Fishery Technology 51 (2014) : 252 - 260

Effect of Binders on the Quality of Restructured Products from Silver Carp (*Hypophthalmichthys molitrix*) Surimi

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Abstract

The present study deals with the use of wheat flour and soya flour as additives in thermal gelification of surimi from silver carp muscle to obtain quality restructured products. Biochemical and mechanical properties of restructured products and changes during storage at -20°C were measured. Water holding capacity of the gel increased significantly when wheat flour and soya flour were added as binders. As regards mechanical properties, the gel strength, hardness and cohesiveness of gel with wheat flour showed intermediate value between control and gel with soya flour. But the springiness of wheat flour-added gel was superior. After 120 days of storage at -20°C, the wheat flour-added gel maintained superior quality amongst others. The study revealed that quality restructured products could be made with thermally set silver carp surimi with or without adding any binder, but wheat flour-incorporated gel maintained superior quality up to a storage period of 120 days.

Keywords: Restructured fish products, silver carp, thermal gel setting, binders, gelification

Received 30 January 2014; Revised 30 July 2014; Accepted 19 September 2014

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Introduction

Surimi technology offers a great opportunity to transform different fish species into high commercial value products. Though surimi is generally produced from coldwater marine species (Kellher et al., 1992), studies on suitability of freshwater species for surimi-based technology have gained importance due to reduction in marine fish landings. Among the cultivated freshwater fishes, silver carp (*Hypophthalmichthys molitrix*) has attracted great attention because of its increasing production, inherent soft texture and less interest by the fish traders in its trading. Surimi possesses functional properties such as gel-forming ability, water-holding capacity (Somjit et al., 2005) and is used as intermediate foodstuff for various texturized products such as crabsticks, crab legs, crab meat, young eel, scallops and others with a long shelf-life (Benjakul et al., 2003; Blanco et al., 2006).

Restructured fishery products are processed from surimi and chopped muscle, usually with added ingredients, to make products with a new appearance and texture. The restructuring process allows commercialization of some low-value fish species with higher profits and trimmings from filleting of commercial fish species (Noriega-Rodriguez et al., 2009). Thermal setting results from the activity of a calcium-dependent endogenous transglutaminase (TGase). This enzyme catalyses the formation of covalent bonds between adjacent proteins, improving gel structure. Transglutaminase catalyses an acyl-transfer reaction between α-carboxyamide groups of glutaminyl residues in proteins (Kumazawa et al., 1993). In this study, wheat flour and soya flour were used as binding agents.

Some investigations have been carried out on the quality of surimi of freshwater fish (Ismond & Tonogai, 1994; Kim et al., 1996). The suitability of several species of marine and fresh water fish for preparation of surimi and surimi-based products has been investigated in detail, but reported works on silver carp are very scanty. Different binding agents are available for the manufacture of value-added fish products from surimi or small pieces of fish muscle. Each of these binding agents work in a different way, as the interaction can be different depending on the kind of ingredients and the type of fish muscle involved in the process. The objective

of this work was to determine the feasibility of obtaining a restructured product from silver carp surimi involving thermal setting using wheat flour and soya flour as binding agent, offering fish processors a new processing alternative.

Materials and Methods

Fresh silver carp for the study was collected from the College of Fisheries fish farm at Lembucherra, Tripura. Length and weight range of fish were 37-49 cm and 636-809 g respectively. Individual fishes were washed with chilled water, gutted, dressed, filleted by hand and minced by employing a mechanical meat mincer (Deb Enterprise, India) with a 3 mm-hole plate. Minced meat was washed in wash tanks maintaining a water temperature of 10°C using a fish mince to water ratio of 1:4 (w/v) three times with five min duration for each wash (twice with potable water and last one with 0.1% NaCl solution to facilitate dewatering). The slurry was stirred for 3 min and allowed to settle for 2 min before water was decanted. Final dewatering was carried out using a screw press (Deb Enterprise, India). The washed mince (surimi) was packed in low density polyethylene (LDPE) pouches (250 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) for development of restructured products within a week.

