Inhibition of Protease Activity in Muscle Extracts and Surimi from Pacific Whiting, *Merluccius productus*, and Arrowtooth Flounder, *Atheresthes stomias*

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Introduction:

Recent increases in consumer demand for seafood have placed added pressure on traditional fishery stocks. Historically, Alaska pollock, *Theragra chalcogramma*, has been the major species of fish utilized for surimi processing, but it is being increasingly used for frozen fillets in various forms. This has resulted in decreased availability of pollock for surimi processing and has created interest in other stocks of fish suitable for surimi preparation.

A fish stock must satisfy several criteria to be suitable for surimi processing. These include relative abundance, mild flavor, and white flesh. In addition to Alaska pollock, there are two species of fish in the Pacific that meet these requirements. One is Pacific whiting, *Merluccius productus*, available on the Pacific coast from California to British Columbia. The second is arrowtooth flounder, *Atheresthes stomias*, which is found on the Pacific coast of the continental United States and Canada and in the Gulf of Alaska along the Aleutian chain and the Bering Sea.

Both Pacific whiting and arrowtooth flounder have been regarded as low-valued species due to a common problem of softening of the flesh during cooking (Patashnik et al., 1982; Dassow et al., 1970; Tsuyuki et al., 1982; Greene and Babbitt, 1990). This softening is the result of protease activity within the muscle tissue of both species. Kabata and Whitaker (1981) suggested that the softening of Pacific whiting during heating was associated with the presence of the myxosporean parasites *Kudoa paniformis* and *Kudoa thyrsitis*. Attempts to estimate the incidence of these parasites in this species produced varying results (Kabata and Whitaker, 1986; Kudo et al., 1987). It has been suggested that only the presence of *Kudoa paniformis* is correlated with soft texture in Pacific whiting (Tsuyuki et al., 1982; Kudo et al., 1987). Although the presence of myxosporean parasites has been reported in arrowtooth flounder, there does not appear to be a relationship between the level of incidence of parasites and autolysis of the flounder muscle. (Greene and Babbitt, 1990).

The enzyme(s) responsible for softening of the flesh of both species during cooking are located within the muscle fibers. It is therefore impossible to select and discard those fillets with high protease activity. As a result, attempts to utilize fillets in individual or block form have been relatively unsuccessful due to extreme variability in cooked texture. Consequently, these species have been marketed only sparingly in traditional forms.

Because surimi is produced in a finely comminuted form with cryoprotectants added prior to freezing, inhibitors can be added with the cryoprotectants during normal processing. Consequently, considerable interest exists for the utilization of Pacific whiting and arrowtooth flounder for surimi. This would serve to alleviate the pressure on the Alaska pollock resource which could be used for the more traditional fillet products.

Several studies have shown chemical inhibitors to be effective against the protease activity in Pacific whiting (Miller and Spinelli, 1982; Konagaya, 1984; Nagahisa et al., 1983). Although effective, these chemicals do not satisfy the necessary requirements as approved food additives. Natural sources of protease inhibitors exist and have varying degrees of specificity toward protease enzymes. Mammalian blood plasma contains inhibitors toward all classes of protease enzymes (Laskowski and Kato, 1980). Bovine plasma is currently used as an inhibitor in Pacific whiting surimi for export. Wasson et al. (1992b) have recently reported bovine plasma to be an effective inhibitor in arrowtooth flounder surimi. However, its acceptability as an ingredient for surimi remains questionable in the United States. Egg white also contains protease inhibitors shown to be effective in Pacific

