Broccoli and Radish Sprouts are Safe and Rich in Bioactive Phytochemicals

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ABSTRACT

Cruciferous sprouts (e.g. broccoli and red radish) are rich source of health-promoting phytochemicals more concentrated than 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 two different temperatures, 5 and 10 °C. Pathogenic microorganisms were absent during shelf-life; nevertheless, the slight growth of *Enterobacteriaceae* organisms, aerobic mesophilic and psychrotrophic bacteria, molds and yeasts was assessed. The temperature of storage highly influenced the quality and content of bioactives in the sprouts, and for practical applications, the storage at 5 ºC is the most suitable option. Moreover, these fresh crucifers remain acceptable for consumers after a long refrigerated storage period (14 d), being an interesting option for consuming fresh and naturally-functional foods.

KEYWORDS: *Brassicaceae, sprouts, microbiology, bioactive compounds.*

INTRODUCTION

Cruciferous sprouts are fairly novel plant foods very interesting because of their rich composition in bioactive compounds compared to adult plants. Germinating seeds could contain unless from 2 to 10-fold increase of phytochemicals depending the species, cultivars, environmental conditions and the time of germination (O'Hare, Wong, Force, & Irving, 2007). According to previous works, seven or eight days old sprouts are of appropriate size for harvest, allowing manipulation and commercialization of this material, maintaining contents of phytochemicals higher than other vegetables (Baenas, Moreno, & Garcia-Viguera, 2012; Pérez-Balibrea, Moreno, & García-Viguera, 2011).

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, Glivetic, Delonga, & Vorkapic-Furac, 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 & 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 broccoli and radish sprouts, such as iberin and erucin, or the indole-3-carbinol, have also showed anticarcinogenic actions (Wagner, Terschluesen, & Rimbach, 2013). Also sulforaphene (SFE), derived from glucoraphenin (GRE) in radish sprouts, has been recently studied because of its cancer preventive effect (Pocasap, Weerapreeyakul, & Barusrux, 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 showed beneficial antioxidant and anti-inflammatory activity for human disease prevention (Teixeira *et al*., 2013).

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. This natural food is an ideal source for microbial growth due to its high nutritional value (Thompson & Powell, 2000) and the high moisture and warm temperatures during sprouting 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, Frías, Gulewicz, Gulewicz, & Vidal-Valverde, 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 (Y. Yang *et al*., 2013) and among the pathogens involved are *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes*. The high initial load of non-pathogenic microorganisms in sprouts cannot be eliminated or reduced by a simple washing (Mohle-Boetani et al., 2001), neither by the application of heat and chemical disinfectants which have shown limited effectiveness (Waje & Kwon, 2007). Nevertheless, it is of high relevance to avoid contamination during sprouting, hence guidelines for specific recommendations have been developed (EFSA, 2011; FDA, 2004) in order to reduce the risk of contamination of sprouts by harmful bacteria and ensure the food quality and safety in sprouts. The sprouts of broccoli and radish are grown organically, hydroponically and marketed in containers filled with a layer of cellulose material, where the germinated seeds are kept refrigerated in perforated plastic boxes until consumed, in this storage period, sprouts 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 ºC, however, some surveys have indicated that more than 40 % of the products stored at grocery refrigerators had a temperature above 7 ºC (Kader & 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 two different temperatures, 5 °C, commonly used in normal household refrigeration, and 10 °C, usually found in grocery refrigerated display cases temperature, in order to evaluate plant foods in terms of optimal content of phytochemicals and safe foods for health-conscious consumers.

MATERIAL AND METHODS

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 24h, then, were distributed in trays lined with cellulose (CN Seeds, UK). 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 $g \cdot L^{-1}$ sodium hypochlorite. Sprouts were treated with 10 ml methyl jasmonate (MeJA) 250 μM per tray, from day four to day seven of germination, as effective strategy in order to provide enriched cruciferous sprouts in bioactive compounds, as previously studied in our research group (Baenas, Villaño, García-Viguera, & Moreno, 2016). Three replicates per sample were rapidly collected at day 8 of germination. One sample was weighted, flashes frozen in liquid nitrogen and stored at −80 ºC prior to analyses. In addition, the remainder samples were storage at 5 or 10 °C, for 7 or 14 d, in a refrigerated chamber with high relative humidity (85 %), in order to simulate the shelf life of these plant foods. After this time all replicates were also frozen and stored prior analyses.

124

125 **Microbiological tests**

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

132 ***Salmonella spp.***

133 The microbiological analysis for *Salmonella* spp. involved a pre-enrichment in PW incubated for
134 24 h at 37 ºC and enrichment in Selenite Cystine Broth (SCB) (Scharlab, Barcelona) incubated
135 for 24 h at 37 ºC. The samples then were plated in Xylose Lysine Deoxycholate Agar (XLD)
136 (Scharlab, Barcelona) and Brilliant Green Agar (BG) (Scharlab, Barcelona) and incubated for 24
137 h at 37 ºC.

