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Food Chemistry 88 (2004) 543–548
Food Chemistry
www.elsevier.com/locate/foodchem

Thermal inactivation characteristics of acid and alkaline phosphatase in fish and shellfish

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Received 27 October 2003; received in revised form 21 January 2004; accepted 21 January 2004

Abstract

To provide information about the roles of acid and alkaline phosphatase (ACP, ALP) activities, as the degree of working indicators of marine foods, the effect of heating (55 and 65 °C) on phosphatase activities, in the edible part of selected species was investigated. Twenty-nine out of 49 fresh fish, shellfish and crustacea samples, such as mackerel *Scomber japonicus*, showed maximum activity at pH 5 or 6 and very low activity at pH 11. The other 20 species showed various ALP activities. Particularly, in the case of gurnard *Lepidotrigla microptera*, sea robin *Chelidonichthys spinosus* and *Glyptocephalus stelleri*, a flounder, the ALP activity was higher than the ACP activity. The phosphatase activities were reduced to 10% or less of the activity in unheated samples by heating at 55 °C for 10 min. The heat-stability of ALP in intestines was far higher than that in dorsal muscle. These results suggest that the measurement of ACP and ALP activities in marine foods can be used as a quality check.

Keywords: Acid phosphatase; Alkaline phosphatase; Marine foods; Thermal inactivation

1. Introduction

Acid phosphatase (ACP, EC 3.1.3.2) and alkaline phoshatase (ALP, EC 3.1.3.1) are widely distributed throughout the living world (Fernley, 1971; Pacova & Kocur, 1978). ALP activity in pasteurized milk generally indicates inadequate pasteurization and the presence of ALP activity may be due to contamination of pasteurized milk with raw milk or post-process bacterial contamination (Mistry, 1989; Pratt-Lowe, Geiger, Richardson, & Barrett, 1988; Scintu, Daga, & Ledda, 2000). In meat products, ACP activity is used as an indicator of pasteurization (el Hadef el Okki, Philippon, & Mouthon, 1987; Incze, Körmendy, Körmendy, & Zsanóczay, 1999; Jung, Ghoul, & de Lamballerie-Anton, 2000).

In many studies of marine foods, phosphatase activities in fish muscles, particularly ATP-, ADP- and IMP-degrading enzymatic activities, have been reported to be related to the *K*-value, an index of freshness (Gill, 1992). In fisheries sciences, changes in phosphatase activities are regarded as indices of growth, illness and spawning (Goldemberg, Paron, & Crupkin, 1987; Matusiewicz & Dabrowski, 1996). We reported that dorsal muscles of many edible fishes have high ACP activity (Kuda, Matsumoto, & Yano, 2002). However, the heat resistances of ACP and ALP of fish muscle are not well established.

The aim of this work was to provide information on the potential roles of ACP and ALP activities as indicators of adequate pasteurization of marine foods. Therefore, we investigated the heat-resistances, at 55 and 65 °C, of ACP and ALP of 49 edible fish and shellfish. Changes in the activities of minced products of sardine, pork belly and chicken breast during the heating at 55, 65, and 75 °C were also investigated.

2. Materials and methods

2.1. Sample preparation

Forty-nine fresh fish, shellfish and crustacea (10 Clupeidae, two Anguillidae, three Belonidae, four

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doi:10.1016/j.foodchem.2004.01.069

Scombridae, three Caranginae, eight Percinae, one Zeidae, one Tetradintidae, two Gadidae, nine Cottidae and six Pleuronectidae), nine mollusks (one Gastropoda, three Plecypoda and five Cephalopoda), five crustaceans (four shrimps and one crab), raw meat of pork Boston butt, pork belly and chicken breast were purchased from retail shops in Ishikawa, Japan. The raw dorsal muscle or other edible parts of the samples were mixed with 9 volumes of 1% Triton X-100 and centrifuged (2220*g* for 10 min). The supernatant was used as a crude enzyme solution. The crude enzyme solutions of intestine, liver, kidney and spleen of six fish (mackerel *Scomber japonicus*, horse mackerel *Trachurus japonicus*, Blanquillos *Xyrichtys dea*, Sea robin *Chelidonichthys spinosus* and, gurnard *Lepidotrigla microptera* and tongue sole *Paraplagusia japonica*) were also prepared.

