DOI: 10.1590/1678-457X.0006

Cytotoxic and genotoxic potential of powdered juices

Marielly de Sousa BEZERRA[1], Geiz de Sousa MALAQUIAS[1], João Marcelo de CASTRO E SOUSA[1], Ana Paula PERON[1]*

Abstract

Powdered juices are widely consumed by the population especially because of their convenient preparation, availability in various fruit flavors and low cost when compared to other industrialized beverages. They have complex formulation, consisting of several classes of food additives. However, there are no scientific studies on the toxicity of these foods. Thus, this study evaluated the toxicity at the cellular level of industrialized powdered juices of orange and guava flavors of three different food companies. This analysis was made using root meristem cells of *Allium cepa* L., at the exposure times of 24 and 48 hours, and two concentrations, 30 g/1000 mL, considered ideal for consumption according to the label of the products, and 30 g/500 mL. Both flavors of juices, of the three companies, in both concentrations and the two exposure times promoted significant antiproliferative effect to root meristem cells and caused a statistically significant number of mitotic spindle changes and micronuclei in cells of the test system used. Therefore, under the studied conditions, all the samples of juice powder exhibited cytotoxic and genotoxic potential.

Keywords: industrialized juice; toxicity at the cellular level; mitotic index; cellular aberrations; *Allium cepa*.

Pratical Application: For the first time it is demonstrated cytotoxicity and genotoxicity solutions from dilution water in industrial juice powder.

1 Introduction

Due to the fast pace of city life, the consumption of powdered or industrialized juices increased significantly worldwide in recent decades (Biral et al., 2013; Longo-Silva et al., 2015). These foods, besides presenting good sensory quality including attractive color, odor and taste, are easily diluted in water and yield several portions (Mörschbächer & Souza, 2012). Also, they are available in various fruit flavors, sold in small packets that facilitates storage, and have prices far lower than other types of processed beverages, such as soft drinks (Ferrarezi et al., 2010).

To achieve the sensory properties currently offered, industrialized powdered juices over two decades have undergone several modifications regarding chemical formulation, including the addition of various classes of food additives such as acidulant, antioxidant, flavoring, coloring, anti-wetting, acidity regulator, foaming and sweetener (Longo-Silva et al., 2015). Such modifications were allowed and standardized by Ordinance 544, of November 16, 1998 – approving the technical regulations for setting the standards of identity and quality of powdered juices – of the Ministry of Agriculture, Livestock and Supply (Brasil, 2006; Ferrarezi et al., 2010).

It is known that food additives have become mandatory in foodstuffs, due to, among other features, the extension of sensory quality and shelf life of processed foods (Marques et al., 2015). However, there are studies associating these compounds with harmful effects to the health of consumers, such as the development of allergies, cancer and changes in the functioning of the digestive tract (Gomes et al., 2013; Oliveira et al., 2013). In this way, Marques et al. (2015) pointed out that the toxicological evaluation, at systemic and cellular levels, of these additives and especially of foods containing such compounds is extremely important in terms of promoting food security of the population and providing a basis for the development or modification of food security strategies of surveillance agencies.

Regarding powdered juices, it is already known that the use of these foods in regular diet, especially of children, has a significant impact, in the long- and medium term, on the increase of overweight, obesity and enhancement of associated chronic diseases, such as diabetes, hypertension and cardiovascular problems (Brasil, 2011; Nogueira & Sichieri, 2009; Longo-Silva et al., 2015). Meanwhile, there is no scientific literature available on toxicological studies regarding the solutions from the dilution of powdered juice in water.

Living organisms are frequently exposed to cytotoxic and genotoxic substances that can damage vital cellular mechanisms, such as duplication and gene transcription, and thus drastically alter the cell division of the affected tissue mechanism and induce the appearance of cellular aberrations, such as the mitotic spindle and chromosome breakage (Valavanidis et al., 2013). Hence, such compounds have the potential to trigger and/or potentiate cancerous processes (Zilifdar et al., 2014). According to Zaineddin et al. (2012), the development of the most common types of cancer results from the interaction

Received 08 Sept., 2015
Accepted 20 Nov., 2015
[1]*Laboratório de Citogenética e Mutagênese – LaCM, Universidade Federal do Piauí – UFPI, Campus Senador Helvídeo Nunes de Barros, Picos, PI, Brasil*
*Corresponding author: anapaulaperon@ufpi.edu.br

between endogenous and environmental factors, highlighting the diet, especially when it consists of excess of processed foods. Also, Bendino et al. (2012) and Louzada et al. (2015) report that over 40% of various cancers occur because of inadequate diets rich in food additives.

