Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v38i1.30475

Toxicity in food flavorings at the cellular level associated with each other at different doses

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ABSTRACT. This study aimed to examine the cytotoxicity and genotoxicity of synthetic flavorings, nature identical, Chocolate, Strawberry and Condensed Milk. This evaluation was performed in root meristem cells of *Allium cepa* L., in exposure times of 24 and 48 hours and using doses of 0.2; 0.4 and 0.6 mL, in combination, in which one of the three doses of a flavoring was combined with a different dose of one of the two other flavor additives studied. Roots were fixed in Carnoy's solution, hydrolyzed in hydrochloric acid, stained with acetic orcein and then analyzed, under light microscopy, 5,000 cells for each treatment. For data analysis, it was used Chi-square test at 5%. All the treatments with combinations between the flavorings Chocolate/Strawberry and Strawberry/Condensed Milk reduced, in both exposure times considered, cell division of *A. cepa* roots, proving to be cytotoxic. In turn, the treatments with the association of Chocolate/Condensed Milk did not change significantly the mitotic index of the cells analyzed. The Strawberry flavoring was the most cytotoxic among the additives tested. None of the evaluated associations was genotoxic under the study conditions.

Keywords: aroma and flavor additives, cytotoxicity, genotoxicity, *A. cepa*.

Toxicidade em nível celular de aromatizantes alimentares associados entre si em diferentes doses

RESUMO. Objetivou-se nesta pesquisa avaliar a citoxicidade e genotoxicidade de aromatizantes alimentares sintéticos de chocolate, morango e leite condensado. Esta avaliação ocorreu por meio das células meristemáticas de raízes de *A. cepa* L., nos tempos de exposição de 24 e 48h e nas doses de 0,2; 0,4 e 0,6 mL, em associação, em que para uma das três doses de um dos aromatizantes associou-se uma dose diferente de um dos outros dois aditivos de aroma em estudo. Em seguida, as raízes foram fixadas em solução de Carnoy, hidrolisadas em ácido clorídrico e coradas com orceína acética. Analisaram-se, em microscópio óptico, 5.000 células para cada grupo tratamento, e utilizou-se o teste estatístico Qui-quadrado a 5% para análise dos dados. A partir dos resultados, verificou-se que todos os tratamentos decorrentes das associações entre chocolate/morango e morango/leite condensado reduziram, nos dois tempos de exposição considerados, a divisão celular das raízes *A. cepa*, mostrando-se citotóxicos. Já os tratamentos provenientes da associação chocolate/leite condensado não alteraram de forma significativa os índices mitóticos das células do tecido em análise. Foi possível inferir que o aditivo de morango foi o mais citotóxico dos aditivos em estudo. Nenhuma das associações avaliadas foi genotóxica nestas condições de estudo.

Palavras-chave: aditivo de aroma e sabor, citotoxicidade, genotoxicidade, *A. cepa*.

Introduction

For several decades, food additives, such as dyes, preservatives, acidulants and flavorings, are essential for the food industry as they allow longer shelf life for processed foods without changing color, flavor, aroma and texture, among other characteristics (Lerner et al., 2015). They are widely used in fast food snacks, frozen meals, industrialized desserts and canned and breaded food products.

Because of odoriferous, aroma and flavor additives provide and/or enhance the aroma and flavor of foods without nutritional purpose (Moodie et al., 2013). They are classified as natural, synthetic nature-identical and artificial synthetic of reaction or processing (Pierce, Albelmann, Spicer, Adams, & Finley, 2014). They have a complex chemical formulation, consisting of eleven classes of compounds - diluents, antioxidants, defoamers, preservatives, emulsifiers, stabilizers, acidity

regulators, flavor enhancers, anti-caking agents, dyes and extraction and processing solvents (Lerner et al., 2015) accepted worldwide by the regulatory agencies Codex Alimentarius Commission, Food and Drug Administration (FDA), Flavour and Extract manufactuers Association (FEMA) and nationally by the *Agência Nacional de Vigilância Alimentar* (Anvisa) (Brasil, 2007; Oliveira, Alves, Lima, Castro, & Peron, 2013). Nevertheless, technical documents of these surveillance agencies do not specify which specific compounds and concentrations are present in these additives, nor the Acceptable Daily Intake (ADI) and tolerable limits of flavorings for each type of food (Gomes, Oliveira, Carvalho, Menezes, & Peron, 2013). This information was also not found on labels of flavoring solutions used by the food industry nor in specialized websites in the marketing of food additives.

