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Use of brillant blue dye on canned cherries

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ABSTRACT

Introduction: The toxicity of erythrosine as well as other photochemical and biochemical degradation products thereof has been addressed in several studies. However, it is often employed in the preparation of canned cherries, since its use is allowed by regulatory agencies such as the FDA. Therefore, it would be important to find less risky replacement dyes for their use in food.

Methodology: canned cherries were produced by a slow confit process, reaching at least 55° Brix, and were then subjected to commercial pasteurization.

Results: Brilliant Blue dyed cherries met the required standard and had a suitable degree of acceptance in the tested population, with the expected parameters being attained in all trials. In addition, the stability test proved that blue dyed cherries remained unchanged, while Erb dyed product suffered an important discoloration.

Conclusion: cherries colored by blue brilliant can be elaborated without problem

KEYWORDS

Erythrosine, Brilliant Blue, food colors, canned cherries.

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ABREVIATIONS:

IDA: Ingesta Diaria Admisible

NOAEL: Nivel sin Efecto Adverso Observado

CAA: Código Alimentario Argentino.

FDA: Food and Drugs Administration

INTRODUCTION

Commercial erythrosine (ErB) mainly consists of the chemical compound 2- (2,4,5,7 - tetraiode -3- oxide-6- oxoxanthen-9-yl) benzoate disodium monohydrate and other subsidiary coloring matters, together with sodium chloride and/or sodium sulphate as adjuvant uncolored components. It is widely used as a colorant in foods, textiles, medicine and cosmetics[1,2,3].

Similarly to other azoados dyes, its toxicity is currently a matter of discussion, since its consumption has been linked to different clinical disorders such as allergies, changes in the thyroid activity, carcinogenicity, DNA damage and neurotoxicity, among other effects in humans and animals[4,5,6].

ErB (tetraiodo fluorescein) is a synthetic food color with a cherry pink tone, and a xantenic and poliyoded structure. This dye is unique in its class and its use in foods has been approved by the Food and Drug Administration (FDA), despite the photochemical and biochemical degradation of ErB can lead to the formation of toxic byproducts[4]. Although its use in food is permitted, the consumption of ErB has been shown to have some impact on children´s behavior[3]. Among the negative effect, it can hinder the thyroid function due to the high content of iodine[5,7], with its excessive consumption leading to diseases such as hyperthyroidism[3].

In vitro studies showed that this additive represents, in some cases, an important cytotoxicical risk[2] as a consequence of the inhibitory effect of the metabolized drug on different enzymatic processes[8] and on protein-protein interactions involved in tumor necrosis factor (TNF)[1]. Drumond Chequer et al[6] proved genotoxic and mutagenic effects in HepG2 cells ErB, coming to the conclusion that ErB effectively represents a health risk, and therefore should be used with caution.

According to Amchova et al.[9], ErB toxicity raises some concerns in the United States because of its use in a wide range of food. On the contrary, both the consumers and the food industry are not particularly alarmed in Europe, despite the stringent regulation on food additives enforced by the European Parliament and the Council (Regulation (EC) N° 1333/2008 published in 2008 and available on the official website of the EU: http://eur-lex.europa.eu/legal -EMC / EN / TXT Telex: 32008R1333). Among other questions, this regulation establishes that the toxicity of food additives evaluated prior to January 2009, should be re-evaluated by EFSA. The program started on 25 March 2010 by Regulation (EC) N° 257/2010, and the objective is to consider new data generated after the approval of different food additives.

In this scenario, Brilliant Blue (triphenyl methane), also known as Blue 1, arises as an interesting alternative to dyes such as ErB, considering that toxicokinetical data confirmed that this dye is poorly absorbed in the gastrointestinal tract and is primarily excreted unchanged in the feces. This compound is an anionic moiety very soluble in water[9]. The NOAEL (No Adverse Effect Level) was determined to be 631 mg/kg body weight per day, according to the assessment of chronic and reproductive toxicity in rats[10]. Based on this value, the European Food Safety Authority (EFSA) changed the new level of IDA to 6 mg/kg body weight per day. This value corresponds approximately to the current estimated intake in Europe. However, latest assessments by the EFSA warned that even in small doses, it can cause hypersensitivity reactions in susceptible individuals[9].

