



Article

Suitability of Hydroponically-Grown *Rumex acetosa* L. as Fresh-Cut Produce

Costanza Ceccanti ¹, Marco Landi ^{1,2,*}, Luca Incrocci ¹, Alberto Pardossi ^{1,2} and Lucia Guidi ^{1,2}

¹ Department of Agriculture, Food and Environment, University of Pisa, 56124 Pisa, Italy; c.ceccanti3@studenti.unipi.it (C.C.); luca.incrocci@unipi.it (L.I.); alberto.pardossi@unipi.it (A.P.); lucia.guidi@unipi.it (L.G.)

² Interdepartmental Research Center Nutrafood “Nutraceuticals and Food for Health”, University of Pisa, 56124 Pisa, Italy

* Correspondence: marco.landì@unipi.it

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Abstract: Sorrel (*Rumex acetosa* L.) is a perennial wild herb appreciated as a folk medicine and for use in folk-traditional cuisines, and its nutraceutical properties are increasingly known and studied. Nowadays, there is a lack of knowledge about the possibility of using this species as fresh-cut produce, and no reports have investigated the physiological/biochemical changes of sorrel leaves upon storage. To test the aforementioned, sorrel seedlings were cultivated in a floating system and two consecutive harvests took place: the first cut at 15 days (C1) and second cut at 30 days (C2) after sowing. Fresh-cut sorrel leaves from C1 and C2 were stored in plastic boxes at 4 °C for 15 days and chlorophylls, carotenoids, total phenols, flavonoids, ascorbic acid, and antioxidant capacity were evaluated during the storage period. During storage, sorrel leaves from the same cut did not show significant changes in total phenolic content and antioxidant capacity, which represents a positive outcome for the maintenance of the nutraceutical value of this species. For this reason, sorrel may be a very promising species as a “new” fresh-cut leafy vegetable. However, some differences were observed between the two cuts, especially in the total flavonoid and the total ascorbic acid contents. While promising, further research will be necessary to standardize the yield and the nutraceutical content of this species in different cuts, which will be necessary to introduce and promote sorrel to consumers.

Keywords: sorrel; cold storage; wild herb; postharvest; bioactive compounds; hydroponic system; shelf-life

1. Introduction

Sorrel (*Rumex acetosa* L.) is a perennial wild edible herb, belonging to the Polygonaceae family, known from ancient times throughout the Mediterranean region. It is appreciated as a folk medicine for its medicinal properties [1–4]. Indeed, some studies have reported that sorrel plants showed medicinal properties for the treatment of cutaneous diseases, jaundice, sore throat, warts and, especially sorrel leaves showed properties for the treatment of fever, diarrhea, lack of appetite, worms, and as a “blood cleanser” [1,2]. Sorrel leaf extracts have also shown anti-mutagenic, cytotoxic, and anti-proliferative activities against human cancer cells [3,4]. In the past, times of famine or food scarcity turned people’s interest to wild herbs as food, but now the use of wild edible products is becoming popular in our modern society [5]. Sorrel is also appreciated for use in folk traditional cuisines such as boiled vegetables, pies, and mixed salads [3]. Numerous studies have reported the presence of phytochemicals in sorrel aerial parts (sinapic acid, vanillic acid, 6-methyl-1,3,8-trichlorodibenzofuran, chrysophanol, physcion/parietin, emodin-8-O-D-glucopiranoside, naphthalene-1,8-diol, catechin/epicatechin, epicatechin-3-O-gallate, vitexine, pulmatin,

gallocatechin/epigallocatechin, procyanidin B2, geraniin, corilagin, ellagic acid, rosmarinic acid, and pyrogallol [1,4–7]).

The demand for a higher variety of vegetable species in the diet can be satisfied by evaluating the suitability of new edible species, including sorrel, for cultivation as a “new” leafy vegetable. In fact, the introduction of cultivation of this species and, consequently, into the market would lead to the diversification of vegetable crops and the widening of the supplies of leafy vegetables.

