究研ノート

Destruction of Phytic Acid in Diet for Germfree Rats and Mice during Autoclaving or Gamma Irradiating

Tsutomu Yoshida, Syoko Shinoda and Satoru Watarai*

Department of Food and Nutrition, Tachikawa College of Tokyo,

*Institute of Laboratory Animals, Funabashi Nohjoh Co. Ltd.

高圧蒸気滅菌または放射線滅菌による無菌ラット・マウス用飼料中フィチン酸の分解

吉田 勉, 篠田 粧子, 渡来 智*

東京都立立川短期大学食物学科, *船橋農場

無菌マウス・ラットの飼育に用いられる実用飼料L-485-Cr（主成分はとうもろこしと脱脂大豆）につき、高圧蒸気滅菌ないしは放射線滅菌を行った際のフィチン酸含量の変化を調べた。放射線滅菌に関しては、生大豆や脱脂大豆についても実験を行った。

乾物中フィチン酸含量は、L-485-Cr が1%, 生大豆が1%, 脱脂大豆が1.6%で、全P中に占めるフィチン酸態Pは、それぞれ、43%, 52%, 62%であった。

高圧蒸気滅菌後における L-485-Cr 中のフィチン酸態Pの分解は、121°C で30分という無菌動物用飼料の滅菌条件では平均7%であった。なお、1ロットの実験であるが、同上飼料を 121°C で30分及び2時間滅菌した際のフィチン酸態Pの分解は、9%及び17%であった。

また、5 Mrad 放射線滅菌後における L-485-Cr 中のフィチン酸態Pの分解は8%であった。

すなわち、無菌動物用飼料に対する実用的滅菌条件下では、飼料 L-485-Cr 中フィチン酸の分解はわずかであると思われる。

一方、生大豆で5及び10 Mrad の γ -線照射、また脱脂大豆で5 Mradの γ -線照射後のフィチン酸態Pの分解は、約17%であった。

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Phytic acid (myo-inositol-1, 2, 3, 4, 5, 6-hexa-kis dihydrogen phosphoric acid) is an important constituent of legumes and cereal grains. Among the mono through hexaphosphates, only the hexaphosphate is detected in extracts of mature seed of the soybean, corn, wheat, and rice (1). It forms an insoluble salt, phytin, with Ca, Mg, and K and may thereby impair the intestinal absorption of these cations (2, 3, 4). Phytic acid has been shown similarly to reduce the availability of a number of essential divalent metals, i.e., Fe (5), Cu, Mn (6) and Zn (7).

Investigations have been conducted in which autoclaving for 60 minutes destroyed rachitogenic activity exhibited by isolated soybean protein (8) and increased the Zn uptake of the sesame meals (9) in the poults. Moreover, autoclaving an isolated soybean protein improved Fe bioavailability

to rats over the unheated sample (10), and steam pelleting increased the absorbability of P by pigs on corn soybean meal diets in the low levels of dietary Ca and P (11).

It may be possible that partial destruction of phytate in the diets after autoclaving improved mineral bioavailability. However, it has been reported that phytate destruction does not occur during autoclaving for 30 minutes (9) or 2 hours (12). On the other hand, no work has been done in order to estimate the influence of gamma irradiation.

The purposes of this work were to affirm the phytate destruction effect of autoclaving a practical diet for germfree mice and rats, and to clarify the same effect of gamma-irradiating the same diet, raw soybeans, and soybean meal.

Experimental

Materials:

The diet used in this experiment was L-485-Cr which has the same composition as diet L-485 (13), but contains an additional 1%, on an air dry basis, Cr_2O_3 (Kanto Kagaku Co., Japan).

The diet was compressed into pellets at Funabashi Nohjoh Co. The diet, raw soybeans, and soybean meal were sterilized either by free-flowing steam for 30 minutes and 2 hours at 121°C , or by accumulation of approximately 5 and 10 Mrad from a ^{60}Co source. After that, they were kept refrigerated until use in the experiment.

Determination of P and phytate P:

Total P was estimated by the colorimetric method of Allen (14). For the determination of phytate P, the method of Oberleas (15) was used with two modifications: The diluted extracts of the sample treated with FeCl_3 solution to precipitate ferric phytate were heated for 30 minutes (16) instead of 15. The precipitates were kept in 1.2% HCl containing Na_2SO_4 for 2 hours with occasional stirring (17) instead of simple washing. Phytate P was determined by the method of Allen (14) as inorganic phosphate P after acid hydrolysis of the ferric salt precipitate (15). Phytic acid was calculated on the assumption that it contained 28.16% of P.

The differences between the raw and sterilized diets were analyzed for significance by paired sample *t*-tests.

Results and Discussion

The effect of autoclaving on phytate P content of diet L-485-Cr is shown in Table 1.