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ABSTRACT—Muscle extracts of Pacific whiting, Merluccius productus*, and arrowtooth flounder,* Atheresthes stomias*, were assayed for proteolytic activity using azocasein as a substrate. Pacific whiting extracts showed maximum activity at pH 5.0-5.2 and a temperature of 50°C, while arrowtooth flounder extracts had maximum activity at pH 5.5 and 55°C. Three sources of inhibitors (potatoes, egg white, beef plasma protein) were evaluated in vitro for inhibition of protease activity. All three were found to be effective inhibitors in crude muscle extracts. Further studies utilizing these inhibitors in surimi showed that potato was equivalent to both egg white and beef plasma protein in preserving the gel-forming characteristics of heated kamaboko in both species.*

whiting surimi (Haga et al., 1980; Nagahisa et al., 1983; Groninger et al., 1985; Chang-Lee et al., 1989; and Wasson et al., 1992b). The use of egg white, however, has not been widely accepted due to its high cost and off-flavors found at the levels required for inhibition. Potatoes are a rich source of protease inhibitors, and various preparations have shown activity in crude enzyme preparations from trout muscle (Kaiser and Belitz, 1973) and Pacific whiting muscle (Nagahisa et al., 1983; Porter et al., 1990).

There is a need for an inhibitor source that is effective, readily available, economical, and acceptable as a food ingredient. This study was conducted to compare the relative efficacy of a potato extract and potato powder with egg white and bovine plasma protein as inhibitors of crude protease preparations from Pacific whiting and arrowtooth flounder. These potential inhibitors were also tested in kamaboko prepared from both species. Kamaboko is a product made by mixing surimi with salt and extruding the resulting paste into a mold such as a casing and heating until gelation occurs. The integrity of the gel is severely impaired by the activity of protease enzymes during the heating step. Therefore, we compared the gel characteristics of cooked kamaboko prepared from surimi containing each of these inhibitors to determine the extent to which inhibition observed in the enzyme studies is reflected in improved gel properties of the kamaboko.

Materials and Methods

Preparation of Enzyme Extracts

Pacific whiting was obtained from Newport, Oreg., and arrowtooth flounder from the Goose Island area off the west coast of Canada. The fish were filleted, plate frozen, vacuum packed, and held at –29°C until used.

Samples of frozen fillets were thawed at room temperature. Then 300 g portions were diced and homogenized with 900 ml of extracting solution (1% NaCl and 0.2% NaN_3) in a Waring[1] blender. The homogenates were held overnight at 2°C, centrifuged at 10,400 *g* for 20 minutes, and the supernatant filtered through glass wool to remove fat. The supernatants were used as crude enzyme preparations.

Assay of Proteolytic Activity

Proteolytic activity was determined using azocasein as substrate (Barrett and Kirschke, 1981). The reaction mixture consisted of 2.0 ml of assay buffer, 0.2 ml of 6% azocasein (Sigma Chem. Co.), an aliquot of crude enzyme extract, and water to bring the total volume to 3.0 ml. All other reagents were combined prior to addition of substrate. Immediately upon substrate addition, the tubes were mixed on a Vortex mixer and placed in a 50°C water bath for 30 minutes. The reaction was terminated by adding 7.0 ml of ice-cold 5% trichloroacetic acid (TCA). After 30 minutes, the samples were filtered through Whatman #1 filter paper and A_{366} determined against a blank. Blanks were prepared by adding TCA prior to the enzyme.

The pH dependence of proteolytic activity was investigated over the range of pH 2.6 to pH 8.0 using two assay buffer systems. A 0.05M citrate-phosphate system was used from pH 2.6 to pH 5.8, while the pH 6.8 to pH 8.0 range was assayed using 0.05M sodium phosphate buffers. Incubation was for 30 minutes at 50° C.

The temperature-dependent activity was determined using a 0.05M citrate-phosphate buffer at pH 5.5. The assay buffer, water, and enzyme were combined and preincubated at the chosen temperature for 10 minutes prior to the addition of substrate, then incubated for an additional 30 minutes.

Inhibitors

Fresh Norgold potatoes were obtained from central Washington and placed in a 4°C cooler. They were either extracted within 2 days or chilled in ice-water, diced into 1/2-inch cubes, frozen and held at –29°C until needed.

The potato extract was prepared according to the method described by Melville and Ryan (1972) with modifications as described by Porter et al. (1990). Potato powder was prepared by freezing diced, fresh-peeled potatoes, then grinding the frozen dices through a meat grinder (3/16-inch die). The frozen ground potatoes were lyophilized and ball-milled to <100 mesh (U.S. screen) powder.