Plant bioassays are highly sensitive and simple for monitoring cytotoxic, genotoxic and mutagenic effects of chemicals (Caritá & Marin-Morales, 2008; Herrero et al., 2012). Among these, the root meristematic zone of *Allium cepa* L. (onion) is regarded by the scientific community as an effective bioassay for the initial screening of acute toxicity at the cellular level (Tabrez et al., 2011; Lacerda et al., 2014). This test organism shows excellent kinetic properties of proliferation, large and few chromosomes (2n=16), which facilitates the detection of chromosomal aberrations and mitotic spindle abnormalities (Herrero et al., 2012; Cardoso et al., 2014). It also reveals changes in cell division or mitotic index, or when exposed to chemical compounds with cytotoxic activity.

Based on the above, this study aimed to evaluate the cytotoxicity and genotoxicity of powdered juices, orange and guava flavors, of three different food companies, using root meristem cells of *A. cepa*. These flavors were chosen as they are highly appreciated by the people.

2 Material and methods

2.1 Samples and concentrations

Samples of Orange and Guava powdered juices of the food companies General Foods Corporation©, *Três Corações Alimentos S/A*© and General Brans© – referred to hereinafter as A, B and C, respectively – were purchased from the retail market in the city of Picos, Piauí State, Brazil. All the products were within the expiration date and their packets were not damaged.

The label of each sample suggested the dilution of the entire content of the packet, 30 g, in one liter of cold water. Thus, the first concentration set for evaluation was 30 g/1000 mL. The second concentration was set at 30 g/500 mL, because dilution is not always made according to label instructions. In this study, the dilution of the powdered juice was performed in mineral water previously chilled in the refrigerator.

2.2 Root meristem cells of A. cepa and cytogenetic analysis

Onion bulbs were placed in bottles with aerated distilled water at room temperature (± 27 °C) until obtaining 2.0 cm long roots. For analysis of each treatment, we defined an experimental group with five onion bulbs. Before placing the roots in contact with their respective treatments (solutions), some roots were collected and fixed to serve as control of the bulb itself. Then, the remaining roots were placed into their respective solutions for 24 hours, a procedure called 24 hour exposure time (ET 24 h).

After 24 hours, some roots were taken and fixed. Subsequently, the remaining roots of each bulb were returned to their respective solutions and remained therein for another 24 hours, which is called 48 hour exposure time (ET 48 h). Thereafter, roots again were collected and fixed. The ET 24 and 48 h were chosen to evaluate the effects of these treatments on more than one cell cycle. Roots were fixed in Carnoy 3: 1 (ethanol: acetic acid) for 24 hours. In each collection, we took, on average, three roots per bulb.

In both exposure times, the flasks with the treatments under study remained under gentle and constant stirring. This procedure was carried out so as to not allow precipitation of the solutions. The ET 24 h and 48 h were defined to evaluate the solutions of powdered juices in more than one cell cycle.

Em todos os tempos de exposição considerados, os frascos com os tratamentos em estudo permaneceram em agitação leve e constante. Tal procedimento foi realizado com o intuito de não permitir a precipitação das soluções. Os TE 24 h e 48 h foram estabelecidos com o intuito de se avaliar as soluções dos preparados sólidos para refresco em mais de uma ciclo celular.

2.3 Preparation and reading of slides, and statistical analysis

On average 03 slides per bulb were prepared following the protocol proposed by Guerra & Souza (2002), and analyzed under an optical microscope using 400x objective lens. We examined 1,000 cells for each onion bulb, totaling 5,000 cells for the control, ET 24 and ET 48 hours of each treatment analyzed.

Cells were observed in interphase, prophase, metaphase, anaphase and telophase. For the calculation of the mitotic index (MI), it was used the following equation: (total number of mitotic cells/total number of cells) × 100. We also evaluated the activity of different concentrations by means of the number of micronucleated cells, colchicine metaphase, anaphase and telophase bridges, gene amplifications, cell adhesions, nuclear buds and multipolar anaphases. For the statistical analysis, we used the Chi-square test ($\chi 2$) with probability level <0.05.

