



Evaluation of ultraviolet irradiation effects on *Aspergillus flavus* and Aflatoxin B1 in maize and peanut using innovative vibrating decontamination equipment

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ABSTRACT

This study investigated the effectiveness of the ultraviolet irradiation of the C region (UV-C) for the decontamination of *Aspergillus flavus* and aflatoxin B1 (AFB1) on artificially inoculated maize and peanut using innovative decontamination equipment that supports vibrations to achieve semi-fluidization of the grain/kernel material and getting equal irradiation of all surfaces of irradiated foods. UV irradiation is a non-thermal alternative to thermal processes commonly used in food processing with a well-known effect on AFB1 degradation. Samples of maize and peanut were exposed to UV-C irradiation with a total dose in the range of 1080–8370 mJ/cm². Analysis by Tracker and ImageJ software confirmed the even distribution of irradiation on all surfaces during the entire duration of exposure. The highest reduction of *A. flavus* count was observed after ten days of incubation and irradiation treatment delivering a dose of 8370 mJ/cm² achieving *A. flavus* count reduction of 4.4 log CFU/g in maize and 3.1 log CFU/g in peanut. Depending on the treatment, AFB1 reduction level in maize ranged from 17 to 43% and in peanut ranged from 14 to 51%. Sensory and physical testing of the peanut samples showed only minimal changes in the evaluated characteristics caused by different levels of the UV-C treatment. Presented results demonstrate a potential for the use of the presented approach as an effective reduction strategy for both *A. flavus* and AFB1 in maize and peanut.

1. Introduction

Aflatoxins (AFs) and the producing fungi *Aspergillus* section *Flavi* are widely known as the most serious and dangerous issue in agricultural products (Perrone, Gallo, & Logrieco, 2014). Produced primarily by the *Aspergillus flavus* and *A. parasiticus*, AFs are a group of about 20 chemically related metabolites sharing structure of difuranocoumarin derivatives, in which a bifuran group is attached at one side of the coumarin nucleus, while a pentanone ring in the case of the AFs-B series, or a six-membered lactone ring in the case of the AFs-G series is attached to the other side (Bennett & Klich, 2003; Nakai et al., 2008). AFs are genotoxic, carcinogenic, and teratogenic for both humans and animals, with aflatoxin B1 (AFB1) occurring at the highest levels in different food

products and being considered as the most potent type (IARC, 2002). Regular consumption of foods contaminated with low levels of AFB1 can result in cancer, immune suppression, stunted growth in children and reduced life expectancy (Shephard, 2008). Food crops are contaminated with AFs both before and after harvesting. Extensive pre-harvest contamination with AFs occurs in maize, peanut and tree nuts (Bennett & Klich, 2003). Post-harvest AFs contamination is caused initially by the infection by aflatoxigenic strains at the pre-harvest stage (Waliyar et al., 2015) and can lead to levels of contamination much higher than those found in the field especially if the phases of drying and storage are poorly managed (Chulze, 2010). This produces a necessity for additional post-harvest prevention strategies as well as detoxification methods to reduce AFs content in contaminated products. Traditionally, the focus

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for AFs risk management is maize and peanut (JECFA, 2017), as these products are the major sources of AFs exposure considering the high occurrence of AFs and high level of consumption (Udovicki et al., 2021; Wu, Stacy, & Kensler, 2013). It is estimated that AFs plays a role in 4.6–28.2% of all global hepatocellular carcinoma cases (Y. Liu & Wu, 2010) and that they cause up to 636,869 DALY (Disability Adjusted Life Year) globally each year (Gibb et al., 2015). Next to these, health effects, it is estimated that the USA maize industry can lose as much as 1.7 billion US dollars annually (Mitchell, Bowers, Hurburgh, & Wu, 2016), while African countries record loss of up to 750 million US dollars annually (Cardwell, Desjardins, Henry, Munkyold, & Robens, 2004) due to AFs contamination.

