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Effect of Godulbaegi (Korean Lettuce, *Ixeris sonchifolia* H.) Kimchi on the *in vitro* Digestibility of Proteins

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Abstract

Extent of reduction in protein digestibility by Godulbaegi (*Ixeris sonchifolia* H.) kimchi powder appeared to be related to the kimchi weight-to-protein weight ratio, the kind of protein sources, part of plants and fermentation period. *In vitro* digestibilities of protein were significantly ($p<0.05$) reduced as the freeze dried kimchi powder weight-to-protein ratio increased from 0.5 : 1.0 to 1.0 : 1.0 for all Godulbaegi samples and protein sources. Overall digestibility of protein sources in the presence of kimchi powder, the reduction ranked in the following order : soybean>casein>beef>squid. Lower ($p<0.05$) reduction occurred for each protein source when raw plant was exchanged for kimchi products. Some greater reduction of digestibility was noted in young plants and leaf samples than ripe or root samples. Trypsin inhibitor, which expressed as soybean trypsin inhibitor, was inversely related (r=-0.8437) to *in vitro* protein digestibility of casein in the presence of Godulbaegi kimchi powder. More than three times of total polyphenols contained in leaves than in roots. Young leaves had 30% more total polyphenols (37.64mg/g sample) than that in ripe ones. Soaking in 5% NaCl solution for 24 hrs was markedly reduced in total polyphenols as 73% for leaves and 33% for roots. Remarkable reduction in total polyphenols was not checked during fermentation followed after soaking. Trypsin inhibitor content correlated well (r=0.8873) with total polyphenols in all of Godulbaegi samples.

Key words : Godulbaegi (Korean lettuce, *Ixeris sonchifolia* H.), kimchi, *in vitro* protein digestibility, trypsin inhibitor, total polyphenols

INTRODUCTION

The great hallmark of traditional Korean cuisine is kimchi, a term which denotes any side dish pickled in brine with garlic and chilies. There are more than 179 varieties of kimchi, with infinitive variations of each, and several types appear at every meal (1,2). Recently attention has been paid to the role of these kimchi varieties in human nutrition. Current interest particularly lies with kimchi, for its high levels of vitamin C and A (3, 4) as well as its importance as a good dietary fiber source, render it a very attractive fermented vegetables for human health. With kimchi seasonings including garlic, green onion, chili and ginger, green-yellow vegetables used in kimchi preparation had some inhibitory effects on mutagenicity and growth of the cancer cells (5,6). In addition to its medicinal effects on the human system (7,8), it has also been considered as a food with high fiber contents which may provide beneficial aspects of gastrointestinal function. Several investigators have observed that a high fiber diet causes a great fecal excretion and alter *in vitro* or *in vivo* digestibility of food proteins (9,10).

Generally, it has been known that protease inhibitors which is widely found in the plant kingdom (11) contributed in reduction of protein digestibility (12-17). Protease inhibitor such as polyphenolic substances have the ability to form insoluble complexes with proteins, inactivation of digestive enzymes, or a combination of these (18). Among the diverse varieties of kimchi, Godulbaegi (Korean lettuce, *Ixeris sonchifolia* H.) kimchi, which is consumed as a delecacy in southern province of Korea, is an only kimchi containing high levels of polyphenols and dietary fiber (19). In our previous investigation (20), the effects of processing conditions on the changes in

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the content of dietary fiber fractions and its physical properties of Godulbaegi kimchi which have yet to be fully assessed the nutritional implications. Therefore, the present study was designed to estimate the relative inhibitory potency of kimchi powder as the source of dietary fiber and polyphenols towards the *in vitro* digestibility of proteins.

MATERIALS AND METHODS

Materials

Characterization of raw Godulbaegi and kimchi preparation used in this investigation has been previously reported (20). To prepare the freeze dried kimchi powder, all ingredients were removed from kimchi mixtures at each stages of fermentation period. Rinsed kimchi samples were freeze dried and then ground to pass 80 mesh screen. Freeze dried samples were stored in the hermetic sample containers for further analysis. Freeze dried ANRC sodium caseinate, squid, beef and soybean served as the protein sources for determining how Godulbaegi kimchi powder affects *in vitro* protein digestibility. Fresh squid (*Todarodes pacificus*) mantle was weighed, placed in a pot with distilled water (squid : water ratio, 1 : 10w/v), and boiled for 2.5min which could show the highest protein digestibility (21). Fifty grams of chopped lean beef packed in test tube (2×20cm), cooked in boiling water for 20 min. This cooking period was selected to achieve maximum protein digestibility in beef (22). Soybeans were mixed with tap water in the proportion of 1 : 3w/v, seeds : water and allow to imbibe water at room temperature for 9hrs. After soaking and draining, soybeans were autoclaved at 110°C (5psi), and freeze dried. All of freeze dried samples were ground to pass through 80mesh screen.

