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COMPARISON OF ANTHOCYANINS EXTRACTED WITH DIFFERENT SOLVENTS AND METHODS IN SELECTED BERRY FRUITS WITH AN AGRO-INDUSTRIAL POTENTIAL

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INTRODUCTION

Studies in anthocyanins and other naturally occurring pigments have made progress in the last years providing a valuable information about many classes of naturally occurring dietary phytochemicals present in foods. Their abundance in many red, purple, and blue color fruits has boosted their application as natural colorants in food, as well showing to be important to food quality and having possible health benefits due to their strong antioxidant activity.

Albania is placed in the Mediterranean Basin, offering enormous opportunities to grow many fruit crops, which have found spontaneous and wild forms of blackberries, raspberries (*Rubus*), strawberries (*Fragaria*) and vacciniums, and are part of a natural ecosystems (Kullaj et al. 2012).

The term “red fruit” or “berry” is used to name the small fruits, sweet or bitter, juicy and intensely colored (usually red, purple or blue) that grow in wild bushes, can be eaten whole, and lack objectionable seeds. The most well-known red fruits are strawberry, raspberry, blueberry, blackberry, and cranberry. Berries, have recently attracted a lot of attention for their antioxidant properties, which are related to the high concentration of polyphenols present in them. In addition, their consumption worldwide has notoriously increased, and red fruits are nowadays not only consumed fresh but also used in cosmetics and dietary supplements (Hidalgo and Almajano 2017). Berry fruits are defined as functional food because of their protective and enhancing effects on health, affected by their various bioactive components, mainly high amounts of anthocyanins, which are water-soluble plant pigments responsible for the blue, purple, and red color of many plant tissues (Galván et al. 2014).

Anthocyanins are common components of the human diet, founded in many foods and especially in berries while ellagitannins and anthocyanins are the most abundant phytochemicals in raspberries. There is increasing interest in the anthocyanin content of foods and nutraceuticals because of possible health benefits.

MATERIALS AND METHODS

This paper aims to compare the anthocyanins content in selected berry fruits such as e blueberry, blackberry, raspberry and strawberry, their extraction affected by different solvents: methanol, ethanol, acetone different ratios 60 & 80 % (solvent/water v/v), and water, and the method of extraction (maceration, ultrasound assisted or not).

For this study were selected berry fruits blueberry (*Vaccinium spp.*), blackberry (*Rubus spp.*), red raspberry (*Rubus idaeus*), and strawberry (*Fragaria ananassa*), collected randomly in 2021 in Tirana market in Albania, and immediately transported to the laboratory for further analyzation. Fruits were pre-selected with uniform maturity, shape, size, color, and free from defects.