Frozen surimi was tempered for about 2 h at 20±2°C until it reached 5±1°C, followed by chopping for 1 min at high speed in a silent cutter (Sun labz Equipments, Chennai, India). Different ingredients were added for different treatments designated as CON (control, without wheat or soya flour), TS-WP (surimi with wheat flour) and TS-SP (surimi with soya flour). Moisture was adjusted to 80% in all the parts by using ice water. Mixing of all ingredients including cryoprotectants (sorbitol-4%, sucrose-4% and sodium tripolyphosphate-0.3%) with the surimi was done in silent cutter and throughout the mixing operation temperature of surimi sol was kept below 10°C. The solubilised paste was stuffed into stainless-steel tubes (1.8 cm inner diameter; 12.5 cm length), which were previously sprayed with commercial vegetable oil to prevent sticking. For thermal setting, tubes were capped before immersion in water at 40°C for 30 min followed by immersion in water at 85°C for 30 min according to the two-step heating method suggested by Luo et al. (2008). After cooking, the tubes were immediately removed, placed in a refrigerated water bath and cooled at 4–5°C for 30 min. All gels were removed from tubes, placed in polystyrene bags and stored overnight at 4°C in a refrigerator. For storage study, the products were stored at -20°C for 120 days and storage changes were analysed at 30 days interval.

Moisture (method #930.15), fat (method #920.39) and protein (method #988.05) content were determined according to AOAC (2005). Water soluble protein (WSP) and salt soluble protein (SSP) contents were extracted from the surimi as per the procedure described by Dyer et al. (1950) and Srikar & Reddy (1991) followed by estimation of nitrogen by Kjeldahl method (AOAC, 2005). Thiobarbituric acid reactive substances (TBARS) were determined as described by Benjakul & Bauer (2001) and results were expressed as mg malondialdehyde kg^{-1}sample. Values of three independent experiments were recorded as mean ± SD. Total volatile base nitrogen (TVBN), peroxide value (PV) and free fatty acid (FFA) values were estimated as described by Conway (1947), Jacob (1958) and Takagi et al. (1984) respectively.

WHC was evaluated by the technique outlined by Barrera et al. (2002). 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 5000xg at 4°C for 15 min (make REMI, India). 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.

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

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

Mechanical properties were determined using a TA-XT2 Stable Micro Systems Texture meter (Surrey, England, UK). Cylindrical samples (1.8 cm x 2.5 cm, d x l) of restructured fish products were equilibrated to room temperature for 30 min in a plastic bag to avoid dehydration before the mechanical properties were measured. The breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyser equipped with a spherical plunger (5 mm diameter) according to the method of Benjakul et al. (2003). The probe was pressed into the cut surface of a gel specimen

perpendicularly at a constant depression speed (60 mmmin^{-1}) until the puncture occurred. The force in gram (g) required to puncture into the gel (breaking force) and the distance (in cm) at which the ball probe punctured into the gel (deformation) were recorded. Six samples were analysed for each treatment. Textural profile analysis (TPA) was performed using an aluminium cylindrical probe (P/50) with 50 mm diameter as described by Bourne (1978). Samples were compressed to 60% of the initial height using a compression speed of 60 mm min^{-1}. Hardness, springiness and cohesiveness were reported for each treatment. Six samples were analysed for each treatment at room temperature (25–27°C). The data obtained from biochemical and mechanical analyses were subjected to one-way ANOVA using SPSS (Statistical Package for Social Systems) windows 16.0 software. The significant differences are indicated as $p<0.05$.

Results and Discussion

The main constituents of the raw sample were moisture (78.53 ± 0.94%), crude protein (16.62 ± 0.77%), fat (2.24 ± 0.2%) and ash (1.57 ± 0.08%) (Table 1). As the proximate analysis shows, the muscle had medium fat and a high proportion of protein. Almost similar proximate composition of silver carp was reported by different workers

Table 1. Changes in biochemical characteristics of restructured products from silver carp mince during frozen storage of at -20°C (mean ± SD)*

