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Mixing chia seeds and sprouts at different developmental stages: A cost-effective way to improve antioxidant vitamin composition

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ABSTRACT

Various approaches can be used to improve chemical food composition avoiding the low acceptance risks that imply the use of transgenic crops. Here, we evaluated the antioxidant vitamin composition of dry and germinating seeds and sprouts of chia and examined the potential of exploiting natural variation of developmental stages to improve vitamin contents in chia-derived foodstuffs. Results showed that dry seeds contained the highest contents of vitamin E, with values 8-fold higher compared to sprouts. Vitamin C contents strongly increased just after seed imbibition, so that germinating seeds contained 5- and 17.5-fold higher values than dry seeds and sprouts, respectively. Sprouts displayed the highest contents of carotenoids (including β-carotene [provitamin A]). We conclude that mixing dry seeds, germinating seeds and sprouts (in a proportion of 1.5:2:1 w/w/w) may be a cost-effective way to obtain an optimal composition of antioxidant vitamins in foodstuffs such as salads.

1. Introduction

Plant-based foods are an essential part of a healthy and balanced diet. This kind of foods are rich in nutrients and bioactive compounds that contribute to maintain our healthy status. In the last years, the demand of foods that not only provide nutrients and satisfy hunger but also promote beneficial health effects has increased (Dinçoğlu & Yeşildemir, 2019). This kind of foods can be defined as functional foods. In this context, according to the Sustainable Development Goals (SDGs), modern agriculture and horticulture will face two main challenges during this century: provide people with high nutritional quality food and minimize the environmental impact of its production. These challenges are not exclusive, and they must be faced as a whole objective. There are different approaches to improve chemical food composition in agriculture: transgenic crops, conventional breeding, and agronomy (Marques et al., 2021). The use of transgenics is not globally accepted since transgenesis is a controversial topic both in terms of legislation and public opinion (Malyska et al., 2016). Furthermore, transgenic crops imply several environmental risks, both direct and indirect. These risks include potential effects on soil and water quality, biodiversity loss, genetic erosion and changes in persistence or invasiveness of crops (Dale et al., 2002). Thus, transgenic crops are not the best approach if we want to guarantee sustainability in food production and global acceptance, so non-transgenic approaches are usually given a priority to them.

Chia (*Salvia hispanica* L.) is a plant originating from the region between northern Mexico and Guatemala which oleaginous seeds have fed humans since the Aztecs era, by the year 3500 BCE (Dinçoğlu & Yeşildemir, 2019). These seeds are known for its desirable nutritional value, and they are highly appreciated in the agri-food industry, so they are considered functional foods. They contain high levels of essential ω-3 and ω-6 fatty acids, fibre, proteins, polyphenolic compounds, and minerals (Dinçoğlu & Yeşildemir, 2019; Kulczyński et al., 2019). Moreover, chia seeds have antioxidant, anti-inflammatory and cardioprotective properties (Ullah et al., 2016). Nevertheless, chia sprouts, which are relatively unknown compared with seeds, have recently sparked the interest of researchers and consumers. It has been demonstrated that these sprouts have invaluable nutritional properties, and they have a huge potential in the agri-food industry. Chia sprouts have higher antioxidant activity and contain more phenolic compounds, flavonoids, essential amino acids and γ-aminobutiric acid (a compound with

Abbreviations: ABA, abscisic acid; GAs, gibberellins; HPLC, high performance liquid chromatography; MeJA, methyl jasmonate; MeSA, methyl salicylate; PGRs, plant growth regulators; RDA, recommended dietary allowance; ROS, reactive oxygen species.

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antidiabetic, anticancer and anti-inflammatory properties) than chia seeds (Abdel-Aty et al., 2021; Gómez-Favela et al., 2017; Mlinarić et al., 2020; Pająk et al., 2019). Therefore, chia sprouts can provide to consumers not only organoleptic properties completely different from seeds but also high contents of bioactive compounds.

Although chia seeds and sprouts have been widely studied, there are still compounds that have not been described yet in this system. Some of these compounds are especially important for human health, such as vitamins C and E which are antioxidants which play essential functions in our body. They are not synthesized by humans so they must be acquired from diet (Granger & Eck, 2018). Vitamin E is a group of eight liposoluble compounds, including four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ). Vitamin E counteracts oxidative stress and prevents cancer and other age-related diseases by modulating cell signalling and gene expression, preserving membrane stability, and avoiding lipoprotein oxidation (Azzi et al., 2004; Clarke et al., 2008). On the other hand, vitamin C is a water-soluble antioxidant which is essential for collagen synthesis and contributes to iron uptake and epigenetic regulation (Grosso et al., 2013; Paciolla et al., 2019). Furthermore, it is a cofactor in cholesterol, catecholamines, amino acids and some peptide hormones synthesis (Grosso et al., 2013). Vitamin C contributes to a proper immune function and reduces inflammation and thus oxidative damage, preventing the onset and progression of several chronic and acute diseases (Grosso et al., 2013). Another important group of antioxidants are carotenoids, such as β-carotene, an important compound for human health, that must also be acquired from the diet. It is the precursor of vitamin A (pro-vitamin A) and, along with other carotenoids, have been previously studied in chia sprouts (Bermejo et al., 2021). Vitamin E, vitamin C and β-carotene do their own specific functions in our organism, but they are known to also act synergistically. It has been widely reported that vitamin C contributes to the regeneration of vitamin E (Chan, 1993; Packer, Slater & Willson, 1979; Sato, Niki & Shimasaki, 1990). It has also been suggested that β-carotene and vitamin E may act synergistically by scavenging radicals at different positions in membranes (Niki et al., 1995). It has also been known for decades that vitamin C, vitamin E and β-carotene offer a synergistic cell protection against reactive nitrogen species (Böhm et al., 1998).

