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Characterization of honey from different floral sources. Its functional properties and effects of honey species on storage of meat

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Abstract

The antioxidative effects of honey species and their related products were evaluated using a lipid peroxidation model system. The antioxidant activities of honey species gradually decreased with passage of time. Buckwheat honey was as effective as 1 mM α-tocopherol. Superoxide-scavenging activities of propolis and royal jelly were strongest among the honey species tested. 1,1-Diphenyl-2-picrylhydrazyl radical scavenging ability of sample species were lower than those of 1 mM ascorbic acid and α-tocopherol. Hydroxyl radical scavenging activity was very high in all honeys (over 77% inhibition). From the results of the bacterial test on storage of meat and muscle, each honey exhibited the inhibition of bacterial growth. In particular, propolis and royal jelly exhibited the strongest inhibitory effects against bacterial growth. This suggests that honey species from different floral sources possess strong antioxidative and antibacterial activities and are scavengers of active oxygen species.

Keywords: Honey species; Royal jelly, Propolis; Antioxidative activity; Scavenging abilities against active oxygen species; Antibacterial activity

1. Introduction

The antibacterial property of honey has long been recognized in vivo (Aristotle, 1910) and in vitro (Sackett, 1919). The antibacterial activity was originally thought to be due the high osmotic properties of honey, but some activity persisted after dilution. Dold, Du, and Dziao (1937) termed this activity, inhibine, and found it to be labile to light and heat. Other workers (White & Subers, 1963; White, Subers, & Shepartz, 1962) concluded that inhibine was hydrogen peroxide generated by the action of glucose oxidase in excess of that which could be accounted for by the action of hydrogen peroxide alone (White & Subers, 1963). Adcock (1962) found antibacterial activity persisting after the removal of hydrogen peroxide by the addition of catalase.

The typical composition of honey is: moisture, 17.7%; total sugars, 76.4%; ash, 0.18%; and total acid (as formic acid), 0.08%. Traditionally, its use in food has been as a sweetening agent. However, several aspects of its use indicate that honey also functions as a food preservative. In Japan its consumption amounts to about 40,000 tons per year. The Japanese most frequently use honey on such items as sandwiches and toast. Honeys contain a number of components to act as preservatives; these include α-tocopherol, ascorbic acid, flavonoids, and other phenolics and enzymes such as glucose oxidase, catalase, and peroxidase (Crane, 1975;

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Ferreres, Garciaviguera, Tomaslorente, & Tomasbarberan, 1993; Ioyrish, 1974). It is suggested that any of these substances owe their preservative properties to their antioxidative activity (Cerutti, 1994).

In meat, amino acids and carbohydrates interact to form intermediates that are converted to meat flavour compounds by oxidation, decarboxylation, condensation, and cyclization. During the first stages of cooking, thermally induced oxidation of lipids produces the desirable flavour of meat. Lipid-derived carbonyl products interact with amino groups, the amino group of phosphatidylethanolamine interacts with sugar-derived carbon, and free radicals from peroxidized lipids interact with Maillard reaction compounds to produce a range of desirable volatiles (Kanner, 1994). The direct addition of honey to turkey breast meat prior to heating had an antioxidative effect on the meat, that has been attributed to Maillard reaction products formed during heating (Antony, Han, Rieck, & Dawson, 2000). The addition of Maillard reaction products formed by heating honey–lysine mixtures also had an antioxidative effect in a linoleic acid model system and in a turkey meat model system (Antony, Han, Rieck, & Dawson, 2002). Moreover, Antony et al. (2002) reported the effect of dry honey on oxidation in turkey breast meat, and they showed that an addition of up to 15% dry honey inhibited the development of oxidative compounds in cooked turkey meat. They suggest that honey can act as a natural antioxidant, which is important with the recent emphasis on decreasing the use of artificial preservation in food and the perception of honey as a healthy sweetener. Thus, although the functional properties of honey species in foreign countries are well studied, properties of Japanese honey species are not fully investigated.

