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Distribution of Trypsin Indigestible Substrate (TI) in Seafoods and Its Changes during Processing

2. Changes in TI and *In Vitro* Apparent Digestibility of Boiled and Dried Anchovy during Processing and Storage

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In order to study the optimal conditions of processing and storage for boiled and dried anchovy (*Engraulis japonica*) with high protein digestibility, the contents of trypsin indigestible substrate (TI) and *in vitro* apparent protein digestibility were determined. Peroxide value (PoV), TBA number and nonenzymatic brown pigments, that accounted for important antinutritional factors, were also measured and confirmed the relationship between those factors and formation of TI or *in vitro* protein apparent digestibility. The results were as follows;

Samples boiled for 5 minutes showed the lower content of TI than the other samples boiled for 0.5 min. or 1 min. Hot air dried products had a lower TI content in comparison with the other dried ones such as sun dried or freeze dried products. It was revealed that the lower temperature (8±1°C) did not affect to a great degree of forming TI and falling *in vitro* digestibility comparing to high temperature (26±1°C) during storage. The lowest TI content (0.173 *mg/g* solid) was noted in the samples for 5 minutes and then sun drying after 56 days storage at 9+1°C.

A rapid decrease of *in vitro* protein digestibility occurred within 0.5 min. of boiling and showed the value 85.3%. Freeze dried samples possessed the highest *in vitro* protein digestibility (85.9%), when compared to sun dried or hot air dried products.

Fat oxidation and nonenzymatic browning were proceeded with the various boiling times, drying methods and storing temperatures. It was noted that boiling for 5 minutes and freeze drying accelerate the fat oxidation significantly. More nonenzymatic brown pigments was developed in samples boiled for shorter time (0.5 min.) and that stored at high temperature (26±1°C) than the other products. Therefore, fat oxidation and nonenzymatic browning assumed to be a major inhibitory reaction in enzyme digestion and those might be an important role in forming TI in boiled and dried anchovy products during processing and storage.

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Introduction

It has heen repeatedly established that heat treatment of foods may cause a decreased digestibility and unavailability of amino acids, resulting in a lower nutritive value of protein. The extent of these changes is dependent upon the conditions and severity of the heat treatment(de Groot, 1963). This opinion has been supported by the many *in vitro* studies(Tanikawa and Suno, 1952; Almquist, 1956; Adachi et al., 1958; Sawant and Magar, 1961; Ford and Salter, 1966; Jeong et al., 1978; Ryu, 1983).

Especially in case of fish proteins that play an important role in the animal protein supplement in Korea, they are usually processed before being consumed for foods because they have a structural weakness and compositional characteristics resulting so many difficulties in prolonged storage.

Therefore, observation of optimal heat treatment conditions for processed fish proteins that can give higher digestibility and lower antinutritional substrates, such as trypsin indigestible substrate (TI), is important work for their efficient utilization.

As the same purpose in part 1 of this series (Lee et al., 1984), it was carried out the formation of TI and the changes of *in vitro* apparent digestibility of boiled and dried anchovy during processing and storage, in order to obtain the fundamental data on the nutritional value of that product which has been consumed and produced popularly in Korea (39,054 M/t, Yearbook of Fisheries in Korea, 1982). Peroxide value (PoV), TBA value and nonenzymatic brown pigment, those could be considered as antinutritional factors, were also measured and the relationship between those factors and TI or *in vitro* apparent protein digestibility was confirmed.

Materials and Methods

1. Samples

1) Fresh anchovy Anchovy(*Engraulis japonica*), 0.3-0.7 gram in average weight of individual and 3-5 cm in mean length, were caught in adjacent seas of Kosung, Kyung-nam, Korea on 27th, 1983.

2) Sun dried products The preparation of sun dried product was employed commercial procedure as follows. Thirty to fifty kg of fresh anchovy was purchased immediately after fishing on ship, and then boiled for 0.5 min., 1 min., and 5 min. in 25 *l* of 8% NaCl solution using open pan. The weight of sample used in each boiling trials was from 700 g to 800g. After boiling, anchovies were scored on the net screen placed at a height of 40 to 50 cm from ground and exposed to bright sun from 10 a.m. to 6 p.m. (max. temp., 33°C ; min. temp., 30°C) with occasional turning of fish. Fresh anchovy samples were also dried under the same conditions as boiled ones and used as control. Both sun dried samples (fresh and boiled) were placed in paper bag assembling commercial package with free circulation of air around each bag and stored at temperature, 9± °C and 26±1°C. Each lot was periodically samples by repeated mixing and ground to pass a 100 mesh screen.

