Fishery Technology 55 (2018) : 24 - 33

Effects of Rosemary Extracts on Textural and Functional Qualities of Surimi Gel from Striped Catfish (*Pangasianodon hypophthalmus*) (Sauvage, 1878) during refrigerated storage

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Abstract

Effects of aqueous and ethanolic extracts of rosemary leaf on textural and functional properties of surimi gel from striped catfish (*Pangasianodon hypophthalmus*) (Sauvage, 1878) during refrigerated storage was evaluated. The properties of surimi gel such as gelling, water holding capacity, protein solubility, texture profile, whiteness, as well as the quality indices like total volatile bases and thiobarbituric acid values were estimated periodically during a refrigerated storage period of 20 days. Increase of water holding capacity in rosemary extract added gels indicated stronger protein network formation, whereas, protein solubility decreased significantly in all the groups as the storage progressed indicating the formation of protein aggregates. Thiobarbituric acid value, indicative of lipid oxidation decreased in treated samples but at different rates, depending upon the concentration of rosemary extract. Gel strength of samples was affected upon incorporation of rosemary extract and treated groups showed higher values than the control, indicating the positive role of rosemary's phenolics in cross linking of protein-protein interaction. Textural attributes were found to be affected most due to addition of rosemary. The control samples tended to have the largest decreasing rate of whiteness during the period of storage, compared to the treated groups (0.25-0.75%). Both the extracts of rosemary at higher concentration (0.75%) showed highest consumer acceptability on day-1 and also at the end of the storage period. On the basis of sensory evaluation the control was acceptable only upto 12 days.

Keywords: Striped catfish, surimi gel, rosemary leaf, gelling properties, texture profile, lipid oxidation

Introduction

Lipid oxidation and microbial propagation are considered as two most important hazards during refrigerated storage of sausage type of products from surimi gel of fatty fish. Lipid oxidation is responsible for reduction in nutritional quality as well as changes in flavour (Aguirrezabal et al., 2000) of products developed out of surimi especially from fatty fish, while microbial contamination can precipitate major public health hazards and economic loss in terms of food poisoning and meat spoilage. Lipid oxidation leads to the development of unpleasant odour, rancid taste and discoloration (Frankel, 2005). Moreover, the compounds resulting from lipid oxidation can modify proteins and amino acids of nutritional interest and decrease the protein functionality due to protein denaturation (Pokorny et al., 1976). The addition of antioxidants is therefore necessary to increase storage stability, sensory quality and nutritional value of fish products. Thus, application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life and preventing economic loss (Yin & Cheng, 2003). The process of gel incorporation and dispersion coating offered a method for extended storage and distribution of catfish patties under chilled conditions (Smruti et al. 2004). Addition of synthetic antioxidants has been restricted because of their health risks and toxicity (Linderschmidt et al., 1986). Hence, the use of natural antioxidants is

Received 04 July 2015; Revised 13 September 2017; Accepted 23 December 2017

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emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences (Frankel, 1998).

Since ancient times, spices and herbs have been added to food, not only as flavoring agents, but also as folk medicine and food preservatives (Nakatani, 1994). Furthermore, certain spices and herbs prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bacteriostatic or bactericidal activity, even with respect to food-borne pathogenic bacteria (Shelef et al., 1980). Among herbs, Rosemary (*Rosmarinus officinalis* L.) was explored commercially as a natural antioxidant (Jadhav et al., 1996). Rosemary extracts containing phenolic derivatives were reported to have strong antioxidant effects on cooked meat (Younathan, 1985; Rhee, 1988), and antimicrobial activity (Wang et al., 2008).

Polyphenolics are one of the compounds that are found in both edible and inedible plants and herbs/ spices and it could be the source of a good antioxidant agent. These can act as reducing agents, free radical scavengers and Fe^{2+}chelators or quenchers of singlet oxygen (Juntachote et al., 2006; Pizzale et al., 2002). Thus phenolics are of increasing interest in the food industry because they retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Wojdylo et al., 2007). The high antioxidant capacity of these plant parts is particularly due to their content of different phenols, anthocyanins and ascorbic acid, which can act as radical scavengers (Pantelidis et al., 2007). The antimicrobial mechanisms of phenolic compounds include the interruption of function of bacterial cell membranes. The -OH groups in phenolic compounds are highly reactive under aqueous conditions and react with several biomolecules, causing deformation of these molecules, which results in retardation of growth and bacterial growth. Phenolic compounds are also involved in protein and cell wall binding, inactivation of bacterial enzymes, and intercalation into the bacterial DNA during replication (Pelczar et al., 1988).

