B-COMPLEX VITAMIN CONTENT OF CHEESE [1, 2]
II. NIACIN, PANTOTHENIC ACID, PYRIDOXINE, BIOTIN, AND FOLIC ACID

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SUMMARY

A large number of samples of 23 varieties of cheese and eight varieties of processed cheese or cheese foods and spreads were assayed for several B vitamins. The cheeses contained on an average 277 μg of niacin, 94 μg of vitamin B_6, 691 μg of pantothenic acid, 1.79 μg of biotin, and 22.3 μg of folic acid per 100 g. There were wide variations in vitamin content among individual samples of the same variety, as well as among the different varieties. The proteolytic type cheeses, Blue, Camembert, etc., had the most niacin, vitamin B_6, pantothenic acid, biotin, and folic acid. The hard varieties, except for biotin and folic acid, were next, followed by semihard or soft-unripened types. The proteolytic varieties were high in vitamin content, possibly because microorganism varieties synthesize vitamins during curing. In general, any cheese variety high in one B-vitamin was high in most of the other B-vitamins studied. The cheese contained 9 to 55% of the vitamin content of milk and, nutritionally, cheese may be considered a fairly good source of B vitamins.

The public is becoming increasingly interested in the vitamin content of food. More information is needed relative to the vitamin content of different foods, to help determine the nutritive value of various diets. The importance of dairy products as a source of vitamins of the B-complex is discussed by Chapman et al. (4) and Sullivan et al. (20). Wokes et al. (21) have reported that vegetarians whose diet contained dairy products showed no apparent ill-effects; whereas, another group of vegetarians who ingested no dairy products developed several illnesses which were alleviated by B_{12}.

Cheese occupies an important place in the American diet. The per capita yearly consumption of ripened and unripened cheeses has risen to more than 14 lb. The literature contains relatively limited information on the vitamin content of cheese. Many of these studies were concerned with the vitamins A, D, ascorbic acid, thiamine, and riboflavin content of cheese (5, 7, 8, 10, 12) and little attention has been given to the other vitamins of the B group (15, 19, 20).

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Aykroyd and Roscoe (2) were perhaps the first to assay cheese for vitamin B_2 content. In 1936, Day and Darby (6) reported that American cheese contained 80 μg of riboflavin per 100 g, Swiss cheese 60 μg, and cream cheese 14 μg. Most of the work on cheese was done during the 1940's (5, 7, 8, 14, 20), because of the meat shortage at that time and the greater importance of cheese from a nutritional point of view. Several of these studies, however, were made on only a few samples and their results differ widely, possibly because of the different assay methods employed. The present study was conducted, therefore, because of the lack of information concerning the content of some of the newer B vitamins in several varieties of cheese commonly used in this country. A report on the riboflavin content of cheese has been published by Hathaway and Davis (14), and in this paper are presented results of assays of several cheese varieties for other B-complex vitamins.

EXPERIMENTAL PROCEDURE

Market samples of various cheeses were obtained from the University of Nebraska dairy and local food stores, and from Omaha and Milwaukee. The vitamin content was determined immediately, or the samples were held frozen until the assay could be made. The cheeses were classed as follows: Soft-unripened varieties, like Cottage and cream cheese; semihard varieties, like brick and Muenster; hard varieties, like Swiss or Cheddar cheese; very

hard varieties, like Parmesan and Romano; high proteolytic varieties, like Blue; and unclassified varieties. The B vitamins determined were: niacin, pantothenic acid, pyridoxine, biotin, and folic acid. In the initial stages of the study, the microbiological methods as described by the Association of Vitamin Chemists (1) and Chapman et al. (4) were tried. However, because of the differences in the pH, and in the solids and protein contents of different cheese varieties, a vitamin assay method for any vitamin could not be applied in exactly the same way to all the different varieties of cheese. Lawrence et al. (18) also observed that an assay method for a vitamin in milk may not be applicable to other products. Therefore, the microbiological assay technique for each vitamin was modified slightly for application to different cheese varieties. The methods in brief are described below.

Niacin. A weighed sample of cheese, ranging between 1.0 to 10.0 g, depending upon the kind of cheese, was blended with approximately 20 times its volume with 1.0 N sulfuric acid. The blend was autoclaved at 15 psi for 30 min, cooled, brought to pH 6.8 with 20% sodium hydroxide, and filtered. The niacin content of the filtrate was determined microbiologically, using *Lactobacillus arabinosus* ATCC 8014 as the assay organism.

