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한국 상용 식품의 엽산 분석에 관한 연구

—제 I 보 채소류의 엽산치 분석—

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=국문초록=

엽산의 결핍은 대혈구성 빈혈(macrocytic and/or megaloblastic anemia)을 일으킨다. 체내에서 엽산은 합성될 수 없는 것으로 알려져, 식품을 통하여 섭취하여야 한다. 이에, 한국인 식생활의 기본을 이루고 있는 쌀, 콩 및 채소류의 식품에 포함된 엽산치를 미생물학적인 측정방법에 따라 분석하였다.

Streptococcus faecalis 보다 Lactobacillus casei 미생물을 사용하여 측정한 방법이, 인간에게 이용될 수 있는 보다 정확한 엽산치로 밝혀졌다. 산화되기 쉬운 형태로 된 labile folate를 보호하기 위한 ascorbic acid의 첨가는, 각 식품에 포함된 엽산치를 증가 시켰다. 결합형으로 존재하는 polyglutamates를 유리형으로 하기 위하여 conjugase enzyme을 사용하였으며, 식품에 따른 화학적 조성은 주로 polyglutamyl form이나 각 식품에 따라 큰 차이가 있는 것으로 나타났다.

Lactobacillus casei을 사용하여 측정된 각 식품의 엽산치는, 배추 34.5, 당근17.8, 오이 25.3, 가지 24.7, 쑥갓 76.5, 마늘 3.1, 파 40.2, 완두콩 68.7, 풋고추 27.1, 강남콩 66.9, 부추 64.1, 상치 39.3, 양파 4.3, 시금치 150.7, 호박 26.1, 무우 40.3, 백미 29.9 ug으로 각기 식품 100 g 중에 함유됨을 보였다.

The Measurement of Folacin Coutent in Korean Foods

—Part I. Folate Distribution in Vegetables—

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Introduction

Folacin is the generic descriptor for folic acid(FA), pteroylmonoglutamic acid(PGA), and related compounds exhibited qualitatively the biologic activity of folic acid. These compounds are folic acid glutamates (N). with N representing the number of glutamic acid residues, or more simply, "polyglutamates". The folic acid molecule contains three portions such as pteridine moiety, para-aminobenzoic acid and glutamic acid. The structure of folic acid(PGA) is shown in Figure 1. Folacin exists approximately 75~80% of natural folates as polyglutamyl folate[1].

The matter of concern with folic acid content of foods has aroused a great deal of interest reporting the effects of a yeast in tropical macrocytic anemia[2]. The recognition of folic acid as a factor required for growth, reproduction, prevention and treatment of anemia in animals, as well as human, made the actual need of studies consequently. The nutritional folate deficiency occurs even in affluent populations, particularly in stress situations such as infancy[3~4], pregnancy[5~7] and alcoholism[8].

Since man seems to be totally unable to synthesize the pteridine ring structure, he is dependent upon dietry sources for his natural supplying of the pteroylglutamates or folate vitamins. Therefore, the measurement of folate activity in food is dependent almost exclusively on the microbiological assay methods, using Lactobacillus casei and Streptococcus faecalis. These two organisms are quite sensitive and

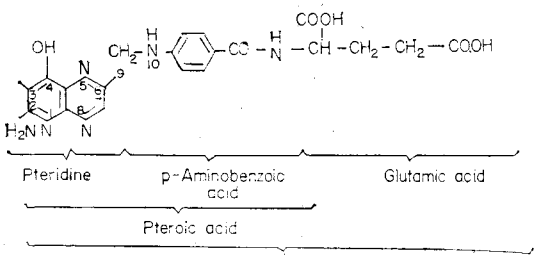

Figure 1. Structure of folic acid(Pteroylmonoglutamic acid: PGA)

capable of indicating minor differences in chemical strucure of folate available in human and animal materials. Table 1 presents the specificity of assay organisms for various forms of folate[9]. L. casei responds to a wide range of conjugated and unconjugated pteroylglutamates[1,9]. It includes N^5 methyl-tetrahydro-PGA and triglutamates which do not support in the growth of other organisms. S. faecalis responds to the unconjugated PGA and formyl derivates.

