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Nutritional Evaluation of Naturally Fermented Soybean and the Enzymatic Activity Changes during the Preparation

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自然醱酵 大豆食品의 営養的 価値와 그의 製造 中 酵素活性変化

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Abstract

The four varieties of Korean soybeans were allowed to undergo natural fermentation for seven days at ambient temperature. The average pH of the product was 3.93 and titratable acidity was 1.94%. For all varieties of soybeans the content of riboflavin increased from 98 to 309.4 *ug*/100 *g* dry-matter, relative nutritive value from 78.66 to 94.59% and available lysine from 6.56 to 7.38 *mg*/*g*N, respectively. During fermentation, the activities of protease and lipase increased, while lipoxygenase and trypsin inhibitor activity decreased markedly. The capacity of water sorption of fermented soybeen flour was increased with progress of proteolysis during fermentation. The cookie and noodle prepared with 20:80 mixture of fermented soybean flour and wheat flour were in the "like" category, but it was desirable to neutralize the sour taste produced by fermentation. Among five kinds of products prepared from the fermented soybean flour pan cake was liked most by rural consumers.

Introduction

From ancient times soybeans have been used as important dietary food stuffs[1] in many Asian countries. However, it is believed that soybeans do not provide the high quality protein that the products originated from animals do[2]. In comparison with animal protein, soybean protein was observed poor in digestibility, biological value, net protein utilization and chemical score.

These may be attributed to several factors[3] such as presence of enzyme inhibitors (trypsin- and chymotyrpsin inhibitors) and toxins like phytohemagglutinins, unbalanced amino acid composition, and presence of raffinose and stachyose, which produce flatulence or intestinal gas[4]. It is, therefore, necessary to optimize the use of soybeans to improve the diet and nutrition of people who utilize the soybean to a great extent. In the Orient, fermentation has been conventionally used to improve nutritional quality and storage characteristics of soybeans. In Korea, typical fermented soybean foods are soybean paste and soybean sauce in which fermentation is carried out by mold, expecially, by

Aspergillus.

Recently, among food researchers, increasing concerns are brought on natural fermentation in which lactic acid microorganisms play a major role. During natural fermentation of soybean, Wang[5] observed increase of relative nutritive value (RNV), available lysine, methionine and tryptophan. Hasim and Fields[6] found increase of various vitamins and amino acids of sorghum. Zamora[7] identified microorganisms and toxins of naturally fermented cowpeas and chickpeas.

Available lysine and vitamins increased during fermentation of various cereals[8] and Kakade[9] reported the presence of trypsin inhibitor and chymotrypsin inhibitors of soybean during natural fermentation.

The objective of this study is to confirm nutritional changes and to determine the changes of enzymatic activities during fermentation. Sorption isotherms[10] of fermented soybean flour were determined and sensory evaluation and preference test[11] were also carried out on the food products prepared from the fermented soybean flour.

Materials and Methods

Materials

Three Korean recommended varieties of soybeans were purchased from the respective provincial Office of Rural Development (ORD). *Bong Eui* was obtained from Gangweon provincial ORD, *Kwang Gyo* from Gyunggi provincial ORD and *Dong Bug Tae* from Chungbuk provincial ORD. *Si Pan* (unknown mixed varieties) was bought from the market in Suweon in April, 1981.

Fermentation

Each variety of soybeans was screened to remove broken, cracked, moldy or insect infested grains. The screened grains were cracked with a Maruroku Mill to two to four grits and dehulled completely by rubbing with hands. Those soybean grits were milled with a Wiley mill to flour to pass 1 *mm* mesh screen. A slurry of the bean flour was made by adding water (1:4, *W/V*) and the mixture was incubated at room temperature (25°C) for 1 to 7 days. During fermentation the slurry was taken out every day and dried at 35-40°C in an oven for 24 hrs. The dried crumb was grounded again with a Wiley mill using 1 *mm* screen. This dehydrated product was stored in a refrigerator at 4°C during the experiment. Controls were prepared by the above procedures without fermentation according to the method of Hamad.[8]