For surimi products, the technique mostly used for obtaining a good gel depends on solubilizing and extracting myofibrillar proteins with 2 to 3 g 100 g^{-1} salt and the solubilized proteins form a continuous matrix and then undergo cross-linking and develop into fine three dimensional solid-like networks resulting in elastic gel. The cross-linking of myosin promoted by a calcium-dependent endogenous transglutaminase (TGase) contained in fish muscle, which catalyses an acyl transfer reaction between γ-carboxyamide groups of glutaminyl residues in proteins. Fish with high content of lipid and myoglobin result difficulties in making high quality surimi (Chen, 2002). To enhance the gel strength of surimi or fish mince, various food-grade ingredients and cross-linking enzymes such as microbial transglutaminase have been used (Benjakul et al., 2004). Due to adverse effects of some ingredients on the surimi gel, particularly on its flavour or colour, the need of natural additives with an ability of protein cross-linking has been paid increasing attention for the surimi industry. The interactions between phenolic compounds and proteins play a very important role in the processing of certain food products. The formation of rigid molecular structures by reactions of ortho-quinones with proteins has been demonstrated by Strauss & Gibson (2004). Interactions of different phenolic acids and flavonoids with soy proteins were reported by Rawel et al. (2002). Significant increase in the gel strength of bigeye snapper surimi was found when oxidised phenolic compounds were added (Balange & Benjakul, 2009).

The gel forming ability of many freshwater species is relatively lower than that of the marine counterparts, but, could be upgraded by manipulating processing techniques. Striped catfish (*Pangasianodon hypophthalmus*) is extensively cultured in India and Bangladesh. The fish has a great aquaculture potential due to its very high growth rate compared to other popular major carps. The abundant catch of striped catfish in peak season might be utilized as an alternative source of surimi raw material for development of restructured products. This study aims to determine the effects of aqueous and ethanol extract of rosemary (*Rosmarinus officinalis* L.) on textural and functional properties of gel from striped catfish surimi during refrigerated storage.

Materials and Methods

To prepare extracts, rosemary leaves were dried in an incubator at temperature 40±2°C. Dried leaves were ground using an electric blender. Twenty grams of the ground material was soaked in 100 ml of hot sterile water for aqueous extract (AE) and 100 ml 90% ethanol for ethanolic extract (EE) and allowed to stand for 48 h. The crude extracts were obtained by filtration. The process was repeated twice and all the filtrates were collected and subjected to evaporation at 50°C (40°C for EE) in rotary vacuum evaporator (OSAKA J.P. Selecta, Spain) followed by drying at temperature 40±2°C. The powdered extracts of rosemary were kept in aluminium pouches and stored at -20°C for future use.

Fresh striped catfish for the study was collected from the local fish farm at Lembucherra, Tripura. Length and weight range of fish were 37– 49 cm and 636–809 g, respectively. Fishes were washed with chilled water, gutted, dressed, filleted by hand and minced by employing a mechanical meat mincer with a 3 mm hole plate. Washing of the minced meat was performed at 10°C using a fish mince to water ratio of 1:4 (w/v) for three times with ten min duration of each wash (twice with potable water and last one with 0.1% NaCl solution to facilitate dewatering). The slurry was stirred for 7 min and allowed to settle for 3 min before water was decanted. Final dewatering was carried out using a screw press (Deb Enterprise, India). Sorbitol (4 g), sucrose (4 g) and polyphosphate (0.3 g) were added to 100 g of dewatered mince as cryoprotective agents and then mixed for 5 min in a silent cutter (Sunlabz, India) at a temperature below 10°C. Surimi was packed in low density polyethylene (LDPE) pouches (150 g per pouch) and quickly frozen at -35°C for 2 h in air blast freezer (Sanyo, Japan) and stored at -20°C in a deep freezer (Vest Frost, Denmark) until further use.

Frozen surimi was tempered for about 2h at 20±2°C until it reached 5±1°C, followed by chopping for 1 min at high speed in a silent cutter. Moisture of surimi was adjusted to 80% using ice water. Salt (NaCl) was added @ 2.5% and mixed in silent cutter for five min. Aqueous and ethanolic extracts of rosemary leaves were added to each 150 g part @ 0.25, 0.5, and 0.75% respectively. Throughout the mixing operation temperature of surimi sol was kept below 10°C. The control (CON) was made without addition of rosemary extract. The surimi paste was then stuffed into vinylidene chloride casing (10 cm length, 2.0 cm diameter). Thermal setting was done according to the two-step heating method suggested by Luo et al. (2008). The casings were immersed in water at 40°C for 30 min followed by immersion in water at 85°C for 30 min. After cooking, the casings were immediately removed, placed in iced water, and cooled at 4–5°C for 30 min. The gels were then stored overnight at 4°C in a refrigerator.