Due to the important role of these three compounds, both individually and synergistically, we examined the chemical composition of antioxidant vitamins in chia seeds (both dry and germinating seeds) and sprouts. To improve vitamin E and vitamin C composition in chia sprouts through non-transgenic approaches, we also tested plant growth regulators (PGRs), a typical approach in plant nutritional value improvement. These PGRs are known to be involved in stress tolerance mechanisms in plants. Thus, we hypothesized that these PGRs may enhance the antioxidant machinery of chia sprouts, which is a strategy commonly used by stressed plants to eliminate reactive oxygen species (ROS). We propose here a cost-effective way to obtain high levels of antioxidant vitamins by exploiting the natural variation of developmental stages of chia and discuss its advantages and limitations.

2. Materials and methods

2.1. Plant material, treatments, and samplings

Chia (*Salvia hispanica* L.) seeds, which were obtained in a local supermarket, were sown on terracotta plates of 15 cm of diameter. Plates were put inside glass casseroles of 1.5 L of capacity with lids not hermetically closed. Previously, 680 mL water were put inside each casserole, so that the plates were half dipped, and the porous material could absorb water. Chia seeds were moistened every 24 h by spraying them with distilled water to guarantee a homogeneous distribution of humidity and make possible a proper seed imbibition, germination, and growth. Seeds remained in darkness for 4 days by covering the casseroles with aluminium foil. On day 5, the plant material received, all of it equally, a light stimulus for 30 min by uncovering the casseroles and exposing them to light. From day 6 to 8, de-etiolated sprouts were exposed to a prolonged light stimulus for 48 h to ensure a proper development of green sprouts. Temperature was kept between 20 °C and 23 °C and relative humidity between 48 % and 55 % throughout the experiments.

To evaluate the antioxidant vitamin composition of dry, germinating seeds and sprouts of chia (experiment 1), five samplings were performed on untreated plants at various developmental stages: day 0 (dry seeds), day 2 (germinating seeds), day 3 (etiolated sprouts), day 6 (de-etiolated sprouts) and day 8 (green sprouts). The germination process started at day 0 and ended at day 8, when chia sprouts reached an optimal developmental stage for consumption.

To evaluate the effects of PGRs on the antioxidant vitamin contents of chia sprouts (experiment 2), the following treatments were tested: (i) controls, (ii) abscisic acid (ABA), (iii) methyl jasmonate (MeJA), (iv) methyl salycilate (MeSA), (v) Promalin® (P), (vi) P + ABA, (vii) P + MeJA, (viii) P + MeSA and (ix) P + ABA + MeJA + MeSA. Promalin® is a commercial product containing gibberellins (GAs) and cytokinins (GA4/GA7 1.9 % w/v + 6-benzyladenine 1.9 % w/v). This product was applied to antagonize a possible germination inhibitory effect of ABA. Treatments were applied twice (on days 2 and 5) by spray, and during these two days no additional distilled water was applied to the plant material. A concentration of 100 μM of each PGR was applied (Suppl. Table 1). In the case of Promalin®, the sum of the two GAs was 100 μM. Tween 80 was used as a surfactant to ensure the correct absorption of PGRs, and methanol was used as a solvent for the different PGRs. The surfactant concentration was always 0.1 % and the concentration of methanol was kept to a minimum but slightly differed in each case depending on the need to dissolve the corresponding PGR (Suppl. Table 1). Promalin®, as a commercial product, did not require any extra additives and it was applied by diluting the commercial product 570 times. All solutions were made with distilled water.

To evaluate the effects of pyrabactin on vitamin E contents of chia sprouts (experiment 3), the following treatments were tested: (i) controls and (ii) pyrabactin. The procedure was as described for experiment 1 but using pyrabactin. The concentration of pyrabactin was 100 μM. Methanol and Tween 80 were also used (Suppl. Table 1).

To evaluate the effects of PGRs (experiments 2) and pyrabactin (experiment 3), only green sprouts (day 8) were collected. In all cases, the plant material that was taken for each sampling was randomly chosen. For each sampling, the plant material was put into small aluminium foil envelopes and frozen with liquid nitrogen. Then, samples were stored at −80 °C until they were analysed.

2.2. Vitamin E analysis

For vitamin E analysis, 50 mg of ground sample was extracted with 100 % cold methanol, subjected to ultrasounds for 30 min and then centrifuged at 10,000 rpm for 10 min. The supernatant was collected and the process was repeated twice, obtaining a final volume of 1 mL (always at 4 °C). Supernatants were pooled, the final extract filtered using single-use hydrophobic 0.45 μm PTFE filter and transferred to glass vials prior to high-performance liquid chromatography (HPLC) analysis. The HPLC system consisted of a Waters 600 controller pump, a Jasco FP-1520 fluorescence detector and a Waters 717 plus autosampler. The mobile phase was composed by *n*-hexane and 1,4-dioxane (95,5:4,5 v/v). Vitamin E compounds (α-, β-, δ- and δ -tocopherol, and α -, β -, δ - and δ -tocotrienol) were separated using an Inertsil 100A column (5 μm, 30 μm × 250 μm, GL Sciences Inc., Tokyo, Japan). Fluorescence detection was performed with an excitation wavelength of 295 nm and emission at 330 nm. The vitamin E compounds were quantified using a calibration curve with authentic standards from Sigma-Aldrich (Steinheim, Germany).