In this way, Lerner et al. (2015) report that the use of aroma and flavor additives raises a number of questions about their toxicity at systemic and cellular level, and the urgent need to conduct research to assess their toxicological potential. Likewise, the agencies Codex Alimentarius Commission, FEMA (Konishi, Hayashi, & Fukushima, 2011) and Anvisa (Brasil, 2007) point out in their regulations about the constant need for acute toxicological studies involving food additives in general. The results of such researches are very important as they determine the need for evaluating chronic toxic effects triggered by such ingredients. The results from these analyses support the preparation or amendment of strategies of regulatory agencies and hence the performance of the professionals responsible for food and nutrition surveillance of the population.

Plant bioassays are highly sensitive and simple in monitoring the toxic effects of chemical compounds (Herrero et al., 2012; Lacerda, Malaquias, & Peron, 2014) and the *A. cepa* L. (onion) test system, through the meristematic region of its roots, is considered efficient for the assessment of acute toxicity at the cellular level (Cardoso, Dantas, Sousa, & Peron 2014; Caritá & Marin-Morales, 2008). This test system has excellent kinetic properties of proliferation, large and few chromosomes (2n = 16) which facilitates the detection of cell aberrations and mitotic spindle abnormalities (Herrero et al., 2012; Neves, Ferreira, Lima, & Peron, 2014). It also allows the verification of changes in the mitotic index, or when exposed to chemical compounds with potential cytotoxic activity (Tabrez et al., 2011).

According to Herrero et al. (2012), the *A. cepa* system is very efficient in assessing cytotoxicity, genotoxicity and mutagenicity of chemicals and in validating these conditions after testing in other bioassays. It still shows, in most cases, a satisfactory similarity to the results obtained with other bioassays (Cardoso et al. 2014). For instance, we can mention the studies performed by Gomes et al. (2013) and Oliveira et al. (2013), who evaluated the toxic potential of synthetic dyes used in the food industry in *A. cepa* root meristematic cells and obtained similar results in animal test systems and cell cultures.

Although flavorings are essential for the food industry, little is known about their specific chemical formulation and toxicological evaluations of marketed solutions are practically nonexistent, this study evaluated the toxicity, in *A. cepa* root meristematic cells, of three flavors, chocolate, strawberry and condensed milk, directly in the solutions marketed by the food industry. These additives were selected for study because of their widespread use in sweet processed foods, often associated with each other by means of different doses (Moura, Santana, Ferreira, Sousa, & Peron, 2016), and because there is no scientific literature discussing the toxicity of such substances.

Material and methods

Food flavorings

Aroma and flavor additives, synthetic, nature identical Chocolate, Strawberry and Condensed Milk flavorings were obtained from a food additive manufacturing industry located in northeastern Brazil, specialized in national and international marketing of synthetic food additives.

Setting the doses

The label of the three flavorings studied suggested the use of 1.0 mL of flavoring to 1.0 kg of dough. The selected onion bulbs weighed, on average, 200 g. Thus, proportionally to that recommended on vial labels, it was initially set, for this study, the volume of flavoring solution (dose) of 0.2 mL. Then, two other doses were set, greater than that considered ideal, 0.4 and 0.6 mL. These doses were analyzed in combination, as described below:

Chocolate doses combined with Strawberry doses:

Treatment group: 0.2 mL Chocolate/0.4 mL Strawberry;

Treatment group: 0.2 mL Chocolate/0.6 mL Strawberry;

Treatment group: 0.4 mL Chocolate/0.2 mL Strawberry;
Treatment group: 0.4 mL Chocolate/0.6 mL Strawberry;
Treatment group: 0.6 mL Chocolate/0.2 mL Strawberry;
Treatment group: 0.6 mL Chocolate/0.4 mL Strawberry;
Chocolate doses combined with Condensed Milk doses:
Treatment group: 0.2 mL Chocolate/0.4 mL Condensed Milk;
Treatment group: 0.2 mL Chocolate/0.6 mL Condensed Milk;
Treatment group: 0.4 mL Chocolate/0.2 mL Condensed Milk;
Treatment group: 0.4 mL Chocolate/0.6 mL Condensed Milk;
Treatment group: 0.6 mL Chocolate/0.2 mL Condensed Milk;
Treatment group: 0.6 mL Chocolate/0.4 mL Condensed Milk;
Strawberry doses combined with Condensed Milk doses:
Treatment group: 0.2 mL Strawberry/0.4 mL Condensed Milk;
Treatment group: 0.2 mL Strawberry /0.6 mL Condensed Milk;
Treatment group: 0.4 mL Strawberry /0.2 mL Condensed Milk;
Treatment group: 0.4 mL Strawberry /0.6 mL Condensed Milk;
Treatment group: 0.6 mL Strawberry /0.2 mL Condensed Milk;
Treatment group: 0.6 mL Strawberry /0.4 mL Condensed Milk;

