LWT - Food Science and Technology 63 (2015) 8–13

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Inhibitory effects of red cabbage and sour cherry pomace anthocyanin extracts on food borne pathogens and their antioxidant properties

Aslıhan Demirdöven, Şeniz Karabıyıklı*, Kader Tokatlı, Nilgün Öncül

Gaziosmanpaşa University, Faculty of Engineering and Natural Science, Food Engineering Department, 60000 Tasliçiftlik, Tokat, Turkey

ARTICLE INFO

Article history:
Received 15 January 2015
Received in revised form
24 March 2015
Accepted 25 March 2015
Available online 2 April 2015

Keywords:
Red cabbage
Sour cherry pomace
Anthocyanin
Antimicrobial
MIC

ABSTRACT

Anthocyanins, known for their antioxidant characteristics, also have antimicrobial effects. Antimicrobial, antioxidant and some physicochemical properties of red cabbage (RC) and sour cherry pomace (SCP) anthocyanin extracts were investigated. Conventional (CE) and ultrasonic extraction (UE) methods were used. The antioxidant activities of samples extracted by UE were higher than those extracted by CE. Antimicrobial effects of the extracts were determined by the detection of minimum inhibitory concentration (MIC) values for *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Bacillus cereus*. The extracts were inoculated (6 and 3 log CFU/mL) with five different pathogens, separately. All the extracts have antimicrobial effects on the tested pathogens and the results ranged depending on the concentration of the extracts and inoculation dose of the pathogen. The extraction method did not affect the inhibitive characteristics of the extract, and it was concluded that the antimicrobial effects of the extracts were mainly dependent on the anthocyanin content of the materials.

1. Introduction

There is an increasing demand for natural, safe and functional foods in the last decade. Consumers are interested in safe foods which have positive contributions to health, but contain no chemical additives or preservatives. However, keeping the food safety stable until the expiration date is not possible without the addition of any preservatives or application of thermal processing, which causes deterioration of functional ingredients. In this view, plant extracts, which could be used for natural colorant, antioxidant, flavoring or antimicrobial agents, are good alternatives for the food industry. Polyphenol rich plants especially are gaining popularity as a good source for anthocyanins. Anthocyanins are bioactive compounds present in many fruits, vegetables and their products. They are a sub-group within the flavonoids, characterized by a C6–C3–C6-skeleton (Patras, Brunton, O'Donnellb, & Tiwari, 2010). Antioxidant and antimicrobial properties of anthocyanins were studied by several researchers (Caillet, Côté, Sylvain, & Lacroix, 2012; Da Silva, Maquiaveli, & Magalhães, 2012; Martin et al., 2012; Oliveira et al., 2013; Park, Biswas, Phillips, & Chen, 2011; Rockenbach et al., 2011; Schved, Henis, & Juven, 1994; Smith-Palmer, Stewart, & Fyfe, 1998). Anthocyanins could fight against chronic diseases, such as neuronal and cardiovascular illnesses and diabetes, among others (Konczak & Zhang, 2004). Anthocyanins may offer anti-inflammatory, anti-viral, and anti-cancer benefits (Ali, Masud, & Abbasib, 2011; Basu, Rhone, & Lyons, 2010; Cassidy et al., 2011; Faria et al., 2010; Ghosh & Konishi, 2007; Prior et al., 2008). Also, they have anti-carcinogenic effects because of their effective protection against oxidative damage by acting as an antioxidant. Antioxidant mechanisms of anthocyanins also make them available to extend the shelf life of industrial food products. The anthocyanin composition of red cabbage is very complex and the dominant structures are: cyanidin-3,5-diglucoside and cyanidin-3-sophoroside-5-glycoside acylated with sinapic acid, ferulic acid, p-coumaric acid, caffeic acid or malonic acid (Dyrby, Westergaard, & Stapelfeldt, 2001). Turkey is one of the biggest sour cherry cultivators in the world (FAOSTAT, 2010) and the pomace of the juice processing is a potential and substantial waste for the food industry. Four types of anthocyanin compounds were determined in sour cherries: cyanidin-3-glucosylrutinoside, cyanidin-3-sophoroside, cyanidin-3-rutinoside and cyanidin-3-