3 Results and discussion

Table 1 presents the number of cells in interphase and at different stages of cell division, and mitotic index values obtained from root meristem cells of *A. cepa* treated with industrialized Orange and Guava juice powders, respectively, at concentrations of 30 g/1000 mL, and 30 g/500 mL of the food companies A, B and C. Cells were analyzed at exposure times of 24 and 48 hours. The table also shows the significant values of $\chi 2$.

Based on the results in Table 1, orange juice powder of the three companies at concentrations of 30 g/1000 mL and 30 g/500 mL and ET 24 and 48 hours promoted a significant reduction in cell division in root meristem tissue of *A. cepa* compared to their respective controls. Also, although not statistically significant, the mitotic index for the ET 48 h of all treatments was lower than those obtained for their respective ET 24 h.

For guava juice powder (Table 1), at a concentration of 30 g/1000 mL, ET 24 hours, of the three food companies, there was a drastic reduction in the cell division of root meristem cells of *A. cepa* in relation to their respective controls. Apparently, the antiproliferative effect was enhanced with increasing exposure time, where the mitotic indices obtained for the ET 48 h of the treatments under study were significantly lower in relation to cell division indices found for their respective ET 24 h. As for the concentration of 30 g/500 mL, the cell division rate was significantly

reduced at ET 24 h in all treatments when compared with their respective controls. However, the cell division indices for the ET 48 h in all treatments with this concentration, although lower, were not significant when compared to their respective ET 24 h.

Therefore, considering the results in Table 1, all the samples of orange and guava juice powder, at the concentrations and exposure times evaluated, were cytotoxic to meristematic cells of *Allium cepa* roots, causing an antiproliferative effect to the tissues

Table 1. Number of root meristem cells of *A. cepa* at each stage of cell division treated with powdered juice, Orange and Guava flavors, at 30 g/1000 mL and 30 g/500 mL, and ET 24 h and 48 h, of the food companies A, B and C.

ORANGE JUICE POWDER 30 g/1000 mL								
Company	ET	TCII	P	M	A	T	TCD	MI (%)
A	CO	4255	287	214	137	107	745	14.9[a]
	24 h	4660	221	78	19	18	336	6.7[b]
	48 h	4732	130	113	24	00	267	5.3[b]
B	CO	4424	260	107	103	106	576	11.5[a]
	24 h	4853	64	41	09	33	147	2.9[b]
	48 h	4865	36	13	14	02	65	1.3[b]
C	CO	4023	581	181	110	105	977	19.5[a]
	24 h	4767	152	69	12	00	233	4.7[b]
	48 h	4930	37	17	16	00	70	1.4[b]
ORANGE JUICE POWDER 30 g/500 mL								
Company	ET	TCII	P	M	A	T	TCD	MI (%)
A	CO	4287	366	158	163	26	713	14.3[a]
	24 h	4692	187	105	14	02	308	6.2[b]
	48 h	4703	195	72	30	00	297	5.9[b]
B	CO	3869	667	199	121	145	1132	22.6[a]
	24 h	4650	118	182	40	10	350	7.0[b]
	48 h	4669	105	127	86	13	331	6.6[b]
C	CO	4281	375	143	185	17	720	14.4[a]
	24 h	4795	96	107	11	01	215	4.3[b]
	48 h	4863	58	74	05	00	137	2.7[b]
GUAVA JUICE POWDER 30 g/1000 mL								
Company	ET	TCII	P	M	A	T	TCD	MI (%)
A	CO	3669	695	541	92	03	1331	26.6[a]
	24 h	4653	347	159	20	03	529	10.6[b]
	48 h	4866	99	24	04	07	134	2.7[c]
B	CO	3813	831	168	153	35	1187	23.7[a]
	24 h	4495	229	219	53	04	505	10.1[b]
	48 h	4887	58	12	07	06	83	1.7[c]
C	CO	4263	632	138	128	139	1037	20.7[a]
	24 h	4884	193	113	114	56	476	9.5[b]
	48 h	4935	11	29	11	14	65	1.3[c]
GUAVA JUICE POWDER 30 g/500 mL								
Company	ET	TCII	P	M	A	T	TCD	MI (%)
A	CO	4309	361	153	164	16	694	13.9[a]
	24 h	4944	50	03	00	03	56	1.1[b]
	48 h	4954	22	23	2	00	47	0.9[b]
B	CO	4388	210	158	111	38	517	10.3[a]
	24 h	4876	57	37	11	09	114	2.3[b]
	48 h	4915	59	23	02	01	85	1.7[b]
C	CO	4266	360	189	170	14	733	14.7[a]
	24 h	4678	93	43	26	14	176	3.5[b]
	48 h	4853	92	40	15	00	147	2.9[b]