Recent *in vitro* studies showed the potential cytotoxic and genotoxic capacity of this dye in cell cultures of human blood lymphocytes. In dose-dependent studies, a decrease in the values of the mitotic index frequencies was observed, together with a concomitant increase in the micronuclei frequency[11]. It has been demonstrated that Brilliant Blue dye can be absorbed by shaved skin, oral mucosa and passes into the bloodstream[12]. However, the International Association of Manufacturers of Color states that the total amount absorbed in this way is more than 3600 times below the accepted daily intake (ADI), as established by the EFSA, and therefore, the risk to human health can be considered as negligible[13].

In Argentina, the law enforces an ADI of 6 mg / kg for the Blue Brillant, which is considerable higher than the IDA for ErB, of 0,1mg / kg (Código Alimetario Argentino). Brilliant Blue dye is a striking and innovative unconventional color for cherries. It offers to the local producers the alternative of a dye with a less restricted IDA, therefore representing a lower level of risk to the health. Anyway, it is expected that the debate will continue, in spite of the fact that, even though its potential toxicity represents a certain risk, it is more acceptable than other options at the moment of choosing a synthetic dye.

OBJETIVE

All things considered, the objective of this research is to evaluate the use of Brillant Blue as a replacement dye to ErB for the elaboration of canned cherries.

METHODOLOGY

Whole, pitted, stemmed, 2.2 cm caliber Rainier *sp* cherries sulphited with 3500 ppm were prepared according to the French method. This method consists of subjecting the product to a series of impregnations with syrups at increasing concentrations from 25° to 65° Brix, to avoid cherries cell plasmolisis. The solution was made with refined white sugar (sucrose). Prior to the candy point, they were desulphited for 24 hours with running water followed by two consecutive water baths immersions at 60° C during 10 minutes, until the complete disappearance of sulfur dioxide (Monier -Williams's method).

Three treatments were assayed by triplicate: T1: colored cherries with ErB at 0.023 g/L cherries, T2: Colored cherries with Brilliant Blue (light blue at 0.023 g/L), and T3: cherries with Brilliant Blue (Dark Blue at 0.046 g/L).

1. Coloration

After the third impregnation and prior to addition of the dyes, the syrup was heated at 50 ± 2° C, and 9 ml of a solution of citric acid 2 % were added to bring the solution to pH about 4.4 to solubilize the ErB (initial pH = 5.75, final pH = 4.45). the The impregnation process was continued, with the pH being adjusted in the last impregnation with a solution of citric acid 2 % until a pH close to 3.80. The solution of sodium bicarbonate 2 % was added then to pH 4,2 and the cherries were finally packaged in hexagonal flasks of 380 g and pasteurized for 20 minutes at 100° C. Different parameters were periodically measured, such as density of the syrup (gravimetric and volume), pH (AOAC 960.10), soluble solids solutions and pulp with refractometer Arcano Model 75 (AOAC 969.38), and humidity[14]. The following variables were also measured on the final products: texture, color and water activity.

2. Cherries Texture

Firmness tests were conducted by the Kramer test, in a Kramer cell 10 Blade, Pert code HDP / KS10, with a load of 5.9 N, on approximately 20 g of halved cherries. Fruit were

subjected to the cutting force of the cell through a feed rate of 3.33 mm/s at room temperature. Maximum firmness (N) corresponding to the peak in the load-time curve was recorded. Differences in the amount of fruit charged were standardized by dividing the total mass of firm fruit charged.

3. Color measurement of cherries:

Color measurements (L *, a * and b *) were evaluated with a colorimeter Konica Minolta CR-400, illuminant D65. Three measurements were taken at different positions on the fruit equatorial surface of three fruits per experimental unit. The instrument was calibrated with a white Minolta calibration plate. It was used as the light source lamp pulsed xenon arc and an area of 8 mm, where L * expresses the brightness or darkness (0 is dark and 100 is bright); -a *, + a * -b * y + b * represents green, red, blue and yellow respectively.

