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Biochemical and organoleptic changes of surimi from the Thai pangas (*Pangasianodon hypophthalmus*) during frozen storage

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ABSTRACT

A study was conducted on the feasibility of preparing myofibrillar protein concentrate (surimi) from the catfish, *Pangasianodon hypophthalmus* with specific aim to assess shelf life of washed mince at −20°C based on the changes in biochemical and functional qualities. The moisture and pH increased significantly in the washed mince. The ash, total lipid and crude protein contents were higher in the unwashed mince, whereas, there was a positive effect on fish quality according to marked content decrease in expressible moisture, volatile basic amines, free fatty acids and thiobarbituric acid reactive substances. During storage of washed mince for 90 days at −20°C, moisture, ash, protein, fat and non-protein nitrogen contents registered decrease, whereas functional attributes of the surimi such as salt soluble protein, water holding capacity and gel strength were found to decrease during this period. While the appearance and odour of the product was acceptable throughout the storage period, the texture of the surimi was found in the border line on day 90. Based on these, the functional shelf life of washed pangas mince under frozen storage condition was estimated to be 90 days.

Keywords: Fish mince, Frozen storage, Gel forming ability, *Pangasianodon hypophthalmus*, Water holding capacity

Introduction

Surimi is mechanically deboned, washed and dewatered minced fish flesh (Okada, 1992) and a semi-purified concentrate of myofibrillar protein from fish muscle. Surimi possesses some functional properties such as gel forming ability, water holding capacity (Somjit *et al.*, 2005), and is used as intermediate foodstuff with a long shelf life and high potential for various texturised products. Both myosin and actomyosin have dominant roles in surimi gelation and show species specificities with regard to gelation properties (Shimizu *et al.*, 1983; Numakura *et al.*, 1985). Of late, the industry is shifting towards development of fish mince based products.

The first step in the surimi preparation involves washing the fish mince in chilled water to remove water soluble and odour bearing compounds comprising enzymes, sarcoplasmic proteins, blood, inorganic salt, certain lipids and other undesirable materials like pigments, which would otherwise interfere with the storage and rheological characteristics (Lee, 1984). This in turn will help to enhance the gel forming ability of the washed proteins (Venugopal, 2006). Washing fish mince decreases its stability but increases the storage tolerance (Shimizu and Fujita, 1985) and the tolerance is species dependent. The mincing of fish flesh and washing of mince also improve taste (Hoke *et al.*, 2000).

Although undesirable changes such as microbial aspects, and other chemical alterations are controlled during frozen storage (Shenouda, 1980), changes do occur in the quality of protein (Reddy and Srikar, 1991) and lipids (Reddy and Srikar, 1996). These changes are mainly caused by alterations in fish myofibrillar proteins during frozen storage as a result of the formation of intermolecular cross linkages, and consequently the aggregation and denaturation of actomyosin (Jiang and Lee, 1985). Although microbial growth and almost all chemical reactions can be temporarily slowed by low temperature, freezing and frozen storage may be responsible for many chemical and physical changes in fish, which can affect the functional and sensory properties of the products (Krivchenia and Fennema, 1988). The retention of functional properties, particularly gel forming ability and water holding capacity (WHC), is important for manufacturing fish based texturised products.

Although surimi is generally produced from coldwater marine species (Kellher *et al.*, 1992), recently the study on suitability of freshwater species for mince based products has gained importance due to the reduction in fish landings from marine sources. The surimi making ability of many freshwater species could be upgraded by manipulating the processing techniques (Onibala *et al.*, 1997). Some investigations have been done on the quality of the mince of freshwater fish for the manufacture of

surimi (Ismond and Tonogai, 1994; Kim *et al.*, 1996). However, the information about the tropical freshwater fish as surimi raw material is very scanty.

In recent years, fish technologists and the fish trade have increasingly prompted more attention to aquaculture techniques as a source of fish and other seafood products (Stickney, 1990). Pangas (*Pangasianodon hypophthalmus*) is an exotic catfish species (Family Pangasiidae) which is gaining importance for aquaculture in India especially in the state of Andhra Pradesh. In spite of year round production, quick growth and omnivorous feeding behaviour, pangas fetch a low market price, mainly due to its low consumer preference. However, the low priced fish is presently evolving towards increased utilisation for production of surimi. The objective of the present study was to analyse the biochemical and organoleptic changes of pangas surimi during storage at -20 ^{0}C in order to assess the shelf life for product development.

