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Polyphenol composition, antioxidant capacity, and antimicrobial activity of the extracts obtained from industrial sour cherry pomace☆

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ABSTRACT

Polyphenol extracts from industrial sour cherry pomaces were characterized on polyphenol composition, antioxidant capacity and antimicrobial activity. Extracts of pomace were purified and freeze-dried. Preparations were characterized by high polyphenol contents including anthocyanins, hydroxycinnamic acids and flavonoids, selected were characterized by high flavanol content and high antioxidant capacity. The antimicrobial effect of sour cherry polyphenol extracts was tested against *Salmonella*, *Escherichia coli* O157:H7 and *Listeria* spp. The bacteriostatic effect was tested by growing the strains in a liquid medium containing the extracts, the bactericide effect was assayed by putting the strains in direct contact in an aqueous suspension of the extract, simulating the disinfection process in the fresh-cut industry. Two of the sour cherry extracts tested reduced the growth of *Salmonella* and *E. coli* O157:H7 at concentrations higher than 2500 μg/mL, and inhibited *Listeria* spp. growth.

1. Introduction

Stone fruit like sour cherries are food products in demand on the European market due to their taste and nutrition values. Mean annual crops of sour cherry in Poland reach 200,000 T (Eurostat, 2010). The fruits are used for the production of juices, nectars, soft and alcoholic drinks, jams, and as additives in the dairy and confectionary industry. Sour cherries are a rich source of polyphenols—phytocomponents characterized by the presence of more than one phenol group in the molecule. Many research publications indicate that the increased consumption of fruits and fruit polyphenols is beneficial for maintaining good human health (Lee et al., 2007; Rissanen et al., 2003). The effect is attributed to a number of factors, including polyphenol components which are characterized by antioxidant properties (Halvorsen et al., 2002) and contribute to many life processes, resulting from their interactions with biomolecules, like enzyme regulators (Lee et al., 2007; D'Ischia et al., 2006). The main phenolics present in sour cherry are anthocyanins, represented by cyanidin glycosides (Chandra et al., 2009; Simunic et al., 2005). Besides anthocyanins, hydroxycinnamic acids, especially caffeoylquinic and *p*-coumaroylquinic acids are present in sour cherry. The presence of quercetin, kaempferol, and isorhamnetin glycosides was detected in sour cherries as well (Kim et al., 2005). Sour cherry stones are an interesting material as well. According to Yılmaz and Gökmen (2013) sour cherry kernel might be utilized as a source of dietary fibers, proteins and lipids, furthermore oil extracted from seed kernel is rich in polyunsaturated fatty acids, tocopherols, β-carotene and phenolic compounds.

In addition, plant phenolics and terpenoids, have been widely used because of their strong antimicrobial properties against foodborne pathogens, and therefore could be applied as novel preservatives in the food industry (Friedman et al., 2002).

The contamination of foods by foodborne pathogens and spoilage microorganisms is a problem that is not yet under adequate control despite a wide range of preservation techniques available. The microbiological safety of food continues to be a major concern to consumers, regulatory agencies and food industries throughout the world. Fresh-cut fruits and vegetables are of particular concern as they do not receive any treatment that guarantees the complete elimination of pathogens. Currently, chlorine, in the

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form of sodium hypochlorite at a concentration of 50–200 ppm is commonly used to disinfect produce (Beuchat, 1998). Nevertheless, concerns about its limited efficacy and the toxicity of chlorination by-products formed in the presence of organic matter, has prompt the search for alternative, safer, more effective and environmentally friendly sanitation agents. As far as microbial preservatives are concerned, traditional antimicrobials such as acetic, benzoic, lactic, propionic and sorbic acids and nitrite and sulphites have been used for many years to control the growth of microorganisms in food (Sofos et al., 1998). The current consumer demands for more natural and fresh-like foods, with fewer synthetic additives but increased safety and shelf life, urges food manufactures to use natural or mild preservation techniques (Negi et al., 2008). Polyphenols extracted from plants and fruits could be an alternative. Several papers have demonstrated the antimicrobial activity of flavonoids extracted from bergamot (Mandalari et al., 2007), *Garcinia* spp. (Negi et al., 2008), pome fruits including apple, pears and quinces (Alberto et al., 2006; Fattouch et al., 2007, 2008) and grapes (Baydar et al., 2004) among others. However, the antimicrobial efficacy of sour cherry extracts has not been evaluated.

The use of sour cherry in the juice industry results in the side production of significant amounts of pomaces, which may reach from 15% to 28% of transformed raw material, depending on the process conditions (Toydemir et al., 2013). The main use of such a by-product is as a fuel, the alternative is its use as an animal food or its further transformation, i.e. the production of dietary fibre and anthocyanin- and other polyphenol-reach extracts.

