Extracts from Organically and Conventionally Cultivated Strawberries Inhibit Cancer Cell Proliferation In Vitro

M.E. Olsson, S.C. Andersson, R.H. Berglund and K.-E. Gustavsson
Department of Crop Science
Swedish University of Agricultural Sciences
SE-230 53 Alnarp
Sweden

S. Oredsson
Department of Cell and Organism Biology, Lund University
SE-223 62 Lund
Sweden

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Abstract

The effects of extracts from strawberries of cv. 'Honeoye', grown in both organic and conventional production systems, on cancer cell proliferation in vitro were investigated. The content of several antioxidants in the strawberry extracts were analysed and compared with the inhibition effect on cancer cell proliferation. The strawberry extracts inhibited cell proliferation in colon cancer cells HT29 and breast cancer cells MCF-7 in a concentration dependent way. Extracts from organically grown strawberries inhibited cell proliferation to a higher extent than conventionally grown at the two highest concentrations. This might indicate that the content of secondary metabolites with anticancer properties were higher in the organically grown strawberries. There were great differences in the content of the analysed antioxidants between the two cultivation methods. The content of ascorbate was 36% higher and the ratio of ascorbate to dehydroascorbate were eight-fold higher in the organically grown strawberries than in the conventionally grown, whereas the content of ellagic acid, total anthocyanidins and total phenolics were lower. No significant differences were found in the content of hydroxycinnamic acid and flavonols between the two production systems. The higher content of ascorbate found in the organically grown strawberries might have affected the higher inhibition of cancer cell proliferation by the extracts from the organically produced strawberries compared to the conventionally grown strawberries. Ascorbate is suggested to act synergistically with other substances in the extracts.

INTRODUCTION

Apart from being a primary food source of some essential nutrients, fruits and vegetables also contain a variety of phytochemicals which might have potential beneficial health effects. The association between a high intake of fruit, berries and vegetables and a lower risk of developing some types of cancer could be attributed to the content of secondary metabolites, as antioxidants or other bioactive compounds. There has been a growing interest in understanding the reason for the cancer protective effect of fruit and vegetables, and to identify the components with the anticancer effect. Several antioxidants, or bioactive compounds, in fruit and vegetables have been suggested to contribute as anticarcinogenic substances. Many epidemiological studies have found protective effects of vitamin C (Block et al., 1992). Flavonoids have been shown to inhibit cancer cell proliferation in vitro (Kunst et al., 1999) and to induce apoptosis in vitro (Birt et al., 2001). Plasma carotenoids have been found to inversely correlate with lipid peroxidation and oxidative DNA damage (Haegele et al., 2000).

Strawberries are an economically important berry crop in Sweden as well as in several other countries. They are also valuable from a nutritional point of view as eg. they are rich in vitamin C, folate and also contain other compounds, which may have physiological effects. Ellagic acid, present in relatively high levels in strawberries (Maas et al., 1991), has been proposed to exert antimutagenic and anticarcinogenic effects and has recently raised much interest (Clifford and Scalbert, 2000; Carlsen et al., 2003).

Hydroxycinnamic acids may prevent human low-density lipoprotein and liposome oxidation (Heinonen et al., 1998). Anthocyanins, present in relatively high amounts in strawberries, have been found to inhibit human cancer cell proliferation (Seeram et al., 2003). Cultivar, growth stage and environmental conditions may affect the content of the different antioxidants (Mozafar, 1994; Parr and Bolwell, 2000; Olsson et al., 2004a).

In recent years, there has been a growing interest in more environmental friendly farming systems to minimize negative impacts of agricultural production. Organic production of today is using many different methods of cultivation, with the common feature that the production is characterized by restrictions against using synthetic pesticides and synthetic fertilizers. Different methods may be used for removing weeds, to reduce pathogens, and different fertilizers may be applied. Nevertheless, many consumers of today believe the organically produced plant food to be healthier, due to expectancy that the products contain more health-promoting components (Magnusson et al., 2001; Zanoli and Naspetti, 2002). However, the results from the studies comparing organic and conventional cultivation are conflicting, although there are still a limited number of investigations. Several investigations have indicated that there might be a higher level of ascorbic acid in horticultural products from organic cultivation (Woese et al., 1997; Magos et al., 2003), whereas the results concerning the content of phenolic compounds have been conflicting (Mikkonen et al., 2001; Carbonaro et al., 2002).

