Fishery Technology 52 (2015) : 42 - 47

Spoilage Indices in Chill-Stored Rohu Steaks Extracted with Different Concentrations of Trichloroacetic Acid

M. R. Raghunath*, H. Umalatha, N. Sridhar, K. Hemaprasanth, B. Gangadhar and P. Jayasankar
Regional Research Centre of Central Institute of Freshwater Aquaculture, P.O. Hesarghattalake, Bengaluru - 560 089, India

Abstract

Spoilage or freshness indices are extensively used in fish processing industry and research. Although frequently employed, the index total volatile basic nitrogen (TVBN) in freshwater fish does not correlate well with storage. Rohu (*Labeo rohita)* steaks treated with chlorine dioxide (ClO_2) and chill-stored in ice for 14 days were extracted with differing strengths of trichloroacetic acid (2, 4, 6 and 8% TCA) and analyzed for TVBN as well as biuret +ve and folin +ve substances. TVBN, biuret +ve and folin +ve substances did not increase significantly during the first 10 days, but increased sharply thereafter in both control and ClO_2 treated steaks. Greater amounts of TVBN and biuret +ve substances were extracted at lower TCA concentrations. Biuret +ve substances extracted by 2% TCA from both treated and control steaks showed significant proportional changes with storage, but decreased in the former and increased in the latter. Sensory changes in the steaks appeared as early as the 4^{th} day and darkening of the lateral line red meat was the most easily noticed and off-odours by the 6^{th} day. Biuret +ve substances extracted at low TCA concentrations may hold promise as spoilage indices, but need further investigation.

Key words: Rohu, spoilage indices, TCA, chilled storage

Introduction

The search for suitable indices of spoilage/freshness in fish and fishery products has a long history and is an ongoing one. While spoilage indices are critical in deciding the shelf-life of fish and fishery products and setting quality standards, freshness indices come to the fore for making decisions on whether or not to purchase (accept) raw material consignments. Such indices may be based on sensory, microbiological or chemical attributes. Sensory methods such as the 'Quality Index methods' (Sykes et al., 2009, Zhu et al., 2012) developed for many species of fish show good correlation with spoilage/storage period, but also entail inherent subjectivity. Microbiological indices require long time for determination and thus are of limited value as field tests. Among the various chemical tests in vogue, the total volatile base nitrogen (TVBN) and trimethyl amine nitrogen (TMA) have been the most popular and are used extensively in industry and research. However, the applicability of TMA and TVBN indices to freshwater fishes has often been debated and extensively reviewed (Monique et al., 2005, Howgate, 2010). The 'K' value freshness index has shown great promise, being applicable to marine and freshwater fish but is determined by HPLC or specialized instruments (Huss, 1995, Itoh et al. 2013), though simpler methods were mooted for its estimation (Uchiyama & Kakuda, 1984). The quantum of soluble fractions remaining after precipitating protein solutions with agents such as Trichloroacetic acid (TCA) vary, depending on the concentration of the precipitating agent used and can range from 330 to 380 daltons (Greenberg & Shipe, 1979). These fractions thus may offer clues to the quantum and nature of the protein degradation products produced during spoilage. This paper describes our efforts to find out whether the formation of TVBN and other low molecular weight products such as biuret +ve and folin +ve substances extracted by a range of TCA concentrations, correlate well with sensory characters of

Received 04 August 2014; Revised 17 January 2015; Accepted 24 January 2015

* *E-mail: raghunathmr@rediffmail.com*

steaks of a freshwater fish Rohu (*Labeo rohita)* during chilled storage.