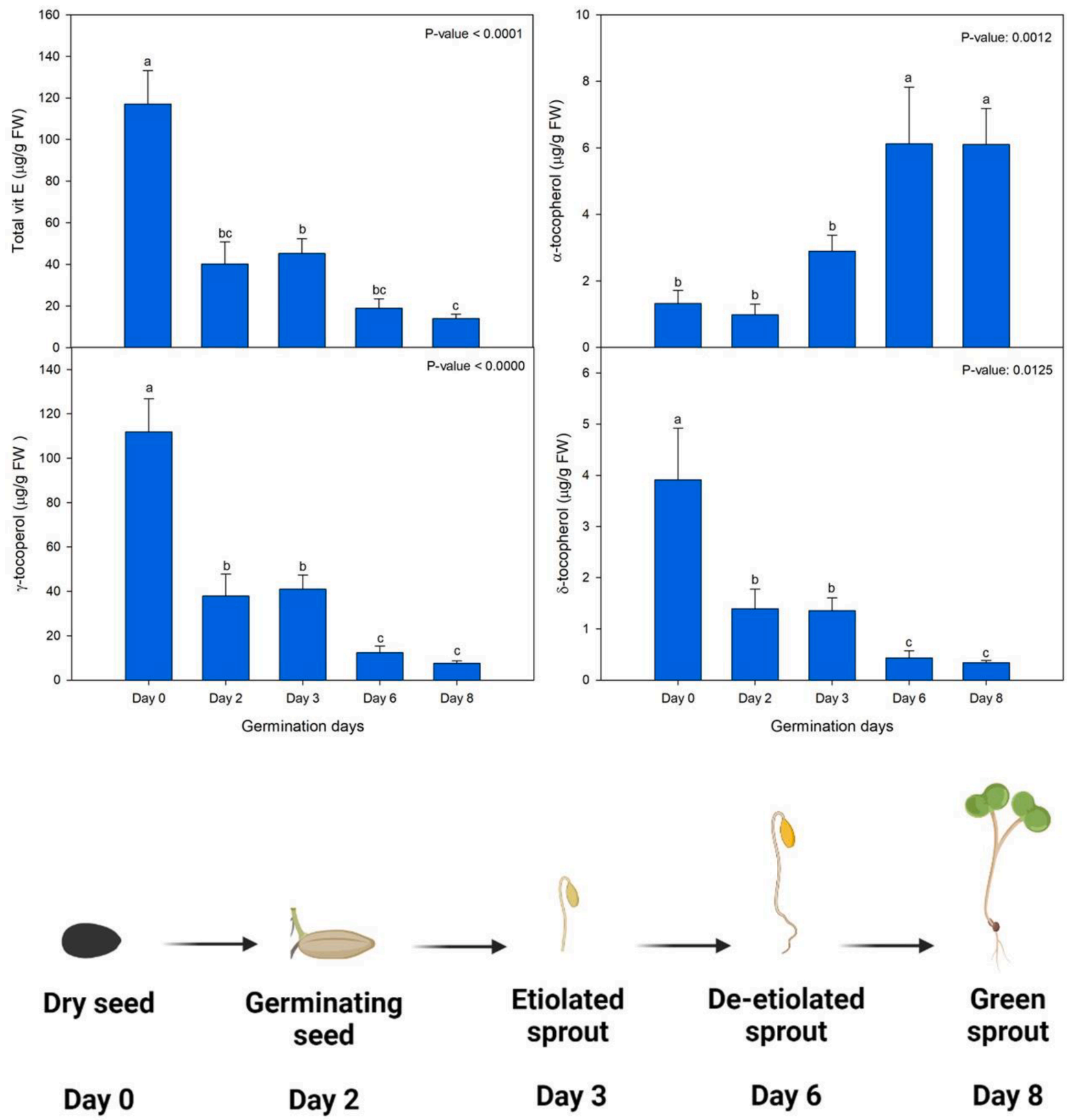

Fig. 1. Vitamin E contents (µg/g fresh weight) in chia during germination. The data is shown as mean ± SE of n = 5 replicates. Different letters indicate significant differences among germination days (P-value < 0.05). Below, graphical representation of each developmental stage during germination (created with biorender.com).

2.3. Vitamin C analysis

For vitamin C analysis, both its reduced form (ascorbic acid (AA)) and oxidized form (dehydroascorbate (DHA)), 50 mg of ground sample was used. The analysis was performed as described (Miret and Munné-Bosch, 2016) with some modifications. In brief, samples were extracted with cold 6 % (w/v) *meta*-phosphoric acid and 0.2 mM diethylene triamine pentaacetic acid as solvents. Samples were subjected to ultrasounds for 30 min (Branson 2510 ultrasonic cleaner; Bransonic, USA) and centrifuged at 10,000 rpm for 10 min (always at 4 °C). The supernatants (350 µL final volume) were transferred to another tube and the vitamin C analysis was performed in triplicate by spectrophotometry (xMArk Microplate Spectrophotometer, Bio-rad, Hercules, CA, USA) using a quartz microplate, following the procedure described by Queval and Noctor (2007). We analysed both total vitamin C (AA + DHA) and vitamin C redox state, which was calculated as AA/(AA + DHA) and expressed as a percentage.

2.4. Carotenoids analysis

Total carotenoids were analysed spectrophotometrically as described by Lichtenthaler and Buschmann (2001). In brief, 50 mg of each sample was extracted with 100 % methanol, subjected to ultrasounds for 30 min and centrifuged at 10,000 rpm for 10 min. Supernatants were pooled, transferred to another tube and centrifuged for 10 min at 10,000 rpm before absorbance was measured at the following wavelengths: 470 nm, 652.4 nm, 665.2 nm and 750 nm. The last wavelength was used to eliminate sample debris of the measure. To quantify total carotenoids, the formula described by Lichtenthaler and Buschmann (2001) for methanolic extracts was used.

Xanthophylls (neoxanthin, violaxanthin, antheraxanthin lutein and zeaxanthin) and β-carotene were also quantified by HPLC as described (Bermejo et al., 2021). In brief, 50 mg of each sample was extracted with 100 % methanol, subjected to ultrasounds for 30 min and centrifuged at 10,000 rpm for 10 min. Supernatants were transferred to another tube. The extraction process was performed three times for each sample. The extracts were filtered using hydrophobic filters with a pore size of 0.45

