Journal of Food Protection, Vol. 59, No. 3, Pages 312–318
Copyright ©, International Association of Milk, Food and Environmental Sanitarians

Proteinase Inhibition of Fish Muscle Enzymes Using Legume Seed Extracts

F. L. GARCÍA-CARREÑO,* M. A. NAVARRETE DEL TORO, M. DÍAZ-LÓPEZ,† M. P. HERNÁNDEZ-CORTÉS, and J. M. EZQUERRA

Centro de Investigaciones Biológicas del Noroeste (CIBNOR), P.O. Box 128, La Paz, BCS, México, 23000

(MS# 95-67: Received 27 March 1995/Accepted 15 June 1995)

ABSTRACT

Seed extracts from indigenous and introduced legumes were prepared and used to search for inhibitors of fish muscle proteinases. Fish flesh extracts were prepared from samples of *Merluccius productus* (Pacific whiting or *merluza*) obtained off the Oregon coast and in the Gulf of California, respectively. The proteinase activity in the fish muscle for the Pacific whiting was the highest, followed by parasitized *merluza*. The lowest proteinase activity was for the nonparasitized *merluza*. Six out of 12 seed extracts reduced the proteinase activity from the fish flesh by more than 50%. The reduction of enzyme activity was higher for samples of fish flesh extracts from the Gulf of California than for the Oregon samples. Seed extracts also reduced the proteinase activity of commercial serine and cysteine proteinases such as trypsin, chymotrypsin, and papain. This inhibitory capacity was maintained even after heating the seed extracts to 90°C for 15 min. Several seed extracts show promise for use as proteinase inhibitors during production of surimi, the intended commercial product of massive fisheries such as Pacific whiting or *merluza*.

Key words: Proteinase inhibitor, protease inhibitor, legume seeds, fish muscle enzymes, surimi

---

Protein hydrolysis by enzymes is an expanding field of research in food science and technology. The inhibition of digestive proteinases by proteinaceous inhibitors in feeds reduces the availability of amino acids in the diet. Inhibitors are widely distributed molecules in plant and animal tissues *(11)*. Since the isolation, characterization, and crystallization of a soybean trypsin inhibitor by Kunitz in 1945 *(7)*, proteinase inhibitors in legume seeds have been studied extensively. Today, legume seed meals are one of the most common protein ingredients for food and feed. Consequently, there is increasing interest in seeds which can be produced in arid lands or irrigated with salty water.

Shrimp farming in arid lands is highly dependent on cheap feeds. The high cost of transporting feed ingredients to remote and arid lands such as the Baja California Peninsula has launched several efforts to include nontraditional meals from legume seeds as a main ingredient of shrimp feeds. However, the feed conversion has usually been inadequate to support a shrimp farm, mainly because of the inhibition of digestive enzymes by inhibitors present in the legume seeds. A knowledge about the physicochemical properties of these inhibitors will lead to more appropriate use of these seed meals.

Sometimes proteolysis in food processing is an unwanted process. This is particularly true in fish species which undergo excessive softening due to proteolytic activity in muscle during cooking or surimi preparation *(9)*. An et al. *(2)* reported that proteinolytic activity in fish flesh extracts can be reduced by using proteinase inhibitors such as egg white, beef plasma protein, potato and tomato leaf extracts.

We are currently investigating new sources of protein for aquaculture feeds by analyzing a dozen legumes that either grow wild or were introduced to Baja California. As a branch investigation, we are looking for added value and profit products from legume seeds. In this study, we used the legume seed extracts to determine the presence and some characteristics of proteinase inhibitors which were capable of reducing the proteolytic activity in fish flesh extracts from fish intended for surimi production.

Surimi production is a way to use massive fish stocks which otherwise would be unexploited because of the softening of the flesh. Massive stocks such as those of *Merluccius productus* (*merluza*) are still useless for food. About 250,000 metric tons were recently discovered off the Pacific coast of Baja California *(3)*.

MATERIALS AND METHODS

Reagents including the enzymes porcine trypsin, bovine chymotrypsin, and papain, and substrate azocasein were supplied by Sigma Chemical Co. (St. Louis, MO, USA).

Seed extracts

Seeds were obtained from legume plants that were either endemic or introduced to Baja California Peninsula. In the summer of 1994, seeds were collected from *tabachín* (*Caesalpina pulcher-*

---

* Author for correspondence. Fax: 112-5-4710; Email: fgarcia@cibnor.conacyt.mx.

