The effect of three organic pre-harvest treatments on Swiss chard (*Beta vulgaris* L. var. *cycla* L.) quality

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Abstract Despite the increasing interest in organic products, our understanding of how different organic treatments affect fruit and vegetable quality is still limited. The effect of three organic pre-harvest treatments [effective microorganisms (EM), a fermented mixture of effective microorganisms with organic matter (EM-Bokashi+EM), and an auxiliary soil product (Greengold®)] on Swiss chard quality was evaluated. The Swiss chard was analyzed 8 and 19 weeks after sowing. The treatments did not notably modify the physical and chemical quality of the chard when compared with control plants. Chard harvested 19 weeks after sowing showed greater differences in nutritional quality than chard harvested 8 weeks after sowing. Control plants had higher water content than the plants treated with EM, EM-Bokashi+EM and Greengold®. Chards treated with EM-Bokashi+EM had lower ascorbic acid content and higher phosphor and magnesium content than control plants. Application of EM to plants induced higher levels of calcium compared with non-treated plants.

Keywords *Organic production · Effective microorganisms · Bokashi · Greengold® · Physical and chemical quality · Nutritional quality · Swiss chard*

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Introduction

In multiple epidemiological studies eating vegetables has been found to protect against several chronic diseases associated with aging such as cardiovascular diseases and some types of cancer [1]. Swiss chard (*Beta vulgaris* L. var. *cycla* L.) is a leafy vegetable highly valued because it is available year round and for the nutritional properties of its leaves which contain considerable amounts of vitamin C, potassium, calcium and magnesium [2-4].

In the recent years, the growing consumer's awareness of health and safe-controlled foods, together with environmental protection plans, have determined a significantly increased interest in organic food [5,6]. Horticultural crops produced organically should behave differently than those produced with chemical fertilizers, pesticides and herbicides. Exposure to different chemicals, nutrients and cultivation techniques will probably affect the physiological response of the product. However, there is a relative scarcity of published research about the effect of organic production on quality of vegetables [7]. Woese et al. [8] presented a review of the literature of comparative studies between organically and conventionally produced foods. However, most of the reviewed material refers to nitrate contents, pesticide residues and other physico-chemical indexes and there is a lack of information regarding the physical, chemical and nutritional quality of organic foods.

Some studies have been developed about the quality of Swiss chard cultivated by organic production systems. The storage shelf life of Swiss chard produced by conventional and organic methods has been investigated [3]. The initial populations and evolution of yeast, molds and psychrotrophic, mesophilic and lactic acid bacteria, water content, chlorophyll content, pH and titratable acidity were similar for organic and conventional chards, stored at 4 ± 2ºC and 98 ± 1% HR for 25 days. However, sensorial

analysis showed that organic chard retained turgidity, color and brightness longer than conventional chard [3,9]. Moreover, the possibility of using essential oils, as an alternative to the use of synthetic chemicals to preserve organically grown Swiss chard, has been evaluated [10].

Organic farming involves different cultivation practices and limited use of non-synthetic fertilizers and conditioners. Among these substances, effective microorganisms (EM) can be pointed out. EM is a fermented mixed culture of three naturally-occurring species of microorganisms: phototrophic bacteria, lactic acid bacteria and yeasts, in acidic medium (pH below 3.5). This mixture is enriched naturally by other species such as filamentous fungi and Actinomycetes [11]. Research and field studies have shown that the inoculation of EM culture to the soil/plant ecosystem can improve growth, yield, and quality of crops and enhance soil physical and chemical properties [12-14]. EM have been also used to improve the beneficial effects of soil and crop management practices in organic production systems [15]. EM is often inoculated to organic matter fermented and it is known as Bokashi. This mixture is called EM-Bokashi and can improve the ability of microorganisms to break down organic matter, thereby providing plant nutrients to make better yield and quality [13,16]. Greengold® is an auxiliary soil product that can act in the recovery of soil texture and in the decomposition of toxic blockages from soil that are formed by the use of fertilizers and pesticides, which can affect to the final quality of the food plants.

The main objective of this study was to evaluate the effect of different organic pre-harvest treatments on the physical, chemical and nutritional quality of Swiss chard. The organic treatments evaluated were: effective microorganisms, a mixture of effective microorganisms with fermented organic matter and an auxiliary soil product made up of

organic salts, organic acids, plant extracts, polysaccharides, minerals and polyelectrolytes.

Materials and methods

Experimental field, crop conditions and plant material

The research study was done under organic production following the production standards established on the Council Regulation (EEC) 2092/91 [6] and its posterior modifications. The experiments were carried out at a 300 m^2 organic field located at Los Silos on the north of Tenerife (Canary Islands, Spain), over the period September 2004 - January 2005. The field was placed 111 m above sea level. The field was used for ecological production for 30 years.

During the study period, the average of the lowest and highest air temperatures were 14 and 20ºC, the average of the relative humidity was between 70 and 84% and the average rainfall and average daily solar radiation were 60 mm and 15500 W/m^2 day, respectively.

The land was tilled and the experiment, which had four treatments (three organic pre-harvest treatments and one control without pre-harvest treatment), was laid out in a random block design with four replicates per treatment (a total of 16 plots, each measuring 7 x 1.8 m).

Bressanne winter Swiss chard (*Beta vulgaris* L. var. *cycla* L.) seeds were planted on September 20, 2004 in straight lines, with 30 cm of space between each seed (138 seeds per plot).