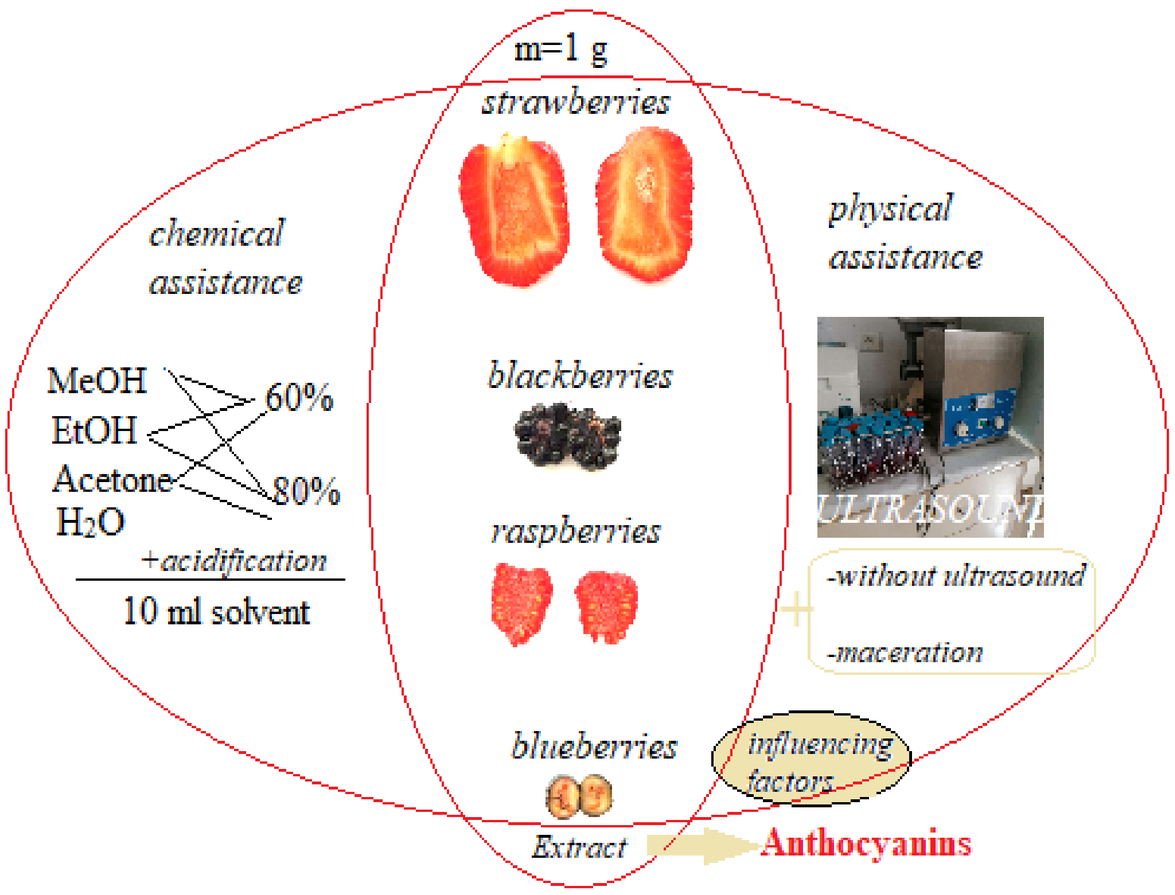

Fig. 1: Scheme of the extraction of anthocyanins from red fruits selected for this study

Extract preparation were performed according to Fig. 1, where 1 (±0.001) g of fruits pure was extracted by using different solvents (10 ml): methanol 60 and 80 % (solvent/water; v/v), ethanol 60 and 80 % (v/v), acetone 60 and 80 % (v/v), and water, all acidulated to enhance extraction, at a 1% amount, by using HCl. Different methods of extraction were applied: a) vortex for 1 min, ultrasound assisted for 15 min (40 KHz) 3500 rmp for 5 min, filtration, supernatant collection; b) same steps without ultrasound assisted; and c) maceration for 1 week at room temperature.

The total anthocyanins content (TAC) was determined using a rapid measurement such as pH differential method (AOAC 2005.02), calculated as cyanidin-3-glucozide (C3G) mg/100 g fresh weight of sample (FW) as it is the most common compound in berries. pH measurements were done using pH meter (Lab 855), standardized with standard buffer solution at pH 4 and pH 7. Color measurement of blended sample were carried out using a portable colorimeter (model NH310) with the CIE L*, a*, b*.

Fruit’s extracts preparation and other parameters determination were done at least in triplicates and presented as Mean ± standard deviation (SD).

RESULTS AND DISCUSSIONS

Result of this study indicate that selected berry fruits are rich in anthocyanins (Fig. 2) containing amounts respectively in blueberry (*Vaccinium spp.*) 29.74-317.65 mg C3G /100 g (FW), blackberry (*Rubus spp.*) 22.82-341.20 mg C3G /100 g (FW), raspberry (*Rubus idaeus*) 12.25-74.33 mg C3G /100 g (FW), and strawberry (*Fragaria ananassa*) 21.25-59.49 mg C3G /100 g (FW). From selected fruits blackberry reached the highest amount, with no significant differences compared to blueberry (7-23% differences in value), while with lesser amounts resulted in strawberry with no significant differences with raspberry (20-42% differences in value), while differences among blackberry, blueberry and strawberry, raspberry are approximately till 80%.

