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Morpho-histological characterization and nutritional properties of prickly pear (*Opuntia ficusindica* L. Mill)

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ABSTRACT

Opuntia ficusindica (L.) is a large genus of succulent shrubs native to Mexico. In 16th century it was introduced into several continents and is now widely grown in the warmer parts of the world. It has been shown that the prickly pear fruit is very rich in vitamins, minerals, amino acids and in sugars. Another important compositional factor of prickly pear is the presence of pigments, such as betalains, which make the fruit and its products particularly attractive. Betalains are vacuolar pigments present in all varieties of reported *O. ficusindica*. Their main function in the plant is to attract animals for pollen transfer facilitating plant propagation and to protect against UV radiation. Two betalain derivatives are present in cactus-pears: betacyanin, which gives the red-purple colour, and betaxanthin, which gives a yellow-orange colour. These pigments have important antioxidant activities without toxic effects to humans. Given the considerable interest aroused by this species, the aim of our research was to study the morphological and cyto-histological characteristics of the fruit from two different varieties of the prickly pear typical of Sicily (Italy):" *Sanguigna*" and "*Muscaredda*" or "*Sciannarina*".Some functional compounds of prickly pear such as the betalains, the carotenoids and chlorophyll (*a* and *b*) were also quantified. There were no cyto-histological differences between the fruits of the two considered varieties; instead the content of betacyanins and chlorophyll was very different.

Keywords : *Opuntia ficusindica*, fruit, betacyanin, chlorophyll

Prickly pear *(Opuntia ficusindica* L.) is a member of the *Cactaceae* family, native to Mexico; in 16th century it was introduced into several continents and is now widely distributed in Mexico, much of Latin America, South Africa, and the Mediterranean area. In Italy and Israel it is planted for fruit consumption; it is also used for ornamental purposes, windbreaks, land reclamation and rehabilitation and erosion control. In Italy, 90% of prickly pear cultivation is in Sicily, which ranks second in the world for producing and exporting prickly pear fruit.In Sicily there are three main production centers, two of which have been awarded the D.O.P. trademark by the European Community.

The nutritional properties of the fresh stems (cladodes) have long been known, and prickly pear is also used in traditional medicine for its hypoglycemic and hypolipidemic effects (Frati *et al.* 1990, Hegwood 1990, Ferdandez *et al.* 1992).

In the industrialized countries of the Mediterranean area, cladodes are not a usual nutritional source for humans, but the fruits are largely consumed. Several researchers have shown that the Prickly pear fruit is very rich in vitamins, minerals, amino acids and sugars (Yahia & Mondragon-Jacobo 2011). It is used as a foodstuff, for medical useand cosmetics.

An important compositional factor of prickly pear is the presence of pigments, such as betalains, which make the fruit and its products particularly attractive. Betalains are vacuolar pigments present in all varieties of reported *O. ficus indica.* Their main function in the plant is to attract of animals for pollen transfer, facilitating, plant propagation and to protect against UV radiation damage. They may function also as osmolytes to support physiological processes, to stabilize subcellular structures, and to reduce nitrogen toxicity and they are an excellent radical scavenger (Chauhan *et al.* 2013).

The betalains functionally replace anthocyanins in 13 taxons grouped in the *Caryophyllales* order. These pigments have important antioxidant activities (Yahia & Castellanos-Santiago 2008) without toxic effects to humans. The major advantages of betalains as dietary antioxidants are their bioavailability, which is greater than most flavonoids, and their superior stability in comparison to anthocyanin.

The antioxidant properties of the phenolic compounds in cactus pear plants make them an important product for protecting human beings against degenerative diseases.There are increasing concerns and recommendations for consumers to use natural antioxidants from plant sources since the use of synthetic antioxidants has been restricted because some of them have been found to be toxic and carcinogenic. In fact, in many epidemiological studies, the frequent consumption of fruit and vegetables high in natural antioxidants has been reported to lower the incidence of certain types of cardiovascular diseases, diabetes and cancer (Namiki 1990, Naveen 2011, Abd El-Razekand Hassan 2011, Laura *et al.* 2012, Prasad *et al.* 2013; Yeddes *et al.* 2013).