138 ***Listeria spp.***

139 The microbiological analysis for *Listeria* spp. involved a pre-enrichment in Half-Fraser (Scharlab,
140 Barcelona) incubated for 24 h at 37 ºC and enrichment in Fraser Broth (FB) incubated for 48 h at
141 37 ºC. The samples then were plated in OXFORD Agar Base (Scharlab, Barcelona) and
142 PALCAM Agar (Scharlab, Barcelona) and incubated for 24 – 48 h at 37 ºC.

143 ***Clostridium perfringens***

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

146 ***Escherichia coli***

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

149 ***Staphylococcus aureus***

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

Enterobacteriaceae

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

Aerobic mesophilic and psychrotrophic bacteria

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

Moulds and yeasts

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

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 ºC 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).

The quantitative analysis of glucosinolates and phenolic compounds was carried out simultaneously by a LC multipurpose method (Francisco *et al*., 2009) in an HPLC-DAD Agilent 1260 Infinity equipped with a binary pump (model G 1312 B), a degasser (model G 1379 B), an autosampler (model G 1313-44510), and a diode array detector, DAD (model G 4212 B) that is controlled by the Agilent software B. 02. 02., according to their UV spectra and order of elution already described for similar acquisition conditions (Baenas *et al*., 2012). Glucosinolates were quantified at 227 nm using sinigrin and glucobrassicin as standard of aliphatic and indole GLS, respectively (Phytoplan, Germany). Sinapic acid and ferulic acid derivatives were quantified at 330 nm using sinapic acid as standard (Sigma, St. Louis, MO, USA). Results are expressed on a fresh weight basis.

Extraction and determination of isothiocyanates

Freeze-dried samples (50 mg) were extracted with 1.6 ml of Milli-Q water, shaken on a Vortex mixer during 1 min and then were kept at room temperature for 24h. Then, samples were shaken again and centrifuged (17 500 × g, 5 min). The supernatants were collected and filtered (0.42 μm Millex-HV13 filter, Millipore, Billerica, MA, USA). Isothiocyanates were analysed following their MRM transitions by UHPLC-QqQ-MS/MS (Agilent Technologies, Waldbron, Germany) according to Dominguez-Perles et al. (Dominguez-Perles *et al*., 2014). Results are expressed on a fresh weight basis.

Statistical methods

Data are calculated as the mean (n=3) ± standard error (SD) and processed using the SPSS 15.0 software package (SPSS Inc., Chicago, USA). Statistical differences were analysed using a one-way ANOVA followed by Tukey´s test (assumption of homogeneity of variance) or Games-Howell test (no assumption of homogeneity of variance) for multiple comparisons. A value of $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Microbiological analysis

Vegetables once harvested are stored in grocery refrigerated display cabinets which recommended temperature is 5 ºC, however, the temperature found is usually higher (> 7 °C), so we performed the experiments in these two temperatures in order to analyse possible negative effects of this common storage practice. The data of the microbiological analysis on broccoli and radish sprouts at 0, 7 and 14 d of storage at 5 ºC and 10 ºC are showed in Tables 1 and 2.

Salmonella spp. and *Listeria* spp. were absent in both kind of sprouts at day 0 and during the time of storage at 5 ºC and 10 ºC. Other microorganisms such as *C. perfringens* and *E. coli* showed <10 CFU/g (<1 log CFU/g) while *S. aureus* showed <100 CFU/g, in all samples. Moreover, counts from 8.98 to 9.60 log CFU/g and 7.98 to 9.63 log CFU/g were obtained for *Enterobacteriaceae* in broccoli sprouts (Table 1) and radish sprouts (Table 2), respectively. No

208 significant differences were found between the counts obtained for *Enterobacteriaceae* at the
209 different storage times in broccoli sprouts while significant differences were obtained for the
210 *Enterobacteriaceae* counts in radish sprouts, showing growth within the storage time, regardless
211 distinct temperatures. For aerobic mesophilic bacteria, counts from 8.83 to 10.04 log CFU/g were
212 obtained in both sprouts and significant differences were observed in the growth of these bacteria
213 during the storage time. Counts for aerobic psychrotrophic bacteria were closed to those obtained
214 for aerobic mesophilic bacteria for both sprouts. Only growth of aerobic psychrotrophic bacteria
215 was observed after 14 d of storage in broccoli sprouts. Finally, also growth of moulds and yeasts
216 was observed after 14 d of storage in both sprouts. In most cases, the counts for the different
217 microorganisms tested obtained at the same storage time were roughly similar at the two
218 temperatures studied (5 ºC and 10 ºC) in both sprouts.
219 The microbial load of broccoli and radish sprouts was high; showing initial counts around 8 log
220 CFU/g after harvesting and reaching values around 10 log CFU/g after 14 d of storage. The results
221 obtained agree with those found in the literature in similar products (Gabriel *et al*., 2007;
222 Martínez-Villaluenga *et al*., 2008; Prokopowich & Blank, 1991). However, the counts obtained
223 for moulds and yeasts were higher than those found in the literature in which the counts obtained
224 were around 4 – 5 log CFU/g (Tournas, 2005). This high level of microorganisms in sprouts could
225 be due to the favourable conditions for microbial growth during sprouting (Feng, 1997) and the
226 leak of nutrients from the germinated seeds or the sprouts (EFSA, 2011) which would lead to the
227 formation of a microbial population in the seeds of sprouts. Despite this, it is important to remark
228 that both sprouts did not show any visible sign of spoilage. Moreover, absence or low level of
229 pathogenic bacteria have been also reported in the literature (Kimanya et al., 2003), fulfilling the
230 Regulation (EC) No 2073/2005 on microbiological criteria for foods stuffs. The lack of
231 pathogenic bacteria could be related to the high level of microbial population in sprouts which
232 may limit the growth of pathogenic bacteria through competition, during sprouting and
233 subsequent storage of the sprouts (EFSA, 2011). Nevertheless, sprouts could be contaminated
234 during production, harvest, storage and transport and once present, pathogenic bacteria are likely
235 to survive for extended periods of time. Therefore, contamination with pathogenic bacteria must