Obtaining the *A. cepa* root meristem cells for cytogenetic analysis

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

After this period of 24 hours, some roots were removed and fixed. Upon the completion of this procedure, the rest of the roots of each bulb were returned to their respective solutions, where they remained for more 24 hours, which was called as 48-hour exposure time. Afterwards, roots were collected and fixed again. The exposure times of 24 and 48 hours were chosen to evaluate the effects of these treatments on more than one cell cycle. Roots were fixed in Carnoy's solution 3: 1 (ethanol: acetic acid) for 24 hours. At each collection, on average, three roots per bulb were taken.

Slide preparation and reading and statistical analysis

On average, 3 slides were mounted per bulb following the protocol proposed by Guerra and Souza (2002) and analyzed under an optical microscope with 40 x objective lens. For each onion bulb, we analyzed 1,000 cells, amounting 5,000 cells for the control, 24 and TE 48h of each treatment analyzed. Cells in interphase, prophase, metaphase, anaphase and telophase were examined. It was calculated the number of cells in interphase and in division for each control and exposure time and determined the mitotic index (MI). Also, it was assessed the effect of flavoring doses by the number of micronucleated cells, colchicine metaphases, anaphase and telophase bridges, gene amplifications, cells adhesions, nuclear buds and multipolar anaphases. For data analysis, we used the statistical test Chi-square (χ^2) at 5%.

Results and discussion

In this study, no dilution was performed when setting the flavoring doses, in other words, we intended to verify the toxicity of these additives associated with each other, on root meristem cells of *A. cepa*, directly from the original solutions in the vials. We did so because the aroma additives have complex chemical formulation - not detailed on their labels or in specialized websites in the marketing of these food additives - and to not change the concentration and the action of compounds present in these ingredients. It is also important to note that the doses of flavoring solutions of 0.4 and 0.6 mL, two and four times higher than the first, were also tested, given the indiscriminate use of these flavorings by the population, as reported by Moura et al. (2016). The 24 and TE 48h were set in order to evaluate the effect of these additives on more than one cell cycle. It is also important to mention that, according to the Technical Regulation on Flavorings/Aroma approved by Anvisa in (Brasil, 1999), the formulation of any synthetic food flavoring is standardized worldwide.

Table 1 lists the number of cells in interphase and in different stages of cell division and mitotic index values obtained from root meristem cells of *A. cepa* treated with water and flavorings Chocolate and

Strawberry in combination with different doses. Cells of these roots were analyzed at 24 and TE 48h. Significant values of χ^2 are also presented.

The mitotic indices obtained for 24 and TE 48h in all combinations (treatments) AC 0.2/AM 0.4; AC 0.2/AM 0.6; AC 0.4/AM 0.2; AC 0.4/AM 0.6; AC 0.6/AM 0.2 and AC 0.6/AM 0.4 were significantly different from cell division indices obtained for their respective controls, proving to be cytotoxic (Table 1). However, the mitotic indices of the TE 24h of these treatments were not statistically different from their specific TE 48h.

Table 2 presents the number of cells in interphase and in different stages of cell division and mitotic index values obtained from root meristem cells of *A. cepa* treated with water and with treatment solutions containing the flavorings Chocolate and Condensed Milk with different doses. These combinations were analyzed at 24 and TE 48 hours. Significant values of χ^2 are also presented.

The mitotic indices of TE 24 and TE 48h of the treatments containing the flavorings Chocolate and Condensed Milk in combination, AC 0.2/ALC 0.4; AC 0.2/ALC 0.6; AC 0.4/ALC 0.2; AC 0.4/ALC 0.6; AC 0.6/ALC 0.2 and AC 0.6/ALC 0.2 were not significantly different from cell division indices of their respective controls (Table 2).

