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Broccoli and radish sprouts are safe and rich in bioactive phytochemicals

Nieves Baenas[a], Isabel Gómez-Jodar[b], Diego A. Moreno[a,c,*], Cristina García-Viguera[a,c], Paula M. Periago[b,c,d]

[a] Phytochemistry Lab. Dept. of Food Science and Technology, CEBAS-CSIC, P.O. Box 164, Espinardo, 30100, Murcia, Spain
[b] Departamento de Ingeniería de Alimentos y del Equipamiento Agrícola, Campus de Excelencia Internacional Regional, Universidad Politécnica de Cartagena (UPCT), Paseo Alfonso XIII 48, Cartagena, Murcia, Spain
[c] Unidad Asociada "CALIDAD Y EVALUACIÓN DE RIESGOS EN ALIMENTOS" UPCT y CEBAS-CSIC, Murcia, Spain
[d] Instituto de Biotecnología Vegetal, Campus de Excelencia Internacional Regional "Campus Mare Nostrum", Universidad Politécnica de Cartagena, Spain

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ABSTRACT

Cruciferous sprouts (e.g. broccoli and red radish) are rich source of health-promoting phytochemicals that are more concentrated than in the adult plant edible organs; however, these tiny microgreens need cold storage conditions to preserve their quality to reach the consumers in microbiologically safe conditions, maintaining their composition and acceptability. In this work, the microbiological status and phytochemical composition of broccoli and radish sprouts were evaluated at harvest (Day 0), and after seven and fourteen days of storage at 5 and 10 °C. Pathogenic microorganisms were absent during shelf-life; nevertheless, the slight growth of *Enterobacteriaceae* organisms, aerobic mesophilic and psychotropic bacteria, molds and yeasts was assessed. The storage temperature influenced the quality and content of bioactives in the sprouts, and for practical applications, storage at 5 °C is the most suitable option. Moreover, these fresh crucifers remain acceptable for consumers after 14 d storage period, being an interesting option for consuming fresh and naturally-functional foods.

1. Introduction

Cruciferous sprouts are novel plant foods because of their rich composition in bioactive compounds compared to adult plants. Germinating seeds could contain from 2 to 10-fold increase of phytochemicals depending the species, cultivar, environmental conditions and the time of germination (O'Hare et al., 2007). Seven or eight days old sprouts are of appropriate size for harvest, allowing post-harvest handling and commercialization of this material, maintaining contents of phytochemicals higher than other vegetables (Pérez-Balibrea et al., 2011; Baenas et al., 2012). Broccoli and radish sprouts are very young plants that continue their highly metabolic activities after harvesting, which affected their shelf life and composition, therefore, storage conditions such as temperature and time, directly affects the physiology and cellular constituents of these plant products, as well as the safety in terms of microbial content (Thompson and Powell, 2000).

The glucosinolates (GLS) are bioactive compounds, almost exclusively found in crucifers, with a common core structure containing a β-D-thioglucose group linked to a sulfonated aldoxime moiety and a variable side chain derived from amino acids; depending this amino acid chain, GLS could be classified in aliphatic (derived from methionine, isoleucine, leucine or valine), indole (derived from tryptophan) or aromatic (derived from phenylalanine or tyrosine) (Radojcic Redovnikovi et al., 2008). These compounds in presence of the enzyme myrosinase (thioglucohydrolase, E.C.3.2.1.147), as a result of tissue disruption by crushing or herbivory/chewing or by the action of the gut microflora upon human ingestion, are hydrolysed into several biologically active products, such as isothiocyanates (ITC) and indoles, widely studied because of their antioxidant, anti-inflammatory and anticarcinogenic activity (Dinkova-Kostova and Kostov, 2012). Sulforaphane (SFN), the major breakdown product from the predominant GLS glucoraphanin (GRA) of broccoli sprouts, is one of the most potent naturally occurring inducers of phase 2 detoxification enzymes. Other ITC present in

* Corresponding author at: Phytochemistry Lab. Dept. of Food Science and Technology, CEBAS-CSIC, P.O. Box 164, Espinardo, 30100, Murcia, Spain.
E-mail address: dmoreno@cebas.csic.es (D.A. Moreno).