236 be minimized by the application of GAP, GHP, GMP, HACCP principles at all steps of the
237 production chain (EFSA, 2011). Furthermore, the different temperatures of storage studied (5 ºC
238 and 10 ºC) showed similar counts of microorganisms for both sprouts. However, an increase in
239 the counts obtained could be observed after 14 d of storage, which means that the storage time is
240 an important factor to take into account.
241
242 **Effect of time and temperature on bioactive compounds**
243 After 7 and 14 d of storage at 5 or 10 ºC, individual and total GLS (Figure 1), ITC (Figure 2) and
244 phenolic compounds (Figure 3) showed significant decreases in broccoli and radish sprouts. Even
245 though these losses, sprouts still content higher or similar concentrations of phytochemicals than
246 mature vegetables. This information should be taken into account in order to estimate the
247 adequacy of shelf-life conditions to these plant foods, maintaining their health-promoters
248 properties.
249
250 ***Glucosinolates***
251 The contents of GLS (Figure 1) in the samples were higher when compared with recent studies
252 on broccoli (Tian, Xu, Liu, Xie, & Pan, 2016; R. Yang *et al*., 2015) or radish sprouts (Yuan,
253 Wang, Guo, & Wang, 2010; Zhou, Zhu, & Luo, 2013) and these differences mainly owned to
254 different seed materials and appropriate germination conditions. After 7 d of storage at 5 ºC, the
255 decrease in total GLS was a 30 % and 20 % in broccoli (A) and radish (B) sprouts, respectively
256 (Figure 1), following with a decrease of 20 % more until day 14. Similar decreases after 7 d of
257 refrigeration (4 – 5 ºC) of *B. oleracea* sprouts were found by Vale *et al*. (2015), while Force *et al*.
258 (2007) after cutting, packaging in perforated bags and stored broccoli, kohlrabi and white radish
259 sprouts at 5 °C for three weeks, showed no statistically significant changes in its tentative results
260 about GLS concentrations. The loss of GLS in broccoli sprouts between 7 and 14 d of storage was
261 no significant (Figure 1A), being the first week of storage more relevant for the GLS content,
262 consistent with Schreiner *et al*. (Schreiner, Peters, & Krumbein, 2006), who observed a decrease
263 of GLS during the first four days of storage of mini broccoli and cauliflower. When the sprouts

were stored at 10 ºC, this decrease in total GLS at day 7 of storage was extremely high, achieving about a 65 % of loss and remained until day 14 in similar values in both sprouts (Figure 1A), and decreasing a 20 % more at day 14 in case of radish sprouts (Figure 1B) compared to the day 0 (control). Temperature had substantial impact on these compounds, some authors observed stable levels of total GLS or glucoraphanin (GRA), the predominant GLS in broccoli, during storage at 4 ºC, but a high decrease during storage at 20 ºC (Rybarczyk-Plonska *et al*., 2016; Vallejo, Tomás-Barberán, & García-Viguera, 2003). When we studied the individual GLS we focused in the predominant quantifiable GLS in the sprouts under study. GRA represent the 65 % of the total in broccoli sprouts, according to different authors (Force *et al*., 2007), and has been widely investigated because its hydrolysis compound the isothiocyanate (ITC) sulforaphane (SFN), having bioactivity against the development of certain cancers (Wagner *et al*., 2013). GRA was better preserved at 5 ºC than at 10 ºC. During the first 7 d of storage at 5 ºC, only a slight loss of 7 % was found, and, in day 14, the loss of GRA was a 20 % more. In case of storage at 10 ºC, the loss of 65 % of GRA at day 7 was maintained until day 14 (Figure 1A). It is noteworthy that GRA content in broccoli sprouts remains quite high on day 7 and day 14 after storage at 4 ºC (1.6 and 1.14 g kg^{-1}, respectively), if compared to broccoli heads (0.4 g kg^{-1}) (Rangkadilok *et al*., 2002), therefore, broccoli sprouts continues being a rich source of this compound during commercialization in spite of the loss of total GLS.