Materials and methods

Preparation of fish mince

Twenty kilograms of pangas (*P. hypophthalmus*) was collected from a local farm and transported in ice to the laboratory. Individual fishes (average weight, 713 ± 62g; average length, 40 ± 4 cm) were washed with chilled water, gutted, dressed, filleted by hand and minced in a mechanical meat mincer having a 3 mm hole plate. Mince was washed using chilled freshwater at 4/1 ratio (v/w, aqueous solution/minced fish). There were three washing cycles each with five minutes duration. In wash water, 0.1% NaCl was used in the last wash to facilitate dewatering. During each wash, mince was agitated for 60% of the total washing time followed by allowing them to settle for rest of the time. Washed minced fish muscle was then dewatered by hand in a thin cloth. After final wash, excess water was removed using screw press. All minced samples were packed in polythene pouches @ 200 g each, sealed and stored at −20°C for study of shelf stability through biochemical and sensory assessment. Sampling was done fortnightly. During sampling, two poly-pouches with mince were removed from the deep freezer (Voltas, India) and thawed in sealed condition before taking sample for biochemical analysis.

Proximate analyses

Moisture, ash, crude protein, non-protein nitrogen and lipid content of the mince were determined according to AOAC (2000). Total volatile base nitrogen (TVBN) was estimated by Conway's micro-diffusion method (Conway, 1947). The free fatty acid (FFA) value was determined according to the methods suggested by Takagi *et al.* (1984). Thiobarbituric acid (TBA) value was determined by the titrimetric method of Tarladgis *et al.* (1960) using thiobarbituric acid standard in 90% glacial acetic acid.

Quality analyses

For analysing pH, 10 g of sample was blended with 10 ml CO_2 free water. The temperature of the prepared sample was adjusted to 25 °C and pH was measured using a digital pH meter (Sartorius).

Salt soluble protein (SSP) was extracted by homogenising 10 g of minced fish with Dyer's buffer (Dyer *et al.*, 1950). The supernatant containing salt soluble protein, *i.e.*, myofibrillar fraction of muscle, was estimated through Kjeldahl distillation following standard method (AOAC, 2000). Results are expressed as g SSP per 100 g minced fish. Water holding capacity of minced meat was determined as expressible moisture content following the method of Suvanich *et al.* (2000).

Folding test for assessment of gel properties

The washed mince of pangas was ground with 3% NaCl and the paste was stuffed into a polythene tube. The tube was heated at 50°C for 2 h prior to heating at 80°C for 30 min in the water bath. Then the gels were cooled immediately in ice water and were stored at 4°C until assessment of gel properties by folding test. For folding test, a spherical disc of 1 mm thick gel was cut off and placed on the index and middle finger of the right hand, the disc was folded first into halves and then quarter with the help of thumb and index finger. The gel was graded using scores presented in the Table 1 as suggested by Lee (1984).

Sensory assessment of the surimi samples

Sensory assessments of the surimi samples (before and after each washing) of *P. hypophthalmus* was performed following the 5-point scale suggested by Lee (1984) and given in Table 2 based on the freshness degree as given in Table 3.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 11.0 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 using Duncan's Multiple Comparison Test. Significance of differences was defined at $p<0.05$.

Table 1. Grades used in the folding test of gel

Grade	Results on folding
AA	No crack visible when disc is folded into quarter
A	No crack when disc is folded into half but one or more cracks of breaks are visible when folded into quarter
B	One or more cracks are visible when disc is folded into half
C	Breaks, but does not split into halves
D	Splits into halves when folded into half
O	Sample too soft to evaluate

Table 2. Sensory assessments of the surimi samples

Characteristics	BW	0 day	15 days	30 days	45 days	60 days	75 days	90 days
Appearance	5d (0.00)	5d (0.00)	4.86d (0.38)	4.71cd (0.49)	4.57cd (0.53)	4.29bc (0.49)	4bc (0.58)	3.57a (0.53)
Odour	5c (0.00)	5c (0.00)	4.71cd (0.49)	4.57bcd (0.53)	4.43bc (0.53)	4.29bc (0.49)	4.14ab (0.38)	3.71a (0.49)
Texture	5d (0.00)	5d (0.00)	5d (0.00)	4.86d (0.38)	4.57cd (0.53)	4.29c (0.49)	3.71b (0.49)	3.29a (0.49)

