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REGULAR ARTICLE

Vitamin C content in leaves and roots of horseradish (*Armoracia rusticana*): Seasonal variation in fresh tissues and retention as affected by storage conditions

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ABSTRACT

Since antiquity, horseradish is known as folk medicinal herb and food condiment. The richness in phytochemicals has recently encouraged its use in the medical field and as functional food. In this study, vitamin C content by HPLC analysis in young and mature leaves and roots of two horseradish accessions collected at different developmental stages was evaluated. The effect of freezing and freeze-drying on vitamin C loss after different storage time was also analyzed. The vitamin C content varied in fresh tissues depending on accessions and developmental stages. Roots contained the highest values of vitamin C when plant approximated to senescence (about 80 mg 100 g^{-1} fw). A great content of vitamin C was found in young leaves during the full developmental stages (up to 350 mg 100 g^{-1} fw) and in mature ones toward plant senescence (up to 280 mg 100 g^{-1} fw). By freezing tissues at −20°C, a monthly vitamin C loss of 5% was estimated, while by freezing at −80°C the losses did not exceed 6% after 8 months of storage. By freeze-drying tissues, a loss of vitamin C of about 40% was found after 4 months of storage at 4°C. Despite the losses associated with food processing, the residual content of vitamin C in horseradish remains still higher compared to other vegetables.

Keywords: Biologically active compounds; *Brassicaceae*; Horseradish; Food preservation; L-Ascorbic acid

INTRODUCTION

Over the last decades, the plant-derived bioactive components (e.g. glucosinolates, polyphenols, vitamins) are gaining interest due to the wide range of clinic and pharmacological properties, as to be of fundamental importance for human nutrition and health (Björkman et al., 2011; Sayeed et al., 2017). In this respect, scientists are focusing on methods and strategies addressed to improve the content of health-affecting compounds of plant foods, either in terms of the amounts present in commercial crops (fruits and vegetables) to maximize benefits for production and nutritional quality, or in minimizing losses before consumption during processing and storage (Davey et al., 2000; Galgano et al., 2007). Among the bioactive molecules, of particular interest is vitamin C (L-ascorbic acid), a universal constituent of fruits and vegetables that has an important role in cellular growth, protecting both the plants and mammals from oxidative stress (Davey et al., 2000; Smirnoff and Wheeler 2000).

Besides species and genotype, climate and soil quality and a wide type of environmental stresses and agronomic practices can affect the vitamin C content. After harvest, many other factors influence the spoilage and vitamin C losses as the postharvest handling conditions, processing techniques and storage conditions (Davey et al., 2000; Galgano et al., 2002; Lee and Kader, 2000). In general, fresh fruits and vegetables contain more vitamin C than those stored (Galgano et al., 2007); thus, the vitamin C losses could be controlled within certain limits by using appropriate postharvest procedures to maintain as much as possible the compositional quality in plant foods. The processing techniques applied to food and vegetables, such as canning, freezing, and dehydration, to provide all-year-round availability of these foods can also result in significant losses of vitamin C. The extent of these losses is highly variable; losses of over 50% are typical for vegetables, but are much less for most fruits and in particular acid fruits (Davey et al., 2000).

Among the cruciferous vegetables, that are generally known for higher vitamin C content and sulfur compounds than

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non-cruciferous vegetables, the horseradish (*Armoracia rusticana* P. Gaertner, B. Meyer & Scherbius), a perennial plant known since antiquity as a folk medicinal herb, natural preservative and dish condiment (Wedelsbäck Bladh and Olsson, 2011), is proving to be a rich source of glucosinolates (Agneta et al., 2014; De Maria et al., 2016), phenolics, flavonoids and other constituents, including vitamins (Calabrone et al., 2015; Majewska et al., 2004). Recently the species, approved as seasoning, spice, and flavouring and affirmed as generally recognized as safe by the Food and Drug Administration (FDA, 2008), is gaining an increasing scientific interest due to the richness in the bioactive compounds that, besides their relevance for human health benefits, are promising candidate for innovative applications in different fields (e.g. cancer-protecting component, natural antibacterial, fungicide) (Nguyen et al., 2013). In the historical overview of Wedelsbäck Bladh and Olsson (2011), it has been reported that the species was believed to alleviate various forms of pains or even cure a range of diseases, but it was most known for treatment of scurvy so that to be described as one of the best remedies. Both leaves and roots were used for this purpose by seamen, who did not have access to fresh vegetables and fruits and therefore lacked vitamin C.