The color of prickly pear fruits varies due to the combination of two betalain pigments: betacyanin, which gives the red-purple color, and betaxanthin, which gives a yellow-orange color. The yellow cultivar is the main cultivar in Sicily, for almost 90% of the plantations, while the red and the white cultivars account for 10% and 2% of the plantations, respectively. However, cactus pear diversity and variability in different parts of the world is very large, and so is the diversity in the contents of the fruit. Variability is expressed in all parts of the plant, including the fruit. Features like skin and pulp color, pulp texture, sugar content, and juice acidity are directly related to the presence, intensity and activity of nutritional and functional compounds

Given the considerable interest aroused by this species, the aim of our research was to study the morphological, cyto-histological and physiological characteristics of the fruits from two different varieties of prickly pear,"*Sanguigna*" (known as 'Red') and "*Muscaredda*" or "*Sciannarina*" (known as 'white'), from San Cono (Sicily, Italy). Some functional compounds of prickly pear such as the betalains, carotenoids and chlorophyll (*a* and *b*) were also quantified.

MATERIALS & METHODS

Plant material-Fully ripe prickly pear fruit from two different varieties, "*Sanguigna*", also known as 'Red',and "*Muscaredda*" or "Sciannarina", also known as 'white', were collected from the private farms in San Cono, Sicily, Italy. The fruits were stored at 4°C from harvest to sample preparation.

Cyto-histological observations-For the cyto-histological observations, portions of the fruits and stemswere fixed in 3% (w/v) glutaraldehyde in 0.075 M phosphate buffer at pH 7.2 for 5h. The samples were then washed four times for 15 min each in 0.075 M phosphate buffer at pH 7.2, post-fixed in 1% (w/v) OsO_4, dehydrated in increasing concentrations of ethanol and, finally, embedded in resin (Epon, 2-dodecenylsuccinic anhydride, and methylnadic anhydride mixture) (Reale et al. 2014). The semi-thin sections (1–2 μm of thickness) were cut with an ultramicrotome (OmU2, Reichert, Heidelberg) equipped with a glass blade. Semi-thin sections of cladodes were stained with 0.5% (w/v) toluidine blue in 0.2 % $NaHCO_3$ bufferand observed under a light microscope (BX53, Olympus, Tokyo, Japan); semi-thin sections of fruits, instead, were stained with 0.025% (w/v) toluidine blue in 0.1 M citrate buffer pH4 to put in evidence the presence of mucilages.

Quantification of betalains- The fruits were washed for 2 min under tap water and the peel was removed manually. Both pulp (without seeds) and peel were cut into small pieces, ground together and filtered. Betacyanins and betaxanthins were extracted from the pulp and peel according to Castellar *et al.* (2003) and Stintzing *et al.* (2005); results are reported in mg equivalents of betacyanins/g fresh matter and mg equivalents of betaxanthins/g fresh matter. Betacyanins were detected at 535 nm and betaxanthins at 484 nm, and their concentration were calculated according to the following equation:

Betacyanins or betaxanthins content [mg/L] = [(A × DF × MW × 1000/Î × l)] where: A = absorbance at 535 or 480 nm, DF = dilution factor, MW = molecular weight, Î = extinction coefficient, l = width of the spectrophotometer cell (1 cm).

For betacyanin the extinction coefficient is 60,000 L/(mol cm) and MW = 550 g/mol.

For betaxanthins the extinction coefficient is 48,000 L/(mol cm) and MW = 308 g/mol.

***Photosynthetic pigment analysis*—**The fruits were washed for 2 min under tap water and the peel was removed manually. Both, pulp (without seeds) and peel were cut into small pieces, ground separately and extracted with 80% v/v acetone. Extracts were maintained at 4 °C until analyses and all manipulations had been performed in dim green safe light to avoid photo-degradation. Absorption spectra (400–750 nm range) of extracts were recorded at room temperature (25 °C) with the spectrophotometer. For Chlorophyll (Chls) and carotenoid determinations, the extracts were measured at 661 nm (Chl a), 644 nm (Chl b), and 470 nm (carotenoids) and pigment concentrations were determined according to the equations proposed by Wellburn (1994) and are reported as mg/g of fresh substance.

Statistical analysis-Data were analysed statistically using analysis of variance (ANOVA); Duncan's ($P<0.05$) multiple comparison tests were used to compare the chlorophyll and carotenoid contents in the two varieties, when the ANOVA ($P<0.05$) results were statisticallysignificant. Instead to compare the betalains content, the T-test was instead used.

RESULTS & DISCUSSION

Plant sources including grains, vegetables, fruit and medicinal plants have received increasing attention for their potential role in the prevention of human diseases. Recently, there has been considerable increase in the interest to find naturally occurring antioxidants to use in foods, cosmetics, or medicinal materials in order to replace synthetic antioxidants, whose use is being restricted due to their carcinogenicity (Abdel-Hameed 2009, Nurliyana *et al*. 2010, Abdel-Hameed *et al*. 2013).

