LAURA TAMKUTĖ

VALORIZATION OF CRANBERRY POMACE BY BIOREFINING INTO VALUABLE FUNCTIONAL FOOD-GRADE INGREDIENTS USING HIGH PRESSURE EXTRACTIONS AND FRACTIONATION

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ABBREVIATIONS

1S – first separator
2S – second separator
AAPH – 2,2'-azobis(2-amidinopropane) dihydrochloride
$ABTS^{\bullet+}$ – 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
AC – anthocyanin content
AMB – aerobic mesophilic bacteria
ANOVA – analysis of variance
BHI – brain-heart infusion broth
BPW – buffered peptone water
CCD – central composite design
CO_2 – carbon dioxide
DMSO – dimethyl sulfoxide
DP – degree of polymerization
DW – dry pomace weight
E – dry extract
EtOH – ethanol
FA – fatty acid
FW – fresh weight
GAE – gallic acid equivalents
GFP – green fluorescence protein
HDL – high-density lipoproteins
HF – heavier fraction
H_2O – water
HPLC – high performance liquid chromatography
LAB – lactic acid bacteria
LDL – low-density lipoproteins
LF – lighter fraction
L-ORAC – oxygen radical antioxidant capacity for lipophilic compounds
MetMb – metmyoglobin
MIC – minimum inhibitory concentration
MOI – multiplicity of infection
MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MUFA – monounsaturated fatty acids
ORAC – oxygen radical absorbance capacity
PAC – proanthocyanidin
PLE – pressurized liquid extraction
PLE-EtOH – cranberry pomace ethanol extract
PLE-H_2O – cranberry pomace water extract
PUFA – polyunsaturated fatty acids
Q_1 – fast acting antioxidants
Q_2 – slow acting antioxidants
Q_T – the sum of Q_1 and Q_2 values

QUENCHER – quick, easy, new, cheap and reproducible
ROS – reactive oxygen species
RSM – response surface methodology
SD – standard deviation
SFE – supercritical fluid extraction
SFE-CO_2 – supercritical fluid extraction with carbon dioxide
SFA – saturated fatty acids
SC-CO_2 – supercritical carbon dioxide
PBS – phosphate buffered saline
PLE – pressurized liquid extraction
PUFA – polyunsaturated fatty acids
TAG – triacylglycerols
TBARS – thiobarbituric acid reactive substances
TE – trolox equivalents
TPC – total phenolic content
TSB – tryptic soy broth
ZIKV– Zika virus
$ZIKV_{GFP}$ – the green fluorescent protein (GFP) expressing strain of ZIKV-MR766

INTRODUCTION

Berries are nutritionally valuable fruits containing various compounds beneficial to health. Many berry species also possess excellent flavor properties and may be consumed both as fresh foods and raw materials for a variety of processed products. Yet, some berry species are not consumed as fresh fruits due to unacceptable taste; however, they also accumulate large amounts of valuable phytochemicals, particularly phenolic antioxidants. So far as fresh berries are highly perishable fruits, major part of their harvest is processed into various longer shelf-life products such as jam, juice, confiture, etc. The processing of fruits into juice generates large amounts of by-products which may reach 20–30% of the initial dry weight (FAO, 2011). These by-products are called pomace, press-cake, or marc. Berry pomace is composed of seeds, skin, pulp residues, and, sometimes, stems. It is sometimes discarded as waste or used inefficiently for composting and animal feeding. Pomace still contains a considerable fraction of beneficial bioactive phytochemicals, such as tocopherols, phytosterols, polyphenols which have been shown to possess numerous health benefits (Venskutonis, 2020). Consequently, there is urgent need of valorization of berry pomace for reducing the losses of valuable nutrients and negative environmental impact of biowaste.

One of the main goals is recovery of valuable phytochemicals from fruit by-products, and it is in line with the increasing interest of the consumers who have been paying more attention to healthy, safe, nutritious foods without chemical additives which may have potential toxicological effects on human health. During the last decade, researchers have been focusing on search for new, natural and valuable bioactive compounds originating from natural sources (berries, vegetables, fruits) which are safe, effective and acceptable as natural preservatives or antioxidants. These bioactives may be used to control the microbial and chemical mechanisms responsible for spoilage of food products. A lot of studies showed that, in the food industry, by-products (waste) (including pomace) retain a huge amount of bioactive compounds which may still be recovered as functional components for food, pharmaceutical and cosmetic industries. Furthermore, the constantly growing food industry produces higher amounts of waste, which has a devastating negative effect on the environment. The modern industry is focused on reducing the environmental impact of industrial by-products and creating added value products. Therefore, the combination of various novel techniques allows reducing the amount of by-products and recovering bioactive compounds.

Environmental aspects represent other important issues in developing efficient technologies for the valorization of food processing by-products and waste. From this point of view, the so-called green processes are preferred in the development of new technologies in food and other industries. Numerous studies apply green chemistry principles for the recovery of valuable phytochemicals from fruit processing by-products. For instance, among various extraction techniques, preference is given to green chemistry-based techniques which do not use harmful organic solvents, and which reduce the use of energy and other resources. Therefore, the development of complex multistep processing methods including novel

techniques, such as supercritical CO_2 and pressurized liquid extraction for the recovery of high added value constituents, may be considered as a highly promising trend in biorefining the by-products. To achieve better results, these extraction methods can be accompanied with various mathematical optimization techniques.

Cranberries (*Vaccinium macrocarpon*) are popular berries, particularly in the Northern countries. Due to their strong sour taste, cranberries are processed into various products, including juice. Cranberries contain various health-beneficial substances and have been used not only for foods but also as a natural remedy (Nemzer, Vargas, Xia, Sintara, & Feng, 2018). For instance, cranberry polyphenols show antimicrobial activity against some pathogenic bacteria (*Staphylococcus, Salmonella, Escherichia, Listeria*) which are responsible for foodborne diseases. The bioactive components of berry pomace may be obtained by using various by-product processing techniques which are applied to enhance the functionality of various food products. Furthermore, every year, the number of natural food products with various bioactive components from berries and their pomace has been increasing. So far as systematic studies on the processing of cranberry pomace have not been performed previously, this study investigates the valorization of cranberry pomace when using multistep processes for biorefining the biomass into high value food-grade functional ingredients with the objective to define their application in meat products. Firstly, supercritical carbon dioxide extraction and pressurized liquid extraction were optimized by using the response surface methodology with the face-centered central composite design for effective isolation of the lipophilic fraction and higher polarity substances. Then, supercritical fluid extraction with carbon dioxide was used to fractionate the cranberry pomace extract under optimal conditions and evaluate the efficiency of fractionation for various bioactive compounds at a low temperature and reduced pressure in separators. Finally, the ethanol extract of cranberry pomace, which is rich in polyphenols, was applied to improve the safety and quality of meat products. Various assays (antioxidant activity, antiviral, antimicrobial) were applied to determine the best polar extract for the value-added ingredient production and their application in food products.

The aim and tasks of the work

The aim of the thesis is to valorize cranberry pomace by applying effective multistep biorefining technologies based on high pressure extraction/fractionation techniques for the production of valuable functional ingredients and evaluation of their phytochemical composition, biological and technological properties.

The following objectives were raised for achieving this aim:

1. By using the Central Composite Design (CCD) based on Response Surface methodology (RSM) to determine the optimal supercritical carbon dioxide extraction (SFE-CO_2) parameters (temperature, extraction time and pressure) so that to obtain the highest possible yield of the lipophilic fraction from cranberry pomace; to investigate extraction kinetics under optimal conditions and to determine the fatty acid composition of the recovered triacylglycerols.

2. To evaluate the possibilities of fractionation of the cranberry pomace lipophilic fraction during SFE-CO_2 with or without co-solvent ethanol in two post-extraction separators by changing the temperature in the range of subcritical CO_2 level at a constant pressure; to characterize the composition of the obtained fractions.

3. To evaluate the possibilities of fractionation of higher polarity non soluble in supercritical CO_2 constituents from defatted cranberry pomace by pressurized liquid extraction with the green solvents ethanol and water. To optimize the process of pressurized liquid extraction with ethanol by using CCD-RSM and to characterize the obtained extracts.

4. To characterize the phytochemical composition, anticancer, antiviral and antimicrobial properties of the polar extracts obtained by pressurized liquid extraction and to quantify individual anthocyanins.

5. To evaluate the antioxidant potential of lipophilic, ethanol and water extracts and extraction residues by various *in vitro* methods.

6. To test the applicability of the ethanol extract additive in pork meat products by investigating its effect on the growth of selected food pathogenic microorganisms, lactic acid bacteria (LAB) and aerobic mesophilic bacteria (AMB) and determine the effect on the antioxidant capacity, physico-chemical and sensory characteristics.

Scientific novelty

1. The concept of the biorefining of cranberry pomace into lipophilic and hydrophilic fractions by using high pressure technologies with green solvents such as supercritical fluid extraction with carbon dioxide (SFE-CO_2) and pressurized liquid extraction (PLE) has been applied for the first time; it was comprehensively evaluated for the isolation of high added value functional ingredients. The parameters of multistep high pressure separation scheme (SFE-CO_2 and PLE) were optimized for obtaining the highest yields.

2. This study has reported for the first time the fractionation of berry pomace lipophilic substances by the on-line SFE-CO_2 (with and without co-solvent ethanol), which resulted in the separation of the different classes of lipids by applying low (< 0 °C) temperatures in the 1st separator for precipitating heavier lipophilic fractions. Moreover, it was shown that different classes of lipophilic compounds which are present in cranberry pomace may be pre-concentrated by using fractionation at freezing temperatures.

3. The polar extracts of cranberry pomace have been tested and evaluated against the Zika virus infection for the first time.

4. The effect of defatted cranberry pomace extracts (ethanol and water), consecutively isolated by pressurized solvents has been tested for the first time on the growth of selected pathogenic and spoilage bacteria in their cultures or in meat products (slurry, burgers and ham) for controlling their stability and for the evaluation of their sensory properties.

Practical significance of the thesis

In the process of juice production, a large amount of by-products including berry pomace is accumulated and usually discarded as waste. The application of high pressure extraction techniques (SFE-CO_2 and PLE) with mathematical modeling tools for cranberry pomace process optimization helped to achieve high efficiency of the recovery of bioactives. The fractionation of cranberry pomace extract by online SFE-CO_2 allows redistributing different classes of lipids (tocopherols, phytosterols, etc.) between separators and thus achieving fractions with superior composition. The extracts obtained by using innovative high pressure extraction methods were used for meat products. This method may be considered as a promising way of increasing the oxidative and microbiological stability of a product.

Publication of the research results

The results of this research have been presented in 3 publications included in the list of the *Clarivate Analytics Web of Science* database and reported at 6 international conferences and 2 national conferences.

Structure and content of the dissertation

The dissertation is written in English. It involves of a list of abbreviations, an introduction, a review of the most relevant literature, materials and methods, a chapter covering the results and discussion, a conclusion, a list of references (in total, 275 references were used), and a list of publications on the topic of the dissertation. The final work contains 145 pages including 25 tables and 33 figures.

Key points of the thesis

1. The application of consecutive extraction with supercritical CO_2 and pressurized liquid extraction with higher polarity solvents allows isolating valuable fractions which possess strong antioxidant, antimicrobial and antiviral activity and a high concentration of bioactives from cranberry pomace; whereas, modeling of SFE-CO_2 and PLE extraction by applying the response surface methodology when using the face-centered central composite design is an effective method to determine the optimal extractions parameters.

2. The application extraction at the supercritical solvent state and fractionation at subcritical CO_2 parameters is an effective method for obtaining higher specificity cranberry pomace fractions with higher concentrations of various classes of lipophilic compounds.

3. Polar extracts of defatted cranberry pomace isolated by pressurized liquid extraction may contain a promising additive for the improvement of the safety and quality of meat products as well as a bioactive ingredient with antiviral potential.

1. LITERATURE REVIEW

1.1. Cranberry – a fruit containing valuable nutrients

1.1.1. Botanical characterization and proximate composition

The cranberry originally comes from North America and is therefore also named as the 'American cranberry'. It is a group of evergreen dwarf shrubs in the subgenus *Macrocarpon* of the genus *Vaccinium* and belongs to the *Ericaceae* family. In Northern and Central Europe, the native species of *Vaccinium oxycoccos* are common, while, in North America, *Vaccinium macrocarpon* is widespread. These berries grow in acidic bogs throughout the cooler regions of the Northern Hemisphere. They are low, creeping shrubs up to 2 meters long and 5–20 centimeters tall (Fröberg, 2011). The shrubs produce round, oblong or pear-shaped edible berries whose color ranges from pink to prominently dark red with an acidic taste which usually overwhelms its sweetness. The ripe berries of the large cranberry (American cranberry) accumulate up to 88% of water, while the dry substances comprise 8–15.1% which are composed of insoluble (3.8–3.87%) and soluble (8.5–10.8%) dry substances. Monosaccharides comprise from 3.4% to 7.1% of the fresh weight (FW). The dominant monosaccharides are glucose and fructose, while the glucose represents about 58.9–65.9% of the total content of monosaccharides. In American cranberries, various vitamins, such as vitamin C, E, B_1, B_2, macro- and micro-components, are present. The amount of various compounds mostly depends on the conditions of growth and species.

Berries can be consumed as fresh fruits; however, due to an extremely short shelf life and/or unfavorable sensory properties, a major part of their harvests is processed into other products. For instance, in 2017, USA produced more than 8.3 million barrels of American cranberries, while less than 4% of it was sold as fresh berries; the rest of the harvest was processed into juice, sauces, jams, or sweetened dried cranberries (USDA-NASS, 2011). Every year, juice pressing generates large amounts of solid residues which are called press-cake, pomace or marc.

American cranberry (*Vaccinium macrocarpon*) pomace is a uniquely rich source of dietary fiber, essential fatty acids, phytosterols, antioxidants (phenolic acids, flavonoids, anthocyanins, tocopherols, and tocotrienols), carotene, minerals, aromatic substances, vitamins, bacterial and viral inhibitory substances (Česoniene & Daubaras, 2016). Of all these components, more than 150 have been identified and studied as bioactive compounds. According to White, Howard and Prior (2010) cranberry pomace is mainly composed of insoluble dietary fiber (65.5%), while the portion of soluble dietary fiber was found to be much lower (5.7%). The majority of the cranberry cell wall was comprised of cellulose, pectin and hemicellulose with a small amount of protein, ash, starch and fat.

1.1.2. Lipophilic constituents

Berries of the American cranberry accumulate up to 0.482 g/100 DW of fats. Cranberry oil is a good source of unsaturated fatty acids, such as α-linolenic acid

(34.3%), linoleic acid (35.3%), oleic acid (23.6%), which are associated with the lowering of the total cholesterol and inhibition of the oxidation of low density lipoproteins. Omega-3 (α-linolenic) and omega-6 (linoleic acid) fatty acids are essential polyunsaturated fatty acids (PUFA) (**Fig. 1.1**) which cannot be synthesized in the human body and must be derived through the diet. Their ratio is commonly used as an index to evaluate the nutritional value of dietary fat which has particular relevance on human health. The *Department of Health* of the United Kingdom determined the recommended the ratio of omega-6/omega-3 which should be below 4 (nearly 3:1 and even better 1:1) (Cordain *et al*., 2005).

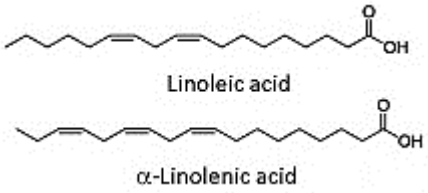


Figure 1.1. Polyunsaturated fatty acids (PUFA) in cranberry oil

The most important lipid-soluble antioxidant in the human body is α-tocopherol, while such lipophilic dietary antioxidants as carotenoids may be obtained from many foods of the plant origin. The dietary intake of α-tocopherol from natural sources is often preferred due to its higher bioavailability. Although γ-tocopherol features lower activity than α-tocopherol, yet, according to the data, it contains better scavenger of nitrogen-containing reactive molecules (Stone and Papas, 2012), and it has even shown anti-inflammatory potential. The oil of cranberries also features a large amount of γ-tocotrienol (180 mg/100g oil). The difference in the chemical structure of tocotrienols if comparing with tocopherols is the presence of three double bonds in the phytyl side chain, and, due to differences in the chemical structure, they possess a more prominent antioxidative and anticancer effect (Stone and Papas, 2012). The content of carotenoids in cranberries is not high; for instance, Lashmanova, Kuzivanova and Dymova (2012) determined 200 μg/100 g DW, and the major contributors to the total content of carotenoids were β-carotene (28%), lutein (23%) and neoxanthin (20%).

Phytosterols, which are found in cranberries (β-sitosterol, stigmasterol, ergosterol, cholestan-3-ol, campesterol), as well as other bioactive compounds, generally have a positive effect on human health. They do not affect the high density lipoprotein (HDL) cholesterol, but bind bile acids and reduce the risk of high blood pressure, inhibit the development of intestinal cancer and show antioxidative and cholesterol-lowering activities (Kritchevsky and Chen, 2005; Mel'nikov, Seijen Ten Hoorn and Eijkelenboom, 2004). The main phytosterol which was found in cranberry oil is sitosterol. It reduces the absorption of cholesterol and helps to maintain a low level of the total cholesterol in peripheral blood.

1.1.3. Polyphenolic phytochemicals

Historically, cranberries have been used in folk medicine. Mostly, health benefits, such as prevention against urinary tract infections, reduced risk of cancer or

cardiovascular disease, antiviral, anticarcinogenic, antiangiogenic, antibacterial, antimutagenic, anti-inflammatory, antitumorigenic and antioxidant properties *in vitro* are associated with polyphenols (Álvarez-Martínez, Barrajón-Catalán, Encinar, Rodríguez-Díaz, & Micol, 2020; Clain *et al*., 2019; Stagos, 2020). Three classes of flavonoids (flavonols, anthocyanins and proanthocyanidins), catechins, hydroxycinnamic, phenolic acids and triterpenoids have been identified. Analysis demonstrated that proanthocyanidins are the leading component (63%) of phenolic compounds. Smaller amounts of anthocyanins (16%), flavonols (14%), hydroxycinnamic acids (7%), and hydroxybenzoic acids (0.05%) were also detected (Kylli *et al*., 2011). Flavonoids are a group of phenolic compounds which naturally occur in various plants and contain the C6-C3-C6 flavan ring (**Fig. 1.2**). In terms of the substitutes in the different positions on the rings, they may be divided into several sub-groups. All these groups are denoted by different chemical and biochemical properties. Additional details about them are provided in the following discussion.

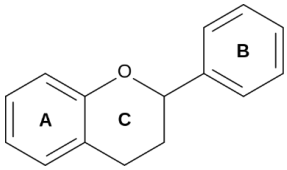


Figure 1.2. Basic flavan 3 ring structure

Anthocyanins are a large group of water-soluble pigments and the most studied components of cranberry fruit (**Fig. 1.3**). They are responsible for the bright red color and are located in the external layers of the berry skin. The six major anthocyanins found in cranberries are 3-monogalactosides, 3-monoarabinosides and 3-monoglucosides of cyanidin and peonidin, which differ in terms of the number and location of the hydroxyl and methoxyl groups on the flavan ring. Wu and Prior (2005) during the HPLC analysis of frozen cranberry fruits identified several new anthocyanins, such as 3-monoarabinosides of delphinidin, 3-monogalactosides of petunidin, 3-monogalactosides and 3-monoarabinosides of malvidin and pelargonidin. The concentration of anthoacyanins varied from 13.6 to 171 mg/100 g FW, and it is strongly dependent on the fruit size, ripeness, variety, and a few other factors. The high level of anthocyanins makes the cranberry a significant dietary source.

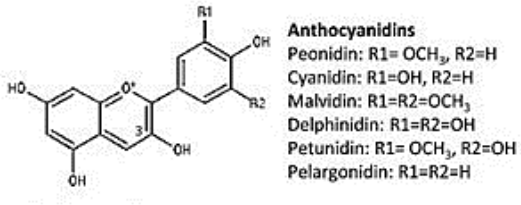


Figure 1.3. Structure and substitution patterns of common anthocyanidins present in cranberries

Flavonols are mostly glycosylated with glucose, galactose, rhamnose or arabinose of quercetin and myricetin (**Fig. 1.4**). In some varieties (but not all), kaempferol was also identified. The predominant form of flavonols observed in the cranberry is quercetin 3-galactoside, but, during recent researches, more than 11 other glycosides were also identified, although in lower concentrations (Côté, Caillet, Doyon, Sylvain and Lacroix, 2010a; Pappas and Schaich, 2009). The total flavonol content in cranberries ranges from 20 to 40 mg/100g of FW, while some researchers reported individual components of cranberries, such as hyperin (7.04 mg/100 g), quercetin (4.16 mg/100 g), myricetin 3-arabinoside (3.75 mg/100 g) and avicularin (3.45 mg/100 g) (Harnly *et al*., 2006; Vvedenskaya and Vorsa, 2004).

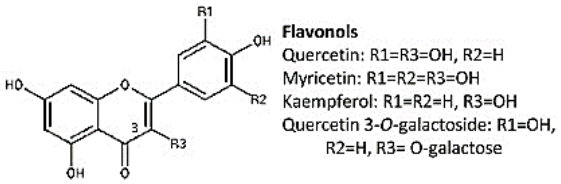


Figure 1.4. Structure and substitution patterns of common flavonols present in cranberries

In cranberries, there is a high amount of flavanols (flavan-3-ols) which are present as monomers, oligomers and polymers (**Fig. 1.5**). They provide 418 mg of total flavanols, including 63–70 mg of oligomers with a different degree of polymerization and 234 mg of polymers, while monomers, dimers and trimers are found in much lower amounts (7.3, 26, and 19 mg, respectively). It has been reported that cranberries contain gallocatechin gallate, catechin, epigallocatechin, epigallocatechin gallate and catechin gallate in amounts of 0.4 mg/100 g, 0.8 mg/100 g, 1.5 mg/100 g, 1.9 mg/100 g, 7.9 mg/100 g, respectively (Harnly *et al*., 2006).

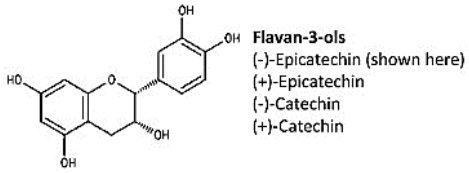


Figure 1.5. Common flavan-3-ols in cranberries. Structure of (-)-epicatechin

Oligomers and polymers are known as proanthocyanidins (PACs) or condensed tannins and represent the bigger part (about 85%) of the total content of flavan-3-ols defined in cranberries (**Fig. 1.6**). PACs are characterized by constitutive units (epicatechin (EC), epigallocatechin (EGC) and catechin), the types of linkage (two B-type linkages (C4→C6 and C4→C8), and the relatively uncommon A-type linkage (C2→*O*→C7)), and the degree of polymerization (DP) (Madrigal-Carballo, Rodriguez, Vega-Baudrit, Krueger and Reed, 2013).

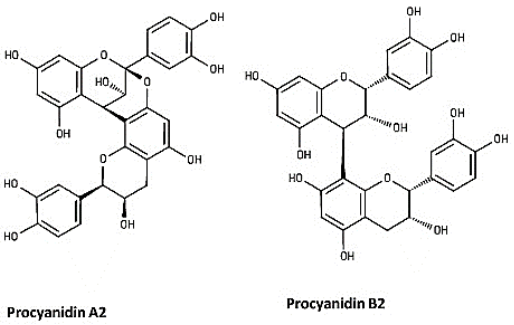


Figure 1.6. Examples of A-type and B-type procyanidin linkages

The structural characteristics of PACs are associated with several functions, when polyphenolic structures increase the antioxidant capacity, and vicinal hydroxyl groups bind metals. The dominant constitutive unit in PACs composition is (2)-epicatechin, while (+)-catechin and (epi)gallocatechins are only found in traces. Cranberry PACs with at least one A-type linkage contain 51–91% of the total PACs (Feliciano, Krueger, Shanmuganayagam, Vestling and Reed, 2012). It means that A-type linkages dominate in cranberries while such linkages are fairly rare in other foods. It has been proven that the difference between the A- and B- types of PACs structures exerts a significant effect on their biological properties. The PACs with the A-type linkage exhibit significantly higher inhibition *in vitro* against *Escherichia coli* bacteria adhesion on uroepithelial cells than the B-type during urinal tract infection (Howell *et al*., 2005).

The cranberry is a rich source of phenolic acids, including hydroxybenzoic and hydroxycinnamic acids (**Fig. 1.7**). The most abundant hydroxybenzoic acid is benzoic acid at levels of 474–557 mg/100 g FW, while the content of 2,4-dihydroxybenzoic, p-hydroxybenzoic and o-hydroxybenzoic acids were much lower and varied from 2 to 4 mg/100 g FW. p-coumaric, sinapic, caffeic and ferulic acids are the main hydroxycinnamic acids in the cranberry fruit. Their contents varied from 8.8 to 25 mg/100 g FW (Wang and Zuo, 2011; Zuo *et al*., 2002). Zheng and Shetty (2000) reported that in cranberry pomace they found gallic, chlorogenic, *p*-hydroxybenzoic and *p*-coumaric acids as major compounds.

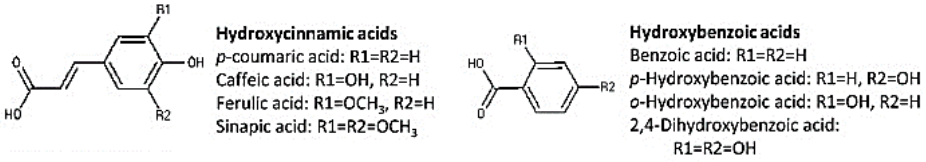


Figure 1.7. Structure and substitution patterns of common hydroxybenzoic and hydroxycinnamic acids present in cranberries

Nonphenolic organic acids are also observed in cranberries; they comprise 570 mg/100 g FW. The main acids are quinic, citric (1.88–6.08 mg/g) and malic acids, while lower amounts of ascorbic (~11.5 mg/100 g), shikimic (0.1–0.9 g/100 mL), galacturonic (0.19 g/100 mL) acids and even traces of tartaric acid and its methyl

ester were identified (Cunningham *et al*., 2003; He and Rui, 2006; Wang and Wang, 2009).

In cranberries, 0.00011% of volatile oils were detected, from which 68 compounds, including terpenes and their derivatives (cineol (0.7%), carvacrol (0.5%), nerol, limonene (0.4%), linalool (0.3%), myrcene, *α*-pinene, *β*-pinene, kaurene (1.1%), pimaradiene (1.0%), manoyloxide (0.3%), and *α*-terpineol (24% of the total volatile oils)) are responsible for their flavor and aroma (Croteau and Fagerson, 1968). Ursolic acid (triterpene) is abundant in cranberries (46–109 mg/100 g FW) (Kondo *et al*., 2011) and used in the traditional herbal medicine (**Fig. 1.8**). It was determined that ursolic acid has strong anti-inflammatory and anticancer effects. Two rare hydroxycinnamic derivatives (cis-3-O-p-hydroxycinnamoyl ursolic acid (12–16 mg/100 g FW) and trans-3-O-p-hydroxycinnamoyl ursolic acid (42–60 mg/100 g FW)) of ursolic acid were isolated from cranberries.

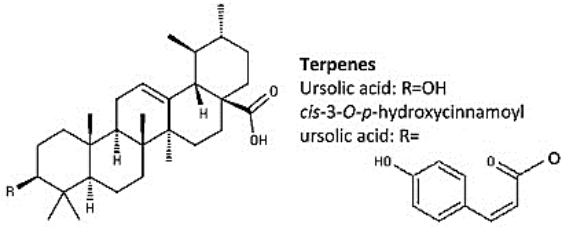


Figure 1.8. Structure and substitution patterns of common terpenes present in cranberries

1.2. Biological activity and putative health benefits of cranberry bioactives

Flavonoids are the main compounds which are found in cranberries. They possess a lot of health benefits. Additional details about the antimicrobial, antiviral, antioxidant and anticancer activities of flavonoids are provided in the following discussion.

1.2.1. Antimicrobial activity

The main foodborne pathogens in the world are *Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus* and *Escherichia coli O157:H7*. *E. coli* can cause hemolytic uremic syndrome (HUS) or hemorrhagic colitis (HC); in 2005, in the United States, there were 73 thousand of confirmed cases of *E. coli O157:H7* infection (US centers for disease control and prevention (US CDC), 2005). *S. typhimurium* may cause salmonellosis, *L. monocytogenes* may trigger listeriosis, which is really dangerous for pregnant women, individuals with a weak immune system, or senior people, *S. aureus* releases an exotoxin which poisons food. Conventionally, chemical additives are used for food preservation, but nowadays the society is demanding natural, healthy and safe food; therefore, an increased demand for natural preservatives has been observed. It is well known that berries are a rich source of bioactive compounds including polyphenols and organic acids, which may inhibit the growth of bacteria. Rauha *et al*. (2000) and Puupponen-Pimiä *et al*. (2001) studied the antimicrobial properties of Finnish plant and berry extracts containing flavonoids and other phenolic compounds. They found that lactic acid

bacteria (LAB) were more resistant than generic *E. coli* or *Salmonella spp.* Also, it was observed that flavones, quercetin and naringenin were very effective in inhibiting the growth of unwanted bacteria.

The American cranberry has a lot of health benefits related with phenolic compounds, such as prevention against urinary tract infections by preventing the adherence of fimbriated *E. coli* to mucosal surfaces, development and progression of cancer and cardiovascular disease (Vattem, Lin, Ghaedian and Shetty, 2005). Wu, Qiu, Bushway and Harper (2008) reported that cranberry concentrate has strong antibacterial effects against foodborne pathogens (*E. coli O157:H7, L. monocytogenes*, *S. Typhimurium* and *S. aureus*) *in vitro*. The antimicrobial compounds of cranberry concentrate (mainly polyphenols) damaged the cell wall and membrane thus causing their destruction. These components easily enter the interior of the cell through the damaged places, and, inside the cell, they can react with the bacterial DNA, which results in its death.

The antimicrobial activity of cranberries was determined for various illnesses caused by such bacteria as *Salmonella*, *S. aureus*, *E. coli*, *Campylobacter*, *Candida albicans* and *Helicobacter pylori* (Feng *et al*., 2013; Puupponen-Pimiä *et al*., 2005; Rane, Bernardo, Howell and Lee, 2014). This would explain their role in preventing some infectious diseases, including stomach ulcers, cancers, etc. The inhibition of undesirable bacteria is associated with the low pH of cranberries and the modest amount of phenolic compounds in them, while Caillet, Côté, Sylvain and Lacroix (2012) obtained results which proved that the antimicrobial properties of the cranberry are not only based on the low pH. They found that the fraction enriched in proanthocyanidins showed a higher antimicrobial activity on *E. coli* O157:H7 EDL 933, *S. typhimurium* SL1344, *L. monocytogenes* HPB 2812 1/2a, *Enterococcus faecium* resistant to vancomycin (ERV), *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 15442 comparing with the fractions enriched with anthocyanins. The inhibition on the growth of the few pathogenic bacteria by proanthocyanidins with at least one A-type linkage might be explained by several mechanisms including the destabilization of the cytoplasmic membrane, the inhibition of extracellular microbial enzymes, the direct actions on microbial metabolism deprivation of the substrates required for microbial growth and the permeabilization of the plasma membrane (Heinonen, 2007).

1.2.2. Antiviral activity

Viruses are very dangerous for humans and animals. Viral infections are distributed all over the world and may cause a wide range of diseases, and, in some cases, when the immune system of a patient is weak, they may become even life threatening as they enter the body and use its metabolism to produce a lot of copies of their genome and proteins. Diseases caused by these viruses are difficult to treat. Patients became resistant to the currently available antivirus drugs; therefore, it is extremely important to find plants/berries/fruits which possess antiviral properties. Studies showed that flavonoids, terpenoids, polyphenolics, and even some essential oils may possess potential antiviral properties. They inhibit the formation of the viral

DNA or RNA, or prevent the activity of virus reproduction (Narayana, Reddy, Chaluvadi and Krishna, 2001). The main reasons for being difficult to fight against viruses are the close proximity of cells multiplying the virus with the rest of the host cells; thus it is very important to use compounds which inhibit the cycle of the viral growth in the infected cell, but are not toxic to the surrounding normal cells. The second problem is that infections of viruses are difficult to diagnose, and that sometimes it is even too late for effective treatment.

Plants rich in polyphenolic compounds were already used in the folk medicine due to their antiviral properties (Haslam, Lilley, Cai, Martin, & Magnolato, 1989). Studies showed that flavonoids, terpenoids, polyphenolics, and even some essential oils may inhibit the formation of viral DNA or RNA, or prevent the activity of virus reproduction (Narayana *et al*., 2001). The strongest antiviral properties are shown by the main class of polyphenols, i.e., flavonoids. They inhibit the reproduction of various viruses including the human immune deficiency virus (HIV), the herpes simplex virus, *retroviridae*, the influenza virus, *hepadnaviridae*, the polio virus, *hespervirides*, the dengue virus, etc. The most important compounds from the group of flavonoids which possess the strongest antiviral properties are quercetin, kaempferol and rutin, though other polyphenols, such as gallic acid, epicatechin, epigallocatechin, catechin and various gallates of phenolic acids, are important as well (Amoros, Simões, & Girre, 1992). There is some evidence that bioactive compounds, such as alkaloids, coumarins, flavonoids, terpenoids, polyphenolics and saponins, exhibit antiviral activity against numerous enveloped RNA viruses including *flaviviruses* (Gaudry *et al*., 2018; Lani *et al*., 2016; Lee & Shin, 2019; Powers & Setzer, 2016), while polyphenols, including epigallocatechin gallate (EGCG), delphinidin and curcumin, exhibit antiviral activity against the Zika virus (Balasubramanian *et al*., 2019; Gaudry *et al*., 2018; Mounce, Cesaro, Carrau, Vallet, & Vignuzzi, 2017; Sharma, Murali, Singh, & Giri, 2017; Vázquez-Calvo, de Oya, Martín-Acebes, Garcia-Moruno, & Saiz, 2017; Wong *et al*., 2017).

Juices and extracts of the fruit of the American cranberry (*V. macrocarpon*) are rich in flavonoids. Several articles have been published about their use as antiviral agents. Studies have been conducted showing their inhibition properties against rotavirus, reovirus (Lipson *et al*., 2007; Lipson, Cohen, Zhou, Burdowski and Stotzky, 2007), enterovirus, human norovirus surrogates, namely, murine norovirus (MNV-1), feline calicivirus (FCV-F9), bacteriophage MS2, bacteriophage 4-X174 (Su, Howell and D'Souza, 2010) and influenza virus (Oiknine-Djian *et al*., 2012; Weiss *et al*., 2005; Yang *et al*., 2014). The study showed that a beverage of *V. macrocarpon* stimulated the immune system of patients because its daily consumption increased the amount of gamma delta T cells (Tγδ) which participate in defense mechanisms against bacterial and viral diseases. The daily consumption of a cranberry beverage did not ensure any significant effect on the incidence of influenza, while the symptoms were significantly lower. This effect may be associated with polyphenols, especially proanthocyanidins (Nantz *et al*., 2013). Although the above outlined studies suggest that the consumption of cranberry products may be useful for the protection of human health from infections caused by

the viruses listed above, but, currently, there is no sufficient clinical data which could scientifically prove this benefit.

1.2.3. Antioxidant activity

Oxygen is one of the most important elements in the nature and is essential for life, but, in some cases, it may act as a toxic substance. Eukaryotic and prokaryotic cells use this element to produce energy via the electron transport chain in the mitochondria, while, for anaerobic bacteria, it is toxic. This element is essential for oxidation-reduction reactions which are very important for biochemical pathways, biosynthesis, and regulation. These reactions are related with compounds accepting an electron and are called oxidizing agents (prooxidants), while the compounds donating an electron are called reducing agents (antioxidants). During the oxidation process, an oxidizing agent accepts an electron, while another substance becomes oxidized and loses an electron. These mechanisms are very important to understand the oxidation process in biological systems and the effect of antioxidants.

Energy generation, toxification of the body, and physical exercises are normal cellular events which provoke the production of reactive oxygen species (ROS) in the human body. There are many types of free radicals or prooxidants which may be produced via various physiological reactions or as exposure to foreign substances (radiation, drugs, smog, chemicals, etc). Examples of ROS-produced radicals in the body include hydroperoxyl ($HOO^{\bullet}$), alkoxyl ($RO^{\bullet}$), superoxide ($O_2^{\bullet}$), peroxyl ($ROO^{\bullet}$), hydroxyl ($OH^{\bullet}$), and one form of singlet oxygen ($^1O_2$) (Ruel, Pomerleau, Couture, Lamarche and Couillard, 2005). Furthermore, free radicals can originate from nitrogen (including nitric oxide ($NO^{\bullet}$) and the peroxynitrile anion ($ONOO^{\bullet}$)) or hydrogen reactive species. Free radicals or prooxidants can damage lipids, proteins, DNA, or even the cell's defense systems. These changes increase the inflammatory processes resulting in a high risk of cardiovascular diseases, cataract, diabetes, and some types of cancers (Khansari, Shakiba and Mahmoudi, 2009; Mayne, 2003).

The bioactive compounds which may prevent or slow down the oxidation process are called antioxidants. They block and eliminate the free radical-initiated chain reactions and prevent the occurrence of oxidative damage in the organism (Côté *et al*., 2010). In the human body, there is endogenous protection provided by various enzymes, such as catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase. Oxidative stress is caused by the imbalance between the production of free radicals and the insufficient endogenous protection of the human body which leads to oxidation damage. Under these conditions, the production of ROS is too intensive, and the activation of ROS systems is excessive. It is not sufficient to maintain the cellular redox balance just with endogenous antioxidants; therefore, exogenous antioxidants must be obtained by the dietary intake. Previously, berries were attributed to their antioxidant compounds (carotenoids, vitamins C and E, polyphenols) which feature strong antioxidant activity (Huang, Ou and Prior, 2005).

Scientific evidence indicates that the cranberry is a rich source of flavonoids (anthocyanins, flavonols and procyanidins) and phenolic acids which are associated with antioxidant properties. The hydroxyl groups and conjugated aromatic ring structures in every group have a huge impact on their antioxidant properties. The inhibition of prooxidants by flavonoids may proceed via various ways, such as enzyme inhibition, free radical chain breaking, metal chelating, or quenching of the singlet oxygen. When polyphenols break the chain and donate a hydrogen atom to neutralize free radicals, then the antioxidant radical is stabilized by delocalizing the unpaired electron around the phenol ring (Shahidi and Zhong, 2010). Some compounds may be effective in suppressing one type of radical, while not as effective against another type. Cranberry extracts have been reported to inhibit LDL oxidation (Yan, Murphy, Hammond, Vinson and Neto, 2002), oxidative and inflammatory damage to the vascular endothelium (Youdim, McDonald, Kalt and Joseph, 2002), to prevent the formation of peroxidation products (Manach, Williamson, Morand, Scalbert and Rémésy, 2005; Ruel *et al*., 2008). Consumption of cranberry products showed a decline of protein, lipid and DNA oxidation products markers (Basu *et al*., 2011; Valentová *et al*., 2007), while experiments with rats showed that the diet enriched with cranberry powder reduced the lipid and protein oxidation (Kim, Kim and Kwak, 2014).

1.2.4. Anticancer properties

Cancer is one of the most dangerous diseases in the world. It may be caused by the uncontrolled division of cells and their invasion into organs and tissues. All cancers are developed due to genes responsible for normal functions of cells (cell growth regulation and cellular DNA repair) and mutations. Numerous epidemiological studies about anticancer properties of flavonoids *in vitro* and *in vivo* have been conducted. Isolated anthocyanins and extracts rich in anthocyanins possess anti-proliferative properties against several different cancer cells, especially against colon cancer cells, while normal colon cells were not damaged *(Seeram et al*., 2006; Yanjun Zhang, Vareed and Nair, 2005). Other studies revealed preventive action of anthocyanins-rich extracts regarding tumor growth and metastasis and the induction of cancer cell apoptosis (Reddivari, Vanamala, Chintharlapalli, Safe and Miller, 2007). Studies with animals (*in vivo*) also showed inhibition of skin, colon, lung and esophageal cancer growth caused by anthocyanins (Afaq, Saleem, Krueger, Reed and Mukhtar, 2005; Ding *et al*., 2006).

Quercetin aglycone (flavonol) has been extensively studied for anticancer properties, and many studies reported its anti-proliferation properties *in vitro*. It inhibits various cancer cells, such as leukemia (inhibits protein kinase C and/or tyrosine protein kinase activity on leukemia cells), colon (inhibits cyclin D1 and survivin expression in colon cancer cells), lung (inhibits the activation of MEK/ERK signaling pathway of lung cancer cells), breast (inhibits the induction of cell cycle arrest at G2/M transition and apoptosis in breast cancer cells), ovarian and prostate (inhibits the androgen receptor in prostate cancer cells) cancers (Nguyen *et al*., 2004; Xing, Chen, Mitchell and Young, 2001). Less studied flavonols myricetin and kaempherol also demonstrate anticancer properties. It was reported that kaempherol

inhibits the growth of pancreatic, lung, ovarian and colon cancer cells, while myricetin suppresses skin, bladder and pancreatic cancer cells (Luo, Rankin, Li, DePriest and Chen, 2011; Phillips *et al*., 2011; Sun *et al*., 2012). Anticancer properties of flavonols are less studied, possibly due to their lower absorption in the human body.

Flavan-3-ols are important dietary flavonoids. Proanthocyanidins (PACs) with the B-type linkage were widely studied in the course of *in vitro* and *in vivo* researches, and many reports evidenced their anticancer properties. The mechanism how they inhibit the growth of cancer cells is different for every type of cancer. The PACs anti-proliferation activity was also determined against breast, lung and colon cancer cells. *In vivo* studies with mice and rats showed that dietary feeding enriched with PACs inhibited or reduced carcinogenesis of skin, mammary, colon or breast cancers (Davis and Hord, 2005; Kim *et al*., 2004). Cranberries contain a higher level of PACs with the A-type than B-type linkage which also exhibits anticancer activity. The anticancer properties of PACs with the A-type linkage were strongly dependent on the degree of polymerization (DP)). Neto *et al*. (2006) found that A-type PACs with DP ranging from 4 to 7 inhibited the growth of colon cancer, leukemia cells and lung cancer, while, in another study, the same type PACs with a higher range of DP (2–8) selectively inhibited proliferation of neuroblastoma, prostate and ovarian cancer cells comparing with the normal cells.

1.3. The importance of waste utilization and application of extractions

Recently, bioactive compounds present in various plants, fruits and berries started attracting a lot of interest, while they may be used as an excellent source to produce functional foods, nutraceuticals, food additives, and various flavor compounds. Mostly, bioactive components are obtained from by-products which are generated during all the stages of food processing (farm production, postharvest operations and distribution). There are four main sources of food waste production: household activities (42% of all food waste), food processing industry (39%), food service sphere (14%), and distribution of food (5%). According to the statistics amassed during the years, it was expected that, in 2020, the amount of food waste is bound to increase to 126 million metric tons if any of the prevention procedures are not enforced (Mirabella, Castellani and Sala, 2014). One of the ways to reduce the amount of food waste is to apply extraction of bioactive compounds including polysaccharides, phytochemicals, proteins, fibers and flavoring compounds which may be used as ingredients for functional foods.

Among numerous materials of plant origin, berries are known as the richest sources of polyphenolic antioxidants which may also possess antimicrobial activity. So far as fresh berries are highly perishable fruits, the major parts of their harvests are processed into various products. Processing generates by-products which are often discarded as waste causing the loss of valuable nutrients and environmental pollution problems (FAO, 2011). For instance, pressing berry juice results in high amounts of pomace which, on average, contains approx. 30% of dry matter present in the whole berry. Moreover, a large fraction of bioactive compounds, such as

polyphenolic antioxidants and antimicrobials, remains in the pomace after pressing (Struck, Plaza, Turner and Rohm, 2016). However, berry pomaces, as by-products, have limited applications in animal feed due to the low content of nutritive proteins and carbohydrates, whereas, when disposed as waste in the soil or landfills, it presents serious environmental problems due to the low pH, a high amount of moisture and high water activity (Vattem and Shetty, 2002). Application of by-products generated during agrorefining of berries is a serious concern for the global industry.

Berry pomace constitutes approx. 20% of the fresh weight and is a highly heterogeneous material containing peels, seeds, some flesh and stems of the fruit which differ in terms of physical properties and chemical composition. It is well known that pomace is a promising source of bioactive compounds which may be used due to the favorable technological and nutritional properties (Aaby, Skrede and Wrolstad, 2005; Sójka and Król, 2009). Therefore, its processing requires selection of the most effective methods and optimization of their parameters. For this purpose, various extraction and fractionation techniques have been applied, including conventional solid-liquid, microwave, ultrasound and enzyme assisted extractions under atmospheric pressure as well as supercritical fluid and pressurized liquid extractions (Brglez Mojzer, Knez Hrnčič, Škerget, Knez and Bren, 2016). Hydrophilic polyphenols are usually separated by using water, polar organic solvents, or their mixtures. In addition, these compounds are more stable at a low pH, which helps them remain neutral and facilitates their extraction into organic solvents (Khoddami, Wilkes and Roberts, 2013).

There are a lot of high pressure extraction techniques, but the most popular ones are supercritical fluid extraction (SFE) with carbon dioxide and pressurized liquid extraction (PLE) which may easily be adapted for a variety of solvents. The advantages of these extraction methods are the shorter extraction time, lower solvent consumption, and higher extraction yields (Corrales, Toepfl, Butz, Knorr and Tauscher, 2008; Jun, 2009), but the main difference between SFE and PLE is the solvent state during extraction.

1.3.1. Supercritical fluid extraction with carbon dioxide (SFE-CO_2)

Supercritical fluid extraction (SFE) is based on the specific state which is achieved when the temperature and pressure of the substance are above the critical point, and a supercritical fluid is obtained (**Fig. 1.9**) which has specific characteristics of gases and liquids (solvation power similar to liquids, yet low viscosity and high diffusivity similar to gases). In the phase diagram of carbon dioxide (CO_2), it is possible to distinguish three states (solid, liquid and gas). These states are separated by the fusion curve (solid-liquid equilibrium), the sublimation curve (solid-vapor equilibrium) and the vaporization curve (liquid-vapor equilibrium). The triple point and the critical point also are extremely important. The triple point shows the conditions when all the three states coexist, while the critical point is at the end of the vaporization curve where liquids and gases form a homogenous fluid phase, and, above this point, the supercritical fluid is obtained (Raventós, Duarte and Alarcón, 2002).

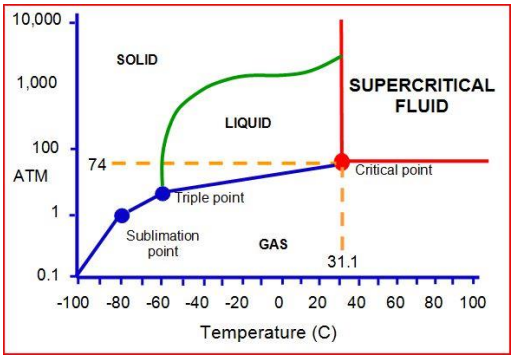


Figure 1.9. Phase diagram of carbon dioxide as a function of temperature and pressure

Comparing with the liquid solvent, supercritical fluid solvents have two main advantages. First, the dissolving power of the supercritical fluid depends on its density which might be based on small changes in pressure and/or in temperature. Secondly, the supercritical fluid has a lower viscosity and surface tension, yet a higher diffusion coefficient, which improves the mass transfer.

A supercritical extraction system is shown in **Figure 1.10**. It should involve a tank of the mobile phase (CO_2), a pump for CO_2 to pressurize gases, an extraction vessel with a heating jacket, a controller to maintain the pressure in the system, and a collecting vessel. First of all, liquid CO_2 is pumped to the heating zone, where it is heated to the supercritical fluid, then it passes into the extraction cell which contains a heating jacket with the temperature control and the inlet/outlet pressure valves to maintain the desired extraction conditions. The supercritical fluid in the extraction cell rapidly diffuses into the sample matrix and dissolves the material which is to be extracted. The fluid and dissolved compounds are transported from the extraction cell into the separator where the power of the fluid is decreasing by a decreased pressure or an increased temperature of the fluid. Finally, the extract can be collected by the valve located in the lower part of the separator, while CO_2 gases can be cooled, recompressed and recycled or discharged to the atmosphere.

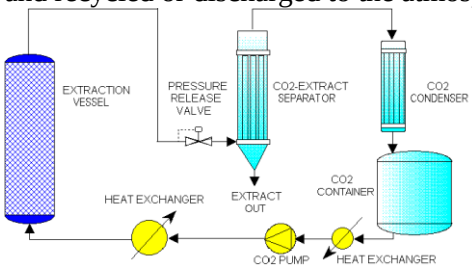


Figure 1.10. Symmetric diagram of SFE apparatus (Laitinen, 1999)

During SFE extraction, it is very important to pay attention to a few factors including a solvent, plant material preparation involving the appropriate particle size, co-solvents (modifiers), extraction conditions (temperature, pressure and extraction time) which may influence the extraction, the flow rate of CO_2, and the solvent-to-feed-ratio (Reverchon and De Marco, 2006).

The selection of the proper solvent is very important. Nowadays, one of the most commonly used solvents for supercritical fluid extraction is carbon dioxide. The main reasons why it is used so extensively are the conditions for CO_2 to reach its critical state (31.06°C, 7.3 MPa, 0.460 g/cm^3). Also, it is non-polluting, non-flammable, non-toxic, odorless, tasteless, inexpensive, inert and completely recoverable (Hurren, 1999). The critical conditions for CO_2 are safe and easy to achieve, so it is appropriate for the extraction of volatile and thermolabile components. Supercritical carbon dioxide is a very good solvent for the extraction of a lower molecular weight (non-polar: essential oils, fats, tocopherols, carotenoids, etc.) compounds, but higher molecular weight bioactive components, such as phenolics, alkaloids and glycosidic compounds are almost insoluble in it. In order to increase the solubility of the polar compounds in supercritical CO_2, polar co-solvents (modifiers) are successfully applied. The modifier has an intermediate volatility compared to the supercritical fluid and compound which should be extracted. During extraction, the modifier is mixed with a supercritical fluid and constitutes a small part of the total fluid composition. Usually, modifiers are liquids including methanol, ethanol, acetonitrile, acetone, water, ethyl ether, and dichloromethane, but, sometimes, modifiers can also be gases. The most commonly used solvent in the food industry is ethanol (up to 10% of the CO_2 flow rate) due to its low toxicity (Ghafoor, Park and Choi, 2010).

Plant material preparation and its particle size are also very important for effective SFE-CO_2 extraction. A fresh material contains a huge amount of water which is not soluble (as low as 0.3%) in supercritical CO_2 and can lead to various mechanical difficulties including clogging due to ice formation and decrease the efficiency of SFE. Therefore, for extraction, it is better to use a dry material or add some absorbents which retain the moisture of a fresh sample. The particle size of extractable substances may influence the extraction process. It is well known that the extraction of the raw material of bigger particles may result in a longer extraction process, while the extraction of smaller particles lasts shorter; yet, on the other hand, it becomes more difficult to maintain the constant flow rate.

The extraction efficiency mainly depends on the solubility of compounds in a supercritical fluid which may be affected by the temperature and density (pressure) of the fluid. It is very important to choose the proper density because it influences the selectivity as well as the solvent power; it is the factor which determines the composition of the extract. The fractionation of extracts is achieved by controlling the density and temperature of the fluid. Another important parameter influencing the extract composition is time. Less polar and lower molecular weight components are extracted faster, when the extraction is controlled by the internal diffusion (Cherchi *et al*., 2001). Changes of the flow rate during extraction did not influence the composition of the extract, although they did increase the yield.

