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# Effect of Heat Treatment on the *In Vitro* Protein Digestibility and Trypsin Indigestible Substrate (TIS) Contents in Some Seafoods

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# 水產蛋白質 消化率에 미치는 加熱處理의 影響

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## 요 약

가열 및 동결저장이 오징어, 굴, 새우 및 명태의 *in vitro* 소화율과 trypsin indigestible substrate (TIS) 함량 변화에 미치는 영향을 실험한 결과는 다음과 같다.

Boiling 했을 때 최고의 *in vitro* 소화율을 나타내는 시간은 오징어 1분, 내장을 제거한 굴 0.5분, 내장을 제거하지 않은 굴은 1분이었고, 명태는 5분 이었으며, 오징어와 굴은 부위에 따라 소화율은 다르게 나타났다. 최고의 *in vitro* 소화율을 나타내는 steaming 조건은 오징어는 100°C에서 1분, 굴은 88°C에서 1분이었으며, 명태는 100°C에서 1~2.5분이었다. TIS는 전시료에서 *in vitro* 소화율과 역상관관계를 가지며 변화하였다. 동결건조한 시료가 다른 건조품보다 현저하게 높은 *in vitro* 소화율을 보였으나, 내장을 제거했거나 지방함량이 낮은 시료를 천일건조했을 때도 동결건조시료에 버금가는 *in vitro* 소화율을 나타내었다. 동결저장은 지방함량이 낮은 시료의 *in vitro* 소화율의 저하 및 TIS함량 증가에 효과적이었으나, 지방함량이 높아 산패가 심하게 진행된 시료에는 현저한 효과를 볼 수 없었다. 지방함량은 수산식품단백질을 가열처리했거나 저장했을 때의 소화율 변화에 결정적인 영향을 미침을 알 수 있었으며, 4가지의 효소를 이용한 multi-enzyme digestion technique은 수산식품단백질의 소화율 변화를 측정함에 있어 아주 민감하고 유용한 방법으로 판명되었다.

## Introduction

Heat processing is one of the most important methods developed by man for extending storage life and increasing the nutritional value of foodstuffs. Increasing the nutritional value of many foods is partly due to the destruction of protease inhibitors and other toxic substances along with the opening of the protein structure through denaturation. However, processing can also cause a decrease in protein digestibility via the nonenzymatic browning and thermal cross-linking reactions(Tannenbaum, 1974).[1] Therefore, it is important to determine the optimum conditions of heat treatment for increasing or maintaining nutritional quality as measured by biological value(BV), protein efficie-

ncy ratio(PER), and protein digestibility.

Seafoods(mainly fish and shellfish) have been considered to be highly digestible with their nutritive value dependent mainly on the essential amino acid(EAA) profile and protein digestibility. Fresh fish is baked, steamed, fried, boiled or dried before being consumed and it plays an impotant role in the protein nutrition of many people, it is valuable to determine the effect of those various processes have on the EAA composition and protein digestibility. Because the determination of *in vivo* protein digestibility in animal and human experiments is time-consuming and expensive, *in vitro* methods have been proposed for the routine evaluation of protein quality in foods(AOAC, 1982).[2]

Since White and Crozier(1911)[3], a number of authors have studied on the *in vitro* protein digestion of fish by enzyme.[4~11] Possibly the oldest assays for estimating protein digestibility related with protein quality are those of Sheffner(1956)[12] which utilized pepsin hydrolysis, and Adachi et al. (1958)[13] which determined by means of the PDR (pepsin digest residue) index, the nature and extent of the nutritive changes that occur in the fish proteins. Sawant and Magar(1961b)[14] also determined the changes in the nutritive value of bombay duck during processing by the PDR index. Ford and Salter(1966)[15] utilized a protease preparation from *Streptomyces griceus* to predict digestibility for cod fillets and Jeong et al. (1978)[16] used the pepsin pancreatin digest residue index(PPDRI) assay of Akeson and Stahmann(1964)[17] for the nutritional evaluation of flounder. Hsu et al. (1977)[18] described a 10 minute multienzyme-automated assay that could successfully predict($r=0.90$) rat apparent protein digestiblity, but they did not use fish samples when developing the assay. Therefore, Satterlee et al. (1979)[19] modified the assay of Hsu et al. (1977)[18] by increasing the sample base to 50 foods, which included fish samples(tuna and salmon) and adding an additional enzyme protease from (*Streptomyces griceus*). Hsu et al. (1977)[18] noted that the *in vitro* assay was sensitive to trypsin inhibitors, as well as the effect of processing or preservation on protein digestibility. Very little research(Florian et al. 1981)[20] has been done on the presence of trypsin or trypsin like enzyme inhibitors in seafoods, yet a large amount of research has been performed on the trypsin inhibitors in plant protein since Pande and Mead(1968).[21]

The objectives of the present study were 1) to evaluate the effect of various heat treatments on *in vitro* protein digestibility, 2) to assay for the presence of trypsin insoluble substrate (TIS) in processed seafoods, and 3) to confirm the application of the four enzyme assay by Satterlee et al. (1979) to the nutritive changes in seafoods.

