Fishery Technology 56 (2019) : 49 - 59

Cryoprotective effect of Carrot (*Daucus carota*) Antifreeze Protein on Surimi from Stripped Catfish (*Pangasianodon hypophthalmus*) (Sauvage, 1878)

Sanjeev Sharma, R. K. Majumdar*, K. Siddhnath and J. Parhi
College of Fisheries, CAU (I), Lembucherra, Tripura - 799 210, India

Abstract

Cryoprotective effect of (*Daucus carota*) antifreeze protein from carrot was compared with the typical cryoprotectant (sucrose-sorbitol-sodium tripolyphosphate) during frozen storage of surimi from Stripped catfish (*Pangasianodon hypophthalmus*) (Sauvage, 1878) for six months at $^{-}$20°C. Carrot concentrated protein (CCP) containing antifreeze protein was used as cryoprotectant either alone or with the typical cryoprotectant. A typical cryoprotectant was made control. Molecular weight of carrot antifreeze protein was 36 kDa. There was a significant ($p<0.05$) decrease of protein solubility, Ca^{2+}ATPase activity and gel strength during the storage. In respect of denaturation and other functional attributes of surimi, treatment T-3 (CCP 0.5% + 50% of typical cryoprotectant) maintained quality of protein significantly higher ($p<0.05$) compared to all the treatments. Further, a synergistic effect of carrot antifreeze protein and typical cryoprotectant could also be suggested.

Keywords: Surimi, cryoprotectant, antifreeze protein, protein denaturation

Introduction

Surimi, a myofibrillar protein concentrate from fish is used for development of different value added products. Mechanically deboned fish meat is water-washed for removal of sarcoplasmic protein, blood, inorganic salt, and some lipid and other undesirable materials like pigments so as to enhance the gel forming ability. Frozen storage of surimi is usually accompanied by several biochemical changes which include formation of intermolecular cross-linkages leading to aggregation and denaturation of acto-myosin due to loss of bound water available to the proteins. Such decrease of bound water results increase in electrolyte concentration and mechanical damage of muscle structures due to ice crystal growth (Sikorski et al., 1976). Cryoprotectants function to stabilize the typical properties of myofibrillar proteins, such as gel-forming ability, water holding capacity, protein solubility etc. during frozen storage by preventing unfolding of the protein molecules (Carpenter & Crowe, 1988) and in this way the proteins exclude the solutes from their surface through their interaction with the surrounding. A typical cryoprotectant consists of sucrose and sorbitol in a 1:1 ratio with sodium tripolyphosphate (STPP) as synergist (MacDonald & Lanier, 1994). But, since sucrose imparts sweetness and high calorie value to surimi (Carvajal et al., 1999), its use is undesirable for some types of food products and consumers especially who avoid sweetness and/or have health concerns such as diabetes.

Recently interest have grown amongst the food scientists to identify other cryoprotectants for surumi which can reduce sweetness, less capable to induce Maillard browning reaction (Park et al., 1987; Sych, 1990) and capable of non-colligative freezing point depression (Knight et al., 1984). AFPs (anti-freeze proteins) inhibit growth and re-crystallization of ice through binding with the small ice crystals, which would otherwise be fatal for protein. Therefore, cryoprotective ability of AFPs may find application to protect surimi during frozen storage of muscle foods by inhibiting recrystallization of ice. AFPs have potential to preserve food texture by reducing cellular damage and minimizing the loss of nutrients by reducing drip (Mueller et al., 1991)

Received 10 April 2018; Revised 06 October 2018; Accepted 24 December 2018

*E-mail: drrkmcof@gmail.com

and their application have been reported in frozen meat (Payne et al., 1994), pre-slaughter lamb (Payne & Young, 1995) and frozen dough (Zhang et al., 2007).

Smallwood et al. (1999) isolated a novel cold-induced antifreeze protein (AFP) with molecular weight 36 kDa from carrot. The carrot concentrated protein (CCP) containing 18% carrot antifreeze protein (*Dc*AFP) was reported by Zhang et al. (2007) and they also demonstrated its anti-recrystallization property when incorporated in frozen dough. Although, most of the studies with carrot's anti-freeze protein have been reported on improvement of the texture property of dough (Zhang et al. 2007; 2008), very few works on the application of AFP to reduce effect of freezing of meat are reported. Payne & Young (1995) reported about reduction of ice crystal size and tissue damage when meat was soaked in a solution of AFP before freezing. Pre-treatment of fish through injection of AFP resulted in freezing point depression in case of rainbow trout (Fletcher et al., 1986).

Since, there are no reports available on the use of CCP as cryoprotective agent during frozen storage of surimi; the present study is an attempt to evaluate the potential of CCP in protecting protein from denaturation during frozen storage of surimi.

Materials and Methods

Locally available carrot (*Daucus carota*) roots were purchased from market to prepare CCP powder. CCP was prepared according to the method of Zhang et al. (2007). Carrot roots were acclimatized at refrigerated temperature (4°C) for 4 weeks as suggested by Ding et al. (2014) for cold acclimatization. Then the carrot roots were washed with ice-cold de-ionized water, cut into small pieces and immediately homogenized with ice-cold 50 mM Tris-HCl buffer (7.4 pH) at a ratio of 1:2 (w/v) using kitchen grinder (MX-AC300, Panasonic). Homogenate was centrifuged at 10,000 xg at 4°C for 30 min. followed by collection of supernatant which was further precipitated with an equal amount (1:1, v/v) of chilled acetone. Precipitate was re-suspended in ice-cold 50 mMTris-HCl buffer (7.4 pH) followed by centrifugation at 3500 xg and the pellet was lyophilized by using freeze-drier.

