Effects of Setting Time on the Physical Properties of Restructured Fish Products from *Oreochromis niloticus* and *Channa striatus*

WOLYNA, P.[1/], CH'NG, S.E.[1/], SAADIAH, I.[2/], MAMOT, S.[1/] and BABJI, A.S.[1/]

[1/] *School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia.* daging@pkrisc.ukm.my
[2/] *Fisheries Research Institute, 11960, Batu Maung, Penang, Malaysia*

Abstract: The restructured fish product of Tilapia (*Oreochromis niloticus*) and Haruan (*Channa striatus*) were subjected to non-setting and setting at 40°C for 0.5, 1, 1.5 and 2 h followed by heating at 90°C for 30 min. Changes in mechanical properties (puncture test), expressible water (EW) and colour (*whiteness)* were evaluated. The rheological properties of restructured fish were analysed by dynamic rheological measurement from 20 - 90°C. Setting resulted in higher hardness for *O. niloticus* and *C. striatus* ($p<0.05$). Setting at 40°C for 1 h also decreased the amount of expressible water in both fishes ($p<0.05$). On the other hand, the whiteness of all samples subjected to setting were higher than that of the non-setting samples after cooking ($p>0.05$). Storage moduli (G') on temperature sweep analysis for all samples were higher than loss moduli (G''). An increase in time of setting also improved the storage moduli (G'). These results suggest that setting temperature of 40°C for 1h provided better textural properties, expressible water and appearance (colour) of *O. niloticus* and *C. striatus* gels.

Keywords: restructured fish product, gelation, setting, rheology, texture

Abstrak: Produk ikan terstruktur semula daripada Tilapia *(Oreochromis niloticus)* dan Haruan *(Channa Striatus)* diberikan perlakuan tanpa set dan set pada 40°C selama 0.5, 1, 1.5 dan 2 jam, diikuti dengan pemanasan 90°C for 30 minit. Perubahan pada ciri-ciri mekanikal (ujian tusukan), air boleh ekstrak dan warna (indeks keputihan) dikaji. Ciri-ciri reologi produk ikan terstruktur semula dianalisis menggunakan pengukuran reologi dinamik pada suhu 20 - 90°C. Set memberikan kekerasan yang tinggi pada gel *O. niloticus* and *C. striatus* ($p<0.05$). Set pada 40°C selama 1 jam turut mengurangkan jumlah air boleh ekstrak bagi kedua-dua jenis ikan ($p<0.05$). Selain itu, nilai warna putih bagi semua sampel yang diberi perlakuan set adalah lebih tinggi berbanding sampel tanpa set selepas dimasak ($p>0.05$). Penyimpanan modulus (G') adalah lebih tinggi berbanding kehilangan modulus (G') daripada analisis hayunan suhu bagi semua sampel. Peningkatan masa set juga memperbaiki penyimpanan modulus (G'). Keputusan ini menunjukkan suhu set pada 40°C selama 1 jam dapat memperbaiki ciri-ciri tekstur, air boleh ekstrak dan warna gel *O. niloticus* dan *C. striatus*.

Introduction

Tilapia is native to Africa and Middle East; it has emerged from mere obscurity to one of the most productive and internationally traded food fish in the world. The farming of tilapias in its crudest form is believed to have originated more than 4,000 years ago from Egypt (Gupta and Acosta, 2004). Tilapia is the common name for nearly a hundred species of cichlid fishes from the tilapiine cichlid tribe within three genera: *Oreochromis*, *Sarotherodon*, and *Tilapia*. The species of greatest importance to aquaculture are the Nile tilapia (*O. niloticus*), Mozambique tilapia (*O. mossambicus*), and blue tilapia (*O. aureus*) and their hybrids (Chapman, 2007). The fish is being farmed in about 85 countries worldwide and about 98% of tilapia produced in these countries is grown outside their original habitats (Shelton, 2002). Tilapia is a fish with mild flavor and white flesh. It is one of the most frequently aquacultured freshwater fish in the world (Yongsawatdigul *et al.*, 2000).

