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

1. Distribution and Post-mortem Changes of TI in Fish Muscle

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魚貝類의 Trypsin活性 沮害物質(TI)의 分布와 加工 中의 變化

1. 魚肉 中의 TI의 分布와 鮮度低下에 따른 變化

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要 約

한국 근해에 서식하고 있는 各種 魚類 中 活魚 및 鮮魚상태의 赤色肉魚 8種(곱상어, 전갱이, 우레기, 고등어, 눈퉁멸, 전어, 방어, 돌고기), 白色肉魚 8種(볼락, 쥐치, 흑돔, 복어, 노래미, 붕장어, 가자미, 조기)을 선택하여 鮮魚肉 中에 있어서의 TI(trypsin활성저해물질, 혹은 trypsin indigestible substrate)의 존재 유무를 확인하고 소화율 정도를 알아 보았다. 또한 이들 선어를 저온저장(−5°C)하여 선도저하에 따른 TI함량 및 소화율의 변화와의 관계를 알아보기 위해 VBN 및 TBA value도 함께 測定하였으며 그 結果는 다음과 같다.

赤色肉어류의 TI함량은 어종별에 따라 다소 차이를 나타내고 백색육어류는 비슷한 분포를 나타내고 있었으며 전체적으로 보아 백색육의 TI함량이 적색육어류의 경우보다 높게 나타났다. 그러나 소화율은 어종별에 따라 별 차이를 내지 않은 83~88 %를 나타내었다.

부위별에 따른 TI함량을 Hamerstrand방법으로 측정하였을 때, 내장에 TI함량이 가장 높았고(0.30 mg/g) 표피에는 거의 없었으며 血合肉이(0.21 mg/g) 普通肉(0.15 mg/g) 보다 다소 높게 나타났다.

저온저장 (−5°C) 중에 있어서의 TI함량 및 소화율변화를 선도저하 및 지방산화와 함께 알아본 결과 저장기간이 경과함에 따라 TI, VBN, TBA value는 증가의 경향을 보였고 소화율은 감소의 경향을 나타내어 선도저하나 지방산화에 의해 상당한 영향을 받음을 알 수가 있었으며 특히 지방함량이 소화율에 가장 많은 영향을 미치는 것으로 나타났다.

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Introduction

Seafoods, mainly fish and shellfish, are generally inexpensive compared with other protein foods. Nevertheless, many consumers and even workers in field of human nutrition continue to regard fish only as a substitute for meat, to be introduced into the menu because of its lower cost, easier availability, or culinary value.

From a nutritional point of view, seafoods have been considered to highly nutritive depending mainly on their levels of the essential amino acid(EAA) and digestibility. A lots of medical surveys have also demonstrated the adequacy of seafoods as the principal source of dietary protein, and repeated and thorough laboratory investigations have confirmed these empirical findings. But as to harvesting, storing and processing, the situation of seafoods greatly differ from that of livestocks. For instance, seafoods bear many difficulties in consuming as foodstuffs due to their high perishability from reducing freshness and undesirable changes, such as fat oxidation, nonenzymatic browning and deterioration caused by microorganisms.

By the way, seafoods are consumed by far-eastern peoples in Japan and Korea as raw state ("sashimi") which has been prepared using traditional recipe. Therefore, it is necessary to evaluate the nutritional value of proteins in raw seafoods and to elucidate the antinutritional factors, such as enzyme indigestible substrates, before consumed.

Many investigators have evaluated the nutritive value of seafood proteins using the amino acid profiles, by means of chemical, microbiological, and biological techniques. However, it is true that lots of data, offered by many researchers, could not be correctly compared one another and be lacked replication of the results owing to the experimental difficulties such as long time and high cost. On the purpose of assessing the nutritional quality of fish proteins, a number of authors have studied and designed the *in vitro* protein digstion of fish enzymes[1~10] since White and Crozier[11] to overcome the difficulties in chemical or biological assays that mentioned above. Satterlee et al[12]. measured accurate *in vitro* apparent protein digestibility of salmon and tuna using four enzyme technique that had been modified from the multienzyme automated assay of Hsu et al[13]. Recently, some researchers[14~16] estimated the *in vitro* apparent protein digestibility of a few kinds of fish meat(raw and processed products) using four enzyme assays.