Table 3. Degree of freshness of surimi

Properties	Scores				
	5	4	3	2	1
Appearance	Golden blonde colour	Colour nearly golden blonde	Colour becomes dark golden blonde	Colour dark, much cuts	Colour is indistinct, and meat crumbles
Odour	Fresh odour	Fresh odour is slightly loosened	Odour slightly stale and bad	Odour stale and bad	Odour evidently putrefied rotten, ammonia odour
Texture	Texture tight, very hard, elasticity breaking is not evident when it is folded to quarter	Tight texture slightly loosened, hard elasticity; breaking is not evident when it is folded to half	Texture slightly soft, suitable elasticity; breaking is slight when it is folded to half	Texture soft, weak elasticity, breaking is excess when it is folded to half	Texture evidently soft, no elasticity, weak texture; there are smashed pieces when pressed by fingers

Results and discussion

Washing to remove the solubles enhanced the overall characteristics of the fish flesh. The data on biochemical changes of minced meat before and after wash and during storage at −20°C are presented in Table 4. The moisture content increased from 73.97 to 82.26% after the wash and gradually decreased throughout the storage period and reached 77.57% on 90th day. The decrease in moisture content of washed mince during the period of storage (90 days) was about 6% ($p>0.05$). This could be explained as increased hydration of protein because of increase in water holding capacity due to removal of sarcoplasmic proteins during washing. In a similar experiment with pink perch surimi, decrease in moisture content with storage was attributed to dehydration (Garg *et al.*, 1982). Lin and Park (1997) 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. A steady decrease of ash content due to wash was observed (from 0.87% before wash to 0.45% after wash) followed by slow decrease during the storage period. Loss of minerals during storage is due to removal of mineral contents with the drip water. Even though, mineral contents decreased due to wash, there was no significant differences ($p > 0.05$) observed during the frozen storage of washed mince.

Table 4. Changes in biochemical characteristics during frozen storage of minced *P. hypophthalmus* at −20°C*

Parameters	BW	Period of storage						
		0	15	30	45	60	75	90
Moisture	73.97^{a} (1.36)	82.26^{c} (3.02)	80.7^{bc} (2.89)	80.81^{bc} (23.17)	78.81^{bc} (2.00)	79.53^{bc} (0.92)	78.82^{bc} (1.27)	77.57^{ab} (1.55)
pH	6.59^{a} (0.16)	6.93^{b} (0.08)	7.08^{bc} (0.10)	7.14^{cd} (0.05)	7.17^{cd} (0.95)	7.32^{de} (0.05)	7.46^{ef} (0.12)	7.51^{f} (0.09)
Ash	0.87^{b} (0.08)	0.45^{a} (0.03)	0.44^{a} (0.02)	0.44^{a} (0.08)	0.43^{a} (0.07)	0.41^{a} (0.04)	0.41^{a} (0.02)	0.39^{a} (0.01)
Protein	16.42^{a} (0.06)	16.25^{a} (0.29)	16.23^{a} (0.53)	15.87^{a} (0.87)	15.68^{a} (0.70)	15.53^{a} (0.51)	15.61^{a} (0.51)	15.54^{a} (0.10)
fat	7.61^{c} (0.71)	3.8^{b} (0.05)	3.13^{a} (0.48)	3.19^{a} (0.39)	2.97^{a} (0.09)	2.7^{a} (0.10)	2.66^{a} (0.95)	2.63^{a} (0.15)
NPN	0.39^{b} (0.07)	0.35^{ab} (0.03)	0.33^{ab} (0.04)	0.33^{ab} (0.01)	0.3^{a} (0.08)	0.29^{a} (0.01)	0.29^{a} (0.02)	0.28^{a} (0.01)
TVB-N	13.7^{ab} (1.6)	10.17^{a} (0.55)	17.53^{b} (2.57)	22.6^{c} (2.69)	25.1^{c} (3.65)	27.2^{c} (3.99)	32.3^{d} (3.57)	39.57^{e} (2.07)
FFA	6.12^{b} (0.63)	4.62^{a} (0.38)	7.65^{c} (0.96)	10.55^{d} (0.53)	13.27^{e} (0.08)	16.72^{g} (0.79)	15.58^{f} (0.64)	19.00^{h} (0.37)
TBA	0.79^{ab} (0.11)	0.56^{a} (0.08)	0.73^{a} (0.10)	0.98^{b} (0.07)	1.36^{c} (0.07)	1.57^{cd} (0.19)	1.78^{e} (0.12)	2.07^{f} (0.20)

