

Binding, Antioxidant and Anti-proliferative Properties of Bioactive Compounds of Sweet Paprika (*Capsicum annuum* L.)

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Abstract The scope of this research was to determine the bioactive composition, antioxidant, binding, and anti-proliferative properties of red sweet paprika growing under artificial light. The amounts of carotenoids, chlorophyll, polyphenols, tannins, and flavonoids in red paprika (RP), cultivated in Korea, before and after light treatments under high pressure sodium (HPS) and lighting emitting plasma (LEP) lamps (RPControl, RPHPS, RPLEP), were analyzed in water (W) and ethanolic extracts (Et). Spectroscopic, radical scavenging assays, fluorescence and cytotoxicity measurements were applied. The results of this study showed that total chlorophyll and carotenes were the highest in RPHPS (10.50 ± 1.02 and

33.90 ± 3.26 $\mu\text{g/g}$ dry weight (DW)). The strongest antioxidant capacity ($\mu\text{M TE/g DW}$) in a 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS⁺) assay was in RPControlEt (24.34 ± 2.36), in a ferric-reducing/antioxidant power (FRAP) assay in RPHPSW (27.08 ± 2.4) and in a cupric reducing antioxidant (CUPRAC) in RPLEPW (70.99 ± 7.11). The paprika ethanolic extracts showed lower values in their bioactivity than the water ones. The binding and cytotoxicity abilities of extracted polyphenols correlated with their amounts. LEP treatment is better for plant growth characteristics than other conventional treatments. The investigated paprika samples can be used as a source of antioxidants.

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Keywords Red sweet paprika · Light treatments · Antioxidants · Fluorescence · Binding · Cytotoxicity

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Introduction

Sweet red bell paprika is one of the sources of ascorbic acid, carotenoids and phenolic compounds, which are important in the human diet [1–4]. The levels of bioactive compounds are very variable and may be affected by ripeness, genotype and cultivation [5]. There is no data describing the changes in the composition of paprika using artificial light. Plant development is strongly influenced by the light quality, which refers to the color or wavelength of the light, which reached the plant surface [6]. Light intensity can positively affect photochemical accumulation [7], but the effects of light quality are more complex, and mixed results are often reported. Paprika (*Capsicum annuum* L.) is widely used as a healthy vegetable having antiproliferative activity, which reduces or prevents chronic disease [8, 9]. Paprika has been mostly consumed as fresh fruit, and as food colorants such as oleoresin or pigment

powder. Limited research was found in connection with the binding, antioxidant and proliferative properties of red paprika after the use of artificial light during the growth. Therefore such aspect was taken under consideration in the present research with high pressure sodium (HPS) and lighting emitting plasma (LEP) lamps during growing period of paprika. Antioxidant assays and three-dimensional fluorescence were used for these studies [10].

Materials and Methods

Chemicals All reagents were of analytical grade and purchased from Sigma Chemical Co., St. Louis, MO, USA and Fluka Chemie, Buchs, Switzerland. The cell lines were purchased from Korean Cell Line Bank.

Samples Artificial light sources HPS (HSE DAYlight model; 315 watt (W); energy consumption = 1.5 ampere (A)/230 volt (V); color rendition index >90; manufacturer Hortilux Schröder, Netherlands) and LEP (Gavita-Pro 300 model; 300 W; energy consumption = 1.3 A/230 V; color rendition index 94; manufacturer Gavita, Netherlands) were placed in the glass greenhouse (Venlo-type) located in the city of Gimje in Jeollabuk-do Province of South Korea in order to investigate the growth and fruit setting properties of paprika (Red, De Ruiter, NL). HPS and LEP lamps were provided from October 12, 2012 till February 28, 2013. These periods required supplemental of lighting because light intensity is very low in winter season in Korea. Then, mature sweet pepper fruits with ≥ 90 % coloration were harvested to investigate their bioactive properties. Control sample was grown at natural light (sunlight). Three replications (HPS, LEP and natural light) were used [8].