A – *General Foods Corporation*; B – *General Brans*; C – Três Corações Alimentos S/A; TCII – Total number of cells in interfase and undifferentiated; ET – Exposure Time; CO – Control; MI – Mitotic Index; TCD – Total number of dividing cells. Within the same treatment, MI values followed by different letters are significantly different at 5% by χ^2 test.

studied. According to Gomes et al. (2013), the reduction in the mitotic index caused by chemicals on normal tissue cells, without any mutation and/or cell alteration, may cause the malfunction of a tissue as a function of not allowing the replacement of cells, changing protein production and consequently resulting in malfunction of the organ.

In Table 2 are listed the mitotic spindle abnormalities and micronuclei found in root meristem cells of *A. cepa* treated with water or industrialized Orange and Guava juice powders, at concentrations of 30 g/1000 mL and 30 g/500 mL of the food companies A, B and C, and exposure times of 24 and 48 hours. The table also presents the significant values of $\chi 2$.

Table 2. Cellular alterations observed in root meristem cells of *Allium cepa* treated with water and industrialized powdered juice, Orange and Guava flavors, of food companies A, B and C, at 30 g/1000 mL and 30 g/500 mL, at ET 24 and 48 hours.

ORANGE JUICE POWDER 30 g/1000 mL							
Company	ET	Colchicine metaphase	Anaphase bridge	Telophase bridge	Micronuclei	Binucleate cell	TAC
A	CO	00	00	00	01	00	01[a]
	24 h	31	14	13	57	01	116[b]
	48 h	13	19	07	71	00	110[b]
B	CO	00	01	00	00	00	01[a]
	24 h	47	12	17	38	00	104[b]
	48 h	22	10	12	69	00	113[c]
C	CO	00	01	00	00	00	01[a]
	24 h	24	08	10	31	02	73[b]
	48 h	18	05	03	18	00	44[b]
ORANGE JUICE POWDER 30 g/500 mL							
Company	ET	Colchicine metaphase	Anaphase bridge	Telophase bridge	Micronuclei	Binucleate cell	TAC
A	CO	01	00	00	00	00	01[a]
	24 h	23	34	13	97	01	167[b]
	48 h	15	17	19	79	00	130[b]
B	CO	00	00	00	01	00	01[a]
	24 h	24	07	19	88	00	138[b]
	48 h	18	11	09	92	00	130[b]
C	CO	00	00	00	01	00	01[a]
	24 h	13	22	02	43	05	85[b]
	48 h	02	08	00	28	00	38[b]
GUAVA JUICE POWDER 30 g/1000 mL							
Company	ET	Colchicine metaphase	Anaphase bridge	Telophase bridge	Micronuclei	Binucleate cell	TAC
A	CO	00	00	00	01	00	01[a]
	24 h	18	22	08	50	00	148[b]
	48 h	09	12	14	28	01	64[b]
B	CO	00	01	00	00	00	01[a]
	24 h	13	04	01	34	00	52[b]
	48 h	04	01	01	14	00	20[b]
C	CO	00	01	00	00	00	01[a]
	24 h	27	21	08	32	01	89[b]
	48 h	09	09	03	19	00	40[b]
GUAVA JUICE POWDER 30 g/500 mL							
Company	ET	Colchicine metaphase	Anaphase bridge	Telophase bridge	Micronuclei	Binucleate cell	TAC
A	CO	01	00	00	00	00	01[a]
	24 h	22	18	13	58	00	111
	48 h	14	05	05	29	00	53
B	CO	00	00	00	01	00	01[a]
	24 h	19	11	07	45	00	82[b]
	48 h	03	00	07	17	02	27[b]
C	CO	00	00	00	01	00	01[a]
	24 h	11	11	17	23	00	62[b]
	48 h	09	01	00	18	01	29[b]

A – *General Foods Corporation*; B – *General Brans*; C – Três Corações Alimentos S/A; C – Concentration; ET – Exposure Time; CO – Control; TCA – Total Number of Cell Alterations. Within the same treatment, TCA values followed by different letters are significantly different at 5% by χ^2 test.