Table 1. Number of cells observed in each cell cycle phase from *Allium cepa* root meristematic tissue treated with synthetic, nature identical flavorings of Chocolate and Strawberry combined with different doses at TE 24 and TE 48h. Doses studied were: 0.2; 0.4 and 0.6 mL.

Chocolate Flavoring/Strawberry Flavoring								
Treatment solution – Combined doses (mL)	TE	TCII	P	M	A	T	TCD	MI (%)
	CO	4.467	195	144	99	95	533	10.7[a]
AC 0.2 / AM 0.4	24h	4.914	43	17	18	08	86	1.7[b]
	48h	4.909	50	19	14	08	91	1.8[b]
	CO	4.362	234	159	135	110	638	12.8[a]
AC 0.2 / AM 0.6	24h	4.724	52	19	11	14	96	1.9[b]
	48h	4.733	41	12	13	11	77	1.5[b]
	CO	4.376	229	229	93	73	624	12.5[a]
AC 0.4 / AM 0.2	24h	4.727	148	99	19	07	273	5.5[b]
	48h	4.797	84	98	12	09	203	4.1[b]
	CO	4.437	321	128	88	26	563	11.3[a]
AC 0.4 / AM 0.6	24h	4.903	59	22	08	08	97	1.9[b]
	48h	4.907	63	18	09	03	93	1.9[b]
	CO	4.546	318	93	40	03	454	9.1[a]
AC 0.6 / AM 0.2	24h	4.807	94	58	22	19	193	3.7[b]
	48h	4.901	52	31	11	05	99	2.0[b]
	CO	4.495	309	113	83	13	505	10.1[a]
AC 0.6 / AM 0.4	24h	4.938	32	14	13	03	62	1.4[b]
	48h	4947	25	18	07	03	53	1.0[b]

TCII – Total number of undifferentiated cells in interphase; TE – Exposure time; CO – Control; MI – Mitotic Index; TCD – Total number of dividing cells; AC – Chocolate Flavoring, AM – Strawberry Flavoring; / - combination. MI values followed by different letters in the same treatment are significantly different by χ^2 test at 5% significance level.

Table 2. Number of cells observed in each cell cycle phase from *Allium cepa* root meristematic tissue treated with synthetic, nature identical flavorings of Chocolate and Condensed Milk combined with different doses at TE 24 and TE 48 h. Doses studied were: 0.2; 0.4 and 0.6 mL.

Chocolate Flavoring/Condensed Milk Flavoring								
Treatment solution – Combined doses (mL)	TE	TCII	P	M	A	T	TCD	MI (%)
	CO	4663	199	68	46	24	337	6.7[a]
AC 0.2 / ALC 0.4	24h	4730	82	120	40	28	270	5.4[a]
	48h	4855	67	47	18	13	145	2.9[a]
	CO	4495	207	140	115	43	505	10.1[a]
AC 0.2 / ALC 0.6	24h	4469	213	144	119	45	531	10.6[a]
	48h	4627	197	95	47	34	373	7.4[a]
	CO	4567	238	62	25	08	433	8.7[a]
AC 0.4 / ALC 0.2	24h	4557	204	140	64	35	443	8.9[a]
	48h	4665	191	95	32	17	335	6.7[a]
	CO	4528	279	84	65	44	472	9.4[a]
AC 0.4 / ALC 0.6	24h	4577	262	79	61	21	423	8.4[a]
	48h	4695	199	47	44	15	305	6.1[a]
	CO	4514	332	75	61	18	486	9,7[a]
AC 0.6 / ALC 0.2	24h	4514	261	97	74	54	486	9,7[a]
	48h	4583	244	72	51	50	417	8,3[a]
	CO	4526	332	81	31	30	474	9.5[a]
AC 0.6 / ALC 0.4	24h	4591	239	88	35	47	409	8.2[a]
	48h	4757	181	40	14	08	243	4.8[a]

TCII – Total number of undifferentiated cells in interphase; TE – Exposure time; CO – Control; MI – Mitotic Index; TCD – Total number of dividing cells; AC – Chocolate Flavoring, ALC – Condensed Milk Flavoring; / - combination. MI values followed by different letters in the same treatment are significantly different by χ^2 test at 5% significance level.

Within each treatment, comparing the mitotic index of 24 and TE 48h, they were not significantly different from each other. However, although not significant, there was a reduction in mitotic indices of these combinations in all TE 48h, compared to their respective controls and TE 24h.