Most Malaysians consider Haruan *(Channa striatus)* as a good source of health food. *C. striatus*, a genus of the Channidae family of snakehead fishes, is a freshwater, air-breather and carnivorous fish indigenous to many tropical and subtropical countries including Malaysia. There are thirty identified species around the world, and eight were reported found in Malaysia. Besides having a very good composition of amino acids and fatty acids, it is regarded as a dietary treatment in ameliorating wound lesions and in post-partum involution (Mohsin and Ambak, 1983).

Restructured fishery products are products made from minced and/or chopped muscle and which are used, with or without ingredient, to make other products with a new appearance and texture (Moreno *et*

al., 2008; Borderías *et al.*, 2005). The reason of restructuring fish muscle is that the supply of high quality fishery products is limited and many are becoming exhausted due to severe over-fishing (Borderías *et al.*, 2005).

The utiltization of fish mince is more preferable than of surimi, because the processing of surimi requires more labor and water, and produces more waste than the preparation of mince. Therefore, fish mince is less costly in operation than surimi and could be suitable for application by the food industry (Yinghong *et al.*, 2003). Moreover, the repeated washing during surimi processing entail elevated requirements of freshwater and high contamination of the wastewater (Guenneugues and Morrissey, 2005; Park and Lin, 2005).

One of the most important attributes of surimi, its gel-forming ability, is affected by the fish species, formulation and cooking procedures (Lee, 1986). Among these factors, cooking procedure has been recognized as one of the critical steps that can be controlled to improve the gel quality of surimi, but its impact may vary depending on the fish species. For some fish species, extended incubation at certain temperatures (generally below 40°C) can enhance the gelation of surimi (defined as setting or suwari), whereas for other species, extended incubation around 60°C may weaken the surimi gel (defined as gel-softening or 'modori') (Shimizu, 1990). Setting or suwari is a well known occurrence in surimi paste during the incubation at temperature lower than 40°C. Gelation of fish paste during setting has been reported to have a close relationship to the formation of cross-linking between myosin heavy chain induced by endogenous transglutaminase (TGase) (Kumazawa *et al.*, 1995; Seki *et al.*, 1990) as well as to the thermal formation of non-covalent bonds and disulfide bonds (Hossain *et al.*, 1998).

Setting response can be varied, depending on fish species (Benjakul and Visessanguan, 2003) and it is related to habitat temperature of fish species (Morales *et al.*, 2001). Generally, setting can be performed at low (0-4°C), medium (25°C) and high (40°C) temperatures (Lanier, 1992). Setting at different temperatures may lead to different gel characteristics, especially in different fish species. Since setting at low-temperature takes a longer time, it is not commonly implemented in the industries (Benjakul *et al.*, 2003). Higher-temperature setting is widely used to improve the gel properties of surimi because a shorter setting time is required (Benjakul *et al.*, 2004).

Gelation of fish protein is the most important step in forming desired textures in many seafood products. Functional properties, notably gel strength, of surimi gels can be affected by many physical conditions as well as protein concentration, setting temperature and setting time (Luo *et al.*, 2004; Camou *et al.*, 1989). Ideal cooking condition for surimi may vary substantially depending on the fish species. To our knowledge, there are no data that characterizes the gelling properties of restructured *C. striatus* and *O. niloticus* fish products. Accordingly, we conducted this study to explore the suitability of these fishes meat for restructured fish product production. Specifically, our objectives were to investigate the effects of various setting time at 40°C on the rheological properties of restructured *C. striatus* and *O. niloticus* fish products.

Materials and Methods

Sampling

Adults Haruan (*C. striatus*) and Tilapia (*O. niloticus*) sized 1-1.5 kg and 400-500 g each were bought from Kajang Market. The fish were brought alive to the laboratory in batches. The fish fillets were obtained by cutting the fish lengthwise along the backbone to gain the maximum amount of flesh without any backbone. Filleting was done manually and kept in ice box. The fillets were then washed under running tap water to remove blood, dirt and slime.