Orange and guava juice powders of the three food companies considered, at concentrations of 30 g/1000 mL and 30 g/500 mL, and in both exposure times, induced a significant appearance of mitotic spindle changes – as colchicine metaphases and anaphase and telophase bridges - as well as chromosome breakages - evidenced by the presence of micronuclei – to root meristem cells of *A. cepa*, proving to be, under the studied conditions, genotoxic (Table 2). According to Türkoğlu (2007), cells with mitotic spindle changes and/or chromosome breakages have great chances to generate daughter cells with different chromosome number. Such changes, if not repaired or discarded, can change the cell cycle control mechanism leading to the inhibition or uncontrolled proliferation of cells.

As mentioned above, there are no studies to date considering toxicological evaluation at the cellular level of industrialized powdered juices. Nevertheless, toxicological assessments are found at the cellular level with some of the chemical constituents of food additives present in the formulation of such foods. However, it is relevant to report that the chemical composition described on the labels of powdered juices, with emphasis on those of the three food companies mentioned here, is allowed and assured by the Brazilian law (Agência Nacional de Vigilância Sanitária, 2007; Brasil, 2006; Ferrarezi et al., 2010).

Thus, in relation to the labels on the packets of the samples, it was found, for orange juice powder, artificial food dyes in the chemical composition including Sunset Yellow and Tartrazine. On the packets of the guava flavor, it was listed artificial dyes Red 40, Bordeaux Red and Bright Blue.

The dyes Sunset Yellow, Tartrazine and Red 40 are azo food additives for containing the azo chemical grouping, a nitrous derivative with the property of producing aromatic amine and sulfanilic acid after metabolized by the intestinal microflora (Sardi et al., 2010). These chemicals have the potential to change the turnover of cells during interphase and the regenerative hyperplasia process, thus contributing significantly to the development of cancer (Polônio & Peres, 2009). In a study conducted by Gomes et al. (2013), it was found that these three azo dyes were significantly cytotoxic and genotoxic to meristematic cells of *Allium cepa* roots.

With regard to non-azo dyes, Sarıkaya et al. (2012) investigated the activity of Bordeaux Red on larvae of *Drosophila melanogaster*, and found that this additive caused somatic mutations in salivary gland cells of this test organism. Mpountoukas et al. (2010) conducted an experiment in human peripheral blood cell cultures to assess the cytotoxic effect of Bordeaux Red, and found that these additives caused significant reduction of the mitotic index and induced the appearance of micronuclei in cells of this test system. In addition, Shimada et al. (2010) evaluated the effect of this dye at various concentrations on colon cells of mice and rats, and observed that all tested doses caused chromosomal aberrations and significant reduction in the cell division rate of the analyzed tissues.

Based on the packets of the samples, one of the anti-wetting agents present in the chemical formulation was sodium diacetyl sulfosuccinate. The group diacetyl is also often found in formulation of food flavorings; in a gene mutation assay in rat lymphoma, this compound caused significant damage to loci on chromosome 11 of these cells, causing loss of expression of genes for thymidine kinase in the animal (Whittaker et al., 2008). This chemical compound also had the potential to replace thymine with guanine in euchromatin regions, and the disruption of hydrogen and disulfide bonds in the tertiary structure of enzymes involved in the cell division process, drastically reducing the proliferative index of cells (More et al., 2012). For other anti-wetting agents cited in packets – tricalcium and silicon dioxide – we did not find work evaluating their toxicity at the cellular level.

The sweeteners listed on the labels of the samples of powdered juices included aspartame, sodium cyclamate, acesulfame potassium and saccharin sodium. A study performed by Van Eyk (2015) verified that these sweeteners were cytotoxic, genotoxic and mutagenic to cell lines Caco-2 (colon cells), HT-29 (colon cells) and HEK-293 cells (kidney cells). Corroborating the results of Van Eyk (2015), Sasaki et al. (2002) employed the comet assay and reported that sodium saccharin and sodium cyclamate were genotoxic and mutagenic to rodent colon cells.

