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Bioactivity and bioavailability of minerals in rats loaded with cholesterol and kiwi fruit ☆

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ABSTRACT

The aim of this study was to compare the content of polyphenols (TP), minerals, ascorbic acid (AA) and total antioxidant capacities (TAC) of conventional and organic kiwifruit 'Hayward' treated with ethylene after harvest and to determine their influence on plasma TAC, mineral content in the liver and bioavailability (RBV — relative bioavailability value) in rats fed diet containing cholesterol. Organic and conventionally grown kiwifruits 'Hayward' as supplementation to rat diet were investigated in vitro for their bioactive compounds (polyphenols, flavonoids, flavanols, tannins, dietary fiber, and ascorbic acid), minerals, trace elements and TAC. In the in vivo investigation, 36 male Wistar rats (111 ± 5 g) were randomly divided into six diet groups, each of 6 rats: control without cholesterol (C) and 5 groups with 1% cholesterol (ch). Four cholesterol groups were supplemented with 5% lyophilized kiwifruit: ethylene treated, organic (chOHE) or conventional (chCHE) and untreated, and organic (chOHC) or conventional (chCHC). During a period of 33 days of ad libitum feeding feed intake, body weight and feed utilization ration (FER) were controlled. In the end of the experiment rats were anesthetized using Narcotan and sacrificed, and blood and liver were assessed. Testing of antioxidant activity in plasma, minerals and trace elements in the diet and in the liver was performed. Ethylene-treated organic kiwifruit (OHE) had the highest content of TP and TAC. Mg content was significantly higher and Mn was lower in organic kiwifruit 'Hayward'. Inverse relationship in the case of Mn in conventional kiwifruit was found. Supplementation of kiwifruit in ch diet groups improved diet palatability, influenced the increase of feed intake and body gain and also improved FER (lower values). Significant increase of plasma TAC for DPPH (19%), FRAP (68%) and ABTS (62%) in rats fed diets with kiwifruits was obtained. Ethylene treated conventional and organic kiwifruit improved (vs ch group) the antioxidant status of the rat body. Bioavailability of Mn and Zn in rats, calculated on the basis of its content in the liver was significantly lower in chOHC in comparison with ch group. Supplementation of ch diet groups with kiwifruit significantly decreased Mg bioavailability. Ethylene treated kiwifruit had no effect on mineral bioavailability in rats. Organic kiwifruit 'Hayward' contains more bioactive compounds, and showed higher antioxidant capacity than the conventional. Ethylene treatment of kiwifruit after harvest increases its bioactivity. Growing conditions of kiwifruit affect their Mg and Mn contents. Supplementation of kiwifruit in atherogenic diet increased plasma antioxidant capacity in rats. Similar effect was estimated for organic and conventional fruits. The ethylene treated kiwifruit significantly decreased magnesium bioavailability determined on the basis of its concentration in the liver. Supplementation of Mg in hypercholesterolemia is an important factor.

1. Introduction

Kiwifruit is a climacteric fruit, particularly sensitive to ethylene postharvest treatment. The research of Park et al. [1–3] indicates that the stimulation of this fruit with ethylene increases their bioactivity, reaching the highest value on the sixth day. During this period, the fruit is ready for consumption, because it contains the highest amount of total polyphenol and flavonoids, which affects the total antioxidant capacities. Nowadays, the interest of health food leads to organic production [4]. In organic system different methods are used to maintain

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[4] This article was written in memory of my dear brother Prof. Simon Trakhtenberg, who died in November 2011, who encouraged me and our entire scientific group during all his life.

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soil fertility, including addition of organic matter to soil and slow release of nutrients to the soil, in contrast to chemical fertilizer [5]. Conventional agriculture practices utilization of pesticides (and fungicides), which can result in a disruption of phenolic metabolites in the plant, having a protective role in plant defense mechanism [6]. These differences may result in the change of plant composition and nutritional quality, which in turn influences storage performance of products. Hassey et al. [7] found that organically grown kiwifruits are firm or firmer than conventionally grown fruits. Benge et al. [8] did not observe difference between these two systems. Worthington et al. [5] showed that organic products contain more vitamin C and mineral elements (Fe, Mg and P) and less nitrates in comparison with fruit and vegetable crops derived from conventional plants.

It is known that citrus and exotic fruits, including kiwifruit are good source of bioactive compounds, which have an important role in prevention of diseases [9,10]. On the other hand it must be underlined that bioactive compounds (polyphenols, mainly tannins, dietary fiber) can influence mineral metabolism and change bioavailability of minerals and trace elements. Bioavailability of elements [11] depends on the physiological status of the organism, diet composition, and interaction between nutrients, which can be synergistic and/or antagonistic. In this context it is interesting to compare kiwifruits from ecological or conventional cultivation on the basis of their composition; bioactivity and also organism reaction loaded cholesterol diet supplemented with kiwifruit. Ripening behavior in a population of kiwifruit at harvest is asynchronous, so a short burst of exogenous ethylene is used to synchronize ripening until control fruit softened to an 'eating-ripe' firmness.