Pyridoxine. A weighed sample of cheese, 0.3 to 0.4 g, was mixed thoroughly with 0.005 N hydrochloric acid and autoclaved at 15 psi for 4 hr. The sample was cooled, adjusted to pH

TABLE 1

Niacin content of a variety of cheeses

Cheese variety	No. of samples	Average solids	Niacin content		
			Range	Mean [a]	Std.[b] dev.
		(%)	(μg/100 g)	(μg/100 g)	(μg)
Highly proteolytic					
Blue	33	61	68-2,346	1,247	577.0
Roquefort	8	63	84-1,044	629	313.0
Camembert	10	51	46-1,124	586	414.1
Liederkranz	8	36	60- 767	428	284.4
Limburger	10	50	35- 390	158	114.1
Beer Kaese	10	65	34- 213	85	27.5
Port Salut	9	52	30- 120	59	28.0
			Avg	664	
Very hard					
Parmesan	10	75	44- 500	290	171.5
Romano	10	70	25- 173	77	53.3
Edam	10	72	19- 64	38	13.2
			Avg	135	
Hard					
Provolone	10	60	50- 314	191	103.0
Swiss	10	59	35- 233	120	219.1
Gruyère	9	57	62- 145	98	35.7
Gouda	10	61	24- 185	63	47.8
Cheddar	30	65	13- 212	58	46.0
			Avg	93	
Semihard					
Husker	10	62	30- 287	99	72.6
Brick	13	60	12- 163	89	45.3
Muenster	11	64	21- 125	67	34.5
Mozzarella	9	51	15- 126	62	33.0
			Avg	80	
Soft					
Cottage-creamed	10	25	22- 233	120	66.0
Cottage-plain [c]	10	26	51- 325	112	90.0
Neufchatel	8	40	29- 158	86	42.6
Cream	20	51	42- 175	83	35.0
			Avg	98	
Total	278		Grand avg	277	

[a] On wet or fresh weight basis.
[b] Standard deviation.
[c] Commercially known as dry Cottage cheese.

TABLE 2

Vitamin B_6 content of a variety of cheeses

Cheese variety	No. of samples	Average solids	Vitamin B_6 content		
			Range	Mean [a]	Std. [b] dev.
		(%)	(μg/100 g)	(μg/100 g)	(μg)
Highly proteolytic					
Camembert	22	51	142-367	248	189.5
Blue	22	61	101-239	200	56.0
Liederkranz	13	36	124-333	189	63.1
Roquefort	20	63	61-210	104	34.8
Limburger	22	50	39-163	89	31.1
Beer Kaese	19	65	42-139	83	22.2
Port Salut	8	52	47- 85	59	12.8
			Avg	146	
Very hard					
Parmesan	15	75	64-114	96	13.2
Edam	20	72	61-111	84	14.9
			Avg	89	
Hard					
Swiss	32	59	51-154	91	30.4
Cheddar	63	65	49-147	84	23.0
Provolone	21	60	58-122	83	18.0
Gruyère	46	57	55-116	81	13.2
Gouda	20	61	49-124	80	20.4
			Avg	84	
Semihard					
Husker	9	62	63- 96	79	11.9
Muenster	10	64	54-100	76	15.3
Brick	28	60	49-105	73	17.0
Mozzarella	10	51	51- 79	64	10.8
			Avg	73	
Soft					
Cottage-plain [c]	14	26	21- 90	56	25.3
Cottage-creamed	32	25	28- 83	53	18.1
Cream	32	51	32- 85	53	12.8
			Avg	53	
Total	478		Grand avg	94	

[a] On wet or fresh weight basis.
[b] Standard deviation.
[c] Commercially known as dry Cottage cheese.

4.5 with sodium hydroxide, and filtered. The filtrate was subjected to the vitamin assay, using *Saccharomyces carlsbergensis* ATCC 9080 as the test organism.