Very little researchers[17~19] have been reported the presence of trypsin inhibitors or trypsin indigestible substrates as antinutritional factors influencing the quality of fish proteins, yet a large amount of researches have been performed on the trypsin inhibitors in plant proteins.

In this study, it was performed the distribution of trypsin indigestible substrate(TI) and the apparent *in vitro* protein digestibility in eight species of dark-fleshed fishes and eight species of white-fleshed fishes to obtain the fundamental data on the nutritional value of protein for fresh fishes that be consumed in Korea popularly.

Materials and Methods

1. Sample Items Used

1) Raw Fishes

The 16 species of fishes used in this study were caught from July to September in 1983 and divided into two groups as dark and white-fleshed fishes. All of them were purchased from Pusan Cooperative Fish Market and Jagalchi Fish Market of Pusan as "live" or could be judged as "good" by sensory evaluation[20]. After purchasing, all samples were quickly chilled to 5°C. Table 1 summerizes the samples analyzed.

2) Stored Fish Samples

Samples used for frozen storage were yellow corvenia(*Nibea aliflora*) and file fish (*Navodan modestus*) for white-fleshed fishes. Two species of round fish, pacific mackerel(*Scomber japonicus*) and striped bonito(*Pungtungis herzi*), were also used for frozen storage. All of them were stored

Table 1. Summary of sample analyzed

Sample	Species	State of sample	Length (cm)	Weight (g)
Horse mackerel	*Trachurus japonicus*	Fresh	20	70
Pacific mackerel	*Scomber japonicus*	Fresh	35	150
Round herring	*Etrumeus micropus*	Fresh	25	60
Sole	*Verasper variegatus*	Live	30	60
Gold porgy	*Semicossyphus reticulatus*	Live	30	80
Rock trout	*Agramnus agramnus*	Live	18	40
Sea eel	*Astronger myriater*	Live	40	60
File fish	*Navodan modestus*	Live	25	40
Dog fish	*Squalis acanthias*	Live	40	90
Rock fish	*Sebastes inermis*	Live	20	30
Gizzard shad	*Konosirus punctatus*	Live	16	20
Uregi	*Brachymystax coregonoides*	Live	25	40
Yellow corvenia	*Nibea albiflora*	Live	35	100
Putter	*Fugu zanthopterus*	Live	20	80
Yellow tail	*Seriola quinquerodiata*	Live	30	120
Striped bonito	*Pungtungis herzi*	Live	30	100

at −10°C for a month.

2. Chemical Analyses

1) Approximate Analyses

Moisture content was determined by drying overnight in a vacuum oven at 105°C (27 inches Hg), total ash by the procedure of AOAC[21], total nitrogen by the micro-Kjeldahl method of AOAC[22], and crude fat by Soxhlet extraction method.

2) Volatile Basic Nitrogen(VBN) and Thiobarbituric Acid(TBA) Value

VBN was determined by micro-diffusion technique[23] and TBA value was performed on the samples according to the procedure of Taradgis[24].

3) Assay of K-value

In order to observe the changes of freshness that occurred in sampling or storing period, nucleotides and their derivatives were extracted from ordinary muscle in fish samples, and those were fractionated through Dowex 1×4 Cl^-(400 mesh) column. Freshness was expressed as K-value described by Kobayashi and Uchiyama[25].

4) Apparent *In Vitro* Protein Digestibility Assay

The *in vitro* protein digestibility values of the various fish samples were determined according to the procedure of AOAC[26].

