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Vitamin B_{12} Content of Fermented Foods in the Tropics

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The Vitamin B_{12} (V. B_{12}) content of fermented foods transported from tropical countries was analysed. Of those, Tempeh was an interesting example, because it is made of soybeans and contained a comparatively large amount of V.B_{12}, namely 4.6 μg/100g. However not all the Tempehs contained such large amounts. For example, a fresh sample of Tempeh which was transported from Indonesia as rapidly as possible contained a very low amount, 0.7 μg/100g, and Tempehs prepared in the laboratory by using the Tempeh-making fungus, *Rhizopus oligosporus*, contained only 0.02～0.06 μg/100g. However the low V. B_{12} content in Tempeh which was transported from Indonesia increased to a value of 8 μg/100g when the sample was incubated at 30°C, unlike in the Tempeh prepared in the laboratory. It is probable that microorganisms accompanied with Tempeh-making fungus were associated with the production of V.B_{12} in Indonesian Tempeh. Further studies will be required to identify the microorganisms capable of producing V.B_{12} and useful for the fermentation food industry. (Received May 9, 1983)

The only primary souce of vitamin B_{12} (V.B_{12}) in nature is the metabolic activity of microorganisms[1]. It is known that certain fermented foods in the tropics contain V.B_{12}[2]. The objective of this study is to isolate microorganisms producing V.B_{12} from tropical fermented foods and utilize them in the fermentation food industry in Japan as well as in tropical countries. The V.B_{12} content of some fermented foods in the tropics determined so far is reported here.

MATERIALS AND METHODS

1. Materials

Some of the fermented foods used in this study were brought from Indonesia and Thailand in Februrary 1982. Others were supplied by the First Laboratory of Applied Microbiology. A form of Fermented soybean, Tempeh, shown in Table 2, was brought from Indonesia in January 1983. Some of the Tempehs were prepared with a starter of Indonesian Tempeh or with *Rhizopus oligosporus* NRRL 2710[3], a Tempeh-making fungus. Samples of 1～3g were used to prepare the test extracts of V.B_{12} assay.

2. Preparation of test extracts for V.B_{12} assay

The preparation was performed according to the method employed for the Japan Food Analysis Center. To each sample of 1～3g, 5 ml of 0.2M acetate buffer (pH 4.5), 0.2 ml of potassium cyanide (0.5 mg/ml), and approximately 30 ml of distilled water were added, and the samples were homogenized. The homogenate was heated at 100°C for 30 min, then cooled and 0.3 ml of a solution of 10% metaphosphoric acid was added. After the solution stood in ice water for a while, distilled water was added to bring the volume to 50 ml and centrifugation was performed. Two aliquots of 20 ml were removed from the supernatant. One portion was adjusted to pH 6.0 and distilled water was added to bring the volume to 40 ml, then the solution was re-centrifuged to supply the test extract A. The other portion was adjusted to pH 11～12 and heated at 120°C for 30 min. Thereafter the pH was adjusted to 6.0 and distilled water was added to reach a volume 40 ml. The solution was re-centrifuged to obtain the test extract B in which V.B_{12} was destroyed.

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3. V.B_{12} assay

V.B_{12} was assayed by applying a biological method using *Lactobacillus leichmannii* ATCC 7830 according to the instructive leaflet[4]. Details were given in the previous report[5] in which the method was re-evaluated because the standard curve obtained by this method showed fluctuations. The assay was performed in 16ϕ x 150 mm tubes with caps. Assay medium (2.5 ml of two fold concentrated basal medium 'Nissui'), standard V.B_{12} or test extracts (0.02 ~0.1 ng/tube) and distilled water were added to the tubes to bring the total bolume of all the tubes to 5 ml. The tubes were capped, sterilized at 120°C for 5 min and cooled as rapidly as possible. One drop of inoculum (OD≒0.25) was added aseptically to all the tubes except for two blanks tubes, then they were incubated at 37°C for 19 hr. After incubation, the tubes were steamed for 5 min in order to interrupt microbial growth, allowed to cool, and the cells were dispersed by shaking. The optical density readings at 600 nm of the standard curve prepared by placing 0, 0. 02 0.04, 0.06, 0.08, and 0.1 ng standard V.B_{12} in the tubes were plotted against the concentration of V.B_{12}. The V.B_{12} values in the unknown test extracts (A and B) were obtained by comparing the optical density with that of the standard curve. The difference between the values in A and B were considered to represent the 'true' content of V.B_{12}. The content of V.B_{12} in the original materials was calculated in taking account of the dilution for the initial preparations and of the factor of standard V.B_{12}.