The developing demand for vegetable crops encourages the introduction of this species into the fresh-cut products market. Fresh-cut products are obtained from minimally-processed leafy vegetables or fruits which are packaged in film or plastic boxes, stored, transported, and sold at temperatures between 0 to 8 °C (usually 4–5 °C), according to Italian Regulation number 77/2011 and Italian Ministerial Decree number 3746/2014 [8]. The shelf life differs among different fresh-cut products due in part to crop-specific enzymatic browning which usually develops during cold storage [9]. In fact, enzymatic browning of a cut surface is a problem for numerous leafy fresh-cut products such as lettuce (*Lactuca sativa*) or cabbage (*Brassica oleracea*) [9–11]. The browning results from the oxidation of phenols to quinones, which is catalysed by polyphenol oxidase and influenced by temperature, pH, and oxygen availability in the tissues [12]. The shelf life of most fresh-cut products has been established in the range of 7–15 days [13]. Currently, little information is available on the use of new edible herbs, such as sorrel, when cultivated in hydroponic systems and on their shelf-life as fresh-cut products. In fact, hydroponic cultivation, especially a floating system, is an efficient cultivation technique which allows modulation of the nutrient solution based on the unique needs of each horticultural species [14,15]. Moreover, harvest by cutting and allowing regrowth of plants is a currently used practice for leafy horticultural crops which will be processed as fresh-cut products (e.g., lettuce [16]).

Therefore, the aim of this work was to determine the quality of hydroponically-cultivated sorrel leaves from two consecutive harvests. For each harvest, the main nutraceutical compounds were measured during 15 days of cold storage. The results will lay a foundation for the introduction of a “new” nutraceutically-valuable leafy species in markets with the goal to enrich the Mediterranean diet.

2. Materials and Methods

2.1. Material Preparation and Experimental Setup

The work was carried out in a greenhouse of the Department of Agriculture, Food and Environment (University of Pisa, Pisa, Italy) from June to July 2018. Seeds of sorrel were purchased from S.A.I.S. S.p.a. (Società Agricola Italiana Sementi, Cesena, Italy) and they were sown in polystyrene trays (120 holes) for germination. Sprouts were grown in greenhouse until they had 4 or 5 true leaves and were ready for transplanting. The transplanting was carried out in the greenhouse in 16-hole polystyrene trays which were placed in tanks with 50 L of nutritive solution, typical of floating system cultivation, composed of: 10 mM NO₃⁻, 0.5 mM NH₄⁺, 1 mM PO₄³⁻, 6 mM K⁺, 4 mM Ca²⁺, 2 mM Mg²⁺, 0.5 mM Na⁺, 3.5 mM SO₄²⁻, 0.5 mM Cl⁻, 0.5 mM HCO₃⁻, 40 µM Fe²⁺, 25 µM BO₃⁻, 1 µM Cu²⁺, 5 µM Zn²⁺, 10 µM Mn²⁺, and 1 µM Mo³⁺. The growing solution was previously optimized for sorrel cultivation by Ceccanti et al. [7]. Electrical conductivity was 1.98 dS m⁻¹; pH was adjusted to 5.7–6.0 with dilute sulphuric acid. The nutrient solution was continuously aerated. At 15 days after transplanting, when seedlings had 15–20 leaves, plants were harvested by cutting the entire aerial part of the plant at the base (C1). At 15 days after C1, when seedlings had re-grown and had 15–20 leaves, plants were again harvested by a second cut of the entire aerial part of the plant at the base (C2). During the post-harvest, leaves obtained from C1 and from C2 were transported to the laboratory. A part of the fresh material from each plant was frozen using liquid nitrogen and stored at –80 °C for biochemical analyses. Another part of the fresh material was immediately stored and packed as a fresh-cut product. Samples of 15 g of fresh sorrel leaves were packed in polyethylene terephthalate (PET) boxes (150 cm³, Comital Cofresco, Volpiano (TO), Italy) and stored for 15 d at 4 °C in the dark. Analyses to determine total phenol, flavonoid, chlorophyll, carotenoid, and ascorbic

acid content as well as the total antioxidant capacity were performed using the fresh material (t0) and at 1, 2, 3, 6, 9, 13, and 15 d of cold storage.