Phytic acid content of raw diet L-485-Cr was 959 mg/100 g dry matter. There was only moderate destruction of phytate (7.1%) in the autoclaved diets. The result obtained from one lot of the diet showed that longer autoclaving time increased the phytate destruction from 9% after 30 minute autoclaving to 17% after 2 hour autoclaving. This is in agreement with the result in which 5–25% losses of phytate in the slurry of rice, wheat and sesame meal was observed with autoclaving for 2 hours at 115°C (18).

Table 1 Phytic acid content in the diet L-485-Cr after autoclaving (on dry weight basis)

	Total P (mg/100 g)	Phytate P (mg/100 g)	(Phytic acid) (mg/100 g)	Phytate P/Total P (%)	Phytate destruction* (%)
Before autoclaving**	623±27	270±25	(957)	43.3±3.8	
After autoclaving** 121°C 30 min	—	256±20†	(888)	40.1±2.8	7.1±0.5
Before autoclaving***	586	247	(877)	42.2	
After autoclaving*** 121°C 30 min	—	225	(799)	38.4	9.0
121°C 2 hr	—	205	(728)	35.0	17.0

* (Phytate before sterilization-phytate after sterilization)/phytate before sterilization.

** Mean and standard deviation of four lots.

*** Analysis of one lot.

† Difference from the diet before sterilization significant at $p < 0.01$ using paired sample t -test.

(33)

Table 2 Phytic acid content in soybeans, soybean meal and the diet L-485-Cr after gamma irradiation (on dry weight basis)

	Total P (mg/100 g)	Phytate P (mg/100g)	(Phytic acid) (mg/100 g)	Phytate P/Total P (%)	Phytate destruction* (%)
[Soybeans***] Before irradiation	561	290	(1,030)	51.7	
After irradiation 5 Mrad	—	242	(859)	43.1	16.6
10 Mrad	—	239	(849)	42.6	17.6
[Soybean meal***] Before irradiation	720	443	(1,573)	61.5	
After irradiation 5 Mrad	—	366	(1,300)	50.8	17.4
[Diet L-485-Cr**] Before irradiation	623±27	270±25	(957)	43.3±3.8	
After irradiation 5 Mrad	—	246±16†	(875)	39.6±2.4	8.3±0.9

*, **, *** and † See table 1.

Lease (9) showed that in the phytate content of sesame meals, there was no change after 2 hour autoclaving at 116°C and about 20% destruction after 4 hour autoclaving. Moreover, Carlson (12) observed that no destruction of phytate occurred during autoclaving of isolated soybean protein for 30 minutes at 120°C. Table 1 shows the disagreement with the results of Lease (9) and Carlson (12).

It is likely that the autoclaving condition used in the experiment of Lease was less effective than that in this experiment, in which sterilization was conducted at a higher temperature.

Other possible reasons for the disagreement of our experiment with those of Lease and Carlson are that the rate of phytate destruction, probably by hydrolysis, is influenced by the protein and cation environment of the diets (18) or their water content, as follows.

Although the phosphate ester bonds in phytate are reasonably stable, they can be broken by simple autoclaving in an aqueous medium (1). The rate of hydrolysis of phytic acid depends to some extent upon the protein with which it is associated. It has been observed that autoclaving an isolated soybean protein for 4 hours at 115°C destroyed most of the phytate (1, 19), but under the same conditions approximately 80% of the phytate in sesame meal remained intact (9).

Toma and Tabekhia (20) observed the effect of the ions on phytate destruction: cooking of rice with domestic tapwater reduced phytic acid content to approximately 2/3, but not with distilled deionized water.

Reddy et al. (21) observed that cooking of beans (black gram) with four parts of water for 5—45 minutes at 116°C resulted in a substantial loss in phytate. Conversely, no decrease was observed in the phytate content of a slurry of sesame meal with deionized water after autoclaving for 2 hours at 116°C, as mentioned above (9). It may be stated that the amount of water added during autoclaving has an effect on the rate of phytate destruction.

The effects of gamma irradiation on the phytate P content of raw soybeans, soybean meal, and diet L-485-Cr are shown in Table 2.

The principal source of P found in soybeans (approximately 3/4) is in the form of phytin, and phytic acid makes up about 1—1.5% of soybeans by weight (22). In the present experiment, phytic acid content of raw soybeans was about 1% on a dry weight basis, and phytate P/total P was 51.7%.

Rackis (23) showed that dehulled and defatted soybean meal contained almost 0.5—0.6% total P, 70—80% of which was present as phytic acid. On the other hand, total P and phytate P contents in soybean meal analyzed by Nwokolo et al. (24) were 0.7% and 0.24%, respectively. Our results on total P and phytate P/total P lay between these two experiments.

The same degrees of phytate destruction were observed in soybeans after 5 Mrad and 10 Mrad irradiation. Therefore, no experiment of 10 Mrad irradiation was carried out on soybean meal and the practical diet, L-485-Cr.

