Protective effects of acerola juice on genotoxicity induced by iron in vivo

Roberta Nunes Horta1,2,*, Vivian Francilia Silva Kahl2,*, Mericlen da Silva Sarmento2, Marisa Fernanda Silva Nunes1,2, Carem Rejane Maglione Porto2, Vanessa Moraes de Andrade4, Alexandre de Barros Falcão Ferraz3 and Juliana Da Silva2

1Centro de Ciências da Saúde, Universidade da Região da Campanha (URCAMP), Bagé, RS, Brazil. 2Laboratório de Genética Toxicológica, Universidade Luterana do Brasil, Canoas, RS, Brazil. 3Laboratório do Farmacognosia e Fitoquímica, Universidade Luterana do Brasil, Canoas, RS, Brazil. 4Laboratório de Biologia Celular e Molecular, Programa de Pós-Graduação em Ciências de Saúde, Unidade de Ciências de Saúde, Universidade do Estado de Santa Catarina, Criciúma, SC, Brazil.

Abstract

Metal ions such as iron can induce DNA damage by inducing reactive oxygen species (ROS) and oxidative stress. Vitamin C is one of the most widely consumed antioxidants worldwide, present in many fruits and vegetables, especially in Malpighia glabra L., popularly known as acerola, native to Brazil. Acerola is considered a functional fruit due to its high antioxidant properties and phenolic contents, and therefore is consumed to prevent diseases or as adjuvant in treatment strategies. Here, the influence of ripe and unripe acerola juices on iron genotoxicity was analyzed in vivo using the comet assay and micronucleus test. The comet assay results showed that acerola juice exerted no genotoxic or antigenotoxic activity. Neither ripe nor unripe acerola juices were mutagenic to animals treated with juices, in micronucleus test. However, when compared to iron group, the pre-treatment with acerola juices exerted antimutagenic activity, decreasing significantly micronucleus mean values in bone marrow. Stage of ripeness did not influence the interaction of acerola compounds with DNA, and both ripe and unripe acerola juices exerted protective effect over DNA damage generated by iron.

Keywords: genotoxicity; comet assay; micronucleus; acerola juice; iron.

Received: June 17, 2015; Accepted: October 07, 2015.

Introduction

Fruits play a prominent role in the prevention of various diseases, such as cancer, cardiovascular and neurodegenerative conditions. People who eat fruits in childhood have 38% less probability to develop cancers (Maynard et al., 2003) and balanced diets can contribute for the genomic stability (Fenech, 2014). More attention has been paid to these foods, since epidemiological evidence has shown that regular consumption of vegetables is associated with reduced mortality and morbidity from some chronic diseases (Strandhagen et al., 2000), and its protective effect has been attributed to the presence of constituents with antioxidant properties such as pholyphenols, carotenoids, and vitamins (Nunes et al., 2011; Kahl et al., 2012; Liu, 2013; Kozłowska and Szotask-Wegierek, 2014).

Malpighia glabra L., popularly called “acerola” in Brazil or “Barbados cherry”, is a native species from tropical America. Acerola compounds, such as vitamin C, carotenoids, precursors of vitamin A, lycopene, among others (Chaves et al., 2004), depends on the cultivars, environmental conditions and the stage of fruit ripeness (Chaves et al., 2004; Nunes et al., 2011).

Despite scientific data reporting benefits from juice consumption (Franke et al., 2006; Leffa et al., 2013), some compounds present in juices have also been identified as being mutagenic or carcinogenic (Spada et al., 2008). Although the genotoxicity of several components of acerola juice have already been evaluated individually, it is important to test the effect of whole juice, a complex mixture, in different biological systems. Genotoxic effects, for example, may be mediated by the interaction of juice compounds with transition metals or by-products of juice auto-oxidation; Vitamin C can act as a pro-oxidant, because its reducing ability, through Fenton and Fenton-like reactions (De Freitas and Meneghini, 2001).

