Biochemistry and Biophysics Reports 26 (2021) 100989

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Carotenoids from mamey (*Pouteria sapota*) and carrot (*Daucus carota*) increase the oxidative stress resistance of *Caenorhabditis elegans*

Marco Antonio González-Peña [a], José Daniel Lozada-Ramírez [b], Ana Eugenia Ortega-Regules [c,*]

[a] Department of Chemical, Food and Environmental Engineering, Universidad de las Américas Puebla, Sta. Catarina Mártir, C.P. 72820, Cholula, Puebla, Mexico
[b] Department of Chemical and Biological Sciences, Universidad de las Américas Puebla, Sta. Catarina Mártir, C.P. 72820, Cholula, Puebla, Mexico
[c] Department of Health Science, Universidad de las Américas Puebla, Sta. Catarina Mártir, C.P. 72820, Cholula, Puebla, Mexico

ARTICLE INFO

Keywords:
Pouteria sapota
Daucus carota
Carotenoids
Oxidative stress
Caenorhabditis elegans

ABSTRACT

Carotenoids are natural pigments and antioxidants found in fruits and vegetables such as carrot, tomato, orange, mango, yellow corn, pumpkin, and mamey. In this study, we evaluated the antioxidant potential of mamey (*Pouteria sapota*) carotenoids and compared them to carrot (*Daucus carota*) carotenoids. The carotenoids were extracted from mamey and carrot, and their antioxidant capacity were determined via *in vitro* (ABTS method) and *in vivo* assays (resistance against oxidative stress in *Caenorhabditis elegans*). The carotenoid contents in mamey and carrot were 4.42 ± 0.12 and 5.47 ± 0.04 mg β-carotene/100 g, respectively. Despite the differences between the carotenoid contents in both products ($p < 0.05$), the *in vitro* antioxidant capacity results showed no significant differences between the extracts ($p > 0.05$). The mamey and carrot carotenoid extracts decreased the oxidative damage in *C. elegans* by 20–30% and 30–40%, respectively. Both extracts increased the resistance and enhanced the survival of the nematodes, and showed better effects than pure β-carotene, probably owing to the complex mixture in the carotenoid extracts. These results suggest that mamey is a good alternative source of carotenoids and that it protects against oxidative stress in *C. elegans*. The protective effect of mamey carotenoids was similar to the effect of carrot carotenoids.

1. Introduction

Carotenoids are a group of natural pigments responsible for the yellow, orange, and red colors in fruits and vegetables, which have been associated with the prevention of diseases because of their functions as vitamin A precursors, antioxidant compounds, and modulators of physiological processes, such as the regulation of immune system, cell development, proliferation, communication, and maintenance, gene expression, hematopoiesis, and apoptosis [1,2].

The nematode *Caenorhabditis elegans* is an organism frequently found in soils that feeds on bacteria and other microorganisms; it is used as a biological model due to its small size, simple anatomy, short lifespan, clear structure, easy propagation, completely sequenced genome, and disposition of mutant strains [3,4]. For the above reasons, *C. elegans* has been used as a research model for understanding the metabolic, pathological, and molecular mechanisms associated with the aging process, development of diseases, function, antioxidant capacity, and toxicity of foods, bioactive compounds, and plant extracts [5,6]. Thus, several researchers have established that its lifespan and/or resistance against oxidative stress increased after the exposure to multiple antioxidants, such as vitamin C [6,7], spinach extracts [8], and phenolic compounds, such as quercetin [9] and resveratrol [4]. Furthermore, the short lifespan of *C. elegans* and its capacity to produce multiple descendants allows to evaluate the effect of bioactive compounds across multiple generations [10].

Environmental contaminants, such as sunlight, smoke, ozone, and herbicides, are sources of reactive oxygen species (ROS). The overproduction of ROS could result in oxidative stress and oxidative damage to proteins, DNA, and membrane lipids [4,11]. The oxidative stress occurs when the balance between pro-oxidant and antioxidant compound is broken [12]. The oxidative stress is associated with premature aging and development and progression of chronic diseases such as diabetes, atherosclerosis, Parkinson's, Alzheimer's, and Huntington's disease, and cancer [11,13]. Carotenoids play a major role in the cell protection against oxidative stress-induced by ROS, reactive nitrogen species (RNS), and lipid peroxides via its singlet oxygen-quenching activity and

* Corresponding author.
E-mail addresses: marco.gonzalezpa@udlap.mx (M.A. González-Peña), jose.lozada@udlap.mx (J.D. Lozada-Ramírez), ana.ortega@udlap.mx (A.E. Ortega-Regules).

https://doi.org/10.1016/j.bbrep.2021.100989
Received 25 November 2020; Received in revised form 9 March 2021; Accepted 16 March 2021

free radical-scavenging activity [1]. In *C. elegans*, the ROS accumulation accelerates aging and shortens the longevity and survival of the nematode. β-Carotene [6] and astaxanthin [14,15] have shown the ability to decrease the oxidative stress damage, neutralize the ROS production and increase the survival of *C. elegans*.

The largest intake of carotenoids in the human diet comes from fruits and vegetables, such as carrots, tomatoes, spinach, and oranges [16,17]; however, those are not the only source of carotenoids. In this regard, Alia-Tejacal et al. [18] and Yahia et al. [2] identified the mamey sapote (*Pouteria sapota*) fruit as an alternative source of carotenoids. Mamey is a tropical fruit native from Mexico and Central America, and it is valued for its sensory characteristics and high nutritional value (carbohydrates, fiber, minerals, vitamins A, C, and E, and antioxidant compounds) [2,19,20]. Mamey is well known for its salmon-red pulp, smooth texture, and sweet flavor, often used as an ingredient in jam, sorbet, ice pop, gelatin, yogurt, desserts and bakery [18].

Although several studies on the characterization and post-harvest handling of mamey have been conducted, there is little evidence concerning the antioxidant potential of mamey carotenoids and no *in vivo* studies have been conducted to evaluate its antioxidant capacity, which is especially important because it is well-known that the antioxidant properties can vary between *in vitro* and *in vivo* conditions [6,21,22]. Therefore, we propose that mamey may be an excellent source of carotenoids with effective antioxidant properties both *in vitro* and *in vivo*, similar to carrot. Hence, the objective of this study was to compare the antioxidant capacity of the carotenoids extracted from mamey with those extracted from carrot. To evaluate the *in vivo* antioxidant capacity, the resistance against oxidative stress in the nematode *C. elegans* was studied.

2. Materials and methods

2.1. Materials

Mamey (*Pouteria sapota*) and carrot (*Daucus carota*) were obtained from a local supermarket in the city of Puebla, Mexico. Both products were washed with water to remove impurities and other contaminants and peeled, and the pulp was homogenized using an immersion mixer (2612, Oster, China). The mamey skin was carefully cleaned to remove traces of pulp and cut into small pieces. The mamey pulp, mamey skin, and carrot were stored in polystyrene bags at −20 °C until further use.