In soybean meal, the rate of phytate destruction after 5 Mrad irradiation was the same as in soybeans.

Phytate destruction in diet L-485-Cr after gamma irradiation was less than that in soybeans and soybean meal. In addition, it was shown that, in diet L-485-Cr, sterilization with 5 Mrad irradiation (a practical dose for the sterilization of germfree animal diets) brought about the same degree of destruction as autoclaving at 121°C for 30 minutes.

Although it may be difficult to confirm any assumption from the present study because of the small number of observations, it seems that the practical conditions for sterilization of diet L-485-Cr cause only moderate destruction of phytate.

Summary

Phytic acid contents in a practical diet for germfree mice and rats (L-485-Cr which is based on maize and soybean meal), raw soybeans, and soybean meal, on a dry matter basis, were about 1%, 1% and 1.6%, and phytate P/total P values were 43%, 52% and 62%, respectively.

The phytate destruction effect of autoclaving and gamma irradiating the practical diet was observed.

Phytate P destruction of diet L-485-Cr after autoclaving at 121°C for 30 minutes, which is a practical procedure for the sterilization of germfree animal diets, was 7%. In the experiment on one lot of the diet, phytate P destruction after autoclaving at 121°C for 30 minutes and 2 hours was 9% and 17%, respectively. Phytate P destruction of diet L-485-Cr after 5 Mrad gamma irradiation was 8%. It seems that the practical conditions for sterilization of the practical diet cause only moderate destruction of phytate.

On the other hand, phytate P destruction in soybeans after 5 and 10 Mrad irradiation and in soybean meal after 5 Mrad irradiation were about 17%.

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References

- 1) O'Dell, B. L. : *Am. J. Clin. Nutr.*, **22**, 1315 (1969)
- 2) Bruce, H. M. and Callow, R. K. : *Biochem. J.*, **28**, 517 (1934)
- 3) McCance, R. A. and Widdowson, E. M. : *J. Physiol.*, **101**, 44 (1942)
- 4) Seeling, M. S. : *Am. J. Clin. Nutr.*, **14**, 342 (1964)
- 5) Widdowson, E. M. and McCance, R. A. : *Lancet*, **1**, 588 (1942)
- 6) Davies, N. T. and Nightingale, R. : *Br. J. Nutr.*, **34**, 243 (1975)
- 7) O'Dell, B. L. and Savage, J. E. : *Fedn. Proc. Fedn. Am. Soc. Exp. Biol.*, **16**, 394 (1957)
- 8) Thompson, O. J., Carlson, C. W., Palmer, L. S. and Olsen, O. E. : *J. Nutr.*, **94**, 227 (1968)
- 9) Lease, J. G. : *Poultry Sci.*, **45**, 237 (1966)
- 10) Steinke, F. H. and Hopkins, D. T. : *J. Nutr.*, **108**, 481 (1978)
- 11) Bayley, H. S., Pos, J. and Thomson, R. G. : *J. Anim. Sci.*, **40**, 857 (1975)
- 12) Carlson, C. W., Saxena, H. C., Jensen, L. S. and McGinnis, J. : *J. Nutr.*, **82**, 507 (1964)

- 13) Kellogg, T. E. and Wostmann, B. S. : *Lab. Anim. Care.*, **19**, 812 (1969)
- 14) Allen, R. J. L. : *Biochem. J.*, **34**, 858 (1940)
- 15) Oberleas, D. : The determination of phytate and inositol phosphates. In *Methods of Biochemical Analysis*, Vol. 20, 87 (1971), Interscience, U. S. A.
- 16) Makower, R. U. : *Cereal Chem.*, **47**, 288 (1970)
- 17) Ellis, R., Morris, E. R. and Philpot, C. : *Anal. Biochem.*, **77**, 536 (1977)
- 18) De Boland, A. R., Garner, G. B. and O'Dell, B. L. : *J. Agr. Food Chem.*, **23**, 1186 (1975)
- 19) Rackis, J. J. : *J. Am. Oil Chemists. Soc.*, **51**, 161A (1974)
- 20) Toma, R. B. and Tabekhia, M. M. : *J. Food Sci.*, **44**, 619 (1979)
- 21) Reddy, N. R., Balakrishnan, C. V. and Salunkhe, D. K. : *J. Food Sci.*, **43**, 540 (1978)
- 22) Lolas, G. M., Palamidas, N. and Markakis, P. : *Cereal Chem.*, **53**, 867 (1976)
- 23) Rackis, J. J. : Biologically active compounds. In Smith, A. K. and Circle, S. J. ed. : "Soybeans" Chemistry and Technology, Vol. 1, 2nd ed., 185 (1978), The Avi Pub. Co., U. S. A.
- 24) Nwokolo, E. N. and Bragg, D. B. : *Can. J. Anim. Sci.*, **57**, 475 (1977)

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