REMOVAL OF GLUCOSINOLATES IN DEFATTED RAPESEED MEAL BY ASPERGILLUS ORYZAE

H. Yoshii(1), T, Hino(2)

(1) Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo, JAPAN 156
(2) TOYO Oil Mills Co. Inc., 230 Shinminato, Chiba, JAPAN 260

INTRODUCTION

Defatted meals of rapeseed have a high protein content and a reasonably well balanced amino acid composition. However the large amount of glucosinolates(thioglucosides) present, which yield 5-vinyl-oxazolidine-2-thion(OZT,goitrin) and isothiocyanates upon enzymatic hydrolysis, limit their use as feed. We have investigated a method for removing toxic factors (goitrins) from defatted rapeseed meal by fungi.

MATERIALS AND METHODS

Materials

Laboratory meals were prepared from Swedish rapeseed obtained from TOYO Oil Mills Co. Inc., removing oil by percolation of pentane-hexane and grinding. Chinese rapeseed meals were obtained from OOTA Oil and Fat Co. Inc. located in Aichi Prefecture.

Analytical methods

Thioglucosides was determined by the thiourea-UV assay of Wetter and Young(1976). Rapeseed meal(100mg) is weighed into a 10ml screw-cap vial. One milliliter of the phosphate-citrate buffer of pH 7.0.containing 5mg of myrosinase and exzactly 5.0 ml of methylene chloride is added to the vial. The capped vial containing one glass bead is shaken on an oscillating shaker for 2hr at room temperature. After completion of the enzyme incubation, the emulsion is broken by centrifuging at ca. 1000Xg. The total isothiocyanate plus OZT content is determined by adding 0.1ml of the methylene chloride extract to 6ml of 20% ammoniacal ethanol and heating for 2hr at 50 C in a water bath. The reaction is carried out in a culture tube equipped with a screw cap. Optical density of the methylene chloride extract was read on a Hitachi spectrophotometer continuously from 230-260nm. The total isothiocyanate content is expressed as mg 3-butenyl isocyanate(3-BITC) per g of meal. OZT content is determined by following the steps outlined above, except that 95% ethanol is substituted for the ammoniacal solution. OZT content is expressed as mg OZT per g of meal.

Myrosinase(Thioglucoside glucohydrolase, EC 2.3.2.1) was prepared according to the method of Nagashima et al.(1959).

Moisture, lipids and total sugar analysis followed Methods of Food Analysis(1982). Protein content was calculated as percent nitrogen X6.25. Nitrogen was determined by KJELTEC system. Amino acids was determined with Formol-titration.

Koji making from rapeseed meal

Rapeseed meal was moistened with tap water(30-40% moisture) and then steam cooked at 105 C for 15 min.. After cooling inoculated with the spore of Asp. sydowi and Asp.

oryzae. The inoculated material is spread into trays and incubated in a humid atmosphere maintained at 30°C. Incubation was favored for 3 to 4 days.

RESULTS AND DISCUSSION

Glucosinolates in rapeseed meal Koji with Asp. sydowi

Glucosinolates in steam cooked meal and finished Koji of Asp. sydowi are given in Table 1. The major glucosinolates in

Table 1. Glucosinolates in meal Koji with Asp. sydowi

		Steam cooked Meal	Finished Koji S-7	Finished Koji S-10
OZT	Free	0.08%(1)	0.37	0.14
	Bound	0.40	0.09(22.5)(2)	0.16(40.0)
	Progoitrin	1.32	0.31(23.5)	0.54(40.9)
	Total	0.48	0.46(95.8)	0.31(64.6)
3-BITC	Free	0.07	0.24	0.11
	Bound	0.46	0.08(17.4)	0.20(43.5)
	Total	0.55	0.33(60.0)	0.30(54.5)
Moisture(%)		31.61	28.95	29.72

(1) % Dry bases
(2) Residual percentage based on steam cooked meal

rapeseed meal are progoitrin and gluconapin. In finished Koji with Asp. sydowi, 60-75% of progoitrin in the meal was hydrolyzed to OZT(goitrin). For total OZT content, no significant difference was observed between meal and finished Koji. In finished Koji, 60-80% of total glucosinolates(Gluconapin and Progoitrin) in the meal were hydrolyzed to 3-BITC. Total 3-BITC content of finished Koji was about 60% of the meal.

Glucosinolates in rapeseed meal Koji with Asp. oryzae S-3

Glucosinolates in steam cooked meal and Koji with Asp. oryzae are given in Table 2. After incubated three days, Progoitrin in the meal was completely converted and OZT(free) increased. However, the content of OZT(total) decreased to 29% of that of the meal. After incubated seven days, OZT(free) decreased to 5% of that of the meal. From these results, it was estimated that in the process of Koji making, Progoitrin was hydrolyzed to give OZT(goitrin) by thioglucosidase of Asp. oryzae. Moreover, secondary OZT were degradated or removed In the same manner, total glucosinolate in the meal were completely hydrolyzed to 3-BITC after incubated three days by thioglucosidase of Asp. oryzae and thereafter secondary the greater part of them were removed. In addition, meal Koji increased amino acids content owing to hydrolysis of protein by the protease of Asp. oryzae.

Table 2. Glucosinolates in meal Koji with Asp. oryzae S-3

		Steam cooked Meal	Incubated period(days) 3	7
OZT	Free	0.05%(1)	0.17	0.03
	Bound	0.53	0	0
	Progoitrin	1.75	0	0
	Total	0.58	0.17(29.3)(2)	0.03(5.2)
3-BITC	Free	0.04	0.16	0.04
	Bound	0.59	0	0.01(1.2)
	Total	0.63	0.16(25.4)	0.05(7.5)
Moisture(%)		32.98	32.45	32.12
Protein(%)		23.15	30.81	30.5
Formol nitrogen(%)		0.05	0.30	0.28

(1) % Dry bases
(2) Residual percentage based on steam cooked meal

Removal of glucosinolates in rapeseed meal Koji with Asp. oryzae

Removal of glucosinolates in rapeseed meal by Koji making with Asp. oryzae are given in Table 3. After inoculating Asp.

Table 3. Removal of glucosinolates in rapeseed meal Koji

		Steam cooked Meal	Finished S-3	Koji SA
OZT	Free	0	0	0
	Bound	0.205(1)	0	0
	Progoitrin	0.678	0	0
3-BITC	Free	0.12	0	0
	Bound	0.964	0	0
Formol nitrogen(%)		0.095	0.702	0.731

(1) % Dry bases

oryzae S-3 or SA(mold starter for Sake) incubated for 96hr at 30 ℃. In finished Koji of rapeseed meal, glucosinolates(Progoitrin,Gluconapin) and their hydrolyzed products(OZT,3-BITC)

were completely removed. Cultivation of Asp. oryzae also increased amino acids or inorganic phosphorous in rapeseed meal owing to the hydrolysis of protein or phytin. From these results, it was estimated that cultivation of Asp. oryzae should improve the nutritionally value for rapeseed meal as feed.

REFERENCES

Methods of Food Analysis.1982. Japanese Soc. for Food Sci. and Technol. ed. Korin, Tokyo. P.5,126,172.

Nagashima,Z. and Uchiyama,M. 1959. Studies on Myrosinase. Part 1. Trials of separating Myrosulfatase and Thioglucosidase , and the method of their activity measurement. J. Agric. Chem. Soc. Jpn. 33:478-483.

Wetter,I.R. and Young,C.G. 1976. A Thiourea-UV Assay for Total Glucosinolate Content in Rapeseed Meals. J. Agr. Oil Chem. Soc. 53:162-164.