One feature of the normal human diet is the simultaneous presence of both essential and toxic metals (Beyers...
mann and Hartwig, 2008). Metal ions generate DNA damage directly or indirectly by formation of reactive oxygen species (ROS) (De Freitas and Meneghini, 2001; Phaniendra et al., 2015). In recent years, many studies have been conducted on the role of ROS in the etiology of various diseases since some free radicals, in a condition of oxidative stress, are not neutralized by antioxidant cell protective mechanisms or antioxidant compounds (Halliwell and Guttridge, 2000; Phaniendra et al., 2015). Vitamin C, present in acerola juice is an example of an antioxidant compound that can chelate metals, thus preventing the generation of ROS (De Freitas and Meneghini, 2001; Phaniendra et al., 2015), apart from playing a role in the regulation of DNA repair enzymes (Jomova and Valko, 2011).

Considering the importance of consumption of fruits and the intense growth of the culture of acerola in Brazil and its widespread use, the aim of this study was to test the genotoxic effects of acerola juice at two ripeness stages associated with metallic agents in vivo in mice, using the comet assay and the micronucleus test, in order to improve our understanding of the role of dietary antimutagens and anticarcinogens in humans.

Material and Methods

Preparation of acerola juice

Acerola samples were collected from an organic culture in May 2008, in the district of Ubajara, CE, Brazil. The fruits were collected randomly across the whole plant, and were sorted according to ripeness using a skin color gradient, as follows: (1) green fruits (unripe), fruits showing a green hue on more than 75% of its skin; and (2) red fruits (ripe), those showing a red or burgundy hue on 100% of the skin (Campelo et al., 1998). After collection, fruits were kept frozen and protected from light in order to preserve their chemical and physical characteristics upon juice preparation (Nunes et al., 2011). Approximately 20 units of acerola fruit were used to produce 150 g of pulp extract, after fruits were peeled and seeds removed. The pulp extract from ripe and unripe fruits were diluted with water (150 g of pulp extract per liter of water), forming the juice used in treatments. A glass of juice consumed by humans usually has 200 mL, and therefore, is composed by around 30 g of pulp extract. The acerola cultivar used in this study was AC-69.

Animals and treatment

Animals used were CF1 mice Mus musculus, aged 5-7 weeks old, weighing 20-40 g, provided by the Fundação Estadual de Produção e Pesquisa em Saúde (FEEPS), Porto Alegre, RS, Brazil. The animals were kept in the central animal house of Lutheran University of Brazil (ULBRA), Canoas, RS, Brazil. The temperature in the experimental room was about 24 °C, and relative humidity was roughly 60%. The light cycle was 12 h light/12 h dark. All animals received commercial standard mouse cube diet (Labina-PURINE) and water ad libitum. All experimental procedures were approved by CONEP 2008-008-A, Brazil (National Commission of Ethics in Research).

Ten mice per group (five females and five males) were used for the treatment (0.1 mL/10 g body weight) divided in six groups: (a) Negative control, water; (b) Positive Control, FeSO₄ (35 mg/kg), (c) Unripe acerola juice; (d) Ripe acerola juice; (e) Unripe acerola juice plus FeSO₄; (f) Ripe acerola juice plus FeSO₄. The dose, exposure time and the administration route of FeSO₄ were based on a previous study (Nunes et al., 2011). Table 1 summarizes the treatment period. Acerola, FeSO₄ and water were administrated by gavage. The test substance was handled as potentially mutagenic, according to the safety procedures required.

Table 1 - Experimental procedures: treatment protocols and blood sampling schedules.

| Group                          | Exposure and sampling schedule | 0h               | 3h               | 24h              | 48h                      |
|-------------------------------|--------------------------------|------------------|------------------|---------------------|-------------------------|
| Control                       | Treatment: water               | Blood sampling   | Blood sampling   | Treatment: water    | Blood and bone marrow sampling |
| FeSO₄                          | Treatment: FeSO₄               | Blood sampling   | Blood sampling   |                      | Blood and bone marrow sampling |
| Unripe acerola juice          | Treatment: unripe acerola juice| Blood sampling   | Blood sampling   | Treatment: unripe acerola juice | Blood and bone marrow sampling |
| Ripe acerola juice            | Treatment: ripe acerola juice  | Blood sampling   | Blood sampling   | Treatment: ripe acerola juice | Blood and bone marrow sampling |
| Unripe acerola juice + FeSO₄   | Pre-treatment: unripe acerola juice |              | -                | Treatment: FeSO₄    | Blood and bone marrow sampling |
| Ripe acerola juice + FeSO₄    | Pre-treatment: ripe acerola juice |              | -                |                      | Blood and bone marrow sampling |
Genotoxicity assays