* Corresponding author. Gaziosmanpaşa University, Faculty of Engineering and Natural Sciences, Department of Food Engineering, 60100, Tokat, Turkey. Tel.: +90 356 2521616x2899; fax: +90 356 2521729.
E-mail addresses: aslihan.demirdoven@gop.edu.tr (A. Demirdöven), senizkarabiyikli@hotmail.com, seniz.karabiyikli@gop.edu.tr (Ş. Karabıyıklı), kader.tokatli@gop.edu.tr (K. Tokatlı), nilgun.oncul@gop.edu.tr (N. Öncül).

http://dx.doi.org/10.1016/j.lwt.2015.03.101
0023-6438/

glucoside (Chandra, Rana, & Li, 2001; Kim, Heo, Kim, Yang, & Lee, 2005).

Food borne illnesses are still a major problem all over the world, even in developed countries. Outbreaks of human infections associated with food consumption increased in frequency during the past decade (Beuchat, 2002; CDC, 2014). It was reported that 6 to 81 million cases of illnesses, and up to 9000 deaths, annually are attributed to food borne pathogens in the USA (Alzoreky & Nakahara, 2003). *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Bacillus cereus* are the most common food borne pathogens (Beuchat, 1996; CDC, 2013). Various chemical antimicrobials have been used to prevent food borne infections/intoxications in the food industry. However, chemical preservatives are discountenanced nowadays as a result of increasing awareness of the consumers on the adverse effects of chemical preservatives on human health. The numbers of the studies on natural alternatives are increasing as a natural consequence of requirement by the food industry. New, available, natural and effective alternatives are required to satisfy the consumers and to supply their demand.

The objective of this study was to investigate the antimicrobial, antioxidant and physicochemical properties of sour cherry pomace and red cabbage anthocyanin extracts, which were extracted by conventional and ultrasonic extraction methods.

2. Materials and methods

2.1. Materials

Whole red cabbages (RC) and sour cherry pomace (SCP) were used as raw material. The red cabbages were obtained from Bafra-Samsun/TURKEY and sour cherry pomace was purchased from Dimes Concentrated Fruit Juice Company (Tokat, TURKEY). They were stored at +1 °C maximum for 48 h before processing.

2.2. Processing methods

2.2.1. Extraction of red cabbage and sour cherry pomace anthocyanins

Sorting, washing, cutting and chopping operations were carried out for red cabbages. Red cabbages were chopped (Moulinex FP 519G-750 W) at 500 rev/min under standardized conditions determined by pre-trials. Sour cherry pomace was used after separation of stones and stems. The extraction process was carried out using solid–liquid extraction method. 1% formic acid was used to acidify the environment during the extractions. An ethanol/water/formic acid mixture was used as the solvent in all extraction applications. Extractions were carried out with conventional and ultrasonic methods. The raw material amount that was determined by pre-trials was mixed with extraction solvent (red cabbage 1/3; m/v and sour cherry pomace 1/15; m/v). The mixture was homogenized at the fourth speed position for 45 s (Ultra-Turrax Ika-Werke, Staufen, Germany). Conventional and ultrasonic extractions were applied after the homogenization process. The obtained mixture was filtered (Whatman no:1, 125 mm) under a vacuum (Milipore, WP6122050, Germany). The extraction solvent was evaporated in a rotary evaporator at 50 °C. Single extraction step was used in all samples and for all methods.

2.2.2. Conventional and ultrasonic extractions

Conventional extractions (CE) were done by using a water bath (Memmert-WB22) whereas ultrasonic extractions (UE) were carried out using an ultrasound bath operating at 37 kHz (Elmasonic-S100H). Temperatures were controlled with a digital thermometer (Shaanxi Taurus, China) during extractions to stabilize the desired temperature.