Table 3 presents the number of cells in interphase and in different stages of cell division and mitotic index values obtained from root meristem cells of *A. cepa* treated with water and with treatment solutions containing the flavorings Strawberry and Condensed Milk with different doses. These combinations were analyzed at 24 and TE 48 hours. Significant values of $\chi^2$are also presented.

In Table 3, for all treatments from the combination of the flavorings Strawberry and Condensed Milk, AM 0.2/ALC 0.4; AM 0.2/ALC 0.6; AM 0.4/ALC 0.2; AM 0.4/ALC 0.6; AM 0.6/ALC 0.2 and AM 0.6/ALC 0.4, the cell division indices of the 24 and TE 48h were significantly different from their controls. Nevertheless, when compared within the same treatment, the TE 24h and 48h were not different from each other.

By analyzing the results in Tables 1, 2 and 3, it can be suggested that the Strawberry flavoring was the most cytotoxic among the tested additives. In Table 1, it is observed that whenever the flavoring dose was greater than 0.2 mL (AC 0.2/AM 0.4; AC 0.2/AM 0.6; AC 0.4/AM 0.6 and AC 0.6/AM 0.4), the cell division index was drastically reduced in the TE 24h in relation to their respective controls. This was also seen in Table 3, where treatments AM 0.4/ALC 0.2; AM 0.4/ALC 0.6; AM 0.6/ALC 0.2 and AM 0.6/ALC 0.4 showed significantly reduced mitotic indices in 24 and TE 48h, when compared to their controls. In treatments where 0.2mL Strawberry additive was used, AC 0.4/AM 02 and AC 0.6/AM 0.2 (Table 1) and AM 0.2/ALC 0.4 and AM 0.2/ALC 0.6 (Table 3), significant inhibition of cell division also occurred, but gradually between the two TE.

In Table 2, treatments from the combination of Condensed Milk and Chocolate flavorings also reduced gradually the cell division of the tissue between the TE, but, this reduction was not statistically significant. None of the treatments combining Chocolate / Strawberry, Chocolate / Condensed Milk and Strawberry / Condensed Milk induced significant number of abnormalities in the mitotic spindle and micronuclei, therefore were not genotoxic under the study conditions. Cell changes observed when combining the flavorings were micronuclei colchicine metaphase, anaphase and telophase bridges.

Unfortunately, we found no description of the chemical composition of these three flavorings. However, there are few studies available in the scientific literature evaluating the toxicity of some constituents of the classes of chemical compounds that can comprise the formulation of the aroma and flavor additives in general.

Table 3. Number of cells observed in each cell cycle phase from *Allium cepa* root meristematic tissue treated with synthetic, nature identical flavorings of Strawberry and Condensed Milk combined with different doses at 24 and TE 48h. Doses studied were: 0.2; 0.4 and 0.6 mL.

	Strawberry Flavoring/Condensed Milk Flavoring							
Treatment solution – Combined doses (mL)	TE	TCII	P	M	A	T	TCD	MI (%)
AM 0.2 / ALC 0.4	CO	4442	287	140	88	43	558	11.1
	24h	4854	92	90	58	26	216	4.3
	48h	4868	98	18	13	03	132	2.6
AM 0.2 / ALC 0.6	CO	4104	434	206	169	87	896	17.9[a]
	24h	4841	155	64	30	10	259	5.1[b]
	48h	4862	63	52	15	08	138	2.7[b]
AM 0.4 / ALC 0.2	CO	4094	456	211	163	76	906	18.1[a]
	24h	4800	91	77	26	06	200	4.0[b]
	48h	4793	46	37	20	04	107	2.1[b]
AM 0.4 / ALC 0.6	CO	4413	251	187	77	62	587	11.7[a]
	24h	4925	28	20	16	11	75	1.5[b]
	48h	4957	24	14	05	03	46	0.9[b]
AM 0.6 / ALC 0.2	CO	4278	538	111	51	22	722	14.4[a]
	24h	4632	99	47	42	20	208	4,1[b]
	48h	4819	90	36	38	17	181	3,6[b]
AM 0.6 / ALC 0.4	CO	4439	341	145	57	18	561	11.2[a]
	24h	4887	56	26	15	16	113	2.2[b]
	48h	4901	28	08	11	02	49	1.0[b]

TCII – Total number of undifferentiated cells in interphase; TE – Exposure time; CO – Control; MI – Mitotic Index; TCD – Total number of dividing cells; AM – Strawberry Flavoring; ALC – Condensed Milk Flavoring; / - combination. MI values followed by different letters in the same treatment are significantly different by χ^2 test at 5% significance level.