Comet assay

The analysis was conducted in accordance with the protocol described by Singh et al. (1998) with some modifications (Nunes et al., 2011). Blood cells (10 μL of whole blood with heparin) were embedded in 90 μL of 0.75% (w/v) low melting point agarose and the mixture added to a microscope slide pre-coated with 1.5% (w/v) of normal melting point agarose and topped with a coverslip. The slides were briefly placed on ice for agarose to solidify and then the coverslips were carefully removed. Next, the slides were immersed in lysis solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0–10.5) containing freshly added 1% Triton X-100 and 10% dimethyl sulfoxide (DMSO) for at least 1 h at 4 °C. Subsequently, the slides were incubated in freshly made alkaline buffer (300 mM NaOH and 1 mM EDTA, pH > 13) for 20 min for DNA unwinding, and electrophoresis was performed in the same buffer. The electrophoresis conditions were 15 min at 300 mA and 25 V (0.7 V/cm). All these steps were carried out under dim indirect light. Following electrophoresis, slides were neutralized in 400 mM Tris buffer (pH 7.5) and fixed (15% w/v trichloroacetic acid, 5% w/v zinc sulfate, 5% glycerol), washed in distilled water and dried overnight. The gels were re-hydrated for 5 min in distilled water, and then stained for 15 min (37 °C) with a solution containing the following sequence: 34 mL of Solution B (0.2% w/v ammonium nitrate, 0.2% w/v silver nitrate, 0.5% w/v tungstosilicic acid, 0.15% v/v formaldehyde, 5% w/v sodium carbonate) and 66 mL of Solution A (5% sodium carbonate). The staining was stopped with 1% acetic acid and the gels were air-dried. To calculate a damage index (DI), cells were visually allocated into 5 classes according to tail size (0 = no tails, to 4 = maximum-length tails) which resulted in a single DNA damage score for each sample and consequently for each group studied. Thus, the damage index (DI) of the group could range from 0 (completely undamaged = 100 cells X 0) to 400 (maximum damage = 100 cells X 4). The damage frequency (DF in %) was calculated for each sample based on the number of cells with tail vs. those without. All slides were coded for blinded analysis.

The results of the potential of acerola juice to modulate the effect of FeSO₄ treatment were expressed as described in the literature (Kapiszewska et al., 2005), as percentage inhibition of damage index (DI) according to the expression: (1%) percentage inhibition of DI = [FeSO₄ DI - DI of the extract with FeSO₄] / [FeSO₄ DI - DI negative control] x 100

Micronucleus test

Each complete test was made according to a report by Mavournin et al. (1990) and OECD (2013). Bone marrow smears were prepared for the 48-h exposure sample, when animals were killed by decapitation. The bone marrow was extracted from the two femurs and the smears were prepared directly on slides, two per animal, with a drop of fetal calf serum. The slides were stained with 10% Giemsa for 5 min, air-dried and coded for blinded analysis. To avoid false negative results and as a measure of toxicity in bone marrow, the polychromatic erythrocytes: normochromatic erythrocytes (PCE/NCE) ratio was scored in 1,000 cells. The incidence of micronuclei was observed in 2,000 PCE for each animal (i.e. 1,000 from each of the two slides prepared from the duplicate), using bright-field optical microscopy at a magnification of 200–1000. The test groups were compared to the respective negative controls by gender, separately and in combination.

Statistical analysis

The normality of variables was evaluated using the Kolmogorov–Smirnov test. Statistical differences between the groups were analyzed using the non-parametric two-tailed Kruskal–Wallis Test with the Dunn correction for multiple comparisons for comet assay and micronucleus test results. Student’s t-test was used to compare damage between genders. The critical level for rejection of the null hypothesis was considered to be a P value of 5%.