4. Relative Weight Loss (rwl)

Before the first impregnation, 25 cherries were placed in a flexible mesh bag tied with nylon thread in order that the syrup could impregnate the fruit. The initial weight of the bagged cherries (P1 = initial weight) was taken and then submerged into the syrup. Every half hour the bags were drained, dried with absorbent paper and weighed on an analytical balance (P2 = weight of the bag with the sample). The parameter rwl was calculated according to the equation:

5. Statistical analysis

Analysis of the variance (ANOVA) was used to establish significant differences ($p<0.05$) between treatments in texture and color. Duncan's Multiple Range Test was used to determine differences among treatments. Microsoft Excel programs and StatGraphics Centurion XVI.I were used for statistical analysis.

6. Sensory analysis

Sensory analysis was performed with an Hedonic scale test of 5 points and reference test with untrained judges[15].

7. Shelf Life

The stability of dyes was performed by placing three samples of each treatment in the darkness for six months, while three other samples were exposed for the same time to the sunlight, at room temperature, at 50 cm from standard window glass (4 mm thick), trying to simulate cherry storage in shelves. Color parameters L *, a* and b * were measured in three samples each.

RESULTS AND DISCUSSION

As in all product with osmotic dehidratation it shows an the decrease in soluble solids contents (Brix) in the syrup, due to the sugar entrance into the cherry matrix in the different impregnation steps. This process started with a concentration of 25° Brix syrup and ended with 63° Brix, when the product was finally stabilized. The different impregnations allowed the osmotic exchange typical in this kind of process: water exit and entry, and soluble solids entrance as sucrose syrup[16.]

A similar behavior was observed in the different treatments (T1, T2 and T3), *i.e.* a decrease in syrup soluble solids (Brix) due to the entrance of sugar into the cherry matrix in each impregnation. The process began with 25° Brix syrup and ended with a stabilized product at 62 ° Brix. The slow decrease in soluble solids was influenced by the room temperature (20 ± 2 ° C) and the concentration of syrup, among other variables. The more relevant decrease in concentration was observed in the first impregnation at an early time, close to the beginning of the process.

The relative weight loss (%) of the fruit performed during the cherries candying process for the two dyes, ErB (T1) and Ligth Blue T2, as assessed at the end of each of the five impregnation steps (T3 evolution, was similar to T2). The total weight loss calculated, 47.29 %, was consistent with previous results showing that the major exchanges occur in the early stages of the treatment[17]. This weight loss represents the balance between the release of water and some solutes into the liquid (syrup) and the entrance of soluble solids into the fruit matrix as a consequence of the diffusion phenomenon, which altogether produces an osmotic dehydration[17,16].

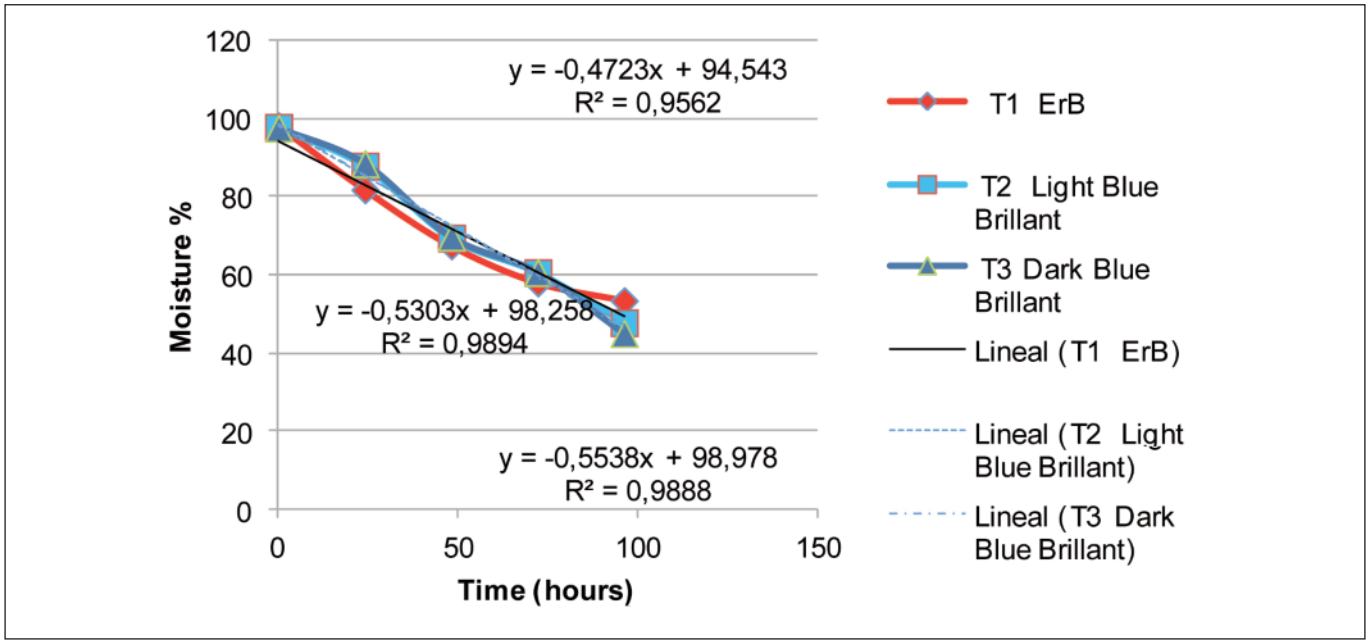
Weight loss in T1 was considerably more pronounced (47.29 %) than those in the treatments with Brilliant Blue (T2 and T3), which rendered similar values at the end of the five impregnations (35.52 % and 34.40 % respectively), this difference was statistically significant ($p<0.05$) as assessed by the ANOVA.