Digestibility procedure

Protein-kimchi powder mixture were prepared at powder weight-to-protein weight ratios of 0.5 and 1.0. Upon combining the protein and kimchi in the dry form, both substances were hydrated for 2hrs at 37°C prior to beginning digestion assay (23). The *in vitro* digestion was conducted following the A.O.A.C. procedure (24) using four enzymes including trypsin (Sigma, 14,6000 BAEE units/mg solid), α-chymotrypsin (Sigma, 41units/mg solid), peptidase (Sigma, 50units/g solid), and bacterial protease (*Streptomyces griceus*, Sigma, 58units/mg solid).

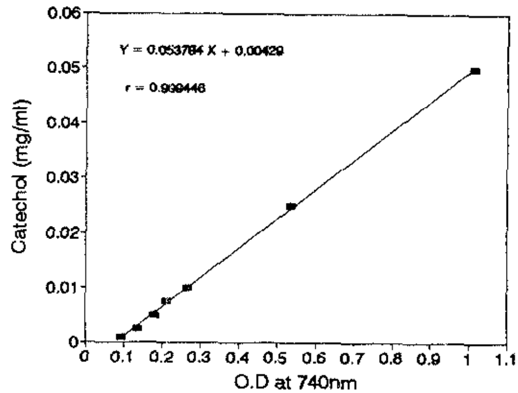

Fig. 1. *Relationships between optical density and catechol concentration.*

Trypsin inhibitor (TI) and total polyphenols

Determination of TI was done by the procedure of Ryu (25). Results were expressed in TI equivalents which equals the mg of purified soybean trypsin inhibitor per gram sample. Total polyphenols were extracted from 1g of freeze dried kimchi powder by stirring for 1hr in 20ml of aqueous ethanol (ethanol : water, 8 : 2v/v). After the insoluble residue had been removed by filtration using Whatman #42 filter paper, the filtrate was condensed to dry and then added 50ml of distilled water. Total polyphenols were determined by the Folin-Denis method described in A.O.A.C. (26) using catechol standard curve (Fig. 1).

RESULTS AND DISCUSSION

Protein digestibility

The *in vitro* digestibilities of protein sources used in this experiments were in agreement with previous establishment utilizing the multienzyme method (21,22,27) except for autoclaved soybean which showed a similar digestibility as soymilk's (28) (Table 1). The main observation is that reduction of *in vitro* digestibility by Godulbaegi related to part of plant and fermentation period of kimchi. The effect of added amount of Godulbaegi on the reduction in digestibility depends on whether this plant was in raw or fermented. Average reduced digestibility by raw plant was greater than that by fermented plant (kimchi), but there was no a stepwise reduction in digestibility by the

Table 1. Reduction of the *in vitro* digestibility of various protein sources digested in the presence of freeze dried Godulbaegi (Korean lettuce) kimchi powder

	Powder : Protein ratio (w/w)	Protein sources			
		Casein	Soybean	Beaf	Squid
Original dig. (%)		90.00	88.80	86.50	85.00
Reduction of dig. (%)[1]					
Raw					
Young leaves	0.5	6.99	5.59	5.97	4.24
	1.0	9.24	10.53	7.55	6.28
Ripe leaves	0.5	4.51	6.92	4.62	3.79
	1.0	6.09	9.17	7.10	5.82
Young roots	0.5	4.28	6.92	3.18	3.57
	1.0	7.44	7.72	4.39	5.15
Ripe roots	0.5	3.60	6.69	3.04	2.89
	1.0	4.96	10.08	4.62	4.70
Kimchi[2]					
Leaves					
20 days	0.5	4.06	5.44	3.71	3.34
	1.0	6.54	8.95	4.29	5.37
40 days	0.5	2.48	5.21	3.49	1.76
	1.0	4.51	8.72	5.29	3.79
240 days	0.5	1.80	5.79	2.02	1.31
	1.0	4.96	6.92	4.50	3.57
Roots					
20 days	0.5	2.03	6.01	1.91	2.77
	1.0	3.60	9.40	3.20	4.47
40 days	0.5	1.80	5.99	1.90	1.31
	1.0	4.06	8.27	3.71	3.34
240 days	0.5	1.35	5.79	1.02	0.89
	1.0	3.83	6.04	3.17	3.31