The present work investigated the effects of extracts from strawberries, grown in both organic and conventional production systems, on cancer cell proliferation. The content of several antioxidants in the strawberry extracts were analysed and compared with the inhibition effect on cancer cell proliferation.

MATERIAL AND METHODS

The strawberries were grown at Rånna research station, Swedish University of Agricultural Sciences, in Sweden. The berries of the cultivar 'Honeoye' were grown both organically and conventionally. The conventionally cultivated strawberries were fertilized with NPK fertilizer at the amount of 44 kg N per hectare, followed by an amount of additional NPK fertilizer, equal to 33 kg N per hectare, or 0.6 g N/plant. The weeds were removed by hand hoeing. For pest control, the plants were sprayed with Gusathion WP, and for control of grey mold, the plants were sprayed with Teldor WG 50 and Rovral. The organically cultivated strawberries were fertilized with fresh and dried poultry manure (Binadan), in amounts equal to 50 kg N per hectare. Further fertilization was applied later, using dried poultry manure (0.5g N/plant) and also Vinasse, a by-product from the production of yeast. The weeds were removed by hand. For pest control, the plants were sprayed with Bioruiskute S (active substance pyrethrine and containing no piperonyl-butoxid).

The berries were at commercial ripening stage when harvested, and were as soon as possible at the harvest day frozen at -20ºC, and shortly after transferred to -80ºC, before they were later used in cancer proliferation experiments and analysed for their content of antioxidants. From each composite sample of strawberries, collected at random in the fields from each of the two cultivation systems, four sub-samples were extracted independently.

Each sample of the berries was homogenized with an Ultraturrax in ethanol-water with 50 mM H_3PO_4, (approximately 20 g FW per 40 ml solvent). The samples were centrifuged at 12,000 g, 4°C, for ten minutes. Aliquots of the supernatants were stored at -80°C before they later were used for cancer cell proliferation tests or analyzed with HPLC.

The extracts used for cell proliferation tests were evaporated to nearly dryness under N_2, and dissolved in 50% ethanol.

Human colon cancer cells HT29 and oestrogen receptor-positive breast cancer cells MCF-7 were used in the cancer cell proliferation tests. The cells were incubated for 24 h at 37°C and in an atmosphere of 95% air / 5% CO_2. The cancer cells were cultured and the cell proliferation tests were performed as in Olsson et al., 2004b. Effects on cell

proliferation rate were determined by the ability of the cells to cleave the tetrazolium salt WST-1 (Roche Diagnostics, Mannheim, Germany) to formazan. Four different concentrations of the strawberry extracts were used; 0.025%, 0.05%, 0.25%, 0.5% of plant dry matter of total weight in the wells (weight approximated to be equal to volume in the wells; i.e. 200 µl). Equal amount of solvent (50% ethanol) was added in the control wells. Three replicates were used for each extract, and the proliferation tests were repeated on three different occasions.

Ascorbate, flavonols, non cell-wall-bound hydroxycinnamic acids and free ellagic acid were analyzed by HPLC as in Olsson et al. (2004b). Anthocyanins were analyzed by HPLC as in Olsson et al. (2004b). Pelargonidin-3-glucoside was used as a standard.

Total phenolics were quantified according to the Folin-Ciocalteu method. 0.1 ml of ethanol-water extract was mixed with 0.2 ml of Folin-Ciocalteu reagent, 2 ml of H_2O and 1 ml of 15% Na_2CO_3 in a cuvette. The samples with the reagents were measured after two hours in room temperature in a spectrophotometer at 765 nm. Gallic acid was used as a standard.

RESULTS AND DISCUSSION

The strawberry extracts inhibited cell proliferation in colon cancer cells HT29 and breast cancer cells MCF-7 in a concentration dependent way. Extracts from organically grown strawberries inhibited cell proliferation to a higher extent than conventionally grown at the two highest concentrations, 0.5% and 0.25%. At the concentration 0.5% for HT29 cells there was a 58.6% inhibition of cell proliferation by the extract from the organically grown strawberries compared to 51.9% inhibition by the extracts from the conventionally grown, and 55.2% inhibition compared to 42.9% inhibition for MCF-7 cells. This might indicate that the content of secondary metabolites with anticancer properties were higher in the organically grown strawberries.