Parameters	Treats	Day-1	Day-30	Day-60	Day-90	Day-120
Moisture (%)	CON	$79.39\pm2.8_{a}^{A}$	$78.99\pm3.7_{a}^{A}$	$78.84\pm3.7_{a}^{A}$	$78.16\pm2.2_{a}^{A}$	$78.12\pm2.3_{a}^{A}$
	TS-WP	$79.76\pm4.7_{a}^{A}$	$79.32\pm2.8_{a}^{A}$	$79.17\pm2.8_{a}^{A}$	$78.82\pm3.7_{a}^{A}$	$78.16\pm2.3_{a}^{A}$
	TS-SP	$79.81\pm4.7_{a}^{A}$	$79.64\pm4.6_{a}^{A}$	$79.60\pm4.5_{a}^{A}$	$78.14\pm2.2_{a}^{A}$	$78.01\pm1.9_{a}^{A}$
CP (%)	CON	$16.12\pm0.6_{a}^{A}$	$15.75\pm0.8_{a}^{A}$	$15.68\pm0.8_{a}^{A}$	$15.61\pm0.7_{a}^{A}$	$15.33\pm0.7_{a}^{A}$
	TS-WP	$16.70\pm0.3_{a}^{A}$	$16.62\pm0.3_{a}^{A}$	$16.46\pm0.2_{a}^{A}$	$16.38\pm0.6_{a}^{A}$	$16.15\pm0.6_{a}^{AB}$
	TS-SP	$17.16\pm1.1_{a}^{A}$	$16.93\pm1.1_{a}^{A}$	$16.83\pm1.0_{a}^{A}$	$16.78\pm1.1_{a}^{A}$	$16.62\pm0.3_{a}^{B}$
Lipids (%)	CON	$0.56\pm0.02_{ab}^{A}$	$0.80\pm0.03_{d}^{A}$	$0.71\pm0.03_{c}^{A}$	$0.58\pm0.02_{ab}^{A}$	$0.53\pm0.02_{a}^{A}$
	TS-WP	$0.81\pm0.03_{bc}^{B}$	$0.78\pm0.04_{ab}^{A}$	$0.85\pm0.03_{c}^{B}$	$0.77\pm0.04_{a}^{bB}$	$0.73\pm0.04_{a}^{B}$
	TS-SP	$1.04\pm0.06_{bc}^{C}$	$1.04\pm0.05_{bc}^{B}$	$0.94\pm0.06_{a}^{C}$	$0.95\pm0.04_{ab}^{C}$	$0.90\pm0.03_{a}^{C}$
SSP (%)	CON	$12.82\pm0.8_{c}^{A}$	$12.21\pm0.8_{c}^{A}$	$11.18\pm0.2_{bc}^{B}$	$10.34\pm0.4_{b}^{B}$	$9.08\pm0.4_{a}^{B}$
	TS-WP	$12.52\pm0.8_{e}^{A}$	$11.44\pm0.2_{cd}^{A}$	$10.11\pm0.4_{bc}^{A}$	$8.17\pm0.4_{a}^{A}$	$7.77\pm0.4_{a}^{A}$
	TS-SP	$13.08\pm0.8_{f}^{A}$	$12.04\pm0.2_{de}^{A}$	$11.37\pm0.2_{cd}^{B}$	$10.64\pm0.4_{bc}^{B}$	$8.69\pm0.4_{a}^{B}$
TVBN (mg100g^{-1}gel)	CON	$14.00\pm0.4_{a}^{B}$	$16.12\pm0.5_{b}^{B}$	$17.30\pm0.6_{bc}^{B}$	$18.40\pm0.5_{bc}^{B}$	$18.80\pm0.5_{bc}^{AB}$
	TS-WP	$13.00\pm0.3_{a}^{A}$	$13.48\pm0.4_{a}^{A}$	$15.10\pm0.4_{b}^{A}$	$16.13\pm0.5_{c}^{A}$	$18.00\pm0.6_{d}^{A}$
	TS-SP	$13.80\pm0.4_{a}^{B}$	$13.80\pm0.3_{a}^{A}$	$14.80\pm0.4_{bc}^{A}$	$16.16\pm0.5_{d}^{A}$	$19.60\pm0.6_{e}^{B}$
FFA (% Oleic acid)	CON	$6.40\pm0.2_{a}^{C}$	$8.40\pm0.3_{b}^{C}$	$9.21\pm0.9_{cd}^{B}$	$9.70\pm0.3_{de}^{C}$	$10.00\pm0.3_{e}^{B}$
	TS-WP	$5.70\pm0.2_{a}^{B}$	$7.70\pm0.2_{b}^{B}$	$8.15\pm0.3_{cd}^{B}$	$8.50\pm0.2_{cd}^{B}$	$8.80\pm0.3_{e}^{A}$
	TS-SP	$5.30\pm0.2_{a}^{A}$	$6.40\pm0.2_{b}^{A}$	$6.96\pm0.3_{c}^{A}$	$8.00\pm0.2_{e}^{A}$	$9.20\pm0.3_{g}^{A}$
PV (meq O_2 kg^{-1} lipid)	CON	$0.70\pm0.03_{a}^{C}$	$6.40\pm0.2_{b}^{C}$	$6.52\pm0.3_{b}^{C}$	$7.60\pm0.2_{c}^{B}$	$7.60\pm0.3_{c}^{A}$
	TS-WP	$0.60\pm0.02_{a}^{B}$	$5.12\pm0.2_{b}^{B}$	$5.83\pm0.2_{c}^{B}$	$6.40\pm0.2_{d}^{A}$	$8.60\pm0.2_{e}^{B}$
	TS-SP	$0.50\pm0.01_{a}^{A}$	$3.84\pm0.1_{b}^{A}$	$5.00\pm0.2_{c}^{A}$	$8.40\pm0.2_{d}^{C}$	$12.60\pm0.3_{e}^{C}$
TBARS	CON	$0.49\pm0.01_{a}^{A}$	$1.63\pm0.04_{b}^{B}$	$1.48\pm0.05_{bc}^{C}$	$1.73\pm0.04_{d}^{A}$	$2.17\pm0.05_{e}^{A}$
(mg malonaldehyde kg^{-1}	TS-WP	$0.48\pm0.01_{a}^{A}$	$1.34\pm0.05_{b}^{A}$	$1.37\pm0.05_{b}^{B}$	$1.87\pm0.05_{c}^{B}$	$2.37\pm0.06_{d}^{B}$
gel)	TS-SP	$0.63\pm0.02_{a}^{B}$	$1.87\pm0.05_{b}^{C}$	$1.26\pm0.04_{c}^{A}$	$1.82\pm0.05_{b}^{AB}$	$2.32\pm0.06_{d}^{B}$