Native of the temperate regions of Eastern Europe and western parts of Russia, the species is today popular nearly worldwide; it is cultivated for its white and tapered root that has a mix of intense pungency and cooling taste. Furthermore, fresh root is mainly used grated or processed into a sauce, ideal to accompany roasted or boiled meat. After the harvest, roots are usually stored in cold room to limit the spoilage and loss of its typical turgidity, or preserved in white vinegar, beet juice, processed in sauces or frozen grated (Kosson and Horbowicz, 2008). Moreover, particularly in Germany, also the leaves are consumed, eaten as a vegetable (for example cooked like spinach) and in salads (Agneta et al., 2013; Duke, 2003). Furthermore, the leaves of horseradish are considered to prevent food spoilage processes (Majewska et al., 2004).

To date, although it is often mentioned that horseradish is rich in vitamin C, whose average content can be almost three times higher than in citrus fruits, very limited are the studies available in literature and referred almost exclusively to the roots, and no published data are reported for leaves. Based on what is reported by several authors (Davey et al., 2000; Raghavan, 2007; Tomsone et al., 2013), the vitamin C content in horseradish varies from about 25 to 300 mg 100 g^{-1} fw. In particular, Kosson and Horbowicz (2008) evaluated that in roots the ascorbic acid varied from 90 to 228 mg 100g^{-1} fw, depending on the type, season and place of cultivation.

The present study aimed at 1) evaluating the vitamin C content in both leaves and roots of two horseradish accessions, grown in Mediterranean area and collected at different developmental stages during two growing cycles; 2) evaluating the effect of freezing preservation at −20 and −80 °C and freeze-drying on vitamin C loss.

MATERIALS AND METHODS

Plant material and sample preparation

The experiment was carried out on two accessions of *Armoracia rusticana*, Corleto and Montemurro (named as Cor and Mon), grown in raised beds, located in Potenza, Italy, (PZ, 40°38'43"N - 15°48'33"E, 819 m a.s.l.). Root cuttings (approximately 20 cm in length and 1.5 cm in diameter) obtained from local nurseries were transplanted (50 cm between rows and 30 cm on the row) on March 6, 2013. Irrigation, plant protection and weed control were carried out according to local practices and weather conditions.

Plants were sampled at different developmental stages during two growing cycles (2013 - 2014): beginning and end of July 2013 (D1 and D2, respectively) during the period of maximum vegetative development of the plant; end of September 2013 (D3) at the beginning of the foliage senescence (close to the traditional period of root harvesting); in March 2014 (D4) at the stage of root dormant and devoid of leaves (when the harvest of roots usually ends); in April 2014 (D5) at the beginning of vegetative re-growth (when young leaves are already formed on the crown of the roots left in the field, and a new growing cycle begins); end of May 2014 (D6) during the vegetative development of the plant; in November 2014 (D7) when the leaves are senescent and the harvest of roots starts again. In the two-year experiment, biometric traits of the above-ground tissues (leaf number, leaf area, fresh weight (fw) and plant height) and below-ground tissues (length, diameters and fresh weight of the main root, side root number and weight) were recorded on four plants during the period of vegetative development of the plants (at D1 and D6) and root harvesting (at D3 and D7), respectively. At each sampling data, plants were separated into roots, young leaves (at D1, D2, D5 and D6) and/or mature ones (at D1, D2, D3, D6 and D7), quickly cleaned with distilled water, dried with paper towels, and weighted for fresh weight (fw). Subsamples of each tissue were dried in ventilated oven at 75 °C until steady weight to determine the dry matter weight (dw).

Analytical procedure for vitamin C determination

At each sampling data, fresh samples (roots, young and/or mature leaves) were quickly analyzed for vitamin C content.

Moreover, samples collected at D1 stage were processed using the following methods: freezing at -20 °C (and analyzed after 2 ½, 5, 8 and 12 months of storage) and at -80 °C (and analyzed after 8 months of storage); freeze-drying (72 - 96 hours at temperature of -50 °C and pressure of 0.035 mbar) and analyzed after 3 days and after 2 ½ and 4 months of storage at 4 °C.