Ultraviolet (UV) irradiation is a non-thermal alternative to thermal processes commonly used in food processing. As a physical preservation method, UV irradiation has a positive consumer image and is widely used in the food industry for disinfection of air, control of contamination on the surface of plant and packaging materials, treatment of liquid foods, post-processing treatment for ready-to-eat meats, and in post-harvest storage of fruits and vegetables (Begum, Hocking, & Miskelly, 2009; Koutchma, Forney, & Moraru, 2009). Within the UV wavelength spectre, the UV-C (200–280 nm) region has a germicidal effect on most micro-organisms, including fungi, yeast, bacteria, viruses, protozoa, and algae. Advantages of this method are its low cost, it does not leave chemical residues and contribute to minimal loss of quality in terms of flavour, colour, and nutritional value (Diao, Li, et al., 2015). The efficiency of UV-C irradiation on decreasing initial fungal load and inactivation of various genera of post-harvest spoilage fungi in the food industry has been previously reported (Begum et al., 2009). Next to dose, being the most important factor, various other factors have an impact on the effect of UV-C irradiation on microorganisms. This effect can vary depending on species, strain, the density of microorganisms, composition and the characteristics of the food (Guerrero-Beltrán & Barbosa-Cánovas, 2004; Wright, Sumner, Hackney, Pierson, & Zoecklein, 2000). While delivering appropriate germicidal dose in liquid media is described by Lambert-Beer law relating the attenuation of light to the properties of the material through which the light is travelling, the same cannot be applied for UV treatment of solid food, especially grains. As UV light penetrates only up to several millimetres in solid foods, the efficacy of the UV treatment is strongly affected by surface morphology, crevices and shadow areas that may shield microorganisms (Shama, 2007). Due to the weak penetration capacity of UV light, it is necessary to form a thin layer of the treated foods to achieve the appropriate effect (Diao, Li, et al., 2015). The photosensitivity of AFB1 and the efficiency of UV irradiation in AFB1 decontamination have been known for a long time (Andrellos, Beckwith, & Eppley, 1967). However, considering limitations regarding hindered effects due to the low penetration capacity of UV light and food surface morphology the practical application in solid foods is considered as limited. Literature review on UV decontamination of AFs has shown only around half dozens of studies that investigated the potential use of UV irradiation in solid foods in the last two decades. The reported efficiency of decontamination varied, and these studies mainly investigated the effect after direct irradiation of food surface or on food in layers of different thicknesses (Diao, Li, et al., 2015; Pankaj, Shi, & Keener, 2018; M.-H.; Shen & Singh, 2021a). Only recent work by Shen et al. (M.-H. Shen & Singh, 2021b) assessed uniformity of irradiation distribution as a factor for AFs decontamination and confirmed its significance.

Based on these considerations, the present study aimed to investigate the effectiveness of UV-C irradiation for the decontamination of *A. flavus* and AFB1 on artificially inoculated maize and peanut using innovative decontamination equipment that supports vibrations to achieve semi-fluidization of the grain/kernel material getting equal irradiation of all surfaces of irradiated foods. Additionally, the effects of UV-C treatment on the sensory and physical attributes of peanut were examined.

2. Materials and methods

2.1. Samples, chemicals, and materials

Maize grains were collected from the local silo while raw, shelled, and skinned Runner-type peanuts, grown in Argentina, were purchased from one big importer in Belgrade, Republic of Serbia. Methanol (HPLC and LC-MS grade) was from J. T. Baker (Fisher Scientific, New Hampshire, USA); formic acid was from Sigma Aldrich (St. Louis, USA); AFB1 standard was from Trilogy (Washington, USA); potato dextrose agar was from TM Media (Titan Biotech Ltd., Rajasthan, India); peptone was from Torlak (Belgrade, Serbia); Tween 80 synthesis grade was from Sharlau (Barcelona, Spain); KH_2PO_4 , KNO_3 , KCl , MgSO_4 were from Centrohem (Stara Pazova, Serbia); NaCl was from Alkaloid (Skopje, Macedonia); 85% glycerol was from Zorka Pharma (Sabac, Serbia); AflaStar™-Immunoaffinity Columns were from Romer Labs Diagnostic GmbH, (Tulln an der Donau, Austria); Celer Afla B1®Techna ELISA test kits were from Tecna S.r.l. (Trieste, Italy); and 45 μm nylon filters were from Amtast (Lakeland, USA).