Preparation of restructured fish products

Fillets were ground in a meat grinder (Beem-Gigant, Germany) using a 4 mm plate. Restructured products were obtained from homogenized fish mince. For each treatment, fish mince samples (0.5 kg) were chopped in a Hobart cutter (Model 55, Hobart Ditosama, France) for 3 min and added with 2% salt. The temperature of the fish paste remained below 15°C throughout the chopping operation for all treatments studied. The sol was then stuffed into cellulose casing (ϕ =2.5 cm) and both ends were sealed tightly. To

prepare gels, the sols were incubated in a temperature controlled water bath at 40°C for 0.5, 1, 1.5 and 2 hour respectively, followed by heating at 90°C for 30 min. Directly heated gels by heating the sol at 90°C for 30 min (without prior setting) was used as the control. The gels were cooled in ice water and stored for 24 h at 4°C prior to further analysis.

Texture analysis

Texture analysis of fish gels was carried out using a texture analyzer AGS-J 500N (Shimadzu, Japan). Cylindrical samples (2.5 × 2.5 cm) of cooked restructured fish product were equilibrated to room temperature for 30 min in a plastic bag to avoid dehydration before testing. A puncture test was performed by compressing samples to 75% of their initial height by using a spherical probe (ϕ=1.0 cm) at the speed of 60 mm/min. Breaking force (g) and deformation (mm) for each treatment, were measured. Six samples were analyzed for each treatment (Ramirez *et al.*, 2007).

Temperature sweep analysis

Rheological properties of fish meat paste were analyzed using rheometer (Anton Paar, MCR300) equipped with a 25 mm-parallel plate geometry at a gap of 1.0 mm. The space between the parallel plate geometry and sample table was covered with paraffin oil to prevent dehydration. Temperature sweep analysis to measure the changes in dynamic rheological parameters including storage moduli (G'), loss moduli (G") and tangent of phase angle (δ) during heating were performed. The constant frequency of 1.0 Hz and amplitude strain of 1% which was within the linear viscoelastic region, from 20 to 90°C at an increasing rate of 1°C/min (Fukushima *et al.* 2007).

Expressible water

The amount of expressible water for each treatment was measured. Samples of 3 ± 0.1 g of cooked gels were weighed and put between two layers of filter paper (Whatman No. 1). Samples were then placed at the bottom of 50 ml centrifuge tubes and centrifuged at 1500 g for 5 min at 15°C. Immediately after centrifugation, the fish sample was removed and weighed (Ramirez *et al.*, 2007). Three samples were analyzed for each treatment and average values were recorded. The amount of expressible water was calculated as follows:

$$\text{Expressible water (\%)} = \frac{\text{Initial Weight-Final Weight}}{\text{Initial Weight}} \times 100$$

Colour attributes

Colour of fish gel (after cooking) was determined using a Minolta Chroma meter (Model C400). L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) were measured. Whiteness of cooked gel was calculated using 100 $[(100\ L^*)^2 + a^{*2}\ b^{*2}]^{1/2}$ (Park 1995). Seven gel measurements per sample were used for colour analysis.

Statistical analysis

The data collected were analyzed by ANOVA using SAS Ver. 6.12. Comparison of means was performed using Duncan Multiple Range Test when F-Value was significant ($p<0.05$).

Results

Breaking force and deformation

Breaking force and deformation of kamaboko gels with prior setting at 40°C for different time are shown in Fig. 1. Using directly heated gels (without setting) as controls, setting fish gels for 0.5, 1, 1.5 and 2 h prior to cooking resulted in significant increase ($p<0.05$) in breaking force of *O. niloticus* and *C. striatus* kamaboko gels. The results showed that the restructured fish products produced from *O. niloticus* and *C. striatus,* incubated at 40°C for 2 h prior to heating at 90°C for 30 min, showed the highest breaking force, 1032.25 g and 1564.24 g respectively. When categorizing fish gels, both *O.niloticus* and *C. striatus* were graded as SSA (breaking force of > 800 g) (Benjakul *et al.* 2004). Benjakul and Visessanguan (2003) found that when setting temperature of 40°C used for *P. tayenus* kamaboko gel, breaking force and deformation increased when setting time increased ($p<0.05$). Klesk *et al.* (2000) demonstrated that tilapia surimi exhibited 'setting' at 40°C. Its gel quality was comparable with the *Alaska Pollack.*

