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ScienceDirect
Food Chemistry 107 (2008) 106–111
Food Chemistry
www.elsevier.com/locate/foodchem

Pacific whiting (*Merluccius productus*) underutilization in the Gulf of California: Muscle autolytic activity characterization

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Received 23 February 2007; received in revised form 13 July 2007; accepted 19 July 2007

Abstract

In México's Northwest coast, Pacific whiting (*Merluccius productus*) is considered an under-utilized species because of its high tendency to become parasitized, thus promoting a high proteolytic activity present in muscle tissue. Sample fish for study were separated in lots by degree of parasitism in "apparent" parasitized (APP) and advanced parasitized (ADP). Thus, pH and temperature conditions of mayor endogenous proteolytic activity in muscle were determined. Maximum activity was detected at 50 °C (pH 3.5–4.0) and at 60 °C (pH 6.75–7.0). Parasitism degree had a significant effect in enzyme activity at acidic pH ($p < 0.05$) being higher in APP at low temperature (30 °C). Higher temperatures (40–50 °C) favored ($p < 0.05$) activity in ADP muscle (same pH range) with the highest ($p < 0.05$) observed at 50 °C at pH 3.5. No much difference was observed at pH 7.0–8.0. Results suggest that pH around physiological conditions at 60 °C could be used as an advantage in fish protein hydrolysate production or as processing aid where a protein hydrolysis is required.

Keywords: Autolysis; Proteolytic activity; Pacific whiting; Parasitism degree

1. Introduction

Marine seafood consumption has consistently improved since it has been recognized as an important source of nutrients for human health. It possesses great quality protein, among other nutrients, that differ from those of land animals (Alasalvar, 2002). Nevertheless many species are under-utilized because of some technological inconvenience, making them no attractive to be used as food and just employed as raw materials for feed production. One of the major problems fish muscle can present is the occurrence of parasites, which promote a high proteolytic activity either by exogenous enzymes from parasites (Moran, Whitaker, & Kent, 1999) or as an "immune response" from the fish to the presence of those parasites (Erickson, Gordon, & Anglemier, 1983). This proteolytic activity results in muscle filament degradation, texture loss and myofibrillar protein functionality, limiting its use by the seafood industry (Martinez de Velasco, Rodero, Zapatero, & Cuellar, 2002). The presence of proteases in fish flesh infected with a Myxosporea parasite is known to affect the texture in species such as arrowtooth flounder (*Atheresthes stomias*) (Wasson, Babbitt, & French, 1992), Yellowfin sole (*Limanda aspera*) (An, Seymour, Morrissey, Peters, & An, 1994) and Pacific whiting (*Merluccius productus*) (An, Seymour, Wu, & Morrissey, 1994; Erickson et al., 1983).

In México's Northwest coast, Pacific whiting (*Merluccius productus*), is considered an under-utilized species because of its high tendency to become parasitized. As its fishery is not yet established in the region, their captures have been kept low and referred solely as by-catches from other commercial fisheries; however, their stocks in the region have been reported to be of about 100,000 tons (Casas-Valdez, 2004).

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0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.07.056

Pacific whiting muscle tissue, infected with myxosporidia (*Kudoa paniformis* and/or *K. thyrsitis*), contains high levels of proteolytic activity (Erickson et al., 1983; Kudo, Barnett, & Nelson, 1987; Patashnik, Groninger, Barnett, Kudo, & Koury, 1982; Tsuyuki, Williscroft, Kabata, & Whitaker, 1982). The proteases involved can hydrolyze fish skeletal muscle and connective tissue proteins, which in turn induce tissue softening thus affecting its marketability (Adlerstein & Dorn, 1998; Kabata & Whitaker, 1981).