Meanwhile, the moisture evolution was similar in all treatments, as shown in figure N° 1

Figure N° 1 shows the evolution of moisture for the different treatments. It can be seen that this parameters decreases gradually in the different impregnation steps by following a linear function, with R^2 ranging from 0.9562 to 0.9894. The moisture in the final step was 53.52 % for treatment T1 treatment, 47.98 % for treatment T2, and 45.14 % for treatment T3. Analysis of the variance showed the existence of a statistically significant difference among the average of the three variables with a 95.0 % of reliability. Fisher test determined that the significant difference occurred between treatment T1 (ErB) and treatments T2 and T3 with Brilliant Blue.

Both moisture and weight loss differences between the samples treated with the two dyes could be related not only to differential chemical structure but also to other variables such as sample purity and physicochemical interactions with the cherry tissue.

Figure 1. Moisture content variation.



In this regards, ErB was of food grade, representing 90 % of purity, with 10 % of volatile impurities as chlorides, sulphate, among other substances. Interestingly, ErB is a molecule shows differential levels of hydrofilicity among the different parts. The presence of sulfonics groups acts as auxocromic group, increasing the solubility in water. Such capacity may also be hampered by other chemical groups present in the molecule. Similar characteristics apply for Brilliant Blue dye, where sulfonic acid groups and the presence of nitrogen favor the hydrophilic interactions. In addition to being much more soluble than Erb (200 g/L vs 10g/L) Brilliant Blue is also more stable than this dye.

The water activity: this parameter was, after the five impregnations, 5 % higher for T1 treatment in comparison to the treatments with Brilliant Blue (T2 and T3). Considering that the lower the water activity, the higher the biological stability of the product, it could be concluded that cherries stained with Brilliant Blue will be more stable than produce stained with ErB. Therefore, since water will be less available in Brilliant Blue-colored cherries (T1 and T2), the development of microbial growth will be more efficiently prevented in these treatments.

The statistical significance of the difference among treatments was assessed with a level of 95.0 % of reliability. Fisher test determined a high level of significance for the difference was between treatment T1 (ErB) and the homogeneous group formed by T2 and T3 Brilliant Blue with an α value of 0.05.

It would be meaningful to evaluate in future studies the effect of the osmotic dehydration with different dyes on different physic-chemical parameters of cherries, by considering variables such as the electric charge of the colorant (Z), the molecular size of the dyes, and the molecular affinity (MA), which is related to the hydrophilicity or hydrophobicity of the dye and its relation to the tissue. This value is calculated in practice as the logarithm of the partition coefficient (log P) of the dye with octanol water. It should be also considered the number of conjugated linkages of the dye, which is related to the size of the aromatic molecule. Other aspects to be consider are the pH and the pectins remaining in the matrix of the fruit, since the acidic conditions could induce the precipitation of ErB in the matrix.