The most extensive assay was performed used standardized methods which were carried out by Toepfer, Zook, Orr and Richardson[10] in U.S. to determine the amounts of free and total folic acid in foods. Although this study carried out prior to the use of ascorbic acid to protect against the oxidation of tetrahydrofolates.

Data available in the literature with inclusion of ascorbic acid is some what limited. Less extensive, the folate composition of foods using ascorbic acid protection are those of Lakshimiah and Ramasastri[11] in the Indian foods, Murata and Miyamoto[12~13] and Taguchi et al[14~15] in Japanese foods. Data on the folate of individual foods in the U.S. have continued by Herbert[16], Dong and Oace[17], Butterfield and Calloway[18] Similar data has reported by Hoppner et al[19] in Canada and Sucke wer[20] in Poland. Elsewhere the data has been reported by Hurdle et al[21], though they did not use conjugase treatment in all instances to free the conjugated folate forms to pteroylglutamic acid. None of the similar Korean data has been available.

Since the human depends solely on the ingested folates for the supply of this B vitamins, the data on folate content of foods are essential for estimating dietary intake. However, information available for assay methods for folate in foods and the concentration of the nutrient in foods are somewhat incomp lete and results are in conflicting interpretation.

With these limitations, this study is to investigate the folate content and pattern of some selected foods on the Korean market and to clarify conflicting data in the literature and to evaluate the sources of dietary folate.

Materials and Methods

Food Samples

The individual foods selected for assay are based on the general diet in which consisted of and consumed almost all the time by Korean. This study includes only certain common foods such as rice, beans, peas and some other vegetables. The food samples were obtained from ordinary commeircal sources in either fresh or dried state and prepared from edible portions of the foods. Dry foods such as rice, peas and beans were softened by soaking overnight in water. The sequence of sampling and analysis

Table 1. Specificity of Assay Organisms for Various Forms of Folate

Assay Organisms	"Free" Folate			Polyglutamyl Folate		
	Methyl	Non-methyl Oxidized	Non-methyl Reduced	Methyl	Non-methyl Oxidized	Non-methyl Reduced
L. Casei	+	+	+	−	−	−
S. Faecalis	−	+	+	−	−	−

+ The organism can utilize fully as indicated chemical form of folate.
− The organism cannot utilize as indicated form to a significant extend. (adopted from Blakley[9])

were reported for a total of four to five sampling for each food representing triplicate assay.

Assay Procedure

The food samples were homogenized in with 0.1 M phosphate buffer(pH 6.4) containing 0.5 per cent ascorbic acid(pH readjusted to 6.4 with 1 N sodium hydroxide), made up to volume, and autoclaved for 15 minutes at 15 lb. pressure, then cooled to the room temperature. The autoclaved mixture was centrifuged and portions of supernatant were frozen in a screw-capped tube until used.

Chicken pancreas enzyme was prepared according to the method described by Santini et al[22] with some modifications. 10 g. of fresh chicken pancreas were homogenized in a blendor with 35 ml. phosphate buffer, pH 6.4. The homogenate was then diluted to 5 ml. with distilled water. The solution was distributed into the test tubes and stored in the freezer. Before use, 1 ml. of the solution was diluted to 10 ml. with distilled water. For standardization of this preparation, blanks using distilled water with diluted enzyme were run parallel with the samples. Aliguots of supernatant(each food sample homogenized in phosphate ascorbic acid buffer) divided into two parts. One part was assayed without conjugate treatment to determine the unconjugated or "free" folate activity. Another part was treated with the conjugate to release the conjugated folates(polyglutamate forms) in which after microbiological assay was carried out to determine the "total" folate activity.

Test organisms, both Lactobacillus casei(ATCC 7469) and Streptococcus faecalis(KFCC 35116) were maintained at 6° to 8°C as stabs in tubes containing 5 ml. nutrient agar. The medium consisted of 1 g. yeast extract, 0.5 g. glucose, 0.5 g. sodium acetate and 1.5g. agar in 100 ml. deionized water. A tube containing sterile single-strength assay medium and the appropriate folate standard was inoculated from a stab culture and was incubated at 37°C overnight. Two drops of this were transfered with sterile technique to a tube containing standard and incubated 4 hours at 37°C. 1ml. of this was diluted to 10 ml. in sterile saline solution*

* 0.8g. sodium chloride per 100ml.

and one drop of this suspension served as the inoculum.