Determination of Bioactive Compounds and Total Antioxidant Capacity Polyphenols were extracted with ethanol and water from previously lyophilized paprika samples and were determined by Folin-Ciocalteu method [11]. Flavonoids, extracted with 5 % NaNO_2 , 10 % $\text{AlCl}_3 \times \text{H}_2\text{O}$ and 1 M NaOH, were measured at 510 nm. Condensed tannins (procyanidins) were extracted with 4 % vanillin solution in EtOH and then the optical density was measured at 500 nm.

Total antioxidant capacity (TAC) was determined with utilization of four complementary assays in order to receive reliable results: (1) 2, 2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS^{•+}) [12]. (2) Ferric-reducing/antioxidant power (FRAP) assay [13]. (3) Scavenging free radical potentials were tested in an ethanolic solution of 1, 1-diphenyl-2-picrylhydrazyl method (DPPH) [14]. (4) Cupric reducing antioxidant (CUPRAC) [15].

Total chlorophylls, chlorophylls a and b and total carotenoids were extracted with 100 % acetone [16]. Total ascorbic

acid was determined by CUPRAC assay in water extract (100 mg of lyophilized sample and 5 ml of water) [17].

Fluorometric measurements were used for the evaluation of binding properties of paprika fruit polyphenol extracts to human serum albumin (HSA). Two dimensional (2D-FL) and three dimensional (3D-FL) fluorescence measurements were recorded [10].

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) Assay

Anti-proliferative activity of methanol and ethyl acetate extracts of the studied plants on human cancer cell line (Calu-6 for human pulmonary carcinoma) was measured using MTT assay [18].

Statistical Analysis To verify the statistical significance, means \pm SD of five independent measurements were calculated. One-way analysis of variance (ANOVA) for statistical evaluation of results was used, followed by Duncan's multiple range test to assess differences between group's means. *P* values of <0.05 were considered to be significant.

Results and Discussion

Bioactive Compounds in Paprika Samples Paprika is considered as an excellent source of phytochemicals with health-promoting effects, such as carotenoids, vitamin C and phenolic compounds. The results of the effect of different light systems (HPS vs. LEP lamps) on the resulting bioactive composition, antioxidant, binding, and anti-cancer properties of sweet paprika are shown in Table 1. The amounts of carotenoids, chlorophyll, polyphenols, tannins, and flavonoids in red paprika before and after light treatment (RPControl, RPHPS, RPLEP) were analyzed in water (W) and ethanolic extracts (Et). Bioactive compounds in ethanolic extracts showed lower values than the water. Total chlorophyll and carotenes were the highest in RPHPS (10.50 ± 1.02 and 33.90 ± 3.26 $\mu\text{g/g DW}$). The strongest antioxidant capacity ($\mu\text{M TE/g DW}$) in a 2, 2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS^{•+}) assay was in RPControlEt (24.34 ± 2.36), in a ferric-reducing/antioxidant power (FRAP) assay in RPHPSW (27.08 ± 2.4) and in a cupric reducing antioxidant (CUPRAC) in RPLEPW (70.99 ± 7.11). Our results were in line with the data of others [19], considering the results of ethanolic extracts of red paprika of chlorophylls, antioxidant capacities in ABTS and DPPH assays. The light spectra in many reported experiments, which were produced by LEDs or fluorescent lamps, were inconsistent, therefore it was difficult to compare our results with the literature data. The light intensity was non-uniform because the

Table 1 (A) The content of total polyphenols, flavonoids, tannins; vitamin C, chlorophylls, xanthophylls + carotenes and antioxidant capacities of sweet pepper (*Capsicum annuum* L.) samples in water and ethanol extracts (per g DW); Three-dimensional fluorescence spectral char-

acteristics of (B) water and ethanol extracts; (C) water and ethanol extracts in interaction with HSA of sweet pepper (*Capsicum annuum* L.); (D) Survival rate of lung cancer (Calu6) cells of paprika fruits of two types of lamps (high pressure sodium (HPS) and lighting emitting plasma (LEP)

