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Phenolic profile evolution of different ready-to-eat baby-leaf vegetables during storage☆

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ABSTRACT

Ready-to-eat baby-leaf vegetables market has been growing and offering to consumers convenient, healthy and appealing products, which may contain interesting bioactive compounds. In this work, the composition and the evolution of the phenolic compounds from different baby-leaf vegetables during refrigerated storage was studied. The phenolic compounds were extracted using pressurized liquid extraction (PLE) and the phenolic profile of each sample was analyzed and quantified by using LC–MS and LC–DAD methods, respectively, at the beginning and at the end of a 10-day storage period. The baby-leaf vegetables studied included green lettuce, ruby red lettuce, swiss chard, spinach, pea shoots, watercress, garden cress, mizuna, red mustard, wild rocket and spearmint samples and a total of 203 phenolic compounds were tentatively identified and quantified. The main naturally phenolic compounds identified correspond to glycosylated flavonoids, with exception of green lettuce and spearmint leaves which had a higher content of hydroxycinnamic acids. Quantification of the main compounds showed a 10-fold higher content of total phenolic content of ruby red lettuce (483 mg g^{-1}) in relation to the other samples, being the lowest values found in the garden cress (12.8 mg g^{-1}) and wild rocket leaves (8.1 mg g^{-1}). The total phenolic content only showed a significant change ($p < 0.05$) after storage in the green lettuce (+17.5%), mizuna (+7.8%), red mustard (−23.7%) and spearmint (−13.8%) leaves. Within the different classes of phenolic compounds monitored, the flavonols showed more stable contents than the hydroxycinnamic and hydroxybenzoic acids, although the behavior of each compound varied strongly among samples.

1. Introduction

The consumer demand for more convenient fresh food products led to a rapid grow of the fresh-cut industry, that became a multi-billion dollar sector worldwide in the last years. Fresh-cut vegetables can meet the consumer demands about the relationship between food, healthy lifestyle and convenience [1]. They are elaborated without additives, by minimal processing methods such as washing, cutting and packaging at chilling temperatures with polymeric films. Baby leaf salads have gained popularity over the traditional fresh-cut salads, by adding more variety to the diet and offering a product that attracts consumers and producers. The baby leaves are mixed, washed and packaged as whole, maintaining an appealing 3-D structure, reduced oxidation damage due to a small stem diameter and greater stability during shelf life [2–4]. Lettuces, rocket, watercress, spinach, and mustard greens are among the most used baby leaves, being sold individually or in salad mixtures [3].

The consumption of fresh vegetables is encouraged, not only due to their micronutrient composition (normally rich in vitamins and minerals), but also due to their phytochemicals, that are believed to protect human health [5]. Some antioxidant, anti-inflammatory and antitumor effects have been attributed to certain phytochemicals, that are also related to the vegetable color and flavor [5,6]. Within the European Union there is no specific regulation related to the presence of phytochemicals, but any nutrition and/or health claim made on the labels must be based on scientific studies that take into consideration the composition of phytochemicals and their qualitative and quantitative characteristics [5,7]. The antioxidant properties of the vegetables are one of the most present label claims due to the high levels of carotenoids, tocopherols and ascorbic acid that have epidemiological evidence of beneficing human health [6]. In the other hand, the antioxidant properties of vegetable intake are also closely related to the presence of phenolic compounds. These are secondary metabolites of

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the plants, characterized by having at least one aromatic ring with one or more hydroxyl groups [6,8]. Polyphenols can range from simple molecules (phenolic acids) to more complex structures (e.g., phenylpropanoids or flavonoids) or even highly polymerized compounds (such as lignins or tannins), with flavonoids representing the most common and widely distributed sub-group [9]. Moreover, different types and numbers of sugars and functional derivatives such as esters or methyl esters can be conjugated to aglycones, forming numerous structures of phytochemicals, being described more than 8000 natural phenolic compounds [6,8,10]. The phenolic content of a plant is affected by several factors like plant species, cultivar, environmental conditions, water availability, light exposure, germination, maturity, processing and storage [5,11]. In minimally processed fresh-cut products, the shredding step can increase the antioxidant capacity associated with wound-induced phenolic compounds [12]. Reyes et al. [13] described major changes in the total soluble phenolic content during the storage of fresh-cut vegetables, influenced by the initial levels of reduced ascorbic acid and phenolic compounds. Also light exposure and temperature of storage can induce the synthesis of certain phenolic compounds.

The importance of phenolic compounds as potential antioxidants and their complex chemical structure, variability and distribution creates a challenge to properly assess their content in food products. Traditionally, techniques to extract phenolic compounds from fresh or freeze-dried vegetables use large amounts of hydro-organic solvent mixtures [14] and are normally very laborious, time-consuming and not very selective. Pressurized liquid extraction (PLE) has been shown to be a more environment friendly alternative to extract bioactive compounds from a vegetal matrix [15]. PLE combines elevated temperature and pressure with the use of minimum amounts of food-grade solvents to achieve a fast and efficient extraction of several compounds, while preserving their bioactivity and chemical structure. A better diffusion of the solvent into the matrix is obtained by maintaining the pressures and temperatures below the critical point of the solvents, due to a higher solubility of the analytes in the solvent and to the decrease of solvent viscosity and surface tension [15]. PLE has been successfully applied to the extraction of phenolic compounds from vegetable matrixes, showing high yields, better recoveries, being more time efficient and economic when compared to the traditional methods [14,16,17].