*Mean values bearing different subscripts (a, b, c…) in a row and different superscripts (A, B, C…) in column are significantly different ($p<0.05$) with respect to period of storage and treatments respectively.

(Taskaya et al., 2009, Asgharzadeh et al., 2010, Buchtova & Jezek, 2011, Majumdar et al., 2012).

The gel was set following 2-step heating process, *viz*., 30 min at 40°C followed by 30 min at 85°C. Generally, a thermal gel is formed in a 2-step heating process to improve gelling characteristics (Lee, 1984). Silver carp showed a setting effect (suwari), texture-enhancing treatment by pre-incubating salted surimi at temperatures lower than 40°C before cooking at high temperature (85°C) (Chen et al., 2000). In another study, Nowsad et al. (1999) also found that silver carp paste did not set at low incubation temperatures till 40°C in one-step heating and the highest setting ability was found at around 50°C. In the present study, the proximate composition of thermally set gel was found as moisture (79.39, 79.76 and 79.81%), lipid (0.56, 0.80 and 1.04%) and protein (16.1, 16.7 and 17.16%) in CON, TS-WP and TS-SP respectively. The salt-soluble protein and TBARS values were found to be 12.82%, 12.52%, 13.08% and 0.49, 0.48, 0.63 in CON, TS-WP and TS-SP respectively. Cardoso et al., (2012) reported the proximate composition of heat-induced gel from sea bream as moisture (73.1%), protein (16.7%), fat (6.2%) and ash (2.9%).

The biochemical characteristic of the gel obtained by thermal setting is given in Table 1. A slight increase ($p>0.05$) of moisture content was noticed in the sample containing wheat flour and soya flour compared to the control. This could be due to interaction of water with starch present in wheat flour and soya flour. The slight increase in crude protein and fat content in TS-WP and TS-SP may be due to inclusion of lipid and protein contents of wheat (lipid~1%, protein~10%) and soya flour (lipid~6.7%, protein~50%) in surimi. Although statistically not found significant, but a slightly less SSP in TS-WP and slightly more in TS-SP compared to control was observed (Table 1). Lower SSP values in TS-WP could be that wheat flour by itself is able to form fiber/protein aggregates large enough to resist extraction (Sanchez-Alonso & Borderias, 2007). Higher ($p<0.05$) TBARS value in TS-SP could be due to higher fat content in the gel compared to other samples.