Nutritional analysis

Titratable acidity[12] was determined by titration with 0.1 *N* NaOH and expressed as lactic acid and pH was measured with a Fisher pH meter. Moisture content was determined by drying at 130°C for 1 hr and riboflavin content[13] was analyzed by electrophotoflourometric method. Nitrogen content was determined by microkjeldahl method. Relative nutritive value (RNV)[14,15] was measured by the procedure of Scott using *Tetrahymena pyriformis W* (ATCC No. 10542). Certified casein manufactured by Fisher Scientific Co. was used as a reference protein. Available lysine content[16,17] was determined by Carpenter's method. Fatty acid[18,19] was extracted from 1 *g* of soybean flour with 25 *ml* extraction solvent (CCl_4/Methanol (2:1, *v/v*) for 2 hrs and centrifuged at 5,000 x *g* for 20 min. The extract was then applied to thin layer chromatography plates coated with silica gel G with 0.5 *mm* thickness. The elution solvent system of ethyl ether/benzene/-ethanol/acetic acid (40:50:2:0.3, *v/v/v/v*) was allowed to migrate 16 *cm*.

Concentration of fatty acid was determined by measuring the triangular area developed on TLC plate and comparing the area to the standard curve.

Enzymatic assay

For assessment of proteolytic activity, enzyme solution was made by extraction 5 *g* of dried fermented soybean flour with 20 *ml* of 0.1 *M* citrate phosphate buffer (pH 5.4). The extraction process included homogenization and centrifuging at 10,000 x *g* for 30 min. The supernatant obtained was used as enzyme solution and the protease activity was measured by the method of Noh *et al.*[20]

For determining lipoxygenase activity[21,23], enzyme solution was made by the same procedures as described above, but 20 *ml* of 0.15 *M* sodium phosphate buffer was used instead as an extraction medium. The enzyme activity was expressed as absorbance change at 235 *nm*.

Trypsin inhibitor solution[9] was obtained by homogenizing 5 *g* of soybean flour with 20 *ml* of 0.01 *m* to 0.05 *N* hydrochloric acid and centrifuging at 10,000 x *g* for 30 min. The supernatant obtained was used as trypsin inhibitor solution. One *ml* of standard stock trypsin solution and 1 *ml* of 0.1 *M* phosphate buffer (pH

7.6) were added to 2 *ml* of 2% casein substrate and incubated at 37°C for 20 min. Trypsin activity[24] was expressed as absorbance change at 280 *nm*. Instead of buffer solution, 1 *ml* of trypsin inhibitor solution was added to trypsin activity assay mixture. Then trypsin activity was measured by the same method as described above. Trypsin inhibitor activity was represented as trypsin activity inhibited by 1 *ml* of trypsin inhibitor solution.

Degree of proteolysis(%) was measured by Malthiolouthi's method[25] and water sorption isotherm was determined by Lang's method[26]. One *g* of soybean flour was placed for 30 days in the apparatus in which relative humidity was controlled by saturated salt at 20°C. After equilibration, moisture content of soybean flour was determined and water activity was calculated from the equilibrated moisture content.

Cookie and noodle made from fermented and nonfermented soybean flour was evaluated by panels composed of 20 graduate students in Department of Food Science and Technology, Seoul National University and staffs in Korea Rural Nutrition Institute.

Preference test was carried out on food products[27] prepared from fermented soybean flour in Wooduk Village, Sangsuhmyun, Booangun, Jeonbuk province from 13 to 14 November, 1981. Fifty two rural residents participated in this preference test.