The literature lacks data on the properties of products derived from sour cherry pomace extracts. Another alternative could be its use as a source of antimicrobial and antioxidant preservatives for the food industry.

The aim of this work was to obtain concentrated polyphenol extracts from industrial sour cherry pomaces by the use of a low pressure chromatography method with solvents acceptable in food production. The qualitative and quantitative composition of the preparations obtained was characterized. The antimicrobial capacity of polyphenol preparations on different foodborne pathogens was determined from two points of view. The bactericidal effect was tested with a view to using the extracts as an alternative to chlorine for a washing-disinfection process of fresh-cut fruits and vegetables, and the bacteriostatic effect to be used as a natural preservative to prevent the growth of foodborne pathogens.

2. Materials and methods

2.1. Chemicals

Ultrapure water (Millipore System, GmBH, Vienna, Austria) and HPLC gradient-grade methanol (J.T. Baker, Deventer, Holland) were used to prepare all solutions. HPLC gradient-grade acetonitrile and formic acid were purchased from J.T. Baker (Deventer, Holland). Cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, peonidin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, quercetin, kaempferol, isorhamnetin, ellagic acid from Extrasynthese (Genay, France) and (+)-catechin, (−)-epicatechin, chlorogenic acid, phloridzin from Sigma-Aldrich Chemie GmBH (Steinheim, Germany) were used as standards for MS spectral comparisons. Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals to determine anti-oxidant activities were purchased from Sigma-Aldrich Chemie GmBH (Steinheim, Germany).

2.2. Plant material

Stoned pomace of sour cherry was a material for the research. The pomace came from fruit processing in 2009 at a modern concentrated juice production plant Alpex at Łęczeszyce, Poland. Fresh pomace collected from the production line was sieved on a manual 5-mm screen to eliminate stones. The seedless part was extracted promptly, while the seed part was dried and used for other experiments (not described herein).

2.3. Preparation of sour cherry extracts

Three kinds of purified extracts, differing in polyphenol contents were obtained from the stoneless pomace fraction. Fresh sour cherry pomaces were subjected to water extraction in three steps, at a temperature of 70–75 °C for 30 min. In the first step the weight ratio of fresh pomace to water was 1:4, while in the second and third extraction step the volume of water was equal to the volume of the extracts resulting from the preceding extraction step. The first and second extracts were collected by soaking the pomace on filtration cloth, while the third extract, after soaking the pomace was pressed on own-manufactured laboratory press (Lodz University of Technology, Poland). The choice of deionized water or optionally condensate of technological vapor as a solvent for extraction resulted from economic factor (inexpensive solvent), safety and availability of water or vapor as a boiler medium. The excess of technological vapor appears in many industrial facilities, in case of shortage the installation for deionized water (e.g. reversed osmosis) can be applied. Unlike technological water, deionized water does not add to extracts any salts, especially calcium and iron salts, which with polyphenols may result in residues or undesired color. Disadvantage of deionized water as a solvent is relatively low yields of some polyphenol groups, in particular flavanols. In presented research the water was heated to 70 °C in order to increase the yields. Determinations of polyphenols in starting material and post-extraction pomaces showed, that the yields of anthocyanins, hydroxycinnamic acids and flavonols after triple extraction was 80%, while the yields of flavanols was 30%. All the 3 extracts were put together and filtered on cellulose. The filtered extract was purified on a 80 × 2.5 cm column (Pharmacia Fine Chemicals, Uppsala, Sweden) filled with Amberlite XAD-7HP (Sigma-Aldrich, Steinheim, Germany). The extract was applied with a flow rate of 250 mL/h. Next, the column was washed with 300 mL of water and 150 mL of 10% ethanol, with a flow rate of 150 mL/h. The elution of polyphenols was carried out with the use of 300 mL of 20%, and 750 mL of 60% ethanol, with a flow rate of 150 mL/h. Seven fractions of 150 mL each were collected. The fractions were joined as follows: 1 + 2, 3 + 4, and 5 + 6 + 7. Ethanol was removed from each of the joined fractions on a laboratory vacuum rotary evaporator (type 350P, Unipan-Scientific Instruments, Warsaw, Poland), at 50 °C, and fractions thus prepared were then freeze-dried. That procedure yielded 3 concentrated preparations from the pomace. The sour cherry extracts were labeled as follow: CHPE1 (1 + 2), CHPE2 (3 + 4), CHPE3 (5 + 6 + 7).

2.4. Sample preparation for quantification

Polyphenol extracts from pomaces were diluted in a 50% solution of methanol, then centrifuged at 6000 rpm (3600 g) for 5 min. The samples prepared as above were used to determine the total polyphenol content (TCP), the antioxidant activity, and the polyphenol, i.e. anthocyanins, hydroxycinnamic acids and flavonols contents, by the HPLC method.