Texture profiles for all the gels studied are shown in Table 2. In thermally set gel breaking force (BF, g) varied from 826.4 to 1041.2. Highest ($p<0.05$) BF was found in sample TS-SP followed by TS-WP and CON. Addition of soya protein is reported to have increased the breaking force of gel but concentration beyond 10% decreased the BF (Luo et al., 2008). The breaking deformation ranged from 1.13 to 1.26 cm and showed no significant difference between the samples. Work of penetration (kgxcm), *viz*., gels strength (GS) varied from 0.904 to 1.305 kgcm with highest in sample TS-SP. The higher gel strength in wheat flour and soya flour incorporated gel than the control could be due to higher protein content in TS-WP and TS-SP as the strength of the gel increases with protein concentration (Mulvihill & Kinsella, 1987; Hermansson et al., 1986). In respect of other textural characteristics of thermally set gels such as hardness, springiness and cohesiveness, the sample TS-SP showed better performance than TS-WP and CON (Table 2). Devatkal & Mendiratta (2001) also reported increased hardness when binding agents were added during restructuring of fish surimi. Soya protein isolate (SPI) modified the textural properties of surimi gels from silver carp (Ramirez et al., 2011). However, adding 100 g kg^{-1} SPI improved the mechanical properties of surimi gels obtained by incubating fish pastes at 50°C for 60 min before heating at 85°C for 30 min (Luo et al., 2006; 2008).

Water holding capacity (WHC) of the gel increased ($p<0.05$) when wheat flour (TS-WP) and soya flour (TS-SP) were added as binders (Table 2). Improvement of WHC of meat upon incorporation of wheat fibre and soya protein has been reported (Sanchez-Alonso et al., 2007; Tsao et al., 2002). Soya protein concentrate has fat and water holding properties (Singh et al., 2008).

Biochemical quality changes during storage of restructured products (RP) at -20°C are given in Table 1. The initial moisture content of all the samples of thermal set RPs decreased slightly which were not significant ($p>0.05$). The decrease could be explained as decrease of WHC of the gel and this occurs mainly due to freeze-denaturation of myofibrillar proteins. However, in all the treatments, the loss of moisture during the storage of RPs was very less (1.6 to 2.2%), and this could be due to the effect of cryoprotectants used before gel setting. Arakawa & Timasheff (1982) reported that cryoprotectants increase the surface tension of water as well as the binding energy, preventing withdrawal of water molecules from the protein, thus stabilizing the protein.

Crude protein content registered a loss during the period of storage for 120 days in all the samples of

Table 2. Changes in mechanical properties (TPA and puncture test) and WHC of restructured products from silver carp mince during frozen storage of at -20°C (mean ± SD)*