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broccoli and radish sprouts, such as iberin and erucin, or the indole-3-carbinol, have also showed anticarcinogenic actions (Wagner et al., 2013). Also sulforaphene (SFE), derived from glucoraphenin (GRE) in radish sprouts, has been recently studied because of its cancer preventive effect (Pocasap et al., 2013). Other phytochemicals also present in *Brassicaceae* sprouts are the phenolic compounds, mainly derivatives of hydroxycinnamic acids (from chlorogenic acids or sinapic acids). These compounds have shown beneficial antioxidant and anti-inflammatory activity for human disease prevention (Teixeira et al., 2013).

Sprouts are an ideal source for microbial growth due to its high nutritional value and the high moisture and warm temperatures during germination, which creates a suitable environment for bacteria (Feng, 1997). Total plate counts as high as 10^8–10^9 CFU/g are frequently reported in sprouts (Gabriel et al., 2007; Martínez-Villaluenga et al., 2008) due to the intrinsic microflora of the seeds. Moreover, although a low level of pathogenic bacteria is generally found in sprouts (Kimanya et al., 2003), they can be contaminated during the sprouting process, harvesting, postharvest handling and distribution. In fact, several outbreaks caused by sprouts consumption have been frequently reported (Yang et al., 2013), being the pathogens involved *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes*, among others. The high initial load of non-pathogenic microorganisms in sprouts cannot be eliminated or reduced by a simple washing (Mohle-Boetani et al., 2001) or by the application of heat and chemical disinfectants (Waje and Kwon, 2007). Nevertheless, guidelines for specific recommendations, avoiding contamination during sprouting, have been developed (FDA, 2004; EFSA, 2011) to reduce the risk of contamination of sprouts by harmful bacteria and ensure the food quality and safety in sprouts. Broccoli and radish sprouts are grown organically, hydroponically and marketed in containers filled with a layer of cellulose material. Then, the germinated seeds are kept refrigerated in perforated plastic boxes until consumed, when did not usually show any change in visual appearance (yellowing, loss of the initial firmness or development of off-odours). Even though *Brassicaceae* sprouts are being widely studied and consumed as novel plant foods rich in bioactive compounds, there are not many data or reports documenting the stability of their phytochemicals during shelf life, as well as the microbial flora contents. Sprouts are treated and consumed as fresh products, the recommended temperature for storage is about 0–2 °C, however, some surveys have indicated that more than 40% of the products stored at grocery refrigerators had a temperature above 7 °C (Kader and Thompson, 2001). In this work, we analysed the microbial contents as well as the contents of glucosinolates, isothiocyanates and phenolic compounds of 8-day-old broccoli and radish sprouts once collected and after 7 and 14 d of storage at 5 °C, commonly used in normal household refrigeration, and 10 °C, usually found in grocery refrigerated display cases, to evaluate plant foods in terms of optimal content of phytochemicals and safe foods for health-conscious consumers.