Results

Table 2 summarizes the comet assay data expressed as damage index (DI) and damage frequency (DF) for blood cells of mice exposed for 3 h, 24 h and 48 h to water, FeSO₄ and acerola juices, at different maturation stages. The FeSO₄ group showed a significant increase in DI, as compared to the negative control, at 24 h (P < 0.01) and 48 h (P < 0.001). After treatment with FeSO₄ at 48 h, the mean DF values were also significantly elevated (P < 0.001). Concerning the acerola juice groups, no genotoxic activity was observed, when compared with the negative control group.

Figure 1 shows that groups pre-treated with acerola presented lower DI than the FeSO₄ group. Nevertheless, no statistically significant difference in antigenotoxic activity of acerola juice was observed in relation to FeSO₄. The pre-treatment with unripe acerola juice presented modulation of DNA damage of 21.33%, while the modulation by ripe acerola juice was 56.65%. Our study also found no differences between juice maturation stage and animal gender in the comet assay results.

Table 3 shows the results of the micronucleus test for bone marrow samples. Bone marrow cells of mice treated with unripe and ripe acerola juice showed no significant increase in micronucleus mean, when compared with negative control, therefore not presenting mutagenicity. FeSO₄ was mutagenic when compared to water (P < 0.001) and acerola juices from ripe (P < 0.01) and unripe (P < 0.001) fruits. There was no significant difference in PCE/NCE ratios, indicating the absence of toxicity. No difference in micronuclei formation was observed between genders.
Bone marrow cells of mice pre-treated with juices from unripe and ripe acerola fruits showed a significant decrease in micronucleus (P < 0.001 and P < 0.01, respectively), when compared with the FeSO₄ group, revealing antimutagenic activity (Figure 2).

**Discussion**

A recent study considers acerola as a functional food since its pulp shows great antioxidant activity and phenolic content and, therefore is consumed to prevent diseases or as

Table 2 - Comet assay parameters (damage index and damage frequency index; mean ± standard deviation) for blood samples of mice treated with unripe and ripe acerola juice. For each group, n = 10 (five males and five females), with 100 cells per animal.

| Groups                  | Gender | Schedule | Per Gender | Per Group | Per Gender | Per Group | Per Gender | Per Group |
|-------------------------|--------|----------|------------|-----------|------------|-----------|------------|-----------|
|                         |        | 3h       | 24h        | 48h       |            |           |            |           |
| Damage Index            |        |          |            |           |            |           |            |           |
| Water                   | Male   | 7.60 ± 3.43 | 6.70 ± 3.94 | 7.80 ± 1.30 | 8.60 ± 2.79 | 10.80 ± 3.27 | 8.50 ± 4.03 |
|                         | Female | 5.80 ± 4.60 | 9.40 ± 3.78 | 44.75 ± 38.86 | 31.00 ± 13.29 |
| FeSO₄                   | Male   | 10.00 ± 4.89 | 10.60 ± 6.39 | 19.50 ± 1.92 | 32.13 ± 28.80 | 4.00 ± 4.96  | 36.00 ± 10.70 |
|                         | Female | 11.20 ± 8.20 | 4.60 ± 3.37 | 14.00 ± 8.66 | 13.33 ± 7.84 | 2.60 ± 3.71  | 2.70 ± 3.83  |
| Unripe acerola juice    | Male   | 3.80 ± 3.03 | 4.60 ± 3.37 | 14.00 ± 8.66 | 13.33 ± 7.84 | 2.60 ± 3.71  | 2.70 ± 3.83  |
|                         | Female | 5.40 ± 3.84 | 12.50 ± 7.59 | 2.80 ± 4.38  |            |           |           |           |
| Ripe acerola juice      | Male   | 6.80 ± 6.05 | 5.10 ± 4.65 | 14.00 ± 5.78 | 13.90 ± 3.98 | 2.80 ± 2.28  | 2.40 ± 1.89  |
|                         | Female | 3.40 ± 2.19 | 13.80 ± 1.48 | 2.00 ± 1.58  |            |           |           |           |
| Damage Frequency         |        |          |            |           |            |           |            |           |
| Water                   | Male   | 6.40 ± 2.60 | 5.00 ± 3.27 | 4.80 ± 2.49  | 5.00 ± 2.35 | 5.40 ± 1.81  | 4.30 ± 2.05  |
|                         | Female | 4.80 ± 3.96 | 5.20 ± 2.49  | 3.20 ± 1.78  |            |           |           |           |
| FeSO₄                   | Male   | 7.80 ± 3.63 | 6.67 ± 3.50 | 9.20 ± 1.00  | 17.13 ± 18.13 | 28.00 ± 4.24 | 25.38 ± 8.71  |
|                         | Female | 5.25 ± 3.20 | 24.75 ± 24.72 | 22.75 ± 11.87 |
| Unripe acerola juice    | Male   | 2.60 ± 1.94 | 3.10 ± 1.96 | 11.60 ± 4.98 | 10.68 ± 4.89 | 1.60 ± 2.30  | 1.50 ± 2.12  |
|                         | Female | 3.60 ± 2.07 | 9.75 ± 5.13  | 1.40 ± 2.91  |            |           |           |           |
| Ripe acerola juice      | Male   | 4.80 ± 4.43 | 3.00 ± 3.55 | 9.20 ± 3.27  | 10.00 ± 3.39 | 1.60 ± 1.14  | 1.60 ± 1.17  |
|                         | Female | 1.20 ± 0.83 | 10.80 ± 3.70 | 1.60 ± 1.34  |            |           |           |           |