The fractionation of various plant materials can be applied for the pure supercritical fluid or its mixtures with higher polarity co-solvents while focusing on the recovery of lipophilic substances and polyphenolic antioxidants. Fractionation can be done via two strategies: the simplest cases involve optimized SFE parameters for different classes of compounds by using separate extractions for each of them or

install separators after the main extractor which operate at different parameter settings (temperature and pressure) for consecutive precipitation of the target substances. Numerous studies demonstrated that SFE provides various options, mainly based on the thermodynamic solubility of various compounds, for the preliminary separation of crude extracts into the fractions with the increased concentration of selected compound classes.

There are some advantages of supercritical fluid extraction. This process can selectively extract and fractionate various components; when the extraction temperature and pressure are changed, the solubility of the fluid changes as well. The supercritical fluid has the density of a liquid, so it can solubilize a solid material like a liquid solvent; solubility increases with a higher pressure when the density of the fluid increases. Furthermore, it is easy to remove the supercritical solvent from the extract without any loses of volatile compounds due to its high volatility. The diffusivity of the supercritical fluid is up to two times higher than other liquids, and, due to its mass transfer, it leads to faster extraction and a higher yield comparing with the traditional extraction methods (Roy, Goto, Kodama and Hirose, 1996). The lower temperature of supercritical CO_2 allows extracting thermolabile compounds. During this extraction, a small amount of a sample can be used compared to the conventional extraction methods and to save some time for the implementation of other experiments. Complete extraction may be achieved by the repeated reflux of a supercritical fluid to the matrix. As well as every other method, SFE also suffers from some disadvantages: extraction must be operated at a high pressure to maintain the supercritical state, CO_2 is not a good solvent for polar compounds, it is immiscible in water (which limits the application for some materials), and it requires substantial investment into the extraction equipment along with its operating costs.

1.3.2. Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE), which is also known as accelerated solvent extraction (ASE), is based on the possibility of keeping low boiling point solvents in the liquid state at a high pressure and increased diffusivity of the analytes from the solid matrix (Kaufmann and Christen, 2002). The solvent is below its critical conditions all the time. The efficiency of the extraction depends on a few factors: the nature of the sample matrix, the analyte which should be extracted, and its location in the matrix. A wide range of temperatures (30–200 °C) and pressures (3.5–20 MPa) can be applied in PLE. PLE is performed by filling a vessel (cell) with a solvent and then heated to the desired temperature; the cell is pressurized to maintain the solvent as a liquid. The elevated temperature improves the diffusivity of the solvent, which results in increased extraction kinetics, while the high pressure keeps the solvent in the liquid state, allows filling the extraction cell faster, and helps the liquid enter the sample matrix. These conditions lead to fast and safe extraction. In most equipment, when the set parameters (temperature and pressure) have been reached, extraction is performed from 10 to 30 min during the different cycles, and, at the end (in order to avoid losses of the extract), the cell with the sample is purged with inert gas (nitrogen) into the collection vial. A typical

schematic diagram of a pressurized liquid extraction system is given in **Figure 1.11**. The increased interest in this extraction method is mainly due to the fact that PLE is automated with a reduced extraction time and solvent consumption, and its setup suits analytes that are oxygen- and light-sensitive.

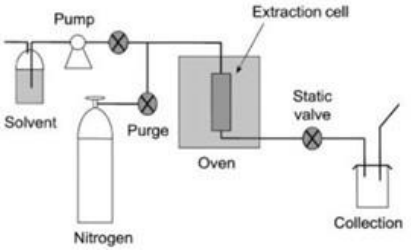


Figure 1.11. Scheme of a pressure liquid extraction (PLE) apparatus (Maarasyid, Muhamad, & Supriyanto, 2014)

There are two types of pressurized liquid extractions: the static mode and the dynamic mode. During the dynamic extraction, the solvent is continuously pumped through the vessel with the sample, and a pump delivers the solvent at a constant flow rate according to the set static time. While the static extraction process consists of one or more extraction cycles when the solvent is replaced between cycles, and the main factors which influence the efficiency of this extraction are temperature and time. The efficiency of extraction mostly depends on the solubility of the target compounds in the used solvent, but the main disadvantage is that complete extraction might not be achieved due to the insufficient amount of the solvent used for it (Ong, Cheong and Goh, 2006; Teo Chye, Tan Ngin, Hong Yong, Hew Sin and Ong Shi, 2010). In order to avoid this disadvantage, the strategy of using more cycles should be applied. The extraction process might be explained in two stages, when the first stage starts as solubility-controlled, and the second stage is a diffusion-controlled phase (Turner, King and Mathiasson, 2001). In this case, when there are strong interactions between the matrix and the analytes, or a long way to pass for the analyte from the sample matrix, the temperature of the solvent or the particle size of the sample are involved, these factors play an important role in the efficiency of extraction. A higher temperature or a smaller particle size reduces the extraction time. In the course of the extraction in the solubility-controlled phase, the interaction of the analyte and the sample matrix is weak, and the rate mostly depends on the distribution of the analyte between the matrix and the extraction solvent. In this case, the yield increases more if the solvent is replaced a higher number of times. PLE could be used for polar and non-polar solutes. The main factors affecting the extraction are the solvent, the extraction time, the temperature, the particle size, and the moisture content in the sample.

In polar solvents, polar analytes dissolve, while, in non-polar solvents, nonpolar analytes dissolve. The choice of the solvent depends on the solubility characteristics and the diffusivity of the desired analyte and the characteristics of the sample which is used for extraction (Pronyk and Mazza, 2009). When the desired analyte has a high solubility in the solvent but other compounds are not soluble or are minimally soluble in the employed solvent, then, the extract is obtained with a

higher purity and selectivity. The solvent should be chosen according to the safety, economy and sustainability aspects, and priority should be given to the less toxic and non-harmful solvents. A new concept as 'green' solvents has emerged, and its aim is to reduce the environmental impact by avoiding the use of harmful solvents for extractions. The solvents which are defined as safe or 'green' are water, simple alcohols including ethanol and methanol, or alkanes, such as heptane or hexane. These solvents are more environmentally friendly comparing to dioxane, formaldehyde, tetrahydrofuran, acetonitrile, or acids. It was observed that solvent mixtures (methanol–water or ethanol–water) are more environmentally friendly than pure alcohols, which thus enhances the extraction yield. The mixtures improve the solubility and interaction of the desired analyte with the used solvent (Arapitsas, Sjöberg and Turner, 2008; Mukhopadhyay and Panja, 2008) and may also increase the efficiency of extraction because one solvent could increase the solubility of the analyte in it, while another solvent improves the desorption of the analyte.

Temperature is one of the most important factors for PLE. A higher extraction temperature may increase the efficiency of extraction because it disrupts the interaction between the analyte and the sample, which is caused by van der Waals forces, hydrogen bonding and dipole attraction (Richter, Jones, Ezzell and Porter, 1996). The thermal energy helps to disrupt cohesive and adhesive interaction between the analyte and the sample matrix, which results in the decrease of the activation energy which is necessary for the desorption process. A higher temperature used for extraction decreases the surface tension of the solvent, the matrix and the solute, and it also improves the solvent access to the sample. The decreased solvent tension permits the analytes to dissolve faster in the solvent. The elevated temperature reduces the viscosity of the solvent, which affects its better penetration into the matrix particles and improves the extraction process and also enhances the diffusion rate. This results in better mass transfer of the molecules in the solvent, and ultimately leads to faster extraction. On the other hand, higher temperatures may lead to a higher amount of co-extracted analytes and decrease the selectivity of extraction. Thermolabile compounds might also be affected by the temperature, which determines their disintegration and hydrolytic degradation (Fernández-González, Concha-Graña, Muniategui-Lorenzo, López-Mahía and Prada-Rodríguez, 2008; Moreno, Reza and Trejo, 2007). The PLE is based on higher diffusion fluids, which improves the extraction rate but decreases the amount of the solvent that is necessary for extraction (Björklund, Nilsson and Bøwadt, 2000).

The usage of **pressure** during PLE allows using the extraction temperature above the solvent's boiling point while the solvent is still maintained in the liquid state. The use of a higher pressure at an elevated temperature and the reduced tension of the solvent surface helps the solvent enter the matrix faster and extract the analyte easier. During the extraction, the pressure inside the cell disrupts the matrix, removes air bubbles inside the sample (air bubbles disturb the ability of the solvent to reach analytes), and this results in more efficient mass transfer of the analyte from the sample to the solvent (Rostagno, Villares, Guillamón, García-Lafuente and Martínez, 2009).

The **extraction time** is very important for the extraction efficiency because longer contact periods between the solvent and the matrix permit enhanced wetting of the sample and improve the penetration of the solvent into the pores of the matrix (sample) with better solvation of its compounds. Due to this particular reason, the solvent can break the specific compound-matrix interactions and extract them. It has been proven that in order to extract the majority of the targeted compounds at a high yield, it is enough for extraction to last from 5 to 30 min. Also, it is worth mentioning that, sometimes, a higher temperature and a long extraction time may result in the degradation of some components or even the entire matrix (Runnqvist *et al*., 2010).

According to Cheah, Heng and Chan (2010), the **particle size** has a significant influence on the PLE process because smaller particles generate a higher percentage of the solid matrix, which leads to a better recovery of bioactive components. Smaller particles not just increase the surface area of the raw material, but also, during the milling process (the sample preparation), the cell walls are disrupted, and this releases a high amount of bioactive compounds (Cheah *et al*., 2010; Del Valle and Uquiche, 2002).

The most appropriate conditions should be selected for each raw material while taking into account the choice of the appropriate solvent for the compounds to be isolated. The extraction static time, the number of cycles of passing the solvent through the matrix, the temperature and the amount of the solvent are usually optimized for applying PLE. This method (PLE) has been successfully used for the extraction of thermally sensitive phytochemicals from various plant sources.

1.4. Process optimization by response surface methodology (RSM)

Optimization helps to improve the process, product or system in order to obtain higher benefits in terms of the outcome. Traditionally, optimization was carried out by monitoring the impact of one variable (factor) on the experimental response, while other parameters were being kept constant. This optimization method is called one-variable-at-a-time. It means that the usage of this technique did not include the interactive effect of the studied variables, and therefore it cannot show the complete effect of all the variables on the response. Also, when applying the one variable optimization method, the number of experiments increases during the research, which results in the long duration of the experiment, increased consumption of reagents, solvents or the processed material (Bezerra, Santelli, Oliveira, Villar and Escaleira, 2008).

In order to avoid this problem, multivariate statistic techniques were started to use for the optimization process. The most popular is the response surface methodology (RSM) which is a collection of mathematical and statistical techniques. This methodology is based on the fit of a polynomial equation of experimental data, which should show the behavior of the data set. This technique may be applied when there is a need to know the impact of several variables on one or a few responses. In order to achieve the best system performance, the levels of the variables should be optimized simultaneously. First of all, before applying RSM, it is necessary to decide which experimental design should be used. There are a few

experimental matrices, and the simplest is the first-order model when a data set is linear (Hanrahan and Lu, 2006). A huge part of the experimental data cannot be described with a linear function; for this purpose, more complicated experimental designs for the quadratic response surface should be used (Box-Behnken, central composite, Doehlert designs, and three-level factorial).

Some stages in the application of RSM as an optimization technique are very important. First of all, it is necessary to choose such independent variables which have a major effect on the system according to the objective of the study and the skills of the researcher. Screening of some studies allows choosing whichever parameters and their interactions have the greatest impact. As the next step, it is still very important to choose the right experiment design and carry out the experiments according to the selected experimental matrix. A linear function is the simplest model of RSM, and it can be applied if the responses are well-fitted with the following equation:

$$y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \varepsilon \quad (1)$$

where β_0 is the constant term; k is the number of independent variables; β_i represents the coefficients of the linear parameters; x_i represents the independent variables; ε is the experiment residue.

Therefore, when using this kind of model, the responses should not have any curvature. In order to evaluate the curvature and the more complicated interactions of the independent variables, a second-order model might be used. This model can be used when it has been identified that second-order effects are significant for the experiment which was carried out. Also, the polynomial model should feature the appropriate terms which describe the interactions between the independent variables (see in the following equation) during the experiment (Bezerra *et al*., 2008).

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (2)$$

where β_{ij} represents the coefficients of the interaction parameters.

In order to determine a critical point (the minimum, the maximum, or the saddle) for various extraction methods, it is important to contain quadratic terms in the polynomial function according to the following equation:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ij} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (3)$$

where β_{ij} represents the coefficients of the quadratic parameter.

In order to estimate and calculate the parameters in the third equation, all the studied independent variables in the experimental design are carried out in at least three factorial levels. In this case, the symmetrical response surface design is available. More known second-order symmetrical designs including the three-level factorial design, Box–Behnken design, central composite design and Doehlert design, differ from each other by their selection of the experimental points, the number of runs, the blocks and the levels for variables (Bezerra *et al*., 2008).

For the first time, the central composite design (CCD) was presented in the 1950s by Box and Wilson (Box and Wilson, 1951). This design involves the fractional factorial or the full factorial design, an additional design, which is similar to the star (the experimental points are at a specific distance (α) from its center) and

the central point. In **Figure 1.12**, there is an illustration of the central composite design of two and three independent variables for their optimization.

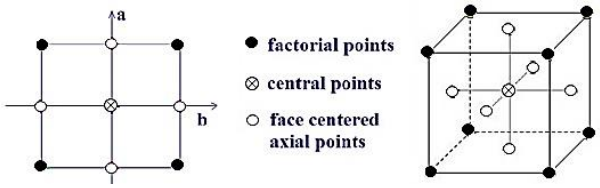


Figure 1.12. Face Centered Central Composite Design

By using the central composite design, each independent variable is analyzed at five levels ($-\alpha$, -1, 0, +1, $+\alpha$), and this model requires an experiment number which could be calculated according to the following equation:

$$N= k^2 +2k+c_p \quad (4)$$

where k is the number of the study variables, and c_p is the repetitions in the central point.

The number of independent variables has influence on the specific distance (α) of the experimental points from the central point. For example, for two, three and four variables, the distance is 1.41, 1.68, and 2.00, respectively. This distance might be calculated according to the following formula:

$$\alpha=2^{(k-p)/4} \quad (5)$$

There are three main varieties of the central composite design: face-centered, rotatable, and inscribed. **Figure 1.12** presents the face-centered CCD for two and three independent variables. The design of two independent variables consists of the center point, four factorial points (the intersection points of the coded variable (±1) bounds), and four axial points (the points whose distance (α) from the center point is equal to 1.0). The center points are the repetitions in the center of the cube/square which are important if we need to estimate the variability of the experimental measurements. The center points may vary from three to six. α determines the geometry of the design region, for instance, when α is 1.0 for two variables, it defines a square, for three variables, it defines a cube, for four variables, it defines a hypercube, etc. When the value of α increases, the axial points are beyond the faces of the square, and the design becomes more spherical.

More often, rotatable CCD for modeling a second-order response surface is used. The design received that name (rotatable) because the variance of the predicted response depends on the distance between the center point and the other points of the design, but not on the direction. The rotatable design provides the same magnitude of the prediction error because all points are at the same distance from the center point (Sahoo, Barman and Davim, 2011). Relative to the α (distance) value of 1.4 in the rotatable design, it may be extended beyond the defined variable bounds. Thus, the predicted responses at or near the axial points are now in the design region. The magnitude of the prediction error increases geometrically with the distance outside the design region. Extending the axial points beyond the defined variable bounds leads to the operation of the process at five level settings of each variable.

The inscribed CCD also uses an α value of 1.4 thus obtaining the rotatable central composite design. The inscribing design restricts the actual design for the independent variable ranges, because, in it, the location of the axial points is in the lower and upper bounds of the variable ranges. The factorial points are inside the interior design space (inscribed) by the distance from the center point, which allows keeping the proportional distance of the factorial points to the axial points.

A number of publications about the application of CCD for various optimization processes have been presented. It can be a highly useful tool to optimize the extraction conditions to extract a higher amount of various bioactive compounds which possess antimicrobial, antiviral, anticancer, anti-inflammatory, antibacterial, and antioxidant properties.

1.5. Application of berry extracts to improve quality and safety of meat products

Meat is an important source of high nutritional value proteins and various essential micronutrients for the human diet, such as vitamins, bioavailable iron and others (Murphy, Spungen, Bi and Barraj, 2011). However, recently, *The International Agency for Research on Cancer* (IARC) recognized processed meat as causing (group 1) and red meat as possibly causing (group 2A) cancer. In addition, meat and its products, particularly ones produced from minced meat, are highly susceptible to microbial spoilage, which reduces the shelf life and increases the risk of food-borne diseases. For instance, raw meat burgers are potentially hazardous products with a very short shelf life. On the other hand, health claim is granted for meat by an EU regulation for its contribution to the improvement of iron absorption (Commission Regulation (EU) No. 432/2012). Consequently, these contradictory health effects cause serious problems for meat producers and raise a dilemma for consumers regarding their choices regarding the consumption of meat products.

During the production and storage of meat products, various oxidative processes take place, and harmful compounds are formed which have a negative effect on the human body. The gastrointestinal tract is exposed to oxidized compounds originating from various meat products and obtained in the process of digestion. A part of these substances is directly absorbed into the bloodstream (Kanner, 2007). Toxic compounds can be generated during the digestion reactions of hydroperoxides in the stomach. The oxidation processes in meat and meat products are caused by the presence of oxygen, metal ions, moisture, heat and light (Bhattacharya, Kandeepan and Vishnuraj, 2016). Oxidation targets are (mainly) amino acids, especially cysteine, tryptophan and tyrosine. Protein carbonyls and oxidized protein products are associated with various diseases, such as cancer, Alzheimer's disease, chronic renal failure, diabetes (Soladoye, Juárez, Aalhus, Shand and Estévez, 2015) and other types of morbidities and mutagenesis (Papuc, Goran, Predescu and Nicorescu, 2017). Recently, a lot of studies have been performed to determine the role of aldehydes generated during lipid peroxidation on the carcinogenesis and other chronic disease. Several researches reported that the consumption of fatty meals containing oxidation products exerts impact on the

vascular function and increases the amount of pro-inflammatory cytokines (Kanner, 2007). For this reason, it is very important to determine the freshness of meat products and to find ways how to protect meat products from oxidation and microbial spoilage.

Various chemical markers may provide important information about the meat freshness/spoilage, e.g., ATP breakdown products, the total volatile basic nitrogen, free fatty acids, peroxide, and TBARS values (markers of lipid oxidation), some biogenic amines (putrescine, cadaverine, histamine and tyramine), volatile sulphur compounds as well as some others. Meat spoilage is also characterized by the growth of natural and pathogenic microorganisms resulting in the formation of unwanted substances (off-odors, gas, slime, toxins) which can cause food-borne diseases, while the oxidation of lipids and pigments generates undesirable flavors and causes discoloration (Sun and Holley, 2012). Various preservation methods have been used to prolong the shelf life of raw meat including modified atmosphere and vacuum packaging, steam and high hydrostatic pressure pasteurization, ionizing radiation, etc. (Aymerich, Picouet, and Monfort, 2008). Meat spoilage may also be controlled by synthetic preservatives and antioxidants; however, consumer's preferences nowadays are closely linked to food 'naturalness', which increases the interest and demand for alternative food additives of the natural origin. Therefore, testing of antioxidants and antimicrobials from spices, fruits, vegetables, oilseeds and medicinal plants in meat and meat products has been steadily increasing during the last few decades, and the results of these studies have been regularly reviewed (Ahmad, Gokulakrishnan, Giriprasad and Yatoo, 2015; Falowo, Fayemi and Muchenje, 2014; Lorenzo *et al*., 2018).

However, studies on the application of berry pomace in meat and other products are rather scarce, except for grape pomace which demonstrated strong antioxidant activity in the tested foods (Ahmad *et al*., 2015; García-Lomillo and González-San José, 2017; Lorenzo *et al*., 2018). Polar extracts of raspberry pomace were also added to meat hamburgers and remarkably decreased their oxidation rate (Kryževičiūtė, Jaime, Diez, Rovira and Venskutonis, 2017). Bearberry extracts significantly reduced the lipid oxidation and had a significant effect in both raw and cooked pork meat during chilled storage (Carpenter, O'Grady, O'Callaghan, O'Brien and Kerry, 2007). The addition of blueberry extract to pork meat resulted in higher product stability during the storage time (Muzolf-Panek, Waśkiewicz, Kowalski and Konieczny, 2016), while pork burger patties enriched with blackberry extracts were tested for oxidation of the muscle proteins and lipids over 12 days (at a temperature of 2 °C) after cooking and showed a significant effect of inhibiting the formation of carbonyls, reducing the TBARs values and the hexanal content (Ganhão, Estévez, Armenteros and Morcuende, 2013; Ganhão, Morcuende and Estévez, 2010). Some studies claim that blackcurrant, cloudberry and strawberry extracts have a significant effect on the reduction of lipid and protein oxidation, respectively, in raw pork patties, cooked pork patties and Frankfurter sausages during chilled storage (Armenteros, Morcuende, Ventanas and Estévez, 2013; Jia, Kong, Liu, Diao and Xia, 2012; Rey, Hopia, Kivikari and Kahkonen, 2005). The obtained results showed that all the extracts which were used for the preparation of

various meat products are promising antioxidants which could enhance the nutritional, safety and sensory properties of these products.

The use of natural antimicrobials, such as berry extracts, could be a good strategy to inhibit microbial spoilage of meat products (Negi, 2012). There are several main mechanisms for berry pomace extracts to demonstrate their antimicrobial effects. The presence of phenolic compounds in the extracts which are used for meat product preparation has an impact on the enzyme activity and/or causes protein denaturation; it causes changes in the permeability of microbial cells and in the functions of the normal activity of cell membranes (electron transfer, protein synthesis, nutrient exchange, nucleic acids and enzymatic activity) (Aminzare, Hashemi, Hassanzad Azar and Hejazi, 2016). Cranberries are an excellent source of flavonols, hydroxycinnamic and other organic acids, vitamins and some other bioactive compounds (Côté, Caillet, Dussault, Sylvain and Lacroix, 2011; Häkkinen *et al*., 1999). Cranberry phytochemicals are well-known for their potential in protecting against several bacterial diseases (Pappas and Schaich, 2009). For instance, it was reported that cranberry preparations reduced the growth of such pathogenic bacteria as *E. coli O157:H7*, *L. monocytogenes* and *S. typhimurium* (Côté *et al*., 2011; Wu *et al*., 2008). Wu, Qiu, de los Reyes, Lin and Pan (2009) applied cranberry concentrate for controlling *E. coli O157:H7* counts in ground beef and related its antimicrobial mechanism to the downregulated carbon starvation (slp) genes, hypothetical protein hdeA and cyclopropane fatty acyl phospholipid synthase. Diarra *et al*. (2020) analyzed the antimicrobial activity of the cranberry pomace extract in cooked chicken breast with different concentrations of cranberry extract (0.4% and 0.8%) additives. The results of the study showed that the cranberry extract exhibited promising *in vitro* antimicrobial activity against *L. monocytogenes* species, while the obtained data indicated that the *L. monocytogenes* strain used in the chicken breast experiments survived in this protein-rich medium. The data obtained during this experiment clearly highlighted the profound influence of the food matrices on the antimicrobial activity, yet this idea still requires further research. However, the studies of the effects of the cranberry pomace fractions on the growth of bacteria and antioxidant effects in foods are rather scarce (Gniewosz and Stobnicka, 2018).

In addition, it may be expected that phytochemicals added with berry pomace extracts to meat products would exert some health benefits, such as antioxidant, anti-inflammatory, detoxifying, and, finally, antimutagenic and anticarcinogenic effects, e.g., by inhibiting the formation of carcinogenic and other hazardous compounds (Xiong, 2017). Consequently, purposive utilization of berry pomace fractions in meat products may provide several benefits, by improving product safety/healthiness and by increasing the effectiveness of by-product and/or waste utilization (Abd El-Khalek and Zahran, 2013).

1.6. Summary of the scientific and technical information and substantiation of research trends

Numerous studies related with the recovery of target compounds or their groups from berry pomace by the application of different extraction techniques have already been published. Any extraction strategy applied to berry pomace generates the main product and residues. The residues still remain neglected, although, they still contain a lot of bioactive compounds. For this reason, preferably by using the biorefining concept, we may valorize berry pomace. Most recently, the biorefining concept was applied for converting blackberry (Kitrytė, Narkevičiūtė *et al*., 2020), chokeberry (Brazdauskas, Montero, Venskutonis, Ibañez, & Herrero, 2016; Grunovaitė, Pukalskienė, Pukalskas, & Venskutonis, 2016; Kitrytė, Kraujalienė, Šulniūtė, Pukalskas, & Venskutonis, 2017), bilberry (Ravi, Breil, Vian, Chemat, & Venskutonis, 2018), raspberry (Kryževičiute, Kraujalis, & Venskutonis, 2016), sea-buckthorn (Dienaitė *et al*., 2020; Kitrytė, Povilaitis, *et al*., 2017), guelder rose berry (Kraujalis, Kraujalienė, Kazernavičiūtė, & Venskutonis, 2017), lingonberry (Kitrytė, Kavaliauskaitė, *et al*., 2020), rowanberry (Bobinaitė, Grootaert, *et al*., 2020; Bobinaitė, Kraujalis, *et al*., 2020), and elderberry (Kitrytė, Laurinavičienė, Syrpas, Pukalskas, & Venskutonis, 2020) pomaces into higher added-value ingredients by multistep processes, including PLE, SFE-CO_2, enzyme assisted extraction and ultrasound assisted extraction methods. It was demonstrated that a combination of different methods leads to fractions containing high-value substances recovered from berry pomace which possess strong antimicrobial, antioxidant and antiproliferative activities. Kryževičiūtė *et al*. (2017) demonstrated that the addition of raspberry pomace extract inhibited the growth of unwanted bacteria (*Enterobacteriaceae*, *B. thermosphacta*, *Pseudomonas spp.*, and lactic acid bacteria) and lipid oxidation during the prolonged storage of beef burgers.

2. MATERIALS AND METHODS

2.1. Research objects and pretreatment

Frozen cranberries (*Vaccinium macrocarpon*) were donated by the local company *Fudo* (Kaunas, Lithuania) in 2016. The berries were thawed, and the juice was immediately pressed by using a Philips HR1880/01 juicer. The pomace was dried at 35 °C in a hot air dryer until reaching the final moisture content of 5.83%, ground in a centrifugal high speed mill Retsch ZM200 (Haan, Germany) while using a 0.2 mm sieve (with an average particle size of 0.2 mm) and stored in hermetically closed glass jars in a dark, dry and cool room (<18 °C). Fresh pork was obtained from *Campofrio* (Burgos, Spain) and stored in the fridge (<5 °C).

2.2. Reagents

Analytical grade methanol was acquired from *Chempur* (Piekary Śląskie, Poland), agricultural origin ethanol (96.6%) was sourced from *MV Group Production* (Kaunas, Lithuania). CO_2, and nitrogen were obtained from *Gaschema* (Jonava, Lithuania). Sodium sulphate (Na_2SO_4) was purchased from *Eurochemicals*

(Vilnius, Lithuania). HPLC grade solvents for chromatographic analyses as well as Folin-Ciocalteu phenolic reagent, gallic acid (>99%), 6-hydroxy, 2,5,7,8-tetramethylchroman-2- carboxylic acid (Trolox, ≥97%), 2,2'-azinobis-3-ethylbenzotiazoline-6-sulfonate (ABTS), microcrystalline cellulose, fluorescein (FL) sodium salt, 2,2-azobis(2-amidino-propane) dihydrochloride (AAPH), dimethyl sulfoxide, anhydrous sodium carbonate (98%), potassium persulfate, potassium dihydrogen phosphate, aqueous sodium hydrogen phosphate were bought from *Sigma-Aldrich* (Saint Louis, MO, USA). Thiobarbituric acid, (±)-α-tocopherol, rac-β-tocopherol, (+)-γ-tocopherol, δ-tocopherol, β-carotene, a mixture of 37 fatty acid methyl esters (FAME) were obtained from *Sigma-Aldrich* (Steinheim, Germany). Cellulose filter paper and diatomaceous earth (DE) dispersant were sourced from *Dionex* (Ahlstrom, USA). Squalene (99%) was purchased from *Supelco Analytical* (Bellefonte, PA, USA). Streptomycin thallous acetate agar (STAA), STA selective supplement, MRS agar, *Campylobacter* blood-free selective agar base, Tryptone soya agar, *Pseudomonas* agar base and their supplements were purchased from *Oxoid LTD* (Hampshire, England), buffered peptone water (BPW), plate count agar (PCA) were bought from *Conda* (Madrid, Spain), *Listeria* agar base, *Listeria* selective supplements, trichloracetic acid (TCA) were acquired from *Sharlaud* (Barcelona, Spain), and glycerol was obtained from *VWR International S.A.S.* (Fontenaysous-Bois, France).

2.3. Flowchart of cranberry pomace biorefining

The integral flowchart of cranberry pomace biorefining is proposed in **Figure 2.1**. The valorization of berry pomace was done by using green chemistry-based extraction technologies (SFE-CO_2 and PLE). First of all, we performed the optimization process to obtain the highest yield during SFE-CO_2. Later, under optimal conditions, we performed online fractionation by SFE-CO_2 with two separators. Defatted cranberry pomace was sequently extracted by PLE with ethanol and water. The composition of the lipophilic fractions was analyzed by different chromatographic assays. The characterization of phytochemical composition, anticancer, antiviral, antioxidant and antimicrobial properties of the polar fractions obtained via pressurized liquid extraction was analyzed by involving various methods. Also, we determined the acceptability of the ethanol extract additive in pork meat products by investigating its effect on the growth of the selected food pathogenic microorganisms, antioxidant capacity, physico-chemical and sensory characteristics.

2.4. Proximate analysis of cranberry pomace

The chemical composition of ground cranberry pomace was analyzed according to the methods established by the *Association of Official Analytical Chemists* for moisture (925.10), crude protein (960.52), crude fat (920.39) and ash (900.02)) content (AOAC, 1995). Carbohydrates were determined according to the difference method by subtracting the other components from 100. All the analyses were performed in triplicate.

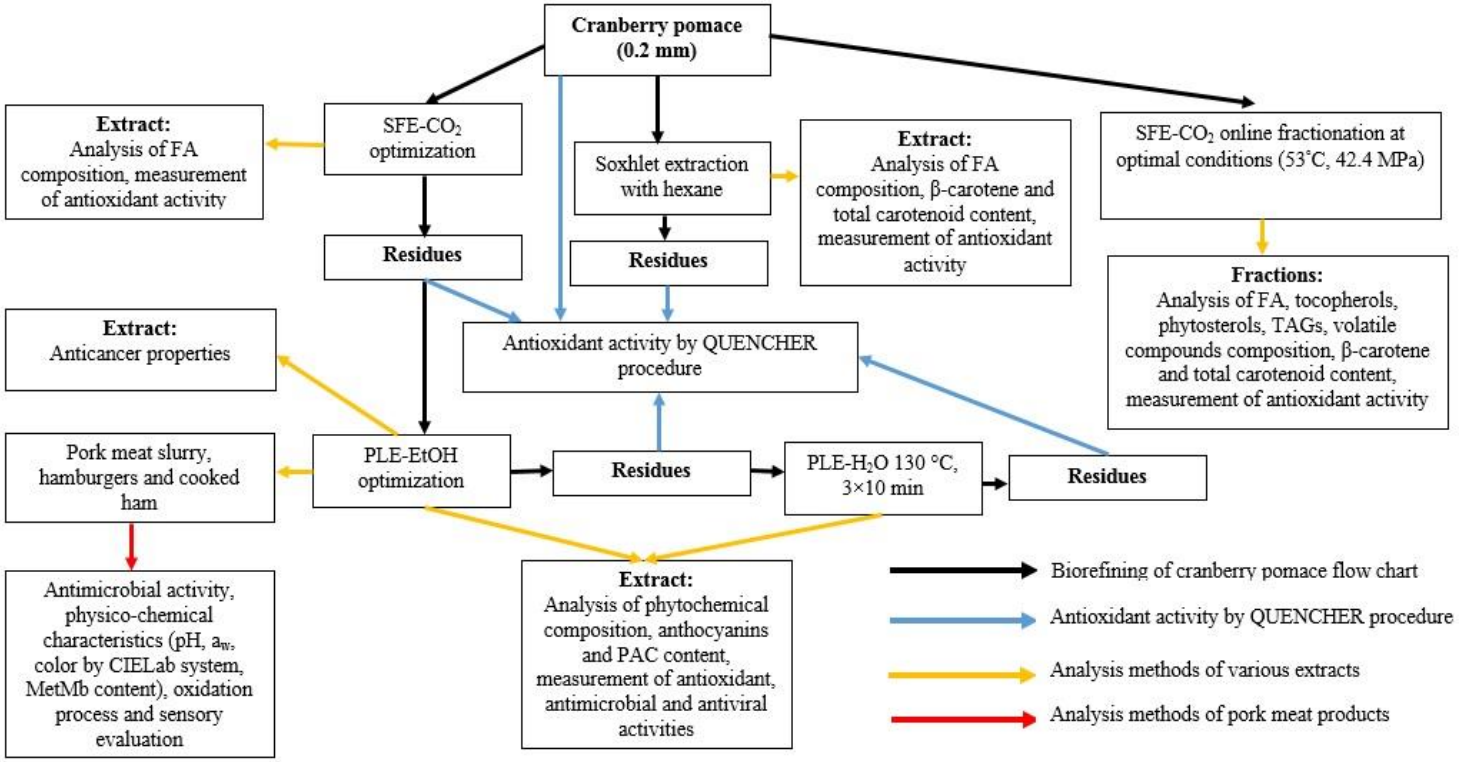


Figure 2.1. Thesis research scheme. FA – fatty acid; TAG – triacylglycerols; PAC – proanthocyanidins; MetMb – metmyoglobin; a_w – water activity; PLE – pressurized liquid extraction; EtOH – ethanol; H_2O – water; SFE-CO_2 – supercritical fluid extraction with carbon dioxide

2.5. Extraction methods

2.5.1. Traditional extraction by Soxhlet

The conventional Soxhlet extraction was used for comparison purposes (920.39) (AOAC, 1995). 20 g of pomace was extracted in a Soxhlet apparatus *EZ100H* (*Behr Labor-Technik*, Düsseldorf, Germany) with n-hexane for 6 h. The solvent was evaporated in a rotary vacuum evaporator at 40 °C, the residue was finally dried at 50 °C.

2.5.2. Supercritical fluid extraction for optimization process

Supercritical fluid extraction (SFE-CO_2) optimization experiment was carried out in a Helix extractor (*Applied Separation*, Allentown, PA, USA) by using 10 g of ground cranberry pomace placed in a 50 mL vessel between two layers of cotton wool at both ends. The temperature was controlled by a surrounding heating jacket, the CO_2 flow rate in the system was controlled manually by using the micro-metering valve. The volume of the consumed CO_2 was measured in standard liters per min (SL/min) under the standard state: P = 100 kPa, T = 20 °C, ρ = 0.0018 g/mL. The extracts were collected in glass bottles and kept at -20 °C until further analysis. A static time of 15 min was included into the total extraction time and was constant.

2.5.3. Extraction and fractionation of cranberry pomace by SFE-CO_2

Each SFE extraction under optimal conditions was performed by using 50 g of ground pomace in a Helix extractor (*Applied Separation*, Allentown, PA, USA) equipped with a 500 cm^3 extraction cell and two separators, S1 and S2 (**Fig. 2.2**). The temperature (53 °C) of the extraction vessel was maintained by a surrounding heating jacket, while the CO_2 flow rate was controlled manually by using a micro-metering valve. The extracts were fractionated by reducing the pressure in S1 to 7 MPa and using different cooling temperatures (0, -10, -20 and -30 °C) which are remarkably lower than the CO_2 critical point. It was expected that heavier substances (further HF) would precipitate due to the decrease of their solubility in liquid CO_2 and will be at least partially separated from the lighter fractions (further LF) which would remain soluble in cooled liquid CO_2 and transferred to S2. Pure CO_2 and its mixture with 5% of co-solvent ethanol (EtOH) were used for extractions. EtOH was introduced into the solvent flow at the level of 0.228 mL/min, while the separation parameters were kept similar. The volume of the consumed CO_2 was measured in standard liters per min (SL/min) at the standard state: P = 100 kPa, T = 20 °C, ρ = 0.0018 g/mL. The extracts and the separated fractions were collected in glass bottles, weighed and kept at -20 °C until further analysis. A static time of 30 min was not included into the total extraction time and was constant.

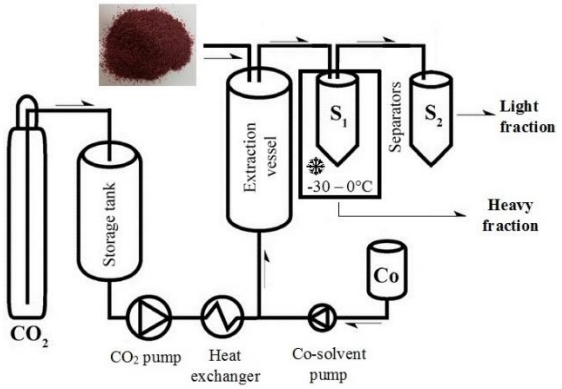


Figure 2.2. Diagram of SFE apparatus for fractionation process

2.5.4. Pressurized liquid extraction

For pressurized liquid extraction (PLE) defatted by SFE-CO_2, pomace powder was mixed with diatomaceous earth in 10 mL extraction cells which were fixed with cellulose filters at the top and at the bottom, and sequentially extracted with ethanol (EtOH) and water in an accelerated solvent extraction apparatus *ASE350* (*Dionex*, Sunnyvale, CA, USA) at constant 10.3 MPa pressure by using three static extraction cycles. EtOH was removed in a rotary evaporator, while H_2O was evaporated by freeze-drying. The extracts were weighed and stored at -20 °C. PLE-EtOH parameters were optimized, while PLE-H_2O conditions (130 °C, 3 cycles, 10 min each) were selected as in the previously reported studies (Basegmez *et al*., 2017; Kraujalienė, Pukalskas, Kraujalis and Venskutonis, 2016).

2.5.5. The experimental design

Face-centered central composite design (CCD) based on the response surface methodology (RSM) was applied to evaluate the effect of three and two independent variables in SFE-CO_2 and in PLE, respectively, by using the extract yield as the main response thus identifying the optimum conditions (**Table 2.1**). The main parameters determining the solubility of the extract during SFE-CO_2 are the pressure and the temperature, but, sometimes, the dynamic extraction time is equally important. Therefore, all of these parameters were chosen to optimize the process. During PLE extraction, the pressure was a constant, therefore, the extraction time and temperature were chosen as the independent variables for the optimization process. This experiment showed the effect of the temperature and the extraction time on the extraction yield when ethanol is used.

Table 2.1. Independent variables used in RSM

Independent variables	Units	Supercritical CO_2 extraction (SFE-CO_2)		
		-1	**0**	**1**
Extraction pressure (p)	MPa	25	40	55
Extraction time (τ)	min	60	120	180
Extraction temperature (T)	ºC	50	65	80
		Pressurized liquid extraction (PLE)		
Independent variables	**Units**	**-1**	**0**	**1**
Extraction time (τ)	min	5	10	15
Extraction temperature (T)	ºC	50	70	90

For SFE-CO_2 extraction of cranberry pomace, three independent variables with three levels for each of them were chosen according to the researches which had been done previously: pressure (25–55 MPa), temperature (50–80 °C), and the dynamic extraction time (60–180 min). CCD involves 20 experimental runs: 8 factorial points, 6 axial points, and 6 center points (**Table 3.1**). According to the experimental data, the multiple regression equation was used to fit the second-order polynomial equation (3). Extractions at every experimental point were performed in triplicate and in random order.

Defatted cranberry pomace obtained under optimal SFE-CO_2 extraction conditions was used for the extraction optimization in PLE with ethanol as a solvent. The central composite design (CCD) was defined by two independent variables: extraction temperature (50–90 °C) and time (5–15 min) with three levels for each of them. The complete design of PLE with ethanol consisted of 13 experimental runs with 4 factorial points, 4 axial points and 5 center points (**Table 3.9**). Extractions at every experimental point were performed in triplicate, and the order of experiments was randomized.

The face centered design using the software Design-Expert free trial version (*Stat-Ease Inc*., Minneapolis, MN, USA) was used for data analysis and quadratic model building. Statistical significance of the model and model variables was determined at the 5% probability level ($p < 0.05$). The adequacy of the model was determined by evaluating the lack-of-fit coefficient and the Fisher test value (the F-value) obtained from the analysis of variance.

2.6. Evaluation of antioxidant potential for extracts and solid materials

2.6.1. Total phenolic content (TPC)

The total phenolic content (TPC) was measured by using the Folin-Ciocalteu reagent (Singleton, Orthofer and Lamuela-Raventos, 1999). Briefly, 150 μL of the appropriate dilution sample or water (blank) was mixed with 750 μL of diluted in distilled water (1:9, v/v) Folin-Ciocalteu reagent (2M), after 3 min of reaction, 600

μL of Na_2CO_3 solution (75 g/L) was added; the sample was left in the dark for 2 h, and the absorbance was measured at 760 nm with a *Spectronic Genesys 8* spectrophotometer (*Thermo Spectronic*, Rochester, NY, USA). The results were expressed as gallic acid equivalents, mg GAE/g of extract (E), and dry pomace weight (DW).

2.6.2. $ABTS^{\bullet+}$ scavenging assay

$ABTS^{\bullet+}$ scavenging was measured by the decolorization assay (Re *et al*., 1999). Firstly, a phosphate buffered saline (PBS) solution (75 mmol/L; pH 7.4) was prepared by dissolving 8.18 g NaCl, 0.27 g KH_2PO_4, 1.42 g Na_2HPO_4 and 0.15 g KCl in 1 L of distilled water. The $ABTS^{\bullet+}$ solution was prepared by mixing 50 mL of ABTS (2 mmol/L PBS) with 200 μL $K_2S_2O_8$ (70 mmol/L) and allowing the mixture to stand in the dark at room temperature for 15–16 h before use. The working solution was prepared by diluting the $ABTS^{\bullet+}$ solution with PBS to obtain the absorbance of 0.700±0.010 at 734 nm. $ABTS^{\bullet+}$ solution (1500 μL) was mixed with 25 μL of the appropriate dilution sample or methanol (blank), the mixtures were left in the dark for 2 h, and the absorbance was measured at 734 nm with a *Spectronic Genesys 8* spectrophotometer. The $ABTS^{\bullet+}$-scavenging capacity was expressed as trolox equivalents, mg TE/g of extract (E) and dry pomace weight (DW).

2.6.3. Oxygen radical absorbance capacity (ORAC) assay

The oxygen radical absorbance capacity (ORAC) was evaluated by using fluorescein as a fluorescent probe (Prior *et al*., 2003). 25 μL of the appropriate dilution sample or MeOH (blank) were mixed with 150 μL of fluorescein solution (14 μmol/L) in 96-well black opaque microplates, pre-incubated for 15 min at 37 °C, followed by a rapid addition of 25 μL of AAPH solution (240 mmol/L). The microplate was immediately placed in a *FLUOstar Omega* reader (*BMG Labtech*, Offenburg, Germany), automatically shaken prior to each reading, and the fluorescence was recorded every cycle (1 min × 1.1); in total, 120 cycles using 485-P excitation and 520-P emission filters were performed. The raw data was exported from the *Mars* software to *Microsoft Excel* 2003 (*Microsoft*, Roselle, IL, USA) for further calculations. The L-ORAC assay was used to measure lipophilic extracts. 10 mg of extract was dissolved in a 1 mL acetone/methanol mixture (1/9, v/v) to solubilize the antioxidants. The measurement was performed as described above. The results were expressed as mg TE/g of extract (E) and dry pomace weight (DW).

2.6.4. Antioxidant capacity by e-BQClab device

The antioxidant capacity was also determined with an e-BQClab device (*Bioquochem*, Asturias, Spain) measuring the redox potential which is expressed in micro Coulombs (μC). It distinguishes among different types of active compounds which may perform as fast or slow antioxidants: the Q_1 value refers to the antioxidant capacity of the compounds with the higher rate of free radical scavenging (e.g., uric and ascorbic acids, vitamin E, carotenoids); whereas the Q_2

value refers to the antioxidant capacity of the compounds with a lower rate of free radical scavenging (e.g., polyphenols, α-lipoic acid, resveratrol, astanxanthin). The device also calculates the Q_T value which is the sum of both above defined values. For this measurement, 40 µL of EtOH and H_2O cranberry pomace extracts (10 mg/mL) was used, and the mean values were calculated from at least 3 replicates.

2.6.5. Antioxidant capacity of solid substances

The antioxidant capacity of solid substances was evaluated with the QUENCHER method (Kitrytė, Šaduikis and Venskutonis, 2015). All the above described methods evaluate the antioxidant potential of the recovered extracts. Some antioxidants may be strongly bound to the solid matrix and are not extracted. The QUENCHER method evaluates the antioxidant capacity directly in the solid material and provides additional important information both on the extraction efficiency and the residual antioxidant capacity of the solids after extraction. The QUENCHER method was applied for the pomace solids while using Folin-Ciocalteau, $ABTS^{\bullet+}$-scavenging and ORAC assays for 10 mg of sample (when necessary, it was diluted with microcrystalline cellulose) or cellulose (blank) as described below.

For TPC, the sample or blank was mixed with 150 µL of distilled water, 750 µL of Folin-Ciocalteu reagent, and 600 µL of Na_2CO_3 solution vortexed for 15 s, shaken for 2 h in the dark, and centrifuged (4500 rpm for 5 min), and the absorbance of the clear supernatant was measured at 760 nm. The TPC content was expressed as gallic acid equivalents, mg GAE/g of the sample and dry pomace weight (DW).

In the $ABTS^{\bullet+}$ scavenging assay, the sample or blank was diluted with 25 µL of methanol and 1.5 mL of the working $ABTS^{\bullet+}$ radical solution vortexed for 15 s, shaken for 2 h in the dark, and centrifuged (4500 rpm for 5 min), and the absorbance of the clear supernatant was measured at 734 nm. The $ABTS^{\bullet+}$-scavenging capacity was expressed as trolox equivalents, mg TE/g of the sample and dry pomace weight (DW).

In the ORAC assay, the sample or blank was mixed with 150 µL of PBS solution, 900 µL of fluorescein, vortexed for 15 s, shaken for 1 h in the dark, and centrifuged (4500 rpm for 5 min). 175 µL of the clear supernatant was transferred to a 96-well plate, preincubated for 15 min at 37 °C, and 25 µL of AAPH solution was added. The results were expressed as trolox equivalents, mg TE/g of the sample and dry pomace weight (DW).

2.7. Chromatographic analysis of SFE-CO_2 extracts

2.7.1. Determination of triacylglycerol (TAG), fatty acids and squalene

TAGs were analyzed as described by Zeb and Murkovic (2010) on a *Waters AQCUITY* ultra performance liquid chromatography system (*Waters Corp*., Milford, MA, USA) equipped with a hybrid *Bruker Daltonics* time-of-flight/quadrupole mass detector (UPLC-Q/TOF) (Bremen, Germany). The UPLC equipment consisted of a binary solvent manager, a sample manager, a column heater interfaced with a mass spectrometer equipped with an ESI source operating in the positive mode.

Instrument control and data processing were performed by using *HyStar* software (*Bruker Daltonics*, Bremen, Germany). Separations were performed on an *Acquity BEH*, C18 column, 2.1 × 50 mm, particle size 1.7 μm (*Waters*, Ireland), the autosampler and column oven were maintained at 20 °C and 40 °C, respectively. The analytes were eluted by using an isocratic solvent system consisting of 18% isopropanol in methanol (0.1% acetic acid) and 0.05% of ammonium acetate. The following parameters were selected: the flow rate was 0.4 mL/min; the separation time was 10 min; the fragmentor's potential was 150 V; the drying gas temperature was 350 °C; the capillary voltage was 4000 V; the m/z range was 200–1000.

The fatty acid composition was analyzed by the AOAC (969.33) method (AOAC, 1995). For the hydrolysis of TAGs and the esterification of the liberated fatty acids, 0.1 g of the extract and 0.8 mL of methanolic NaOH (0.5 M) were poured into a round-bottomed flask and heated with a condenser until the disappearance of the fatty phase. Afterwards, 1 mL of 24% boron trifluoride/methanol complex was added, boiled for 2 min and cooled to room temperature. The sample was diluted with 1 mL n-hexane, well-shaken and left until the layers separated. The top hexane phase was collected and stored at -20 °C until analysis. For the analysis, 100 μL of the hexane phase was diluted with 900 μL pure GC-grade hexane. Fatty acid methyl esters (FAME) were analyzed on a *HRGC 5300* (*Mega Series*, Carlo Erba, Milan, Italy) equipped with a flame ionization detector and a 100 m length 0.25 mm internal diameter and 0.20 μm film thickness fused silica capillary column *SPTM-2560* (*Supelco*, Bellefonte, PA, USA). The injector's temperature was 220 °C, the detector's temperature was 240 °C; the oven temperature was programed from 80 °C to 240 °C at 4 °C/min and maintained for 5 min. Helium was used at a flow rate of 20 cm^3/s, the volume of the injected sample was 1 μL. The compounds were identified by comparing their retention times with those of the FAME mixture. Squalene was determined together with FAMEs, it was identified by using a reference compound. Triplicate GC runs were performed, and the results have been presented as a mean.

2.7.2. Determination of tocopherols and sterols

Saponification was used to convert tocopherol and sterol esters to their free forms prior to HPLC analysis as described elsewhere (Slavin and Yu, 2012). The separation of compounds was carried out on a *Waters Acquity UPLC* consisting of a solvent delivery system, an autosampler, and a column manager (*Waters*, Milford, MA, USA). An *Acquity BEH C18* column (50 × 2.1 mm, 1.7 μm; *Waters*, Milford, MA, USA) was used for the separation of compounds. Separation was performed while using the following gradient: starting with 30% A (0.1% formic acid in water) and 70% B (0.1% formic acid in acetonitrile), then changing to 100% B in 1 min and maintaining at 100% B for 5 min. After that, the gradient was returned to the initial conditions in 0.5 min. The flow rate of the eluents was 0.4 mL/min, the injection volume was 2 μL, and the column temperature of 30 °C was maintained. Before each run, the column was equilibrated for 2 min. The mass spectra were recorded on a *MAXIS 4G Q-TOF* mass spectrometer (*Bruker Daltonics*, Bremen, Germany), operating in the APCI positive mode. All the spectral data was collected in the full

scan mode in the m/z range from 200 to 1200 at a 3 Hz rate. The following parameters of the mass spectrometer were maintained: capillary voltage – 2000V; corona current – 3000 nA; end plate offset – -500V; vaporizer temperature – 400 °C; nebulizer gas pressure – 1.6 bar; drying gas flow rate – 8 L/min; drying gas temperature – 200 °C. All the analyzed compounds were quantified by their corresponding protonated ion peaks $[M+H]^+$, and single ion chromatograms were extracted with the accuracy of 0.01 m/z.

2.7.3. Analysis of total carotenoids and β-carotene content

The total content of carotenoids was determined spectrophotometrically by using a calibration curve prepared with the β-carotene standard as described elsewhere (Zymone *et al*., 2018). Briefly, 0.1 g of the sample was dissolved in 1 mL of hexane, and the absorbance was measured at 450 nm. The total content of carotenoids was expressed as mg β-carotene equivalents in 100 g of the extract. The content of β-carotene was determined by the chromatographic method while using a *Waters 2695* liquid separation module (*Water Corporation*, USA). The elution of materials was monitored by a UV-Visible detector (UV-Vis, 2489, *Water Corporation*, USA); β-carotene was quantified at 450 nm. Chromatographic separations were performed on a *RP-C30* column (5 μm, 250 × 4.0 mm, *YMC Europe*, Dinslaken, Germany) connected to a *C30* guard column (5 μm, 10 × 4.0 mm, *YMC Europe*, Dinslaken, Germany) while using a flow rate of 0.65 mL/min. The temperature of the column was 25 °C. The mobile phase used in the study consisted of methanol (solvent A) and methyl tert-butyl ether (solvent B). The samples were injected at 40% B (held 5 min), and the gradient then changed to 83% B in 50 min; then, the gradient was changed to 100% B in 5 min (maintained for 10 min) and to 40% B in 5 min (maintained for 10 min). The recovery of β-carotene and the total content of carotenoids was evaluated according to the results, i.e., we seek to determine how many mg of these compounds were extracted during the fractionation process, and then we compared the obtained results with Soxhlet extraction. The recovery of β-carotene and the total carotenoids was expressed by percentage.