2. Material and methods

2.1. Germination and storage of sprouts

Seeds of broccoli (*Brassica oleracea* L. var *italica*) and red radish (*Raphanus sativus* cv. Rambo) were provided from Intersemillas, S.A. (Valencia, Spain). Sprouts germination was carried out under environmentally friendly practices (ES-ECO-024-MU) according to previous conditions (Baenas et al., 2012). Briefly, seeds were activated by hydration and aeration for 24 h, then, were distributed in trays lined with cellulose (CN Seeds, UK). A tray containing 25 g of sprouts was considered a replicate. Three trays per sample, in order to have triplicates, were introduced in a controlled dark chamber for three days for increase stem elongation, then, were transferred to an environment controlled chamber for 5 more days. All trays were irrigated everyday with water with 5 g L^{-1} sodium hypochlorite. Sprouts were treated with 10 ml methyl jasmonate (MeJA) 250 μM per tray, from day four to day seven of germination, in order to provide enriched cruciferous sprouts in bioactive compounds, as an effective strategy previously studied in our research group (Baenas et al., 2016). Three replicates per sample were rapidly collected at day 8 of germination. Samples were weighed, flash-frozen in liquid nitrogen and stored at −80 °C. Prior to analyses all samples were lyophilized at −50 °C (Christ Alpha 2–4D, Christ, Osterode am Harz, Germany). In addition, the remaining samples were stored at 5 or 10 °C, for 7 or 14 d, in a refrigerated chamber with high relative humidity (85%), to simulate the shelf life of these plant foods. After this time all replicates were also freeze-dried and stored prior analyses.

2.2. Microbiological tests

Twenty-five grams of each cruciferous sprouts tray (replicate) were aseptically placed into a sterile stomacher bag with 225 ml of Buffered Peptone Water (PW) (Scharlab, Barcelona) and homogenized in a Stomacher. Samples were then analysed for *Salmonella* spp., *Listeria* spp., *Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacteriaceae*, aerobic mesophilic bacteria, aerobic psychrotrophic bacteria and moulds and yeasts at 0, 7 and 14 d of storage at 5 °C and 10 °C.

Microbiological analysis for *Salmonella* spp. involved a pre-enrichment in PW incubated for 24 h at 37 °C and enrichment in Selenite Cystine Broth (SCB) (Scharlab, Barcelona) incubated for 24 h at 37 °C. The samples then were plated in Xylose Lysine Deoxycholate Agar (XLD) (Scharlab, Barcelona) and Brilliant Green Agar (BG) (Scharlab, Barcelona) and incubated for 24 h at 37 °C.

Microbiological analysis for *Listeria* spp. involved a pre-enrichment in Half-Fraser (Scharlab, Barcelona) incubated for 24 h at 37 °C and enrichment in Fraser Broth (FB) incubated for 48 h at 37 °C. The samples then were plated in OXFORD Agar Base (Scharlab, Barcelona) and PALCAM Agar (Scharlab, Barcelona) and incubated for 24–48 h at 37 °C.

Sulfite Polymyxine Sulfadiazine Agar (SPS) (Scharlab, Barcelona) was used for *C. perfringens* analysis and incubated in anaerobic conditions for 48 h at 37 °C.

Triptone Bile X-Glucuronide Agar (TBX) (Scharlab, Barcelona) was used for *E. coli* analysis and incubated for 18–24 h at 44 °C.

Baird-Parker Agar (BP) (Scharlab, Barcelona) was used for *Staphylococcus* analysis and incubated for 24 h at 37 °C.

Violet Red Bile Glucose Agar (VRBG) (Scharlab, Barcelona) was used for *Enterobacteriaceae* analysis and incubated for 24 h at 37 °C.

Plate Count Agar (PCA) (Scharlab, Barcelona) was used for mesophilic and psychrotrophic bacteria analysis and incubated for 24–48 h at 30 °C and for 5–7 d at 5 °C, respectively.

Rose Bengal Chloramphenicol Agar (RB) (Scharlab, Barcelona) was used for moulds and yeasts and incubated for 5 d at 25 °C.

2.3. Extraction and determination of glucosinolates and phenolic compounds

Freeze-dried samples (50 mg) of broccoli and radish sprouts were extracted with 1 ml of methanol 70% V/V, then were heated at 70 °C for 30 min in a bath, shaking every 5 min, and centrifuged (17 500 × *g*, 5 min). The supernatants were collected and the extractant was removed using a rotary evaporator. The dry material obtained was re-dissolved in Milli-Q water and filtered (0.45 μm Millex-HV13 filter, Millipore, Billerica, MA, USA).