Moisture, ash, protein and fat content of striped catfish surimi were determined according to AOAC (2000). For determination of the pH, a digital pH-meter (Sartorius, USA) was used. Gel (0.5 g) was homogenised in 10 ml of 0.6 M KCl in 50 mM pH 7.4 tris–HCl buffer for 1 min in a tissue homogenizer (IKA, Germany). The homogenate was centrifuged at 10000 rpm for 10 min at 4°C (Remi, India). The supernatant was diluted ten-fold with 0.6 M KCl and protein determination was performed by Biuret method (Gornall et al., 1949). Analyses were performed in triplicate and the protein solubility was expressed in mg of soluble protein 100 mg^{-1} of gel.

WHC was evaluated by the technique outlined by Barrera et al. (2002). Texture profiles of gel was determined using a TA-XT2 Stable Micro Systems Texturometer (Surrey, England, UK). Surimi gels were removed from the casings and equilibrated to room temperature for 30 min in a plastic bag to avoid dehydration before the mechanical properties were measured. Textural profile analysis (TPA) was performed using an aluminium cylindrical probe (P/50) with 50 mm diameter. Samples were compressed to 60% of the initial height using a compression speed of 60 mm min^{-1}. Hardness, cohesiveness, adhesiveness, springiness and gumminess were reported for each treatment. Three samples were analysed for each treatment at room temperature (25–27°C).

Sensory evaluation was performed by a panel of six judges. The panel evaluated each treatment within each replication in triplicate, and the evaluation was performed with the samples at room temperature. The panel judges were trained on the attributes of gelled fish products such as aroma, flavour, meat colour, juiciness, tenderness and taste. Based on those attributes they were instructed to evaluate overall acceptability using ten-point Hedonic Scale suggested by Majumdar et al. (2015). A score below 6 was considered as rejected.

The data obtained were analyzed using analysis of variance (ANOVA), and when significant differences were found, comparisons among means were carried out by using Duncan's Multiple Comparison Test ($p<0.05$) by Statistical Package for Social Sciences (SPSS, version 11.0 for windows).

Results and Discussion

Proximate composition of striped catfish (*Pangasianodon hypophthalmus*) were moisture 74.04 ± 0.25%, protein 16.39 ± 0.34%, fat 7.57 ± 0.14% and ash 1.09 ± 0.02%. The result shows that the fish had low moisture and high protein and moderate fat content. Lower moisture and higher lipid content in striped catfish muscle have been reported textural quality and gel strength (Tanuja et. al 2014) and (Hossain et al. 2004). There are influences of various factors such as nutrition, living area, fish size, catching season, seasonal and sexual variation as well as other environmental condition on the proximate composition of fish species. The surimi had moisture 79.57 ± 0.18%, protein 14.68 ± 0.27%, fat 1.33 ± 0.04% and ash 3.36 ± 0.14%. Washing removed fat and sarcoplasmic proteins and increased concentration of myofibrillar proteins which improved water holding capacity of protein.

Thermal setting results from the activity of a calcium-dependent endogenous transglutaminase (TGase). However, addition of any cross linking agent interferes in protein-protein interaction and influences gelling properties in particular and overall textural properties in general. The result showed that the gel strength (GS) of treated samples increased significantly ($p<0.05$) compared to that of control sample (CON) (Fig. 1). On Day-1, the GS of rosemary aqueous extract (AE) treated surimi gels (Fig. 1c) were found to be higher than the control and ethanolic extract (EE) treated samples (Fig. 1f). The GS changed during the refrigerated storage of surimi gels. In control, the GS of Day-1 (179.72) reduced to 134.55 on Day-20. Whereas, the initial GS of AE and EE treated samples were 199.59 (RMAE-0.25), 211.09 (RMAE-0.5), 218.42 (RMAE-0.75) and 184.32 (RMEE-0.25), 192.42 (RMEE-0.5), 204.27 (RMEE-0.75), respectively. In all the aqueous extract treated samples, the GS reduced during the period of storage, whereas, in case of ethanolic extract treated gels, the GS showed increase initially and decreased in the later part of storage period. Gel strength is a function of breaking force and breaking deformation. In both control and aqueous extract treated samples, the breaking force (Fig. 1a) decreased during the storage period, but reverse was observed in case of all the samples treated with EE (Fig. 1d). No significant change in the breaking deformation (Fig. 1b, 1e) was observed in the treated samples with the progress of storage period.