2.7.4. Evaluation of volatile aroma compounds

The composition of SFE-CO_2 extracts was analyzed on a Gas Chromatography-Time-of-Flight Mass Spectrometry (GC×GC-TOF-MS) *LECO Pegasus 4D* system consisting of an *Agilent 7890A GC*, a *GERSTEL Multipurpose Sampler MPS* (*Gerstel GmbH*, Mülheim an der Ruhr, Germany) coupled with a high-speed *TOF-MS* detector (*LECO*, St. Joseph, MI, USA). Solid phase microextractions (SPME) were performed automatically with an *MPS-2* autosampler (*Gerstel*, Mülheim an der Ruhr, Germany) with a divinylbenzene/carboxen/polydimethylsiloxane fiber (*Supelco*, Bellefonte, PA, USA) from 100 mg of extracts which were placed in a 20 mL vials. The headspace bottle was equilibrated at 40 °C for 15 min, and, afterwards, the fiber was exposed in it at 40 °C for 30 min. The chromatographic system was made up of a primary

column *BPX-5* (30 m, 0.25 mm i.d., 0.25 μm film thickness) (*SGE Analytical Science*, Australia) linked with a secondary column, *BPX-50* (2.0 m, 0.10 mm i.d., 0.1 μm film thickness). The primary oven temperature program was as follows: 40 °C (1 min) then ramped to 250 °C at 7 °C/min (maintained for 1 min) with a modulator offset temperature of +15 °C. The transfer line temperature was 250 °C. The GC injector port was set at 150 °C and then ramped to 250 °C at (720 °C/min) rate, the desorption time was 5 min. The TOF-MS acquisition rate was 10 spectra/s, the mass range used for the identification ranged from 30 to 550 m/z units. The detector voltage was set at 1550 V, and the ion source temperature was set at 250 °C. The data from the system was collected with *ChromaTOF* software v.4.22 (LECO) after a solvent peak delay of 360 s. Tentative identification of volatile substances was assigned by comparison of the obtained experimental mass spectra against the spectra of the *Adams*, *NIST*, *MainLib*, and *Replib* mass spectral libraries, and only matches with the signal-to-noise ratio higher than 10 and with minimum similarity of 800 were considered acceptable. Moreover, the retention indexes of compounds were calculated by referring them to the retention times of C7–C30 n-alkanes under the same conditions and compared with those found in chemical databases such as *NIST* (NIST (*National Institute of Standards and Technology*), 2017), *Flavornet* (Acree & Arn, 2004) or *Pubmed*.

2.8. Determination of proanthocyanidins and anthocyanins content in PLE extracts by spectrophotometric analysis

The assay for proanthocyanidin content (PAC) is based on 4-dimethylaminocinnamaldehyde (DMAC) reaction with procyanidin A cycle at C8 of the terminal units of the molecules. The PAC values were determined by using the BQC kit according to the manufacturer's recommendations (*Bioquochem*, Asturias, Spain). Ten μL of EtOH or H_2O cranberry pomace extracts (10 mg/mL), 230 μL of reagent A and 10 μL of DMAC reagent were put in 96-well clear bottom plates. Then, the plates were left for agitation for 15 min, and the absorbance was measured at 640 nm in a FLUOstar Omega reader.

The content of anthocyanins AC was measured by using the BQC kit according to the manufacturer's recommendations (*Bioquochem*, Asturias, Spain). 20 μL of EtOH or H_2O cranberry pomace extracts (10 mg/mL) was mixed with 220 μL of Reagent A or 220 μL of Reagent B in 96-well clear bottom plates. Then, the plates were shaken at 200 rpm, left for 10 min, and the absorbance was measured at 510 and 700 nm while using FLUOstar Omega. The PAC and AC values were expressed as the means of at least 3 replicate measurements.

2.9. Phytochemical analysis of PLE extracts

A *Waters AQCUITY* ultra performance liquid chromatography system (*Waters Corp*., Milford, MA, USA), equipped with a hybrid *Bruker Daltonics* (Bremen, Germany) time-of-flight/quadrupole mass detector (UPLC-Q/TOF) was used for phytochemical analysis. The UPLC equipment consisted of a binary solvent manager, a sample manager, a column heater interfaced with a mass spectrometer

equipped with an ESI source operating in the negative mode for phenolic compounds and in the positive mode for anthocyanins. The instrument control and data processing were performed by using *HyStar* software (*Bruker Daltonics*, Bremen, Germany). Separations were performed on an *Acquity BEH*, C18 column (2.1×100 mm, particle size 1.7 μm) (*Waters Corp*., Ireland), the autosampler and the column oven were maintained at 10 °C and 40 °C, respectively. The mobile phase consisted of solvents A (1% or 0.4% formic acid in ultra-pure water for anthocyanins and flavonoids/phenolic acids, respectively), and B (100% acetonitrile).

The preparation of cranberry pomace for anthocyanins was performed as follows. Anthocyanins were extracted from 2 g of pomace with 2 × 15 mL C_2H_5OH/H_2O (30:70, v/v) acidified with 0.1 M HCl, sonicated for 5 min and mixed on a magnetic stirrer (60 min at 200 rpm). The extracts were filtered and centrifuged, and then the solvent was removed in a rotary evaporator at 25 °C and diluted to 25 mL in a measuring flask with C_2H_5OH acidified with 0.1 M HCl solution. 1 mL of mixture was dried with a flow of nitrogen, diluted with HPLC grade methanol, filtered and chromatographed on a UPLC-QTOF.

Anthocyanins in extracts and cranberry pomace were analyzed as previously described by Grunovaitė, Pukalskienė, Pukalskas and Venskutonis (2016). The following gradient was used for phenolic compounds: B was raised from 5% to 16% in 7 min, then increased to 100% in 9 min, and, during the next 2 min, returned to the initial conditions. The column was equilibrated for 3 min before the next analysis. The flow rate was 0.4 mL/min, the injection volume was 2 μL. The nebulizer pressure was 2.0 bar, and the nitrogen flow rate was 10 L/min. The mass spectra were determined by full scan acquisition covering m/z 100–1200. The ionization conditions were adjusted at +4000 V and 8 eV for the capillary voltage. For the fragmentation study, a data-dependent scan was performed by deploying collision induced dissociation (CID) while using nitrogen as a collision gas at 35eV. All the operations, data acquisition, and analyses were controlled with *HyStar* software. Anthocyanins were quantified by using cyanidin-3-glucoside, whereas the concentration of some other identified compounds was determined by using the references of quinic acid, catechin, and chlorogenic acid. Standard stock solutions were prepared in methanol and subsequently diluted to working concentrations. The amounts of individual identified compounds were expressed in mg/100 g of the extract, while the recovered amounts of anthocyanins were also calculated for 100 g DW of cranberry pomace.

2.10. Determination of PLE-EtOH extract effect on cancer cell viability

Human colorectal carcinoma DLD-1 and HCT-116 cell lines were obtained from the *American Type Culture Collection* (Rockville, Maryland, USA). Both cell lines were maintained in an *RPMI-1640* cell culture medium which was supplemented with 10% fetal bovine serum (*Gibco*, Germany), 100 UI/mL penicillin (*Sigma Aldrich*, USA), and 0.1 mg/mL streptomycin (*Sigma Aldrich*, USA). Cell culturing was performed at 37 °C in a 5% CO_2 humidified incubator. The cranberry

pomace extract was prepared in stock by dissolving it in RPMI with the concentration of 1000 µg/mL, filtered through a 0.22 µm sterile filter; fetal bovine serum and antibiotics supplements were added.

2.10.1. Cell viability assay

In order to determine the effect of the combination therapy of the cranberry extract on colorectal cancer cell proliferation, an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was deployed as previously described by Skeberdytė *et al*., 2018. DLD-1 and HCT-116 cells were seeded in 96-well plates at the density of 3×10^3 cells per well in 200 µL of an RPMI-1640 culture medium in order to reach 70–80% confluence. After 24 h of incubation at 37 °C, the cells culture medium was replaced with the extract at different concentrations (1000, 200, 40 µg/mL). The viability of cells was evaluated after 24 and 48 h. First of all, the cells were washed with 90 µL of PBS, then 10 µL of MTT reagent (5 mg/mL) was added, and the sample was incubated at 37 °C for 4 h. Formazan crystals were dissolved in 100 µL of DMSO, and the absorbance of each well was measured with a plate reader *MultiskanGo* at a test wavelength of 570 and 630 nm. The absorbance values of each concentration of the cranberry extract were expressed as a percentage change of that of the control sample.

2.10.2. Flow cytometry assay

DLD-1 and HCT-116 cells were seeded in a 12-well plate at a density of 5×10^4 cells per well in an RPMI-1640 culture medium as previously described by Skeberdytė *et al*., 2018. Next day, the cells culture medium was replaced with the extract at different concentrations (1000, 200, 40 µg/mL). After 24 h of incubation, the cells were washed with PBS, trypsinized with 0.025% trypsin-EDTA solution, and collected by using centrifugation at 500 rpm for 5 min. Then, the supernatant was removed, the cells were resuspended in 75 µL of PBS with 75 µL *Guava Nexin* ® (*Millipore*, Burlington, MA, USA) reagent and incubated for 20 min in the dark. The samples were quantified by using a *Guava* easy *Cyte HT* flow cytometer (*Millipore*, Burlington, MA, USA). The data was analyzed with the *Guava Nexin* software.

2.10.3. Clonogenic (a.k.a. colony forming) assay

DLD-1 and HCT-116 cells were seeded in a 12-well plate at a density of 100 cells per well in an RPMI-1640 culture medium as previously described by Skeberdytė *et al*., 2018. Next day, the cells culture medium was replaced with the extract at different concentrations (1000, 200, 40 µg/mL). After 7 days, the colonies were fixed with 4% paraformaldehyde (PFA) for 15 min and stained with crystal violet. The stained cells were photographed with a *Syngene G-Box*, the count of colonies and their area were estimated by using the *GeneTools* analysis program (a true colony is considered to consist of more than 50 cells).

2.11. Antiviral activity of PLE extracts against Zika virus (ZIKV)

2.11.1. Cell cultures, virus and sample preparation

Ethanol and water cranberry pomace extracts were solubilized in dimethyl sulfoxide (40 mg/mL) and stored at +5 °C while being protected from light. Cell-based assays were performed while using maximum 1% of dimethyl sulfoxide (DMSO).

Human lung epithelial A549 cells (*ATCC*, CCL-185, Manassas, VA, USA) and Vero cells (*ATCC*, CCL-81) were cultured in the minimum essential medium (*MEM: Gibco/Invitrogen*, Carlsbad, CA, USA) supplemented with 10% or 5% of heat inactivated Fetal Bovine Serum (FBS), 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 100 U/mL of penicillin, 0.1 mg/mL of streptomycin and 0.5 µg/mL of fungizone (*PAN Biotech*, Aidenbach, Germany) under 5% CO_2 atmosphere at 37 °C. The clinical isolate PF-25013-18 of ZIKV (ZIKV-PF13) has been described previously (Frumence *et al*., 2016; Gadea *et al*., 2016). ZIKV-MR766 and the green fluorescent protein (GFP)-expressing strain of ZIKV-MR766 ($ZIKV_{GFP}$) are molecular clones of the strain MR766 of ZIKV (Bos *et al*., 2018). The cells were infected with various viruses at multiplicity of infection (MOI) of 1, unless stated otherwise.

2.11.2. MTT assay

In order to confirm that the observed inhibitory effect was not a result of a change in cellular conditions, the cytotoxicity of cranberry pomace ethanol and water extracts in human lung cells, A549 was assessed according to Haddad *et al*., 2020 with some modifications. 100 µL of two-fold dilutions of cranberry pomace EtOH and H_2O extracts ranging from 800 to 25 µg/mL were used to treat human lung cells A549 at a density of 2×10^4 per cultured well in a 96-well culture plate. As the control, we used MEM 5%. After an incubation period of 24 h at 37 °C under 5% CO_2 atmosphere, the supernatant was removed, and then 120 µL of 5% MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) solution was added. The plates were incubated for 2 h at 37 °C under 5% CO_2 atmosphere. After two hours, MTT was removed from the plates, and the formazan crystals were solubilized by addition of 50 µL of DMSO. The absorbance of the plate was measured at 570 nm with a reference wavelength at 690 nm. CC_{50} was determined by using a nonlinear regression on the *Graphpad* prism software (version 5.00, La Jola, USA).

2.11.3. Screening of antiviral activity of cranberry pomace extracts against ZIKV

Human lung cells A549 were used for the primary evaluation of the anti-ZIKV effect of cranberry pomace extracts as previously described by Haddad *et al*., 2020. The cells were seeded the previous day in a 96-well culture plate at a density of 2×10^4 cells per well and incubated at 37 °C with 5% CO_2 for 24 h prior to infection. The cells were infected by 100 µL of the culture medium (MEM 5%) mixed with the

GFP-expressing strain of $ZIKV_{GFP}$ at a density of 2.5×10^4 PFU/mL and treated with 1 µL (40 mg/mL) of each cranberry extract (EtOH or H_2O) per well. The final concentration of the extracts was 400 µg/mL per well. The same amount of virus was incubated with the medium without cranberry extracts as the virus control. The medium without extracts and virus was considered as the control medium. The 96-well plate was incubated for 24 h at 37 °C under 5% CO_2 atmosphere. After the incubation period, the medium with the virus and the extract was removed, and the cells were rinsed once with 50 µL of PBS. As the next step, the bonds between the cells were interrupted by addition of 20 µL of trypsin 0.25%/EDTA. The cells were fixed by addition of 80 µL of 3.7% PFA/MEM 5% mixture and left for 10 min. Then, the 96-well plate was submitted to flow cytometry analysis using *CytoFLEX* (*Beckman Coulter*, Brea, CA, USA).

2.11.4. Dose-dependent assay on antiviral activity of water extract against ZIKV

Human lung cells A549 had been seeded the previous day in a 96-well culture plate at a density of 2×10^4 cells per well and incubated at 37 °C with 5% CO_2 for 24 h prior to infection as previously described by Haddad *et al*., 2020. 100 µL of two-fold dilutions of cranberry pomace H_2O extract ranging from 400 to 12.5 µg/mL were used to treat human lung cells A549, which then were infected with $ZIKV_{GFP}$ at MOI=1. The density of $ZIKV_{GFP}$ used for infection was 2×10^4 PFU/mL. The same amount of the virus was incubated with the medium (MEM 5%) without the cranberry extract as the control virus. The media without the extract and the virus was considered as the control media. The 96-well plate was incubated for 24 h at 37 °C under 5% CO_2 atmosphere. After the incubation period, the medium with the virus and the extract was removed, and the cells were rinsed once with 50 µL of PBS. As the next step, the bonds between the cells were interrupted by addition of 20 µL of trypsin 0.25%/EDTA. The cells were fixed by addition of 80 µL of 3.7% PFA/MEM 5% mixture and left for 10 min. Then, the 96-well plate was submitted to flow cytometry analysis using *CytoFLEX* (*Beckman Coulter*, Brea, CA, USA). The IC_{50} was determined by using nonlinear regression on the *Graphpad* prism software (version 5.00, La Jola, USA). According to the results, the selectivity index (SI) was calculated.

2.11.5. Time-of-drug-addition assay

Human lung cells A549 were used to determine in which stage the cranberry pomace extract acts against the Zika virus. The cells had been seeded the previous day in 96-well plates at a density of 2×10^4 cells per well and incubated at 37 °C with 5% CO_2 for 24 h prior to infection as previously described by Haddad *et al*., 2020.

Inactivation assay. 10 µL of cranberry pomace water extract (800 µg/mL) was mixed with $ZIKV_{GFP}$ (5×10^4 PFU) in a 48-well plate and incubated for 2 h at 37 °C with 5% CO_2. Simultaneously, the same amount of virus was incubated with the medium without the plant extract as a control sample. The mixture (extract-virus or medium-virus) was then diluted 15-fold with MEM containing 5% FBS. The 15-fold

dilution served to titrate the water extract below its effective dose and prevent meaningful interactions with the host cell surface. Then, the mixtures were subsequently added to a human lung A549 cell monolayer seeded in 96-well plates and incubated for 24 h at 37 °C with 5% CO_2.

Entry assay. $ZIKV_{GFP}$ free particles (2×10^4 PFU) were incubated with 100 µL of the cranberry pomace water extract (400 µg/mL) for 2 h at 37 °C. As a control test, $ZIKV_{GFP}$ was incubated with the medium (MEM 5%). After 2 h of incubation at 37 °C, the supernatant was discarded, the cells were washed twice with PBS and then incubated with a fresh medium (MEM 5%) for 24 h at 37 °C before being subjected to the flow cytometry assay.

Replication assay. $ZIKV_{GFP}$ free particles (2×10^4 PFU) were incubated with 100 µl of medium (MEM 5%) for 2 h at 37 °C. As a control test, $ZIKV_{GFP}$ was incubated with the medium. After 2 h of incubation at 37 °C, the supernatant was discarded, the cells were washed twice with PBS and then incubated with 100 µL of the cranberry pomace water extract (400 µg/mL) for 24 h at 37 °C before being subjected to the flow cytometry assay.

After the incubation period (24 h), the medium with the virus and the extract was removed from all the plates (inactivation, entry and replication assays), the cells were rinsed once with 50 µL of PBS. As the next step, the bonds between the cells were interrupted with the addition of 20 µL of trypsin 0.25%/EDTA. The cells were fixed by addition of 80 µL of 3.7% PFA/MEM 5% mixture and left for 10 min. Then, the 96-well plates were submitted to flow cytometry analysis using *CytoFLEX* (*Beckman Coulter*, Brea, CA, USA).

2.11.6. Virus binding assay

Virus binding was carried out as described by Haddad *et al*. (2020). A549 cells had been seeded the previous day in 96-well plates at a density of 2×10^4 cells per well. The cells were incubated with $ZIKV_{GFP}$ free particles (2×10^4 PFU) at MOI of 1 in the presence or absence of 100 µL of the cranberry pomace water extract (400 µg/mL) for 1 h at 4 °C. After 1 h, the plate was incubated for 20 min at 37 °C to shift the temperature. Then, the inoculum was removed, the cells were washed with 50 µL of PBS, and then the plates were incubated with 100 µl of fresh medium for 24 h at 37 °C. After the incubation period, the medium was removed, and the cells were rinsed once with 50 µL of PBS. As the next step, the bonds between the cells were interrupted by addition of 20 µL of trypsin 0.25%/EDTA. The cells were then fixed by addition of 80 µL of 3.7% PFA/MEM 5% mixture and left for 10 min. Then, the 96-well plates were submitted to flow cytometry analysis by using *CytoFLEX* (*Beckman Coulter*, Brea, CA, USA).

2.11.7. Plaque forming unit assay

The plaque forming unit (PFU) assay was used to quantify the release of infectious viral particles as previously described by Haddad *et al*., 2020. Human lung cells A549 had been seeded the previous day in a 24-well plate at a density of 1.2×10^5 cells per well and incubated at 37 °C with 5% CO_2 for 24 h prior to

infection. 500 µL of two-fold dilutions of cranberry pomace H_2O extract ranging from 400 to 12.5 µg/mL were used to treat human lung cells A549 which then were infected with ZIKV-PF13 at MOI=1. The density of ZIKV-PF13 used for infection was 2.4×10^5 PFU/mL. The same amount of the virus was incubated with the medium (MEM 5%) without the cranberry extract as the control virus. The media without the extract and the virus was considered as the control media. The 24-well plate was incubated for 24 h at 37 °C under 5% CO_2 atmosphere. After the incubation period, the supernatants were collected and kept at -80 °C until further analysis.

The vero cells had been seeded the previous day in 24-well culture plates at a density of 7×10^4 cells per well and incubated at 37 °C with 5% CO_2 for 24 h prior to infection. The cells were infected by 30 µL of ten-fold dilutions of supernatants. After 3 h of incubation at 37 °C, addition of 0.2 mL of the culture medium was performed, and the medium was supplemented with 5% fetal bovine serum (FBS) and 0.8% carboxymethylcellulose sodium salt (*Sigma-Aldrich*, Saint-Quentin-Fallavier, France). The incubation extended for 96 h at 37 °C. After the incubation, the medium was removed, and the cells were rinsed with 200–300 µL of PBS, fixed with PFA 3.7% dissolved in PBS and stained with 0.5% of crystal violet (*Sigma-Aldrich*, Saint-Quentin-Fallavier, France) diluted in 20% ethanol. The plaques were counted and expressed as plaque-forming units per mL (PFU/mL).

2.11.8. Immunofluorescence assay

The viral protein production was evaluated by immunofluorescence analysis using an antibody which recognizes the viral protein E as previously described by Haddad *et al*., 2020. Human lung cells A549 had been seeded the previous day in a 24-well plate on the coverslips at a density of 1.2×10^5 cells per well and incubated at 37 °C with 5% CO_2 for 24 h prior to infection. 500 µL of two-fold dilutions of the cranberry pomace H_2O extract ranging from 400 to 12.5 µg/mL were used to treat human lung cells A549 which were then infected with ZIKV-MR766 at MOI=1. The density of ZIKV-MR766 used for infection was 1.2×10^5 PFU/mL. The same amount of the virus was incubated with the medium (MEM 5%) without the cranberry extract as the control virus. The media without the extract and the virus was considered as the control media. The 24-well plate was incubated for 24 h at 37 °C under 5% CO_2 atmosphere.

The cells grown on coverslips were rinsed twice with PBS and fixed with 3.7% paraformaldehyde (PFA) at room temperature for 10 min. For permeabilization, the cells were incubated with 0.1% of Triton X-100 in PBS and stained for 1 h at room temperature in the dark for ZIKV by using *4G2-Alexa Fluor 594* (1:1000 in PBS-BSA 1%). The nucleus morphology was determined by DAPI staining. The coverslips were mounted in *Vectashield* (*VectorLabs*), and fluorescence was observed by using a *Nikon Eclipse E2000-Umicroscope*. A *Hamamatsu ORCA-ER* camera and *NIS-Element AR* (*Nikon*) imaging software were used to capture images.

2.11.9. Evaluation of cranberry pomace water extract toxicity in zebrafish

Adult wildtype zebrafish (*Danio rerio*; 3–6 months of age, male) were kept under standard conditions at a temperature of 28 °C, photoperiod (14/10 h light/dark), and conductivity (400 μS). Every day, the fish were fed 3 times with commercially available food from *Planktovie* (*Gemma Micro ZF 300*). For intraperitoneal injections, the fish were anesthetized with 0.02% tricaine (MS-222; REF: A5040, *Sigma-Aldrich*) and injected with the respective vehicle (MEM 5%) or cranberry pomace water extract (400 μg/g of body weight). After the injection, the fish were immediately placed back in the water and carefully observed for detecting any striking signs of stress. All the animal experiments were conducted in accordance with the French and European Community Guidelines for the Use of Animals in Research (86/609/EEC and 2010/63/EU) and approved by the local *Ethics Committee* for animal experimentation of *CYROI* (APAFIS #2019052910002738_v4). At the end of the procedure, the animals were sacrificed with an overdose of tricaine.

2.12. Antimicrobial activity of cranberry pomace extracts in bacterial cultures

Firstly, 6.6% ethanol and water extract solutions were prepared which were further diluted to 3.3, 1.65, 0.83 and 0.42% concentration by using a sterile broth corresponding for each microorganism (**Table 2.2**).

The day before the experiment, one single isolated microorganism colony was transferred to a tube with 5 mL of the corresponding broth. The tubes were incubated overnight (18 h), and the microorganism counts were determined by measuring absorption at 620 nm in a *Hitachi U-1900* spectrophotometer (Tokyo, Japan). Serial decimal dilutions were prepared by using the corresponding broth. For microplate preparation, 495 μL of various concentration ethanol and water extracts were inoculated with the final concentration of 10^4 or 10^8 cfu/mL of each microorganism. Three microplate wells were filled with 150 μL of the corresponding concentration inoculated extracts, broth (a blank) or inoculums (10^4 and 10^8 cfu/mL) as controls and incubated in *MultiscanGo* (*Thermo Fisher Scientific Brand*, UK). After 48 h duplicate sets of Petri plates (*Eurolab*, Barcelona, Spain) with the adequate culture, the media were inoculated aseptically with 1 or 0.1 mL aliquots from the appropriate dilutions and incubated (**Table 2.2**). The numbers of the colonies which were present in the plates were counted, and the data was transformed into log cfu/mL. For testing the effect of heating the centrifuge tubes with 2 and 1.65% concentration, the ethanol extracts were kept in a thermostated water bath at 72 °C for 2 h, and were afterwards cooled down to room temperature. The control samples were kept in the fridge. Inoculum and microplates were prepared by using *L. monocytogenes* colonies as described above. An effect of cranberry phytochemicals can be bacteriostatic (when the extract inhibits the bacterial growth), bactericidal (the concentration of the extract which completely inhibits the visible growth of the organisms), or insignificant. In our study, the strong, medium and low antibacterial effects were considered when the inhibition range reached 80–100%, 40–80%, and 1–40%, respectively.

Table 2.2. Culture media and incubation temperature for various microorganisms (incubation period for all microorganisms: 48 h)

Microorganisms	Media	Broth	Temperature, °C
Aerobic mesophilic bacteria	PCA agar		30
Brochothrix thermosphacta	STAA agar	TSB	25
Pseudomonas putida	*Pseudomonas* agar	TSB	30
Leuconostoc mesenteroides	MRS agar	MRS broth	30
Weissella viridescens	MRS agar	MRS broth	30
Listeria monocytogenes	*Listeria* agar	BHI	37
Campylobacter jejuni	*Campylobacter* blood-free selective agar	BHI	42

2.13. Preparation of pork meat slurry, hamburgers and cooked ham and methods of their analysis

2.13.1. Pork meat slurry

For each batch, 200 g of pork meat was homogenized with 400 mL of sterile water by using a laboratory blender *Stomacher 400C* (*Seward*, London, UK), and 200 mL of the homogenate was transferred into a sterile bottle. Four different batches were prepared, namely, without inoculum (C), with inoculum (CI), with 2% extract (CL), and with 2% extract and inoculum (L). For the samples, CI and L 1% of *L. monocytogenes*, *B. thermosphacta* and *P. putida* inoculum was added. The final concentration of *L. monocytogenes* was 10^2 cfu/g, that of *P. putida* and *B. thermosphacta* was 10^3 cfu/g. All the batches were stored at 4–5 °C until their analysis. The samples were analyzed after 0, 2, 4, 7 and 16 days of storage.

2.13.2. Pork meat hamburgers, their packaging and storage

Pork meat was minced to a particle size of 8 mm and mixed by using a vacuum machine (*Talleres Cato*, Cassà de la Selva Girona, Spain) with (per kg): 160 g water, 20 g soya, 10 g corn starch, 10 g pea fiber (control, without extract); in the second sample, 20 g of water was replaced with 20 g of the extract. After mixing, the samples were divided into 4 batches: without inoculum (RC), with inoculum (RCI), with extract (RCL), and with extract and inoculum (RL). The samples RCI and RL were mixed with 1% of the inoculum consisting of 4 *L. monocytogenes* serotypes (ILSI – 18, ILSI – 29, C_{170}, C_{3771}), *B. thermosphacta* and *P. putida*. The final concentration of *L. monocytogenes* was 10^2 cfu/g, the final concentration of *P. putida* and *B. thermosphacta* was 10^4 cfu/g. For the non-inoculated samples (RC and RCL), 1% of sterile water was used. Two pork burgers (125 g each) were placed in a polyethylene tray for each day of analysis. Then, the trays were packed by using modified atmosphere consisting of 20% CO_2 and 80% N_2 in a *Carburos Metálicos S.A.* apparatus (Barcelona, Spain). The packages were heat-sealed in a packer (*Efabind*, Murcia, Spain) by using a high barrier film. The gas content in each pack and the residual O_2 were controlled with a gas analyzer *OxyBaby 6.0* (*Witt-Gasetechnik GmbH & Co KG*, Witten, Germany). All the samples were stored at 4

°C, while, after 7 days of storage, the temperature was raised to 8 °C. The samples were analyzed after 0, 2, 5, 7, 9, 13 and 16 days of storage.

2.13.3. Formulation, processing, packaging and storage of cooked ham

All the cooked ham samples were manufactured on the same day by using a similar technology. Lean pork meat was minced and mixed with (per kg): 18 g sodium chloride, 4.5 g polyphosphates, 0.15 g sodium nitrite, 5 g carrageenan, 10 g caseinate, 0.8 g monosodium glutamate, 1.5 g flavor enhancer, 1 g meat aroma, 5 g starch (*Moguntia*, Germany), 284.7 g water (control, without extract); in the second sample, 20 g of water was replaced with 20 g of the extract (2%). The mixtures were stuffed into polyamide casings and boiled in a universal chamber *Ellermatic/H* (*Eller SRL*, Merano, Italy) operating at 99 °C and 90–100% relative humidity until 72 °C was reached in the middle of the ham pieces. After cooking, the samples were cooled and divided into 4 batches: without inoculum (CC), with inoculum (CCI), with extract (CCL), and with extract and inoculum (CL). The cooked hams were cut, and two pieces (approx. 125 g each) were randomly selected and placed on polystyrene trays. Inoculation and other handling was performed as described in Section 2.13.2 by using a mixture of 4 *L. monocytogenes* serotypes. All the samples were stored at 4 °C, while, after 13 days of storage, the temperature (in order to accelerate microbiological and oxidative processes) was raised to 8 °C. The samples were analyzed after 0, 7, 15, 22, 27, 32, 36 and 40 days of storage. The entire experiment was replicated twice.

2.13.4. Microbiological assays of slurry, hamburgers and cooked ham

Every day of analysis, 1 mL of slurry was taken directly from the bottles and used for 7–9 serial decimal dilutions in sterile BPW (2 replicate samples). Depending on the rate of growth of bacteria, 1 or 0.1 mL of the diluted samples was selected and spread in Petri dishes which were filled with the appropriate media for *L. monocytogenes*, *P. putida*, *B. thermospacta*, AMB and LAB (**Table 2.2**). The samples of hamburgers or cooked ham (25 g each) were aseptically transferred into a sterile blender bag with a full surface filter (*BagPagem*, Brussels, Belgium) and homogenized for 120 s in a laboratory blender with 225 mL of BPW. The further steps of this analysis were similar to those of slurry; however, for cooked ham, *P. putida* and *B. thermosphacta* were excluded.

2.13.5. Physico-chemical analysis of hamburgers and cooked ham

pH was measured with a pH-meter *Micro Ph 2001* (*Crison*, Barcelona, Spain) by placing an electrode into the sample and using phosphate buffer solutions (pH 4.0 and 7.0) for calibration. Three measurements were done by changing the electrode insertion place.

Color characteristics (L^*, a^*, b^*) were measured on a spectrophotometer *Minolta CM-2600d/2600d* (Osaka, Japan) by using the Commission Internationale de l'Eclairage (CIELab) space with D65 illuminant at the standard observer position

10° immediately after opening the package. L^* indicates lightness, a^* ranged from redness (a^*) to greenness ($-a^*$), and b^* ranged from yellowness (b^*) to blueness ($-b^*$). The colorimeter was calibrated before use with a white tile. The measurement was repeated on 6 randomly selected locations of each hamburger or piece of cooked ham and averaged for statistical analysis.

The water activity was measured with a *CX-2m* device (*Decagon Devices, Inc*. Pullman, Washington, USA). The sample was placed in a fully sealed measuring chamber in which the air is humidified or dehumidified to achieve equilibrium humidity. Three replicate measurements were done.

2.13.6. Determination of metmyoglobin content in hamburgers

The samples (5 g) were weighed in plastic tubes surrounded with silver foil so that to prevent pigment changes, homogenized with 25 mL of Na/ K phosphate buffer (40 mM, pH 6.8) for 10 s at 13500 rpm by using an *Ultra-Turex* device (*IKA*, Staufen, Germany), kept 1 h at 4 °C and centrifuged at 5000 rpm for 30 min at 4 °C (*Ependorf AG*, Hamburg, Germany). The supernatant was filtered, and the absorbance of the filtrate was measured at 700, 572 and 525 nm by using a *U-1900* spectrophotometer (*Hitachi*, Tokyo, Japan). The total metmyoglobin content was calculated according to the following equation: MetMB (%)={1.395-[(A572-A700)/ (A525-A700)]×100} (Krzywicki, 1982). Three replicate measurements were done for each sample.

2.13.7. Thiobarbituric acid reactive substances (TBARS)

TBARS was measured by using a modified method of Nissen, Byrne, Bertelsen and Skibsted (2004). 6 mL of 7.5% TCA was added to 2 g of meat, the mixture was homogenized in an *Ultra-Turax IKA18* homogenizer (*IKA*, Staufen, Germany), the slurry was centrifuged at 3000 rpm for 5 min at 10 °C and filtered. 2 mL of TBA (0.02 M) was added to 2 mL of the filtrate, the mixture was heated in a boiling water bath for 40 min and subsequently cooled. The absorbance was read at 530 nm, and the values were expressed in mg of malondialdehyde (MDA) per kg of product.

2.14. Sensory assessment of pork burgers and cooked ham

Sensory evaluation was conducted by eight panelists selected from the staff members of the University of Burgos. The panel members were in individual booths in a light-controlled room; they received the samples in a randomized order. The uncooked burgers and cooked ham samples consisting of 3 cm side cubes were presented at room temperature in white dishes. The samples were individually labeled with three-digit random numbers. The panelists were asked to evaluate the following descriptors: homogeneity, superficial humidity, intensity of color, discoloration, intensity of odor, intensity of flavor, and juiciness. The two final aspects were used to describe pieces of cooked ham. The intensity of every attribute was expressed on a structured scale from 1 (sensation not perceived) to 5 (maximum of the sensation). In addition, off-colors, off-odors and off-flavors were evaluated.

The analyses were performed according to the European Standard ISO 13299:2016 (2016).

2.15. Statistical analysis

The mean values and standard deviations were calculated by using *MS Excel 2016*. All the analyses were carried out in triplicate, unless specified otherwise. Significant differences among the means were determined by one-way ANOVA by using the statistical package *Statgraphics 18-X64*. Fisher's Least Significant difference (LSD) and Tukey's range test were used to determine significant difference among the treatments at $p < 0.05$. IC_{50} and CC_{50} were calculated by using the Graphpad prism.

3. RESULTS AND DISCUSSION

3.1. Optimization of the supercritical fluid extraction of cranberry pomace[1] and evaluation of product composition

3.1.1. Proximate analysis of cranberry pomace

Proximate analysis of cranberry pomace was performed before the application of biorefining processes. The composition of pomace highly depends on the berry cultivar and preparation method. During this research, cranberry pomace was mainly comprised of seeds, skin, and residual pulp. The content of moisture in cranberry pomace was 5.83±0.05%, which is slightly higher than previously reported by White *et al*. (2010) (4.5%). The crude protein content was 7.79±0.17%, which is 3.5-fold higher than previously reported (2–2.2%) (Bajerska *et al*., 2018; White *et al*., 2010). The total ash content was 1.11±0.02%, which is very close to the results of previous studies (Bajerska *et al*., 2018; White *et al*., 2010). Lipids are accumulated in the skin and seeds of berries which remain in the pomace after juice pressing. The content of lipids was 11.00±0.59%, while other authors reported from 10.5% (pomace) to 23.1% (seeds) of lipids (Bajerska *et al*., 2018; Bhagdeo, Ashraf-Khorassani, & Taylor, 2006; White *et al*., 2010). Other macrocomponents most likely consisted mainly of carbohydrates.

3.1.2. Optimization of the supercritical fluid extraction of cranberry pomace

Previous researches with SFE-CO_2 revealed that the particle size had influence on the extraction yield (Kraujalis and Venskutonis, 2013a; Povilaitis, Šulniūtė, Venskutonis and Kraujalienė, 2015). Consequently, the smallest fraction (< 0.2 mm) was selected for cranberry pomace optimization experiments (**Table 3.1**). The application of CCD so that to optimize three independent variables (pressure, temperature and dynamic extraction time) at three different levels allowed obtaining

[1] This section was prepared with reference to Tamkutė, Liepuoniūtė, Pukalskienė and Venskutonis (2020)

response surface plots (**Fig. 3.2**), which show the effect of these variables on the extract yield.

Table 3.1. Experimental data for the oil yield from cranberry pomace obtained by SFE-CO_2 from CCD

Experiment No.	p, MPa	τ, min	T, ºC	Yield (g/100g DW)	
				Experimental	Predicted
1	25	60	50	5.53±0.08^{b}	5.61
2	55	60	50	8.93±0.28^{d}	9.68
3	25	180	50	10.65±0.12^{fg}	10.67
4	55	180	50	10.98±0.13^{gh}	10.89
5	25	60	80	3.54±0.08^{a}	3.78
6	55	60	80	10.82±0.25^{fgh}	11.48
7	25	180	80	5.69±0.31^{b}	5.10
8	55	180	80	8.91±0.08^{d}	8.96
9	25	120	65	7.76±0.11^{c}	7.31
10	55	120	65	11.05±0.10^{h}	11.28
11	40	60	65	9.95±0.11^{e}	9.71
12	40	180	65	11.16±0.17^{h}	10.98
13	40	120	50	10.50±0.14^{f}	10.74
14	40	120	80	9.72±0.31^{e}	8.86
15	40	120	65	10.65±0.07^{fg}	10.59
16	40	120	65	10.95±0.16^{gh}	10.59
17	40	120	65	10.08±0.23^{e}	10.59
18	40	120	65	10.61±0.06^{fg}	10.59
19	40	120	65	10.52±0.07^{f}	10.59
20	40	120	65	10.66±0.03^{f}	10.59

*DW – dry pomace weight; ^{a,b} Different letters in the same column indicate statistical differences (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of triplicate determinations

SFE is based on the ability of CO_2 to dissolve various substances depending on its pressure and temperature. Therefore, these parameters should be properly selected for the recovery of the target compounds. Several other parameters are also important for SFE-CO_2 effectiveness, while, in this study, RSM was applied for determining the optimal pressure (p), temperature (T), and time (τ) for obtaining the highest possible extract yield from cranberry pomace. The effect of variables on the response was tested for significance by ANOVA (**Table 3.2**).

The analysis of the quadratic regression model with the R^2 value of 0.98 proved that model is significant, and the 'lack of fit' was not significantly relative to the pure error with the p-value of 0.4. The predicted values of the extract yield were calculated by the regression model and compared with the experimental results (**Fig. 3.1**). The experimental yields obtained at the selected extraction parameters were in a wide range, from 3.54% to 11.16% (**Table 3.1**). All the parameters had impact on the extract yield in the following significance order: P ($p < 0.0001$, F-value = 221.78), T ($p < 0.0001$, F-value = 45.15), τ ($p < 0.0001$, F-value = 53.62). The interactions between P- τ, τ -T and P-T also exerted significant impact on the yield

($p < 0.05$) with the F-values of 46.10, 43.30, and 41.57, respectively. Finally, the second order model terms p^2 and T^2 were significant factors ($p < 0.0073$) as well with the F values of 42.30 and 11.28.

Table 3.2. Analysis of the variance table for RSM experimental design of SFE-CO_2

Source	Sum of squares	df	Mean square	F value	p-value Prob > F
			SFE-CO_2		
Model	87.04	9	9.67	69.96	< 0.0001*
Pressure (P)	30.66	1	30.66	221.78	< 0.0001*
Time (τ)	7.41	1	7.41	53.62	< 0.0001*
Temperature (T)	6.24	1	6.24	45.15	< 0.0001*
Pτ	6.37	1	6.37	46.10	< 0.0001*
PT	5.75	1	5.75	41.57	< 0.0001*
τT	5.99	1	5.99	43.30	< 0.0001*
P^2	5.85	1	5.85	42.30	< 0.0001*
τ^2	0.26	1	0.26	1.89	0.1993
T^2	1.56	1	1.56	11.28	0.0073*
Residual	1.38	10	0.14		
Lack of Fit	0.98	5	0.20	2.43	0.1757
Pure Error	0.40	5	0.081	0.40	5
Total	88.42	19		88.42	19

*- significant

Determination coefficient $R^2 = 0.98$ proves the relevance of the model; it shows good fit of the model to the experimental data. The predicted R-squared value of 0.897 was in reasonable agreement with the adjusted R-squared of 0.97 and indicated that this design could be used for modeling the design space. The model can be considered as reasonably reproducible because the coefficient of variance (CV) was 3.94%. An empirical relationship between the dependent response and independent variables (P, τ, T) is described by the following equation (Eq. 6):

$$Y = 10.69+1.75\cdot P+0.86\cdot\tau-0.79\cdot T-0.89\cdot P\cdot\tau+0.85\cdot P\cdot T-0.86\cdot\tau\cdot T-1.46\cdot P^2-0.31\cdot\tau^2 0.75\cdot T^2 \quad (6)$$

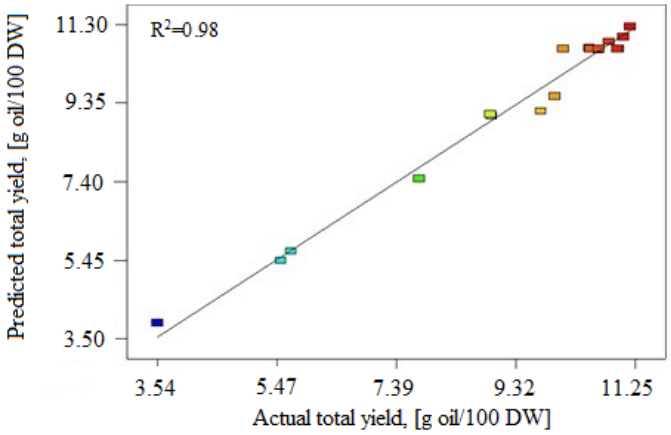


Figure 3.1. Comparison of the actual and predicted values for the lipophilic extract yield in SFE

3D plots (**Fig. 3.2**) show the impact of the independent variables and their interactions on the extract yield.

The pressure was the most important factor: its increase from 25 to 40 MPa resulted in the increase of the extract yield up to 11.16% (w/w). Temperature and time were less important parameters. In addition, a strong quadratic effect of all the independent variables (p and T) on the extract yield may be observed in **Figure 3.2**, A and B. It is well established that the solubilizing capacity of CO_2 depends on its density and diffusivity, while the increasing temperature has a different effect on these properties of the solvent. Similar SFE results were reported in many previously performed studies, e.g., in the case of optimizing oil extraction from amaranth seeds (Kraujalis and Venskutonis, 2013a). Plot C in **Figure 3.2** shows the interaction between the temperature and time; the changes of the extract yield were in a rather narrow range, from 9.72% to 11.16%, which indicates a weaker impact of time and temperature on the extract yield in the selected range of these variables. Thus, the optimal conditions for the highest extract yield (11.10±0.15%) were 42.4 MPa pressure, 53 °C temperature and 158 min extraction time. A similar yield (11.00±0.59%) was obtained in a Soxhlet extraction with hexane in 360 min. Basegmez *et al.* (2017) and Kryževičiutė *et al*. (2016) reported higher yields from raspberry and black currant pomace, respectively, under optimized SFE parameters, while Grunovaitė *et al*. (2016) recovered less than 3% from chokeberry pomace. These comparisons indicate that extraction processes should be developed individually for each natural material. To the best of our knowledge, two studies have applied SFE for cranberry pomace (Kühn and Temelli, 2017) and seeds previously (Bhagdeo *et al*., 2006). The seeds separated from other cranberry parts under optimized SFE conditions (45 MPa, 45 °C, 90 min, 1 mL/min CO_2 flow rate) yielded 21.4% of oil, which was slightly lower than in Soxhlet extraction (23.1%) (Bhagdeo *et al*., 2006). It is not surprising because the major part of berry oil is located in berry seeds. Kühn and Temelli (2017) reported a 5.52% yield from the

whole cranberry pomace at 40 MPa and 50 °C, which is 2 times lower than the values obtained in our study.

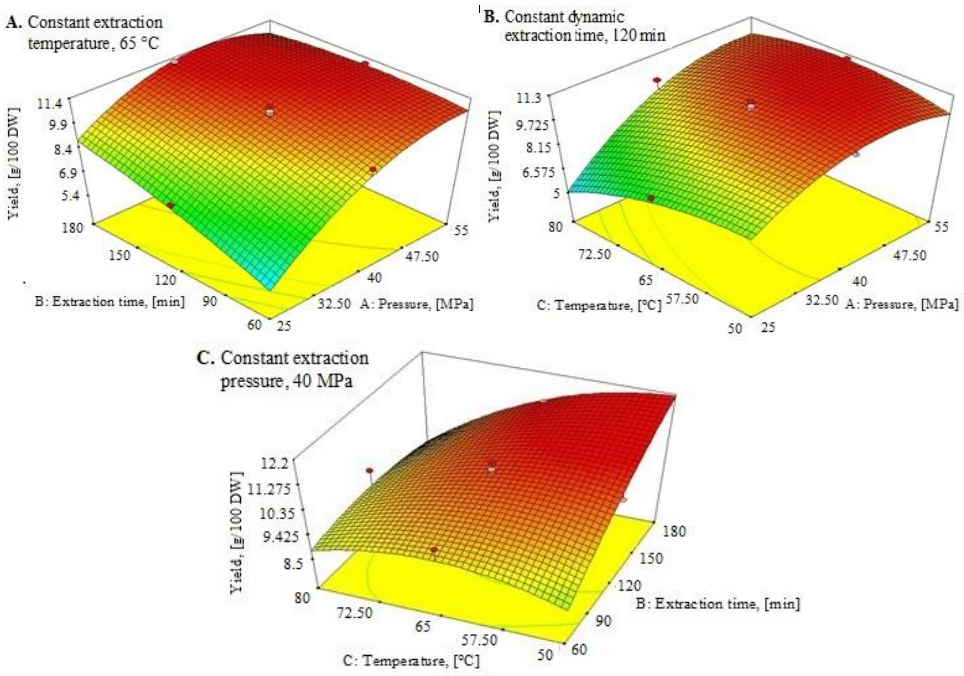


Figure 3.2. 3D response surface plots showing the effects of independent variables on extract yields during SFE: (A) time and pressure; (B) temperature and pressure; (C) time and temperature

The effect of the extraction time was additionally checked by the preliminary evaluation of extraction kinetics at the theoretically established optimal conditions (**Fig. 3.3**).

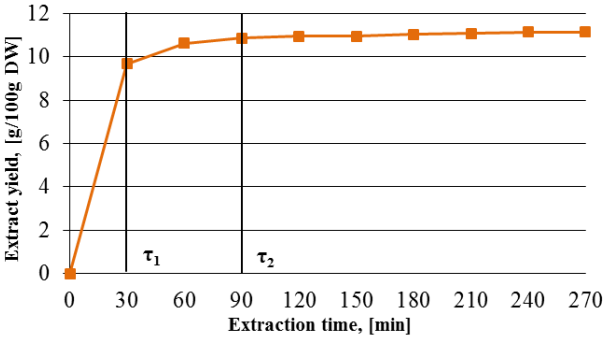


Figure 3.3. Kinetics of cranberry pomace SFE-CO_2 process at optimal extraction parameters (42.4 MPa, 53 °C). The shown results are means ± SD of three independent experiments

It should be noted that, despite the RSM-suggested concrete set of optimal parameters for the highest yield, it may be observed that, at 25 MPa and 50 °C, a

highly similar, although statistically lower, yield (10.65%) was obtained. The modeling of extraction kinetics was beyond the scope of this study; however, the extraction curve may be briefly commented based on the previously developed models (Pardo-Castaño, Velásquez and Bolaños, 2015; Sovová, 2017). It may be observed that the cranberry pomace extraction curve consists of an initial straight line and a transition segment until reaching a plateau at the 30 min extraction point. Sovová observed two distinct extraction phases when studying SFE of oil from ground grape seeds (Sovová, Kucera and Jež, 1994). This 2-step simplified extraction curve reflects the initial fraction (G=1) of extract in open cells (Sovová, 2012). In fact, 3 zones of SFE of cranberry pomace may be distinguished: the first zone (0–30 min), the fastest one, when the solubility is the dominating factor; the second zone (30–90 min) reflects the transition between solubility and mass transfer dominions; and the third zone (90+ min), the slowest one, is the segment in which the mass transfer from the inner particle part dominates. Therefore, the curve may also be divided into 3 extraction phases by separating a phase between τ_1 and τ_2 when G<1. However, there is no sharp transition between the second and the third parts of the curve, and, according to Sovová (2012), the division into three parts would not be necessary. Lipid adsorption on the biomass matrix may also be an important factor in slowing down the extraction rate; however, for vegetable oils, adsorption is not strong, e.g., if compared with algae (Sovová, Nobre and Palavra, 2016). Consequently, simplified models for SFE of natural matter might also be applicable for the kinetics of SFE of cranberry pomace. Therefore, for practical applications, the SFE time of cranberry pomace could be remarkably reduced if compared with the theoretically proposed optimal time by the RSM-generated model. Similar extraction kinetics was reported for the SFE of raspberry (Kryževičiutė *et al*., 2016) and blackcurrant (Basegmez *et al*., 2017) pomace: the major part of the extract was recovered during the first 0–60 min of extraction, while further extraction resulted in a very small increase of the yield. The selection of a different extraction pressure and temperature within a similar range of yields may be suggested for more detailed studies of SFE kinetics and thermodynamic solubility for berry pomaces. To the best of our knowledge, the modeling of solubility of berry lipophilic substances, mainly consisting of triacylglycerols, in SC-CO_2 have not been reported previously; however, such studies have been performed for other plant materials, e.g., green coffee beans (Cornelio-Santiago, Gonçalves, de Oliveira and de Oliveira, 2017), and grape seeds (Duba and Fiori, 2016). The thermophysical properties of triacylglycerols were estimated by using such a widely accepted thermodynamic approach as the Peng-Robinson equation of state. For instance, Duba and Fiori (2016) reported that the solubility of grape seed oil measured in a wide range of temperatures (40–80 °C) and pressures (20–50 MPa) was from 0.64–1.45 (80 °C, 20 MPa) to 15.99–37.32 g/kg (80 °C, 50 MPa). Considering that SFE exhaustively recovers lipophilic substances at optimal conditions: 42.4 MPa, 53 °C and 158 min (similar yields as in conventional extraction with hexane) without using toxic organic solvents and at a lower temperature, it may be considered as a promising method for the isolation of the nonpolar fraction from berry pomace.

3.1.3. Fatty acid profile of cranberry pomace oil obtained by SFE

The lipophilic fraction of berry pomace consists of macro- (triacylglycerols) and micro-components (free fatty acids, tocopherols, carotenoids, phytosterols, squalene and other compounds). As far as the fatty acid composition of oils is the most important characteristic of their nutritional quality, the fatty acid composition was determined for cranberry oil isolated at optimal SFE parameters: 42.4 MPa, 53 °C and 158 min (**Table 3.3**).

