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Effect of aqueous and ethanolic extract of sweet lemon peel (*Citrus sinensis*) in refrigerated storage life of pangas (*Pangasianodon hypophthalmus*) surimi gel

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Abstract

Effect of aqueous and ethanolic extract of sweet lemon peel (*Citrus cinensis*) on surimi gel from Striped Catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) was studied and a positive effect was observed in the sense of storage life, gel strength and sensory attributes. Different levels of extract (0.5, 1.0, 1.5 and 2.0%, w/w) were used. Water holding capacity of the treated gels were found to be higher compared to control and higher values were observed in respect of treatments with 2.0% of ethanolic extract. Gel strength of treated samples increased significantly ($p < 0.05$) from the control and maximum was found with sweet lemon peel ethanolic extract at 2.0% level. In order to avoid any chemical gel enhancer or preservative for checking lipid oxidation in sausage type of products, use of natural antioxidant and antimicrobial is a good option. From consumers acceptance point of view this type of products would fetch a high level of consumers in city and big towns who are health conscious.

Keywords: striped catfish, gel strength, sensory attributes, antioxidant, antimicrobial

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. Lipids oxidation is responsible for reduction in nutritional quality as well as changes in flavour [1] 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 will lead to the development of unpleasant odour, rancid taste and discoloration [2]. 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 [3]. Addition of synthetic antioxidants has been restricted because of their health risks and toxicity [4]. Hence, the use of natural antioxidants is emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences [5]. Since ancient times, spices and herbs have been added to food, not only as flavouring agents, but also as folk medicine and food preservatives [6]. Furthermore, certain spices and herbs prolong the storage life of foods by preventing rancidity through their antioxidants activity or through bacteriostatic or bactericidal activity, also even with respect to food-borne pathogenic bacteria [7].

Sweet lemon peel essential oils (EO) consisted of monoterpenes exclusively, with limonene as the major component, contributing 90.94% of the total volatile organic compounds. Other significant constituents present in oil were b-myrcene (4.75%) and a-pinene (1.30%); whereas, b-pinene, a-phellandrene, d-3-cerene, b-phellandrene, (Z)-b-ocimene, a-terpinolene, octanal, cis-limonene oxide, trans-limonene oxide, decanal, linalool, octanol, 4-terpineol, neral, a-terpieol and geranial were found as trace components in sweet lemon peel EO. Limonene was the major component present in the citrus fruit essential: 85.5% in lemon (*C. sinensis*) EO, 59.1% in lemon (*Citrus lemon*) EO and 74.4% in mandarin (*Citrus reticulata*) EO [8]. Espina *et al.* [8] demonstrated that 74.4% limonene was present in mandarin (*C. reticulata*) and had strong antibacterial action against *S. enteritidis*, *S. aureus*, *P. aeruginosa*, *E. coli* and *L. monocytogenes*.

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 Fe²⁺ chelators or quenchers in the formation of singlet oxygen^[9]. 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^[10]. The potentially 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.

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 salt and the solubilised expanding proteins form a continuous matrix and then undergo thermal aggregation, 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 -carboxamide groups of glutamyl residues in proteins. Fish with high content of lipid and myoglobin results difficulties in making high quality surimi^[11]. To enhance the gel strength of surimi, various food-grade ingredients and cross-linking enzymes such as microbial transglutaminase have been used^[12]. The formation of rigid molecular structures by reactions of ortho-quinones with proteins has been demonstrated by Strauss and Gibson^[13]. This study aims to determine the effects of aqueous and ethanol extract of sweet lemon peel (*Citrus sinensis*) as natural preservative and improving texture in refrigerated storage of gel from Thai pangas surimi.

Materials and Methods

Raw material

Thai pangas was procured from the Battala fish market located at Agartala, West Tripura (Distt.), brought to the laboratory in iced condition in plastic polystyrene insulated containers within 1h and used for this study. The average length and weight of fish were 45.5±5.27 cm and 2500.3±17.60 g respectively.

Preparation of surimi

Immediately after reaching Fish processing laboratory (Department of Fish Processing Technology and Engg, College of Fisheries, Lembucherra) the raw material fish, i.e., Pangasius was washed with ice cold potable water to remove dirt, sand and unwanted material. Immediately the fishes were gutted, dressed, filleted by hand and minced by employing a mechanical meat mincer with a perforated plate having 3 mm-dia hole. Washing of the minced meat was performed in wash tanks maintaining a water temperature of 8-10°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 wash with 0.1% NaCl solution to facilitate dewatering). The slurry was stirred for 4 min and allowed to settle for 6 min before water was decanted. Final dewatering was carried out using a screw press. 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. The washed mince (surimi) was packed in low density polyethylene (LDPE) pouches (500 g per pouch) and immediately frozen at -35°C for 2 h in air blast freezer and stored at -20°C in a deep freezer for preparation of surimi gel within a week.