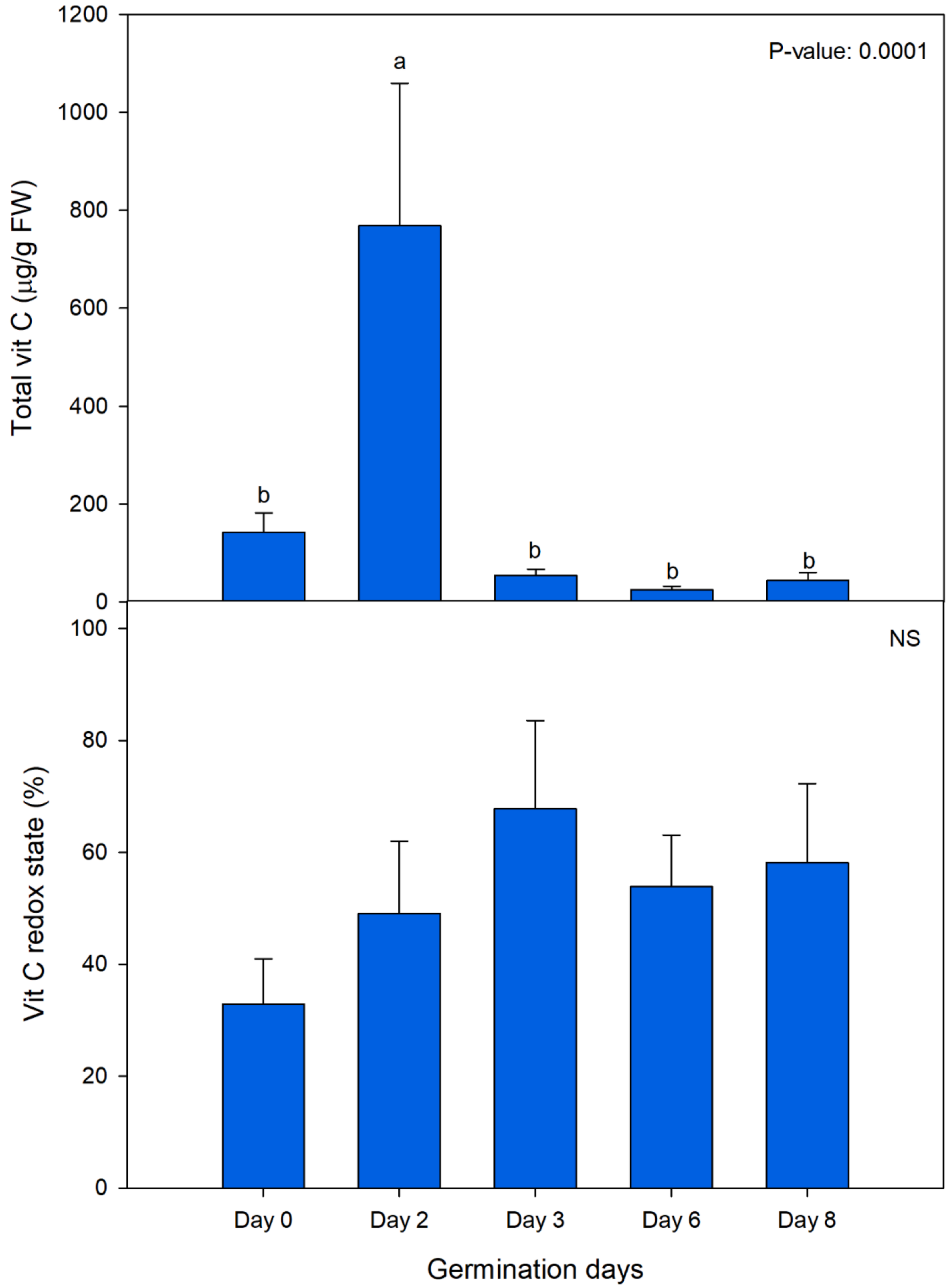

Fig. 2. Total vitamin C (μg/g fresh weight) and vitamin C redox state (%) in chia during germination. The data is shown as mean ± SE of n = 5 replicates. Different letters indicate significant differences among germination days (P-value < 0.05). NS indicates no significant differences (P-value > 0.05).

μm. Then, 300 μL of each extract was transferred to glass vials. The samples were analysed by reverse-phase HPLC, using an Agilent 1100 Series HPLC system with a Zorbax ODS-5 μm column (4.6 mm × 250 mm). The mobile phases were methanol:ethyl acetate (68:32, v/v) and acetonitrile:methanol (85:15, v/v). Quantification of each compound was made by calculating the pmol/area ratios for each compound at 445 nm. The conversion factors from peak-area units to pmol per injection were those used by Thayer and Björkman (1990).

2.5. Fresh weight and germination rate

Sprouts fresh weight was measured by weighing 20 representative sprouts from each replicate on the last day of germination (day 8). Germination rate was calculated by counting the non-germinated and germinated seeds from each replicate with ImageJ 1.53c software on germination day 3 (considering all seeds from each replicate).

2.6. Statistical analysis

Means and standard errors (SE) were calculated using Excel (2012 version) and STATGRAPHICS (1.18.13 version). All statistical tests were performed with STATGRAPHICS with a $\alpha = 0.05$ (95 % confidence). Analysis of variance (ANOVA) were performed using Fisher's LSD test as a post-hoc. When normality of residues was rejected (Shapiro-Wilk test), data were log transformed and ANOVA tests performed again. If normality of residues of log transformed data was still rejected, the non-parametric Kruskal-Wallis test applying Bonferroni as a post-hoc. All graphs were made using Sigma-plot (System Software, California, USA).

3. Results and discussion

3.1. Antioxidant vitamin composition in chia

Germination affected vitamin E composition of chia. Total vitamin E decreased during chia germination (Fig. 1). The maximum contents of vitamin E were found on dry seeds, which were 8-fold higher compared to sprouts (P-value < 0.0001). This vitamin E was composed by 95.5 % γ-tocopherol, which decreased progressively during development together with δ -tocopherol. In seeds, very low amounts of α-tocopherol were found, but in this case, contents increased during germination (P-value = 0.012), reaching the highest values in sprouts, but still in low concentrations compared to γ-tocopherol accumulated by seeds. It is well-known that α-tocopherol is the most important vitamin E compound for human health. This vitamin E isoform has not only antioxidant activities in our body but also pro-oxidant, cell signalling and gene regulatory roles (Tucker & Townsend, 2005). However, γ-tocopherol (the immediate α -tocopherol precursor in plants) also has beneficial properties for humans, both from a nutritional and clinical point of view. In fact, this compound has been shown to be better than α-tocopherol in some activities. For instance, γ-tocopherol eliminates reactive nitrogen species more efficiently than α -tocopherol and it is stronger than α-tocopherol in protection against some cancers and inflammation processes (Jiang et al., 2022). Furthermore, it has beneficial effects in patients with kidney disease and asthma and it has been shown to be potentially better than α-tocopherol against Alzheimer's disease (Jiang et al., 2022; Pahrudin Arrozi et al., 2020). In plants, it is common to find γ-tocopherol accumulated in seeds, whereas α-tocopherol usually accumulates in photosynthetic tissues such as leaves. The accumulation of γ-tocopherol also occurs at comparable amounts in other edible seeds, like sesame (Haji et al., 2008).

