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Comparison of the ionizing radiation effects on cochineal, annatto and turmeric natural dyes

Helio M. Cosentino [a], Patricia Y.I. Takinami [b], Nelida L. del Mastro [b,*]

[a] Mackenzie Presbyterian University, R. da Consolação 930, 01302-907 SP, Brazil
[b] Center of Radiation Technology, IPEN-CNEN/SP, Av. Prof. Lineu Prestes, 2242, 05508-900 SP, Brazil

HIGHLIGHTS

- Comparison of radiosensitivity of food colors was performed.
- Carmine showed the highest resistance to radiation.
- Annatto and turmeric behaved sensitive to radiation when diluted.
- Turmeric was the most affected by ionizing radiation.

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ABSTRACT

As studies on radiation stability of food dyes are scarce, commercially important natural food grade dyes were evaluated in terms of their sensitivity against gamma ionizing radiation. Cochineal, annatto and turmeric dyes with suitable concentrations were subjected to increasing doses up to 32 kGy and analyzed by spectrophotometry and capillary electrophoresis. The results showed different pattern of absorbance versus absorbed dose for the three systems. Carmine, the glucosidal coloring matter from the scale insect *Coccus cacti* L., Homoptera (cochineal) remained almost unaffected by radiation up to doses of about 32 kGy (absorbance at 494 nm). Meanwhile, at that dose, a plant-derived product annatto or urucum (*Bixa orellana* L.) tincture presented a nearly 58% reduction in color intensity. Tincture of curcumin (diferuloylmethane) the active ingredient in the eastern spice turmeric (*Curcuma longa*) showed to be highly sensitive to radiation when diluted. These data shall be taken in account whenever food products containing these food colors were going to undergo radiation processing.

1. Introduction

Useful colors of plant or animal origin have been known for a long time and not only are still widely used but also the use of natural-type food colors continues to increase (Downham and Collins, 2000).

Carmine was used extensively before the advent of synthetic coloring materials. Since then it has been used only when a natural pigment is required in the food industry, for cosmetics and pill coatings and water-soluble drug preparations. The coloring principle of the extract is carminic acid, a hydroxyanthraquinone linked to a glucose unit, comprising 10% of the cochineal and 2–4% of its extract (Fig. 1). Treatment of carminic acid with an aluminum salt produces carmine, the soluble aluminum lake. Carmine is normally 50% or more carminic acid. Typical applications are at a dosage levels ranging from 0.1 to 0.5% (FAO, 2014). Carminic acid and particularly carmine aluminum lake are permitted and widely used in the food industries in North and South America and Western Europe. In Japan, carminic acid rather carmine is employed by the food industry.

Among natural colorants, extracts obtained from annatto have been used in many processed foods, especially dairy products. Annatto, a plant-derived product, is the carotenoid-based dye extracted from the seeds of the tropical tree *Bixa orellana*, known as *achiote* in Spanish and *urucum* in Portuguese. The pigment is made up of bixin (both *cis* and *trans*) with traces of norbixin, bixin dimethyl ester and other apocarotenoids, several of them lycopene cleavage products (Giuliano et al., 2003). Bixin which is a carotenoid with two carboxylic groups, one of which is esterified, is the major pigment present in annatto extract (Fig. 2). Norbixin, which is derived from bixin by hydrolysis of the ester group, is also

* Corresponding author.
E-mail address: nlmastro@ipen.br (N.L. del Mastro).

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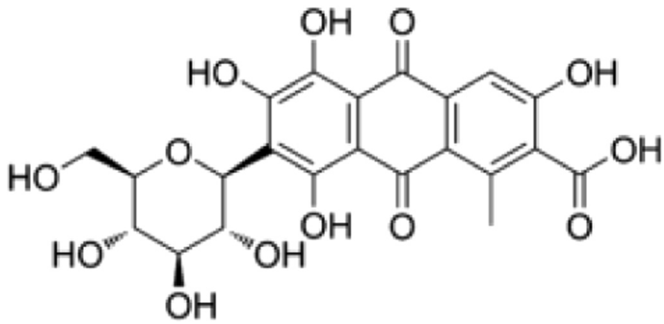

Fig. 1. Chemical structure of carminic acid (*Source*: Merck Index, 11th Edition, 1850).

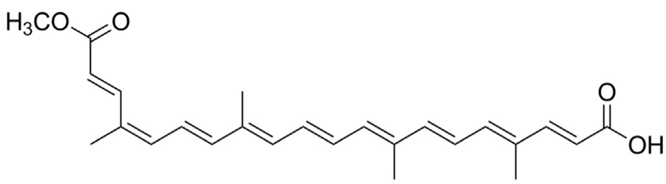

Fig. 2. Chemical structure of bixin (*Source*: http://ntp.niehs.nih.gov/testing/status/background/execsumm/b/bixin/index.html#selection).

sold as a food pigment, and this molecule is water soluble, whereas bixin is oil-soluble.

