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Nutritional quality of leaf proteins prepared from crops containing phenolic compounds and polyphenolase

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Abstract

Italian ryegrass, red clover, sorghum, and alfalfa were used for leaf protein preparation. Fresh leaves were pulped in the presence or absence of a reducing agent(sodium ascorbate or $NaHSO_4$) and green juice was heated and washed with acetone. The biological evaluation of leaf proteins was carried out by the growth method with male rats weighing about 45g. Italian ryegrass, red clover, and sorghum were brown when leaves were pulped in the absence of a reducing agent. On the other hand, alfalfa had neither o-diphenolics nor polyphenolase, and hence the alfalfa leaf protein did not brown during pulping ever in the absence of a reducing agent. The brown leaf protein from Italian ryegrass had lower digestibility than the leaf protein protected from browning, although there were no difference in growth-promoting effect and protein efficiency ratio(PER) between the two leaf protein.

The feeding of brown leaf protein from red clover resulted in the lowering of weight gain, digestibility, and PER, and all the measurement including diet intake were lowered by feeding the brown leaf protein from sorghum. In the case of alfalfa leaf protein, there were no difference in nutritional quality between the two leaf protein made with and without an attempt to prevent browning.

The results mentioned above indicate that the occurrence of phenolics and polyphenolase in a crop in responsible for the browning of leaf protein and that the browning of leaf protein caused its nutritional impairment.

Key words : leaf protein, diphenolic compound, polyphenolase, browning, Italian ryegrass, red clover, sorghum, alfalfa

Introduction

Leaf protein prepared by green-crop fractionation has been studied by many researchers many countries, and it has been indicated that leaf protein affords good potential as a protein supplement[1,2,3]. It has been noted, however, that there are some marked differences among crops in nutritional quality of leaf protein in spite of a similarity of amino acid composition among leaf proteins from different crops. Horigome and Kantatsu[4] showed that brown caseins that were produced by the interaction of milk casein with phenolic compounds undergoing enzymatic oxidation had lower nutritional value that the original casein. It was also showen that many crops contained phenolic compounds[5] and polyphenolase[6]. Accordingly, it can be assumed that the browning of leaf protein is attributable to phenolics and that such browning depresses its nutritional quality. The primary aim

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of the investigation reported here was to determine whether the browning of leaf protein affects its nutritional quality.

Materials and methods

Leaf protein preparation

The forage crops used for preparation of leaf proteins were alfalfa(*Medicago sativa* L.), Italian ryegrase(*Lolium multiflorum* Lam.) red clover (*Trifolium pratense* L.), and sweet sorghum(*Sorghum rulgare*). These crops were harvested before flowering or before earing. Diphenolic[7] and tannin[8] contents and polyphenolase(diphenol ; oxygen oxidoreductase)activity[5] in the leaves of these crops were determined. Laboratory protein preparation was performed as follows : Fresh leaves were pulped with a small amount of water and fractionated into green juice and pressed leaves. To prevent the browning of protein, either sodium ascorbate(20g/ℓ) or $NaHSO_3$(5g/ℓ)was added as a reducing agent during pulping. The green juice was adjusted to pH 4 with 10% HCl and then heated to 80℃. This treatment gave a voluminous coagulam of protein. The coagulam was separated from brown juice and washed repeatedly with acetone. This acetone-washed leaf protein was subjected to further experiments.

Amino acid analysis

Protein(10-20mg) was weighed into 14×120mm Pyrex tube and mixed with 10ml of constant-boiling HCl. The air above the mixture was displaced with nitrogen and the tube was sealed under reduced pressure. Hydrolysis were carried out in an autoclave at 110℃ for 24 hours. An aliquot of the hydrolysate was analyzed with a Yanaco Model LC 11 amino acid analyzer. Methionine and cysteic acid, respectively, after performic acid oxidation of leaf protein according to Moore.[9]

Biological test

Growth experiments were performed with weanling male rats of the Wistar strain weighing about 45g each. The basal protein-free diet contained(g/100g) ; cellulose powder, 1.0 ; cane sugar, 10.0 ; mineral mixture, 4.0 ; vitamin mixture, 1.0 ; methionin, 0.2 ; corn oil, 10.0 ; and corn starch, 73.8. Each leaf protein was incorporated into the basal diet in replacement of an equal weight of corn starch to provide about 8% protein(N×6.25). Methionine was added to all diets, since it is the first limiting amino acid of leaf proteins. Each diet group included five rats ; they were allowed *ad-libitum* access to the diets for a 14 day period. Protein efficiency ratio (PER) was calculated after 14 days as g body weight gain/g protein eaten. The apparent digestibility of leaf protein was determined by analyzing the nitrogen intake and fecal nitrogen excreted during the last 5 days of the period.