Results and Discussion

pH and titratable acidity

Fig. 1 illustrates the treds in pH and titratable acidity of fermenting soybeans. Up to the second day of fermentation pH of fermented soybean liquor was in the range of 3.6 to 4.1 in which food poisoning bacteria coud be inhibited on their growth. From the chicken embryo bioassay test[7] it is verified that no toxicological substance existed in the fermented soybean sample. The titratable acidity increased continuously during the fermentation and reached the value of 0.4 to 0.8% on the 7th day of fermantation. Changes in pH and titratable acidity indicated that fermentation in this experiment was lactic acid fermentation.

Riboflavin content

Riboflavin content of fermented soybean increased rapidly from 98 to 300 μg/100 *g* dry-matter during the 3 or 4 days of fermentation but decreased slowly thereafter (Fig. 2). Fig. 2 indicates that riboflavin synthesis activity of microorganisms was vigorous in the initial step of fermentation but the acidity was inhibited somehow probably either by changes in conditions of fermented liquor including pH and acidity or by changes in microorganisms' flora.

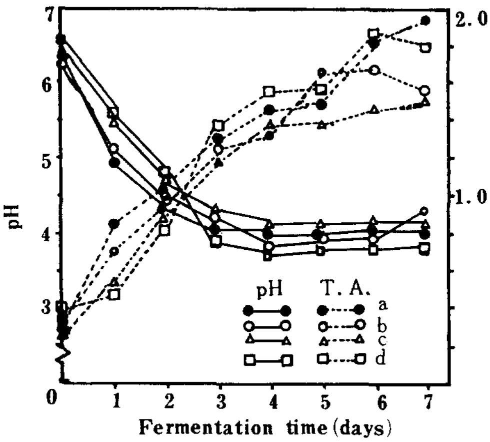

Fig. 1. Changes of pH and titratable acidity of soybean slurry during fermentation
Sample a; *Si Pan*, b; *Bong Eui*, c; *Kwang Gyo*, d; *Dong Bug Tae*

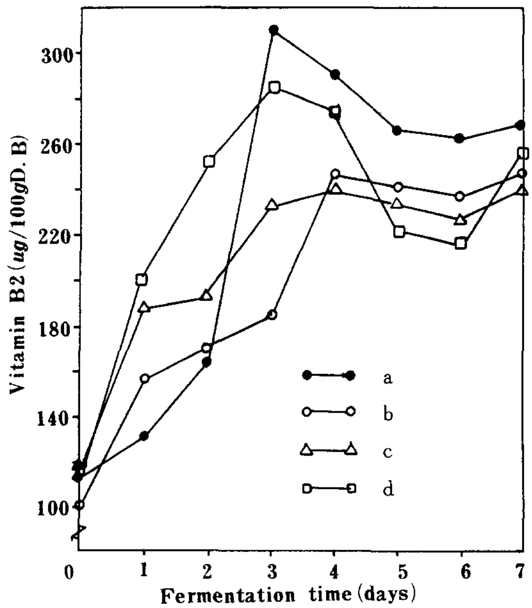

Fig. 2. Changes in riboflavin content of fermented soybean
Sample a; *Si Pan*, b; *Bong Eui*, c; *Kwang Gyo*, d; *Dong Bug Tae*

Available lysine and relative nutritive value

Available lysine increased about 12.5% on the 4th day of fermentation and then decreased (Table 1). The results indicate that the optimum period of fermentation is 3 to 4 days in consideration of nutritional changes such as riboflavin content, available lysine content and relative nutritive value. The RNV of fermented soybeans increased by as much as 20.3%, compared to nonfermented samples. Zamora et al[12] postulated that there might be an improvement in the amino acid balance of the fermented cowpeas and chickpeas. It could be considered that fermentation of soybean could improve the nutritional quality by improving the amino acid balance.

Table 1. Changes in available lysine content and relative nutritive value during *Si Pan* soybean fermentation

Fermentation time (day)	0	2	4	6
Available lysine (*mg/gN*)	6.56	6.99	7.38	6.13
Relative nutritive value (%)	78.66	−	94.59	−

− : undetermined

Lipid changes

As shown in Fig. 3, triglyceride of soybean lipid decreased, while diglyceride and monoglyceride increased with the progress of fermentation. We also observed that the content of free fatty acid increased linearly with the fermentation time in *Si Pan* soybean. This showed that triglyceride had been decomposed into diglyceride, monoglyceride and free fatty acid by lipase.