At the present, because of its white muscle color, mild flavor and low fat content, Pacific whiting is extensively used by the USA fish industry as raw material for surimi production. However, the use of protease inhibitors is strongly recommended if surimi from this species is going to be produced since the high proteolytic activity present in the muscle could produce a low-quality surimi due to degradation of myosin needed to form the surimi gel (Morrisey, Wu, Lin, & An, 1993; Seymour, Peters, Morrissey, & An, 1997).

Many types of endogenous proteases (acid, neutral, or alkaline) have been investigated as possible agents of post-mortem degradation of fish muscle (Haard, 1992; Wasson, 1992) Among them, the so-called alkaline proteases have been implicated in the textural degradation of fish meat gels at relatively high temperatures and pH (50–70 °C, pH 8.0) as well as those enzymes which are optimally active at post-mortem pH of fish muscle (Wasson, 1992).

Both, pH and temperature for optimal protease activity are influenced considerably by substrate used (An et al., 1994). Substrate sources such as casein (Crapo, Himelbloom, Pfutzenreuter, & Lee, 1999; Erickson et al., 1983) and urea-denatured hemoglobin (Chang-Lee, Pacheco-Aguilar, Crawford, & Lampila, 1989) have been used to measure the proteolytic activity present in the fish muscle. However, as the development of mushy texture reflects hydrolysis of structural muscle proteins, it is more meaningful and more practical, from the point of view of producing functional hydrolysates, to measure protease activity against myofibrillar proteins from the species themselves. Hence, the main objective of the present study was to determine the optimal pH and temperature conditions where the highest autolytic activity in Pacific whiting muscle take place in order to evaluate the use of such information for protein hydrolysate production from the same species.

2. Materials and methods

2.1. Fish sample

Pacific whiting (*M. productus*) were harvested off the Gulf of California coast by a commercial fishing vessel from the fishing camp "El Desemboque del Seri", Sonora, México, during March and May of 2004. Whole fish samples were obtained from two sampling trips (40 and 100 kg/each) (lot 1 and 2, respectively) and transported in portable coolers in alternate layers of fish and ice to the Seafood Laboratory at CIAD. Fish was processed within 24 h post catch. Three sub-lots ($n = 3$) of 10 fishes/each were obtained from each lot. Each fish was manually eviscerated, headed and washed thoroughly in iced water. Dressed fish was butterfly opened and mechanical deboned in a Bibun deboner model NDX103 (Bibun Corp. Fekuyama, Japan). The minced flesh from every sub-lot was packed in polyethylene bags (~500 g) and kept frozen at −20 °C until used.

2.2. Chemical analysis

Moisture, ash, fat, and protein of minced muscle, was determined following the AOAC (1993) methodologies (Sec 950.46, 938.08, 960.39 and 955.04, respectively). Non-protein nitrogen (NPN) extracts from minced muscle were obtained according to Woyewoda, Shaw, Ke, and Burns (1986). All analyses were carried out in duplicates.

2.3. Degree of parasitism

As mentioned elsewhere Pacific whiting muscle has been characterized for presenting a high proteolytic activity related to infestation with Myxosporidian parasites. Specimens used for this study were not the exception. A remarkable difference of infestation degree was detected between lots, defined as apparent infestation for fishes of lot 1 and advanced for fishes of lot 2. Apparent parasitism (APP) was defined as "non evident" signs of parasites (samples presenting yellow-cream muscle spots and slightly soft texture), while advanced parasitism (ADP) was defined as evident signs of parasites showing dark streaks (black pseudocysts) in flesh as a result of parasite breakdown products accumulation (Abollo, Novoa, & Figueras, 2005; Morrissey, Hartley, & An, 1995). Comparison was made as such APP vs. ADP. No identification of parasites was carried out, since it was out of the scope of present study.