2.2. Effect of 55 and 65 °C heating for 10 min on acid and alkaline phosphatase activities in fresh marine foods

One ml of the crude enzyme solutions of the samples was transferred into a micro tube (1.5 ml, PP, As One, Tokyo). Then the tubes were submerged in water maintained at 55 and 65 °C in water baths and held for 10 min. The tubes were then cooled in ice water. Phosphatase activities, at pHs ranging 4–11 in the crude enzyme solutions, were then measured.

2.3. Change of acid phosphatase activity of minced sardine, minced pork belly and minced chicken muscle during heating at 55, 65 and 75 °C

Each 50 g portion of surimi (fish paste, Okada, 1992) of sardine *Sardinops melanostictus*, pork belly or chicken breast was put into a pouch (ONy/CPP, Meiwa R-1, Osaka) and the pouches were heat-sealed. Then the pouches were heated at 55, 65 and 75 °C in a water bath for 8 min. After 0.5 min and each 1 min interval of heating, acid phosphatase activity at pH 5.0 was measured.

2.4. Assay of phosphatase activity

To assay phosphatase activities, *p*-nitrophenol phosphatase disodium salt hexahydrate (PNP, Wako Pure Chemical Industries, Ltd, Osaka) was used (Berberianm & Beauge, 1992; Houk & Hardy, 1987). The phosphatase activity was determined by the microplate assay (Kuda et al., 2002), modified slightly; 0.15 ml of working solution, containing the 15 mmol/l PNP, 1 mmol/l $MgCl_2$ and buffer solution, was put into a well. The buffer solution was adjusted to pH 4.0, 5.0 and 6.0 by 0.1 mol/l of CH_3COOH–CH_3COONa, to pH 7.0, 8.0 and 9.0 by 0.1 M of Tris–HCl and to pH 10.0 and 11.0 by 0.05 mol/l of $NaHCO_3$–0.1 mol/l NaOH. Then 0.025 ml of the crude enzyme solutions was added. After mixing, the microplate was incubated at 37 °C for 15, 30 or 60 min. To stop the reaction, 0.05 ml of 0.5 M NaOH was added. Absorbance at 405 nm was measured by the microplate reader (Model 550, Bio-Rad, CA). The absorbance at 655 nm was also measured as reference.

3. Results and discussion

3.1. Effect of 55 and 65 °C heating on phosphatase activities in edible part of fish

The effects of heating for 10 min, at 55 and 65 °C, on phosphatase activities, at pHs ranging from 4 to 11 in the edible part of fish, molluscs, crustaceans and meat (chicken and pork) are summarized in Fig. 1 which shows the activities in typical samples.

Twenty-nine samples, among 49 fresh fish samples, showed maximum activity at pH 5 or 6 and very low activity at pH 11, such as mackerel (Fig. 1(a)). The ACP activity ranged from 120 to 440 nmol *p*-nitrophenol (PN)/min/g tissue and the activity was higher in red-flesh fish samples rather than in white-flesh fish samples. These results agree with our previous report (Kuda et al., 2002). Some reports have indicated that the IMP-degrading enzyme activity in dark muscle is higher than that in ordinary muscle and, for the emzyme in dark muscle, the optimal pH is 6 (Nakagawa & Nagayama, 1994). The ACP activity was suppressed, by 55 and 65 °C heating for 10 min, to 10% or less of the activity in intact samples.