Prickly pear cactus is a good source of antioxidant and a promising alternative for the food industry. Many reports concerning the importanceof this plant all over the world (Felker *et al.* 2005, Talia *et al.* 2005, El-Samahy *et al.* 2006) encouraged the authors to study two prickly pear cactus cultivars growing in San Cono, Italy, in regard to some cyto-histological and physiological characteristics.

***Cyto-histological observations*—**In both varieties, tangential fresh sections of stem showed anomocytic sunken stomata (Fig. 1A). In transversal sections, a layer of epidermal cells covered with cutin and wax was observed (Fig. 1B, C, D); below this layer there was a mechanical ipoderm, constituted by two/three layers of sub-epidermal cells with a thick cell wall. The underlying parenchyma was characterized by the presence of chloroplasts, above all in the more external portion. In almost all epidermal cells and in a few parenchymatic cells, crystals of calcium oxalate were also observed; they are clearly visible as striking crystal rosettes (Fig. 1D). Calcium plays an important role in water retention in succulent tissues to regulate the osmotic pressure in the cells andit has been shown that oxalate crystal size increases in relation to maturation (Rectamal *et al.* 1987). There were no differences in the cladode structure of the"*Sanguigna*" and "*Muscaredda*" varieties.

Prickly pear fruits consists of an edible portion, which develops from the ovary, and a non-edible peel portion that develops from the dicotyledonous stem. This non-edible peel portion is composed of aparenchyma, a green chlorenchyma and an overlying epidermis. The epidermal layer is green in the "*Muscaredda*" fruit and partially red in the "*Sanguigna*" fruit; this red color normally occurs on the side of the cactus fruit that faces outwards from the plant towards the sun (Fig. 2A). Felker *et al.* (2008) suggested that the accumulation of pigment observed in the epidermis of cactus pear is similar to that in grape. As the grape matures and the sugar concentration increases, anthocyanin production also increases. Similarly, in cactus pear, the peel becomes pigmented when the sugar concentration is maximal and the epidermis becomes pigmented as a result of light exposure. In both varieties, the color of the parenchyma is similar to the edible portion (Fig. 2B): in "*Sanguigna*" fruit the color of the parenchyma is red as is the edible portion, while in "*Muscaredda*" all tissues are white/green. The external portion of the fruit appeared very similar to the stem, with the epidermal layer coated with cutin and waxes, and the mechanical ipoderm (Fig. 3A, B). The peripheral