3) Hot air dried products Fresh anchovies were iced on boad and brought to laboratory within 5 hours. Hot air drying was performed in hot air cross-flow dryer (Shirakawa, velocity, 3 m/sec.) at 55±5°C for 4hours. The conditions of storage and sampling were controlled as same as sun dried products.

4) Freeze dried products The transported anchovy samples were held at −30 °C for 6 hours and prepared the freeze dried samples by using Virtis freeze dryer for 24 hours at 1-10.2 *m* Torr. vacuum degree. Sampling and storing were carried out under the same conditions as described in case of sun dried ones.

2. Chemical Analyses

1) Proximate analyses : Moisture, nitrogen, curude lipid and crude ash were determined by the methods of AOAC(1980).

2) Apparent *in vitro* protein digestibility

The *in vitro* digestibility values of various anchovy samples were determined according to the procedure of AOAC(1982).

3) Trypsin indigestible substrate (TI) assay

The content of TI in all samples was determined using Rhinehart method described in the report of Ryu(1983) and the results were expressed in trypsin inhibitor equivalents, which equals the mg of purified soybean trypsin inhibitor (Sigma, 10,000 BAEE units/mg protein) per gram solid. The standard curve used in measuring TI content was shown in Figure 1. In addition, Hamerstrand's method(1981) was employed to determine the TI content quantitatively.

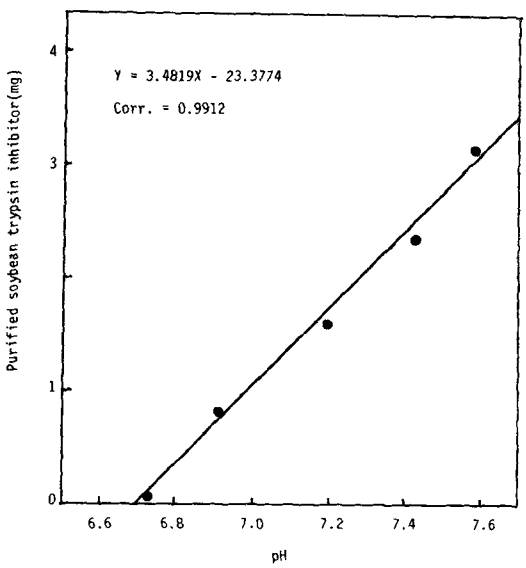

Fig. 1. Relationship of pH at 10 minute to purified soybean trypsin inhibitor (TI) concentration.

4) Peroxide value(PoV) and TBA value PoV was determined by the procedure of AOAC (1980) and TBA value was performed on the samples according to the procedure of Tarladgis(1960).

5) Nonenzymatic brown pigment The procedure introduced by Saltmarch (1979) was used in determination of nonenzymatic brown pigments.

Results and Discussion

1. Proximate composition of raw anchovy

Whole anchovy used in this study contained 74.5 g of crude protien (N×6.25) and 7.6 g of diethyl ether extractable lipid per 100g of solid. 15.9 g of total ash was measured in 100g of solid. The content of crude protein was higher than the results in earlier reports (Office of Rural Development in Korea, 1977), though the crude fat was severely lower than the result described in that report.

Therefore, those results indicated that the small size, as well as caught in summer season, so they contained lower fat content in comparison with the result cited above. Those fact could be supported by the suggestions of Jacquot (1961) and Shibata (1980) also. By the way, it seems that the whole anchovy is a good protein and mineral source in diet of oriental peoples from the results of proximate composition.

2. Effect of boiling times on the *in vitro* protein digestibility and TI content in anchovy products after 8 hour sun drying

Changes in the TI content and *in vitro* protein digestibility under various boiling time are presented in Figure 2. From the figure, there was not noticeable differences in digestibilty between boiling times and the values were revealed 85% around. But in case of control, it showed comparatively drop in digestibility about 7% than that of processed samples. It may be that desirable denaturation had been occurred and this leading to more susceptible to enzyme hydrolysis with the heat treatment in boiling salt solution within 5 minutes. On the other hand, TI was decreased steadily throughout the boiling period. It was suspected that the substances which enable to form TI, such as lipids or active carbohydrate residues, might be extracted into boiling salt solution

Table 1. Differences in the *in vitro* protein digestibilities and trypsin indigestible substrate(TI) contents of boied anchovy* as a function of the drying process