Table 3.3. Composition of fatty acids (g/100 g) in pomace oil extracted with SFE-CO_2

No.	Fatty acid	SFE-CO_2
1	Palmitic acid (C 16:0)	4.36±0.01[b]
2	Stearic acid (C 18:0)	0.85±0.03[a]
3	Oleic acid (C 18:1n9c)	21.79±0.07[c]
4	Linoleic acid (C 18:2n6c)	36.58±0.25[e]
5	Arachidic acid (C 20:0)	0.34±0.09[a]
6	α-Linolenic acid (C 18:3n3)	32.44±0.79[d]
7	Heneicosanoic acid (C 21:0)	0.35±0.04[a]
8	cis-11,14,17-Eicosadienoic acid (C 20:3n3)	0.47±0.02[a]
9	Lignoceric acid (C 24:0)	0.54±0.25[e]
10	cis-4,7,10,13,16,19-Docosahexaenoic acid (C 22:6n3)	0.09±0.03[a]
	∑ Monosaturated fatty acids	21.79±0.07
	∑ Polyunsaturated fatty acids	69.58±0.27
	∑ Saturated fatty acids	6.44±0.08

[a,b] Different letters indicate statistical differences (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of triplicate determinations

The fatty acid composition of oils isolated at other extraction parameters might slightly differ due to the variations in the solubility of triacylglycerols esterified with different fatty acids; however, the determination of such differences was beyond the scope of the present study. The main task was to recover all the supercritical CO_2-soluble lipids. Thus, SC-CO_2-extracted oil consisted mainly of polyunsaturated (PUFA, 69.58 g/100 g) and monounsaturated (MUFA, 21.79 g/100 g) fatty acids, while saturated fatty acids (SFA) constituted as little as 6.44 g/100g. Linoleic (n-6) and α-linolenic (n-3) acids were the major PUFA, more than 30% each, while oleic acid was the main MUFA. A high percentage of α-linolenic acid in the cranberry SC-CO_2 extract shows that this product is an excellent natural source of dietary n-3 PUFA, which is an essential nutrient in the human diet. Moreover, the ratio of n-6 to n-3 fatty acids in the extract is very low (1.1:1), which is very important in defining the nutritional value of dietary fats in terms of the current recommendation of reducing the ratio of n-6 to n-3 in the human diet. A number of studies demonstrated that a lower n-6/n-3 ratio may reduce the risk of cancer and/or heart diseases, or even improve the bone health (Aronson *et al*., 2001; Hu, 2001; Maillard *et al*., 2002). In general, this data is in agreement with the previously reported data for the cold-pressed cranberry seed oil (Parker, Adams, Zhou, Harris and Yu, 2003);

however, the latter contained a higher content of palmitic and stearic acids (SFA, 9.74 g/100 g) comparing with the SC-CO_2 extract.

3.2. Fractionation of cranberry pomace lipids by supercritical carbon dioxide extraction and on-line separation of extracts at low temperatures[2]

3.2.1. Yields of extracts and fractions and their antioxidant capacity

SFE is recognized as an excellent alternative for obtaining high value specialty oils from various seeds (Catchpole *et al*., 2009). Standard Soxhlet extraction with hexane yielded 11.00±0.59% of oil from cranberry pomace (**Table 3.4**). SFE with pure CO_2 gave a 21% lower yield, while the addition of 5% co-solvent EtOH increased the yield to the Soxhlet extraction level. In general, the differences between the sum of fractions and the crude extract yields were not considerable, and, in most cases, almost equal. In the case of SFE with pure SC-CO_2, 61–74% of the total extract was collected in 1S (HF), the remaining 26–39% (LF) was collected in 2S. Significant differences were found only in the case of -30 °C (for both fractions) and 0 °C for HF. In the case of SFE with SC-CO_2 + 5% EtOH, the effects of separator cooling were more temperature-dependent. The content of LF at 0 and -10 °C was higher than in case of pure SC-CO_2 by 67–68%; while, at -20 °C and -30 °C, it was higher by 2.9 and 3.5 times, respectively. The content of HF at 0 and -10 °C was similar in the case of pure SC-CO_2 and SC-CO_2/EtOH, while, at -20 °C and -30 °C, the addition of EtOH reduced the content of HF by 2.2 times. In SFE with 5% EtOH, the content of HF was 2.7–3 times higher than that of LF. It is known that a co-solvent may increase the solubility selectively or non-selectively by an increase of the solvent density and/or intermolecular interactions (Güçlü-Üstündağ and Temelli, 2005).

Polar co-solvent EtOH extracts some higher polarity compounds together with soluble in SC-CO_2 nonpolar lipids. It seems that, at 0 and -10 °C, these extra compounds precipitate together with SC-CO_2 soluble LF. However, the solubility behavior and phase equilibria of a complex system becomes completely different when the separator is cooled to lower separation temperatures, namely, to -20 °C and -30 °C. It would be fairly difficult to explain this phenomenon from the results of this study due to the complexity of the system consisting of numerous compounds belonging to different chemical classes and therefore possessing different properties; however, these preliminary findings with the real plant material encourage studies featuring simplified models, e.g., consisting of fewer SC-CO_2 and EtOH soluble compounds present in berry pomace.

[2] This section was prepared with reference to Tamkutė, Pukalskas, Syrpas, Urbonavičienė and Veskutonis (2020)

Table 3.4. Fraction yields of cranberry pomace SFE-CO_2 extracts and their oxygen radical scavenging capacity (ORAC)

Fraction	EtOH, %	Separator temperature, ℃	Yield, %	ORAC, mg TE/g extract	ORAC, mg TE/g DW
Soxhlet extraction with hexane					
			11.00±0.59^{i}	11.84±0.71^{bc}	1.30±0.08^{c}
SFE-CO_2					
Total	0	-	8.71±0.01^{h}	19.96±1.04^{d}	1.74±0.09^{d}
Total	5	-	10.88±0.02^{i}	33.84±1.65^{ef}	3.68±0.18^{h}
LF	0	0	2.90±0.46^{a}	37.68±1.66^{fg}	1.09±0.05^{bc}
		-10	2.78±0.11^{bc}	33.17±1.54^{e}	0.92±0.04^{b}
		-20	2.79±0.02^{bc}	31.33±1.19^{e}	0.87±0.03^{b}
		-30	2.23±0.01^{ab}	39.21±0.94^{g}	0.87±0.02^{b}
LF	5	0	4.83±0.02^{d}	79.69±3.74^{i}	3.85±0.18^{h}
		-10	4.67±0.13^{d}	51.84±4.14^{h}	2.42±0.19^{e}
		-20	8.18±0.03^{gh}	42.16±2.71^{e}	3.45±0.22^{f}
		-30	7.91±0.02^{g}	40.15±2.49^{g}	3.18±0.20^{g}
HF	0	0	6.86±0.46^{f}	5.68±0.37^{a}	0.39±0.03^{a}
		-10	5.94±0.11^{e}	6.02±0.61^{a}	0.36±0.04^{a}
		-20	5.93±0.02^{e}	5.94±0.32^{a}	0.35±0.02^{a}
		-30	6.48±0.01^{ef}	8.34±0.64^{ab}	0.54±0.04^{a}
HF	5	0	6.05±0.02^{e}	8.40±0.68^{ab}	0.51±0.04^{a}
		-10	6.21±0.13^{e}	14.51±0.90^{c}	0.90±0.06^{b}
		-20	2.69±0.03^{bc}	14.22±0.60^{c}	0.38±0.02^{a}
		-30	2.96±0.02^{c}	13.36±0.59^{c}	0.40±0.02^{a}

*HF – heavier fraction; *LF – lighter fraction; DW – dry pomace weight; ^{a,b} Different letters in the same column indicate statistical differences (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of triplicate determinations

The antioxidant capacity of extracts and fractions was measured by using an L-ORAC assay which measures the peroxyl radical scavenging capacity of antioxidants which may donate a hydrogen atom (Prior *et al*., 2003). This assay was selected as more relevant to oxidation processes taking place in biological systems than, for instance, the widely used DPPH radical, ABTS radical cation scavenging and ferric radical antioxidant power assays which are based on single electron transfer reactions. First of all, it may be noted that the ORAC values of SC-CO_2 extracts were remarkably higher than those of Soxhlet extracts. Moreover, co-solvent EtOH increased ORAC by 70% compared to SFE with pure SC-CO_2. It is not surprising; a polar co-solvent may add to the extract polar antioxidants, e.g., polyphenolic compounds. For instance, CO_2/EtOH/H_2O mixtures yielded cranberry pomace extracts with a high total phenolic content and antioxidant activity (Kühn and Temelli, 2017). LFs were several times stronger antioxidants than HFs both in the case of SFE with pure SC-CO_2 and co-solvent; meanwhile, the fractions isolated with a co-solvent were remarkably stronger antioxidants compared to the fractions

obtained under similar separation parameters with pure SC-CO_2. The highest ORAC values were determined for the extracts which precipitated at 0 °C (79.69±3.74 mg TE/g extract). In fact, the major part of the oxygen radical absorbing compounds was collected in the LF. However, it should be noted that the sums of the ORAC values of LFs and HFs separated at 0 and -20 °C were higher than the ORAC value measured for the crude extract. It may be assumed that the differences in the complex composition of antioxidants present in the crude extract and fractions may have an effect on the reactions used in the ORAC assay. Also, the redistribution of susceptible to oxidation polyunsaturated fatty acids may contribute to these differences.

3.2.2. Composition of triacylglycerols (TAGs) and fatty acids

The oily fraction of berry seeds is composed mainly of TAGs. The molar mass and the unsaturation level of the fatty acid molecules characterize their physical, chemical and nutritive properties and are important for the uses in the food and other industries. For instance, the content of solids as a function of temperature is important for margarines and culinary fats, while the content of n-3 and n-6 polyunsaturated fatty acids as well as their ratio is important in terms of the nutritional value. The solubility of oils decreases by increasing their molecular mass (Catchpole *et al*., 2009).

Highly unsaturated TAGs were majorly found in the extracts and fractions. The LLLn constituted 22.14–23.46%, OLnL 21.26–22.23%, LLnLn 13.00–14.93% and OLL 13.69–15.59% (**Table 3.5**). The differences in the content of the two major TAGs, LLLn and OLnL, were not high, although ANOVA indicated significant differences between many values presented in **Table 3.5**. The tendency of a slightly higher content of highly unsaturated LLLn and, more remarkably, a higher amount of LnLnLn may be observed in LF than in HF. On the contrary, the percentage of stearic acid containing SLL and SLO was higher in all HFs. The content of OLL was higher in HFs as well. Most likely, it may be explained by the larger molecular mass and the higher melting point of saturated and monounsaturated acids containing TAGs; however, more precise data may be obtained only by studying the thermodynamic solubility of pure TAGs. Studies of high TAGs are fairly scarce, while the differences in the oil solubility of oils of various origins may be remarkable. For instance, Soares, Gamarra, Paviani, Gonçalves and Cabral (2007) reported that in the SFE range of 40–80 °C/20–35 MPa, the solubility of babassu, uccuuba (both rich in C12:0 and C14:0 fatty acids), sunflower and corn (both rich in C18:1 and C18:2 fatty acids) were in the ranges of 4.8–51.4; 3.17–38.0; 0.2–9.9; and 0.7–11.4 g/kg, respectively. The solubility of grape seed oil containing a high percentage of free fatty acids, mono- and diglycerides, was 2 times larger than the solubility of refined blackcurrant seed oil, and it decreased after most of the free fatty acids had been extracted (Sovová, Zarevúcka, Vacek and Stránský, 2001).

Table 3.5. Composition of triacylglycerols (TAGs, %) of cranberry pomace oil in SFE-CO_2 extracts and fractions

TAG	Pure SFE-CO_2								
	Not fractionated	0ºC		-10		-20		-30	
		HF	LF	HF	LF	HF	LF	HF	LF
LLLn	22.19±0.30^{a}	22.40±0.20^{a}	23.46±0.16^{b}	22.25±0.07^{a}	23.46±0.09^{b}	22.14±0.21^{a}	23.36±0.06^{b}	22.48±0.18^{a}	23.59±0.18^{b}
LnLnLn	5.08±0.04^{c}	4.87±0.15^{ab}	5.81±0.11^{f}	4.85±0.09^{ab}	5.68±0.01^{ef}	4.72±0.06^{a}	5.54±0.08^{de}	4.93±0.07^{bc}	5.47±0.09^{d}
NK1	3.81±0.07^{a}	3.97±0.09^{ab}	4.41±0.08^{e}	3.94±0.03^{ab}	4.33±0.02^{de}	4.16±0.08^{cd}	4.39±0.05^{e}	4.02±0.09^{bc}	4.09±0.11^{bc}
SLO	1.99±0.06^{d}	1.92±0.06^{cd}	1.42±0.08^{a}	1.92±0.03^{cd}	1.46±0.01^{a}	1.90±0.06^{cd}	1.51±0.04^{ab}	1.84±0.05^{c}	1.60±0.07^{b}
PLS	0.65±0.02^{d}	0.60±0.04^{bcd}	0.54±0.05^{ab}	0.62±0.02^{cd}	0.53±0.02^{ab}	0.65±0.04^{d}	0.55±0.02^{abc}	0.64±0.04^{d}	0.51±0.03^{a}
LLnLn	13.56±0.09^{b}	13.25±0.12^{ab}	14.93±0.19^{e}	13.24±0.18^{ab}	14.75±0.07^{de}	13.00±0.15^{a}	14.44±0.11^{cd}	13.21±0.17^{ab}	14.29±0.22^{c}
OLL	15.40±0.08^{d}	15.51±0.20^{d}	13.69±0.09^{a}	15.59±0.17^{d}	13.99±0.11^{ab}	15.59±0.17^{d}	14.23±0.08^{bc}	15.45±0.12^{d}	14.39±0.14^{c}
PLL	4.35±0.07^{a}	4.47±0.07^{abc}	4.67±0.07^{d}	4.49±0.07^{abcd}	4.59±0.06^{bcd}	4.66±0.11^{ed}	4.59±0.15^{bcd}	4.54±0.05^{abcd}	4.43±0.03^{ab}
PLnLn	1.83±0.07^{a}	1.92±0.02^{ab}	2.25±0.02^{e}	1.92±0.03^{ab}	2.21±0.03^{de}	2.04±0.03^{c}	2.19±0.04^{de}	2.01±0.03^{bc}	2.11±0.07^{cd}
OLnL	21.88±0.19^{bc}	22.23±0.22^{c}	21.26±0.27^{a}	22.20±0.24^{c}	21.36±0.18^{a}	22.19±0.05^{c}	21.42±0.29^{ab}	22.14±0.23^{c}	21.60±0.14^{ab}
PLO	2.21±0.18^{b}	2.10±0.06^{ab}	2.03±0.09^{ab}	2.15±0.03^{ab}	2.01±0.02^{a}	2.21±0.06^{b}	2.07±0.04^{ab}	2.11±0.05^{ab}	1.99±0.05^{a}
SLL	6.90±0.04^{e}	6.76±0.06^{de}	5.53±0.04^{a}	6.82±0.09^{e}	5.63±0.10^{ab}	6.74±0.08^{de}	5.77±0.05^{bc}	6.62±0.03^{d}	5.89±0.13^{c}
TAG	SFE-CO_2 + 5% EtOH								
	Not fractionated	0ºC		-10		-20		-30	
		HF	LF	HF	LF	HF	LF	HF	LF
LLLn	22.68±0.18^{b}	22.50±0.17^{b}	23.29±0.02^{c}	22.48±0.19^{b}	23.57±0.12^{c}	21.23±0.12^{a}	23.28±0.12^{c}	21.38±0.33^{a}	22.81±0.28^{b}
LnLnLn	5.08±0.09^{bc}	5.09±0.12^{bc}	5.44±0.14^{c}	5.03±0.12^{b}	5.36±0.12^{bc}	4.27±0.21^{a}	5.27±0.20^{bc}	4.54±0.26^{a}	5.18±0.21^{bc}
NK1	4.09±0.05^{ab}	4.08±0.02^{ab}	4.20±0.04^{b}	4.14±0.08^{ab}	4.14±0.10^{ab}	4.20±0.16^{ab}	3.95±0.09^{a}	4.20±0.21^{ab}	4.18±0.05^{ab}
SLO	1.84±0.05^{bc}	1.81±0.06^{bc}	1.67±0.04^{ab}	1.88±0.09^{c}	1.57±0.01^{a}	2.36±0.06^{d}	1.69±0.02^{abc}	2.27±0.19^{d}	1.69±0.04^{abc}
PLS	0.63±0.02^{cd}	0.65±0.02^{d}	0.57±0.04^{ab}	0.59±0.01^{abcd}	0.54±0.03^{a}	0.82±0.04^{e}	0.56±0.01^{a}	0.64±0.03^{bcd}	0.58±0.02^{abc}
LLnLn	13.46±0.18^{bcd}	13.29±0.35^{bc}	13.80±0.25^{cde}	13.12±0.19^{b}	14.08±0.19^{e}	12.04±0.22^{a}	14.01±0.13^{de}	12.21±0.30^{a}	13.71±0.20^{cde}
OLL	15.23±0.07^{cd}	15.42±0.12^{d}	14.52±0.08^{a}	15.48±0.11^{d}	14.53±0.04^{ab}	16.84±0.19^{e}	14.88±0.12^{bc}	16.59±0.32^{e}	14.84±0.08^{ab}
PLL	4.56±0.05^{bc}	4.55±0.04^{bc}	4.52±0.07^{bc}	4.66±0.07^{cd}	4.43±0.08^{ab}	4.87±0.17^{d}	4.31±0.05^{a}	4.81±0.12^{d}	4.69±0.03^{cd}
PLnLn	1.96±0.04^{bc}	1.99±0.04^{bc}	2.06±0.06^{c}	2.00±0.03^{bc}	2.07±0.03^{c}	1.79±0.11^{a}	2.03±0.06^{bc}	1.89±0.12^{ab}	2.05±0.04^{c}
OLnL	21.71±0.35^{a}	21.90±0.24^{a}	21.77±0.24^{a}	21.73±0.07^{a}	21.83±0.21^{a}	22.07±0.32^{a}	21.84±0.05^{a}	22.33±0.38^{a}	21.81±0.22^{a}
PLO	2.14±0.04^{a}	2.11±0.03^{a}	2.03±0.05^{a}	2.18±0.11^{a}	1.98±0.09^{a}	2.51±0.23^{b}	1.97±0.04^{a}	2.18±0.12^{a}	2.10±0.03^{a}
SLL	6.61±0.09^{d}	6.63±0.07^{d}	6.12±0.03^{ab}	6.66±0.04^{d}	6.04±0.07^{a}	7.87±0.11^{f}	6.20±0.03^{bc}	7.61±0.04^{e}	6.36±0.08^{c}

*HF – heavier fraction; *LF – lighter fraction; L – linoleic acid; Ln – linolenic acid; O – oleic acid; P – palmitic acid; S – stearic acid; NK1 – not identified TAG; [a,b] Different letters in the same row indicate statistical differences (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of duplicate determinations

Table 3.6. Composition of fatty acids (g/100 g) and squalene (%) of cranberry pomace oil in SFE-CO_2 extracts and fractions

Fatty acid	Pure SFE-CO_2								
	Not fractionated	0ºC		-10		-20		-30	
		HF	LF	HF	LF	HF	LF	HF	LF
C 16:0	4.36±0.01^{ab}	4.18±0.04^{a}	6.16±0.64^{c}	4.88±0.54^{ab}	4.84±0.06^{ab}	4.22±0.14^{a}	5.26±0.07^{abc}	4.56±0.19^{ab}	5.48±0.10^{bc}
C 18:0	0.85±0.03^{abc}	0.81±0.07^{a}	0.78±0.00^{a}	0.97±0.01^{d}	0.81±0.02^{ab}	0.93±0.01^{cd}	0.86±0.02^{abc}	0.92±0.01^{bcd}	0.84±0.01^{abc}
C 18:1n9c	21.79±0.07^{bc}	21.48±0.09^{b}	18.70±0.47^{a}	23.12±0.11^{d}	21.49±0.08^{b}	21.11±0.47^{b}	20.90±0.01^{b}	22.50±0.17^{cd}	21.42±0.21^{b}
C 18:2n6c	36.58±0.25^{b}	35.28±0.07^{ab}	34.38±0.94^{a}	36.11±0.47^{b}	36.70±0.08^{b}	33.99±0.04^{a}	36.64±0.04^{b}	35.20±0.07^{ab}	36.51±0.39^{b}
C 20:0	0.34±0.09^{ab}	0.48±0.00^{ab}	0.52±0.12^{b}	0.36±0.04^{ab}	0.36±0.03^{ab}	0.47±0.02^{ab}	0.33±0.00^{ab}	0.51±0.06^{ab}	0.29±0.03^{a}
C 18:3n3	32.44±0.79^{d}	30.96±0.02^{bc}	28.66±0.02^{a}	29.88±0.31^{ab}	31.76±0.03^{cd}	29.08±0.42^{a}	31.29±0.02^{cd}	29.53±0.27^{a}	31.04±0.34^{bc}
C 21:0	0.35±0.04^{a}	0.98±0.11^{b}	0.45±0.19^{a}	0.30±0.10^{a}	nd	1.06±0.02^{b}	nd	0.45±0.01^{a}	nd
C20:3n3	0.47±0.02^{ab}	0.60±0.05^{ab}	5.24±0.83^{c}	nd	1.50±0.05^{b}	nd	1.16±0.01^{ab}	nd	1.14±0.08^{ab}
Squalene	0.54±0.25^{ab}	0.91±0.09^{bc}	0.30±0.00^{a}	nd	nd	nd	nd	1.19±0.09^{c}	nd
C 22:6n3	0.09±0.03^{a}	0.13±0.03 ^{a}	1.01±0.06^{b}	0.55±0.33^{ab}	0.19±0.00^{a}	2.02±0.17^{c}	0.36±0.04^{a}	0.52±0.04^{ab}	0.29±0.02^{a}
Fatty acid	SFE-CO_2 + 5% EtOH								
	Not fractionated	0ºC		-10		-20		-30	
		HF	LF	HF	LF	HF	LF	HF	LF
C 16:0	4.51±0.27^{abcd}	3.66±0.66^{a}	4.60±0.22^{abcd}	3.79 ±0.06^{ab}	5.66±0.39^{d}	4.06±0.09^{abc}	5.03±0.29^{bcd}	3.97±0.17^{ab}	5.24±0.30^{cd}
C 18:0	0.76±0.02^{ab}	0.81±0.04^{ab}	0.78±0.03^{ab}	0.80±0.01^{ab}	0.81±0.02^{ab}	0.96±0.05^{b}	0.86±0.03^{b}	0.63±0.14^{a}	0.84±0.01^{b}
C 18:1n9c	20.54±0.02^{bc}	19.01±1.74^{b}	21.39±0.20^{bc}	20.13±0.01^{b}	20.29±0.32^{bc}	22.69±0.01^{c}	20.71±0.07^{bc}	13.44±0.01^{a}	20.93±0.17^{bc}
C 18:2n6c	36.34±0.08^{cd}	29.58±0.93^{b}	36.58±0.13^{d}	32.27±0.08^{bcd}	35.11±0.71^{cd}	32.14±0.58^{bc}	36.17±0.13^{cd}	25.00±0.02^{a}	36.37±0.37^{cd}
C 20:0	0.30±0.02^{a}	0.36±0.03^{ab}	0.46±0.01^{abc}	0.41±0.05^{ab}	0.56±0.03^{bcd}	0.87±0.09^{e}	0.72±0.12^{de}	nd	0.70±0.05^{cde}
C 18:3n3	33.17±0.27^{c}	24.77±0.19^{b}	32.21±0.15^{de}	27.79±0.23^{bc}	29.64±0.17^{cd}	25.18±0.57^{b}	30.17±0.52^{cde}	20.62±0.14^{a}	30.43±0.14^{cde}
C 21:0	0.15±0.00^{a}	0.72±0.05^{b}	nd	0.67±0.09^{b}	nd	1.15±0.10^{c}	nd	1.27±0.15^{c}	nd
C 22:0	0.45±0.00^{a}	1.37±0.29^{b}	0.46±0.06^{a}	1.05±0.02^{b}	0.84±0.19^{ab}	1.24±0.02^{b}	0.88±0.10^{ab}	3.41±0.23^{c}	0.86±0.05^{ab}
C20:3n3	0.31±0.10^{a}	nd	0.84±0.18^{ab}	nd	1.41±0.33^{ab}	nd	1.31±0.56^{ab}	nd	1.67±0.25^{b}
Squalene	1.11±0.00^{ab}	8.67±0.94^{cd}	0.54±0.14^{a}	4.57±0.14^{ab}	1.41±0.50^{ab}	4.75±0.09^{bc}	0.98±0.17^{ab}	12.09±0.67^{d}	1.00±0.14^{ab}
C 22:6n3	0.13±0.08^{a}	1.79±0.07^{bc}	nd	1.11±0.06^{ab}	0.73±0.31^{ab}	1.26±0.11^{ab}	0.57±0.27^{ab}	3.27±0.05^{c}	0.43±0.10^{ab}

*HF – heavier fraction; *LF – lighter fraction; *nd – not detected; ^{a,b} Different letters in the same row indicate statistical differences (one-way ANOVA, $p < 0.05$). Palmitic (C 16:0), Stearic (C 18:0), Oleic (C 18:1n9c), Linoleic (C 18:2n6c), Arachidic (C 20:0), Linolenic (C 18:3n3), Heneicosanoic (C 21:0), Behenic (C 22:0), 11,14,17-Eicosadienoic (C20:3n3), 4,7,10,13,16,19-Docosahexaenoic (C 22:6). The results are expressed as mean ± standard deviation of triplicate determinations

Oleic, linoleic and linolenic acids were major components in cranberry oil (**Table 3.6**). So far as fatty acids are determined as the components of TAGs after hydrolysis and esterification, it would not be possible to discuss their distribution between separators based on the thermodynamic solubility data of pure fatty acids. However, some tendencies may still be observed. In the case of pure SC-CO_2, significantly lower percentages of palmitic acid were determined in LF, except for -10 °C. Significantly, although not remarkably, lower percentages of linoleic acid in LF were also determined, while the content of the second major, linolenic, acid in LF was higher, except for separation at 0 °C. Most remarkable differences were found for minor acids; for instance, the percentage of C20:3n3 was almost 9 times higher in LF, while, at other separation temperatures, it was not detected in HF at all. The content of C22:6n3 was between 0.13–2.02% and 0.19–1.01% in HFs and LFs, respectively. Some specific tendencies may also be observed in the case of the extraction with co-solvent EtOH. The content of palmitic, linoleic and linolenic acids was significantly, and, in some cases, remarkably, higher in all the LFs. Co-solvent EtOH remarkably increased the content of squalene in the extracts. Moreover, this valuable bioactive component was effectively separated; its content in HF was up to 16 times higher than in LF. Hoed *et al*. (2009) reported 617.5 mg of squalene in 100 g of cranberry seed oil. It is a highly unsaturated hydrocarbon (a constituent of the waxy fraction), and the liquid phase of CO_2 was reported as an effective on-line method to eliminate waxes from crude SC-CO_2 extracts of various plant materials (Perrut and Perrut, 2018).

3.2.3. Total content of carotenoids and β-carotene

Carotenoids are important plant pigments and valuable bioactive compounds providing various physiological benefits to human health. Although carotenoids are lipophilic compounds, their recovery by SFE is not an easy task; usually, higher recovery rates are achieved by using standard extraction with hexane. The solubility of carotenoids in SC-CO_2 depends on the solubility of the main system's component (TAGs) and may be facilitated by higher ratios of carotenoids-to-oil, as it was recently demonstrated in the SFE studies of microalgae (Sovová and Stateva, 2019). In this study, the concentration of β-carotene and the total amount of carotenoids in hexane extracts were used for comparison purposes (**Table 3.7**). It may be observed that less than half of carotenoids was isolated with pure SC-CO_2, however, co-solvent EtOH remarkably increased the efficiency of carotenoid extraction. It was reported that the solubility of β-carotene decreases by decreasing SC-CO_2 pressure and temperature, while EtOH co-solvent, depending on SFE temperature and pressure, remarkably increases its solubility (Catchpole *et al*., 2009; Sovová *et al*., 2001). The pattern of β-carotene and the total carotenoid redistribution in the separators is quite complicated; however, some tendencies may still be observed. First of all, remarkably higher concentrations of these pigments were detected in the HFs in the case of SFE with pure SC-CO_2. For instance, the concentration of β-carotene and the total carotenoids in the fractions collected in 2S was remarkably higher than in LF. Moreover, HFs obtained at 0, -20 and -30 °C contained higher β-

carotene concentration than the hexane extracts. When EtOH was added, the concentration of β-carotene was similar in both fractions, except for the temperature of -10 °C. It seems that this temperature is somewhat critical for carotenoids extracted with SC-CO_2; their concentration in LF was considerably lower than in the fractions collected at other temperatures. In the case of pure SC-CO_2, it was also lower in HF, although the differences were less remarkable. However, a decrease of the separator's temperature to -20 and -30 °C increased the concentration of carotenoids in LF.

Table 3.7. Content of β-carotene and total carotenoids in cranberry pomace extracts and fractions

Fraction	EtOH, %	Separator temperature, °C	β-carotene, mg/100 g extract	β-carotene recovery, %	Total carotenoids, mg/100 g extract	Total carotenoids recovery, %
Soxhlet extraction						
			6.07 ± 0.08^{gh}	100^{i}	23.72 ± 0.02^{h}	100^{i}
SFE-CO_2						
Total	0	-	3.48 ± 0.09^{cd}	45.39 ± 1.19^{f}	11.98 ± 0.47^{cde}	39.97 ± 1.56^{fg}
Total	5	-	4.02 ± 0.25^{de}	65.56 ± 4.06^{g}	15.89 ± 0.00^{efg}	66.25 ± 0.02^{h}
LF	0	0	2.38 ± 0.20^{bc}	10.33 ± 0.85^{ab}	5.92 ± 0.59^{ab}	6.58 ± 0.65^{ab}
		-10	1.69 ± 0.14^{ab}	7.02 ± 0.58^{a}	3.98 ± 0.36^{a}	4.24 ± 0.39^{a}
		-20	3.02 ± 0.12^{cd}	12.63 ± 0.50^{abc}	6.34 ± 0.17^{abc}	6.78 ± 0.18^{ab}
		-30	4.75 ± 0.11^{ef}	15.85 ± 0.38^{abc}	11.02 ± 0.57^{bcde}	9.42 ± 0.49^{ab}
LF	5	0	3.06 ± 0.17^{cd}	22.14 ± 1.21^{cd}	6.96 ± 0.15^{abc}	12.87 ± 0.28^{abc}
		-10	1.06 ± 0.00^{a}	7.43 ± 0.01^{ab}	3.83 ± 0.05^{a}	6.84 ± 0.09^{ab}
		-20	3.40 ± 0.15^{cd}	41.68 ± 1.85^{ef}	9.09 ± 0.03^{abcd}	28.49 ± 0.08^{def}
		-30	3.47 ± 0.23^{cd}	41.17 ± 2.70^{ef}	8.04 ± 0.40^{abcd}	24.37 ± 1.21^{cde}
HF	0	0	7.48 ± 0.65^{i}	76.87 ± 6.70^{h}	22.88 ± 3.08^{h}	60.12 ± 8.10^{h}
		-10	5.57 ± 0.38^{fg}	49.51 ± 3.37^{f}	16.25 ± 1.93^{efg}	36.99 ± 4.39^{efg}
		-20	7.07 ± 0.18^{hi}	62.81 ± 1.61^{g}	20.11 ± 0.25^{gh}	45.68 ± 0.58^{g}
		-30	7.01 ± 0.55^{hi}	68.00 ± 5.34^{gh}	18.57 ± 1.22^{fgh}	46.11 ± 3.04^{g}
HF	5	0	3.47 ± 0.31^{cd}	31.34 ± 2.83^{de}	12.61 ± 3.99^{de}	29.22 ± 9.24^{def}
		-10	3.14 ± 0.12^{cd}	29.17 ± 1.15^{d}	11.63 ± 0.25^{cde}	27.67 ± 0.60^{def}
		-20	3.49 ± 0.12^{cd}	14.05 ± 0.47^{abc}	13.53 ± 1.17^{def}	13.94 ± 1.21^{abc}
		-30	4.01 ± 0.51^{de}	17.78 ± 2.28^{bc}	16.26 ± 1.94^{efg}	18.45 ± 2.20^{bcd}

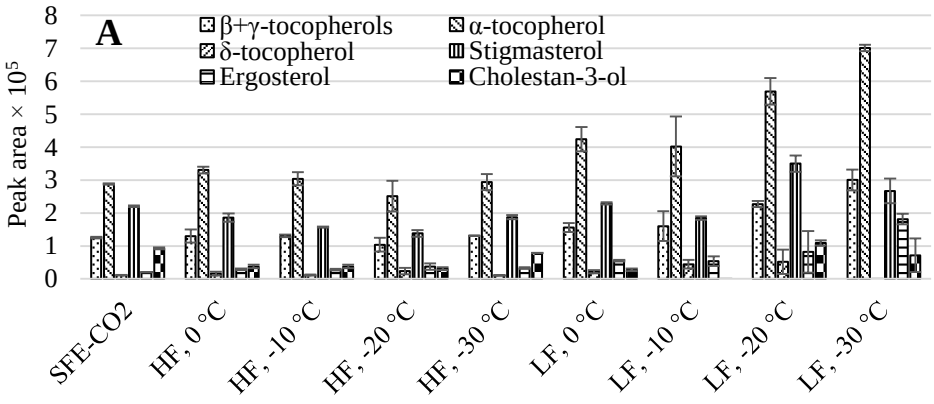
*HF – heavier fraction; *LF – lighter fraction; ^{a,b} Different letters in the same column indicate statistical differences (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of triplicate determinations

Based on the obtained results, it would be difficult to explain the variations in the carotenoid redistribution between the fractions obtained at different cooling temperatures. The thermodynamic solubility of individual compounds, the molecular interactions between them, the phase equilibria and even the influence of compositional changes on the analytical procedure (β-carotene is determined by HPLC, while the total amount of carotenoids is detected by the spectrophotometric method) may have some impact. The data correlating the solubility behavior of minor lipid components in SC-CO_2 (Güçlü-Üstündağ and Temelli, 2004), the

findings on the solubility behavior of model systems of lipids, co-solvents and SC-CO_2 (Güçlü-Üstündağ and Temelli, 2005), as well as the solubility data of individual carotenoids (Shi, Mittal, Kim and Xue, 2007) may provide the basis for studying multicomponent lipid mixtures; however, the solubility data of such substances in liquid CO_2 at freezing temperatures is not available.

3.2.4. Composition of tocopherols and phytosterols

Tocopherols are valuable lipophilic antioxidants, while phytosterols also are denoted by various other properties and health benefits. Therefore, natural products with high concentrations of these bioactive constituents are of interest in developing functional foods and nutraceuticals. Four tocopherols (sometimes also called *E-vitamers*) and 4 phytosterols were preliminarily quantified by their peak areas in the extracts and fractions (**Fig. 3.4**). α-Tocopherol was dominating in cranberry pomace oil, yet it was followed not by chromatographically separated β+γ-vitamers and δ-tocopherol. Based on the chromatographic peak areas, the content of β-sitosterol (**Fig. 3.5**) was many times higher than the content of other detected phytosterols, namely, stigmasterol, ergosterol and cholestan-3-ol. β-Sitosterol was reported as the major phytosterol in several other seed oils including cranberry (Hoed *et al*., 2009).



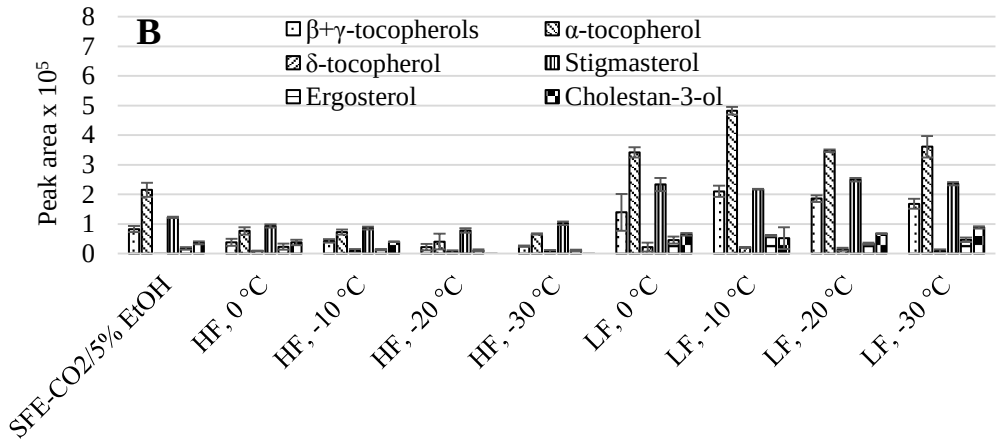


Figure 3.4. Changes of the content of tocopherols and phytosterols during cranberry pomace fractionation with pure SC-CO_2 (A) and SC-CO_2/5% EtOH (B). *HF – heavier fraction; *LF – lighter fraction. [a,b] Different letters in the same color column indicate statistical differences (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of duplicate determinations

It may be observed that, in the case of pure SC-CO_2, the concentration of tocopherols was higher in LFs, while the content of phytosterols was slightly higher in HF. When 5% EtOH was introduced, the distribution of these compounds between the separators was different: LFs contained a remarkably higher content of tocopherols and phytosterols. For instance, the concentration of α-tocopherol in LF separated at -10 °C in 2S was 7 times higher than in HF precipitated in 1S; the content of β-sitosterol, depending on the separation temperature, was approximately 2.2 to 3.8-fold higher. The decreasing pressure in the separators enabled to produce fractions with a higher concentration of tocopherols from amaranth seeds (Kraujalis and Venskutonis, 2013b).

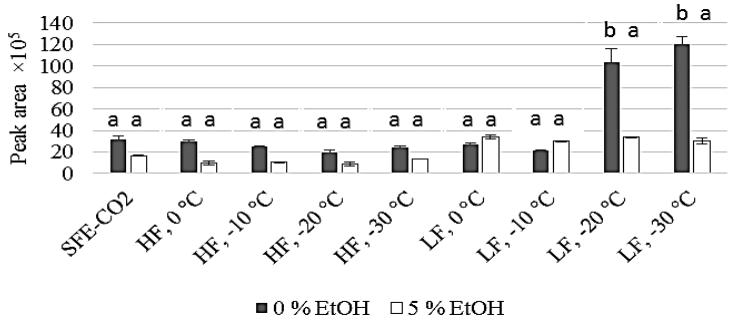


Figure 3.5. β-sitosterol content changes during cranberry pomace fractionation with SFE-CO_2 with or without co-solvent (5% EtOH). *HF – heavier fraction; *LF – lighter fraction. The results are expressed as mean ± standard deviation of duplicate determinations. a,b Different letters in the same column color indicate statistical differences (one-way ANOVA, $p < 0.05$)

3.2.5. Volatile constituents

Cranberry pomace SC-CO_2 extracts and, particularly, LFs possessed strong and pleasant cranberry-like aroma. Therefore, it was of interest to evaluate the redistribution of volatile aroma compounds between the separators. Several volatile compounds, such as alcohols, acids, terpenoids, and aldehydes, which are developed during the fruit maturation and ripening are known to contribute to the overall aroma characteristics of cranberries (Zhu *et al*., 2016). The list of detected and identified in cranberry pomace extract compounds is presented in **Table 3.8** which also includes the peaks showing a high (≥800) mass spectral similarity as compared to the libraries and whose retention index variation did not exceed 10 comparing with the previously reported data. The application of low temperatures in 1S demonstrated substantial variation both in the qualitative and, mainly, quantitative composition of volatiles. As it is evident from the representative chromatograms (**Fig. 3.6, A–F**), the isolation of aroma compounds in the collected HFs was extremely low, whereas, in all the cases, LFs were characterized by the presence of various aroma compounds. It is characteristic that, for most of the detected compounds, the peak areas in LFs were by the levels of magnitude higher than in HFs. The volatile profile of all LFs was similar, with small variations observed in the quantitative manner among the various applied separation temperatures.

A distinctive peak appearing in all the fractions was the compound eluting at 990 s with a measured mass of 122.0368; it was identified as benzoic acid. Not surprisingly, naturally occurring benzoic acid which has a sweet, honey-like aroma has been reported to be a major phenolic substance in cranberry fruits and juices (Chen, Zuo and Deng, 2001; Zuo *et al*., 2002). It should also be noted that benzoic acid is a strong antimicrobial agent. Other aromatic compounds, such as benzaldehyde and benzyl alcohol, were present primarily in the light SC-CO_2 extracts. Benzyl alcohol, which has a mild pleasant, sweet, flowery odor, has been reported as one of the main aroma contributors of cranberry juices or wines (Zhang, Chen, Chen, Kilmartin and Quek, 2019). Other aroma active substances, which were identified in previous studies as well, include aldehydes (decenal, decadienal) and alcohols (phenylethyl alcohol, nonanol) (Jambrak *et al*., 2017; Zhu *et al*., 2016). Monoterpenes, including α and β-terpineols, α-pinene, β-caryophyllene and limonene, have been previously reported in cranberry juices (Jambrak *et al*., 2017; Zhu *et al*., 2016). Overall, it should be noted that the application of low temperatures in 1S during SC-CO_2 extraction leads to production of fractions retaining a substantial amount of aroma substances. It may be expected that an aroma volatiles-rich fraction might be of great interest as natural aroma for foods and even as a fragrance for cosmetics and perfumery.

Table 3.8. Volatile constituents detected in heavy and light fractions of SC-CO_2 cranberry pomace extracts

No	RI-E	RI-R	Name	Heavier fraction								Lighter fraction					
				SFE-CO_2				SFE-CO_2+5% EtOH				SFE-CO_2		SFE-CO_2+5% EtOH			
				0	-10	-20	-30	0	-10	-20	-30	0	-10	0	-10	-20	-30
1	801	802	2,3-Butanediol	-	+	+	+	-	+	+	+	+	+	+	+	+	+
2	854	856	2-Methylbutanoate	-	-	-	-	-	-	-	-	+	+	+	-	+	-
3	939	928	Butyrolactone	-	-	-	-	-	-	-	-	+	+	+	+	+	+
4	985	981	Hexanoic acid	-	-	+	-	+	-	-	+	+	+	+	+	+	+
5	990	990	Benzaldehyde	-	+	-	-	-	+	+	+	+	+	+	+	+	+
6	999	998	2-Pentyl furan	-	-	-	-	-	-	-	+	+	+	+	+	+	+
7	1040	1039	p-Cymene	-	-	-	-	-	+	-	-	+	-	+	-	+	-
8	1043	1039	Limonene	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	1047	1032	β-Phellandrene	-	-	-	-	-	-	-	-	+	+	-	-	-	+
10	1049	1049	Eucalyptol	+	+	-	-	-	-	-	-	+	+	-	-	-	-
11	1060	1053	Benzyl alcohol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	1117	1108	Nonanal	-	-	-	-	-	-	+	+	+	+	+	+	+	+
13	1121	1126	2-Ethyl hexanoate	-	-	-	-	-	-	-	-	+	+	+	+	+	+
14	1140	1139	Phenylethyl alcohol	-	-	-	-	-	+	-	+	+	+	+	+	+	+
15	1171	1173	β-Terpineol	+	+	+	-	-	-	-	-	+	+	+	+	+	+
16	1182	1183	Nonanol	-	-	-	-	-	-	+	-	+	+	-	-	-	-
17	1187	1188	4-Ethylphenol	-	+	+	-	-	+	-	+	+	+	+	+	+	+
18	1193	1191	Benzoic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	1199	1200	Dodecane	-	-	-	-	+	-	+	-	-	+	-	-	-	-

Table 3.8. (continuation). Volatile constituents detected in heavy and light fractions of SC-CO_2 cranberry pomace extracts

No	RI-E	RI-R	Name	Heavier fraction								Lighter fraction					
				SFE-CO_2				SFE-CO_2+5% EtOH				SFE-CO_2		SFE-CO_2+5% EtOH			
				0	-10	-20	-30	0	-10	-20	-30	0	-10	0	-10	-20	-30
20	1220	1207	α-Terpineol	+	+	+	-	-	+	-	+	+	+	+	+	+	+
21	1280	1279	(Z)-2-Decenal	-	-	-	-	-	-	-	+	+	+	+	+	+	+
22	1291	1280	Nonanoic acid	-	-	-	-	-	+	-	-	+	+	+	+	+	+
23	1312	1308	Thymol	-	-	-	-	-	+	-	+	+	-	-	+	+	+
24	1342	1340	2,4-Decadienal	-	-	-	-	-	-	-	-	+	+	-	-	-	-
25	1442	1435	Vanillin	-	-	-	-	-	+	-	+	+	+	+	+	+	+
26	1450	1453	β-Caryophyllene	+	+	-	-	-	+	+	+	+	+	+	+	+	+
27	1461	1461	Geranyl acetone	-	-	-	-	-	+	-	+	+	+	+	+	+	+
28	1487	1481	Humulene	-	-	-	-	-	-	-	-	+	+	-	-	-	-
29	1528	1526	α-Bulnesene	+	+	-	-	-	+	+	+	+	-	+	+	+	+
30	1552	1546	*cis*-Calamenene	-	+	-	-	-	+	+	+	+	+	+	+	+	+
31	1697	1700	Heptadecane	+	+	+	+	+	+	+	+	+	-	-	-	-	-
32	1793	1795	Octadecene	-	+	-	-	-	+	-	-	+	+	+	-	-	-
33	1934	1930	methyl hexadecanoate	-	-	-	-	-	+	+	+	+	-	-	-	-	-
34	2000	1997	Ethyl palmitate	-	-	-	-	-	-	-	-	+	-	-	+	+	+

RI-E and RI-R: retention indexes determined experimentally and provided in the database, respectively; + detected; – not detected

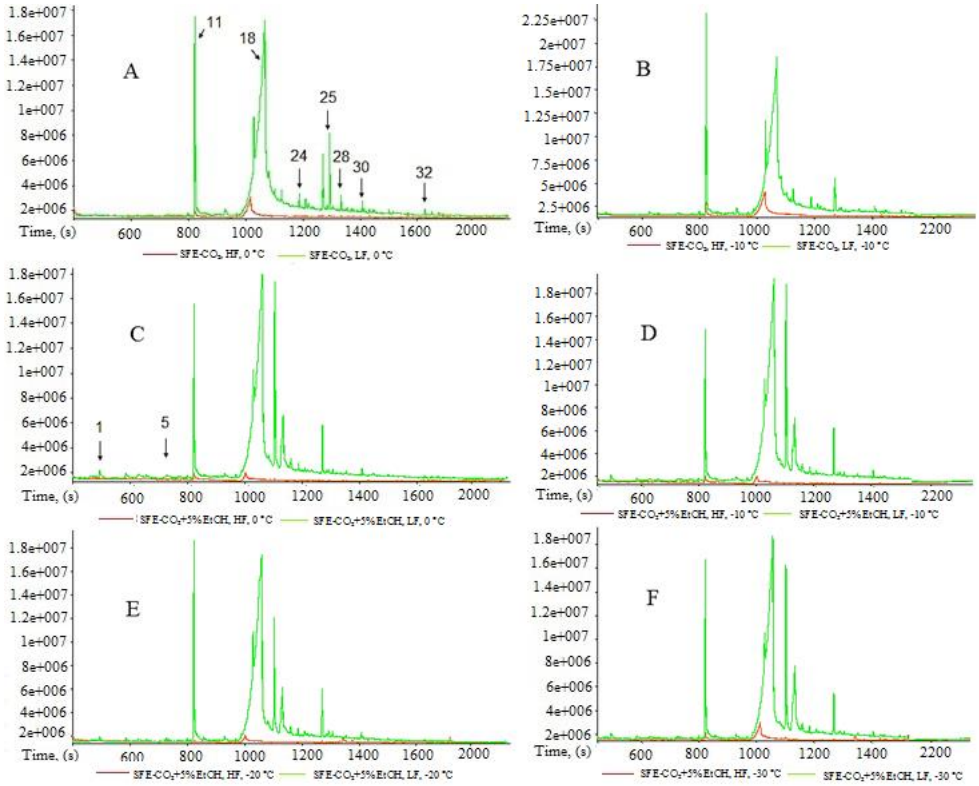


Figure 3.6. Chromatograms comparing volatile constituents in heavier (the red bottom line) and lighter (the green upper line) fractions of cranberry pomace extracts: A, SC-CO_2 0 °C; B, SC-CO_2 at -10 °C; C, SC-CO_2 with 5% EtOH at 0 °C; D, SC-CO_2 with 5% EtOH at -10 °C; E, SC-CO_2 with 5% EtOH at -20 °C; F, SC-CO_2 with 5% EtOH at -30 °C (selected peak numbers as in **Table 3.8**)

3.3. Optimization of pressurized liquid extraction (PLE)[1] and characterization of product antioxidant properties and their composition

Berry pomace is a heterogeneous material consisting of skins, seeds, and pressed flesh. The properties and the composition of all these anatomical parts of the fruit are different: the seeds contain a high amount of lipids, while other parts of pomace contain more polar (hydrophilic) components, including polyphenols. Therefore, after SFE of the whole ground cranberry pomace, a large amount of CO_2-insoluble residues (approx. 89% of the pomace) remained as the extraction by-product. For the recovery of higher polarity compounds from the SFE residue, another food-grade protic solvent, ethanol, was applied in PLE. A remarkably faster extraction process using lower amounts of solvent and, in many cases, higher extract

[1] This section was prepared with reference to Tamkutė, Liepuoniūtė, Pukalskienė and Venskutonis (2020)

yields comparing to the conventional extraction have been reported as the main advantages of PLE (Brazdauskas, Montero, Venskutonis, Ibañez and Herrero, 2016; Grunovaitė *et al*., 2016; Kaufmann and Christen, 2002; Kitrytė, Povilaitis *et al*., 2017; Machado, Pasquel-Reátegui, Barbero and Martínez, 2015). So far as in the commercially available extractors the pressure cannot be changed, e.g., in *ASE300*, it is 10.3 MPa by default, the time and temperature are usually used as independent variables for optimizing the PLE process. These parameters were optimized for the highest yield in PLE-EtOH of cranberry pomace SFE residue (**Table 3.9**).

Table 3.9. Experimental design for the optimization of PLE-EtOH of cranberry pomace using three consecutive extraction cycles after SFE-CO_2 and their yields

Experiment No.	τ, min	T, °C	Yield, g/100g DW	
			Experimental	Predicted
1	5	50	$53.45\pm0.46^{a, A}$	53.58
2	15	50	$55.02\pm0.60^{a, B}$	54.79
3	5	90	$55.41\pm0.32^{b, A}$	55.69
4	15	90	$56.62\pm0.63^{b, B}$	57.44
5	5	70	$55.05\pm0.01^{b, A}$	55.65
6	15	70	$56.72\pm0.01^{b, C}$	57.14
7	10	50	$54.56\pm0.02^{a, AB}$	54.34
8	10	90	$56.43\pm0.60^{b, B}$	56.74
9	10	70	$56.45\pm0.06^{b, B}$	56.58
10	10	70	$56.50\pm0.04^{b, B}$	56.58
11	10	70	$56.33\pm0.05^{b, B}$	56.58
12	10	70	$56.32\pm0.13^{b, B}$	56.58
13	10	70	$56.45\pm0.40^{b, B}$	56.58

*DW – dry pomace weight; ^{a,b} Different letters in the same column indicate statistical differences between different extraction times (one-way ANOVA, $p < 0.05$); ^{A,B} Different letters in the same column between the used extraction temperatures indicate significant differences, $p < 0.05$. The results are expressed as mean ± standard deviation of triplicate determinations

It may be observed that, in the selected range of independent variables, their effect on extract yield was not considerable, although, for some parameter sets, the differences were significant. Thus the yields between 13 experimental runs varied from 53.45 to 56.72 g/100g DW (**Table 3.9**). For comparison, Kryževičiutė *et al*. (2016) reported more than 2 times lower PLE yields from raspberry pomace. ANOVA determined that the model is significant ($p < 0.0001$) with an F-value of 238.17; the 'lack-of-fit' was not significantly related to the pure error with an F-value of 2.35 (**Table 3.10**). Both time ($p < 0.0001$, F-value = 483.12) and temperature ($p < 0.0001$, F-value = 324.47) had a significant impact on the extract yield, whereas their interaction was not significant ($p > 0.05$, F-value = 3.19). The second order model terms τ^2 and T^2 were also significant factors ($p < 0.0001$) with F-values of 191.67 and 55.03, respectively. The adequacy of the model was

evaluated by the total determination coefficient (R^2) value of 0.99, which indicates a reasonable fit of the model to the experimental data. The adjusted coefficient of determination (adj-R^2) was 0.99, which is in agreement with the predicted coefficient of determination (pred-R^2) of 0.96.