The results revealed that phenolic compounds at the optimum concentration were effective in increasing gel strength of striped catfish surimi. The phenolic compounds present in plant extracts have been reported to enhance protein-protein interaction which results in the enhancement of GS. Naturally derived plant phenolic compounds have been shown to be the potential protein cross-linker (Rawel et al., 2002; Wanh et al., 2017). Phenolic phytochemicals present in natural spices contain sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with the proteins and other macromolecules. Rosemary leaves are rich source of different phenolic compounds (Bubonja-Sonje et al., 2011) which consists of phenolic acids (caffeic acid, rosmarinic acid, caffeoyl derivatives), phenolic diterpenes (carnosic acid, carnoso l, epirosmanol) and different flavonoids (Shan et al., 2005).

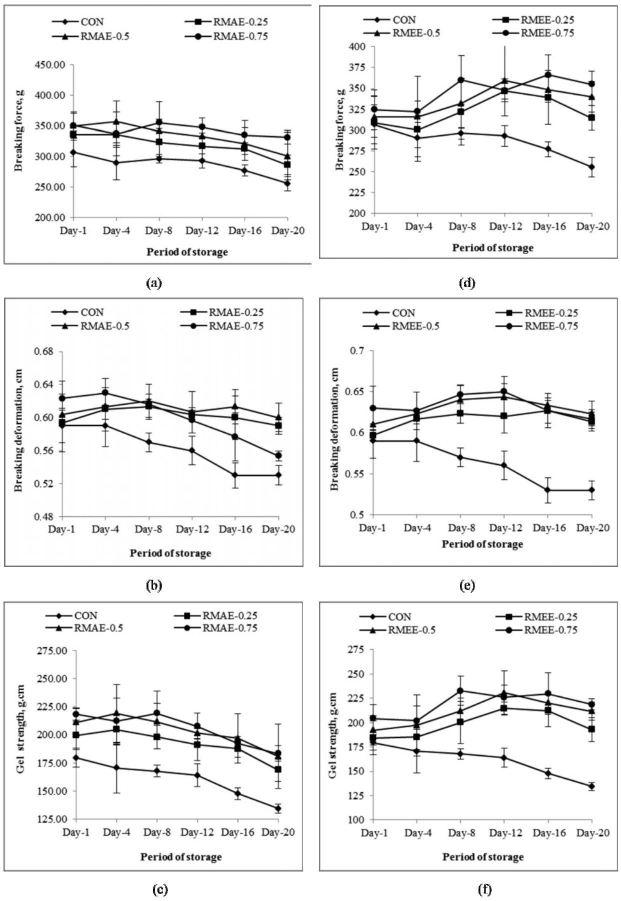

Fig. 1. Changes in gelling properties of aqueous (RMAE) and ethanolic (RMEE) extract of rosemary incorporated surimi gel during refrigerated storage (a & d - breaking force of RMAE & RMEE, b & e- breaking deformation of RMAE & RMEE, and, c & f- gel strength of RMAE & RMEE)

The GS of the gel was significantly increased by the addition of AE, with the maximum value for the RMAE containing the highest AE concentration of 075%. This may be due to retention of more phenolic compounds in aqueous extract compared to the ethanolic extract. Interaction of phenolic compounds with protein enhance protein-protein crosslinks, resulting increased gel strength. Significant increase in gel strength in surimi due to addition of different phenolic acids have been reported previously (Rawel et al., 2002; Binsi et al., 2016). A significant increase in the gel strength of big eye snapper surimi upon addition of oxidised phenolic compounds were reported (Balange & Benjakul, 2009).

