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EFFECT OF SHELLFISH PROTEIN HYDROLYSATE (SPH) OF PEARL OYSTER MEAT ON THE STATE OF WATER AND DENATURATION OF MYOFIBRILS DURING DEHYDRATION PROCESS

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ABSTRACT

Pearl oyster meat, considered as the waste product of pearl industry, can be transformed to a useful product through hydrolysis using protease enzyme. Protein hydrolysate of pearl oyster meat (SPH) was added to myofibrils (Mf) from lizard fish at certain ratios 2.5 - 12.5 g/100 gr Mf, and the changes in the state of water in myofibrils associated with dehydration were analyzed on the basis a water sorption isotherm curve and isosteric sorption heat. Involvement of these change in the denaturation of myofibrils was investigated with respect to Ca-ATPase activity.

The addition of SPH resulted a decreased of water activity, an increase in the amount of monolayer or multilayer water and increase in the isosteric sorption heat, indicative the changes in the state of water in myofibrils. Furthermore, it was found that SPH has suppressive effects on the denaturation of myofibrils during dehydration process.

Key words : Shellfish Protein Hydrolysate, Myofibrils, Water activity, Ca-ATPase activity.

I. INTRODUCTION

There are fifty pearl culture enterprises of various industrial scales in Indonesia. At the pearl harvest, the activities around the factory is highly dense. The survey of pearl harvest revealed that the waste of shell of pearl oyster had been used directly as road hardener and building construction, while most of the meat had not been used and thrown away to the water. This disposal causes not only the odor around the factory and beach which disturbs the work activity, but also fading of water color which endangers ecosystem around the factory and beach.

The protein of meat will denature in a short time if it is not treated properly, due to the activity of bacteria, enzyme and chemical process in the oyster meat it self. On the contrary, if protein is hydrolyzed by acid, alkali or enzyme treatments, it will produce useful amino acid for human beings.

Addition of SPH as foodstuff (either for man, fish or other cattle) will increase the quality of the food. Therefore, it is necessary to study how the addition of SPH effect the form and nature of the foodstuff.

Water is a very important component in the foodstuff. It does not only affects taste, texture, and flavor, but also determines acceptability, freshness and durability of the food. There are several different states of water in the foodstuff, according to the degree of affinity. Nozaki *et. al.* (1991) reported that the state of water plays an important role in the dehydration of fish myofibrillar protein. Adjustment and control of water have now become important measures of quality assurance in the food processing industry. However, only in recent decades has been recognized that the chemical, physical and biological properties enhance, quality and stability food product are related directly to relative humidity or water activity. Dehydration property in volves not only a measure of control by artifice over the removal of water, but also the preparation of an improved type of product, quite different from traditional commodities, being greatly altered in appearance, odor, flavor and texture by a prolonged curing and drying process (Rockland and Stewart, 1981).

Myofibril protein, the protein that forms myofibrils, contains myosin, actin and regulating proteins as tropomyosin, troponin and actinin and so on. Myofibril protein accounts for 66 - 77% of total protein in fish meat, and plays an important role in coagulation and gel formation when fish meat is processed (Suzuki, 1981). With dehydration myofibril protein that has long period, the functional properties such as capacities in emulsifying, lipid binding, water holding and forming can be lowered (Matsinmoto, 1979). There are many methods of measuring protein denaturation during dehydration such as solubility, of myofibrillar protein, viscosity, Ca-ATPase activity and electron microscopy (Cleland *et. al.*, 1986). Nozaki *et. al.* (1993) used the Ca-ATPase activity as an indicator of quality of myofibrillar protein during frozen storage and dehydration process.

II. METHODOLOGY

The research materials of pearl oyster meat was processed with protease endoenzyme produced by *Bacillus Substillus* and exoenzyme produced by *Aspergillus.* Meanwhile, the myofibrillar protein (Mf) was prepared by isolating lizardfish meat.