2.2. Carotenoids

The extraction of carotenoids was based on the method described elsewhere [23] with some modifications. Samples of 10 g (mamey pulp, mamey skin, or carrot) were mixed with 50 mL of a hexane: acetone: ethanol solution (70 : 15: 15 v/v/v, respectively). The mixture was magnetically stirred for 1 h; afterwards, 5 mL of 40% KOH in methanol was added and incubated at room temperature in the dark for 2 h until saponification. Then, 30 mL of hexane was added, the mixture was shaken vigorously, and the upper layer was collected. The extraction was repeated with the lower layer; then, the upper layer was collected again. Both supernatants were mixed and filtered through Na_2SO_4 powder to remove traces of water. Later, the supernatant was concentrated in a rotary evaporator (R-124, Büchi, Switzerland) at 35 °C, dissolved in ethanol, and spectrophotometrically analyzed at 450 nm (Genesys 10S UV–Vis spectrophotometer, Thermo Fisher Scientific, USA). A calibration curve was prepared using β-carotene (0–20 ppm) (purity >97%, Sigma-Aldrich, Mexico) in ethanol, using pure ethanol as the blank. The results were expressed as mg of β-carotene/100 g of fresh weight. Determinations were performed in triplicate.

2.3. In vitro antioxidant capacity

The antioxidant capacities of the carotenoid extracts were determined by the method developed by Re et al. [24] with some modifications. First, 5 mL of an aqueous solution of 7 mM 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Sigma-Aldrich, Mexico) was prepared. The $ABTS^{\bullet+}$ radical cations were produced by reacting the ABTS stock solution with 2.45 mM $K_2S_2O_8$ and allowing the mixture to stand at room temperature in the dark for 16 h. Then, the solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm (Genesys 10S UV–Vis spectrophotometer, Thermo Fisher Scientific, USA) and recorded as the initial absorbance. Then, 980 μL of the diluted $ABTS^{\bullet+}$ was mixed with 20 μL of the carotenoid extract, and the absorbance was measured after 6 min. The percentage of inhibition was calculated using Eq. (1):

$$\text{Inhibition percentage} = [(\text{Initial absorbance} - \text{Final absorbance}) / (\text{Initial absorbance})] \times 100\% \quad (1)$$

A calibration curve was prepared by dissolving Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid; Sigma-Aldrich, Mexico) in ethanol. Trolox solutions (0–1500 μM) were subjected to the same treatment with $ABTS^{\bullet+}$, as previously described using pure ethanol as the blank. The Trolox concentration was plotted against the inhibition percentage to obtain the calibration curve. The results were expressed as micromole of Trolox equivalent antioxidant capacity (TEAC)/100 g of fresh weight. Determinations were performed in triplicate.

2.4. In vivo antioxidant capacity

The biological model used was the wild strain of *C. elegans*, Bristol N2, which feeds on the auxotrophic uracil bacteria *Escherichia coli* OP50. The nematodes were maintained at 22 ± 2 °C in nematode growth medium (NGM) plates supplemented with 200 μL of *E. coli* OP50 [25]. Both organisms were obtained through the Chemical and Biological Sciences Department of the Universidad de las Américas Puebla.

The eggs were obtained via synchronization to ensure that all nematodes were in the same larval stage for the experimental assays [9]. For this purpose, the nematodes were taken at the adult stage (third day), washed with M9 solution (6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 5 g/L NaCl, 0.215 g/L $MgSO_4{\cdot}7H_2O$) to eliminate the bacteria, and centrifuged at 4600 rpm and 4 °C for 1 min (centrifuge Z 366K, HERMLE Labortechnik, Germany). The supernatant was removed, 1 mL of M9 solution was added, and centrifuged again under the same conditions. The supernatant was removed, 1 mL of 1 M NaOH was added, and then it was vortex-shaken (Vortex-Gene 2 G560, Scientific Industries, USA) for 30 s and centrifuged under the same conditions. The supernatant was removed, and 500 μL of 1 M NaOH and 500 μL of 1 M NaOH: 5% NaClO (60 : 40 v/v) were added; it was then vortex-shaken for 60 s and centrifuged under the same conditions. The supernatant was removed, washed two times with 1 mL of M9 solution and centrifuged between each wash, increasing the centrifugation speed to 5600 rpm. Finally, the supernatant was removed, and the residue was placed on new NGM plates with fresh *E. coli* OP50. The plates were incubated at 22 ± 2 °C.

The synchronized nematodes were divided into the following groups for oxidative stress resistance assays: control group (without antioxidant), antioxidant group (225 μg/mL of Trolox) and carotenoid groups

(20, 30 and 40 μg/mL of carotenoid extracts or β-carotene).

For the oxidative stress resistance assays, the methodology described elsewhere [21] was used with some modifications. First, 60 ± 5 nematodes in the L4 stage (2–2.5 days), which were previously exposed to the antioxidants, were transferred to new NGM plates with 400 μM of juglone (5 hydroxy-1,4-naphthoquinone; Sigma-Aldrich, Mexico), which induces lethal oxidative stress. The survival of *C. elegans* was evaluated every hour for 8 h and the nematodes were scored as dead if they failed to respond upon stimulation with a platinum wire [4].

Simultaneously, the nematodes in the L4 stage were synchronized, and the eggs obtained were placed in new NGM plates with bacteria and antioxidants and incubated at 22 ± 2 °C until the L4 stage was reached again. The survival was evaluated following the methodology above. This procedure was repeated until the oxidative stress resistance of three different generations (F1, F2, and F3) were evaluated for each condition. Experiments were performed in triplicate.

2.5. Statistical analysis

The means and standard deviations (SD) from all determinations performed in triplicate were reported. Data were analyzed using the ANOVA test and means comparison routines (Fisher, $p < 0.05$). The survival assays of *C. elegans* were analyzed using the Kaplan–Meier methodology and log-rank test to determine significant differences ($p < 0.05$). All analyses were performed using the Minitab Statistical Software (18th version, Minitab Inc., USA).

3. Results

3.1. Carotenoid extraction and antioxidant capacity

Carotenoids are natural compounds responsible for the typical colors of mamey and carrot because they provide red, orange, and yellow tonalities, according to their type and content. In this regard, the mamey pulp is typically associated with red–orange color tonalities, whereas orange is the most common color for carrot. Table 1 shows the carotenoid contents and carotenoid antioxidant capacities for the mamey pulp, mamey skin, and carrot. We decided to investigate the carotenoid content in the mamey skin, although the skin is not used in the food industry and is frequently discarded. The highest content of carotenoids was found in carrot ($p < 0.05$). The highest antioxidant capacity corresponds to the carrot carotenoid extract (CCE), followed by the mamey pulp carotenoid extract (MPCE), whereas the mamey skin carotenoid extract (MSCE) had the lowest antioxidant capacity. The inhibition percentages were between 60% (MPCE) and 70% (CCE), whereas for the MSCE it was near 25%. These differences are associated with the type and content of carotenoids found in each sample. Although the carotenoid content in carrot was higher than in mamey pulp ($p < 0.05$), no significant differences were observed amongst the antioxidant capacities of the extracts ($p > 0.05$).

Table 1
Contents and antioxidant capacities of mamey and carrot carotenoids.

Sample	Carotenoids (mg of β-carotene/100 g)	Antioxidant capacity (micromole TE/100 g)
Mamey pulp	4.42 ± 0.12^{b}	87.75 ± 5.74^{d}
Mamey skin	0.85 ± 0.20^{c}	53.53 ± 11.61^{e}
Carrot	5.47 ± 0.04^{a}	90.24 ± 11.66^{d}

Data shown correspond to means ± standard deviations (n = 3). Different letters between samples indicate significant differences ($p < 0.05$) between carotenoid contents and antioxidant capacities of mamey and carrot. TE: Trolox equivalents.