2.4. Total proteolytic activity

Total proteolytic activity (TPA) in minced muscle was determined by the method described by Tsuyuki et al. (1982) with modifications. Briefly, 30 g of minced muscle were homogenized with 120 mL of the respective cold (0–5 °C) buffer (ratio 1:4, muscle:buffer) for 30 s in a mixer at high velocity with temperature never exceeding 10 °C. Buffers used were as follows: 0.05 M citrate for pH 3.0, 3.5 and 4.0; 0.1 M phosphate for pH 7.0, 7.5, and 8.0 and 0.1 M NaCl for normal pH of muscle. Incubation temperatures were 30, 35, 40, 50 and 60 °C, for acidic conditions (pH 3.0, 3.5 and 4.0); 50 and 60 °C for neutral and alkaline conditions (pH 7.0, 7.5 and 8.0); finally at 60 °C for the actual non-adjusted fish pH (6.75) as in Pacheco-Aguilar and Crawford (1994). Then, 5 mL aliquots of homogenate (~32 mg prot/mL) were put in an 18 ×

150 mm test tube and incubated for an hour at conditions mentioned before. Control was held in ice for the same period. Reaction was stopped with 5 mL of 10% trichloroacetic acid (TCA) cold (0–5 °C) solution, keeping tubes on ice. After 30 min in ice, samples were filtered using Whatman paper No. 1. Protein content of filtrates (hydrolysis products) were determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951), using a Perkin–Elmer spectrophotometer Lambda 3B (UV/VIS) (Perkin Elmer, Mexico) at 500 nm. A tyrosine (Sigma Chemical Co., St. Louis, MO) standard curve was done using a 200 μg/mL stock solution. Results were reported as total proteolytic activity expressed as μmole of tyrosine released per g of protein in 60 min of incubation. All analyses were carried out in triplicate.

2.5. Statistical analysis

As mentioned elsewhere, lot 1 (APP) and lot 2 (ADP) were divided in three sub-lots of 10 fishes/each ($n = 3$) and the correspondent samples taken for further analyses. The effect of the degree of parasite infestation (APP vs. ADP) on the TPA present was evaluated. Data was analyzed by one-way analysis of variance (ANOVA) and multiple comparison when necessary (SAS System for windows, version 8.01). All statistics were carried out at $\alpha = 5\%$.

3. Results and discussion

3.1. Sample characteristics

Remarkably, sampled fish from both lots displayed a significant difference in their degree of infestation. Derived from that fact and as described in Section 2, fish from lot 1 was defined as APP, and those from lot 2 ADP. Hence, it was necessary to evaluate the results accordingly and separately. Pacific whiting used in the present study had an average weight of 1167 ± 608 g, ranging from 550 to 1700 g, and an average size of 49 ± 9 cm, ranging from 40 to 56 cm. According to Inada (1981), the age of the specimens used in our study ranged from 6 to 7 years old.

In the present study the average yield for the edible portion obtained with a mechanical deboner was $45 \pm 0.7\%$ of the whole fish weight, a higher value if compared to the 32–35% reported from Chang-Lee (1988), Crawford, Law, Babbit, and Mcgill (1972) and Pacheco-Aguilar, Crawford, and Lampila (1989) for the same species. It is well documented that the edible portion varies among species from 30% to 60%, depending of biological factors such as shape, age and physiological condition of fish before capture (Suzuki, 1981). If mechanical deboned, an additional 15–30% yield could be obtained as compared with the manual processed (Bycowski, 1990). The higher yield obtained in this study could be explained in terms of the difference in fish sizes since these researchers used smaller animals (approximately 20 cm shorter) and to the manual removal of the triangular spinal bone located below the dorsal fin previous to deboning allowing for a better meat extraction.