Before vitamin C extraction, 20 g of fresh tissue were quickly frozen in liquid nitrogen, and then grounded into fine powder using a laboratory mill, whereas 20 g of frozen samples were directly milled. In order to prevent vitamin degradation and reduce dehydroascorbic acid to ascorbic acid, prior to the grinding step, each sample was added with potassium metabisulfite (0.2%). The procedure was conducted under dim light and amber glassware was used. About 1 g of sample was weighted in a 10 ml volumetric flask and brought up to volume using 20 mM NaH_2PO_4 acidified at pH 2.14 with 1N HCl. The flask was then vortexed 5 times for 30 s, with intervals of 1 min. As regards freeze-dried samples, the powder was added with potassium metabisulfite (0.2%) and about 0.5 g were submitted to vitamin C extraction with the same procedure. Then the extracts were filtered through a 0.2 µm cellulose acetate filter and stored in the dark at −20 °C until analysis. For analytical purposes, two extractions were carried out for each sample. Vitamin C content was assessed by HPLC according to Galgano et al. (2002) (Fig. 1), checking the linearity of the UV detector over a vitamin C concentration ranging from 25.6 to 256.0 µg ml^{-1}. Chemicals of suitable analytical grade and ascorbic acid standard were purchased from Sigma-Aldrich (Milan, Italy).

Statistical analysis

Statistical analysis was performed by using M-STAT software (version 2.00). Variations of vitamin C content in fresh tissues throughout the growing cycles were tested by applying a two-way ANOVA (accession × developmental stage) followed by least significant difference (LSD) to compare the means ($p \leq 0.05$). Losses of vitamin C in tissues frozen at -20 °C and stored for different time intervals, as well as in freeze-drying tissues analyzed after different time of storage at 4 °C, were performed by using multifactorial ANOVA (accession × tissue × time of storage). Similarly, differences in losses of vitamin C content in tissues frozen at -20 and -80 °C and analyzed at 8 months of storage were performed by using multifactorial ANOVA (accession × tissue × temperature) followed by LSD to compare the means ($p \leq 0.05$). Before ANOVA, the values expressed in percentage were normalized using arcsine transformation.