A								
Indices	Control	LEP	HPS					
Polyph W, mgGAE	23.93 ± 2.21 ^a	22.09 ± 2.12 ^b	20.52 ± 2.09 ^b					
Polyph Et, mgGAE	6.84 ± 6.07 ^a	6.47 ± 0.54 ^a	6.28 ± 0.61 ^a					
Tannins W, mgCE	1.58 ± 0.15 ^a	1.82 ± 0.14 ^a	1.52 ± 0.14 ^a					
Tannins Et, mgCE	1.95 ± 0.16 ^b	2.16 ± 0.21 ^a	2.31 ± 0.21 ^a					
Vit C, mgAA	2.23 ± 0.21 ^a	2.08 ± 0.18 ^a	1.72 ± 0.12 ^b					
Chlorophyll a, µg	3.65 ± 0.29 ^b	1.74 ± 0.15 ^c	6.27 ± 0.52 ^a					
Chlorophyll b, µg	3.81 ± 0.28 ^a	1.85 ± 0.13 ^b	4.21 ± 0.41 ^a					
Chlorophyll a + b, µg	7.58 ± 0.72 ^b	3.62 ± 0.28 ^c	10.50 ± 1.02 ^a					
Xanth + Carotenes, µg	27.30 ± 2.18 ^b	29.40 ± 0.24 ^{ab}	33.90 ± 3.26 ^a					
Flavon W, mgCE	0.52 ± 0.04 ^a	0.54 ± 0.04 ^a	0.64 ± 0.04 ^a					
Flavon Et, mgCE	1.13 ± 0.11 ^a	1.13 ± 0.09 ^a	1.09 ± 0.09 ^a					
ABTS W, µMTE	66.42 ± 6.51 ^a	64.16 ± 6.12 ^a	64.02 ± 6.22 ^a					
ABTS Et, µMTE	24.34 ± 2.36 ^a	21.69 ± 2.12 ^{ab}	20.72 ± 2.03 ^b					
DPPH W, µMTE	18.35 ± 1.76 ^a	19.08 ± 1.72 ^a	18.65 ± 1.65 ^a					
DPPH Et, µMTE	5.19 ± 0.43 ^a	5.35 ± 0.52 ^a	4.86 ± 0.42 ^a					
FRAP, W, µMTE	19.34 ± 1.76 ^b	25.55 ± 2.43 ^{ab}	27.08 ± 2.4 ^a					
FRAP Et, µMTE	12.70 ± 1.21 ^a	11.90 ± 1.11 ^{ab}	10.70 ± 1.01 ^b					
CUPRAC W, µMTE	67.35 ± 6.38 ^{ab}	70.99 ± 7.11 ^a	64.76 ± 6.32 ^b					
CUPRAC, Et, µMTE	20.89 ± 2.18 ^a	22.13 ± 2.12 ^a	21.78 ± 2.45 ^a					
B								
Peaks		CW	HPS W	LEPW	CEt	HPS Et	LEPEt	
a	$\lambda_{ex}/\lambda_{em}^*$	247/431	245/431	247/432	283/332	282/332	283/332	
	FI**	242.23	189.50	243.54	227.73	259.42	262.07	
b	$\lambda_{ex}/\lambda_{em}$	344/422	344/417	347/422	339/412	342/422	337/414	
	FI	708.88	418.74	729.84	239.31	247.72	189.45	
b1	$\lambda_{ex}/\lambda_{em}$	–	–	–	257/417	–	–	
	FI	–	–	–	124.92	–	–	
c	$\lambda_{ex}/\lambda_{em}$	282/349	282/350	282/350	261/421	259/406	257/417	
	FI	149.13	144.61	128.51	125.02	112.63	152.67	
d	$\lambda_{ex}/\lambda_{em}$	–	–	–	406/670	410/670	403/672	
	FI	–	–	–	47.70	167.62	46.52	
f	$\lambda_{ex}/\lambda_{em}$	–	–	–	283/645	283/645	283/645	
	FI	–	–	–	42.61	46.89	48.61	
C								
Peaks		CW	HPS W	LEPW	CEt	HPS Et	LEPEt	HSA
a	$\lambda_{ex}/\lambda_{em}$	278/364	280/359	278/362	228/350	228/344	229/352	226/344
	FI	482.13	549.23	482.50	233.02	212.23	220.02	718.72
b	$\lambda_{ex}/\lambda_{em}$	347/426	346/424	344/421	276/357	280/354	280/354	280/352
	FI	478.63	424.18	522.66	640.70	689.59	669.79	856.74
c	$\lambda_{ex}/\lambda_{em}$	255/431	255/431	255/424	339/421	339/421	337/422	280/674
	FI	230.16	208.37	247.12	147.64	131.28	152.70	112.17
d	$\lambda_{ex}/\lambda_{em}$	–	–	–	280/670	278/674	280/676	–
	FI	–	–	–	75.17	92.86	81.19	–
e	$\lambda_{ex}/\lambda_{em}$	–	–	–	–	413/672	413/672	–
	FI	–	–	–	–	100.21	30.11	–