*a*For more details see Table 1.

*b*Significant difference in relation to water (negative control) at P < 0.01, Kruskall-Wallis test.

*c*Significant difference in relation to water (negative control) at P < 0.001, Kruskall-Wallis test.

Table 3 - Detection of micronucleus mean (± standard deviation) in bone marrow polychromatic erythrocytes (MNPCE) of mice individuals exposed to acerola juice. Each group, n = 10 (five males and five females), with 2000 cells/animal.

| Groups                  | Gender | Bone marrow (MNPCE) | Ratio (PCE:NCE) |
|-------------------------|--------|---------------------|-----------------|
|                         |        | Per gender | Per group | Per gender | Per group |
| Water                   | Male   | 1.80 ± 0.83 | 2.50 ± 1.35 | 1.09 ± 0.28 | 1.14 ± 0.29 |
|                         | Female | 3.20 ± 1.48 | 1.19 ± 0.32 |            |           |           |
| FeSO₄                   | Male   | 7.33 ± 1.52 | 7.28 ± 1.49 | 1.18 ± 0.27 | 1.26 ± 0.23 |
|                         | Female | 7.25 ± 1.70 | 1.33 ± 0.21 |            |           |           |
| Unripe acerola juice    | Male   | 2.00 ± 1.00 | 1.90 ± 0.87 | 0.86 ± 0.11 | 0.91 ± 0.13 |
|                         | Female | 1.80 ± 0.83 | 0.95 ± 0.14 |            |           |           |
| Ripe acerola juice      | Male   | 4.60 ± 1.14 | 4.00 ± 1.70 | 0.94 ± 0.23 | 0.93 ± 0.16 |
|                         | Female | 3.40 ± 2.07 | 0.92 ± 0.09 |            |           |           |

*P < 0.001 in relation to unripe acerola juice, Kruskall-Wallis test;*  
*P < 0.01 in relation to ripe acerola juice, Kruskall-Wallis test.  
*Significant at P < 0.001 in relation to water, Kruskall-Wallis test.*
adjuvant in treatment strategies (Paz et al., 2015). Acerola shows complex traits, interacting with biological organisms in many ways. In this study, genotoxicity and anti-genotoxicity of acerola juices in different stages of ripeness in relation to FeSO4 DNA damages were evaluated in blood cells of mice using the comet assay and in bone marrow cells using the micronucleus test. The choice of these two types of fruit stage is due to the fact that the physico-chemical characteristics of acerola undergo changes as the fruit ripens (Chaves et al., 2004; Nunes et al., 2011; Paz et al., 2015).

Acerola juice from unripe or ripe juices did not show genotoxic and mutagenic potential in mice after in vivo treatment. Our previous studies have shown that acerola samples of lyophilized fruit pulp collected in two Brazilian states (São Paulo and Ceará) were not genotoxic in mice blood cells ex vivo (Nunes et al., 2012). A similar result was observed for genotoxicity ex vivo also when two ripeness stages of acerola were used (Nunes et al., 2011). The genotoxicity of juices of different fruits and vegetables, such as orange, guava, Pterodon emarginatus and carrot has been studied, also showing no genotoxicity (Franke et al., 2006; Fernandéz-Bedmar et al., 2011; Lee et al., 2011).

