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Effects of Germination on Antinutritional Oligosaccharides of Mung Beans

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녹두발아시 항영양 과당류의 변화

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요 약

녹두발아중 항영양 과당류의 변화를 알아보기 위하여 실온에서 15시간 침지시킨 녹두(*Phaseolus aureus*)를 25°C에서 7일간 발아시키면서 실리카박층크로마토그라피로 당의 패턴을 분석하였다. 녹두의 수분함량은 15시간 침지이후 발아 3일까지 65%로 유지되다가 4일째부터는 82%로 거의 안정하였다. 항영양 과당류인 raffinose와 stachyose는 박층크로마토그라피상에서 발아 1~2일째 급격히 감소하여 미량 존재하다가 3일째부터는 거의 소멸되었다.

Introduction

On a worldwide basis, legumes make on important contribution to human food. Compared to oilseeds, dry beans have received less attention in the past in western countries. A renewed interest in dry beans as a human food is evident in recent years. In orient, mung beans used to be widely available and consumed as rice-mung bean cake, mung bean gelatin, mung bean noodle, mung bean porridge, mung bean pan cake, mung bean sprouts, etc. as well as soybeans.

Utilization of dry beans as human food is below their potential, partly due to the presence of several antinutritional factors (trypsin, chymotrypsin, amylase inhibitors), low protein quality (deficiency of sulfur contaning amino acids) and flatulence causing agents (oligosaccharides) of dry beans. Since the protein content of mung beans is 20-27% and essential amino acid levels comparable to those of soybeans,[1)2)] mung beans are suitable for supplementing cereal-based products, espcially rice and barley ones due to their amino acid pattern (lysine, threonine and tyrosine, which are short in rice and barley, are sufficient in mung beans)[1)]. Inactivation and/or removal of antinutritional and flatulence producing factors are desirable to help improve dry bean utilization.

Of the several processing methods used for dry bean processing, germination is a relatively simple method, does not require intensive energy input and also yields natural product. Germination of legume seeds is accompanied by the metabolism of the reserve proteins stored in protein bodies in the cotyledons. Changes in storage proteins of several legumes during germination have been reported.[3)4)] Enzyme inhibitory activities in beans have been investigated to be reduced during germination to a

variable degree.[5]

The production of flatulence is associated with various legume seeds and their components are well known. Most legumes contain about 60% carbohydrates, including oligosaccharides of the raffinose family, which are known for the flatulence problem in men and animals.[6~9] The raffinose family sugars such as stachyose and raffinose escape digestion, due to the lack of α-galactosidase activity in mammalian mucosa, and they are not absorbed into the blood. Consequently, bacteria in the lower intestinal tract metabolize them to form large amounts of CO_2, H_2 and small amounts of methane gases, which lower the pH(4.5). The flatus produced by fermentation in lower intestine may cause nausea, cramps, diarrhea and discomfort.

Although changes in storage proteins phytic acid or enzyme inhibitoryactivities of several legume seeds during germination have been investigated,[3~5] and studies on the change of antinutritional oligosaccharides of various legumes are reported,[10~13] but works particularly of the mung bean sprouts are scanty.

The persent report concerns the change of antinutritional oligosaccharides of mung beans (*Phaseolus aureus*) germination.

Experimental

1) Bean samples

Mung beans (*Phaseolus aureus*) were supplied by Office of Rural Development, Suwon. Some deformed grains were discarded before use. The moisture content of the seeds was 8.9%. Unless mentioned otherwise, all chemicals used were reagent grade.

2) Germination

Each 20 gm of mung beans was soaked in distilled water (200 ml) for 15 hr at 25°C and germinated in the dark at 25°C for 7 days on filter paper lined a flowpot (diameter 20 cm). Sodium azide at a concentration of 0.01% (w/v) was added to distilled water used for soaking and germination in order to prevent microbial growth. During germination distilled water containing sodium azide was sprinkled on the seeds 3~4 times per day ad libitum. Seeds germinated for 1, 2, 3, 4, 5, 6 and 7 days were immediately used.

3) Flour preparation

Whole dry beans were ground in a grinding mill fitted with a 40 mesh screen and served as control

4) Moisture determination

Moisture content of samples of dry bean flour, of soaked beans and of germinated beans were determined using oven-drying method at 110°C for 6 hr and the means were taken.