Preparation of surimi gel

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% by using ice water. Salt (NaCl) was added @ 2.5% and mixed in silent cutter for five min. Sweet lemon peel extracts (both aqueous and ethanolic) were added at different concentration (0.5, 1.0, 1.5 and 2.0%, w/w) to each 500 g part and The control (CON) was made without addition of plant extract (only 2.5% NaCl).

Throughout the mixing operation temperature of surimi sol was kept below 10°C. The surimi paste was stuffed into poly vinylidene chloride (PVC) casing (10 cm length, 2.0 cm diameter). Thermal setting was done according to the two-step heating method suggested by Luo *et al.*^[14]. 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 stored overnight at 4-5°C in a refrigerator. For storage study, the gels were stored at 4-5°C in a refrigerator for 20 days and storage changes were analysed at 4 days interval.

Preparation of aqueous extracts (AQ) and ethanolic extracts (EE) of sweet lemon peel

To prepare extract, sweet lemon (*C. sinensis*) peel were collected, washed and dried in an hot air oven at temperature 40±2°C. Dried materials were ground using an electric blender. Twenty grams of the ground material was soaked in 100 ml of hot sterile water 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 in rotary vacuum evaporator. The powdered aqueous extract of garlic was kept in aluminium pouch and stored at -20°C for future use. Similarly for ethanolic extracts hot sterile water was replaced by 90% ethanol and vacuum evaporation was done at 40°C.

Analyses of moisture, ash, protein, fat content and pH

Moisture, ash, protein and fat content of Thai pangas, surimi and were determined according to AOAC^[15]. For determination of the pH, 10 g of sample was homogenized with 50 ml distilled water and pH value was measured by a digital pH-meter (Sartorius, PB-20).

Determination of total volatile base nitrogen (TVBN)

Total volatile basic nitrogen was estimated by Conway^[16] method. The volatile nitrogenous substances present in the sample was distilled over and trapped by the standard H₂SO₄ solution. The remaining acid can be back titrated with the standard NaOH.

Determination of thiobarbituric acid reactive substances (TBARS)

The 2-thiobarbituric acid (TBA) assay was carried out according to the procedure of Schmedes and Holmer^[17]. Surimi gel sample (10 g) was mixed with 25 ml of trichloroacetic acid solution (200 g/l of TCA in 135 ml/l phosphoric acid solution) and homogenized in a blender for 30 s. After filtration, 2 ml of the filtrate were added to 2 ml TBA solution (3 g/l) in a test tube. The test tubes were incubated at room temperature in the dark for 20 h; then the absorbance was measured at 532 nm by using UV-VIS spectrophotometer (Shimadzu, Japan). A standard curve was

constructed using malondialdehyde (MDA), and results were expressed as mg malondialdehyde per kg of surimi gel.

Determination of protein solubility (PS) and water holding capacity (WHC)

Gel (0.5 g) were 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 10,000 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 [18]. Analyses were performed in triplicate and the solubility was expressed in mg of soluble protein/100 mg of gel.

WHC was evaluated by the technique outlined by Barrera *et al.* [19]. A portion of 5 g of each gel was weighed and placed on 8 layers of filter paper (Whatman No. 1). Samples were placed in 50 ml centrifuge tubes and centrifuged at 5000×g at 4 °C for 15 min. immediately after centrifugation; the gels were removed and re-weighed. WHC was expressed as the weight of the centrifuged gels relative to the original weight of samples.

$$\text{WHC (\%)} = (\text{W2/W1}) \times 100$$

Where, W1 represents the weight of the gel before centrifugation and W2 represents the weight of the gel after centrifugation.

Analysis of gel strength (GS)

Heat induced gels were cut into 3 cm high cylindrical slices. Puncture tests were carried out using a 5.0 mm dia spherical head stainless steel plunger attached to a 50 N cell connected to the crosshead of a TA-XT2 Stable Micro Systems Texturometer (Surrey, England, UK). Breaking force (g), breaking deformation (cm) and work of penetration, i.e., gel strength (g.cm) were determined from force deformation curves obtained at a crosshead speed of 0.2 mm sec⁻¹. Each measurement was replicated 3 times.

Sensory evaluation

Sensory evaluation was performed by a panel of 6 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 the restructure fish products such as appearance, flavour, taste and texture. Based on those attributes they were instructed to evaluate overall acceptability using 9-point Hedonic Scale (like extremely-9, like very much-8, like moderately-7, like slightly-6, neither like nor dislike-5, dislike slightly-4, dislike very much-3, dislike moderately-2, dislike slightly-1). A score below 5 was considered as rejected.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 16.0 for windows). Analysis of variance (one way - ANOVA) was performed to determine the differences between experimental periods of maturation. The tests for differences were done by using Duncan's Multiple Comparison Test. Significance of differences was defined at $p < 0.05$.