The find out the effects of SPH on the state of water and denaturation of myofibrillar protein during dehydration process, SPH was added to myofibrils at certain ratios, 2.5 - 12.5 g/100g Mf. Moisture content, water activity, Ca-ATPase activity were determined.

Moisture content

The moisture content was determined as the loss of water in a sample by oven drying at 105^{0}C for 16 to 18 hours.

Water activity

Water activity (Aw) is an index to represent mobility of water molecules in a substance and expressed as a ratio of the partial water vapor pressure of

the substance to that of the saturated water vapor pressure at the same temperature. The partial water vapor pressure was measured with oil manometer according to Akiba equation (1961).

$$Aw = \frac{P}{Po}$$

With the above equation if :

Pure water $P = Po \rightarrow Aw = 1$

Un hydrous substance $P = 0 \rightarrow Aw = 0$

The water activity of hydrous substance $1>Aw>0$

Aw = Water activity
P = Sample vapor pressure
Po = Water vapor pressure

Ca-ATPase activity

Effect of SPH againt denaturation of myofibril during dehydration process was studied through change of Ca-ATPase activity. The Ca-ATPase activity was indicated by micro moles per minute of the inorganic phosphate in the presence of 1 mM ATP, 100 mM KCl and 5 mM $CaCl_2$ at pH 7.0 with 25 mM tris - maleate buffer. The concentration of protein of myofibrils was determined by the biuret method with bovine serum albumine as a standard.

Data Analysis

Analysis of water sorption isotherm

Moisture contents of myofibrils during dehydration were plotted against water activity at a certain temperature yield water sorption isotherm. Each of the isotherms was divided into three sections as demarcated by M_1 and M_2 which appeared as flexing point on isotherm. The water up to M_1 is regarded as in a state of monolayer, then between M_1 and M_2 mutilayer, and then above M_2 capillary, respectively.

The monolayer water (M_1) was determined using the formula Brunauer *et. al.* (1968) as below ;

$$\frac{1}{V} \times \frac{Aw}{1-Aw} = \frac{1}{Vm \times c} + \frac{C-1}{Vm \times c} \times Aw$$

Aw : Water activity
Vm : Monolayer Water content (g/g of dried matter)
V : Volume of sorbed water
C : Constant

$$M_1 (\%) = \frac{100 \times Vm}{1 + Vm}$$

M_1 : Moisture content (%) for upper limit of monolayer water

The multilayer water (M_2) was determined using Bull's method (1944) and the sorption surface area (S) or 1g water in monolayer water was determined by using the formula of Brunauer *et. al.*, (1968) as bellow ;

$$S (m^2/mg) = \frac{Vm \times Sm \times N}{M \times 10^3}$$

N : Avogadro's numbers (6.023×10^{23}/mol)
M : Molecule weight of H_2O (H_2O : 18 g/mol)
Sm : Cross section of water molecule (10.45×10^{-6} cm^2)

Isosteric sorption heat ($-\Delta H$) of myofibril during dehydration process was calculated by using the formula of clausius clapeyron (Chirife and Iglesias, 1978) as below ;

$$-\Delta H \text{ (cal /mol)} = \frac{RT_1T_2}{T_1 - T_2} \times \text{Ln} \frac{Aw_1}{Aw_2}$$

$-\Delta H$ = isosteric sorption heat (cal/mol)
R = gas constant (1.986 cal/mol)
Aw_1 = water activity at T_1
Aw_2 = water activity at T_2
T_1, T_2 = absolute temperature (K)

Analysis of Ca-ATPase activity.

