lett. Appl. Microbiol. 34: 27–31.

Murthy, L. N., Rao B. M., Asha, K. K., and Prasad, M. M. 2015. Nutritional composition, product development, shelf-life evaluation and quality assessment of pacu *Piaractus brachypomus* (Cuvier, 1818). Indian J. Fish. 62: 101–109.

Ozyurt, A. G., Ozkutuk, S., Simsek,A., Yesilsu, F. A., and Erguven, M. 2015. Quality and shelf life of cold and frozen rainbow trout (*Oncorhynchus mykiss*) fillets: Effects of fish protein-based biodegradable coatings. Int. J. Food Prop. 18: 1876–1887.

Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., Chessa, M., Cerri, R., and Casanova, J. 2002. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. Flavour Frag. J. 17: 15–19.

Prescott, L. M., Harley, J. P., and Klein, D. A. 2005. Microbiology, 6th Edn. New York: McGraw-Hill. Pp. 119–124.

Rao, B. M., Murthy, L. N., and Prasad, M. M. 2013. Shelf life of chill stored pangasius (*Pangasianodon hypophthalmus*) Fish fillets: Effect of vacuum and polyphosphate. Indian J. Fish. 60: 93–98.

Roy, B. C., Ando, M., Itoh, T., and Tsukamasa Y. 2012. Structural and ultrastructural changes of full-cycle cultured Pacific bluefin tuna (*Thunnus orientalis*) muscle slices during chilled storage. J. Sci. Food Agric. 92: 1755–1764.

Song, Y., Liu, L., Shen, H., You, J. and Luo, Y. 2011. Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (*Megalobrama amblycephala*). Food Control 22: 608–615.

Suarez, M., Abad, M, Ruiz-Cara, T., Estrada, J. and Garcıa-Gallego, M. 2005. Changes in muscle collagen content during post mortem storage of farmed sea bream (*Sparus aurata*): Influence on textural properties. Aquacult. Intl. 13: 315–325.

Szczesniak, A. S. 2002. Texture is a sensory property. Food Qual. Pref. 13: 215–225.

Taylor, R., Fjaera, S., and Skjervold, P. 2006. Salmon fillet texture is determined by myofiber-myofiber and myofiber-myocommata attachment. J. Food Sci. 67: 2067–2071.

Van De Braak, S. A. A. J., and Leijten, G. C. J. J. 1999. Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, p. 116.

Viji, P., Tanuja, S., Ninan, G., Lalitha, K. V., Zynudheen, A. A., Binsi, P. K., and Srinivasagopal, T. K. 2015. Biochemical, textural, microbiological and sensory attributes of gutted and ungutted sutchi catfish (*Pangasianodon hypophthalmus*) stored in ice. J. Food Sci. Technol. 52: 3312–3321.

Wang, J. X., Liu, Q. H., and Teng, Y. 1994. Research on coatings of frozen mussel flesh. Food Science 2: 70–72.

Wanstedt, K. G., Seideman, S. C., and Donnelly, L. S. 1981. Sensory attributes of precooked, calcium alginate-coated pork patties. J. Food Prot. 44: 732–735.

Yu, X. L., Li, X. B., and Xu, X. L. 2008. Coating with sodium alginate and its effect on the functional properties and structure of frozen pork. J. Muscle Foods 19: 333–351.

Zeng, Q., and Xu, Q. 1997. Study on preservation techniques of fish, shrimp, scallop of edible coating. J. Dalian Fish. 12: 37–42.