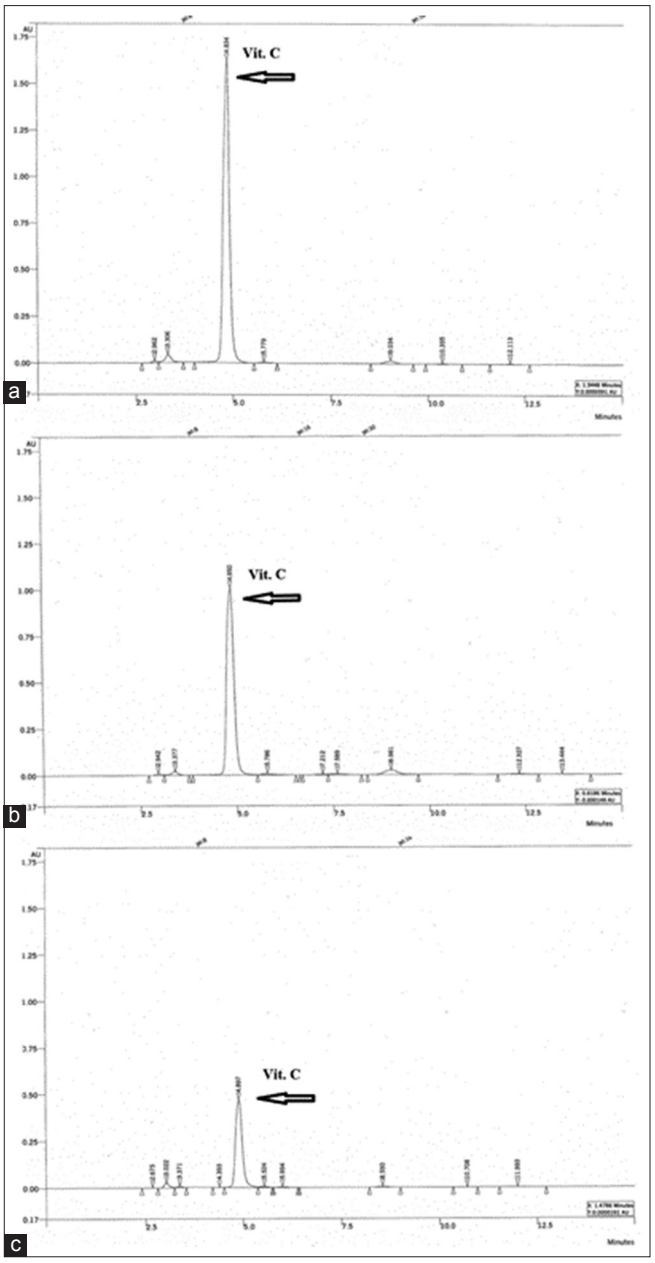

Fig 1. Vitamin C chromatograms in root, young and mature leaf samples (a, b and c respectively). Vitamin C was identified and quantified by reference to a standard.

RESULTS AND DISCUSSION

Biometric and morphological characteristics

Biometric characteristics of the horseradish plants are shown in Table 1. The first five traits describe the above-ground tissues of the plants during the vegetative growing stages (as average value of D1 and D6 stages), whereas the others are related to the roots collected during the period of root harvesting (as average value of D3 and D7).

The accessions analysed showed similar characteristics, the plants reached a height of about 60 cm, with a green leaf

Table 1: Biometric characteristics of the above and below-ground tissues of two horseradish accessions (Cor and Mon) during the vegetative development of the plants and at the root harvesting, respectively

Trait and unit	Accession	
	Cor	Mon
Above-ground tissues		
Plant height (cm)	60.3±2.4	60.4±1.0
Total leaves (n.)	36±9	50±12
Leaf area (cm^2)	7538±1250	7325±1226
Fresh weight (g plant-1)	568±108	621±118
Water content (%)	74±1.6	76±1.5
Below-ground tissues		
Main root length (cm)	19±1.4	18±1.4
Main root Ø at the top (cm)	4.3±0.3	5.4±0.6
Main root Ø at the base (cm)	2.8±0.2	4.0±0.4
Main root fresh weight (g $plant^{-1}$)	189±18	293±49
Side roots (n.)	16±2	14±1
Total root fresh weight (g $plant^{-1}$)	323±27	424±60
Water content (%)	68±0.8	69±0.4

Values are means (n=8) ± SE

area of about 7430 cm^2 and a leaf fresh weight varying from 568 in Cor accession to 621 g in Mon. Similarity of green leaf area in spite of differences in leaf number is due to different leaf shape of the two accessions (Cor showed wider leaves tending to elliptical shape, while Mon had tapered leaves, similar to narrow ellipses). Likewise, the differences in root shape between the two accessions reflected the differences in weight and diameters. Indeed, at the stage of root harvesting, the total root fresh weight was 323 in Cor and 424 g in Mon, with the main root representing about 59 and 69% in Cor and Mon, respectively. The diameter at the top was by about 5 cm in both accessions with a basal diameter of 2.8 in Cor and 4.0 cm in Mon having the former accession a root with a nearly cylindrical shape, and the other a frustoconical shape. The water content in roots was about 68%.

Variation of vitamin C content in leaves and roots

In the above and below-ground tissues above described, the vitamin C content was quantified in fresh tissues at different developmental stages during the two growing cycles (Fig. 2). Referring to the leaves, significant differences among the accessions ($p \leq 0.01$), developmental stages ($p \leq 0.01$) and the interaction accession x developmental stage ($p \leq 0.001$) for both young leaves (Fig. 2a) and mature ones (Fig. 2b) were detected. A surprising amount of vitamin C was found in young leaves (from about 190 up to 350 mg 100 g^{-1} fw) particularly during the stages of full vegetative development of the plant (at D1 and D6), tending to be higher in Cor accession compared to Mon (Fig. 2a). In mature leaves, the vitamin C varied from 140 up to 280 mg 100 g^{-1} fw, when the plants approximated to senescence (at D3 and D7) (Fig. 2b). The vitamin C has