		Period of storage	Control	Sun drying	Hot air drying	Freeze drying
In vitro. dig. (%)		0 day	78.50	84.59	84.24	85.94
		56 days	71.94	79.74	79.54	82.97
TI(*mg/g* solid)	R	0 day	38.37	5.11	10.33	12.15
		56 days	49.69	19.77	18.38	20.67
	H	0 day	0.230	0.113	0.163	0.180
		56 days	0.370	0.228	0.225	0.238

* Anchovy was boiled for 1 minute and stored at 26±1°C after drying.
R: Rhinehart method(1975) H: Hamerstrand method(1981)

within 5 minutes. This tendency of decreasing TI was found to be in close agreement with the effect of boiling times on pollock fillet that reported by Ryu(1983). Therefore, marketable and desirable sun dried anchovy products with high digestibility could be made with boiling for from 1 minute to 5 minutes, from the results revealed in Figure2.

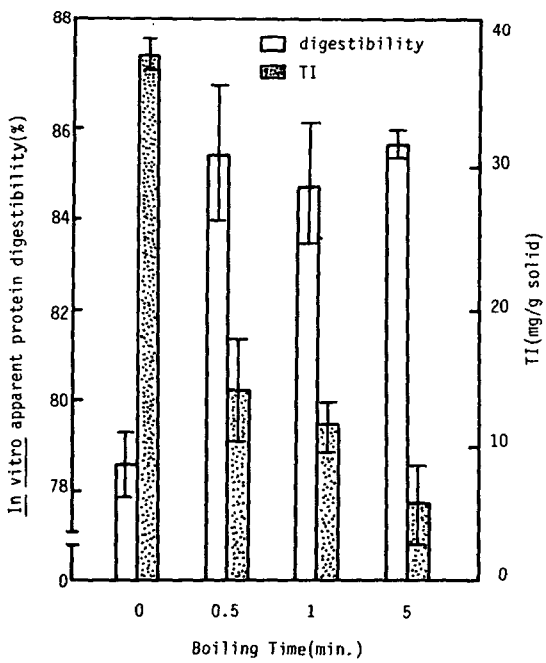

Fig. 2. Effect of boiling times on the content of TI and *in vitro* protein digestibility of anchovy. TI was measured using Rhinehart method(1975).

3. Determination of optimal drying method for dried anchovy products

In an attempt to study the effect of various drying conditions on the nutritional value of boiled anchovy, three methods of drying were tried on anchovy boiled for 1 minute, which was produced under the same conditions as Korean traditional anchovy production. The comparative results of the *in vitro* protein digestility and TI content in two kinds of stored samples (initial and 56 days stored at 26±1°C) were shown in Table 1. At initial stage of storage, the digestibility of freeze dried anchovy was higher than that of hot air dried and sun dried ones, while the results, that the *in vitro* digestibilty of sun dried fish meats were not changed markedly comparing with freeze dried ones, could be seen in the reports of Jeong et al. (flounder, 1978) and Ryu (squid, shrimp and pollock, 1983). After 56 days storage at 26±1 °C, the 5 % drop of digestibility was noted in both sun and hot air dried products, while it showed some little drop (3 %) of digestibility in freeze dried ones. It was also suggested that the sun dried products held for long time (56 days) was also able to give lower digestibility as the severe heat damaged products (hot air dried products). Instead, the TI content was higher for freeze dried product than the other dried ones at initial stage. Those results might be due to the hygroscopic structure formed in freeze dried product

Table 2. Changes in nonenzymatic brown pigments, TBA number and peroxide value (POV) of various boiled anchovies during storage at 26±1°C**

Storage time (days)	TBA number(OD/*g* solid)				PoV(meq./*kg* lipid)				NEB*(OD/*g* solid)			
	Boiling time(minutes)				Boiling time (minutes)				Boiling time (minutes)			
	C	0.5	1	5	C	0.5	1	5	C	0.5	1	5
0	0.098	0.164	0.179	0.188	9.7	13.1	15.4	19.4	0.156	0.049	0.050	0.049
14	0.216	0.343	0.456	0.467	19.3	25.6	32.7	34.5	0.213	0.058	0.064	0.057
35	0.373	0.464	0.514	0.543	53.3	44.8	60.5	64.4	0.276	0.073	0.071	0.071
56	0.459	0.418	0.524	0.618	24.9	26.5	29.8	34.6	0.338	0.079	0.076	0.075

*NEB: nonenzymatic brown pigments C: control(fresh and sun dried)
**All anchovies were sun dried after boiling.

resulting the increased active surface area and then TI was determined more easily than the other products. But after 56 days storage, there was negligible variation of TI within dried products, and the level of TI in all dried and stored samples were half of that in control.