2.2. Total Phenolic and Total Flavonoid Contents

Leaf samples (1 g) were homogenised in 4 mL 80% (*v/v*) methanol solution, then were sonicated using a sonicator (Digital ultrasonic Cleaner, DU-45, Argo-Lab, Modena, Italy) for 30 min and centrifuged (MPW-260R, MWP Med. instruments, Warsaw, Poland) at 10,000× *g* for 15 min at 4 °C. The supernatants (2 mL) were then centrifuged for 3 min at 7000× *g*. Extracts were used for the analysis of the total phenol and flavonoid content and the antioxidant capacity assay.

Total phenol determination was performed using the method of Dewanto et al. [17] with some modifications. Extracted samples (10 µL) were added to a solution of 115 µL deionized water and 125 µL Folin-Ciocalteu reagent. Blank solutions were performed with 10 µL distilled water, instead of the extract. Samples were stirred and, after 6 min, 1.25 mL 7% (*w/v*) Na₂CO₃ were added. Samples were incubated for 90 min at room temperature and the increase in absorbance at 760 nm using an Ultrospec 2100 Pro spectrophotometer (GE Healthcare Ltd., Little Chalfont, UK) was measured against a blank solution. Using a gallic acid standard curve, the results were expressed as mg gallic acid equivalents per g FW (mg GAE g⁻¹ FW).

Flavonoid determination was performed using the method of Du et al. [18] with some modifications. An aliquot of sample extract (100 µL) was added to a solution of 440 µL deionized water and 30 µL NaNO₂ 5% (*w/v*). Samples were stirred and then held for 6 min. Then, 30 µL 10% (*w/v*) AlCl₃ were added to samples which were stirred again. After 6 min, 400 µL NaOH 4% (*w/v*) were added to samples and the mixture was stirred and incubated for 15 min at room temperature. The increase in absorbance at 510 nm was measured spectrophotometrically against a blank solution made with distilled water. Using a catechin standard curve, the results were expressed as mg catechin equivalents per g FW (mg CAE g⁻¹ FW).

2.3. Total Chlorophyll and Total Carotenoid Contents

Chlorophyll and carotenoid contents were determined using the spectrophotometric method described by Porra et al. [19] with some modifications. An aliquot of 0.3 g of fresh material was extracted with 20 mL 80% (*v/v*) acetone and stirred for 72 h at 4 °C in the dark. Spectrophotometrically, the increase in absorbance at 663, 648, and 470 nm were detected to calculate chlorophyll *a*, chlorophyll *b*, and carotenoid content against a blank solution (only made by 80% (*v/v*) aqueous acetone). The results were expressed as mg chlorophyll or carotenoid per g FW (mg CHL or CAR g⁻¹ FW).

2.4. Total Ascorbic Acid Content

Ascorbic acid content was measured spectrophotometrically using the method described by Kampfenkel et al. [20] with some modifications. Extractions were carried out with the homogenization of 0.3 g fresh material with 1 mL 6% (*v/v*) trichloroacetic acid followed by centrifuging for 10 min at 10,000× *g* at 4 °C. Immediately, the analysis was performed by adding 50 µL supernatant to 50 µL 10 mM dithiothreitol (DTT) and to 100 µL 0.2 M Na-P buffer (pH 7.4). Samples were stirred and incubated for 15 min at 42 °C in a water bath. Then, 50 µL 0.5% (*w/v*) *N*-ethylmaleimide (NEM) were added and samples were stirred again. After 1 min of stirring, 250 µL 10% (*v/v*) trichloroacetic, 200 µL 42% (*w/v*) orthophosphoric acid, 200 µL 4% (*w/v*) 2,2'-dipyridil (diluted in 70% (*v/v*) ethanol 70% (*v/v*)), and 100 µL 3% (*w/v*) FeCl₃ were added to samples. The increase in absorbance at 525 nm was measured against blank solution (with 6% (*v/v*) trichloroacetic acid, instead of supernatant) after 40 min of incubation at 42 °C in a water bath. Using an ascorbic acid standard curve, the results were expressed as mg ascorbic acid per g FW (mg ASA g⁻¹ FW).