Fresh Stripped catfish (*P. hypophthalmus*) was procured from the local fish market and brought to the laboratory with ice within one hour. The average length and weight of fish were 42.6±2.76 cm and 2530.9±182.60 g respectively. Surimi was made following the method of Majumdar et al. (2015). Typical cryoprotectants (sorbitol-4%, sucrose-4% and polyphosphate-0.3%) as well as CCP were mixed as per schedule given in Table 1 with each 500 g surimi in a silent cutter (Sunlabz Equipments, Chennai, India) at temperature below 10°C. Finally, the surimi was packed in low density polyethylene (LDPE) pouches (500 g per pouch), 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 further study.

Table 1. Different treatments

Treatments	CCP	Conventional cryoprotectants
C	-	4% sucrose, 4% sorbitol and 0.3% STPP
T-1	1%	-
T-2	0.5%	-
T-3	0.5%	2% sucrose, 2% sorbitol and 0.15% STPP

Frozen surimi was tempered at 20±2°C until it reached to 5±1°C, followed by chopping in a silent cutter for 1 min at high speed. The surimi was adjusted to a moisture content of 80% by using ice water by mixing in silent cutter for 5 min with 2.5% NaCl taking care to maintain the temperature below 10°C. The surimi sol was stuffed into vinylidene chloride casing (10 cm length, 2.0 cm Ø). Two step thermal setting was followed, in which casings were first immersed in water bath at 40°C for 30 min followed by immersion in another water bath at 85°C for 30 min. Following cooking, the casings were immediately removed from the waterbath, placed in iced water, and cooled to 4–5°C for 30 min. The cold gels were stored overnight at 4°C in a refrigerator and textural and sensory properties were analyzed in the next day.

Proximate composition of samples, i.e., moisture, ash, pH, protein and fat contents were done according to AOAC (2000). Methods suggested by Conway (1947) and Tarladgis et al. (1960) were followed to determine total volatile basic nitrogen (TVBN) and thiobarbituric acid reactive substances (TBARS) respectively. TBARS values were expressed in mg malonaldehyde kg^{-1}.

Protein solubility of the surimi was determined according to the method of Benjakul & Bauer (2000). The precipitated protein was solubilised in 0.5 M NaOH for estimation of protein content following Biuret method (Gornall et al. 1949). Water holding capacity (WHC) of surimi samples was estimated following the technique described by Barrera et al. (2002). WHC was expressed as (%) the weight of the centrifuged gels relative to the original weight of samples.

The method suggested by Noguchi & Matsumoto (1970) was followed to estimate myosin ATPase activity and expressed as microgram of inorganic phosphorus (Pi)/mg protein/min at 27°C. 1 g of surimi was macerated with 10 ml 50 mM glycine-NaOH buffer, pH 9.2. The slurry was filtered through Whatman No. 1 filter paper, and filtrate was used as an enzyme solution. The reaction mixture containing 0.06 ml of ATP solution (0.05 M), 0.4 ml $CaCl_2$ (0.1 M), 2 ml buffer (0.05 M glycine-NaOH, pH 9.2) and 0.4 ml of meat extract was made up to 4.0 ml with buffer and incubated at 27°C for 5 min. The reaction was stopped by the addition of 2 ml of 15% TCA. The blank was carried out by adding 15% TCA before the extract was added. The mixture was filtered through Whatman No. 1 filter paper. To 3 ml of filtrate, 2 ml of freshly prepared ferrous sulphate-ammonium molybdate solution was added and intensity of colour developed was read at 660 nm after 30 min (Tausky & Shorr, 1952).

ATPase activity was calculated as described in following equation:

$$\text{ATPase activity (microgram Pi/mg protein/min)} = \frac{A - B}{5 \times \text{Protein (mg)}}$$

Where, A and B represent the absorbance values of the sample and Sample blank at 660 nm, respectively

Antioxidant activity was determined according to the method of Kulkarni & Aradhya (2005) with some modifications. 1 g of sample was mixed with 9 ml of 100 mM Tris- HCl buffer (pH 7.4) to which 1ml of DPPH (0.500 mM in ethanol) was added. The control sample was prepared in a similar way instead of sample. The mixtures were shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (Perkin-Elmer, USA). The reaction mixture without DPPH was used for the background correction. The antioxidant activity was calculated using following equation:

$$\text{Antioxidant activity (\%)} = [1- (\text{OD (sample)} / \text{OD (control)})] \text{ X } 100 \text{ \%}$$

The molecular weight distribution of CCP was studied by SDS-PAGE as suggested by Laemmli (1970) using 7.5% polyacrylamide separating gel and 4% stacking gel. A sample buffer was prepared by mixing 2.5 ml 0.5 M Tris-HCl (pH 6.8), 4 ml 10% SDS, 2 ml glycerol, 1 ml 1% b-mercaptoethanol, 0.03 ml 0.002% bromophenol blue and the final volume was made to 10 ml. Electrophoresis (Bio-RAD Mini Protein System, USA) was carried out at a constant voltage of 95 V (for 90 min at a constant current of 400 mA) using an electrophoretic buffer of Tris-glycine containing 0.1 g/100 ml SDS. After electrophoresis, the gels were stained with 0.05 g Coomassie brilliant blue R-250 in 15% methanol and 5% acetic acid, and destained with destaining solutions [solution-1 (50% methanol and 7.5% acetic acid) and solution-2 (5% methanol and 7.5% acetic acid)]. The molecular weight was estimated using protein standard (10-245kDa) (HiMedia, India).