4. Changes in nonenzymatc brown pigments, TBA value and peroxide value (PoV) of various boiled anchovy products during storage at 26±1°C

To assess the deterioration of boiled anchovy products, rancid fat (expressed as TBA number and PoV) and nonenzymatic brown pigments were determined, and the results were give in Table 2. On the TBA number, there was not significant differences within boiled products while control showed lower value at initial stage of storage. Oxidative rancidity occurred remarkably in all samples along with storing period, especially in control. It suggested that those continuous heat treatment, such as boiling, sun drying and high aging temperature proceeded fat oxidation rapidly even if the fat had been extracted and the amount of fat content were lowered with boiling process. PoV of all samples showed the similar tendency as the changes of TBA number but there was sharp drop of that value between 36 days and 56 days storage. The results were accordance with the published report of Han et al. (1973). Nonenzymatic browning through the Maillard reaction is a major deteriorative reaction in dried products(Labuza et al., 1972) and identification of those pigments which formed during storage and processing would be useful in establishing the desirable methods of preservation and heat treatments. From the results given in Table 2, control had a great amount of brown pigments comparing with all sun dried products after boiling. As the boiling time prolonged to 5 min., development of nonenzymatic brown pigment was decreased. It was true that the extractable nonproteinous nitrogen and free proteinous nitrogen participated in forming the brown pigments (Fujimoto, 1968; Suyama, 1970; Lee et al., 1982). The reason that brown pigments decreased as boiling time increased was most likely due to the water extractable sugars and nitrogenous compounds which play an important role in nonenzymatic browning (Warmbier et al., 1976; Kim et al., 1982).

5. Effect of drying methods on the deterioration of boiled anchovy

In order to obtain a dried anchovy products with good quality, three kinds of drying methods performed on the anchovy boiled for 1 minute. As shown in Table 3, the patterns of developing PoV, TBA number and nonenzymatic brown pigment were almost the same that during storage, so that the period prolonged, the values were greater. But there were some differences within drying methods as the TBA number of freeze dried products showed higher than those of sun dried or hot air dried ones. Those were thought that heating the fish muscle caused a

Table 3. Effect of drying methods on the deterioration of anchovies boiled for 1 minute during storage at 26±1°C

Storage time (days)	TBA number(OD/*g* solid)				PoV(meq./*kg* lipid)				NEB*(OD/*g* solid)			
	C	S	H	F	C	S	H	F	C	S	H	F
0	0.098	0.179	0.173	0.192	9.7	15.4	14.9	13.9	0.156	0.050	0.056	0.036
14	0.216	0.456	0.446	0.462	19.3	32.7	29.4	38.2	0.213	0.064	0.059	0.058
35	0.373	0.514	0.501	0.518	53.3	60.5	62.3	62.7	0.276	0.071	0.072	0.067
56	0.459	0.524	0.416	0.554	24.9	29.8	36.9	37.9	0.338	0.076	0.076	0.076

*NEB: nonenzymatic brown pigment C: control(fresh and sun dried)
S: sun drying H: hot air drying F: freeze drying

some drop in TBA number and/or nonenzymatic brown pigments; whether that is a polymerization reaction or a reaction between malonaldehyde and some portion of the fat or protein has not been ascertained as mentioned by Sinnhuber and Yu(1957). It was also suspected that there was a possibility for a high content of nonenzymatic brown pigments occurred in hot air dried products owing to accelerating browning under high temperature condition (55°C) as the report of Warmbier et al. (1976).

6. Effect of storing temperature on the protein quality of boiled and sun dried anchovy

Changes in the *in vitro* protein digestibility, TI, PoV, TBA number and nonenzymatic brown pigments of boiled and sun dried anchovy were tried. The results were presented in Table 4 for 5 minutes boiled and Figure 3 for 1 min. boiled

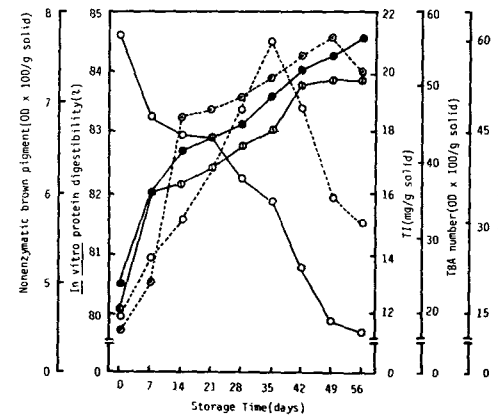

Fig. 3. Changes in the *in vitro* protein digestibility (○—○), TI(●—●), PoV(○—○, TBA number (⊙—⊙) and nonenzymatic brown pigments (◑—◑) of anchovy boiled for 1 minute during storage at 26±1°C.