Organic pre-harvest treatments

The four pre-harvest treatments which were evaluated consisted of: 1, control (without pre-harvest treatment); 2, effective microorganisms (EM); 3, EM-Bokashi+EM; and 4, Greengold®.

EM is available in a dormant state [EM-1® (Emiko, Swisttal-Heimerzheim, Germany)] and requires activation before application. Activation involves the preparation of a solution containing 3% EM-1® and 3% organic molasses (Emiko) in water. Molasses were dissolved with warm water before adding to make the preparation easier. The fermentation process took place away from direct sunlight at ambient temperatures for 7-14 days. The pH was always below 4.0. For EM application, the EM solution was diluted 1:500 and sprayed at a rate of 1.6 l/m^2 (equivalent to 3.2 ml EM/m^2). EM was spray-applied at 1-week intervals (application 19 times).

The treatment EM-Bokashi+EM consisted in the application of the product Eminent-Fertigbokashi® (Eminent natural GbR, Wildpoldsried, Germany) which is a mixture of EM with organic matter (wheat bran and chicken manure) fermented in an anaerobic way. This mixture was applied to the soil as fertilizing material and then EM was periodically added. The composition of the mixture EM-Bokashi was: total nitrogen, 2.1% N; ammonium nitrogen, 0.6% NH_4; total phosphate, 2.2% P_2O_5; soluble potassium, 2.0% K_2O; sulfur, 0.21% S; organic matter, 45%; dry matter, 52%. Two weeks before sowing, 0.40 kg bokashi/m^2 and a solution of EM (dilution 1:100, 1.6 l/m^2, equivalent to 16 ml EM/m^2) were applied to the plots. Therefore, EM was weekly (application 19 times) sprayed at a rate of 1.6 l/m^2 (dilution 1:1000, equivalent to 1.6 ml EM/m^2).

Greengold® (Naturgerecht, Bonn, Germany) is made up by organic salts (calcium, magnesium and potassium phosphates, and pebbles), organic acids, plant extracts,

polysaccharides, minerals (iron, cobalt, cupper, molybdenum, manganese, selenium, zinc, and boron), and polyelectrolytes. Greengold® was diluted 1:500 and applied two weeks after sowing at a rate of 1.2 l/m^2 (equivalent to 2.4 ml Greengold®/m^2). Then, it was sprayed at 6-weeks intervals (application 3 times).

The control plots were sprayed weekly with a volume of water equal to the volume of solution applied in EM-treated plots (1.6 l/m^2, application 19 times). Extreme caution was taken to avoid contamination between treatments. No fertilizers were applied and in the absence of rainfall the plots were irrigated when the top layer of soil (at a depth of 15 cm) was dry.

Sampling

Two samplings were done: 8 weeks after sowing (November 16 2004) and 19 weeks after sowing (January 31 2005). Each of the four plots used per treatment was divided in four sub-plots and chard samples (0.5 kg) were harvested from each of these sub-plots (n=16 for each pre-harvest treatment evaluated). Swiss chard was manually harvested by collecting leaves with an adequate size (length of the leaves: 24 ± 9 cm or 27 ± 4 cm for chard harvested 8 or 19 weeks after sowing respectively; width of the leaves: 8 ± 4 cm or 14 ± 2 cm for chard harvested 8 or 19 weeks after sowing respectively). Harvested leaves were transported about 45 km by ventilated car to the laboratory and immediately selected on the basis of integrity, and lack of evident defects or diseases.

Determination of physical and chemical parameters indicators of quality in Swiss chard

The effect of the different pre-harvest treatments assayed was evaluated by determining physical, chemical and nutritional quality of Swiss chard. Physical and chemical quality of chard was characterized by respiration rate, ethylene production, color, and taste [total soluble solids (TSS), pH, and titratable acidity]. Except for analysis of respiration rate and ethylene production (intact leaves were used), stems were removed and the green tissue was used for the other determinations. All analyses were done in triplicate (n=48 for each pre-harvest treatment evaluated).

Respiration (ml CO_2/kg h) and ethylene (µl C_2H_4/kg h) production were measured using two or three intact chard leaves (30-40 g) placed in sealed containers of 2.3 l for 1h and samples of 1 ml were obtained from the headspace of the containers. Carbon dioxide and ethylene were determined by infrared analysis and gas chromatography, respectively as recently reported [17].

Color was measured in three different points of each chard leave with a Minolta Chroma Meter CR-300 (Minolta Corp., Ramsay, USA) color difference meter, using attributes lightness (L), Hue and chromaticity (Chroma). TSS was determined using an Atago ATC-1 (Tokyo, Japan) hand refractometer and pH was measured by a WTW (Izasa, Madrid, Spain) pH-meter. After determination of pH, titratable acidity (mg malic acid/100 g fresh weight) was measured with 0.1 mol/l sodium hydroxide standard solution (Merck, Darmstadt, Germany) up to pH 8.1 [18].

Determination of nutritional parameters indicators of quality in Swiss chard

Nutritional quality of Swiss chard was characterized by water, proteins (total and soluble), ascorbic acid, and minerals (phosphor, sodium, potassium, calcium, magnesium, and iron) contents. Stems of the chard leaves were removed and the green tissue was used for the analyses. Previous to the soluble proteins and vitamin C determination, an aliquot of chard was frozen into liquid nitrogen and stored at -80ºC. Other aliquot of chard was desiccated in a forced-draft oven (65ºC) until constant weight and ground in a grinder until mineral analyses were carried out. All analyses were done in triplicate (n=48 for each pre-harvest treatment evaluated) and the results were expressed on the basis of fresh weight.