† Author Díaz-López is a posdoctoral fellow at CIBNOR and is supported by funding from Universidad de Almería, his permanent address.

rima), *palo blanco* (*Lysiloma candida*), *guamuchil* (*Pithecellobium dulce*), *palo verde* (*Cercidium floridum* subsp. *peninsulare*), *guaje* (*Leucaena* spp.), *mezquite* (*Prosopis articulata*), *palo fierro* (*Olneya tesota*), soybean (*Glycine max*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), *gandul* (pigeon peas, *Cajanus cajans*), and garbanzo (chickpea, *Cicer arietinum*). Seeds were stored dry at 4°C for several months.

Seed extracts were obtained as follows: dry seeds were ground in a coffee mill. The powder was extracted with about three volumes of 50 mM Tris-HCl, pH 7.5, by shaking for 120 min at room temperature, then for 22 h at 4°C. The extracts were recovered by using a two-step centrifugation: (i) for 10 min at 2,500 × *g* and (ii) for 30 min at 5,000 × *g*, both at 5°C. The clear extracts were stored at 4°C.

Fish muscle extracts

Pacific whiting enzyme preparations were obtained from juice extracted from fish flesh, provided by Dr M. Morrissey, SeaFood Lab, OSU, Astoria, Oregon, USA. Another Pacific whiting called *merluza* from the Gulf of California was provided by Dr R. Pacheco, CIAD, Hermosillo, Sonora, México. Two samples of *merluza* were analyzed, (i) fish flesh with parasites and (ii) parasite-free fish flesh. The parasites were identified as *Kudoa paniformis*. Flesh juice extracts were prepared and assayed for enzyme activity as reported by An et al *(2)*. The flesh was skinned, chopped, and minced in a chilled mortar. After centrifuging at 5,000 × *g* for 30 min at 5°C, the supernatant (juice) was then stored at 4°C for several months in 2-ml portions. Samples were thawed for assays. No reduction in the enzyme activity was noted during storage for this time period. The water used for all the assays was prepared by reverse osmosis and had a conductivity lower than 10 μmhos.

Proteinase inhibition assay

The general assay for proteinase inhibition was that reported by García-Carreño *(4)*. It was used to address the inhibition ability of seed extracts for the commercial enzymes trypsin, chymotrypsin, and papain. The assay for proteinase activity was as follows: 500 μl of 50 mM Tris-HCl (pH 7.5) buffer and 20 μl of enzyme preparation were mixed. The buffer included 40 mM of $CaCl_2$ when trypsin or chymotrypsin were assayed, or 4 mM L-cysteine and 2 mM EDTA when papain was assayed. For the inhibitory assay, 20 μl of the inhibitor preparation were added and the mixture of buffer, enzyme, and inhibitor was incubated for 60 min at 25°C. Five hundred microliters of 1% azocasein in Tris-HCl was poured into the mixture and incubated for various time periods. The enzyme reaction was stopped by adding 500 μl of 20% TCA. The TCA-soluble peptides were separated by centrifuging at 12,500 × *g* for 5 min and the supernatant absorbance recorded at 366 nm. Blanks were prepared by adding the TCA solution before the substrate. Table 1 shows the scheme for the assay.

Remaining activity of fish extracts. The fish juice was heated for 3 min at 60°C and centrifuged at 12,500 × *g* for 20 s; the precipitate was discarded, and the supernatant was used to determine activity as described by An et al. *(2)*. The enzyme preparation was incubated with an equal volume of seed extract for 60 min at 55°C and centrifuged at 12,500 × *g* for 20 s. The supernatant was used for the measurement of remaining activity. An assay mixture containing 51 ml of 0.2 M phosphates, 0.1 M citrates, and 1 mM of sodium azide buffer, pH 5.5, 32.7 ml of water, and 16.7 ml of 1% azocasein in water was adjusted to pH 5.5 and heated to 55°C. The assay mixture 1,250 μl was incubated with 50 μl of seed enzyme extract preparation for 15 min at 55°C. The reaction was stopped by adding 200 μl of 50% TCA. Then the samples were incubated for 10 min at 4°C, centrifuged at 12,500 × *g* for 5 min, and the absorbancy of the supernatant recorded at 366 nm. The control for 100% of enzyme activity was prepared by using 25 μl of the enzyme preparation. Blanks were prepared by adding 50% TCA before the enzyme or enzyme-inhibitor preparation. The enzyme activity was determined as the increase in absorbance per minute.