5) Trypsin Indigestible Substrate(TI) Assay

The content of TI in all samples was determined using the Rhinehart method described in the reports of Ryu[19] 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 sample (dry basis). The standard curve

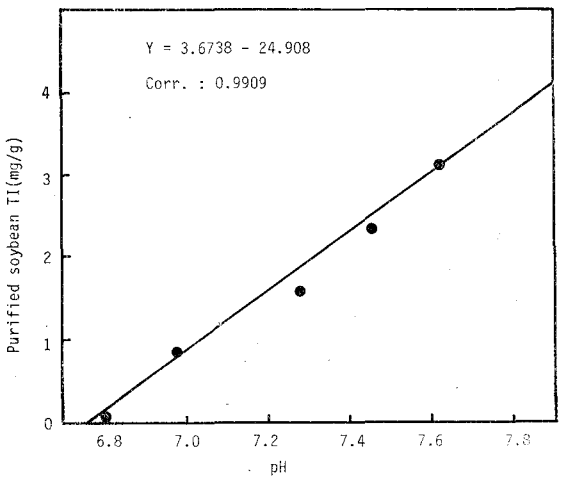

Fig. 1. Relationship of pH at 10 min. to purified soybean trypsin inhibitor concentration.

used in measuring TI content was shown in Fig. 1. In addition, Hamerstrand procedure[27] was also employed to determine the TI content quantitatively.

Results and Discussion

1. Proximate Composition of Fish Samples

As shown in Table 2, moisture contents were about 75 % around for all dark-fleshed fishes except dog fish showed about 81 % and white-fleshed fishes generally contained more moisture from 75 to 80 % than those of dark-fleshed fishes. All the fish samples used in this study varied in nitrogen content from 16 to 23 % and there was not significant difference between white and dark-fleshed fishes. Dark-fleshed fishes contained between 2.1 % (gizzard shad) and 11 % (dog fish) of crude fat, while it showed the value from 6.0 % (yellow corvenia) to 0.3 %(file fish and putter) for white-fleshed fishes. It was reasonable to assume that the great difference in fat content in dark-fleshed fishes is due to the seasonal variation as mentioned by previous investigators[28,29].

Table 2. Proximate composition of fish meat

Sample	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude ash (%)
White-fleshed fishes				
Rock fish	71.66	22.35	2.6	1.4
File fish	82.20	17.35	0.4	1.5
Gold porgy	72.68	23.62	3.1	1.1
Putter	78.47	19.87	0.3	1.0
Rock trout	79.50	17.42	1.1	1.4
Sea eel	74.50	16.23	4.0	1.7
Sole	75.49	20.85	2.0	1.2
Yellow corvenia	80.11	19.81	6.0	1.4
Dark-fleshed fishes				
Dog fish	80.72	21.02	11.0	1.6
Horse mackerel	73.18	18.02	5.2	1.4
Uregi	75.74	18.75	2.4	1.8
Pacific mackerel	75.28	21.14	4.0	1.7
Round herring	75.52	20.44	2.7	1.8
Gizzard shad	70.47	19.18	2.1	2.3
Striped bonito	73.19	23.96	2.8	1.9
Yellow tail	76.94	22.14	3.0	1.4

2. Freshness of Sample

The term freshness is used rather than spoilage because a measurement of freshness implies that the product may still be marketable or edible whereas spoilage implies that the product is no longer edible or spoiled. In this study, the freshness of all samples were indicated as VBN content and K-value, and the results were shown in Table 3. Although the difference of freshness could be revealed between species, most of samples showed the value of 10 mg/100 g below for VBN and 10.5 below for K-value. Comparing with the proposed range of freshness[25] for raw fish, the freshness of all samples could be considered as "good" state respectively. The fish samples purchased as "fresh" such as dog fish, horse mackerel and round herring showed lower freshness than the "live" samples. But in case of yellow corvenia, its freshness was comparable with "fresh" samples even if it was purchsed as "live". It was thought that the higher fat content in yellow corvenia than the other fish samples, as shown in Table 2, had been played