Pantothenic acid. A weighed sample of cheese (0.5 to 1.0 g) was mixed with 50 ml of water, adjusted to pH 6.8-7.0, and autoclaved at 15 psi for 15 min. After cooling, the bound vitamin in the sample was released by incubating it 24 hr either with mylase P or the double enzyme system (alkaline phosphatase and pigeon liver extract). After incubation, the mixture was filtered and made to volume, and pantothenic acid was determined, using *L. plantarum* (formerly *L. arabinosus* 17-5) as the assay organism.

Biotin. A 20- to 30-g sample of cheese was blended with 60 ml of distilled water, and 10 ml of the blend was mixed with 25 ml of 2.0 N sulfuric acid and autoclaved 1 hr at 15 psi. The autoclaved sample was diluted to 100 ml and extracted twice with ethyl ether. Following the extraction, a 10-ml aliquot of the sample was diluted to about 80 ml with distilled water in a 100-ml volumetric flask, its pH adjusted to 6.8, made up to volume with water, and used for the vitamin assay. The assay organism used was *L. arabinosus* ATCC 8014.

Folic acid. One-half gram of the cheese sample was mixed with 5.0 ml of 0.2 M phosphate buffer, pH 7.0, and heated 5 min at 100 C. The sample was cooled, mixed with 20 mg acetone-extracted chicken pancreas, and incubated at 37 C 24 hr. Following the incubation, the sample was placed in a boiling water–bath for 5 min, cooled, extracted with ethyl ether, and filtered. The vitamin content was determined in the filtrate, using *Strepto-*

coccus faecalis ATCC 8043 as the assay organism.

RESULTS

Niacin content of several varieties of cheese. A total of 278 samples of 23 cheese varieties were assayed for niacin, and the results are presented (Table 1). The average niacin content for all the cheese samples was 277 μg per 100 g. There were wide variations in the vitamin content of different samples of cheese of the same variety, as well as of different varieties and groups. The proteolytic cheeses contained the highest concentration, with very hard, soft-unripened, hard, and semihard being next in decreasing order. The standard deviations of the vitamin content of the different varieties of cheese varied widely, ranging from 13 for Edam to 577 for Blue cheese.

When the data were recalculated on the dry or moisture-free solids basis, essentially the same relationship was observed between the niacin content and types of cheese.

Pyridoxine (B_6) content of cheese. A total of 478 samples of 21 varieties of cheese were assayed for B_6, and the results are presented (Table 2). The grand average of the vitamin B_6 content of all the cheeses assayed was 94 μg per 100 g. In general, the vitamin concentration was of a decreasing order in the proteolytic, very hard, hard, semihard, and soft-unripened groups.

As observed for niacin, there were wide variations in the vitamin B_6 content of different samples of the same variety of cheese. However, the differences in the B_6 content of several groups of cheese were not as wide as in niacin. The standard deviations of all the varieties ranged between 11 and 190.

When the data were calculated on a dry solids basis, it became evident, however, that the soft-unripened group was the highest in the concentration of B_6, with the proteolytic group being very close to it. The hard, semihard, and very hard were the next, in decreasing order.

Pantothenic acid content of cheese. As pre-

TABLE 3

Pantothenic acid content of a variety of cheeses

Cheese variety	No. of samples	Average solids	Pantothenic acid content		
			Range	Mean [a]	Std. [b] dev.
		(%)	(μg/100 g)	(μg/100 g)	(μg)
Highly proteolytic					
Blue	13	61	1,004-3,416	2,046	234.0
Liederkranz	8	36	1,252-2,622	1,985	467.8
Roquefort	15	63	1,193-3,634	1,953	646.1
Camembert	15	51	464-6,578	1,398	2,037.0
Limburger	14	50	196-2,781	1,277	647.9
Beer Kaese	12	65	224-1,683	695	386.9
			Avg	1,523	
Very hard					
Edam	11	72	102-1,255	282	327.0
Hard					
Gruyère	5	57	544- 680	614	54.2
Provolone	6	60	29-1,025	476	377.3
Swiss	14	59	175-1,673	441	62.9
Gouda	15	61	65- 740	340	201.9
Cheddar	29	65	111- 711	331	140.0
			Avg	388	
Semihard					
Brick	16	60	108- 643	293	166.4
Husker	6	62	37- 349	240	108.6
			Avg	279	
Soft					
Cream	35	52	95- 645	271	96.7
Cottage-creamed	22	25	79- 416	223	92.4
Cottage-plain [c]	8	26	21- 873	144	290.3
			Avg	239	
Total	244		Grand avg	691	

[a] On wet or fresh weight basis.
[b] Standard deviation.
[c] Commercially known as dry Cottage cheese.