Dried egg white was obtained from ENER-G Foods, Inc., Seattle, Wash. Bovine plasma protein (AMP-600) was obtained from American Meat Protein Corp., Ames, Iowa.

Inhibition Studies

In the first experiment, water suspensions were made of potato extract (0.1%), egg white (3.0%), and bovine plasma protein (1.0%) and allowed to stand overnight at 2°C. The suspensions were centrifuged at 12100 *g* for 20 minutes and the clear supernatants used as inhibitors.

Assay mixtures consisted of 2.0 ml of 0.05M citrate-phosphate buffer (pH 5.5), a suitable aliquot of crude enzyme, inhibitor solution, and water to bring the volume to 2.8 ml. The samples were preincubated for 10 minutes at 50°C, then 0.2 ml 6% azocasein substrate was added. The samples were incubated for an additional 15 minutes and the reaction was stopped by the addition of 7.0 ml of 5% TCA. Controls were treated in the same manner with water replacing the inhibitor solution. The results were presented as a percentage of the activity of the controls (100%=no inhibitor).

A separate experiment was conducted using water suspensions of three concentrations of dried potato powder (2%, 4%, and 6%) along with potato extract (0.1%). These inhibitor solutions were prepared as described above and the assays conducted in the same manner.

Preparation of Surimi

Pacific whiting were caught off the west coast of the U.S.-Canadian border in early evening by mid-water trawl. They were immediately placed in champagne ice and transported to shore within 2-4 hours. Upon landing, the fish were transferred into iced bins and held overnight. The following morning they were filleted by machine (skin-on), packed in plastic bags, immersed in ice

[1]Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

and flown to Seattle. The fillets were transported to the NMFS pilot plant and processed into surimi starting at 24-30 hours after catching. The surimi was prepared as described by Scott et al. (1988). Temperatures were maintained at <10°C throughout transport and processing.

Arrowtooth flounder were caught near Kodiak, Alaska, in the evening, delivered to a local processing plant, and stored in ice overnight. The following morning they were hand-filleted, skinned by machine, packed in plastic bags, iced, and transferred to the NMFS pilot plant at Kodiak. The fillets were ground through a meat grinder with a 3/16-inch plate and washed twice with ice water at a 3:1 (water:mince) ratio. Fat was skimmed off the top of the wash tank before each dewatering step. The dewatering was accomplished with a Model 4000 Brown Finisher equipped with a 1 mm screen and a gap of 3 mm between the paddles and screen at a speed of 500 rpm. After washing, the flesh was run through a Bibun Model 16 strainer to remove connective tissue and any remaining bone fragments and then through a Fukoku screw press (Model FKC, 1 mm screen, 2.5 rpm) to remove excess water. The presscake was blended with cryoprotectants (4% sugar, 4% sorbitol, 0.3% sodium tripolyphosphate) in a ribbon blender, placed in plastic bags, and frozen at –40°C. The surimi was shipped to Seattle, vacuum packaged, and stored at –29°C until analyzed.

Evaluation of Kamaboko

Frozen surimi in vacuum-packed cartons was tempered in running tap water to between –5°C and –3°C. The tempered surimi was sliced into 1/2-inch slices and placed in a Stephan vertical cutter mixer (VCM-12-3/2 Model 12486) with an ice-water jacket. Inhibitor concentrations used were: Potato extract (0.06%, 0.1%, 0.2%, 0.3%); potato powder (3.0%, 4.0%, 5.0%); egg white (3.0%, 4.0%, 5.0%); bovine plasma (1.0%, 1.5%, 2.0%). To standardize the surimi concentration in all samples, the difference between the inhibitor concentration in a given test and 5.0% (i.e., the highest level used), was made up by addition of sucrose. Sucrose was chosen because it does not contribute to gel properties. In addition, 5% ice was added to each sample to make up for the added solids and 3% salt was added to solubilize the surimi. The same lot of surimi was used within each experiment for both species.