Extraction conditions of RC anthocyanins were optimized by Response Surface Method (RSM). A Box-Behnken design was used in designing the CE and UE treatments of three variables with six center points (Design Expert 7.0.0 STAT-EASE, 2005). Extraction conditions of SCP anthocyanins were determined by pre-trials.

After production of anthocyanin extracts, all samples were stored at −80 °C to prevent the deterioration and interaction between the compounds of extracts until analyses. All samples were held at +4 °C to thaw for a night before analyzing. The soluble solid content of the extracts was adjusted based on the natural soluble solid content values of the materials before the analyses (6 °Bx for RC and 12 °Bx for SCP).

2.3. Methods of analysis

2.3.1. Physicochemical analysis

Total anthocyanin contents (TAC) of samples were detected by pH-differential method using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). TAC of samples were determined at 535 nm and (mg cyanidin-3-glucoside/100 mL of extract) were calculated with molar absorptivity of cyanidin-3-glucoside (26.900) (Glassgen, Wray, Dieter, Metzger, & Seitz, 1992). Total phenolic content (TPC) was detected at 760 nm using the Folin-Ciocalteu reagent, diluted 10 fold before use (Sigma Chemical Co., St. Louis, MO) with gallic acid (3,4,5-trihydroxybenzoic acid) as the standard and measured after reaction (2 h) (Franke, Chless, Silveria, & Robensam, 2004). Antioxidant activities of the samples were determined by the ABTS [2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] assay (Re et al., 1999).

Soluble solid contents (SSC) of samples were measured with a refractometer at 20 °C (RFM 330, U.K.) (AOAC, 1995). Total titrable acidity (TA) was determined by means of a potentiometric titration of the acidity of the samples by placing 10 g of sample into 90 mL of deionized water. After filtration, 10 mL filtrate was titrated up to pH 8.1 (pH meter, WTW InoLab, Weilheim, Germany) with 0.1 N NaOH. The results were expressed as g/100 mL (citric acid) (AOAC, 1995). pH values of samples were determined by pH-meter at 20 °C (AOAC, 1995). Water activity (a_w) values of the samples were measured by AquaLab Model Series 3 TE, USA (AOAC, 1980).

2.3.2. Microbiological analysis

The microflora of the extracts was controlled before MIC analyses by enumeration of total mesophilic aerobic bacteria (TMAB, FDA-BAM online, 2001a) and yeast & mold (Y&M, FDA-BAM online, 2001b). The safety of the extracts were checked out by *E. coli* (FDA-BAM online, 2013), *S. aureus* (ISO 6888, 2004), *L. monocytogenes* (ISO 11290, 1996), *Salmonella* spp. (FDA-BAM online, 2014) and *B. cereus* (FDA-BAM online, 2012) analyses.

The antimicrobial effects of the RC and SCP extracts were tested against some food borne pathogens. *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *L. monocytogenes* (ATCC 19115), *Salmonella* Typhimurium (ATCC 14028) and *B. cereus* (ATCC 10876) were used as test microorganisms. Two different inoculum doses were applied: low (~3 log CFU/mL) and high (~6 log CFU/mL) to detect the responses of the RC and SCP extracts for different contamination levels. Each of the test cultures were grown at 37 °C for 18–24 h in Brain Heart Infusion Broth (BHI, pH 7.4 ± 0.2, LabM-LAB049), and then diluted with 0.1% sterile peptone water (PW, pH 6.3 ± 0.2, Oxoid-L37) to achieve a final inoculum of 6.0 log CFU/mL (high inoculum dose) and 3.0 log CFU/mL (low inoculum dose), appropriately. The MIC value of the each extract was determined by modification of the method described by LaBombardi, Sotos, Allen, and Sullivan (2008).