As a part of this study on the functional properties of Japanese honey species, properties such as the antioxidative effects, the scavenging abilities of superoxide anion radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, and hydroxyl radical of Japanese honey species and their related products are evaluated. We also investigate the effects of honey species on storage of meats.

2. Materials and methods

2.1. Samples

Commercially available honey (Chinese milk vetch) was obtained from Suzuya SY Ltd. (Gifu, Japan). Royal jelly, propolis, honey species from the following sources: Chinese milk vetch, acacia, Japanese bee, buckwheat, and mixed-breed were obtained from Inoue Yohojo Bee Farm Inc. (Hyogo, Japan) and used in this study. Fresh beef, pork, chicken meats, and fish fillets (Horse mackerel *Trachurus japonicus*) were purchased from local market in Shimonoseki City, Yamaguchi Prefecture, Japan and were used in the following tests.

2.2. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2-deoxy-D-ribose, 2-thiobarbituric acid (TBA), 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), linoleic acid, α-tocopherol, xanthine, ascorbic acid, and nitroblue tetrazolium salt were purchased from Wako Chemicals Co., Ltd. (Osaka, Japan). Xanthine oxidase from butter milk (0.33 U/mg powder) was obtained from Oriental yeast Co., Ltd. (Tokyo, Japan). Yeast extract and agar were obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). Peptone was obtained from Kyokuto Pharmacy Co., Inc. (Tokyo, Japan). Other reagents used were of an analytical grade.

2.3. Preparation of sample solution

Each honey sample was diluted with distilled water and propolis with ethanol. Royal jelly was melted with 0.01 M NaOH. Each solution (50% (v/v) solution) was used as the sample solution for antioxidative activities, superoxide anion scavenging, DPPH, and hydroxyl radicals. In the storage test, 5% (v/v) sample solutions were used.

2.4. Measurement of antioxidation activity

The antioxidant activity was assayed by the method of Nagai and Inoue 2004. A 0.083 ml of sample solution and 0.208 ml of 0.2 M sodium phosphate buffer (pH 7.0) were mixed with 0.208 ml of 2.5% (w/v) linoleic acid in ethanol. The preoxidation was initiated by the addition of 20.8 μl of 0.1 M AAPH and carried out at 37 °C for 200 min in the dark. The degree of oxidization was measured according to the thiocyanate method (Mitsuda, Yasumoto, & Iwai, 1966) for measuring peroxides by reading the absorbance at 500 nm after colouring with $FeCl_2$ and ammonium thiocyanate. A control was performed with linoleic acid but without sample solution. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive controls. Distilled water was used as the negative control.

2.5. Scavenging ability against superoxide anion radicals

Superoxide anion radical scavenging was evaluated by the method of Nagai, Myoda, and Nagashima (2005). Briefly, this system contained 0.48 ml of 0.05 M sodium carbonate buffer (pH 10.5), 0.02 ml of 3 mM xanthine, 0.02 ml of 3 mM ethylenediaminetetraacetic acid disodium salt (EDTA), 0.02 ml of 0.15% bovine serum albumin, 0.02 ml of 0.75 mM NBT, and 0.02 ml of sample solution. After preincubation at 25 °C for

10 min, the reaction was started by adding 6 mU XOD and carried out at 25 °C for 20 min. After 20 min the reaction was stopped by adding 0.02 ml of 6 mM CuCl. The absorbance of the reaction mixture was measured at 560 nm and the inhibition rate was calculated by measuring the amount of formazan that was reduced from NBT by the superoxide. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive controls. Distilled water was used as a negative control.

2.6. DPPH radical scavenging

DPPH radical scavenging was evaluated by the method of Okada and Okada (1998) with a slight modification. The assay mixture contained 0.3 ml of 1.0 mM DPPH radical solution in ethanol, 2.4 ml of 99% ethanol, and 0.3 ml of sample solution. The solution was rapidly mixed and scavenging ability was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid (0.1 and 1.0 mM) and α-tocopherol (1 mM) were used as positive controls. Distilled water was used as a negative control.