Table 3. Contents of volatile basic nitrogen(VBN) and K-value of samples

Dark-fleshed fishes	(VBN (mg/100 g)	K-value	White-fleshed fishes	VBN (mg/100 g)	K-value
Dog fish	12.19	14.48	Rock fish	10.24	9.66
Horse mackerel	13.02	10.11	File fish	6.81	7.57
Uregi	6.14	8.26	Gold porgy	7.59	9.57
Pacific mackerel	13.53	13.22	Putter	9.80	5.64
Round herring	13.19	7.08	Sea eel	4.02	6.41
Gizzard shad	8.74	7.08	Rock trout	7.33	8.64
Striped bonito	6.67	6.92	Sole	7.51	6.74
Yellow tail	7.95	9.12	Yellow corvenia	10.88	11.41

important role in the rapid drop of its freshness.

3. Distribution of TI Content and Apparent *In Vitro* Protein Digestibility

As shown in Table 4, TI content in dark-fleshed fishes ranged from 0.02 mg/g for round herring to 0.17 mg/g for dog fish while that in white-fleshed ones ranged from 0.10 to 0.26 mg/g using Hamerstrand procedure[27] and variation of TI content within species also noted using the Rhinehart procedure described in Ryu's reports[19], which contained the results of TI content in various freeze dried seafoods using both procedures. As for fresh fishes, white-fleshed fishes had a tendency to contain more TI, especially in case of file fish, than dark-fleshed ones respectively. There was not appreciable variations of *in vitro* protein digestibility of fresh fishes within species ranged from 83 % to 88 %, but the digestibility of white-fleshed fishes was higher than that of dark-fleshed ones generally. This might suggests that 1) the degradation rate of tissue in white-fleshed fishes by enzymes is faster than that of dark-fleshed fishes owing to those weak structure of meat and it enhance the digestibility and that 2) the antinutritional factors, such as

Table 4. Apparent *in vitro* protein digestibility and contents of trypsin indigestible substrate(TI) in fish samples

Sample	TI (mg/g sample)		*In vitro* protein digestibility(%)
White-fleshed fishes			
Rock fish	15.05	0.12	86.40
File fish	20.82	0.26	88.65
Gold porgy	17.60	0.12	83.01
Putter	15.92	0.11	83.01
Rock trout	18.65	0.22	84.36
Sea eel	17.86	0.19	85.49
Sole	16.30	0.11	87.07
Yellow corvenia	17.88	0.16	85.72
Dark-fleshed fishes			
Dog fish	17.49[R]	0.17[H]	85.04
Horse mackerel	11.86	0.02	85.94
Uregi	16.55	0.10	85.27
Pacific mackerel	15.54	0.11	85.72
Round herring	12.28	0.02	84.37
Gizzard shad	13.87	0.08	83.46
Striped bonito	12.24	0.09	84.42
Yellow tail	15.14	0.12	85.27

R: Rhinehart method[19] H: Hamerstrand method[27]

lipid protein trapped complexes were formed during the period of sample preparation or white-fleshed fish meat had a larger sarcoplasmic proteins that can inhibit enzyme degradation.[18] In summerizing, it could be said that the *in vitro* protein digestibliity of white-fleshed fishes is higher than that of dark-fleshed ones as reported by previous authors[15,16,19].

4. Anatomical Difference in TI Content

Within the body, and also between individual specimens, the composition of fresh tissue varies with the anatomical location, it could be expected that their protein nutritional value is also varies with different parts of fish. Therefore, to elucidate the distribution of TI, pacific mackerel, one of dark-fleshed fishes, was splitted into various parts such as gills, viscera, dark muscle and ordinary muscle. As shown in Table 5, TI content was 0.20 mg/g above in gill and dark muscle while ordinary muscle contained 0.15 mg/g by Hamerstrand procedure[27]. Gills had a generally high TI content measured by both methods. It could be explained that the enzyme activity was reduced by a large amount of chromoprotein in gills. On the other hand, TI content in dark muscle was higher than that in ordinary muscle. It was reasonable to assume that the higher TI content in dark muscle was derived from the plentiful fat in dark muscle resulting the formation of TI as reported in previous study[30]. It was revealed that viscera had a most abundant TI comparing with the other parts and that results was similar to the reports of other investigators[17,19].