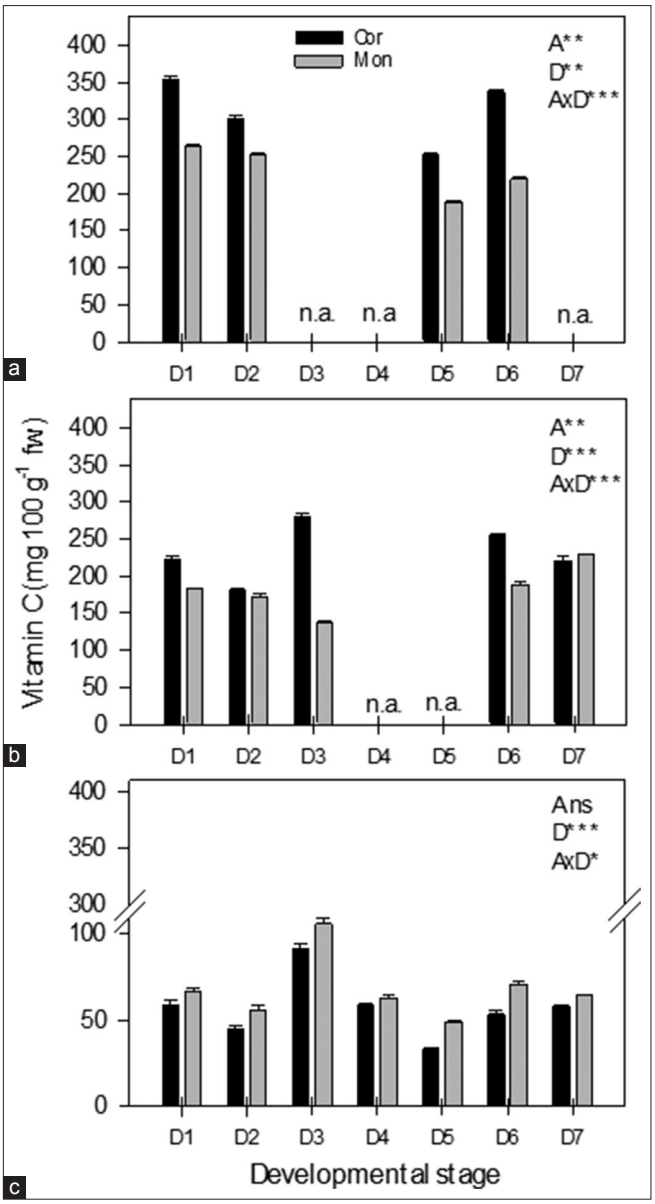

Fig 2. Vitamin C content in young and mature leaves (a and b, respectively) and roots (c) of two horseradish accessions (Cor and Mon) collected at different developmental stages (D1 - D7). Values are means (n=4) ± SE. For each portion data were analysed independently by two-way ANOVA followed by LSD test at $p \leq 0.05$; LSD values were 9.95, 11.82 for young and mature leaves respectively and 6.18 for roots. In each graph, ns, *, **, ***, mean not significant and significant at $p \leq 0.05$, 0.01 and 0.001, respectively; A = accession; D = developmental stage; n.a. = not available at the phenological stage.

a key role in regulating cell division and plant growth; in leaves it is affected by light and photosynthetic activity (Davey et al., 2000; Lee and Kader, 2000; Smirnoff and Wheeler, 2000). The age and the leaf surface light intensity can influence the endogenous vitamin C level, with greater concentrations in young actively growing tissue at the top of the plant (Logan et al., 1996). In horseradish, differences in vitamin C content could be also associated with leaf foliage density: the accession Cor while having the same

leaf area of Mon has fewer and farther leaves, allowing a greater exposure to the light resulting in a higher amount of vitamin C in both young leaves and mature ones.

Conversely to the leaves, in roots (Fig. 2c) the vitamin C content was unaffected by accession, while statistical differences were observed during the developmental stages of the plants, with the highest amount (100 mg 100 g^{-1} fw) detected at the end of September (D3 stage) and the lowest (40 mg 100 g^{-1} fw) in April (D5 stage, at the beginning of vegetative re-growth when tiny leaves are already formed on the crown of the roots left in the field, and a new growing cycle begins). Overall, leaves contained an amount of vitamin C of about 4-fold higher than roots. Vegetables show a wide range of vitamin C contents, with the *Brassicaceae* species generally containing the highest levels ranging from 50 to > 100 mg 100 g^{-1} fw (Davey et al., 2000). Referring to the vitamin C content in the roots of horseradish, our results are consistent with the range (from 25 up to 300 mg 100g^{-1} fw) reported in literature (Davey et al., 2000; Raghavan, 2007; Tomsone et al., 2013), while to our knowledge there is no information for leaves. It is known that more than one pathways exist in plants for vitamin C formation and turnover but still little is known about the regulation of its biosynthesis in plants. Plant senescence comprises a highly regulated series of cytological and biochemical events to co-ordinate the degradation of macromolecules and remobilization of nutrients from senescing tissues into reproductive and young organs as well as storage tissues (Barth et al., 2006). Probably in horseradish, the vitamin C was mobilized from the leaves to the roots during leaf senescence (D3) and back at the beginning of plant vegetative re-growth (D5). Variations of the content of other bioactive compounds, as the glucosinolates, throughout plant development of the two accessions tested in this study (Cor and Mon) have been reported by De Maria et al., (2016) and Rivelli et al., (2016a; 2016b), as well as by Ciska et al., (2017) in other horseradish landraces.