To determine water content (g/100 g) 50 g of Swiss chard were weighed and desiccated in a forced-draft oven at 65ºC until a constant weight was obtained. Then, the weight loss was used to calculate the water content.

Proteins (g/100 g) were quantified by using the Kjeldahl method [19] and the protein content was calculated using a nitrogen factor of 6.25. Soluble protein concentrations (mg/100 g) were measured by the Bradford method [20]. Spectrophotometric measurements were made on a Shimadzu (Kyoto, Japan) UV-visible 160A double-beam recording spectrophotometer at 595 nm. Bovine serum albumin was used as a standard (Sigma, Madrid, Spain).

For vitamin C quantification (mg ascorbic acid/100 g fresh weight), 1.0 g of frozen pulverized samples were mixed with 5 ml of 3% metaphosphoric acid and 8% acetic acid. The mixture was homogenized in an ice cooled Politron PT 6000 blender at 18000 g (in darkness) for 1 min and then centrifuged at 9000 g (refrigerated at 4ºC) for 20 min [21]. It was determined that this procedure must be repeated three times (data not

shown) and the three resulting supernatants were mixed together. All the operations were performed under reduced light and at 4ºC. Ascorbic acid (AA) was determined by the AOAC's official titrimetric method [18]. Because the AOAC method may overestimate the AA content, due to the presence of oxidizable species other than AA, all extracts were tested for interferences such as basic substances (using pH indicator thymol blue) and reducing ions Fe^{2+}, Sn^{2+} and Cu^{2+} (using indicators methylene blue and indigo carmine) before AA determination. None of the extracts contained interfering substances so titrimetric method could be applied to determine AA in Swiss chard. AA standard was obtained from Sigma. Calibration equation for AA was constructed by plotting the volume of indophenol solution against the AA concentration at seven concentration levels (analyzed in triplicate). Indophenol volume (y) over a concentration (x) range of 10 to 10 mg/l was linear ($y = 0.0311 + 0.0004$) with a regression coefficient (r^2) of 0.988. Detection limit was 2.5 mg/l. The relative standard deviation (RSD) for repeatability (11 consecutive analyses of a standard solution containing 50 mg/l of AA) and inter-day reproducibility (five parallel determination carried out for five consecutive days) were 5.0 and 5.2%.

To analyze minerals an amount accurately weighed at 0.5 g of dry ground chard was incinerated in a muffle furnace at 450ºC for 2 h. The ashes were treated with hot hydrochloric acid (1:1). Phosphor (mg P/100 g) was determined by spectrophotometry with a Technicon AutoAnalyzer II at 420 nm. Sodium (mg Na/100 g), potassium (mg K/100 g), calcium (mg Ca/100 g), magnesium (mg Mg/100 g), and iron (mg Fe/100 g) were determined by atomic absorption spectrometry, using a Perkin Elmer AAnalyst 100 atomic absorption spectrometer. To mask interferences in the determination of Na and K or Ca and Mg it was necessary to add 0.1% cesium or 1% lanthanum, respectively. All sample dilutions were made with deionized water of 18 MΩ/cm

resistivity obtained from a Milli-Q water purification system. Certified atomic absorption spectroscopic standard solutions (1 mg/ml) for minerals were purchased from Merck and working standard solutions were prepared by appropriate dilution of the stock solutions.

Statistical analysis

Data analysis was carried out with the Statgraphics Plus software version 5.1 (Statistical Graphics, Rockville, USA). Grubbs' test was applied to detect outliers in the data set. Analysis of variance (ANOVA) was used to evaluate the effect of the organic pre-harvest treatments on physical, chemical and nutritional quality of Swiss chard and the effect of sampling dates on quality of Swiss chard. Fisher's Least-Significant-Difference test, at the 5% significance level, was applied to experimental results to asses intra-pair significant differences.

Results and discussion

Physical and chemical quality of Swiss chard

Vegetable's maturity is associated with respiration because over maturity increases metabolism activity as a decay signal. There were no significant differences in the respiration rate for any of the chard treated with the organic pre-harvest treatments evaluated in this study at both sampling dates. However, average respiration rate was higher (for all the treatments) in the chard harvested 19 weeks after sowing (152 ± 19 ml CO_2/kg h) than in the collected 8 weeks after sowing (123 ± 23 ml CO_2/kg h). There was not detected ethylene production for any of the treatments evaluated.

The application of EM-Bokashi+EM to chard plants caused lower values of lightness (L) and chromaticity (Chroma) and higher values of Hue than those from the other treatments in leaves analyzed 8 weeks after sowing (Fig. 1). No significant differences in color attributes were observed between the vegetables treated with the different organic pre-harvest treatments when the samples were collected 19 weeks after sowing. Moreover, L and Chroma diminished (9-20% for L and 37-43% for Chroma) and Hue increased (3.1-5.3%) in the second sampling related to the first sampling for all the plants treated with the evaluated treatments. Lower values of L and Chroma parameters indicate a color less bright and vivid, which is related with the more dark green (higher Hue values) that showed chard treated with EM-Bokashi+EM 8 weeks after sowing and chard from all the treatments evaluated in the second sampling (19 weeks after sowing) related to the first sampling.