Table 1. V.B_{12} content of fermented foods.

№	Sample name	(Country)	V.B_{12} (μg/100g)
1	Soy sauce mush	(Tha)	0.1
2	Fish sauce, 3-month fermantation	(Tha)	2.4
3	Fish sauce	(Tha)	0.2
4	Oyster flavoured sauce	(Tha)	0.7
5	Fish sauce	(Tha)	1.3
6	Soy sauce	(Tha)	0.2
7	Kung-jom, Fermented shrimp	(Tha)	2.5
8	Ka-pi, Shrimp paste	(Tha)	5.3
9	Tempeh, Fermented soy beans	(Ind)	4.6
10	Tauco, Miso	(Ind)	0.1
11	Ketchup, Soy sauce	(Ind)	0.1
12	Fish sauce	(Jpn)	1.0
13	Fish sauce	(Tha)	0.4
14	Tai pochona food	(Tha)	0.4
15	Anchov fish extract, Fish sauce	(Tha)	0.8
16	Oyster Flavoured sauce	(Tha)	0.1
17	Shin-shin ami, Fermented shrimp	(Sin)	0.3
18	Soy sauce mush	(Sin)	0.1
19	Sufu, Chinese cheese	(Sin)	1.1
20	Neli com patis, Fish sauce	(Phi)	0.1
21	Diners patis, Fish sauce	(Phi)	0.2
22	Dalisay patis, Fish sauce	(Phi)	0.3
23	Mariz patis, Fish sauce	(Phi)	0.1
24	Dalisay patis, Fish sauce	(Phi)	0.1
25	Natto, Fermented soy beans	(Tha)	1.5

Tha; Thailand Ind; Indonesian Sin; Singapore Phi; Philippjne
Jpn; Japan

RESULTS AND DISCUSSION

Table 1 shows the $V.B_{12}$ content of the fermented foods. The daily requirement for man is estimated to be 3 μg[6]. The fermented foods which contain an appreciable quantity of $V.B_{12}$ were No. 2 Fish sauce (3-month fermentation), No. 7 Kung-jom, No. 8 Ka-pi, No. 9 Tempeh, and No. 25 Thai Natto, with values of 2.4, 2.5, 5.3, 4.6 and 1.5 μg/100g, respectively. However the $V.B_{12}$ detected in samples No. 2, 7 and 8 may not have been produced by microorganisms, since the samples contained shrimp or fish. Important samples are No. 9 Tempeh and No. 25 Thai Natto which are made of soybeans. Their content in $V.B_{12}$ is considered to be increased through fermentation, since soybeans do not contain any $V.B_{12}$[7].

Although the $V.B_{12}$ content of Tempeh shown in Table 1 was 4.6 μg/100g, not all of the Tempehs contain such a large amount of $V.B_{12}$. Table 2 shows that fresh Tempeh transported as rapidly as possible from Indonesia contained a very low amount of $V.B_{12}$, namely 0.7 μg/100g. The Tempehs prepared in the laboratory with a starter of Indonesian Tempeh and with *Rhizopus oligosporus* contained less than 0.1 μg/100g of $V.B_{12}$. The value less than 0.1 μg/100g has no significance regarding the source of $V.B_{12}$, because if the requirements in $V.B_{12}$ for man were to be fulfilled only by foods with these levels, more than 3 kg of such foods would have to be consumed each day.

Table 2. $V.B_{12}$ content of Tempeh from Indonesia and Tempeh prepared in the laboratory.

Sample	$V.B_{12}$ (μg/100g)	Note
Tempeh[1)] (The first sample)	4.6	Transported slowly from Indonesia. Smell was bad.
Tempeh[2)] (The second sample)	0.7	Transported quickly from Indonesia. Smell was good. Sweet-like smell.
Tempeh	0.05	Prepared with a Tempeh starter transported from Indonesia.
Tempeh	0.06 0.02 0.03	Prepared with *Rhizopus oligosporus* NRRL 2710.
Japanese Natto	0.01 ~0.08	Reference 7.