## Materials and Methods

### 1. Food Items Studied

This study represents an attempt to determine the influence of boiling, steaming and dehydration on the *in vitro* protein digestibility and trypsin indigestible substrate content of four different seafoods. The seafoods were divided into four groups: fish(pollock), cephalopoda(squid), crustaceans(shrimp), and mollusks(oyster). Table 1 summarizes the samples analyzed which were purchased as individually quick frozen(IQF) product. After the heat treatment, samples were ground in a micromill

**Table. 1. Summary of sample analyzed**

| Sample | Species | Product[a] description | Unit size |
|---|---|---|---|
| Squid | *Loligo vulgaris* | IQF (whole) | 5-6/pound[b] |
| Oyster | *Ostera gigas* | IQF (whole) | 45-50/340g package[c] |
| Shrimp | *Pandalus jordani* | IQF (peeled and deveined) | 32-35/pound |
| Pollock | *Gadius virens* | IQF (fillet) | 6 fillets/5 pound package |

[a]IQF-individually quick frozen
[b]Average length - 26 cm
[c]Average length - 6 cm

(JANK & KUKEL, TYPE A10S1) with dry ice in order to avoid thermal denaturation of proteins. All milled products were able to pass through a 100-mesh standard sieve. To avoid water absorption or oxidation, the samples were sealed under an atmosphere of $N_2$ in glass ampoules as described by Carpenter et al. (1962).[22]

### 2. Heat Treatment

1) Squid: Frozen squids were thawed in a continuous stream of cold tap water for 2 hours. The thawed squids were skinned immediatly and then eviscerated, except the "warmed squid" which was prepared by dipping in 55°C water bath for 5 min and then skinning. Boiled squid was held in a boiling water bath(98±1°C) then immediatly placed in ice water and drained. In an attempt to further denature the proteins and to increase the protein digestibility, samples were steamed for periods from 10 second to 20 minutes at a temperature 100 °C. Sundried squids were dehydrated for 18 hours at 14±1°C. Squids were vacuum dried for 18 hours at 73°C(27 inch Hg). Freeze dried samples were prepared by using a Virtis Freeze Drier for 24 hours at 0.5~0.75 mmHg.

2) Oyster: Frozen oysters were thawed using cold tap water and sectioned into adductor muscle, viscera or kept as whole oyster. *In vitro* protein digestibility was determined on whole oyster, adductor muscle, viscera, whole minus adductor muscle, whole minus viscera, and whole minus viscera and adductor muscle. To determine the optimal heat treatment, 45~50 grams of whole and eviscerated oysters were cooked in 98±1°C water bath for periods from 10 seconds to 10 minutes and steamed at 88~132°C for 1 minute. Freeze dried and vacuum dried oysters were treated as the squid samples were. Sundried samples were prepared by incising the whole oyster. The incised oysters were dried for 36~48 hours at 15~18°C.

3) Pollock: Frozen pollock fillets(375~400 grams, Iceland Seafood Co., PA) was thawed in 4°C cold room for 4~5 hours and then cut into 0.5×4×7cm slices. Boiling was accomplished in 98±1°C water bath for periods from 30 seconds to 20 minutes and steaming was performed on 40~50 grams slices at 88°C to 132°C from 30 seconds to 20 minutes. Freeze dried, vacuum dried and oven dried samples treated as the other samples. Sundrying of pollock was performed for 24~26 hours at 18~20°C. The processing conditions for hot air blast dried samples were not known since it was a commercial sample purchased from Korea. This samples was only used as a reference when comparing the *in vitro* protein digestibility and trypsin indigestible substrate(TIS) to the other processed pollock samples.

4) Shrimp: Peeled and deveined salad shrimp (IQF) were purchased from Young's Market Co., CA. The raw shrimps were dipped in boiling water peeled, broken and then deveined. Freeze drying of shrimps was performed as same as for squid or oyster. Vacuum drying was performed for 5 hours at 100°C(27 inches Hg) and oven drying was carried on for 5 hours at 100°C also.