Eight samples, among the fresh fish samples, particularly three among the six flounders, Pleuronectida showed maximum activity at pH 5 or 6 and low activity at pH 9 and higher as in ayu sweetfish *Plecoglossus altivelis* (Fig. 1(b)). In the case of muscles in gurnard, sea robin and *Glyptocephalus stelleri*, a flounder, the ALP activity was higher than the ACP activity (Fig. 1(c)). Especially, the ALP activity (at pH 10) in gurnard was 632 nmol PN/min/g tissue. The ALP and also the ACP activity were suppressed by heating to 10% or less of the activity in intact samples.

Since whole bodies of Japanese icefish *Salangichthys microdon* and juveniles of Japanese sculpin *Cottus pollux* are edible, their crude enzyme solutions contained their internal organs. They indicated high activity of ALP (262 and 509 PN/min/g) and the ALP was heat-stable at 55 °C (Fig. 1(d)).

The sample of freshwater clam *Corbicula leana* contained digestive organs and the ACP and ALP activities were high (721 and 617 PN/min/g, respectively). The ALP activity indicated heat-resistance (Fig. 1(e)). In the other three shellfish samples, which contained only ordinary muscle, the phosphatase activities were not so high. The activities of squid and octopus muscles were low (140 PN/min/g and lower) compared with those of

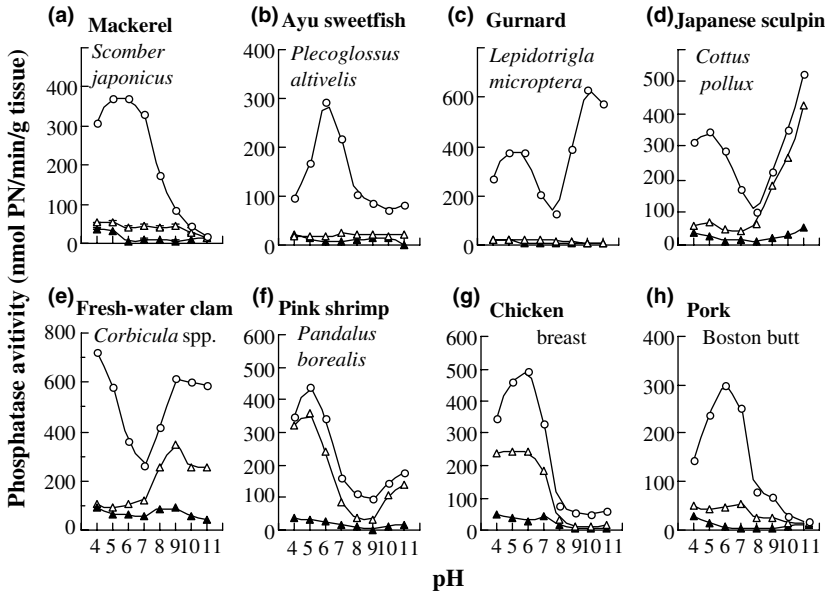

Fig. 1. Effect of heat-treatment on phosphatase activities in dorsal muscle of mackerel *Scomber japonicus*, ayu sweetfish *Plecoglossus altivelis* and gurnard *Lepidotrigla micoptera*, hole body of Japanese sculpin *Cottus pollux* and fresh-water clam *Corbicula* spp., peeled pink shrimp *Pandalus borealis* and meat of chicken breast and pork Boston butt. The activities of crude enzyme solutions were measured before (open circle) and after heating at 55 °C (open triangle) or 65 °C (closed triangle) for 10 min. Values are mean and SE ($n = 3$).

almost all fish samples. The samples of some shrimps, such as pink shrimp *Pandalus borealis*, also contained digestive organs and their phosphatase activities were tolerant to 55 °C heating (Fig. 1(f)). In the club flesh (*Ovalipes punctatus*), high ACP activity and instability to heating were seen, as for mackerel.

Raw meat products of chicken breast and pork Boston butt indicated high ACP activities of about 490 and 300 PN/min/g, respectively (Figs. 1(g) and (h)). The ACP of chicken meat was partially heat-stable at 55 °C. The thermal inactivation characteristics of the meats agree with previous reports (el Hadef el Okki et al., 1987; Lyon, Davis, Windham, & Lyon, 2001).