2.7. Hydroxyl radical scavenging

2-Deoxyribose is oxidized by ·OH that is produced by the Fenton reaction and degraded to malonaldehyde. The reaction mixture contained 0.45 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM $FeSO_4$–EDTA, 0.15 ml of 10 mM H_2O_2, 0.525 ml of H_2O, and 0.075 ml of sample solution in an Eppendorf tube. The reaction was started by the addition of H_2O_2. After incubation at 37 °C for 4 h, the reaction was stopped by adding 0.75 ml of 2.8% (w/v) trichloroacetic acid and 0.75 ml of 1.0% (w/v) of TBA in 50 mM NaOH, the solution was boiled for 10 min, and then cooled in water. The absorbance of the solution was measured at 520 nm. Hydroxyl radical scavenging ability was evaluated as the inhibition rate of 2-deoxyribose oxidation by hydroxyl radicals (Chung, Osawa, & Kawakishi, 1997). Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive controls. Distilled water was used as a negative control.

2.8. Storage test

After meat samples (or muscle sample) were treated with 5% (v/v) honey species and its related products solutions at 10 °C for 30 min, they were taken out, wrapped in polyethylene film, and stored in the refrigerator at 10 °C. The refrigerated samples were used in storage tests. Meat samples (or muscle sample) (strips of the muscles, approximately 15 cm long and weighing about 10 g) were prepared by homogenizing in a ratio of 1:9 with sterile 0.85% NaCl solution in a Waring Blender. Microbial populations were determined by plating 10-fold serial dilutions of homogenates in duplicate. The media for bacterial growth was composed of 0.5% peptone, 0.25% yeast extract, 0.1% glucose, and 1.5% agar, pH 7.1. One milliliter of sample solution was poured on the plate. Total viable counts were estimated using standard plate count and results reported as colony forming units per gram sample (CFU/g).

2.9. Statistical analysis

All results were statistically analyzed by analysis of variance (ANOVA) followed by Fisher's PLSD test. Differences were considered significant for $p < 0.05$.

3. Results and discussion

3.1. Antioxidative activities of honey species and their related products

The thiocyanate method was used to measure the amount of peroxide during the initial stages of lipid oxidation. The antioxidative effects of honey species and their related products on the peroxidation of linoleic acid were investigated. These results are shown in Table 1. The antioxidative activity of the control was suddenly decreased over the duration of this study. This pattern was the same as those obtained using commercially available honey (Chinese milk vetch) and 1 mM ascorbic acid (Table 1). There were no differences in antioxidative activities among these honey species such as pure honey (Chinese milk vetch and acacia), propolis, and royal jelly. In particular, buckwheat honey was the most active, followed by mixed-breed honey and Japanese bee honey. The activity of buckwheat honey was similar to that of 1 mM α-tocopherol. On the other hand, mixed-breed honey and Japanese bee honey exhibited half the activity of both 1 mM α-tocopherol and 5 mM ascorbic acid. α-Tocopherol showed a high antioxidative activity from the initial stages of the peroxidation to 200 min.

3.2. Superoxide-scavenging activities of honey species and related products

Superoxide-scavenging activities of honey species and related products were measured using a xanthine–xanthine oxidase system (NBT method). These results were reported as the superoxide productivity. Each honey and its related products showed superoxide-scavenging activity and this activity decreased in the order: royal jelly > propolis > buckwheat honey > honey (Japanese bee) > mixed-breed honey > pure honey (Chinese milk vetch) > pure honey (acacia) > commercially available honey (Fig. 1). The strongest superoxide-scavenging activity was detected in propolis and royal jelly.

Table 1
Antioxidant abilities of honey species and its related products as measured by the thiocyanate method

Time (min)	Absorbance at 500 nm											
	Sample											
	A	B	C	D	E	F	G	H	I	J	K	CN
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
50	0.008	0.072	0.037	0.017	0.028	0.022	0.048	0.020	0.022	0.016	0.006	0.379
100	0.083	0.081	0.096	0.041	0.034	0.032	0.087	0.054	0.135	0.032	0.025	0.715
200	0.232	0.141	0.165	0.060	0.039	0.067	0.122	0.109	0.469	0.090	0.028	1.406

(A) Commercially available honey (Chinese milk vetch); (B) pure honey (Chinese milk vetch); (C) pure honey (acacia); (D) mixed-breed honey; (E) buckwheat honey; (F) honey (Japanese bee); (G) propolis; (H) royal jelly; (I) 1 mM ascorbic acid; (J) 5 mM ascorbic acid; (K) 1 mM α-tocopherol; (CN) control. Values are average of three replicates. Significant difference ($p < 0.05$).