It was observed that germination strongly changed chia seed and sprout chemical composition. Vitamin C increased significantly (P-value = 0.0001) just after seed imbibition (Fig. 2). When chia seeds have been germinating for 2 days (germinating seeds), they contained 5- and 17.5-fold more vitamin C than dry seeds and sprouts, respectively. This result means that vitamin C increase is transient in chia. Vitamin C accumulation is related with metabolically active cells, as this antioxidant can eliminate ROS through both enzymatic and non-enzymatic reactions (Rajjou et al., 2012). Germination, which is a key process for plant-based food production, implies reactivation of seed metabolism. Previous work has shown a vitamin C increase during germination in other edible species, such as flax (Wang et al., 2015), soybean (Xu, Dong, & Zhu, 2005), wheat (Yang et al., 2001), cowpeas (Doblado et al., 2007) and maize (Liu et al., 2017). If we carefully observe the data in the case of flax, soybean and wheat, we can appreciate that vitamin C peaks first to decrease later slightly. Stasolla and Yeung (2001) found that vitamin C and ascorbate peroxidase, which are both key elements for the elimination of ROS in plant cells, increased during germination. Thus, results suggest that chia seeds may trigger vitamin C production to face an abrupt increase of ROS during germination, so that oxidative balance is restored to avoid cell damage. Interestingly, vitamin C values obtained in germinating chia seeds are higher than those found in other plant-based foods such as lemons, oranges, strawberries, spinaches, potatoes, and tomatoes (U.S. Department of Agriculture, 2019), thus being a potentially excellent dietary source of vitamin C if we can incorporate germinating chia seeds in salads and other food preparations (Table 1).

Table 1
Vitamin C content (mg/100 g fresh weight) among different developmental stages of chia compared to that of other vegetables. All data was obtained from U. S. Department of Agriculture (2019), except for chia (present study). Note that vitamin C contents in chia germinating seeds are higher than those reported for oranges or strawberries.

Vegetable	Vitamin C (mg/100 g FW)
Chia dry seed	14.2
Chia germinating seed	76.8
Chia sprout	4.3
Lemon	45.0
Orange	53.0
Strawberry	58.8
Spinaches	28.1
Potato	17.8
Tomato	19.7

Total carotenoids increased during the progression of seed development into sprouts (P-value < 0.0001), as it was expected (Fig. 3), thus confirming previous results (Bermejo et al., 2021). The main carotenoid found in sprouts was lutein, followed by neoxanthin, violaxanthin, β-carotene, zeaxanthin and antheraxanthin. Dry seeds and germinating seeds contained very low levels of carotenoids, and β-carotene was not detected on their composition. In fact, β-carotene content of green sprouts was higher than total carotenoid content of dry and germinating seeds. Carotenoids, as pigments, are part of the photosynthetic machinery of plants. They are synthesized during photomorphogenesis, a process in which light induces the expression of enzymes involved in carotenoid synthesis as well as chloroplast differentiation (Rodríguez-Villalón et al., 2009). Thus, light triggers the maturation of photosynthetic machinery, leading an accumulation of carotenoids in chia. As shown here, carotenoids accumulation during seed development parallels α -tocopherol increases, despite amounts are not comparable.

3.2. Vitamin E improvement of chia sprouts with plant growth regulators

Vitamin E levels were improved in chia sprouts with PGRs application (P-value = 0.0009), specially with ABA, followed by P + ABA + MeJA + MeSA and P + ABA (Fig. 4). Total vitamin E content increased 3-fold in chia sprouts treated with ABA. Positive effects were observed for both γ-tocopherol and δ-tocopherol, but not for α-tocopherol. ABA is a PGR which role in stress tolerance mechanisms in plants has been widely studied (Mehrotra et al., 2014). Vitamin E plays an essential role in stress tolerance mechanisms in plants, as it protects membranes from oxidative damage caused by ROS (Muñoz & Munné-Bosch, 2019). It is known that ABA-dependent vitamin E synthesis occurs in plants. In rice, it was identified an ABA-responsive element (ABRE) in the promoter regions of genes involved in vitamin E synthesis, specifically *OsHPPD*, *OsγTMT* and *OsMPBQMT* (Chaudhary & Khurana, 2009). ABREs are regulatory regions which are found in ABA-responsive genes. In suspension cultures of *Eucalyptus gunnii,* ABA exogenous application enhanced the expression of tocopherol cyclase, an enzyme which transforms 2,3-dimethyl-6-phytyl-1,4-benzoquinone into γ-tocopherol (El Kayal et al., 2006). Thus, results suggest that ABA triggered vitamin E biosynthesis in chia sprouts. Despite this, according to the role of ABA as a germination inhibitor, its application strongly reduced chia germination rate (Suppl. Table 2). Promalin® application partly solved this problem in P + ABA and P + ABA + MeJA + MeSA treatments, although it had a negative effect on sprouts fresh biomass production. Indeed, ABA can inhibit shoot growth in plants and ABA-dependent reduction in growth appeared to be more apparent with Promalin®. Thus, ABA treatment is preferable to P + ABA and P + ABA + MeJA +