Iron plays an essential role in metabolism, since it participates in the transport of oxygen and xenobiotic metabolizing enzymes (De Freitas and Meneghini, 2001; Cooper and Groves, 2011). However, exposure to excessive amounts of iron can damage cells and organs, since the metal takes part in reactions which generate reactive oxygen species (ROS) (Franke et al., 2006; Beyersmann and Hartwig, 2008; Jomova and Valko, 2011). The World Health Organization (WHO, 2002) has established as the recommended dietary allowance (RDA) between 8 and 18 mg/day iron, depending on the gender. The tested dose in this study corresponds to about 12% of the LD50 for FeSO4 and was genotoxic 24 h after treatment, inducing DNA damage in the comet assay (24 h and 48 h) and micronucleus test (48 h). The evolution of systemic effects and the peak of body iron intoxication occur 4-6 h after administration of large quantities of the compound (SACN, 2010), which can justify the non-genotoxic effect 3-h after exposure. It is likely that the DNA damage was generated early after exposure, as a consequence of the iron peak in the blood and inside the cells. Despite the DNA damage observed after 24 h and 48 h, it is not clear whether iron was free or bound in blood. Although free iron is not likely to exist in biological systems, it is well known that the presence of trace amounts of “free” iron (i.e., weakly bound) is involved in the generation of oxidative stress (Koskenkorva-Frank et al., 2013). Similarly to the present work, a
previous study demonstrated analogous results in mice exposed to FeSO₄ in the comet assay (Franke et al., 2006).

When pre-treatment was evaluated, acerola juice in both maturation stages did not significantly reduce DNA damage evaluated by the comet assay, though unripe and ripe acerola juices showed an interesting modulation of 21.33% and 56.65%, respectively, in relation to FeSO₄ treatment only, apart from antimutagenic activity in the micronucleus test. Thus, pre-treatment with acerola juice protected the blood cells against DNA damage generated by FeSO₄. Although the main positive aspect of acerola fruit is its high vitamin C content, it also presents large quantities of antioxidants, such as carotenoids, flavonoids, and anthocyanins (Chaves et al., 2004; Nóbrega et al., 2014; Silva et al., 2014). Some studies stated that the antioxidant potential of acerola juice depended on its content of phenolic compounds and vitamin C (Leffa et al., 2014; Paz et al., 2015). Vitamin C has been studied for its protective action against different diseases (Folchetti et al., 2014; Ives et al., 2014; Koike et al., 2014). The mechanisms by which vitamin C acts include antioxidative activity as well as bio-antimutagenic and/or desmutagenic actions (De Freitas and Meneghini, 2001; Franke et al., 2006). Although many other factors are involved in the differences observed, considerable evidence demonstrated that acerola juice has beneficial potential to DNA, protecting it against damages caused by FeSO₄.

In a previous study from our laboratory (Nunes et al., 2011) we observed that ripe acerola showed antigenotoxicity activity against damage caused by hydrogen peroxide ex vivo. In the present study, we observed modulation of damage caused by FeSO₄ to both unripe and ripe acerola juice. Furthermore, pre-treatment showed significant antimutagenic activity. Despite the differences in antigenotoxic activities between unripe and ripe fruits (Nunes et al., 2011), in the present study we did not observe this effect, probably due to the biological response of metabolism in vivo and the complex mixture of nutrients of acerola juice. The chemical composition of fruits evaluated by HPLC (the same samples as in the previous study) demonstrated that unripe acerola showed higher levels of vitamin C (8,104 mg/100 g of acerola sample) than ripe acerola (4,447.6 mg/100 g of acerola sample). Quantification analysis shows that the ripe acerola has a higher content of flavonoids (15.3 ± 0.35%) in comparison to unripe acerola (8.7 ± 0.21%). For rutin and quercetin no significant differences were observed (150 mg of rutin/100 g of acerola sample and 57 mg of quercetin/100 g of acerola sample). It was also observed in this previous study (Nunes et al., 2011) that the amount of extract needed to capture the DPPH free radical is lower in unripe than in ripe acerola, and that the antioxidant capacity of unripe fruit at IC₅₀ was two times higher than that of ripe sample. In conclusion, both unripe and ripe acerola juice exerted protective effect on DNA damage generated by FeSO₄. Stage of ripeness did not influence the interaction of acerola compounds with DNA, showing that consumption of acerola juice, combined with a healthy and balanced diet, can lead to a protective effect.