3.2. Evaluation of in vivo antioxidant capacity

To compare and evaluate the antioxidant capacities of the mamey and carrot carotenoid extracts, a compound that serves as a control antioxidant, Trolox (vitamin E analog), was included at a concentration of 225 μg/mL. The above concentration was chosen to mimic the percentage of inhibition of the *in vitro* assays exerted by the carotenoid extracts. The nematodes treated with Trolox (Fig. 1) significantly increased their resistance against oxidative stress compared to untreated nematodes ($p < 0.05$) in all three generations (F1, F2, and F3). Furthermore, the resistance increased with each generation (F3 > F2 > F1) because the antioxidant effect was enhanced, indicating the existence of a hereditary effect (Table S1).

The nematodes treated with pure β-carotene (Fig. 2) showed a significant increase in their resistance against oxidative stress compared to the untreated nematodes ($p < 0.05$), similar to the Trolox-treated nematodes. Moreover, higher concentrations of pure β-carotene were more effective than lower concentrations. The percentage of survivors are shown in Table S1. The number of survivors increased, particularly in the F3 generation, through an inherited effect caused by the pure β-carotene.

The nematodes treated with the MPCE (Fig. 3) also increased significantly ($p < 0.05$) their resistance against oxidative stress and the number of survivors (Table S1). The survivors increased with each passing generation (F3 > F2 > F1). The same inherited effect seen with Trolox and β-carotene was shown. Additionally, the lowest concentration of the extract (20 μg/mL) was as effective as that of higher concentrations (30 and 40 μg/mL).

The nematodes treated with the MSCE showed different responses compared to the other carotenoid extracts. The *in vitro* results showed that the MSCE was not capable of reversing the radical formation. Regardless, the *in vivo* assays revealed that the MSCE exhibited a protective effect against oxidative stress (Fig. 4), which was very similar to the effect of the other carotenoids. The resistance of the F1 nematodes increased as the MSCE concentration increased (20 μg/mL > 30 μg/mL > 40 μg/mL) (Fig. 4a). However, the resistance of the F2 nematodes decreased at higher concentrations ($p < 0.05$) (Fig. 4b). Moreover, the F3 nematodes were less resistant to the oxidative stress ($p < 0.05$) (Fig. 4c), although some survivors were found at the concentration of 20 μg/mL (Table S1).

Finally, the nematodes treated with the CCE showed a significant increase in the resistance compared with untreated nematodes ($p < 0.05$), which was higher at a concentration of 40 μg/mL (Fig. 5). The survivors increased with each passing generation (F3 > F2 > F1) (Table S1). Again, it was shown that the enhanced resistance of *C. elegans* is caused by an inherited effect of the carotenoids. However, unlike the MPCE, the higher concentration (40 μg/mL) was more effective than the other concentrations.

4. Discussion

4.1. Carotenoid composition and antioxidant activity

Color is a key factor to determine the appearance and acceptability of food and is a testimony that pigments with antioxidant activity, such as carotenoids, are present in the product. For example, Yahia et al. [2] identified β-carotene as the main carotenoid (~98%) in mamey. Meanwhile, other carotenoids, such as sapotexanthin, cryptocapsin, β-cryptoxanthin, neoxanthin, and lutein, have also been identified but in smaller quantities [19,26]. In contrast, β-carotene, α-carotene, and lutein have been identified as the predominant carotenoids in carrots [17].

The results of the carotenoid contents in mamey pulp and carrot are consistent with those reported by multiple authors who found values of 1.13 ± 0.01 [2], 2.60 to 3.40 [18], and 3.79 ± 1.25 to 8.08 ± 2.31 [26] mg of β-carotene/100 g for mamey and 6.00 ± 0.39 to 12.52 ± 0.49

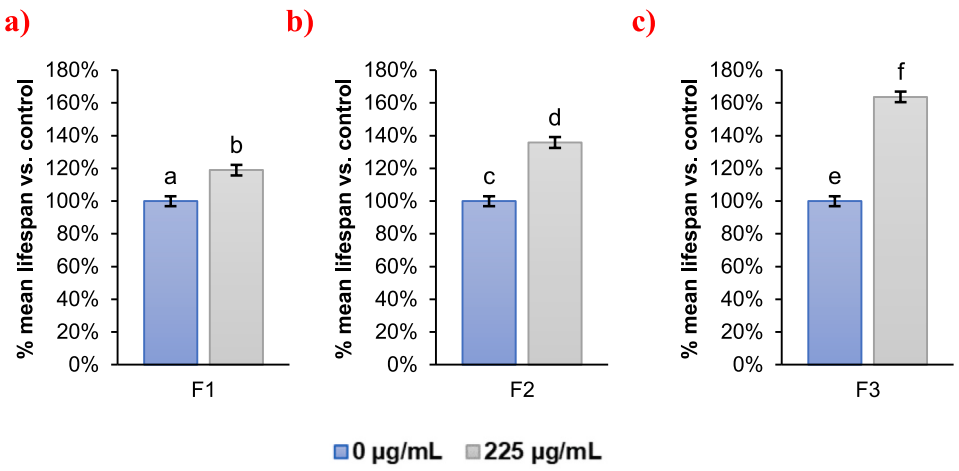

Fig. 1. Oxidative stress resistance of Trolox-treated nematodes. The results are expressed as the percentage of half-life time of nematodes treated with Trolox (225 µg/mL) when they were exposed to oxidative stress (400 µM juglone) compared to the control (0 µg/mL). a) First generation (F1); b) Second generation (F2); c) Third generation (F3). The data shown correspond to means ± standard deviations from three independent measurements. Different letters indicate significant differences ($p < 0.05$) between treated and untreated nematodes in each generation.

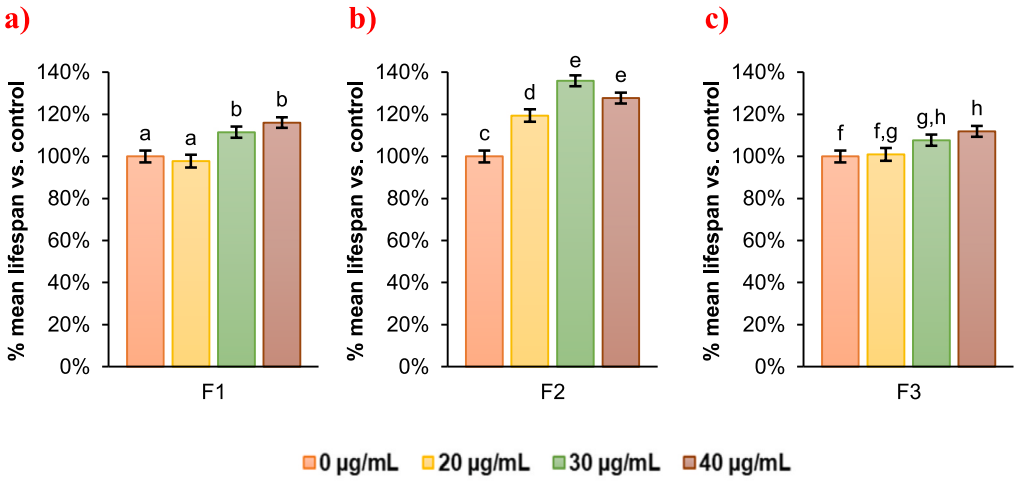

Fig. 2. Oxidative stress resistance of nematodes treated with β-carotene. The results are expressed as the percentage of half-life time of nematodes treated with β-carotene (20, 30, and 40 µg/mL) when they were exposed to oxidative stress (400 µM juglone) compared to the control (0 µg/mL). a) First generation (F1); b) Second generation (F2); c) Third generation (F3). The data shown correspond to means ± standard deviations from three independent measurements. Different letters indicate significant differences ($p < 0.05$) between treatments in each generation.

