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Food Chemistry 75 (2001) 237–240

Food Chemistry

www.elsevier.com/locate/foodchem

Antioxidative activities of some commercially honeys, royal jelly, and propolis

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Received 4 January 2001; received in revised form 28 March 2001; accepted 28 March 2001

Abstract

The antioxidative effects of some honeys, royal jelly, and propolis were evaluated using a lipid peroxidation model. Activities gradually decreased over time. Buckwheat honey, pure honey (Chinese milk vetch), and propolis were the most active among honeys. Excepting royal jelly and propolis, the activities of all honeys were drastically decreased by heat treatment. The superoxide-scavenging activities of each honey, royal jelly, and propolis decreased in the order: propolis > royal jelly > buckwheat honey > honey (Japanese bee) > mixed-breed honey > pure honey (acacia) > commercially available honey > pure honey (Chinese milk vetch).

Keywords: Antioxidative activity; Honey; Scavenging ability of superoxide anion radical; Health food

1. Introduction

In recent years, the physiological functionality of foods has receieved much attention, due to the increasing interest in human health, and it has been studied in vitro and in vivo by many researchers. Antioxidative action, one of the important physiological functions of foods, is supposed to protect living organisms from oxidative damage, resulting in the prevention of various diseases such as cancer, cardiovascular diseases, and diabetes (Ames, Shigenaga, & Hagen, 1993; Gutteridge & Halliwell, 1994). The importance of protective defence systems in living cells, against damage caused by reactive oxygen, is well known. Free radicals and other oxidants are of great importance in the mechanism of action of many toxins. Their involvement in the aging process, and in diseases, has recently been investigated. These oxygen radicals induce oxidative damage in biomolecules such as carbohydrates, proteins, lipids, and nucleic acids, that would lead to the damage of cell organelles and cause aging with or without diseases (Ceruti, 1994; Dean, Gieseg, & Davies, 1993; Diplock, Rice-Evans, & Burdon, 1994; Willett, 1994). Living tissues protect themselves from oxidative damage by antioxidative enzymes, such as superoxide dismutase (SOD), catalase, peroxidase, and low molecular weight compounds such as tocopherol, ascorbic acid, and polyphenols.

Honey has been used since ancient times and has gained appreciation as the only concentrated form of sugar available worldwide (FAO, 1996). Traditionally, its use in food has been as a sweetening agent. However, several aspects of its use indicate that it also functions as a food preservative. In Japan its consumption amounts to about 40 000 t per year. Some honey is consumed with bread and pancakes. On the other hand, honeys contain a number of components known 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; Ferreres, Garciaviguera, Tomaslorente, & Tomasbarberan, 1993; Ioyrish, 1974). It is suggested that many of these substances owe their preservative properties to their antioxidative activity.

The purpose of the present study was to examine the antioxidative effects of some honeys and related products, such as royal jelly and propolis, and to investigate the scavenging abilities of the superoxide anion radical (O_2^-).

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0308-8146/01/$ - see front matter
PII: S0308-8146(01)00193-5

2. Materials and methods

2.1. Samples

Commercially available honey (Chinese milk vetch) was obtained from Suzuya SY Ltd. (Gifu, Japan). Honeys from the following sources: Chinese milk vetch, acacia, Japanese bee, buckwheat, and mixed-breed were obtained from Inoue Bee Farm Inc. (Hyogo, Japan). Royal jelly and propolis also were from Inoue Bee Farm Inc. (Japan).

2.2. Chemicals

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 was from Oriental yeast Co. Ltd. (Tokyo, Japan). Other reagents were of analytical grade.

2.3. Preparation of sample solution

Each honey was diluted with distilled water and propolis was diluted with ethanol. Royal jelly was melted with 0.5 M NaOH. Each solution (5, 10, and 25% solution) was used as the sample solution for antioxidative activity and scavenging ability of superoxide anion radical tests.