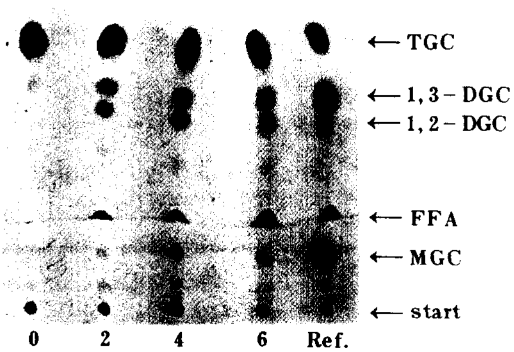

Fig. 3. Thin layer chromatogram of lipids of the fermented soybeans
TGC; tryglyceride, 1,3-DGC; 1,3-diglyceride, 1,2-DGC; 1,2-diglyceride, FFA; free fatty acid, MGC; monoglyceride, Ref; reference sample

Protease activity

Protease activity increased slightly in the initial step of fermentation, but increased rapidly from 1.3 to 2.0 units after the 3rd day of fermentation (Fig. 4). It could be interpreted that protease had been secreted actively by microorganisms from the 3rd day of fermentation. The proteolysis will improve the digestibility and nutritional quality of soyprotein.

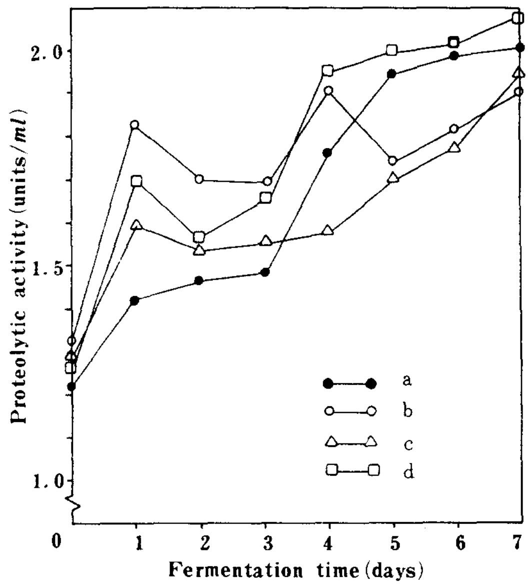

Fig. 4. Relationship between protease activity and fermentation time of soybean
Sample a; *Si Pan*, b; *Bong Eui*, c; *Kwang Gyo*, d; *Dong Bug Tae*

Lipoxygenase activity

Lipoxygenase activity decreased rapidly from 700 to 200 units on the 1st day of fermentation and from the 2nd day its decrease was slow as shown in Fig. 5. This might be due to the high sensitivity of lipoxygenase to heat.

The results indicate that lipoxygenase will not cause the flavor deterioration so much during fermentation. Moreover, since the degree of oxygen supply under the condition of fermentation is very low, the oxidative deterioration by this enzyme will be minimum.

Trypsin inhibitor

It was observed that activities of trypsin inhibitor were slightly different among soybean varieties tested in this study. In general, they appear to decrease significantly in linear relationship with the fermentation time of soybean (Fig. 6). This indicates that the