Result and Discussion

Proximate analyses of fish muscle and surimi

The moisture, crude protein, total lipid and ash contents of Thai pangas was determined as 74.4±0.25, 16.9±0.34,

7.7±0.14 and 1.09±0.02 respectively. Hossain *et al.* [20] reported almost similar proximate composition of pangas as moisture- 78.6±2.14, ash- 0.78±0.06, protein- 16.5±0.88, lipid- 6.8±0.39 and NPN- 0.35±0.04. However, the composition of fish muscle depends upon various factors such as sex, size, stages of maturity and season [21].

After washing the moisture content of mince increased from 74.4 to 79.57%. This could be explained as increased hydration of protein because of increase of water holding capacity due to removal of sarcoplasmic proteins during washing. Lin & Park [22] reported that removal of fat and water-soluble constituents, such as blood, pigments, proteins, and salts, by washing resulted in increased hydration of the mince meat. Protein decreased from 16.9% in raw fish meat to 14.68% in surimi. This content decrease produced during the washing process can be easily explained by means of partial solubilization of the sarcoplasmic protein into the washing solution [23]. Lipid is very important, as far as surimi is concerned, because of its interference with the gel formation. The lipids in surimi products may bring about an adverse effect on the surimi quality, because the oxidized lipids interact with proteins, causing denaturation, polymerization and changes in functional properties [24]. Pangas is considered as fatty fish (> 5% lipid) and the total lipid of the muscle was found to be 7.7% which reduced to 1.33% in surimi due to washing of mince.

Table 1: Proximate composition of raw fish and surimi*

	Raw fish	Surimi
Moisture	74.4±0.25	79.57±0.18
Protein	16.9±0.34	14.68±0.27
Lipid	7.7±0.14	1.33±0.04
Ash	1.09±0.02	3.36±0.14

* The result is mean ± SD of three determinations

Changes in pH, WHC and protein solubility (PS %)

pH was found 7.67, 7.96; 7.66, 7.87; 7.66, 7.83; 7.64, 7.80 and 7.66, 7.80 on first and last day of storage in respect of control and treatments LmAE-0.5, LmAE-1.0, LmAE-1.5 and LmAE-2.0 respectively. Similarly in ethanolic extract (LmEE) treated samples pH was found 7.65, 7.86; 7.65, 7.82; 7.64, 7.79; and 7.66, 7.79 on first and last day of storage in respect of treatments LmEE-0.5, LmEE-1.0, LmEE-1.5 and LmEE-2.0 respectively. A gradual increase ($p < 0.05$) of pH was observed in all the samples with the progress of storage period. This indicated protein degradation during refrigerated storage. Decomposition products such as volatile bases could lead to a pH rise during storage of surimi gel. These changes in pH could cause loss of myofibrillar protein solubility [25].

WHC was found 80.18, 71.81; 82.47, 73.33; 82.87, 75.84; 83.31, 75.78 and 83.15, 77.43 on first and last day of storage in respect of control and treatments LmAE-0.5, LmAE-1.0, LmAE-1.5 and LmAE-2.0 respectively. Similarly in ethanolic extract (LmEE) treated samples WHC was found 82.80, 73.67; 83.20, 76.50; 83.64, 76.12 and 83.81, 77.43 on first and last day of storage in respect of treatments LmEE-0.5, LmEE-1.0, LmEE-1.5 and LmEE-2.0 respectively. The result suggested that as the storage progressed, the WHC decreased in all the treatments. 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.

PS were determined 82.37, 69.22; 82.61, 70.83; 82.73, 73.28; 83.37, 73.78 and 83.62, 71.89 on day-1 and day-20 of storage in respect of control and treatments LmAE-0.5, LmAE-1.0,

LmAE-1.5 and LmAE-2.0 respectively. Similarly in ethanolic extract (LmEE) treated samples the values of PS were determined 83.27, 70.83; 82.50, 74.95; 84.03, 74.11 and 83.62, 70.89 on first and last day of storage in respect of treatments LmEE-0.5, LmEE-1.0, LmEE-1.5 and LmEE-2.0 respectively. Usually the protein solubility of gel from muscle foods experience less protein solubility since during heating, proteins underwent denaturation and aggregation to form a three dimensional structure. The decrease in solubility suggests the formation of protein aggregates during storage as a result of protein denaturation. In the present study, protein solubility was found to be decreased significantly ($P < 0.05$) in all the groups in as the storage progressed indicating the formation of protein aggregates. The formation of disulphide bond which results in the aggregation of proteins [26] might have contributed to low solubility of proteins.