2.4. Antioxidative activity

The antioxidative activity was assayed by using a linoleic acid system. Sample (0.2 ml) plus 0.5 ml of 0.2 M sodium phosphate buffer (pH 7.0) were mixed with 2.5% (w/v) linoleic acid in ethanol (0.5 ml). The preoxidation was initiated by the addition of 50 μl of 0.1 M AAPH and carried out at 37°C for 200 min in the dark. The degree of oxidation was measured according to the thiocyanate (FTC) method (Mitsuda, Yasumoto, & Iwami, 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. α-Tocopherol (1 mM) and ascorbic acid (5 mM) were used as positive controls.

2.5. Scavenging ability of superoxide anion radical

Scavenging ability of superoxide anion radical was evaluated by the method of Rosa, Duncan, Keen, and Hurley (1979). Briefly, this system contained 1.2 ml of 0.05 M sodium carbonate buffer (pH 10.5), 0.1 ml of 3 mM xanthine, 0.1 ml of 3 mM ethylenediaminetetraacetic acid disodium salt (EDTA), 0.1 ml of 0.15% bovine serum albumin, 0.1 ml of 0.75 mM nitroblue tetrazolium (NBT), and 0.1 ml of sample solution. After holding at 25°C for 10 min, the reaction was started by adding 6 mU xanthine oxidase (XOD) and carried out at 25°C for 20 min. After 20 min, the reaction was stopped by adding 0.1 ml of 6 mM CuCl. The optical density of the reaction mixture was measured at 560 nm and the inhibition rate was calculated by measuring the amount of the formazan that was reduced from NBT by superoxide.

3. Results and discussion

3.1. Antioxidative activities of some honeys, royal jelly, and propolis

The FTC method was used to measure the amount of peroxide in initial stages of lipid oxidization. The antioxidative effects of some honeys and propolis on the peroxidation of linoleic acid were investigated, and these results are shown in Fig. 1A–D. The antioxidative activity of commercially available honey (Chinese milk vetch) gradually decreased with the passage of time (Fig. 1A). This pattern was the same as that of propolis (Fig. 1D). Although there were no large differences in antioxidative activities between these honeys, royal jelly, and propolis, mixed-breed honey was active. In particular, buckwheat honey, pure honey (Chinese milk vetch), and propolis were most active among these samples (Figs. 1B–D). In comparison with α-tocopherol and ascorbic acid, pure honey (Chinese milk vetch) and propolis showed the same activity as 1 mM α-tocopherol without reference to sample concentrations (Figs. 1C–D). On the other hand, buckwheat honey exhibited an activity intermediate between α-tocopherol and ascorbic acid (Fig. 1B). Ascorbic acid showed a high antioxidative activity from an initial stage of the peroxidation to 200 min.

3.2. Effect of heat treatment on antioxidative activity

The effect of heat treatment on antioxidative activity was investigated in some commercial honeys, royal jelly, and propolis. After each sample (5, 10, and 25% solutions) was heated at 100°C for 10, 20 and 30 min, the antioxidative activity was measured. As a result, honey (Japanese bee), pure honey (acacia), mixed-breed honey, and buckwheat honey showed the same activity as the control, and these rapidly lost activities (data not shown). Commercially available honey (Chinese milk vetch) and pure honey (Chinese milk vetch) exhibited the same pattern from an initial stage of the peroxidation to a latter period of the peroxidation with reference to the sample concentration (Figs. 2A–B). On the other hand, royal jelly and propolis maintained the high activities during the period of the peroxidation tested (Figs. 2C–D).