Similar results were found in case of the aliphatic GLS glucoiberin, representing the 13 % of the total. The four indole GLS 4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin, accounted for 47 % of the total GLS present in broccoli sprouts, however, the aliphatic glucoerucin was only found at day 0, and degraded during storage. On the other hand, the aliphatic glucoraphenin (GRE) and dehydroerucin (DER), also called glucoraphasatin, were the predominant GLS in radish sprouts (accounting each one around 40 % of the total). The loss of GRE after 7 and 14 d of storage at 5 ºC was similar to that in broccoli for GRA, being a 7 and 30 % of the total, respectively. Decreases of this compound by 60 and 80 % were found after 7 and 14 d of storage at 10 ºC. The loss of DER was higher, being around 30 and 60 % after 7 d of storage at 5 and 10 ºC, respectively, and a 30 % more at day 14. Even though similar amounts

292 of GRE and DER were found after storage at 5 ºC in this radish cultivar, some authors have shown
293 considerable variation in individual GLS among radish samples (Force et al., 2007). In terms of
294 bioactivity, GRE has appeared to have better potency than that derived from DER, therefore, it
295 should be noted that after 14 d of storage at 5 ºC, the amount of GRE (2.3 g kg^{-1}) was higher than
296 DER (1.4 g kg^{-1}). After 14 d of storage at low temperature, total GLS of radish sprouts (4 g kg^{-1})
297 were at least 8-fold higher than those found in radish mature taproots (~ 0.5 g kg^{-1}) (Yi *et al*.,
298 2016).
299 The three indole GLS present in radish sprouts (4-hydroxiglucobrassicin, methoxyglucobrassicin
300 and neoglucobrassicin), accounted for the 30 % of the total, and also decreased to a large extent
301 during storage at 10 ºC than at 5 ºC (Figure 1B).
302 Even though other authors showed a clear influence of the genetics on the glucosinolates stability
303 during refrigerated storage (Force *et al*., 2007; Vale *et al*., 2015), our results showed similar losses
304 of GLS at day 7 of storage (~25 %) in both cruciferous sprouts, demonstrating the great influence
305 of storage temperature, as at 5 °C a 35 % less of degradation took place. On the other hand, fresh
306 sprouts are an interesting source of ITC after consumption, as yield of GLS conversion to ITC of
307 raw vegetables is higher than cooked plants with denatured myrosinase because high temperatures
308 (Rungapamestry, Duncan, Fuller, & Ratcliffe, 2007).
309
310 ***Isothiocyanates***
311 Generally, studies about quality of *Brassicaceae* vegetables representing their effect in health
312 promotion are based on the content of GLS; however, the chemopreventive and anti-inflammatory
313 activities of these species are attributed to the hydrolysis compounds of GLS, the isothiocyanates
314 (ITC). The amount of these compounds could change depending on the GLS chemical structure
315 and the presence in the plant material of epithiospecifier proteins (ESP), ascorbic acid or Fe^{2+}, as
316 well as environmental conditions (Gu *et al*., 2012). Total ITC content in broccoli sprouts was 0.11
317 g kg^{-1}, being sulforaphane (derived from glucoraphanin) the predominant ITC (90 % of total ITC),
318 in accordance to Guo *et al*. (Guo, Guo, Wang, Zhuang, & Gu, 2013). Also iberin and indole-3-
319 carbinol (I3C) (hydrolyzed from glucoiberin and glucobrassicin, respectively) were analysed,

being only the 8 and 1 %, respectively (Figure 2A). Erucin (derived from glucoerucin) was not found in broccoli samples, may be because the quick degradation of GER in the samples. These values are in concordance with other authors who presented values of SFN in broccoli sprouts and florets ranging 0.03 – 0.2 g kg^{-1} depending on the variety and pre-harvest factors (Tian *et al*., 2016; R. Yang *et al*., 2015). Regarding radish sprouts, the predominant ITC found was sulforaphene (SFE), accounting for the 84 % of the total ITC analysed (11.75 mg ·$100g^{-1}$ F.W.) (Figure 2B). Raphasatin (derived from dehydroerucin) was not measured in the samples, as we could not find the pure standard, however, it is reported that this compound is very unstable, and it is rapidly degraded to less active compounds during hydrolysis in aqueous media (Kim, Kim, & Lim, 2015). Comparing to other works, similar amounts of ITC were described in radish taproot, but in these radish sprouts, higher concentrations of SFE were found (0.2 – 0.4 g kg^{-1}) (Hanlon & Barnes, 2011).

The ITC present in sprouts suffered a marked reduction during storage (Figure 2), being this decrease more than 90 % in all samples except for broccoli sprouts after 7 d of storage at 5 ºC, where the concentration of SFN was the 50 % of the initial amount (0.05 g kg^{-1}) (Figure 2A). These results are not in concordance with the contents of total GLS reported before in the sprouts, therefore, the hydrolysis of GLS to ITC due to the presence of the enzyme myrosinase in the sprouts could be decreased because cold temperatures of storage, decreasing the formation of ITC (Lim, Lee, & Kim, 2015). On the other hand, Campas-Baypoli *et al*. (2015), studied also a gradual decrease in SFN concentration up to day 14, showing that SFN is very unstable and tends to degrade rapidly in the food matrix even during refrigerated storage (4-5 ºC).

The I3C experienced also a significant and strong decrease of 85 % during the first 7 d of storage at both temperatures, however, remained unchanged until day 14 in both varieties, being the predominant indol derivative found at the end of storage (Figure 2). To the best of our knowledge, very little information was available regarding the changes in the ITC and indoles in *Brassicaceae* sprouts during storage and these results may be on practical applications to give recommendation on shelf-life conditions in terms of temperature and time of storage.