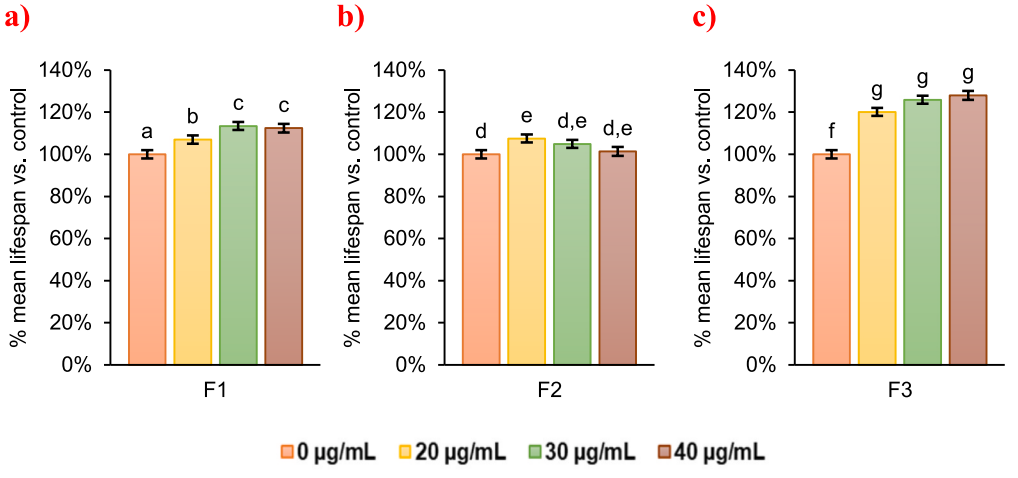

Fig. 3. Oxidative stress resistance of nematodes treated with the mamey pulp carotenoid extract (MPCE). The results are expressed as the percentage of half-life time of nematodes treated with the MPCE (20, 30, and 40 µg/mL) when they were exposed to oxidative stress (400 µM juglone) compared to the control (0 µg/mL). a) First generation (F1); b) Second generation (F2); c) Third generation (F3). The data shown correspond to means ± standard deviations from three independent measurements. Different letters indicate significant differences ($p < 0.05$) between treatments in each generation.

[27], 2.00 to 10.00 [28], and 14.82 [29] mg of β-carotene/100 g for carrot. The differences between the contents can be explained by multiple factors, such as the genotype, the harvesting time, the ripening stage, the fruit size, the growing weather conditions, the soil properties, the crop geolocation, the post-harvest handling, the storage conditions, the extraction method, and the fiber contents [2,30]. Interestingly, although the carotenoid content in the mamey pulp is slightly inferior compared to carrot, the findings suggest that mamey is a significant source of carotenoids, as previously mentioned by Moo-Huchin et al. [23] and Murillo et al. [19].

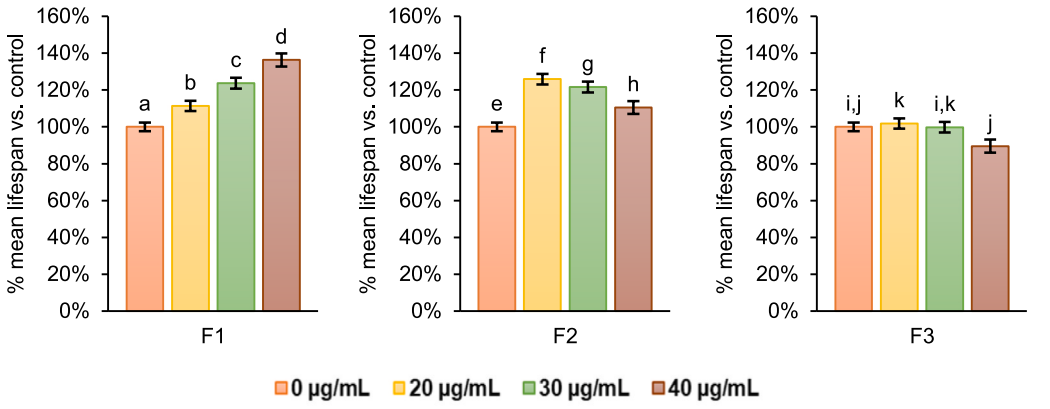

Fig. 4. Oxidative stress resistance of nematodes treated with the mamey skin carotenoid extract (MSCE). The results are expressed as the percentage of half-life time of nematodes treated with the MSCE (20, 30, and 40 µg/mL) when they were exposed to oxidative stress (400 µM juglone) compared to the control (0 µg/mL). a) First generation (F1); b) Second generation (F2); c) Third generation (F3). The data shown correspond to means ± standard deviations from three independent measurements. Different letters indicate significant differences ($p < 0.05$) between treatments in each generation.

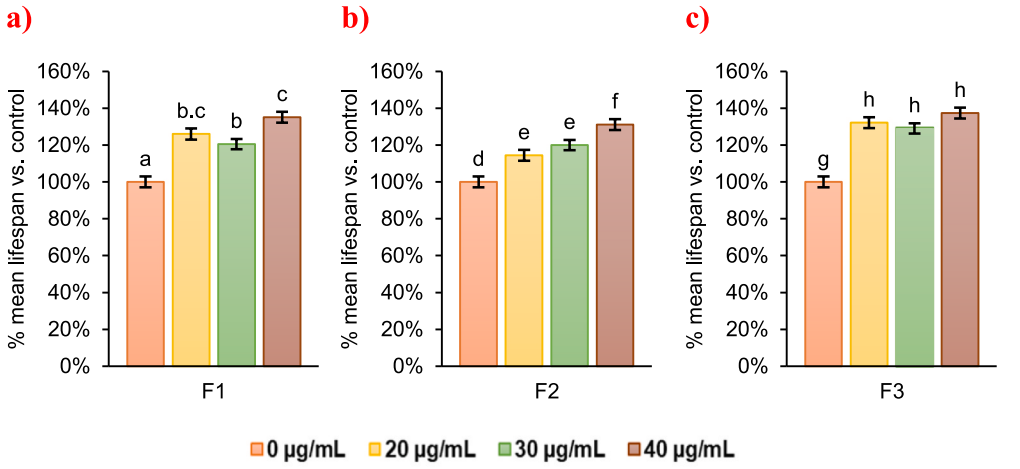

Fig. 5. Oxidative stress resistance of nematodes treated with the carrot carotenoid extract (CCE). The results are expressed as the percentage of half-life time of nematodes treated with the CCE (20, 30, and 40 µg/mL) when they were exposed to oxidative stress (400 µM juglone) compared to the control (0 µg/mL). a) First generation (F1); b) Second generation (F2); c) Third generation (F3). The data shown correspond to means ± standard deviations from three independent measurements. Different letters indicate significant differences ($p < 0.05$) between treatments in each generation.