2.2. UV-C decontamination equipment and irradiation protocol

Lab-scale UV-C decontamination equipment was specially designed for the treatment of grain/kernel type foods (Fig. 1). The decontamination surface was constructed in a way to support vibrations to achieve semi-fluidization of the grain/kernel material intended for decontamination. This surface has a 140 mm wide round glass plate on which grain material was placed.

Used UV-C equipment had three 15W UV-C stationary lamps, with a dominant wavelength of 254 nm, that can be combined into three irradiation intensity (irradiance) levels, namely 12, 18 and 31 W/m^2 . The distance of the decontamination surface from UV lamps was 142 mm. For the experimental setup, irradiances were combined with irradiation time (15 min, 30 min, and 45 min) in a complete full factorial design achieving total fluence (UV dose) in the range from 1080 to 8370 mJ/cm^2 depending on the factor combination.

2.2.1. Defining optimal vibrational parameters

As particle shapes have an impact on UV treatment, to define optimal vibrational parameters, 30 ± 1 g peanut and maize were half coloured in black and recorded for 1 min to analyse average exposure to light. This amount enabled the spreading of peanuts and maize on one layer occupying $50.55\% \pm 4.75\%$ (peanuts) and $55.03\% \pm 3.39\%$ (maize) of the disk area which was calculated measuring surfaces of 10 samples of peanuts and 10 samples of maize using ImageJ software (Image processing and analysis in Java). Frames were further processed using Tracker (Video analysis and modelling tool built on the Open Source Physics (OSP) Java framework) and ImageJ software. Such dynamic behaviour of rotating grains/kernels confirmed the even distribution of light on all surfaces during the entire duration of exposure. This was confirmed when peanuts and maize were exposed to light in the UV-C chamber showing that when directional light from the sources placed at the same position as UV-C lamps was intersected with the objects (grains), shadows were dividing the grain surface in half (observed also through the bottom of the glass plate). This was checked separately for peanuts and separately for maize. In parallel, observation of the vibrating bed showed that two neighbouring grains were never at the same height (due to their difference in size/mass) so shadowing of the neighbouring grain was very limited. As a rationale, all grains were half coloured in black to assume further exposure to UV-C light. The shape of peanuts was assumed as cylindric (Akcali, Ince, & Guzel, 2006) and at each moment $33.16\% \pm 6.65\%$ of peanut kernels' surfaces is exposed to UV-C light. Maize grains' shapes were considered round with lower sphericity (Karababa & Coşkun, 2007). At each moment, $37.39\% \pm 7.60\%$ of maize grains' surface is exposed to UV-C light.