Acknowledgments

We thank Nutrilite Farm, Ceará, Brazil, for help and assistance. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Universidade Luterana do Brasil (ULBRA).

References

Beyersmann D and Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 82:493-512.

Campelo E, Martins M, Carvalho I and Pedrosa E (1998) The role of vitamin C in polyps of acerola (Malpighia glabra L.) congeladas. Bol CEPPA 6:107-113 (in Portuguese).

Chaves MCV, de Gouveia JPG, Almeida FAC, Leite JCA and da Silva FLH (2004) Caracterização físico-química do suco da acerola. Rev Biol Ciência Terra 4:1-10 (in Portuguese).

Cooper HL and Groves JT (2011) Molecular probes of the mechanism of cytochrome P450. Oxygen traps a substrate radical intermediate Arch Biochem Biophys 507:111-118.

De Freitas JM and Meneghini R (2001) Iron and its sensitive balance in the cell. Mutat Res 475:153-159.

Dias FM, Leffa DD, Daumann F, Marques SO, Luciano TF, Possato JC, Santana AA, Neves, RX, Rosa JC, Oyama LM, et al. (2014) Acerola (Malpighia emarginata DC) juice intake protects against alterations to proteins involved in inflammatory and lipolysis pathways in the adipose tissue of obese mice fed a cafeteria diet. Lipids Health Dis 13:e24.

Fenech M (2014) Nutriomes and personalised nutrition for DNA damage prevention, telomere integrity maintenance and cancer growth control. Cancer Treat Res159:427-441.

Fernández-Bedmar Z, Anter J, de la Cruz-Ares S, Muñoz-Serrano A, Alonso-Moraga A and Pérez-Guisado J (2011) Role of citrus juices and distinctive components in the modulation of degenerative processes: genotoxicity, antigenotoxicity, cytotoxicity and longevity in Drosophila. J Toxicol Environ Health A 74:1052-1066.

Folchetti LD, Monfort-Pires M, Barros C, Martini L and Ferreira S (2014) Associations of fruits and vegetables consumption and related-vitamins with inflammatory and oxidative stress markers in prediabetic individuals. Diabetol Metab Syndr 6:e22.

Franke SIR, Prá S, Dias JF, Giulian R, Yoneama,ML, Da Silva J, Erdtmann B, and Henriques JAP (2006) Influence of orange juice in the levels and in the genotoxicity of iron and copper. Food Chem Toxicol 44:425-435.

Halliwell B and Guttridge JMC (2000) Free radicals in biology and medicine, 4rd edition. Oxford University Press, New York, 704 p.

Ives SJ, Harris RA, Witman MAH, Fjeldstad AS, Garten RS, McDaniel J, Wray WD and Richardson RS (2014) The role of redox balance. Hypertension 63:459-467.

Jomova K and Valko M (2011) Advances in metal-induced oxidative stress and human disease. Toxicology 283:65-87.
Kahl VFS, Reyes JM, Sarmento MS and Da Silva J (2012) Mitigation by vitamin C of the genotoxic effects of nicotine in mice, assessed by comet assay and micronucleus induction. Mutat Res 744:140-144.

Kapiszewska M, Soltys E, Visioli F, Cierniak A and Zajac G (2005) The protective ability of the Mediterranean plant extract against the oxidative DNA damage. The role of the radical oxygen species and the polyphenol content. J Physiol Pharmacol 56:183-197.

Koike K, Ishigami A, Sato Y, Hirai T, Yuan Y, Kobayashi E, Tobino K, Sato T, Sekiya M, Takahashi K, et al. (2014) Vitamin C prevents cigarette smoke-induced pulmonary emphysema in mice and provides pulmonary restoration. Am J Respir Cell Mol Biol 50:347-357.

