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Genotypic, storage and processing effects on compositional and bioactive components of fresh sprouts

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ABSTRACT

Fresh sprouts constitute an appealing, nutritional and functional food product. The present study profiles the nutritional and bioactive components of quality of fresh sprouts of five select species (barley, lentil, mung bean, radish and wheat) against their postharvest performance during 4° C cold storage. Moreover, the impact of lyophilisation followed by 30-day ambient storage was assessed on sprouts composition and quality. The highest proteins, P and K contents were observed in radish sprouts. Moreover, radish sprouts demonstrated higher hydrophilic and lipophilic antioxidant activities (HAA and LAA) by 185% and 95%, respectively, than the other species examined. Compositional and quality attributes were however influenced by storage: total soluble solids increased on average by 1.0 \textdegree Brix after day 0, while LAA activity decreased by 33% after day 1. Dry matter, proteins and nitrate contents, juice pH and HAA remained stable. Lyophilisation preserved the compositional and quality attributes of fresh sprouts as no significant differences in proteins, nitrate, K and P contents, HAA and LAA were observed compared to fresh sprouts. The current findings highlight genotypic variation and postharvest performance of fresh sprouts with respect to their functional quality and the impact of lyophilisation as an alternative processing application for their preservation.

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1. Introduction

Consumer perception of functional foods as pivotal in supporting human health and longevity has been entrenched during the last decade [\(Ebert, 2012\)](#page--1-0). Accordingly, consumers, food scientists, nutritionists, and producers alike, are questing for fresh, eminently healthy, inexpensive and convenient food products [\(Kyriacou et al.,](#page--1-0) [2016](#page--1-0)). Sprouts, which are basically germinated seeds of legumes, cereals, pseudocereals, oilseeds, vegetables and herbs fall in this category and are gaining popularity all over the world as a hot new culinary trend [\(Ebert, 2012\)](#page--1-0). Aside from their unique taste, consumption of sprouts is on the rise due to their low fat content and richness in health-promoting phytochemicals such as phenols, flavonoids, vitamins, amino acids, and also minerals (Pásko et al., [2009](#page--1-0)). Moreover, sprouting involves physiological changes which mobilize and augment the nutritional value of seeds, including

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catabolism of macromolecules such as proteins and lipids into more readily absorbed and digested forms (Peñas, Gó[mez, Frías,](#page--1-0) & [Vidal-](#page--1-0)[Valverde, 2009](#page--1-0)). Sprouts therefore constitute excellent functional food candidates for providing natural fortification against multiple ailments ([P](#page--1-0) a[sko et al., 2009](#page--1-0)).

Sprouts are commonly marketed and consumed fresh, although they are highly perishable and susceptible to spoilage and microbial proliferation if not handled and stored properly ([Singh, Kumar,](#page--1-0) & [Singh, 2014](#page--1-0)). At common retail display and domestic refrigerator temperature regimes (3–5 \degree C) sprouts shelf-life is less than four days ([Suslow](#page--1-0) & [Cantwell, 2000](#page--1-0)). Preservation of the nutritional and nutraceutical value and overall quality of fresh sprouts is aided by packing in vented clamshell plastic containers or perforated film pouches and storage at low temperatures ($<$ 5 °C). The nutritional quality of sprouts is at its highest at the time of harvest, however few studies are available concerning their postharvest storage performance and related physicochemical and phytochemical changes ([Force, O'Hare, Wang,](#page--1-0) & [Irving, 2007;](#page--1-0) S[wieca](#page--1-0) & [Gawlik-](#page--1-0)[Dziki, 2015;](#page--1-0) [Swieca, Surdyka, Gawlik-Dziki, Z](#page--1-0)łotek, & [Baraniak,](#page--1-0)

Processing methods that extend the limited postharvest life and marketability of sprouts are highly desirable, particularly when nutritional and functional quality is preserved. Lyophilisation presents a promising processing application for sprouts. The fresh product is fast frozen and subjected to reducing atmospheric pressure to near vacuum conditions (<100 mbar) whereby lyophilisation or freeze-drying is achieved through sublimation of chemically non-bound tissue moisture directly from the solid to the gas phase ([Acton, 2013\)](#page--1-0). Lyophilisation has been widely used in the production of pharmaceuticals and for improving stability over extended storage. Furthermore, lyophilisation has been adopted as an efficient dehydration technique in manufacturing high value fruit snacks and also to facilitate easy handling of several important fruits such as plum and coconut [\(Michalska, Honke, Lysiak,](#page--1-0) & [Andlauer, 2016; Rattanaburee, Amnuaikit, Radenahmad,](#page--1-0) & [Puripattanavong, 2014\)](#page--1-0). Accordingly, the application of lyophilisation as an alternative preservation technique to cold storage of fresh sprouts necessitates the evaluation of potential changes in their compositional and functional quality.

Therefore, the aims of the current study were: (1) to evaluate the composition and quality attributes of select sprouts belonging to the families of Brassicaceae (radish), Fabaceae (lentil and mung bean) and Poaceae (barley and wheat), and (2) to assess the effect of 4° C storage for 0–4 days and the effect of lyophilisation followed by 30 days of ambient storage on proteins, soluble solids and macrominerals contents and on the antioxidant activity of sprouts. The reported findings may guide species selection for production of premium quality sprouts based on nutritive and functional quality traits, and also provide a baseline reference for species-specific postharvest performance of fresh sprouts under cold storage and their response to lyophilisation.