Table 1 (continued)

D Treatment	Survival rate of lung cancer (Calu6) cell				
	50 mg L	100 mg L	200 mg L	400 mg L	800 mg L
Control	58.20b	60.19b	54.95b	53.30b	48.78c
HPS	71.79a	69.92a	65.89a	65.05a	59.40b
LEP	71.16a	65.16ab	66.40a	67.70a	67.27a

Mean \pm SD (standard deviation) of five measurements. Average in rows marked with different letters differ significantly ($P < 0.05$). Abbreviations: *ABTS*, 2, 2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; *FRAP* ferric-reducing/antioxidant power; *DPPH* 1, 1-diphenyl-2-picrylhydrazyl; *CUPRAC* cupric reducing antioxidant capacity; *Polyph* polyphenols; *Vit C* vitamin C; *Flavon* flavonoids; *CE* catechin equivalent; *GAE* gallic acid equivalent; *DW* dry weight; *TE* Trolox equivalent; *C* Control; *Xanth* xanthophylls; * nm/nm; ** Fluorescence intensity, arbitral units; *HSA* 2 mM $\times 10^{-5}$, pepper extracts in ethanol 100 μ l from 25 mg/ml (0.83 mg/ml); *HSA* human serum albumin; *W* water; *Et* ethanol

investigators were unable to precisely modulate and quantify the spectral energy parameters [20]. The results obtained in this study showed that the treatment of paprika affected the level of bioactive compounds (carotenoids and polyphenols) in sweet bell paprika fruits. HSP bell paprika fruits contained significantly more chlorophylls and carotenes. Total phenolics, tannins and antioxidants capacities determined by four scavenging methods slightly differ in all samples with preference for Control. Our results can be compared only with the same variety of the paprika, because the bell paprika variety also affected the level of antioxidant compounds in fruits [5]. The constituents responsible for the hydrophilic antioxidant activity in paprika [5] were primarily phenolic compounds and ascorbic acid, whereas chlorophylls were the main antioxidant in the lipophilic fraction. The period in which fruits are harvested has been shown to affect the quality parameters, such as phenolic compounds and the ascorbic acid content [21]. Carotenoid content increased in LEP (7.7 %) and HPS (24.2 %) peppers in comparison with the Control sample, especially in HPS (Table 1A). Total carotenoids increased by the red light-emitting diode (LED) light (660 nm) in the flavedo of Satsuma mandarin [22]. Our results showed that to achieve solely a positive effect is complicated, because metabolism of antioxidant properties in paprika depended on variety, light quality or seasonality. The total phenolic contents in raw pungent peppers [3] were in the range previously reported (200–7820 μ g/g) for Mexican type peppers, but lower than in our findings. The obtained results about flavonoids can be compared with Bae et al. [23], where maximum flavonoids in paprika were extracted in ethanol. Soil-less green and red peppers showed maximum vitamin C contents of 52 and 80 mg/100 g FW, with the highest content of total carotenoids in the soil-less red peppers, of 148 mg/100 g FW and total phenolic content from 1.2 to 4.1 mg/100 g FW. Bioactivity was significantly affected by the harvest time but not by the production system assayed [24]. The majority of carotenoids are usually ignored [25], using conventional scavenging assays. A main limitation is that few methods allow for the