Parameters	Treats	Day-1	Day-30	Day-60	Day-90	Day-120
Breaking force (g)	CON	$826.4\pm42.6_{a}^{C}$	$780.7\pm21.8_{b}^{C}$	$759.5\pm24.6_{c}^{B}$	$731.3\pm32.4_{cd}^{B}$	$713.7\pm46.9_{d}^{C}$
	TS-WP	$974.3\pm32.5_{a}^{B}$	$967.3\pm33.2_{ab}^{B}$	$941.7\pm41.3_{b}^{A}$	$876.7\pm33.6_{c}^{A}$	$859.6\pm38.7_{d}^{A}$
	TS-SP	$1041.2\pm28.7_{a}^{A}$	$987.6\pm43.4_{b}^{A}$	$912.4\pm52.4_{c}^{A}$	$864.2\pm42.7_{d}^{A}$	$812.4\pm28.6_{e}^{B}$
Breaking deformation (cm)	CON	$1.13\pm0.08_{a}^{B}$	$1.03\pm0.11_{ab}^{C}$	$0.97\pm0.08_{bc}^{B}$	$0.94\pm0.12_{c}^{B}$	$0.89\pm0.07_{cd}^{B}$
	TS-WP	$1.19\pm0.06_{a}^{AB}$	$1.17\pm0.05_{a}^{AB}$	$1.12\pm0.07_{a}^{A}$	$1.05\pm0.06_{b}^{A}$	$1.01\pm0.07_{bc}^{A}$
	TS-SP	$1.26\pm0.17_{a}^{A}$	$1.21\pm0.11_{a}^{A}$	$1.15\pm0.09_{b}^{A}$	$1.08\pm0.12_{bc}^{A}$	$1.02\pm0.18_{bc}^{A}$
Work of penetration (kgcm)	CON	$0.904\pm0.29_{a}^{C}$	$0.807\pm0.17_{ab}^{C}$	$0.755\pm0.204_{c}^{B}$	$0.683\pm0.125_{cd}^{B}$	$0.641\pm0.09_{d}^{B}$
	TS-WP	$1.176\pm0.211_{a}^{B}$	$1.124\pm0.134_{a}^{AB}$	$1.067\pm0.188_{ab}^{A}$	$0.923\pm0.163_{b}^{A}$	$0.859\pm0.11_{c}^{A}$
	TS-SP	$1.305\pm0.197_{a}^{A}$	$1.216\pm0.206_{ab}^{A}$	$1.039\pm0.155_{c}^{A}$	$0.917\pm0.118_{d}^{A}$	$0.831\pm0.14_{e}^{A}$
Hardness (kgf)	CON	$3.12\pm0.24_{d}^{B}$	$3.44\pm0.18_{b}^{B}$	$3.56\pm0.27_{a}^{B}$	$3.22\pm0.21_{c}^{C}$	$2.98\pm0.19_{e}^{C}$
	TS-WP	$3.71\pm0.43_{b}^{A}$	$3.92\pm0.32_{a}^{A}$	$3.85\pm0.26_{ab}^{A}$	$3.69\pm0.19_{bc}^{A}$	$3.48\pm0.23_{d}^{A}$
	TS-SP	$3.94\pm0.33_{a}^{A}$	$3.85\pm0.27_{ab}^{A}$	$3.78\pm0.34_{ab}^{A}$	$3.34\pm0.22_{c}^{B}$	$3.06\pm0.15_{d}^{B}$
Springiness (mm)	CON	$0.567\pm0.08_{c}^{C}$	$0.577\pm0.05_{ab}^{C}$	$0.581\pm0.03_{a}^{C}$	$0.558\pm0.07_{c}^{C}$	$0.531\pm0.11_{d}^{C}$
	TS-WP	$0.667\pm0.15_{c}^{A}$	$0.684\pm0.08_{ab}^{A}$	$0.692\pm0.11_{a}^{A}$	$0.677\pm0.06_{c}^{A}$	$0.642\pm0.08_{d}^{A}$
	TS-SP	$0.624\pm0.06_{a}^{B}$	$0.626\pm0.09_{a}^{B}$	$0.611\pm0.05_{b}^{B}$	$0.589\pm0.06_{c}^{B}$	$0.556\pm0.08_{d}^{B}$
Cohesiveness	CON	$0.128\pm0.02_{b}^{C}$	$0.132\pm0.04_{a}^{C}$	$0.121\pm0.02_{c}^{C}$	$0.117\pm0.02_{d}^{C}$	$0.094\pm0.01_{e}^{C}$
	TS-WP	$0.157\pm0.03_{c}^{B}$	$0.168\pm0.02_{b}^{A}$	$0.174\pm0.01_{a}^{A}$	$0.153\pm0.02_{d}^{A}$	$0.142\pm0.02_{e}^{A}$
	TS-SP	$0.169\pm0.01_{a}^{A}$	$0.157\pm0.02_{b}^{B}$	$0.143\pm0.02_{c}^{B}$	$0.127\pm0.01_{d}^{B}$	$0.109\pm0.02_{e}^{B}$
WHC (%)	CON	$80.06\pm1.49_{a}^{C}$	$76.62\pm1.84_{b}^{C}$	$68.24\pm0.88_{c}^{B}$	$65.56\pm2.21_{d}^{C}$	$57.75\pm1.05_{e}^{C}$
	TS-WP	$85.26\pm1.68_{a}^{A}$	$83.84\pm1.44_{b}^{A}$	$76.52\pm1.13_{c}^{A}$	$73.64\pm0.87_{d}^{A}$	$68.48\pm0.95_{e}^{A}$
	TS-SP	$83.26\pm2.72_{a}^{AB}$	$79.14\pm1.67_{b}^{B}$	$75.59\pm0.69_{c}^{A}$	$71.38\pm1.47_{d}^{B}$	$61.64\pm1.92_{e}^{B}$

*Mean values bearing different subscripts (a, b, c…) in a row and different superscripts (A, B, C…) in column are significantly different ($p<0.05$) with respect to period of storage and treatments respectively.

both thermal and cold set RPs, but the decreases were not significant ($p>0.05$). Moreover, the differences in crude protein content between the treatments in each sampling day were not significant ($p>0.05$). However, in the control (without any binder) of thermal set RPs, the protein content on day-120 was found to be 15.33% (4.9% decrease from the initial value). Whereas, in treatments TS-WP and TS-SP, the decrease of protein contents were 3.3% and 3.15% respectively. Such differences between the control and binder added samples could be due to the effect of binding agents like wheat and soya.