2.5. Antioxidant Capacity Assay

The antioxidant capacity was measured spectrophotometrically by using the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging assay [21]. The same extract used for phenol and flavonoid determination was used. The increase in absorbance was measured at 515 nm after 30 min of incubation of 10 μ L extract added to 900 μ L DPPH solution (0.024 g in 200 mL 80% (v/v) methanol). Using a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard curve, the results were expressed as mg Trolox equivalents per g FW (mg Trolox g^{-1} FW).

2.6. Statistical Analyses

To determine the effect of the two consecutive cuts and of the storage time, a two-way ANOVA was performed using GraphPad (GraphPad, La Jolla, CA, USA) as the statistical software. Mean values \pm standard deviation (SD) of 3 replicates for each assay were compared by the least significant differences test at $p = 0.05$ to identify significant differences among treatments and significant interactions between factors.

3. Results and Discussion

The total phenolic content was significantly lower in leaves of *R. acetosa* derived from C2 than those from C1, and this difference remained constant during storage (Figure 1a). This may suggest that carbon dedicated to the biosynthesis of secondary metabolites (such as phenols) was devoted to structural polymers of new cells during the regrowth of the plants after C1 instead of to secondary metabolites. Others have reported that the effect of a first harvest resulted in higher photosynthetic rate and plant yield but did not induce enhancement of secondary metabolite content [16]. However, only a few studies have reported on the harvest effect on secondary metabolite profile of leafy vegetables, and this aspect requires further investigation. During cold storage, total phenolic content in leaves obtained from C1 and from C2 remained constant, except for a slight increase after 9 days in leaves derived from C1. This last aspect is also evident in the flavonoid pattern, where a moderate increase after 9 days of cold storage occurred (Figure 1b).

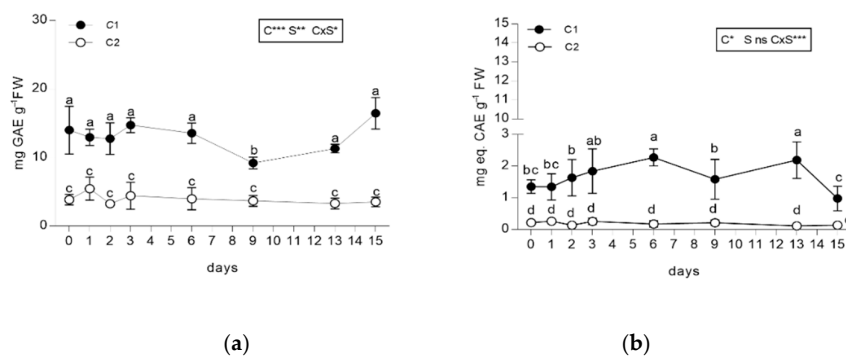


Figure 1. Total phenolic content (a) and flavonoid content (b) of *Rumex acetosa* stored at 4 °C for 15 days as fresh-cut products. Closed and open symbols represent first (C1) and second harvests (C2), respectively. Each value is the mean (\pm SD) of three replicates. Means having the same letter are not significantly different at $p = 0.05$ following two-way ANOVA with storage (S) and cut (C) as variables. F values for main effects and their interaction significant at $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***), or not significant (ns). Total phenol content values and flavonoid content values were expressed as gallic acid equivalents (GAE) $mg\ g^{-1}$ FW and as catechin equivalents (CAE) $mg\ g^{-1}$ FW, respectively.

However, similar to total phenolic content, total flavonoid content of leaves obtained from C2 had a lower content than leaves obtained from C1. The flavonoid content in leaves from C2 was more stable than that of leaves obtained from C1. The pattern of total phenolic content during storage is in agreement with studies of other species such as lettuce, rocket, and escarole [22–25], even though the phenolic amount was lower than that found in sorrel, especially in leaves obtained from C1. In

contrast, Castaner et al. [26] showed that the flavonoid content in lettuce decreased during cold storage because of the enzymatic browning reaction, activated at the cut by the oxidation of phenols present in photosynthetic tissues.

Among pigments, chlorophyll content did not change significantly during storage, similarly to total phenol and total flavonoid content, whilst carotenoid content showed some slight but significant fluctuations (Figure 2).