Table 3.10. Analysis of variance table for RSM experimental design of PLE-EtOH

Source	Sum of squares	df	Mean square	F value	p-value prob > F
			PLE-EtOH		
Model	12.11	5	2.42	238.17	< 0.0001*
Time (τ)	4.91	1	4.91	483.12	< 0.0001*
Temperature (T)	3.30	1	3.30	324.47	< 0.0001*
τT	0.032	1	0.032	3.19	0.1175
τ^2	1.95	1	1.95	191.67	< 0.0001*
T^2	0.56	1	0.56	55.03	0.0001*
Residual	0.071	7	0.010		
Lack of fit	0.045	3	0.015	2.35	0.2141
Pure error	0.026	4	0.0645		
Total	12.18	12			

*- significant

The optimization of PLE parameters by RSM resulted in the following second order polynomial regression model, which is an empirical relationship between the dependent variables and the independent test variables:

$$\text{Yield (g/100g DW)} = 56.39+0.91\cdot\tau+0.74\cdot T-0.84\cdot\tau^2-0.45\cdot T^2 \quad (7)$$

The predicted values, which were calculated by using a second order polynomial equation (7), fitted the experimental results well (**Fig. 3.7**), while the response surface 3D plot shows the effect of the extraction time and temperature on the product yield at a constant pressure of 10.3 MPa (**Fig. 3.8**).

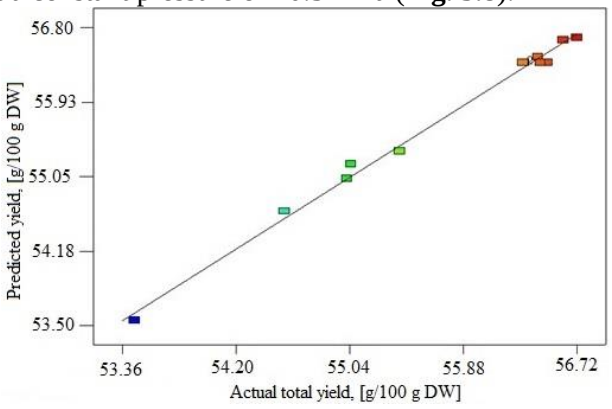


Figure 3.7. Comparison of actual and predicted extract yields in PLE-EtOH

It may be observed that the process temperature and time have some impact on the extraction yield. Thus, the optimal conditions providing the highest yield (55.89±0.5%) with ethanol were 83 °C and 3 cycles, 15 min each. It may be noted that the model suggested the longest extraction as the optimal one, and it may be hypothesized that further increase would offer even higher yields. Therefore, theoretically, the optimal extraction time in this case should be regarded as a conditional value, i.e., within the range of the selected marginal values of time as a variable. On the other hand, PLE-EtOH resulted in very high yields from cranberry pomace compared with the previously reported values for PLE of berry pomace (Basegmez *et al*., 2017; Brazdauskas *et al*., 2016; Grunovaitė *et al*., 2016; Kitrytė, Povilaitis *et al*., 2017; Kryževičiūtė *et al*., 2016; Machado *et al*., 2015).

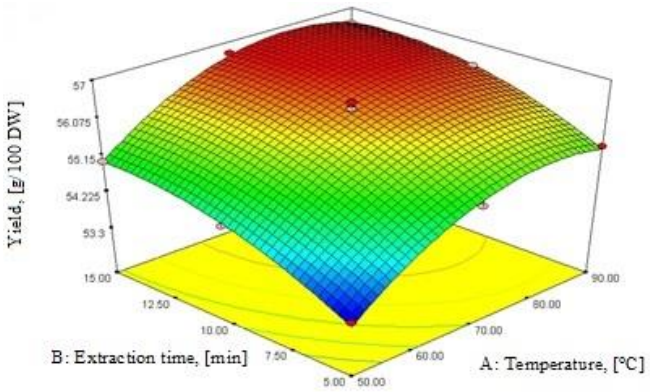


Figure 3.8. 3D response surface plot showing the effects of independent variables on the extract yields during PLE using three consecutive extraction cycles when the independent variables are time and temperature

Consequently, the residue after SFE and PLE-EtOH was approx. 30% of the initial cranberry pomace. This residue was subjected to PLE-H_2O and gave 6.50±0.07% of extract. Due to the high efficiency of PLE-EtOH, it was decided not to apply RSM for the optimization of the last PLE step with water. The final residue constituting approx. 25% of the initial cranberry pomace may be considered as a good source of dietary fiber; however, its characterization was beyond the scope of this study.

3.2.1. Characterization of ethanol extracts obtained by PLE optimization

Most important characteristics of PLE-EtOH of cranberry pomace are provided in **Table 3.11**. The values were determined for extracts (E) and afterwards calculated for pomace dry weight (DW). Both characteristics are important, and the first one evaluates the potency of the extract (the product value), while the second one shows the recovery of bioactive compounds, which is related with the process efficiency. It may be observed that TPC and the antioxidant capacity of PLE-EtOH products increased with the increasing extraction time, and the highest values were achieved after 3 cycles, 15 min each, at 83 °C. These findings are in agreement with

the previously reported results for the extraction of phenolic compounds from carob pulp (Benchikh and Louailèche, 2014) and butterhead lettuce (Viacava, Roura and Agüero, 2015). On the other hand, a longer extraction time may increase the risks of degradation and/or lead to other undesirable changes of the most sensitive constituents; therefore, the optimal parameters should be selected for each plant material individually, also while taking into account the properties of the target substances under extraction.

The range of 50–90 °C was selected for evaluating the effect of temperature on the efficiency of the PLE-EtOH process. In this case, the selection was based on the boiling point of EtOH and the possible heat effects on plant phytochemicals. It may be observed (**Table 3.11**) that the highest TPC, $ABTS^{\bullet+}$-scavenging and ORAC values of the extracts were determined at the highest applied in PLE temperature (90 °C); these values were 43.43 mg GAE/g E, 179.0 mg TE/g E and 270.2 mg TE/g E, respectively; although the ORAC of the extract obtained at 70 °C was not significantly lower. The values calculated for the pomace DW were also the highest at the maximal values selected for the time and temperature parameters, i.e., 15 min × 3 times, 90 °C. It may be assumed that heat enhances the recovery of phenolic and other bioactive compounds from cranberry pomace. Heat increases the diffusivity of the extracting solvent into plant cells, and then enhances the desorption of phenolic compounds from cells (Cacace and Mazza, 2003; Vongsangnak, Gua, Chauvatcharin and Zhong, 2004). In addition, heating at high temperatures decreases the viscosity of solvents thus allowing their better penetration into the solid particles and thereby enhancing the extraction rate (Richter, Jones, Ezzell, Porter *et al*., 1996). However, it should be noted that not only phenolic compounds may participate in the single electron transfer (TPC and $ABTS^{\bullet+}$-scavenging) and peroxyl/hydroxyl radical scavenging (ORAC) reactions, while a higher temperature increases the rate of the formation of heat-generated compounds which can also act as reducing agents. Therefore, it was decided not to use higher temperatures in PLE.

Table 3.11. Antioxidant capacity, the content of proanthocyanidins (PAC) and anthocyanins (AC) of extracts obtained by PLE-EtOH

Parameters	TPC, mg GAE/g		ABTS, mg TE/g		ORAC, mg TE/ g		mg PAC/g		mg AC	
	E	DW	E	DW	E	DW	E	DW	g E	100 g DW
5 min × 3, 50 °C	29.99± 1.07^{c}	14.25± 0.51^{b}	91.52± 2.99^{b}	43.49± 1.42^{a}	138.2± 7.5^{a}	65.67± 3.55^{a}	157.6± 6.9^{ab}	74.87± 3.27^{a}	2.04± 0.03^{a}	97.10± 1.60^{a}
10 min × 3, 50 °C	29.33± 0.53^{bc}	14.15± 0.26^{b}	95.19± 5.92^{b}	45.92± 2.86^{a}	144.4± 4.6^{a}	69.66± 2.22^{a}	176.2± 6.4^{cd}	85.01± 3.09^{bc}	3.81± 0.07^{d}	183.6± 3.3^{cde}
15 min × 3, 50 °C	37.50± 0.83^{e}	18.34± 0.41^{e}	112.3± 5.7^{c}	54.95± 2.77^{abc}	176.7± 9.1^{b}	86.41± 4.45^{b}	173.4± 4.1^{cd}	84.81± 2.03^{b}	3.71± 0.38^{cd}	181.5± 18.4^{cde}
5 min × 3, 70 °C	25.70± 0.67^{a}	12.81± 0.33^{a}	82.41± 5.93^{a}	41.06± 2.95^{a}	133.8± 7.7^{a}	66.69± 3.83^{ab}	148.9± 6.3^{a}	74.18± 3.15^{a}	2.69± 0.06^{ab}	133.8± 2.8^{b}
10 min × 3, 70 °C	29.81± 1.04^{c}	14.96± 0.52^{c}	119.6± 6.0^{cd}	60.01± 3.04^{ab}	191.0± 9.0^{b}	95.86± 4.54^{c}	167.5± 6.5^{bc}	84.05± 3.26^{b}	3.81± 0.03^{d}	191.1± 1.7^{e}
15 min × 3, 70 °C	41.50± 1.01^{f}	20.96± 0.51^{f}	163.0± 8.5^{e}	82.34± 4.29^{d}	262.5± 13.2^{e}	132.6± 6.7^{d}	225.2± 3.1^{e}	113.7± 1.5^{d}	3.20± 0.08^{bc}	161.7± 4.0^{bcd}
5 min × 3, 90 °C	28.49± 0.33^{b}	14.03± 0.16^{b}	124.6± 6.8^{d}	61.40± 3.36^{bc}	224.7± 21.5^{c}	110.7± 10.6^{c}	186.6± 18.7^{d}	91.93± 9.24^{c}	3.18± 0.08^{bcd}	156.4± 3.9^{bc}
10 min × 3, 90 °C	31.72± 1.55^{d}	16.05± 0.78^{d}	127.8± 5.8^{d}	64.70± 2.93^{c}	241.2± 9.6^{d}	122.0± 4.8^{d}	171.8± 0.6^{bc}	86.93± 0.28^{bc}	3.09± 0.10^{bc}	156.5± 5.0^{bc}
15 min × 3, 90 °C	43.32± 1.17^{g}	22.15± 0.60^{g}	179.0± 9.2^{f}	91.52± 4.69^{d}	270.2± 10.5^{e}	138.2± 5.4^{d}	220.2± 8.4^{e}	112.6± 4.3^{d}	3.67± 0.34^{cd}	181.8± 4.5^{de}

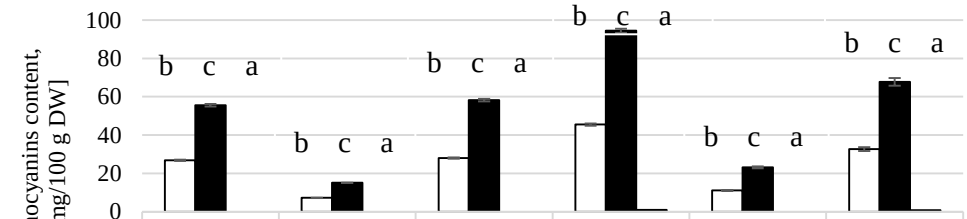
*E – extract; *DW – dry pomace weight; ^{a,b} Different letters in the same column indicate statistical differences (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of triplicate determinations

Proanthocyanidins and anthocyanins are among the most important bioactive phytochemicals in many berries, including cranberries. It may be observed that the dependence between the process parameters and the content of these compound groups in the extracts as well as their recovery from the pomace was more complicated (**Table 3.11**). For instance, the increasing extraction time from 10 to 15 min at 50 °C did not have any significant effect on the PAC content in the extracts, while, at 70°C and 90 °C, the highest PAC values were obtained when each cycle time was 15 min: 225.2 and 220.2 µg/g E, respectively. In the case of AC at 50 °C, the increasing extraction cycle time from 5 to 10 min resulted in 2 times higher AC recovery values, 97.10 vs. 183.6 mg/100g DW; while the longer cycle time (15 min) did not increase the recovery. It may be assumed that anthocyanins are fully recovered from the pomace during 3 cycles, 10 min each. However, different results were obtained during PLE-EtOH at 70 °C and 90 °C: at 70 °C, the highest AC recovery values were obtained after 3 extraction cycles, 10 min each (191.1 mg/100g DW), which decreased when the cycle time was increased to 15 min; whereas, at 90 °C, the longest extraction time resulted in the highest AC recovery values. These fluctuations may be explained by the possible side effects of heat-generated changes of the extracts, e.g., the formation of non-anthocyanin structure compounds which might interfere in the spectrophotometric measurement procedure. It should also be noted that anthocyanins are among the most sensitive to heat and media pH berry phytochemicals; excessively high exposure time and temperature may result in the anthocyanin yield decrease (Vongsangnak *et al*., 2004). According to Cacace and Mazza (2003) the optimal temperature for the extraction of anthocyanins from black currant is 30 °C, while higher temperatures had a negative effect on the content of anthocyanins.

When comparing TPC and PAC values, it may be observed that the latter are several times higher. It may be preliminarily explained by the remarkably higher molecular weight of proanthocyanidins comparing to the smaller phenolic compounds, e.g., gallic acid. For instance, the molecular weight (MW) of cranberry procyanidin B2 (a dimer) is 578.5 Da, whereas MW of gallic acid is 170 Da. However, the differences in assay procedure and reaction mechanism may play the most important role.

3.3.2. Quantification of individual anthocyanins in ethanol and water extracts at optimal conditions

Anthocyanins are natural pigments providing a range of colors to berries, from red to dark blue. Their color depends on the substitutes in the phenyl ring of the molecule. Therefore, it is important to know the anthocyanin composition in different plant sources. UPLC-MS/MS analysis of PLE-EtOH and PLE-H_2O extracts of cranberry pomace enabled to identify and quantify 6 anthocyanin glycosides (**Fig. 3.9**), namely, cyanidin and peonidin galactosides, arabinosides and glucosides.



	Cyanidin-3-galactoside	Cyanidin-3-glucoside	Cyanidin-3-arabinoside	Peonidin-3-galactoside	Peonidin-3-glucoside	Peonidin-3-arabinoside
Pomace	26.78	7.27	28.06	45.46	11.13	32.66
PLE-EtOH	55.59	15.09	58.25	94.36	23.11	67.79
PLE-H2O	0	0	0	0.96	0	0.86

Figure 3.9. Content of anthocyanins in cranberry pomace extracted by the standard method with PLE-EtOH and PLE-H_2O determined by UPLC-MS/MS. [a,b] Different letters for the same anthocyanin indicate statistical differences (one-way ANOVA, $p < 0.05$)

The identification was based on the mass spectra and the elution order from the chromatographic column (Heffels, Weber and Schieber, 2015; Mildner-Szkudlarz *et al*., 2016). Peonidin-3-galactoside was (quantitatively) the major anthocyanin, followed by peonidin-3-arabinoside in all the analyzed samples. Their concentrations in cranberry pomace, PLE-EtOH and PLE-H_2O extracts were 45.46, 94.36, 0.96 (peonidin-3-galactoside), and 32.66, 67.79, 0.86 (peonidin-3-arabinoside), mg/100g, respectively. In general, the values determined for anthocyanins in our study are in agreement with the previously reported data for cranberries (Heffels *et al*., 2015). The low amount of these compounds in the PLE-H_2O extract indicates that PLE-EtOH effectively recovered anthocyanins from cranberry extracts. In general, the chromatographic determination of anthocyanins was in agreement with the spectrophotometric results (**Table 3.11**). However, the content of proanthocyanidins (PAC) in the PLE-H_2O extract was 2.7 times higher than in the PLE-EtOH extract, i.e., 532.2 vs 198.5 µg/g E, although PLE-H_2O, due to the remarkably lower extract yield, recovered a several times lower amount of proanthocyanidins from cranberry pomace than PLE-EtOH (**Table 3.12**).

Table 3.12. Content of proanthocyanidins and anthocyanins and antioxidant capacity measured by e-BQClab device in defatted cranberry pomace extracts under optimal conditions (83 °C, 3 × 15 min)

Extract	PCA mg/100 g		Anthocyanins, mg /100 g		Antioxidant capacity, µC		
	extract	DW	extract	DW	Q_1, µC	Q_2, µC	Q_T, µC
PLE – EtOH	198.5± 2.3[a]	91.55± 1.06[b]	902± 1.11[b]	316.3± 51.3[b]	21.10± 1.41[a]	13.50± 0.71[a]	34.60± 2.12[a]
PLE – H_2O	532.2± 18.0[b]	14.79± 0.50[a]	42± 0.04[a]	2.42± 0.23[a]	36.15± 1.62[b]	34.90± 2.68[b]	71.05± 4.31[b]

*DW – dry pomace weight; [a,b] Different letters in the same column indicate statistical differences (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of triplicate determinations

Most likely, due to the high PAC PLE-H_2O value, the extract demonstrated higher antioxidant capacity values when measured with a BQClab device. Moreover, the differences in Q_2 values which indicate slow antioxidant activity between the H_2O and EtOH extracts were considerably larger than in Q_1 values, which indicates fast antioxidant activity; proanthocyanidins as large polyphenolic compounds demonstrate slow antioxidant effects.

3.3.3. Phytochemical composition of defatted cranberry pomace extracts

Polyphenols are the most important bioactive compounds in cranberries. The structures of flavonoids and organic acids were elucidated by the interpretation of their m/z (molecular ions and fragments) measured by Q-TOF/MS while using the data available in various sources (**Table 3.13**). Organic acids 1, 2, 3, 8 were detected in all the analyzed extracts. Thus, the identity of citric and quinic acids (2 and 3) was based on their [M–H]− m/z=191 and the comparison with reference compounds. The structure of chlorogenic acid (8) was confirmed by the authentic standard and fragmentation patterns. Different amounts of the extracted compounds, such as catechin, quinic and chlorogenic acids, were determined in PLE-EtOH and PLE-H_2O extracts. For instance, the concentration of catechin and chlorogenic acid in PLE-EtOH extracts was 60.3 and 88.6 mg/100 g, respectively, while these compounds were not detected in the PLE-H_2O extract. Quinic acid was the major compound found in both analyzed extracts. The amount of quinic acid in the PLE-H_2O extract was 869.4 mg/100 g, while, in the PLE-EtOH extract, the concentration of quinic acid was lower, specifically, 572.9 mg/100 g. Variations in the distribution of quinic acid between different extracts may depend on its solubility in different polarity solvents, and formation of its esters with hydroxycinnamic acids via hydrolysis at higher temperatures is possible.

Compounds 11 and 13 were tentatively identified as quercetin-pentoside and quercetin-hexoside; their [M–H]−m/z were 433.0406 (fitting MW=$C_{20}H_{17}O_{11}$) and 463.0886 (fitting MW=$C_{21}H_{19}O_{12}$), respectively. In the MS/MS mode, these compounds gave a similar distinct fragment ion at m/z 301.0350 (fitting MW=$C_{15}H_5O_8$), which corresponds to aglycon flavonol quercetin; whereas, the neutral loss of [M-162] and [M-132] corresponds to hexosyl and pentosyl units, respectively. Myricetin galactoside was assigned to compound 10; MS/MS fragmentation patterns revealed the presence of a fragment at m/z 316.0219, which indicates the loss of a galactosyl moiety from the precursor ion at m/z 479.0833 (Romani, Campo and Pinelli, 2012).

Some detected peaks (4, 5, 7, 12, 14) were not identified due to the lack of evidence for proposing a proper structure even after performing MS/MS analysis. These compounds are listed in **Table 3.13.** with their retention time, proposed molecular formula, and MS/MS fragments.

Table 3.13. Proposed compounds detected in PLE-EtOH and PLE-H_2O of cranberry pomace extracts by UPLC-QTOF/MS

Compound No.	RT (min.)	Precursor ion $[M–H]^-$	Ion formula	Negative ions MS^2 m/z (% in MS)	Structural assignment	PLE-EtOH extract	PLE-H_2O extract
						mg/100g	
1	0.60	133.0144[a]	$C_4H_5O_5$	-	Malic acid	+	+
2	0.70	191.0562[a]	$C_7H_{11}O_6$	-	Quinic acid	572.9	869.4
3	0.90	191.0197 [a]	$C_6H_7O_7$	-	Citric acid	+	+
4	1.90	161.0455	$C_6H_9O_5$	143.0217 115.0247	Unknown	+	+
5	2.00	219.0512	$C_8H_4O_7$	157.1020 109.1032	Unknown	+	+
6	2.05	577.1371[a]	$C_{30}H_{25}O_{12}$	407.0768 289.0709 245.0815	Procyanidin B3	+	+
7	2.10	417.0839	$C_{20}H_{17}O_{10}$	-	Unknown	+	-
8	2.20	353.0875[a]	$C_{16}H_{17}O_9$	191.0559 179.0342 135.0446	Chlorogenic acid	88.6	-
9	2.28	289.0706[a]	$C_{15}H_{13}O_6$	221.0819 151.0405 123.0453	Catechin	60.3	-
10	2.32	479.0833 [b,c]	$C_{20}H_{29}O_{13}$	316.0219	Myricetin-galactoside	+	-
11	2.60	433.0785 [b,c]	$C_{20}H_{17}O_{11}$	301.0350	Quercetin-pentoside	+	-
12	2.75	359.1352 [b,c]	$C_{16}H_{17}O_9$	313.0154 197.0822	Unknown	+	+
13	2.90	463.0886[b,c]	$C_{21}H_{19}O_{12}$	301.0350	Quercetin-hexoside	+	+
14	3.15	447.0946 [b,c]	$C_{21}H_{19}O_{11}$	-	Unknown	+	-
15	3.21	431.2290 [b,c]	$C_{21}H_{35}O_9$	165.0924	Coumaroyl-hexoside	+	-

a – confirmed by standard; ***b*** – confirmed by reference: ***c*** – conformed by the experimental mass of the parent ion by using a free chemical database (Chemspider);

3.4. Bioactivities of cranberry pomace ethanol extract

Cranberry pomace ethanol extract due to its higher content of polyphenols (Section 3.3.3.), strong antioxidant (Section 3.6.) and antimicrobial (Section 3.7.1.) properties was added to pork meat products: hamburgers and cooked ham (Sections 3.7.4.–3.7.9.), but *The International Agency for Research on Cancer* (IARC) recognized processed meat as causing (group 1) cancer. For this reason, cranberry pomace ethanol extract was evaluated on colon cancer cells to determine if this extract would have an effect in inhibiting the growth of cancer cells.

3.4.1. Effect on cancer cell viability and clonogenic activity

The incidence of colon cancer in Asia and western countries is steadily increasing. These findings can be associated with many epidemiological studies which indicated that the consumption of red meats possibly increase the risk of colon cancer. Recently, studies related with the food rich in flavonoids showed a reduced risk of colorectal cancer. A number of laboratory and animal studies have shown that berry phytochemicals have anticancer properties. The biological activity of the cranberry is associated with the high content of bioactives including flavonoids, tannins, sugars and phenolic acids.

Cancer cell viability and colony formation were assessed in HCT-116 and DLD-1 cancer cells, while cytotoxicity was evaluated by using CRL-1381 (human skin) cells. The cranberry pomace ethanol extract at final concentrations of 40, 200 and 1000 μg/mL was tested for its capacity to decrease the cell viability of HCT-116 and DLD-1 colon cancer cells. Comparing the effect of the extract containing phenolic compounds on two cell lines after 24 and 48 h, it can be shown that the extract was the most effective at inhibiting the growth of HCT-116 cell lines, while it was not active in inhibiting the growth of DLD-1 cell lines (**Fig. 3.10**). In order to assess whether PLE-EtOH inhibits the normal cell growth, CRL-1381 derived from human skin cells was exposed to the same concentrations of cranberry extract. This study showed that the growth of normal human CRL-1381 cells was increased more than 20% by exposure to 40–1000 μg/mL of the extract for 48 h. The results indicated that the cranberry pomace extract can inhibit the growth of colon cancer cells without affecting the growth of normal skin cells *in vitro*. Therefore, the cranberry pomace extract was able to specifically inhibit the growth of colonic cancer cells, but not normal cells.

HPLC analysis of the PLE-EtOH extract illustrated that it is a mixture of phenolic compounds (**Table 3.13** and **Fig. 3.9**) which possess anticancer properties against colon cancer. Colon cancer cells HCT-116 exposed to PLE-EtOH demonstrated more than 30% growth inhibition within 24 h of exposure on 200 and 1000 μg/mL concentration, while, after 48 h, just 1000 μg/mL showed a significant effect on the inhibition of growth. It was reported that anthocyanins (cyanidin, delphinidin, etc.), compared with other flavonoids, significantly inhibited the growth of HCT-15 intestinal cancer cells and colon cancer cells HT-29 (Zhao, Giusti, Malik, Moyer and Magnuson, 2004). Chlorogenic acid, flavonols (quercetin, myricetin), flavanols (catechin, epicatechin, proanthocyanidins) also possess anticancer

properties. It is known that skin and prostate cancer cells were suppressed by quercetin (Paliwal, Sundaram and Mitragotri, 2005), while prostate cancer was suppressed by myricetin (Knekt *et al*., 2002). Several cranberry extracts, juice, the fraction of proanthocyanidins, a flavonoid-rich fraction, and the total polyphenolic fraction inhibited the viability of colon cancer cells. The most effective compounds against HCT-116 colon cancer cells were proanthocyanidins and ursolic acid, while LS-513 colon cancer cells were sensitive to the exposure of the total polyphenolic fraction and anthocyaninins (Seeram, Adams, Hardy, and Heber, 2004; Seeram *et al*., 2006; Vu *et al*., 2012). Vu *et al*. (2012) reported that water soluble phenolic extracts of the cranberry and its products (mainly phenolic acids and their derivatives) effectively inhibited the proliferation of HT-29 and LS-513 colon cancer cells.

In general, it may be claimed that the low pH value of the extract (2.5) had a different effect in inhibiting the growth of HCT-116 cells comparing with the previously reported data. Vu *et al*. (2012) reported that the extracts from the cranberry fruit at pH 7.0 were effective at inhibiting the growth of colon cancer cells comparing with the extract at pH 2.5.

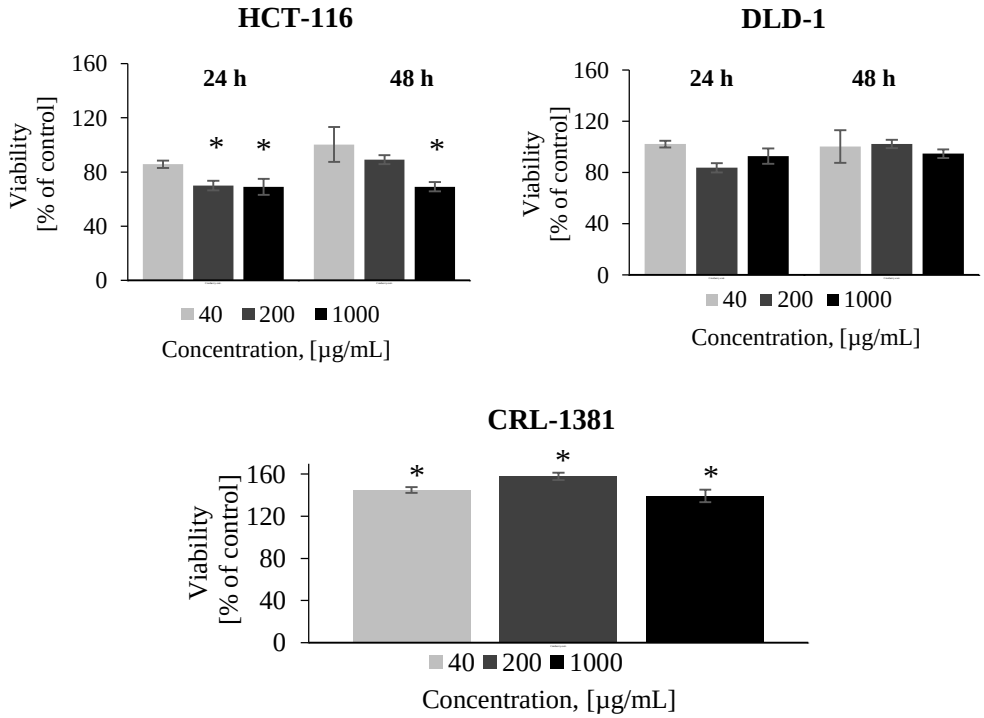


Figure 3.10. The impact of ethanol cranberry pomace extract on the viability of HCT-116, DLD-1 and CRL-1381 cells. * Indicates statistical differences (one-way ANOVA, $p < 0.05$). The shown results are means ± SD of three independent experiments

Similar effects were obtained when analyzing the changes in the cell count and their viability after the exposure to the cranberry extract by flow cytometry (**Fig.**

3.11). The most prominent effect of the cranberry pomace extract was on HCT-116 colon cancer cells, while it had no effect on DLD-1 colon cancer cells after exposure to all the tested concentrations of the cranberry extract. The number of HCT-116 viable cells decreased by 9%, 27% and 18% comparing with the control sample, when 40, 200 and 1000 μg/mL of cranberry ethanol extract was used, respectively. The highest concentration of the extract (1000 μg/mL) significantly ($p < 0.05$) increased the early (dying cells) and late apoptotic cell (already dead) populations after 24 h exposure in HCT-116 cell lines compared with the control sample, while, in DLD-1, it did not have any effect. In addition, HCT-116 cells were more sensitive to the ethanol extract than DLD-1 cells. At 200 and 1000 μg/mL of the extract, 7% of the cells exhibited early apoptosis, whereas, at the same time, 1000 μg/mL concentration induced 10% of cells in late apoptosis, while, in the control sample, it reached less than 5% (early and late apoptosis). Proanthocyanidins are very important compounds as they determine the proapoptotic activity in the cranberry extract. The richness of proanthocyanidins in an extract is not directly related to proapoptotic activity, while the polymer concentration and the polymerization degree are the main criteria which determine their activity. Some studies showed that more polymerized proanthocyanidins showed stronger proapoptotic activities. *In vitro* proapoptotic activity could also be linked with their chemical structures which probably play an important role as well (Minker *et al*., 2015).

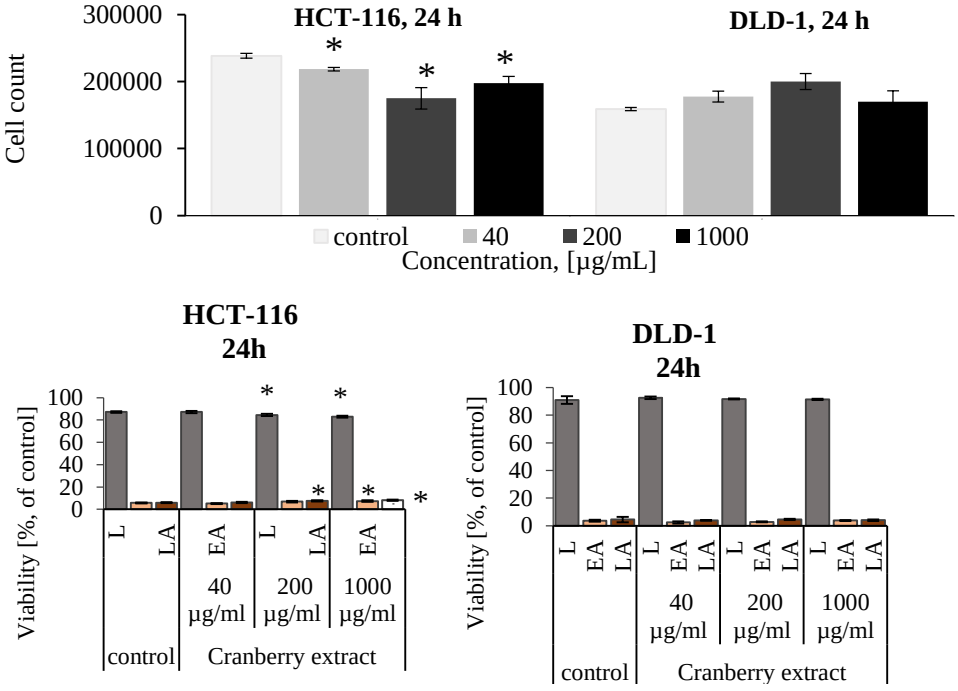


Figure 3.11. Estimation of HCT-116 and DLD-1 cell count and viability by the flow cytometry method after 24 h exposure to cranberry extract; L – alive cells; EA – early apoptosis; LA – late apoptosis. * Indicates statistical differences (one-way ANOVA, $p < 0.05$). The shown results are means ± SD of three independent experiments

In vitro cell viability after the exposure to the cranberry extract was also assessed by the colony formation or clonogenic analysis assay based on the ability of a single cell to form a colony. In DLD-1 cells, a different concentration of the ethanol extract caused a reduction from 10% to 22% in terms of cell colony formation comparing with the control value (**Fig. 3.12**). A higher concentration resulted in higher inhibition of colony formation. However, a slightly more prominent inhibitory effect was observed in HCT-116 cells. Colony formation of these cells was inhibited by 8%, 17% and 33% comparing with the control value when 40, 200 and 1000 μg/mL concentrations of the extract, respectively, were used. Although cranberry extracts at different test concentrations did not have a significant effect on the HCT-116 and DLD-1 cell colony count, yet, 1000 μg/mL of the cranberry extract reliably reduced the HCT-116 cell colony area. The colony formation and cell colony area of colon cancer cells, such as HT-29 and HCT-116, can be affected by ursolic acid and the PAC fraction from the cranberry extract in a dose-dependent manner. The PAC fraction composed from trimers through hexamers of epicatechin with both A and B-type linkages was more effective (over 50%) in the inhibition of the colony formation in HCT-116 than the whole polyphenolic extract (Liberty, Hart and Neto, 2007).

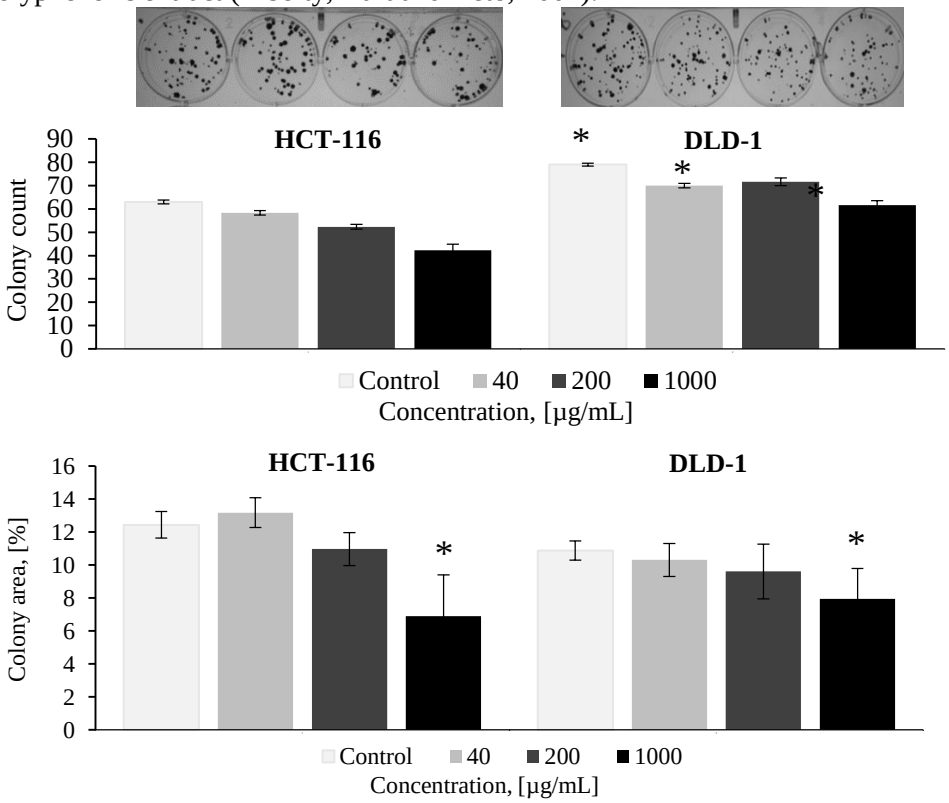


Figure 3.12. Effects of the cranberry extract on HCT-116 and DLD1 colony formation. * Indicates statistical differences (one-way ANOVA, $p < 0.05$). The shown results are means ± SD of three independent experiments

In general, MTT and flow cytometry assays revealed that all the tested cell lines (DLD-1, HCT-116) were not so sensitive to the treatment with the cranberry pomace extract (40–1000 µg/mL) in reducing the cancer cell viability and proliferation. The clonogenic assay showed that the extract at a higher concentration was more effective in the suppression of the HCT-116 cancer cell colony formation. Our data was in agreement with the previously reported results that the extract with a higher total phenolic content was not so effective in inhibiting the growth of colon cancer cells, while the fractions featuring a better composition had a lower TPC (Vu *et al*., 2012). Taken together, our results suggest that the cranberry pomace extract exhibits anticancer activity, yet it is lower comparing with other studies. This might be due to the low pH of the extract and the variety of compounds which do not show any synergistic effect against the tested cells.

3.5. Inhibitory activity of polyphenol-rich PLE extracts of cranberry pomace against Zika virus infection

The expanding distribution area of the Zika virus makes Zika fever an emerging disease which has already affected millions of people from all over the world in the recent years (Haddow *et al*., 2012; Yun & Lee, 2017). Scientists have already conducted a lot research as to how to inhibit the infection of the Zika virus, but still there is lack of natural, safe and effective extracts or compounds against it. Therefore, it is highly important to investigate the potential antiviral effect of various plants or berries as natural sources of nutraceuticals which could be used to prevent this viral infection.

3.5.1. Effect of cranberry pomace extracts on ZIKV infection in A549 cells

Prior to evaluating the antiviral activity against the Zika virus (ZIKV) of cranberry pomace ethanol (PLE-EtOH) and water (PLE-H_2O) extracts, we determined non-cytotoxicity doses on human epithelial cell lines A549 by MTT assay which shows cell metabolic activity (**Figure 3.13 A**). The measurement of the cell viability against various concentrations (25–800 µg/mL) of PLE-EtOH and PLE-H_2O extracts exposed concentration-dependent cytotoxicity in A549 cells. The concentration which inhibited 50% of the cell viability (CC_{50}) was 766.6 µg/mL (PLE-EtOH) and 797.5 µg/mL (PLE-H_2O) (**Table 3.14**). The viability of the cells maintained at 95–100% for both extracts was at the concentration of 400 µg/mL (the maximal non-cytotoxic concentration (MNTC)) (**Figure 3.13 A**); therefore, this concentration was chosen to test the potential antiviral activity against ZIKV for both extracts. For this experiment, we chose a molecular clone of the African strain of the Zika virus expressing a GFP reporter gene ($ZIKV_{GFP}$) which can efficiently replicate in A549 cells. Thus, both cranberry pomace extracts (ethanol and water) were infected for 24 h with $ZIKV_{GFP}$ in the presence of 400 µg/mL concentration (the data is not shown). The obtained results showed that the PLE-EtOH extract cannot be used for further analysis because it reduced the GFP positive cells by less than 49%, which means that the PLE-EtOH extract is not cytotoxic for cells, but it does not possess antiviral properties, either. These findings may be related with the

differences in the phytochemical composition of this extract. Completely different results were obtained with the PLE-H_2O extract, when, after the monitoring of the viral infection with the flow cytometer, the results showed that GFP-positive cells were fewer than 5%, so, for further investigations, we chose the PLE-H_2O extract.

The potential antiviral effect of the cranberry pomace water extract was determined against the Zika virus *in vitro* in A549 human cells. These cells were infected at multiplicity of infection (MOI) of 1 in the sense of increasing concentrations of PLE-H_2O (**Figure 3.13 B**). At the highest non-cytotoxic concentration (400 µg/mL) of the extract, the production of the virus was almost stopped (the share of GFP-positive cells was 4.5%). At 200 µg/mL, the cranberry pomace extract reduced the GFP-positive cells by 83%, which means that the virus progeny production was efficiently inhibited and still acceptable (more than 80% of the GFP-positive cells were inhibited). Further reduction of the concentration (12.5–100 µg/mL) showed a significant increase of virus production from 53% (100 µg/mL) to 93% (12.5 µg/mL). The concentration which inhibited 50% of the virus growth (IC_{50}) was obtained when using nonlinear regression. The IC_{50} determined for the cranberry pomace water extract was 103.3 µg/mL (**Table 3.15**).

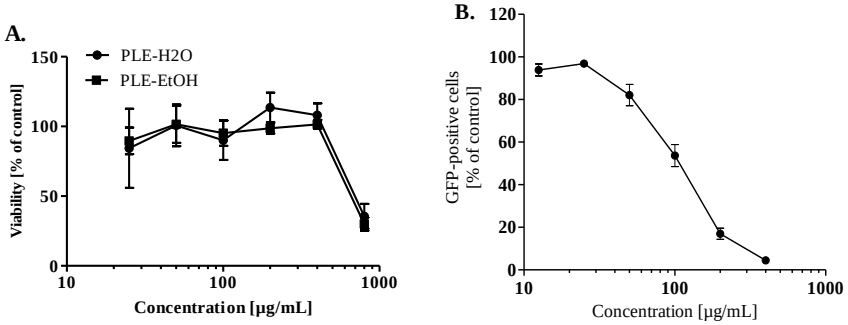


Figure 3.13. Cranberry pomace water extract exhibits antiviral activity against Zika virus (ZIKV). (**A**) The cytotoxicity of cranberry pomace ethanol and water extracts on A549 cells. A549 cells were treated with different concentrations (12.5–800 µg/mL) of extract for 24 h, cell viability was determined by MTT assay. The results are means ± SD of three independent experiments and are expressed as a relative value compared to the vehicle (**B**). The antiviral activity of the cranberry pomace water extract against $ZIKV_{GFP}$ attachment on A549 cells by dose-depended manner. The shown results are means ± SD of three independent experiments and are expressed as relative value compared to untreated infected cells

Based on the obtained cytotoxicity and antiviral activity results, we calculated the selectivity index (SI) which was 7.7. SI is a ratio measuring the window between cytotoxicity (CC_{50}) and antiviral activity (IC_{50}) and shows that the drug with a higher ratio (cytotoxic activity at very high, but antiviral activity at very low concentrations) theoretically is more effective and safe for *in vivo* studies.

Table 3.14. Cytotoxicity and antiviral activity of cranberry pomace PLE-EtOH and PLE-H_2O extracts against ZIKV

Extract	CC_{50} (µg/mL)[a]	IC_{50} (µg/mL)[b]	SI[c]
PLE-EtOH	766.6±7.6	-	-
PLE-H_2O	797.5±14.1	103.3±2.4	7.7

[a] Concentration that inhibited cell viability by 50%; [b] concentration that inhibited infection by 50%; [c] selectivity index (CC_{50}/IC_{50})

3.5.2. Effect of cranberry pomace water extract on inhibition of clinical strain of ZIKV

Human lung epithelial A549 cells can support the infection of the Asian epidemic strain PF-25013-18 (PF13) of ZIKV, therefore, PF13-infected A549 cells at the multiplicity of infection (MOI) of 1 were used to evaluate the quantification of the viral growth by the plaque-forming assay. The results showed that ZIKV-PF13 was sensitive to various concentrations of the PLE-H_2O extract (**Figure 3.14 A**). At the maximal non-cytotoxic concentration of 400 µg/mL, the cranberry pomace water extract reduced the viral progeny production by at least 6-logs. ZIKV-PF13 progeny production was inhibited in a concentration-dependent manner (**Figure 3.14 A**).

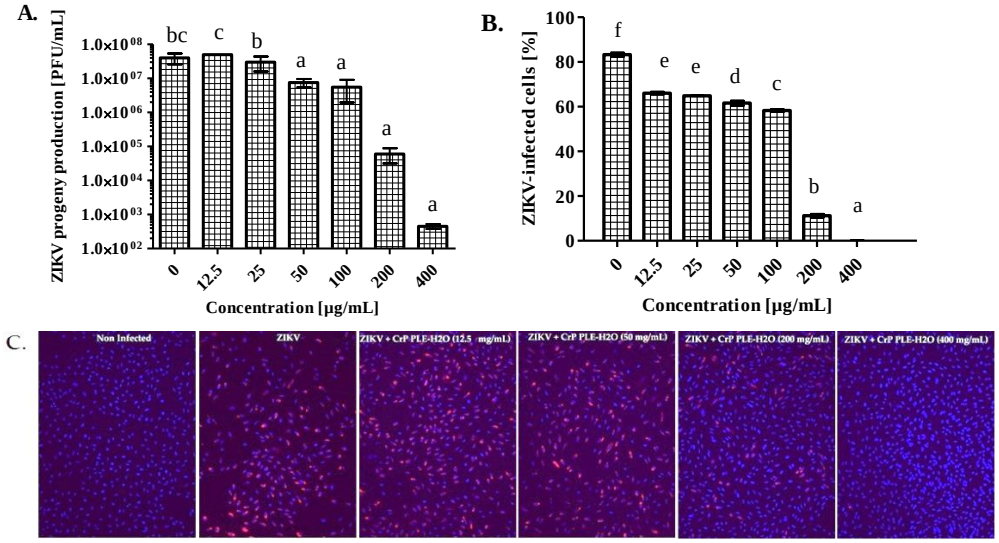


Figure 3.14. Cranberry pomace water extract prevents infection of A549 cells by epidemic strains of Zika virus (ZIKV). (**A**) Plaque-forming assay was used to quantify ZIKV-PF13 progeny production in presence of various concentrations of cranberry pomace PLE-H_2O extract (12.5, 50, 200 and 400 µg/mL). The shown results are means ± SD of two independent experiments. (**B**) Quantification of the number of ZIKV-MR766 infected A549 cells from the experiment represented in the images (**C**). (**C**) Immunofluorescence analysis of viral protein expression in ZIKV-MR766-infected A549 cells. ZIKV E protein (red) and nuclei (blue) were visualized by fluorescence microscopy. Scale bars are 50 µm. [a,b] Different letters indicate statistical differences between concentrations

Viral protein production was evaluated by immunofluorescence analysis 24 h post infection by using an antibody which recognizes viral protein E (anti-flavivirus E mAb 4G2) (**Figure 3.14 B, C**). To determine whether the cranberry pomace water extract exhibited antiviral activity against another viral strain, African lineage ZIKV-MR766 was chosen for this analysis. The obtained results showed that the cranberry pomace PLE-H_2O extract severely restricted ZIKV infection in A549 cells. The percentage of ZIKV-infected cells was reduced down to 0.06% (400 µg/mL), 11.22% (200 µg/mL), 61.59% (50 µg/mL) and 64.92% (12.5 µg/mL) in the presence of the PLE-H_2O extract, while non-treated cells were infected by 83.32% (**Figure 3.14 B**). As a consequence, the production of viral protein E in A549 cells was affected by the addition of the PLE-H_2O extract even at lower concentrations (**Figure 3.14 C**). According to the results of the plaque-forming assay and immunofluorescence analysis, the cranberry pomace water extract has a potential antiviral effect against both (i.e., African and Asian) strains of ZIKV. Our data from all the conducted experiments demonstrated that this extract efficiently inhibited ZIKV infection in A549 cells in a dose-dependent manner and can be provided as a natural source of phytochemicals possessing antiviral properties.

3.5.3. Mechanism of cranberry pomace water extract prevention against ZIKV infection in A549 cells

The time-of-drug addition approach was used to determine in which stage of the Zika virus infection the PLE-H_2O extract worked (**Figure 3.15 A**). For this experiment, we used the Zika virus strain expressing a GFP reporter gene in order to monitor viral replication in A549 cells. Cranberry water extract was added to A549 cells throughout the infectious life cycle, concomitantly with the virus input for 2 h (adsorption) and 2 h post-viral infection. The number of GFP positive cells decreased more than 90% compared with the non-treated control cells when 400 µg/mL of cranberry pomace water extract was added throughout the experiment. Similar results were obtained when water extract was added at the ‘adsorption’ stage **(Figure 3.15 B**), and the intensity of fluorescence was detected in less than 2% of the cells. However, the addition of cranberry pomace water extract (400 µg/mL) at 2 h post-adsorption did not show any effect on virus replication and assembling (**Figure 3.15 B**). From these results, it can be concluded that the addition of the extract prevented Zika virus infection in the early steps of the viral infectious cycle but did not have any effect on inhibiting the intracellular replication, polyprotein processing and synthesis of the Zika virus during the replication stage (post-adsorption).

In order to determine whether cranberry pomace water extract affects the viral particle attachment to the cell membrane, prechilled $ZIKV_{GFP}$ and PLE-H_2O extract were mixed in so that to prevent virus entry, but still allow virus binding (**Figure 3.15 C**). The mixture was allowed to bind A549 cells at 4 °C for 1 h, then, the cells were rinsed, and the number of the attached ZIKV particles after 24 h was evaluated by the flow cytometry assay. The obtained results demonstrated that the percentage in ZIKV-infected A549 cells after 24 h post infection was strongly affected by the

addition of the extract and resulted in 2.8% of GFP-positive cells. It means that cranberry pomace water extract significantly inhibits ZIKV attachment to the cell membrane and suggests that the post-attachment step of the infectious virus cycle could not be altered

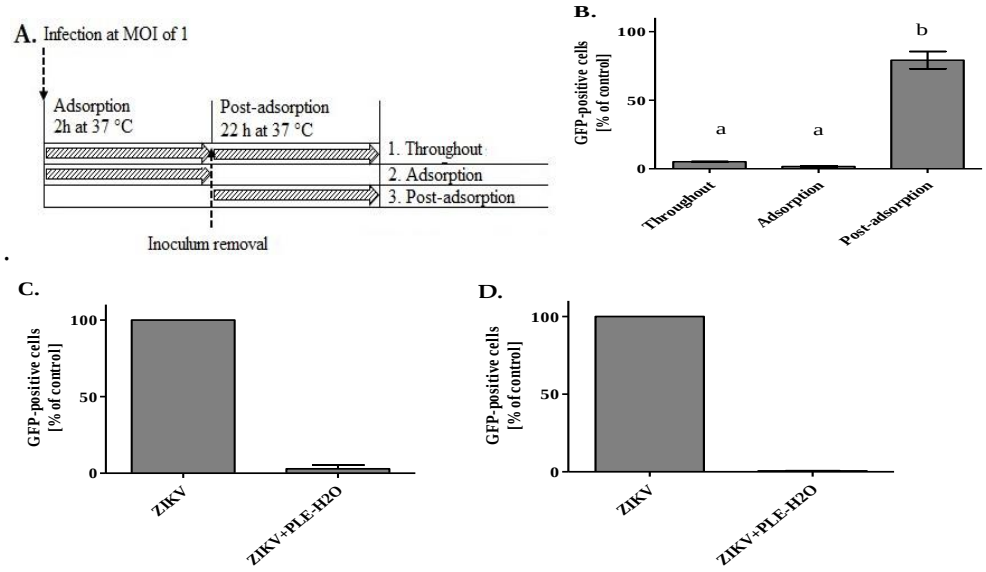


Figure 3.15. Cranberry pomace PLE-H_2O extract targets the early stage of ZIKV infection. (**A**) Schematic representation of time-of-drug-addition assay used to characterize the antiviral activity of cranberry pomace water extract at 400 µg/mL. The arrow indicates the presence of the extract. (**B**) Flow cytometric analysis of GFP expression in A549 infected with $ZIKV_{GFP}$ during 24 h at MOI of 1 under different experimental conditions. ^{a,b} Different letters between columns indicate statistical differences. (**C**) Flow cytometric analysis of GFP expression in A549 cells infected with ZIKV at MOI of 1 for 1 h at 4 °C with or without cranberry pomace water extract (400 µg/mL). (**D**) Viral inactivation assay. $ZIKV_{GFP}$ was incubated with 400 µg/mL of cranberry pomace water extract or mock-treated at 37 °C for 2 h, and the mixture was assessed for viral infectivity on A549 cells at MOI of 1. 24 h post infection, the percentage of GFP-positive cells was determined by flow cytometric assay. The results are mean ± of three independent experiments and are expressed as the relative value compared to untreated infected cells

Furthermore, it was important to find out whether cranberry pomace water extract directly affects the cell free virions so that to eliminate viral infection in the host cell (**Figure 3.15 D**). For this reason, $ZIKV_{GFP}$ particles were incubated with cranberry pomace water extract (400 µg/mL) for 2 h at 37 °C and then diluted 15-fold prior to the infection of A549 cells. This dilution reduces the concentration which was used for infection below its therapeutic concentration and prevents the potential interactions of the host cell surface. The flow cytometry assay showed that ZIKV infectivity was strongly affected by the addition of the cranberry pomace water extract and resulted in 99.5% reduction of GFP-positive cells after 24 h post infection compared with the control test (cells infected with untreated $ZIKV_{GFP}$). Thus, cranberry pomace PLE-H_2O extract could irreversibly interact with the virus

particles of ZIKV to prevent the infection. These results suggest that the phytochemicals which are present in the water extract could disassemble ZIKV particles (the virucidal effect) and/or neutralize the virus infectivity (the virostatic effect). The same effect was detected when using natural antivirals, such as epigallocatechin gallate (EGCG) (Carneiro, Batista, Braga, Nogueira, & Rahal, 2016; Sharma *et al*., 2017), delphinidin (Vázquez-Calvo *et al*., 2017), curcumin (Mounce *et al*., 2017) and medical plants (Clain *et al*., 2019, 2018; Haddad, Koishi, *et al*., 2019).