### 3. Analytical Procedures

Nitrogen was determined by the Kjeldahl method (AOAC, 1980).[23] Moisture content was determined by drying overnight in vacuum oven at 105°C(27 inches Hg). Fat was extracted from samples using diethyl ether in a Soxhlet-type extractor for 5 hours. Fat content was determined by the AOAC (1975)[24] procedure, using a Goldfisch apparatus. The *in vitro* protein digestibility of the various seafoods was determined using the AOAC(1982)[2] procedure. Trypsin indigestible substrate was determined using the procedure of Rhinehart(1975)[25] and Hamerstrand et al. (1981).[26]

## Results and Discussion

### 1. Effect of Cooking on the *In Vitro* Protein Digestibility of Squid

1) Varieties in the *in vitro* digestibility of different squid's parts

The effect of various cooking methods upon the *in vitro* protein digestibility of squid is given in

**Table. 2. *In vitro* digestibilities (%) of various parts of the squid**

| | Whole | Mantle | Tail | Arm |
|---|---|---|---|---|
| Raw | 84.4 | 86.9 | 88.0 | 80.0 |
| Warmed | 85.6 | 84.7 | 89.2 | 83.8 |
| Boiled | 85.8 | 87.2 | 84.4 | 86.5 |
| Steamed | 89.9 | 89.9 | 81.3 | 88.4 |

Table 2. The *in vitro* digestibility of 84.4% for whole raw squid was only slightly lower than that observed when using pepsin digestion of 86.22% as reported by Tanikawa and Suno(1952).[7] An increase in the degree of *in vitro* digestibility of squid was noted when the raw squid was warmed, boiled and steamed, in ascending order of magnitude. But in case of tail meat, the highest digestibility(89.2 %) was for warmed, not boiled or steamed tail. The tail's protein may be more sensitive to heat treatment than the other portions of squid. The results shown in Table 2 were about 10~15% lower than the true digestibility of 98.8% reported for whole squid by Matsuno and Iwaya(1971).[27] True protein digestibility is always higher than apparent digestibility[28,29] because the true protein digestibility takes into account the metabolic nitrogen excreted, which is not of dietary origin.

2) Effect of steaming and boiling time on *in vitro* digestibility of whole squid

Changes in the *in vitro* protein digestibility of squid processed by various boiling and steaming conditions are presented in Figure 1. After 5 minutes of processing, no significant increase in digestibility occurred. The maximum digestibility occurred after 1 minute of heat treatment to the

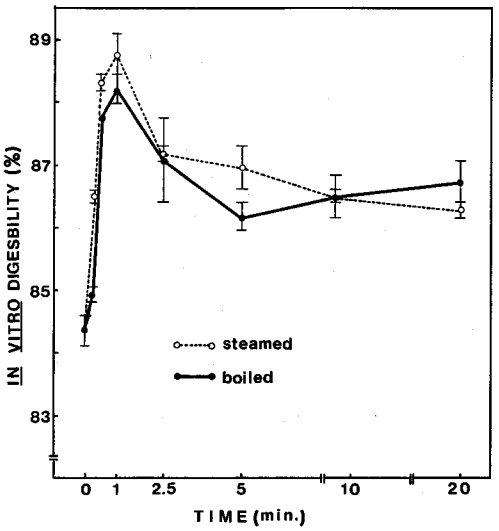


**Fig. 1. Effect of steaming and boiling time on the *in vitro* protein digestibility of squid.**

squid which is in close agreement with that reported by Tanikawa and Suno(1952).[7]

3) Effect of drying method upon the *in vitro* digestibility of whole squid

In Korea and Japan where squid mest is widely consumed, most people underestimate squid's nutritive value and regard it as almost indigestible (Takahashi 1965).[30] It is true that squid meat, when dried or steamed for long periods of time, become hard due to damaged proteins which are difficult to digest, as is shown in Table 3. Drying can cause damage, with the damage being proportional to the temperature and length of heating

**Table. 3. Water, nitrogen, TIS content and *in vitro* protein digestibility of the dried squid**

| | Sundried | | | Vacuum dried | | | Freeze dried | | |
|---|---|---|---|---|---|---|---|---|---|
| | Raw | Boiled | Steamed | Raw | Boiled | Steamed | Raw | Boiled | Steamed |
| Water(%) | 15.13 | 14.14 | 12.62 | 10.14 | 8.49 | 9.59 | 8.57 | 7.87 | 7.12 |
| Nitrogen(%) | 12.92 | 12.72 | 12.86 | 13.29 | 13.39 | 13.51 | 13.37 | 13.70 | 13.23 |
| *In vitro* dig. (%) | 83.0 | 86.3 | 86.7 | 81.4 | 83.8 | 86.0 | 84.7 | 88.1 | 89.0 |
| TIS(mg/g) | 12.8[R] | 7.55 | 6.82 | 11.37 | 6.72 | 6.21 | 67.68 | 10.89 | 7.97 |
| | 0.56[H] | 0.41 | 0.47 | 0.54 | 0.25 | 0.25 | 0.58 | 0.41 | 0.44 |

[R] Rhinehart method (1975)
[H] Hamerstand method (1981)