Gel strength of the samples were determined using a texturometer (TA-XT2 Stable Micro Systems, UK). Puncture tests were performed using a 5 mm diameter cylindrical stainless steel plunger attached to a 50 N load-cell of the texture analyzer at a crosshead speed of 0.2 mm s^{-1}. Breaking force (g), breaking deformation (cm) and work of penetration (g.cm) were determined. 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. Three samples were analyzed for each treatment at room temperature (25–27°C).

Colour of surimi was determined in triplicate using spectrocolourimeter (Colourflex EZ, Hunter Associates Laboratory, Inc., Reston, VA) with illuminant of D 65/10°. This instrument was calibrated with black and white reference titles before analysis. A thin layer of surimi (approx. 5 mm) was placed above the light sources and post processing L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) values were recorded. The CIELAB (L*, a*, b*) colour scale was used for the study. Whiteness was calculated as described by Lanier et al. (1991) as follows:

$$\text{Whiteness} = 100-[(100-L^*)^2 + a^{*2}+b^{*2}]^{1/2}$$

Sensory evaluation for acceptability of surimi gel was done by a panel of eleven members based on the attributes such as colour, flavour, taste and texture of the surimi gel. The 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) was used for scoring. Scores below 6 were considered as rejected.

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 22.0 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 ($p<0.05$).

Results and Discussion

The moisture content of stripped catfish (*P. hypophthalmus*) and surimi prepared was estimated to be 73.65±1.82% and 77.61±0.58% respectively. On dry weight basis, the protein, lipid and ash content of raw fish muscle was found to be 63.15±0.68%, 26.98±0.11% and 12.26±0.49% respectively. The result showed that the fish had low moisture and moderate protein and high fat content (Table 2). Lower moisture and higher lipid content in pangas muscle was also reported by Silverstein et al. (2000); Hossain et al. (2004) and Majumdar et al. (2015). Several factors which influence the proximate composition of fish include nutrition, habitat, size, catching season, seasonal and sexual variation as well as other environmental conditions (Sankar & Ramachandran, 2001). The surimi used for this study had protein (66.81±0.5%), fat (6.12±0.13%) and ash (4.78±0.93%) on dry weight basis. Total protein content was found to be reduced (wet weight basis) in surimi as most of the sarcoplasmic protein which makes up to 20 to 25% of total protein of fish muscles are usually lost during washing. Majumdar et al. (2013) reported proximate composition of Thai pangas surimi as moisture (79.57%), protein (14.68%), fat (1.33%) and ash (3.36%) on wet weight basis. Freshness quality of fish, initial composition, washing cycles and also the method used for removal of water influences final protein and lipid contents in the surimi. Removal of sarcoplasmic proteins may be attributed for increased concentration of myofibrillar proteins.

Table 2. Biochemical composition of raw fish and surimi*

	Raw fish (*P. hypophthalmus*)	Surimi
Moisture	73.65±1.82	77.61±0.58
Protein	63.15±0.68	66.81±0.5
Lipid	26.98±0.11	6.12±0.13
Ash	12.26±0.49	4.78±0.93

*The result is dry weight basis, mean ± SD of three determinations

The moisture and protein content of CCP powder from locally available carrot (*Daucus carota*) was estimated as 9.81±0.15% and 81.22±0.32% respectively (Table 3). Similar observation was reported regarding protein and moisture content of CCP as 84.2±5.4% and 9.5±0.3% respectively by Zhang et al. (2008). The molecular weight of CCP was determined by SDS-PAGE and a single band of *Dc*AFP with mw 36 kDa was detected (Fig. 1). This result was in agreement with the report of Smallwood et al. (1999). Other researchers reported molecular weight of *Dc*AFP as 36kDa (Wang et al., 2002), 36.8 kDa (Meyer et al., 1999; Zhang et al., 2008).

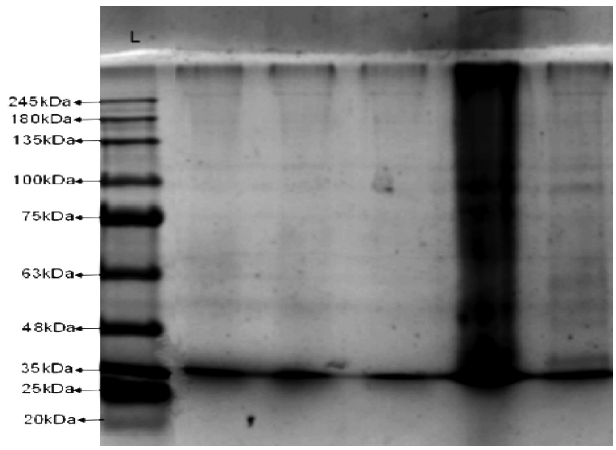

Fig. 1. SDS-PAGE photograph of CCP powder. Lanes 2, 3, 4, 5 and 6 are CCP powder. Speciûcally, aliquots of 15, 20, 20, 50 and 25mg/ml of CCP powder was loaded in lanes 2, 3, 4, 5 and 6 respectively. Lane 1 is the protein ladder standard.