3.5.4. Toxicity of cranberry pomace extract in zebrafish model

In order to investigate *in vivo* toxicity of cranberry pomace water extract, we decided to use the zebrafish, which is widely applied as a relevant physiological model during development and adulthood. High genomic homology with humans (>70%) as well as many physiological processes common with mammals makes zebrafish of exceptional interest for toxicity studies (Howe *et al*., 2013; Williams & Hong, 2011). Consequently, the potential acute toxicity of cranberry pomace water extract was determined in adult zebrafish. The same experiment was recently applied for *Ayapana triplinervis* essential oil, *Phyllanthus phillyreifolius* extract and geraniin (Haddad *et al*., 2020; Haddad, Picard *et al*., 2019). For this purpose, intraperitoneal injection of cranberry pomace water extract was performed corresponding to the maximum non-cytotoxic concentration (400 µg/g of body weight) estimated *in vitro*. The experiment was carried out for 5 days, and we did not detect any sign of suffering, stress or abnormal behavior. The fish survival was monitored several times a day throughout all the experiment (**Table 3.15**). In addition, the obtained results showed that, from the moment of injection to day 5, the water extract-injected fish displayed 100% survival similar to the control group. Taken together, this data suggests that cranberry pomace water extract at the maximum non-cytotoxic concentration does not exhibit acute toxicity *in vivo*.

Table 3.15. Survival of the fish injected with cranberry pomace water extract from 1 day post injection to 5 days post injection (dpi)

	Number of fish alive						
	Number of injected fish	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	Survival rate at 5 dpi (%)
MEM 5% (vehicle)	8	8	8	8	8	8	100
Cranberry pomace water extract	11	11	11	11	11	11	100

MEM 5% – minimum essential medium supplemented with 5% of heat inactivated fetal bovine serum

3.6. Antioxidant activity of extracts and solid residues

Finally, the biorefining process was evaluated by measuring the antioxidant potential of the extracts isolated by different methods and directly of the solid powder (the QUENCHER method), i.e., cranberry pomace and residues after each extraction step (**Table 3.16**).

Thus, all the measured values for lipophilic fractions were rather low; however, Soxhlet extracts demonstrated higher TPC and $ABTS^{\bullet+}$-scavenging than the SC-CO_2 extract. On the contrary, the ORAC assay of lipophilic extracts resulted in a similar antioxidant capacity. Tocopherols are the main fat-soluble antioxidants, which act by inhibiting peroxyl radicals, and a previous study showed that these antioxidants are even more efficiently recovered by SC-CO_2 than Soxhlet-hexane (Kraujalis and Venskutonis, 2013b). On the other hand, hexane is a more effective solvent than CO_2 for the recovery of carotenoids which constitute another type of antioxidants. Antioxidant capacity values after removing lipophilic substances, including fat-soluble antioxidants, was found to be reduced in all the assays by approx. 6–23% except for the product ORAC value after SFE. PLE-EtOH and PLE-H_2O recovered the majority of polar polyphenolic antioxidants. PLE-H_2O extracts were remarkably stronger antioxidants than PLE-EtOH extracts; however, due to the very high yields (**Table 3.9**), EtOH recovered the major part of antioxidants as it was calculated for the whole plant DW. For instance, TPC in the residue after PLE-EtOH was 3.4-fold lower than in the initial cranberry pomace (with moisture) and 11-fold lower when calculated for pomace DW. This data proves the efficiency of the multistep biorefining procedure. Moreover, it enables obtaining 3 products, namely PUFA, and, most likely, the tocopherol-rich lipophilic fraction, a high yield EtOH-soluble antioxidant fraction, and H_2O-soluble very strong antioxidant fraction, which, in addition, contains a high concentration of healthy proanthocyanidins. For instance, in the ORAC assay, the antioxidant capacity of the PLE-H_2O extract was similar to the antioxidant capacity of the pure synthetic antioxidant *Trolox*, i.e., 1084.33 TE/g. Consequently, each fraction may be adapted to foods or other products while taking into account their chemical composition, physical properties and the possible impact on the sensory quality. For instance, PLE-EtOH has already been tested in meat burgers and proven to be an effective additive improving the oxidative stability and microbiological safety of the meat. As it was mentioned above, the final residue, most likely, contains valuable dietary fiber; however, its characterization was beyond the scope of this study. Therefore, the results of this study have created a promising platform for ‘zero waste’ processing of cranberry pomace at the industrial scale.

Table 3.16. Total phenol content and antioxidant potential of extracts, pomace and residues after extractions

Sample	TPC, mg GAE/g		$ABTS^{\cdot+}$, mg TE/g		ORAC, mg TE/g	
	Residue	DW	Residue	DW	Residue	DW
RESIDUES						
Cranberry pomace	19.23±1.61^{d}	20.42±1.71^{e}	84.12±4.91^{d}	89.32±5.21^{e}	40.55±0.52^{e}	43.06±0.55^{e}
After SFE-CO_2 (0.2mm)	18.06±0.60^{c}	16.05±0.53^{d}	76.47±5.19^{c}	67.98±4.61^{d}	46.09±3.69^{d}	40.97±3.28^{d}
After Soxhlet extraction	15.80±1.07^{b}	14.06±0.95^{c}	74.71±0.45^{c}	66.49±0.40^{c}	32.92±1.95^{c}	29.30±1.74^{c}
After PLE-EtOH	5.60±0.52^{a}	1.85±0.17^{b}	39.91±2.09^{b}	13.21±0.69^{b}	10.44±0.65^{b}	3.45±0.21^{b}
After PLE-EtOH-H_2O	4.24±0.99^{a}	1.12±0.26^{a}	11.52±0.63^{a}	3.81±0.20^{a}	4.33±0.15^{a}	1.15±0.04^{a}
EXTRACTS						
SFE-CO_2 (0.2mm)	6.21±0.52^{a}	0.69±0.06^{a}	11.43±0.50^{a}	1.27±0.06^{a}	14.42±1.26^{a}	1.60±0.14^{a}
Soxhlet extraction	12.06±0.61^{b}	1.33±0.07^{b}	27.77±2.26^{b}	3.05±0.25^{b}	11.85±0.71^{a}	1.30±0.08^{a}
PLE-EtOH	52.98±0.31^{c}	26.32±0.28^{c}	130.40±3.3^{c}	60.16±1.52^{d}	345.0±16.4^{b}	159.2±7.6^{c}
PLE-EtOH-H_2O	188.90±5.3^{d}	5.25±0.15^{d}	362.6±13.8^{d}	10.08±0.39^{c}	1084±78^{c}	30.14±2.17^{b}

*DW – dry pomace weight; ^{a,b} Different letters in the same column indicate statistical differences between residues or extracts (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of triplicate determinations

3.7. Effect of cranberry pomace extracts isolated by pressurized ethanol and water on the inhibition of food pathogenic/spoilage bacteria and the quality of pork products[3]

3.7.1. Antimicrobial activity of defatted cranberry pomace extracts in bacterial cultures

High polarity pressurized water and ethanol solvents were used for the fast extraction of bioactive cranberry components which are mainly strong antioxidant phenolic compounds. It was observed that the antimicrobial activity of extracts depends on the applied solvent, its concentration and the numbers of the colony forming units of microorganisms (see **Table 3.17**). The inhibitory action of the extracts was evaluated against Gram-positive (*W. viridescens*, *L. mesenteroides*, *L. monocytogenes*, *B. thermosphacta*) and Gram-negative (*P. putida* and *C. jejuni*) bacteria.

The extracts exhibited the most significant activity against two Gram-positive (*L. monocytogenes* and *B. thermospacta*) and Gram-negative (*P. putida* and *C. jejuni*) bacteria; almost in all the cases, higher concentrations (6.6% and 3.3%), independently on the solvents and the numbers of microorganisms, fully (100%) inhibited the growth of these bacteria. Diarra *et al*. (2013) reported that the fraction of the ethanol extract of cranberry pomace rich in phenolic acids and their derivatives, anthocyanins and flavonol glycosides demonstrated the strongest inhibitory effect against *S. aureus*, mainly by affecting peptidoglycan biosynthesis in bacteria cells. However, the two other Gram-positive bacteria tested in our study, *W. viridescens* and *L. mesenteroides*, were more resistant to the extracts at the all applied concentrations. Actually, the extracts did not inhibit the growth of LAB-related microorganisms; on the contrary, they even slightly stimulated their growth. It is in agreement with the results of Puupponen-Pimiä *et al*. (2005) showing that pure phenolic compounds and berries grown in the Northern countries do not inhibit LAB, while Molan, Lila, Mawson and De (2009) reported the prebiotic activity of water-soluble blueberry extracts. It should be noted that many members of the *Weissella* genus were originally classified as *Leuconostoc* or *Lactobacillus*; however, the application of molecular methods enabled to separate these phylogenetically closely related bacteria into another genus (Dušková, Kameník and Karpíšková, 2013).

When comparing ethanol and water extracts, it may be observed that the former was a stronger agent against *C. jejuni* and *P. putida*, while the latter required a lower concentration (1.65%) to inhibit 100% of *L. monocytogenes*. The inhibitory effect against *B. thermosphacta* was similar for both types of extracts. It should also be noted that the inhibitory effect was more pronounced in the case of using the cultures with lower cfu numbers (4 log cfu/mL). At higher values (8 log cfu/mL), the inhibitory effect of the ethanol extract was more dependent on its concentration; the antimicrobial potential significantly decreased with the decreasing

[3] This section was prepared with reference to Tamkutė, Melero Gil, Rovira Carballido, Pukalskienė and Venskutonis (2019)

Table 3.17. Effect of ethanol and water extracts of defatted cranberry pomace on the count of different bacteria

Sample	*L. monocytogenes*		*C. jejuni*		*P. putida*		*B. thermosphacta*		*L. mesenteroides*		*W. viridescens*	
	Inoculum (log cfu/mL)											
	4	8	4	8	4	8	4	8	4	8	4	8
	log cfu/mL											
Control	$^{c}9.20_{B}$	$^{c}8.77_{A}$	$^{e}8.22_{A}$	$^{e}8.11_{A}$	$^{ef}8.75_{B}$	$^{b}8.98_{A}$	$^{c}8.10_{A}$	$^{e}8.44_{A}$	$^{abc}8.13_{A}$	$^{ef}8.01_{A}$	$^{c}9.06_{B}$	$^{c}7.93_{A}$
EtOH, 6.6%	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{bcd}8.47_{A}$	$^{h}9.03_{B}$	$^{ab}8.63_{A}$	$^{e}8.98_{B}$
EtOH, 3.3%	$^{a}0_{A}$	$^{c}8.61_{B}$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{cd}8.60_{A}$	$^{fg}8.49_{A}$	$^{a}8.56_{A}$	$^{d}8.53_{A}$
EtOH, 1.65%	$^{bc}8.56_{B}$	$^{bc}8.22_{A}$	$^{b}1.93_{A}$	$^{b}1.93_{A}$	$^{a}3.15_{A}$	$^{c}7.46_{B}$	$^{a}0_{A}$	$^{b}5.48_{B}$	$^{de}8.81_{B}$	$^{efg}8.25_{A}$	$^{ab}8.71_{B}$	$^{d}8.50_{A}$
EtOH, 0.83%	$^{bc}8.53_{A}$	$^{c}8.88_{A}$	$^{d}6.81_{B}$	$^{c}5.68_{A}$	$^{ef}8.44_{A}$	$^{ef}9.00_{B}$	$^{c}7.82_{A}$	$^{c}7.54_{A}$	$^{ab}8.05_{B}$	$^{cd}7.22_{A}$	$^{abc}8.83_{B}$	$^{bc}7.69_{A}$
EtOH, 0.42%	$^{b}8.09_{A}$	$^{c}8.85_{A}$	$^{e}8.32_{B}$	$^{e}8.00_{A}$	$^{ef}8.37_{A}$	$^{f}9.01_{B}$	$^{c}7.83_{A}$	$^{cd}7.67_{A}$	$^{ab}8.10_{B}$	$^{b}6.45_{A}$	$^{abc}8.78_{B}$	$^{c}7.78_{A}$
W, 6.6%	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{a}0$	$^{e}9.31_{B}$	$^{gh}8.59_{A}$	$^{ab}8.61_{A}$	$^{de}8.68_{A}$
W, 3.3%	$^{a}0$	$^{a}0$	$^{a}0_{A}$	$^{b}1.83_{B}$	$^{c}7.13_{A}$	$^{d}8.31_{B}$	$^{a}0$	$^{a}0$	$^{abc}8.11_{A}$	$^{de}7.76_{A}$	$^{ab}8.72_{A}$	$^{de}8.75_{A}$
W, 1.65%	$^{a}0$	$^{a}0$	$^{c}2.96_{A}$	$^{c}5.39_{B}$	$^{cd}7.46_{A}$	$^{d}8.22_{B}$	$^{b}3.15_{B}$	$^{a}0_{A}$	$^{ab}8.00_{B}$	$^{cd}7.29_{A}$	$^{ab}8.74_{B}$	$^{a}7.40_{A}$
W, 0.83%	$^{bc}8.57_{A}$	$^{b}7.85_{A}$	$^{e}7.88_{B}$	$^{d}7.54_{A}$	$^{de}7.98_{A}$	$^{d}8.26_{A}$	$^{c}8.27_{B}$	$^{cde}7.82_{A}$	$^{a}7.60_{B}$	$^{bc}6.91_{A}$	$^{bc}8.89_{B}$	$^{ab}7.49_{A}$
W, 0.42%	$^{bc}8.68_{A}$	$^{c}8.64_{A}$	$^{e}8.22_{A}$	$^{e}8.23_{A}$	$^{f}8.75_{A}$	$^{e}8.98_{A}$	$^{c}8.43_{A}$	$^{de}8.35_{A}$	$^{abc}8.23_{B}$	$^{a}4.44_{A}$	$^{ab}8.70_{B}$	$^{bc}7.34_{A}$

^{a,b} Different letters in the same column indicate statistical differences (one-way ANOVA, $p < 0.05$); $_{A,B}$ Different letters in the same line between the used concentrations for each microorganism indicate significant differences, $p < 0.05$. The results are expressed as mean ± standard deviation of triplicate determinations and are given in log cfu/mL

concentrations. When considering the remarkably higher yield and, in some cases, the slightly stronger antibacterial activity against the tested strains, the ethanol extract was selected for further studies with pork meat.

The antimicrobial activity of cranberries depends on various compounds and their mixtures. For instance, organic acids affect the release of lipopolysaccharides from the outer membrane of Gram-negative bacteria, which increases the membrane permeability and causes its disruption. As a result, they invade the cell, inhibit the transcription of genes and prevent the biosynthesis of proteins which are necessary for the growth of bacteria (Wu *et al*., 2009). The information about the mechanisms of cranberry extract on Gram-positive bacteria is rather scarce. It may be observed that Gram-negative *P. putida* and *C. jejuni* were slightly more resistant than Gram-positive *B. thermospacta* and *L. monocytogenes*. These results could be explained by the differences between the cell walls, which plays an important role in the osmotic cellular protection. It was also hypothesized that berry phenolics could bind the outer cell wall membrane and disrupt the permeability barrier in Gram-negative bacteria; therefore, Gram-positive and Gram-negative bacteria may undergo different damage from antimicrobial compounds (Puupponen-Pimiä *et al*., 2001). The wall of Gram-negative bacteria contains a thin layer of peptoglycan and the outer membrane which is not present in Gram-positive bacteria. Due to the lack of this membrane, the permeability of bioactive compounds into cells may increase (Tian *et al*., 2009).

Flavan-3-ols, especially catechins, are known for their *in vitro* antibacterial activity against several bacterial species, such as *Vibrio cholerae*, *Streptococcus mutans, C. jejuni, Clostridium perfringes*, and *E. coli* (Ahn, Kawamura, Kim, Yamamoto and Mitsuoka, 1991; Diker, Akan, Hascelik and Yurdakök, 1991). Flavonols (rhamnetin, myricetin, morin, and quercetin) also demonstrated remarkable activity against Gram-positive and Gram-negative bacteria (Cushnie, Hamilton, Chapman, Taylor, & Lamb, 2007). Puupponen-Pimiä *et al*. (2001) reported that pure myricetin inhibited Gram-negative *E. coli*, but *S. typhimurium* was not affected. Proanthocyanidins, which are present in cranberries, were active against *E. coli*, *S. mutans*, *S. aureus* bacteria (Dixon, Xie and Sharma, 2005; Heinonen, 2007): these authors suggested several possible inhibitory mechanisms by A-type proanthocyanidins, e.g., the destabilization and permeabilization of the membrane, the inhibition of enzymes, the disruption of metabolic processes, and the chelation of metals which are important to the growth of microorganisms. Gallic, caffeic, and ferulic acids inhibited Gram-positive and Gram-negative bacteria, while chlorogenic acid was not active against Gram-positive bacteria (Saavedra *et al*., 2010).

3.7.2. Effect of heating on the antimicrobial activity of ethanol extract

Heating may influence the antimicrobial properties of extracts by changing the structures of heat-sensitive compounds. Consequently, in some cases, heating may reduce the effectiveness of extracts. For instance, garlic completely lost its inhibitory properties after heating at 100 °C for 20 min (Witkowska, Hickey, Alonso-Gomez and Wilkinson, 2013). So far as the majority of finished meat products are obtained

by thermal processing, it was decided to assess the effect of heating on the antimicrobial properties of the ethanol extract.

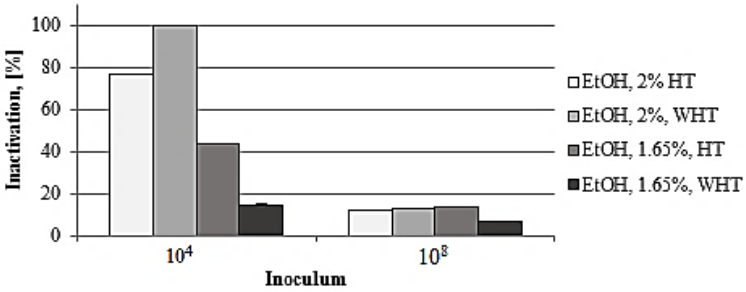


Figure 3.16. Inactivation of *L. monocytogenes* with/without heat treatment.*HT – heat treatment; * WHT – without heat treatment. The results are expressed as mean ± standard deviation of triplicate determinations

It may be observed that, in the case of 10^4 cfu/mL inoculum, *L. monocytogenes* inactivation by 2% ethanol extract after heating at 72 °C reduced from 100% to 77% (**Fig. 3.16**), while the inhibitory activity of 1.65% ethanol extract after heating increased from 14% to 43%. A similar tendency was determined for the latter extract concentration in the case of 10^8 cfu/mL. It is difficult to explain this finding; possibly, due to the chemical complexity of the crude ethanol extract, some antimicrobial compounds lose their activity, while some new antimicrobials may form during heating, and the final effect depends on their concentrations, interactions and antimicrobial potential. The effect of 2% of the ethanol extract on inoculum of 10^8 cfu/mL was similar before and after heating.

3.7.3. Antimicrobial activity of the extract during pork slurry storage

The assessment of the antimicrobial potential of the cranberry pomace ethanol extract was continued by measuring *L. monocytogenes*, *B. thermosphacta*, *P. putida* and AMB in pork slurry (**Fig. 3.17**). Pathogenic *L. monocytogenes* did not grow in C and CL samples (without inoculum) during all the storage time, which indicates that cross-contamination was avoided. In the inoculated samples CI and L, the growth of *L. monocytogenes* was significantly inhibited by the addition of the pomace extract; at the end of the storage, the number of these bacteria in L was 0.15 log cfu/mL, in comparison to CI 4.6 log cfu/mL. It was previously reported that 7.5% additive of the pomegranate peel extract also exhibited the bactericidal effect against *L. monocytogenes* in meat pâté, and this effect could be due to the activity of phenolic compounds (Hayrapetyan, Hazeleger and Beumer, 2012).

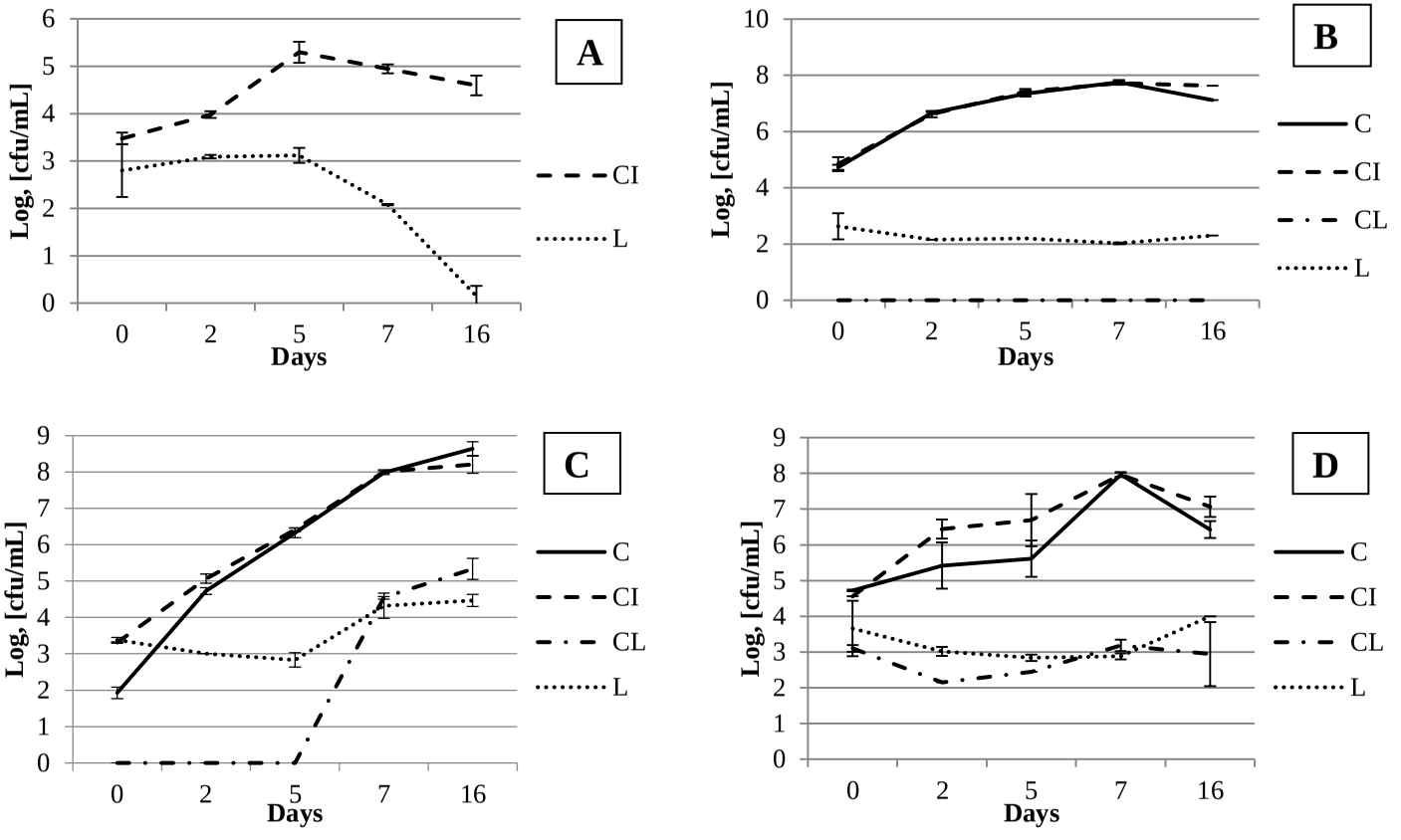


Figure 3.17. Growth of *L. monocytogenes* (A), *B. thermosphacta* (B), *P. putida* (C), aerobic mesophilic bacteria (D) in pork slurry with 2% ethanolic extract during 16 days of storage. Control without inoculum (C); control with inoculum (CI); control with 2% of extract (CL), and sample with 2% of extract and inoculum (L). The results are expressed as mean ± standard deviation of duplicate determinations

The ethanol extract demonstrated bacteriostatic effect against *B. thermosphacta*; the numbers of these bacteria in the inoculated sample with extract (L) were several times lower than in the control sample and, at the end, reached 2.2 log cfu/mL (**Fig. 3.17 B**). No significant differences were observed between the samples without the extract; at the end of the storage, the numbers of *B. thermosphacta* were 7.6 (CI) and 7.1 log cfu/mL (C). It should be noted that, for this experiment, the meat was not sterile, and therefore, at day 0, the control sample contained >4 log cfu/mL of bacteria, while in the control sample with the extract during the whole experiment these bacteria were not detected. It may be assumed that the extract had bactericidal effects on *B. thermosphacta* and, therefore, when it was applied, the counts were not detected. Also, in this particular case, the effects of inoculation and extract addition might not demonstrate linear dependencies. However, a more precise explanation of this finding would require additional experiments.

The addition of the extract also has a significant effect on the growth of *P. putida*. In the sample with extract (CL), the growth of *Pseudomonas* was inhibited by 100% until the 5^{th} – 7^{th} day of storage, and only afterwards started growing until reaching 5.3 log cfu/mL (**Fig. 3.17 C**). *Pseudomonas spp.* was slightly inhibited in the inoculated sample with extract (L); however, after 7 days of storage, these bacteria started to grow and reached 4.46 log cfu/mL (**Fig. 3.17 C**). The growth of *Pseudomonas spp.* in the control sample with (CI) and without (C) inoculum was observed during all the storage time, and, at the end, no significant differences were found between CI and C; however, their log cfu/g values were two times higher than in the samples with the extract.

The numbers of AMB may be linked to the total bacterial count (**Fig. 3.17 D**). The high initial values of AMB between all the samples could be due to the initial contamination of the meat and inoculated bacteria. It may be observed that, in controls without extract, no significant differences were observed, and, at the end of the storage, the counts of these bacteria reached 7 log cfu/mL. The addition of the extract inhibited their growth: in the control sample (CL) and the inoculated sample (L), during the entire experiment, the amount of AMB was similar, and, at the end of the storage, it reached 3–4 log cfu/mL. Recently, Stobnicka and Gniewosz (2018) studied the effects of cranberry fruit and pomace extracts isolated with 40% ethanol on *S. aureus*, *L. monocytogenes*, *S. enteritidis*, and *E. coli* and reported that 2.5% addition of extracts to minced pork meat reduced the number of pathogenic bacteria by 4 log cycles after 4 days of refrigerated storage. To conclude, the cranberry pomace ethanol extract at the applied concentration (2%) demonstrated significant effects on the microbiological processes in pork slurry.

3.7.4. Effect of extract on the microbial growth in hamburgers during storage

Meat is an excellent medium for microorganisms which are uncontrolled in terms of their ability to grow and reproduce on meat surfaces. Moreover, minced meat is more sensitive to oxidation and the growth of microorganisms. In addition, *L. monocytogenes* can survive under relatively extreme physicochemical conditions,

such as low and/or high temperature, low pH and high salt content. *B. thermosphacta* and *P. putida* are the main spoilage microbial flora of meat stored at refrigerated conditions. A properly selected modified atmosphere may restrict the growth of *Pseudomonas spp.* in meat; in this case, LAB and *B. thermosphacta* may become the major spoilage factors. *Pseudomonas spp.* is denoted by proteolytic activity, which may cause a variety of odor and flavor defects, production of slime, and other undesirable changes, such as the formation of green pigments on the surface of meat stored at refrigeration temperature.

The inhibitory effect of extract additives on the growth of *L. monocytogenes* is evident (**Fig. 3.18 A**); their numbers in inoculated control (RCI) increased from 3 log cfu/g (the initial value) to 6.5 log cfu/g on day 16; while the growth of pathogenic bacteria in an inoculated sample with extract (RL) was inhibited during the whole period of storage (2.72 log cfu/g after 16 days). It was determined that polyphenols were effective antimicrobials for *L. monocytogenes*, and their effectiveness increased as pH was reduced from 6.5 to 4.5 (Wen, Delaquis, Stanich and Toivonen, 2003). The numbers of *L. monocytogenes* did not increase in non-inoculated (RC and RCL) samples, which indicates the absence of cross-contamination.

The initial values determined for *B. thermosphacta* were from 2.3 to 3.1 log cfu/g (**Fig. 3.18 B**) indicating that non-inoculated samples were naturally contaminated with these bacteria. Again, the application of 2% extract remarkably reduced the growth of *B. thermosphacta* during storage: their numbers in the samples without the extract increased to 7 log cfu/g, while in the samples with the extract they reached only approx. 3 log cfu/g. The ethanol extract was also very effective against *P. putida* (**Fig. 3.18 C**): the counts of *Pseudomonas spp.* in the samples without extracts (RC and RCI) increased from 3.5 log cfu/g (the initial value) to 7.1 log cfu/g (day 16), whereas extract addition inhibited the growth of *P. putida*, and there was no significant difference between the non-inoculated and inoculated samples.

LAB also significantly influenced the quality of meat and meat products as it caused sour off-flavors and off-odors, slime, pack swelling and/or greenish discoloration. The effect of the extract on LAB was not as remarkable as for the other tested microorganisms (**Fig. 3.18 D**); although, significant differences between the samples without and with the extract were observed. At the end of the storage, the LAB values in the control samples were almost 10 log cfu/g, while in the samples with extracts they were approx. 8 log cfu/g. Again, there was no significant difference between the non-inoculated and inoculated samples.

The effect of the extract on the growth of AMB was notably similar to that on LAB (**Fig. 3.18 E**). The inhibitory effect of the extract appeared after 2–5 days of the storage, while, at the end of the storage time, the counts reached approx. 10 log cfu/g (without the extract) and 8 log cfu/g (with the extract). Based on the European Commission regulation No. 1441/2007, hamburgers without the cranberry pomace extract were not suitable for food after 5 days of storage, while the extract additive increased their shelf life to 9 days, which is an achievement of essential importance.

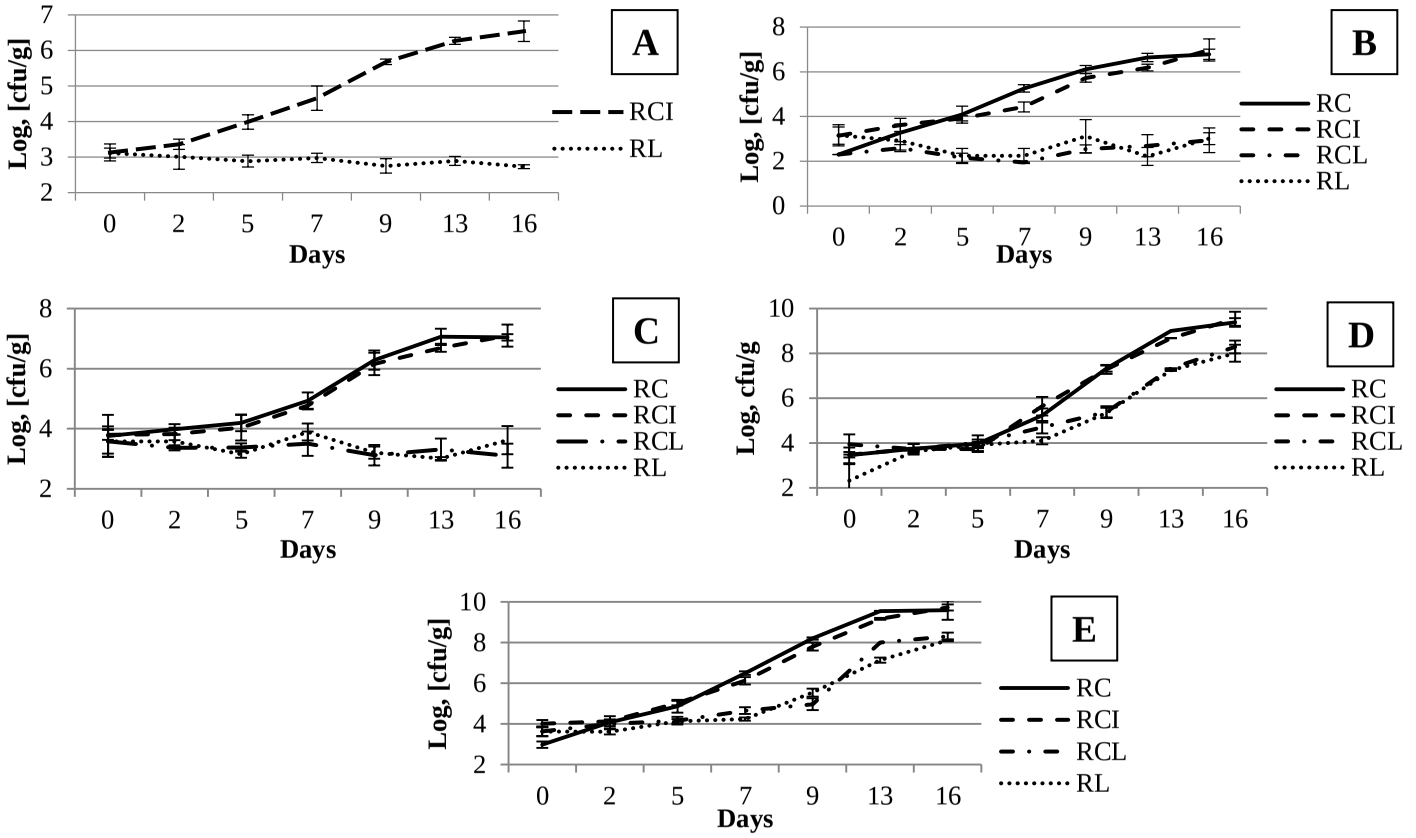


Figure 3.18. Growth of *L. monocytogenes* (A), *B. thermospacta* (B), *P. putida* (C), lactic acid bacteria (D), *aerobic mesophilic bacteria* (E) in hamburgers with 2% ethanolic extract during 16 days of storage. Control without inoculum (RC); control with inoculum (RCI); control with 2% of extract (RCL) and sample with 2% of extract and inoculum (RL). The results are expressed as mean ± standard deviation of duplicate determinations

3.7.5. Effect of extract on the physico-chemical characteristics of hamburgers

3.7.5.1. Gas composition in packages

The modified atmosphere packaging (MAP) is a technique which is widely used to extend the shelf life and to improve the quality of perishable foods including meat and meat products stored at refrigeration temperatures or below. The color stability, the oxidative status of lipids and the microbial counts are the most important quality criteria during the storage of raw red meat. The aim of MAP is to stabilize the color and to inhibit oxidation and microbial growth. It is proposed that 80% N_2 and 20% CO_2 is the preferable gas composition for MAP of meat. It should be noted that oxygen is required for maintaining the oxygenated form of myoglobin, the principal proteinaceous meat pigment providing to it the bright cherry-red color (Mancini and Hunt, 2005); however, the presence of oxygen also increases the rate of lipid oxidation, which causes undesirable changes in the flavor and color.

In the packages of minced pork meat hamburgers, the concentrations of O_2 (0–0.5%), CO_2 (19–27%), and N_2 (71–80%) were adequately similar in all the samples throughout the entire experiment. No leakage was detected in the packages. The main causes for the change in the gas composition may be the result of microbial growth, the permeability of the packaging material, and the respiration of the product or the gas absorption by the food (Sørheim, Ofstad and Lea, 2004).

3.7.5.2. Color changes during the storage of burgers

Color is an important factor for consumer's acceptance of meat and its products. The shelf life and quality of the final products are strongly influenced by the initial meat quality, additives, packaging parameters, and storage conditions (Ozer and Sariçoban, 2010). The effect of the cranberry pomace ethanol extract on the color and its changes during storage was evaluated by the CIELab system (see **Table 3.18**). The changes in parameter L^* were negligible during storage in all the samples. However, the addition of the cranberry extract significantly influenced the a^* and b^* values of hamburgers. The initial changes of the a^* and b^* values are due to the intensive color of the added extract, while the loss of redness (a^*) was the most important change of the hamburger's color during storage: the progressive decrease of a^* was observed in all the samples. However, the redness of burgers with the ethanol extract was more intense than that of the control samples. Parameter b^* (yellowness) decreased from the first day of storage in the control sample (RC) without the extract, while in the product with the extract (RCL) it increased and reached 6.35. According to Esmer, Irkin, Degirmencioglu and Degirmencioglu (2011), the loss of meat redness and the transition to the brownish red color via the formation of metmyoglobin also results in the decrease of the b^* value.

3.7.5.3. Metmyoglobin (MetMb) changes during storage

The meat color depends on the amount of nitrosomyoglobin (NOMb), myoglobin and its forms, oxymyoglobin (MbO_2), metmyoglobin (MetMb) and deoxymyoglobin (Mb) (Karamucki, Jakubowska, Rybarczyk and Gardzielewska,

2013). The meat color changes by conversion of MbO_2 to MetMb due to low pH, salt concentration, spice additives, packaging, and the meat source (King and Whyte, 2006). MetMb was formed during the hamburger's storage, which indicates Mb oxidation. The initial values of MetMb in the samples without (RC) or with the extract (RCL) were similar, about 45%, while as early as during the second day of storage, the MetMb content in RC increased from 45.10% to 52.61%. During the further storage, the content of MetMb decreased until the initial value, and any further changes were not significant. During the storage of RCL hamburgers, the amount of MetMb increased, and, at the end of the experiment, reached 65%. The reason might be low pH because the amount of MetMb increased at pH 5.5 or became lower and thus decreased at pH 6; higher pH values were measured for RC than for RCL.

3.7.5.4. pH changes during storage

pH of meat products is associated with their safety, sensory properties, and texture. Pork hamburgers with 2% ethanol extract had a lower ($p < 0.05$) pH than the control samples during the storage at 4±1 °C (**Table 3.18**). This can be attributed to the low pH (2.5) level of the extract. However, during the 16-day storage, the changes of pH in RCL were very low although significant ($p < 0.05$). Such changes were more noticeable in the control samples without the cranberry extract. For instance, in RC, after 16 days, pH decreased from 6.04 to 5.51. It may be attributed to the accumulation of LAB metabolites; higher numbers of these bacteria were determined in the samples without the extract. It was reported that the grape pomace extract did not have significant effect on the pH of pork burgers during 6-day storage at 4 °C (Garrido, Auqui, Martí and Linares, 2011). Similar effects were reported for raw pork burgers produced with the grape extract and bearberry and stored for 12 days at 4 °C (Carpenter *et al*., 2007). The product pH may also be reduced due to the hydrolysis of lipids and the formation of free fatty acids.

3.7.5.5. Water activity (a_w) changes during storage of burgers

Water activity is an important factor of microbial growth. In general, the differences in a_w were fairly negligible (0.988–0.997), and the pomace extract did not have significant effect on a_w ($p > 0.05$) of raw burgers (**Table 3.18**). However, some significant a_w increase ($p < 0.05$) was indicated by the statistical data handling during the storage period except for the measurements performed on days 7 and 9 when the differences were not significant ($p > 0.05$). A burger's a_w may increase due to the high moisture content under storage conditions (Biswas, Keshri and Bisht, 2004). Generally, at the end of the storage, all the values were slightly although significantly lower as compared to the control sample.

Table 3.18. Changes of physico-chemical parameters of pork burgers during storage

Day	Sample	pH	a_w	Color, L^*	Color, a^*	Color, b^*	MetMb,%	Malondialdehyde, mg/kg
0	RC	6.04±0.02^{bc}	0.997±0.002^{d}	57.03±5.80^{b}	5.02±1.91^{ab}	15.31±1.35^{abc}	45.00±1.44^{ab}	0.351±0.152^{a}
	RCL	4.96±0.02^{b}	0.995±0.001^{b}	46.06±4.03^{ab}	10.59±1.40^{d}	3.86±0.46^{b}	45.86±0.89^{d}	0.063±0.063^{a}
2	RC	6.13±0.04^{d}	0.992±0.003^{bc}	53.77±3.48^{ab}	7.49±1.38^{d}	16.91±1.42^{d}	52.61±2.82^{d}	0.397±0.034^{a}
	RCL	5.02±0.02^{cd}	0.993±0.002^{ab}	44.76±4.79^{a}	8.31±1.78^{c}	2.33±0.35^{a}	64.09±0.77^{a}	0.098±0.045^{a}
5	RC	6.07±0.03^{c}	0.994±0.001^{bcd}	55.86±4.84^{ab}	6.80±2.13^{cd}	16.51±1.90^{cd}	45.96±1.53^{cd}	0.403±0.013^{a}
	RCL	5.06±0.03^{d}	0.995±0.001^{b}	49.28±5.33^{bc}	7.53±0.60^{bc}	4.79±0.64^{bc}	60.60±2.64^{c}	0.117±0.090^{ab}
7	RC	6.09±0.04^{cd}	0.988±0.003^{a}	55.97±4.74^{ab}	6.50±1.25^{bcd}	14.39±3.37^{ab}	48.13±1.81^{bc}	0.479±0.055^{a}
	RCL	5.04±0.04^{d}	0.991±0.002^{a}	46.56±2.46^{ab}	7.40±0.74^{cd}	4.62±0.56^{bc}	56.19±2.96^{b}	0.129±0.030^{ab}
9	RC	5.99±0.05^{b}	0.991±0.003^{ab}	51.40±5.52^{a}	6.56±0.82^{cd}	13.70±1.15^{a}	47.67±2.62^{bc}	0.814±0.178^{b}
	RCL	5.06±0.01^{d}	0.993±0.001^{ab}	47.27±2.51^{abc}	7.18±1.14^{cd}	5.12±0.98^{c}	57.64±2.12^{bc}	0.200±0.047^{b}
13	RC	5.50±0.03^{a}	0.995±0.001^{cd}	53.84±2.62^{ab}	5.23±0.47^{bc}	13.69±1.58^{a}	43.68±0.70^{a}	0.944±0.011^{b}
	RCL	4.99±0.03^{bc}	0.991±0.002^{a}	53.71±2.39^{d}	5.38±0.47^{a}	6.37±1.12^{d}	65.12±2.60^{d}	0.152±0.025^{ab}
16	RC	5.51±0.05^{a}	0.993±0.003^{bc}	53.64±2.56^{ab}	4.51±0.33^{a}	13.57±2.42^{a}	49.90±2.06^{cd}	1.394±0.097^{c}
	RCL	4.78±0.03^{a}	0.991±0.002^{a}	50.88±2.86^{cd}	6.70±0.49^{b}	6.35±1.17^{d}	65.00±1.43^{d}	0.206±0.030^{b}

^{a,b} Different letters in the same column indicate statistical differences in one sample during the storage (one-way ANOVA, $p < 0.05$); Control without inoculum (RC) and control with 2% of extract (RCL). The results are expressed as mean ± standard deviation of triplicate determinations

3.7.5.6. Effect of cranberry pomace extract on oxidation of hamburgers

The ethanol extract is a very strong antioxidant: the value of the total phenolic content measured with the Folin-Ciocalteu reagent was 52.98±0.31 mg gallic acid equivalents per g of extract, the trolox equivalent antioxidant capacity (TEAC) measured in the ABTS radical cation assay was 130.40±3.3 mg/g, and the oxygen radical absorbance capacity (ORAC) was 345±16.4 mg TE/g. Therefore, it was important to determine its antioxidant potential in meat products. Malondialdehyde (MDA), which forms colored products in the course of reaction with TBA, is undesirable in meat products because it is a secondary product of lipid peroxidation. The effect of the extract on MDA accumulation during storage is evident (**Table 3.18**). The MDA value of the control burger increased from 0.351 mg MDA/kg (day 0) to 1.394 mg MDA/kg (day 16), while for the burgers with the 2% extract, this value increased from 0.063 to 0.206 mg MDA/kg, which indicates a strong antioxidative effect provided by the compounds extracted from cranberry pomace. It is in agreement with the strong antimicrobial activity of the extract (see Section 3.7.1.). On the other hand, it should be noted that, according to Wood *et al*. (2004), the values up to 2.00 mg of MDA/kg product are not perceived by the consumers; as manifested by the data, none of the tested samples reached this value during 16 days of storage.

3.7.6. Sensory evaluation of hamburgers

It is extremely important that plant origin additives would not have negative effect on the sensory quality of meat products. Therefore, the sensory evaluation of products was performed by assessing their homogeneity, superficial humidity, intensity of color, discoloration and intensity of odor (**Tables 3.19** and **3.20**). It may be observed that the addition of 2% of the cranberry pomace extract had a significant effect only on the color intensity; according to the panelist's comments, the extract increased pink or light purple color tones. At the beginning of the storage, the color changes were not observed for any of the samples, while, after a longer storage time, discoloration progressively increased in both products and was detected by 50% of the panelists on day 9 and by all of them on days 13 and 16.

Table 3.19. Effect of the type of product (without or with extract) on the sensory properties of raw burgers

Sample	Homogeneity	Superficial humidity	Intensity of color	Discoloration	Intensity of odor
RC	3.00±1.08^{a}	2.32±1.00^{a}	2.22±0.92^{a}	2.10±1.22^{a}	2.85±1.32^{a}
RCL	3.20±0.76^{a}	2.53±0.86^{a}	2.82±0.83^{b}	2.28±1.06^{a}	2.95±0.84^{a}

^{a,b} Different letters in the same column indicate statistical differences (one-way ANOVA, $p < 0.05$); Control without inoculum (RC) and control with 2% of extract (RCL). The results are expressed as mean ± standard deviation of eight determinations

All other evaluated sensory parameters were changing except for superficial humidity. Berry, wine and acid odor notes were reported for the hamburger with the extract. During storage, the acid odor note was detected in this product, being

remarkable for 75% of the panelists on day 16. There were no other indications of inferior odor for the burgers with the extract. However, in the control samples without the extract which did not have any off-odors on day 0, from day 9, the intensity of the spoilage odor notes such as putrid was steadily increasing.

Table 3.20. Effect of time (in days) of storage on the sensory properties of raw burgers

Characteristic	0	2	5	7	9	13	16
Homogeneity	3.88± 0.80^{d}	3.43± 0.51^{cd}	3.42± 0.51^{cd}	3.14± 0.77^{bc}	2.86± 0.77^{abc}	2.64± 1.08^{ab}	2.29± 0.99^{a}
Superficial humidity	2.69± 1.07^{a}	2.50± 1.16^{a}	2.17± 0.94^{a}	2.79± 0.89^{a}	2.21± 0.8^{a}	2.43± 0.85^{a}	2.14± 0.77^{a}
Intensity of color	2.94± 0.99^{c}	2.86± 0.95^{c}	2.58± 0.79^{bc}	2.50± 0.85^{bc}	2.86± 0.77^{c}	2.07± 0.73^{ab}	1.79± 0.80^{a}
Discoloration	1.12± 0.34^{a}	1.64± 0.84^{ab}	1.92± 0.90^{b}	2.21± 1.13^{b}	2.29± 0.99^{bc}	2.93± 0.92^{cd}	3.36± 1.15^{d}
Intensity of odor	2.31± 1.14^{a}	2.71± 1.20^{ab}	2.92± 1.24^{abc}	2.57± 1.01^{a}	2.85± 0.95^{ab}	3.64± 0.84^{c}	3.43± 0.85^{bc}

^{a,b} Different letters in the same row indicate statistical differences (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of eight determinations

3.7.7. The effect of extract on the microbiological quality of cooked ham

The effectiveness of the antimicrobial activity of the ethanol extract was evaluated in cooked ham which was inoculated with four strains of *L. monocytogenes* and stored at 4 °C for 40 days. No pathogen growth was observed in the non-inoculated samples both with (CCL) and without (CC) extract (the data is not shown). The counts of *L. monocytogenes* in the inoculated control (CCI) sample was rapidly growing during 22 days of storage (from 3.04 to 8.26 log cfu/g); afterwards, the increase was slower (**Fig. 3.19 A**). The cranberry pomace extract inhibited *L. monocytogenes* in cooked ham very efficiently; during 32 days of storage, the counts of these bacteria were almost the same, in the range of 2.78–3.13 log cfu/g. An increase in the number by 1.3 log cfu/g was observed after 36 days of storage, while, on the last day of storage, the population of *L. monocytogenes* was higher by 3.97 log cfu/g comparing to the initial value.

LAB is also undesirable in thermally processed meat products because it is the main bacterial group associated with spoilage (Samelis, Kakouri and Rementzis, 2000). *Leuconostoc spp.* and *Leuconostoc*-like microorganisms, such as *W. viridescens*, may cause meat products to turn green due to the formation of hydrogen peroxide which oxidizes nitrosomyochromogen as a consequence of the exposure of meat to O_2 (Dušková *et al*., 2013). LAB (**Fig. 3.19 B**) and AMB (**Fig. 3.19 C**) counts increased rapidly in the control samples with (CCI) and without (CC) inoculum. Higher counts of these bacteria were found in the control sample with inoculum. The extracts (CCL and CL samples) inhibited the growth of both LAB and AMB during storage.