successful measurement of antioxidant activity in lipophilic fractions and most of carotenoids are found in lipophilic fraction. Since CUPRAC assay was used in this study, which is active for both hydrophilic and lipophilic antioxidants and perfectly works in acetone solution, therefore carotenoids were included in the total antioxidant capacity (Table 1A). The total antioxidant capacities found by four different scavenging assays were higher in water extract, probably depending on other phenolics and bioactive compounds such as carotenoids, chlorophyll, and total phenols. Our results can be compared with Cervantes-Paz et al. [26], where the profile of pigments (carotenoids and chlorophylls) and antioxidant activity were determined in raw peppers at three intermediate stages of ripening (brown, 50 % red, and 75 % red). Our results were slightly higher because of 100 % red in our samples. Tested peppers showed a more complex/abundant pigment content and higher antioxidant activity than those typically reported for red paprika.

Binding Properties The interaction of polyphenols with HSA quenched the emission characteristics of HSA. The observed emission spectral shifts proved the effective use of fluorescent data for the determination of binding properties. The changes in the fluorescence intensity of treated and Control samples correlated with the changes of polyphenol compounds (Table 1A). 3D-fluorescence spectra and corresponding contour maps of water extracts of red paprika of RControl, RPHPS and RPLEP (Fig. 1A, B, C, and Fig. 2A, B, C, suppl.) and ethanol extracts of RControl, RPHPS and RPLEP (Fig. 1D, E, F and Fig. 2D, E, F suppl.) showed peaks with different fluorescence intensity and maximum location of the peaks (Table 1B). The samples in two extracts have similar peaks **a**, **b** and **c**. But the ethanolic extracts showed additional **b1** peak only in the Control sample and **d** and **f** peaks in all three samples. The fluorescence intensity in these peaks was relatively low (Table 1B, Fig. 1D, E, F, suppl.). The fluorescence intensity of peak **b** in all water samples was about