Myofibrils, one of the main muscle components, has an enzymatic activity to split ATP. Therefore, by measuring this activity during dehydration process, it was hoped to provide a measure of denaturation (Kamal *et. al.*, 1989). The Ca-ATPase activity was analyzed by the method of Katoh *et. al.*, (1977). With the using formula is :

$$\text{Ln}\left(1 - Pi \times \frac{6}{5} \times \frac{1}{31}\right) \times \frac{1}{5} \times 1 \times \frac{5}{A}$$

Pi : Acquired by the equation of the regression of phosphorus standard solution
A : Acquired by the equation of the regression of biovine serum albumine as a standard

III. RESULT AND DISCUSSION

3.1. Water Sorption Isotherm

A water sorption isotherm of materials represents relationship between moisture content and water activity of myofibrils at a particular temperature. In accordance with this relation, the water activity of myofibrils with 2.5 - 12.5% SPH at 10°C or 20°C during dehydration process was plotted as a function of the moisture content in Fig 1 and 2. All of the water sorption isotherm shows a sigmoidal curve which has two bendings within 0.05 - 0.15 and 0.7 - 0.85 ranges of water activity. Water activity of myofibril with all concentration of SPH decreased remarkably throughout dehydration process and of which were lower than that without SPH (control) for the same of moisture content. Likewise the water activity of myofibrils in total water content decreased with increasing SPH concentration.

In order to evaluate the characteristics of state of water in myofibril from above water sorption isotherm, the amount of monolayer water (M_1) and the amount of multilayer water (M_2) in myofibrils were calculated by the BET method (Brunauer *et. al.*, 1968) and Bull's method (Bull, 1944), respectively. The M_1 and the M_2, water activity-Aw_1 and Aw_2 at the M_1 and the M_2 points, remaining relative Ca-ATPase activity (described for following item) - $T_{\partial 1}$ and $T_{\partial 2}$ at the M_1 and the M_2 points, the ratio of M_2 to M_1, sorption surface area - S of sample are show in Table 1. for myofibrils in the presence of 2.5 - 12.5% SPH. As shown in Table 1, M_1, M_2, the $T_{\partial 1}$, $T_{\partial 2}$ and S in myofibrils with 2.5 - 12.5% SPH were greater than those in myofibrils without SPH (control). Specially, the $T_{\partial 2}$ and $T_{\partial 1}$ at the 12.5% SPH was decreased lower than 2.5 - 10% SPH. It was also show that the M_1 and M_2 and S in the myofibrils increased with increasing concentration of SPH. The composition of amino acid, which influence the binding capacity of molecules in myofibrillar protein were different by to the kinds or concentration of SPH. Nozaki *et. al.* (1991) reported that various amino acids have different effect on monolayer water and

multilayer water in myofibrils protein during the dehydration process. This research showed that a measured temperature effects monolayer water and multilayer water in myofibrils. In this study, for example at 10^0C, the water activity of myofibrils with Pearl oyster SPH was 0.70 at a moisture content of 22%, whereas at 20^0C, the same water activity was observed at a moisture content of 18%. This research predicts that if the temperature is relatively lower, the mobility of one molecule to another in myofibrillar protein will be different, do it is similar to water activity at different moisture contents. Chirite and Iglesias (1978) reported that the water sorption isotherm of a wide variety of foods have a temperature dependence which has a theoretical thermodynamic consideration. This theoretical consideration shows that the effect of the increase in temperature is to shift the water sorption isotherms upwards, namely, its increase water activity at the same moisture content.

Water activity is an index of the availability of water in a food for microbial growth. The water activity of food ingredients will directly effect the quality that food. Therefore, a decrease in water activity slows down all types of chemical deterioration reactions and microbial growth etc. Until at a certain level, and all reaction are almost completely inhibited except for the chemical oxidation of lipid, which strongly favors by a decrease in water activity (Rockland and Stewart, 1981).

A change in the isosteric sorption heat amount is an indication that there is a change in the state of water in myofibrillar protein. Isosteric sorption heat depends on the amount of water activity at various temperatures. The results of this research show in Fig. 3, the isosteric sorption heat of various concentration SPH did not clearly change in sorption water content at 30%. When sorption water content is < 30%, a slight change in sorption water content will greatly increase isosteric sorption heat. When sorption water content is < 20%, a very slight decrease in sorption water content will also highly increase the isosteric sorption heat. This factor is caused by different chemical composition of various concentration of SPH. Nozaki *et. al.*, (1993) reported that the influence of 23 amino acids on isosteric sorption heat in myofibril protein during the dehydration process, to show that each amino acids has a different isosteric sorption heat.

