Food Chemistry 132 (2012) 959–967

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of different cranberry extracts and juices during cranberry juice processing on the antiproliferative activity against two colon cancer cell lines

Khanh Dang Vu [a], Hélène Carlettini [a], Janie Bouvet [a], Jacinthe Côté [a], Gilles Doyon [c], Jean-François Sylvain [b], Monique Lacroix [a,*]

[a] INRS-Institut Armand-Frappier, Research Laboratory in Sciences Applied to Food, 531 Boulevard des Prairies, Laval, Québec, Canada H7V 1B7
[b] Atoka Cranberries Inc., 3025 Route 218, Manseau, Québec, Canada G0X 1V0
[c] Agriculture and Agri-Food Canada, Food Research Centre, 3600 Casavant West, Saint-Hyacinthe, Québec, Canada J2S 8E3

ARTICLE INFO

Article history:
Received 10 June 2011
Received in revised form 19 September 2011
Accepted 15 November 2011
Available online 25 November 2011

Keywords:
Cranberry fruit
Juice processing
Phenolic extracts
Antiproliferative activity
Colon cancer cells

ABSTRACT

The effect of cranberry extracts and juices during cranberry juice processing on the antiproliferative properties against colon cancer cells was investigated. Two colon cancer cell lines HT-29 and LS-513 were treated with different concentrations of cranberry phenolic extracts from fruits, puree, depectinised puree and pomace and different concentration of three juices (raw, filtered and concentrated juices). The phenolic extracts consisted of water-soluble phenolic compounds, apolar phenolic compounds and anthocyanins. These phenolic extracts and juices were tested against two cell lines at pH 2.5 (natural juice pH) and at pH 7.0 (physiological pH). All cranberry extracts and juices could inhibit the growth of both cell lines with the IC_{50} values (the concentration of phenolic content required to inhibit 50% the growth of cancer cells) varied from 3.8 to 179.2 μg gallic acid equivalent/ml. It was found that three types of extracts from fruit at pH 7.0 were the most effective at inhibiting the growth of HT-29 cell line. Extracts containing anthocyanins from fruit and from pomace were the most and the least efficient, respectively, in inhibiting the growth of both cancer cell lines. Further, three juices at natural pH (pH 2.5) were more effective at inhibiting the growth of two cell lines as compared to juices at pH 7.0. Concentrated juice at both pH values was the most effective at growth inhibition of two cancer cell lines compared to two other juices.

1. Introduction

Colon cancer is one of the most prevalent cancers throughout the world and especially in the western countries. Many epidemiological studies indicated that western-style diet such as consumption of red meats is possibly associated with a high colon cancer incidence (Boateng et al., 2007). There is an increase in the research area of novel source of bioactive compounds to prevent colon cancer. In this direction, bioactive compounds of natural origin, particularly from a dietary source, are of significant interest (Patil et al., 2009). In recent years, juices or extracts of the American cranberry (*Vaccinium macrocarpon* (Ericaceae)) have been paid particular attention to, owing to their ability to provide multitude health benefits (Côté, Caillet, Doyon, Sylvain, & Lacroix, 2010a, 2010b).

Juice and fruits of cranberries are known to contain many bioactive compounds such as low molecular weight phenolic acids, organic acids, sugars, anthocyanins, proanthocyanidins (condensed tannins) and flavonol glycosides (Côté et al., 2010a, 2010b; Seeram, Adams, Hardy, & Heber, 2004). These bioactive compounds are considered as excellent sources for human health benefits such as inhibition of low-density lipoprotein oxidation (Wilson, Porcari, & Harbin, 1998), antioxidant activities (Seeram et al., 2004), prevention of microbial adhesion in urinary tract infections and reduction of biofilm formation (Howell, Vorsa, Der Marderosian, & Foo, 1998), as well as, antimicrobial activity against food pathogens (Côté et al., 2011) and *in vitro* anticancer effects (Murphy et al., 2003; Neto, 2007; Sun, Chu, Wu, & Liu, 2002; Yan, Murphy, Hammond, Vinson, & Neto, 2002). Therefore, consumption of juice or fruits of cranberries could be an useful way in maintaining and improving human health and even in prevention of cancer.