Thermostability of proteinase inhibitors. Seed extracts were heated in a water bath at 60 and 90°C for 30 and 15 min, respectively. Then the inhibition assay was done as previously described.

TABLE 1. *General assay for activity and inhibitory effect on proteases*

Sequence: component or procedure	Assay[a] (volumes μl) for: Inhibition	Activity	Enzyme extract control[b]	Inhibitor solvent control[c]
Buffer	500	500	500	500
Enzyme extract	20	20	20	20
Inhibitor solution	10	NA	NA	NA
Inhibitor solvent	NA[d]	NA	NA	10
Incubation	25°C — 60 min			
TCA	NA	NA	500	NA
Substrate + 1% azocasein	500	500	500	500
Incubation	25°C — 60 min			
TCA (20%)	500	500	500	500
Centrifuge	12,500 × *g* — 5 min			
$Absorbance_{366\ nm}$	record			

[a] Three replicate tubes each.
[b] Some crude extracts could absorb at 366 nm because of the presence of carotenoids or other yellow substances.
[c] Some inhibitor solvents could modify the activity of the enzyme.
[d] NA, nothing added.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) characterization of seed inhibitors

The assay was done according to García-Carreño *(5)*. Seed extract samples for SDS-PAGE were prepared by mixing (1:4) with sample buffer containing SDS but no mercaptoethanol. The mixtures were not boiled. Samples were electrophoresed in 12% polyacrylamide gels at 15 mA per plate at 5°C. Gels were stained for (i) protein using 1% Commassie blue R-250 in 40% methanol, 10% acetic acid solution, and (ii) inhibition activity as follows: after electrophoresis, gels were soaked for 30 min at 5°C in an enzyme solution. Then the gels were washed with water, soaked for 90 min in a 2% casein solution containing 50 mM Tris-HCl, pH 7.5, at 25°C, fixed and stained for protein, and dried. The enzyme solutions included 40 mM $CaCl_2$ when 0.5 mg/ml of trypsin or 0.5 mg/ml of chymotrypsin was assayed, or 4 mM L-cysteine and 2 mM EDTA when 0.1 mg/ml of papain was assayed.

All the assays were repeated at least three times and included five replicates. The data were analyzed using the program STATISTICA for PCs (Microsoft Co., Tulsa, OK). Analysis of variance and the Tukey HSD test were used to compare the remaining proteinase activity from the Pacific Whiting and *merluza* flesh juices and commercial enzymes. The effect of temperature on seed inhibitors was also analyzed by these methods. For SDS-PAGE assays, the typical data are presented.

RESULTS

The *merluza* flesh with parasites rendered less juice than the nonparasitized flesh. The pH of the flesh juice was 6.9,

TABLE 2. *Protein activity, protein content, and pH in fish flesh juices from Pacific whiting and* merluza

Flesh juice source	Activity[a]	Protein content[b] (mg/ml)	pH	Specific activity (activity/[protein])
Pacific whiting	0.937A[c]	13.7 (31)[d]	6.55	2.736
Merluza				
parasitized	0.453B	25.6	6.6	0.708
unparasitized	0.321C	24.4	6.9	0.526

[a] Activity was defined as the change in absorbance.
[b] Protein content of the supernatant after heating at 60°C for 3 min and centrifuging.
[c] Values in the a column with different letters are significantly different ($P < 0.05$). The standard deviations were between 0.001 and 0.05.
[d] Parentheses: the protein content of the fish juice before heating.

6.6, and 6.55 for the nonparasitized merluza, parasitized merluza, and Pacific whiting, respectively.

The three flesh juices had azocaseinolytic activity. This activity increased about 8% when the juice was heated for 3 min at 60°C before the proteinase assay. Fish flesh juices showed different proteinase activities when assayed for azocasein. Table 2 shows the proteinase activity in the flesh juices from Pacific whiting and *merluza.* The activity of the juice from Oregon Pacific whiting was the highest. The proteinase activity of parasitized *merluza* flesh juice was higher than the nonparasitized *merluza* flesh juice. During heating of Pacific whiting juice, a protein precipitate was rendered. After centrifuging, the protein content in the supernatant was reduced by half, increasing the specific activity in the enzyme preparation.