Fig. 1 Characterization of Swiss chard color using the attributes lightness (L), Hue and chromaticity (Chroma). Values followed by different lower or upper case letters present significant differences (p<0.05) between organic pre-harvest treatments or sampling dates, respectively

FIGURE 1.EPS

Fig. 2 shows the results of the quality parameters chosen to describe Swiss chard taste (TSS, pH, and titratable acidity). There were significant differences in the mean TSS content on the different samples according to the time of sowing or the organic treatments used. 8 weeks after sowing, chard treated with EM-Bokashi+EM and with Greengold® presented the highest TSS content (4.5 ± 0.7 ºBrix and 4.3 ± 0.4 ºBrix, respectively), while in the second sampling plants treated with Greengold® showed the highest TSS content (7.5 ± 0.3 ºBrix). TSS content of control plants (6.6 ± 0.4 ºBrix) was similar to the content of plants treated with EM and EM-Bokashi+EM (6.6 ± 0.7 ºBrix and 6.7 ± 0.2 ºBrix, respectively). TSS content was higher in the plants sampled 19 weeks after sowing than in those sampled 8 weeks after sowing. In the first sampling, pH of the chard treated with the treatments with effective microorganisms

(EM and EM-Bokashi+EM) was higher than pH of the vegetables treated with the other two treatments, but in second sampling there were no significant differences in pH in the different samples. 8 weeks after sowing, the titratable acidity was equal for all the treatments evaluated. Nevertheless, 19 weeks after sowing, chard treated with EM-Bokashi+EM presented the highest titratable acidity (267 ± 77 mg malic acid/100 g). Except for control plants, where titratable acidity did not change, this parameter increased between samplings. TSS content obtained for the evaluated pre-harvest treatments 19 weeks after sowing was similar to the TSS content described by Roura et al. [22] (7.0 ± 0.1 ºBrix). However, Moreira et al. [3] established a lower pH (6.1 ± 0.1 for organic chard leaves and 6.1 ± 0.02 for conventional chard leaves) and Roura et al. [22] found a higher titratable acidity (360 ± 50 mg malic acid/100 g).

Fig. 2 Characterization of Swiss chard taste using total soluble solids (TSS) content, pH, and titratable acidity. Values followed by different lower or upper case letters present significant differences (p<0.05) between organic pre-harvest treatments or sampling dates, respectively

FIGURE 2.EPS

Nutritional quality of Swiss chard

The mean and standard deviations (obtained from three replicates of each of the 16 samples analyzed) for the nutritional quality parameters studied on Swiss chard, treated with the different organic pre-harvest treatments evaluated in this study, are shown in Table 1. Similar levels of water content were found in the chard treated with the different treatments and harvested 8 weeks after sowing. However, this content changed significantly between samplings, being lower in the second sampling. Consequently, dry matter was higher in the chard harvested 19 weeks after sowing (10.4 ± 0.7% for control leaves and 11.1 ± 0.6% for the chard treated with the other treatments) than in the harvested 8 weeks after sowing (8.1 ± 1.1%). Because water content in chard leaves

harvested 19 weeks after sowing was lower than that in leaves from the first sampling, the differences in chard composition found between samplings can be correlated with a higher concentration of substances (pigments related with color, sugars and acids related with taste, phosphor, potassium, calcium and magnesium) in the leaves. Moreira et al. [3] reported an average water content of 92 ± 1% and 91 ± 1% for organic and conventional chard leaves, respectively, while USDA [4] found higher amounts: 93%.

Table 1
Nutritional composition of Swiss chard obtained by using different organic pre-harvest treatments

TABLE 1.DOC

There were no significant differences in protein (total and soluble) content for any of the organic pre-harvest treatments evaluated in this study at both sampling dates. Fibrous proteins, which form tissue structure, represent 68 ± 7% (first sampling) and 74 ± 5% (second sampling) of total proteins in chard. When the results obtained were compared with those described by other authors [2] differences were found: 2.9 ± 0.3 mg/100 g. USDA [4] established a protein content of 1.80, lower than that obtained in this study.

No significant differences were found between the ascorbic acid (AA) content of control plants and Swiss chard treated with EM and Greengold® at plants harvested 8 and 19 weeks after sowing. Nevertheless, application of EM-Bokashi+EM to plants reduced AA content at both sampling dates. Lisiewska et al. [23] have described how nitrogen fertilizers seem to decrease AA concentration. Since plant growth is generally enhanced by nitrogen fertilization it is possible that the nutrients in the plant tissues may be relatively diluted. Moreover, nitrogen fertilizers are also known to increase plant foliage, thereby reducing the light intensity and accumulation of AA in shaded plants [24]. Although chemical fertilizers were not used in this study, the different organic pre-harvest treatments that were evaluated may have different nitrogen compositions. It is