Effect of storage conditions on vitamin C content

Frozen tissues

Several studies showed the health potential of phytochemical compounds in horticultural products, mainly in relation to cancer prevention (Sayeed et al., 2017). Since the human body is unable to synthesize these compounds, it is necessary to supplement it by a regular intake of fruits and vegetables. However, fruits and vegetables are seasonal and undergo quite rapid changes if stored untreated at ambient temperature. Therefore, they are often processed (e.g., by heating, freezing, dehydration) in order to prolong their shelf life. In this context, it is interesting to assess which are the most suitable storage conditions to preserve the great vitamin C content in horseradish. In our study, we evaluated the effect of frozen and freeze-drying on vitamin C losses. In Fig. 3, the vitamin C loss in the tissues (young and mature leaves and roots) of Cor and Mon accessions *versus* time of storage at -20 °C (upon to 12 months) is reported. Since the relationships among the parameters above-mentioned did not statistically differ among accessions and/or type of tissues (data not shown), data were fitted by one linear regression line (Fig. 3). Regardless of the treatments, a high correlation ($r^2 = 0.95$, $p \leq 0.001$) between vitamin C loss and freezing time at -20 °C was found. Labuza (1982) suggested a criterion to evaluate the shelf life of frozen vegetables, taking into account the vitamin C losses of 10, 25 or 50% as a function of storage time. Considering such criterion, in horseradish a decrease of vitamin C of about 10% occurred after 60 days of storage for roots, young and mature leaves, and a loss of 50% was achieved at 10 months, with an estimated monthly loss of vitamin C of 5% for all tissues throughout the storage period. Similar results have been reported in the literature for other vegetables (Galgano et al., 2007; Howard et al., 1999; Wu et al., 1992). In root of horseradish, Kosson and Horbowicz (2008) recorded a vitamin C decline of about 20 - 30% after 10 months of storage at 0 - 1 °C, highlighting that the residual content could be considered still high ranging from 74 to 156 mg 100 g^{-1} fw. However, the vitamin C retention reported for fruits and vegetables after freezing is highly variable depending on several factors. A loss more than 97% was recorded for green beans and peppers within 1 month of freezing at -22 °C; however, such loss was limited by packaging under vacuum of the products (Oruna-Concha et al., 1998). Veberic et al. (2014) reported a vitamin C loss of about 50% after 7 months of storage at -20 °C in blackberry cultivars. The same authors found that a better retention resulted in the

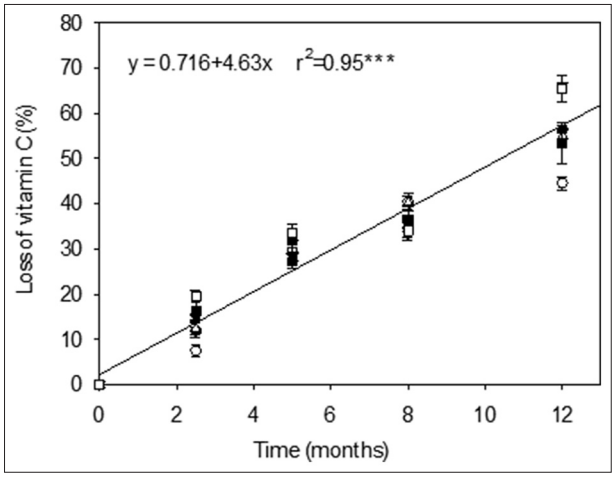

Fig 3. Kinetic of degradation of vitamin C in horseradish tissue at -20 °C (circle, triangle and square indicate, respectively, young and mature leaves and roots of Cor and Mon accessions in black and white, respectively). Values are means (n=4) ± SE.