3.2. Ca-ATPase activity

It has been shown that there is a correlation between the activity of enzyme and the moisture content of the food. A certain minimal amount of water is necessary for enzyme activity which increases with increasing moisture content. Inada *et. al.* (1992) reported that the Ca-ATPase activity was measured as an indicator of the denaturation of myofibrils protein. The Ca-ATPase activity relative to that just before dehydration of myofibrils in the presence of 2.5 - 12.5%. SPH during dehydration was plotted as a function of the water activity as 20^0C and 10^0C (Fig. 4 and 5). The Ca-ATPase activity of myofibrils without SPH (control) decreased rapidly with the decreasing water activity until the latter reached 0.70, when the former was in the range from initial value. There after, in the range from multilayer to capillary layer the decrease of the Ca-ATPase activity became slowed down. The Ca-ATPase activity of myofibrils in the presence of

various concentration SPH also decreased with the decreasing water activity, at a greater rate than that of the control in all region of water activity. These results show that the changing of Ca-ATPase activity is an indication that there has been changing structure and changing state of water in myofibrillar protein during dehydration process. Matsumoto *et. al.* (1980) reported that the denaturation of myofibrils protein (actomyosin and myosin) during dehydration and frozen storage was a result of coagulation caused by the progressive increase of intermolecular cros-linkage due to formation of hydrogen, ionic, hydrophobic and/or disulfhydryl bonds.

IV. CONCLUSIONS

The effects of shellfish protein dehydrolysate of pearl oyster meat on the state of water and denaturation of myofibrils during dehydration process are concluded as follows ;

1. During the dehydration process, the existence of SPH results in differences water sorption isotherm. Increasing the SPH concentrations is parallel with the increase in water sorption isotherms of myofibrils protein.
2. During the dehydration process, the sorption water content drastically decreases and cannot be compared with the increase of isosteric sorption heat. But under certain condition, the sorption water content decreased does not mean that it cannot be compared with the increase isosteric sorption heat.
3. During dehydration process, of various concentration SPH, the same sorption water content was found the have different isosteric sorption heat. With higher concentration of SPH higher isosteric sorption heat.
4. During dehydration process, of various concentration SPH, different amounts of monolayer and multilayer water were found. An increase in SPH concentration is parallel with an increase in monolayer and multilayer water.
5. The adding of SPH suppresses the decrease of Ca-ATPase activity. Specially when water activity 0.80, Ca-ATPase activity drastically falls in a concentration of 12.5%.
6. The adding of SPH resulted in a decrease in water activity, an increase in the amount of monolayer water or multilayer water and an increase in the isosteric sorption heat, indicating the changes in the state of water in myofibrillar protein.

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Table 1. Amount of monolayer and multilayer water, sorption surface area of lizardfish myofibrils added of various concentration pearl oyster SPH from water sorption isotherm at 10^0C, and remaining CA-ATPase activity of the myofibrils corresponding with monolayer and multilayer water.

Pearl oyster SPH	Monolayer water * 1		Aw_1*2	Ta_1*3	Multilayer water * 4		Aw_2*5	Ta_2*6	M_2/M_1	S*7
	M_1*8	Md*9			M2*8	Md_2*9				
Control	6.36	0.067	0.118	2.94	15.53	0.184	0.616	7.04	2.44	0.242
2.5%	8.05	0.087	0.120	5.65	16.10	0.191	0.621	9.13	2.00	0.314
5%	10.06	0.111	0.125	9.13	17.09	0.206	0.627	14.39	1.70	0.401
7.5%	10.75	0.119	0.134	11.30	21.90	0.256	0.660	17.39	2.04	0.430
10%	10.84	0.121	0.137	11.35	22.05	0.282	0.670	17.45	2.03	0.437
12.5%	10.85	0.122	0.138	3.02	23.51	0.307	0.670	7.82	2.17	0.440