5) Extraction and identification of oligosaccharides

Samples of dry bean flour, of soaked beans and of germinated beans were submitted to oligosaccharide extraction according to Tanaka et al.[15] The samples were ground with mortar and suspended in 30 ml of 80% ethanol. The suspension was refluxed for 1 hr and filtered through a Whatman No. 1 filter paper. The residue was stirred in 20 ml of distilled water for 30 min and filtered again. The combined filtrate was concentrated to 1 ml with a rotary evaporator below 50°C. The concentrated sugar extracts were taken for analysis.

The oligosaccharides were separated and identified by a thin-layer chromatography according to Cruz and Park.[16] Vloumes of 15 μl of the final extracts were applied to a kieselgel 60 sheet (Merck, Art. 5748) and developed by double ascending chromatography using a solvent system, ethylacetate, acetic acid water (3:1:1, v/v). Thymol-sulfuric acid reagent containing 5 ml of sulfuric acid, 95 ml of ethanol and 0.5 gm thymol was sprayed to detect sugars. Color was visualized by heating the sheet at 120°C for 10 min.

The sugars were identified by comparison with the reference sugar standards obtained from Kanto Chemical Co., Japan (xylose, glucose, fructose and raffinose), Junsei Chemical Co., Japan(sucrose)

East man Kodak, USA (glactose), Merck Chemical Co., Germany (maltose) and Sigma Chemical Co., USA (stachyose).

Results and Discussion

1) Moisture content

The moisture content of mung bean sprouts are presented on Fig. 1. The water level of dry mung beans(8.9%) was increased greatly with soaking followed by stabilization along to the 3rd day of germination (65%). The level was increased slightly on the 4th day and thereafter stabilized (82%). Moisture content of mung bean sprouts was figured as 95.2% and 88.8% by Tsou and Hsu[17] and by Bowes and Church,[18] respectively. The differences among the levels seem to be due to length of sprouts depending on the conditions of germination.

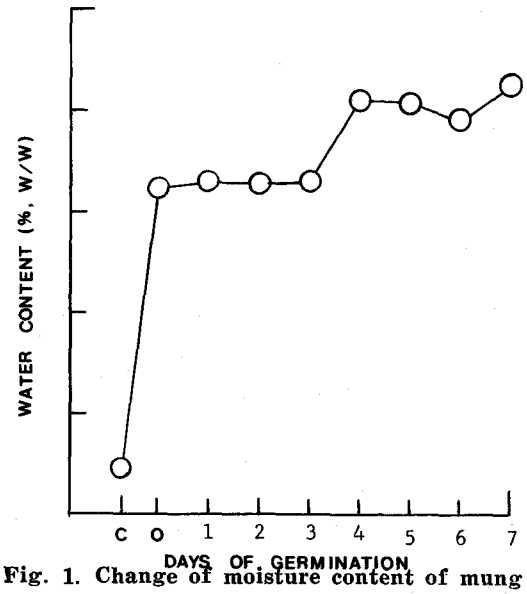

Fig. 1. Change of moisture content of mung beans during germination.
C: dry seeds
O: soaked seeds

2) Oligosaccharide pattern

The changes of oligosaccharides in mung beans after soaking and germination are revealed on Fig. 2. Mung beans contained large amounts of stachyose and sucrose and small amounts of raffinose and glucose. The level of raffinose was increased without changes of other sugars after soaking for 15 hr. Quantitatively, the levels of sucrose, raffinose and stachyose in mung beans were reported by Tanaka et al.[15] as high as 0.93 gm, 0.44 gm and 1.97 gm per 100 gm of the seeds, respectively. Tanusi et al.[11] showed similar amounts. And Silvia and Braga[13] studies with *Phaseolus vulgaris*, green beans to show that the original levels of raffinose and stachyose in the beans (0.71% and 3.77% on dry weight basis, respectively)were decreased after soaking for 24 hr by 32.4% and 13.5%, respectively. However it is shown on Fig. 2. that the level of raffinose was increased after soaking for 15 hr, and that some reduction in raffinose level without change in stachyose level were noticed on the 1st day of germination. The difference of raffinose change after soaking between Silva's and the present data could be explained to be attributed to a slow metabolic progress occurred in this experiment as well as the short time of soaking for 15 hr in this experiment instead of 24 hr in Silva's. Solubility of sugars in water could not explain solely the removal of the sugars because only 19.5% of sucrose (most easily soluble sugar) was extracted in soaking water,[13] so it need not be accounted much for consideration.

