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Chilled Storage Study of Value Added Black Clam (*Villorita cyprinoides)* (Gray, 1825) products in two different Packaging Materials

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Abstract

Black clam (*Villorita cyprinoides)* (Gray, 1825*)*, a nutrient rich bivalve was used for preparing two products *viz.*, clam cutlet and clam bonda, and packaged in two different packaging materials, 200 gauge low density polyethylene and 12 μ polyester/ 200 gauge polyethylene laminate. The biochemical and microbiological parameters of the products were analyzed to study its storage stability in chilled condition. Parameters like pH, color (L*, a*, b*), total volatile base nitrogen (TVBN), thiobarbituric acid (TBA), peroxide value (PV) and free fatty acid (FFA) were within permissible limit during the period of observation. The aerobic plate count, after 24 days of storage of the products were in the range of 6.81 to 7.41 $\log_{10}$ cfu g^{-1} and 6.3 to 7 $\log_{10}$ cfu g^{-1} for LDPE and PES/PE respectively. *Escherichia coli* and Enterobacteriaceae were absent throughout the storage period. The study showed that biochemical and microbiological parameters were greater for the products packed in LDPE compared to PES/PE laminate, indicating that the laminate film was more efficient in preserving the quality of the products when compared to monolayer films.

Keywords: Low density polyethylene (LDPE), polyester polyethylene laminate (PES/PE), value added clam products, chilled storage studies

Introduction

Bivalves, comprising of clams, cockles, oysters and mussels, form a valuable fishery throughout the marine and estuarine sector of India. Among the exploited bivalve resources, clams are widely distributed and abundant (Narasimham et al., 1993). Black clam is the most exploited clam species in India which contributes about 80% of national clam production. It has good potential for trade in domestic markets, and value addition is one way to diversify demand.

Fish and fishery products are highly perishable due to their large moisture content and availability of nutrients for microbial growth (Brody, 1989). Spoilage is mainly accomplished by enzymatic, bacterial and chemical action on the fish product (Gram & Dalgaard, 2002). It is known that the activity of micro-organism can be limited, lowered or even retarded by proper handling and immediate reduction of temperature. Chilling is one of the most common means of preservation by reducing temperatures to below ambient temperature, which results in efficient short term storage of food materials by reducing many of the microbial and biochemical reactions accomplished with food spoilage and deterioration. Proper chilling along with use of suitable packaging material can prolong, the shelf life of fishing products. A range of different packaging materials and packaging technologies have been developed to facilitate quality improvement and preservation of packaged foods. Polyolefins are most commonly used packaging material in food processing industry due to ease of use, availability and lower costs (Marsh & Bugusu, 2007). Monoflexible films rarely fulfill all the requirements of a specific packaging application, but it is possible by laminating two or more materials to obtain the right combination of properties to suit almost any application (Maani et al., 2013; Vasile, 2000). The present study evaluates the shelf life of value added black clam products, cutlet and bonda, in low

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density polyethylene and polyester/polyethylene laminate packaging materials.

Materials and Methods

Live clams collected from Perumbalam Island, Alappuzha district, Kerala were transported to the Institute pilot plant, washed and loaded in perforated plastic trays (6 kg of whole clam per tray) and immersed in well aerated water tanks (600 l capacity) for depuration of about 15 h.

After complete draining, the clams were removed and steam shucked at 110°C for 15 min in a steaming chamber. The meat and shell were separated by using mechanical meat- shell separator (Sreejith et al., 2018). The meat was washed in water and the products were prepared using standard recipes.

Two flexible packaging materials namely low density polyethylene (LDPE) and polyester/polyethylene (PES/PE) laminate were used for packing the products (4 pieces of each product in one packet). After proper sealing and labeling, the products were stored in an insulated ice box with flake ice at 1:1 ratio. Products were stored in a chill room at a temperature of 2±1°C. Melt water was drained every day and supplemented with fresh flake ice. Biochemical and microbiological analyses were performed at an intervals of four days.