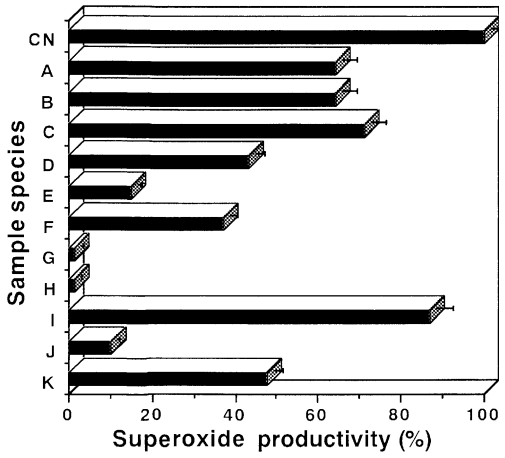

Fig. 1. Effects of honey species and its related products on the superoxide anion radical in xanthine–xanthine oxidase system by the NBT method. (CN) Control; (A) commercially available honey (Chinese milk vetch); (B) pure honey (Chinese milk vetch); (C) pure honey (acacia); (D) mixed-breed honey; (E) buckwheat honey; (F) honey (Japanese bee); (G) propolis; (H) royal jelly; (I) 1 mM ascorbic acid; (J) 5 mM ascorbic acid; (K) 1 mM α-tocopherol.

3.3. DPPH radical scavenging by honey species and related products

The DPPH scavenging activities of honey species and related products are shown in Table 2. The activity of 1 mM ascorbic acid was the highest and followed by that of 1 mM α-tocopherol. Buckwheat honey exhibited the strongest scavenging activity, of the honey products followed by mixed-breed honey, honey (Japanese bee), royal jelly, and pure honey (Chinese milk vetch). Ascorbic acid at lower concentration (0.1 mM) showed activity similar to commercially available honey and pure honey (acacia). DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples (Furuta et al., 2003; Hatano, 1995; Hatano et al., 1989, Hatano, Takagi, Ito, & Yoshida, 1997; Huang, Huang, Tso, Tsai, & Chang, 2004; Nagai, Inoue, Inoue, & Suzuki, 2002, 2005; Ohba et al., 2003; Yoshida et al., 1989). In the present experiment, it was found that many honey species showed DPPH radical scavenging activities. Amakura, Umino, Tsuji, and Tonogai (2000) reported that DPPH radical scavenging activities of nine fruit berries were associated with the contents of the total phenolics. This suggests that buckwheat honey, which showed strongest DPPH radical scavenging activity, may contain high content of total phenolics.

3.4. Hydroxyl radical scavenging by honey species and related products

The scavenging activities of honey species and related products against hydroxyl radical inhibition, reviewed

Table 2
DPPH radical scavenging activities of honey species and its related products

Time (min)	Relative activity (%)											
	Sample											
	A	B	C	D	E	F	G	H	I	J	K	CN
0	100	100	100	100	100	100	100	100	100	100	100	100
1	97.8	96.3	96.7	78.6	69.1	84.4	95.6	87.8	94.6	5.3	9.4	98.7
2	96.9	94.3	96.1	71.1	58.2	78.6	94.0	82.1	94.4	5.3	5.3	98.7
3	96.3	92.9	95.3	66.0	51.4	75.4	92.9	78.8	94.4	5.3	5.3	98.7
5	95.4	91.3	94.3	59.8	42.2	70.8	91.8	74.1	94.4	5.3	5.3	98.6
10	94.3	88.5	92.8	49.9	29.2	64.2	90.7	66.3	94.4	5.3	5.3	98.5

(A) Commercially available honey (Chinese milk vetch); (B) pure honey (Chinese milk vetch); (C) pure honey (acacia); (D) mixed-breed honey; (E) buckwheat honey; (F) honey (Japanese bee); (G) propolis; (H) royal jelly; (I) 0.1 mM ascorbic acid; (J) 1 mM ascorbic acid; (K) 1 mM α-tocopherol; (CN) control. Values are average of three replicates. Significant difference ($p < 0.05$).