The preservatives represent a class that inhibits microbial growth in food including potassium benzoate, sodium benzoate and potassium nitrate (Brasil, 1999), which according to Mpountoukas et al. (2010) and Zequin, Yüzbaşioğlu, Unal, Yilmazand, and Aksoy (2011), were clastogenic, mutagenic, and cytotoxic to normal human peripheral blood cells. This class also includes boric acid, citric acid, potassium citrate and sodium citrate (Brasil, 1999), which, according to Türkoğlu (2007), causes a significant reduction in cell division index of *A. cepa* root meristem cells, proving to be cytotoxic.

For the class of diluents, important compounds for maintaining uniformity and facilitating the incorporation and dispersion of concentrate flavors in food products, Demir, Kocaoglu, and Kaya (2010) reported that benzoic alcohol at high concentrations led to significant damage to the mitotic spindle and therefore for cell division in human peripheral blood cells. The diluent diacetyl (2,3- butanedione) in lymphoma gene mutation assay in rats caused significant damage to loci on chromosome 11 of these cells, causing loss of gene expression for thymidine kinase enzyme in these animals (Whittaker, Clarke, San, Begley, & Dunkel, 2008). Besides that, it has been found that the diluent diacetyl has the potential to replace thymine with guanine in euchromatin regions, causes the breaking of hydrogen and disulfide bonds in tertiary structure of enzymes involved in cell division (More, Raza, & Vince, 2012).

Although very significant, the data obtained by these researchers cannot be directly attributed to the results obtained herein for the Chocolate, Strawberry and Condensed Milk flavorings, as we have no information on which chemicals are representing the preservatives and the solvents in chemical composition of these ingredients. Importantly, on average, thirty-chemical compounds are representing the classes comprising the additives (Brasil, 1999; 2007). Among them, the one with restricted use for some of its constituents is the extraction solvent, where the agaric acid, aloin, beta-azorona, berberine, coumarin, hydrocyanic acid, hypericin, pulegone, quassina, safrole and isosafrole, santonin and alpha and beta tuyona have maximum tolerable limits to compose the food flavorings (Brasil, 1999).

Thus, Konishi et al. (2011) point to the need for toxicological evaluations of constituent classes and/or flavoring classes to which they belong, so that together with the research on toxicity at the cellular level already made, one can rethink and/or reorganize the content of technical documents of the bodies responsible for the regulation of these food additives. These authors also remind that food flavorings sparteine, allylhexanoate and quinine were banned by these regulators in the early 80 according to the results of studies of acute and long-term toxicity tests carried out in different systems.

It is also worth mentioning that the Anvisa (Brasil, 2007), although not mentioning which studies, compounds and concentrations, nor which flavorings determined such a conclusion, declares in its technical document on the regulation of flavorings that high doses of synthetic additives cause irritant and narcotic actions to the body and can produce toxicity to the digestive tract when used chronically and indiscriminately. Meanwhile, Salinas (2002) and Gultekin, Doguc, & Kular (2013) state in their manuscripts that the use of flavorings at low doses is not dangerous to human health. However, when the doses are higher than recommended, these authors report that these ingredients may cause irritant, narcotic actions and chronic cellular toxicity in the long term. Meanwhile, the same way as the national surveillance authority, these studies do not specify which doses are considered high or low and do not determine which flavorings have this action, nor the test organisms used to obtain this information.

Conclusion

All treatments with combination of flavorings reduced cell division of *A. cepa* root meristem cells, but were not genotoxic to this test organism. The Strawberry flavoring showed the greatest toxicity to the cells of the tissue analyzed.

The results of this study, together with those previously described in the literature, allow us to state that, although the use of food flavorings is permitted by food surveillance agencies worldwide, it is necessary the constant analysis of the toxic potential of these compounds (or their chemical constituents) to determine with property the real cytotoxicity of these additives, with emphasis on Chocolate, Strawberry and Condensed Milk flavorings, always valuing the welfare of consumers. Other test systems, with animals and normal cell cultures, must also be used for evaluating toxicity of these three aroma and flavor additives.

Acknowledgements

To the Institutional Program of Scientific Initiation Scholarships (PIBIC). To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Received on January 4, 2016.
Accepted on March 2, 2016.