Comparing the content of ITC in broccoli and radish sprouts (Figure 2), even though similar amounts of SFN and SFE were found, respectively; these results are not definitive in order to justify the bioactivity of these cruciferous sprouts, because radish do not present epithiospecifier proteins (ESP) (Hanlon & Barnes, 2011; O'Hare et al., 2007), which presence in broccoli contributes to the formation of SFN-nitrile, a hydrolysis compound substantially less potent than SFN as an inducing agent of phase II detoxification enzymes (Matusheski & Jeffery, 2001), therefore, radish sprouts could show higher bioactivity than broccoli sprouts. Understanding the changes in the formation of ITC due to myrosinase activity in cruciferous sprouts during postharvest cold storage, as well as after consumption of these sprouts, is important for improving or maintaining their health benefits, as the content in their promoters GLS continues high after 7d at 5 ºC.

Phenolic compounds

Several authors have shown the polyphenolic profiling of cruciferous vegetables mainly composed of flavonol glycosides, and also quantificable amounts of chlorogenic, sinapic and ferulic acid derivatives (Cartea, Francisco, Soengas, & Velasco, 2011). Seeds and sprouts of those vegetables have usually a higher content of hydroxycinnamic acids, especially derivatives of sinapic acid. In this work, we have found only sinapic acid derivatives in broccoli (1.78 ± 0.1 g kg^{-1}) and radish (1.28 ± 0.01 g kg^{-1}) sprouts (Figure 3). Other authors showed very little concentrations of flavonols in broccoli and radish sprouts (0.005-0.01 g kg^{-1}) (Pajak, Socha, Galkowska, Roznowski, & Fortuna, 2014). The quality of the seeds, either for sprouting or for plant production, as well as the species or cultivars, are determinant factors to affect the phenolic compounds composition, such as in broccoli, where the concentration could vary little more than 0.3 up to more than 3 g kg^{-1} (Podsędek, 2007). Pérez-Balibrea *et al*., (2011) showed higher amount of flavonols than sinapic acid derivatives in broccoli sprouts, when using seeds for production of adult plants (cv. Nubia, Marathon and Viola), totally different than the varieties of broccoli and radish for sprouting purposes that we studied in this experiment.

These compounds were affected by time and temperature of storage (Figure 3). After 7 and 14 d of storage the concentration of sinapic and ferulic acid derivatives were lower than at day 0, nevertheless, the changes were not statistically different in broccoli sprouts (Figure 3A), but radish sprouts showed a significant decrease from day 7 to day 14 of storage (Figure 3B). At day 7 of storage at 5 °C, phenolic compounds were better maintained in radish than in broccoli sprouts, however, similar contents were found in both species (1.2 g kg^{-1}). The decrease in these compounds was about 70 % up to day 14 at 10 °C in the two varieties.

Vallejo *et al*., (2003) also reported high losses of hydroxycinnamic acids derivatives in broccoli inflorescences during transport (cold storage at 1 °C) and retail sale period (15 °C). Regarding changes according to temperature, phenolic compounds were better preserved at 5 ºC than at 10 ºC. To maintain the quality of sprouts during shelf-life, it is crucial to store the foods at low temperatures as soon as possible after harvesting, during commercialization, and at home. In spite of the progressive loss of phenolic compounds over time, their presence in sprouts is higher than amounts found in other broccoli sprouts (Vale *et al*., 2015), radish sprouts (Pajak *et al*., 2014), broccoli inflorescences (Vallejo, Tomás-Barberán, & García-Viguera, 2003) and radish mature leaves and taproots (Goyeneche *et al*., 2015). In spite of the loss of bioactive compounds and the microbial contents reported, no differences were appreciated in the aspect of sprouts after 7 and 14 d of storage. Bioactive compounds as well as nutrients present in broccoli and radish sprouts could be subjected to biotransformation by the microbial population in the seeds or sprouts, being responsible of the decrease reported in phytochemicals, among other factors such as metabolism and physiological changes in the plant.

CONCLUSIONS

As a global recommendation, the present study indicates that storage of broccoli and radish sprouts should be carried out at 4 - 5 °C, as recommended for domestic refrigerators (Kennedy, *et al*., 2005), in order to avoid extreme losses of bioactive compounds, and could be consumed up to 14 d in refrigeration maintaining a high amount of phytochemicals. On the other hand, sprouts could be considered safe fresh produce regarding their microbiological content, since no

pathogenic bacteria were found, even after long refrigerated storage (14 d). Both sprouts species may help in the design of more robust clinical studies to better evaluate the protective effects of crucifers in disease prevention and could be an appreciated healthy dietary alternative for consumers to enhance the concentration of health-promoting bioactive compounds in the diet.

ACKNOWLEDGEMENTS

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562 **FIGURE CAPTIONS:**

563

564 Figure 1. Individual and total glucosinolates present in 8-day-old broccoli (A) and radish (B)
565 sprouts (day 0) and after 7 and 14 d of storage at 5 °C and 10 °C. Mean values (n=3) and standard
566 deviations (± SD, error bars) are represented. Different lowercase letters (a-e) indicate statistically
567 significant differences among time points ($p<0.05$). Abbreviations: DER: Dehydroerucin; GB:
568 glucobrassicin; GER:glucoerucin; GIB: glucoiberin; GRA: glucoraphanin; GRE: clucoraphenin;
569 HGB: 4-hydroxyglucobrassicin; MET: 4-methoxyglucobrassicin; NEO: neoglucobrassicin.