The texture of the red cherries (T1 assay) was similar to that of the light blue cherries; while the treatment with the highest concentration of Light Blue (dark blue cherries) rendered the least firm fruit.

The average texture values of cherries was 15.60 N/g, 15.18 N/g, and 12.34 N/g for ErB, T1, and T2 treatments respectively. The ANOVA showed no significant difference among the different treatments with a level of reliability of 95.0 %.

The values obtained by Minolta Colorimeter. Lightness average values (L*)27.86 were higher for fruit colored with light Blue (T2) 26.22 and ErB (T1) in relation to the dark Blue colored (T3) 24.66, with the difference being statistically significant (α = 0.05) according to the multiple range test. In turn, Fisher test (α = 0.05) determined the existence of two homogeneous groups.

In the case of a* value, which is related to red colour, cherries treated with ErB (T1) 15.70 and Light Blue (T2) had

positive values: 0.77, while cherries submitted to treatment with Dark Blue (T3) showed negative values: -1.13, which indicated a tendency towards green colour. In turn, b* values, which indicates a tendency toward yellow colour were statistically different among treatments (α = 0.05) according to the multiple range test, forming two homogeneous groups according to Fisher test. It is important to mention that the tendency towards yellow colour was higher for T1-8.58 and T2: -9.05, while T3: 4.04 treatment showed a tendency toward blue.

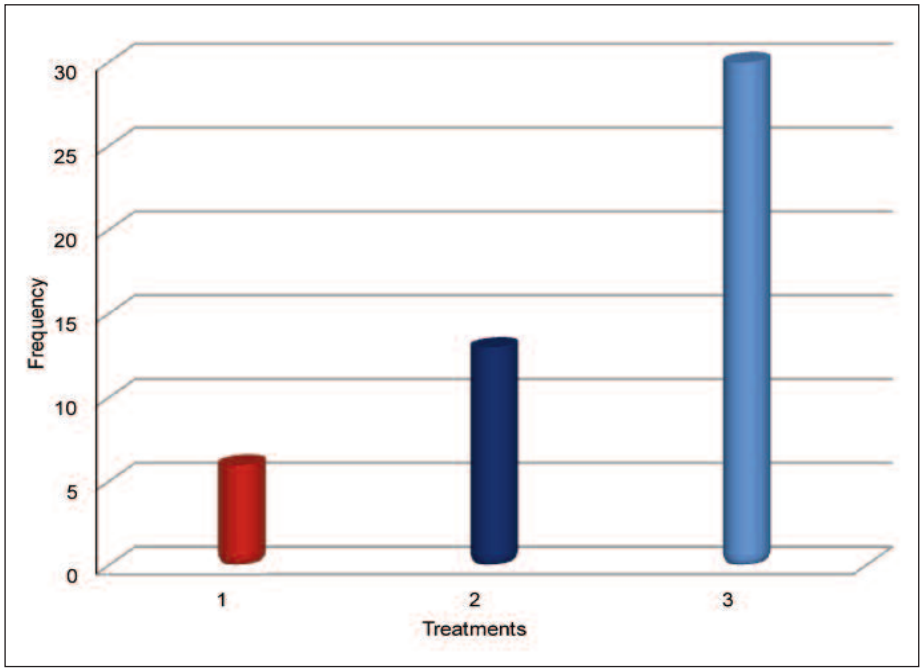
The results of the sensory analysis, which was carried out by 49 untrained testers, whose average age was 23. This information was important because it is well-known that young people adapt themselves more quickly to the inclusion of new colors in food products, which will be important for future markets.

Figure N° 2 presents the histogram of frequencies of the emotional acceptance and preference test for the different treatments: Light Blue cherries (T2) were most accepted (30 preferences) than Dark Blue cherries (T3; 13 preferences), followed in turn by ErB cherries (T1; 6 preferences). This test clearly proves that the color was innovative, having a positive impact and being able to lead the tester's preference in relation to red cherries, which were rather preferred in the past as they are generally linked to the image of ripe fruit.

Picture 1 depicts that cherries subjected to the different treatments (Light Blue -T2- Dark Blue –T3-). Red cherries protected from light suitably preserved the stability of color for a period of 6 months(above).