TABLE 4

Biotin content of a variety of cheeses

Cheese variety	No. of samples	Average solids	Biotin content		
			Range	Mean [a]	Std. [b] dev.
		(%)	(μg/100 g)	(μg/100 g)	(μg)
Highly proteolytic					
Camembert	20	51.1	1.92-17.84	5.70	4.00
Limburger	19	50.4	1.30- 6.59	2.26	2.20
Beer Kaese	9	64.7	1.06- 5.61	2.09	1.40
Liederkranz	11	36.3	0.63- 5.51	1.96	1.45
Blue	17	61.4	0.99- 3.65	1.64	0.80
Roquefort	15	62.9	1.02- 2.81	1.49	0.56
Port Salut	14	52.3	0.53- 3.61	1.18	0.74
			Avg	2.51	
Very hard					
Parmesan	10	75.2	0.90- 3.78	1.70	6.20
Edam	16	72.4	0.75- 5.14	1.52	1.11
Romano	15	70.2	0.55- 4.75	1.34	1.10
			Avg	1.50	
Hard					
Provolone	9	59.6	1.11- 2.19	1.79	0.47
Cheddar	57	65.0	0.98- 2.95	1.73	0.53
Gouda	20	61.4	0.65- 2.50	1.68	5.20
Swiss	25	59.0	0.35- 2.30	0.94	0.56
Gruyère	20	56.7	0.37- 1.92	0.84	0.30
			Avg	1.44	
Semihard					
Mozzarella	13	50.9	0.61- 2.33	1.62	0.49
Brick	30	59.9	0.87- 2.45	1.59	0.49
Muenster	11	64.0	0.96- 3.24	1.39	0.86
Husker	14	62.4	0.49- 1.49	1.18	0.41
			Avg	1.48	
Soft					
Cottage-plain [c]	9	26.3	1.44- 2.94	1.99	0.88
Cottage-creamed	21	25.4	0.48- 2.48	1.96	0.67
Neufchatel	11	39.8	0.79- 2.30	1.93	0.72
Cream	20	51.8	0.99- 3.72	1.64	0.77
			Avg	1.85	
Total	406		Grand avg	1.79	

[a] On wet or fresh weight basis.
[b] Standard deviation.
[c] Commercially known as dry Cottage cheese.

sented in Table 3, the pantothenic acid content in 244 samples of 17 varieties ranged between 144 and 2,046 μg per 100 g for plain Cottage and Blue cheese, respectively. The grand average of all the samples was 691 μg of pantothenic acid per 100 g of cheese. Here again, there were wide variations in the pantothenic acid content of individual samples of the same variety, as well as in different varieties of cheese. The vitamin content was highest in the proteolytic group and the lowest in the soft group. However, on the dry solids basis, the vitamin content of the soft-unripened group of cheese was higher than that of the hard groups.

Biotin content of different cheeses. As presented (Table 4), the biotin content of 406 samples of 23 varieties of cheese ranged from 0.84 μg per 100 g for Gruyère to 5.70 for Camembert cheese. The grand average biotin content of all the samples was 1.79 μg per 100 g of cheese. The proteolytic cheeses as a group were the highest in biotin content, and soft, very hard, semihard, and hard were next, in that order. However, when the vitamin content was calculated on a dry solids basis, the soft-unripened group was found to be the highest, with the proteolytic group being next to it. The semihard, hard, and very hard were the next, in decreasing order.

Folic acid content of cheese. A total of 716 samples of 23 varieties of cheese were assayed and the data are presented (Table 5). The folic acid content ranged between 6.4 and 120.6 μg

per 100 g for Swiss and Liederkranz, respectively, and the grand average for all cheeses was 22.3 μg per 100 g. The proteolytic group as a whole was the most potent source of the vitamin, and the hard cheese group was the lowest. Essentially the same relationship was observed when the vitamin content was calculated on a moisture-free basis.