5. Changes in TI content and Apparent *In Vitro* Protein Digestibility during Storage at −10°C

In order to determine the influence of frozen

Table 5. Contents of trypsin indigestible substrate (TI) in the parts of the fresh pacific mackerel

Parts	Crude protein (%)	Crude fat (%)	TI(mg/g sample)	
			R	H
Gills	10.11	1.50	22.46	0.25
Viscera	14.41	5.94	26.57	0.30
Ordinary muscle	21.04	3.54	14.51	0.15
Dark muscle	14.71	9.94	23.66	0.21
Skin	8.20		4.80	trace

R: Rhinehart method[19] H: Hamerstrand method[27]

Table 6. Changes in TI content and apparent *in vitro* protein digestibility of white and dark-fleshed fishes during frozen storage

Sample	Period(days)					
	0	5	10	15	20	25
White-fleshed fishes						
Yellow corvenia						
Digestibility(%)	84.7	84.2	84.0	83.8	83.9	81.6
TI(mg/g)*	15.5	18.3	18.4	19.7	21.3	22.5
File fish						
Digestibility(%)	84.1	82.5	83.0	82.2	80.3	79.2
TI(mg/g)*	19.6	21.6	22.3	23.6	25.0	26.2
Dark-fleshed fishes						
Pacific mackerel						
Digestibility(%)	85.1	84.2	82.3	80.0	80.9	79.0
TI(mg/g)*	14.2	16.0	16.2	16.4	18.4	20.3
Striped bonito						
Digestibility(%)	85.2	83.6	81.2	80.6	80.0	79.8
TI(mg/g)*	15.9	16.7	20.6	21.6	22.2	23.4

* Results were determined using the procedure described by Ryu[19]

storage on the *in vitro* digestibility and TI content of fresh fishes, two white-fleshed fishes (yellow corvenia, and file fish) and two dark-fleshed fishes(pacific mackerel and striped bonito) were stored at −10°C for a month. As shown in Table 6, the results showed the inverse relationship which exists between the degree of digestibility and TI content. Namely, 2 times above of TI was formed in fresh fishes during frozen storage on the basis of an initial stage. Especially in case of file fish, a significant TI(5.5 times on the basis of an initial stage)was formed after one month frozen storage. *In vitro* protein digestibility of white-fleshed fishes was not altered drastically but that of dark-fleshed fish fell about 6~8 % as storage period prolonged to one month.

6. Relationship between Freshness, TI and Apparent *In Vitro* Protein Digestibility during Frozen Storage

Changes in VBN, TBA value, TI content and digestibility of yellow corvenia, file fish, pacific mackerel and striped bonito during frozen storgage at −10°C and the results were illustrated in Fig. 2 and 3. A rapid freshness (expressed as VBN content) drop was found along the all period of frozen storage and decrease of *in vitro* digestibility was also noted. If the freshness of protein foods is decreased, it has been considered that enzyme can do more easily and then digestibility may be increased due to the weakness or susceptible state of protein structure, but protein can have more chances to trap with lipid or the other antinutritional substrates so that digestibility could be drop on contrary. In addition, as fish meat started its degradation through autolysis after severe drop of freshness, protein degraded into amino acids, the amount of substrate that enable to be attacted by proteolytic enzymes will be reduced and then the *in vitro* digestibility which is measured by four proteolytic enzymes will drop also. As illustrated in Fig. 2 and 3, the increase in TBA value of white-fleshed fish was negligible, while that of dark-fleshed fishes was considerable[31,32]. The severe increase of TI