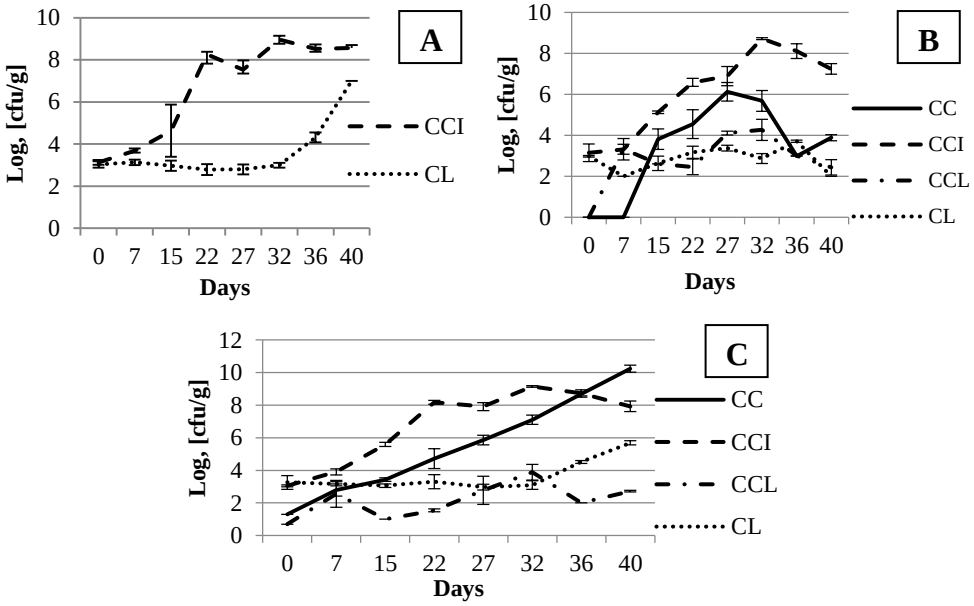


Figure 3.19. Growth of *L. monocytogenes* (A), lactic acid bacteria (B), *aerobic mesophilic bacteria* (C) in cooked ham with 2% ethanolic extract during 40 days of storage. Control without inoculum (CC); control with inoculum (CCI); control with 2% of extract (CCL) and sample with 2% of extract and inoculum (CL). The results are expressed as mean ± standard deviation of duplicate determinations

3.7.8. Physico-chemical characteristics of cooked ham

3.7.8.1. Gas composition changes in the packages

The oxygen concentration was maintained from 0.1% to 16.5% during storage in all the samples (the data is not shown). A higher concentration of oxygen led to a lower concentration of CO_2 which decreased in all the samples during the storage. However, the highest changes were observed in the CCI sample after 15 and 22 days of storage when the concentration of CO_2 decreased to 7.5% and 7.8%, respectively. The changes in the gas composition may have occurred due to the microbial growth (spoilage), the permeability of the film or the solubility in cooked ham (Esmer *et al*., 2011). For instance, the increase in the CO_2 concentration during the storage of burgers may be manifested due to the growth of such microflora as LAB which consumes oxygen and produces CO_2 (Honikel, 2008; Jakobsen and Bertelsen, 2002).

3.7.8.2. pH and water activity changes during storage

The shelf life, texture and the color of ham also depends on pH. A lower pH was observed in the sample with 2% cranberry pomace extract (5.56) compared to the control sample (without the extract). The pH values of cooked ham with the extract may be lower due to the presence of organic acids, such as chlorogenic and benzoic acids, which are extracted from cranberry pomace with 96% ethanol

(Gniewosz and Stobnicka, 2018). During the storage of cooked hams, pH slightly increased in all the samples; however, higher pH values were in the sample without ($p < 0.05$) the extract during all the storage time.

The water activity of the cooked hams without and with 2% extract was similar, 0.980 and 0.977, respectively. During the storage of cooked hams, the water activity remained similar for both samples. This result showed that the addition of the extract in the ham formulation did not exert any influence on the water activity in the product.

3.7.8.3. Color changes during storage

On the first day of storage, the L^* values (lightness) were lower in cooked ham with the extract, which means that these samples were darker than the control sample (**Table 3.21**). During storage (7, 15, 22, 27, 32, 36, 40), this value decreased by 29% for CC and by 23% for CCL and both of them was fairly similar. The addition of 2% extract imparted higher redness (a^*); the differences remained throughout the entire period of storage. However, the redness value decreased both in the samples with the extract and without it, especially during the 15 initial days of storage at refrigerated temperature. At the end of the storage, a^* decreased in both samples by 32% comparing with the initial values. These results could be due to the oxidation of NOMb to MetMb during the storage. Cooked hams with the extract demonstrated lower values of yellowness (b^*) than the control sample. This may be due to the fact that the extract features an intensive red color and has a lower impact on the b^* value. The yellowness was reduced during the storage in control hams, while the changes of b^* in hams with the extracts were not significant ($p > 0.05$).

Table 3.21. Effect of the type of product (without or with extract) on the physico-chemical properties of cooked ham

Day	Sample	pH	a_w	Color, L^*	Color, a^*	Color, b^*
0	CC	5.95±0.20^{a}	0.980±0.001^{a}	91.87±5.74^{b}	6.41±1.17^{c}	9.53±0.77^{cd}
	CCL	5.56±0.01^{a}	0.977±0.002^{bc}	70.05±3.43^{c}	10.43±0.47^{d}	7.47±1.13^{a}
7	CC	6.10±0.03^{b}	0.981±0.002^{ab}	62.93±3.00^{a}	5.01±0.82^{b}	9.09±0.63^{cd}
	CCL	5.66±0.05^{cd}	0.978±0.002^{c}	54.52±2.83^{ab}	8.02±0.72^{c}	7.98±0.71^{a}
15	CC	6.08±0.05^{b}	0.981±0.001^{ab}	64.95±3.21^{a}	3.16±0.77^{a}	9.12±0.67^{cd}
	CCL	5.65±0.01^{bcd}	0.978±0.001^{c}	51.65±2.32^{a}	7.01±0.52^{ab}	7.22±0.53^{a}
22	CC	6.06±0.01^{ab}	0.980±0.000^{a}	63.28±2.45^{a}	5.33±0.63^{b}	8.74±0.91^{bc}
	CCL	5.69±0.04^{d}	0.975±0.001^{a}	55.41±2.20^{b}	6.78±0.55^{a}	7.63±0.51^{a}
27	CC	6.03±0.01^{ab}	0.981±0.003^{ab}	64.82±2.66^{a}	4.33±1.05^{b}	9.96±0.96^{d}
	CCL	5.60±0.01^{ab}	0.980±0.001^{d}	54.44±3.54^{ab}	6.65±0.73^{a}	7.60±0.92^{a}
32	CC	6.01±0.03^{ab}	0.983±0.001^{b}	63.65±2.39^{a}	5.15±0.81^{b}	8.03±0.63^{ab}
	CCL	5.60±0.01^{ab}	0.978±0.001^{c}	54.66±2.72^{ab}	6.95±0.59^{ab}	7.58±0.67^{a}
36	CC	6.04±0.02^{ab}	0.981±0.001^{ab}	63.13±2.69^{a}	5.04±1.30^{b}	8.07±0.87^{ab}
	CCL	5.62±0.01^{bc}	0.975±0.001^{ab}	53.79±2.91^{ab}	7.60±0.47^{bc}	8.08±0.58^{a}
40	CC	6.06±0.01^{ab}	0.981±0.001^{ab}	63.99±2.83^{a}	4.40±0.42^{b}	7.77±0.60^{a}
	CCL	5.64±0.02^{cd}	0.977±0.001^{c}	54.29±3.03^{ab}	7.18±0.51^{ab}	7.82±0.79^{a}

^{a,b} Different letters in the same column indicate statistical differences in one sample during the storage (one-way ANOVA, $p < 0.05$); Control without inoculum (CC) and control with 2% of extract (CCL). The results are expressed as mean ± standard deviation of triplicate determinations

3.7.9. Effect of extract on the sensory evaluation of cooked ham

The color and odor of cooked ham are the primary sensory characteristics for consumers. The evaluation of homogeneity, superficial humidity, the intensity of color, discoloration, the intensity of odor, flavor and juiciness of cooked ham samples as well as their changes during the storage are summarized in **Tables 3.22** and **3.23**. The cranberry pomace extract did not have significant effect ($p < 0.05$) on the discoloration, odor and flavor intensity and juiciness of cooked ham; however, the samples with the extract additive had higher superficial humidity and color intensity and lower homogeneity comparing with the control without the extract (**Table 3.22**). For instance, the extract imparted to ham some color tones, which were described by the panelists as 'light purple'.

Table 3.22. Effect of the type of product (without or with extract) on the sensory properties of cooked ham

Sample	Homoge-neity	Superfi-cial humidity	Intensity of color	Discolora-tion	Intensity of odor	Intensity of flavor	Juici-ness
CC	3.82± 0.83[b]	2.32± 0.84[a]	2.44± 0.79[a]	1.50± 1.21[a]	3.09± 0.99[a]	3.62± 0.89[a]	3.32± 0.77[a]
CCL	2.71± 1.30[a]	3.20± 0.99[b]	2.86± 0.69[b]	1.77± 1.30[a]	3.17± 0.86[a]	3.38± 1.18[a]	3.25± 1.39[a]

[a,b] Different letters in the same column indicate statistical differences (one-way ANOVA, $p < 0.05$); Control without inoculum (CC) and control with 2% of extract (CCL). The results are expressed as mean ± standard deviation of eight determinations

Flavor intensity and juiciness was evaluated only for freshly prepared products due to lack of samples for assessing these sensory characteristics (**Table 3.22**). According to the obtained results, the intensity of flavor and juiciness was slightly more acceptable in the control sample without the extract (3.75 and 3.75, respectively) than in the sample with the extract (3.38 and 3.25, respectively). At the end of the experiment (40 days of storage), the homogeneity scores were reduced for all the samples and were significantly different for different batches. Superficial humidity scores were higher in the samples with the extract during 15 days of storage and on the last day of the experiment, while, after 27 and 36 days, the differences were not significant. The intensity of the color significantly decreased during 15 days of storage of the CCL sample, specifically, from 3.25 to 2.86. Further analyses did not show any significant differences between the samples. Discoloration increased with the storage time, whereas, after 15 days of storage in the CC and CCL samples, the scores increased to 1.57 and 2.14, respectively. Afterwards, for the CCL sample, the scores remained similar, except for the last day, when it increased to 2.63. The score for the control sample without the extract increased steadily, and, at the end of the experiment, it reached 2.5. The intensity of odor did not differ between the stored samples; however, on day 15, the panelists noted a slightly acidic taste for ham with the extract, whereas, in the control samples, it appeared after 27 days. In general, the results indicated that the added

cranberry pomace extract did not have any negative effect on the sensory quality of cooked hams.

Table 3.23. Effect of time of storage on sensory properties of cooked ham

Day	Sample	Homoge-neity	Superficial humidity	Intensity of color	Discolo-ration	Intensity of odor
0	CC	4.13±0.64[b]	2.38±1.06[a]	2.50±0.76[a]		3.38±1.06[a]
	CCL	3.13±1.55[a]	3.50±1.06[b]	3.25±0.46[b]		2.88±0.83[a]
15	CC	3.85±1.07[b]	2.29±0.95[a]	2.86±1.07[a]	1.57±0.79[a]	2.71±0.95[a]
	CCL	2.43±1.27[a]	3.71±0.76[b]	2.86±0.69[a]	2.14±0.90[a]	3.14±0.90[a]
27	CC	3.80±1.09[b]	2.40±0.89[a]	2.40±0.55[a]	1.40±0.90[a]	3.40±1.14[a]
	CCL	3.17±1.17[a]	2.83±0.75[a]	2.67±0.82[a]	2.16±1.16[a]	2.67±0.52[a]
36	CC	3.67±1.03[b]	2.33±0.82[a]	2.33±0.82[a]	2.17±1.16[a]	2.83±0.75[a]
	CCL	2.50±1.52[a]	2.16±0.75[a]	2.83±0.75[a]	2.17±0.75[a]	3.50±0.84[a]
40	CC	3.63±0.52[b]	2.25±0.70[a]	2.13±0.64[a]	2.50±0.93[a]	3.13±1.13[a]
	CCL	2.38±1.06[a]	3.50±0.93[b]	2.63±0.74[a]	2.63±1.18[a]	3.63±0.92[a]

[a,b] Different letters in the same column indicate statistical differences between samples on each featured sampling day (one-way ANOVA, $p < 0.05$). The results are expressed as mean ± standard deviation of eight determinations

3.8. Perspectives for the application of cranberry pomace

Consequently, the findings of this study create a systematic platform for 'zero waste' processing of cranberry pomace via an effective biorefining scheme using consecutive supercritical carbon dioxide and pressurized liquid extractions (SFE and PLE). However, SFE uses a nonpolar solvent (CO_2) which may effectively dissolve only lipophilic substances, but the effectiveness of this extraction highly depends on various process parameters, mainly, pressure, temperature, and extraction time; therefore, we applied RSM to determine the optimal extraction conditions for cranberry pomace. Experimental studies revealed that lipophilic extracts obtained by SFE after SFE-CO_2 extraction or fractionation accumulate various bioactive components, such as polyunsaturated fatty acids, tocopherols, phytosterols, squalene, carotenoids and other components. For the recovery of polyphenols, we applied PLE extraction; therefore, the use of polar solvents (ethanol and water) allows obtaining higher yield of phenolic compounds from defatted cranberry pomace. Ethanol and water extracts were rich in PCA, anthocyanins, various polyphenols and organic acids which possess various health benefits. Polar extracts possess strong antioxidant, antimicrobial and antiviral activities.

The high effectiveness of the ethanol extract additive against pathogenic *Listeria monocytogenes* and some other tested bacteria in pork slurry, burgers and cooked ham during refrigerated storage suggests that the ethanol extract of defatted cranberry pomace may be a promising natural ingredient of meat products increasing their microbiological safety. Oxidation reactions are responsible for several

undesirable changes occurring during meat processing and the subsequent storage of the product which result in off-flavors, lipid oxidation, and the discoloration of meat products. In addition, it may be expected that the enrichment of meat products with bioactive berry phytochemicals improves their quality, oxidative stability, microbiological safety, and, on top of that, may also provide some health benefits. Further studies should focus on the extraction residues which contain mainly proteins, insoluble carbohydrates and minerals.

CONCLUSIONS

1. The conditions for supercritical fluid extraction with carbon dioxide have been optimized, and the highest cranberry pomace oil yield (11.10 g oil/100 g DW) was obtained at 42.4 MPa pressure and 53 °C temperature in 158 min of extraction. The extraction rate was 4 times faster comparing with the traditional extraction (Soxhlet). The extract of cranberry pomace is rich in monosaturated (21.79 g/100g oil) and polyunsaturated (69.58 g/100g oil) fatty acids.

2. It has been found that, by modifying the supercritical extraction solvent and changing the parameters of the system separators, it is possible to produce fractions of lipophilic substances of various compositions. Fractionation of cranberry pomace with pure supercritical CO_2 determined a higher yield in the 1st separator (5.93–6.86%), while the addition of ethanol increased the content of the extract in the heavier fraction (6.05% and 6.21%) at 0 and -10 °C, but, at -20 and -30 °C, it remarkably increased in the lighter fraction (7.91–8.18%). Lighter fractions (especially with EtOH) possessed a significantly ($p < 0.05$) higher antioxidant capacity measured by the L-ORAC assay. There are no remarkable differences between the fractions in TAGs and the composition of fatty acids. The highest amount of squalene (4.51–12.09%) from cranberry pomace was achieved in the heavier fraction at all the test temperatures and after adding 5% of ethanol. The concentration of microcomponents, including phytosterols, tocopherols and volatile aroma compounds, was remarkably higher in the lighter fractions, especially in the case of SC-CO_2/EtOH.

3. Pressurized liquid extraction (PLE) may be applied for the isolation of polyphenols from defatted cranberry pomace. The optimal conditions to obtain the highest yield (55.89%) were 3 extraction cycles, 15 min each, at the temperature of 83 °C with EtOH. The results of the PLE-EtOH process in the range of 50–90 °C (temperature) and 5–15 min (extraction time) during 3 cycles showed the highest TPC (43.43 mg GAE/g E), $ABTS^{\bullet+}$ (179.0 mg TE/g E) and ORAC (270.2 mg TE/g E) values of extracts at the highest temperature (90 °C). The highest PAC values were obtained when each cycle time was 15 min at 70 °C and 90 °C, 225.2 and 220.2 mg/g E, respectively. Higher anthocyanin recovery was at 50 °C, when increasing the extraction cycle time from 5 to 10 min, 97.10 vs 183.6 mg/100g DW, respectively. The remaining residue after PLE with EtOH was further extracted with water at 130 °C (3 extraction cycles, 10 min each) and yielded 6.50%.

4. Peonidin-3-galactoside and peonidin-3-arabinoside were quantitatively the major anthocyanins in PLE-EtOH (94.36 and 67.79 mg/100g, respectively) and PLE-H_2O (0.96 and 0.86 mg/100g, respectively) extracts. Phytochemical analysis showed that mallic, quinic and citric acids, procyanidin B3 and quercetin-hexoside were identified in both extracts. The ethanol extract did not possess strong anticancer properties against human colon cancer cells (HCT-116 and DLD-1) and antiviral properties against the Zika virus. The water extract efficiently inhibited Zika virus infection on human lung A549 cells and was not cytotoxic *in vitro* and *in vivo*.

5. The extracts isolated from defatted cranberry pomace were remarkably stronger antioxidants when using ORAC, TPC and $ABTS^{\bullet+}$ assays comparing to the lipophilic extracts obtained by SFE-CO_2 and Soxhlet extraction. The extract isolated with water had a stronger antioxidant capacity by all *in vitro* assays than the extract obtained with ethanol. TPC, ORAC and $ABTS^{\bullet+}$ values obtained in the water extract were 188.9 mg GAE/g of extract, 1084 and 362.6 mg TE/g of extract, while, in the ethanol extract, the values were 52.98 mg GAE/g of extract, 130.4, 345 mg TE/g of extract, respectively. The antioxidant potential of the cranberry pomace evaluated by the QUENCHER method was approximately the same after removing the lipophilic fraction, but remarkably decreased (by 95–97% comparing with the initial values) after the application of polar solvents (ethanol and water); it suggests that a considerable amount of antioxidants was efficiently recovered from cranberry pomace after extractions.

6. Cranberry pomace extracts (ethanol and water) effectively inhibited the growth of undesirable microorganisms (foodborne pathogens and meat spoilage causing bacteria). When higher concentrations (6.6% and 3.3%) were used, these bacteria were not detected at all. The ethanol extract was also very effective in pork slurry, burgers and ham against all the tested bacteria. The addition of the extract inhibited from 5 to 7 times the formation of malondialdehyde (a lipid oxidation product) comparing with the control sample without the extract during prolonged storage of pork burgers. The effect of the ethanol extract on other quality characteristics of meat products was not as significant as the effect on the color characteristics. The values of the redness (a^*) of meat products with the extract were 2 times higher than the control sample without the extract. In addition, the extract affected the sensory evaluation of burgers and cooked ham only negligibly.

REFERENCES

1. Aaby, K., Skrede, G., & Wrolstad, R. E. (2005). Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*). *Journal of Agricultural and Food Chemistry*, *53*(10), 4032–4040. https://doi.org/10.1021/jf048001o

2. Abd El-Khalek, H. H., & Zahran, D. A. (2013). Utilization of fruit by-product in ground meat preservation. *Food Science and Quality Management*, *11*, 2224–6088. https://doi.org/2225-0557

3. Afaq, F., Saleem, M., Krueger, C. G., Reed, J. D., & Mukhtar, H. (2005). Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-κB pathways and inhibits skin tumorigenesis in CD-1 mice. *International Journal of Cancer*, *113*(3), 423–433. https://doi.org/10.1002/ijc.20587

4. Ahmad, S. R., Gokulakrishnan, P., Giriprasad, R., & Yatoo, M. A. (2015). Fruit-based natural antioxidants in meat and meat products: a review. *Critical Reviews in Food Science and Nutrition*, *55*(11), 1503–1513. https://doi.org/10.1080/10408398.2012.701674

5. Ahn, Y. J., Kawamura, T., Kim, M., Yamamoto, T., & Mitsuoka, T. (1991). Tea polyphenols: Selective growth inhibitors of *Clostridium spp*. *Agricultural and Biological Chemistry*, *55*(5), 1425–1426. https://doi.org/10.1271/bbb1961.55.1425

6. Álvarez-Martínez, F. J., Barrajón-Catalán, E., Encinar, J. A., Rodríguez-Díaz, J. C., & Micol, V. (2020). Antimicrobial capacity of plant polyphenols against gram-positive bacteria: a comprehensive review. *Current Medicinal Chemistry*, *27*(15), 31. https://doi.org/10.2174/0929867325666181008115650

7. Aminzare, M., Hashemi, M., Hassanzad Azar, H., & Hejazi, J. (2016). The use of herbal extracts and essential oils as a potential antimicrobial in meat and meat products; A review. *Journal of Human, Environment, and Health Promotion*, *1*(2), 63–74. https://doi.org/10.29252/jhehp.1.2.63

8. Amoros, M., Simoes, C. M. O., & Girre, L. (1992). Synergistic effect of flavones and flavonols against herpes simplex virus type 1 in cell culture. Comparison with the antiviral activity of propolis. *Journal of Natural Products*, *55*(12), 1732–1740. https://doi.org/10.1021/np50090a003

9. AOAC. (1995). Official methods of analysis of AOAC international (16th edn) official and standardized methods of analysis (3rd edn.). *Trends in Food Science & Technology*, *61*, 1995.

10. Arapitsas, P., Sjöberg, P. J. R., & Turner, C. (2008). Characterisation of anthocyanins in red cabbage using high resolution liquid chromatography coupled with photodiode array detection and electrospray ionization-linear ion trap mass spectrometry. *Food Chemistry*, *109*(1), 219–226. https://doi.org/10.1016/j.foodchem.2007.12.030

11. Armenteros, M., Morcuende, D., Ventanas, S., & Estévez, M. (2013). Application of natural antioxidants from strawberry tree (*Arbutus unedo L.*) and dog rose (*Rosa canina L.*) to Frankfurters subjected to refrigerated storage. *Journal of Integrative Agriculture*, *12*(11), 1972–1981. https://doi.org/10.1016/S2095-3119(13)60635-8

12. Aronson, W. J., Glaspy, J. A., Reddy, S. T., Reese, D., Heber, D., & Bagga, D. (2001). Modulation of omega-3/omega-6 polyunsaturated ratios with dietary fish oils in men with prostate cancer. *Urology*, *58*(2), 283–288. https://doi.org/10.1016/S0090-4295(01)01116-5

13. Aymerich, T., Picouet, P. A., & Monfort, J. M. (2008). Decontamination technologies for meat products. *Meat Science*, *78*(1–2), 114–129. https://doi.org/10.1016/j.meatsci.2007.07.007

14. Bajerska, J., Chmurzynska, A., Mildner-Szkudlarz, S., Drzymała-Czyż, S., Górnaś, P., Waśkiewicz, A., … Woźniewicz, M. (2018). Effects of unextruded and extruded cranberry pomace on selected metabolic parameters in high-fat diet fed rats. *Acta Scientiarum Polonorum Technologia Alimentaria*, *17*(1), 91–100. https://doi.org/10.17306/j.afs.0542

15. Balasubramanian, A., Pilankatta, R., Teramoto, T., Sajith, A. M., Nwulia, E., Kulkarni, A., & Padmanabhan, R. (2019). Inhibition of dengue virus by curcuminoids. In *Antiviral Research* (Vol. 162). https://doi.org/10.1016/j.antiviral.2018.12.002

16. Basegmez, H. I. O., Povilaitis, D., Kitrytė, V., Kraujalienė, V., Šulniūtė, V., Alasalvar, C., & Venskutonis, P. R. (2017). Biorefining of blackcurrant pomace into high value functional ingredients using supercritical CO_2, pressurized liquid and enzyme assisted extractions. *Journal of Supercritical Fluids*, *124*, 10–19. https://doi.org/10.1016/j.supflu.2017.01.003

17. Basu, A., Betts, N. M., Ortiz, J., Simmons, B., Wu, M., & Lyons, T. J. (2011). Low-energy cranberry juice decreases lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome. *Nutrition Research*, *31*(3), 190–196. https://doi.org/10.1016/j.nutres.2011.02.003

18. Benchikh, Y., & Louailèche, H. (2014). Effects of extraction conditions on the recovery of phenolic compounds and in vitro antioxidant activity of carob (*Ceratonia siliqua L.*) pulp. *Acta Botanica Gallica*, *161*(2), 175–181. https://doi.org/10.1080/12538078.2014.909325

19. Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, *76*(5), 965–977. https://doi.org/10.1016/j.talanta.2008.05.019

20. Bhagdeo, M., Ashraf-Khorassani, M., & Taylor, L. T. (2006). Analysis of supercritical CO_2 extractables from cranberry seeds. *ACS Symposium Series*, *926*, 36–50. https://doi.org/10.1021/bk-2006-0926.ch003

21. Bhattacharya, D., Kandeepan, G., & Vishnuraj, M. R. (2016). Protein oxidation in meat and meat products. *Journal of Food and Health Science*, (June), 171–183. https://doi.org/10.3153/jfhs16018

22. Biswas, A. K., Keshri, R. C., & Bisht, G. S. (2004). Effect of enrobing and antioxidants on quality characteristics of precooked pork patties under chilled and frozen storage conditions. *Meat Science*, *66*(3), 733–741. https://doi.org/10.1016/j.meatsci.2003.07.006

23. Björklund, E., Nilsson, T., & Bøwadt, S. (2000). Pressurised liquid extraction of persistent organic pollutants in environmental analysis. *TrAC - Trends in Analytical Chemistry*, *19*(7), 434–445. https://doi.org/10.1016/S0165-9936(00)00002-9

24. Bobinaitė, R., Grootaert, C., Van Camp, J., Šarkinas, A., Liaudanskas, M., Žvikas, V., … Rimantas Venskutonis, P. (2020). Chemical composition, antioxidant, antimicrobial and antiproliferative activities of the extracts isolated from the pomace of rowanberry (*Sorbus aucuparia L.*). *Food Research International*, *136*(May). https://doi.org/10.1016/j.foodres.2020.109310

25. Bobinaitė, R., Kraujalis, P., Tamkutė, L., Urbonavičienė, D., Viškelis, P., & Venskutonis, P. R. (2020). Recovery of bioactive substances from rowanberry pomace by consecutive extraction with supercritical carbon dioxide and pressurized solvents. *Journal of Industrial and Engineering Chemistry*. https://doi.org/10.1016/j.jiec.2020.01.036

26. Bos, S., Viranaicken, W., Turpin, J., El-Kalamouni, C., Roche, M., Krejbich-Trotot, P., … Gadea, G. (2018). The structural proteins of epidemic and historical strains of Zika virus differ in their ability to initiate viral infection in human host cells. *Virology*, *516* (December),

265–273. https://doi.org/10.1016/j.virol.2017.12.003

27. Box, G. E. P., & Wilson, K. B. (1951). On the experimental attainment of optimum conditions. *Journal of the Royal Statistical Society*, *13*(1), 1–45. https://doi.org/10.1016/0169-2070(93)90088-5

28. Brazdauskas, T., Montero, L., Venskutonis, P. R., Ibañez, E., & Herrero, M. (2016). Downstream valorization and comprehensive two-dimensional liquid chromatography-based chemical characterization of bioactives from black chokeberries (*Aronia melanocarpa*) pomace. *Journal of Chromatography A*, *1468*, 126–135. https://doi.org/10.1016/j.chroma.2016.09.033

29. Brglez Mojzer, E., Knez Hrnčič, M., Škerget, M., Knez, Ž., & Bren, U. (2016). Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules (Basel, Switzerland)*, *21*(7). https://doi.org/10.3390/molecules21070901

30. Cacace, J. E., & Mazza, G. (2003). Mass transfer process during extraction of phenolic compounds from milled berries. *Journal of Food Engineering*, *59*(4), 379–389. https://doi.org/10.1016/S0260-8774(02)00497-1

31. Caillet, S., Côté, J., Sylvain, J. F., & Lacroix, M. (2012). Antimicrobial effects of fractions from cranberry products on the growth of seven pathogenic bacteria. *Food Control*, *23*(2), 419–428. https://doi.org/10.1016/j.foodcont.2011.08.010

32. Carneiro, B. M., Batista, M. N., Braga, A. C. S., Nogueira, M. L., & Rahal, P. (2016). The green tea molecule EGCG inhibits Zika virus entry. *Virology*, *496*, 215–218. https://doi.org/10.1016/j.virol.2016.06.012

33. Carpenter, R., O'Grady, M. N., O'Callaghan, Y. C., O'Brien, N. M., & Kerry, J. P. (2007). Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Science*, *76*(4), 604–610. https://doi.org/10.1016/j.meatsci.2007.01.021

34. Catchpole, O. J., Tallon, S. J., Eltringham, W. E., Grey, J. B., Fenton, K. A., Vagi, E. M., … Zhu, Y. (2009). The extraction and fractionation of specialty lipids using near critical fluids. *Journal of Supercritical Fluids*, *47*(3), 591–597. https://doi.org/10.1016/j.supflu.2008.10.008

35. Česoniene, L., & Daubaras, R. (2016). Phytochemical composition of the large cranberry (*Vaccinium macrocarpon*) and the small cranberry (*Vaccinium oxycoccos*). In *Nutritional Composition of Fruit Cultivars*. https://doi.org/10.1016/B978-0-12-408117-8.00008-8

36. Cheah, E. L. C., Heng, P. W. S., & Chan, L. W. (2010). Optimization of supercritical fluid extraction and pressurized liquid extraction of active principles from *Magnolia officinalis* using the Taguchi design. *Separation and Purification Technology*, *71*(3), 293–301. https://doi.org/10.1016/j.seppur.2009.12.009

37. Chen, H., Zuo, Y., & Deng, Y. (2001). Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. *Journal of Chromatography A*, *913*(1–2), 387–395. https://doi.org/10.1016/S0021-9673(00)01030-X

38. Chen, P.-N., Chu, S.-C., Chiou, H.-L., Chiang, C.-L., Yang, S.-F., & Hsieh, Y.-S. (2005). Cyanidin 3-glucoside and peonidin 3-glucoside inhibit tumor cell growth and induce apoptosis *in vitro* and suppress tumor growth *in vivo*. *Nutrition and Cancer*, *53*(2), 232–243. https://doi.org/10.1207/s15327914nc5302

39. Cherchi, G., Deidda, D., De Gioannis, B., Marongiu, B., Pompei, R., & Porcedda, S. (2001). Extraction of *Santolina insularis* essential oil by supercritical carbon dioxide:

influence of some process parameters and biological activity. *Flavour and Fragrance Journal*, *16*, 35–43. https://doi.org/10.1002/ffj.1224

40. Choi, J. A., Kim, J. Y., Lee, J. Y., Kang, C. M., Kwon, H. J., Yoo, Y. D., … Lee, S. J. (2001). Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *International Journal of Oncology*, *19*(4), 837–844. https://doi.org/10.3892/ijo.19.4.837

41. Clain, E., Haddad, J. G., Koishi, A. C., Sinigaglia, L., Rachidi, W., Desprès, P., … El Kalamouni, C. (2019). The polyphenol-rich extract from *Psiloxylon mauritianum*, an endemic medicinal plant from Reunion island, inhibits the early stages of dengue and Zika virus infection. *International Journal of Molecular Sciences*, *20*(8), 1–16. https://doi.org/10.3390/ijms20081860

42. Clain, E., Sinigaglia, L., Koishi, A. C., Gorgette, O., Gadea, G., Viranaicken, W., … El Kalamouni, C. (2018). Extract from *Aphloia theiformis*, an edible indigenous plant from Reunion Island, impairs Zika virus attachment to the host cell surface. *Scientific Reports*, *8*(1), 1–12. https://doi.org/10.1038/s41598-018-29183-2

43. Commission regulation (EC) No 1441/2007. (2007). *Official Journal of the European Union*, *322*(1441), 2–29.

44. Commission Regulation (EU) No. 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. (2015). *Official Journal of the European Union*, *136*, 1–40.

45. Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., … Brand-Miller, J. (2005). Origins and evolution of the western diet: Health implications for the 21st century. *American Journal of Clinical Nutrition*, *81*(2), 341–354. https://doi.org/10.1093/ajcn.81.2.341

46. Cornelio-Santiago, H. P., Gonçalves, C. B., de Oliveira, N. A., & de Oliveira, A. L. (2017). Supercritical CO_2 extraction of oil from green coffee beans: Solubility, triacylglycerol composition, thermophysical properties and thermodynamic modelling. *Journal of Supercritical Fluids*, *128*, 386–394. https://doi.org/10.1016/j.supflu.2017.05.030

47. Corrales, M., Toepfl, S., Butz, P., Knorr, D., & Tauscher, B. (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison. *Innovative Food Science and Emerging Technologies*, *9*(1), 85–91. https://doi.org/10.1016/j.ifset.2007.06.002

48. Côté, J., Caillet, S., Doyon, G., Sylvain, J. F., & Lacroix, M. (2010). Analyzing cranberry bioactive compounds. *Critical Reviews in Food Science and Nutrition*, *50*(9), 872–888. https://doi.org/10.1080/10408390903042069

49. Côté, J., Caillet, S., Dussault, D., Sylvain, J. F., & Lacroix, M. (2011). Effect of juice processing on cranberry antibacterial properties. *Food Research International*, *44*(9), 2922–2929. https://doi.org/10.1016/j.foodres.2011.06.049

50. Croteau, R. J., & Fagerson, I. S. (1968). Major volatile components of the juice of American cranberry. *Journal of Food Science*, *33*(4), 386–389. https://doi.org/10.1111/j.1365-2621.1968.tb03635.x

51. Cunningham, D. G., Vannozzi, S. A., Turk, R., Roderick, R., O'Shea, E., & Brilliant, K. (2003). Cranberry phytochemicals and their health benefits. *ACS Symposium Series*, *222*, 35–51. https://doi.org/10.1021/bk-2004-0871.ch004

52. Cushnie, T. P. T., Hamilton, V. E. S., Chapman, D. G., Taylor, P. W., & Lamb, A. J. (2007). Aggregation of *Staphylococcus aureus* following treatment with the antibacterial

flavonol galangin. *Journal of Applied Microbiology*, *103*(5), 1562–1567. https://doi.org/10.1111/j.1365-2672.2007.03393.x

53. Davis, C. D., & Hord, N. G. (2005). Nutritional "omics" technologies for elucidating the role(s) of bioactive food components in colon cancer prevention. *The Journal of Nutrition*, *135*(11), 2694–2697. https://doi.org/10.1093/jn/135.11.2694

54. Del Valle, J. M., & Uquiche, E. L. (2002). Particle size effects on supercritical CO_2 extraction of oil-containing seeds. *JAOCS, Journal of the American Oil Chemists' Society*, *79*(12), 1261–1266. https://doi.org/10.1007/s11746-002-0637-9

55. Diarra, M. S., Block, G., Rempel, H., Oomah, B. D., Harrison, J., McCallum, J., … Malouin, F. (2013). *In vitro* and *in vivo* antibacterial activities of cranberry press cake extracts alone or in combination with β-lactams against *Staphylococcus aureus*. *BMC Complementary and Alternative Medicine*, *13*(90), 1–14.

56. Diarra, M. S., Hassan, Y. I., Block, G. S., Drover, J. C. G., Delaquis, P., & Oomah, B. D. (2020). Antibacterial activities of a polyphenolic-rich extract prepared from American cranberry (*Vaccinium macrocarpon*) fruit pomace against *Listeria spp*. *LWT – Food Science and Technology*, *123*, 109056. https://doi.org/10.1016/j.lwt.2020.109056

57. Dienaitė, L., Pukalskas, A., Pukalskienė, M., Pereira, C. V., Matias, A. A., & Venskutonis, P. R. (2020). Phytochemical composition, antioxidant and antiproliferative activities of defatted sea buckthorn (*Hippophaë rhamnoides L.*) berry pomace fractions consecutively recovered by pressurized ethanol and water. *Antioxidants*, *9*(4). https://doi.org/10.3390/antiox9040274

58. Diker, K. S., Akan, M., Hascelik, G., & Yurdakök, M. (1991). The bactericidal activity of tea against *Campylobacter jejuni* and *Campylobacter coli*. *Letters in Applied Microbiology*, *12*(2), 34–35. https://doi.org/10.1111/j.1472-765X.1991.tb00496.x

59. Ding, M., Feng, R., Wang, S. Y., Bowman, L., Lu, Y., Qian, Y., … Shi, X. (2006). Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. *Journal of Biological Chemistry*, *281*(25), 17359–17368. https://doi.org/10.1074/jbc.M600861200

60. Dixon, R. A., Xie, D. Y., & Sharma, S. B. (2005). Proanthocyanidins - A final frontier in flavonoid research? *New Phytologist*, *165*(1), 9–28. https://doi.org/10.1111/j.1469-8137.2004.01217.x

61. Duba, K. S., & Fiori, L. (2016). Solubility of grape seed oil in supercritical CO_2: Experiments and modeling. *Journal of Chemical Thermodynamics*, *100*, 44–52. https://doi.org/10.1016/j.jct.2016.04.010

62. Dušková, M., Kameník, J., & Karpíšková, R. (2013). *Weissella viridescens* in meat products – A review. *Acta Veterinaria Brno*, *82*(3), 237–241. https://doi.org/10.2754/avb201382030237

63. Esmer, O. K., Irkin, R., Degirmencioglu, N., & Degirmencioglu, A. (2011). The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat. *Meat Science*, *88*(2), 221–226. https://doi.org/10.1016/j.meatsci.2010.12.021

64. Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. *Food Research International*, *64*, 171–181. https://doi.org/10.1016/j.foodres.2014.06.022

65. FAO. (2011). Global food losses and food waste – Extent, causes and prevention.

66. Feliciano, R. P., Krueger, C. G., Shanmuganayagam, D., Vestling, M. M., & Reed, J. D.

(2012). Deconvolution of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry isotope patterns to determine ratios of A-type to B-type interflavan bonds in cranberry proanthocyanidins. *Food Chemistry*, *135*(3), 1485–1493. https://doi.org/10.1016/j.foodchem.2012.05.102

67. Feng, G., Klein, M. I., Gregoire, S., Singh, A. P., Vorsa, N., & Koo, H. (2013). The specific degree-of-polymerization of A-type proanthocyanidin oligomers impacts *Streptococcus mutans* glucan-mediated adhesion and transcriptome responses within biofilms. *Biofouling*, *29*(6), 629–640. https://doi.org/10.1080/08927014.2013.794456

68. Fernández-González, V., Concha-Graña, E., Muniategui-Lorenzo, S., López-Mahía, P., & Prada-Rodríguez, D. (2008). Pressurized hot water extraction coupled to solid-phase microextraction-gas chromatography-mass spectrometry for the analysis of polycyclic aromatic hydrocarbons in sediments. *Journal of Chromatography A*, *1196–1197*(1–2), 65–72. https://doi.org/10.1016/j.chroma.2008.05.006

69. Foo, L. Y., Lu, Y., Howell, A. B., & Vorsa, N. (2000). The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli in vitro*. *Phytochemistry*, *54*(2), 173–181. https://doi.org/10.1016/S0031-9422(99)00573-7

70. Fröberg, L. (2011). New flora of the british isles. In C. Stace (Ed.), *Systematic Biology* (3rd edition, Vol. 60). https://doi.org/10.1093/sysbio/syq066

71. Frumence, E., Roche, M., Krejbich-Trotot, P., El-Kalamouni, C., Nativel, B., Rondeau, P., … Desprès, P. (2016). The South Pacific epidemic strain of Zika virus replicates efficiently in human epithelial A549 cells leading to IFN-β production and apoptosis induction. *Virology*, *493*, 217–226. https://doi.org/10.1016/j.virol.2016.03.006

72. Gadea, G., Bos, S., Krejbich-Trotot, P., Clain, E., Viranaicken, W., El-Kalamouni, C., … Desprès, P. (2016). A robust method for the rapid generation of recombinant Zika virus expressing the GFP reporter gene. *Virology*, *497*, 157–162. https://doi.org/10.1016/j.virol.2016.07.015

73. Ganhão, R., Estévez, M., Armenteros, M., & Morcuende, D. (2013). Mediterranean berries as inhibitors of lipid oxidation in porcine burger patties subjected to cooking and chilled storage. *Journal of Integrative Agriculture*, *12*(11), 1982–1992. https://doi.org/10.1016/S2095-3119(13)60636-X

74. Ganhão, R., Morcuende, D., & Estévez, M. (2010). Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chill storage. *Meat Science*, *85*(3), 402–409. https://doi.org/10.1016/j.meatsci.2010.02.008

75. García-Lomillo, J., & González-SanJosé, M. L. (2017). Applications of wine pomace in the food industry: approaches and functions. *Comprehensive Reviews in Food Science and Food Safety*, *16*(1), 3–22. https://doi.org/10.1111/1541-4337.12238

76. Garrido, M. D., Auqui, M., Martí, N., & Linares, M. B. (2011). Effect of two different red grape pomace extracts obtained under different extraction systems on meat quality of pork burgers. *LWT – Food Science and Technology*, *44*(10), 2238–2243. https://doi.org/10.1016/j.lwt.2011.07.003

77. Gaudry, A., Bos, S., Viranaicken, W., Roche, M., Krejbich-Trotot, P., Gadea, G., … El-Kalamouni, C. (2018). The flavonoid isoquercitrin precludes initiation of Zika virus infection in human cells. *International Journal of Molecular Sciences*, *19*(4), 1–13. https://doi.org/10.3390/ijms19041093

78. Ghafoor, K., Park, J., & Choi, Y. H. (2010). Optimization of supercritical fluid extraction

of bioactive compounds from grape (*Vitis labrusca B.*) peel by using response surface methodology. *Innovative Food Science and Emerging Technologies*, *11*(3), 485–490. https://doi.org/10.1016/j.ifset.2010.01.013

79. Gniewosz, M., & Stobnicka, A. (2018). Bioactive components content, antimicrobial activity, and foodborne pathogen control in minced pork by cranberry pomace extracts. *Journal of Food Safety*, *38*(1). https://doi.org/10.1111/jfs.12398

80. Grunovaitė, L., Pukalskienė, M., Pukalskas, A., & Venskutonis, P. R. (2016). Fractionation of black chokeberry pomace into functional ingredients using high pressure extraction methods and evaluation of their antioxidant capacity and chemical composition. *Journal of Functional Foods*, *24*, 85–96. https://doi.org/10.1016/j.jff.2016.03.018

81. Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., Holden, J., Haytowitz, D., & Prior, R. L. (2003). Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *Journal of Agricultural and Food Chemistry*, *51*(25), 7513–7521. https://doi.org/10.1021/jf034815d

82. Güçlü-Üstündag, Ö., & Temelli, F. (2004). Correlating the solubility behavior of minor lipid components in supercritical carbon dioxide. *Journal of Supercritical Fluids*, *31*(3), 235–253. https://doi.org/10.1016/j.supflu.2003.12.007

83. Güçlü-Üstündağ, Ö., & Temelli, F. (2005). Solubility behavior of ternary systems of lipids, cosolvents and supercritical carbon dioxide and processing aspects. *Journal of Supercritical Fluids*, *36*(1), 1–15. https://doi.org/10.1016/j.supflu.2005.03.002

84. Haddad, J. G., Grauzdyte, D., Koishi, A. C., Viranaicken, W., Venskutonis, P. R., Duarte dos Santos, C. N., … El Kalamouni, C. (2020). The geraniin-rich extract from reunion island endemic medicinal plant *Phyllanthus phillyreifolius* inhibits Zika and dengue virus infection at non-toxic effect doses in zebrafish. *Molecules*, *25*(10), 1–17. https://doi.org/10.3390/molecules25102316

85. Haddad, J. G., Koishi, A. C., Gaudry, A., Nunes Duarte Dos Santos, C., Viranaicken, W., Desprès, P., & El Kalamouni, C. (2019). *Doratoxylon apetalum*, an indigenous medicinal plant from Mascarene Islands, is a potent inhibitor of Zika and Dengue virus infection in human cells. *International Journal of Molecular Sciences*, *20*(10), 1–13. https://doi.org/10.3390/ijms20102382

86. Haddad, J. G., Picard, M., Bénard, S., Desvignes, C., Desprès, P., Diotel, N., & El Kalamouni, C. (2019). *Ayapana triplinervis* essential oil and its main component thymohydroquinone dimethyl ether inhibit Zika virus at doses devoid of toxicity in zebrafish. *Molecules*, *24*(19), 1–12. https://doi.org/10.3390/molecules24193447

87. Haddow, A. D., Schuh, A. J., Yasuda, C. Y., Kasper, M. R., Heang, V., Huy, R., … Weaver, S. C. (2012). Genetic characterization of Zika virus strains: Geographic expansion of the asian lineage. *PLoS Neglected Tropical Diseases*, *6*(2), 1477. https://doi.org/10.1371/journal.pntd.0001477

88. Häkkinen, S., Heinonen, M., Kärenlampi, S., Mykkänen, H., Ruuskanen, J., & Törrönen, R. (1999). Screening of selected flavonoids and phenolic acids in 19 berries. *Food Research International*, *32*(5), 345–353. https://doi.org/10.1016/S0963-9969(99)00095-2

89. Hanrahan, G., & Lu, K. (2006). Application of factorial and response surface methodology in modern experimental design and optimization. *Critical Reviews in Analytical Chemistry*, *36*(3–4), 141–151. https://doi.org/10.1080/10408340600969478

90. Harnly, J. M., Doherty, R. F., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Bhagwat, S., & Gebhardt, S. (2006). Flavonoid content of U.S. fruits, vegetables, and nuts. *Journal of*

Agricultural and Food Chemistry, *54*(26), 9966–9977. https://doi.org/10.1021/jf061478a

91. Haslam, E., Lilley, T. H., CAi, Y., Martin, R., & Magnolato, D. (1989). Traditional herbal medicines – The role of polyphenols. *Planra Medica*, *55*, 1–8.

92. Hayrapetyan, H., Hazeleger, W. C., & Beumer, R. R. (2012). Inhibition of *Listeria monocytogene*s by pomegranate (*Punica granatum*) peel extract in meat paté at different temperatures. *Food Control*, *23*(1), 66–72. https://doi.org/10.1016/j.foodcont.2011.06.012

93. He, X., & Rui, H. L. (2006). Cranberry phytochemicals: isolation, structure elucidation, and their antiproliferative and antioxidant activities. *Journal of Agricultural and Food Chemistry*, *54*(19), 7069–7074. https://doi.org/10.1021/jf061058l

94. Heffels, P., Weber, F., & Schieber, A. (2015). Influence of accelerated solvent extraction and ultrasound-assisted extraction on the anthocyanin profile of different *Vaccinium* species in the context of statistical models for authentication. *Journal of Agricultural and Food Chemistry*, *63*(34), 7532–7538. https://doi.org/10.1021/acs.jafc.5b02255

95. Heinonen, M. (2007). Antioxidant activity and antimicrobial effect of berry phenolics - a Finnish perspective. *Molecular Nutrition and Food Research*, *51*(6), 684–691. https://doi.org/10.1002/mnfr.200700006

96. Hoed, V. V. A. N., Clercq, N. D. E., Echim, C., Andjelkovic, M., Leber, E., Dewettinck, K., & Verhe, R. (2009). Berry seeds: a source of specialty oils with high content of bioactives and nutritional value. *Journal of Food Lipids*, *16*, 33–49.

96. Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science*, *78*(1–2), 68–76. https://doi.org/10.1016/j.meatsci.2007.05.030

97. Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., … Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*(7446), 498–503. https://doi.org/10.1038/nature12111

98. Howell, A. B. (2002). Cranberry proanthocyanidins and the maintenance of urinary tract health. *Critical Reviews in Food Science and Nutrition*, *42*, 273–278. https://doi.org/10.1080/10408390209351915

99. Howell, A. B., Reed, J. D., Krueger, C. G., Winterbottom, R., Cunningham, D. G., & Leahy, M. (2005). A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. *Phytochemistry*, *66*(18), 2281–2291. https://doi.org/10.1016/j.phytochem.2005.05.022

100. Hu, F. B. (2001). The balance between ω-6 and ω-3 fatty acids and the risk of coronary heart disease. *Nutrition*, *17*(9), 741–742. https://doi.org/10.1016/S0899-9007(01)00620-7

101. Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*, 1841–1856. https://doi.org/10.1007/s11418-014-0862-8

102. Hurren, D. (1999). Supercritical fluid extraction with CO_2. *Filtration and Separation*, *36*, 25–27.

103. ISO 13299:2016. (2016). General guidance for establishing a sensory profile.

104. Jakobsen, M., & Bertelsen, G. (2002). The use of CO_2 in packaging of fresh red meats and its effect on chemical quality changes in the meat: A review. *Journal of Muscle Foods*, *13*(2), 143–168. https://doi.org/10.1111/j.1745-4573.2002.tb00326.x

105. Jambrak, A. R., Šimunek, M., Petrović, M., Bedić, H., Herceg, Z., & Juretić, H. (2017). Aromatic profile and sensory characterisation of ultrasound treated cranberry juice and nectar. *Ultrasonics Sonochemistry*, *38*, 783–793.

https://doi.org/10.1016/j.ultsonch.2016.11.027

106. Jia, N., Kong, B., Liu, Q., Diao, X., & Xia, X. (2012). Antioxidant activity of black currant (*Ribes nigrum L.*) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. *Meat Science*, *91*(4), 533–539. https://doi.org/10.1016/j.meatsci.2012.03.010

107. Jun, X. (2009). Caffeine extraction from green tea leaves assisted by high pressure processing. *Journal of Food Engineering*, *94*(1), 105–109. https://doi.org/10.1016/j.jfoodeng.2009.03.003

108. Kanner, J. (2007). Dietary advanced lipid oxidation endproducts are risk factors to human health. *Molecular Nutrition and Food Research*, *51*(9), 1094–1101. https://doi.org/10.1002/mnfr.200600303

109. Karamucki, T., Jakubowska, M., Rybarczyk, A., & Gardzielewska, J. (2013). The influence of myoglobin on the colour of minced pork loin. *Meat Science*, *94*(2), 234–238. https://doi.org/10.1016/j.meatsci.2013.01.014

110. Kaufmann, B., & Christen, P. (2002). Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. *Phytochemical Analysis*, *13*, 105–113. https://doi.org/10.1002/pca.631

111. Khansari, N., Shakiba, Y., & Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Patents on Inflammation & Allergy Drug Discovery*, *3*(1), 73–80. https://doi.org/10.2174/187221309787158371

112. Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, *18*(2), 2328–2375. https://doi.org/10.3390/molecules18022328

113. Kim, H., Hall, P., Smith, M., Kirk, M., Prasain, J. K., Barnes, S., & Grubbs, C. (2004). Chemoprevention by grape seed extract and genistein in carcinogen-induced mammary cancer in rats is diet dependent. *The Journal of Nutrition*, *134*(12), 3445S-3452S. https://doi.org/10.1093/jn/134.12.3445s

114. Kim, M. J., Kim, J. H., & Kwak, H. K. (2014). Antioxidant effects of cranberry powder in lipopolysaccharide treated hypercholesterolemic rats. *Preventive Nutrition and Food Science*, *19*(2), 75–81. https://doi.org/10.3746/pnf.2014.19.2.075

115. King, N. J., & Whyte, R. (2006). Does it look cooked? A review of factors that influence cooked meat color. *Journal of Food Science*, *71*(4), 31–40. https://doi.org/10.1111/j.1750-3841.2006.00029.x

116. Kitrytė, V., Kavaliauskaitė, A., Tamkutė, L., Pukalskienė, M., Syrpas, M., & Rimantas Venskutonis, P. (2020). Zero waste biorefining of lingonberry (*Vaccinium vitis-idaea L.*) pomace into functional ingredients by consecutive high pressure and enzyme assisted extractions with green solvents. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2020.126767

117. Kitrytė, V., Kraujalienė, V., Šulniūtė, V., Pukalskas, A., & Venskutonis, P. R. (2017). Chokeberry pomace valorization into food ingredients by enzyme-assisted extraction: Process optimization and product characterization. *Food and Bioproducts Processing*, *105*, 36–50. https://doi.org/10.1016/j.fbp.2017.06.001

118. Kitrytė, V., Laurinavičienė, A., Syrpas, M., Pukalskas, A., & Venskutonis, P. R. (2020). Modeling and optimization of supercritical carbon dioxide extraction for isolation of valuable lipophilic constituents from elderberry *(Sambucus nigra L.*) pomace. *Journal of*

CO_2 Utilization, *35*, 225–235. https://doi.org/10.1016/j.jcou.2019.09.020

119. Kitrytė, V., Narkevičiūtė, A., Tamkutė, L., Syrpas, M., Pukalskienė, M., & Venskutonis, P. R. (2020). Consecutive high-pressure and enzyme assisted fractionation of blackberry (*Rubus fruticosus L.*) pomace into functional ingredients: Process optimization and product characterization. *Food Chemistry*, *312*. https://doi.org/10.1016/j.foodchem.2019.126072

120. Kitrytė, V., Povilaitis, D., Kraujalienė, V., Šulniūtė, V., Pukalskas, A., & Venskutonis, P. R. (2017). Fractionation of sea buckthorn pomace and seeds into valuable components by using high pressure and enzyme-assisted extraction methods. *LWT - Food Science and Technology*, *85*, 534–538. https://doi.org/10.1016/j.lwt.2017.02.041

121. Kitrytė, V., Šaduikis, A., & Venskutonis, P. R. (2015). Assessment of antioxidant capacity of brewer's spent grain and its supercritical carbon dioxide extract as sources of valuable dietary ingredients. *Journal of Food Engineering*, *167*, 18–24. https://doi.org/10.1016/j.jfoodeng.2014.12.005

122. Knekt, P., Kumpulainen, J., Järvinen, R., Rissanen, H., Heliövaara, M., Reunanen, A., … Aromaa, A. (2002). Flavonoid intake and risk of chronic diseases. *American Journal of Clinical Nutrition*, *76*(3), 560–568. https://doi.org/10.1093/ajcn/76.3.560

123. Kondo, M., Mackinnon, S. L., Craft, C. C., Matchett, M. D., Hurta, R. A. R., & Neto, C. C. (2011). Ursolic acid and its esters: occurrence in cranberries and other *Vaccinium* fruit and effects on matrix metalloproteinase activity in DU145 prostate tumor cells. *Journal of the Science of Food and Agriculture*, *91*(5), 789–796. https://doi.org/10.1002/jsfa.4330

124. Kraujalienė, V., Pukalskas, A., Kraujalis, P., & Venskutonis, P. R. (2016). Biorefining of *Bergenia crassifolia L.* roots and leaves by high pressure extraction methods and evaluation of antioxidant properties and main phytochemicals in extracts and plant material. *Industrial Crops and Products*, *89*, 390–398. https://doi.org/10.1016/j.indcrop.2016.05.034

125. Kraujalis, P., Kraujalienė, V., Kazernavičiūtė, R., & Venskutonis, P. R. (2017). Supercritical carbon dioxide and pressurized liquid extraction of valuable ingredients from *Viburnum opulus* pomace and berries and evaluation of product characteristics. *Journal of Supercritical Fluids*, *122*, 99–108. https://doi.org/10.1016/j.supflu.2016.12.008

126. Kraujalis, P., & Venskutonis, P. R. (2013). Optimisation of supercritical carbon dioxide extraction of amaranth seeds by response surface methodology and characterization of extracts isolated from different plant cultivars. *Journal of Supercritical Fluids*, *73*, 80–86. https://doi.org/10.1016/j.supflu.2012.11.009

127. Kraujalis, P., & Venskutonis, P. R. (2013). Supercritical carbon dioxide extraction of squalene and tocopherols from amaranth and assessment of extracts antioxidant activity. *Journal of Supercritical Fluids*, *80*, 78–85. https://doi.org/10.1016/j.supflu.2013.04.005

128. Kritchevsky, D., & Chen, S. C. (2005). Phytosterols-health benefits and potential concerns: a review. *Nutrition Research*, *25*(5), 413–428. https://doi.org/10.1016/j.nutres.2005.02.003

129. Kryževičiūtė, N., Jaime, I., Diez, A. M., Rovira, J., & Venskutonis, P. R. (2017). Effect of raspberry pomace extracts isolated by high pressure extraction on the quality and shelf-life of beef burgers. *International Journal of Food Science and Technology*, *52*(8), 1852–1861. https://doi.org/10.1111/ijfs.13460

130. Kryževičiute, N., Kraujalis, P., & Venskutonis, P. R. (2016). Optimization of high pressure extraction processes for the separation of raspberry pomace into lipophilic and hydrophilic fractions. *Journal of Supercritical Fluids*, *108*, 61–68.

https://doi.org/10.1016/j.supflu.2015.10.025

131. Krzywicki, K. (1982). The determination of haem pigments in meat. *Meat Science*, *7*(1), 29–36. https://doi.org/10.1016/0309-1740(82)90095-X

132. Kühn, S., & Temelli, F. (2017). Recovery of bioactive compounds from cranberry pomace using ternary mixtures of CO_2+ethanol+water. *Journal of Supercritical Fluids*, *130*(March), 147–155. https://doi.org/10.1016/j.supflu.2017.07.028

133. Kylli, P., Nohynek, L., Puupponen-Pimiä, R., Westerlund-Wikström, B., Leppänen, T., Welling, J., … Heinonen, M. (2011). Lingonberry (*Vaccinium vitis-idaea*) and European cranberry (*Vaccinium microcarpon*) proanthocyanidins: isolation, identification, and bioactivities. *Journal of Agricultural and Food Chemistry*, *59*(7), 3373–3384. https://doi.org/10.1021/jf104621e

134. Laitinen, A. (1999). Supercritical fluid extraction of organic compounds from solids and aqueous solutions. *VTT Publications*, (403), 2–58.