Miller et al. 1965)[31]. When raw squid was dried in a vacuum oven (for 18 hours at 73°C), the *in vitro* digestibility was less than that for sundried or freeze dried squid. The trypsin indigestible substrate content of vacuum oven dried products was lower than that of the other ones. Long exposure to hot air (73°) caused structural changes in squid proteins forming enzyme resistant bonds. In all cases of dried products, steamed meat's *in vitro* digestibility was higher than boiled squid which was similar to the results by Sawant and Magar (1961b),[14] but TIS content was low. It is thought that some TIS or trypin like inhibitors have been extracted in boiling water. The *in vitro* digestibility and TIS content of sundried squid were not changed significantly compared with the freeze dried sample. Similar results had been obtained by Jeong et al. (1978)[16] using PPD index for dried flounder. The mild treateatment (14-16°C, 18 hours) may not caused the formation of enzyme resistant bonds in squid protein. However, when squid is dried by exposure to direct sun light for a long time, digestibility drops severely (Tanikawa and Suno 1952)[7]. Freeze drying is a mild heat treatment and does not modify the nutritive value of food proteins.[32~34] As presented in Table 3, freeze dried squid gave higher values for digestibility than did squids dried by other methods, which is in accord with previously published results.[31,35]

4) Effect of particle size on the *in vitro* digestibility of freeze dried squid

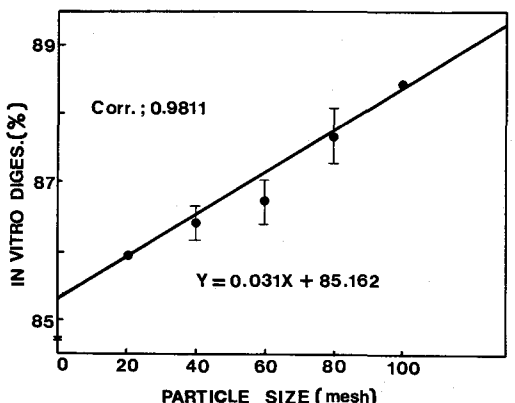


**Fig. 2. Influence of particle size on the *in vitro* protein digestibility of steamed and freeze dried squid.**

Figure 2 illustrates how the *in vitro* digestibility increased steadily from samples having a 20 mesh particle size to those having a 100 mesh particle size. Lakesvela et al. (1966)[36] reported that finely ground materials may on occasion have a reduced nutritive value due to overgrinding causing heat damage of the protein. In the present study dry ice was added when the sample was ground preventing heat damage. Small particles have more surface area accessible to the enzymes than do larger particles which explain the increased digestibility.

## 2. Effect of Heat Treatment on the *In Vitro* Digestibility of Oyster

1) Variations in crude protein, crude fat, TIS content, and *in vitro* digestibility of dried oyster

Crude protein (N×6.25), crude fat, TIS content and *in vitro* digestibility of the oyster and its various, parts are given in Table 4. Close agreement was found in the observed protein level reported by other authors[37~39], but the crude fat was lower (9.1%) than that reported by Gordon and Roberts (1977).[40] Hatanaka (1939)[37] mentioned the seasonal variation in fat content of oyster to range from 11.0 to 15.95%. The highest protein content of any one oyster component was found in the adductor muscle, which also had the lowest TIS and fat content. There was a remarkable difference in fat and TIS content between viscera and the other portions of the oyster. As shown in Table 4, *in vitro* digestiblity increased as the fat level of the sample decreased. This relationship could be due to oxidized fats formed during processing or storage, which may be formed enzyme indigestible substrate with proteins. Oxidized unsaturated lipids in seafood tend to bind to proteins and form insoluble lipid-protein complexes.[21,41~45] Heat treated oysters, oysters stored for long periods would possess lower *in vitro* protein digestibility. Upon vacuum drying the adductor muscle which has the lowest fat content, possessed the highest digestibility. The fat content of seafood may be an important factor which affects the

**Table. 4. Crude protein(N x 6.25), crude fat, TIS and *in vitro* digestibility of dried oyster parts**

| | Whole (W) | Adductor muscle(A) | Viscera (V) | W-A | W-V | W-A-V |
|---|---|---|---|---|---|---|
| Freezedried | | | | | | |
| Crude protein(%) | 46.26 | 89.86 | 31.04 | 42.67 | 62.13 | 44.26 |
| Crude fat(%) | 9.10 | 4.31 | 17.88 | 8.96 | 8.32 | 8.28 |
| TIS (mg/g) | 19.45[R] | 7.34 | 60.77 | 20.37 | 15.18 | 14.88 |
| | 0.53[H] | 0.29 | 1.02 | 0.41 | 0.24 | 0.16 |
| *In vitro* dig.(%) | 81.0 | 91.8 | 72.2 | 80.0 | 83.6 | 81.7 |
| Vacuum dried | | | | | | |
| *In vitro* dig.(%) | 78.7 | 94.1 | 78.1 | 74.2 | 82.2 | 83.0 |
| Sun dried | | | | | | |
| *In vitro* dig.(%) | 74.8 | 88.4 | 70.0 | 79.3 | 82.6 | 83.4 |

[R] Rhinehart method (1975)
[H] Hamerstand (1981)

digestion of proteins.