Since most of the fish muscle samples used in this study showed peaks of ACP that were inactivated by heating (as was pork meat) we believe that the ACP activity can be used as an indicator of thermal treatment in fish foods. As ALP in many fish shows low activity, it cannot be used as a thermal indicator, though the activity is high in some fishes, such as sea robin and gurnard.

3.2. Thermal inactivation characteristics of acid and alkaline phosphatase in fish organs

Thermal inactivation characteristics of ACP and ALP in organs of six fish samples are shown in Figs. 2 and 3. The intestinal ALP indicated high activity (3000 to 17000 PN/min/g tissue) in the fish, except for tongue sole. Although the ALP activities of mackerel and blanquillos were heat-stable at 55 °C, the heat stabilities of horse mackerel, sea robin and gurnard were not so high.

The liver ACP activity was high in mackerel, horse mackerel, blanquillos and tongue sole (1600–3700 PN/min/g tissue). Though the ACP in mackerel showed heat tolerantce at 55 °C and also 65 °C, the ACP in other fish samples was not high. The ALP activity in blanquillos, sea robin and gurnard was high (1200–30,000 PN/min/g tissue). The ALP in blanquillos showed heat-resistance at 55 °C, the ALP activities in sea robin and gurnard were destroyed by heating. Effects of pH and heating on ACP and ALP activities of kidney in mackerel, blanquillos, sea robin and gurnard were similar to those of liver. The kidney ALP activities in horse mackerel and tongue sole were high (14,000 PN/min/g tissue) and they showed heat stability at 55 °C.

High ACP activity (5000–19,000 PN/min/g tissue) in spleen was shown in fish other than gurnard. Most of the ACP activity was removed by 55 °C heating. The spleen ALP activity was high in blanquillos, sea robin and gurnard. The ALP of blanquillos showed heat-tolerance.

In mammals, the relative distribution of the activity of ALP has been reported as: intestinal mucosa = placenta > kidney = bone > liver > lung > spleen (Fernley, 1971). As shown in Fig. 1(d), the marine food containing internal organs had high ALP. Thus, it is thought that the ALP activity of fish muscle, particularly in red-flesh fish, can be used as an indicator of leaching from the internal organs. This exudation is not good for quality, for example, contamination by poisons and