The *in vitro* anticancer effects of cranberry juice or extracts were demonstrated in several cancer cell lines. For example, it was found that the soluble free extract of cranberry had the highest antiproliferative activity against HepG2 human liver cancer cells which demonstrated through the lowest median effective dose (EC_{50}) of 14.5 ± 0.5 mg/ml, followed by lemon (30.6 ± 0.8 mg/ml), apple (49.4 ± 1.6 mg/ml), strawberry (56.3 ± 1.5 mg/ml), red grape (71.0 ± 2.2 mg/ml), banana (110.1 ± 2.5 mg/ml), and grapefruit (130.1 ± 4.5 mg/ml) (Sun et al., 2002). Yan et al. (2002) have

* Corresponding author. Tel.: +1 450 687 5010x4489; fax: +1 450 687 5792.
E-mail address: Monique.Lacroix@iaf.inrs.ca (M. Lacroix).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.11.078

observed the IC_{50} values (the concentration required to inhibit 50% the growth of cells) of a chloroform/methanol extract from whole cranberry fruits against human chronic myelogenous leukaemia (K562) and colon cancer cells (HT-29) were in the range of 16–125 μg/ml. Also, the authors further isolated the active compounds from the chloroform/methanol extract and two compounds were identified as cis- and trans-isomers of 3-*O*-*p*-hydroxycinnamoyl ursolic acid. The authors found that there was a greater activity of cis-isomer of 3-*O*-*p*-hydroxycinnamoyl ursolic acid in inhibiting the growth of nine cancer cell lines, with 50% growth inhibition (GI_{50}) values of approximately 20 μM in MCF-7 breast, ME180 cervical and PC3 prostate tumour cell lines. Moreover, quercetin was found to be less active than cis-isomer of 3-*O*-*p*-hydroxycinnamoyl ursolic acid, while cyanidin 3-galactoside exhibited much lower cytotoxicity, with GI_{50} greater than 250 μM in all cell lines under study, following Murphy et al. (2003).

It should be emphasised here that during processing of cranberry juice, the available bioactive compounds can be charged negatively or positively. This fact may lead to the decrease in their potential biological benefits. For example, variable effects of domestic processing and storage were observed in quercetin, myricetin and kaempferol contents of five types of berries most commonly consumed in Finland (Häkkinen, Kärenlampi, Mykkänen, & Törrönen, 2000). The same authors found that when juices were made by common domestic processing methods, considerable losses of flavonols were observed. Cold-pressing was superior to steam extraction in extracting the flavonols. Moreover, they also claimed that crushing the berries resulted in a considerable loss of quercetin. They also suggest that myricetin and kaempferol are more susceptible than quercetin to losses during processing and storage of berries (Häkkinen et al., 2000). Lee, Durst, and Wrolstad (2002) also found that there was considerable loss of blueberry anthocyanins and polyphenolics in treatments and control during thawing, crushing, depectinisation and pressing steps of juice and concentrate processing. These authors demonstrated that heat and SO_2 treatments yielded higher anthocyanin content in each processing step, and should offer attractive attributes to traditional blueberry juice processing methods for increasing anthocyanin recovery. Thus, it is possible that changes have occurred in the bioactive compounds (soluble phenolic compounds, apolar phenolic compounds and anthocyanins, etc.) of cranberries during juice processing, and these changes may affect the biological properties (for example, the antiproliferative activity against cancer cells) of cranberry juices or extract. However, there are scarce data on this subject mentioned in the literature.

The aim of this study was to evaluate the effect of different cranberry extracts and juices during cranberry juice processing on the *in vitro* antiproliferative properties against two colon cancer cell lines. Different extracts from cranberry whole fruits and cranberry processing products (puree, depectinised puree, pomace, raw juice, filtered juice and concentrated juice) were used to evaluate their antiproliferative properties. Three different groups of bioactive compounds (water-soluble phenolic compounds, apolar phenolic compounds and anthocyanins) were extracted from frozen cranberries and cranberry solids (mash, depectinised mash and pomace) using three different solvents.