2) Effect of boiling(98±1°C) and steaming(88 °C) time on the *in vitro* digestion and TIS content of oyster

Figures 3 and 5 illustrate the differences in the *in vitro* protein digestibility and TIS content of whole and eviscerated oyster after various boiling and steaming times. A significant increase of digestibility occurred within one minute boiling, especially in case of eviscerated oyster. At the end of one minute, the *in vitro* digestibiliety even though TIS in whole oyster was extracted into boiling water. The protein was extremely sensitive to thermal destruction. The different times for optimal protein digestion between whole and eviscerated oyster could be due to the presence of indigestible substrates by enzyme hydrolysis in the viscera which was not extracted within one minute boiling time. No appreciable change in TIS content of whole oyster occurred during steaming at 88°C. The susceptibility of oyster proteins to enzymic digestion was increased rapidly within one minute steaming time for both oyster samples, and then heat damage occurred in the eviscerated oyster protein between 1 and 2.5 minute. This did not occur in the whole oyster, which indicated that the *in vitro* protein digestibility of steamed oyster is primarily affected by the level of protein denaturation.

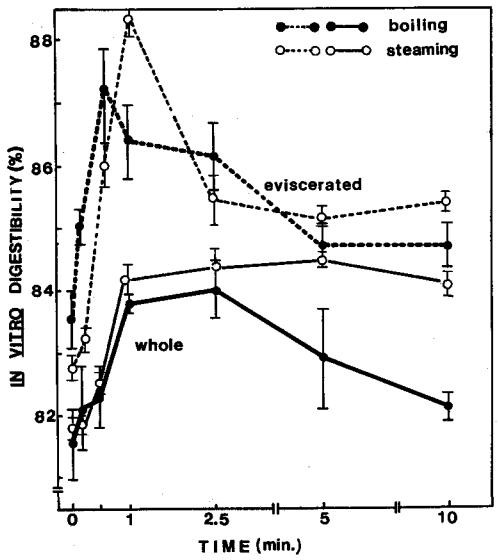


**Fig. 3. Changes in the *in vitro* digestibility of whole and eviscerated oyster after steaming for various times.**

3) Effect of steaming temperature on the *in vitro* digestibility and TIS content of oytser

Figures 4 and 5 show that the changes in the *in vitro* digestibility and TIS content resulting from different steaming temperatures. The *in vitro* digestibility was increased rapidly from raw state after heating to 88°C, but TIS content did not formed significantly. These results contrasted with the findings of Nomura (1953)[8] who reported that shellfish proteins were readily digested with

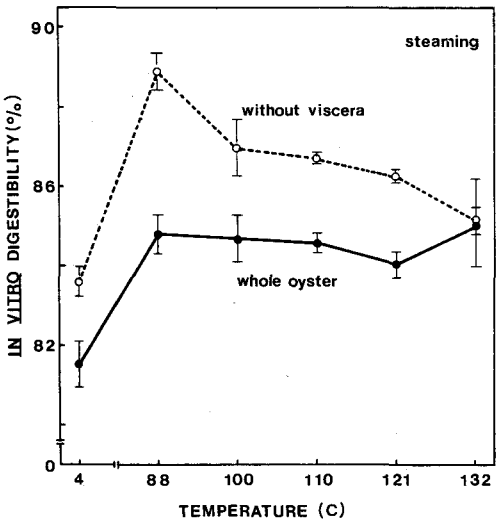


**Fig. 4. Changes in the *in vitro* protein digestibility of whole and eviscerated oyster as a result of differing steam temperature.**

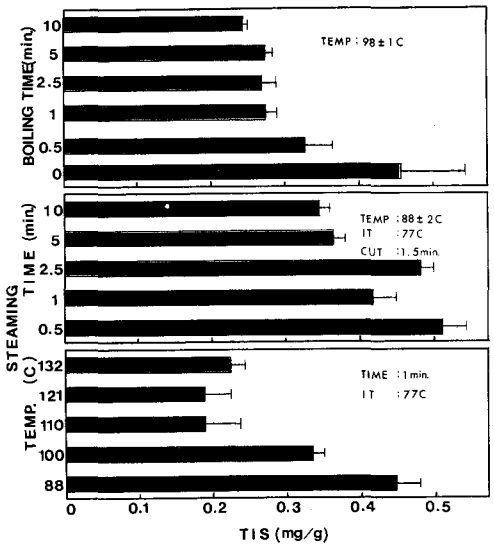


**Fig. 5. Effect of boiling time, steaming time and temperature, on the retention of TIS in whole oyster**

pancreatin in the raw stage than when cooked. The denaturation and enhancement of oyster protein digestibility was apparent at 88°C, but at higher temperatures, TIS content increased as the temperature rose.