Materials and Methods

Rohu (*Labeo rohita)* weighing between 1 to 1.25 kg were harvested by drag-netting from aquaculture ponds and immediately dressed by removing of scales, fins and viscera. Dressed fish were washed in potable quality water before cutting into steaks of 1-1.5 cm thickness and divided into 2 equal batches. One batch was washed with ice cold water (1:2) for 5 min and drained for 5 min (Control). The other batch was washed in ice cold water (1:2) containing 10 ppm ClO_2 for 5 min and drained for 5 min. The steaks were then packed in polystyrene trays (12.5x10.5x4.0 cm, LxWxD) as described earlier in Chitra et al. (2008), stored in insulated iceboxes with ice (1:2) and sampled at 0, 2, 4, 6, 8, 10 and 14 days of chilled storage. Re-icing was done daily to compensate for melted ice. Before sampling, temperature inside the containers was recorded with a thermometer. Post sampling, representative pieces of steak were photographed and evaluated for smell and texture. Steaks were then dressed to remove bones and homogenized. Duplicate homogenized samples were analyzed for moisture (AOAC, 1980). Samples (10 g) for further chemical analysis were prepared in duplicate by grinding in a mortar with 10 ml water to a fine paste before adding 10 ml of 6, 12, 18 or 24% trichloroacetic acid (TCA), further ground for 2 min and allowed to react for 30 min. The treated samples were then filtered through Whatman no.1 filter paper to obtain extracts of the muscle at final TCA concentrations of 2, 4, 6 and 8% respectively and stored at 4°C till further analysis. TCA extracts were analyzed for TVBN, Biuret +ve substances and Folin +ve substances as described by Conway (1962), Gornall et al. (1949) and Herriott (1955) respectively. All chemical analyses were completed within 2 months. 't' test and regression analysis was carried out using Microsoft Excel®.

Results and Discussion

Temperature of steaks inside the containers varied from 2 to 6°C, decreasing towards the end of storage. The steaks did not lose or gain moisture significantly during the storage, although there was a slight increase during the last part of the storage period. There were also no significant ($p<0.05$) differences between control and ClO_2-treated steaks in their moisture content (Table 1).

Table 1. Changes in moisture content (%) of control and chlorine dioxide treated rohu steaks during chilled storage.

Storage, d	Control	Chlorine dioxide Treated
0	75.20[a]	74.79[a]
2	77.62[a]	76.89[a]
4	75.84[a]	76.42[a]
6	76.84[a]	76.83[a]
8	75.41[a]	77.27[a]
10	77.49[a]	75.27[a]
14	79.13[a]	77.95[a]

[a]Rows and columns with same superscripts are not significantly different (<0.05) from each other

In both control and ClO_2 treated steaks, TVBN did not increase significantly ($p<0.05$) during the first 10 days of storage, but increased sharply and significantly at 14d (from ~16 to 44-60 mg%N). The TVBN content of ClO_2 treated steaks during the final stage (14 d) of storage were however, much lesser (43.66 *vs* 60.66 mg%N) compared to controls, indicating the effectiveness of ClO_2 in inhibiting the spoilage.

It was also seen that when lower concentrations of TCA were used for extraction, higher amounts of TVBN were extracted from the tissue regardless of the storage time (Fig.1), in both control and ClO_2 treated steaks. The relatively small changes in

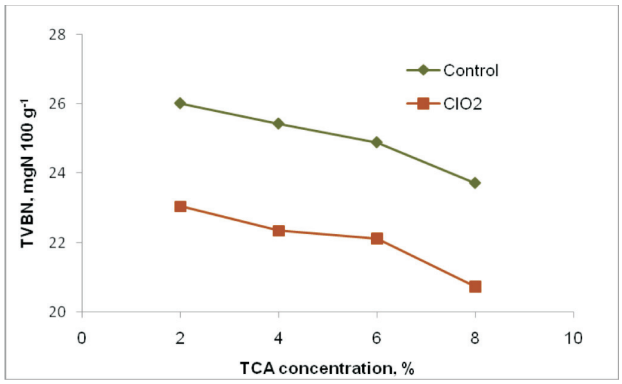

Fig. 1. Differences in total volatile base nitrogen (TVBN) extracted from chill-stored control and ClO_2 treated rohu steaks due to the final concentration of trichloroacetic acid (TCA) achieved during extraction. TVBN concentrations in extracts at a particular TCA concentration were averaged across storage periods to arrive at the figures.

TVBN of both control and treated steaks till 10 d of storage, irrespective of the final TCA concentration at which the tissue was extracted or the treatment given, indicate that TVBN cannot be a sensitive index of freshness or spoilage in rohu.