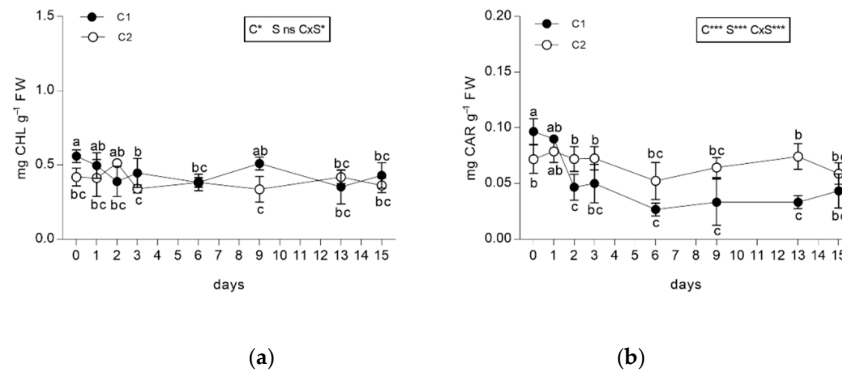


Figure 2. Chlorophyll content (a) and carotenoid content (b) of *Rumex acetosa* stored at 4 °C for 15 days as fresh-cut products. Closed and open symbols represent first (C1) and second harvests (C2), respectively. Each value is the mean (\pm SD) of three replicates. Means having the same letter are not significantly different at $p = 0.05$ following two-way ANOVA with storage (S) and cut (C) as variables. F values for main effects and their interaction significant at $p < 0.05$ (*); $p < 0.001$ (***), or not significant (ns). Chlorophyll (CHL) and carotenoid (CAR) content values were both expressed as mg g⁻¹ FW.

These results are partially in agreement with the behaviour of pigment content in fresh-cut rocket (*Eruca vesicaria*) and chicory (*Cichorium intybus*) found by others [27]. In fact, the total chlorophyll content remained unchanged from the beginning of experiment until the end of the storage period, whereas carotenoid content decreased upon storage, which is quite similar to our results [27]. It has been shown that the change in chlorophyll content is very different if leaves were stored under light conditions. The explanation is that the action of chlorophyllase degrades chlorophyll. Chlorophyllase is an enzyme which is present in the envelope membrane of the chloroplast and, therefore, is separated from chlorophyll as well as is involved in chlorophyll metabolism [28]. The synthesis and the activity of this enzyme has been associated with the presence of chlorophyll-protein complexes. Light conditions improve the formation of light-harvesting chlorophyll *a-b*-protein complex inside the thylakoids of chloroplast. This means that this enzyme does not remain in contact with chlorophyll until thylakoid membranes are degraded. Accordingly, the activity of chlorophyllase should be correlated with the degradation of membranes [28].

Similarly to phenolic and flavonoid contents, ascorbic acid content was higher in sorrel leaves obtained from C1 than those obtained from C2 (Figure 3) and averaged 0.45 mg g⁻¹ FW in C1 at harvest and 0.37 mg g⁻¹ in C2.

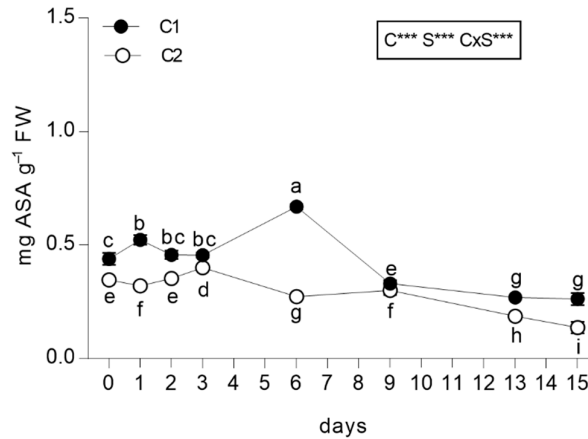


Figure 3. Ascorbic acid content of *Rumex acetosa* stored at 4 °C for 15 days as fresh-cut products. Closed and open symbols represent first (C1) and second cut (C2) during the growth, respectively. Each value is the mean (\pm SD) of three replicates. Means having the same letter are not significantly different at $p = 0.05$ following two-way ANOVA with storage (S) and cut (C) as variables. F values for main effects and their interaction significant at $p < 0.001$ (***). Ascorbic acid content values were expressed as ascorbic acid (ASA) mg g⁻¹ FW.