Results and Discussion

Polyphenolase is also known as phenol oxidase, tyrosinase, o-dephenol oxidase, catechol oxidase, polyphenol oxidase and chlorogenic acid oxidase. Polyphenolase (EC 1.10.3.1) is a copper-containing enzyme which catalyzes either one or two reactions involving molecular oxygen[10]. Diphenolic and tannin contents and polyphenolase activity of forage crops are shown in Table 1. Alfalfa had neither diphenolics nor polyphenolase, and hence the leaf protein was not brown even when no reducing agent and red clover had diphenolic content and polyphenolase activity, so browning occurred in the leaf proteins of both crops when the leaves were pulped in the absence of a reducing agent. In particular, red clover contained approximately 2.6 times the phenolic content of Italian ryegrass, and the red clover leaf protein was very brown. Sorghum had very low activity of polyphenolase, but the sorghum leaf protein also became brown in the absence of a reducing agent during pulping. When a reducing agent was added during pulping, the leaf protein obtained from the forage crops tested was not

Table 1. Phenolic compound content and polyphenolase activity of leaves

	o-Diphenol[1)]	Condensed tannin[2)]	polyphenol-ase[3)]
Alfalfa	0	0	0
Italian ryegrass	0.36	trace	20.1
Red clover	0.95	trace	21.6
Sorghum	0.75	〈0.06	6.0

[1)]Expressed in term of chlorogenic acid (% of dry matter).
[2)]% of dry matter.
[3)]mg purpurogallin produced/g leaf acetone power.

browned. In addition, it can be seen from Table 1 that every forage crop had a negligible or very small amount of tannin. Plant polyphenols include phenolic acids, flavonoids and tannins. They are widely distributed in leaves, steams, roots, flowers, fruits and seeds and almost universally present in animal diets derived from plant[11)]. The different classes of polyphenol have somewhat different nutritional or physiological activities. The tannins may reduce protein digestivility[12)] and perhaps the bioavailabitily of other nutrients. Table 2 shows the results of nutritional evaluation of leaf proteins. In the case of alfalfa leaf protein, the presence or absence of a reducing agent during pulping did not affect the nutritional quality of the leaf protein. On the other hand, Italian ryegrass leaf protein that was not protected from browning was significantly lower in digestibility than leaf protein protected from browning. In red clover, weight gain and RER of rats fed brown protein were depressed as compared to those of rats fed protein from browning. Digestibility of brown protein was lower as well. These results indicate that the nutritional quality of leaf protein is lowered by the browning reaction and that a large amount of diphenolic and a high activity of polyphenolase in a forage crop depressed nutritional quality.

In spite of very low activity of polyphenolase in sorghum leaves, however, a large depression of nutritional quality occurred in sorghum leaf protein that was not protected from browning. It is particularly note worthy that intake by rats fed on the sorghum leaf protein exposed to browning was low. The cause of this low intake could not be determined from the data of these experiments, but the results suggest that, in addition to the amount of phenolics present in leaves, the type

Table 2. Food intake, weight gain, digestibility and protein efficiency(PER) of rats given a diet containing leaf protein.

	Alfalfa		Italian ryegrass		Red clover		Sorghum	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
Food intake (14 day)	97.4±0.2	105.6±4.3	113.7±0.2	113.7±0.1	114.9±0.1	114.9±0.1	113.3±0.4	82.0±5.7**
Weight gain (14 day)	26.3±2.2	29.6±2.8	36.9±1.0	36.2±2.0	42.3±2.1	37.2∓1.7*	32.4±1.8	14.9±4.4**
Digestibility of protein(%)	79.5±1.9	81.4±1.8	86.4±1.4	80.9±0.6**	86.3±0.4	79.4±1.4**	77.6±0.8	67.0±0.9**
PER g gain/ g protein intake	3.27±0.13	3.37±0.30	3.85±0.11	3.74±0.20	4.65±0.23	3.95±0.18**	3.30±0.17	2.10±0.48**

(A) leaf protein with attempt to prevent browning.
(B) leaf protein with no attempt to prevent browning.
*Significantly different from the corresponding value of (A) at the 0.01 level
**Significantly different from the corresponding value of (A) at the 0.001 level

Table 3. Amino acid composition of protein sources (Expressed as mg amino acid per nitrogen g)