Samples were drawn and ground well into a fine paste. Triplicates of the samples of each product packed in the two packaging materials (LDPE and PES/PE) were taken for biochemical analysis. The parameters studied were PH (Cao et al., 2009), color (Robertson, 1977), Total volatile base nitrogen (TVBN) (Conway, 1962), Thiobarbituric acid value (TBA) (Tarladgis et al., 1960), Free fatty acid, FFA (Olley & Lovem, 1960) and Peroxide value (PV) (Jacobs, 1958). Similar product sample triplicates were also used for microbiological analysis.

Results and Discussion

Changes in pH of clam products in the two packaging materials, LDPE and PES/PE laminate, are shown in Fig. 1 & 2. The pH of cutlet and bonda prior to packaging was 6.22 and 6.25 respectively. The pH of these products when packed showed a gradually increasing trend and the increase is more in products packed in LDPE than PES/PE laminate. The results are similar to the observations by Yerlikaya et al. (2005) and Kilinc (2007) in fish patties; Tokar et al. (2006) and Coben (2013) in fish fingers and Mahmoudzadeh et al. (2010) in fish burger; Rathod & Pagarkar (2013) and Talab (2013) in fish cutlet. The reasons for rise in pH could be due to the release of free oxygen and hydrogen by the activity of micro-organisms and enzymes, which results the buildup of hydroxyl ion, thus causing an increment in pH level (Turhan et al., 2001), or the breakage of hydrogen bond and electrostatic interactions (Dhanpal et al., 2012). In fishery products the pH between 6.8 and 7 are considered as acceptable and values above 7 treated as spoiled (Kose et al., 2006; Ozyurt et al., 2007). In this study, the pH varies from 6.22 to 6.45 in cutlet packed in both packaging materials whereas in bonda it ranged from 6.25 to 6.46, both of which are within the acceptable limit recommended.

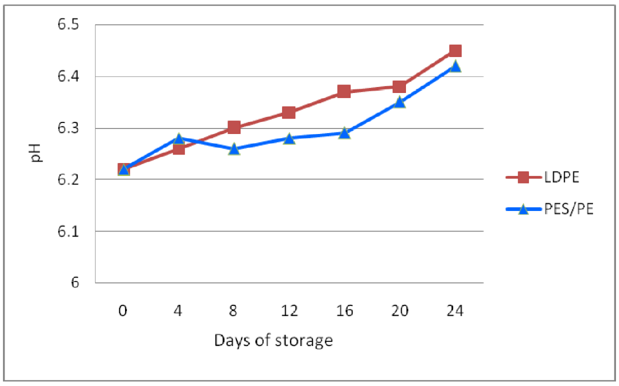

Fig. 1. Changes in pH of clam cutlet during chilled storage in two different packaging materials

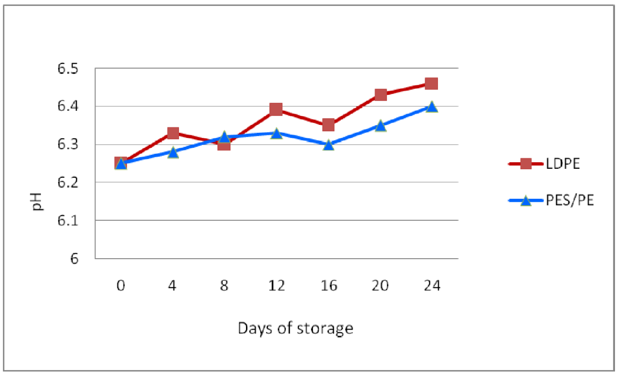

Fig. 2. Changes in pH of clam bonda during chilled storage in two different packaging materials