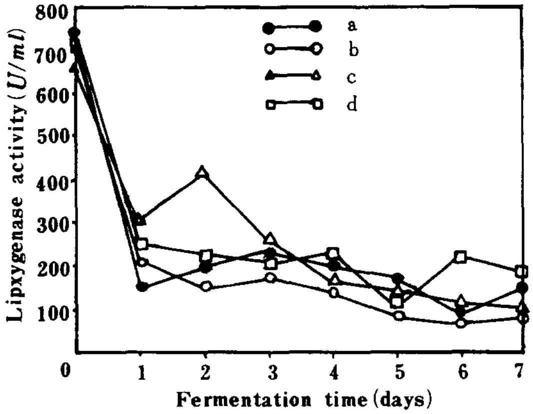

Fig. 5. Changes of lipoxygenase activity during soybean fermentation
Sample a; *Si Pan*, b; *Bong Eui*, c; *Kwang Gyo*, d; *Dong Bug Tae*

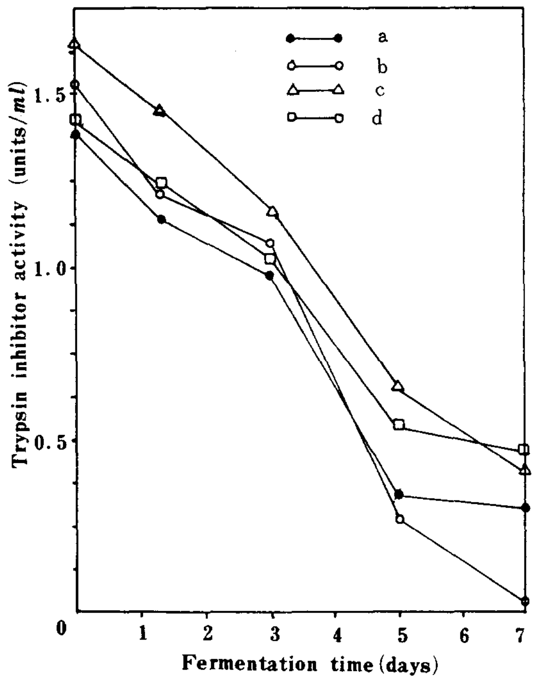

Fig. 6. Changes of trypsin inhibitor activity of soybean during fermentation
Sample a; *Si Pan*, b; *Bong Eui*, c; *Kwang Gyo*, d; *Dong Bug Tae*

digestibility of protein in the fermented products may be greater compared to the nonfermented beans.

Water sorption isotherm

Fig. 7 showed that equilibrium moisture content (EMC) was increased by fermentation.

Malthlouthie *et al*[25] reported that capacity of water sorption of cheese depends upon the degree of proteolysis. Proteolysis expressed as % soluble nitrogen of total nitrogen showed rapid increase during the initial 2 days and it was somewhat slow afterward as shown in Fig. 8. From the results we can summerized that the proteins undergo a hydrolysis during the fermentation and this hydrolysis releases more and more hydrophillic groups which leads to the increasing of the amount of bound water to these groups.