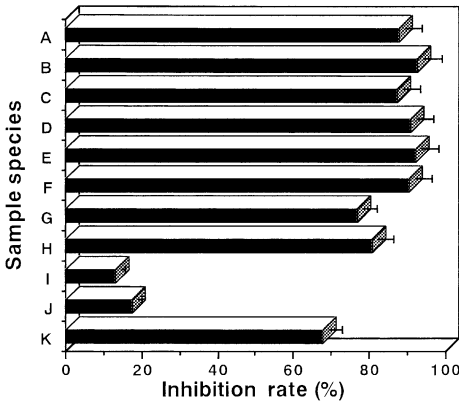

Fig. 2. Hydroxyl radical scavenging activities of honey species and its related products. (A) Commercially available honey (Chinese milk vetch); (B) pure honey (Chinese milk vetch); (C) pure honey (acacia); (D) mixed-breed honey; (E) buckwheat honey; (F) honey (Japanese bee); (G) propolis; (H) royal jelly; (I) 1 mM ascorbic acid; (J) 5 mM ascorbic acid; (K) 1 mM α-tocopherol.

using Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {\cdot}OH$), is shown in Fig. 2. Each honey sample showed hydroxyl radical scavenging activity and this activity was decreased in the order pure honey (Chinese milk vetch) > buckwheat honey > mixed-breed honey > honey (Japanese bee) > commercially available honey (about 90%) (Fig. 2). Surprisingly, propolis and royal jelly showed lower activities (76.7% and 80.7%) in tested samples. Hydroxyl radicals are shown to be capable of abstracting hydrogen atoms from membranes and bring about peroxide reactions of lipids (Kitada, Igarashi, Hirose, & Kitagawa, 1979). It is therefore thought that honey species demonstrate antioxidant effects against lipid peroxidation on meat or muscle membrane by scavenging the hydroxyl radicals and superoxide anions at the stage of initiation and termination of peroxy radicals.

3.5. Effects of honey species and related products on storage of meat and muscle

The effects of honey species and related products on storage of meat and muscle were investigated. As shown in Table 3, when beef meat was treated without honey species, total viable counts were in the order of 3.0×10^3 CFU/g after 3 days at 10 °C. Moreover, viable counts increased to 10^4 CFU/g after storage for 7 days. On the other hand, the total viable counts were of the order of 2.0×10^3 CFU/g after 7 days when the meat was treated with all honey species except for commercially available honey (3.0×10^3 CFU/g). Propolis and royal jelly exhibited the strongest inhibitory effects against bacterial growth: bacteria were not detected in any sample except for fish muscle treated with propolis after 3 days. The same results were obtained in pork, chicken, and fish muscle treated without honey species (Table 3). It is possible that the use of ethanol and NaOH as dilluents may affect the bacterial counts in meat samples containing propolis and royal jelly. However, it has been reported that propolis and its constituents have antimicrobial activities (Bosio, Avanzini, D'Avolio, Ozino, & Savoia, 2000; Koo et al., 2000). In fish muscle it seems that honeys such as pure honey (Chinese milk vetch), pure honey (acacia), and mixed-breed honey exhibited not only the inhibition of bacterial growth but also bactericidal effects. In general, it is well known that total viable counts of fresh fish such as sardine and skipjack tuna are in the order of 10^{3-4} CFU/g (Fujii, 1995). It was reported that total viable counts of fresh horse mackerel were in the order of 10^{4-5} CFU/g, and increased to 10^6 CFU/g when it was stored at 5 °C for 2 days (Sato, Fujii, Masuda, & Okuzumi, 1994). Moreover, its total viable counts were drastically increased to the order of 10^{8-9} CFU/g after storage for 6 days (Sato et al., 1994). This tendency was the same as that of mammalian meat. This suggests