A linear period for time versus proteinase activity was assessed in order to find a correlation coefficient higher than 0.9. Trypsin was assayed for activity, taking samples from 3 to 15 min; chymotrypsin from 20 to 60 min; papain from 5 to 30 min; and Pacific whiting juice from 3 to 15 min. The reduction of the enzyme preparation activity by the inhibitors in the seed extracts was assessed by using the period in which the azocaseinolytic reaction was linear.

Seed extracts showed inhibitory activity for trypsin and chymotrypsin (serine proteinase) and papain (cysteine proteinase). Table 3 shows the percentage of remaining activity of the challenged enzymes after incubation for 60 min with the corresponding seed extract. The table also shows the activity of the seed extracts after storage for 30 days at 4°C. Four of the twelve seed extracts reduced the trypsin activity by 95%, i.e., the remaining activity of the enzyme was about 5%, while sorghum, wheat, *guaje* and *palo fierro* reduced the trypsin activity as little as 10%. The chymotrypsin activity

TABLE 3. *Remaining activity of commercial enzymes after incubation with seed extracts*

	Remaining activity (%) on day[a]:					
	Trypsin		Chymotrypsin		Papain	
Seed extract	0	30	0	30	0	30
Sorghum	98		96		50	
Guamuchil	2		30		18	
Wheat	96		95		46	
Mezquite	35		75		72	
Tabachin	34		52		104	
Guaje	82		87		51	
Palo blanco	0	2	8	7	60	59
Palo fierro	89	89	63	61	61	52
Soybean	2	2	20	31	33	59
Gandul	3	2	68	71	44	64
Palo verde	96	95	91	93	67	65
Garbanzo	27	28	61	52	47	36

[a] Seed extracts were obtained at day zero and stored at 4°C for one month. Those extracts with inhibition ability higher than 50% were evaluated after 30 days of storage at 4°C. The standard deviations were between 1.5 and 2.0.

TABLE 4. *Remaining proteinase activity in fish flesh juices after incubation with seed extracts*

	Remaining proteinase activity[a] (%) on day:				
	Pacific whiting			Merluza	
Seed extract	(0)	(30)	Heated (30)	Parasitized (30)	Unparasitized (30)
Sorghum	68				
Guamuchil	85				
Wheat	49				
Mezquite	57				
Tabachin	86				
Guaje	64				
Palo blanco	32	27	26	0	0
Palo fierro	24	26	20	13	11
Soybean	28	32	25	32	0
Gandul	43	46	42	30	10
Palo verde	45	46	35	10	0
Garbanzo	50	24	25	13	6

[a] The standard deviations were between 1.27 and 3.15.

TABLE 5. *The effect of temperature on inhibitors from seed extracts*

	Remaining enzyme activity (%)[a] after incubation[b] at (°C):											
	Trypsin			Chymotrypsin			Papain			Pacific whiting		
Seed extract[c]	25	60	90	25	60	90	25	60	90	25	60	90
PF	66	69	71	59	56	64	96	90	97	16	18	30
PB	7	2	91	11	11	93	96	103	97	42	18	31
SB	1	2	7	20	23	34	88	90	94	32	34	31
GA	2	2	2	73	75	80	101	99	102	47	50	53
PV	85	88	90	88	87	87	100	100	99	38	38	28
GB	21	29	85	14	68	91	96	103	103	56	56	43

[a] The standard deviations were between 1.07 and 2.42.
[b] The seed extracts were incubated 30 min at 25 and 60°C, and 15 min at 90°C.
[c] Seed extracts: PF, *palo fierro,* 17 mg/ml; PB, *palo blanco,* 28.6 mg/ml; SB, soybean, 40 mg/ml; GA, *gandul,* 17 mg/ml; PV, *palo verde,* 5.8 mg/ml; GB, garbanzo, 18 mg/ml.