The surimi plus all additives were mixed under vacuum (<20 bar) and chopped until the temperature reached 9.8°C (6-8 minutes). The paste was stuffed into 3 cm diameter polyvinylidene chloride casings to make kamaboko sausages and heated for 40 minutes at 90°C in a water bath. The sausages were cooled in ice water for 10 minutes then reimmersed in the 90°C bath for 5 seconds to remove any wrinkles in the casings.

The kamaboko sausages were stored at ambient temperature overnight and evaluated the following day. Gel-strength was determined as described by Scott et al. (1988), except that a 5mm round probe was used in the present study. Each final value represents a mean of 20 determinations.

Results and Discussion

Enzyme preparations from both Pacific whiting and arrowtooth flounder showed similar activity against azocasein substrate with respect to temperature dependence (Fig. 1). Both exhibited measurable activity at 20°C which rose rapidly to an optimum of 50°C for Pacific whiting and 55°C for arrowtooth flounder. Activity was completely eliminated at 65°C for whiting and 70°C for the flounder.

Results of this study differ slightly from those of Greene and Babbitt (1990). They observed no activity below 40°C when measuring autolysis of arrowtooth flounder muscle, whereas in the current study, activity was found at temperatures as low as 20°C. Although activity was not measured at temperatures below 20°C, it is conceivable that the use of longer assay times may have demonstrated activity at lower temperatures.

Pacific whiting appeared to have a slightly lower pH optimum at pH 5.0-5.2 compared to pH 5.5 for arrowtooth flounder (Fig. 2). This observation is in agreement with the optimum pH observed for the autolysis of arrowtooth flounder muscle (Green and Babbitt, 1990). However, Wasson et al. (1992a) recently described a partially purified enzyme preparation from arrowtooth flounder that exhibited optimum activity at pH 3.0 using acid-denatured hemoglobin as substrate and pH 6.0-7.0 using casein. Using an enzyme prepared from Pacific whiting sarcoplasmic fluid, Erickson et al. (1983) observed optimum activity at pH 7.6 and 55°C using casein as a substrate. Differing methods of enzyme preparation and the use of different substrates for assaying activity are the most probable explanation for these variations and make a direct comparison of the results difficult.

During inhibition experiments using an enzyme extract from Pacific whit-

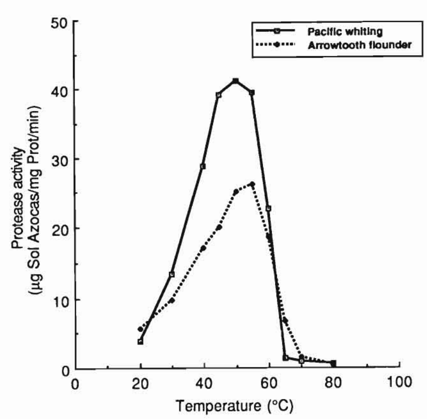

Figure 1.—Effect of temperature on protease activity of Pacific whiting and arrowtooth flounder.

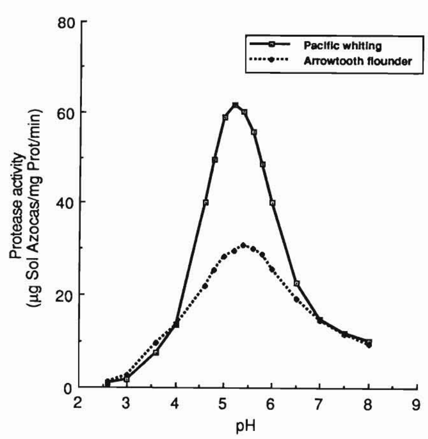

Figure 2.—Effect of pH on protease activity of Pacific whiting and arrowtooth flounder.

ing, the potato extract (0.1%) was a more effective inhibitor than either egg white (3.0%) or bovine plasma (1.0%) regardless of the amount of inhibitor solution added (Fig. 3). However, all were effective against this enzyme preparation, and, as expected, inhibition increased with increasing inhibitor concentration. For the enzyme preparation from Pacific whiting, the inhibition from potato extract leveled off at about 90%, whereas the addition of the same volume of egg white achieved about 70-75% inhibition and the bovine plasma around 80%. This clearly demonstrates that the inhibitors in potato are very effective as inhibitors of endoprotease activity in Pacific whiting extracts using azocasein as substrate.