1) Shown in Table 1. It was exposed at around 25°C for 3 days before taking a sample.
2) It was brought from Indonesia as quickly as posible. However it was exposed at around 25°C for 8 hr before taking a sample.

As mentioned above, the amount of $V.B_{12}$ content of Tempehs transported from Indonesia were very different (Table 2). The first sample, which was transported from Indonesia slowly therefor not so fresh, contained a large amount of $V.B_{12}$. The second sample, which was transported from Indonesia rapidly therefor fresher than the former sample, contained a low amount of $V.B_{12}$. Fig. 1 shows the reason why the former sample contained much more amount of $V.B_{12}$ than the latter sample. When the fresher sample was placed at a temperature of 30°C, its content in $V.B_{12}$ increased. The $V.B_{12}$ content which amounted to 0.9 μg/100g (the $V.B_{12}$ content had already increased from 0.7 to 0.9 when the experiment started) at the beginning of the experiment increased to 8 μg/100g at 30°C in 7 days. It is probable that a few microorganisms producing $V.B_{12}$ in the fresh sample increased in number when the sample was maintained at a temperature of 30°C. It may be postulated that in the first sample the microorganisms producing $V.B_{12}$ had propagated during the transportation. Tempeh prepared by using *Rhizopus oligosporus* only (Fig. 1) did not show an increase in $V.B_{12}$ content, because of the absence of microorganisms producing $V.B_{12}$ from the on set.

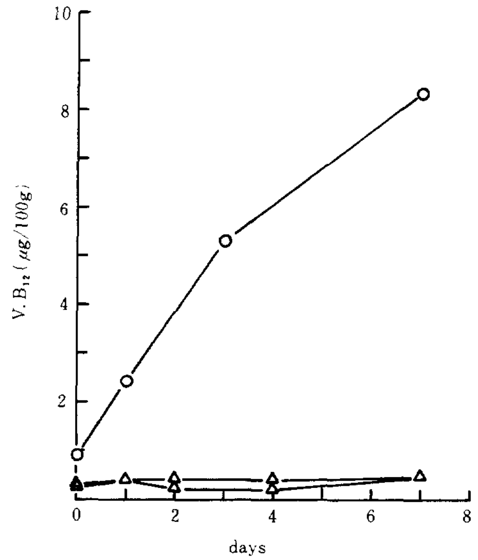

Fig. 1 Change of $V.B_{12}$ content in Tempeh.
○ : Tempeh transported quickly from Indonesia (The second sample).
△ : Tempeh prepared with *Rhizopus oligosporus* NRRL 2710.
Samples were put in the plastic bag and placed at 30°C.

Based on the above results it was evident that *Rhizopus oligosporus*, a Tempeh-making fungus, did not produce enough $V.B_{12}$ to become a useful source of $V.B_{12}$. It is probable that microorganisms accompanied with Tempeh-making fungus were associated with the production of $V.B_{12}$ in Indonesian Tempeh. Further studies will be required to identify the microorganisms capable of producing $V.B_{12}$ and useful for the fermentation food industry.

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熱帯産発酵食品のビタミンB_{12}含量

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熱帯産の発酵食品の$V.B_{12}$含量を調べた。この中でテンペは，大豆を原料としかつ$V.B_{12}$を4.6μg/100gとかなり含んでおり興味がもたれた。しかし，テンペのすべてが$V.B_{12}$をこのように含んでいるのではなかった。たとえば，インドネシアから急輸した新鮮なテンペは0.7μg/100gで非常に低い値であった。またテンペ製造用糸状菌*Rhizopus oligosporus*による実験室製造のテンペは僅か0.02～0.06μg/100gしか含んでいなかった。しかし，30°Cで7日間保つと，インドネシア産は$V.B_{12}$が8μg/100gまで増加したが，実験室製造のものには増加が認められなかった。テンペ中の$V.B_{12}$の生成は*Rhizopus oligosporus*に随伴している他の菌が関与していると考えられる。発酵食品工業にとり害のない有用な$V.B_{12}$生産菌を見つけるため，さらに実験を継続する必要がある。