A sensitive index should ideally show a good correlation with storage time and a belated response in the index at a late stage, when most of the deteriorative changes have already occurred, would be of little value. Uchiyama et al. (1970), who tested 104 commercial fish samples for volatile base nitrogen, trimethylamine nitrogen and the 'K' value, concluded that both the former two indices could not distinguish between freshly killed fish, good quality tuna and material of medium quality. Previous studies on chill-stored rohu have shown similar results (Chitra et al., 2008). A study conducted to compare methods for chemical quality assessment of fish, recommended that while TVBN could be an indicator of spoilage of some fish species such as red fish, flat fish, gadoids, hake and Atlantic salmon, it cannot be used as a freshness indicator due to its constant level during the first days of iced storage and inability to reflect the mode of spoilage (Monique et al., 2005). Common carp fillets stored at ~2°C under MAP had lower (15 mg%) than acceptable levels (20 mg%) of TVBN even after 15 d of storage, whereas acceptability of the fillets was reached on 7th day itself as judged by sensory attributes (Buchtova, 2007).

The study also tested two other indices for possible use as freshness/spoilage indicators in chill-stored rohu steaks. Different concentrations of TCA were used in this experiment as TCA at lower concentrations does not completely precipitate proteins and these TCA soluble fractions of low molecular weight (Greenberg & Shipe, 1979), might provide a clue to the degradation of tissue proteins during spoilage of fish. Biuret method is based on the chelation reaction of peptide bonds in proteins with copper ions and hence most of the low molecular weight peptides also react with it (Kanaya & Hiromi, 1987). As the analytes in our study were TCA soluble peptides rather than proteins, they were not quantified with reference to a standard protein such as bovine serum albumin. Changes in the Biuret +ve substances during chilled storage of rohu steaks are presented in Fig. 2 (a) and (b).

Although the amount of Biuret +ve substances increased significantly ($p<0.05$) with storage time in

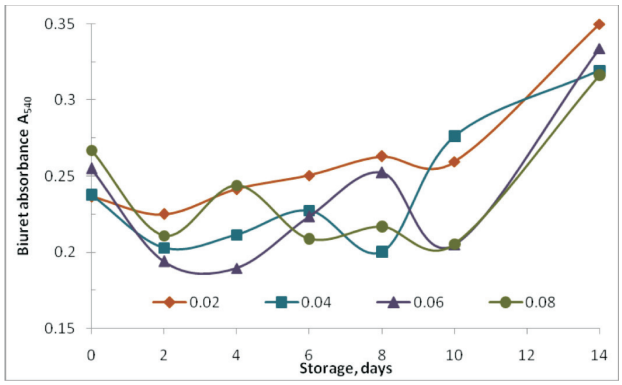

Fig. 2 (a). Changes in biuret +ve substances of chill-stored control rohu steaks extracted with various strengths of trichloroacetic acid.

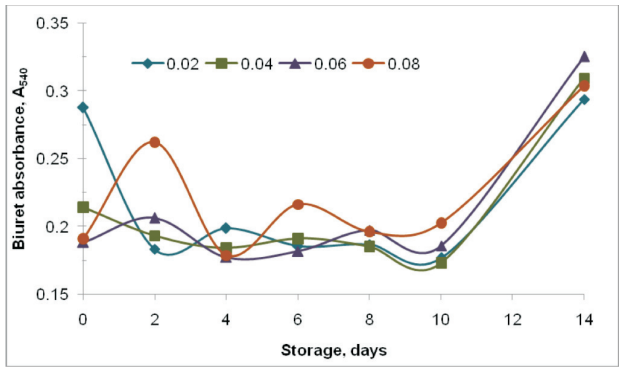

Fig. 2 (b). Changes in biuret +ve substances of chill-stored ClO_2 treated rohu steaks extracted with various strengths of trichloroacetic acid.

both control and ClO_2 treated steaks,the increase was not linear. Changes till the 10 d of storage were minimal and not significant ($p<0.05$), but increased sharply at 14 d as in the case of TVBN. Poor and non-significant ($p<0.05$) regressions between storage time and Biuret +ves (r^2=0.4696 and 0.2405 respectively for control and ClO_2) were obtained for the 14 d storage period. However, there appeared to be a more significant and proportional regression between storage time and Biuret+ves, only when TCA extracts of low strength (2%) were examined till 10 d of storage (r^2=0.7758 and 0.5022 for control and ClO_2 treated steaks respectively). However, while the Biuret+ves increased with time in control steaks, they decreased in ClO_2 treated steaks. Although higher Biuret +ves were extracted with lower TCA concentrations (Fig. 3), the trend was not uniform and even reversed at higher TCA concentrations. Hence, Biuret +ves extracted at low TCA concentrations may be of some promise as indices of spoilage/freshness.