Interestingly, the scientific literature does not contain toxicity studies at the cellular level with food flavorings used in processed juices. However, the Food Inspection Agency – ANVISA (Agência Nacional de Vigilância Sanitária, 2007) states that these food additives, when overused in foodstuffs, especially directed to the child population, cause irritant and narcotic effects, besides cytotoxic, genotoxic and mutagenic activities on cells of the human digestive tract, but does not cite in its regulation which studies, concentrations and flavorings determined such a conclusion. Considering the antioxidants, there were also no studies examining the cytotoxic and genotoxic action thereof. Meantime, Eskandani et al. (2014) reported that antioxidants used in the formulation of industrialized foods, emphasizing those found in beverages, have great potential to alter the gastrointestinal metabolism of rodents, causing toxic reactions to the liver and potentiating the development of some cancers, such as the colon, for example.

For other classes of food additives present on the labels of the samples of powdered juice – acidulant (citric acid), acidity regulator (potassium citrate and sodium citrate), thickener (guar gum, xanthan gum and carboxymethyl cellulose), inorganic colorant (titanium dioxide), foaming (quillaia extract) – after an extensive search in various databases, we found no toxicity studies at the cellular level involving these chemical compounds.

4 Conclusion

All samples of industrialized powdered juices analyzed were cytotoxic and genotoxic to root meristem cells of *A. cepa*, including the concentration of 30 g/1000 mL, suggested as ideal for consumption on the labels of juice packets evaluated.

Still, there is an urgent need for more researches assessing the toxicity at the cellular level of the solutions obtained from diluting the powdered juices in water, considering that, as discussed, there are compounds with cytotoxic and genotoxic potential in chemical composition of these foods.

Our findings combined with those of cellular level toxicity of food additives that make up the formulation of industrialized powdered juices demonstrate the great need for more effective participation of food surveillance agencies as to regulation of these foods and/or compounds relative to their possible toxicological risks to consumers.

References

Agência Nacional de Vigilância Sanitária – ANVISA. (2007). Resolução da Diretoria Colegiada RDC nº 05, de 15 de janeiro de 2007. Aprova a Resolução da Diretoria Colegiada que aprova o regulamento técnico sobre aditivos aromatizantes. *Diário Oficial da União*.

Bendino, N. I., Popolim, W. D., & Oliveira, C. R. A. (2012). Avaliação do conhecimento e dificuldades de consumidores frequentadores de supermercado convencional em relação à rotulagem de alimentos e informação nutricional. *Journal of the Health Sciences Institute*, 30(3), 261-265.

Biral, A. M., Taddei, J. A., Passoni, D. F., & Palma, D. (2013). Dental caries and food practices among children attending daycare centers in the city of São Paulo. *Revista Brasileira de Nutrição*, 26(1), 37-48. http://dx.doi.org/10.1590/S1415-52732013000100004.

Brasil, Ministério da Agricultura, Pecuária e Abastecimento. (2006). Instrução normativa nº 01, de 7 de janeiro de 2000. Regulamento técnico geral para fixação dos padrões de identidade e qualidade para suco de fruta. *Diário Oficial da União*.

Brasil, Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Análise de Situação de Saúde. (2011). *Plano de ações estratégicas para o enfrentamento das doenças crônicas não transmissíveis (DCNT) no Brasil 2011-2022*. Brasília.

Cardoso, G. H. S., Dantas, E. B. S., Sousa, F. R. C., & Peron, A. P. (2014). Cytotoxicity of aqueous extracts of *Rosmarinus officinalis* L. (Labiatae) in plant test system. *Brazilian Journal of Biology*, 74(4), 886-889. http://dx.doi.org/10.1590/1519-6984.07313. PMid:25627599.

Caritá, R., & Marin-Morales, M. A. (2008). Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. *Chemosphere*, 72(5), 722-725. http://dx.doi.org/10.1016/j.chemosphere.2008.03.056. PMid:18495201.

Eskandani, M., Hamishehkar, H., & Dolatabadi, J. E. N. (2014). Cytotoxicity and DNA damage properties of tert-butylhydroquinone (TBHQ) food additive. *Food Chemistry*, 153, 315-320. http://dx.doi.org/10.1016/j.foodchem.2013.12.087. PMid:24491735.