Curcumin (diferuloyl methane) is a bright orange-yellow crystalline powder used as food coloring. It is the product obtained by solvent extraction of turmeric i.e., the ground rhizomes of *Curcuma longa* L. (*Curcuma domestica* Valeton) and purification of the extract by crystallization. Curcumin is chemically a diarylheptanoid, which incorporates several functional groups (Fig. 3). The ring systems, which are phenols, are connected by two α, β unsaturated carbonyl groups. The diketones form stables enols and are readily deprotonated to form enolates, so, the α, β carbonyl groups undergoes nucleophilic addition. In a way, that sort of compound could indicate a good radical scavenging capacity (Duque et al., 2013).

There are numerous reports in the literature about environmental applications of ionizing radiation like bleaching of dyes solutions, some of them from our own laboratory (Borrely et al., 1998). On the other hand, decontamination of food by ionizing radiation is a safe and efficient process for the elimination of potentially pathogenic bacteria (Farkas, 1998). The processes of cochineal insects, urucum seeds and turmeric roots imply a number of phases from the field to the industry where high risks of contamination do exist. Then, the aim of this work was to establish and compare the radiosensitivity of natural food colors: carmine (cochineal), annatto (urucum) and turmeric (curcumin) using spectrophotometry and also capillary electrophoresis.

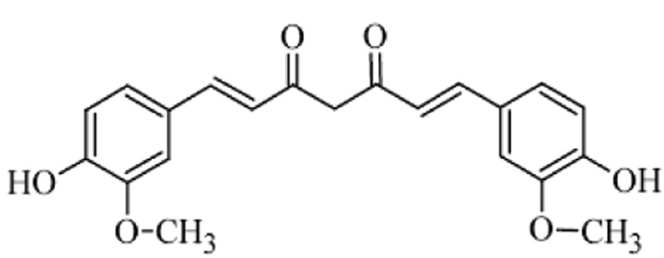

Fig. 3. Chemical structure of Curcumin (*Source*: http://tools.niehs.nih.gov/cebs3/ntpviews/index.cfm?action=testarticle.properties&cas_number=458-37-7).

2. Experimental

2.1. Material

Cochineal carmine: as powder, aluminum-calcic lake, minimum of 52% dry weight carminic acid; as tincture, minimum of 3 g/100 ml carminic acid as ammoniac solution, pH 10.2–10.8, soluble in water.

Urucum dye: as powder, minimum of 30% dry weight of norbixin (expressed as total carotenoids), soluble in water.

Curcumin: as powder, minimum of 30% dry weight of curcuminoids; as tincture, minimum of 30% of curcuminoids in a hydroalcohol solution.

For each one of the food colors a series of samples were prepared as follows:

Carmine powder commercially available containing 5% humidity; commercially available liquid carmine tincture and two tincture dilutions at 5% and 10% v/v.

Commercially available liquid tinctures of urucum and curcumin and their dilutions at 50%, 10% and 5% v/v.

Sample preparations were made according to Codex (1975) and Brazilian norms carmine (Brasil, 1996a) urucum (Brasil, 1996b) and curcumin (Brasil, 1996c). All samples were analyzed in triplicate (spectrophotometry) or duplicate (capillary electrophoresis) and the results expressed as mean and standard deviation.

2.2. Irradiation

Samples (4 concentrations from each food color) were gamma irradiated in a Co-60 Gammacell 220 (AECL), dose rate about 5.2 kGy/h with doses of 0, 1, 2, 4, 8, 16 and 32 kGy, dose uniformity factor: 1.13.

2.3. Spectrophotometry

A VARIAN, model 280, UV/vis was employed.

2.4. Capillary electrophoresis

Capillary electrophoresis separates ions based on their electrophoretic mobility with the use of an applied voltage. It is dependent upon the charge of the molecule, the viscosity, and the atom's radius. The rate at which the particle moves is directly proportional to the applied electric field; neutral species are not affected. Capillary electrophoresis is used most predominately because it gives faster results and provides high-resolution separation (Camilleri, 1997). A BECKMAN P/ACE 5510 (Beckman Coulter Instruments, Fullerton, CA, USA) equipment was utilized with a silica capillary tube, 75 pm internal diameter and 47 cm length model P/ACE 5510, equipped with a variable UV–vis, software for data acquisition and treatment (Beckman P/ACE System Gold Software). Determinations were made at 25 °C under a ddp of 25 kV and injection rate of 0.5 psi/s.