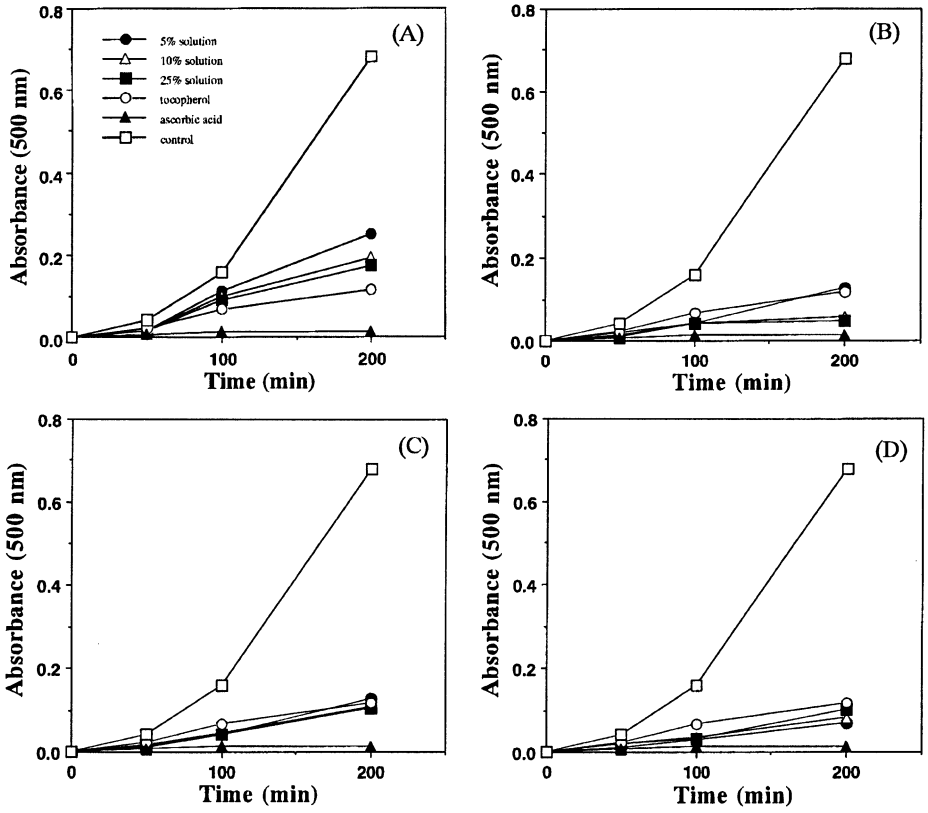

Fig. 1. Antioxidative activities of some honeys and propolis as measured by the thiocyanate method. (A) commercially available honey (Chinese milk vetch); (B) buckwheat honey; (C) pure honey (Chinese milk vetch); (D) propolis.

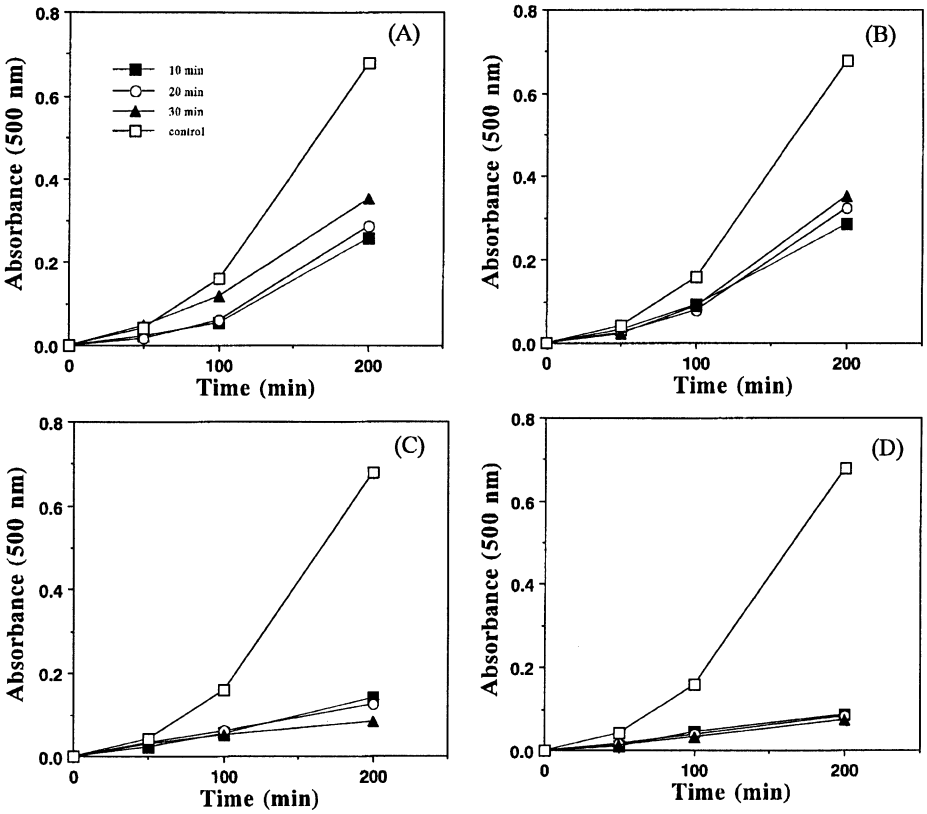

Fig. 2. Effect of heat treatment on antioxidative activities of some honeys, royal jelly, and propolis. (A) commercially available honey (Chinese milk vetch); (B) pure honey (Chinese milk vetch); (C) royal jelly; (D) propolis.