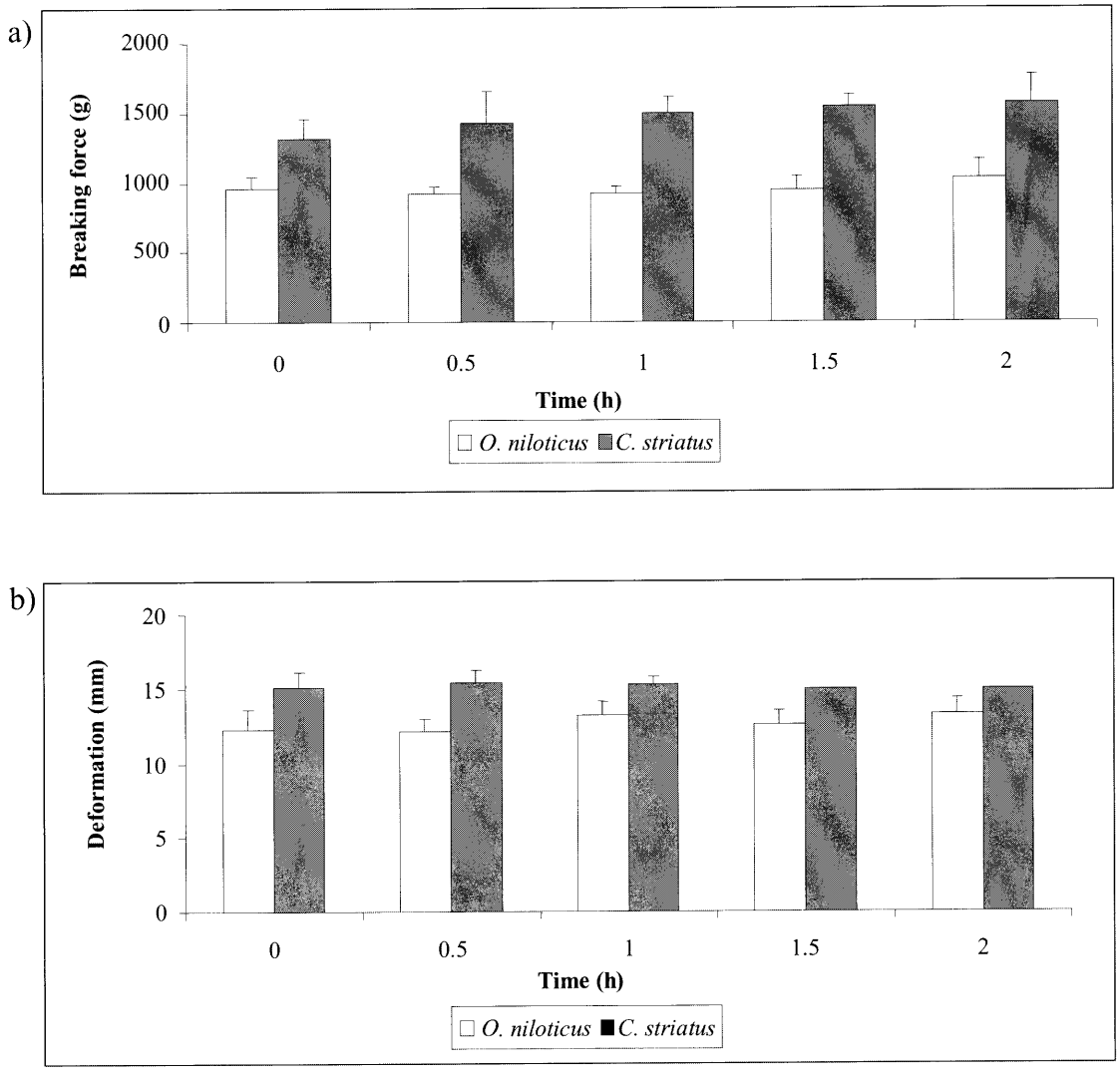

Figure 1: a) Breaking force and b) deformation of kamaboko (restructured product) from *O. niloticus* and *C. striatus* prepared by setting at 40°C for different times, prior heating to 90°C. Bars indicate the standard deviation of six determinations

Deformation of *O. niloticus* varied from 12.71 to 13.31 mm whereas it was from 14.93 to 15.34 mm for *C. striatus*. The results showed that, no significant differences in deformation were observed between gels with different setting times ($p>0.05$). Setting temperature appeared to have a greater effect on breaking force than on deformation. Ramirez *et al.* (2003) also reported that setting affected shear stress (breaking force) to a larger extent than shear strain (deformation). When comparing the properties of kamaboko gels between two species, it was found that *C. striatus* had much higher breaking force and deformation than *O. niloticus* ($p<0.05$). The different setting responses observed between *C. striatus* and *O.niloticus* were possibly due to differences in activity and thermal stability of endogenous Tgase.