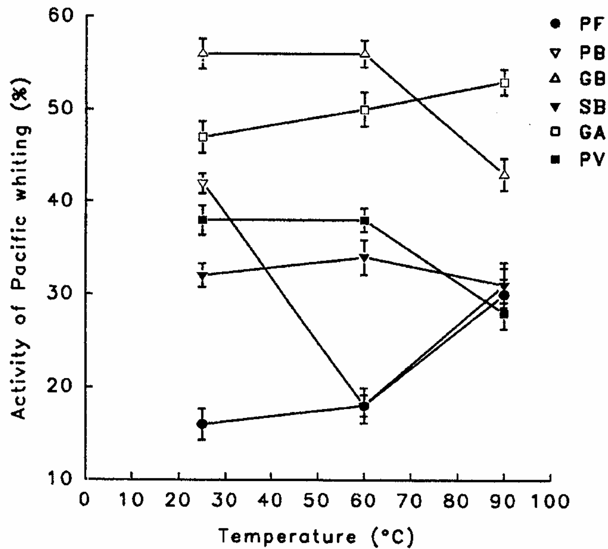

FIGURE 1. *The effect of temperature on seed inhibitors. Seed inhibitors were treated for 30, 30, and 15 min at 25, 60, and 90°C respectively and then assayed for evaluation of remaining activity of Pacific whiting protease. Abbreviations: PF,* palo fierro; *PB,* palo blanco; *SB, soybean; GA,* gandul; *PV,* palo verde; *GB, garbanzo.*

remaining ranged from 8% after treatment with *palo blanco* extract to 96% after sorghum extract treatment. Papain was better inhibited by *guamuchil* extract, which reduced the activity to 18%. The most striking result is that all the legume seed extracts inhibited the serine and cysteine proteinase activities to some extent. Sorghum and wheat extracts, which were included as nonlegume controls, were the poorest inhibitors of the enzymes tested.

The enzymes responsible for softening the fish muscle were identified as cathepsins, mainly cathepsin L *(8)*. This is a cysteine proteinase closely homologous to papain and one of the most active lysosomal endopeptidases. It also plays an important role in extensive protein turnover *(1)*. Several seed extracts reduced the proteinase activity of *merluza* and Pacific whiting juices. Table 4 shows the remaining activity of fish flesh juices after incubation with the seed extracts tested. Six out of twelve seed extracts reduced the Pacific whiting proteinase activity at least 50%, those being from legume plants. These six samples were selected for further investigation and to be challenged for *merluza* extracts. Table 4 also shows the inhibition of the six selected extracts after 30 days of storage at 4°C. In general, the enzyme activity from nonparasitized *merluza* was better inhibited than that from the parasitized *merluza* and the Pacific whiting. The Pacific whiting enzymes were better inhibited by the seed extracts after heating.

Table 5 shows the effect of temperature on the capacity for enzyme inhibition of six seed inhibitors. Although the six seed extracts reduced the azocaseinolytic activity in Pacific whiting at least 50%, they did not reduce the papain activity to the same extent. In fact, the inhibitory activity for papain was the poorest. Figure 1 shows the ability of seed extracts to reduce the fish enzyme activity after treatment at different temperatures. Three of the six seed inhibitors were positively affected. Garbanzo, *palo blanco,* and soybean inhibi-