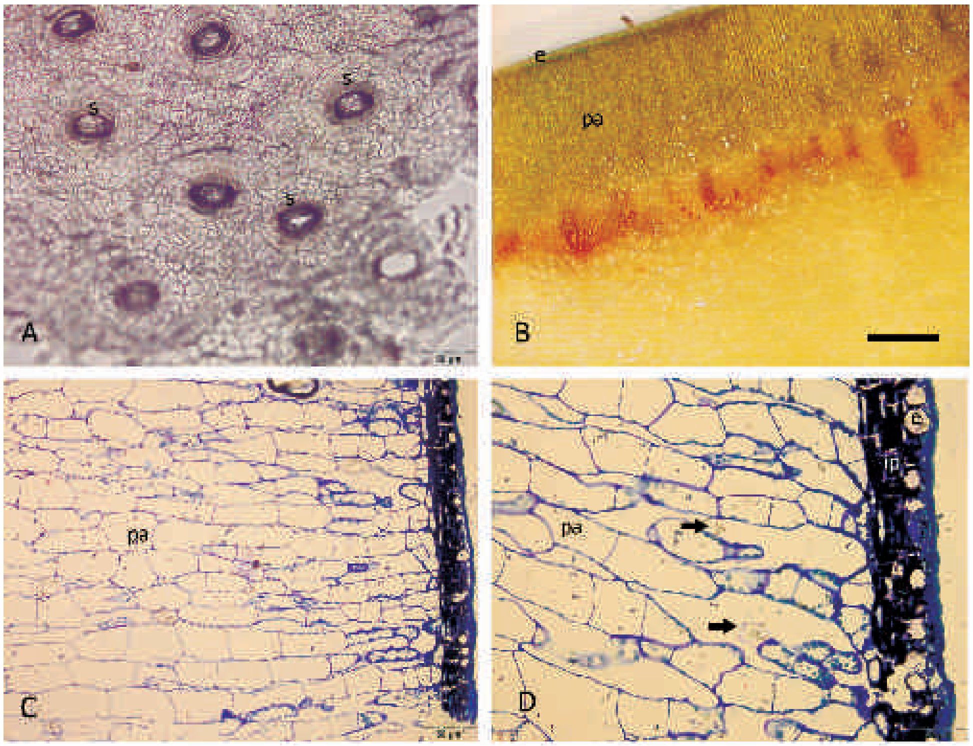

Fig. 1— *Opuntia* stem: **A.** Tangential fresh section of epidermis without staining, anomocytic sunken stomata (s) are evident; **B.** Transversal fresh section of a stem, the epidermis can be seen (e) and the chlorophyll parenchyma (pa); **C-D.** Transversal semi-thin sections stained with toluidine blue, the epidermis can be seen (e) covered with cutin and waxes, the mechanical ipoderm (ip) and the parenchyma (pa) with chloroplast in the external portion, crystals of calcium oxalate are also evident (black arrows).

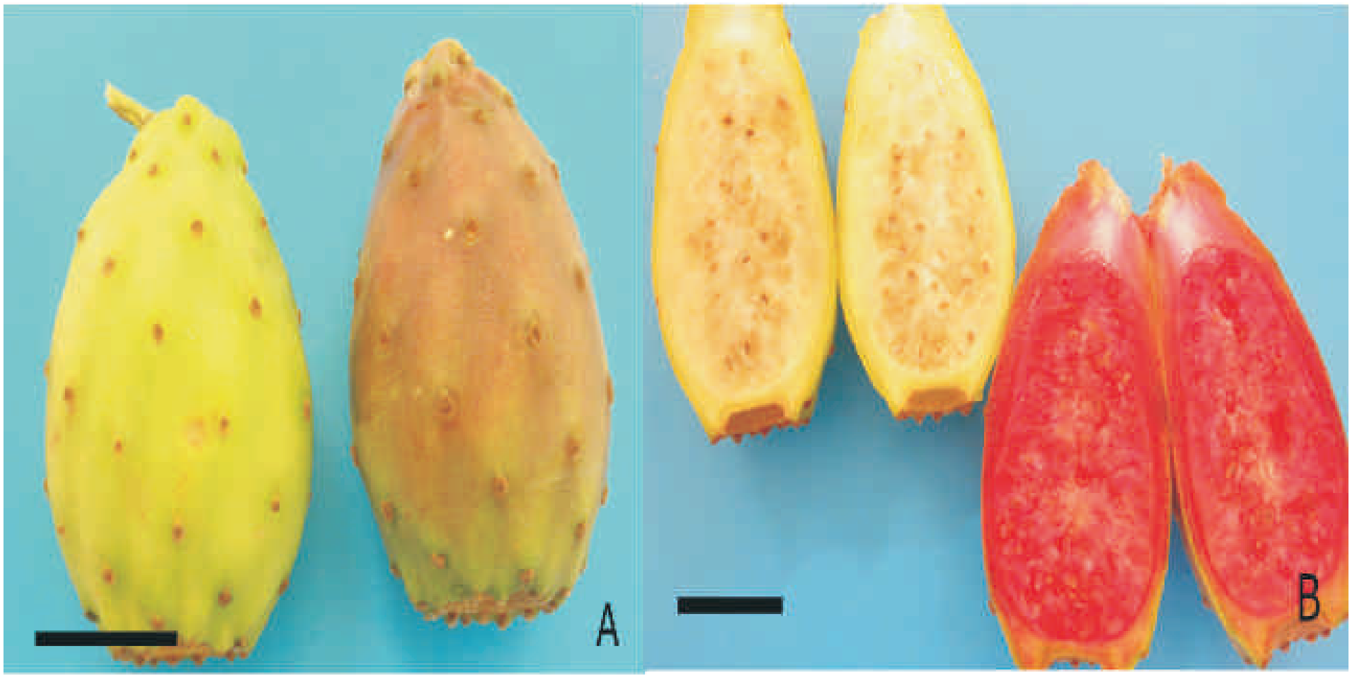

Fig. 2— A. Fruit "*Muscaredda*" (on the left side) and "*Sanguigna*" (on the right side) B. cut in half.