3.2. Chemical analysis

Moisture, protein and ashes content of Pacific whiting ground muscle, did not show a remarkable variation between the samplings, giving values of $82.6\% \pm 1.0\%$, $16.1\% \pm 0.9\%$ and $1.1\% \pm 0.05\%$, respectively. These results agreed with previous work of Pacheco-Aguilar (1990), who reported similar values for the same species. Respect to the fat content, Pacific whiting used in the present study, showed a $0.3 \pm 0.1\%$ which agrees with the value reported in the literature (<1.5%) (Pacheco-Aguilar et al., 1989; Perkins, 1992). NPN content was $0.3\% \pm 0.03\%$, below the levels of 0.4–0.8% reported by Simidu (1961) for the same species. Literature reports a high postmortem muscular proteolytic activity in this species that rapidly increase the NPN content if the muscle is not properly handled (Erickson et al., 1983; Tsuyuki et al., 1982). Our results suggested that fish was properly handled (adequate times and temperatures), thus delaying the enzymatic activity that would increase the NPN content.

3.3. Total proteolytic activity (TPA) in muscle

The average TPA obtained for both sampling lots are showed in Fig. 1. Maximum activity zones were detected at pH 3.5–4.0 (50 °C) and pH 6.75–7.0 (60 °C), with average values of 56 and 45 μmole Tyrosine/g protein/60 min, respectively. Results indicated activity of both acidic and alkaline enzymes in Pacific whiting muscle samples at temperatures commonly used by the food processing industry. The active enzymatic fraction at pH 3.0 was inhibited above 40 °C, while temperatures above 50 °C were required to inhibit the activity at pH 3.5–4.0. This behavior agrees with Koury, Spinelly, and Wieg (1971), whom pointed out that muscular enzymatic activity in Pacific whiting generally is greater at acid pH, tending to diminish when temperature exceeds 50 °C. At neutral and alkaline pH

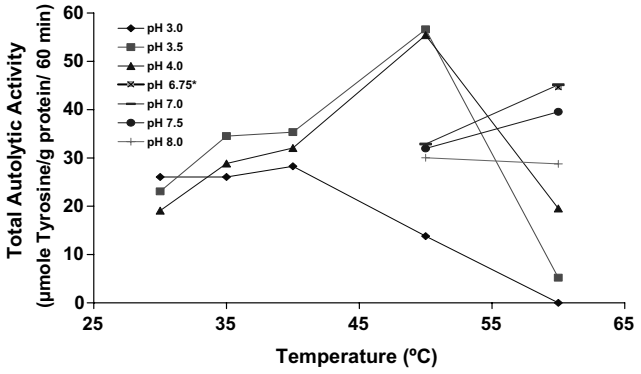

Fig. 1. Total autolytic activity average at various pH and temperatures assayed in Pacific whiting muscle. See Section 2 for conditions used. Points are the means of two samplings. * Non-adjusted muscle pH.

activity increased at temperatures above 50 °C, except for pH 8.

As stated before, fish used in the present study showed muscular parasitism being more noticeable in the second lot, which showed an advanced infection manifested by dark streaks in the flesh (Patashnik et al., 1982; Tsuyuki et al., 1982). To evaluate the effect of parasitism, data was also analyzed comparing the degree of parasite infestation (APP vs. ADP) on TPA. Fig. 2A shows the TPA at 30 °C in the acidic 3–4 pH range. Higher activities were observed for APP throughout this pH range with the highest being at pH 3.0 and a declining tendency towards higher pHs. ADP muscle showed constant values ($p \geqslant 0.05$) throughout this pH range. However, when the temperature was increased to 35 (data not shown) and 40 °C (Fig. 2B), the TPA was greater ($p < 0.5$) for ADP with higher values at pH 3.5 (45–47 μmole Tyrosine/g protein/60 min). Results indicate that this increment in temperature had a higher impact in the ADP muscle, suggesting that the TPA at acidic pH (3–4) was mainly due to parasite infestation. Tsuyuki et al. (1982) reported an optimum temperature for the proteolytic activity of enzymes secreted by *Kudoa* species of 35–40 °C at pH 3.8.

Enzymatic activity detected at pH 3 decreased significantly ($p < 0.05$) at temperatures greater than 50 °C for both sampling lots (Fig. 3A and B). On the contrary, ADP muscle autolytic activity at pH 3.5 and 4.0 increased significantly at 50 °C ($p < 0.001$) as compared to the APP muscle (Fig. 3A). This result was the most remarkable difference detected in the acidic pH range between lots showing the effect of parasitism degree in muscle proteolysis.