2.5 ml of the double-strength assay medium[23] was contained in each tube. After making proper dilution of samples, aliquots which were taken in triplicate tubes was added to phosphate ascorbate buffer to bring the final volume to 5 ml. Tubes werer stirred, capped and placed in a boiling water-bath for 20 minutes and then cooled to room temperature. Tubes were incubated at 37°C overnight to check for sterility. The following day, each tube received one drop of inoculum. Tubes were stirred and then incubated for 20 hours at 37°C. For the determination of "total" folate activity, one drop of the diluted chicken pancreas enzyme extract was added to each tube and the tubes were incubated for 18 hours at 37°C. For the determination of "free" folate activity, the conjugase treatment was omitted only. And then the same procedure followed as for both "free" and "total" folic acid. The turbidity of tubes were read at 650mu in a spectrophotometer which had been zeroed on an uninoculated medium blank.

Results and Discussion

The folic acid content of some selected foods assayed by microorganisms is presented in Table 2. The Lactobacillus casei values are significantly higher at the different fraction with all food items than Streptococcus faecalis. This applies the former responds to oxidized and reduced folates including methyl folates, while the latter can not utilize methyl folates as pointed out in Table 1.[9] The folic acid content of foods assayed by L. casei ranged free 0.4 ~48.3, total 3.1~150.7 ug per 100 g. Among the individual foods, Garland Chrysanthemum 76.5, green peas 68.7, kidney beans 66.9 and leek 64.1 had considerably higher folate activity levels. Garlic 3.1 contained the least and spinach 150.7 contained extremely much folate activity out of the individual foods.

Table 3 shows the distribution of the chemical forms of folate in individual foods. The figures were recalculated from the data in Table 2 by taking into consideration of the specifications about the microorganisms. The considerable increase in folate activity released indicated that a large proportion of the folate

Table 2. Folic Acid Content of Individual Foods

Food	Number of Assay*	L. casei ug/100 g		S. faecalis ug/100 g	
		Without Conjugase Treatment	With Conjugase Treatment	Without Conjugase Treatment	With Conjugase Treatment
Cabbage	4	11.3	34.5	6.0	18.1
Carrot	5	7.4	17.8	3.8	10.6
Cucumber	5	2.0	25.3	0.2	2.1
Egg plant	5	6.2	24.7	2.0	6.6
Garland Chrysanthemum	4	6.0	76.5	0.8	7.2
Garlic	4	0.4	3.1	0.1	0.6
Green onion	5	15.0	40.2	7.2	18.4
Green peas	5	22.6	68.7	8.7	24.2
Green peppeer	4	2.1	27.1	0.3	2.4
Kidney beans	5	9.2	66.9	2.2	10.1
Leek	5	10.6	64.1	2.7	11.4
Lettuce	5	9.2	39.3	3.1	11.4
Onion	4	1.2	4.3	0.9	3.3
Spinach	4	48.3	150.7	24.2	68.7
Squash	5	4.8	26.1	1.5	7.8
Radish	4	14.6	40.3	5.3	16.1
Rice, highly milled	4	4.5	29.9	1.0	4.7

* Each assay represents triplicate

in those individual foods is in the polyglutamyl from [1,21] though the variation of individual foods is very great. Santini et al[22] noticed that the different amount of the enzyme preparation did not alter the total folic acid values, suggesting that there are conjugates which are not split by the enzyme when inhibitors are present. They also pointed out three to four-fold enhancement of the total folic acid activity if the extracts were filtered prior to treatment with the enzyme conjugase. Again it indicates that food residues may contain an inhibitor of the glutamate conjugase. This could be due to the fact that monoglutamate bind more readily than polyglutamates to fibers such as the cellulose present in foods[24].