Inhibition studies with enzyme extract from arrowtooth flounder showed potato extract (0.1%) and egg white (3.0%) leveled off at about 85% inhibition and bovine plasma (1.0%) reached ≈90% inhibition at the highest level of addition (Fig. 4). In these experiments the 400µl level of addition of potato extract solution (0.1%) was slightly less effective than bovine plasma (1.0%) at the same level. Nonetheless, it is still a very potent inhibitor of arrowtooth flounder enzyme extract. Further, these data confirmed the efficacy of both egg white and bovine plasma as inhibitors against the endoprotease activity of these enzyme preparations.

Concentrated potato extract solution (0.1%) was more effective in inhibiting enzyme activity in both species than solutions made from whole potato powder (Fig. 5, 6). As observed with egg white and bovine plasma, inhibition increased with increasing concentrations. These data illustrate that inhibitor activity is retained in dehydrated whole potato.

The dried forms of the various inhibitors were mixed with surimi to determine whether their addition provided sufficient inhibition in the surimi matrix to prevent breakdown of the kamaboko during heating. The gel characteristics of kamaboko made from Pacific whiting surimi with added dried inhibitors are presented in Table 1. As seen by the control data , the presence of an inhibitor was necessary to retain any gel-forming properties. The concentrated potato extract was effective at very low concentrations, peaking at 0.2%. Increasing the concentration from 0.2% to 0.3% did not achieve additional gel strength. Potato powder showed effectiveness at all levels added (3%, 4%, 5%). However, although the gel strength increased slightly at the higher concentrations, the deformation decreased slightly indicating that the kamaboko may simply become firmer and less elastic. This suggests that adding quantities above that adequate for enzyme inhibition is not necessary.

Egg white proved to be effective in stabilizing the gel-forming characteristics of surimi at the 3-5% level. This confirms the work of several other investigators (Haga et al., 1980; Nagahisa et al., 1983; Groninger et al., 1985; Chang-Lee et al., 1989) who have shown egg white to be an effective inhibitor in Pacific whiting surimi. Bovine plasma was also effective in the 1-2% range. Higher concentrations of bovine plasma were not used because of unfavorable contributions to flavor and color.

The same inhibitor preparations were added to arrowtooth flounder surimi to determine their effect on kamaboko gels formed during heating (Table 2). The results showed that the inhibition provided by each of the inhibitors was effective in preserving the gelation properties of kamaboko. The control sample containing no inhibitor had essentially no gel-forming properties. Again, as observed in the Pacific whiting experi-

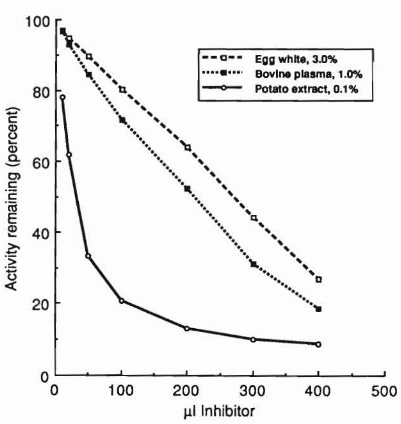

Figure 3.—Inhibition of Pacific whiting muscle enzyme by potato extract, egg white, and bovine plasma

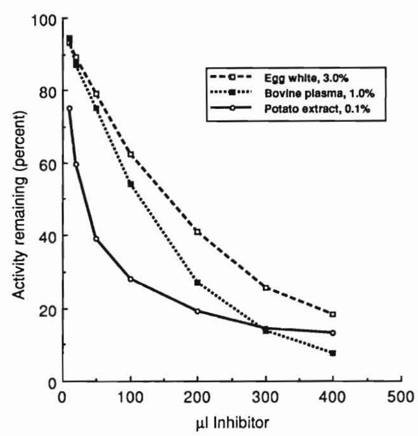

Figure 4.—Inhibition of arrowtooth flounder muscle enzyme by potato extract, egg white, and bovine plasma.