In thermally set RPs, salt soluble protein decreased significantly ($p<0.05$) in all treatments throughout the storage period, *viz*., 29.17, 37.94 and 33.56% in CON, TS-WP and TS-SP respectively. SSP in control was significantly higher than the other groups ($p<0.05$) on all sampling days. After 30 days of storage, the SSP content of TS-WP was found to have a significant ($p<0.05$) than CON and TS-SP. The reason could be explained as interference of added binders in extraction of SSP due to formation of binder-protein aggregates and also as a result of freeze denaturation of protein during storage at -20°C. According to Sanchez-Alonso & Borderias (2007), a possible explanation for the lower values found in the SSP could be that dietary fibre by itself is able to form fiber-protein aggregates large enough to resist extraction. In this study, the restructured product with added wheat flour as binding agent suffered more in respect of extraction of SSP.

Total volatile basic nitrogen (TVBN) increased significantly ($p<0.05$) in all the samples during storage. In thermally set RPs, the increase of TVBN at the end of 120 days was 34% in control, whereas in TS-WP and TS-SP the increases of same were 38.5% and

42.0% respectively. However, the values on 120th day was 18.00 mg 100 g^{-1} and 19.6 mg 10 0g^{-1} in TS-WP and TS-SP respectively (Table 1). Most workers recommended TVB-N of 20 mg 100 g^{-1} meat as the beginning of spoilage and 30 mg 100 g^{-1} of TVB-N as spoiled, while the acceptability limit was between 18 and 24 mg 100 g^{-1} for frozen stored pink perch surimi (Reddy et al., 1995). TVB-N content should be considered a very unreliable indicator of frozen storage quality loss (Kyrana et al., 1997). In this study, the TVBN value on 120th day did not reach the level of unacceptability.

Accumulation of FFA is said to contribute to off-flavour of the product and cause textural alterations by complexing with proteins (Mai & Kinsella, 1980). In thermally set RPs, the FFA value on 1st day was between 5 and 6% as oleic acid and thereafter showed a slow but gradual increase with the progress of storage period and the value on 120thday reached 10.0 (>56% increase) in CON, 8.80 (>54% increase) in TS-WP and 9.2 (>73% increase) in TS-SP. On each day of sampling, the differences in FFA values between the samples were significant ($p<0.05$). A marked FFA increase with time in restructured fish product during frozen storage could be explained as a result of hydrolytic enzymes present in the surimi and in gel, which remain active during frozen storage at -20°C. Similar observation was reported by different authors during frozen storage of fish surimi (Kaneniwa et al., 2000; Sikorski & Kolakowski, 2000). Though formation of FFA itself does not lead to nutritional losses and that the FFA values in the product on 120th day were not high, its assessment is deemed important when considering the development of rancidity. Because, a pro-oxidant effect of FFA on lipid has been proposed and explained on the basis of a catalytic effect of the carboxyl group on the formation of free radicals by the decomposition of hydro peroxides (Aubourg, 2001). In addition, FFA has shown to interact with proteins leading to fish texture deterioration during frozen storage (Mackie, 1993).

Rancidity development was measured by means of primary (PV) and secondary (TBARS) lipid oxidation compound formation. PV registered a steady increase ($p<0.05$) in all the samples during the period of storage. The PVs on 1st day were 0.70, 0.60 and 0.50 meq O_2kg^{-1} lipid in CON, TS-WP and TS-SP, while on 120th day they were 7.60, 8.60 and 12.60 mmoles O_2kg^{-1} lipid in CON, TS-WP and TS-SP respectively. The PV of different samples on each sampling day was significantly ($p<0.05$) different from each other. Initial PV was negligible which could be due to less fat contents of the products and thereafter increased due to oxidative degradation of fat. The increase of PV during frozen storage is indicative of oxidative deterioration (Srikar et al., 1989). Siddaiah et al. (2001) reported increase of PV from 16.93 to 145.54 m eq of O_2kg^{-1} of fat during frozen storage of silver carp surimi and similar observations were also made by Reddy & Srikar (1996) during frozen storage of pink perch surimi. However, in the present study, in both thermally set RPs, the PVs on 120th day seemed to be very low and within the limit of acceptability.