Other fruits, especially tropical fruits, are recognized as important sources of carotenoids. This is the case of mango, a tropical fruit with traits similar to mamey, rich in β-carotene and α-carotene [31]. Varakumar, Kumar, and Reddy [32] determined the carotenoid content in seven varieties of mango (*Mangifera indica*) and found out that it ranged from 0.98 ± 0.15 to 5.81 ± 0.22 mg of β-carotene/100 g. Moreover, other tropical fruits similar to mamey such as sapodilla (*Manilkara sapota*), dragon fruit (*Hylocereus undatus*), green star apple (*Chrysophyllum cainito*), mamoncillo (*Melicoccus bijugatus*), and black sapote (*Diospyros digyna*) have carotenoid contents of 1.69 ± 0.06, 2.93 ± 0.73, 4.25 ± 1.45, 3.85 ± 0.08, and 7.99 ± 0.38 mg of β-carotene/100 g, respectively [23].

Regarding the antioxidant activity, Moo-Huchin et al. [23] found that mamey exhibits an antioxidant activity of 393.81 ± 0.36 µmol TEAC, which is considerably higher than the one found in this study. Regardless, this is expected because the researchers obtained a carotenoid content of 36.12 ± 1.24 mg β-carotene/100 g, which is almost ten times more than the value in the present work. In contrast, the ABTS radical inhibition percentage of 5 µM β-carotene reported by You et al. [22] was 40%. During the current study, the percentages of inhibition obtained were near 70% for both mamey and carrot extracts, thus confirming that a combination of compounds in both extracts (besides β-carotene) generates a synergistic effect that enhances the antioxidant activity of the carotenoids.

4.2. Oxidative stress resistance

C. elegans can undergo experimental oxidative stress upon being exposed to certain pro-oxidant compounds, such as H_2O_2, *tert*-butylhydroperoxide, arsenite, paraquat, and juglone. This leads to increased levels of O_2 and ROS, shortening the nematode lifespan and survival [33,34]. *C. elegans* has been a suitable model for understanding biological responses to various synthetic and natural compounds and their influence on aging, lifespan, and gene regulation [5,35]. This characteristic allowed us to investigate the antioxidant capacities of the MPCE, MSCE, and CCE in *C. elegans*.

Trolox, a water-soluble vitamin E analog, is a well-known antioxidant that has been previously used to protect against the oxidative damage in nematodes. In this sense, Zhang, Xue, Yang, Ma, Han, and Qin [36] and Kim et al. [37] demonstrated that Trolox and vitamin E were capable of reversing the oxidative damage in *C. elegans*, thus prolonging their lifespan. These results are consistent with the results of this study, supporting the value of *C. elegans* as a biological model to assess the antioxidant activity of many substances and the capacity of Trolox to reduce the oxidative stress damage.

In the nematodes treated with β-carotene and MSCE, the continuous exposure to carotenoids has proven to be harmful and the number of survivors decreased. According to You et al. [22], this can be attributed to the degradation of β-carotene into oxidized products that are harmful to the nematode, shortening its lifespan, such as 5,6-epoxy-β-carotene, 5,8-epoxy-β-carotene, 5,6,5′6′-diepoxy-β-carotene, 5,6-epoxy-β-ionone, β-cyclocitral, β-ionone, among others [38]. Moreover, several researchers have suggested that high levels of antioxidants generate a pro-oxidant and toxic effect on the organism, whereas lower concentrations display a protective effect [4,7,14,39,40]. Interestingly, although higher MSCE concentrations demonstrated to be toxic, survival

was superior to that of untreated nematodes. This likely was caused by the enhancement of the antioxidant defense mechanisms and the stress response, a phenomenon called hormesis, in which the continuous exposure to mild oxidative conditions, such as low concentrations of pro-oxidant and/or antioxidant compounds, temperatures between 30 and 35 °C and dietary restriction, increases the lifespan and survival of the nematode under adverse conditions [12,34].

On the other hand, MPCE and CCE increased the resistance against oxidative stress, the effect was superior to the β-carotene but inferior when compared to Trolox. Nevertheless, these results revealed the antioxidant potentials of mamey and carrot carotenoids on living organisms through their capacity for singlet oxygen quenching and deactivation of free radicals [1,6,14]. Some researchers point out that the resistance against oxidative stress is enhanced when natural sources of antioxidants, such as orange juice [6], spinach polyphenol extracts [8], or phenolic extracts from marigold flowers (*Tagetes erecta*) [41] are used instead of pure compounds. This may be attributed to the complex mixture of compounds in the extracts or the food matrix, either enhancing their activity through synergistic interactions or affecting their bioavailability [6,21]. Furthermore, Yazaki et al. [14] stated that the lifespan is influenced by both environmental factors (nutrients, oxygen, antioxidants, toxic substances, and pathogens) and heritage (20–50%). This was consistent with the results of the oxidative stress resistance assays and survival fractions of *C. elegans* treated with Trolox and carotenoids in later generations (F2 and F3).

The increases in the oxidative stress resistance of nematodes treated with carotenoids agrees with the findings reported by other researchers. For example, Pons et al. [6] showed that the continuous exposure to β-carotene (3 and 30 μg/mL) for seven days increased the resistance of the nematodes more than 50%. Similarly, You et al. [22] reported that the synthetic carotenoids BAS and BTS (0.1 mM) increased the size, fertility, and longevity of *C. elegans*. Moreover, Lashmanova et al. [42] found that fucoxanthin (0.3–10 μM) also increased the longevity and the oxidative stress resistance of *C. elegans*. However, β-carotene had no effect on the nematodes, which could be attributed to its poor absorption rate.

Over the past few years, several studies that employed *C. elegans* have shown that the protective effect of the antioxidants (including carotenoids) is not solely determined by their ability to scavenge free radicals and counter the ROS production. The oxidative stress resistance also involves the regulation of the antioxidant defenses, the expression of antioxidant enzyme genes, the modulation of transcription factors and signaling pathways, and the reduction of mitochondrial ROS production, all of which have an impact on the growth, metabolism, and survival of *C. elegans* [14,15,22]. In a previous work, the continuous exposure to Trolox increased the oxidative stress resistance of *C. elegans* offspring. However, the offspring of the nematodes that were not exposed to Trolox beyond the first generation, were still resistant to the oxidative damage and slightly increased their survival rate, suggesting an adaptive mechanism that enhances the survival following the exposure to the antioxidant [10].

C. elegans exhibits defense mechanisms against oxidative stress similar to those of humans, such as antioxidant enzymes, glutathione system, and stress response signaling pathways [11]. A key regulator of the oxidative stress response is DAF-16, a forkhead class transcription factor homologous to the mammal FOXO protein family, which is regulated by the insulin/IGF-1 signaling (IIS) pathway [35,43]. The IIS pathway is an evolutionary conserved pathway that regulates aging, longevity, and stress response in mammals and *C. elegans* [12,44]. In *C. elegans*, the IIS comprises an insulin/IGF-1 receptor (DAF-2), which regulates the activity of a phosphorylation cascade that modulates DAF-16 [45,46]. Under normal conditions, the IIS pathway downregulates the DAF-16 activity by preventing its translocation into the nucleus [11]. Environmental conditions such as stress, heat, nutrient depletion, heavy metals, and ultraviolet irradiation stimulate DAF-16 translocation into the nucleus, activating the expression of genes involve in the oxidative stress resistance and longevity [12]. DAF-16/FOXO is responsible for the activation of genes implicated in the antioxidant defense mechanisms such as superoxide dismutase-3 (*sod-3*), catalase-1 (*ctl-1*), and small heat shock protein-16.2 (*hsp-16.2*) [39,43]. Many bioactive compounds in foods can extend the lifespan of nematodes via DAF-16 pathway. For example, açai (*Euterpe oleracea*) extract increased the expression of CTL-1 expression via DAF-16, thus prolonging the lifespan and increasing the oxidative stress resistance in *C. elegans* [47].