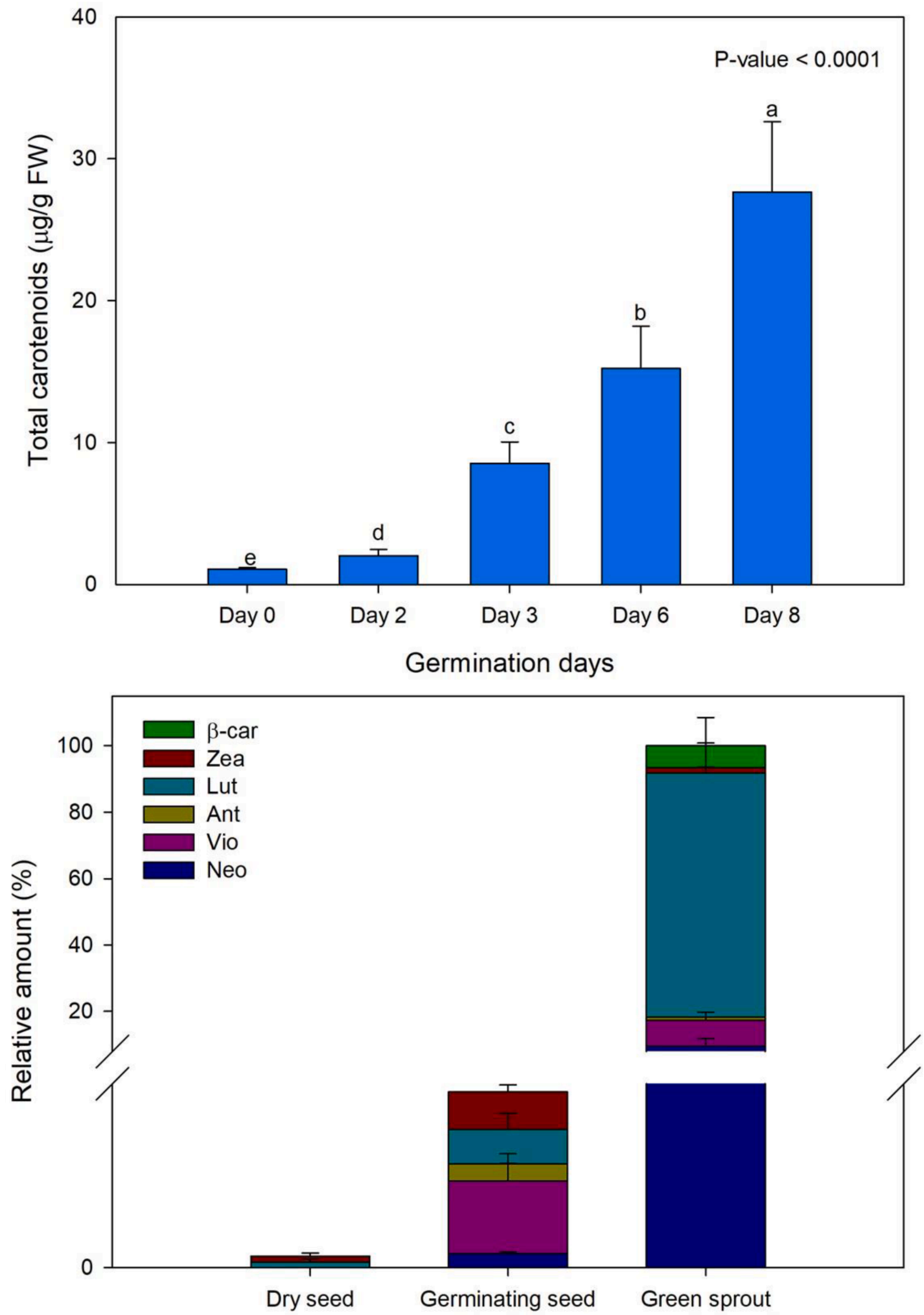

Fig. 3. Total carotenoids (μg/g fresh weight) in chia during germination. The data is shown as mean ± SE of n = 5 replicates. Different letters indicate significant differences among germination days (P-value < 0.05). Below, relative amount (%) of each carotenoid (xanthophylls and β-carotene) in chia dry seeds (day 0), germinating seeds (day 2) and green sprouts (day 8). Carotenoid content of green sprouts were fixed as 100 % and all the values were represented considering that. The data is shown as mean ± SE of n = 3 replicates. Ant = antheraxanthin, β-car = β-carotene, Lut = lutein, Neo = neoxanthin, Vio = violaxanthin, Zea = zeaxanthin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MeSA treatments for chia sprouts production once germination has been achieved. However, dry seeds are still a much better source of total vitamin E than sprouts.

In contrast to what occurred with vitamin E, PGRs could not improve vitamin C composition of chia sprouts (Suppl. Fig. 1). The applied treatments had no significant effects on total vitamin C content and vitamin C redox state. According to the results obtained both in vitamin E and vitamin C, PGRs do not appear to be an effective strategy for improving antioxidant vitamins composition in chia.

Pyrabactin reduced both γ-tocopherol and δ-tocopherol, but not α -tocopherol (Suppl. Fig. 2). Pyrabactin is a synthetic sulfonamide that can act as an ABA agonist or antagonist (Melcher et al., 2010). In this case, pyrabactin acted as an antagonist, providing clues of which may a be a possible mechanism of action for ABA-dependent vitamin E biosynthesis in chia sprouts (Suppl. Fig. 3). ABA functions through a family of PYR/PYL receptors and it is an antagonist of PYL2, PYL3, PYR2, PYR4, PYR5, PYR8 (Gupta et al., 2020; Kreszies, 2019; Melcher et al., 2010), suggesting that ABA-dependent vitamin E biosynthesis (and most particularly up to γ -tocopherol biosynthesis) could be triggered by these specific receptors. However, more research is needed to elucidate the mechanism of action.