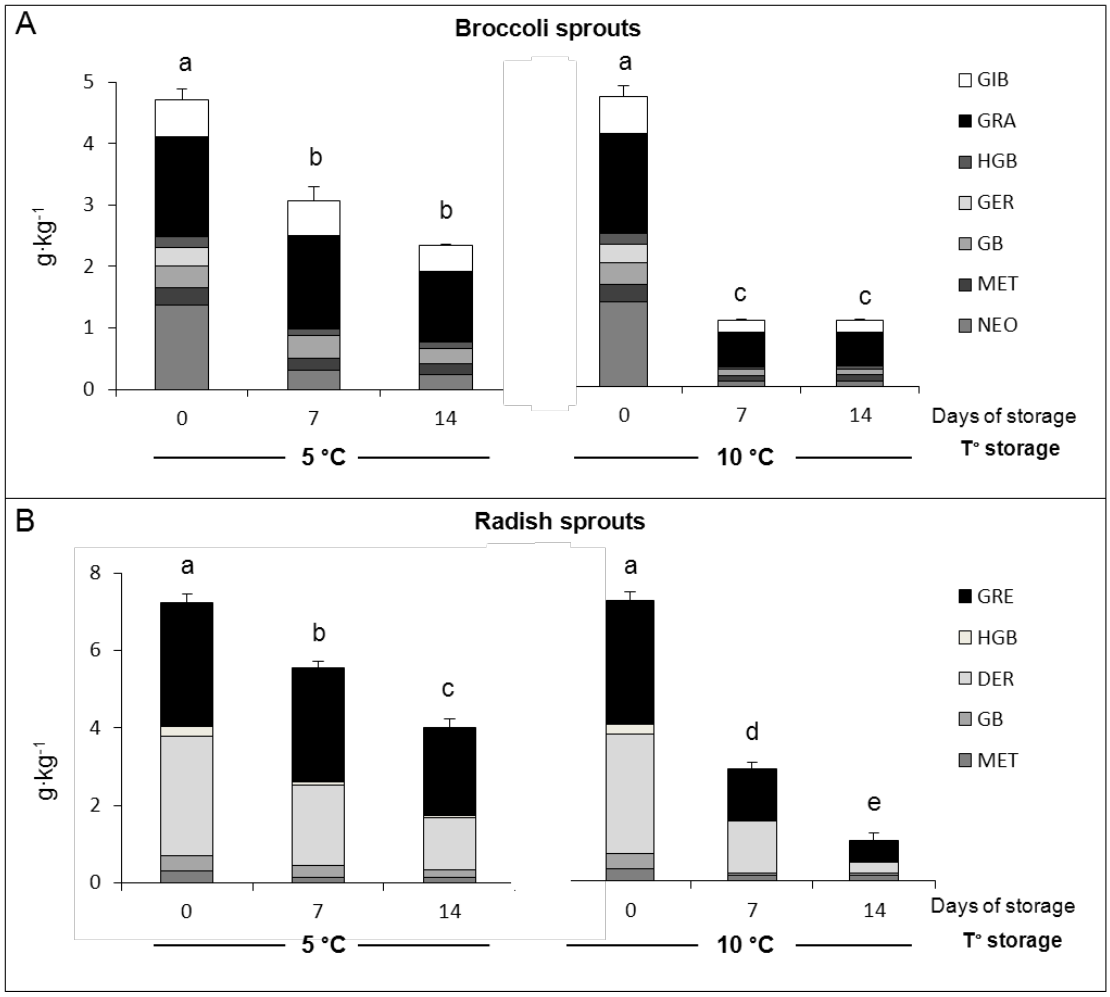
570

571 Figure 2. Individual and total isothiocyanates present in 8-day-old broccoli and radish sprouts
572 (day 0) and after 7 and 14 d of storage at 5 °C and 10 °C. Mean values (n=3) and standard
573 deviations (± SD, error bars) are represented. Different lowercase letters (a-e) indicate statistically
574 significant differences among time points ($p<0.05$). Abbreviations: IB: iberin; I3C: indole-3-
575 carbinol; SFN: sulforaphane; SFE: sulforaphene.