Vitamin content of processed cheese, spreads, and special cheeses. Recently, new varieties of processed cheese, cheese spreads, and special cheeses have appeared on the market, and information concerning the vitamin content of such cheese varieties is not available. Therefore, several processed cheeses and cheese spreads were assayed for the various B vitamins, and the results are tabulated (Table 6). Also, although not presented in the table, ten samples of Velveeta and 31 samples of Kaukauna Klub were assayed for pantothenic acid, and they contained 786 and 985 μg of pantothenic acid per 100 g, respectively. In general, the water-soluble vitamins content in these types of processed cheese and spreads was comparable to that observed in natural hard cheeses. This may be because mostly natural cheeses are used in the manufacture of processed cheese and, since most of these vitamins are heat stable, no great loss occurs during processing. Also, while there were some variations in the individual vitamin contents of different types of cheese, there was little variation between different samples of the same type of cheese. This should be expected, since the methods for the

TABLE 5

Folic acid content of a variety of cheeses

Cheese variety	No. of samples	Average solids	Folic acid content		
			Range	Mean [a]	Std. [b] dev.
		(%)	*(μg/100 g)*	*(μg/100 g)*	*(μg)*
Highly proteolytic					
Liederkranz	21	36	75-248	120.6	49.5
Camembert	18	51	34- 96	62.2	37.5
Limburger	25	50	19- 97	57.5	26.5
Roquefort	24	63	20- 82	49.0	38.0
Blue	34	61	21- 45	36.4	5.2
Beer Kaese	20	65	8- 33	22.2	13.0
Port Salut	19	52	8- 34	18.2	8.0
			Avg	51.5	
Very hard					
Edam	41	72	6- 35	16.2	9.4
Parmesan	20	75	2- 15	7.3	3.5
Romano	24	70	2- 20	6.8	4.9
			Avg	11.4	
Hard					
Gouda	20	61	8- 36	20.9	11.6
Gruyère	51	57	5- 14	10.4	8.7
Provolone	15	60	4- 18	10.4	4.3
Cheddar	112	65	4- 21	10.4	4.5
Swiss	26	59	3- 15	6.4	1.3
			Avg	10.9	
Semihard					
Brick	30	60	5- 39	20.3	17.0
Muenster	25	64	3- 30	12.1	8.4
Husker	34	62	5- 19	10.4	5.1
Mozzarella	21	51	5- 17	9.9	5.1
			Avg	13.4	
Soft					
Cottage-plain [c]	27	26	21-105	42.9	29.0
Cottage-creamed	40	25	9- 36	23.6	12.0
Cream	56	51	5- 27	13.6	5.0
Neufchatel	13	40	7- 16	11.3	2.8
			Avg	22.1	
Total	716		Grand avg	22.3	

[a] On wet or fresh weight basis.
[b] Standard deviation.
[c] Commercially known as dry Cottage cheese.

TABLE 6

Vitamin content of a variety of processed and special cheeses

Processed cheese or spread	Average solids	Niacin			Pyridoxine			Biotin			Folic acid		
		No. of samples	Mean	Std. dev.	No. of samples	Mean	Std. dev.	No. of samples	Mean	Std. dev.	No. of samples	Mean	Std. dev.
	(%)		(µg/ 100 g)	(µg)		(µg/ 100 g)	(µg)		(µg/ 100 g)	(µg)		(µg/ 100 g)	(µg)
Hot processed													
American	61.5	12	81.1	41.1	15	82.4	11.5	15	1.7	0.46	26	7.8	1.2
Cheese-bacon spread	47.9	3	316.3	84.8	8	83.1	32.5	10	2.7	0.81	14	6.4	1.6
Garlic	53.3	8	116.6	60.5	:	.....	.....	14	4.0	0.99	18	12.5	9.2
Jalapenos	54.6	7	114.6	53.0	6	114.3	12.5	14	3.9	0.61	16	9.1	3.1
Velveeta	53.2	14	148.3	70.0	20	122.7	12.5	15	6.2	1.34	18	8.2	2.9
Cold processed													
Kaukauna Klub	57.3	15	51.8	23.5	26	141.3	25.5	27	4.9	1.10	21	5.4	1.6
Old English	65.5	10	41.5	16.5	7	76.0	13.2	13	2.6	1.15	22	16.0	6.8
Unclassified													
Ski Queen	87.4	5	813.4	247.8	:	.....	.....	25	16.6	1.52	8	4.6	2.4

manufacture of these types of cheese are highly standardized.