Koskenkorva-Frank TS, Weiss G, Koppenol WH and Burckhardt S (2013) The complex interplay of iron metabolism, reactive oxygen species, and reactive nitrogen species: insights into the potential of various iron therapies to induce oxidative and nitrosative stress. Free Radical Biol Med 65:1174-1194.

Kozłowska A and Szotask-Wegierek D (2014) Flavonoids - food source and health benefits. Rocz Panstw Zakl Hig 65:79-85.

Lee HJ, Park YK and Kang MH (2011) The effect of carrot juice, β-carotene supplementation on lymphocyte DNA damage, erythrocyte antioxidant enzymes and plasma lipid profiles in Korean smoker. Nutr Res Pract 5:540-547.

Leffa DD, da Silva J, Daumann F, Dajori ALF, Longaretti LM, Damiani AP, de Lira F, Campos, F, Ferraz ABF, Côrrea DS and Andrade VM (2013) Corrective effects of acerola (Malpighia emarginata DC) juice intake on biochemical and genotoxic parameters in mice fed on a high-fat diet. Mutat Res 770:144-152.

Liu RH (2013) Health-promoting components of fruits and vegetables in the diet. Adv Nutr 4:348S-392S.

Mavournin KH, Blakey DH, Cimino MC, Salamone SF and Heddle JA (1990) The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 239:29-80.

Maynard M, Gunnell D and Emmett P (2003) Fruit, vegetable and antioxidant in childhood and risk of adult cancer: the Boyd Orr cohort. J Epidemiol Comm Health 57:218-225.

Nóbrega EM, Oliveira EL, Genovese M and Correia RTP (2014) The impact of hot air drying on the physical-chemical characteristics, bioactive compounds and antioxidant activity (Malpighia emarginata) residue. J Food Process Preserv 39:131-141.

Nunes RS, Kahl VFS, Sarmento MS, Richter MF, Costa-Lotufo LV, Rodrigues FAR, Abin-Carriquiry JA, Martinez MM, Ferronatto S, Ferraz ABF and Da Silva J (2011) Antioxidant and antigenotoxicity activity of acerola fruit (Malpighia glabra L.) at two stages of ripeness. Plant Foods Hum Nutr 66:129-135.

Nunes RS, Kahl VFS, Sarmento MS, Richter MF, Abin-Carriquiry JA, Martinez MM, Ferraz ABF and Da Silva J (2012) Genotoxic and antigenotoxic activity of acerola (Malpighia glabra L.) extract in relation to the geographic origin. Phytother Res 27:1495-1501.

OECD (2013) Organization for Economic Cooperation and Development. Guidelines for the testing of chemicals: in vitro mammalian chromosome aberration test, revised and new guidelines. Paris, 20 p.

Paz M, Gullon,P, Barroso MF, Carvalho AP, Domingues VF, Gomes AM, Becker H, Longhinotti E and Delerue-Matos C (2015) Brazilian fruit pulps as functional foods and additives: evaluation of bioactive compounds. Food Chem 172:462-468.

Phaniendra A, Jestadi DB and Perivasamv L (2015) Free radicals: properties, sources and their implication in various diseases. Indian J Clin Biochem 30:11-26.

SACN (2010) Iron and Health, Scientific Advisory Committee on Nutrition. London, 374 p.

Silva LMR, Figueiredo EAT, Ricardo NMPS, Vieira I, Figueiredo R, Brasi I and Gomes C (2014) Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil. Food Chem 143:398-404.

Singh NP, McCoy MT, Tice RR and Schneider EL (1998) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184-191.

Spada PDS, de Souza GGN, Bortolini GV, Henriques JAP and Salvador M (2008) Antioxidant, mutagenic, and anti-mutagenic activity of frozen fruits. J Med Food 11:144-151.

Strandhagen E, Hansson PO, Bossaeus I, Isaksson B and Eriksson H (2000) High fruit intake may reduce mortality among middle-aged and elderly men. The Study of Men Born in 1913. Eur J Clin Nutr 54:337-341.

WHO (2002) World Health Organization, Food and Agriculture Organization of the United Nations. 2002. Human Vitamin and Mineral Requirements. Food and Nutrition Division. FAO Rome. Report of a joint FAO/WHO expert consultation Bangkok, Thailand. Rome, 303 p.

Associate Editor: Catarina S. Takahashi