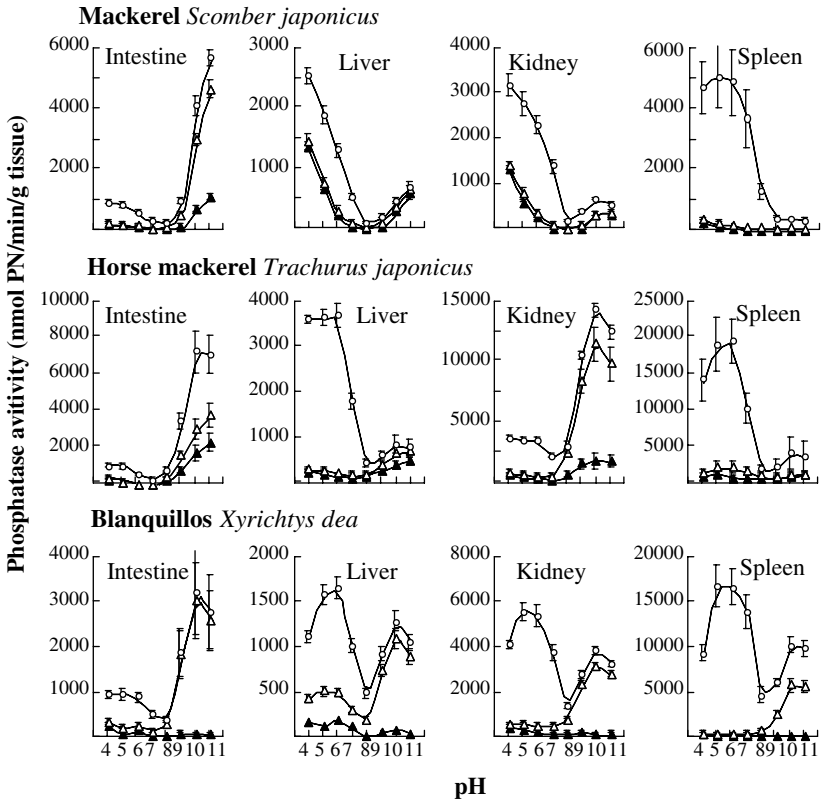

Fig. 2. Effect of heat-treatment on phosphatase activities in internal organs of mackerel (*Scomber japonicus*), horse mackerel (*Tranchurus japonicus*) and blanquillos (*Xyrichtys dea*). The activities of crude enzyme solutions were measured before (open circle) and after heating at 55 °C (open triangle) or 65 °C (closed triangle) for 10 min. Values are means and SE ($n = 3$).

bitter-tasting ingredients accumulated in the internal-organs (Hatano, Marumoto, & Hashimoto, 1976; Nagashima et al., 1999; Noguchi et al., 1987) or by microbes from the intestinal tract (Lartseva, Rogatkina, & Bormotova, 1997; Shalaby, 1996). We have reported that ALP in dorsal muscle of horse mackerel and gurnard increase during spoilage (Kuda et al., 2002).

In general, the ALP of intestine is stable to heat-treatment at 55 °C (Asgeirsson, Hartemink, & Chlebowski, 1995; Sandhu & Mahmood, 1990). Although the intestinal ALP of sea robin and gurnard, white-flesh bottom fish, was unstable toward heating, the activities of ALPs of other fish remained stable. Asgeirsson et al. (1995) indicated that the ALP from the pyloric caeca of Atlantic cod was unstable toward heating above 40 °C. However, the remaining ALP of the intestine was far higher than that of muscle, not only in the red-flesh fish but also in sea robin and gurnard.

3.3. Change of ACP activity of minced sardine, pork belly and chicken breast on heating

Changes of the ACP activity at pH 5 of minced sardine, pork belly and chicken breast, during heating at 55, 65 and 75 °C, are shown in Fig. 4. After 55 °C heating for 8 min, the ACP activities of the minced sardine, pork belly and chicken breast were 20%, 50% and 90%, respectively. After heating at 75 °C for one min, the ACP of the minced sardine and chicken breast were reduced to 3% or lower. In the case of the minced pork, the ACP activity was reduced to 10% after heating at 75 °C for 3 min. In meat products, ACP activity is used as an indicator of pasteurization (el Hadef el Okki et al., 1987; Incze et al., 1999; Jung et al., 2000). From the results obtained in this study, it can be concluded that the ACP activity can be used as a heat treatment marker in marine foods, such as surimi (fish paste) products (Belibagli, Speers, & Paulson, 2003; Benjakul, Chantarasuwam, & Visessanguan, 2002), as it is for meat products.

In conclusion, when the heat-stable ALP activity is detected in dorsal muscle of fish, possibilities of leak of drip containing the enzymes from internal organs and the growth, during storage or processing, of microorganisms having a heat-stable ALP (Ansai et al., 1998; Rosenthal, Bernstein, & Rosen, 1996) would have to be considered. The ACP activity can be used as marker of adequate heat-treatment in various marine foods, as in meat products. In any case, the existence of heat-stable ACP and ALP, not only in cooked products but also in raw marine foods, indicates a possible problem of product quality. The phosphatase test, using PNP, can