Table 3
Effects of honey species and its related products on bacteriological storage of meats and muscle

Sample	Storage days											
	Beef (CFU/g)			Pork (CFU/g)			Chicken (CFU/g)			Fish (CFU/g)		
	0	3	7	0	3	7	0	3	7	0	3	7
	1.5×10^3	3.1×10^3	1.1×10^4	4.8×10^3	5.1×10^3	9.5×10^3	1.4×10^3	1.5×10^3	9.6×10^3	<300	1.6×10^3	8.2×10^3
A	<300	1.9×10^3	3.0×10^3	2.0×10^3	2.4×10^3	5.1×10^3	<300	1.3×10^3	2.4×10^3	<300	<300	2.8×10^3
B	<300	1.7×10^3	1.9×10^3	<300	1.9×10^3	2.3×10^3	<300	1.2×10^3	2.2×10^3	<300	<300	<300
C	<300	4.0×10^2	1.7×10^3	4.0×10^2	1.6×10^3	2.3×10^3	<300	<300	1.9×10^3	<300	<300	<300
D	<300	8.0×10^2	2.0×10^3	< 300	1.7×10^3	1.9×10^3	<300	<300	7.5×10^2	<300	<300	4.0×10^2
E	<300	<300	1.8×10^3	<300	<300	6.0×10^2	<300	<300	2.0×10^3	<300	<300	2.2×10^3
F	<300	1.4×10^3	1.8×10^3	4.0×10^2	5.0×10^2	1.7×10^3	<300	<300	3.0×10^2	<300	<300	3.0×10^3
G	<300	<300	<300	<300	<300	<300	<300	<300	<300	<300	<300	<300
H	<300	<300	<300	<300	<300	<300	<300	<300	<300	<300	<300	<300

(A) Commercially available honey (Chinese milk vetch); (B) pure honey (Chinese milk vetch); (C) pure honey (acacia); (D) mixed-breed honey; (E) buckwheat honey; (F) honey (Japanese bee); (G) propolis; (H) royal jelly; (CN) control. Values are average of 3 replicates. Significant difference ($p < 0.05$).

that the natural substances in honey species with dark colour possess strong inhibitory effects against bacterial growth. It is obvious from the present studies that each honey species inhibited the bacterial growth. It also seems that they owed this to their high sugar concentration (hygroscopic effect). The bactericidal effect of honey was confirmed by White (1975), who attributed it to the presence of substances called inhibine that result from the accumulation of hydrogen peroxide that is produced by the natural glucose-oxidase system in honey. The variation of the antibacterial activity may be apparently due to many factors that are beyond the control of honey keepers, such as differences in soil and atmospheric conditions as well as in the type and physiology of each plant. It also may result from differences in the chemical composition which could attributed to the floral sources and environmental conditions. In fact, it was found that honey species from different floral sources varied in their antibacterial activity: this is due to varying levels of hydrogen peroxide (Allen, Molan, & Reid, 1991). It was reported also that the antibacterial activity persisted after dilution of honey species (Russell, Molan, Wilkins, & Holland, 1990).

The problem of food spoilage has plagued humans since ancient times. In earlier times food was preserved with salt, sugar, spices, and wood smoke. With the development of new products, chemical antimicrobial agents and many organic acids were relied on to achieve a longer shelf life and greater assurance of protection from microbial spoilage. With growing concern over the presence of chemical residues in foods the demand for nontoxic natural preservatives is increasing. Many natural substances in honey species with different plant origins may play an important role in functional properties such as antioxidative and antibacterial activities.

In conclusion, higher antioxidative activity and scavenging activities against active oxygen species were observed in honey species with dark colour such as buckwheat honey and mixed-breed honey in comparison with light coloured pure honeys (acacia and Chinese milk vetch). Honey species from different floral sources also possess antibacterial activities and are scavengers of active oxygen species.

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