Amino acid	Alfalfa		Italian ryegrass		Red clover		Sorghum	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
Lysine	424± 11	427± 10	441± 12	429± 11	440± 10	418± 9	354± 10	362± 8
Histidine	171± 7	166± 6	158± 4	152± 6	169± 6	154± 5	136± 5	128± 5
Arginine	418± 10	422± 11	439± 1	437± 12	411± 9	416± 9	389± 11	398± 10
Aspartic acid	676± 20	653± 19	611± 18	598± 19	633± 15	637± 12	601± 9	611± 11
Threonine	345± 9	340± 8	328± 8	326± 7	325± 10	327± 7	306± 6	311± 8
Serine	305± 9	290± 9	281± 12	291± 10	262± 9	271± 8	280± 10	284± 11
Grutamic acid	742± 19	740± 21	730± 20	750± 20	808± 20	809± 17	767± 13	778± 14
Proline	337± 8	321± 11	332± 6	339± 10	317± 6	305± 6	341± 8	329± 7
Glysine	369± 9	358± 9	334± 8	384± 9	365± 8	352± 7	357± 6	359± 5
Alanine	404± 9	404± 10	448± 8	451± 11	400± 7	385± 8	430± 6	434± 7
Cystine	87± 5	66± 6	82± 5	63± 6	74± 4	63± 5	69± 5	69± 4
Valine	460± 10	485± 11	455± 9	451± 11	441± 9	438± 8	428± 8	420± 8
Methione	145± 7	144± 7	158± 5	154± 6	136± 6	127± 6	161± 6	167± 5
Isolucine	393± 9	395± 12	380± 10	378± 11	358± 12	363± 12	351± 11	341± 10
Leucine	637± 15	645± 15	630± 14	628± 17	653± 13	649± 14	670± 9	656± 10
Tyrosine	326± 9	322± 9	312± 10	308± 11	287± 6	292± 6	245± 10	231± 5
Phenyl alanine	419± 10	426± 10	430± 9	425± 10	407± 9	414± 10	392± 8	385± 6

(A) leaf protein with attempt to prevent browning.

(B) leaf protein with no attempt to prevent browning.

(or kind) of phenolics may be an important factor in the nutritional impairment of leaf protein. On the other hand, Brown discoloration of edible mountain herb and its concentrate was found to be related to the enzymatic browning that take place before or during processing[13, 14]. Ham[15] was investigated some properites of polyphenol oxidase from *Spuriopimpinella bracycarpa, Aster scaber* and *Ligularia fischeri*. The results of amino acid analyses showed Table 3 that amino acids of leaf proteins were scarcely damaged by browning. Lysine, histidine, and cystein, were very slightly impaired in red clover leaf protein prepared with no attempt to prevent browning. Therefore, amino-acid composition, as determined by the methods presented in this paper, does not provide an explanation for the nutritional impairment of leaf protein by the browning reaction. Hurrell et al[16] reported that polyphenol browning reaction, like Maillard browning reaction, can reduce the biologically-avaiable lysine content of protein. The nutritional implications of the Maillard reaction, because its great importance during food processing have been widly studies[17, 18] Few nutritional studies however have been made on the polyphenol browing reactions. These reactions include enzymic browning and protein-polyphenol reaction are O_2 dependent and could be of importance during the preparation of vegetable-protein concentrates. Jones and Lyttleton[19] studies fraction 1 leaf protein isolated from red clover, therefore, it is important to inhibit the formation of polyphenol dxidation products.

In conclusion, the results presented have demonstrated that the occurrence of phenolics and polyphenolase in a crop is responsible for browning of leaf protein and

that browning causes nutritional impairment. However, identification of phenolic compounds present is important for elucidating the mechanism of nurtritional impairment.

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초록 : Phenolic compound와 polyphenolase 함유 작물로 부터 조제한 녹엽단백질의 영양가

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녹엽단백질 조제 원료 목초중 diphenol 및 polyphenolase 의 측정결과 alfalfa에는 존재 하지 않고, Italian ryegrass, red clover, sorghum 에는 존재가 확인 되었다. 이러한 목초로 부터 단백질 추출은 용매를 물만으로 한것과 물에 환원제를 첨가한 것을 사용하여 갈변단백질과 갈변방지단백질을 조제하여 Wistar계 숫컷 흰쥐에 14일간 급여하여 체중증가량, 소화율, PER에 대하여 조사하였다. 그 결과 alfalfa 녹엽단백질의 경우 갈변단백질과 갈변방지단백질 간에 증체량, 소화율, PER은 차가 인정되지 않았다. Italian ryegrass 녹엽단백질의 경우 갈변단백질과 갈변방지단백질의 경우 소화율에서 유의차가 인정 되었다. 그러나, 체중증가량, PER은 차가 인정되지 않았다. red clover 녹엽단백질의 경우 갈변단백질이 소화율, 증체량, PER은 갈변단백질 보다 유의하게 저하 하였다. 이러한 것은 녹엽단백질의 영양가가 갈변반응에 의해 손상 되어지고 원료 목초중의 diphenol량, polyphenolase 활성이 높을수록 갈변반응에 의해 영양가의 저하가 현저하였다. 녹엽단백질의 아미노산 조성은 갈변에 의해 거의 손상 되지 않았지만, red clover 녹엽단백질의 경우 lysine, histidine 이 조금 감소 하였다. 녹엽단백질의 아미노산 손실은 갈변에 의한 것이 아닌 것으로 생각되어지고 유효성 아미노산에 대하여 금후 검토를 요하는 과제이다.