The increase of hardness in fish after setting was related to the formation of protein cross-linking. Gel properties of surimi generally determined by the setting condition (Lanier, 1992). Setting played an important role in cross-linking of gel network, especially by non-disulfide covalent bonds induced by

endogenous transglutaminase (TGase) (Kumazawa *et al.*, 1995; Seki *et al.*, 1990). Setting at 40°C for different durations of time possibly caused different changes in protein conformation, resulting in differences in exposure of reactive groups (glutamine and lysine) involved in the cross-linking reaction via TGase (Benjakul *et al.*, 2004). Benjakul *et al.* (2003) found that the the optimum conditions for setting of surimi sol from lizardfish *(Saurida tumbil)* were 40°C for 30 min. After setting, heating process has been known to induce protein aggregation due to hydrophobic interaction and disulfide bond (Benjakul *et al.*, 2001).

Temperature sweep analysis

Dynamic rheological measurement determines changes in rheological properties of samples serially and nondestructively by variables such as time and temperature (Egelandsdal *et al.*, 1995). The storage modulus G' reflects the elastic component of viscoelasticity, namely, a measure of the elastically stored and recovered energy per cycle of deformation. The loss modulus G'' reflects the viscous component, namely, a measure of the energy dissipated as heat (Ikeda and Foegeding, 2005).

Changes in storage modulus (G') have been used to monitor gelation of proteins including structural proteins (Venugopal *et al.*, 2002). G' indicates an elastic element of surimi gel. High elasticity is desired for good quality surimi (Fukushima *et al.*, 2007). Fig. 2 shows storage modulus (G') changes of *O. niloticus* and *C. striatus pastes* during heating at 20 to 90°C. Storage modulus (G') on temperature sweep analysis for all samples was higher than loss modulus (G'') (data not shown). This is an indication of the formation of viscoelastic gel network. Generally, both fishes at different setting time show better formation of viscoelastic gel as compared to the control sample (without setting). Maximum increases in storage modulus (G') were obtained at 90°C, when setting at 2 h (8.98×10^5 Pa) for *O. niloticus* and 1 h (8.02×10^5 Pa) for *C. striatus*. The higher rate of increase in G' indicates that proteins underwent ordered aggregation and formation of a 3-dimensional network with entrapment of water in the matrix (Hamann, 1992).

Expressible water (EW)

The water holding capacity (WHC) is directly associated with the percent of water expressed by centrifugation (the lowest percentage of water extracted means the highest water holding capacity) (Ramirez *et al.*, 2007). Expressible moisture content of gel with different setting times is shown in Fig. 3. The percentage of water expressed varied in the range of 1.68% to 4.51%. Kamaboko gels from *O. niloticus* and *C. striatus* had the lowest expressible water value after setting at 40°C for 1 h ($p<0.05$) compared to other treatments. After the appropriate setting, heating is required to induce the aggregation of protein, in which gel matrix formed can imbibe the water (Benjakul *et al.*, 2008). Different expressible moisture content suggested the differences in water holding capacity of gel network. In general, low expressible moisture content of the gels suggested the more water retained in the gel network (Niwa, 1992).