TPA based on the recognition of texture as a multi-parameter attribute, is an objective method of sensory analysis pioneered by Szczesniak (1963). Texture profile of sausage from surimi is usually influenced by the gelling properties and it changes during low temperature mostly due to protein denaturation. But addition of cross linking agents like plant phenolics also influence the texture profile of surimi gel due to enhanced protein-protein interaction (Buamard et al., 2017). Texture parameters like hardness, springiness, cohesiveness and gumminess were determined (Fig. 2) for both the treatments. Among textural attributes, hardness is the most important textural attribute to the consumers, as it decides the commercial value of the meat (Chambers & Bowers, 1993). The hardness (the resistance at maximum compression during the 1^{st} compression) was found higher in control than the treated samples. This may be related with the higher breaking deformation as observed in the treated samples compared to the control. In control, the day-1 value of hardness (752.7 g) reduced ($p<0.05$) 21% and reached to 592.0 in day-20. The values of hardness in day-1 were 644.7, 700.5 and 739.0 gf in treatments RMAE-0.25, RMAE-0.5 and RMAE-0.75, respectively. Whereas, in case of EE treated samples, the day-1 hardness values were 694.5, 718.9 and 708.8 gf, respectively in treatments RMEE-0.25, RMEE-0.5 and RMEE-0.75. The differences in hardness between the treatments may be due to different phenolic compounds, their concentration and mode of interaction with protein.

In AE treated samples, the hardness gradually reduced as the storage period progressed, whereas, a slight increase in the initial period of storage followed by gradual decrease during the period of storage was observed in case of EE treated surimi gels. The other textural attributes like springiness and cohesiveness showed a slow but gradual decreasing trend during the storage period. Whereas, the gumminess experienced a reverse trend. But the

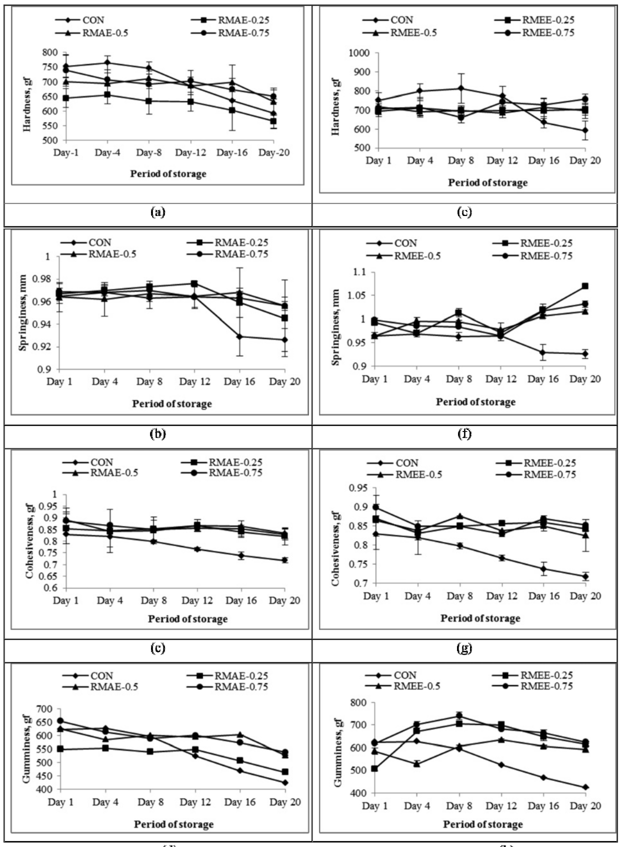

Fig. 2. Changes in texture profiles of aqueous (RMAE) and ethanolic (RMEE) extract of rosemary incorporated surimi gel during refrigerated storage (a & e - hardness of RMAE & RMEE, b & f- springiness of RMAE & RMEE, c & g- cohesiveness of RMAE & RMEE and, d & h- gumminess of RMAE & RMEE)

rate of changes of such textural parameters has been found to be less in control compared to the treated gels. These parameters are dependent on the force-time graph, and basically ratio of different length and area of the graph. So any changes in force during compression results changes in those parameters. This may be due to the denaturation of protein during freezing and frozen storage (Kugino & Kugino, 1994; Sreenath et al., 2009) as well as tissue depletion due to enzyme catalyzed reactions. According to Ganesh et al. (2005) the denaturation of the fish muscle proteins, particularly the actin and myosin and also lipid oxidation could contribute to the change of textural properties during low temperature storage. Textural differences as observed between the treatments may be explained as the quantitative differences in phenolic compounds present in the extracts. Changes in textural parameters during refrigerated storage may be attributed to the modification of protein-phenolics interaction due to protein denaturation. Moreover, interaction of phenolic compounds with the lipid and protein oxidized products may also be considered as a factor responsible for changes in texture profiles during storage. Denaturation of protein during low temperature storage of surimi gel has been reported by several authors (Kugino & Kugino, 1994; Sreenath et al., 2009).