However,below, in the Picture 1 shows that when exposed to the light for the same period, only Light Blue and Dark Blue cherries were stable in color, while Red cherries suffered a discoloration process as a consequence of the dye being photosensitive. In addition to the impact on visual appearance, Mittall et al.[4] stated that degradation products from Erb are also toxic. In the present study, ErB-colored-cherries began to fade approximately within the first two weeks (data not shown), while Light and Dark Blue cherries remained stable for a time considerably longer than this period.

Figure 2. Sensory Analysis: trial histogram.



The discoloration phenomenon can be also appreciated in the color parameters L*, a*and b* (See Table Nº 1) which showed a greater variability in the case of Red cherries (T1) in comparison to both T2 and T3 treatments. Table N° 2 shows that particularly in the case of Brightness (L*), the former treatment had a considerably higher decrease during shelf life simulation test (the higher the L* value, the higher the brightness). It is important to mention that the instability of ErB was so prominent in fruit exposed to light, that the product was almost totally discolored, turning into white in

Table 1. Life test. Values of L*; a*, b*.

Color Average	Erythrosine cherry		Light Blue cherry		Dark Blue cherry	
	LIght	***Darkness***	***LIght***	***Darkness***	***LIght***	***Darkness***
*L**	35.53	27.90	26.22	25.21	24.66	24.04
*a**	2.85	15.65	-1.13	0.03	0.77	1.34
*b**	5.44	4.04	-9.05	-7.94	-8.58	-5.41

Picture 1. Life test.

1- T3 Light Blue Brillant before life test. 2- T3 Light Blue Brillant after life test. 3- T2Dark Blue Brillant before life test. 4- T2Dark Blue Brillant after life test. 5- T1 ErB before life test. 6- T1ErB after life test.

appearance. ANOVA showed that the difference was statistically significant at the level of 5 %. In turn, Fischer test also confirmed the statistically significant difference in the brightness of the Red cherries between fruit exposed to light and darkness.

Regarding the other color parameters, cherries Red cherries kept in the dark showed the highest value of a*, followed by Dark Blue cherries. In turn, Light Blue cherries values were close to zero with a tendency towards green tone, as indicated by the negative values (-a*). ANOVA showed that the difference was statistically significant at a level of 5 % and in turn, Fischer test detected the existence of five homogeneous groups.

In the case of b* value, no statistically significant differences was found between Dark Blue cherries exposed to light and those kept in the darkness, whereas the opposite happened in the case of Red cherries. Table 4 shows the b* values of fruit subjected to the different treatments after 168 hours of storage. Positive b* values indicate a tendency towards yellow colors, while negative values point towards blue tones. According to this analysis, Red cherries attained the highest positive value, while Dark Blue fruit had the highest negative values. ANOVA indicated the existence of statistically significant differences at a level of 5 %, while multiple range test detected the existence of three homogeneous groups. No significant differences were found between Light Blue cherries exposed to light and those kept in the dark, and the same happened in the case of Red cherries (data not shown).

CONCLUSIONS

Results from this research proved the feasibility of developing innovative canned cherries in syrup by staining the product with artificial dye Brilliant Blue. This product successfully withstanded a storage period of up to six month even in challenging conditions such as light exposure, with almost no change in color, which reflects the high stability of the dye. Conversely, red cherries (ErB) were totally discolored when exposed to light for the same period. In addition to the better visual stability it is also important to notice the lower IDA of this additive (6 mg/kg), considerably lower than other synthetic dyes, especially erythrosine. Although this last dye provides the food industry with the most efficient technological alternative for dying foods, including canned cherries, the low value IDA (0.1mg/Kg) present a certain safety risk to consumers.

In contrast, the high stability and low toxicity of Brilliant Blue offers a suitable alternative for the manufacturing of canned cherries or similar products. Sensory analyses included in the present study proved the suitability and feasibility to use this dye for the processing of canned cherry, which were confirmed by perception test with untrained sensory panel, whose preference was clearly oriented towards Blue Cherries.