The fish smells and tastes rancid when the PV value exceeds 20 meq of O_2kg^{-1} of fat (Lakshmanan, 2002). Rancidity was explained as a result of the presence of pro-oxidant enzymes and pro-oxidant molecules in the surimi (Sikorski & Kolakowski, 2000). Rancid odour could not be perceived by the panellists throughout the storage period. The result of this study suggests that there seems to be less influence of binders like wheat and soya flours on lipid oxidation during low temperature storage.

TBARS value, an indicator of the degree of secondary lipid oxidation showed an increase ($p<0.05$) in all the samples during storage study. The initial TBARS value in all the samples of thermally set RPs were between 0.48 and 0.63 mg malonaldehyde kg^{-1} gel and the values on day-120 reached to 2.17, 2.37 and 2.32 in CON, TS-WP and TS-SP respectively. When TBA value exceeds 2.0 mg malonaldehyde kg^{-1} meat, fish smells and tastes rancid (Lakshmanan, 2002). TBARS value of different samples in each sampling day was found significantly ($p<0.05$) different from each other. Increase of TBARS during frozen storage of fish surimi has been reported (Majumdar et al., 2012; Hoke et al., 2000). The protein solubility was reduced when the TBARS value increased during frozen storage of surimi gel. 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). In the present study, the salt soluble protein contents were found to have decreased during the storage period and simultaneous increase in TBARS value could be one of the many possible reasons.

Mechanical properties of restructured products including WHC showed changes during storage at

-20°C (Table 2). Breaking force (g) reduced significantly ($p<0.05$) in all the treatments by 13, 11 and 22% in CON, TS-WP and TS-SP respectively. Accordingly, gel deformation as well as gel strength registered decrease during the frozen storage period. Maximum decrease ($p<0.05$) of gel strength was observed in case of TS-SP (36%) followed by TS-WP (27%) and CON (29%). This could be due to protein denaturation during frozen storage, as it is evidenced by the gradual decrease of SSP content and increase of lipid degraded products with the progress of storage period.

Similarly, all the texture profile parameters showed decrease ($p<0.05$) during the storage period (Table 2). However, the quality of TS-WP was observed to be superior amongst the treatments. Hardness varied from 2.98 to 3.48 kgf. TS-WP showed higher ($p<0.05$) hardness value then TS-SP and control. Springiness and cohesiveness of RS-WP showed values higher ($p<0.05$) than TS-SP and control. Positive role of wheat fibre and wheat glutein in improving the quality of RP has been reported (Sanchez-Alonso et al., 2007).

Indices such WHC are often used to assess textural quality of the RPs and it also indicates the deterioration of protein quality during frozen storage. All treatments experienced a decrease ($p<0.05$) of WHC during the period of storage for 120 days at -20°C. The WHC estimated on 120th day were maximum in TS-WP (68.48%) followed by TS-SP (61.64%) and CON (57.75%). In surimi-based product technology, water-holding capacity is directly correlated with gel strength (Honikel & Hamm, 1994). Improvement of WHC of protein by incorporating wheat fibre or wheat glutein has been reported by Sanchez-Alonso et al. (2007). Soya protein has high potential to be incorporated as binder for restructured meat products because of its high binding ability with muscle proteins (Tsao et al., 2002; Renkema & van Vliet, 2002). Decrease in WHC of protein during frozen storage is indication of loss of functional properties of myofibrillar protein.

The study indicated that less water was imbibed in the gel matrix as a result of an increase in protein denaturation due to extended frozen storage leading to lower water affinity and accordingly, a decrease in WHC. Study also indicates that quality restructured products could be made with thermally set silver carp surimi with or without adding any binder, but the quality changes during frozen storage period. Products with added wheat flour maintain superior quality up to a storage period of 120 days.

Acknowledgement

The authors like to express their sincere thanks to the authority of College of Fisheries (Central Agricultural University), Lembucherra, Tripura and technical staff of the Department of Fish Processing Technology for all assistance.

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