Fig. 2 depicts the changes in biochemical and functional properties of frozen surimi with cryoprotectants during storage. Total volatile base nitrogen (TVBN) increased significantly ($p<0.05$) in all the treatments including control, but the final values were found below the acceptable limit of 24

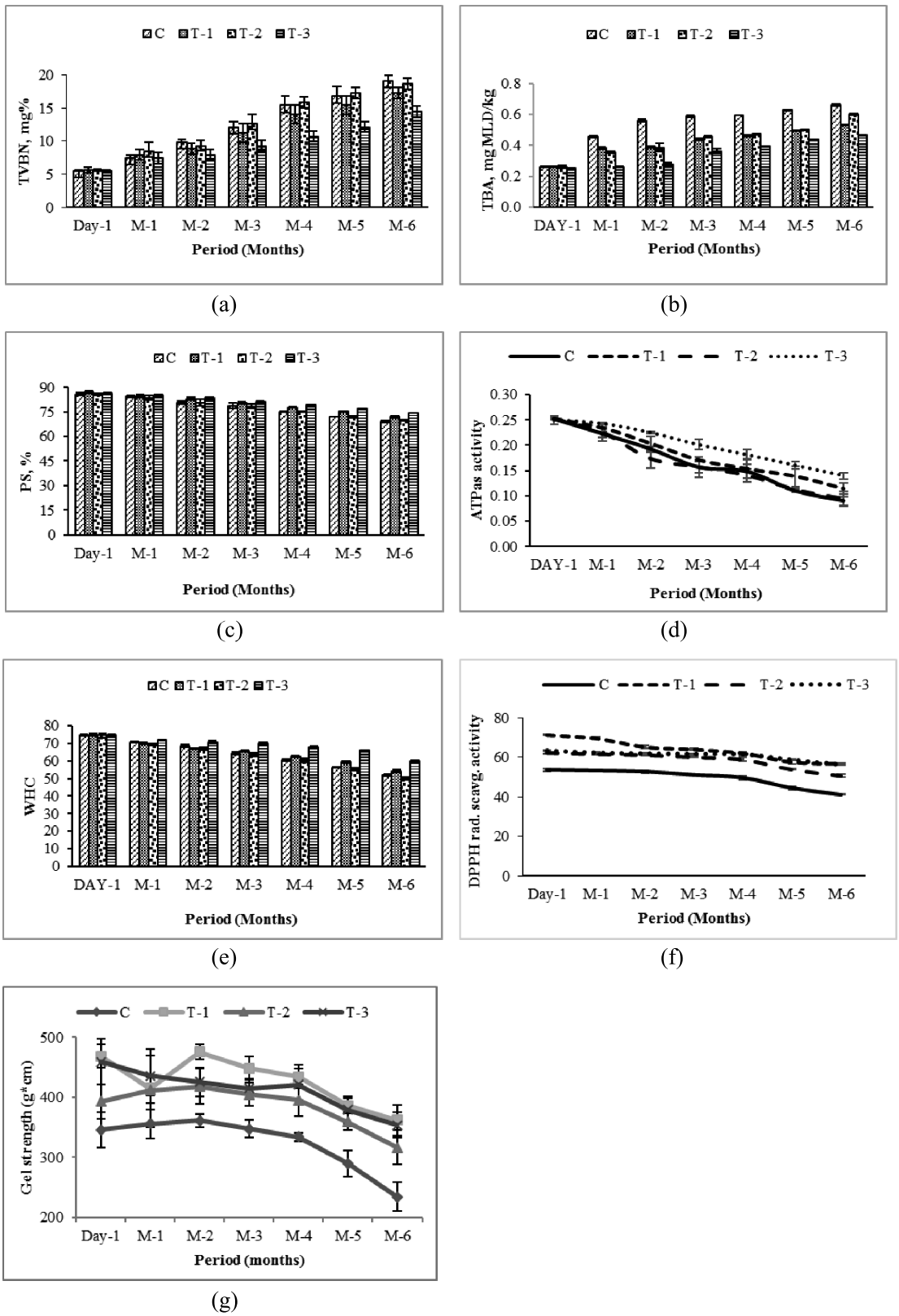

Fig. 2. Changes of biochemical and functional properties of surimi during frozen storage at -20°C, TVBN (a), TBA (b), protein solubility (c), ATPase activity (d), WHC (e), antioxidant activity (f) and gel strength (g)

Table 3. Composition of carrot concentrated protein (CCP)

	Carrot concentrated protein (CCP)
Moisture (%)	9.81±0.15
Crude Protein (%)	81.22±0.32
Antioxidant activity (%)	72.82±0.16

* The result is mean ± SD of three determinations

mg 100 g^{-1} for frozen stored pink perch (Reddy et al. 1997). Increase of TVBN level during frozen storage of surimi indicates the formation of ammonia and other volatile amines. The slow increase of TVBN in CCP treated samples compared to the control may be attributed to the inhibitory effect of CCP on protein degradation and such effect might have increased when typical cryoprotectants was used with CCP, since they protect the primary layer of water surrounding the protein (Fig. 2a).

Frozen storage accelerates the accumulation of secondary oxidative products and their subsequent release upon destruction of cell membrane by ice crystals leads to facilitate release of pro-oxidants, especially haem-iron (Benjakul & Bauer, 2001). The secondary lipid oxidative products, measured as TBA value, showed significant ($p<0.05$) increase during frozen storage (Fig. 2b). The initial values of TBA (0.26) increased to 0.66, 0.53, 0.60 and 0.47 mg malonaldehyde kg^{-1} in treatments control, T-1, T-2 and T-3 respectively at the end of the storage. Several studies also demonstrated the ability of proteins to inhibit lipid oxidation in foods (Shantha et al., 1994; Faraji et al., 2004). In this experiment, slow increase of TBA values was recorded in the CCP treated samples compared to the control.

Changes in protein solubility of surimi as a function of frozen storage time is given in Fig. 2c. Protein solubility (PS) decreased significantly ($p<0.05$) from initial values in all treatments, namely 19.5, 15.9, 18 and 13.7% in control, T-1, T-2 and T-3 respectively during storage. Loss of protein solubility during frozen storage of fish, mince or surimi is the result of protein aggregation/denaturation as explained by many researchers (Leelapongwattana et al., 2005; Ganesh et al., 2005). Higher solubility was observed when typical cryoprotectant (sorbitol-4%, sucrose-4% and SSTP-0.3%) was replaced by 1% CCP and further increase was recorded when both CCP (0.5%) and typical cryoprotectant (50%) were used. Benjakul et al. (2005) reported that the protein solubility of surimi made from some tropical fish in Thailand mixed with sorbitol and sucrose was in the range 17.50–90% during 25 weeks of frozen storage. The decrease in protein solubility was found to be concurrent with the decrease in Ca^{2+}ATPase activity, indicative of state of muscle protein.