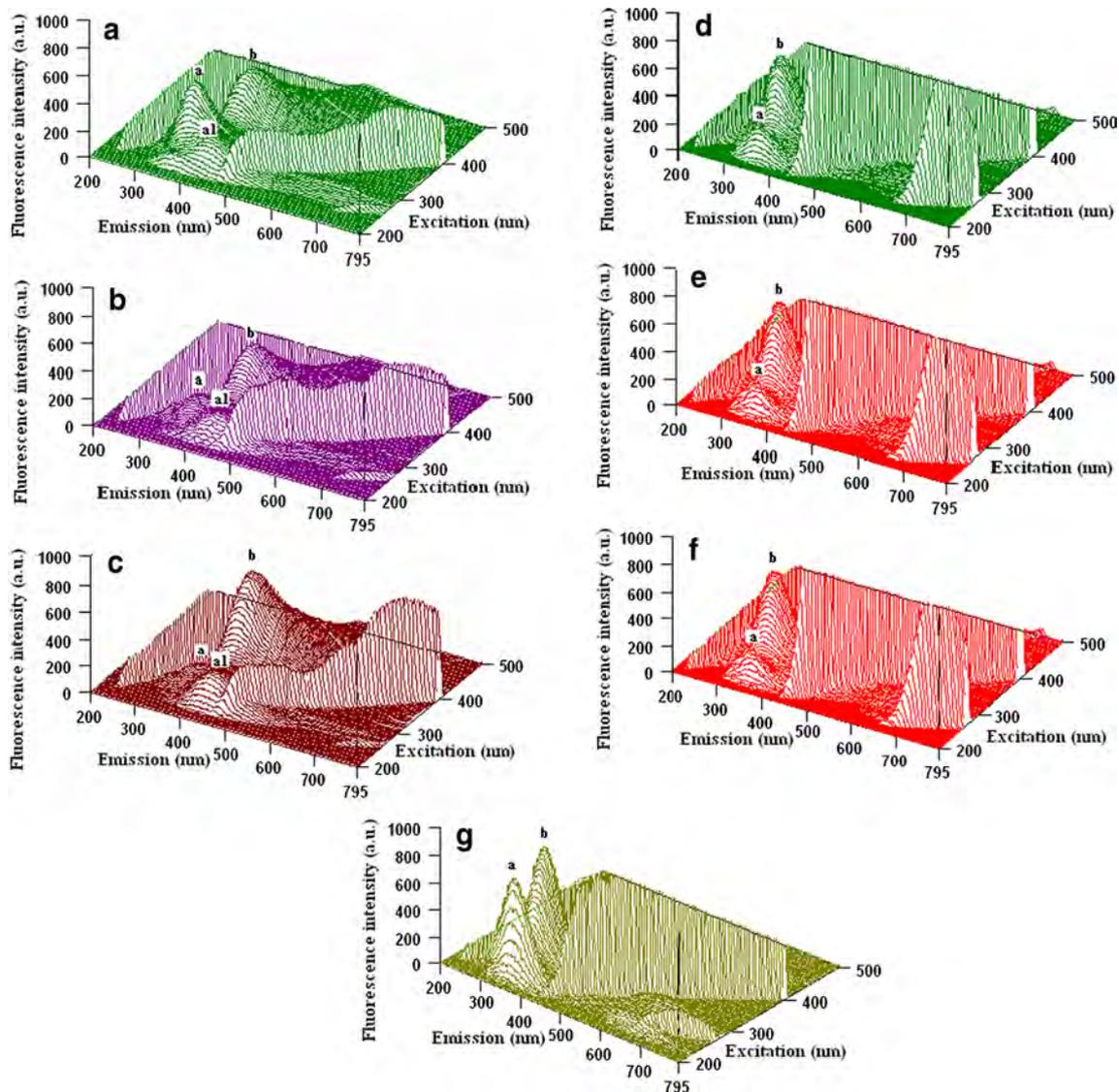


Fig. 1 3D fluorescence spectra of different extracts of pepper samples in interaction with HSA: **a, b, c, d, e, f, g**, RPControlW, RPHPSW, RPLEPW, RPControlEt, RPHPEt, RPLEPEt. HSA. Abbreviations: RP, red paprika; C, Control; HPS, LEP, high pressure sodium and

lighting emitting plasma lamps; W, water extracts; Et, ethanol extracts; HSA, human serum albumin. Excitation wavelength scan: 200–500 nm. Emission wavelength scan: 200–795 nm. In each sample of water and ethanol extracts several peaks are shown (see Table 1C)

three times higher than in ethanol (Table 1B, Figs. 1A–F, suppl.). The interaction of paprika polyphenols with HSA in water (Fig. 1a, b, c and 2a, b, c) and in ethanol (Figs. 1d, e, f and 2d, e, f) showed different binding ability (Table 1C). Peak **a** ($\lambda_{ex}/\lambda_{em} = 228\text{--}229/344\text{--}352$ nm), which is the main showing the decrease in the intensity during binding of polyphenols to HSA, was the smallest for paprika ethanol extracts (Table 1C, Fig. 2d, e, f) in comparison with the water samples (Table 1C, Fig. 2a, b, c). Peak **b** was the lowest in water extracts. The overall binding ability (%) decreased in the following order: RPCEt (46.4)>RPLEPEt (45.6)>RPHPEt (45.0)>RPCW (43.0)>RPHPSW (41.6)>RPLEPW (40.7). Control samples in two extractions were slightly higher in the binding

ability than the treated. Fluorescence measurements showed a difference between the investigated samples, especially in their antioxidant status. The quenching properties of these samples are correlated with their antioxidant properties and the amount of polyphenols and the decrease in fluorescence intensity.