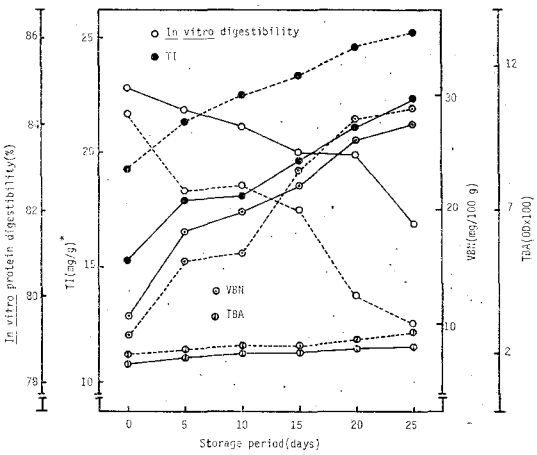

Fig. 2. Relationship between the apparent *in vitro* digestibility and TI content, VBN and TBA valut of the file fish(···) and yellow corvenia(—) during frozen storage at −10°C.
*TI contents were determined by Rhinehart method[19]

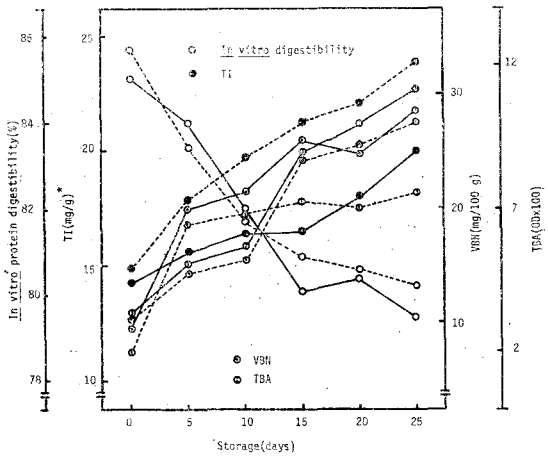

Fig. 3. Relationship between the apparent *in vitro* digestibility and, TI content, VBN and TBA value of the pacific mackerel (—) and striped bonito (···) during frozen storage at −10°C.
*TI contents were determined by Rhinehart method[19]

content noted in all case of fish samples but *in vitro* digestibilities were drop, especially in dark-fleshed fishes, during frozen storage. It could be explained that lipids in fish meat cause the

decrease in digestibility due to development of indigestible protein as demonstrated the previous reports[19,33].

Summary

To obtain the fundamental data on the nutritional value of protein for fresh meat, it was performed the distribution of TI(trypsin indigestible substrates) and the apparent *in vitro* protein digestibility in 8 species of dark-fleshed fishes and 8 species of white-fleshed fishes which were consumed in Korea popularly. It was also investigate the changes in VBN and TBA value during frozen storage at −10°C on the purpose of assaying the antinutritional factors that affect on apparent *in vitro* protein digestibility or TI forming.

TI content in dark-fleshed fishes were varied with their species, ranged from 0.02 to 0.17 mg/g. using the method by Hamerstrand, while that in white-fleshed fishes was almost same, ranged from 0.10 to 0.26 mg/g.

For all the fresh fish samples, however, the apparent *in vitro* protein digestibility were showed the value from 83 to 88%. In comparison with the parts of pacific mackerel, viscera had the most abundant TI content as much as 0.3m g/g, while a trace was noted for skin and dark muscle had more TI content than odrinary muscle based on the method by Hamerstrand.

The apparent *in vitro* protein digestibility for all samples was dropped but the changes of VBN and TBA were revealed the similar tendency with the increasing TI content during frozen storage at −10°C.

Therefore, it could be concluded that TI contbnt and apparent *in vitro* protein digestibility were affected by its freshness and fat oxidation and that, especially, fat was assumed to play an important role on apparent *in vitro* protein digestibility.

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