### 3. Effect of Drying Method on the *In Vitro* Digestion and TIS Content of Peeled and Deveined Shrimp

Changes in the *in vitro* digestibility and TIS content of peeled and deveind shrimp was investigated. *In vitro* digestiblity was affected by the variation in the method of drying, so freeze dried shrimp had a higher protein digestibility compared to oven dried shrimp. The TIS content was lower for the freeze dried product than the other processed products. These results were in agreement with the observation made by Shrinivas et al. (1974)[46] who reported that freeze dried shrimp was more susceptible to proteolytic action(pepsin-trypsin-erepsin, pepsin-papain) compared to air dried shrimp (65-70°C. 8-10 hours). But these data were not in agreement with the variation in TIS content of oyster or squid as a result of different drying methods. However, the question arises to why the digestibility was affected to such a greater degree when compared to squid or oyster. This may be explained as following: 1) shrimp protein is more easily denatured and thus susceptible to enzyme hydrolysis than that of squid or oyster between the temperatures of 65°C and 100°C or 2) fat oxidation occurred more rapidly during the drying period due to the higher concentration (18.5%) of polyunsaturated fatty acids(Donovan 1982).[47] Others have reported that fat levels averaged around 10~11%[48~50] in shrimp(not considering seasonal and species variation), but the fat content of shrimp used in this study was 3.2%. Oven drying temperature and exposure to air brought about marked alteration in shrimp's fat, tending to render the shrimp less digestible than freeze dried samples. The deterioration of shrimp protein during oven and air drying was more serious than that for squid or oyster. Shrimp was more susceptible to quality deterioration drying than the other seafoods.

### 4. Changes in the *In Vitro* Digestibility and TIS Contents in Pollock under the Various Processing Conditions

1) *In vitro* digestion and TIS content of pollock versus exposure time to boiling and steaming

The effect of different boiling and steaming times on the *in vitro* digestibility and TIS content was studied. Pollock is used as a starting material for kamaboko, frozen minced meat(surimi) and textured fish protein concentrate(marine beef) in Japan and Korea (Suzuki 1981).[51] The maximum *in vitro* digestibility was obtained when pollock muscle was boiled for 5 minutes at 98±1°C (88.9%) and the TIS decreased throughout the boiling period(Fig. 6 and 8). Miller et al. (1965)[31] reported that *in vivo* protein digestibility of cod muscle was 96% when heated for 4.5 minutes at 99°C, but lowered to 90% when held at 105°C for 27 hours. Pollock protein exhibited marked differences in the heat denatured pattern, when compared to oyster(100°C, 30 sec) and squid(100°C, 1 min). TIS in pollock muscle was extracted into boiling water within five minutes. Digestibility increased up to 1 minute of steaming, but did not significant change from 1 min to 5 min. Progressive heat damage took place on continued steaming after 5 min. The difference in optimum time between steaming and boiling could be accounted for by differences in heat penetration rates. The heat treatment curves from these data showed that too much heat could be as detrimental to digestibility(and presumably protein availability) as could too little heat. As the protein was heated to extremes, polymerization resulted in a drop in protein digestibility possibly as a result of physical changes in protein structure which render the protein unavailable. The initial rapid drop in TIS content corresponded to the initial sharp increase in protein digestibility. The greater loss of TIS by boiling than by steaming was due to the fact that TIS of pollock was primarily in the water soluble fraction.

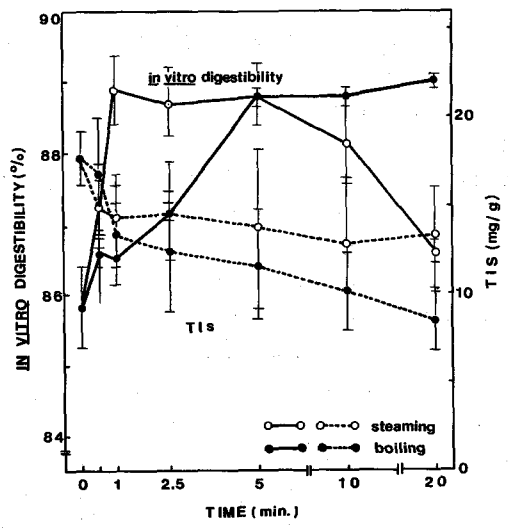


**Fig. 6. Variation in the *in vitro* digestibility and TIS content of pollock following the various boiling and steaming time.**

**Table. 5. Differences in the *in vitro* protein digestibilities and TIS contents of the peeled and deveined shrimp as a result of the drying**

| | Freeze dried | Vacuum dried | Oven dried |
|---|---|---|---|
| *In vitro* digestibility (%) | 89.3 | 86.8 | 84.8 |
| TIS (mg/g) | 19.33[R] | 17.03 | 15.63 |
| | 0.36[H] | 0.41 | 0.45 |

[R] Rhinehart method (1975)
[H] Hamerstrand method (1981)