Anti-proliferative Properties The survival rate of lung cancer of paprika fruit with two extractions (ethanol and water) and two types of lamps are shown in Table 2A (Suppl.). For survival rate of lung cancer, there was no significant difference between distilled water and ethanol, using different extracting methods from 50 to 100 mg L⁻¹. The survival rate of lung cancer cells of paprika fruits of two extract methods is shown

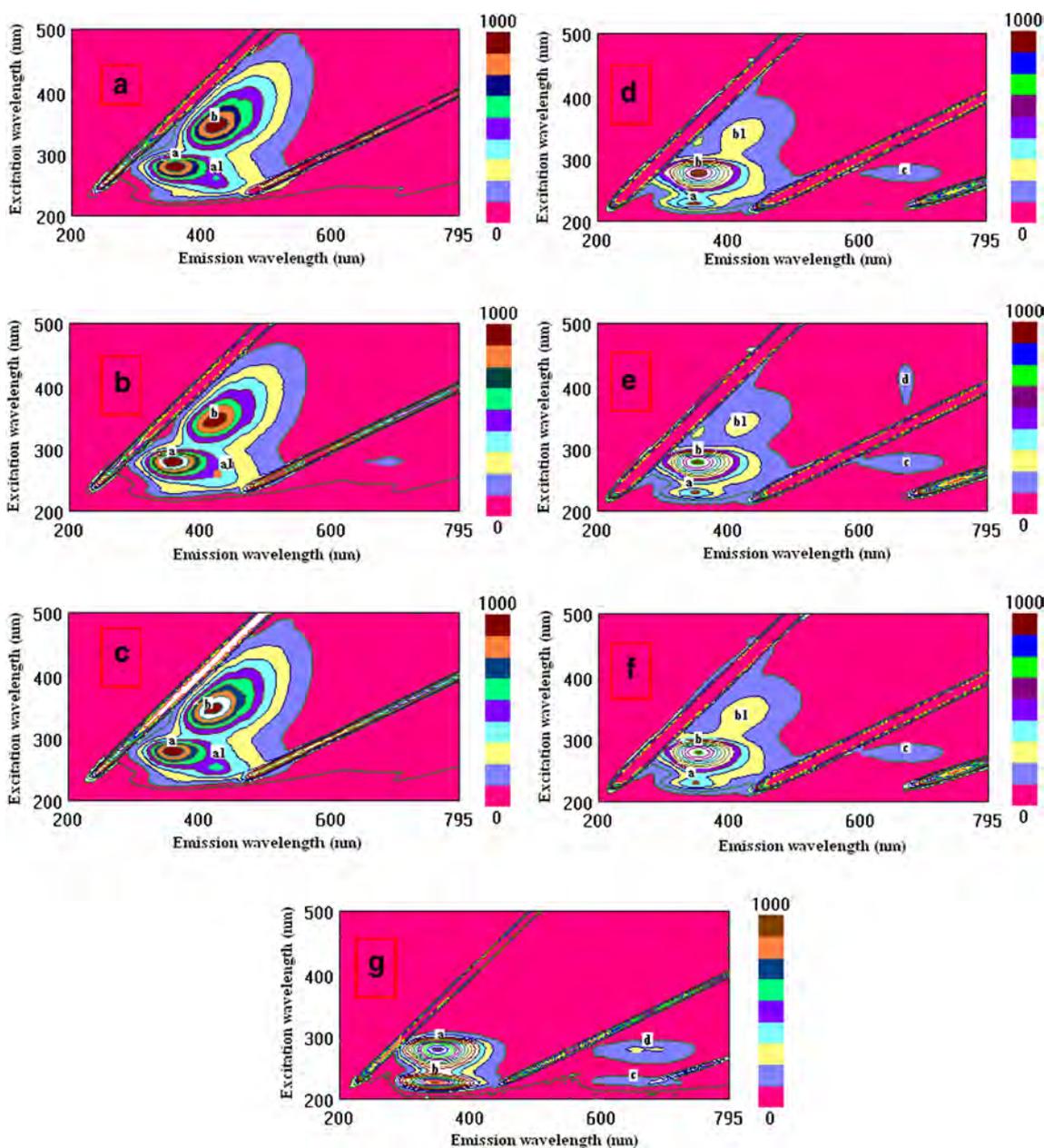


Fig. 2 Corresponding contour maps of three-dimensional fluorescence spectra (Fig. 1) of different extracts of pepper samples with interaction with HSA: **a, b, c, d, e, f, g**, RPControlW, RPHPSW, RPLEPW, RPControlEt, RPHPSEt, RPLEPEt, HSA. Abbreviations: C, Control; HPS, LEP, high pressure sodium and lighting emitting plasma lamps; HSA, human serum albumin; Fluorescence intensity, arbitral units;

Excitation wavelength scan: 200–500 nm. In each sample of water and ethanol extracts several peaks are shown (see Table 1C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article). HSA $2 \text{ mM} \times 10^{-5}$, pepper extracts in ethanol or water $100 \mu\text{l}$ from 25 mg/ml (0.83 mg/ml). The reaction was during 1 h at room temperature

on Table 2B (Suppl.). From 200 mg L^{-1} onwards, survival rate of lung cancer of paprika fruit with ethanol extract was higher than those of distilled water extract. This means that edible paprika fruit was expected to enhance anti-lung cancer effect than using ethanol. The survival rate of lung cancer cells of paprika fruits of two types of lamps is shown on Table 1D. For survival rate of lung cancer of two types of lamps (Table 1D) was that two other treated samples were higher than those of control, indicating that supplementary light during winter

