C – Phytochemicals and Antioxidants

Antioxidant Activity of Acerola Extracts

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Abstract: West Indian cherries or acerola fruits (*Malpighia glabra* L.) are very rich in ascorbic acid, and also contain flavonoids and anthocyanins. Therefore, their antioxidant activity is interesting. Aqueous and methanolic acerola extracts increased the stability of β-carotene-linoleic acid emulsions against oxidation. Flavonoids and anthocyanins obviously enhanced the effect of ascorbic acid. The effect of natural acerola antioxidants was comparable to that of phenolic antioxidants, such as ferulic acid. During the determination of antioxidant activity in emulsions, carotene was destroyed following a complex kinetics in the beginning of oxidation, but the zeroth order kinetics in later stages of oxidation.

Keywords: acerola; anthocyanins; antioxidants; ascorbic acid; West Indian cherries

INTRODUCTION

Acerola (*Malpighia glabra* L.) is a subtropical bush originating from Central America, therefore, its fruits are often called West Indian or Barbados cherries. The fruit really resembles cherries, it is red, with three segments containing stones [1]. Unripe fruits are extremely rich in ascorbic acid, containing 3% or more in green fruits, but the content gradually decreases during the ripening [2]. The ascorbic acid content depends on the particular *Malpighia* species [3]. Ascorbic acid losses may occur during pasteurization and storage [4]. The losses are very low at frozen and refrigerated storage, but substantially higher at ambient temperature [5]. Carotene belongs to oxidation inhibitors of acerola pulp and juice, too [6]. The antioxidant activity of ascorbic acid present in acerola cherries is increased by the presence of several natural anthocyanins, whose content varies between 38 and 597 mg/kg [7]. They are mostly present as glycosides, and their destruction is evident from colour changes.

EXPERIMENTAL

Chemicals. β-Carotene (min. 97%, Fluka, Buchs, Switzerland); Tween 20, linoleic acid (99%), butylated hydroxyanisole (BHA), di-tert. butylated hydroxytoluene (BHT), gallic acid, rutin trihydrate, ferulic acid, L-ascorbic acid (all Sigma Chemical Co., St. Louis, MO, USA); chloroform (p.a., Lach-Ner, Neratovice, Czech Republic); methanol (p.a., Penta, Chrudim, Czech Republic).

Material. Acerola fruits and leaves were harvested at Okinawa prefecture (Acerola Fresh Ltd., Okinawa, Japan) and were soaked and washed by water. Purees were prepared by grinding of fruits and removing seeds. 100 kg of acerola puree was extracted using the same amount of water or of 5% (w/w) lemon juice for 1 h at 20°C. After filtration through gauze, extracts were freeze-dried and sterilized with isothiocyanate and stored at –20°C until further use. Acerola leaves were dried and powdered (yield 20%) and then extracted with the same amount of hot water or hot 5% (w/w) lemon

juice for 1 h at 90°C. The extracts were spray dried after filtration and they were stored at –20°C until further use. The industrial extracts were prepared by Cosmo Foods Co. Ltd (Hokkaido, Japan), and furthermore the extracts of fruits were prepared in the laboratory. The list of samples is given in Table 1.

Methanolic extracts of samples A, B, C and D were prepared as follows: in a conic flask, 10 g of dry sample were mixed with 100 ml of methanol, and the suspension was left in the dark at ambient temperature for 2 h under occasional stirring. The suspension was filtered and the extraction continued with two successive extraction steps under the same conditions. The combined filtrates were then concentrated, and filled up with methanol to 100 ml. Methanolic extracts of samples E and F were obtained from 1 g of powder which was extracted with three batches of 10 ml of methanol, and the final volume was adjusted to 25 ml. The yields were between 88–89% for extracts A and B, about 80% for extracts E and F, and between 12–13% in the case of extracts C and D. The methanolic extracts and their composition are summarized in Table 2. Extracts were stored in a refrigerator until further use.

Apparatus. The measurement of antioxidant activity was performed on spectrophotometer Cary 100 (Varian Inc., Palo-Alto, CA, USA) provided with 10 mm polystyrene cells PLASTIBRAND® (BRAND GmbH, Wertheim, Germany). Thermostat Ecocell 55 (BMT, Brno, Czech Republic) was used for heating the samples.

Analytical methods. The dry matter content was determined by drying the samples at 40°C under reduced pressure till the constant mass. Total amount of phenolic compounds was determined spectrophotometrically after reaction with a standard Folin-Ciocalteu reagent [8]. Ascorbic acid and tocopherols were determined using the RP-HPLC

Table 1. Powdered aqueous acerola extracts used in the experiments

Sample code	Method of the preparation	Ascorbic acid (mg/g)
A	Aqueous extract of acerola fruit puree	97.9
B	Lemon juice extract of acerola fruit puree	99.0
C	Aqueous extract of acerola leaves	not detected
D	Lemon juice extract of acerola leaves	not detected
E	Aqueous extract of acerola fruit puree prepared in the laboratory	186.8
F	Lemon juice extract of acerola fruit puree prepared in the laboratory	194.8

Table 2. Composition of methanolic extracts obtained from the samples A-F

Sample code	Extraction No.	Dry matter (g/l)	Phenolic substances (mg/l)[a]	α-Tocopherol (mg/l)	γ-Tocopherol (mg/l)	δ-Tocopherol (mg/l)	Ascorbic acid (mg/l)
A	1	88.6	8095	0.24[d]	8.14[d]	0.41[d]	3691.9[d]
A	2	89.2	7292	[b]	[b]	[b]	8194.5[e]
B	1	88.4	8136	0.30[d]	9.02[d]	0.41[d]	2685.7[d]
B	2	89.9	7224	[b]	[b]	[b]	8563.2[e]
C	1	11.4	595	[c]	[c]	[c]	[c]
C	2	12.8	628	[c]	[c]	[c]	[c]
D	1	13.5	575	[c]	[c]	[c]	[c]
D	2	12.4	581	[c]	[c]	[c]	[c]
E	1	32.1	4412	[b]	[b]	[b]	6387.5[e]
F	1	32.7	3970	[b]	[b]	[b]	6740.4[e]

[a]expressed as mg of gallic acid equivalent; [b]not determined; [c]not detected; [d]determined after 4 months of storage; [e]determined 24 h after extraction

procedure [9] and [10, 11], respectively, with electrochemical detection and certain modifications.