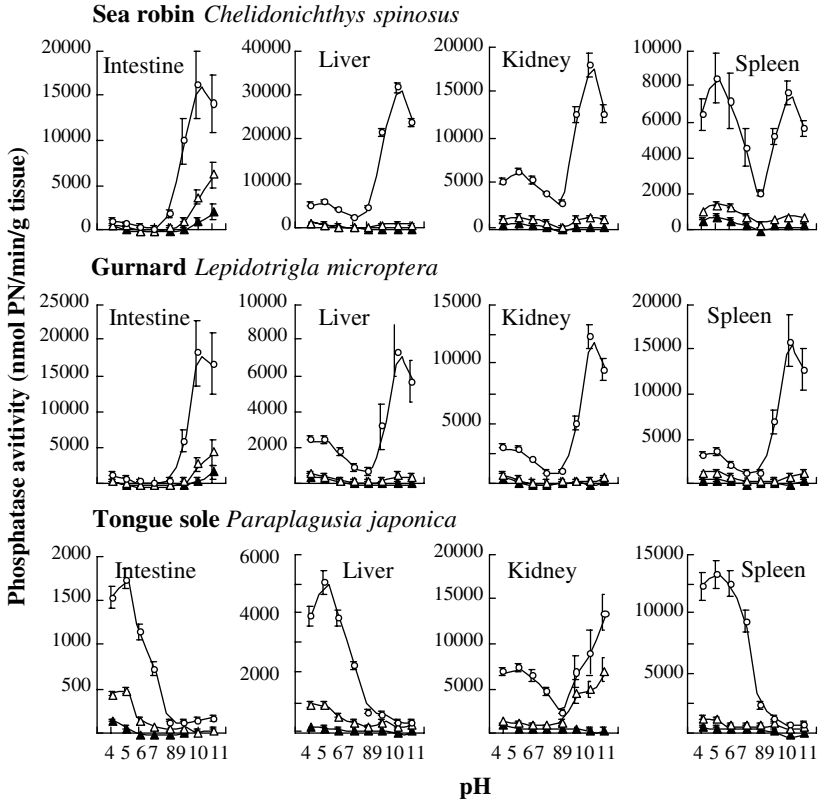

Fig. 3. Effect of heat-treatment on phosphatase activities in internal organs of sea robin (*Chelidonichthys spinosus*), gurnard (*Lepidotrigla microptera*) and tongue sole (*Paraplagusia japonica*). The activities of crude enzyme solutions were measured before (open circle) and after heating at 55 °C (open triangle) or 65 °C (closed triangle) for 10 min. Values are means and SE ($n = 3$).

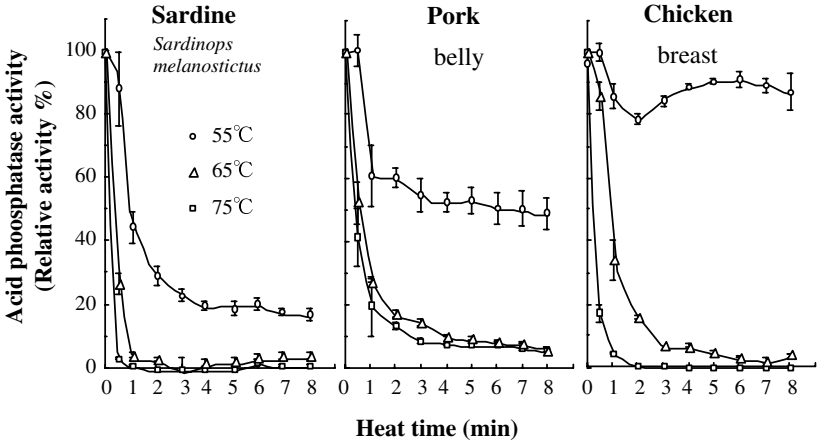

Fig. 4. Effect of heat-treatment on minced products of sardine *Sardinops melanostictus*, pork belly and chicken breast. Portions (50 g) of the minced meat were heated in pouches in water baths maintained at temperatures of 55, 65 and 75 °C, respectively. Values are means and SE ($n = 3$).

assay tens or hundreds of samples per hour easily and routinely, and has been used extensively in food safety and medical checks (Kissemehi, Treptau, Kottwitz, & Plattner, 1997). It is thought that the rapid and simple check for the existence of ALP and ACP, as for dairy and meat products, can be of value in assuring the quality of marine products.

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