Another key genetic pathway that modulates the oxidative stress response and longevity in *C. elegans* is SKN-1 [46]. SKN-1 is the nuclear factor erythroid 2-related factor 2 (Nrf2) mammalian ortholog, which regulates phase II detoxification genes and modulates lifespan [13,44]. Nrf2/SKN-1 defends against age-related diseases such as neurodegenerative, chronic inflammation, and cancer [45]. SKN-1 activity is inhibited by IIS kinases under normal conditions [46,48]. Under oxidative stress conditions, however, SKN-1 is phosphorylated and activated via the p38 MAPK, PMK-1 [49]. The phosphorylation of SKN-1 promotes its translocation into the nucleus, binds to antioxidant response element (ARE) sequences in the upstream promoter regions of diverse antioxidant genes, and promotes the expression of phase II detoxification enzymes such as glutathione S-transferase 4 (GST-4) [13,39,50]. For example, Yue et al. [46] showed that *p*-coumeric acid was capable of reducing the oxidative damage and extended the lifespan of *C. elegans* via SKN-1. Hydralazine also extended the lifespan of *C. elegans*, increased the locomotion, reduced the superoxide concentration, and increased the expression of GST-4 via SKN-1 pathway [13].

Carotenoids have demonstrated the ability to scavenge free radicals and counter the ROS production. However, carotenoids can also regulate and activate the antioxidant defense mechanisms to protect against the oxidative damage [14]. In this sense, the leaf extract of cashew tree (*Anacardium occidentale*) containing β-carotene, lutein, and polyphenols, reduced the intracellular ROS levels, promoted the expression of SOD-3, and increased the survival rate of *C. elegans* [39]. Liu et al. [15] and Yazaki et al. [14] also reported that astaxanthin increased the nuclear translocation of DAF-16 via IIS pathway, decreasing the oxidative damage and prolonging the lifespan of *C. elegans*. On the other hand, lycopene and adonixanthin reduced the ROS production and protected against cell death induced by light exposure in 661 W cells via Nrf2 [51]. Moreover, the pasteurized red orange (*Citrus sinensis* L. Osbeck) juice, rich in violaxanthin, zeaxanthin, lycopene, and β-carotene, reduced the ROS levels, increased the survival rate, and promoted the oxidative stress resistance in *C. elegans* via SKN-1 and DAF-16 by increasing the expression of *gst-4* and *sod-3* genes, respectively [52].

The results from the *in vivo* antioxidant activity assays evidenced the potential of Trolox and carotenoids from mamey and carrot to neutralize the oxidative damage in *C. elegans*. The increases in the survival and oxidative stress resistance involve the direct antioxidant mechanisms as well as the activation of antioxidant genes, probably, through the DAF-16/FOXO and/or SKN-1/Nrf2 pathways. Further studies are needed to elucidate the signaling pathways involved in the lifespan extension and oxidative stress resistance of *C. elegans* treated with carotenoids.

5. Conclusions

In summary, the carotenoid content of carrot was higher than that of mamey, even so the fruit can be regarded as an important source of carotenoids and antioxidant compounds with equal effectiveness in both *in vitro* and *in vivo* studies. The MPCE and CCE increased the survival of *C. elegans* exposed to oxidative stress conditions. The protective effect was superior compared to that of β-carotene. The resistance was enhanced in the next generations after the exposure to carotenoids, either through the DAF-16 and/or SKN-1 signaling pathways. Future perspectives should involve the elucidation of the molecular and biochemical pathways of the carotenoids responsible for increasing the survival and resistance against oxidative stress of *C. elegans*, as well as

the identification of the compounds present in the carotenoid extracts.

Although the consumption of fruits rich in phytochemical compounds has been associated with health benefits, the results of this study are limited to the ability of carotenoids to overcome the oxidative damage in *C. elegans*, a model organism. The results cannot be scaled to human beings. However, there is a strong possibility that the antioxidant activity of the carotenoids is connected to their ability to scavenge ROS as well as to activate the antioxidant defense mechanisms through conserved signaling pathways in both nematodes and humans.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

M.A.G.P. thanks to Universidad de las Américas Puebla (UDLAP) and Consejo Nacional de Ciencia y Tecnología (CONACYT) for the scholarship granted to complete his doctoral degree.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrep.2021.100989.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] R.K. Saini, S.H. Nile, S.W. Park, Carotenoids from fruits and vegetables: chemistry, analysis, occurrence, bioavailability and biological activities, Food Res. Int. 76 (2015) 735–750, https://doi.org/10.1016/j.foodres.2015.07.047.

[2] E.M. Yahia, F. Gutiérrez-Orozco, C. Arvizu-de Leon, Phytochemical and antioxidant characterization of mamey (*Pouteria sapota* Jacq. H.E. Moore & Stearn) fruit, Food Res. Int. 44 (2011) 2175–2181, https://doi.org/10.1016/j.foodres.2010.11.029.

[3] S. Brenner, The genetics of *Caenorhabditis elegans*, Genetics 77 (1974) 71–94.

[4] W. Chen, L. Rezaizadehnajafi, M. Wink, Influence of resveratrol on oxidative stress resistance and life span in *Caenorhabditis elegans*, J. Pharm. Pharmacol. 65 (2013) 682–688, https://doi.org/10.1111/jphp.12023.

[5] A. Bouyanfif, S. Jayarathne, I. Koboziev, N. Moustaid-Moussa, The nematode *Caenorhabditis elegans* as a model organism to study metabolic effects of ω-3 polyunsaturated fatty acids in obesity, Adv. Nutr. 10 (2019) 165–178, https://doi.org/10.1093/advances/nmy059.

[6] E. Pons, B. Alquézar, A. Rodríguez, P. Martorell, S. Genovés, D. Ramón, M. J. Rodrigo, L. Zacarías, L. Peña, Metabolic engineering of β-carotene in orange fruit increases its *in vivo* antioxidant properties, Plant Biotechnol. J. 12 (2014) 17–27, https://doi.org/10.1111/pbi.12112.

[7] D. Desjardins, B. Cacho-Valadez, J.-L. Liu, Y. Wang, C. Yee, K. Bernard, A. Khaki, L. Breton, S. Hekimi, Antioxidants reveal an inverted U-shaped dose-response relationship between reactive oxygen species levels and the rate of aging in *Caenorhabditis elegans*, Aging Cell 16 (2017) 104–112, https://doi.org/10.1111/acel.12528.

[8] D. Fan, D.M. Hodges, J. Zhang, C.W. Kirby, X. Ji, S.J. Locke, A.T. Critchley, B. Prithiviraj, Commercial extract of the Brown seaweed *Ascophyllum nodosum* enhances phenolic antioxidant content of spinach (*Spinacia oleracea* L.) which protects *Caenorhabditis elegans* against oxidative and thermal stress, Food Chem. 124 (2011) 195–202, https://doi.org/10.1016/j.foodchem.2010.06.008.

[9] F. Surco-Laos, J. Cabello, E. Gómez-Orte, S. González-Manzano, A.M. González-Paramás, C. Santos-Buelga, M. Dueñas, Effects of O-methylated metabolites of quercetin on oxidative stress, thermotolerance, lifespan and bioavailability on *Caenorhabditis elegans*, Food Funct 2 (2011) 445, https://doi.org/10.1039/c1fo10049a.