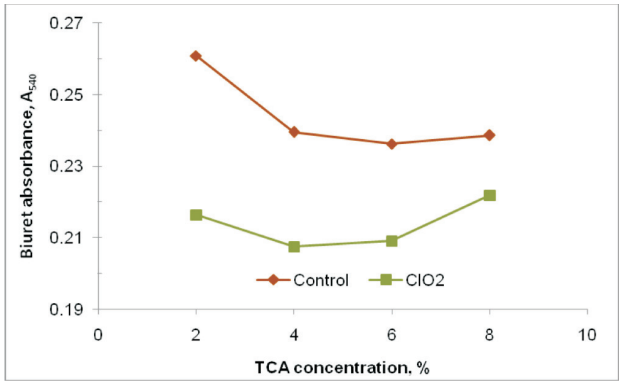

Fig. 3. Differences in Biuret +ve substances extracted from chill-stored control and ClO_2 treated rohu steaks due to final concentration of trichloroacetic acid (TCA) achieved during extraction.

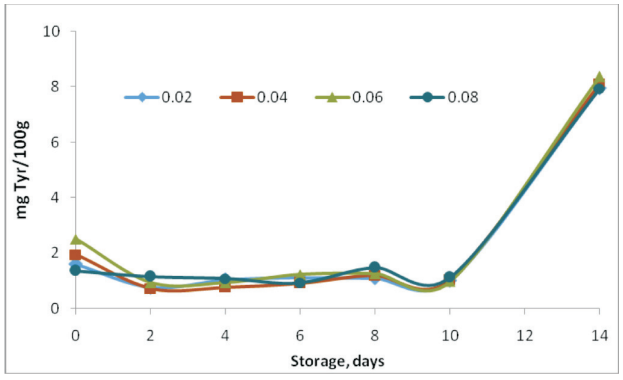

Fig. 4 (a). Changes in Folin +ve substances of chill-stored control rohu steaks extracted with various strengths of trichloroacetic acid.

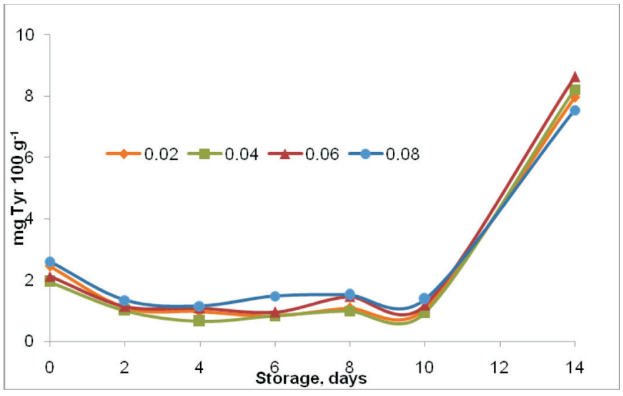

Fig. 4 (b). Changes in Folin +ve substances of chill-stored ClO_2 treated rohu steaks extracted with various strengths of trichloroacetic acid.

Changes in the Folin +ve substances during the chilled storage are shown in Fig. 4a & b. Folin reagent reacts with aromatic amino acid residues that are ubiquitous and thus are expected to be present in any protein breakdown products of spoilage. There were no significant changes in Folin +ve substances in either control or ClO_2 treated steaks till 10 d of storage, but increased sharply thereafter. Further, in spite of highly significant ($p<0.01$) differences between the amounts of Folin+ve substances extracted at different TCA concentrations, there was no discernible relationship between them (Fig. 5). It would thus appear that Folin+ve substances did not serve as an useful spoilage index during storage.

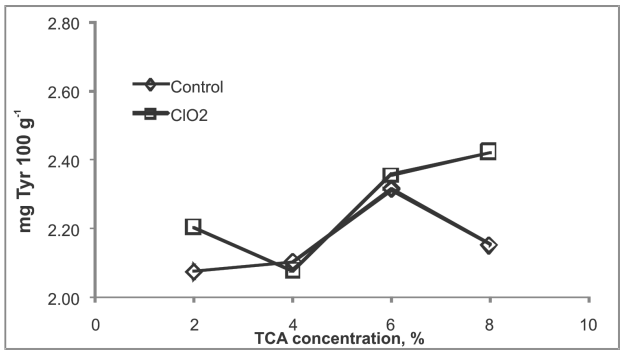

Fig. 5. Differences in Folin +ve substances extracted from chill-stored control and ClO_2 treated rohu steaks due to final concentration of trichloroacetic acid (TCA) achieved during extraction. Folin +ve substance concentrations in extracts at a particular TCA concentration were averaged across storage periods to arrive at the figures.