*Values are mean ± SD. Mean values bearing different superscripts (a, b, c, *etc*.) in a row are significantly different ($p< 0.05$) with respect to sampling

The pH of mince (6.59) was found to increase slightly after wash (6.93) and thereafter slowly increased to 7.51 on 90th day of storage. Decomposition products such as volatile bases could lead to a pH rise during storage of fish mince. Bennour *et al.* (1991) reported less than one unit increase in pH of mackerel (*Scomber scombrus*) during storage in ice. Rodger *et al.* (1980) found changes in pH of minced cod during storage and the declining pH values during early storage was attributed to formation of lactic acid from glycogen; whereas, rising pH later during storage was correlated to formation of dimethylamine (DMA) from trimethylamine oxide (TMAO). Present results suggest that these possibilities (but in this case volatile bases other than DMA) would be applicable in pangas mince because pH showed a rising trend during storage. The pH and TVB-N was found to be positively correlated ($p<0.01$) with the progress of storage period (Table 5).

The protein content of unwashed pangas mince registered a decrease of 1.03% ($p>0.05$) in the washed mince and then slowly decreased to 15.54% during the period of storage. However, no difference ($p>0.05$) during frozen storage period could be outlined. Washing can cause sarcoplasmic protein, which makes up to 20 to 25% of total protein of fish muscle, to exit; hence, the amount of protein in surimi is less than that of mince (Taşkaya *et al.*, 2003). Majumdar *et al.* (2012) reported that the protein content of silver carp mince decreased significantly immediately after wash (from 17.21% of unwashed mince to 15.78% of washed mince) and then slowly decreased to 13.48% at the end of 90 days when stored without cryoprotectants. In the present study with *P. hypophthalmus*, the higher lipid content in muscle may be responsible for less removal of crude protein (sarcoplasmic fraction) due to washing.

The NPN content decreased from 0.39% (before wash) to 0.35% (after wash) and then gradually decreased to 0.28% on 90th day of storage (Table 4). A 10% decrease of the NPN content of pangas mince after wash was observed and this may be because of loss of low molecular weight compounds during washing cycles. Although, there was chance of increase of NPN due to the activities of proteolytic enzymes (Abraham *et al.*, 1992), but the loss of such low molecular weight compounds with drip was more than their generation and so, overall NPN content of the mince showed a decreasing trend. Concerning the TVB-N value, the washing process led to a remarkably lower ($p<0.05$) content, according to value obtained before and after such treatment (13.7±1.6 and 10.17±0.55, respectively) which gradually increased up to 39.57% on 90th day of storage. Lower TVB-N values in washed mince could have resulted from removal of free amino acids, sarcoplasmic protein, or N-containing compounds of non-protein nature during washing (Suvanich *et al.*, 2000). In the present study with warm water fish *P. hypophthalmus*, it was found that

Table 5. Correlation matrix showing relationships among the parameters during frozen storage study of pangas (*P. hypophthalmus*) mince

Treatment		pH	SSP	FFA	TBA	WHC	TBVN	Appear	Odour	Texture
Treatment	1	.92**	-.86**	.96**	.93**	.81**	.94**	-.96**	-.97**	-.92**
pH	.92**	1	-.64**	.84**	.81**	.62**	.83**	-.84**	-.87**	-.79**
SSP	-.86**	-.64**	1	-.91**	-.91**	-.92**	-.88**	.88**	.88**	.87**
FFA	.96**	.84**	-.91**	1	.95**	.84**	.93**	-.93**	-.95**	-.88**
TBA	.93**	.81**	-.91**	.95**	1	.93**	.92**	-.95**	-.94**	-.94**
WHC	.81**	.62**	-.92**	.84**	.93**	1	.86**	-.89**	-.85**	-.90**
TBV-N	.94**	.83**	-.88**	.93**	.92**	.86**	1	-.94**	-.96**	-.91**
Appearance	-.96**	-.84**	.88**	-.93**	-.95**	-.89**	-.94**	1	.98**	.98**
Odour	-.97**	-.87**	.88**	-.95**	-.94**	-.85**	-.96**	.98**	1	.94**
Texture	-.92**	-.79**	.87**	-.88**	-.94**	-.90**	-.91**	.98**	.94**	1