313 necessary to point out that the chard treated with EM-Bokashi+EM had the highest
314 number of leaves per plant, the longest average leaf length and the greatest yield (data
315 not shown). Except for chard treated with EM-Bokashi+EM, AA content increased with
316 sampling date. Agüero et al. [25] established AA content of 4.5 ± 0.3 mg/100 g in
317 summer chard (variety Lyon), which were similar to the results that were found in this
318 study for chard harvested 8 weeks after sowing. However, Moreira et al. [3] found an
319 AA content of 25 ± 5 and 23 ± 6 mg/100 g for organic and conventional winter chard
320 leaves (variety Bressanne) which also differed from the results that we indicate. These
321 differences could be attributed to the differences on pre-harvest factors, which largely
322 affect AA content of vegetables [24].
323 Phosphor (P) and sodium (Na) content of Swiss chard were similar regardless of pre-
324 harvest practices at both sampling dates. However, phosphor content increased from
325 first to second sampling. In this study the P and Na amount found were higher than that
326 obtained by USDA [4] 46 mg P/100 g and 213 mg Na/100 g, and by Macias et al. [2] 41
327 ± 5 mg P/100 g and 235 ± 14 mg Na/100 g. Similar to other vegetable foods [4],
328 potassium (K) was the most abundant mineral in Swiss chard. Statistical analysis did
329 not show significant differences in the content of potassium (K) as a function of pre-
330 harvest treatments 8 weeks after sowing. However, in the second sampling, the plants
331 treated with EM-Bokashi+EM showed higher concentrations of K (505 ± 52 mg/100 g)
332 than control plants (438 ± 84 mg/100 g). These results were similar to that described by
333 Macias et al. [2] for this mineral: (493 ± 19 mg/100 g), but higher than found by USDA
334 [4]. Except for control plants, K content increased in plants harvested 19 weeks after
335 sowing regarding to plants collected 8 weeks after sowing. In the first sampling, there
336 were no significant differences on calcium (Ca) and magnesium (Mg) content of the
337 chard leaves analyzed. The results indicate that the differences were statistically

338 significant for both minerals 19 weeks after sowing within the chard leaves from the
339 different pre-harvest treatments. Plants treated with EM showed higher Ca content (177
340 ± 20 mg/100 g) than the plants of the rest of the treatments, whereas the plants with the
341 highest Mg content were those treated with EM (126 ± 14 mg/100 g) and EM-
342 Bokashi+EM (136 ± 19 mg/100 g). Ca and Mg content changed significantly between
343 samplings, being higher in the plants harvested 19 weeks after sowing. USDA [4]
344 described Ca and Mg contents of 51 mg/100 g and 81 mg/100 g, respectively. Macias et
345 al. [2] established a Ca level of 101 ± 8 mg/100 g and a Mg level of 52 ± 3 mg/100.
346 Although, there were slight differences on iron (Fe) content between chard leaves from
347 the different pre-harvest treatments 8 weeks after sowing, there were no significant
348 differences on this mineral content (2.3 ± 0.6 mg Fe/100 g) between treatments 19
349 weeks after sowing. Fe levels were similar to those described for other authors [2,4].
350 Nutritional quality of Swiss chard treated with the organic pre-harvest treatments
351 evaluated in this study was similar to the quality of control plants (without any pre-
352 harvest treatment) 8 weeks after sowing. However, 19 weeks after sowing, slight
353 differences were found on nutritional quality of chard. Control leaves showed higher
354 water content than the chard leaves from the plants treated with EM, EM-Bokashi+EM
355 and Greengold®. Chards treated with EM-Bokashi+EM had lower AA content and
356 higher P and Mg content than control plants. Application of EM to plants induced
357 higher levels of Ca in chard leaves than when plants were not treated.
358 Table 2 shows the contribution of the consumption of 100 g of Swiss chard to the
359 daily dietary intake of the analyzed nutrients in relation to the Dietary Reference Intake
360 (DRI) values [26-30] or Dietary Reference Values (DRVs) [31]. DRI values are based
361 on Recommended Dietary Allowances (RDAs) or Adequate Intakes (AIs) when there is
362 not adequate scientific evidence to establish RDA values. The contribution of a serving

363 chard of 100 g to the protein intake in humans is low, but if it is compared with the
364 contribution of other fruits and vegetables it was considerable (3.4-3.8% and 4.1-4.6%
365 of the RDA for men and women, respectively). The consumption of chard has a
366 relatively low contribution to the intake of vitamin C, being this contribution lower in
367 the vegetables treated with EM-Bokashi+EM (6.4% and 7.7% of the RDA for men and
368 women, respectively, and 9.7% of the DRV) than in the chard treated with the other pre-
369 harvest treatments evaluated. The highest contribution was for Swiss chard treated with
370 EM, representing approximately 13% for men and 16% for women of the RDA and
371 20% of the DRV. The contribution of Swiss chard to the intake of minerals such as P,
372 Na, K, Ca, Mg, and Fe is, in general, very important. The K contribution was the lowest
373 among the analyzed minerals, with 9.1% of the AI for control chard and 9.8% of the AI
374 for the plants treated with EM-Bokashi+EM. However, the highest contribution was for
375 Mg and Fe, although it can be highlighted the important contribution of Ca to the daily
376 intake. Mg contributes to the intake of this mineral with a 25-37% of the RDA
377 (depending on the organic pre-harvest treatment, and sex or age of the reference
378 population) and 35-39% of the DRV. Although the contribution to the intake of Fe is
379 considerable (11-29% of the RDA and 14-16% of the DRV), it is not nutritionally
380 important because of the low bioavailability of Fe from leafy vegetables. It is important
381 to highlight the high contribution of a serving of 100 g of chard to the Na intake (19-
382 21% of the AI). Moreover, the contribution of Na to the Tolerable Upper Intake Level
383 (UL), defined as the highest level of daily nutrient intake that is likely to pose no risk of
384 adverse health effects to almost all individuals in the general population, is very high
385 (14%). These contributions must be considered because it is known that increased
386 sodium chloride intake increases blood pressure, and it is associated with an increased
387 risk of cardiovascular outcomes, and possibly with an increased risk of asthma and

388 gastric cancer [30]. For those analyzed nutrients (vitamin C, P, Ca and Fe) which UL
389 has been determined, the contribution of a chard serving of 100 g to UL is very low:
390 0.60% for vitamin C, 2.7% for P, and 5.1% for Ca and Fe.