* 1 Estimated by B.E.T. analysis
* 2 Water activity of the sample at the M_1 point
* 3 Remaining myofibril relative Ca-ATPase activity (%) of the sample at the M_1 point
* 4 Estimated by Bull's analysis
* 5 Water activity of the sample at the M2 point
* 6 Remaining myofibril relative Ca-ATPase activity (%) of the sample at the M_2 point
* 7 Sorption surface area (m^2/mg)of sample
* 8 Moisture content (g/100g of sample)
* 9 Moisture content (g/g of dried matter)

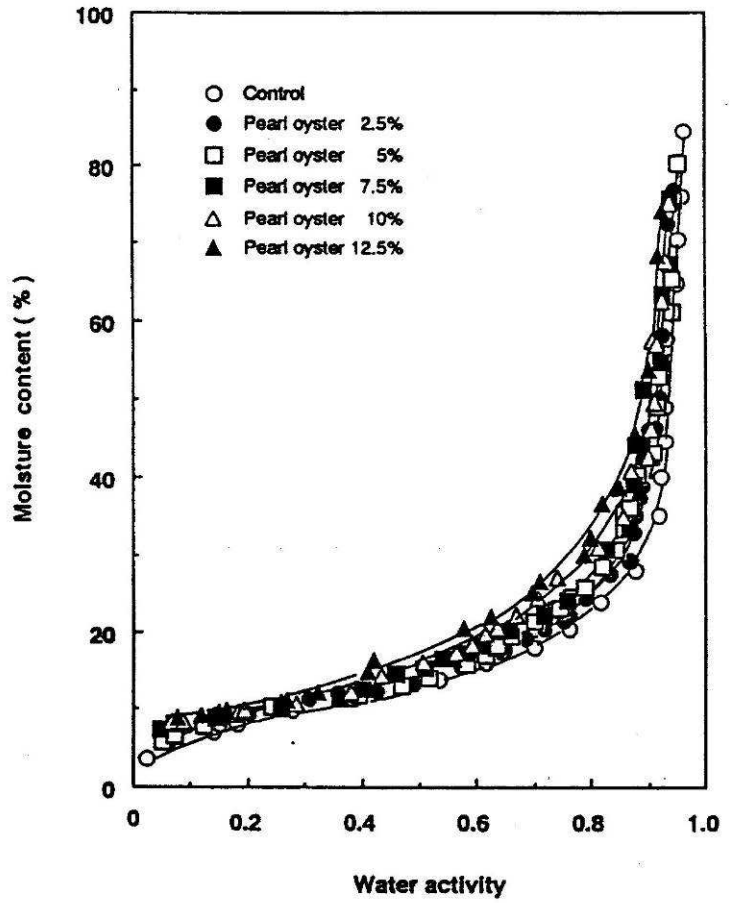

Fig. 1 The desorption isotherms to show the effect of various concentration of pearl oyster protein hydrolysate added to lizardfish myofibrils at 20℃.