[10] M.A. González-Peña, J.D. Lozada-Ramírez, A.E. Ortega-Regules, Trolox protection against oxidative stress in *Caenorhabditis elegans*, in: S. Picazo-Vela, L.R. Herández (Eds.), Technology, Science and Culture - A Global Vision, Volume II, InTech, 2020, ISBN 978-1-78984-306-4, pp. 73–82.

[11] G. Camargo, A. Elizalde, X. Trujillo, R. Montoya-Perez, M. Luisa Mendoza-Magana, A. Hernandez-Chavez, L. Hernandez, Inactivation of GABA(A) receptor is related to heat shock stress response in organism model *Caenorhabditis elegans*, Cell Stress Chaperones 21 (2016) 763–772, https://doi.org/10.1007/s12192-016-0701-9.

[12] P. Back, B.P. Braeckman, F. Matthijssens, ROS in aging *Caenorhabditis elegans*: damage or signaling? Oxid. Med. Cell. Longev. (2012) https://doi.org/10.1155/2012/608478.

[13] E. Dehghan, Y. Zhang, B. Saremi, S. Yadavali, A. Hakimi, M. Dehghani, M. Goodarzi, X. Tu, S. Robertson, R. Lin, et al., Hydralazine induces stress resistance and extends *C. elegans* lifespan by activating the NRF2/SKN-1 signalling pathway, Nat. Commun. 8 (2017) 2223, https://doi.org/10.1038/s41467-017-02394-3.

[14] K. Yazaki, C. Yoshikoshi, S. Oshiro, S. Yanase, Supplemental cellular protection by a carotenoid extends lifespan via ins/IGF-1 signaling in *Caenorhabditis elegans*, Oxid. Med. Cell. Longev. 2011 (2011) 1–9, https://doi.org/10.1155/2011/596240.

[15] X. Liu, Q. Luo, Y. Cao, T. Goulette, X. Liu, H. Xiao, Mechanism of different stereoisomeric astaxanthin in resistance to oxidative stress in *Caenorhabditis elegans*, J. Food Sci. 81 (2016) H2280–H2287, https://doi.org/10.1111/1750-3841.13417.

[16] B. Beltrán-de-Miguel, R. Estévez-Santiago, B. Olmedilla-Alonso, Assessment of dietary vitamin A intake (retinol, α-carotene, β-carotene, β-cryptoxanthin) and its sources in the national survey of dietary intake in Spain (2009–2010), Int. J. Food Sci. Nutr. 66 (2015) 706–712, https://doi.org/10.3109/09637486.2015.1077787.

[17] K.D. Sharma, S. Karki, N.S. Thakur, S. Attri, Chemical composition, functional properties and processing of carrot—a review, J. Food Sci. Technol. 49 (2012) 22–32, https://doi.org/10.1007/s13197-011-0310-7.

[18] I. Alia-Tejacal, R. Villanueva-Arce, C. Pelayo-Zaldívar, M.T. Colinas-León, V. López-Martínez, S. Bautista-Baños, Postharvest physiology and technology of sapote mamey fruit (*Pouteria sapota* (Jacq.) H.E. Moore & stearn), Postharvest Biol. Technol. 45 (2007) 285–297, https://doi.org/10.1016/j.postharvbio.2006.12.024.

[19] E. Murillo, D. Giuffrida, D. Menchaca, P. Dugo, G. Torre, A.J. Meléndez-Martinez, L. Mondello, Native carotenoids composition of some tropical fruits, Food Chem. 140 (2013) 825–836, https://doi.org/10.1016/j.foodchem.2012.11.014.

[20] A. Torres-Rodríguez, Y. Salinas-Moreno, S. Valle-Guadarrama, I. Alia-Tejacal, Soluble phenols and antioxidant activity in mamey sapote (*Pouteria sapota*) fruits in postharvest, Food Res. Int. 44 (2011) 1956–1961, https://doi.org/10.1016/j.foodres.2011.04.045.

[21] J.S. Sangha, D. Fan, A.H. Banskota, R. Stefanova, W. Khan, J. Hafting, J. Craigie, A. T. Critchley, B. Prithiviraj, Bioactive components of the edible strain of red alga, *Chondrus crispus*, enhance oxidative stress tolerance in *Caenorhabditis elegans*, J. Funct. Foods 5 (2013) 1180–1190, https://doi.org/10.1016/j.jff.2013.04.001.

[22] J.S. You, S. Jeon, Y.J. Byun, S. Koo, S.S. Choi, Enhanced biological activity of carotenoids stabilized by phenyl groups, Food Chem. 177 (2015) 339–345, https://doi.org/10.1016/j.foodchem.2015.01.062.

[23] V.M. Moo-Huchin, I. Estrada-Mota, R. Estrada-León, L. Cuevas-Glory, E. Ortiz-Vázquez, M.de L. Vargas y Vargas, D. Betancur-Ancona, E. Sauri-Duch, Determination of some physicochemical characteristics, bioactive compounds and antioxidant activity of tropical fruits from Yucatan, Mexico, Food Chem. 152 (2014) 508–515, https://doi.org/10.1016/j.foodchem.2013.12.013.

[24] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237, https://doi.org/10.1016/S0891-5849(98)00315-3.

[25] T. Stiernagle, Maintenance of *C. elegans*, WormBook, 2006, https://doi.org/10.1895/wormbook.1.101.1.

[26] T. Chacón-Ordóñez, R.M. Schweiggert, A. Bosy-Westphal, V.M. Jiménez, R. Carle, P. Esquivel, Carotenoids and carotenoid esters of orange- and yellow-fleshed mamey sapote (*Pouteria sapota* (Jacq.) H.E. Moore & stearn) fruit and their postprandial absorption in humans, Food Chem. 221 (2017) 673–682, https://doi.org/10.1016/j.foodchem.2016.11.120.

[27] C. Nicolle, G. Simon, E. Rock, P. Amouroux, C. Rémésy, Genetic variability influences carotenoid, vitamin, phenolic, and mineral content in white, yellow, purple, orange, and dark-orange carrot cultivars, J. Am. Soc. Hortic. Sci. 129 (2004) 523–529, https://doi.org/10.21273/JASHS.129.4.0523.

[28] A.P. Mestry, A.S. Mujumdar, B.N. Thorat, Optimization of spray drying of an innovative functional food: fermented mixed juice of carrot and watermelon, Dry. Technol. 29 (2011) 1121–1131, https://doi.org/10.1080/07373937.2011.566968.

[29] M.M. Al-Dabbas, M.I. Saleh, K. Al-Ismail, Preservation methods impacted phenolic, flavonoid and carotenoid contents and antioxidant activities of carrots (*Daucus carota* L.): antioxidant activities of processed carrots, J. Food Process. Preserv. 39 (2015) 1618–1625, https://doi.org/10.1111/jfpp.12391.

[30] J.M. Villarreal-Fuentes, I. Alia-Tejacal, E. Hernández, C. Pelayo-Zaldivar, O. Franco-Mora, Caracterización poscosecha de selecciones de zapote mamey (*Pouteria sapota* (Jacq.)H. E. Moore & Stearn) procedentes del Soconusco, Chiapas, Ecosistemas Recur. Agropecu. 2 (2015) 217–224.

[31] K. Gul, A. Tak, A.K. Singh, P. Singh, B. Yousuf, A.A. Wani, Chemistry, encapsulation, and health benefits of β-carotene - a review, Cogent Food Agric. 1 (2015), https://doi.org/10.1080/23311932.2015.1018696.