During germination, oligosaccharides were reduced significantly and glucose and sucrose increased (Fig. 1). On the 2nd day of germination, stachyose and raffinose were diminished definitely along with the increments of sucrose, glucose and others, which were probably appeared from the metabolism of large molecular weight carbohydrates during germination. On the 4th day, a decrease of glucose but the same amount of sucrose were shown. It was implicated that glucose was metabolized further to unknown metabolites (presumably xylose, ribose, trioses, etc.), but the supply of glucose from sucrose was limited. On the 5th day, something of larger molecule than that of sucrose was detected, to which large molecular carbohydrates were metabolized probably and to sucrose and glu-

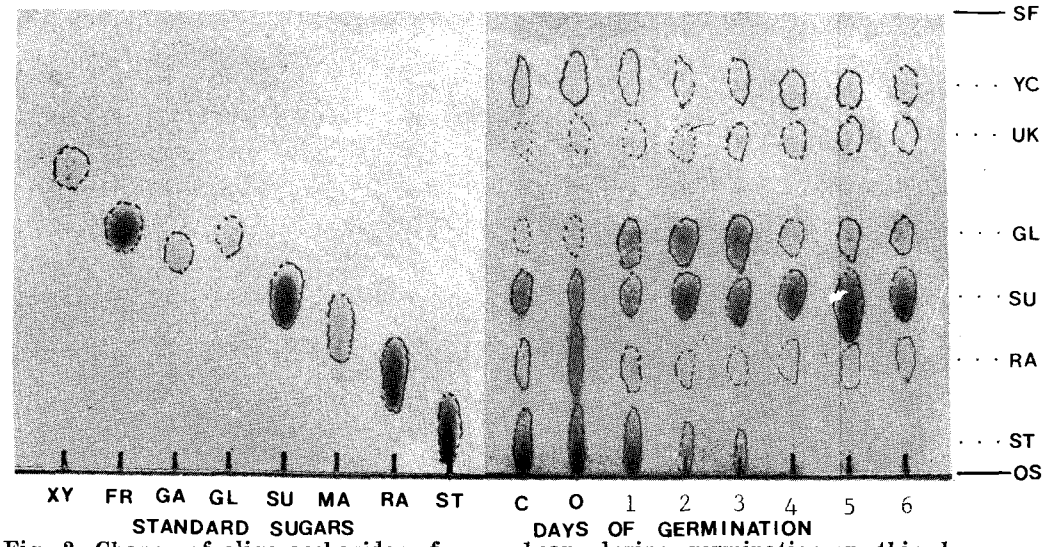

Fig. 2. Change of oligosaccharides of mung beans during germination on thin layer chromatography.

TLC was developed for 4 hr using solvent system, ethylacetate, acetic acid and water(3:1:1, v/v) by double ascending technique.

Sugars were detected by thymol-sulfuric reagent containing sulfuricacid 5 ml, ethanol 95 ml and thymol 0.5 gm and visualized by heating at 120°C for 10 min.

XY: xylose, FR: fructose, GA: galactose, GL: glucose, SU: sucrose, MA: maltose, RA: raffinose, ST: stachyose, YC: yellow color, UK: unknown, SF: solvent front, OS: original start, C: extract of dry seeds, O: extract of soaked seeds, 1~6: extracts of mung beans germinated for 1~6 days, respectively.

cose consequently. These results are in agreement with those reported by Ko and Park[14] in terms of a great decrease of total sugar content and an increase of reduding sugars in mung beans during germination.

Sathe et al.[3] reported that the highest removal (76.6%) in stachyose+raffinose in Great Northern beans had shown at the end of the 3rd day of germination. Others revealed that the removal in raffinose in red gram[19] and red kidney beans[20] had been maximum at the 2nd and 4th day of germination, respectively, with some different degrees. In this study, the largest reductions of stachyose and raffinose were demonstrated on the 2nd and 1st day of germination. These differences might be partly due to origins and cultivar conditions as well as the levels of endogenous α-galactosidase activity in different seeds.

Summary

To examine changes of antinutritional oligosaccharides, particularly raffinose and stachyose, of mung beans during germination, mung beans (*Phaseolus aureus*) were germinated at 25°C for 7 days after soaking for 15 hr. The 80% ethanol extracts of mung bean sprouts were analyzed by Kieselgel thin-layer chromatography using a solvent system, ethylacetate, acetic acid and water (3:1:1, v/v).

The moisture level was increased greatly after soaking followed by stabilization along to the 3rd day of germination. The level was increased slightly on the 4th day and thereafter stabilized.

Antinutritional oligosaccharides, raffinose and stachyose was diminished significantly on a thin-layer chromatogricadtly on the 1st and 2nd day of germination, and disappeared almost thereafter.

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