period may have negative effect on anti-lung cancer. Antioxidant and anti-proliferative activities are in direct correlation with bioactive compounds in paprika. This was as well reported by others [27], where the anti-proliferative action of four different colored (red, orange, yellow, and green) methanol extracts of bell peppers (*Capsicum annuum* L.) was tested on different line of cells (HT-29). In our research water and ethanol extracts were tested on CALU-6 cells. Our results were in line of the conclusion that it was found a positive

correlation between total polyphenols, total antioxidant activity, carotenoids and cytotoxicity. Our results also in correspondence with Molnar et al. [28], where carotenoid fractions were extracted from red spice paprika. Paprika extracts showed slightly higher cytotoxic activity against three human tumor cell lines and human promyelocytic leukemic HL-60 cells than against three normal human oral cells, suggesting a tumor-specific cytotoxic activity. Our results were in line with Motohashi et al. [29], where *Capsicum annuum* L. var. *angulosum* Mill. (Solanaceae) was extracted with hexane, acetone, methanol and 70 % methanol. These extracts showed relatively higher cytotoxic activity against two human oral tumor cell lines (HSC-2, HSG) than against normal human gingival fibroblasts (HGF), suggesting antitumor potential of ‘Anastasia Red’ of sweet pepper.

Conclusions

Changes in bioactive compounds after the growth of red paprika under artificial light were determined. Spectroscopic, fluorescence and anti-proliferative measurements were applied. Plant growth and development stage (not quality) of LEP or HSP treatments were greater for paprika growth than control treatment, because paprika plants require light during winter season. LEP treatment is better for plant growth characteristics than other conventional treatments. Organic growing and variety increased the level of antioxidant compounds such as carotenoids, phenolic compounds and vitamin C in sweet bell pepper. The binding of pepper polyphenol extracts to HSA strongly correlated with their antioxidant properties.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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