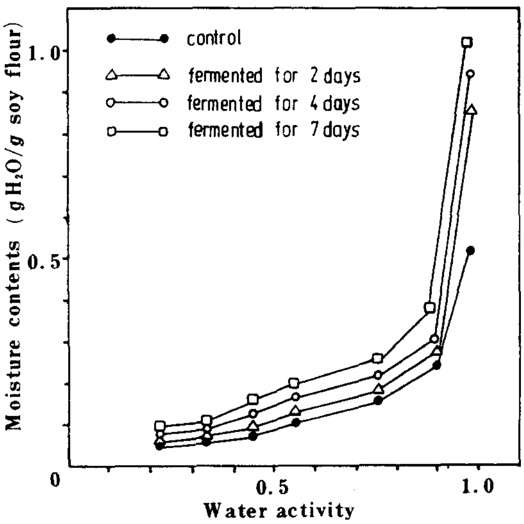

Fig. 7. Water sorption isotherms of fermented soybean at 20°C
Sample: *Si Pan*

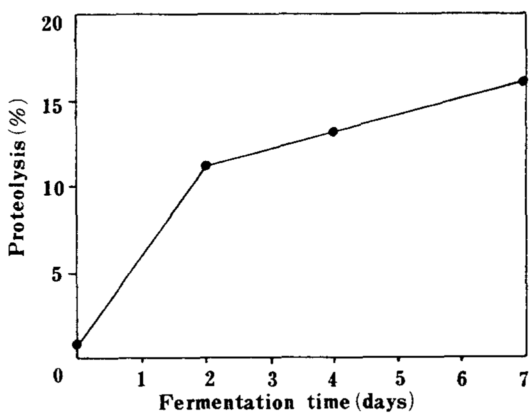

Fig. 8. Effect of fermentation on the degree of proteolysis
Sample: *Si Pan*

Sensory evaluation

Cookie and noodle prepared from mixed flour of fermented soybean and wheat flour in the ratio of 1 to 4 *(w/w)* were subjected to the panel test. The test was carried out by scoring method; maximum score; 5, minimum; 1. From the rsult shown in Table 2, varietal difference was hardly observed both in cookie and noodle except *Dong Bug Tae* in cookie. Therefore, it could be sugested that the quality of fermented soybean products was more affected by the fermentation process than the varietal difference of soybean.

Table 2. Sensory evaluation of fermented soybean food by the scoring method

Sample	Variety \ Sense	Odor	Color	Taste	Texture	Mean
Cookie	*Si Pan*	3.4	3.4	3.5	3.6	3.5
	Bong Eui	3.4	3.7	3.7	3.5	3.6
	Kwang Gyo	3.2	3.8	3.6	3.6	3.6
	Dong Bug Tae	2.8	2.1	2.6	2.3	2.4
Noodle	*Si Pan*	3.2	3.6	3.1	3.2	3.3
	Bong Eui	3.4	3.8	3.3	3.3	3.4
	Kwang Gyo	3.0	3.4	3.0	3.1	3.1
	Dong Bug Tae	3.5	3.8	3.3	3.3	3.5

Grade 1; dislike very much, grade 2; dislike moderately, grade 3; neither like nor dislike, grade 4; like moderately, grade 5; like very much.

Preference test

Five kinds of food prepared from fermented soybean were subjected to the preference test by 52 residents in Wooduk village Jeonbuk province, Korea. All of the raw materials for the preparation of these foods were able to be purchased from the village. More than 70% rural people answered 'good' or 'very good' for pan cake among 5 kinds of food.

In conclusion, we could improve the quality of soybeans by fermentation, and if we could enhance the taste of fermented soybean products slightly, this process of fermentation could be acceptable in the rural areas.

Table 3. Preference on various kinds of fermented soybean food.

	Very Good	Good	Fair	Bad	Very Bad
Pan Cake	24 (46.16)	14 (26.92)	10 (19.23)	3 (5.77)	1 (1.92)
Noodle	11 (21.15)	11 (21.15)	12 (23.08)	15 (28.85)	3 (5.77)
Vegetable fried	22 (42.31)	13 (25.00)	11 (21.15)	5 (21.15)	1 (1.92)
Bread	14 (26.92)	18 (34.62)	11 (21.15)	9 (17.31)	-
Leaf Onion fried	8 (15.38)	18 (34.62)	15 (28.85)	7 (13.46)	4 (7.69)

Numbers; Persons

( ); % of total people

要 約

韓國産 大豆 4品種을 25℃에서 1日에서 7日까지 自然醱酵시키며, 영양가와 효소활성의 변화 및 水分活性度를 조사하고 食味檢査를 하였다. 大豆의 자연발효 중 pH는 6.48에서 3.93으로 떨어졌고, 적정산도는 0.3%에서 1.94%로 증가하였다. 또는 riboflavin, relative nutritive value, available lysine 含量은 각각 98 에서 309.4μg/100g D. B, 78.66에서 94.59%, 6.56에서 7.38 mg/gN으로 상당한 증가를 나타내었다. 프로테아제(protease) 力價는 2~3日 후 급격한 증가를 보였으며 리파아제(lipase) 역가도 거의 직선적으로 증가하였는데 反하여 trypsin inhibitor와 리폭시게나아제(lipoxygenase)의 역가는 발효가 진행됨에 따라 현저하게 감소하였다.