The pH of the surimi gel in day-1 was found 7.67 in control which slightly reduced ($p<0.05$) in treated samples (7.63 to 7.65). pH showed gradual increase ($p<0.05$) in all the samples during the period of storage. On 20th day, the pH were recorded as 7.83, 7.83 and 7.81 in treatments RMAE0.5, RMAE0.75 and respectively. Whereas, almost similar increases of pH were observed in EE treated surimi gels. Decomposition products such as volatile bases could lead to a pH rise during storage of surimi gel (Rodger et al., 1980).

Total volatile basic nitrogen (TVBN) which are breakdown products of endogenous nitrogenous compounds, increased ($p<0.05$) in control as well as in the treated samples during storage, but the rate of increase in treated samples were lower than the control. In control, the TVBN (mg%) increased from initial value of 5.17 to 8.67 mg% on day-20 (Fig. 3), whereas, in the treated samples the TVBN did not exceed 7.4% during this period. The protective action of spice extracts on protein degradation may be the probable explanation of the slow increase of TVBN value in treated gels compared to control.

Indices such as water holding capacity (WHC) is often used to assess the textural quality of the surimi gels and it also indicates the deterioration of protein quality during low temperature storage. In control, the initial WHC recorded on day-1 as 80.18% was decreased to 71.18% on day-20. In this study, all the treatments experienced increase ($p<0.05$) of WHC in day-1compared to control. The result suggested that as the storage progressed, the WHC decreased ($p<0.05$) in all the treatments. In AE treated gels, maximum value of WHC was found in treatment RMAE0.75 (76.05%) followed by RmAE0.5 (74.39%) and RmAE0.25 (72.72%). Almost similar trend was observed in respect of EE treated samples. The increase in WHC on day-1 observed in treated surimi gels may be explained on the basis of the formation of stronger three dimensional network

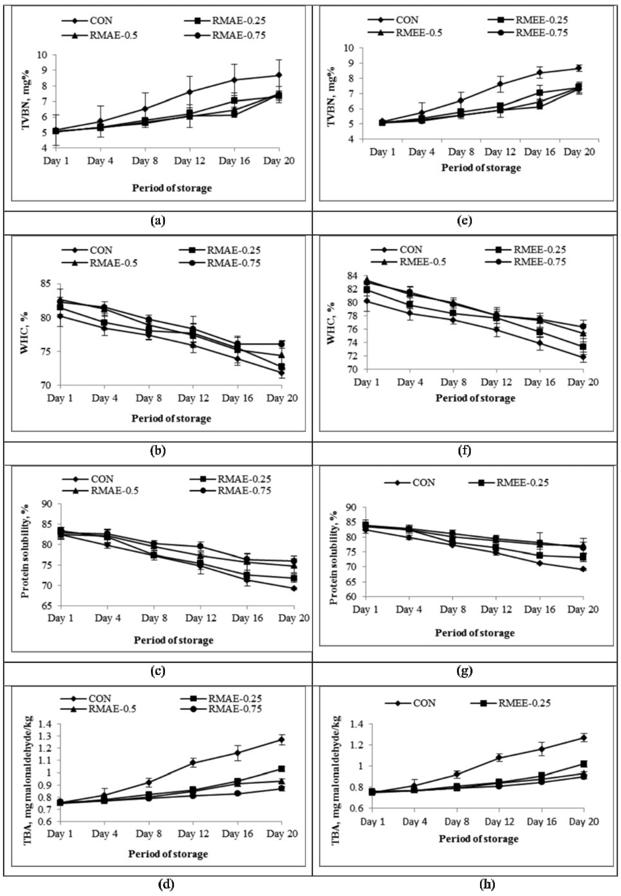

Fig. 3. Changes in TVBN (a & e), WHC (b & f), protein solubility (c & g) of aqueous (RMAE) and ethanolic (RMEE) extract of rosemary incorporated surimi gel during refrigerated storage

induced by polyphenols, which might facilitate better entrapment of water in the three dimensional matrix. However, the variation in WHC between the treatments may be due to differences in the concentration of phenolics in different treatments. From day-4 onwards, the differences between the treatments were significant ($p<0.05$). This may be explained as the result of protein denaturation induced by refrigerated storage leading to low affinity for water and it was accompanied by gradual loss of protein solubility. Moreover, modification of protein-phenolics interaction with the gradual denaturation and/or degradation of protein during storage may also be responsible for changes in WHC of protein as observed in this study. The texture of gel is also dependent on WHC which affect or influence sensory acceptability. So WHC is important to maintain at higher level during the storage period for better sensory quality.