The aim of this study was to determine the bioactive compounds, minerals and antioxidant potentials of organic and conventional kiwifruit 'Hayward', treated with ethylene after harvest, and to compare the antioxidant activity in plasma and bioavailability of minerals in the body of rats fed atherogenic diet. As far as we know no results of such investigations were published before.

2. Experimental

2.1. Samples and preparation

'Hayward' kiwifruits (organic and conventional) at their commercial maturity stage were harvested in orchard (Heanam Country, Jeonnam province, South Korea, 2010). Samples of kiwifruit organic ethylene treated (OHE) and conventional ethylene treated (CHE) were treated with 100 ppm of ethylene for 24 h at 20 °C in a growth chamber (Percival Scientific Inc. Perry, IA, USA). The samples were put into an 18 l glass jar and ventilated with humidified flow air mixed with ethylene at 300 ml min^{-1}. The samples of kiwifruit organic (OHC) and conventional (CHC) were put into 18 l glass jar and ventilated with humidified flow of air. Then the ethylene and air-treated kiwifruits were ripened separately using the same conditions, at 20 °C, in a growth chamber (Percival, USA) for 10 days.

All fruits were cleaned with tap water, and dried, using five replicates of five fruits each. The peeled fruits (without using steel knives) were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 10–324), and the dry weight was determined. The samples were ground to pass through a 0.5 mm sieve and stored at −20 °C until the bioactive substances were analyzed.

2.2. Determination of composition of kiwifruit samples

Polyphenols were extracted from lyophilized fruits with 50% dimethyl sulfoxide (DMSO) (concentration 25 mg/ml) at room temperature twice during 3 h. The new solvent system was chosen on the basis of our previous investigations where various extracts were studied [1–3]. Therefore it was interesting to extract phenolic compounds with DMSO. Polyphenols were determined by Folin–Ciocalteu method with measurement at 750 nm using a spectrophotometer (Hewlett-Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DM [12]. Flavonoids, extracted with 5% $NaNO_2$, 0.1 ml of 10% aluminum chloride hexahydrate ($AlCl_3$), and 1 M NaOH, were measured at 510 nm. The total flavanols were estimated using the p-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was measured [13]. The extracts of condensed tannins (procyanidins) with 4% vanillin solution in MeOH were measured at 500 nm. (+) Catechin served as a standard for flavonoids, flavanols and tannins, and the results were expressed as catechin equivalents (CE). Total ascorbic acid was determined by CUPRAC assay in water extract (100 mg of lyophilized sample and 5 ml of water) [14].

2.2.1. Determination of minerals (Se, Mn, Cu, Zn, Mg, Fe, Ca)

Samples of lyophilized kiwifruit (CHC, CHE, OHC, OHE) were placed in Teflon vessels, and 5 ml of HNO_3 and 1 ml of H_2O_2 were added. The samples were mixed and allowed to stand for 24 h. Mineralization was carried out in the microwave Milestone Ethos 900, USA–Italy. The elements were determined by flame atomic absorption spectrometry in a Perkin-Elmer 1100 B, using cathode ray wavelengths appropriate for the analyzed elements: 422.7, 285.2, 428.3, 324.8, 213.9 and 279.5 for Ca, Mg, Fe, Cu, Zn and Mn, respectively. Selenium was determined using starter with the method of MHS-10 hydride (NaBH4).

2.3. Determination of total antioxidant capacity (TAC)

The TAC was determined by three complementary assays: (1) 2, 2′-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) was generated by the interaction of ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm [15]. (2) Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm [16]. (3) Scavenging free radical potentials were tested in a methanolic solution of 1, 1-diphenyl-2-picrylhydrazyl method (DPPH). In its radical form, DPPH has an absorption band at 515 nm which disappears upon reduction by antiradical compounds. DPPH solution (3.9 ml, 25 mg/l) in methanol was mixed with the sample extracts in DMSO (0.1 ml), then the reaction progress was monitored at 515 until the absorbance was stable [17].

2.4. Rats and diets

The Animal Care Committee of the Warsaw Agricultural University, Warsaw, Poland approved this study. The mean weight of the male Wistar rats ($n = 36$) at the beginning of the experiment was 111 ± 5 g. The rats were divided into six diet groups, each six and named Control (C), ch, chCHC, chCHE, chOHC and chOHE. During first 5 days of adaptation all groups were fed the basal diet (BD), which included wheat starch, casein, soybean oil, cellulose, vitamin (AIN-93-VX Vitamin Mix Cat. No. 960402) and mineral mixtures (AIN-93-MX mineral mix Cat. No. 960400) of the American Institute of Nutrition for laboratory animals. The rats were housed in metabolic cages (TECNIPLAST S.p. A, 21020, Italy). The rats of the Control group during the 28 days of the experiment received the BD only, and the diets of the other groups were supplemented with 1% of cholesterol (ch), 1% of cholesterol and 5% of lyophilized kiwifruit for chCHC, chCHE, chOHC and chOHE, respectively. All rats were fed once a day at 10.00 h ad libitum, having unrestricted access to drinking water. The feed intake and body gains were monitored daily every week. At the end of the experiment after 24 h of starvation, the rats were anesthetized using Halothane (Narcotan–Zentiva), and the blood samples were taken from the left atrium of the heart.