391 Table 2
392 Contribution to daily dietary intake of the adult population of water, proteins, vitamin C and minerals
393 (sodium, potassium, calcium, magnesium and iron) for the consumption of 100 g of Swiss chard obtained
394 by using different organic pre-harvest treatments

395 TABLE 2.DOC

396 The comparison of the nutritional quality of Swiss chard (*Beta vulgaris* L. var. *cycla*
397 L.), 19 weeks after sowing, with beet (*Beta vulgaris* L. var. *crassa* (Alef.) J. Helm),
398 other vegetable of the same specie, indicates that chard has higher contents of nutrients
399 [4] such as proteins (beet content, 1.6 ± 0.1 g/100 g), vitamin C (4.9 ± 1.5 mg/100 g), P
400 (40 ± 5 mg/100 g), Na (78 ± 10 mg/100 g), K (325 ± 15 mg/100 g), Ca (16 ± 2 mg/100
401 g), Mg (23 ± 2 mg/100 g), and Fe (0.80 ± 0.20 mg/100 g) than beet. From the viewpoint
402 of nutrition, Swiss chard has considerable amounts of vitamin C, K, Ca and Mg. If it is
403 compared with other leafy green vegetables, chard harvested 19 weeks after sowing
404 provides more K and Fe than lettuce (romaine lettuce, 247 ± 8 mg K/100 g and 0.97 ±
405 0.08 mg Fe/100 g; iceberg lettuce, 141 ± 3 mg K/100 g and 0.41 ± 0.04 mg Fe/100 g;
406 green leaf lettuce, 194 ± 10 mg K/100 g and 0.86 ± 0.12 mg Fe/100 g) and watercress
407 (330 mg K/100 g and 0.20 mg Fe/100 g), and more Ca and Mg than lettuce (romaine
408 lettuce, 33 ± 1 mg Ca/100 g and 14 ± 0.3 mg Mg/100 g; iceberg lettuce, 18 ± 0.4 mg
409 Ca/100 g and 7 ± 0.2 mg Mg/100 g; green leaf lettuce, 36 ± 2 mg Ca/100 g and 13 ± 1
410 mg Mg/100 g), spinach (99 ± 5 mg Ca/100 g and 79 ± 5 mg Mg/100 g), and watercress
411 (120 mg Ca/100 g and 21 mg Mg/100 g) [4].
412

Conclusions

The organic pre-harvest treatments do not notably modify the quality of Swiss chard compared to control plants (without any pre-harvest treatment). Moreover, the differences found on physical and chemical quality were not very important from a sensorial viewpoint. It can be highlighted that applying EM-Bokashi+EM reduced ascorbic acid content in plants harvested 8 and 19 weeks after sowing. This may be due to higher nitrogen content in this organic pre-harvest treatment enhancing plant growth, thus producing a relative dilution of ascorbic acid in plant tissues. However, although both samplings were done after the Swiss chard's growing cycle was completed (between 55-80 days) the greatest difference in chard quality was registered between these samplings. Moreover, greater differences in nutritional quality were found in chard harvested 19 weeks after sowing than in that harvested 8 weeks after sowing. In the second sampling, control plants showed higher water content than the plants treated with EM, EM-Bokashi+EM and Greengold®. Chard treated with EM-Bokashi+EM had lower ascorbic acid content and higher phosphor and magnesium content than control plants. Application of EM to plants induced higher levels of calcium in chard leaves than when plants were not treated.

From the viewpoint of nutrition, the consumption of Swiss chard contributes significantly to the intake of vitamin C and minerals such as potassium, calcium, magnesium and iron. It is also important to emphasize that chard has a great deal of sodium, due to the negative effects that elevated intakes of this mineral has on human health.

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Table 1
Nutritional composition of Swiss chard obtained by using different organic pre-harvest treatments