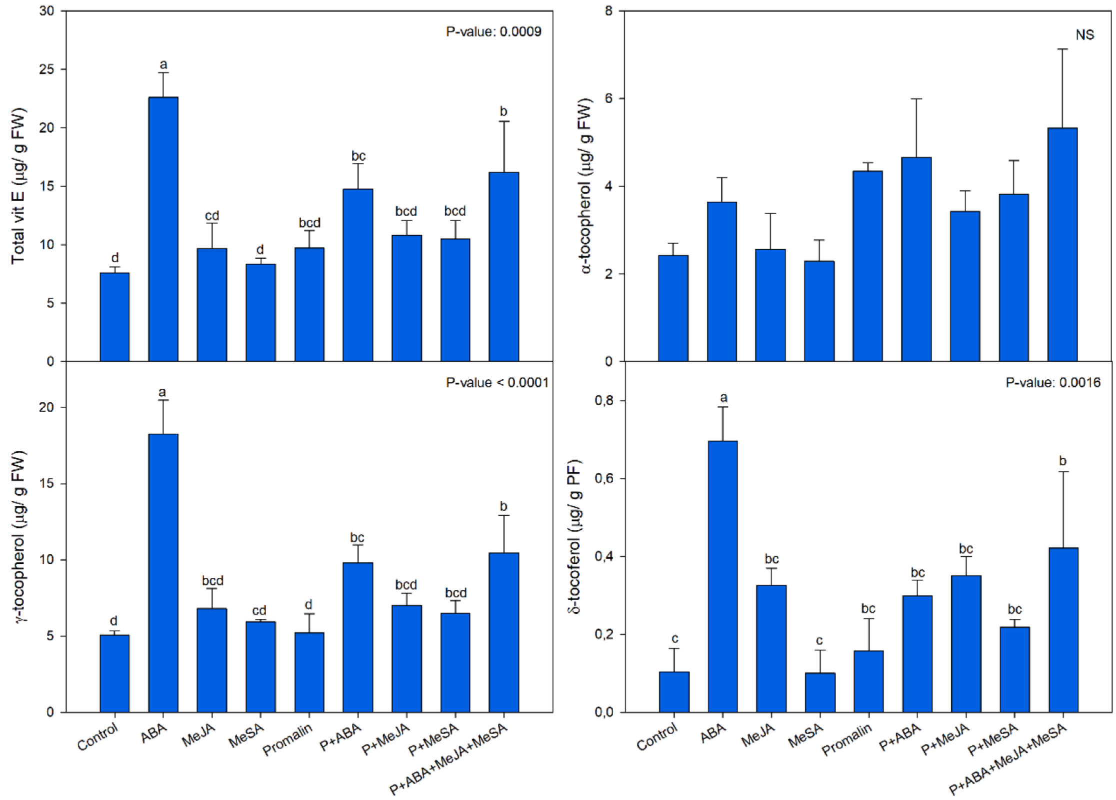

Fig. 4. Vitamin E contents (μg/g fresh weight) in chia sprouts obtained after 8 days of germination. The data is shown as mean ± SE of n = 3 replicates. Different letters show significant differences among treatments (P-value < 0.05). NS indicate no significant differences (P-value > 0.05). ABA = abscisic acid, MeJA = methyl jasmonate, MeSA = methyl salicylate, P = Promalin®.

3.3. Optimal antioxidant vitamin contents in chia food preparations

Total vitamin E content (with 95.5 % γ -tocopherol) of dry seeds was 8-fold higher than in sprouts, vitamin C content of germinating seeds was 5- and 17.5-fold higher than dry seeds and sprouts, respectively, and carotenoids (including pro-vitamin A) peaked in green sprouts, together with α -tocopherol. Although ABA application increased γ-tocopherol content in chia sprouts, results indicate that exploiting natural composition variation of the different developmental stages is a much better tool to optimize antioxidant vitamin contents in chia food preparations. Chia dry seeds are a very rich source of vitamin E and a simple two-day germination can be a cost-effective approach that strongly improves vitamin C in chia, and no exogenous substances or transgenesis are needed. Natural physiological processes of plants can modify their chemical composition through several metabolic, transcriptional, and hormonal changes, leading to transgenic- and chemical-free plant-based food improvement. Germination has a beneficial effect on the antioxidant activity and/or nutritional value of many edible plant species, such as millet (Sharma, Jan, & Riar, 2021), wheat, mustard, radish, soybean, kale, broccoli, garden cress (Abdel-Aty et al., 2019; Cevallos-Casals & Cisneros-Zevallos, 2010) and several legumes (Dueñas et al., 2009; López-Amorós et al., 2006; Sallam et al., 2021). In the case of chia, as high contents of antioxidant vitamins are found at different developmental stages, an optimal solution could be using a mixture of dry seeds, germinating seeds and green sprouts in food preparations, so that contents of vitamin E, vitamin C and β-carotene, respectively, are the highest (Fig. 5A). For adults, the Recommended Dietary Allowance (RDA) for vitamin C is 90–75 mg/day and 1.8–1.4 mg/day for β-carotene (Krinsky et al., 2000). For vitamin E, there is no RDA established for the γ-tocopherol form. If we take as reference the established RDA for α-tocopherol, it would be 15 mg/day (Krinsky et al., 2000). Thus, considering the results obtained in this work, the optimum proportion of the chia mixture to be used in food preparations would be approximately 1.5/2/1 (dry seed/germinating seed/green sprout, w/w/w).

Mixing different types of food has been widely studied as a strategy to improve nutritional quality in food preparations. It has been found that mixing pumpkin puree with wheat flour increases protein, calcium, carotene and vitamin C content of biscuit (Gurung, Ojha & Subba, 2016). Other example is the addition of onion to heated apple juice, which was reported to increase flavonoid (Lee et al., 2016). In the case of chia, its incorporation into gluten-free products for celiacs has shown positive nutritional effects (Coorey, Grant & Jayasena, 2012; Costantini et al., 2014; Menga et al., 2017; Sung et al., 2020). Chia has been widely used as a fortifying agent of food products, such as yogurt and bread (Eker & Karakaya, 2020; Romankiewicz et al., 2017). It is important to observe that mixing food products to improve nutritional value has never been proposed within the same species. Although it was already known that the incorporation of chia to food preparations can increase their nutritional quality, exploiting the characteristic composition of different developmental stages within chia has never been proposed. As shown in the present study, taking advantage of naturally occurring developmental processes and mixing different developmental stages within the same plant-based food can be a simple approach to obtain an optimal composition of antioxidant vitamins.