Ca^{2+}ATPase activity is a good indicator for monitoring the denaturation of myofibrillar proteins during iced and frozen storage (Benjakul et al., 1997). The initial Ca^{2+}ATPase activity (0.25) experienced a steady decline ($p<0.05$) during storage (Fig. 2d). Rate of decrease was recorded as 64, 56, 64 and 44% respectively in control, T-1, T-2 and T-3. The present study revealed the potential of *Dc*AFP in maintaining integrity of acto-myosin during frozen storage of surimi and it was more than the conventional cryoprotectant (sucrose-sorbitol-SSTP mixture). The lowest rate of decrease as observed in treatment T3 suggests the possible synergistic role of conventional cryoprotectant by minimizing the movement of protein's monolayer of water. Involvement of myosin head in the fish myosin aggregation through rearrangement of protein via protein-protein interactions in myosin during frozen storage may contribute to loss of ATPase activity (Benjakul & Bauer, 2000). Decrease of Ca^{2+}ATPase activity is accompanied by conformational change and oxidation of sulfhydryl groups of actomyosin during frozen storage (Yongsawatdigul & Park, 2002) lead to alteration of myosin heads resulting protein denaturation. The excessive Ca^{2+}ATPase activity phenomenon was also reported with several cryoprotectants (Carvajal et al., 1999) and its loss is associated with the proteolysis.

The water holding capacity of surimi decreased significantly ($p<0.05$) during storage (Fig. 2e). This may be due to protein denaturation induced by frozen storage leading to gradual loss of protein solubility. In this study, sample T-1 and T-3 exhibited minimum loss of WHC during the six months of frozen storage compared to control and T-2. The result indicated that *Dc*AFP was more effective than the typical cryoprotectant in retaining WHC of protein and which may be due to formation of stronger protein-protein network induced by *Dc*AFP which imbibed more water.

Several studies demonstrated the ability of proteins and peptides to inhibit lipid oxidation in foods (Balti et al., 2011; Loizzo et al., 2012). Carboxyl groups in