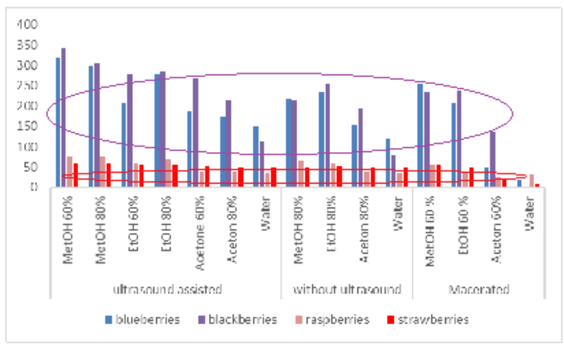

Fig. 2: Comparison of anthocyanins content in selected berry fruits

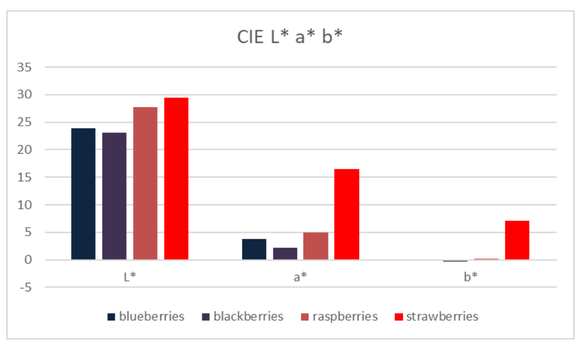

Fig. 4: CIE L* a* b* values of selected berry fruits

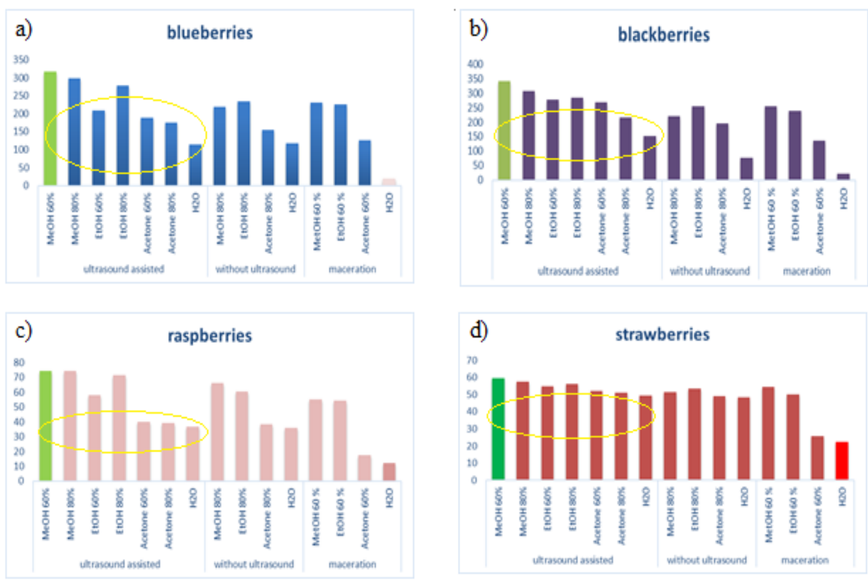

Fig 3: Comparison of extraction method effect on the anthocyanins

From results comparison, may be concluded that extractions performed using acidulated solvents such as methanol, ethanol, acetone, and water (Fig.3 a, b, c, d) affected the anthocyanins extraction by contributing in their content according to the order: ultrasound assisted> without ultrasound> maceration.

Extraction yields of anthocyanins obtained with 60% methanol at room temperatures were about till 28% higher than the yields of extraction using 60% ethanol, till 46 % higher compared to 60 % acetone, and till 67 % compared to water extracts; also, the 60% methanol solvent yielded extraction till 10 % higher compared to 80% methanol. In this study effectiveness of solvents followed the order: MeOH 60%> MeOH 80 %> EtOH 80%> EtOH 60%> acetone 60%> acetone 80%> H2O.

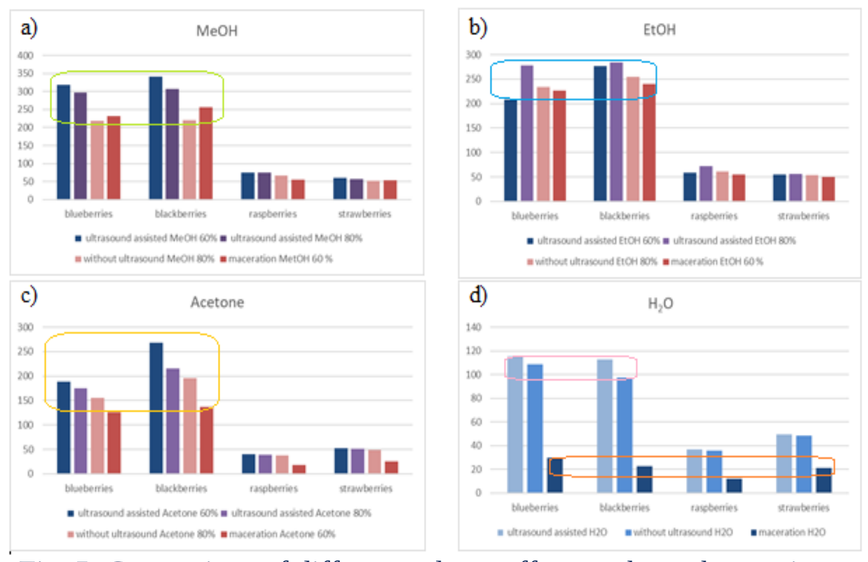

Fig. 5: Comparison of different solvent effect on the anthocyanins

CONCLUSIONS

From selected fruits blackberry reached the highest amount, and was followed the order: blackberries>blueberries>raspberries>strawberries. From the comparison of extraction methods was noted that ultrasound assisted contributed more in the anthocyanin extraction, from 12 % to 28% compared to method without ultrasound assisted, and much more if it is compared to maceration from 27% to 79 %, and the same trend when comparison was made between the solvent extraction without ultrasound assisted and maceration. The extraction method affected anthocyanins by contributing in their content according to the order: ultrasound assisted> without ultrasound> maceration. In this study effectiveness of solvents by contributing anthocyanins content followed the order: MeOH 60%> MeOH 80 %> EtOH 80%> EtOH 60%> acetone 60%> acetone 80%> H2O. From this study may be concluded that selected fruits had very high anthocyanin content, and considering the high content of anthocyanins, blueberry, blackberry, raspberry and strawberry may be utilized as good source of natural colorant with agro-industrial potential, also for food value addition.

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