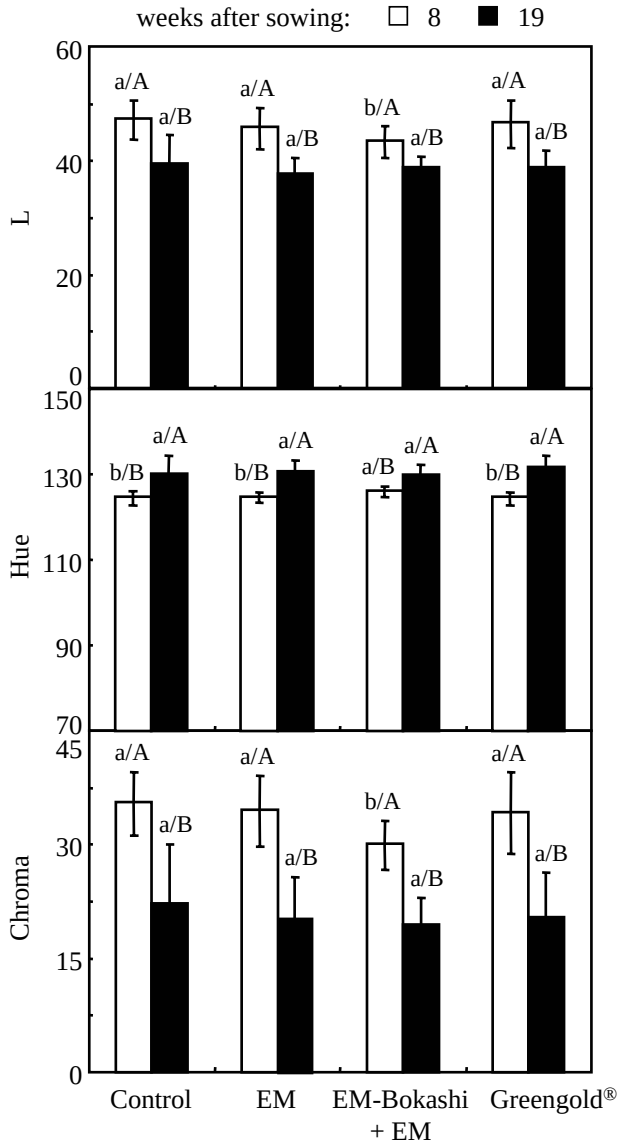
Nutritional parameter[a]	Time after sowing (weeks)	Control	EM	EM-Bokashi + EM	Greengold®
water (g/100 g)	8	92 ± 2 $^{a/A}$	92 ± 1 $^{a/A}$	92 ± 1 $^{a/A}$	92 ± 1 $^{a/A}$
	19	90 ± 1 $^{a/B}$	89 ± 1 $^{b/B}$	89 ± 1 $^{b/B}$	89 ± 1 $^{b/B}$
proteins (g/100 g)	8	1.7 ± 0.3 $^{a/B}$	2.0 ± 0.3 $^{a/A}$	2.0 ± 0.4 $^{a/A}$	1.9 ± 0.4 $^{a/B}$
	19	2.0 ± 0.3 $^{a/A}$	2.2 ± 0.5 $^{a/A}$	2.2 ± 0.4 $^{a/A}$	2.4 ± 0.5 $^{a/A}$
soluble proteins (mg/100 g)	8	579 ± 176 $^{a/A}$	563 ± 127 $^{a/A}$	671 ± 186 $^{a/A}$	599 ± 180 $^{a/A}$
	19	582 ± 126 $^{a/A}$	556 ± 203 $^{a/A}$	533 ± 116 $^{a/B}$	617 ± 218 $^{a/A}$
vitamin C (mg ascorbic acid/100 g)	8	5.4 ± 1.2 $^{a/B}$	5.3 ± 1.1 $^{a/B}$	4.1 ± 0.7 $^{b/A}$	5.3 ± 1.1 $^{a/B}$
	19	11 ± 6 $^{a/A}$	14 ± 9 $^{a/A}$	6.4 ± 2.1 $^{b/A}$	11 ± 6 $^{a/A}$
phosphor (mg P/100 g)	8	70 ± 27 $^{a/B}$	68 ± 28 $^{a/B}$	81 ± 34 $^{a/B}$	85 ± 41 $^{a/B}$
	19	119 ± 45 $^{a/A}$	121 ± 30 $^{a/A}$	137 ± 45 $^{a/A}$	125 ± 44 $^{a/A}$
sodium (mg Na/100 g)	8	300 ± 52 $^{a/A}$	301 ± 71 $^{a/A}$	292 ± 75 $^{a/A}$	276 ± 35 $^{a/A}$
	19	327 ± 60 $^{a/A}$	322 ± 42 $^{a/A}$	293 ± 42 $^{a/A}$	295 ± 53 $^{a/A}$
potassium (mg K/100 g)	8	415 ± 33 $^{a/A}$	412 ± 53 $^{a/B}$	413 ± 71 $^{a/B}$	412 ± 39 $^{a/B}$
	19	438 ± 84 $^{b/A}$	481 ± 63 $^{ab/A}$	505 ± 52 $^{a/A}$	468 ± 43 $^{ab/A}$
calcium (mg Ca/100 g)	8	71 ± 10 $^{a/B}$	76 ± 13 $^{a/B}$	76 ± 24 $^{a/B}$	79 ± 21 $^{a/B}$
	19	144 ± 35 $^{b/A}$	177 ± 20 $^{a/A}$	131 ± 21 $^{b/A}$	137 ± 32 $^{b/A}$
magnesium (mg Mg/100 g)	8	88 ± 15 $^{a/B}$	95 ± 15 $^{a/B}$	94 ± 21 $^{a/B}$	89 ± 9 $^{a/B}$
	19	121 ± 14 $^{b/A}$	126 ± 14 $^{ab/A}$	136 ± 19 $^{a/A}$	120 ± 16 $^{b/A}$
iron (mg Fe/100 g)	8	2.0 ± 0.6 $^{ab/A}$	2.4 ± 1.1 $^{a/A}$	1.5 ± 0.8 $^{b/B}$	1.8 ± 0.5 $^{b/B}$
	19	2.3 ± 0.8 $^{a/A}$	2.3 ± 0.6 $^{a/A}$	2.2 ± 0.5 $^{a/A}$	2.4 ± 0.6 $^{a/A}$