** correlation is significant at 0.01 level (t - tailed)
** correlation is significant at 0.05 level (t - tailed)

although the TVB-N level of washed mince reached up to 39.57 mg 100 g^{-1}, no considerable offensive odour up to 90th day of storage at −20°C was perceived by the judges. However, previous studies account for a TVB-N content increase during the frozen storage of minced muscle (Suvanich *et al.*, 2000; Siddaiah *et al.*, 2001). Chakraborti (1984) observed the same trend in frozen muscle of Indian major carp.

There are several reports regarding acceptability limit of TVB-N in ice or frozen stored fish *viz.,* 35-45 mg100 g^{-1} meat (Connell, 1975); 19.5 mg 100 g^{-1} for tilapia and 25.2 mg 100 g^{-1} for Spanish mackerel (Al-Kahtani *et al.*, 1996) and 22.2 to 23.1 mg 100 g^{-1} for mackerel (Bennour *et al.*, 1991). Reddy *et al.* (1995) reported the acceptability limit of TVB-N for frozen stored pink perch mince between 18 and 24 mg 100 g^{-1} meat. From the study of correlation (Table 5) it was revealed that TVB-N was positively correlated with period of storage ($p<0.01$) and WHC ($p<0.01$).

Lipid is very important, as far as surimi is concerned, because of its interference with the gel formation. Almost 50% of the initial fat was reduced from the mince due to washing and thereafter, the fat content decrease throughout the storage period was not significant ($p>0.05$). The previous washing process has led to a FFA content decrease ($p<0.05$) according to values observed before and after such treatment (6.12 ± 0.63 and 4.62 ± 0.38, respectively). The FFA content increased significantly ($p<0.05$) from 4.62 to 19% of total lipid as oleic acid at the end of 90 days storage period indicating extensive hydrolysis of lipids. Accumulation of FFA is said to contribute to off-flavour of the product and cause textural alterations by complexing with proteins (Mai and Kinsella, 1980). A marked FFA increase with time in minced meat during frozen storage was explained as a result of hydrolytic enzymes present in the minced muscle, which remain active during frozen storage at -18°C (Kaneniwa *et al.*, 2000; Sikorski and Kolakowski, 2000).

Rancidity development was measured by means of secondary lipid oxidation compound formation (TBA). The TBA (mg malonaldehyde kg^{-1} fish) showed significant decrease (0.79 and 0.56 before and after washing respectively) as a result of washing, showing a higher ($p<0.05$) oxidation stage in the unwashed material before undergoing the frozen storage step. This lower TBA ($p<0.05$) for washed mince showed a gradual increase ($p<0.05$) throughout the frozen storage period and finally reached 2.07 on the 90th day of storage. Scott *et al.* (1992) reported that TBA value above 3-4 mg malonaldehyde kg^{-1} indicates quality loss in the product. The protein solubility decreased when the TBA value increased during frozen storage of washed pangas mince. This could be explained by interaction between protein and lipid oxidation products, causing a decline of protein solubility (Siddaiah *et al.*, 2001; Alzagtat and Alli, 2002). The correlation coefficient (r) between changes in TBA and period of storage was 0.92 ($p<0.01$).