In general, the hot processed cheese varieties did not differ too greatly in vitamin content from the cold processed varieties, except in the case of niacin content, which was somewhat lower in the cold-processed varieties. Ski Queen, a whey cheese which constitutes the third group of unclassified or special cheese, was unusually high in the niacin, biotin, and folic acid content.

DISCUSSION

The average values of several vitamins in a large number of cheese varieties assayed were fairly typical of the few reported in the literature. Sullivan et al. (20) assayed 12 varieties of cheese and noted their niacin, pantothenic acid, and biotin contents ranged from 30 to 1,600, 130 to 960, and 1.1 to 7.6 µg per 100 g of cheese, respectively. Hardinge and Crooks (11) have reported that in Cheddar, Cottage, and processed cheeses, the pantothenic acid content ranged from 280 to 480; the biotin ranged from 3.6 to 4.6; folic acid ranged from 11 to 31 µg per 100 g; and Cheddar cheese contained 66 µg of B_6 per 100 g. The results of the present study revealed that the average niacin, B_6, pantothenic acid, biotin, and folic acid contents in a large number of cheeses were 277, 94, 691, 1.79, and 22.3 µg per 100 g, respectively.

In general, there were wide variations in vitamin content not only in different varieties and classes but also in different samples of the same variety. This might be due partly to the differences in the vitamin content of the milk used in the manufacture of the cheese. In addition, as pointed out by Burkholder et al. (3), the vitamin content of cheese depends in large measure on the organisms and methods used in its manufacture and the subsequent curing period.

The proteolytic types contained the highest concentrations of the vitamins. Perhaps the microorganisms present in these types synthesize vitamins during curing, as was observed by others (3, 19, 20). Pilot studies were carried out in this laboratory to determine the rate of synthesis of vitamin B_6 during the curing of certain types of cheese. Using the same lot of milk, Coulommier, Romadur, and Camembert cheeses were made and cured at 55 to 60 F. At the end of 5 days' curing period, Coulommier, Romadur, and Camembert contained 57.2, 55.0, and 65.7 µg of B_6 per 100 g of cheese, respectively. At the end of 64 days' curing, the vitamin content of cheeses increased to 131.6, 123.0,

and 215.0 μg per 100 g, respectively, indicating that during curing the organisms synthesize vitamin B_6. The organisms in Camembert cheese were able to synthesize the vitamin more rapidly than the organisms of the two other varieties.

Except for the biotin and folic acid content, the very hard or hard varieties of cheese were the next highest in vitamin content, followed by the semihard or soft-unripened types. The low folic acid content of the hard cheeses might result from the high processing temperature used in their manufacture, since folic acid is more heat-labile than B vitamins in general (1), particularly in an acid medium.

Ski Queen cheese variety was very high in niacin, biotin, and folic acid. It is made from cow's or goat's milk whey and contains more than 87% solids. Also, Velveeta cheese, in the manufacture of which whey solids are included, was relatively high in the B vitamins. The high concentration of the vitamins present in these cheeses might be because the whey solids contain a major part of the water-soluble vitamins present in the original milk. These observations are in harmony with the results of Sullivan et al. (20).

Since most of the cheese samples assayed were purchased from the market, no information was available regarding the vitamin content of the milk used in their manufacture. Therefore, the values for the B vitamins content of milk as reported in the literature were taken to calculate theoretically the retention of the vitamins in cheese from milk. The literature records average values for the niacin, B_6, pantothenic acid, biotin, and folic acid content of milk as 910, 700, 3,100, 4.7, and 60 μg per 100 g, respectively (4, 9, 11, 13, 17, 18). On the basis of the average values for these vitamins found in the cheese samples, it was observed that the cheeses contained 9 to 55% of the vitamin content of milk.

The significance of B vitamins in nutrition is well established. While they have been shown to be essential in the metabolism of carbohydrates, proteins, and fats in the human diet, the daily requirements for an adult have been established for only a few of the B vitamins. Day and Darby (6) have reported that a 4-oz portion of American cheese would provide approximately one-third of the daily requirement of riboflavin. The present data reveal that cheese is fairly rich in most of the B vitamins.

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