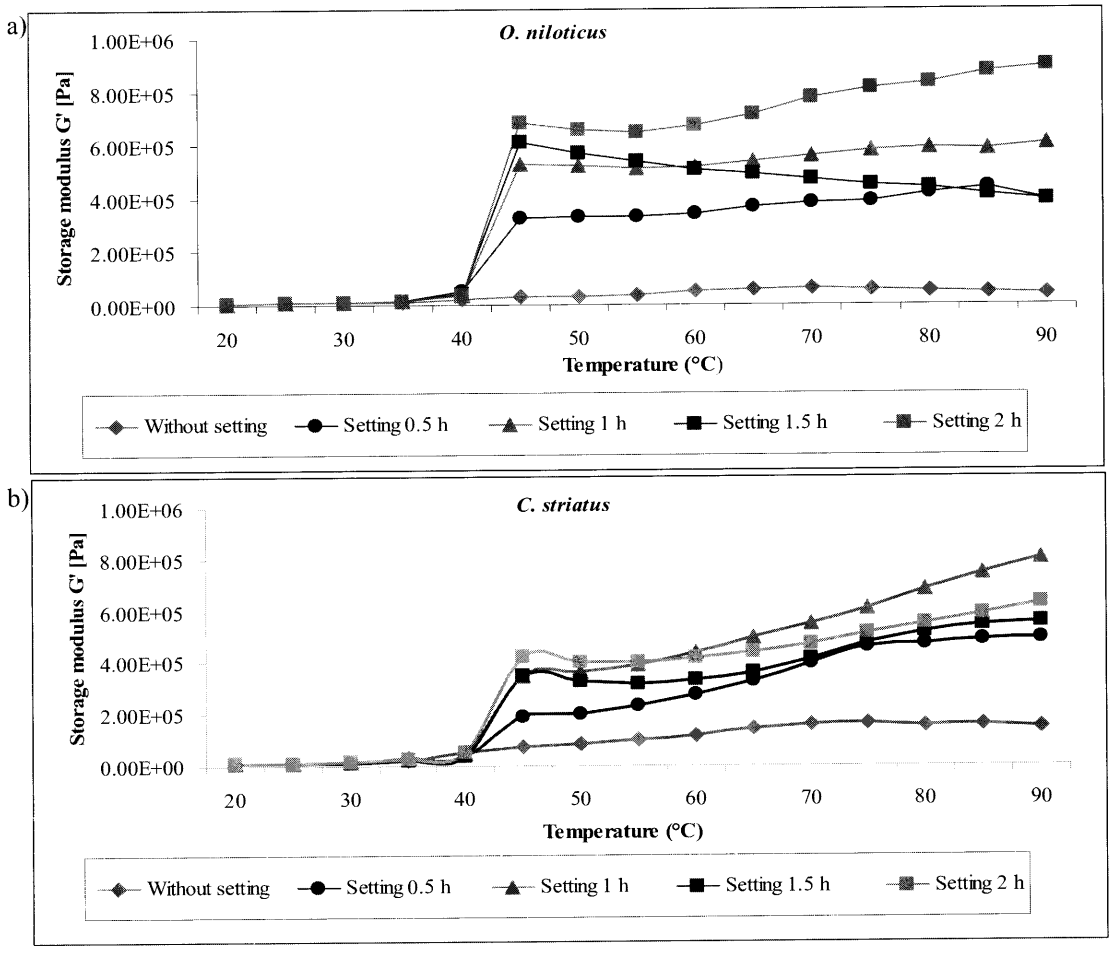

Figure 2: Changes in storage modulus (G') on temperature sweep analysis for fish meat pastes from a) *O. niloticus* and b) *C. striatus* upon thermal treatment with different setting time