2. Materials and methods

2.1. Plant material

In the present study sprouts of crucifers: radish (Raphanus sativus L. var. radicula 'Cherry belle', La Rosa Emanuele Sementi, Italy), legumes: lentil (Lens culinaris Medik. 'Santo Stefano', Slowfood, Italy) and mung bean (Vigna radiata L. Wilczek 'Berken', Co.Pro.Sem.El., Italy), and cereals: barley (Hordeum vulgare L. 'Rondo', Società Italiana Sementi, Italy) and wheat (Triticum durum L. 'Creso', Cereal Sorrentino, Italy) were chosen. The five examined species were selected according to overall popularity and consumers' demand and based on the results of a preliminary experiment carried out on 12 species including also anise, basil, buckwheat, chickpea, flax, sunflower and turnip. Radish, lentil, mung bean, barley and wheat were characterized by high germination rate and short germination time, thus were selected for the present study.

2.2. Sprouting conditions, postharvest storage and sample preparation

Seeds of the above five species were sterilized in 5 mL/L of sodium hypochlorite for 15 min, then drained and washed with distilled water to attain neutral pH. Seeds were subsequently soaked in distilled water at room temperature (25 \degree C) for 12 h in darkness and agitated every 30 min to accelerate germination. The imbibed seeds were then spread on 25 cm diameter steel sieves and allowed 4 days (radish, lentil and mung bean) to 6 days (barley and wheat) for germination in a dark plant growth chamber held at 25 \degree C and 90% relative humidity (Termaks series 6000, Bergen, Norway). On each steel sieve 1 kg of barley, lentil or mung bean seeds was spread (c. 20,000 seeds), 0.8 kg wheat (c. 16,000 seeds), or 0.4 kg radish (c. 40,000 seeds). Sprouts were watered daily with distilled water. Sprouts of the five species were harvested at the 3 cm stage and rinsed twice in tap water and then in distilled water to remove all ions and substances from their surface.

Freshly harvested sprout samples were subdivided into two subsamples. One sub-sample was frozen at -40 °C for 24 h, then lyophilised for 48 h under conditions of -55 °C and 3.5 Pa pressure (Heto Powerdry PL6000-55, Thermo Fisher Scientific, Waltham, USA) and stored at room temperature in sealed plastic boxes for 30 days. A fraction of this sub-sample was then used for reconstitution in distilled water at ambient conditions. The other sub-sample of fresh sprouts was packed in vented plastic boxes of 100 g net fresh weight, randomly allocated to storage intervals of 0, 1, 2, 3 and 4 days at $4 \degree C$ in dark, simulating domestic refrigerator and retail display conditions. Part of the fresh samples was used for determining the juice pH and total soluble solids contents. The rest of the fresh samples were dried in a forced-air oven for mineral analysis and also lyophilised for the quantification of the antioxidant activities. The entire experiment was replicated three times.

2.3. Juice pH and soluble solids analysis

A homogenate prepared under low speed using a commercial blender and filtered through double cheesecloth was obtained from the fresh sprout samples. The total soluble solids (TSS) content at 20 \degree C of the filtered juice was determined using an Atago N1 refractometer (Atago Co. Ltd., Japan). The pH of the juice was measured with a pH electrode (HI-9023, Hanna Instruments, Padova, Italy).

2.4. Dry matter, proteins and macrominerals contents analysis

The sprouts dry matter (DM) content was determined by oven drying at $65 °C$ to constant weight according to the official method 934.01 of the Association of Official Analytical Chemists ([AOAC,](#page--1-0) [2005\)](#page--1-0). Dried sprouts were ground in a Wiley Mill (IKA Werke MF 10 Basic, Germany) to pass through a 841 μ m screen, and then portions of the dried tissues were analyzed for the following minerals: Nitrogen (N), Phosphorus (P), Potassium (K) and nitratenitrogen (N-NO₃). Nitrogen (total N) concentration in the sprouts was determined by the Kjeldahl method following mineralization with concentrated sulphuric acid (H₂SO₄, 96%, Carlo Erba Reagents, Cornaredo, Milan, Italy) in the presence of potassium sulphate and a low concentration of copper catalyst ([Bremner, 1965\)](#page--1-0). Total proteins content was assessed based on the Kjeldahl method previously described, with nitrogen-to-protein conversion factor of 6.25 (official method 976.05; [AOAC, 2000](#page--1-0)). Nitrate content was determined on aqueous extracts of the dried sprout samples, based on the cadmium reduction method ([Sah, 1994](#page--1-0)) using a Hach DR 2000 spectrophotometer (Hach Co., Loveland, Colorado, USA). The absorbance of the solution was determined at 550 nm wavelength.

Phosphorus and K were extracted after dissolution of the dried samples by nitric-perchloric acid digestion. After acid digestion, the solution was appropriately diluted and P analysis was performed in triplicate according to the ascorbic acid method [\(Jhohn, 1970](#page--1-0)). The absorbance of the solution was detected at 880 nm wavelength using a spectrophotometer Hach DR 2000. Potassium was determined by Atomic Absorption Spectrophotometry (AAS) according to the method described by [Walinga, van der Lee, van Vark, and](#page--1-0) [Novozamsky \(1995\).](#page--1-0) The AAS analyses were carried out using a Varian AA 250 Plus Atomic Absorption Spectrometer (Varian Inc., Mulgrave, Victoria, Australia).

2.5. Analysis of hydrophilic and lipophilic antioxidant activities

Two different cation assays were applied on lyophilised samples

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