blackberry cultivars with a lower vitamin C content at the harvest and that the reduction was more pronounced in fast-frozen than slow-frozen fruits (Veberic et al., 2014). Smaller changes of the vitamin C content were reported in broccoli and cauliflower after 12 months of frozen-storage at -30 °C (reduced by 15 - 18% and 6 - 13%, respectively) (Lisiewska and Kmiecik, 1996). In our study, a comparison between horseradish tissues frozen and stored until 8 months at -20 and -80 °C was also performed (Fig. 4). The interactions tissue × temperature, and accession × temperature (both at $p \leq 0.05$) were significant. The lowering of the temperature reduced considerably the vitamin C loss that was more than 35% at -20 °C and no more than 6% at -80 °C (3.1, 5.5 and 6.0% for young and mature leaves and roots, respectively). Despite results clearly showed the lowest reduction in vitamin C content in the tissues stored at -80°C, quality benefits must be balanced against the increased cost of storing products at such temperature. Indeed, at industrial, commercial and domestic level the storage of frozen food is usually carried out at temperatures not lower than -20 °C.

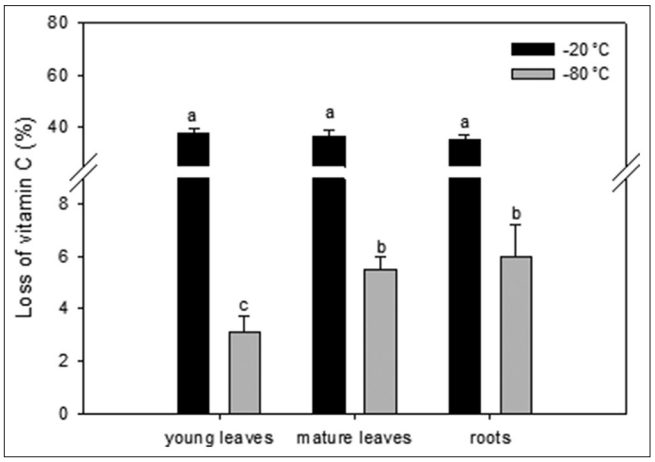

Fig 4. Vitamin C loss in frozen (-20 and -80 °C) horseradish tissues (young and mature leaves and roots) stored 8 months. Values are means (n=4) ± SE. Different letters above the bars indicate significant differences for $p \leq 0.05$ according to LSD test.

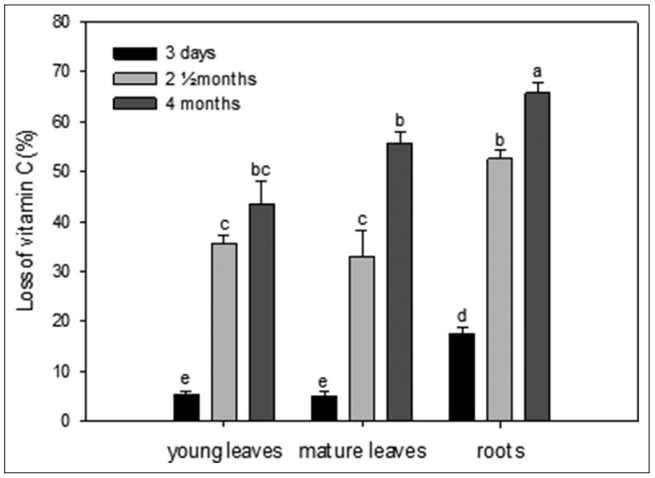

Fig 5. Vitamin C loss in freeze-dried horseradish tissues (young and mature leaves and roots) analyzed after 3 days and 2½ and 4 months of storage at 4°C. Values are means (n=4) ± SE. Different letters above the bars indicate significant differences for $p \leq 0.05$ according to LSD test.