Using a chia mixture in food preparations has some advantages, including the avoidance of transgenesis and application of exogenous substances. Global acceptance of public and legislation are guaranteed since germination is a naturally occurring process. The methodology used for chia germination and development is sustainable, easy and cost-effective, given that it only needs regular moisture and darkness, and light at the end of the process. Thus, the improvement of the antioxidant vitamin composition (including vitamins C and E, and pro-vitamin A) of chia food preparations through mixing different developmental stages can be more sustainable, globally accepted, and easier than transgenic

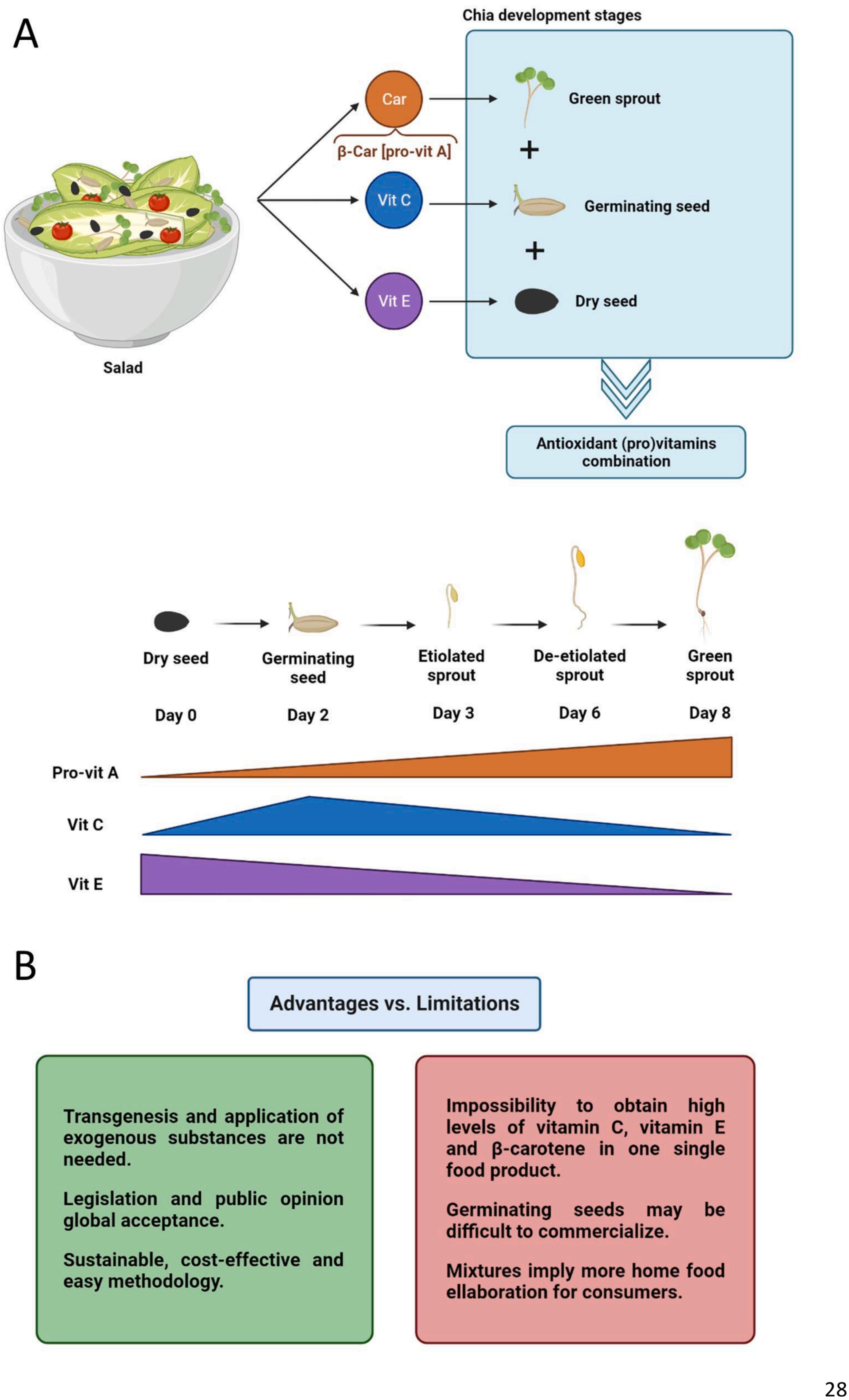

Fig. 5. **(A)** Graphical representation of a mixture of the three developmental stages of chia for the intake of a combination of antioxidant (pro)vitamins. There are represented chia sprouts, germinating seeds and dry seeds for β-carotene, vitamin C and vitamin E intake, respectively. Below, graphical representation of the variation of each compound throughout chia germination (created with biorender.com). **(B)** Advantages and limitations of mixing different developmental stages of chia as a non-transgenic approach for antioxidant vitamin composition improvement (created with biorender.com).

strategies. However, this approach is not fully devoid of limitations (Fig. 5B). In this case, a mixture of three developmental stages of chia is needed, so that the need of a mixture means more home food preparation. Although the incorporation of plant-based food into diet is a functional food trend (Sloan, 2014), and in this respect, chia seeds and sprouts are marketable as food products, germinating chia seeds have never been commercialized. Indeed, imbibition is a critical process in seed germination and there are some issues that need to be considered, especially in terms of conservation. Moisture increase in seed storage may lead to the growth of microbial populations, which may be a handicap to market this kind of food. In this case, we propose that germinating seeds must be immediately freeze-dried prior to be incorporated in food preparations.

4. Conclusions

Chia seeds and sprouts are functional foods with desirable properties for the agri-food industry. The nutritional improvement of chia sprouts through the application of PGRs have been possible for vitamin E, but results are not comparable to exploiting natural variation. Although ABA could improve vitamin E levels in chia sprouts, amounts were not comparable to those found in dry seeds. In contrast, germination resulted to be an effective and simple approach to improve vitamin C contents, so that germinating seeds contained 5- and 17.5-fold higher vitamin C contents than dry seeds and sprouts, respectively. Given that total carotenoids, including β-carotene (provitamin A), peak in green sprouts, a chia mixture is proposed as the best option for optimizing the intake of antioxidant vitamins in food preparations. Considering the established RDAs for each compound, the mixture should have a proportion of 1.5/2/1 (dry seed/germinating seed/green sprout, w/w/w) for optimal intake.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134880.

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