Changes in Total volatile basic nitrogen (TVBN mg %)

The day-1 and day-20 values of TVBN were found to be 5.17, 8.67; 5.31, 7.27; 5.30, 6.84; 5.29, 6.73 and 5.29, 6.63 in respects of control and treatments LmAE-0.5, LmAE-1.0, LmAE-1.5 and LmAE-2.0 respectively. Whereas, in ethanolic extract (LmEE) treated samples the TVBN values were observed as 5.31, 7.26; 5.30, 6.83; 5.29, 6.72 and 5.29, 6.62 in respect of treatments LmEE-0.5, LmEE-1.0, LmEE-1.5 and LmEE-2.0 respectively. TVBN which indicates the breakdown of endogenous compounds into non-protein N-compounds increased ($p < 0.05$) in treated samples compared to control. The TVBN increased during the period of storage, but the rate of increase in treated samples were lower than the control. According to Connell [27], 35-45 mg/100 g meat of TVB-N content is the limit of acceptability. In this study, the TVBN value on 20th day did not reach to the value on the basis of which a muscle product is rejected.

Changes in thiobarbituric acid reactive substances (TBARS mg malonaldehyde /kg)

The day-1 and day-20 values of TBA were found to be 0.75, 1.27; 0.75, 1.16; 0.76, 0.98; 0.75, 0.95 and 0.75, 0.91 in respects of control and treatments LmAE-0.5, LmAE-1.0, LmAE-1.5 and LmAE-2.0 respectively. Whereas, in ethanolic extract (LmEE) treated samples the TBA values were observed as 0.75, 1.04; 0.76, 0.97; 0.74, 0.93 and 0.75, 0.91 in respect of treatments LmEE-0.5, LmEE-1.0, LmEE-1.5 and LmEE-2.0 respectively. The rate of increase of TBA values was found to be less in treated gels compared to control. Also, the rate of increase was reduced with the higher concentration of extracts. Antioxidant potential of sweet lemon peel has long been documented. Presence of -OH groups in phenolic compounds are largely responsible for their antioxidative activity.

Changes in gel strength (GS)

Gel strength were determined 179.72, 134.55; 194.25, 147.77; 218.87, 181.58; 226.30, 193.64; and 234.87, 218.33 on day-1 and day-20 of storage in respect of control and treatments LmAE-0.5, LmAE-1.0, LmAE-1.5 and LmAE-2.0 respectively. Similarly in ethanolic extract (LmEE) treated samples the values of gel strength were determined 215.70, 150.60; 225.40, 190.00; 222.90, 202.40; and 238.20, 211.30 in respects of treatments LmEE-0.5, LmEE-1.0, LmEE-1.5 and LmEE-2.0 respectively. The phenolic compounds present in plant extracts have been reported to enhance protein-protein interaction which results GS enhancement. Naturally derived plant phenolic compounds, especially in the oxidised

form, have been shown to be the potential protein crosslinker [28].

Changes in sensory characteristics

The values of overall acceptability was found to be 7.50, 4.00; 7.67, 4.47; 7.83, 6.50; 8.33, 7.17 and 7.67, 6.50 on day-1 and day-20 of storage in respect of treatments LmAE-0.5, LmAE-1.0, LmAE-1.5 and LmAE-2.0 respectively. Similarly in ethanolic extract (LmEE) treated samples the values were found to be 7.67, 6.17; 8.00, 7.00; 8.50, 7.50 and 7.83, 6.67 in respects of treatments LmEE-0.5, LmEE-1.0, LmEE-1.5 and LmEE-2.0 respectively. 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 5 in all the attributes including overall acceptability in day-16, the product was considered to be of acceptable up to 12 days. The overall acceptability scores of the samples were assigned based on the attributes such as appearance, flavour, taste and texture. In case of lemon peel extract treated samples LmAE-0.5 was found acceptable only up to Day-16 as per overall acceptability was concerned, however, in respect of all remaining treated samples, they were acceptable upto day-20. In case of EE treated surimi gels, all sensory attributes were found acceptable upto the end of the storage on day-20. The EE treated samples scored slightly higher than AE treated ones in respect of all sensory attributes.

Conclusion

The interactions between phenolic compounds and proteins play a very important role in the processing of certain food products. Antioxidants are therefore necessary to incorporated in order to increase storage stability, sensory quality and nutritional value of fish products. Beneficial effect of suitable agents possessing both antioxidant and antimicrobial activities for maintaining meat quality, extending shelf-life and preventing economic loss. Since, addition of synthetic antioxidants has been restricted because of their health risks and toxicity. In case of sweet lemon peel extract treated samples LmAE-0.5 was found acceptable only up to Day-16 as per overall acceptability was concerned, however, in respect of all remaining treated samples, they were acceptable upto day-20. In case of EE treated surimi gels, all sensory attributes were found acceptable upto the end of the storage on day-20. The EE treated samples scored slightly higher than AE treated ones in respect of all sensory attributes.

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