Table 4. Influence of storage temperatures upon the quality of anchovies boild for 5 minutes and then sun dried.

	Storage time (days)	Stored at 26±1°C		Stored at 9±1°C	
		Control	5 min.	Control	5 min.
In vitro dig. (%)	0	78.50	85.27	78.50	85.27
	56	71.94	80.64	73.54	82.27
TI(*mg*/*g* solid) R	0	38.37	5.11	38.37	5.11
	56	49.69	15.91	48.83	15.50
H	0	0.230	0.113	0.230	0.113
	56	0.270	0.174	0.264	0.173
PoV(meq./*g* lipid)	0	9.7	19.4	9.7	19.4
	56	24.9	34.6	32.0	43.1
TBA(OD/*g* solid)	0	0.098	0.188	0.098	0.188
	56	0.259	0.618	0.183	0.568
NEB*(OD/*g* solid)	0	0.156	0.049	0.156	0.049
	56	0.338	0.075	0.215	0.054

NEB*: nonenzymatic brown pigments
R: Rhinehart method(1975) H: Hamerstrand method (1981)

one. Those two kinds of samples were stored at 26±1 °C and 9±1 °C, and the results were gained after 56 days storage. As shown in table and figure, digestibility fell about 4—5 % as storing period prolonged for both boiled samples, while 3 times of TI in samples at initial stage were formed after 56 days measured by Rhinehart method. The high aging temperature resulted that higher PoV, TBA number and nonenzymatic brown pigment concentration than low aging temperature. That might be indicated the oxidized fatty acids trapped protein to resist enzyme hydrolysis through a severe fat oxidation as mentioned by Roubal and Tappel (1966) and Ryu (1983). Nonenzymatic brown pigment was an important factor influenced on the decreasing digestibility and forming TI during storage also.

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魚貝類의 Trypsin活性 沮害物質의 分布와 加工中의 變化

2. 煮乾멸치 加工貯藏中의 Trypsin活性 沮害物質과 *In Vitro* Apparent Digestibility의 變化

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소화율이 우수한 煮乾멸치의 加工條件을 究明하기 위하여 煮熟時間과 乾燥方法을 달리하여 製造한 멸치를 低溫(9±1°C) 및 高溫(26±1°C)에서 貯藏했을 때의 trypsin 活性沮害物質(TI)과 試驗管的 蛋白質槪算消化率의 變化를 측정하고, 동시에 이에 영향을 미친다고 생각되는 脂肪의 酸敗 및 褐變度의 변화를 分析 檢討하였다.

煮熟時間에 따른 TI의 生成정도는 5分間 煮熟한 試料가 가장 적었고, 乾燥方法을 달리 했을 때는 熱風乾燥試料가 日乾 및 凍結乾燥試料보다 낮은 값을 나타내었다. 그리고, 低溫(9±1°C)에서 貯藏한 試料는 高溫(26±1°C) 貯藏試料보다 낮은 값을 보였으며, 5分間 煮熟 後 天日乾燥한 試料의 경우는 低溫貯藏 56日에 0.173 *mg/g*의 TI를 나타내어 全 試料 中에서 가장 낮은 값을 보였다.

소화율은 0.5分間 煮熟하여 天日乾燥한 경우는 85.3%, 1分間 煮熟 後 凍結乾燥했을 때 85.9%로 가장 높았으며, 低溫貯藏(9±1°C)한 것이 高溫貯藏(26±1°C)한 것 보다 감소정도가 적어 貯藏 56日 後에도 2.5% 밖에 減少하지 않았다.

脂肪의 酸敗는 煮熟條件別로 볼 때 5分間 煮熟한 試料에서, 乾燥方法別로는 凍結乾燥한 試料에서 가장 促進되었고, 褐變은 生試料 또는 0.5分間 煮熟하여 日乾한 뒤 高溫(26±1°C)에서 貯藏한 試料가 가장 促進되었다.

소화율은 저장기간이 경과함에 따라 저하하는 반면, TI의 生成程度 脂肪의 酸敗정도 및 非酵素的 褐變度도 增加하였다. 結果的으로 脂肪의 酸敗 및 非酵素的 褐變反應이 TI의 生成 및 소화율의 저하에 깊이 관여하는 것으로 생각되었다.