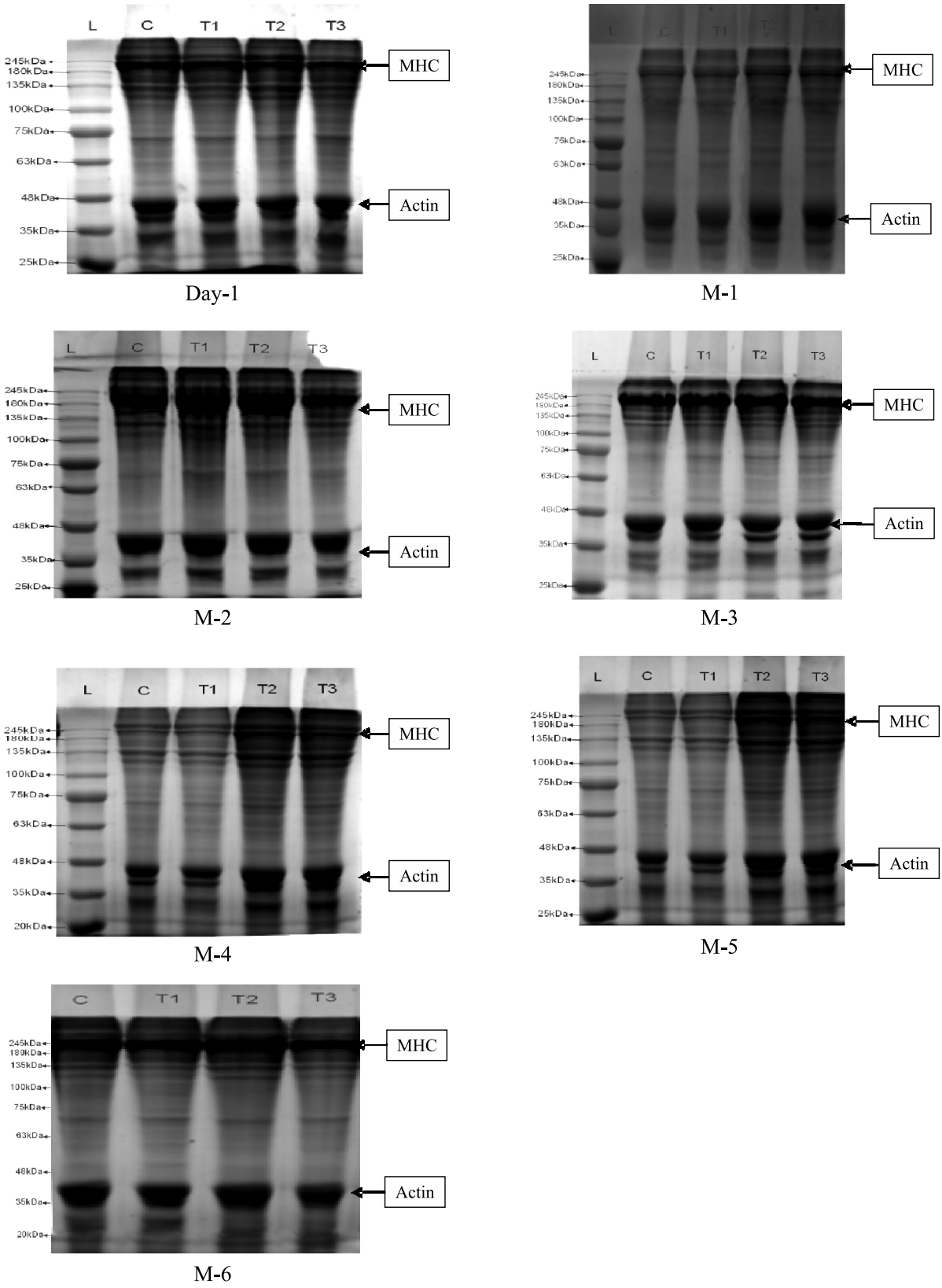

Fig. 3. Changes of protein pattern of surimi during frozen storage at -20°C

the side chains of proteins are capable to scavenge free radicals and chelate metal ions (Loizzo et al., 2012). In this study, DPPH radical scavenging activity of surimi and CCP was found to be 53.63 and 72.82% respectively. Some food proteins are unique and can potentially act as multifunctional antioxidants, which can inhibit several different lipid oxidation pathways (Elias et al., 2006). Although, there is no report on the antioxidant activity of stripped catfish surimi, but the CCP treated surimi samples exhibited antioxidant activity and given in (Fig. 2f). Initially, the DPPH radical scavenging activity increased upon mixing of CCP with surimi and thereafter showed slow but gradual reduction along the progress of the storage. This may be attributed to the gradual denaturation/ aggregation of *Dc*AFP during frozen storage. Retention of antioxidant activity at the end of the frozen storage was maximum in T-3 followed by T-1, T-2 and control. This may be considered as the additional advantage of using CCP as cryoprotective agent compared to typical sucrose-sorbitol, besides other benefits.

The gel strength (g.cm) of the surimi is given in (Fig. 2g). Initially, the gel strength increased ($p<0.05$) upon addition of CCP and continued steadily during the storage. The ability of the *Dc*AFP to reduce the size of ice crystals in frozen meat (Payne et al., 1994) and strong anti-recrystallization ability (Zhang et al., 2004) may be attributed to the enhancement of gel strength of the surimi as observed in the present study. AFPs bind to small ice crystals to inhibit growth and re-crystallization of ice during frozen storage, thus preserving food texture by reducing cellular damage and minimizing the loss of nutrients by reducing drip. During the frozen storage, gel strength showed steep reduction and may be explained as the result of denaturation and aggregation of myofibrillar proteins.

Myosin heavy chain (MHC, 212 kDa) and actin (45 kDa) were two major components visible in the protein pattern throughout the frozen storage (Fig. 3). The band intensity of MHC in all the samples increased up to 3rd month and thereafter decreased. A slight reduction in band intensity of actin was also observed in all the samples from 4th month onwards. The decrease in the intensity of the MHC and actin bands was due to protein denaturation especially actomyosin during frozen storage. Disappearance of the MHC band in surimi without a cryoprotectant was also reported by Sultanbawa & Li-Chan (1998) in ling cod surimi. In the present study, MHC in T-3 was found to be more compact compared to other treatments including control. Retention of MHC during frozen storage indicates effectiveness of cryoprotectants in preventing the aggregation and subsequent insolubility of myosin and actin.

Generally, surimi with high lightness (L*), low yellowness (b*), and high whiteness (W) are preferred by consumers (Hsu & Chiang, 2002). Interaction of CCP with protein influenced the whiteness of gel. In control, the whiteness value was observed as 73.83 on day-1 which reduced ($p<0.05$) to 66.95 at the end of the storage (Fig. 4). Whereas, the initial values in CCP treated surimi was found to be 61.07, 64.19 and 64.03 which gradually reduced to 58.35, 59.44 and 57.44 in T-1, T-2 and T-3 respectively. Since the CCP is naturally dark in colour, the addition of those compounds may be responsible for darkening of the final products. One possible explanation for gradual decrease of whiteness in CCP treated surimi may be the presence of some carotenoid pigments in the CCP and its oxidation during frozen storage.

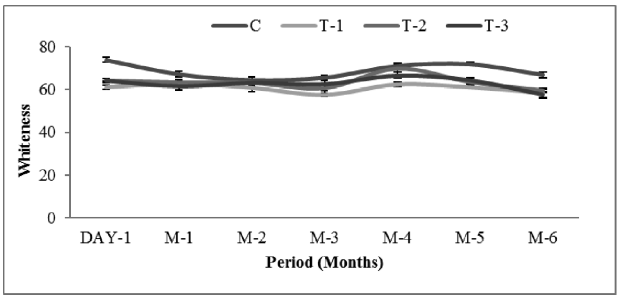

Fig 4. Changes in whiteness of surimi during frozen storage

The acceptability of the surimi based on appearance, flavour, taste and texture, during storage as affected by different cryoprotectants is presented in Fig. 5. Sensory attributes of frozen stored CCP incorporated surimi including control showed higher mean values initially which decreased during storage. The acceptance at the end of the storage period scored 5.33, 6.87, 6.23 and 7.29 for samples C, T-1, T-2 and T-3 respectively. The quality deterioration was found to be rapid from month 3 onwards. On 6th month, the control was not found acceptable due to some off smell as perceived by the panel judges. Although, according to the sensory evaluation, all CCP treated surimi samples were found acceptable

at the end of the storage, but of which, T-3 showed superiority over others.