In leaves obtained from C1, an increase in ASA content was observed during the first six days of cold storage; then, ASA content decreased and remained unchanged until the end of cold storage when the content was lower as compared to the harvest date (Figure 3). In leaves obtained from C2, ASA content decreased during cold storage, declining by 60% at the end of storage as compared to values at harvest (Figure 3). However, ASA levels in this species were close to those found in leafy vegetables considered a good source of vitamin C, like spinach (*Spinacia oleracea*) [29–31]. The content of ASA in leaves is related also to the season of cultivation and, in this sense, Phillips et al. [30] reported that in spinach the ASA content averaged 0.44, 0.30, 0.18, and 0.18 mg g⁻¹ FW when grown in winter, spring, summer, and fall, respectively. In contrast, different results were found by Bergquist et al. [29] who showed that spinach leaves had a lower ASA content in winter than in summer (0.14 against 0.46 mg g⁻¹ FW).

Antioxidant capacity showed a more constant level in sorrel leaves obtained from C2 than that observed in leaves obtained from C1, even though values of antioxidant capacity in leaves from C2 were significantly lower than those from C1 (Figure 4). Leaves obtained from C1 showed a substantial decrease after nine days of storage which paralleled the decrease in phenols, flavonoid, and ASA contents, which suggests the importance of these metabolites in antioxidant capacity. Notably, sorrel leaves from C1 and from C2 exhibited an antioxidant capacity higher than several vegetables as red cabbage (*B. oleracea*), carrots (*Daucus carota*), onions (*Allium cepa*), and baby cos lettuce (*L. sativa*) [32,33].

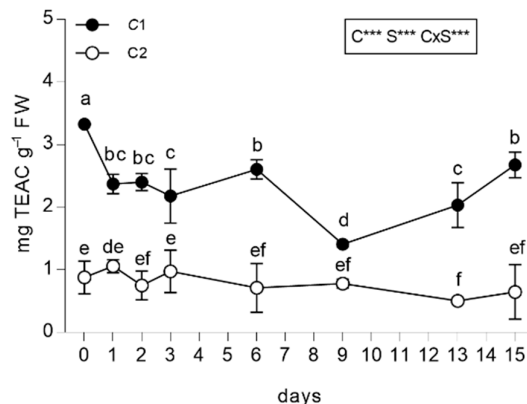


Figure 4. The antioxidant capacity of *Rumex acetosa* stored at 4 °C for 15 days as fresh-cut products. Closed and open symbols represent first (C1) and second cut (C2) during the growth, respectively. Each value is the mean (\pm SD) of three replicates. Means having the same letter are not significantly different at $p = 0.05$ following two-way ANOVA with storage (S) and cut (C) as variables. F values for main effects and their interaction significant at $p < 0.001$ (***). The antioxidant capacity values were expressed as Trolox equivalents (TEAC) g^{-1} FW.

4. Conclusions

The present dataset revealed that sorrel leaves had a higher antioxidant capacity and a higher level of bioactive compounds than those commonly observed in other leafy species traditionally used as fresh-cut products. This was also associated with a good shelf-life in terms of nutraceutical stability during cold storage. Therefore, *R. acetosa* could be an interesting “new” leafy species to be used as a leafy, fresh-cut product. In addition, there was a higher level of nutraceuticals in leaves obtained from C1 than in leaves obtained from C2, even though leaves from C2 showed a more stable pattern of nutraceutical compound content during cold storage. Future studies are therefore needed to reduce the nutraceutical variability between consecutive cuts in order to standardize the yield and the biochemical profile of sorrel leaves and to determine if the light conditions of storage (as the fresh-cut produce is usually stored) or the modification of composition of the gas in the headspace of the packaging may promote changes in biochemical attributes of sorrel leaves.

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