Although the launch of a new product to market encompasses a series of steps, from conception to market performance, results from the present study can be considered as a relevant contribution, as shown by the sensory analysis, which were highly satisfactory, with an acceptance and preference of the tasters for blue cherries of 88% (the remaining 12% of the panel preferred cherries red with erythrosine). This finding encourages the development of further research to design a market strategy for this striking product, which in addition to offer the consumer with an innovative product, it also represents a valid alternative to prevent the exposure of consumers to the risk of a compound with a high level of toxicity such as erythrosine.

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REFERENCES

1. Ganesan, L, Margolles-Clark E, Song Y, Buchwald P. The food colorant erythrosine is a promiscuous protein–protein interaction inhibitor. Biochem Pharmacol. 2011; 81: 810–18.
2. Mpountoukas, P, Pantazaki, A, Kostareli, E, Christodoulou P, Kareli D, Poliliou S. Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. Food Chem Toxicol; 2010; 48: 2934–44.
3. Silbergeld, EK y Anderson, SM. Artificial food colors and childhood behavior disorders. Bull NY Acad Med, 1982; 58: 275–95.
4. Mittal, A, Mittal J, Kurup L, Singh, AK. Process development for the renewal and recovery of hazardous dye erythrosine from wastewater by waste materials bottom ash and de-oiled soya as absorbent. J Hazard Mater, 2006; B138: 95-105.
5. Jennings, AS, Schwartz, SL, Balter, NJ, Gardner, D, Witorsch, RJ. Effects of oral erythrosine (2´, 4´, 5´, 7´-tetraiodofluorescein) on the pituitary–thyroid axis in rats. Toxicol Appl Pharma, 1990; 103:549-56.
6. Drumond Chequer, FM, Vinícius de Paula, V, Pires Bianchi, ML, Greggi Antunes, LM. Genotoxic and mutagenic effects of erythrosine B, a xanthene food dye, on HepG2 cells. Food Chem Toxicol, 2012; 50: 3447–51.
7. Bora, SS, Radichevich, I, Werner, SC. Artifactual elevation of PBI from an iodinated dye used to stain medicinal capsules pink. J Clin Endocrinol Metab, 1969; 29:1269–71.
8. Mizutani, T. Toxicity of xanthene food dyes by inhibition of human drug-metabolizing enzymes in a noncompetitive manner. J Environ Public Health, 2009; Article ID 953952. 9pp.
9. Amchova P, Kotolova H, Ruda-Kucerova J. Health safety issues of synthetic food colorants. Regul Toxicol Pharmacol, 2015; 73 (3): 914-22.
10. Borzelleca, JF, Depukat, K, Hallagan, JB. Lifetime toxicity/carcinogenicity studies of FD & C Blue No. 1 (Brilliant Blue FCF) in rats and mice. Food Chem Toxicol, 1990; 28: 221-34.
11. Kus, E. y Eroglu, HE. Genotoxic and cytotoxic effects of Sunset Yellow and Brilliant Blue, colorant food additives, on human blood lymphocytes. Pak J Pharm Sci, 2015; 28: 227-30.
12. Lucova, M, Hojerova, J, Pazourekova, S, Klimova, Z. Absorption of triphenylmethane dyes Brilliant Blue and Patent Blue through intact skin, shaven skin and lingual mucosa from daily life products. Food Chem Toxicol, 2013; 52: 19-27.
13. Codrea SA, Comment on "Absorption of triphenylmethane dyes Brilliant Blue and Patent Blue through intact skin, shaven skin and lingual mucosa from daily life products" by Lucova et al. (2013). Food Chem Toxicol, 2013; 52: 19-27.
14. A.O.A.C. Official Methods of Analysis of the Association of Official Agricultural Chemists. 16° Edition. 1995.
15. Anzaldúa-Morales A. La evaluación sensorial de los alimentos en la teoría y la práctica. Editorial Acribia. España. 220 pp. 1994
16. Barbosa Canovas, G. V. Deshidratación de alimentos. Editorial Acribia. España. 314 pp. 2000.
17. Ceballos Chan, G. Estudios en papaya mínimamente procesada por deshidratación osmótica. Tesis Doctoral. Universidad Politécnica de Valencia. Departamento de Tecnología de Alimentos. Valencia. España. 196 p. 2005.