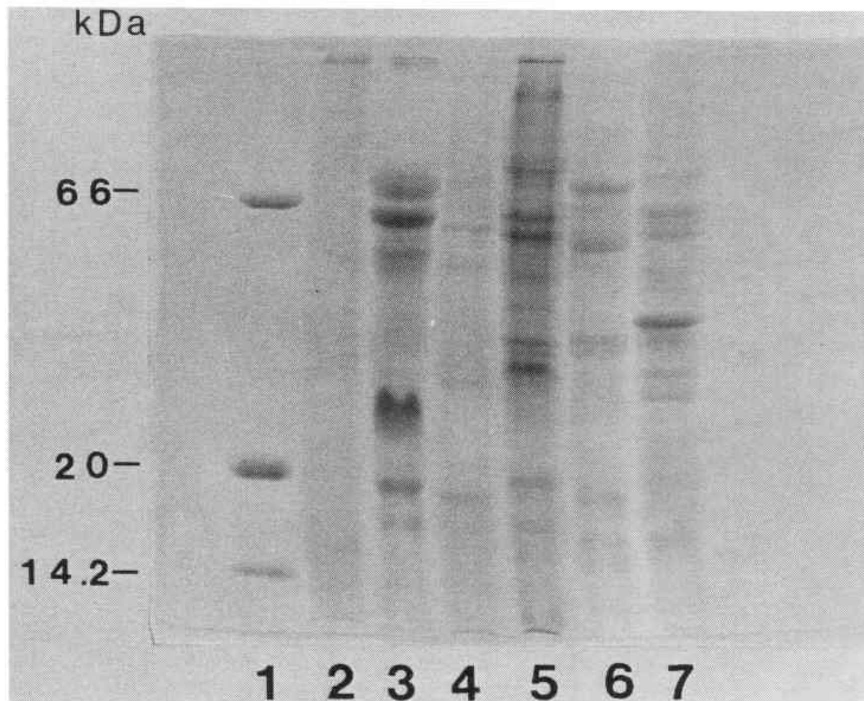

FIGURE 2. *SDS-PAGE of seed extracts for protein composition. Lanes: 1, Molecular weight markers (15 μl); 2,* palo fiero *(15 μl); 3,* palo blanco *(10 μl); 4,* palo verde *(15 μl); 5, soybean (10 μl); 6,* gandul *(10 μl); and 7, garbanzo (10 μl). Sample volumes were adjusted for protein content.*

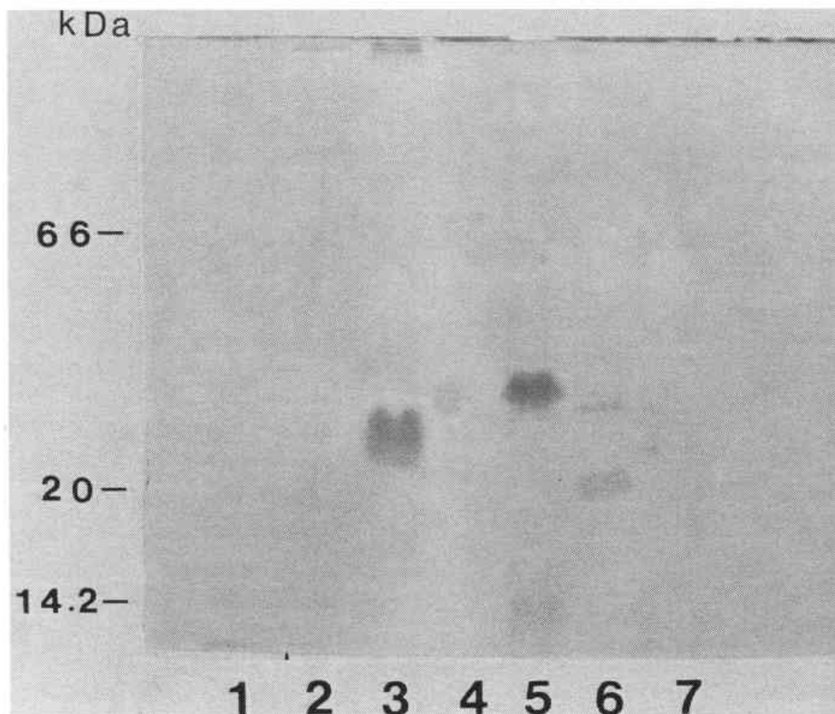

FIGURE 3. *SDS-PAGE of seed extracts for inhibition of trypsin. Lanes as in Figure 2.*

tors were activated by heating, which rendered higher inhibition for the Pacific whiting proteinase activity. The other three seed extracts were affected negatively by the same treatment; however, the remaining activity of the fish enzyme was always lower than 50%.

SDS-PAGE of the seed inhibitors provided an easy technique for assessing the molecular mass and composition of the proteins responsible for the inhibition of serine and cysteine proteinases. Figure 2 shows the protein composition of the seed extracts. Figures 3 to 5 show the bands