[32] S. Varakumar, Y.S. Kumar, O.V.S. Reddy, Carotenoid composition of mango (*Mangifera indica* L.) wine and its antioxidant activity, J. Food Biochem. 35 (2011) 1538–1547, https://doi.org/10.1111/j.1745-4514.2010.00476.x.

[33] D. Gems, R. Doonan, Antioxidant defense and aging in *C. elegans* : is the oxidative damage theory of aging wrong? Cell Cycle 8 (2009) 1681–1687, https://doi.org/10.4161/cc.8.11.8595.

[34] K.I. Zhou, Z. Pincus, F.J. Slack, Longevity and stress in *Caenorhabditis elegans*, Aging 3 (2011) 733–753.

[35] L.P. Arantes, M.L. Machado, D.C. Zamberlan, T.L. da Silveira, T.C. da Silva, I.B. M. da Cruz, E.E. Ribeiro, M. Aschner, F.A.A. Soares, Mechanisms involved in anti-aging effects of Guarana (*Paullinia cupana*) in *Caenorhabditis elegans*, Braz. J. Med. Biol. Res. 51 (2018), e7552, https://doi.org/10.1590/1414-431X20187552.

[36] J. Zhang, X. Xue, Y. Yang, W. Ma, Y. Han, X. Qin, Multiple biological defects caused by calycosin-7-O-β-d-Glucoside in the nematode *Caenorhabditis elegans* are

associated with the activation of oxidative damage, J. Appl. Toxicol. 38 (2018) 801–809, https://doi.org/10.1002/jat.3588.
[37] J. Kim, M. Takahashi, T. Shimizu, T. Shirasawa, M. Kajita, A. Kanayama, Y. Miyamoto, Effects of a potent antioxidant, platinum nanoparticle, on the lifespan of *Caenorhabditis elegans*, Mech. Ageing Dev. 129 (2008) 322–331, https://doi.org/10.1016/j.mad.2008.02.011.
[38] D. Ribeiro, M. Freitas, A.M.S. Silva, F. Carvalho, E. Fernandes, Antioxidant and pro-oxidant activities of carotenoids and their oxidation products, Food Chem. Toxicol. 120 (2018) 681–699, https://doi.org/10.1016/j.fct.2018.07.060.
[39] C. Duangjan, P. Rangsinth, X. Gu, M. Wink, T. Tencomnao, Lifespan extending and oxidative stress resistance properties of a leaf extracts from *Anacardium occidentale* L. in Caenorhabditis elegans, Oxid. Med. Cell. Longev. (2019) 1–16, https://doi.org/10.1155/2019/9012396.
[40] M.J. Jara-Palacios, S. González-Manzano, M.L. Escudero-Gilete, D. Hernanz, M. Dueñas, A.M. González-Paramás, F.J. Heredia, C. Santos-Buelga, Study of Zalema Grape pomace: phenolic composition and biological effects in *Caenorhabditis elegans*, J. Agric. Food Chem. 61 (2013) 5114–5121, https://doi.org/10.1021/jf400795s.
[41] C. Moliner, L. Barros, M. Dias, V. López, E. Langa, I. Ferreira, C. Gómez-Rincón, Edible flowers of *Tagetes erecta* L. As functional ingredients: phenolic composition, antioxidant and protective effects on *Caenorhabditis elegans*, Nutrients 10 (2018) 2002, https://doi.org/10.3390/nu10122002.
[42] E. Lashmanova, E. Proshkina, S. Zhikrivetskaya, O. Shevchenko, E. Marusich, S. Leonov, A. Melerzanov, A. Zhavoronkov, A. Moskalev, Fucoxanthin increases lifespan of *Drosophila melanogaster* and *Caenorhabditis elegans*, Pharmacol. Res. 100 (2015) 228–241, https://doi.org/10.1016/j.phrs.2015.08.009.
[43] F.-X. Gillet, C. Bournaud, J.D.A. de Souza Júnior, M.F. Grossi-de-Sa, Plant-parasitic nematodes: towards understanding molecular players in stress responses, Ann. Bot. 119 (2017) 775–789, https://doi.org/10.1093/aob/mcw260.
[44] Y. Ye, Q. Gu, X. Sun, Potential of *Caenorhabditis elegans* as an antiaging evaluation model for dietary phytochemicals: a review, Compr. Rev. Food Sci. Food Saf. (2020), https://doi.org/10.1111/1541-4337.12654.
[45] B. Onken, M. Driscoll, Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* healthspan via AMPK, LKB1, and SKN-1, PloS One 5 (2010) 1–13, https://doi.org/10.1371/journal.pone.0008758.
[46] Y. Yue, P. Shen, Y. Xu, Y. Park, *p*-Coumaric acid improves oxidative and osmosis stress responses in *Caenorhabditis elegans*, J. Sci. Food Agric. 99 (2019) 1190–1197, https://doi.org/10.1002/jsfa.9288.
[47] L. de F. Bonomo, D.N. Silva, P.F. Boasquivis, F.A. Paiva, J.F. da C. Guerra, T.A. F. Martins, Á.G. de Jesus Torres, I.T.B.R. de Paula, W.L. Caneschi, P. Jacolot, N. Grossin, F.J. Tessier, E. Boulanger, M.E. Silva, M.L. Pedrosa, R. de Paula Oliveira, A. Nazir, Açaí (*euterpe oleracea* mart.) modulates oxidative stress resistance in *Caenorhabditis elegans* by direct and indirect mechanisms, PloS One 9 (2014), e89933, https://doi.org/10.1371/journal.pone.0089933.
[48] S. González-Manzano, A.M. González-Paramás, L. Delgado, S. Patianna, F. Surco-Laos, M. Dueñas, C. Santos-Buelga, Oxidative status of stressed *Caenorhabditis elegans* treated with epicatechin, J. Agric. Food Chem. 60 (2012) 8911–8916, https://doi.org/10.1021/jf3004256.
[49] C.-W. Wu, A. Deonarine, A. Przybysz, K. Strange, K.P. Choe, The Skp1 homologs SKR-1/2 are required for the *Caenorhabditis elegans* SKN-1 antioxidant/detoxification response independently of P38 MAPK, PLoS Genet. 12 (2016) 1–30, https://doi.org/10.1371/journal.pgen.1006361.
[50] C. Espinosa-Diez, V. Miguel, D. Mennerich, T. Kietzmann, P. Sánchez-Pérez, S. Cadenas, S. Lamas, Antioxidant responses and cellular adjustments to oxidative stress, Redox Biol. 6 (2015) 183–197, https://doi.org/10.1016/j.redox.2015.07.008.
[51] Y. Inoue, M. Shimazawa, R. Nagano, Y. Kuse, K. Takahashi, K. Tsuruma, M. Hayashi, T. Ishibashi, T. Maoka, H. Hara, Astaxanthin analogs, adonixanthin and lycopene, activate Nrf2 to prevent light-induced photoreceptor degeneration, J. Pharmacol. Sci. 134 (2017) 147–157, https://doi.org/10.1016/j.jphs.2017.05.011.
[52] R.B. de Oliveira-Caland, C.O.M. Cadavid, L. Carmona, L. Peña, R. de Paula-Oliveira, Pasteurized orange juice rich in carotenoids protects *Caenorhabditis elegans* against oxidative stress and β-amyloid toxicity through direct and indirect mechanisms, Oxid. Med. Cell. Longev. (2019), https://doi.org/10.1155/2019/5046280. Article ID 5046280.