576

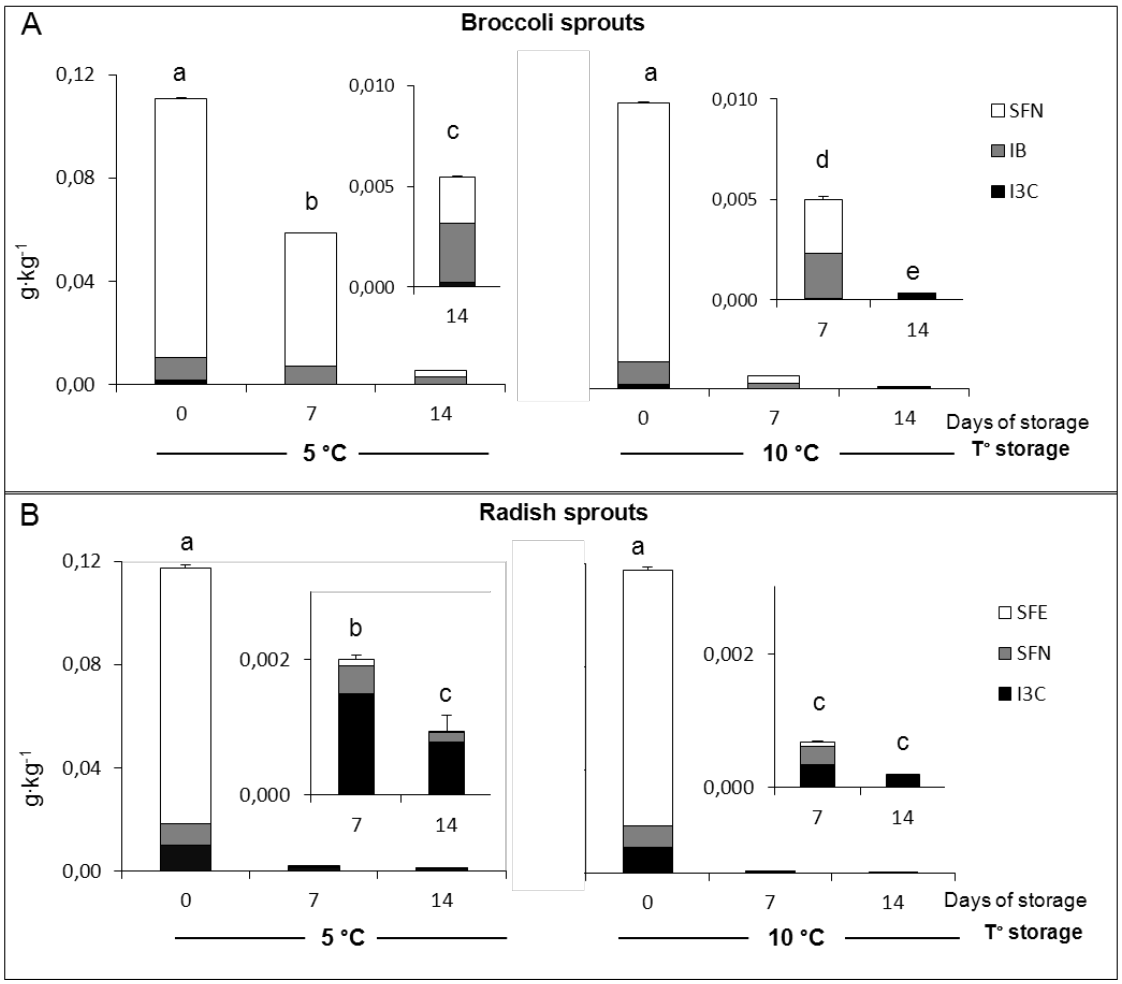
577 Figure 3. Total phenolic compounds as sinapic acid derivatives, present in 8-day-old broccoli and
578 radish sprouts (day 0) and after 7 and 14 d of storage at 5 °C and 10 °C. Mean values (n=3) and
579 standard deviations (± SD, error bars) are represented. Different lowercase letters (a-c) indicate
580 statistically significant differences among time points ($p<0.05$).