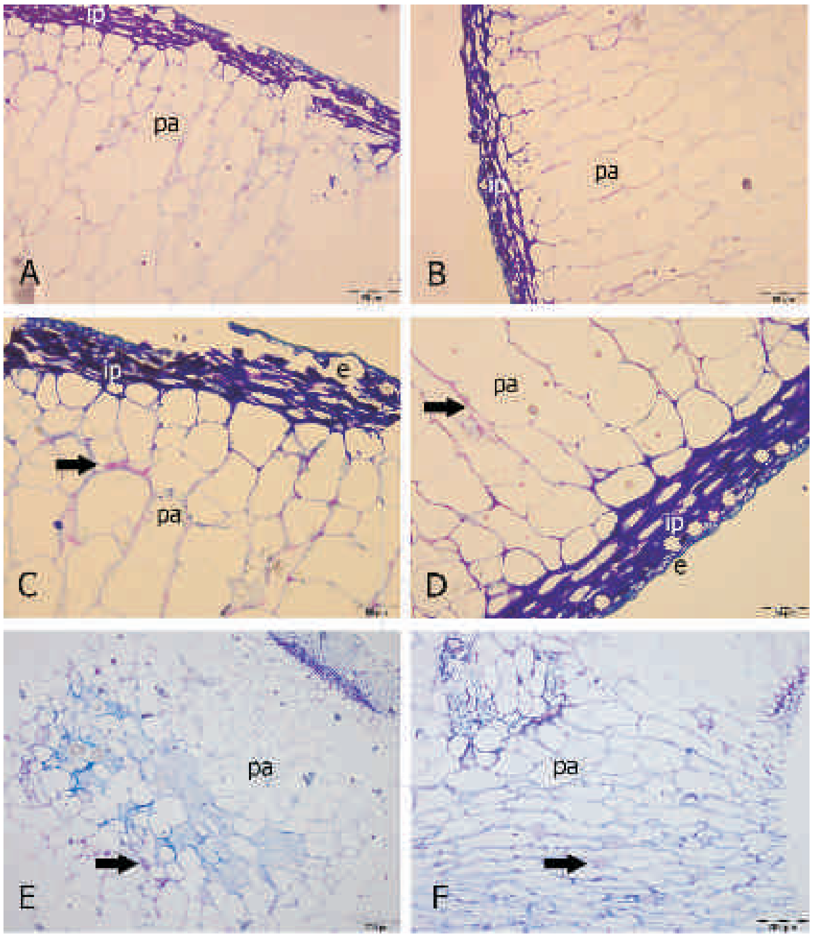

Fig. 3—A, C, E-Semi-thin sections of "*Sanguigna*" **B, D,** F- "*Muscaredda*" fruits stained with toluidine blue O. (epidermis (e), the mechanical ipoderm (ip) and the parenchyma with chloroplast (pa) are evident; mucilages (black arrows) accumulated in the intercellular spaces can also be seen. In the internal portion **E, F** parenchymatic cells were bigger and with more intercellular spaces; mucilages are also present also in this portion.

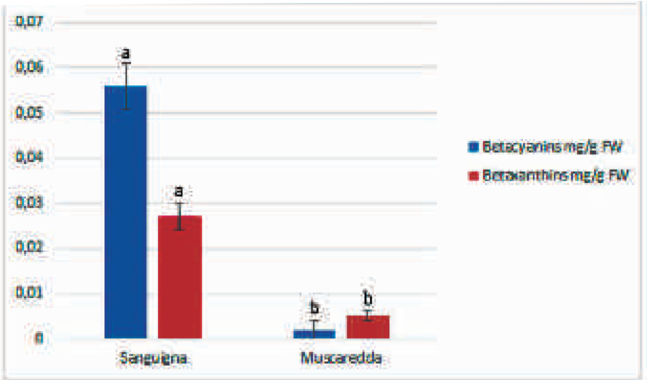

Fig. 4—Graph showing the Chlorophyll *a* and *b* and carotenoids contents of "*Sanguigna*" and "*Muscaredda*" fruits. Different letters (a-d) in the columns with the same color indicate significant differences between means (P<0.05).