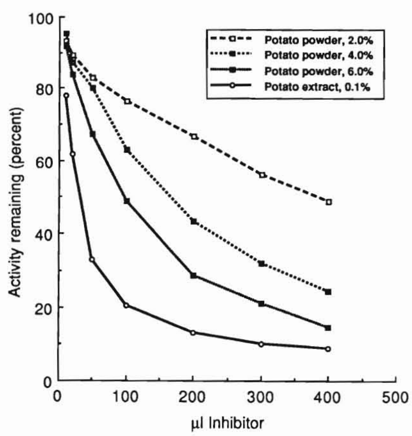

Figure 5.—Inhibition of Pacific whiting muscle enzyme by potato extract and potato powder.

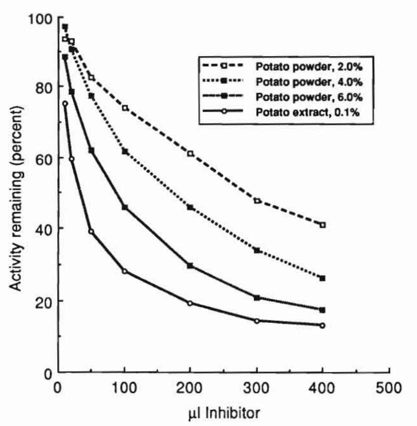

Figure 6.—Inhibition of arrowtooth flounder muscle enzyme by potato extract and potato powder.

Table 1.—Effect of various dried inhibitors upon the gel characteristics of kamaboko made from Pacific whiting surimi.

		Gel strength (g x cm)		Load (g)		Deformation (cm)	
Inhibitor	Percent	Avg.	(S.E.)	Avg.	(S.E.)	Avg.	(S.E.)
Control		66	(3)	136	(40)	0.48	(0.01)
Potato extract	0.06	525	(24)	474	(12)	1.10	(0.02)
	0.10	531	(22)	463	(10)	1.14	(0.03)
	0.20	602	(16)	532	(8)	1.13	(0.02)
	0.30	571	(24)	517	(9)	1.10	(0.03)
Potato powder	3.0	511	(59)	487	(35)	1.05	(0.05)
	4.0	559	(79)	546	(48)	1.05	(0.06)
	5.0	589	(95)	599	(59)	0.98	(0.06)
Egg white	3.0	538	(117)	503	(64)	1.06	(0.09)
	4.0	566	(92)	547	(56)	1.03	(0.07)
	5.0	674	(107)	637	(64)	1.05	(0.07)
Bovine plasma	1.0	419	(61)	384	(34)	1.09	(0.07)
	1.5	502	(93)	445	(52)	1.12	(0.08)
	2.0	542	(98)	485	(53)	1.11	(0.07)

Table 2.—Effect of various dried inhibitors upon the gel characteristics of kamaboko made from arrowtooth flounder surimi.

		Gel strength (g x cm)		Load (g)		Deformation (cm)	
Inhibitor	Percent	Avg.	(S.E.)	Avg.	(S.E.)	Avg.	(S.E.)
Control		38	(1)	90	(2)	0.41	(0.01)
Potato extract	0.06	231	(10)	241	(6)	0.95	(0.02)
	0.10	266	(12)	275	(7)	0.96	(0.02)
	0.20	250	(14)	259	(8)	0.95	(0.02)
	0.30	290	(8)	302	(5)	0.96	(0.01)
Potato powder	3.0	295	(11)	294	(7)	1.00	(0.06)
	4.0	329	(12)	354	(7)	1.02	(0.08)
	5.0	370	(15)	401	(10)	0.91	(0.07)
Egg white	3.0	229	(9)	275	(7)	0.83	(0.08)
	4.0	276	(14)	307	(10)	0.89	(0.09)
	5.0	336	(13)	369	(9)	0.91	(0.06)
Bovine plasma	1.0	212	(21)	215	(6)	0.98	(0.02)
	1.5	237	(19)	255	(5)	0.93	(0.02)
	2.0	331	(17)	329	(10)	0.99	(0.02)

ment, the concentrated potato extract was effective at very low concentrations in arrowtooth flounder whereas the potato powder was required at somewhat higher concentrations to be equally effective (3-5%). Egg white (3-5%) and bovine plasma (1-2%) were equally effective at the same concentrations used in Pacific whiting surimi, confirming the observations of Wasson et al. (1992b).