Table 2 lists the sensory changes in the rohu control and ClO_2 treated steaks during chilled storage, while Fig. 6 shows the changes in their appearance in photographs. It can be seen that the earliest change was darkening of the initial light pink colour of the white meat and more notably of the dark meat at the lateral line. The darkening of the red meat deepened and extended along the backbone and lateral line. Some general yellow discoloration of flesh was also observed in later stages. The white meat became progressively darker by 14 d, with appearance of reddish discoloured patches. Off-odours were evident by 6 d, while the muscle texture lost its springiness on 8th day. Thus it is evident that prime condition of the steaks lasted only till 4th day of storage and treatment of the rohu steaks with 10 ppm ClO_2 did not extend their period of freshness. Quality index methods (QIM) which have been developed for a number of whole (round) fish and shell fish chiefly rely on sensory inputs such as colour and appearance of skin, eyes and gills

Table 2. Changes in sensory characters of rohu steaks during chilled storage.

Storage period, days	Control	ClO_2 treated
0	Fresh fish odour, flesh firm	Fresh fish odour, flesh firm
2	Neutral odour, flesh firm	Neutral odour, flesh firm
4	Formaldehyde type odour, slightly soapy to touch, flesh firm	Formaldehyde type odour, flesh firm, slightly soapy to touch, flesh firm
6	Flesh firm, slight off-odour, dry appearance	Formaldehyde and rancid type odour,
8	Light spoiled odour, dry appearance, steak does not spring back when pressed	Light spoiled odour, dry appearance, steak does not spring back when pressed
10	Spoiled ammonical odour, flesh softer, dry and dull appearance	Spoiled ammonical and rancid odour, flesh softer, dry, dull and dark appearance
14	Distinct spoiled odour, very soft and disintegrating flesh, brown, slimy surface	Distinct spoiled odour, Yellow, very soft and disintegrating flesh, slimy surface.

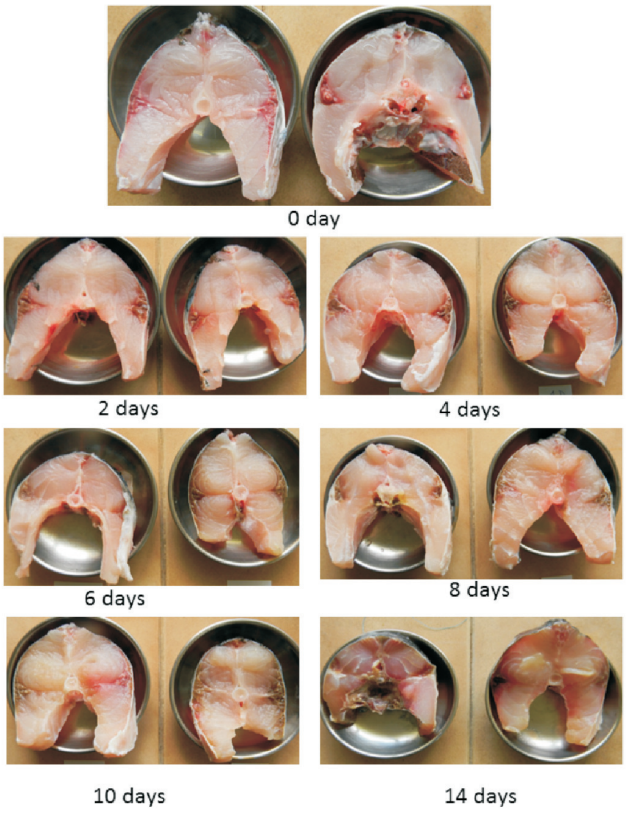

Fig. 6. Photographs showing the appearance of the rohu steaks during chilled storage. In each photograph the control steaks are on the left and ClO_2 treated steaks on the right.

as well as the texture (firmness) of flesh and smell (Barbosa & Vaz-Pires, 2004; Sykes et al., 2009). But in fish portions such as fillets and steaks, fewer sensory inputs are available, like skin mucus, flesh colour & brightness and gaping which would require trained persons for evaluation (Bonilla et al., 2007). This may make the QIM methods a little more difficult to adopt, especially in view of the subtle changes in colour and smell as observed in this study.