Ferrarezi, A. C., Santos, K. O. D., & Monteiro, M. (2010). Avaliação crítica da legislação brasileira de sucos de fruta, com ênfase no suco de fruta pronto para beber. *Revista de Nutrição*, 23(4), 667-677. http://dx.doi.org/10.1590/S1415-52732010000400016.

Gomes, K. M. S., Oliveira, M. V. G. A., Carvalho, F. R. S., Menezes, C. C., & Peron, A. P. (2013). Citotoxicity of food dyes sunset yellow (E-110), bordeax red (E-123), and tatrazine yellow (E-102) on *Allium cepa* L. root meristematic cells. *Food Science and Technology*, 33(1), 218-223. http://dx.doi.org/10.1590/S0101-20612013005000012.

Guerra, M., & Souza, M. J. (2002). *Como observar os cromossomos: um guia de técnicas em citogenética vegetal, animal e humana*. Ribeirão Preto: FUNPEC.

Herrero, O., Martín, J. P., Freire, P. F., López, L. C., Peropadre, A., & Hazen, M. J. (2012). Toxicological evaluation of three contaminant of emerging concern by use of *Allium cepa* test. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 743(1-2), 20-34. http://dx.doi.org/10.1016/j.mrgentox.2011.12.028. PMid:22249112.

Lacerda, L. P., Malaquias, G. de S., & Peron, A. P. (2014). Antiproliferative action of aqueous extracts of *Hymenaea stigonocarpa* Mart. (Fabaceae) on the cell cycle of *Allium cepa* L. *Anais da Academia Brasileira de Ciências*, 86(3), 1147-1150. http://dx.doi.org/10.1590/0001-3765201420130163. PMid:25029361.

Longo-Silva, G., Toloni, M. H., Menezes, R. C. E., Asakura, L., Oliveira, M. A. A., & Taddei, J. A. D. A. C. (2015). Introdução de refrigerantes e sucos industrializados na dieta de lactentes que frequentam creches públicas. *Revista Paulista de Pediatria*, 33(1), 34-41. http://dx.doi.org/10.1016/j.rpped.2014.06.009. PMid:25662561.

Louzada, M. L. D. C., Martins, A. P., Canella, D. S., Baraldi, L. G., Levy, R. B., Claro, R. M., Moubarac, J. C., Cannon, G., & Monteiro, C. A. (2015). Ultra-processed foods and the nutritional dietary profile in Brazil. *Revista de Saúde Pública*, 49, 38. http://dx.doi.org/10.1590/S0034-8910.2015049006132. PMid:26176747.

Marques, G. S., Silva, S. I. O., Sousa, J. M. C., Ferreira, P. M. P., & Peron, A. P. (2015). Cytotoxicity and mutagenic potential of liquid synthetic food flavoring evaluated individually and in association. *Food Science and Technology*, 35. In press.

More, S. S., Raza, A., & Vince, R. (2012). The butter flavorant, diacetyl, forms a covalent adduct with 2-deoxyguanosine, uncoils DNA, and leads to cell death. *Journal of Agricultural and Food Chemistry*, 60(12), 3311-3317. http://dx.doi.org/10.1021/jf300180e. PMid:22385266.

Mörschbächer, A. P., & Souza, C. F. V. D. (2012). Determinação do teor de aditivos em preparados sólidos para refresco sabor abacaxi comercializados na região do Vale do Taquari, RS. *Destaques Acadêmicos*, 3(4), 49-54.

Mpountoukas, P., Pantazaki, A., Kostareli, E., Christodoulou, P., Kareli, D., Poliliou, S., Mourelatos, C., Lambropoulou, V., & Lialiaris, T. (2010). Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. *Food and Chemical Toxicology*, 48(10), 2934-2944. http://dx.doi.org/10.1016/j.fct.2010.07.030. PMid:20667460.

Nogueira, F. A., & Sichieri, R. (2009). Association between consumption of soft drinks, fruit juice, and milk and body mass index among public school students in Niterói, Rio de Janeiro State, Brazil. *Cadernos de Saúde Pública*, 25(12), 2715-2724. http://dx.doi.org/10.1590/S0102-311X2009001200018. PMid:20191162.