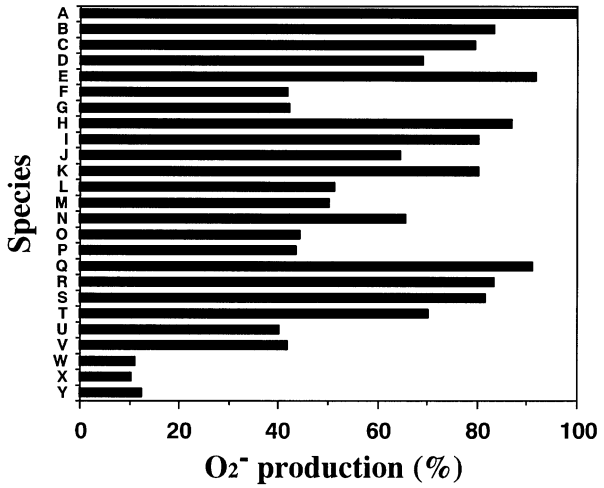

Fig. 3. Scavenging activities of some honeys, royal jelly, and propolis on the superoxide anion radical in xanthine–xanthine oxidase system by the NBT method. (A) control; (B) 1% commercially available honey (Chinese milk vetch); (C) 10% commercially available honey (Chinese milk vetch); (D) 25% commercially available honey (Chinese milk vetch); (E) 1% Japanese bee; (F) 10% Japanese bee; (G) 25% Japanese bee; (H) 1% acacia; (I) 10% acacia; (J) 25% acacia; (K) 1% mix-breed; (L) 10% mix-breed; (M) 25% mix-breed; (N) 1% buckwheat; (O) 10% buckwheat; (P) 25% buckwheat; (Q) 1% Chinese milk vetch; (R) 10% Chinese milk vetch; (S) 25% Chinese milk vetch; (T) 1% royal jelly; (U) 10% royal jelly; (V) 25% royal jelly; (W) 1% propolis; (X) 10% propolis; (Y) 25% propolis.

3.3. Superoxide-scavenging activities of some honeys, royal jelly, and propolis

Superoxide-scavenging activities of some honeys, royal jelly, and propolis were measured using the xanthine–xanthine oxidase system (NBT method). These results were indicated as the superoxide productivity. Each honey, royal jelly, and propolis showed the superoxide-scavenging activity and this activity was decreased in the order propolis > royal jelly > buckwheat honey > honey (Japanese bee) > mixed-breed honey > pure honey (acacia) > commercially available honey > pure honey (Chinese milk vetch; Fig. 3). Except for propolis, this activity tended to increase with increasing sample concentration (Fig. 3).

In conclusion, antioxidative activity was demonstrated in all commercially available honeys, royal jelly, and propolis. In particular, buckwheat honey showed the highest antioxidative activity. It is proposed that high antioxidative activity occurs in honeys with dark colour, such as buckwheat, and weak activity with light colour, as pure honey (acacia) and honey (Japanese bee). As a result of heat treatment, the antioxidative activity was drastically decreased in these honeys, suggesting not only the decomposition of vitamins such as vitamin B_1, B_2, and C but also the destruction of the integrity of the enzymes catalase and peroxidase. Although there is a amount of vitamin C in honey (Crane, 1975; Ferreres et al., 1993; Ioyrish, 1974), the majority of the antioxidant capacity of honeys may be from compounds other than vitamin C. Particularly, strong antioxidative activity in propolis may be due to flavonoids, such as quercetin, flavones, isoflavones, flavonones, anthocyanins, catechin, and isocatechin.

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