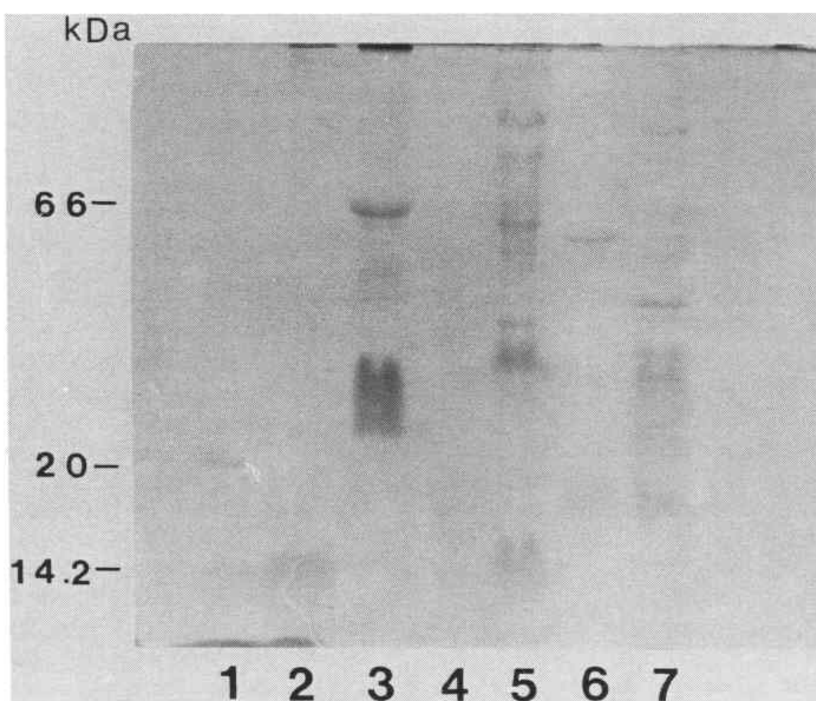

FIGURE 4. *SDS-PAGE of seed extracts for inhibition of chymotrypsin. Lanes as in Figure 2.*

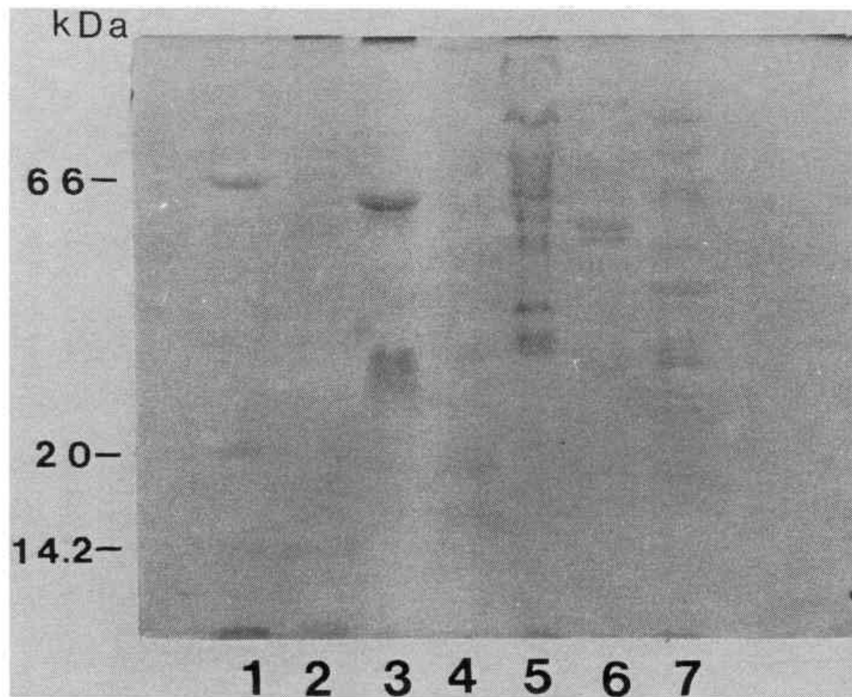

FIGURE 5. *SDS-PAGE of seed extracts for inhibition of papain. Lanes as in Figure 3.*

which had inhibitory activity for trypsin, chymotrypsin and papain. The molecular weight of the bands involved in the inhibition of trypsin, chymotrypsin and papain were from 14,200 to 66,000, some of them like the soybean trypsin inhibitor. The one-step technique we used to evaluate the molecular weight allowed us determine these molecular characteristics of the inhibitors. Figures 1 to 5 show (i) the composition of seed extracts and (ii) the protein bands which were resistant to the hydrolysis of trypsin, chymotrypsin, and papain. A pair of bands with molecular weight of roughly 20,000 were resistant to the hydrolysis of the three commercial enzymes. These bands seem to share the inhibition capability for enzymes belonging to either the serine or cysteine classes.

DISCUSSION

The results herein show significant differences among the proteinase activity from the fish flesh juices. The highest activity was for the Pacific whiting flesh, then the parasitized *merluza* flesh, and the lowest for the nonparasitized *merluza* flesh.