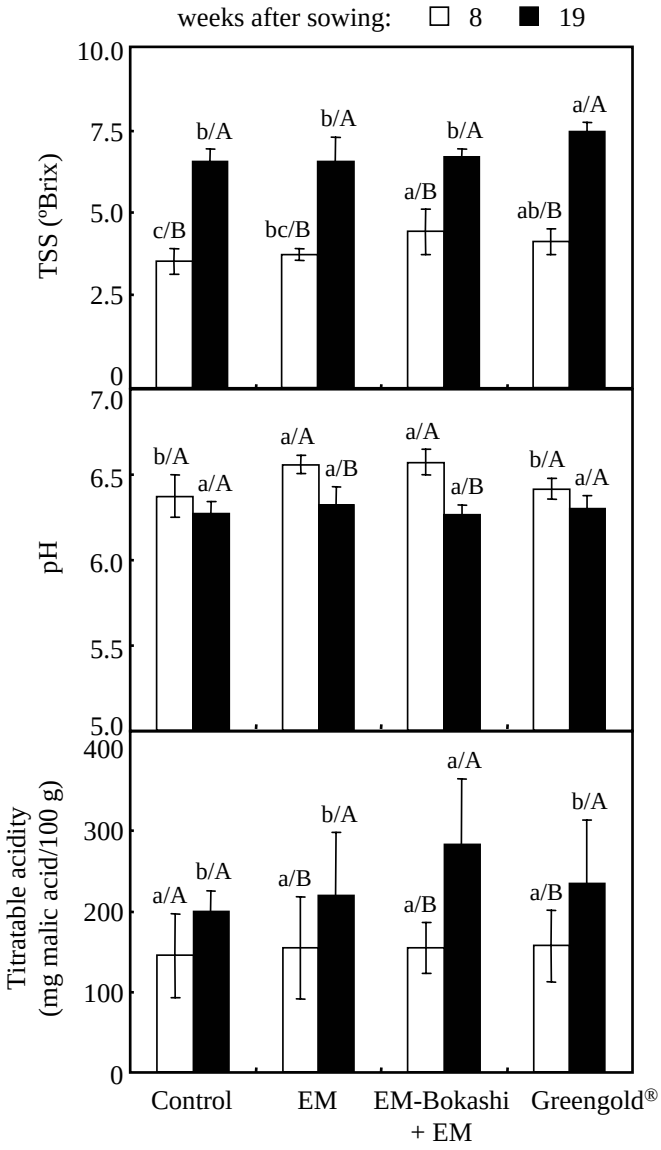
Within a row (lower case letters) or a column (upper case letters), different letters denotes significant differences (p<0.05) between organic pre-harvest treatments or sampling dates, respectively.
[a] Mean ± standard deviation (n=48).

Table 2
Contribution to daily dietary intake of the adult population of water, proteins, vitamin C and minerals (sodium, potassium, calcium, magnesium and iron) for the consumption of 100 g of Swiss chard obtained by using different organic pre-harvest treatments

Nutrient	DRI[a] or DRV[b] (mg/day)[c]	Control		EM		EM-Bokashi + EM		Greengold®	
		intake (mg/day)[c]	% of DRI or DRV	intake (mg/day)[c]	% of DRI or DRV	intake (mg/day)[c]	% of DRI or DRV	intake (mg/day)[c]	% of DRI or DRV
water[d]	**703 (513)**[e]	91	13 (18)[e]	90	13 (18)[e]	90	13 (18)[e]	90	13 (18)[e]
proteins	56 (46)[e]	1.9	3.4 (4.1)[e]	2.1	3.8 (4.6)[e]	2.1	3.8 (4.6)[e]	2.1	3.8 (4.6)[e]
vitamin C	90 (75)[e] 60[b]	10	11 (13)[e] 17[b]	12	13 (16)[e] 20[b]	5.8	6.4 (7.7)[e] 9.7[b]	10	11 (13)[e] 17[b]
phosphor	700 800[b]	94	13 12[b]	94	13 12[b]	109	16 14[b]	106	15 13[b]
sodium	**1500**	313	21	312	21	292	20	286	19
potassium	**4700**	427	9.1	447	9.5	459	9.8	443	9.4
calcium	**1000-1200** 800[b]	109	11-9.1 14[b]	128	13-11 16[b]	103	10-8.6 13[b]	108	11-9.0 14[b]
magnesium	400-420 (310-320)[e] 300[b]	105	26-25 (34-33)[e] 35[b]	111	28-26 (36-35)[e] 37[b]	116	29-28 (37-36)[e] 39[b]	105	26-25 (34-33)[e] 35[b]
iron	8 (8-18)[e] 14[b]	2.2	28 (28-12)[e] 16[b]	2.3	29 (29-13)[e] 16[b]	1.9	24 (24-11)[e] 14[b]	2.1	26 (26-12)[e] 15[b]

[a] DRI, Dietary Reference Intake based on Recommended Dietary Allowances or Adequate Intakes (indicated in bold).
[b] DRV, Dietary Reference Value.
[c] mg/day, except for water or proteins that data correspond to ml/day or g/day, respectively.
[d] DRI data correspond to 19% of the total adequate intake of water (amount that is provided by water contained in food).
[e] The values for women are indicated in parentheses.


weeks after sowing: 8 19
L
Hue
Chroma
60
40
20
0
150
130
110
90
70
45
30
15
0
a/A
a/B
b/A
b/B
Control
EM
EM-Bokashi + EM
Greengold®


weeks after sowing: 8 19
TSS (°Brix)
10.0
7.5
5.0
2.5
0
c/B
b/A
bc/B
b/A
a/B
b/A
ab/B
a/A
pH
7.0
6.5
6.0
5.5
5.0
b/A
a/A
a/A
a/B
a/A
a/B
b/A
a/A
Titratable acidity
(mg malic acid/100 g)
400
300
200
100
0
a/A
b/A
a/B
b/A
a/B
a/A
a/B
b/A
Control
EM
EM-Bokashi + EM
Greengold®