[1]Reduction value is the % digestibility of corresponding protein-% digestibility of proteins plus Godulbaegi kimchi powder at specified weight : weight ratio

[2]Young Godulbaegi (Korean lettuce) samples were harvested in September and kimchi was prepared through fermentation at 4°C using ripe plants harvested in November

increased plant-to-protein weight ratio. This indicates that the fiber constituents in raw Godulbaegi were more active in interfering with protein sources when compared to fermented plants (kimchi). Rather, their effect appears related to specific polymer characteristics such as the polymer branching and/or the presence of ionizable acidic groups. Fermented Godulbaegi samples may have slight branching and contain a small amount of hemicellulose and lignin than in raw plants (20). Lignin and undefined hemicellulose probably reduced digestibility remarkably (29). The nature of the hemicellulose contained in the preparation in this study was not known. However, the result may suggest the fiber matrix slightly retarded the substrate-enzyme reaction when the freeze dried kimchi powder was tested at the higher levels.

Trypsin inhibitor

Data relating trypsin inhibitor content in Godulbaegi kimchi to *in vitro* digestibility of ANRC casein are presented in Table 2. The trypsin inhibitor (TI) content in two raw root samples was not significantly different, each containing about 26mg/g dry matter. Ripen samples resulted in a decrease in TI compared with that of young samples, especially in raw leaf Godulbaegi samples (about 13%) respectively. The effect of fermentation on TI was dependent on the fermentation period and part of samples used in kimchi preparation. Longer fermentation period (240 days) resulted in a similar decrease in TI of ripe roots (31%) and leaves (27%). The changes in TI content of ripe leaves with fermentation period was similar to that of ripe roots. The effect of fermentation on Godulbaegi TI content may be explained by an increase in solubility of TI and soft-

Table 2. Effect of trypsin inhibitor (TI) in Godulbaegi (Korean lettuce)[1] on the *in vitro* protein digestibility of ANRC casein

Sample	TI (mg/g solid)	*In vitro* Dig. (%)
Raw		
Young leaves	31.58	80.76
Ripe leaves	27.50	83.91
Young roots	27.79	82.56
Ripe roots	25.14	85.04
Kimchi[2]		
Ripe leaves		
20 days	23.92	83.46
40 days	22.87	85.49
240 days	19.96	85.04
Ripe roots		
20 days	23.20	86.40
40 days	20.96	85.94
240 days	17.39	86.17

[1]Young Godulbaegi (Korean lettuce) samples were harvested in September

[2]Godulbaegi kimchi was prepared through fermentation at 4 °C using ripe plants harvested in November

ened fiber matrix with extended fermenting time. Water soluble TI extracted into kimchi juice during fermentation process thus lowering TI content. The extraction of water soluble TI into kimchi juice during fermentation was supported by the fact that the detected total polyphenol in Godulbaegi kimchi leaves was just around 20% of total polyphenols in raw leaf samples (Table 3). The TI in raw young samples exhibited a relatively high inhibitory capacity against *in vitro* digestibility of ANRC casein. A slight, but no significant ($p > 0.05$) decrease in the trypsin-inhibitory activity was found when raw ripe samples had been added to ANRC casein prior to the addition of the enzymes. The Godulbaegi kimchi, prepared from ripe leaves and roots, was also found to reduce the digestibility of protein. The degree of decrease in digestibility was correlated with TI content ($r = -0.8473$).