TBA is an indicator of degree of rancidity which is a secondary breakdown product of lipid oxidation (Aubourg, 1999; Baldarati et al., 1982). The TBA values of clam products exhibit an increasing trend throughout the storage period (Fig. 3 and 4). This increasing trend was evident up to the 20th day of storage in cutlet packed in LDPE and PES/PE laminate; whereas bonda in LDPE and PES/PE laminate, TBA decreased from 16th and 20th day of storage respectively. The decrease in TBA could be because of the retardation of lipid oxidation by products of lipid hydrolysis and interaction of malonaldehyde with proteins (Castell et al., 1966). The acceptable limit of TBA in fish is 1-2 mg malonaldehyde kg^{-1} (Lakshmanan, 2000). In the current study, the TBA values were well within the permissible limit throughout the storage period for both products.

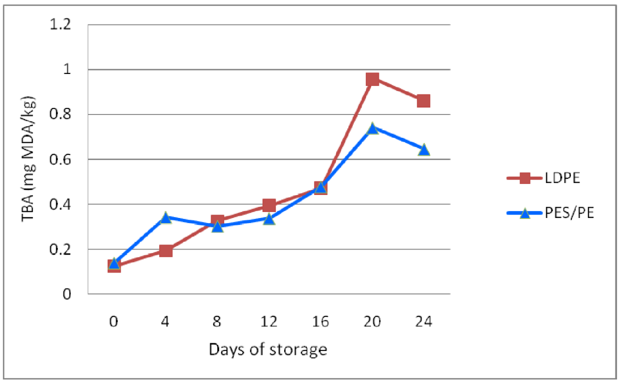

Fig. 3. Changes in TBA of clam cutlet during chilled storage in two different packaging materials

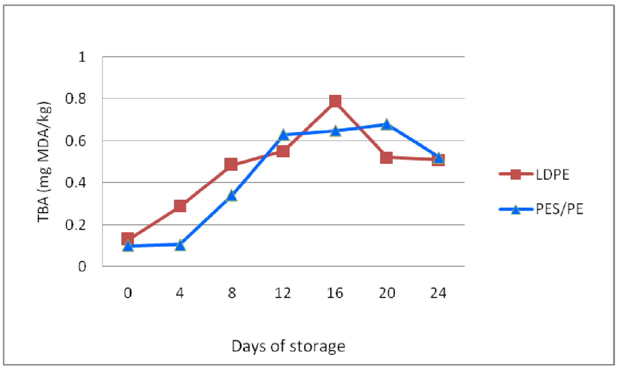

Fig. 4. Changes in TBA of clam bonda during chilled storage in two different packaging materials

Peroxide value (PV) is a measure of degree of oxidation in fat (Gopakumar, 2002). The PV is used for quantifying the production of primary oxidative products during storage. Oxidative rancidity is accompanied by the formation of hydro peroxides, which is formed by the interaction of oxygen with unsaturated fat in the products that results in unfavorable rancid flavor (Kolekar & Pagarkar, 2013). The changes in the peroxide value of clam products during chilled storage are shown in Fig. 5 & 6. During the initial stage of the storage period, the PV was absent in both products. It developed gradually and increased to 7.23 and 5.84 meq O_2 kg^{-1} of fat for cutlet in LDPE and PES/PE laminate and 12.34 and 9.01 for bonda in LDPE and PES/PE laminate respectively. Tokur et al. (2004) also observed the same during the frozen storage study of fish burger. Ninan et al. (2010) reported a hike in PV value during frozen storage study of fish cutlet for a period of 12 weeks. The acceptable limit of PV in fishery product is considered as 10-20 meq O_2 kg^{-1} (Connell, 1995) and the present study also the limits were acceptable.

