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Note

Polyamine Content of Ordinary Foodstuffs and Various Fermented Foods

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Soybeans, tea leaves, and mushrooms were conspicuously rich in spermidine, while oranges contained a large amount of putrescine. Among the fermented foods, soy sauces were rich in putrescine and histamine, while Japanese sake contained plenty of agmatine. These polyamines are thought to be produced from amino acids during fermentation with amino acid decarboxylases formed by the microorganisms.

Key words: polyamine; food polyamine; histamine; agmatine

It has been well documented that polyamines (putrescine, spermidine, and spermine) are essential components of all living cells and play diverse roles in cellular metabolism and growth. The regulation of polyamine biosynthesis is complex and the key biosynthetic enzyme, ornithine decarboxylase, which produces putrescine from ornithine, is probably one of the most highly regulating enzymes.[1–3] Precise regulation prevents overproduction and deficiency of polyamines in animals, and excessive amounts of polyamines are known to be harmful.[2,4]

Despite the importance of polyamines and exogenous polyamine uptake,[5] the influence of dietary polyamines on the human body and health are completely unknown.[6] As the first step to elucidate the precise biological effect of exogenous polyamines on humans, we planned to determine the polyamine content of commonly consumed foodstuffs, which have not previously been studied in any detail except for the reports by Bardócz *et al.*,[5] and then to examine any apparent relationship between polyamine intake and human health through animal experiments and an epidemiological

Table I. Polyamine Content of Ordinary Foods

Food	Polyamine content (nmol/g)								
	put	spd	spm	cad/hit	agm	c. put	c. spd	c. spm	c. cad/hit
Starchy foodstuff									
Rice, polished	<10	27	<20	ND	ND	ND	10	34	ND
Wheat flour	17	66	26	ND	ND	22	15	18	ND
Corn	980	240	ND	ND	ND	46	13	<5	ND
Potato	200	93	ND	12	ND	ND	ND	ND	ND
Vegetables									
Spinach	29	120	<5	820	<5	42	22	ND	140
Carrot	40	55	ND	ND	ND	ND	ND	ND	ND
Tomato	120	12	ND	45	ND	ND	ND	ND	ND
Soybean, dried	470	1430	340	84	26	ND	ND	53	ND
Mushroom shiitake	29	890	ND	<5	ND	ND	24	<5	ND
honshimeji	210	480	68	ND	<5	ND	26	ND	ND
enokidaka	15	600	<5	<10	ND	12	46	ND	39
Fruit									
Orange	1330	13	8	ND	ND	ND	ND	ND	ND
Apple	ND	7	ND	ND	ND	ND	ND	ND	ND
Tea									
Green tea dried leaf	547	851	524	ND	ND	89	58	80	<5
extract[a]	17	32	<5	ND	ND	53	11	ND	ND
Meat									
Pork, lean	13	32	140	<5	ND	19	ND	21	ND
Beef, lean	6	18	140	100	ND	<10	14	77	<5
Chicken	<5	20	310	<10	ND	30	11	<10	<5
Tuna fish	ND	30	34	ND	ND	ND	ND	33	<5
Egg (hen)[b]	<5	<10	<5	<5	ND	ND	ND	ND	ND
Milk (cow)	ND	ND	ND	ND	ND	ND	ND	ND	ND

Each food sample was raw unless indicated otherwise.

[a] Each value is expressed as the amount of extracted polyamine/g of dried tea leaf.

[b] Raw egg and boiled egg were analyzed after removing their shells. Boiled egg yolk was subjected to an ether extraction to remove lipids before the polyamine analysis.

put, putrescine; spd, spermidine; spm, spermine; cad, cadaverine; hit, histamine; agm, agmatine; c. put, conjugated putrescine; c. spd, conjugated spermidine; c. spm, conjugated spermine; c. cad, conjugated cadaverine; c. hit, conjugated histamine; cad/hit, cad or hit.

ND: not detected.

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investigation.

