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Monitoring of phytochemicals in fresh and fresh-cut vegetables: A comparison

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ABSTRACT

Bearing in mind that fresh-cut market is currently the fastest growing subsector in the food industry, a comparison of the levels of phytochemicals in fresh and fresh-cut vegetables has been carried out. Thus, several families of phytochemicals, such as phenolic acids, isoflavones, flavones, flavonols and glucosinolates were determined in fresh and fresh-cut samples including tomato, carrot, grape, eggplant and broccoli. Both type of products have potential and similar beneficial properties, regarding its content as phytochemicals, except tomato, which should be consumed as fresh. Other factors such as commercial presentation (sliced, grated, diced) and storage conditions (temperature and light) were evaluated observing that in eggplant, the content of phenolic acids is statistically different depending on the presentation. On the other hand, the content of phytochemicals was higher when fresh-cut carrots were stored at 4 °C regardless of the presence or absence of light. Multivariate analysis, based on cluster analysis was used as a first approach to distinguish between fresh and fresh-cut samples, obtaining good results except for eggplant and carrot.

1. Introduction

It is well known that fruit and vegetables are important components of a healthy diet, and their daily consumption could help to prevent major diseases, such as cardiovascular diseases (Bhupathiraju & Tucker, 2011; Ness et al., 2005) and certain cancers (Soerjomataram et al., 2010; Wicki & Hagmann, 2011). These beneficial effects of fruits and vegetables have been attributed to non-essential food constituents, which are known as phytochemicals or bioactive compounds, that possess a relevant bioactivity when they are frequently consumed as a part of a regular diet (Mudgal, Madaan, Mudgal, & Mishra, 2010). In general, these compounds could possess antioxidant capacity (AOC) (Kim, Padilla-Zakour, & Griffiths, 2004), antiinflammatory (González-Gallego, García-Mediavilla, Sánchez-Campos, & Tuñón, 2010; Vincent, Bourguignon, & Taylor, 2010), lipid profile modification (Perez-Vizcaino & Duarte, 2010; Wang, Melnyk, Tsao, & Marcone, 2011) and antitumor effects (Collins, 2005; Pietta, Minoggio, & Bramati, 2003; Stan, Kar, Stoner, & Singh, 2008).

Nowadays, there is an increased interest in health and consumers are concerned with the role of food for maintaining and improving human well-being. In this sense, the practical advantages and convenience that the fresh-cut products provide to the consumers makes this market currently the fastest growing subsector in the food industry, and it still has a high potential of growth worldwide (Ragaert, Verbeke, Devlieghere, & Debevere, 2004). Currently, minimal processing is applied to fruit and vegetables, involving at industrial scale initial rinsing, peeling, slicing, washing, packaging and storage (Laurila & Ahvenainen, 2000).

Therefore, it is important to investigate if this minimal processing may result in loss of important phytochemicals. There are several studies that evaluate the effects of minimally processing on phenolic acids, vitamin C and carotenoids in fresh-cut carrot (Alasalvar, Al-Farsi, Quantick, Shahidi, & Wiktorowicz, 2005; Simões, Allende, Tudela, Puschmann, & Gil, 2011; Simões, Tudela, Allende, Puschmann, & Gil, 2009) or lettuce (Martínez-Sánchez et al., 2012; Selma et al., 2012), but there are few studies regarding flavonoids (Gil, Ferreres, & Tomás-Barberán, 1998; Selma et al., 2012) or glucosinolates (Jones, Faragher, & Winkler, 2006) in other fresh-cut vegetables or fruits such as tomato (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008), eggplant (Mishra, Gautam, & Sharma, 2012), grape (Costa et al., 2011) and broccoli (Jones et al., 2006).

In general, it was observed that the content of phenolic acids increased in fresh-cut products. This fact could be explained considering that when a sharp blade was used for cutting, it produced a lower release of phenolic acids and lowered the polyphenol

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oxidase (PPO) activity compared to when a knife was used. This cutting process is known to be a key player in the browning process of various raw and cut fruit and vegetables (Mayer, 2006; Mishra et al., 2012). Furthermore, the abiotic stress (Cisneros-Zevallos, 2003), such as altered O_2 and CO_2 levels in controlled and modified atmospheres packaging (MAP), or C_2H_4 gassing for ripening and degreening, could also affect phytochemical accumulation. For example, in the phenylpropanoid pathway, the phenylalanine ammonialyase (PAL) generates an increase in phenolic compounds in certain fresh-cut products (Kenny & O'Beirne, 2010). Moreover, in some cases, natural additives, such as ascorbic acid or citric acid (Robles-Sánchez, Rojas-Graüb, Odriozola-Serrano, González-Aguilar, & Martín-Belloso, 2009; Son, Moon, & Lee, 2001) were added to enhance the shelf life of the fresh-cut product, and therefore it is possible that the phytochemicals content remained constant or even increased.

In contrast to fresh products, the shelf life of fresh-cut products usually extend upto 16 days at 4 °C, 12 days at 10 °C and 5 days at 26 °C storage temperatures, although this depends on the type of matrix (Mishra et al., 2012). In relation to the evolution of phytochemical in fresh-cut products, some studies reported that total phenolic indices significantly decreased after 5 days of storage at 2–4° C in apple (Roossle, Wijngaard, Gormley, Butler, & Brunton, 2010), although some studies reported by Simões et al. (2011), indicate that the phenolic compounds, mainly chlorogenic acid, increased twofold in baby carrots stored under MAP, where the concentration of the gases was used as an important parameter. Furthermore, Odriozola-Serrano et al. (2008), reported that a minimal processing maintains the main antioxidant compounds of sliced tomatoes for 21 days at 4 °C, preserving their initial nutritional value. Finally, Cisneros-Zevallos (2003) reported that the total phenolic content and the antioxidant capacity of the tissue increased in sliced orange carrots and purple potatoes stored during 2 days at 20 °C.