As the popularity of ready-to-eat baby-leaf vegetables increases, there is an urgent need to understand how the profile of important components of these more immature vegetables evolves during storage. In this sense, it has been already demonstrated how some fat- and water-soluble free vitamin losses may be produced during refrigerated storage of these products [18]. The purpose of this work was to study the evolution of the phenolic compounds of a wide group of ready-to-eat baby-leaf vegetables during storage, including green lettuce, ruby red lettuce, swiss chard, spinach, watercress, garden cress, mizuna, red mustard, wild rocket, peashoots and spearmint. To do that, a PLE method was optimized to extract the phenolic compounds from these vegetables and the obtained extracts were analyzed by HPLC-DAD–MS. The information available so far focuses more on the identification of the phenolic compounds present in this baby leaves [19–22]. Moreover, there are only a few publications on the changes of the phenolic compound during the storage of baby-leaf samples, mainly for spinach leaves [23–25], wild rocket [26] and lettuces [27].

2. Materials and methods

2.1. Chemicals and standard solutions

Methanol was of HPLC-grade and acquired from LabScan (Dublin, Ireland) whereas ethanol was purchased from Scharlab (Barcelona, Spain). Folin–Ciocalteau phenol reagent and sodium carbonate (Na_2CO_3) were acquired from Merck (Darmstadt, Germany) and the water used was Milli-Q Water (Millipore, Billerica, MA, USA). Formic acid, gallic acid, 4-hydroxybenzoic acid, sinapic acid, syringic acid, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, quercetin, kaempferol and catechin were supplied by Sigma–Aldrich (Madrid, Spain). The others phenolic standards, i.e., vanillic acid, rosmaniric acid, quercetin-3-rutinoside, quercetin-3-rhamnoside, quercetin-3-glucoside, quercetin-3-galactoside, luteolin-7-glucoside, apigenin-7-glucoside, kaempferol-3-glucoside, apigenin and diosmetin were acquired from Extrasynthese (Genay, France).

Individual phenolic standard solutions were prepared in 70% MeOH solution with the following concentrations: 0.2 mg mL^{-1} for caffeic acid, p-coumaric acid, quercetin, apigenin, apigenin-7-glucoside and kaempferol-3-glucoside; 0.5 mg mL^{-1} for syringic acid; 0.7 mg mL^{-1} for luteolin-7-glucoside, kaempferol and diosmetin; 1.0 mg mL^{-1} for sinapinic acid, rosmarinic acid, salicylic acid, p-hydroxybenzoic acid, vanillic acid, gallic acid, quercetin-3-rhamnoside, quercetin-3-rutinoside and catechin; and 1.4 mg mL^{-1} for chlorogenic acid, ferulic acid, quercetin-3-galactoside and quercetin-3-0-glucoside. All standard solutions were kept under refrigeration at 4 °C until analysis. During development of HPLC-DAD–MS method, a mixture of phenolic standards was prepared by dilution of the individual phenolic stock solutions with 70% MeOH solution.

2.2. Samples

Samples were supplied by a producer (Odemira, Portugal) of minimally processed vegetables: the minimally processed baby leaf vegetables were washed, packaged and sent to our laboratory using the conditions normally employed by the company for processing, packaging, distribution and commercialization of fresh-cut products. The samples comprised 4 of the most common baby leaves used in ready-to eat salads, namely, green lettuce, ruby red lettuce, swiss chard and spinach, 5 baby-leaf vegetables from the Brassicaceae family characterized by their peppery flavor, namely, watercress, garden cress, mizuna, red mustard and wild rocket, a baby-leaf recently introduced in salad mixtures, pea shoots, and a fresh-cut aromatic herb that can be added to ready-to-eat salads, such as spearmint. Each sample was divided into two batches, corresponding to the two sampling times studied (day 1 and day 10). After sampling, the baby-leaf samples were freeze-dried (Telstar Cryodos-80, Terrassa, Spain) until analysis. The 10-day refrigerated storage (3 ± 1 °C) period was monitored with an EL-USB 2 (Lascar Electronics, Salisbury, UK). The freeze-dried leaves were reduced to a fine powder in a knife mill (GM 200, Retsch, Haan, Germany) and stored protected from light, oxygen and high temperatures. This procedure intended to exclude individual differences and ensure the representativeness of the test sample.

2.3. Pressurized liquid extraction (PLE)

The extractions were performed using an accelerated solvent extraction system (ASE 200, Dionex, Sunnyvale, CA) equipped with a solvent controller. The PLE method was first optimized using pea shoots and spearmint as test samples. Methanol/water and ethanol/water mixtures in three different proportions (50%, 70% and 90%) were the solvents tested to extract phenolic compounds from freeze dried vegetable leaves. The extraction temperature (70 °C), pressure (10 MPa), flush volume (60% of cell volume using extraction solvent), sample quantity (0.5 g) and dispersion (2 g of sea sand) were maintained constant during this study. The static extraction time was of 20 min, including an additional 5 min heat-up step prior to any extraction. After choosing the solvents,