2) Effect of steaming temperature on *in vitro* digestibility and TIS content of pollock muscle

The effect of various steam temperatures on the *in vitro* digestibility is shown in Fig. 7. The changes in TIS content of pollock subjected to various steam temperatures is presented in a histogram in Fig. 8. As illustrated in Fig. 7, the *in vitro* digestibility was increased when heated to 100°C, but decreasing when heated above 100°C. On the other hand, the loss of TIS content occurred up to 121°C, as seen in Fig. 8. Steamed pollock muscle's *in vitro* digestibility was higher than that for boiled meat (Fig. 6). Results were similar to those obtained by Nesheim and Carpenter(1976)[52] and Miller et al. (1965).[31] Steaming at 100°C gave an optimum susceptibility of pollock protein to enzyme hydrolysis even if the TIS content did not showed the

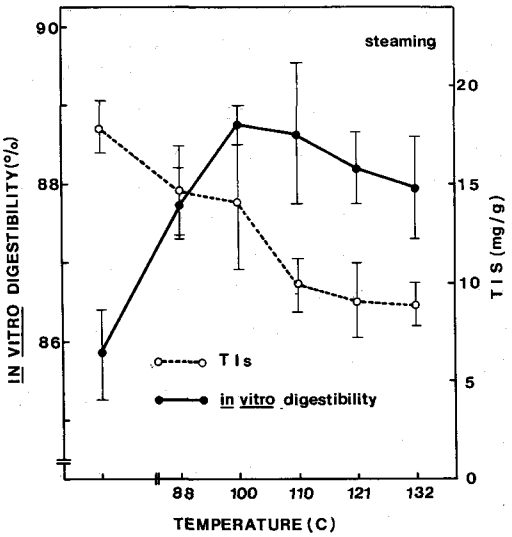


**Fig. 7. Effect of steaming temperature on the *in vitro* digestibility and TIS content of pollock.**

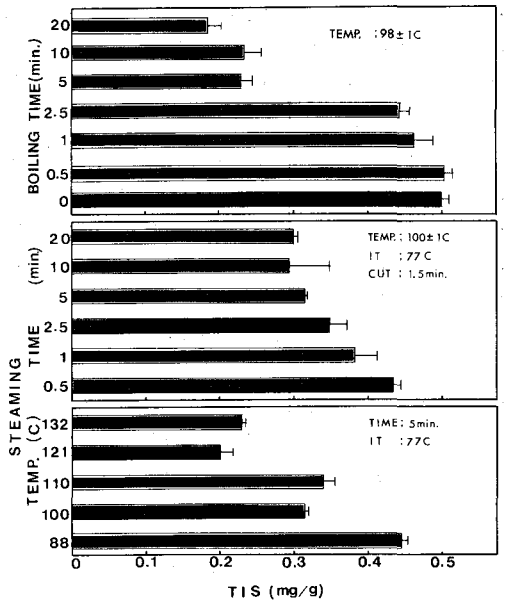


**Fig. 8. Changes in the TIS content of pollock subjected to the various heating times and temperatures.**

lowest value.

3) Determination of optimum drying method for pollock muscle

To obtain a dried pollock product with the highest digestibility possible, several methods of drying were used on the raw, boiled and steamed pollock. The comparative values for *in vitro* digestibility and TIS content are shown in Table 6. All of the oven and hot air dried pollock's digestibility dropped to below 85% while the TIS content showed the lowest value. The drop in digestibility of those samples occurred as the temperature or time exposure to air increased. The digestibility of the freeze dried samples increased with moderate heating(73°C, 18 hours for vacuum dried). The digestibility values fell as heating time was increased, indicating a high level of protein destruction or refolding. Richardson and Catsimpoolas(1979)[53] observed this refolding or protein polymerization effect of protein as denaturation by heat became excessive.

**Table. 6. Differences in the *in vitro* digestibilities and TIS content of pollock as a function of the drying process**

| | Raw | | | Boiled | | | Steamed | | |
|---|---|---|---|---|---|---|---|---|---|
| | *In vitro* dig. (%) | TIS(mg/g) R | TIS(mg/g) H | *In vitro* dig. (%) | TIS(mg/g) R | TIS(mg/g) H | *In vitro* dig. (%) | TIS(mg/g) R | TIS(mg/g) H |
| Freeze dried | 85.8 | 17.84 | 0.50 | 88.8 | 11.66 | 0.23 | 88.9 | 14.28 | 0.23 |
| Vacuum dried | 87.0 | 21.15 | 0.41 | 87.4 | 13.38 | 0.15 | 88.3 | 11.26 | 0.31 |
| Sun dried | 84.7 | 28.99 | 0.43 | 86.5 | 15.21 | 0.19 | 86.7 | 13.23 | 0.21 |
| Oven dried | 84.0 | 13.02 | 0.22 | 84.7 | 6.41 | 0.07 | 85.2 | 9.27 | 0.10 |
| Hot air blast dried | 84.7[a] | 11.13 | 0.25 | | | | | | |