It should be noted that the absolute gel strength values for arrowtooth flounder were lower than those observed for the Pacific whiting. This may be due in large part to the fact that the flounder was washed only twice whereas the whiting mince received three washes during surimi preparation. Okada (1964) has shown that increasing the number of washes of arrowtooth flounder flesh had a positive effect on the gel-formation of cooked kamaboko.

By specifically inhibiting the protease activity, concentrated potato extract allows investigators to study the intrinsic gel-forming characteristics of Pacific whiting and arrowtooth flounder surimi. The contributions of significant quantities of additional ingredients that may affect gel-strength, e.g., starch (potato powder) and heat-coagulable protein (egg albumin and bovine plasma), are eliminated.

In conclusion, this study demonstrates that the inhibitors present in potato are potent inhibitors of the protease enzyme(s) extracted from both Pacific whiting and arrowtooth flounder muscle. This study confirms that egg white and bovine plasma protein also inhibit the protease enzyme(s) extracted from both species. Cooked kamaboko made from surimi without inhibitors had essentially no gel-strength (Pacific whiting, 66; arrowtooth flounder, 38). Surimi of both species required the addition of an inhibitor to preserve gelation properties during heating. Addition of either potato extract or potato powder preserved the gel forming properties of the muscle proteins. The effect of potato-derived inhibitors is comparable to that obtained by the addition of egg white and bovine plasma. This illustrates the efficacy of potato inhibitor either as an extract or in the form of potato powder as an alternative to egg white and bovine plasma for use in Pacific whiting and arrowtooth flounder surimi.

Literature Cited

Barrett, A. J. and H. Kirschke. 1981. Methods in enzymology, vol. 80. Proteolytic enzymes, pt. C. *In* L. Lorand (Editor), Acad. Press., N.Y. p. 542-543.

Chang-Lee, M. V., L. E. Lampila, and D. L. Crawford. 1990. Yield and composition of surimi from Pacific whiting (*Merluccius productus*) and the effect of various protein additives on gel strength. J. Food Sci. 55(1):83-86.

________, R. Pacheco-Aguilar, D. L. Crawford, and L. E. Lampila. 1989. Proteolytic activity of surimi from Pacific whiting (*Merluccius productus*) and heat-set gel texture. J. Food Sci. 54(5):1116-1124.

Dassow, J. A., M. Patashnik, and B. J. Koury. 1970. Characteristics of Pacific hake, *Merluccius productus*, that affect its suitability for food. U.S. Dep. Inter., Fish Wildl. Serv., Circ. 332, p. 127-136.

Ericksen, M. C., D. T. Gordon, and A. F. Anglemeier. 1983. Proteolytic activity in the sarcoplasmic fluids of parasitized Pacific whiting (*Merluccius productus*) and unparasitized true cod (*Gadus macrocephalus*). J. Food. Sci. 48(4):1315-1319.

Greene, D. H., and J. K. Babbitt. 1990. Control of muscle softening and protease-parasite interaction in arrowtooth flounder, *Atheresthes stomias*. J. Food Sci. 55(2):579-580.

Groninger, H., G. Kudo, R. Porter, and R. Miller. 1985. Preparation and evaluation of surimi from Pacific whiting (*Merluccius productus*). *In* R. L. Martin and R. L. Collette (Editors), Proceedings of the International Symposium on Engineered Seafood Including Surimi, p.199-210. Seattle, Wash.