In conclusion, sensory characteristics appear to be better indices of freshness and spoilage than the chemical indices of TVBN, Biuret +ve and Folin +ve substances in chill-stored rohu steaks. Biuret +ve substances extracted at low TCA concentrations however appear to hold promise, but needs further investigation.

Acknowledgements

The authors wish to thank the Director, Central Institute of Freshwater Aquaculture, Bhubaneswar, for permission to publish this paper.

References

AOAC (1980) Official methods of Analysis, 13th edn., (Horwitz, W. Ed), AOAC, Washington, DC

Barbosa, A. and Vaz-Pires, P. (2004) Quality index method (QIM): development of a sensorial scheme for common octopus (*Octopus vulgaris*). Food Control. 15: 161-168

Bonilla, A. C., Sveinsdottir, K. and Martinsdottir, E. (2007) Development of quality index method (QIM) scheme for fresh cod (*Gadus morhua)* fillets and application in shelf life study. Food Control. 18: 352-358

Buchtova, F. J. H. (2007) Physical and Chemical Changes in Fresh Chilled Muscle Tissue of Common Carp (*Cyprinus carpio* L.) Packed in a Modified Atmosphere. Acta Vet. Brno. 76: 83-92

Chitra, C. P., Ganesh, K., Vasudev, V., Sridhar, N. Hemaprasanth and Raghunath, M. R. (2008) Efficacy of sanitizers on the shelf life of Rohu steaks stored at two refrigeration temperatures. Fish. Technol. 45(2): 197-208

Conway E. J. (1962) Determination of volatile amines. Micro diffusion analysis and volumetric error. 5th edn., pp 195-200 Crosby Lockwood, London UK

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

Greenberg, N. A. and Shipe, W. F. (1979) Comparison of the abilities of trichloroacetic, picric, sulfosalicylic and tungustic acids to precipitate protein hydrolyzates and proteins. J. Food Sci. 44: 735-737

Herriott, R. M. (1955) Swine pepsin and pepsinogen. In: Methods in Enzymology, Vol.II. (Colowick, S. P. and Kaplan, N. O., Eds) pp 3-7, Academic Press, London

Howgate, P. (2010) A critical review of total volatile bases and Trimethylamine as indices of freshness of fish. Part 1 Determination. Elec. J. Env. Agricult. Food Chem. 9(1): 29-57

Huss, H. H. (1995) Quality and quality changes in fresh fish. FAO Fisheries Technical paper-348 FAO-UN, Rome

Itoh, D., Koyachi, E. Yokokawa, M., Murata, Y., Suzuki, H. (2013) Micro-device for On-site fish freshness checking based on K-value measurement. Anal. Chem. 85(22): 10962-10968

Kanaya, K and Hiromi, K. (1987) Determination of low concentrations of protein by the biuret method using the 'stopped-flow time difference analysis' technique. Agric. Biol. Chem. 51(7): 1885-1892

Monique, E., Ifremer. and Nantes. (2005) Traceability: Valid methods for chemical quality assessment: Volatile Amines as Criteria for Chemical Quality Assessment. European Project SEAFOOD plus, 21p

Sykes, A. V., Oliveira, A. R., Domingues, P. M., Cardoso, C.M., Andrade, J.P. and Nunes, M.L. (2009) Assessment of European cuttlefish (*Sepia officinalis,* L.) nutritional value and freshness under ice storage using a developed Quality Index Method (QIM) and biochemical methods. LWT-Food Sci. Technol. 42: 424-432

Uchiyama, H., Ehira, S., Kobayashi, H. and Shimiza, W. (1970) Significance in measuring volatile base and trimethyl amine nitrogen and nucleotides in fish muscle as indices of freshness of fish. Bull. Japan Soc. Sci. Fish. 36: 177-187

Zhu, Z., Ruan, Z., Li, B., Meng, M. and Zeng, Q. (2012) Quality loss assessment of crisp grass carp (*Ctenopharyngodon idellus*) fillets during ice storage. J. Food Process Pres. 37(3): 254-261