The alteration of protein extractability is a useful factor which may be used to determine the textural quality of fish muscle, as protein aggregation is accompanied by a significant decrease in their solubility (Badii & Howell, 2002). Protein solubility (PS) was estimated to be 82.37% in control on day-1, which gradually reduced ($p<0.05$) to 69.22% on day-20. There was no significant difference ($p>0.05$) in day-1 between treatments and control. Usually the protein gels from muscle foods exhibit less solubility, since during heating proteins undergo denaturation and aggregation to form a three dimensional structure. In the present study, protein solubility (%) was found to be decreased significantly ($p<0.05$) in all the groups as the storage progressed indicating the formation of protein aggregates. The decrease in solubility suggests the formation of protein aggregates during storage as a result of protein denaturation. The formation of disulphide bond which results in the aggregation of proteins (Lim & Haard, 1984) might have contributed to low solubility of proteins. Hydrogen bonds might involve in the interactions between hydroxyl groups of phenolic compounds and the nitrogen or oxygen of amino acids. From the result, the decreased solubility indicated the aggregation as well as denaturation of proteins caused by low temperature storage (Viji et al., 2015). Protein denaturation, water holding capacity and protein solubility (PS) are factors which are interdependent and changes during low temperature storage.

The thiobarbituric acid (TBA) value is the measure of secondary oxidative degradation of lipid. It gives an idea about the formation of aldehyde compounds like malonaldehyde during oxidation of lipid and generally it is produced after degradation of peroxides. In control there was an increase ($p<0.05$) in TBA value reaching 1.27 mg malonaldehyde kg^{-1} on 20^{th} day of refrigerated storage period. With the progress of storage period, the TBA values increased ($p<0.05$) in all the treatments. Lipid oxidation occurred during refrigerated storage might cause the denaturation of proteins. Many lipid degradation products are also capable of cross-linking polypeptides and thus are responsible for the generation of insoluble protein aggregate (Buttkus, 1966). This possibly resulted in the loss in solubility of protein, especially when the storage time increased. The protein solubility decreased when the TBA value increased during refrigerated storage of surimi gels. This could be explained by interaction between protein and lipid oxidation products, causing a decline of protein solubility (Alzagtat & Alli, 2002; Siddaiah et al., 2001).

The rate of increase of TBA values was found to be less in rosemary leaf extract treated gels compared to control. Also, the rate of increase was reduced with the higher concentration of extracts. On the 20^{th} day, no considerable difference was found in the TBA values of both aqueous and ethanolic extracts. The auto-oxidation of fat poses a major problem leading to deterioration in the quality of the foods in which they are contained which finally reduce their nutritional value (Esterbauer, 1993; Kubow, 1992). Safeguarding fats against oxidation is normally done by restricting the access of oxygen or adding antioxidants. Antioxidant potential of plant extracts has long been documented. Antioxidant compounds in herbs are mainly comprised of phenolic acids (Cao & Cao, 1999) and presence of -OH groups in phenolic compounds are largely responsible for their antioxidative activity. Two major diterpenes, carnosic acid and carnosol together with rosmarinic acid, hydroxycinnamic acid ester, carnosol, rosmanol and epi-rosmanolare considered to be the main antioxidant compounds present in rosemary (Wellwood et al., 2004; Penuelas et al., 2005). The phenolic derivatives present in rosemary extracts have shown strong antioxidant effects on cooked meat. TBA values of lamb patties treated with rosemary extracts have been reported to be retarded significantly ($p<0.01$) compared to control patties. Rosemary extract has been reported to possess antioxidant activity comparable to that of commercial blend of BHA/BHT (Angelo et al., 1990; Barbut et al., 1985).

In control, the whiteness value was recorded as 76.65 on day-1 which was reduced ($p<0.05$) to 66.53 on day-20. The result indicated that the interaction of phenolic compounds with protein influenced the whiteness of gel. The whiteness values showed a reduction on day-1 compared to control and proportionate to the concentration of extract in both AE and EE treated surimi gels. Since the plant phenolic compounds naturally have dark colour, the addition of those compounds might have caused the darkening of the final products. From the result, it was revealed that the addition of plant extracts decreased the whiteness of resulting gels. On day-20, the whiteness of all the treated samples particularly at higher concentration of extract were found to be lower ($p<0.05$) than the day-1. The control samples tended to have the largest decreasing rate of whiteness during the period of storage, compared to the treated groups. Such observation may be related with the interaction between the free amino groups and lipid oxidized products, the formation of which was more in control and lower in treated groups, as both the denaturation o protein and lipid oxidation were more in control. There possibility of interaction of different plant phenolics with the muscle pigments during thermal setting and low temperature storage might be responsible for different degree of whiteness of gels is treatments. Since, whiteness of surimi gel tends to be an important sensory attributes, therefore, optimization of plant extract's concentration in surimi gel on the basis of their total phenolics is essential.