[a] Alaska pollock (*Theragra chalcogramma*) from Korea
R Rhinehart method (1975)
H Hamerstrand method (1981)

They reported that a drop in digestibility following heat induced protein polymerization. The same results were given by Yanez et al. (1970)[54] who reported the true digestibility of hake was practically the same for freeze dried and dried at 105°C, but the digestibility of the more intensely heated (170°C) product was decreased by 25%. Freeze drying tend not to modify nutritive properties of protein. On the other hand, heat-drying causes damage that is proportional to temperature and length of heating(Miller et al. 1965).[31] Sundried (18~20°C, 24~28 hours) pollock has comparable *in vitro* digestibility with freeze dried pollock if it is boiled or steamed(86.5% for boiled and 86.7% for steamed). Jeong et al. (1978)[16] also reported that PPI of sundried flounder was not significantly lower than that of the freeze dried ones.

### 5. Changes in the *In Vitro* Digestibility and TIS Content during Frozen Storage

To determine the influence of frozen storage on the *in vitro* digestibility and TIS content of precooked and defatted seafoods, all samples placed in plastic containers covered with polyethylene film and stored at −20°C with free circulation of air around each container. As shown in Table 7, the digestibility values of oyster and shrimp fell about 4~5% as storage continued. TIS content of shrimp or defatted oyster were increased, indicating a high level of fat oxidation occurred and the oxidized fatty acid trapped protein which made resistant to enzyme hydrolysis. Almquist(1956)[9] noted the evidence of this phenomenon with California sardine stored at room temperature from 4 to 331 days. He observed the severe decrease of pepsin-digestible protein occurred in higher fat content samples. Squid showed comparatively little drop in digestibility and a little rise in TIS content even if its crude fat content was higher than the other samples. The higher crude fat content in squid meat could be derived from pigment which has not relation to fat oxidation. Sawant and Magar(1961a)[11] reported that the drop of PDR index of mackerel and pomfret during frozen storage was due to the loss in total amino acids which had been affected severely by unsaturated fatty acid level in samples. Morey et al. (1982)[55] reported the 9.4% drop in the *in vitro* protein digestibility of rockfish which was stored for 14 days in air was due to the decreased extractable protein. During the frozen storage, as shown in Table 7, digestibility and TIS content of low fat samples and defatted samples were not changed.

## Summary

In an attempt to determine the optimum heat treatment, the changes in TIS content and *in vitro* protein digestibility of squid, shrimp, oysterand pollock under various heating conditions were studied. The effect of drying method and cold storage on the *in vitro* digestibility and TIS content were also studied. Optimal boiling conditions were 1 min. for squid, 0.5 min. for oyster(eviscerated), 1 min.

**Table. 7. Changes in the *in vitro* digestibilities and TIS contents of precooked and freeze dried seafood samples stored at −20°C.**

| Sample | Heat treatment | Crude fat (%) | Storage time (months) 0 Dig. (%) | 0 TIS (mg/g) | 3 Dig. (%) | 5 Dig. (%) | 5 TIS (mg/g) |
|---|---|---|---|---|---|---|---|
| Squid | Steamed(100°C, 1 min.) | 11.96 | 88.4 | 0.44 | 88.5 | 87.6 | 0.55 |
| | Defatted after heating | | 89.3 | 0.15 | 88.4 | 87.4 | 0.19 |
| Oyster | Heated(88°C, 1 min.) | 9.10 | 84.6 | 0.44 | 80.2 | 79.3 | 0.69 |
| | Defatted after heating | | 90.3 | 0.20 | 87.6 | 86.5 | 0.22 |
| Shrimp | Commercial peeled and deveined | 3.18 | 89.3 | 0.36 | 88.1 | 85.5 | 0.42 |
| | Defatted after processing | | 90.6 | 0.14 | 89.4 | 88.7 | 0.15 |
| Pollock | Steamed(100°C, 2.5 min.) | 1.19 | 88.9 | 0.32 | 86.2 | 85.2 | 0.33 |
| | Defatted after heating | | 88.6 | 0.10 | 88.9 | 87.2 | 0.11 |

for whole oyster, and 5 min. for pollock. Steaming times that yieled products with the highest *in vitro* digestibility value were: 1 min. at 100°C for squid, 1 min. at 88°C for oyster and 1~2.5 min. at 100°C for pollock. All of freeze dried samples showed the highest *in vitro* digestibility value and sundried one were comparable to freeze dried samples except high fat level or noneviscerated samples. Fat content was the main inhibitory factor of the seafood enzymic digestion during processing and storage. The multi-enzyme assay, used to predict the quality change of dried seafoods stored in a cold room for long periods or raw seafoods treated with various heating methods, offers many advantages over the conventional methods of determining protein quality.

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