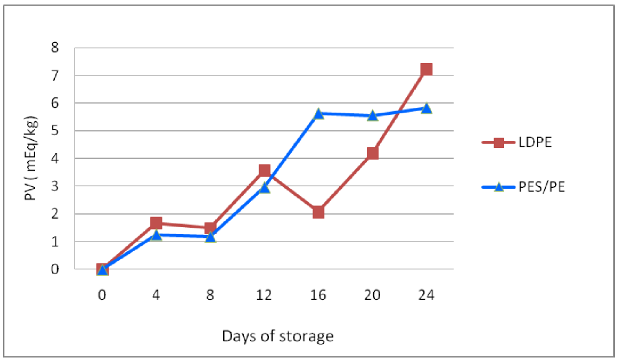

Fig. 5. Changes in PV of clam cutlet during chilled storage in two different packaging materials

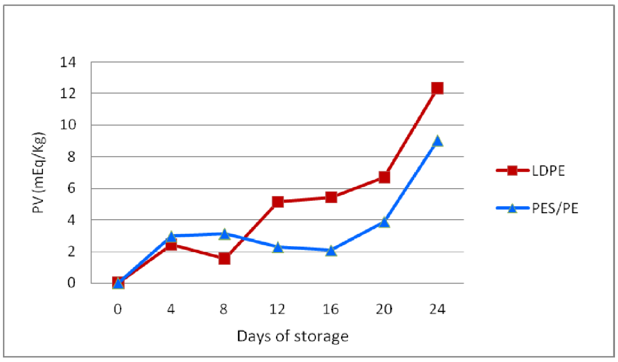

Fig. 6. Changes in PV of clam bonda during chilled storage in two different packaging materials

Free fatty acids are developed during storage as the result of oxidative hydrolysis of lipids which deteriorate the quality of meat (Huss, 1971). During frozen storage, free fatty acids are liberated either by enzymatic degradation of phospholipids or by non enzymatic hydrolysis of lipids. The trend of change in free fatty acid content of clam products in LDPE and PES/PE laminate are represented in Fig. 7 and 8. The increase in tendency of FFA is evident throughout period of study. The FFA content increased to 3.37 and 2.89 for cutlets packed in LDPE and Polyester/polyethylene laminate respectively. In case of bonda the respective values were 3.96 and 3.47. Observations in the present study concurs with result of Ninan et al. (2010) in tilapia fish cutlets, Pawar et al. (2013) in catla fish cutlet and Raju et al. (2000) in crab sticks cutlets during frozen storage.

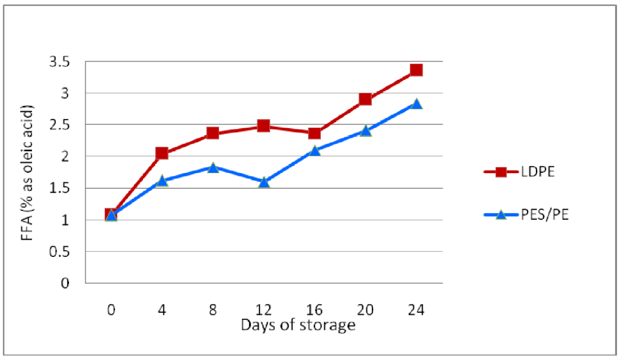

Fig. 7. Changes in FFA of clam cutlet during chilled storage in two different packaging materials

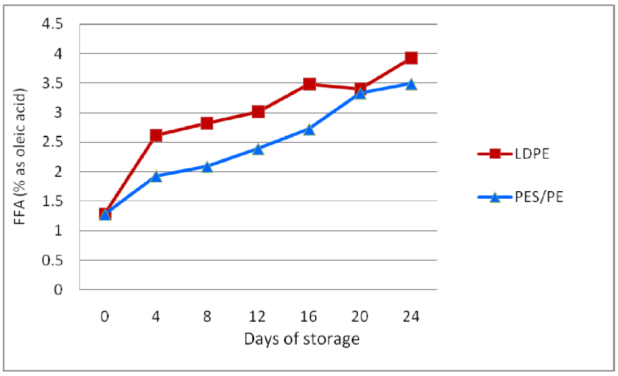

Fig. 8. Changes in FFA of clam bonda during chilled storage in two different packaging materials