Determination of antioxidant activity. The method proposed by Taga *et al.* [12] was used for the determination of antioxidant activity, with slight modifications: into a round-bottom flask, 200 mg of Tween 20 were weighed, and 1 ml of a solution of β-carotene in chloroform was added; the concentration of the β-carotene solution was adjusted to such a value that the initial absorbance of the emulsion attains the value of about 0.8. The solvent was evaporated in a rotating evaporator at a temperature not exceeding 40°C. To the residue, 20 mg of linoleic acid were added. After 1 min, 50 ml of deionized water were added, and the mixture was thoroughly shaken to form a stable emulsion. In the same way, a control emulsion without β-carotene was prepared, in order to determine the effect of the coloration of the initial extract on the process.

For the measurement, methanolic extracts after 2 months of refrigerated storage were used. When using original aqueous extracts, the powdered sample was dissolved in deionized water (0.425% w/v). For comparative purposes, methanolic solutions of the following antioxidants were prepared: BHA, BHT, rutin (conc. 2.50 g/l); ferulic acid, gallic acid (conc. 5 g/l). Ascorbic acid was added in concentrations corresponding to its level in the samples. Each time 260 µl of the analyzed sample was pipetted into the spectrophotometric cell, and 3.25 ml of the linoleic acid emulsion were added. The control without antioxidant was prepared in the same way, 260 µl of methanol or water being pipetted into the cell. The absorbance of the reaction mixture was read at 470 nm, using deionized water as the blank, immediately after addition of the emulsion, and later in 15–20 min intervals for 3–4 h, depending on the rate of decolorization of the control emulsion devoid of antioxidant. The samples were kept either at ambient temperature (25°C) or in a thermostated oven at 40°C.

RESULTS AND DISCUSSION

The reaction course of β-carotene decomposition is evident from the examples given in Figure 1. The decrease of the absorbance did not follow any defined kinetics, especially at the beginning of the reaction. It approached the zeroth order at later stages. Therefore, the activity was determined after 180 min of the reaction.

Antioxidant activity of acerola extracts was generally lower than that of synthetic antioxidants BHA and BHT, and comparable to the activity of rutin and gallic and ferulic acids in tested concentrations.

In experiments at 25°C, the following sequence of activities was obtained for methanolic acerola extracts: A ≅ B > E > D > C, i. e. leaf extracts were less active than fruit extracts. However, in the case of aqueous extracts of acerola, the following sequence of activities was obtained: C > E > A. Ascorbic acid (added as a methanolic solution of the same concentration as in natural extracts of acerola fruits) was less active than methanolic extracts from fruits.

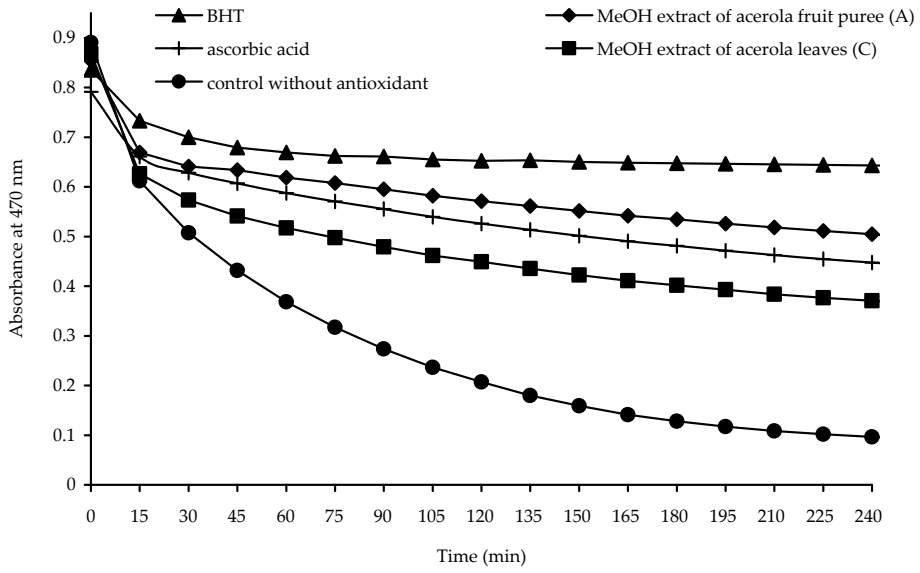

Figure 1. Inhibition of β-carotene bleaching in the presence of different methanolic extracts at 25°C

Results of experiments at 40°C using methanolic acerola extracts were different, corresponding to the sequence: D > C > B > A > E ≅ F, but differences among the activities of individual extracts were only moderate. The activity of ascorbic acid was again much lower than that of methanolic extracts from acerola fruits.

From the results obtained in our experiments, it is evident, that other substances than ascorbic acid contribute to the antioxidant activity of acerola extracts in emulsions. The work on identifying the most likely compounds responsible for the antioxidant activity is proceeding. The activity of acerola extracts will be tested in further experiments also in other systems in order to evaluate the antioxidant potential of this material.

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