발효대두의 等溫吸濕曲線을 실험에 의해 구하였고 발효에 의한 단백질 분해정도가 증가함에 따라 水分吸收能이 증가하였다. 발효대두를 이용하여 제조한 과자류 및 국수류의 식미검사 결과 성적이 양호하였으나 신맛의 개선이 요구되었고 5종의 農村型食品은 농민들의 좋은 반응을 얻었다.

References

1. Kim, S.W. and Byun, S.M.: *Korean J. Agri. Chem. Soc.*, **7**, 85 (1966)
2. Zamora, A.F. and Fields, M.L.: *J. Food Sci.*, **44**, 930 (1979)
3. Sathe, S.K. and Salunkhe, D.K.: *J. Food Sci.*, **46**, 626 (1981)
4. Calloway, D.H., Hickey, C.A. and Murphy, E.L.: *J. Food Sci.*, **36**, 251 (1971)
5. Wang, Y.Y. and Fields, M.L.: *J. Food Sci.*, **43**, 1113, (1978)
6. Hasim, N.B. and Fields, M.L.: *J. Food Sci.*, **44**, 936 (1979)
7. Zamora, A.F. and Fields, M.L.: *J. Food Sci.*, **44**, 928 (1979)

8. Hamad, A.M. and Fields, M.L.: *J. Food Sci.*, **44**, 456 (1979)
9. Kakade, M.L., Rackis, J.J., McGhee, J.E. and Puski, G.: *Cereal Chem.*, **51**, 376 (1974)
10. Young, J.F.: *J. Appl. Chem.*, **17**, 241 (1969)
11. Larmond, E.: *Laboratory Methods for Sensory* Evaluation of Food, Research Branch Canada Depart Agriculture Publication 1637 (1977)
12. Zamora, A.F. and Fields, M.L.: *J. Food Sci.*, **44**, 234 (1979)
13. A.O.A.C.: *Methods of Analysis of the A.O.A.C.*, 11th ed.(1970)
14. Stoff, J.A., Smith, H. and Rosen, G.D.: *Brit. J. Nutr.*, **17**, 227 (1963)
15. Warren, R.M. and Labuza, T.P.: *J. Food Sci.*, **42**, 429 (1977)
16. Carpenter, K.J.: *Biochem. J.*, **77**, 604 (1960)
17. Raghavendar, R.S., Carter, F.L. and Frampton, V.L.: *Anal. Chem.*, **35** (12), 1927 (1963)
18. Bennett, G.A., Freer, S. and Shotwell, O.L.: *J. Am. Oil. Chem. Soc.*, **53**, 52 (1976)
19. Price, P.B., Usda, A. and Parson, J.G.: *J. Am. Oil Chem. Soc.*, **52**, 490 (1975)
20. Noh, B.S. and Park, K.H.: *Korean J. Food Sci. Technol.*, **12**, 209 (1980)
21. Stebens, F.C., Brown, B.M. and Smith, E.L.: *Arch. Biochem. Biophy.*, **136**, 413 (1970)
22. Smith, W.L. and Lands, W.E.: *J. Biol. Chem.*, **247**, 1038 (1972)
23. Wolf, W.J.: *J. Agri. Food Chem.*, **23**, 136 (1975)
24. Rackis, J.J.: *Food Technol.*, **20**, 1482 (1966)
25. Mathilouthi, M., Michel, J.F. and Maitenaz, P.C.: *Lebensmittel. Wiss. U. Technol.*, **14**, 163 (1981)
26. Lang, K.W., Mocune, T.D. and Steinberg, M.P.: *J. Food Sci.*, **46**, 936 (1981)
27. Min, Y.K., Park, I.S. and Baek, O.H.: *Annual Research Report of Korea Rural Nutrition Institute*, p. 201 (1980)