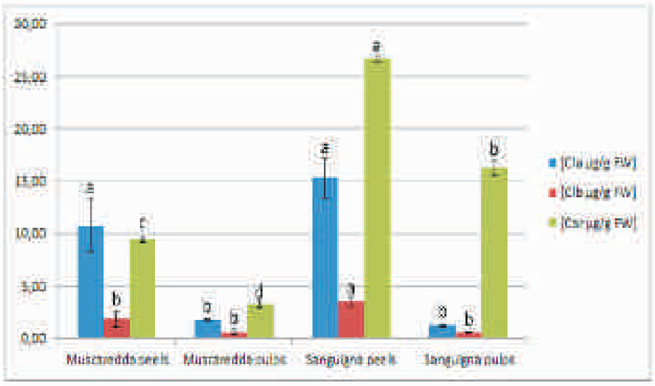

Fig. 5—Betacyanin and betaxanthin contents of "*Sanguigna*" and "*Muscaredda*" fruit. Different letters (a-d) in the columns with the same color indicate significant differences between means (P<0.05).

portion of the mesophyll was constituted by parenchymatic cells very adpressed with few intercellular spaces (Fig. 3C, D). In the internal portion, the parenchymatic cells were bigger than those in the peripheral portion with more intercellular spaces (Fig. 3E, F). In the internal and external portion of the parenchyma, mucilage accumulated in intercellular spaces. The physiological role of the plant mucilage is to regulate the cellular water content during prolonged drought and to regulate calcium fluxes of the plant. Mucilage from *Opuntia ficus-indica* is an interesting ingredient for the food industry because of its viscosity (Sepulveda *et al*., 2007). It can create gels that retain a great quantity of water (Medina-Torres *et al.* 2003, Saenz *et al*. 2004) and it also has good emulsifying properties (Medina-Torres *et al*. 2003, Sáenz *et al*. 2004, Bernardino-Nicanor *et al*. 2013) for use in the production of edible films (Del-Valle *et al*. 2005, Espino-Díaz *et al*. 2010) and it can be used to encapsulate both *Saccharomyces boulardii* (Zamora-Vega *et al*. 2012) and gallic acid (Medina-Torres *et al*. 2013). As in the cladodes, there were also crystals of calcium oxalate in the fruits. There were no differences between "*Sanguigna*" (Fig. 3A, C, E) and "*Muscaredda*" (Fig. 3B, D, F) varieties.

Pigments characterization– Chlorophyll *a* and *b* and carotenoids were extracted from the pulp and peel; all these pigments were more abundant in the peel than in the pulp (Fig. 3). Chlorophyll *a* was much higher than Chlorophyll *b* in both varieties, as also in observed Mexican cactus pear (Yahia & Mondragon-Jacobo 2011), but unlike the Mexican varieties, which have relatively low amounts of total carotenoids, carotenoids in "*Sanguigna*" and "*Muscaredda*" were generally much higher than Chlorophyll *a* and *b.* The only exception was the carotenoid content in "*Muscaredda*" peels, which was similar to the amount of Chlorophyll *a*. In the peels, Chlorophyll *a* was more abundant in "*Sanguigna*" than in "*Muscaredda*", while in pulp it was higher in "*Muscaredda*", but the differences were not statistically significant. Carotenoids were higher in "*Sanguigna*" than in" *Muscaredda*", both in the pulp and peels.

The pigments of *Opuntia ficus-indica* fruit, which are derived from betalain rather than from the anthocyanin pathway, have an extraordinary range of color from lime green, orange, red to purple. This is a result of the varying concentrations and proportions of about half a dozen betaxanthins and betacyanins. The yellow-orange betaxanthins are derived from the spontaneous condensation of betalamic acid with amines or amino acids. The reddish-purple betacyanins are enzymatically formed from betalamic acid and cyclo-dihydroxyphenylalanine (DOPA) yielding betanidin and further glycosylated on either of the two hydroxyls of the cyclo-DOPA moiety.

Betacyanins and Betaxanthins were extracted from the peel and pulp of "*Sanguigna*" (red fruits) and "*Muscaredda*"(white fruits) and their concentration were determined; Felker *et al.* (2008) did not find a purple-fruited variety that has betacyanins but no betaxanthins, or a yellow-fruited variety without betacyanins. In contrast, in beets, there are cultivars with only yellow pigments and the fruit of purple pitaya are known to show only traces of betaxanthins (Stintzing & Carle 2008).We observed that betacyanins and betaxanthins were higher in "*Sanguigna*" than in "*Muscaredda*"; in particular the betacyanins, which give the red-purple color, were the highest in "*Sanguigna*".

CONCLUSION

Although "*Sanguigna*" and "*Muscaredda*" have different components (especially in pigment types and contents), they are both characterized by important nutritional and health components. However, "*Sanguigna*" can be considered as having better nutritional characteristics compared to "*Muscaredda*"; "*Sanguigna*" had the highest amount of betalains and carotenoids, which have an important antioxidant activity. These results may be important to postharvest physiologists and technologists, nutritionists, marketing authorities and consumers in general as well as plant breeders.

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