3. Results and discussion

3.1. Carmine

In Table 1 are shown the variation of absorbance at 494 nm due to the application of different absorbed doses for all the carmine samples: original carmine powder, and 5%, 10% and 50% aqueous solutions. The hydroxyanthraquinone absorption spectrum corresponding to most diluted samples showed notorious decrease, but as a rule, carmine samples remained quite stable against radiation treatment. It was reported that carminic acid extracts, the

Table 1
Color variation (absorbance at 494 nm) of irradiated cochineal carmine samples.

Dose (kGy)	Carmine powder	Carmine tincture	10% C. tincture	5% C. tincture
0	0.666 ± 0.017 100%	0.208 ± 0.008 100%	0.022 ± 0.001 100%	0.015 ± 0.000 100%
1	0.671 ± 0.022 101%	0.209 ± 0.002 100%	0.024 ± 0.001 109%	0.011 ± 0.000 73%
2	0.683 ± 0.027 103%	0.209 ± 0.005 100%	0.024 ± 0.001 109%	0.010 ± 0.001 67%
4	0.681 ± 0.005 102%	0.195 ± 0.009 94%	0.022 ± 0.000 100%	0.009 ± 0.000 60%
8	0.653 ± 0.008 98%	0.194 ± 0.003 93%	0.022 ± 0.001 100%	0.007 ± 0.000 47%
16	0.651 ± 0.029 98%	0.190 ± 0.004 91%	0.021 ± 0.001 95%	0.006 ± 0.000 40%
32	0.647 ± 0.015 97%	0.188 ± 0.009 90%	0.021 ± 0.000 95%	0.006 ± 0.000 40%

glucosydal coloring matter from cochineal, essential constituent of carmine, display good stability to heat, light and oxygen (FAO, 2014) being the first communication about their gamma radiation stability made by Cosentino et al. (2005).

3.2. Urucum

Present results on the stability of urucum, annatto dye, against ionizing radiation showed also a limited stability as can be seen in Table 2. The absorbance at 453 nm is strongly affected by the increase of the absorbed dose (up to 32 kGy). Nevertheless, a noticeable increase of color is perceived for the lower radiation doses, 1 and 2 kGy, followed by a decrease afterward.

The stability of annatto dye against heat was established as being limited and depending of the processing conditions (Rao et al., 2005). Annatto carotenoids present antioxidant activity (Alvarez-Parrilla et al., 2014; Santos et al., 2014) and as such are considered protectors against ionizing radiation (Weiss and Landauer, 2003) and are also listed among natural compounds for potential skin cancer treatment (Chinembiri et al., 2014).

Antioxidant activity of carotenoids is related to oxygen concentration, the chemical structure and the presence of other antioxidants (Krinsky, 1993; Palozza, 1998). Carotenoids can act as chain breaking antioxidants, by scavenging and deactivating free radicals both in vitro and in vivo by quenching the harmful singlet oxygen. Then, the lack of stability of urucum against free radical generated by radiation can be attributable to that sort of mechanism evidenced at the higher absorbed doses employed. On the other hand, carotenoids, in spite of behave as antioxidants, they present a pro-oxidant character in the absence of other additives (Palozza, 1998). Carotenoids, being eminent electron donators to reactive radicals, could favorably act as electron acceptors too. That dual behavior could be involved on the perceived increase on absorbance observed in all urucum samples when submitted to the lower absorbed doses.

Table 2
Color variation (absorbance at 453 nm) of irradiated urucum samples.

Dose (kGy)	Urucum Tincture	50% U. tincture	10% U. tincture	5% U. tincture
0	1.429 ± 0.004 100%	0.714 ± 0.025 100%	0.178 ± 0.009 100%	0.095 ± 0.003 100%
1	1.585 ± 0.070 111%	0.988 ± 0.033 138%	0.230 ± 0.003 129%	0.118 ± 0.039 124%
2	1.542 ± 0.056 108%	0.943 ± 0.027 132%	0.200 ± 0.007 112%	0.107 ± 0.003 113%
4	1.509 ± 0.038 106%	0.671 ± 0.049 94%	0.108 ± 0.003 61%	0.080 ± 0.002 84%
8	1.442 ± 0.063 101%	0.624 ± 0.021 87%	0.095 ± 0.001 53%	0.054 ± 0.002 57%
16	1.337 ± 0.023 94%	0.528 ± 0.012 74%	0.058 ± 0.001 33%	0.032 ± 0.001 34%
32	0.824 ± 0.009 58%	0.506 ± 0.012 71%	0.026 ± 0.001 15%	0.018 ± 0.001 19%

3.3. Curcumin

It was described that a remarkable difference in color strength when different extracts of irradiated and un-irradiated turmeric powder were used to dye irradiated and un-irradiated fabric (Batti et al., 2011). Curcumin or turmeric was described as stable at high temperatures and in acids, but unstable in alkaline conditions and in the presence of light (ftp://ftp.fao.org/es/esn/jecfa/cta/CTA_61_Curcumin.pdf). The stability of the curcumin tincture solutions against radiation is very poor at high doses, as it is evident looking at the results presented in Table 3. Nevertheless, the tincture itself showed quite good resistance to radiation treatment at 2 kGy, and the samples still preserve certain radiation stability.