Considering that current approaches have been based on the evaluation of a few family of compounds in some matrices, the purpose of this work was the comparison of the content of several families of phytochemicals (phenolic acids, flavonols, flavones, glucosinolates and isoflavones) in fresh and fresh-cut products stored under MAP, such as tomato, eggplant, grape, carrot and broccoli, in order to get a comprehensive view of the presence of phytochemicals in fresh and fresh-cut products. Other variables such as type of cut and shelf life were also evaluated.

2. Materials and methods

2.1. Chemicals and reagents

Commercial phenolic compound standards such as progoitrin, gluconasturtin and glucoraphanin were supplied by PhytoLab GmbH & Co (Vestenbergsgreuth, Germany). Glucotropaeolin, glucoerucin and glucoiberin were purchased from Scharlab (Barcelona, Spain). Other standards as genistein, apigenin, quercetin, quercetin-3-*O*-glucoside, gallic acid, sulforaphane, ferulic acid, baicalein, gallic acid and caffeic acid were purchased from Sigma–Aldrich (Steinheim, Germany). Other standards as daidzein, glycitein, luteolin-4-*O*-glucoside, luteolin-7-*O*-glucoside, apigenin-7-*O*-neohesperoside, kaempferol, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, luteolin, luteolin-6-*C*-glucoside, luteolin-8-*C*-glucoside, apigenin-7-*O*-glucoside, apigenin-6-*C*-glucoside, apigenin-8-*C*-glucoside, quercetin-3-*O*-ramnoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-ramnoside, quercetin-3-*O*-galactoside, isorhamnetin, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, apigenin-7-*O*-rutinoside and tamarixetin were purchased from Extrasynthese (Genay, France). Stock standard solutions of individual compounds (with concentrations between 200 and 300 mg/l) were prepared by exact weighing of the powder and dissolved in 10 ml of HPLC grade methanol or in a mixture of methanol:water (50:50, *v*/*v*). Then they were stored at −20 °C in dark bottles. A multicompound working standard solution at a concentration of 5 mg/l of each compound was prepared by appropriate dilutions of the stock solutions with methanol and stored in screw-capped glass tubes at −20 °C. The solutions were prepared each 6 months. Ultrapure water was obtained from a Milli-Q Gradient water system (Millipore, Bedford, MA, USA). Ammonium acetate was purchased from Panreac (Barcelona, Spain). Formic acid (purity >98%), HPLC-grade methanol was provided by Sigma (Madrid, Spain). Millex-GN nylon filters of 0.20-μm were provided by Millipore (Millipore, Carrightwohill, Ireland).

2.2. Apparatus and software

Chromatographic analyses were carried out using an Agilent series 1290 RRLC instrument (Agilent, Santa Clara, CA, USA) equipped with a high-performance autosampler (G4226A), a binary pump (G4220A), a column compartment thermostat (G1316C) and an autosampler thermostat (G1330B). The system was coupled to an Agilent triple quadrupole mass spectrometer (6460A) with a Jet Stream ESI ion source (G1958-65138). For the chromatographic separation of the extracts, a Zorbax Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 μm particle size) from Agilent was used. The injection volume was 5 μl and column temperature was set at 30 °C. Chromatographic separation was carried out using a gradient elution with methanol as eluent A, and an aqueous solution of ammonium acetate (30 mM), adjusted to pH 5 with formic acid, was used as eluent B. The elution started at 5% of eluent A for 1.5 min, and then it was increased to 30% in 2.5 min. After that, it was increased to 100% at 4 min. This composition was kept constant during 2 min, before being returned to the initial conditions after 0.5 min, keeping this composition during 1.5 min prior to the next analysis, obtaining a total run time of 12 min. The flow rate was set at 0.2 ml/min.

The Jet Stream ion source parameters were: drying gas temperature and sheath gas temperature at 325 °C and 400 °C respectively; drying gas flow and sheath gas flow at 7 and 12 ml/min respectively; nebulizer pressure at 40 psi; capillary voltage was set at 4000 and 3500 V in positive and negative acquisition respectively.

An Agilent Mass Hunter Quantitative analysis (Agilent Technologies, Inc.) was used for data acquisition and quantification of samples.

Statistical analysis, analysis of variance and cluster analysis were carried out with JMP v9 (Cary, NC, USA).

Lyophiliser Alpha from Martin Christ (Osterode, Germany) was also used; an analytical balance AB204-S from Mettler Toledo (Greifensee, Switzerland), a Reax-2 rotary agitator from Heidolph (Schwabach, Germany), and vacuum pump from Vacuubrand (Wertheim, Germany) were also utilised.

2.3. Extraction procedure

Samples were homogenised and they were transferred to a Petri dish, and weighed and cooled to −18 °C. Then, all samples were processed according to the following procedure: 150 mg of lyophilized sample was weighed in a 15 ml polypropylene centrifuge tube and 3 ml of a mixture of methanol:water (80:20, *v*/*v*) was added. The mixture was agitated for 30 min with a rotary shaker. After that, the extract was filtered and 100 μl were transferred into a vial containing 400 μl of a mobile phase (50:50 *v*/*v* of eluent A and B), and 5 μl were injected into the chromatographic system