The predominant chemical form of folate shows variable, even among similar foods. For example, total folate is ten times higher in green onion than in onion while green onion actually contains less nonmethyl folate than does in onion. Therefore, the proportions of different folate components from total folate data can not be predicted, suggesting the variation of folate pattern in individual foods.

Comparison with published values of folate content of individual foods is given in Table 4. Regardless of assay organisms with and without conjugase treatment, using ascorbic acid shows much increased the foods folate values. Our data actually corresponds the results in which was pointed out many fold differences from other reports[13,19,21] as they used ascorbic acid in assay. This results are doubtless due to the improvement in methodology, primarily the inclusion of ascorbic acid in a buffer solution to protect the labile form of folate againts oxidation of tetrahydrofolates. In this way, labile folates are protected when autoclaved. Applying destructive oxidation procedures, Ghitis and Carmen Candarosa[25] found that free folic acid activity is made up of a labile and a stable fraction, plus a frcation of intermediate lability. Hurdle et al[21] showed that the use of ascorbic acid with and without conjugase treatment increased the folate values in foods 1.2 to 40 times than that reported with the use of conjugase. He stated that this presumably implies that heat labile sample folates are often present in greater amount than complex

Table 3. Calculated Distribution of Folates in Individual Foods*

Food	Total Folate #			Free Folate #			
	All Forms ug/100 g	Methyl %	Non-methyl %	All Forms % of Total	ug/100 g	Methyl %	Non-methyl %
Cabbage	34.5	47.5	52.5	32.8	11.3	46.9	53.1
Carrot	17.8	40.4	59.6	41.6	7.4	48.6	51.4
Cucumber	25.3	91.7	8.3	7.9	2.0	90.0	10.0
Egg plant	24.7	73.3	26.7	25.1	6.2	67.7	32.3
Garland Chrysanthemum	76.5	90.6	9.4	7.8	6.0	86.7	13.3
Garlic	3.1	80.6	19.4	12.9	0.4	75.0	25.0
Green onion	40.2	54.2	45.8	37.3	15.0	52.0	48.0
Green peas	68.7	64.8	35.2	32.9	22.6	61.5	38.5
Green pepper	27.1	91.1	8.9	7.7	2.1	85.7	14.3
Kidney beans	66.9	84.9	15.1	13.8	9.2	76.1	23.9
Leek	64.1	82.2	17.8	16.5	10.6	74.5	25.5
Lettuce	39.3	71.0	29.0	23.4	9.2	66.3	33.7
Onion	4.3	23.3	76.7	27.9	1.2	25.0	75.0
Spinach	150.7	54.4	45.6	32.1	48.3	49.9	50.1
Squash	26.1	70.1	29.9	18.4	4.8	31.2	31.2
Radish	40.3	60.0	40.0	36.2	14.6	36.3	36.3
Rice, highly milled	29.9	84.3	15.7	15.1	4.5	22.2	22.2

* The data calculated from that of the data in Table 2 and based on the forms of folate presneted in Table 1.

Calculations: Total folate

All forms(ug/100g)=LCa

$$\text{Methyl}(\%)=\frac{LCa-SFa}{LCa}\times 100$$

Non-methyl(%)=100−Methyl(%)

Free folate

All forms(ug/100g)=LCb

$$\text{All forms}(\%)=\frac{LCb}{LCa}\times 100$$

$$\text{Methyl}(\%\text{ of free})=\frac{LCb-SFb}{LCb}\times 100$$

Non-methyl(%)=100−Methyl(%)

Where

LC=Lactobacillus casei

SF=Streptococcus faecalis

a=With chicken pancreas conjugase treatment

b=Without chicken pancreas conjugase treatment

polyglutamates that require deconjugation before L. casei. Also there was no significant difference between the effects of varying the amount of ascorbic acid concentration from 150 mg./100 ml. up to 10 g./100 ml.

Our data assayed by L. casei with and without conjugase agrees closely with the reports[13,19] in each individual food. In contrary, the report[14] shows much higher figures of which similar assay technique was employed. Accordingly, it can say that these occasions were presented to be considered about differences such as the kind of food, active derivates of food itself, method of keeping food, soil being produced food, differences affected by season, especially differences caused by the condition of measurement.