2. Materials and methods

2.1. Materials

Chemicals such as gallic acids, Folin–Ciocalteu reagent, methanol, acetone and acetic acid were purchased from Sigma–Aldrich (Oakville, ON, Canada). Cancer cell culture media (RPMI and DMEM-Ham's F12), foetal bovine serum and other supplements were obtained from Fisher Scientific Limited (Ottawa, Ontario, Canada). Cell proliferation WST-1 was purchased from Roche Diagnostic (Laval, QC, Canada).

2.2. Raw material and cranberry juice processing

Frozen cranberry fruits (*V. macrocarpon*) and six cranberry processing products (puree depectinised puree, pomace, raw juice, filtered juice and concentrated juice) were used to determine their antiproliferative activity against colon cancer cells *in vitro*. These samples were provided by Atoka Cranberries Inc. (Manseau, QC, Canada) and were stored at −80 °C until used. Cranberry juice processing: The first step to make juice was to mill frozen cranberries to form puree or mash using a fruit miller. Then, heat treatment of puree was conducted at 55 °C for extracting the valuable colour (plasmolysis). The heated puree was then allowed to macerate under minimal agitation in a mixer. At the same time, pectinase enzymes were added to provide acceptable yields and throughputs on the press and improve colour extraction. The aim of the enzyme treatment of puree was to degrade soluble pectin. The recovery of raw juice from depectinised puree was carried out using a fruit press at 1.90 bars. During the juice pressing step, high amounts of press cake were obtained. Cranberry pomace was the main byproduct of the cranberry juice processing. It is composed primarily of skin, seeds and stems which are residuals after pressing the depectinised puree. The obtained raw juice was then filtered through a cross-flow membrane filtration to remove colloids and produce a clear juice. Finally, the filtered juice was concentrated by evaporation process under vacuum condition to obtain a concentrated juice at 50° Brix.

2.3. Extraction of phenolic compounds and sample preparation for antiproliferative assay

The extraction of phenolic compounds was conducted in a mild condition to avoid oxidation, thermal degradation and other chemical or biochemical changes in the sample. Extraction of phenolic compounds from frozen cranberry fruits and cranberry solids (puree, depectinised puree and pomace) was conducted using three different solvent solutions to obtain three different extracted fractions. The most water soluble phenolic compounds were extracted with water/methanol solvent (85:15, v/v) (Seeram et al., 2004), the most apolar phenolic compounds (flavonols, flavan-3-ols and proanthocyanidins) were extracted with acetone/methanol/water solvent (40:40:20, v/v), modified from a method described by Neto et al. (2006), and the anthocyanins were extracted with methanol/water/acetic acid solvent (85:15:0.5, v/v/v) as described by Wu and Prior (2005). Frozen cranberries fruits were crushed at 4 °C for 40 s in a Waring commercial blender (Waring Laboratory, Torrington, CT) to obtain a fine powder. Extraction process was immediately performed at 4 °C under agitation and nitrogen for 40 min by macerating of 300 g of fruit powder in the extracting solvents. Three successive extractions in each extracting solvent were performed using the same procedure. The first extraction was done using 700 ml of solvent, but for the two last ones, 500 ml was used. The solvent containing the phenolic compounds was recovered after each extraction and the solvents from the successive extractions were combined, then filtered on Whatman paper No. 4 (Fisher Scientific, Nepean, ON, Canada). The filtrate was concentrated by evaporation of the solvent using the SpeedVac automatic evaporation system (Savant System, Holbrook, NY). Finally, the dry matter was obtained by freeze-drying the concentrated extracts for 48 h using a Virtis Freeze mobile 12 EL lyophilizator (The Virtis Co., Gardiner, NY) and was stored at −80 °C until used.

Prior to each assay, the freeze-dried extracts were weighed and dissolved in 10% (v/v) specific solvents which were used to extract them to obtain stock solutions of 50 mg/ml. The stock of each