TVBN is a frequently used spoilage index to determine the quality of fish. Total volatile base nitrogen (TVBN) is produced by bacterial spoilage and endogenous enzyme action in fishery products (EEC, 1995). Microbial enzymes lead to hydrolysis and putrefaction process, which results in the production of volatile bases and its exponential increase leads to the deteriorated odour and flavor. The TVBN prior to packaging was 4.2 and 5.6 for cutlet and bonda respectively. At the end of the storage study, it rose to 27 and 24 for cutlet packed in LDPE and PES/PE laminate and in the case of bonda the values were 28.89 and 26 respectively, which were within acceptable limit of below 35 mg 100 g^{-1} in fishery products (Marrakchi et al., 1990; Harpaz et al., 2003). Adebona (1978) reported that rise in TVBN with storage period is primarily due to the production of ammonia. Increase in TVBN values during storage is associated to bacterial spoilage, action of endogenous enzymes and decomposition of tissue proteins (Chomnawang et al., 2007).

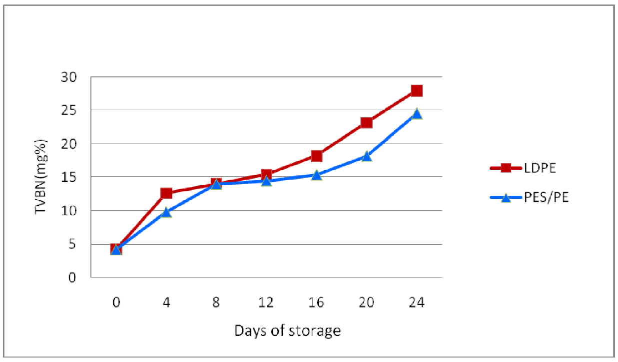

Fig. 9. Changes in TVBN of clam cutlet during chilled storage in two different packaging materials

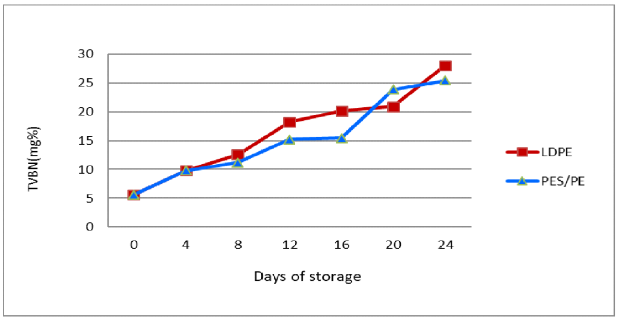

Fig. 10. Changes in TVBN of clam bonda during chilled storage in two different packaging materials

Colour is one of the significant parameter used to determine the freshness of fishery products (Ocano-Higuera et al., 2009). Haard (1992) stated that the initial colour of the fishery products altered during storage which affect the quality. The color of clam products packed in both packaging materials showed a gradual increase throughout the storage period. The reason may be due to the degradation of pigments in the products during storage (Alonso et al., 2016)

The aerobic plate count (APC) is a widely used quality indicator in fishery products. It evaluates shelf life and potential for the growth of micro-organism (Buchanan et al., 1992). The changes in APC

Table 1. Changes in color of clam cutlet during chilled storage

Days of storage	L* LDPE	L* PES/PE	a* LDPE	a* PES/PE	b* LDPE	b* PES/PE
0	42.27	47.19	0.99	1.41	29.43	29.59
4	47.23	48.33	0.89	1.42	31.23	30.22
8	48.19	48.29	1.31	1.41	30.2	20.01
12	49.18	48.31	1.45	1.42	31.32	31.09
16	49.55	49.55	1.48	1.41	30.2	31.58
20	50	49.69	1.69	1.69	32.05	31.72
24	50.9	50.28	1.97	1.81	33.62	32.82