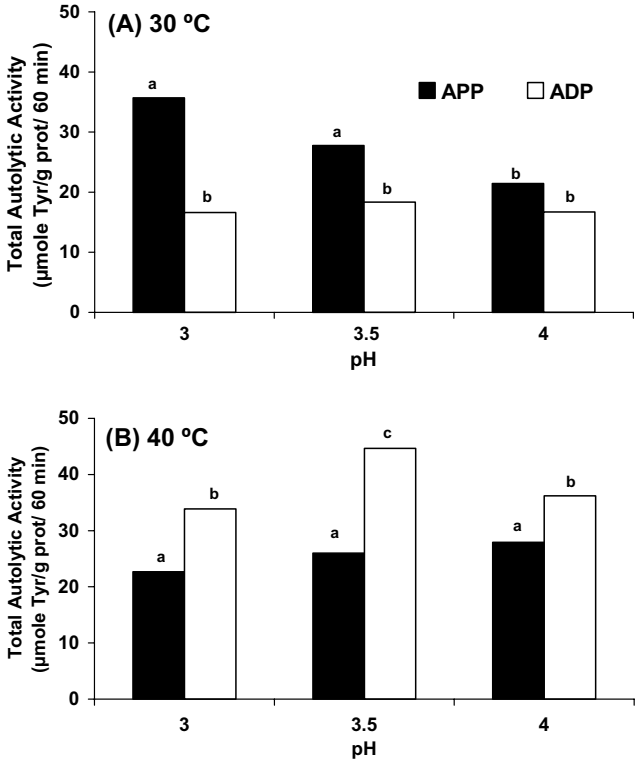

Fig. 2. Total autolytic activity in Pacific whiting muscle at 30 °C (A) and 40 °C (B) at acidic pH. Bars with different letters are statistically different ($p < 0.05$). APP: apparent parasitized; ADP: advanced parasitized.

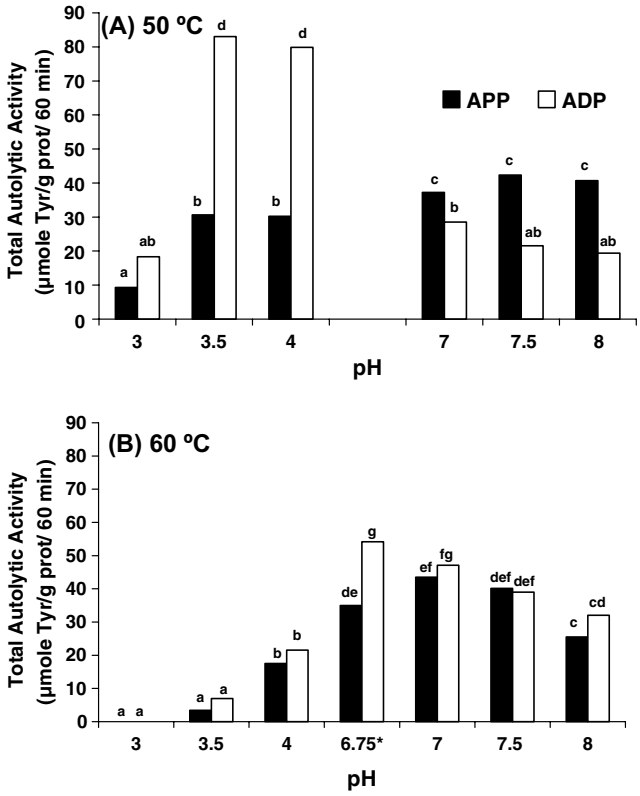

Fig. 3. Total autolytic activity in Pacific whiting muscle at 50 °C (A) and 60 °C (B) throughout all pHs measured. Bars with different letters are statistically different ($p < 0.05$). APP: apparent parasitized; ADP: advanced parasitized. *Non-adjusted muscle pH.