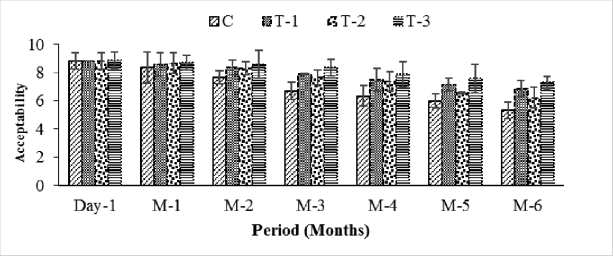

Fig. 5. Changes in acceptability of surimi during frozen storage at -20°C

This study suggests that carrot antifreeze protein as a cryoprotectant could maintain protein from denaturation more efficiently, however, enhanced protection of protein during frozen storage was observed when 50% of typical cryoprotectant was replaced by CCP (0.5%). Recently, some undesirable effects of typical cryoprotectant (sorbitol-sucrose-SSTP) like sweetness as well as some gastrointestinal diseases restrict their use in health- sensitive societies. The outcome of the present preliminary study may be useful for its commercial application with further refinement. The future study may also be aimed towards complete replacement of typical cryoprotectant, i.e., sorbitol, sucrose, STPP as well as improvement of whiteness of the surimi.

Acknowledgements

The authors would like to thank the Dean, College of Fisheries (CAU), Lembucherra, Tripura (W), India, for providing financial support to conduct this research.

References

AOAC (2000) Official Methods of Analysis. Association of Official Analytical Chemists (Horwitz, W., Ed) 17th edn., Section 13.32 and 13.33. Gaithersburg, Maryland, USA

Balti, R., Bougatef, A., Hadj Ali, N. E., Ktari, N., Kemel, J., Nedjar-Arroume,N., Dhulster, P. and Nasri1, M. (2011) Comparative study on biochemical properties and antioxidative activity of cuttlefish (*Sepia officinalis*) protein hydrolysates produced by alcalase and *Bacillus licheniformis* NH1 proteases. J. Amino Acid. Article ID 107179, 11p

Barrera, A. M., Ramirez, J. A., Gonzalez-Cabriales, J. J. and Vazquez, M. (2002) Effect of pectins on the gelling properties of surimi from silver carp. Food. Hydrocol. 16: 441-447

Benjakul, S. and Bauer, F. (2000) Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze–thaw cycles. J. Sci. Food Agric. 80: 1143-1150

Benjakul, S. and Bauer, F. (2001) Biochemical and physicochemical changes in catfish (*Silurus glanis* Linne) muscle as influenced by different freeze–thaw cycles. Food. Chem. 72: 207-217

Benjakul, S., Seymour, T. A., Morrissey, M. T. and An, H. (1997) Physicochemical changes in Pacific whiting muscle proteins during iced storage. J. Food Sci. 62: 729-733

Benjakul, S., Visessanguan, W., Thongkaew, C. and Tanaka, M. (2005) Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. Food. Hydrocol. 19: 197-207

Carpenter, J. F. and Crowe, J. H. (1988) The mechanism of cryoprotection of proteins by solutes. Cryobiol. 25: 244-55

Carvajal, P. A., Macdonald, G. A. and Lanier, T. C. (1999) Cryostabilization mechanism of fish muscle proteins by maltodextrins. J. Cryobiol. 38: 16-26

Conway, E. J. (1947) Microdiffusion analysis and volumetric error; 4th edn., Van Nostrad Co. Inc., New York, USA

Ding, X., Hui Zhang, H., Liu, W., Wang, L., Qian, H. and Qi, X. (2014) Extraction of carrot (*Daucus carota*) antifreeze proteins and evaluation of their effects on frozen white salted noodles. Food Bioprocess. Technol. 7: 842-852

Elias, R. J., Bridgewater, J. D., Vachet, R. W., Waraho, T., Mcclements, D. J. and Decker, E. A. (2006) Antioxidant mechanisms of enzymatic hydrolysates of *â*-lactoglobulin in food lipid dispersions. J. Agric. Food Chem. 54: 9565-9572

Faraji, H., Decker, E. A. and Aaron, D. K. (2004) Suppression of lipid oxidation in phosphatidylcholine liposomes and ground pork by spray dried porcine plasma. J. Agric. Food Chem. 39: 1288-1290

Fletcher, G. L., Kao, M. H. and Forney, R. M. (1986) Antifreeze peptides confer freezing resistance to fish. Can. J. Zool. 64(9): 1897-1901

Ganesh, A., Dileep, A. O., Shamasundar, B. A. and Singh, U. (2005) Gel-forming ability of common carp fish (*Cyprinus carpio*) meat, effect of freezing and frozen storage. J. Food Biochem. 30: 342-361

Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751-766

Hossain, M. I., Kamal, M. M., Shikha, F. H. and Hoque, M. S. (2004) Effect of washing and salt concentration

on the gel forming ability of two tropical fish species. Int. J. Agric. Bio. Engg. 6(5): 762-766

Hsu, K. C. and Chiang, B. H. (2002) Effects of water, oil, starch, calcium carbonate and titanium dioxide on the colour and texture of threadfin and hairtail surimi gels. Int. J. Food Sci. Technol. 37(4): 387-393

Knight, C. A., De Vries, A. L., and Oolman, L. D. (1984) Fish antifreeze protein and the freezing and recrystallization of ice. Nature. 308: 295-296

Kulkarni, A. P. and Aradhya, S. M. (2005) Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food Chem. 93(2): 319-324

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-685

Lanier, T. C., Hart, K. and Martin, R. E. (1991) A manual of standard methods for measuring and specifying the properties of surimi. Washington, DC: National Fisheries Institute

Leelapongwattana, K., Benjakul, S., Visessanguan, W. and Howell, N. (2005) Physicochemical and biochemical changes in whole lizardfish (*Saurida micropectoralis*) muscles and fillets during frozen storage. J. Food Biochem. 29: 547-569