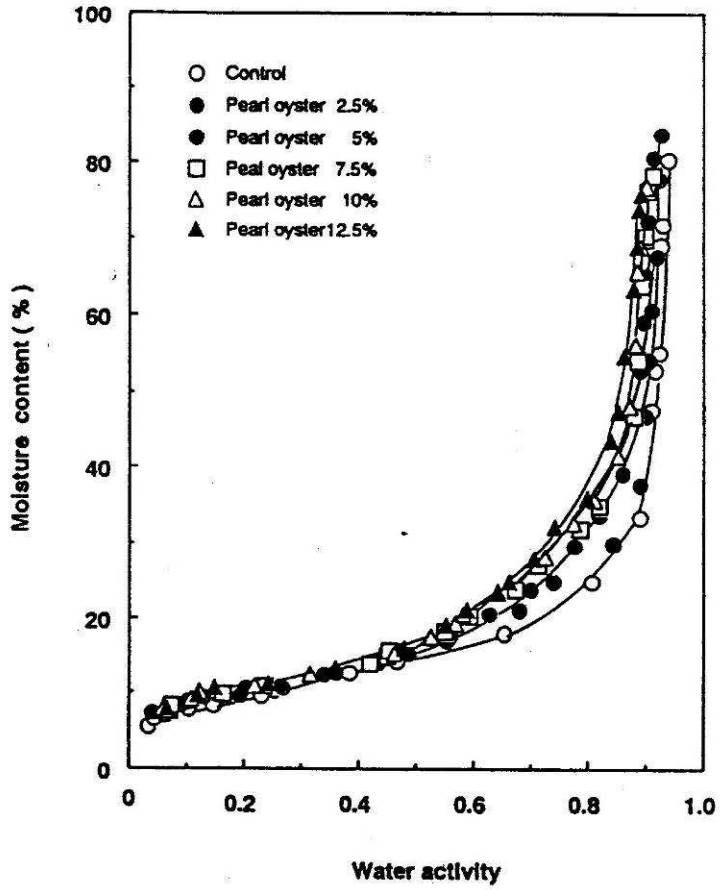

Fig. 2 The desorption isotherms to show the effect of various concentration of pearl oyster protein hydrolysate added to lizardfish myofibrils at 10℃.

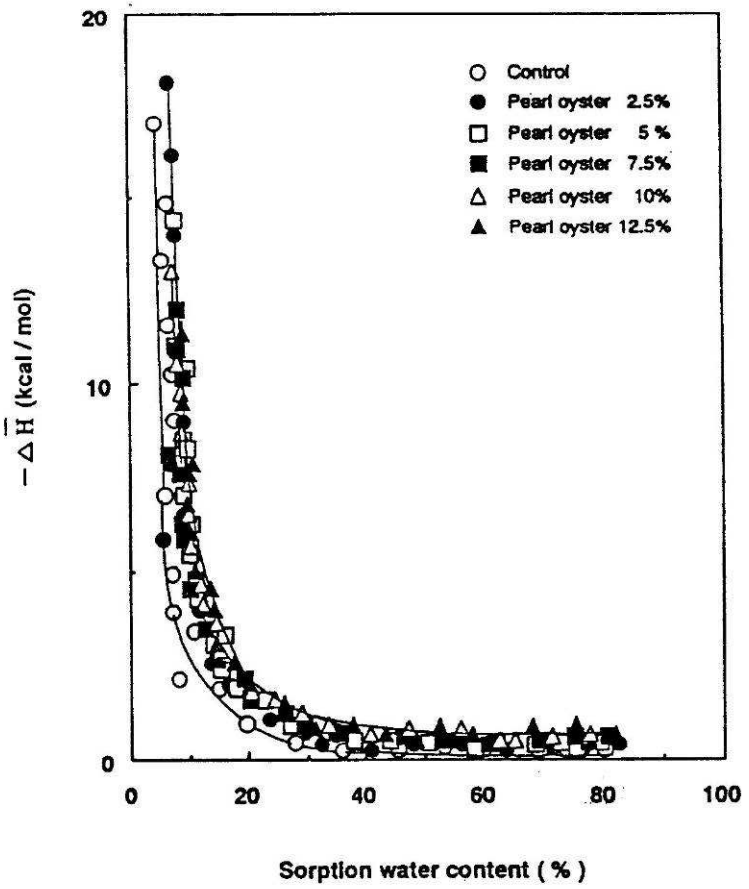

Fig. 3 The isosteric sorption heat to show the effect of various concentration of pearl oyster protein hydrolysate added to lizardfish myofibrils during dehydration process.