Oliveira, M. V. A., Alves, D. D. L., Lima, L. H. G. M., Castro, J. M. C., & Peron, A. P. (2013). Cytotoxicity of erytrosine (E-127), brilliant blue (E-133) and red 40 (E-129) food dyes plant test system. *Acta Scientiarum Biolpgical Sciences*, 35(4), 557-562. http://dx.doi.org/10.4025/actascibiolsci.v35i4.18419.

Polônio, M. L. T., & Peres, F. (2009). Consumo de aditivos alimentares e efeitos a saúde: desafios para a saúde pública brasileira. *Cadernos de Saúde Pública*, 25(8), 1653-1666. http://dx.doi.org/10.1590/S0102-311X2009000800002. PMid:19649407.

Sardi, M., Haldemann, Y., Nordmann, H., Bottex, B., Safford, B., Smith, B., Tennant, D., Howlett, J., & Jasti, P. R. (2010). Use of retailer fidelity card schemes in the assessment of food additive intake: sunset yellow a case study. *Food Additives and Contaminants*, 27(11), 1507-1515. http://dx.doi.org/10.1080/19440049.2010.495728. PMid:20672203.

Sarıkaya, R., Selvi, M., & Erkoç, F. (2012). Evaluation of potential genotoxicity of five food dyes using the somatic mutation and recombination test. *Chemosphere*, 88(8), 974-979. http://dx.doi.org/10.1016/j.chemosphere.2012.03.032. PMid:22482698.

Sasaki, Y. F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K., Taniguchi, K., & Tsuda, S. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 519(1-

2), 103-119. http://dx.doi.org/10.1016/S1383-5718(02)00128-6. PMid:12160896.

Shimada, C., Kano, K., Sasaki, Y. F., Sato, I., & Tsudua, S. (2010). Differential colon DNA damage induced by azo food additives between rats and mice. *The Journal of Toxicological Sciences*, 35(4), 547-554. http://dx.doi.org/10.2131/jts.35.547. PMid:20686341.

Tabrez, S., Sahkil, S., Urooj, M., Damanhouri, G. A., Abuzenadah, A. M., & Ahmad, D. (2011). Genotoxicity testing and biomarker studies on surface water: an over view of the techniques and their efficacies. *Journal of Environmental Science Health*, 29(3), 250-275. http://dx.doi.org/10.1080/10590501.2011.601849. PMid:21929382.

Türkoğlu, S. (2007). Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 626(1-2), 4-14. http://dx.doi.org/10.1016/j.mrgentox.2006.07.006. PMid:17005441.

Valavanidis, A., Vlachogianni, T., Fiotakis, K., & Loridas, S. (2013). Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. *International Journal of Environmental Research and Public Health*, 10(9), 3886-3907. http://dx.doi.org/10.3390/ijerph10093886. PMid:23985773.

Van Eyk, A. D. (2015). The effect of five artificial sweeteners on Caco-2, HT-29 and HEK-293 cells. *Drug and Chemical Toxicology*, 38(3), 318-327. http://dx.doi.org/10.3109/01480545.2014.966381 . PMid:25317478.

Whittaker, P., Clarke, J. J., San, R. H., Begley, T. H., & Dunkel, V. C. (2008). Evaluation of the butter flavoring chemical diacetyl and a fluorochemical paper additive for mutagenicity and toxicity using the mammalian cell gene mutation assay in L5178Y mouse lymphoma cells. *Food and Chemical Toxicology*, 46(8), 2928-2933. http://dx.doi.org/10.1016/j.fct.2008.06.001. PMid:18585428.

Zaineddin, A. K., Buck, K., Vrieling, A., Heinz, J., Flesch-Janys, D., Linseisen, J., & Chang-Claude, J. (2012). The association between dietary lignans, phytoestrogen-rich foods, and fiber intake and postmenopausal breast cancer risk: a German case-control study. *Nutrition and Cancer*, 64(5), 652-665. http://dx.doi.org/10.1080/01635581.2012.683227. PMid:22591208.

Zilifdar, F., Alper-Hayta, S., Yilmaz, S., Kaplan-Özen, Ç., Foto, E., Aydoğan, Z., Yildiz, I., Aki, E., Yalçin, I., & Diril, N.. (2014). Genotoxic potentials and eukaryotic DNA topoisomerase I inhibitory effects of some benzoxazine derivatives. *Medicinal Chemistry Research*, 23(1), 480-486. http://dx.doi.org/10.1007/s00044-013-0658-5.