Influence of frozen storage on salt soluble protein, water holding capacity as expressible moisture contents and gel strength is given in Table 6. The salt soluble protein (SSP) in washed mince (12.84%) was higher ($p<0.05$) than the unwashed mince (10.15%) and during the period of storage, a reduction ($p<0.05$) of SSP was observed and the value reached 6.37% during 90 days of storage (Table 6). Protein denaturation could have caused the reduction of SSP during storage. This loss could play an important role in reducing gel-forming ability of pangas mince. Moreover, the SSP content of pangas

mince and water holding capacity were highly correlated (r=−0.92, p<0.01), confirming findings of Cheng *et al*. (1979). Frozen storage of threadfin bream for 12 weeks at −18 ºC resulted in a significant (p<0.05) decrease in salt soluble proteins (Sarma *et al.*, 2000). The SSP values were negatively correlated with other attributes (Table 5) such as storage period (p<0.01) and TVB-N (p<0.01).

The expressible moisture content was found to decrease after wash from 31.14 to 25.10% (p<0.05) and thereafter increased (p<0.05) to 39.33% on the 90th day of storage (Table 6). This indicated that less water was imbibed in the gel matrix as a result of increase in protein denaturation by extended frozen storage leading to lower water affinity and accordingly, to a decrease in WHC (Visessanguan *et al.*, 2005). The increase in expressible moisture of mince during frozen storage may be attributed to a change in microstructure of myofibrillar proteins from a continuous filamentous matrix to a globular matrix (Smith, 1987). Cheng *et al.* (1979) stated that the loss of water-holding capacity of tissues during frozen storage was correlated with a decrease in myofibrillar protein solubility. In the present study, this phenomenon was supported by the observed decrease of SSP during the period of frozen storage of mince. Expressible moisture of pangas mince was found correlated with salt soluble protein content (r = − 0.92, p<0.01) (Table 5).

According to the result of folding test, mince was graded 'AA' initially which gradually decreased to 'B' on day 75, then 'C' and 'D' on day 90 and day 105 respectively (Table 6). The folding test result indicated that gel forming ability of the mince improved due to washing. The gel strength showed gradual decrease during the frozen storage and was accompanied by significant change in expressible moisture content (increased) and salt soluble protein (decreased). The gel-forming ability of surimi of various fish species gradually decreased during frozen storage (Atsumi *et al.*, 1995), as observed in the present study. Since, gel strength is an important functional property of fish mince for development of paste products. Gradual loss of gel forming ability of pangas mince with the progress of frozen storage period was reflected in the organoleptic quality of the surimi. Prabhu *et al.* (1986) observed significant decrease in the kamaboko forming ability of the fish sausage prepared from lesser sardine mince during frozen storage.

Changes in the organoleptic characteristics of the surimi during storage are given in Table 2. Appearance in respect of colour changed from characteristic red of unwashed mince to light pink of washed mince which gradually became lighter and faded to pale whitish to whitish with the progress of frozen storage period. The whiteness of pink perch surimi, however, increased during frozen storage (Singh and Balange, 2005). Texture remained unchanged up to 15th day but other characteristic attributes showed gradual decrease with the progress of storage period. It was observed that, the lowest values of appearance, odour and texture were 3.65, 3.85 and 3.24, respectively up to 90th days of storage. The sensory attributes *viz.*, appearance, odour and texture of raw pangas mince under study decreased significantly (p<0.05) during the storage. While the appearance and odour of the product was acceptable throughout the storage period, the texture of the surimi was found in the border line on day 90 and that was considered to be the functional shelf life of mince (frozen stored without any cryoprotectants) for preparation of paste products which essentially needs gel forming ability of mince. The appearance, odour and texture were found negatively correlated with all the quality attributes including period of storage (p<0.01).

Table 6. Changes in the salt soluble protein (SSP), water holding capacity (expressed as expressible moisture content) and gel strength during frozen storage of minced *P. hypophthalmus* at −20°C.

Days of storage	Parameters		
	Salt soluble protein*	Expressible moisture content*	Gel strength
BW	10.15e(1.02)	31.14c (1.60)	NA
0	12.84g (0.30)	25.10a (0.56)	AA
15	11.52f (0.52)	27.07ab (0.62)	AA
30	9.69de (0.39)	29.6bc (0.92)	A
45	8.78cd (0.39)	31.02c (0.32)	A
60	7.92bc(0.64)	34.1d (3.03)	A
75	7.02ab (0.19)	37.41e (2.16)	B
90	6.37a (0.52	39.33e (1.03)	C
105	NA	NA	D

*Mean values followed by different letters in row denote significant differences (p<0.05) as a result of the frozen storage time
NA - not assessed.

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