There were great differences in the content of the analysed antioxidants between the two cultivation systems. The content of ascorbate was 36% higher and the ratio of ascorbate to dehydroascorbate were eight-fold higher in the organically grown strawberries than in the conventionally grown, whereas the content of ellagic acid, total anthocyanidins and total phenolics were lower (9% lower, 62% lower and 12% lower respectively). No significant differences were found in the content of hydroxycinnamic acid and flavonols between the two production systems.

Previous investigations have shown that the effects of organic or sustainable cultivation of strawberries in terms of content of antioxidants have been conflicting. Compost as a soil supplement was found to increase the level of the ascorbic acid (Wang and Lin, 2003). In organically cultivated strawberries, one investigation found no differences in the content of vitamin C in three cultivars compared to conventionally cultivated (Hakala et al., 2002), whereas in another investigation the average of all harvest dates showed no differences, but a higher number of the harvest dates had a higher level of vitamin C in the organically cultivated (6 harvest dates) than in the conventionally cultivated (3 harvest dates) (Cayuela et al., 1997). Higher level of ascorbic acid was also found in sustainable grown strawberries than in conventionally cultivated (Asami et al., 2003). The higher content of ascorbic acid in organically produced strawberries of cv. 'Honeoye' found in this investigation thus corresponds to the results found in several previous investigations, although there are also investigations were no difference was found.

Higher levels of total phenolics were found in organically and sustainably grown strawberries and marionberries (Asami et al., 2003). Similar levels of flavonols and phenolic acids were found in two strawberry cultivars when different cultivation techniques were used, but in a third cultivar the organically cultivated berries had a higher content of ellagic acid (Häkkinen and Törrönen, 2000). The lower levels of several phenolic substances found in this investigation thus make the picture even more complex.

Increased nitrogen fertilization has lead to different result in the content of ascorbic acid depending on the investigated species in different vegetables, fruit and

berries, but a decrease in the content has been found to be a somewhat more common result, as for instance in strawberries (Mozafar, 1994). Flavonoid content has been found to be influenced by nutrient availability. The content of flavonols in leaves of tomato plants increased in nitrogen deficient plants, while phosphorus had no effect (Stewart et al., 2001). Also the content of anthocyanins decreased in nitrogen deficient plants (Bongue-Bartelsman and Phillips, 1995). Differences in the available nitrogen may have influenced the content of antioxidants in the present investigation, although it may be difficult to estimate the actual availability of nitrogen to the strawberry plants during the whole growing season, since this is not only due to the actual amount present in the fertilizer, but also depends on factors, for example, nutrient leakage from the soil, presence of mycorrhizza and by soil pH.

Other factors than mineral nutrients might also have influenced the concentrations of antioxidants as well as other secondary metabolites. Environmental stress factors as pathogen invasion, herbicide action or drought influence the antioxidative defence system in plants and may result in changes in the concentrations of antioxidants as well as other secondary metabolites (Pastori and Foyer, 2002; Blokhina et al., 2003). Especially phenolic compounds have been implicated to take an active role in the direct defence against plant pathogens. Phenolic-storing cells have been suggested both to be able to seal off infections or injuries at pathogen attack and to promote a secondary line of defence participating in metabolic changes (Beckman, 2000). These factors may vary between the two production systems and may therefore have affected the content of the antioxidants.

The higher content of ascorbate found in the organically grown strawberries might have affected the higher inhibition of cancer cell proliferation by the extracts from the organically produced strawberries compared to the conventionally grown strawberries. In a previous investigation, a correlation was found between the content of ascorbate in extracts of 10 different species of fruit and berries, and inhibition of cancer cell proliferation. A synergistic effect between ascorbate and other components in the extracts was suggested to exert the inhibitory effect on the cancer cell proliferation (Olsson et al., 2004b). The results in this investigation correspond to this conclusion.

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