APP muscle showed nearly 36% enzymatic activity (pH 3.5–4.0/50 °C) from that observed for ADP muscle (Fig. 3A). The inverse behavior, however with lower values of activity, was detected at neutral to alkaline pH (same temperature), where the TPA detected in APP was significantly higher ($p < 0.05$). This same behavior was reported by Erickson et al. (1983) in sarcoplasmic fluid from Pacific whiting with different parasitism degree, using hemoglobin as substrate. Literature reports that this difference in activity is related to parasitism degree and to the kind of parasite that infest the Pacific whiting muscle; while enzymes secreted by *Kudoa thyrisitis* increase its activity at acidic pH, *Kudoa paniformis*' enzymes increase its activity at both, acidic and neutral pH (Adlestein, 1991). Remarkably Fig. 3B shows the effect of temperature over the TPA in muscle, as a 10 °C temperature increase reduced and even suppressed the activity detected in the acidic pH range.

Similar TPA was detected independently of the parasitism degree in the 6.75–8.0 pH range at 60 °C (Fig. 3B). No significant difference ($p \geqslant 0.05$) was observed for the TPA between sampling lots for each pH in the 7.0–8.0 range. No difference ($p \geqslant 0.05$) was found also when comparing sampling lots (APP vs. ADP) at pH 7.0–7.5. These results

suggest the presence of thermo-stables alkaline proteases from viscera contamination due to the removal of internal organs during the dressing operation (Martinez & Gildberg, 1988; Su, Lin, & Lanier, 1981). Washing of visceral cavity before mechanic separation of muscle, could help in the reduction or elimination of this contamination. Further work is necessary to validate this fact.

Overall results suggest that the enzymatic activity detected at acidic pH and temperatures lower than 50 °C came from the combination of endogenous catheptic enzymes (An et al., 1994, An, Peters, Seymour, & Morrissey, 1995), and secreted enzymes by the parasites and/or by the immunological response to this infestation (Kudo et al., 1987; Patashnik et al., 1982; Seymour et al., 1994; Wasson, 1992). The results of this study for ADP muscle agreed with those of Tsuyuki et al. (1982) who found two optimum temperatures ranges for the proteolytic activity of enzymes secreted by *Kudoa* species, one (Fig. 3A) at 35–40 °C (pH 3.8) and the other (Fig. 3B) at 55–60 °C (pH 6.7).

Complementary, Fig. 3B shows that temperatures above 50 °C had a similar effect on the TPA regardless of the degree of parasite infestation, suggesting that this enzymatic activity comes mainly from an endogenous source. It has been stated that the enzymatic activity at neutral and alkaline pH and temperatures higher than 50 °C could be the result of alkaline muscular proteinases (Asghar & Bhatti, 1987; Wasson, 1992) and/or visceral fluid contamination characterized by a high proteolytic activity (Martinez & Gildberg, 1988; Su et al., 1981).

4. Conclusions

The enzymatic activity detected in Pacific whiting muscle from the Gulf of California, similar to that reported for the same species caught in the northeast pacific ocean, could also considerably reduced the possibility of its use for direct human consumption as fresh, frozen-fresh and canned products among others. Because of this inconvenience, technology applications such as production of surimi and/or functional hydrolysates generate alternatives of its use as food ingredients. Further work is currently under way in our laboratory for the production and evaluation of functional hydrolysates controlled for different degree of hydrolysis. Since freezing could disrupt tissue and destroy cells it is necessary to evaluate the possible effect of freezing and prolonged frozen storage over the TPA of APP and ADP muscles, comparing frozen and unfrozen fish samples. All samples in this study were frozen-stored (−20 °C).

Acknowledgement

Authors wish to thank Science and Technology National Council (CONACyT) from the Republic of México for supporting this research.

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