135. Lang, Q., & Wai, C. M. (2001). Supercritical fluid extraction in herbal and natural product studies – a practical review. *Talanta*, *53*, 771–782. https://doi.org/S0039-9140(00)00557-9

136. Lani, R., Hassandarvish, P., Shu, M. H., Phoon, W. H., Chu, J. J. H., Higgs, S., … Zandi, K. (2016). Antiviral activity of selected flavonoids against Chikungunya virus. *Antiviral Research*, *133*, 50–61. https://doi.org/10.1016/j.antiviral.2016.07.009

137. Lashmanova, K. A., Kuzivanova, O. A., & Dymova, O. V. (2012). Northern berries as a source of carotenoids. *Acta Biochimica Polonica*, *59*(1), 133–134. https://doi.org/10.18388/abp.2012_2188

138. Lee, J. K., & Shin, O. S. (2019). Advances in Zika virus – host cell interaction: Current knowledge and future perspectives. *International Journal of Molecular Sciences*, *20*(5). https://doi.org/10.3390/ijms20051101

139. Li, W., Du, B., Wang, T., Wang, S., & Zhang, J. (2009). Kaempferol induces apoptosis in human HCT116 colon cancer cells via the ataxia-telangiectasia mutated-p53 pathway with the involvement of p53 upregulated modulator of apoptosis. *Chemico-Biological Interactions*, *177*(2), 121–127. https://doi.org/10.1016/j.cbi.2008.10.048

140. Liberty, A. M., Hart, P. E., & Neto, C. C. (2007). Ursolic acid and proanthocyanidins from cranberry (*Vaccinium macrocarpon*) inhibit colony formation and proliferation in HCT-116 and HT-29 colon and MCF-7 breast tumor cells. *National Meeting of the American Chemical Society*, 233. Chicago.

141. Lipson, S. M., Sethi, L., Cohen, P., Gordon, R. E., Tan, I. P., Burdowski, A., & Stotzky, G. (2007). Antiviral effects on bacteriophages and rotavirus by cranberry juice. *Phytomedicine*, *14*(1), 23–30. https://doi.org/10.1016/j.phymed.2006.11.009

142. Lipson, Steven M, Cohen, P., Zhou, J., Burdowski, A., & Stotzky, G. (2007). Cranberry cocktail juice, cranberry concentrates, and proanthocyanidins reduce reovirus infectivity titers in African green monkey kidney epithelial cell cultures. *Molecular Nutrition and Food Research*, *51*(6), 752–758. https://doi.org/10.1002/mnfr.200600276

143. Lorenzo, J. M., Pateiro, M., Domínguez, R., Barba, F. J., Putnik, P., Kovačević, D. B., … Franco, D. (2018). Berries extracts as natural antioxidants in meat products: A review. *Food Research International*, *106*, 1095–1104. https://doi.org/10.1016/j.foodres.2017.12.005

144. Luo, H., Rankin, G. O., Li, Z., DePriest, L., & Chen, Y. C. (2011). Kaempferol induces apoptosis in ovarian cancer cells through activating p53 in the intrinsic pathway. *Food Chemistry*, *128*(2), 513–519. https://doi.org/10.1016/j.foodchem.2011.03.073

145. Maarasyid, C., Muhamad, I. I., & Supriyanto, E. (2014). Potential source and extraction of vitamin E from palm-based oils: a review. *Jurnal Teknologi*, *69*(4), 43–50. https://doi.org/10.11113/jt.v69.3172

146. Machado, A. P. D. F., Pasquel-Reátegui, J. L., Barbero, G. F., & Martínez, J. (2015). Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus L.*) residues: A comparison with conventional methods. *Food Research International*, *77*, 675–683. https://doi.org/10.1016/j.foodres.2014.12.042

147. Madrigal-Carballo, S., Rodriguez, G., Vega-Baudrit, J. R., Krueger, C. G., & Reed, J. D. (2013). MALDI-TOF mass spectrometry of oligomeric food polyphenols. *International Food Research Journal*, *20*(5), 2023–2034.

148. Maillard, V., Bougnoux, P., Ferrari, P., Jourdan, M. L., Pinault, M., Lavillonnìre, F., … Chajès, V. (2002). n-3 and n-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in tours, France. *International Journal of Cancer*, *98*(1), 78–83. https://doi.org/10.1002/ijc.10130

149. Malik, M., Zhao, C., Schoene, N., Guisti, M. M., Moyer, M. P., & Magnuson, B. A. (2003). Anthocyanin-rich extract from *Aronia meloncarpa* E. induces a cell cycle Block in colon cancer but not normal colonic cells. *Nutrition and Cancer*, *46*(2), 186–196. https://doi.org/10.1207/S15327914NC4602_12

150. Manach, C., Williamson, G., Morand, C., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition*, *81*, 230–242. https://doi.org/10.1093/ajcn/81.1.230s

151. Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science*, *71*(1), 100–121. https://doi.org/10.1016/j.meatsci.2005.03.003

153. Mayne, S. T. (2003). Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. *The Journal of Nutrition*, *133*(3), 933S-940S. https://doi.org/10.1093/jn/133.3.933s

154. Mel'nikov, S. M., Seijen Ten Hoorn, J. W. M., & Eijkelenboom, A. P. A. M. (2004). Effect of phytosterols and phytostanols on the solubilization of cholesterol by dietary mixed micelles: an *in vitro* study. *Chemistry and Physics of Lipids*, *127*(2), 121–141. https://doi.org/10.1016/j.chemphyslip.2003.09.015

155. Mildner-Szkudlarz, S., Bajerska, J., Górnaś, P., Segliņa, D., Pilarska, A., & Jesionowski, T. (2016). Physical and bioactive properties of muffins enriched with raspberry and cranberry pomace powder: a promising application of fruit by-products rich in biocompounds. *Plant Foods for Human Nutrition*, *71*(2), 165–173. https://doi.org/10.1007/s11130-016-0539-4

156. Minker, C., Duban, L., Karas, D., Järvinen, P., Lobstein, A., & Muller, C. D. (2015). Impact of procyanidins from different berries on caspase 8 activation in colon cancer. *Oxidative Medicine and Cellular Longevity*, *2015*. https://doi.org/10.1155/2015/154164

157. Mirabella, N., Castellani, V., & Sala, S. (2014). Current options for the valorization of food manufacturing waste: a review. *Journal of Cleaner Production*, *65*, 28–41. https://doi.org/10.1016/j.jclepro.2013.10.051

158. Mittal, A., Elmets, C. A., & Katiyar, S. K. (2003). Dietary feeding of proanthocyanidins from grape seeds prevents photocarcinogenesis in SKH-1 hairless mice: Relationship to decreased fat and lipid peroxidation. *Carcinogenesis*, *24*(8), 1379–1388. https://doi.org/10.1093/carcin/bgg095

159. Molan, A. L., Lila, M. A., Mawson, J., & De, S. (2009). *In vitro* and *in vivo* evaluation of the prebiotic activity of water-soluble blueberry extracts. *World Journal of Microbiology and Biotechnology*, *25*(7), 1243–1249. https://doi.org/10.1007/s11274-009-0011-9

160. Moreno, E., Reza, J., & Trejo, A. (2007). Extraction of polycyclic aromatic hydrocarbons from soil using water under subcritical conditions. *Polycyclic Aromatic Compounds*, *27*(4), 239–260. https://doi.org/10.1080/10406630701462916

161. Mounce, B. C., Cesaro, T., Carrau, L., Vallet, T., & Vignuzzi, M. (2017). Curcumin inhibits Zika and Chikungunya virus infection by inhibiting cell binding. *Antiviral Research*, *142*, 148–157. https://doi.org/10.1016/j.antiviral.2017.03.014

162. Mukhopadhyay, M., & Panja, P. (2008). Recovery of phytochemicals from kokum (*Garcinia indica choisy*) using pressurized hot water. *International Journal of Food Engineering*, *4*(8), 1–20. https://doi.org/10.2202/1556-3758.1327

163. Murphy, M. M., Spungen, J. H., Bi, X., & Barraj, L. M. (2011). Fresh and fresh lean pork are substantial sources of key nutrients when these products are consumed by adults in the United States. *Nutrition Research*, *31*(10), 776–783. https://doi.org/10.1016/j.nutres.2011.09.006

164. Muzolf-Panek, M., Waśkiewicz, A., Kowalski, R., & Konieczny, P. (2016). The effect of blueberries on the oxidative stability of pork meatloaf during chilled storage. *Journal of Food Processing and Preservation*, *40*(5), 899–909. https://doi.org/10.1111/jfpp.12668

165. Nantz, M. P., Rowe, C. A., Muller, C., Creasy, R., Colee, J., Khoo, C., & Percival, S. S. (2013). Consumption of cranberry polyphenols enhances human γδ-T cell proliferation and reduces the number of symptoms associated with colds and influenza: a randomized, placebo-controlled intervention study. *Nutrition Journal*, *12*(1), 1–9. https://doi.org/10.1186/1475-2891-12-161

166. Narayana, K. R., Reddy, M. S., Chaluvadi, M. R., & Krishna, D. R. (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian Journal of Pharmacology*, *33*(1), 2–16.

167. Negi, P. S. (2012). Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *International Journal of Food Microbiology*, *156*(1), 7–17. https://doi.org/10.1016/j.ijfoodmicro.2012.03.006

168. Nemzer, B., Vargas, L., Xia, X., Sintara, M., & Feng, H. (2018). Phytochemical and physical properties of blueberries, tart cherries, strawberries, and cranberries as affected by different drying methods. *Food Chemistry*, *262*(April), 242–250. https://doi.org/10.1016/j.foodchem.2018.04.047

169. Nesaretnam, K. (2008). Multitargeted therapy of cancer by tocotrienols. *Cancer Letters*, *269*(2), 388–395. https://doi.org/10.1016/j.canlet.2008.03.063

170. Neto, C. C., Krueger, C. G., Lamoureaux, T. L., Kondo, M., Vaisberg, A. J., Hurta, R. A. R., … Reed, J. D. (2006). MALDI-TOF MS characterization of proanthocyanidins from cranberry fruit (*Vaccinium macrocarpon*) that inhibit tumor cell growth and matrix metalloproteinase expression *in vitro*. *Journal of the Science of Food and Agriculture*, *86*(1), 18–25. https://doi.org/10.1002/jsfa.2347

171. Nguyen, T. T. T., Tran, E., Nguyen, T. H., Do, P. T., Huynh, T. H., & Huynh, H. (2004). The role of activated MEK-ERK pathway in quercetin-induced growth inhibition and apoptosis in A549 lung cancer cells. *Carcinogenesis*, *25*(5), 647–659. https://doi.org/10.1093/carcin/bgh052

172. Nissen, L. R., Byrne, D. V., Bertelsen, G., & Skibsted, L. H. (2004). The antioxidative

activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. *Meat Science*, *68*(3), 485–495. https://doi.org/10.1016/j.meatsci.2004.05.004

173. Oiknine-Djian, E., Houri-Haddad, Y., Weiss, E., Ofek, I., Greenbaum, E., Hartshorn, K., & Zakay-Rones, Z. (2012). High molecular weight constituents of cranberry interfere with influenza virus neuraminidase activity *in vitro*. *Planta Medica*, *78*(10), 962–967. https://doi.org/10.1055/s-0031-1298579

174. Ong, E. S., Cheong, J. S. H., & Goh, D. (2006). Pressurized hot water extraction of bioactive or marker compounds in botanicals and medicinal plant materials. *Journal of Chromatography A*, *1112*(1–2), 92–102. https://doi.org/10.1016/j.chroma.2005.12.052

175. Ozer, O., & Sariçoban, C. (2010). The effects of butylated hydroxyanisole, ascorbic acid, and α-tocopherol on some quality characteristics of mechanically deboned chicken patty during freeze storage. *Czech Journal of Food Sciences*, *28*(2), 150–160.

176. Paliwal, S., Sundaram, J., & Mitragotri, S. (2005). Induction of cancer-specific cytotoxicity towards human prostate and skin cells using quercetin and ultrasound. *British Journal of Cancer*, *92*(3), 499–502. https://doi.org/10.1038/sj.bjc.6602364

177. Pappas, E., & Schaich, K. M. (2009). Phytochemicals of cranberries and cranberry products: Characterization, potential health effects, and processing stability. *Critical Reviews in Food Science and Nutrition*, *49*(9), 741–781. https://doi.org/10.1080/10408390802145377

178. Papuc, C., Goran, G. V, Predescu, C. N., & Nicorescu, V. (2017). Mechanisms of oxidative processes in meat and toxicity induced by postprandial degradation products: a review. *Comprehensive Reviews in Food Science and Food Safety*, *16*(1), 96–123. https://doi.org/10.1111/1541-4337.12241

179. Pardo-Castaño, C., Velásquez, M., & Bolaños, G. (2015). Simple models for supercritical extraction of natural matter. *Journal of Supercritical Fluids*, *97*, 165–173. https://doi.org/10.1016/j.supflu.2014.09.044

180. Parker, T. D., Adams, D. A., Zhou, K., Harris, M., & Yu, L. (2003). Fatty acid composition and oxidative stability of cold-pressed edible seed oils. *Journal of Food Science*, *68*(4), 1240–1243. https://doi.org/10.1111/j.1365-2621.2003.tb09632.x

181. Perrut, M., & Perrut, V. (2018). Towards ingredients by combining supercritical fluids with other processes. *Journal of Supercritical Fluids*, *134*, 214–219. https://doi.org/10.1016/j.supflu.2017.11.019

182. Phillips, P. A., Sangwan, V., Borja-Cacho, D., Dudeja, V., Vickers, S. M., & Saluja, A. K. (2011). Myricetin induces pancreatic cancer cell death via the induction of apoptosis and inhibition of the phosphatidylinositol 3-kinase (PI3K) signaling pathway. *Cancer Letters*, *308*(2), 181–188. https://doi.org/10.1016/j.canlet.2011.05.002

183. Povilaitis, D., Šulniute, V., Venskutonis, P. R., & Kraujaliene, V. (2015). Antioxidant properties of wheat and rye bran extracts obtained by pressurized liquid extraction with different solvents. *Journal of Cereal Science*, *62*, 117–123. https://doi.org/10.1016/j.jcs.2014.11.004

184. Powers, C. N., & Setzer, W. N. (2016). An in-Silico investigation of phytochemicals as antiviral agents against Dengue fever. *Combinatorial Chemistry & High Throughput Screening*, *19*(7), 516–536. https://doi.org/10.2174/13862073196661605

185. Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., … Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *Journal*

of Agricultural and Food Chemistry, *51*(11), 3273–3279. https://doi.org/10.1021/jf0262256

186. Pronyk, C., & Mazza, G. (2009). Design and scale-up of pressurized fluid extractors for food and bioproducts. *Journal of Food Engineering*, *95*(2), 215–226. https://doi.org/10.1016/j.jfoodeng.2009.06.002

187. Puupponen-Pimiä, R., Nohynek, L., & Meier, C. (2001). Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology*, *90*(4), 494–507. https://doi.org/10.1046/j.1365-2672.2001.01271.x

188. Puupponen-Pimiä, Riitta, Nohynek, L., Hartmann-Schmidlin, S., Kähkönen, M., Heinonen, M., Määttä-Riihinen, K., & Oksman-Caldentey, K. M. (2005a). Berry phenolics selectively inhibit the growth of intestinal pathogens. *Journal of Applied Microbiology*, *98*(4), 991–1000. https://doi.org/10.1111/j.1365-2672.2005.02547.x

189. Rane, H. S., Bernardo, S. M., Howell, A. B., & Lee, S. A. (2014). Cranberry-derived proanthocyanidins prevent formation of *Candida albicans* biofilms in artificial urine through biofilm- and adherence-specific mechanisms. *Journal of Antimicrobial Chemotherapy*, *69*(2), 428–436. https://doi.org/10.1093/jac/dkt398

190. Rauha, J.-P., Susanna, R., Heinonen, M., Hopia, A., Kahkonen, M., Kujala, T., … Vuorela, P. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology*, *56*, 3–12.

191. Raventós, M., Duarte, S., & Alarcón, R. (2002). Application and possibilities of supercritical CO_2 extraction in food processing industry: An overview. *Food Science and Technology International*, *8*(5), 269–284. https://doi.org/10.1106/108201302029451

192. Ravi, H. K., Breil, C., Vian, M. A., Chemat, F., & Venskutonis, P. R. (2018). Biorefining of bilberry (*Vaccinium myrtillus L.*) pomace using microwave hydro-diffusion and gravity , ultrasound assisted and bead milling extraction. *ACS Sustainable Chemistry and Engineering*, *6*(3), 4185–4193. https://doi.org/10.1021/acssuschemeng.7b04592

193. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*(9/10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

194. Reddivari, L., Vanamala, J., Chintharlapalli, S., Safe, S. H., & Miller, J. C. (2007). Anthocyanin fraction from potato extracts is cytotoxic to prostate cancer cells through activation of caspase-dependent and caspase-independent pathways. *Carcinogenesis*, *28*(10), 2227–2235. https://doi.org/10.1093/carcin/bgm117

195. Reverchon, E., & De Marco, I. (2006). Supercritical fluid extraction and fractionation of natural matter. *Journal of Supercritical Fluids*, *38*(2), 146–166. https://doi.org/10.1016/j.supflu.2006.03.020

196. Rey, A. I., Hopia, A., Kivikari, R., & Kahkonen, M. (2005). Use of natural food/plant extracts: cloudberry (*Rubus Chamaemorus*), beetroot (*Beta Vulgaris "Vulgaris"*) or willow herb (*Epilobium angustifolium*) to reduce lipid oxidation of cooked pork patties. *LWT – Food Science and Technology*, *38*(4), 363–370. https://doi.org/10.1016/j.lwt.2004.06.010

197. Richter, B. E., Jones, B. A., Ezzell, J. L., & Porter, N. L. (1996). Accelerated solvent extraction: a technique for sample preparation. *Analytical Chemistry*, *68*(6), 1033–1039. https://doi.org/10.1021/ac9508199

198. Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N., & Pohl, C. (1996). Accelerated solvent extraction: a technique for sample preparation. *Analytical Chemistry*, *68*(6), 1033–1039. https://doi.org/10.1021/ac9508199

199. Romani, A., Campo, M., & Pinelli, P. (2012). HPLC/DAD/ESI-MS analyses and anti-radical activity of hydrolyzable tannins from different vegetal species. *Food Chemistry*, *130*(1), 214–221. https://doi.org/10.1016/j.foodchem.2011.07.009

200. Rostagno, M. A., Villares, A., Guillamón, E., García-Lafuente, A., & Martínez, J. A. (2009). Sample preparation for the analysis of isoflavones from soybeans and soy foods. *Journal of Chromatography A*, *1216*(1), 2–29. https://doi.org/10.1016/j.chroma.2008.11.035

201. Roy, B. C., Goto, M., Kodama, A., & Hirose, T. (1996). Supercritical CO_2 extraction of essential oils and cuticular waxes from peppermint leaves. *Journal of Chemical Technology & Biotechnology*, *67*(1), 21–26. https://doi.org/10.1002/(sici)1097-4660(199609)67:1<21::aid-jctb522>3.3.co;2-s

202. Ruel, G., Pomerleau, S., Couture, P., Lamarche, B., & Couillard, C. (2005). Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption. *Metabolism: Clinical and Experimental*, *54*(7), 856–861. https://doi.org/10.1016/j.metabol.2005.01.031

203. Ruel, G., Pomerleau, S., Couture, P., Lemieux, S., Lamarche, B., & Couillard, C. (2008). Low-calorie cranberry juice supplementation reduces plasma oxidized LDL and cell adhesion molecule concentrations in men. *British Journal of Nutrition*, *99*(2), 352–359. https://doi.org/10.1017/S0007114507811986

204. Runnqvist, H., Bak, S. A., Hansen, M., Styrishave, B., Halling-Sørensen, B., & Björklund, E. (2010). Determination of pharmaceuticals in environmental and biological matrices using pressurised liquid extraction-Are we developing sound extraction methods? *Journal of Chromatography A*, *1217*(16), 2447–2470. https://doi.org/10.1016/j.chroma.2010.02.046

205. Saavedra, M. J., Borges, A., Dias, C., Aires, A., Bennett, R. N., Rosa, E. S., & Simões, M. (2010). Antimicrobial activity of phenolics and glucosinolate hydrolysis products and their synergy with streptomycin against pathogenic bacteria. *Journal of Medical Chemistry*, *6*, 174–183.

206. Sahoo, P., Barman, T., & Davim, J. P. (2011). *Fractal analysis in maschining* (First edition). https://doi.org/10.1007/978-3-642-17922-8

207. Samelis, J., Kakouri, A., & Rementzis, J. (2000). Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4°C. *Food Microbiology*, *17*(3), 329–340. https://doi.org/10.1006/fmic.1999.0316

208. Seeram, N. P., Adams, L. S., Hardy, M. L., & Heber, D. (2004). Total cranberry extract *versus* its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. *Journal of Agricultural and Food Chemistry*, *52*(9), 2512–2517. https://doi.org/10.1021/jf0352778

209. Seeram, N. P., Adams, L. S., Zhang, Y., Lee, R., Sand, D., Scheuller, H. S., & Heber, D. (2006). Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells *in vitro*. *Journal of Agricultural and Food Chemistry*, *54*(25), 9329–9339. https://doi.org/10.1021/jf061750g

210. Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*, *39*(11), 4067–4079. https://doi.org/10.1039/b922183m

211. Sharma, N., Murali, A., Singh, S. K., & Giri, R. (2017). Epigallocatechin gallate, an active green tea compound inhibits the Zika virus entry into host cells via binding the envelope protein. *International Journal of Biological Macromolecules*, *104*, 1046–1054.

https://doi.org/10.1016/j.ijbiomac.2017.06.105

212. Shi, J., Mittal, G., Kim, E., & Xue, S. J. (2007). Solubility of carotenoids in supercritical CO_2. *Food Reviews International*, *23*(4), 341–371.

213. Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods of Enzymology*, 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

214. Skeberdytė, A., Sarapinienė, I., Aleksander-Krasko, J., Stankevičius, V., Sužiedėlis, K., & Jarmalaitė, S. (2018). Dichloroacetate and salinomycin exert a synergistic cytotoxic effect in colorectal cancer cell lines. Scientific Reports, 8(1), 1–13. https://doi.org/10.1038/s41598-018-35815-4

215. Slavin, M., & Yu, L. L. (2012). A single extraction and HPLC procedure for simultaneous analysis of phytosterols, tocopherols and lutein in soybeans. *Food Chemistry*, *135*(4), 2789–2795. https://doi.org/10.1016/j.foodchem.2012.06.043

216. Soares, B. M. C., Gamarra, F. M. C., Paviani, L. C., Gonçalves, L. A. G., & Cabral, F. A. (2007). Solubility of triacylglycerols in supercritical carbon dioxide. *Journal of Supercritical Fluids*, *43*(1), 25–31. https://doi.org/10.1016/j.supflu.2007.03.013

217. Sójka, M., & Król, B. (2009). Composition of industrial seedless black currant pomace. *European Food Research and Technology*, *228*(4), 597–605. https://doi.org/10.1007/s00217-008-0968-x

218. Soladoye, O. P., Juárez, M. L., Aalhus, J. L., Shand, P., & Estévez, M. (2015). Protein oxidation in processed meat: mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety*, *14*(2), 106–122. https://doi.org/10.1111/1541-4337.12127

219. Sørheim, O., Ofstad, R., & Lea, P. (2004). Effects of carbon dioxide on yield, texture and microstructure of cooked ground beef. *Meat Science*, *67*(2), 231–236. https://doi.org/10.1016/j.meatsci.2003.10.010

220. Sovová, H. (2012). Steps of supercritical fluid extraction of natural products and their characteristic times. *Journal of Supercritical Fluids*, *66*, 73–79. https://doi.org/10.1016/j.supflu.2011.11.004

221. Sovová, H. (2017). Broken-and-intact cell model for supercritical fluid extraction: Its origin and limits. *Journal of Supercritical Fluids*, *129*, 3–8. https://doi.org/10.1016/j.supflu.2017.02.014

222. Sovová, H., Kucera, J., & Jež, J. (1994). Rate of the vegetable oil extraction with extraction of grape oil. *Chemical Engineering Science*, *49*(3), 415–420.

223. Sovová, H., Nobre, B. P., & Palavra, A. (2016). Modeling of the kinetics of supercritical fluid extraction of lipids from microalgae with emphasis on extract desorption. *Materials*, *9*(6), 1–19. https://doi.org/10.3390/ma9060423

224. Sovová, H., & Stateva, R. P. (2019). New developments in the modelling of carotenoids extraction from microalgae with supercritical CO_2. *Journal of Supercritical Fluids*, *148*, 93–103. https://doi.org/10.1016/j.supflu.2019.03.002

225. Sovová, H., Zarevúcka, M., Vacek, M., & Stránský, K. (2001). Solubility of two vegetable oils in supercritical CO_2. *Journal of Supercritical Fluids*, *20*(1), 15–28. https://doi.org/10.1016/S0896-8446(01)00057-2

226. Stagos, D. (2020). Antioxidant activity of polyphenolic plant extracts. *Antioxidants*, *9*(1). https://doi.org/10.3390/antiox9010019

227. Stobnicka, A., & Gniewosz, M. (2018). Antimicrobial protection of minced pork meat with the use of swamp cranberry (*Vaccinium oxycoccos L*.) fruit and pomace extracts. *Journal of Food Science and Technology*, *55*(1), 62–71. https://doi.org/10.1007/s13197-017-2770-x

228. Stone, W. L., & Papas, A. (2012). Tocopherols, tocotrienols and vitamin E. In *Lipids for Functional Foods and Nutraceuticals*. https://doi.org/10.1533/9780857097965.53

229. Struck, S., Plaza, M., Turner, C., & Rohm, H. (2016). Berry pomace – a review of processing and chemical analysis of its polyphenols. *International Journal of Food Science and Technology*, *51*(6), 1305–1318. https://doi.org/10.1111/ijfs.13112

230. Su, X., Howell, A. B., & D'Souza, D. H. (2010). The effect of cranberry juice and cranberry proanthocyanidins on the infectivity of human enteric viral surrogates. *Food Microbiology*, *27*(4), 535–540. https://doi.org/10.1016/j.fm.2010.01.001

231. Sun, F., Zheng, X. Y., Ye, J., Wu, T. T., Wang, J. L., & Chen, W. (2012). Potential anticancer activity of myricetin in human T24 bladder cancer cells both *in vitro* and *in vivo*. *Nutrition and Cancer*, *64*(4), 599–606. https://doi.org/10.1080/01635581.2012.665564

232. Sun, X. D., & Holley, R. A. (2012). Antimicrobial and antioxidative strategies to reduce pathogens and extend the shelf life of fresh red meats. *Comprehensive Reviews in Food Science and Food Safety*, *11*, 340–354. https://doi.org/10.1111/j.1541-4337.2012.00188.x

233. Sung, K. J., Ki, W. L., Byun, S., Nam, J. K., Sung, H. L., Heo, Y. S., … Dong, Z. (2008). Myricetin suppresses UVB-induced skin cancer by targeting Fyn. *Cancer Research*, *68*(14), 6021–6029. https://doi.org/10.1158/0008-5472.CAN-08-0899

234. Teo Chye, C., Tan Ngin, S., Hong Yong, J. W., Hew Sin, C., & Ong Shi, E. (2010). Pressurized hot water extraction (PHWE). *Journal of Chromatography A*, *1217*(16), 2484–2494. https://doi.org/10.1016/j.chroma.2009.12.050

235. Tian, F., Li, B., Ji, B., Yang, J., Zhang, G., Chen, Y., & Luo, Y. (2009). Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*:The polarity affects the bioactivities. *Food Chemistry*, *113*(1), 173–179. https://doi.org/10.1016/j.foodchem.2008.07.062

236. Turner, C., King, J. W., & Mathiasson, L. (2001). Supercritical fluid extraction and chromatography for fat-soluble vitamin analysis. *Journal of Chromatography A*, *936*(1–2), 215–237. https://doi.org/10.1016/S0021-9673(01)01082-2

237. US centers for disease control and prevention (US CDC). (2005). *Escherichia coli* O157:H7.

238. USDA-NASS. (2011). Statistics by Subject. *NASS – Statistics By Subject*, (202), 2157.

239. Valentová, K., Stejskal, D., Bednář, P., Vostálová, J., Číhalík, Č., Večeřová, R., … Šimánek, V. (2007). Biosafety, antioxidant status, and metabolites in urine after consumption of dried cranberry juice in healthy women: a pilot double-blind placebo-controlled trial. *Journal of Agricultural and Food Chemistry*, *55*(8), 3217–3224. https://doi.org/10.1021/jf0636014

240. Vattem, D. A., Lin, Y. T., Ghaedian, R., & Shetty, K. (2005). Cranberry synergies for dietary management of *Helicobacter pylori* infections. *Process Biochemistry*, *40*(5), 1583–1592. https://doi.org/10.1016/j.procbio.2004.06.024

241. Vattem, Dhiraj A., & Shetty, K. (2002). Solid-state production of phenolic antioxidants from cranberry pomace by *Rhizopus oligosporus*. *Food Biotechnology*, *16*(3), 189–210. https://doi.org/10.1081/FBT-120016667

242. Vázquez-Calvo, Á., de Oya, N. J., Martín-Acebes, M. A., Garcia-Moruno, E., & Saiz,

J. C. (2017). Antiviral properties of the natural polyphenols delphinidin and epigallocatechin gallate against the flaviviruses West Nile Virus, Zika Virus, and Dengue Virus. *Frontiers in Microbiology*, *8*(JUL), 1–8. https://doi.org/10.3389/fmicb.2017.01314

243. Venskutonis, P. R. (2020). Berries. In C. M. Galanakis (ed.), *Valorization of fruit processing by-products* (1st ed., 95–126). https://doi.org/10.1017/CBO9781107415324.004

244. Viacava, G. E., Roura, S. I., & Agüero, M. V. (2015). Optimization of critical parameters during antioxidants extraction from butterhead lettuce to simultaneously enhance polyphenols and antioxidant activity. *Chemometrics and Intelligent Laboratory Systems*, *146*, 47–54. https://doi.org/10.1016/j.chemolab.2015.05.002

245. Vongsangnak, W., Gua, J., Chauvatcharin, S., & Zhong, J. J. (2004). Towards efficient extraction of notoginseng saponins from cultured cells of panax notoginseng. *Biochemical Engineering Journal*, *18*(2), 115–120. https://doi.org/10.1016/S1369-703X(03)00197-9

246. Vu, K. D., Carlettini, H., Bouvet, J., Côté, J., Doyon, G., Sylvain, J. F., & Lacroix, M. (2012). Effect of different cranberry extracts and juices during cranberry juice processing on the antiproliferative activity against two colon cancer cell lines. *Food Chemistry*, *132*(2), 959–967. https://doi.org/10.1016/j.foodchem.2011.11.078

247. Vvedenskaya, I. O., & Vorsa, N. (2004). Flavonoid composition over fruit development and maturation in American cranberry, *Vaccinium macrocarpon* Ait. *Plant Science*, *167*(5), 1043–1054. https://doi.org/10.1016/j.plantsci.2004.06.001

248. Wang, C. Y., & Wang, S. Y. (2009). Effect of storage temperatures on fruit quality of various cranberry cultivars. *Acta Horticulturae*, *810*, 853–862.

249. Wang, C., & Zuo, Y. (2011). Ultrasound-assisted hydrolysis and gas chromatography-mass spectrometric determination of phenolic compounds in cranberry products. *Food Chemistry*, *128*(2), 562–568. https://doi.org/10.1016/j.foodchem.2011.03.066

250. Weiss, E. I., Houri-Haddad, Y., Greenbaum, E., Hochman, N., Ofek, I., & Zakay-Rones, Z. (2005). Cranberry juice constituents affect influenza virus adhesion and infectivity. *Antiviral Research*, *66*(1), 9–12. https://doi.org/10.1016/j.antiviral.2004.12.011

251. Wen, A., Delaquis, P., Stanich, K., & Toivonen, P. (2003). Antilisterial activity of selected phenolic acids. *Food Microbiology*, *20*, 305–311. https://doi.org/10.1016/S0740-0020(02)00135-1

252. White, B. L., Howard, L. R., & Prior, R. L. (2010). Proximate and polyphenolic characterization of cranberry pomace. *Journal of Agricultural and Food Chemistry*, *58*(7), 4030–4036. https://doi.org/10.1021/jf902829g

253. Williams, C. H., & Hong, C. C. (2011). Multi-step usage of *in vivo* models during rational drug design and discovery. *International Journal of Molecular Sciences*, *12*(4), 2262–2274. https://doi.org/10.3390/ijms12042262

254. Witkowska, A. M., Hickey, D. K., Alonso-Gomez, M., & Wilkinson, M. (2013). Evaluation of antimicrobial activities of commercial herb and spice extracts against selected food-borne bacteria. *Journal of Food Research*, *2*(4), 37. https://doi.org/10.5539/jfr.v2n4p37

255. Wong, G., He, S., Siragam, V., Bi, Y., Mbikay, M., Chretien, M., & Qiu, X. (2017). Antiviral activity of quercetin-3-β-O-D-glucoside against Zika virus infection. *Virologica Sinica*, *32*(6), 545–547. https://doi.org/10.1007/s12250-017-4057-9

256. Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., … Enser, M. (2004). Effects of fatty acids on meat quality: A review. *Meat Science*, *66*(1), 21–32. https://doi.org/10.1016/S0309-1740(03)00022-6

257. Wu, Vivian C H, Qiu, X., de los Reyes, B. G., Lin, C. S., & Pan, Y. (2009). Application of cranberry concentrate (*Vaccinium macrocarpon*) to control *Escherichia coli* O157:H7 in ground beef and its antimicrobial mechanism related to the downregulated slp, hdeA and cfa. *Food Microbiology*, *26*(1), 32–38. https://doi.org/10.1016/j.fm.2008.07.014

258. Wu, Vivian Chi Hua, Qiu, X., Bushway, A., & Harper, L. (2008). Antibacterial effects of American cranberry (*Vaccinium macrocarpon*) concentrate on foodborne pathogens. *LWT – Food Science and Technology*, *41*(10), 1834–1841. https://doi.org/10.1016/j.lwt.2008.01.001

259. Wu, X., & Prior, R. L. (2005). Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. *Journal of Agricultural and Food Chemistry*, *53*(7), 2589–2599. https://doi.org/10.1021/jf048068b

260. Xing, N., Chen, Y., Mitchell, S. H., & Young, C. Y. F. (2001). Quercetin inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells. *Carcinogenesis*, *22*(3), 409–414. https://doi.org/10.1093/carcin/22.3.409

261. Xiong, Y. L. (2017). Inhibition of hazardous compound formation in muscle foods by antioxidative phytophenols. *Annals of the New York Academy of Sciences*, 1–10. https://doi.org/10.1111/nyas.13368

262. Yan, X., Murphy, B. T., Hammond, G. B., Vinson, J. A., & Neto, C. C. (2002). Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *Journal of Agricultural and Food Chemistry*, *50*(21), 5844–5849. https://doi.org/10.1021/jf0202234

263. Yang, Z. F., Bai, L. P., Huang, W. B., Li, X. Z., Zhao, S. S., Zhong, N. S., & Jiang, Z. H. (2014). Comparison of in vitro antiviral activity of tea polyphenols against influenza A and B viruses and structure-activity relationship analysis. *Fitoterapia*, *93*, 47–53. https://doi.org/10.1016/j.fitote.2013.12.011

264. Youdim, K. A., McDonald, J., Kalt, W., & Joseph, J. A. (2002). Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *Journal of Nutritional Biochemistry*, *13*(5), 282–288. https://doi.org/10.1016/S0955-2863(01)00221-2

265. Yun, S. I., & Lee, Y. M. (2017). Zika virus: An emerging flavivirus. *Journal of Microbiology*, *55*(3), 204–219. https://doi.org/10.1007/s12275-017-7063-6

266. Zeb, A., & Murkovic, M. (2010). Analysis of triacylglycerols in refined edible oils by isocratic HPLC-ESI-MS. *European Journal of Lipid Science and Technology*, *112*(8), 844–851. https://doi.org/10.1002/ejlt.201000064

267. Zhang, J., Chen, D., Chen, X., Kilmartin, P., & Quek, S. Y. (2019). The influence of vinification methods and cultivars on the volatile and phenolic profiles of fermented alcoholic beverages from cranberry. *Antioxidants*, *8*(144), 1–19. https://doi.org/10.3390/antiox8050144

268. Zhang, Yanjun, Vareed, S. K., & Nair, M. G. (2005). Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. *Life Sciences*, *76*(13), 1465–1472. https://doi.org/10.1016/j.lfs.2004.08.025

269. Zhang, Yuqing, Chen, A. Y., Li, M., Chen, C., & Yao, Q. (2008). Ginkgo biloba extract kaempferol inhibits cell proliferation and induces apoptosis in pancreatic cancer cells. *Journal of Surgical Research*, *148*(1), 17–23. https://doi.org/10.1016/j.jss.2008.02.036

270. Zhao, C., Giusti, M. M., Malik, M., Moyer, M. P., & Magnuson, B. A. (2004). Effects

of commercial anthocyanin-rich on colonic cancer and nontumorigenic colonic cell growth. *Journal of Agricultural and Food Chemistry*, *52*(20), 6122–6128. https://doi.org/10.1021/jf049517a

271. Zheng, W., & Wang, S. Y. (2003). Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *Journal of Agricultural and Food Chemistry*, *51*(2), 502–509. https://doi.org/10.1021/jf020728u

272. Zheng, Z., & Shetty, K. (2000). Solid-state bioconversion of phenolics from cranberry pomace and role of *Lentinus edodes* β-glucosidase. *Journal of Agricultural and Food Chemistry*, *48*(3), 895–900. https://doi.org/10.1021/jf990972u

273. Zhu, J., Chen, F., Wang, L., Niu, Y., Chen, H., Wang, H., & Xiao, Z. (2016). Characterization of the key aroma volatile compounds in cranberry (*Vaccinium macrocarpon Ait*.) using gas chromatography-olfactometry (GC-O) and odor activity value (OAV). *Journal of Agricultural and Food Chemistry*, *64*(24), 4990–4999. https://doi.org/10.1021/acs.jafc.6b01150

274. Zuo, Y., Wang, C., & Zhan, J. (2002). Separation, characterization, and quantitation of benzoic and phenolic antioxidants in American cranberry fruit by GC-MS. *Journal of Agricultural and Food Chemistry*, *50*(13), 3789–3794. https://doi.org/10.1021/jf020055f

275. Zymone, K., Raudone, L., Raudonis, R., Marksa, M., Ivanauskas, L., & Janulis, V. (2018). Phytochemical profiling of fruit powders of twenty *Sorbus* L. cultivars. *Molecules*, *23*(10), 1–17. https://doi.org/10.3390/molecules23102593

LIST OF PUBLICATIONS ON THE TOPIC OF DISSERTATION

List of scientific publications on the topic of the dissertation published in the Clarivate Analytics WOS database:

1. **Tamkutė, Laura**; Pukalskas, Audrius; Syrpas, Michail; Urbonavičienė, Dalia; Viškelis, Pranas; Venskutonis, Petras Rimantas. Fractionation of cranberry pomace lipids by supercritical carbon dioxide extraction and on-line separation of extracts at low temperatures // Journal of Supercritical Fluids. Amsterdam: Elsevier, 2020. (I.F. 3.744 (2019)).

2. **Tamkutė, Laura**; Liepuoniūtė, Rūta; Pukalskienė, Milda; Venskutonis, Petras Rimantas. Recovery of valuable lipophilic and polyphenolic fractions from cranberry pomace by consecutive supercritical CO_2 and pressurized liquid extraction // Journal of Supercritical Fluids. Amsterdam: Elsevier, 2020, vol. 159, pp. 1–11. ISSN: 0896-8446. (I.F. 3.744 (2019)).

3. **Tamkutė, Laura**; Melero Gil, Beatriz; Rovira Carballido, Jord; Pukalskienė, Milda; Venskutonis, Petras Rimantas. Effect of cranberry pomace extracts isolated by pressurized ethanol and water on the inhibition of food pathogenic/spoilage bacteria and the quality of pork products // Food Research International. Amsterdam: Elsevier. 2019, vol. 120, pp. 38–51. ISSN: 0963-9969. (I.F. 4.972 (2019)).

List of other scientific publications published in the Clarivate Analytics WOS database:

1. Eisinaitė, Viktorija; **Tamkutė, Laura**; Vinauskienė, Rimantė; Leskauskaitė, Daiva. Freeze-dried celery as an indirect source of nitrate in cold-smoked sausages: effect on safety and color formation // LWT – Food Science and Technology. Amsterdam: Elsevier. 2020, vol. 00, pp. 1–10. ISSN: 0023-6438. (I.F. 4.006 (2019)).

2. Kitrytė, Vaida; Kavaliauskaitė, Asta; **Tamkutė, Laura**; Pukalskienė, Milda; Syrpas, Michail; Venskutonis, Petras Rimantas. Zero waste biorefining of lingonberry (*Vaccinium vitis-idaea* L.) pomace into functional ingredients by consecutive high pressure and enzyme assisted extractions with green solvents // Food Chemistry. Oxford: Elsevier. 2020, vol. 322, pp. 1–8. ISSN: 0308-8146. (I.F. 6.306 (2019)).

3. Bobinaitė, Ramunė; Kraujalis, Paulius; **Tamkutė, Laura**; Urbonavičienė, Dalia; Viškelis, Pranas; Venskutonis, Petras Rimantas. Recovery of bioactive substances from rowanberry pomace by consecutive extraction with supercritical carbon dioxide and pressurized solvents // Journal of Industrial and Engineering Chemistry. New York: Elsevier. 2020, vol. 85, pp. 152–160. ISSN: 1226-086X. (I.F. 5.278 (2019)).

4. Kitrytė, Vaida; Narkevičiūtė, Aistė; **Tamkutė, Laura**; Syrpas, Michail; Pukalskienė, Milda; Venskutonis, Petras Rimantas. Consecutive high-pressure and enzyme assisted fractionation of blackberry (*Rubus fruticosus* L.) pomace into functional ingredients: process optimization and product characterization // Food Chemistry. Oxford: Elsevier. 2020, vol. 312, pp. 1–11. ISSN: 0308-8146. (I.F. 6.306 (2019)).

5. Raudienė, Edita; Gailius, Darius; Vinauskienė, Rimantė; Eisinaitė, Viktorija; Balčiūnas, Gintautas; Dobilienė, Justina; **Tamkutė, Laura**. Rapid evaluation of fresh chicken meat quality by electronic noise // Czech Journal of Food Sciences. Prague: Czech Academy of Agricultural Sciences. 2018, vol. 36, iss. 5, pp. 420–426. ISSN: 1212-1800. (I.F. 0.932 (2019)).

List of presentations in international conferences on the dissertation topic

1. **Tamkutė, Laura**; Melero Gil, Beatriz; Rovira Carballido, Jordi; Pukalskienė, Milda; Venskutonis, Petras Rimantas. Defatted cranberries pomace extract improves the quality of pork burgers // 13th Baltic conference on food science and technology "Food. Nutrition. Well-Being" and NEEFOOD 2019 5th North and East European congress on food, May 2–3, 2019, Jelgava, Latvia. Abstract book/ Faculty of Food Technology. Jelgava: Latvia University of Life Sciences and Technologies, 2019, p. 132. ISBN: 978-998-44-8317-7.

2. Venskutonis, Petras Rimantas; **Tamkutė, Laura**; Liepuoniūtė, Rūta. Biorefining of cranberry pomace into valuable fractions by supercritical carbon dioxide and pressurized liquid extraction // 17th European meeting on supercritical fluids. 7th European meeting high pressure technology, April 8–11, 2019, Ciudad Real, Spain. Abstract book/ Institute of Chemical and Environmental Technology (ITQUIMA). Ciudad Real: Universidad de Castilla-La Mancha, 2019. p. 260–261. ISBN: 978-840-91-0484-0.

3. **Tamkutė, Laura**; Melero Gil, Beatriz; Rovira Carballido, Jordi; Venskutonis, Petras Rimantas. Recovery of bioactive fractions from cranberry pomace and their use for increasing functional properties of meat products // International conference and exhibition on nutraceutical and functional foods (ISNFF-2018), October 14–17, 2018, Vancouver, BC, Canada. Vancouver: ISNFF, 2018, p. 43.

4. **Tamkutė, Laura**; Melero Gil, Beatriz; Rovira Carballido, Jordi; Venskutonis, Petras Rimantas. Effect of ethanolic extracts of cranberry pomace isolated by higher pressure extraction on the quality and shelf life of pork hamburgers // 5th international ISEKI Food conference "The food system approach: new challenges for education, research and industry", July 3–5, 2018, Stuttgart, Germany. Abstract book/ University of Hohenheim. Vienna: ISEKI Food Association, 2018, p. 347. ISBN: 978-390-09-3234-3.

5. **Tamkutė, Laura**; Melero Gil, Beatriz; Rovira Carballido, Jordi; Venskutonis, Petras Rimantas. Effect of ethanolic extracts of cranberry pomace on pork hamburgers // 12th Baltic conference on food science and technology "Food R&D in the Baltics and beyond" (FoodBalt-2018), May 17–18, 2018, Kaunas, Lithuania. Abstract book/ Department of Food Science and Technology. Kaunas: Kaunas University of Technology, 2018, p. 51. eISBN: 978-609-02-1462-6.

6. **Tamkutė, Laura**; Liepuoniūtė, Rūta; Venskutonis, Petras Rimantas. Optimization of supercritical fluid and pressurized liquid extraction of cranberries pomace using response surface methodology // 12th Baltic conference on food science and technology "Food R&D in the Baltics and beyond" (FoodBalt-2018), May 17–18, 2018, Kaunas, Lithuania. Abstract book/ Department of Food Science

and Technology. Kaunas: Kaunas University of Technology, 2018, p. 61. eISBN: 978-609-02-1462-6.

7. **Tamkutė, Laura**; Venskutonis, Petras Rimantas. Optimization of supercritical fluid extraction of cranberries pomaces using response surface methodology // 4th North and East European congress on food (NEEFood 2017), September 10–13, 2017, Kaunas, Lithuania. Abstract book/ Department of Food Science and Technology. Kaunas: Kaunas University of Technology, 2017, p. 52. ISBN: 978-609-02-1373-5.

Other presentations

1. **Tamkutė, Laura**; Venskutonis, Petras Rimantas; Liepuoniūtė, Rūta. Can cranberry pomace extract mitigate the carcinogenicity of processed meat? // "Technorama 2019": from vision to innovation!, May 9, 2019. Kaunas, Lithuania. Innovation catalogue/ Santaka Valley. Kaunas: Kaunas University of Technology, 2019, p. 6.

2. **Tamkutė, Laura**; Melero Gil, Beatriz; Rovira Carballido, Jordi; Venskutonis, Petras Rimantas. Defatted cranberry pomace extract increases safety and antioxdant potential of pork burgers during in vitro gastrointestinal digestion // Chemistry and chemical technology: Lithuanian chemists conference, May 16, 2019, Vilnius, Lithuania. Conference book/ Lithuanian Academy of Sciences. Vilnius: Vilnius University, 2019, p. 127. eISBN: 978-609-07-0167-6.

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