Loizzo, M. R., Tundis, R., Bonesi, M., Menichini, F., Mastellone, V., Avallone, L. and Menichini, F. (2012) Radical scavenging, antioxidant and metal chelating activities of Annonacherimola Mill. (Cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents. J. Food Comp. Anal. 25: 179-184

Macdonald, G. A. and Lanier, T. C. (1994) Carbohydrates as cryoprotectants for meats and surimi. Food. Technol. 45: 152-154

Majumdar, R. K., Deb, S., Dhar, B. and Priyadarshini, B. M. (2013) Chemical changes in washed mince of silver carp *(Hypophthalmichthys molitrix*) during frozen storage at -20^{0}C with or without cryoprotectants. J. Food. Proc. Preserv. 37(5): 952-961

Majumdar, R. K., Saha, A., Dhar, B., Maurya, P. K., Roy D., Shitole, S. and Balange, A. K. (2015) Effect of garlic extract on physical, oxidative and microbial changes during refrigerated storage of restructured product from Thai pangas (*Pangasianodon hypophthalmus*) surimi. J. Food Sci. Technol. 52: 7994-8003

Meyer, K., Keil, M. and Naldrett, M. J. (1999) A leucine-rich repeat protein of carrot that exhibits antifreeze activity. FEBS Letters. 447(2): 171-178

Mueller, G. M., McKown, R. L., Corotto, L. V., Hague, C., and Warren, G. J. (1991) Inhibition of recrystallization in ice by chimeric proteins containing antifreeze domains. J. Bio. Chem. 266(12): 7339-7344

Noguchi, S. and Matsumoto, J. J. (1970) Studies on the control of the denaturation of the fish muscle proteins during the frozen storage 1. Preventive effect of Na-glutamate. Bull. Jap. Soc. Sci. Fish. 36: 1078-1087

Park, J. W., Lanier, T. C., Swaisgood, H. E., Hamann, D. D. and Keeton, J. T. (1987) Effects of cryoprotectants in minimizing physicochemical changes of bovine natural actomyosin during frozen storage. J. Food Biochem. 11(2): 143-161

Payne, S. R., Oliver, J. E. and Upreti, G. C. (1994) Effect of antifreeze proteins on the motility of ram spermatozoa. Cryobiol. 31(2): 180-184

Payne, S. R., Oliver, J. E., and Upreti, G. C. (1994) Effect of antifreeze proteins on the motility of ram spermatozoa. Cryobiol. 31(2): 180-184

Payne, S. R. and Young, O. A. (1995) Effects of pre-slaughter administration of antifreeze proteins on frozen meat quality. Meat Sci. 41(2): 147-155

Reddy, G. V. S., Srikar, L. N., Ravikiran, K., Sarma, J. and Khuntia, B. K. (1997) Keeping quality of frozen stored pink perch mince. Environ. Ecol. 15: 497-502

Sankar, T. V. and Ramachandran, A. (2001) Changes in Biochemical Composition in Indian Major Carp in relation to size. Fish. Technol. 38(1): 22-27

Shantha, N. C., Crum, A. D. and Decker, E. A. (1994) Conjugated linoleic acid concentrations in cooked beef containing antioxidants and hydrogen donors. J. Food Lipids. 2: 57-64

Sikorski, Z. E., Olley, J., Kostuch, S. and Olcott, H. S. (1976) Protein changes in frozen fish. Crit. Rev. Food Sci. Nutr. 8(1): 97-129

Silverstein, J. T., Wolters, W. R., Shimizu, M. and Dickhoff, W. W. (2000) Bovine growth hormone treatment of channel catfish: strain and temperature effects on growth, plasma IGF-I levels, feed intake and efficiency and body composition. Aquacult. (1): 77-88

Smallwood, M., Worral, D., Byass, L., Elias, L., Ashford, D., Doucet, C. J. and Bowles, D. J. (1999) Isolation and characterization of a novel antifreeze protein from carrot (*Daucus carota*). Biochem. J. 340(2): 385-391

Sultanbawa, Y. and Li-Chan, E. Y. (1998) Cryoprotective effects of sugar and polyol blends in ling cod surimi during frozen storage. Food Res. Int. 31(2): 87-98

Sych, J., Lacroix, C., Adambounou, L. T. and Castaigne, F. (1990) Cryoprotective effect of lactitol, palatinit and polydextrose on cod surimi proteins during frozen storage. J. Food Sci. 55: 356-360

Tarladgis, B. G., Watts, B. M. and Younathen, M. T. (1960) A distillation method for quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44-48

Tausky, H. H. and Shorr, E. (1952) A microcolorimetric method for the determination of inorganic phosphorus. J. Biol. Chem. 202: 675-685

Wang, L. H., Wusteman, M. C., Smallwood, M. and Pegg, D. E. (2002) The stability during low-temperature storage of an antifreeze protein isolated from the roots of cold-acclimated carrots. Cryobiol. 44(3): 307-310

Yongsawatdigul, J. and Park, J. W. (2002) Biochemical and conformational changes of actomyosin from threadfin bream stored in ice. J. Food Sci. 67(3): 985-990

Zhang, C., Zhang, H. and Wang, L. (2007) Effect of carrot (*Daucus carota*) antifreeze proteins on the fermentation capacity of frozen dough. Food Res. Int. (6): 763-769

Zhang, C., Zhang, H., Wang, L. and Guo, X. (2008) Effect of carrot (*Daucus carota*) antifreeze proteins on texture properties of frozen dough and volatile compounds of crumb. LWT-Food Sci. Technol. 41(6): 1029-1036

Zhang, D. and Hamauza, Y. (2004) Phenolic compounds and their antioxidant properties in different tissues of carrots. Food Agric. Environ. 2: 95-100