581 **Figure1.**



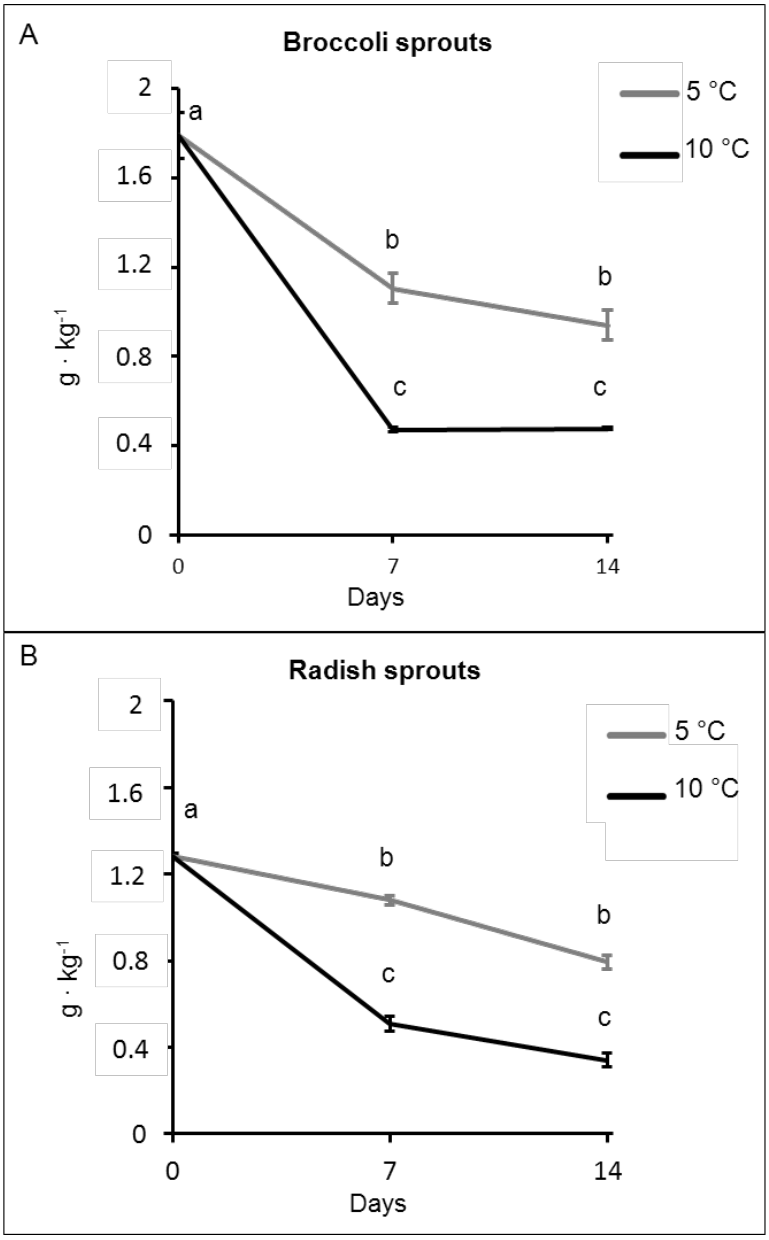
582

583 **Figure 2.**



584

585 **Figure 3.**



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588 **Tables**

589 **Table 1.** Mean value of log CFU/g for *Salmonella* spp., *Listeria* spp., *Clostridium perfringens*,
590 *Escherichia coli*, *Staphylococcus aureus*, *Enterobacteriaceae,* aerobic mesophilic bacteria,
591 aerobic psychrophilic bacteria and moulds and yeasts in broccoli sprouts at day 0, 7 and 14 d of
592 storage at 5 and 10 ºC.

593

Microorganism	t_0	5 ºC		10 ºC	
		t_{7d}	t_{14d}	t_{7d}	t_{14d}
Salmonella spp.	A	A	A	A	A
Listeria spp.	A	A	A	A	A
C. perfringens	<1	<1	<1	<1	<1
E.coli	<1	<1	<1	<1	<1
S.aureus	<2	<2	<2	<2	<2
Enterobacteriaceae	8.98 ± 0.01[a]	9.02 ± 0.35[a]	9.15 ± 0.62[a]	9.00 ± 0.56[a]	9.60 ± 0.53[a]
Aerobic mesophilic bacteria	8.90 ± 0.21[a]	9.53 ± 0.03[b]	10.06 ± 0.24[c]	9.60 ± 0.10[b]	10.04 ± 0.18[c]
Aerobic psychrophilic bacteria	8.94 ± 0.01[a]	9.30 ± 0.01[ab]	9.58 ± 0.01[b]	9.39 ± 0.16[ab]	10.19 ± 0.17[b]
Moulds and yeasts	7.87 ± 0.09[a]	7.73 ± 0.05[a]	8.47 ± 0.56[b]	8.15 ± 0.57[a]	8.59 ± 0.11[b]

[Ψ]A: absence in 25 g of sample
[a-c]Mean values (n=3) and standard deviations (± SD, error bars) are represented followed by the same letter within the row are not significantly different ($p<0.05$).

594

595 **Table 2.** Mean value of log CFU/g for *Salmonella* spp., *Listeria* spp., *Clostridium perfringens*,
596 *Escherichia coli*, *Staphylococcus aureus*, *Enterobacteriaceae,* aerobic mesophilic bacteria,
597 aerobic psychrophilic bacteria and moulds and yeasts in radish sprouts at day 0, 7 and 14 d of
598 storage at 5 and 10 ºC.

Microorganism	t_0	5 ºC		10 ºC	
		t_{7d}	t_{14d}	t_{7d}	t_{14d}
Salmonella spp.	A[Ψ]	A	A	A	A
Listeria spp.	A	A	A	A	A
C. perfringens	<1	<1	<1	<1	<1
E.coli spp.	<1	<1	<1	<1	<1
S.aureus	<2	<2	<2	<2	<2
Enterobacteriaceae	7.98 ± 0.05[a]	9.19 ± 0.38[b]	9.63 ± 0.02[c]	9.10 ± 0.34[b]	9.47 ± 0.35[c]
Aerobic mesophilic bacteria	8.83 ± 0.15[a]	9.83 ± 0.01[b]	9.93 ± 0.05[b]	9.98 ± 0.13[b]	10.10 ± 0.02[b]
Aerobic psichrophilic bacteria	8.83 ± 0.01[a]	9.54 ± 0.04[a]	9.28 ± 0.82[a]	9.71 ± 0.01[a]	10.23 ± 0.22[a]
Moulds and yeasts	7.70 ± 0.19[a]	7.62 ± 0.63[a]	8.60 ± 0.14[b]	8.11 ± 0.12[a]	8.75 ± 0.22[b]

[Ψ]A: absence in 25 g of sample
[a-c]Mean values (n=3) and standard deviations (± SD, error bars) are represented followed by the same letter within the row are not significantly different ($p<0.05$).

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