The acceptability scores of the samples were assigned based on the attributes such as appearance, flavour, taste and texture. The acceptability of the product over the storage time as affected by different concentration of rosemary's aqueous and ethanolic extracts are presented in Fig. 4 and 5, respectively. The acceptability of all the samples registered a slow and gradual decrease ($p<0.05$) during the study period of 20 days under refrigerated condition. All the treated groups were acceptable upto the end of the storage, however, they were scored in the order of 0.75% > 0.5% > 0.25% > CON

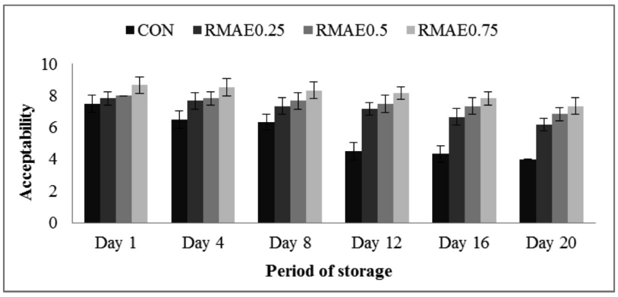

Fig. 4. Changes in acceptability of Rosemary AE incorporated gel during refrigerated Storage

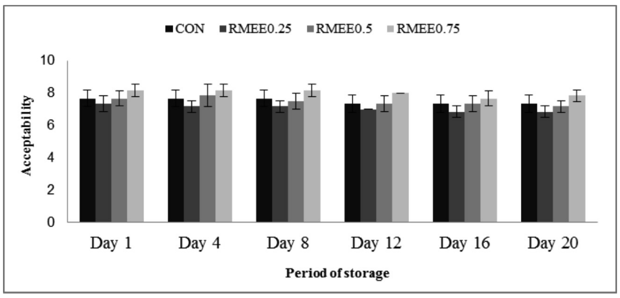

Fig. 5. Changes in acceptability of Rosemary EE incorporated gel during refrigerated Storage

in respect of both aqueous and ethanolic extracts, respectively. Although, ethanolic extract added gel scored higher than the aqueous extract added ones. In case of control, there was a steady decrease ($p<0.05$) of all the sensory attributes and on this basis, since the gel scored below 4 as per consumers' acceptability in day-16, the product was considered to be of acceptable up to 12 days. The consumers' acceptability is based on the cumulative effect of tenderness, juiciness, flavour, colour (whiteness), mouthfeelness and finally taste of the product, that basically encompasses all the sensory and textural attributes as well as functional characteristics especially water holding capacity and protein solubility. Textural properties of sausage type of products, which is regarded as an important criterion from consumer's acceptability, is of prime concern. In this study, the gel strength as well as hardness of gels containing 0.75% rosemary extract was found to be the highest amongst the samples. The present study for a limited storage period concluded that surimi gel incorporated with rosemary extract (aqueous or ethanolic) could easily be stored under refrigeration for a period of 20 days without losing any significant sensory attributes. Further, this study also indicated that higher gel strength with lower hardness improves the sensory acceptability of the surimi gel type products.

Since present day consumers are very much sensitive to the presence of synthetic antioxidants and gelling agents in food, use of plant phenolics in sausage type of products seems to be a step towards development of healthy food. This study revealed the potential of rosemary extracts in preventing lipid auto-oxidation as well as improving gel and textural properties of sausage type of products. According to this study, the surimi gel incorporated with rosemary extract (aqueous or ethanolic) could easily be stored under refrigeration for a period of 20 days without losing any significant sensory attributes. It was also observed that combined effects of two major textural properties such as gel strength and hardness decide the consumers' acceptability of the sausage type of products.

Acknowledgements

The authors like to express their sincere thanks to the Technical Staff of the Dept. of Fish Processing Technology & Engg., College of Fisheries (Central Agricultural University), Lembucherra, Tripura for all assistance rendered by them.

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