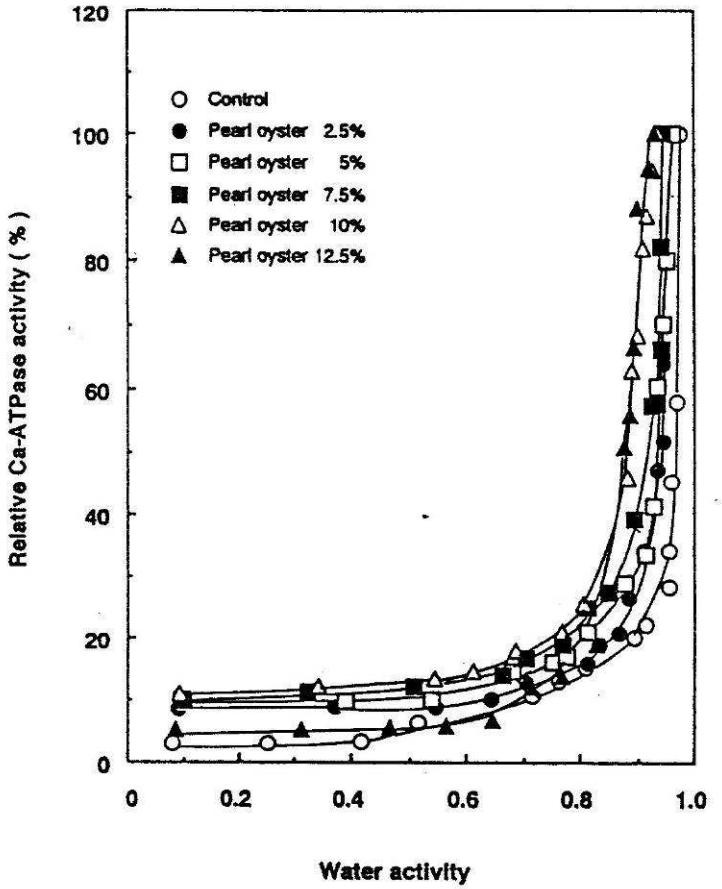

Fig. 4 The relative Ca-ATPase activity and water activity (20℃) to show the effect of various concentration of pearl oyster SPH added to lizardfish myofibrils during dehydration process.

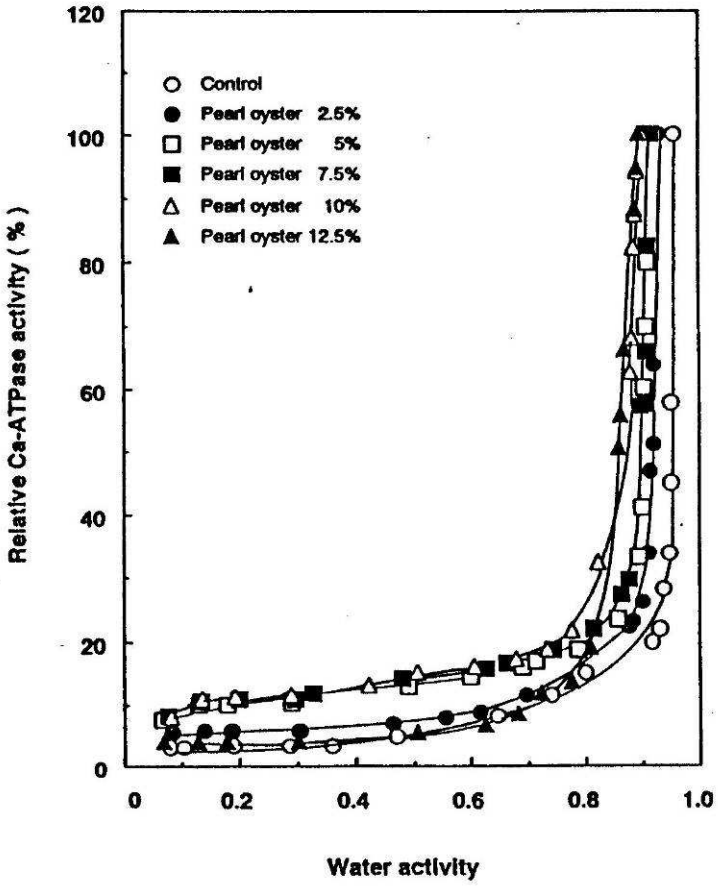

Fig. 5 The relative Ca-ATPase activity and water activity (10℃) to show the effect of various concentration of pearl oyster protein hydrolysate added to lizardfish myofibrils during dehydration process.