Freeze-drying tissues

Besides freezing, the processing techniques most relevant to fruits and vegetables are canning and dehydration. Among this latter, freeze-drying is considered one of the best methods of drying in order to preserve the quality attributes of the products, because the combination absence of liquid water/low temperature stops many degradation reactions. This latter treatment is considered the most expensive dehydration process, due particularly to the energy cost of the sublimation step. On the other hand, the storage of the obtained products is inexpensive, compared to other preservation techniques, such as freezing. The root of horseradish is generally used grated to flavor food preparations, thus the possibility to store it as a freeze-dried powder could be interesting. In this context, the vitamin C loss in the freeze-dried plant tissues, analyzed after 3 days and 2 ½ and 4 months of storage at 4 °C was performed (Fig. 5). The interactions accession × time of storage (at $p \leq 0.05$), tIssue 10 time of storage and tissue × accession (both at $p \leq 0.01$) were significant. At the beginning of storage, the vitamin C loss was lower in mature and young leaves (about 5%) and more marked in roots (17.6%), indicating a higher sensibility of this latter tissue to the freeze-drying process. During the storage, all the freeze-dried samples showed a consistent vitamin C loss that in roots reached a value of 66%. We hypothesized that the higher vitamin C loss in freeze-dried than frozen samples could be attributed to the higher exposure to the atmospheric oxygen of the powdered freeze-dried samples, and probably to a high vitamin C sensibility to the temperature of storage (4 °C), considering the better vitamin C preservation at -80 °C than -20 °C.

Traditional dehydration process can be very destructive for vitamin C, reaching losses up to 75% (Mishkin et al., 1984). This latter technique determines the greatest loss of vitamin C, more than canning and much more than freezing (Davey et al., 2000). A considerable part of this loss can be attributed to the thermal treatment necessary to remove water. However, no studies are available in literature regarding the vitamin C evaluation in horseradish in freeze-dried samples. Tomsone et al. (2013) compared different storage methods (freezing at -20 °C, microwave-vacuum drying, freeze-drying) for preserving phenolic compounds and antioxidant activity of horseradish roots. They stated that technological process has a significant influence on the content of phenolic compounds. For most of individual polyphenols the highest content has been found in the fresh samples, but someone were highest in the frozen samples.

In the freeze-dried tissues, total polyphenols, individual polyphenols and antioxidant activity were considerably lower than frozen and fresh samples. Despite the lack of data in horseradish tissues, several studies are available on the effect of freeze-drying on vitamin C retention for several fruits and vegetables. Chang et al. (2006) reported that the vitamin C content in tomato did not vary significantly in freeze-dried tomatoes with respect to the fresh ones; Shofian et al. (2011) obtained similar results on some tropical fruits. On the other hand, Marquez et al. (2006) by applying the freeze-drying process to several tropical fruits found that the retention of vitamin C was about 29% for guava and over 60% for the other fruits. The ascorbic acid retention is affected by numerous operative parameters, as well as by the matrix. Drying time is one of the most important parameters, together with water activity and temperature. The mechanism by which water controls the degradation reaction seems to be very complex (Goula and Adamopoulos, 2006; Santos and Silva, 2008), and this could explain the differences found by applying the freeze-drying at different vegetables.

CONCLUSIONS

The roots and leaves of horseradish (*Armoracia rusticana*) represent an interesting source of vitamin C due to the great amount in plant. Overall, leaves contain a vitamin C level up to 4-fold higher than roots, which are the portion of the plant usually consumed. However, a variability in terms of vitamin C was found in tissues as a function of the accession (higher values in leaves for Cor and in roots for Mon) and developmental stage of the plant. Interestingly, the higher amount of vitamin C in roots coincides with the beginning of the traditional period of root harvesting for fresh market, while in leaves it remains generally high throughout the vegetative growing cycle. The great vitamin C content found in leaves could also be exploited industrially for the extraction of this nutrient, which is widely used in the formulation of fortified foods, food additives, and in the formulation of food supplements and cosmetics, besides also promoting the use of fresh leaves.

The vitamin C content in the fresh product can be preserved by reducing the storage temperature. The freezing was found to be a good method to reduce the vitamin C degradation. The lowering of the temperature of freeze storage (-80 °C) significantly reduced the vitamin C loss that did not exceed 6%. This result may be useful for pharmacological utilization of vitamin C from natural source than for food production and preservation since the high energy cost of storage at -80 °C. In our operating conditions, the freeze-drying is found to be a less valid technique. Despite the losses observed with the processing techniques analyzed, the residual vitamin C content in horseradish remains still higher compared to other vegetables.

Authors' contributions

A.R. Rivelli designed and performed the experiment. S. De Maria performed data collection and statistical analysis. M.C. Caruso and F. Galgano performed the experimental design and the chemical analysis. All authors revised and approved the final manuscript.

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