Haga, J., Shigeoka, and T. Yamauchi. 1980. Method for processing fish contaminated with sporozoa. U.S.Patent Off., Wash., D.C., Patent No. 4,207,354.

Kabata, Z., and D. J. Whitaker. 1981. Two species of *Kudoa* (Myxosporea: Multivalda) parasite in the flesh of *Merluccius productus* (Ayres, 1855) (Pices: Teleostei) in the Canadian Pacific. Can. J. Zool. 59:2085-2091.

________ and ________. 1986. Distribution of two species of *Kudoa* (Myxozoa: Multivalvulida) in the offshore population of the Pacific hake, *Merluccius productus* (Ayres, 1855). Can. J. Zool. 64:2103-2110.

Kaiser, K.-P., and H.-D. Belitz. 1973. Specificity of potato isoinhibitors towards various proteolytic enzymes. Z. Lebensm. Unters.-Forsch. 151(1):18-22.

Konagaya, S. 1984. Studies on the jellied meat with special reference to that of yellowfin tuna. Chapter IV. Jellied meat in other species of fish. Section 2. Pacific hake. Bull. Tokai Reg. Fish. Res. Lab. 114 (Nov.):1-110.

Kudo, G., H. J. Barnett, and R. W. Nelson. 1987. Factors affecting cooked texture quality of

Pacific whiting, *Merluccius productus*, fillets with particular emphasis on the effects of infection by the myxosporeans *Kudoa paniformis* and *Kudoa thyrsitis*. Fish. Bull. 85(4):745-756.

Laskowski, M., Jr., and I. Kato. 1980. Protein inhibitors of proteinases. Ann. Rev. Biochem. 49:593-626.

Melville, J. C., and C. A. Ryan. 1972. Chymotrypsin inhibitor I from potato. J. Biol. Chem. 247(11):3445-3453.

Miller, R., and J. Spinelli. 1982. The effect of protease inhibitors on proteolysis in parasitized Pacific whiting, *Merluccius productus*, muscle. Fish. Bull. 80(2):281-286.

Nagahisa, E., S. Nishimuro, and T. Fujita. 1983. Kamaboko-forming ability of the jellied meat of Pacific hake. Bull. Jpn. Soc. Sci. Fish. 49(6):901-906.

Okada, M. 1964. Effect of washing on the jelly forming ability of fish meat. Bull. Jpn. Soc. Sci. Fish. 30(3):255-261.

Patashnik, M., H. S. Groninger, Jr., H. Barnett, G. Kudo, and B. Koury. 1982. Pacific whiting *Merluccius productus*: 1. Abnormal muscle texture caused by myxosporidian-induced proteolysis. Mar. Fish. Rev. 44(5):1-12.

Porter, R. W., B. J. Koury, and G. Kudo. 1990. Method for treating fish meat contaminated with sporozoa and potato product for improving heat gelation of fish muscle. U.S. Patent Off., Wash., D.C., Patent No. 4,935,192.

Scott, D. N., R. W. Porter, G. Kudo, R. Miller, and B. Koury. 1988. Effect of freezing and frozen storage of Alaska pollock on the chemical and gel forming properties of surimi. J. Food Sci. 53(2):353-358.

Tsuyuki, H., S. N. Williscroft, Z. Kabata, and D. J. Whitaker. 1982. The relationship between acid and neutral protease activities and the incidence of soft cooked texture in the muscle tissue of pacific hake, *Merluccius productus*, infected with *Kudoa paniformis* and/or *K. thyrsitis*, and held for various times under different pre-freeze chilled storage conditions. Fish. Aquat. Sci. Can. Tech. Rep. 1130.

Wasson, D. H., J. K. Babbitt, and J. S. French. 1992a. Characterization of a heat stable protease from arrowtooth flounder, *Atheresthes stomias*. J. Aquat. Food Prod. Technol. 1(3/4):167-182.

________, K. D. Reppond, J. K. Babbitt, and J. S. French. 1992b. Effects of additives on proteolytic and functional properties of arrowtooth flounder surimi. J. Aquat. Food Prod. Technol. 1(3/4):147-165.