Total polyphenols

The total polyphenols including tannins from various plant origins are known to inhibit digestive enzymes through their ability to bind with proteins (14,16,30,31). The changes in total polyphenols during soaking and fermentation are present in Fig. 2. Soaking Godulbaegi leaves in water for 24hrs prior to kimchi preparation and fermentation resulted in a significant ($p < 0.05$) decrease in the quantity of polyphenols retained in raw ones but there

Table 3. Relationships between trypsin inhibitor (TI) contents and total polyphenols in Godulbaegi (Korean lettuce)[1]

Sample	TI	Total polyphenols (mg/g)
Raw		
Young leaves	31.58	37.64
Young roots	27.79	10.39
Ripe leaves	27.50	28.06
Ripe roots	25.14	6.98
Kimchi[2]		
Ripe leaves		
20 days	23.92	5.34
40 days	22.87	4.98
Ripe roots		
20 days	23.20	4.09
40 days	20.96	2.91

[1]Young Godulbaegi (Korean lettuce) samples were harvested in September

[2]Godulbaegi kimchi was prepared through fermentation at 4 °C using ripe plants harvested in November

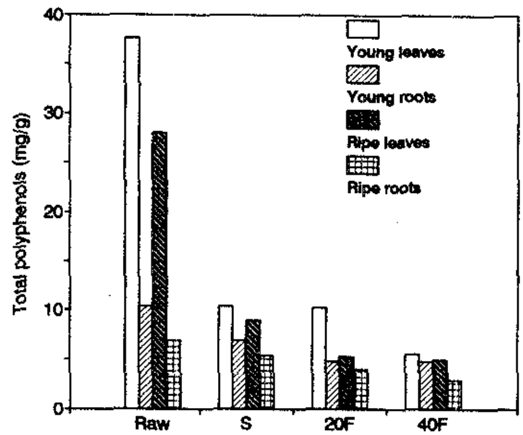

Fig. 2. Changes in total polyphenols during Godulbaegi (*Ixeris sonchifolia* H.) kimchi processing.
Young samples were harvested in September and ripe samples were harvested in November.
S : Soaked in 5% NaCl solution for 24hrs, 20F : Fermented at 40°C for 24hrs, 40F : Fermented at 4°C for 24hrs.

was no severe decrease in total polyphenols during fermentation. In case of root samples the levels of total polyphenols were lowered gradually, but not significantly, through soaking and fermentation. All of total polyphenol content in leaf samples was higher than that in root samples as the exerted data for TI (Table 2, 3). Another results were revealed from Table 3, total polyphenols did not play an important role as a trypsin inhibitor in case of Godulbaegi roots. In spite of those difference in total polyphenols content between root and leaf samples, the data for raw and kimchi showed a high correlation between TI and total polyphenols (r=0.8873).

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고들빼기 김치가 단백질 소화율에 미치는 영향

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요 약

단백질 소화율 저하에 미치는 고들빼기 김치의 영향은 고들빼기 김치 분말과 단백질량, 단백질원의 종류, 고들빼기 김치의 부위와 김치 발효기간에 따라 달라지는 결과를 나타냈다. 단백질 소화율은 고들빼기 김치시료와 단백질원의 비율을 0.5 : 1에서 1 : 1 (wt/wt)로 높여, 37°C에서 2시간 반응시켰을 때 유의적으로 감소되었다. 고들빼기 김치분말을 첨가할 경우 여러 단백질원들의 소화율 감소 경향은 대두>casein>쇠고기>오징어 순으로 나타났다 ($p<0.05$). 생시료에 비해 김치시료가 단백질 소화율을 전체적으로 적게 감소시켰으며, 잎부분이 뿌리 부분 보다, 어린 시료가 성숙한 시료 보다 소화율을 크게 감소시켰다. 고들빼기 김치분말 중의 trypsin 활성 저해물질과 단백질 소화율과는 역상관 관계를 나타냈다 (r=-8473). 총 polyphenols의 함량은 뿌리에 비해 잎에 3배 이상 함유되어 있었으며 성숙한 잎에 비해 어린잎에 30% 정도 더 많이 함유되어 있었다. 5% NaCl용액에 24시간 침지시킨 시료에서는 총 polyphenols 함량이 생시료에 비해 잎에서는 73% 정도, 뿌리에서는 33% 정도로 크게 감소하였다. 김치 발효 중에는 총 polyphenols 함량이 큰 변화가 없었으며, trypsin inhibitor 함량과 총 polyphenols 함량은 모든 고들빼기 시료에서 비교적 높은 상관관계를 보였다 (r=0.8873).