Microbiological assay methods are unable to tell how much of the food folic acid activity for a given microrganism is available to man. Conversely, foods contain complex derivatives which are not useful for the microorganism but it might be active for man[26,27]. Ghitis and Carmen Candarosa[25] stated that a ratio of activity for L. casei active for man expressed PGA equivalence can be defined in spite of the factor that only assay

Table 4. Comparison of Published Values of Folate Content of Individual Food

Food	Folate Content	LC, AsA (This study) ug/100 g	LC, AsA (Taguchi, 1972) ug/100 g	LC, AsA (Hoppner, 1972) ug/100 g	LC, AsA (Miyamoto, 1973) ug/100 g
Cabbage	Fr	11.3	39.8	25.1	5.0
	To	34.5	110.8	30.0	30.0
Carrot	Fr	7.4	21.8	14.6	6.0
	To	17.8	67.3	18.3	13.0
Cucumber	Fr	2.0	30.5	9.6	—
	To	25.3	102.2	15.6	—
Egg plant	Fr	6.2	12.8	7.8	2.0
	To	24.7	50.9	13.4	21.0
Garland Chrysanthemum	Fr	6.0	59.8	—	8.0
	To	76.5	138.2	—	78.0
Garlic	Fr	0.4	29.2	—	—
	To	3.1	35.2	—	—
Green onion	Fr	15.0	73.4	45.7	9.0
	To	40.2	143.8	58.0	35.5
Green peas	Fr	22.6	120.0	21.1	13.0
	To	68.7	352.0	33.4	19.0
Green pepper	Fr	2.1	—	8.2	2.0
	To	27.1	—	19.2	12.0
Kidney beans	Fr	9.2	—	23.5	9.0
	To	66.9	—	132.8	37.0
Leek	Fr	10.6	35.4	—	11.0
	To	64.1	102.2	—	61.0
Lettuce	Fr	9.2	63.5	24.1	4.0
	To	39.3	111.8	23.7	13.0
Onion	Fr	1.2	14.0	—	1.0
	To	4.3	57.8	—	3.0
Spinach	Fr	48.3	124.0	175.6	33.5
	To	150.7	235.1	203.5	145.5
Squash	Fr	4.8	36.0	23.1	—
	To	26.1	90.1	31.3	—
Radish	Fr	14.6	37.8	18.1	15.0
	To	40.3	91.5	24.0	49.0
Rice, highly milled	Fr	4.5	40.0	—	4.9
	To	29.9	123.2	—	31.0

Fr="Free" folic acid To="Total" folic acid
LC=Lactobacillus casei
AsA=Ascorbic acid

of human is valid in determing the available folate for man. Thus, in spite of the factors discussed above, it becomes clear to conclude that the data may require reevaluation depending upon further defined assay methods developed and showed to be used only as an approximate guide at the present time.

Summary

Folic acid is needed for normal hematopoiesis and must be furnished from diet. In order to estimate availability of food folate to man, the individual foods were selected for assay, based upon the general pattern of Korean diet in which consisted of and consumed almost all the time by Korean. Only certain common foods such as rice, beans, peas and some other vegetables were measured in this study by the method of microbiological assay using Lactobacillus casei and Streptococcus faecalis with and without chicken pancreas conjugase treatment.

The folate content of foods assayed by L. casei are significantly higher in all individual foods than

released to the assay organisms especially to L. casei by conjugase treatment indicates that a large proportion of the folate in foods is in the polyglutamyl form.

Although, the predominant chemical form of folate in the individual foods shows variable, even among similar foods. The use of ascorbic acid in assay to protect the labile form of folate against oxidation of tetrahydrofolates shows much increased folate contents at the different level in all of individual foods. These data were compared with some published values.

Total folate contents of foods assayed by L. casei were: cabbage 34.5, carrot 17.8, cucumber 25.3, egg plant 24.7. Garland Chrysanthemum 76.5, garlic 3.1, green onion 40.2, green peas 68.7, green pepper 27.1, kidney beans 66.9, leek 64.1, lettuce 39.3, onion 4.3, spinach 150.7, squash 26.1, radish 40.3 and highly milled rice 29.9 ug per 100 g.

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