Table 2. Changes in color of clam bonda during chilled storage

Days of storage	L* LDPE	L* PES/PE	a* LDPE	a* PES/PE	b* LDPE	b* PES/PE
0	53	53.18	2.57	2.23	28.09	28.93
4	53.1	53.21	2.65	2.42	29.23	29.51
8	53.3	53.21	2.66	2.57	29.24	29.66
12	53.4	53.21	2.71	2.75	30.02	30.02
16	53.4	54.56	2.91	2.75	30.92	30.11
20	54.91	55.32	2.95	3.58	30.93	30.51
24	55.92	56.08	3.38	2.56	31.1	31

during study were enumerated and the results are shown in Fig. 11 and 12. APC of both products showed a gradual increasing trend during the study period. The initial APC values for clam cutlet in LDPE and Polyester/polyethylene laminate were 5.03 $\log_{10}$ cfu g^{-1} and 5.08 $\log_{10}$ cfu g^{-1} respectively. After 24 days of storage cutlets packed in LDPE and Polyester/polyethylene laminate were 7.41 $\log_{10}$ cfu g^{-1} and 7 $\log_{10}$ cfu g^{-1} whereas the respective values for bonda in LDPE and Polyester/polyethylene laminate were 6.81 $\log_{10}$ cfu g^{-1} and 6.3 $\log_{10}$ cfu g^{-1}. Aerobic plate count of fish products did not exceed the maximum level of 7 $\log_{10}$ cfu g^{-1} (Jay, 1996). Bacteria such as *E.coli* and Enterobacteriaceae were absent in both the products throughout the study. The studies of Taskaya et al. (2003) in fish burger, Kilinc (2007) in fish patties, Baygar et al. (2008) in rainbow trout meat ball and Joseph et al. (1984) in fish cutlets revealed that there is a rise in the bacterial count during refrigerated and chilled storage.

The study showed that the shelf life of the two clam products *viz.*, cutlet and bonda in chilled condition, were upto 24 days when packed in LDPE and above

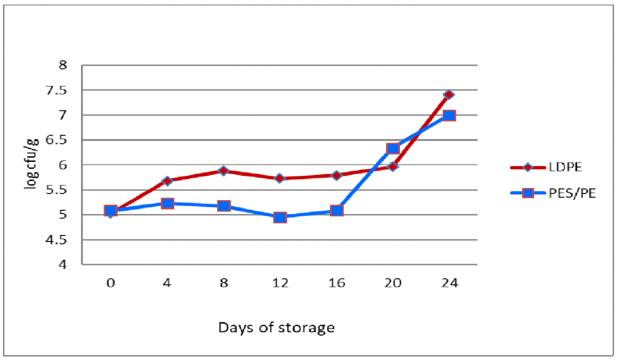

Fig.11. Changes in APC of clam cutlet during chilled storage in two different packaging materials

24 days when packed in PES/PE laminate. The values of biochemical and microbiological parameters were more for LDPE compared to PES/PE laminate which proved that laminate film was more efficient in preserving the quality of the products when compared to monolayer films. This information will benefit fishers and vendors to extend the shelf life of clam products using suitable packaging materials.

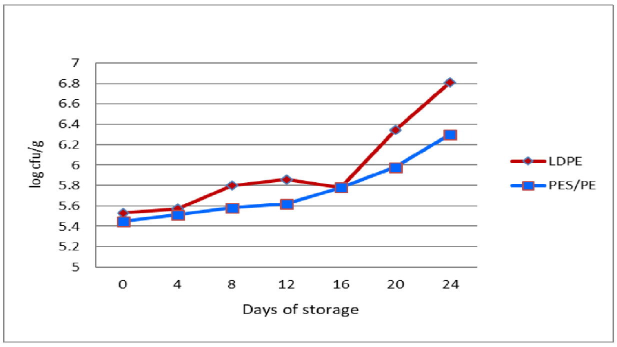

Fig. 12. Changes in APC of clam bonda during chilled storage in two different packaging materials

Acknowledgments

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