The foods analyzed were obtained from local and special markets. The polyamines were analyzed by a combination of several methods described previously.[7-9] Briefly, each food was homogenized in 5% trichloroacetic acid and then centrifuged. An aliquot of the supernatant was applied to a weak cationic ion-exchange resin column (Muromac CR-70, Muromachi Kagaku) to eliminate the amino acids and peptides which would interfere with the analysis. The polyamines were eluted with 6 N HCl which was subsequently removed by evaporation. The eluate was subjected to a high-performance liquid chromatographic (HPLC) analysis. The conjugated polyamines were determined from the difference in the free polyamine contents between a hydrolyzed and non-hydrolyzed sample solution. HPLC was conducted with a TSK-gel Aminopak column (Tosoh) at 65°C. The polyamine elution was monitored with a spectrofluorometer after reacting with an *o*-phthalaldehyde solution. The samples containing histamine and/or cadaverine which were eluted at the same retention time were enzymatically identified by using putrescine oxidase which oxidizes cadaverine, but not histamine.[8] The differences in the values obtained from 5 separate analyses were within 15% for one sample. The values shown in the tables are the mean amounts of polyamines measured at least twice for at least 2 different samples of the same type of food.

As shown in Table I, many ordinary foods contain only small amounts of polyamines, while soybeans and green tea were found to contain large amounts of polyamines. Soybeans are the raw material for manufacturing many kinds of food in Japan and in many Asian countries. Tofu, a soybean protein curd, had little polyamine content, suggesting that polyamines are not bound to proteins (data not shown). Tea leaves, especially green tea, were rich in polyamines. Although oolong tea and black tea are referred to as fermented, fermentation usually proceeds *via* cellular enzymes in the leaves, unlike other fermented foods in which fermentation proceeds by microorganisms (Table II). In contrast to the large polyamine content of tea leaves, a hot-water extract, which had been prepared by adding about 50 volumes of hot water to dried leaves at 85°C for 4 min without homogenization, contained only a few percent of the total amount of polyamines contained in the leaves, indicating that the tea we usually drink contains only small amounts of polyamines. However, Japanese powdered tea is usually drunk as a suspension and is subjected to low pH in the stomach, so that polyamines will be released into solution.

Spinach is rather unique in its large histamine content which was identified by the enzymatic method already described. Most of the spermidine in dry shiitake appeared to be in the bound form, since little was found in the immersion water extract (data not shown). The putrescine content of oranges without peel and

Table II. Polyamine Content of Fermented Foods

Food		Polyamine content (nmol/g)								
		put	spd	spm	cad/hit	agm	c. put	c. spd	c. spm	c. cad/hit
Fermented soybean products										
Soy sauce[a]	koikuchi	540	100	<5	3730	ND	170	<5	ND	150
	saishikomi	1420	190	<5	4030–8010	<10	960	57	<10	820
	tamari	1640–5530	84–250	11	1310–21200	<10		90–160	<5	810–4180
	shiro	310	<5	<5	130–960	ND	ND	<5	<10	ND
Miso[b]	barley	450	30	ND	<10	88	58	140	23	26
	rice	310	130	ND	42	ND	58	83	ND	<10
	soybean	230	81	10	30	42	38	110	ND	27
Natto		110	190–680	41	69	ND	ND	ND	ND	ND
Tempe		520	750	29	ND	ND	ND	ND	ND	ND
Vinegar	rice	<10	<10	ND	<10	ND	<5	<5	ND	ND
	apple	ND	ND	ND	ND	ND	ND	ND	ND	ND
Alcoholic beverages										
Beer		54	<5	ND	<10	37	14	<10	ND	ND
Sake		<10	<5	ND	ND	880	<5	<5	ND	ND
Liquor	distilled	<5	<10	ND	ND	ND	<10	ND	ND	ND
Wube	white	<10	<5	<5	<5	ND	ND	ND	ND	ND
	red	33	<5	ND	11	ND	<5	ND	ND	<5
Fermented milk products										
Yogurt		<5	ND	ND	ND	ND	<5	ND	ND	ND
Cheese	camembert	ND	<10	ND	ND	ND	ND	ND	ND	ND
	gouda	ND	ND	ND	ND	ND	ND	ND	ND	ND
	blue	77	140	ND	ND	ND	25	25	ND	ND
Fermented tea										
Oolong tea	leaf	100–350	140–330	58	<10	ND	81	<10	66	16
	extract[c]	24	10	ND	ND	ND	31	15	14	<5
Black tea	leaf	80	130	94	<5	<5	18	11	35	<5
	extract[c]	12	12	<10	<5	ND	<5	<10	ND	<5