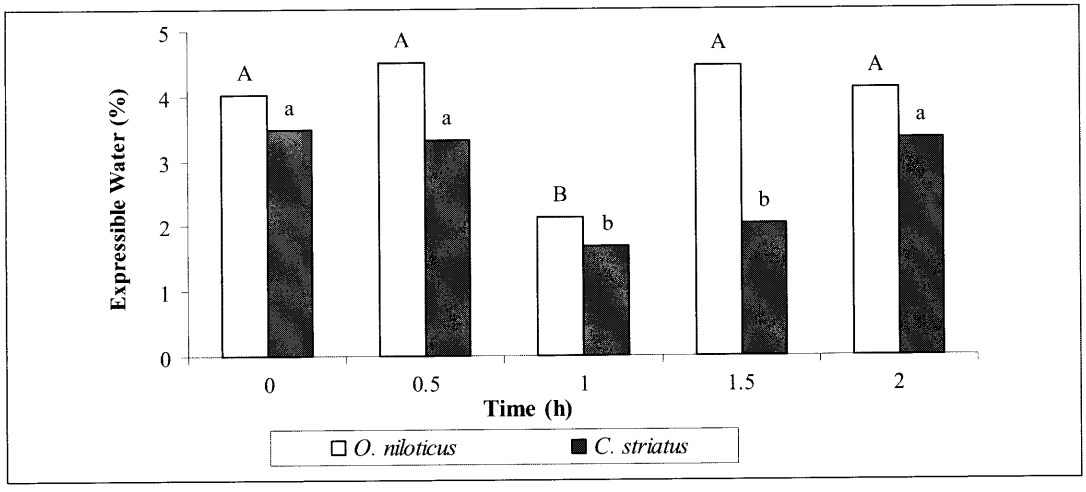

Figure 3: Changes in water extracted from kamaboko (restructured fish products) as affected by setting at 40°C for different times, prior heating to 90°C. Bars indicate the standard deviation of six determinations. Different letters within the same fish species indicate significant differences ($p<0.05$)

Fig. 4 shows the results of whiteness values of gels from *O. niloticus* and *C. striatus*. From the result, both *O. niloticus* and *C. striatus* whiteness were affected by setting time ($p<0.05$). Slightly higher whiteness value was observed in gels when setting times were increased. Increased whiteness can be related to degree of protein degradation, for instance, with cooking (Hwang *et al.*, 2007). Generally, the demand is higher for surimi gels with a high L^* value, low b^* value and high whiteness (Hsu and Chiang, 2002). Thus, setting at 40°C is a potential approach for increasing gel strength and exhibited no adverse effect on whiteness of resulting gel.

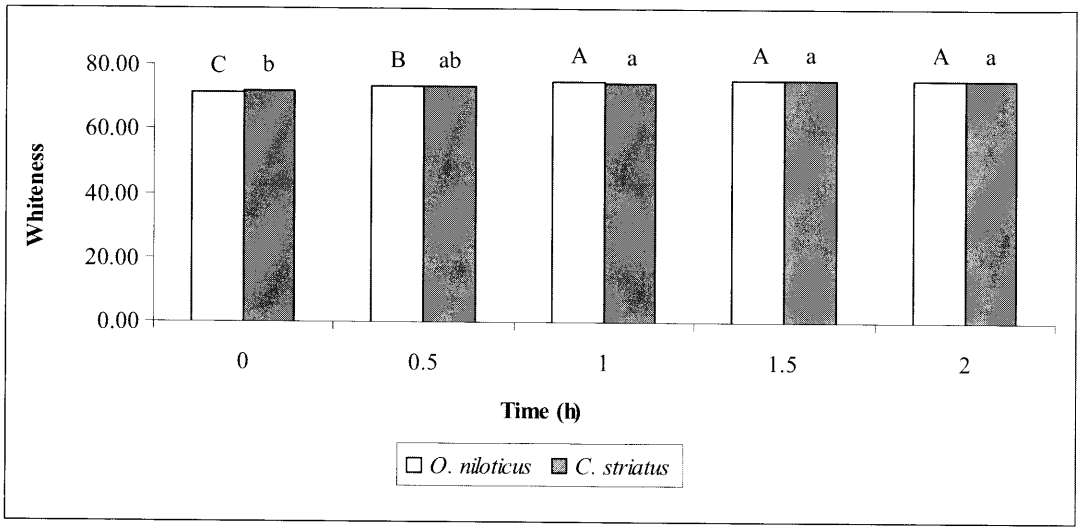

Figure 4: Whiteness values of gels from *O. niloticus* and *C. striatus* as affected by setting at 40°C for different times, prior heating to 90°C. Bars indicate the standard deviation of six determinations. Different letters within the same fish species indicate significant differences ($p<0.05$)

Conclusion

The gelation of restructured fish products from *C. striatus* and *O. niloticus* were time dependent. Pre-incubation at 40°C for 2 h followed by cooking at 90°C for 30 min seemed to give the best gel strength for both fish. Kamaboko gels from *O. niloticus* and *C. striatus* had the lowest expressible water value after setting at 40°C for 1 h ($p<0.05$) compared to other treatments. Thus, *C. striatus* exhibited higher physical properties as compared to *O. niloticus*. Setting at 40°C for an optimum time is a promising means for improving the gel quality of restructured fish products from freshwater fish.

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