The principal coloring components of curcumin exhibit antioxidative, antimutagenic and antibacterial activities (Parvathy et al., 2009). Curcumin has been found to exert a dual mode of

Table 3
Color variation (absorbance at 428 nm) of irradiated curcumin samples.

kGy	Curcumin tincture	50% C. tincture	10% C. tincture	5% C. tincture
0	0.553 ± 0.012 100%	0.332 ± 0.005 100%	0.158 ± 0.005 100%	0.078 ± 0.000 100%
1	0.550 ± 0.010 99%	0.330 ± 0.005 99%	0.157 ± 0.003 99%	0.075 ± 0.000 96%
2	0.549 ± 0.003 99%	0.274 ± 0.008 83%	0.131 ± 0.002 83%	0.065 ± 0.000 83%
4	0.530 ± 0.005 96%	0.167 ± 0.006 50%	0.080 ± 0.000 51%	0.039 ± 0.000 50%
8	0.530 ± 0.009 96%	0.020 ± 0.001 6%	0.009 ± 0.000 6%	0.005 ± 0.000 6%
16	0.518 ± 0.015 94%	0.011 ± 0.000 3%	0.005 ± 0.000 3%	0.003 ± 0.000 4%
32	0.480 ± 0.008 87%	0.005 ± 0.000 2%	0.003 ± 0.000 2%	0.001 ± 0.000 1%

Table 4
Comparison among integration areas of non-irradiated and irradiated (32 kGy) carmine, urucum and curcumin electropherograms.

	0 kGy	32 kGy	Decrease after irradiation (%)
Carmine powder	4.163.960	2.972.090	29
Tincture	2.761.160	1.448.400	48
10% Tincture	694.080	691.98	90
5% Tincture	346.485	3.505	99
Urucum Tincture	4.103	2.749	33
50% Tincture	2.616	1.170	55
10% Tincture	599	0	100
5% Tincture	311	0	100
Curcumin Tincture	6.512	4.884	25
50% Tincture	3.406	222	94
10% Tincture	699	0	100
5% Tincture	315	0	100

action after irradiation depending on its dose. It has been reported to protect various study systems against the deleterious effects induced by ionizing radiation and to enhance the effect of radiation (Jagetia, 2007). In a case of radiosensitizer behavior in vivo, the authors ascribed that to the increased reactive oxygen species production and overactivation of the mitogen-activated protein kinase (Prashanthi et al., 2008).

Capillary electrophoresis (CE) is applied for the determination of food additives and colorants as the analyte mobility at a defined pH is unique and identifying (https://www.beckmancoulter.com/ucm/idc/groups/public/documents/webasset/paceset1_1.pdf; Goltz et al., 2012). In CE the movement of analytes in an electrical field is the combination of the analyte's induced charge with respect to its mass. In the present work CE was employed just in order to evidence the action of ionizing radiation on that relation induced charge/mass. For all the food colors assayed a strong decrease of the integrated surface area of the electropherograms versus absorbed dose was found (Table 4). Further elements would be needed to arrive to a correct interpretation of this phenomenon.

4. Conclusion

Although carmine, urucum and curcumin are used for the same purpose, i.e. coloring food, they have different chemical structure and consequently diverse stability against chemical or physical treatments. It is possible to perceive that carmine looks extraordinary radiation stable, except at the highest dilution. Urucum carotenoid behaves generally as radiation sensitive characterized by a decline in absorbance with absorbed dose, although an increase in the absorbance can be perceived for the lowest ones, 1 and 2 kGy. In a certain way, carmine presents also a mild increase of absorbance for the lower radiation doses. Curcumin tincture is notoriously sensitive to radiation when diluted. The small increase in color development observed mainly for urucum carotenoid samples when irradiated with low doses, although significant, would be not enough per se to recommend irradiation with that purpose. Nevertheless, as ionizing radiation treatment can be successfully apply either to raw materials or to ready to eat foods, the knowledge provided by the present study about the radiosensitivity of food colors represents a great contribution for practical applications and an stimuli for further researches on the radiation chemistry involved.

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