Legume seed extracts showed inhibition of the azocaseinolytic activity of trypsin, chymotrypsin, papain, Pacific whiting, and *merluza.* The fish proteinase responsible for muscle softness has been identified with cathepsin L activity, which is a cysteine proteinase. Seed extracts showing inhibition to serine (trypsin and chymotrypsin) as well as cysteine proteinase (papain) reduced the muscle proteinase activity. In general, *merluza* was better inhibited by the legume inhibitors. *Palo blanco* reduced almost all the azocaseinolytic activity of *merluza,* regardless of whether the sample had parasites. However, most of the seed extracts reduced the enzyme activity in the nonparasitized *merluza* to a greater extent. Konagaya and Aoki *(6)* have found a correlation between the degree of infection and proteolytic activity. The reason for the better inhibition by seed extracts could be the amount of the enzymes; however, alternative hypotheses should be addressed, such as differences in the inhibition kinetics, i.e., the inhibition constant: K_i. In any case, the effect of reducing the proteolytic activity in fish flesh by seed inhibitors needs to be better related with the viscoelastic properties of the soles and the gel strength in surimi production.

The inhibitory capacity of the twelve seed extracts for the serine and cysteine proteinases trypsin, chymotrypsin, and papain was done to determine the class of the proteinase inhibited in the fish flesh. However, this approach did not provide much information because the seed inhibitors seemed to be nonspecific for a proteinase class. To solve the contradictory results, two working hypotheses are being evaluated: (i) different molecules may bear specific inhibition for serine or cysteine enzymes, and (ii) one molecule may be responsible for inhibition of both proteinase classes. The SDS-PAGE indicates one to two bands involved in the enzyme inhibition. However, more molecules with similar molecular weights could be involved.

The six seed inhibitors selected for further studies were thermostable and reduced fish enzyme activity by more than 50% even when treated at 90°C for 15 min, which is the typical temperature during gel formation in surimi production. Several seed extracts show promise for use as a proteinase inhibitor during surimi production, provided they can be characterized for (i) molecular and physicochemical properties such as molecular weight and thermostability, (ii) inhibition of muscle enzymes when processing for surimi

production, and (iii) differentiation between inhibition and pseudosubstrate activity such as has been reported for BSA *(10)*.

ACKNOWLEDGMENTS

The authors thank MC José Luis León de la Luz for identifying the legume plants and seeds, Drs. M. Morrissey, OSU, R. Pacheco, CIAD for kindly supplying the fish samples, Dr. Roy Bowers for English review and B. Francisco Magallón for launching this study. The study was supported in part by Grant 3589-N from CONACyT, México, given to author García-Carreño.

REFERENCES

1. Agarwal, S. 1990. Proteases cathepsins—a view. Biochem. Edu. 18:67–72.
2. An, H., T. Seymour, J. Wu, and M. Morrissey. 1994. Assay system and characterization of Pacific whiting (*Merluccius productus*) protease. J. Food Sci. 59:277–281.
3. Balart, E., and J. Castro-Aguirre. 1995. Estimación del impacto de la depredación de merluza sobre la langostilla, p. 139–162. *In* D. Aurioles and E. Balart (ed.), La langostilla: biología, ecología y aprovechamiento. CIBNOR, La Paz, BCS, México.
4. García-Carreño, F. L. 1992. Protease inhibition in theory and practice. Biotechnol. Edu. 3:145–150.
5. García-Carreño, F. L., N. Dimes, and N. Haard. 1993. Substrate-gel electrophoresis for composition and molecular weight of proteinases or proteinaceous proteinase inhibitors. Anal. Biochem. 214(1):65–69.
6. Konagaya, T., and T. Aoki. 1981. Jellied condition in Pacific hake in relation to myxosporidian infection and protease activity. Bull. Tokai Reg. Fish. Res. Lab. 105:1–16.
7. Kunitz, M. 1944. Crystallization of a trypsin inhibitor from soybean. Science 101:668.
8. Masaki, T., Shimomukai, M. Miyauchi, Y. Ono, S. Tuchiya, T. Mastuda, T. Akasawa, H. and Soejima, M. 1993. Isolation and characterization of the protease responsible for jellification of Pacific hake muscle. Nippon Suisan Gakkaishi 59:683–690.
9. Morrissey, M., J. Wu, D. Lin, and H. An. 1993. Protease inhibitor effect on torsion measurements and autolysis of pacific whiting surimi. J. Food Sci. 58:1050–1054.
10. Saymoure, T., V. Weerasinghe, and H. An. 1995. Characterization of food grade protease inhibitors. Paper presented at Fish. Technol. 46th Annu. Meet. Mazatlan, Sin., México, 7 Feb.
11. Vogel, R., I. Trautschold, and E. Werle. 1968. Natural proteinase inhibitors. Academic Press, New York.