[a] Koikuchi is the most basic soy sauce; that is, soybeans and wheat are fermented with various microorganisms such as *Zygosaccharomyces rouxii* and *Pediococcus halophilus*. Tamari is a type of soy sauce in which almost all of the fermentation material is soybean. Saishikomi is a refermented soy sauce; *i.e.*, soybean is fermented by adding soy sauce instead of salt water. Shiro soy sauce is made by fermenting wheat grain with a small amount of soybean, resulting in rapid maturation.

[b] Miso is prepared by growing halophilic microbes on highly salted cooked soybeans. *Aspergillus oryzae* was grown on rice for rice-miso or on barley for barley-miso, before these cultures were added to the fermentation mixture.

[c] Each value is expressed as the amount of extracted polyamine/g of dried tea leaves.

The abbreviations used are the same as those in Table I.

seeds was conspicuously high. Other foods of animal origin such as egg and milk were found to lack polyamines.

The polyamine contents of fermented foods are summarized in Table II. Miso and soy sauce are typical fermented foods made mainly from soybean and were found to contain high levels of putrescine and spermidine, but almost no spermine. Interestingly, considerable amounts of the conjugated form of polyamines and large amounts of histamine (several thousands nmol/g) were found in soy sauce. The polyamine profile of soy sauces was quite different from that of soybeans, indicating that those polyamines were produced through the fermentation process. The large amounts of putrescine and histamine in soy sauce are presumed to be produced through simple decarboxylation of amino acids during fermentation by the decarboxylases of some microorganisms. No histamine was detected in miso, and there was no marked difference among the 3 kinds of miso investigated (rice, barley and soybean), which differs from the result for soy sauces. This may be accounted for by the fact that the percentage of soybeans in the fermentation materials of the 3 kinds of miso was similar, unlike the case of the different soy sauces, and liquefaction of the materials did not proceed as much as it did in the soy sauces, resulting in similar amounts of solubilized amino acids in the fermentation mixtures.[10]

Natto and tempe, relatively simple forms of fermented soybean compared with soy sauce and miso, reflected the polyamine content of soybeans, but contained much lower amounts, suggesting the degradation of some polyamines during fermentation.

Vinegar and various kinds of alcoholic beverages hardly contain any polyamines, except for putrescine in beer and red wine, and agmatine in sake and beer. Sake uniquely contained large amounts of agmatine (about 900 nmol/g) which was probably produced from arginine present in the fermentation mixture during the fermentation process by arginine decarboxylase that had been secreted by lactic acid bacteria and nitric acid-reducing bacteria.[11] Although the intake of small amounts of these amines may have no effect, the repeated intake of alcoholic beverages in extraordinary amounts might have some influence on humans. Fermented products from milk contained no polyamines, except for some types of cheese such as blue cheese (Table II) and chedder.[5]

It should be noted that fermented foods which use soybeans as a starting material are relatively rich in polyamines. This is probably due to the fact that soybeans contain relatively large amounts of polyamines and also that soybeans are rich in proteins (40–50%) compared with other representative fermentation materials like wheat or rice (8–13% and 6–9%, respectively), which are hydrolyzed to form amino acids, the precursor of polyamines, through the fermentation process.[10] Another reason may be that the production of soy sauce and miso requires a complicated process with many kinds of microorganisms and a long maturation period, which result in the presence of various amino acid decarboxylase-producing bacteria in the fermentation mixture and allows sufficient time for enzymatic reactions to occur. This may also be the case for Japanese sake which contained a large amount of agmatine.

Considerable amounts of conjugated polyamines were detected in tea leaves, soy sauce, and miso. These foods, which are comparatively rich in conjugated polyamines, also contained large amount of the non-conjugated forms of polyamines. An analysis with acetylpolyamine amidohydrolase[12] showed more than half of the conjugated putrescine in soy sauce were in the acetylated form, while the conjugated polyamines in tea leaves were not acetyl derivatives. An analysis of conjugated polyamines in miso was not possible due to interfering substances.

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