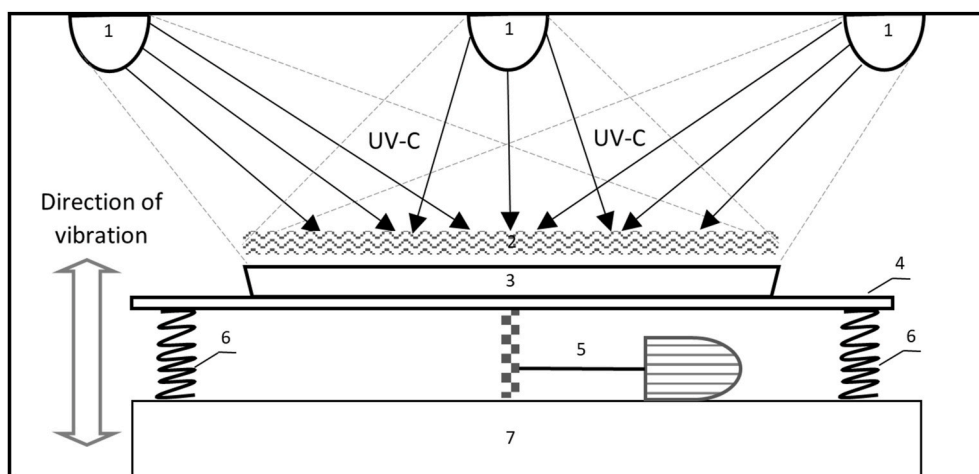


Fig. 1. Simplified scheme of the vibrating device for UV-C treatment.

(Legend: 1 – UV-C lamps; 2 – fluidized bed of treated grain; 3 – Round glass plate; 4 – Vibration plate; 5 – Vibrating mechanism (electric motor with eccentricity); 6 – Springs; 7 – Fixture body).

2.3. *Aspergillus flavus* inoculation, incubation, and determination of fungal number on the UV-C treated maize and peanut

Aflatoxin-producing *A. flavus* strain was provided by the Maize Research Institute “Zemun-Polje”, Belgrade, Serbia. The spore suspension was obtained by growing the fungal strain in shaking cultures in 500 mL Erlenmeyer flasks containing 250 mL of liquid medium (2 g KH_2PO_4 , 2 g KNO_3 , 1 g KCl, 1 g MgSO_4 , 1 g dextrose, trace FeSO_4 , FeCl_3 , MnSO_4 , ZnSO_4 added to 1 L distilled water) inoculated with pieces of potato dextrose agar containing a pure culture of strain MRIZP 4050. After six days of incubation, the flask contents were filtered through two layers of cheesecloth to obtain a spore suspension. Inoculum concentration (1×10^6 spores/mL) was measured with a hemacytometer (Neubauer, Germany).

Autoclaved and a_w -adjusted (0.98) samples of maize and peanut were inoculated with *A. flavus* spore suspension to obtain approximately 1×10^4 to 2×10^4 spores/g and incubated for 3 h at 25 °C to allow attachment to grains/kernels. After incubation, subsets of 10 g of the samples were subjected to the UV-C treatment protocol described in Chapter 2.2. All treatments were done in three replications. After the treatment, the samples were stored in dark at 30 °C to promote the growth of the fungi. Containers containing glycerol/water solutions (1/99, v/v) of the same water activity as the grains were placed in the incubators to maintain an atmosphere with the same equilibrium relative humidity (Velluti, Sanchis, Ramos, Turon, & Marín, 2004). Samples were kept in this regime for five and ten days. After the incubation, fungi were isolated from the maize and peanut by shaking 10 g of the samples with 90 mL of the solution containing 0.85% NaCl, 0.1% peptone and 0.1% Tween 80 for 30 min (Czembor, Stepien, & Waskiewicz, 2015). From this dilution, further serial dilutions were made, and 0.1 mL of inoculum was spread plated in potato dextrose agar. Plates were further incubated at 30 °C for 72 h (due to the rapid growth of the used *A. flavus* isolate). After this period, total colonies were counted.

2.4. AFB1 decontamination and determination by LC-MS/MS

2.4.1. Sample preparation and irradiation

Samples were spiked with an AFB1 working solution to a level of at least 5 and 8 ng/g of AFB1, for maize and peanut, respectively. Lower concentrations were chosen to imitate lesser contaminated samples which are to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs as stated in EU Regulation 1881/2006 (2006b). From the stock standard solution (25 µg/mL), a working solution was prepared by diluting AFB1 in methanol

to a concentration of 1000 ng/mL. As no blank samples in needed amounts were available, spiking was performed using samples with low AFB1 concentration (based on the ELISA analysis by Celer Afla B1®Techna ELISA test kits with LOD of 1 ng/g). Therefore, the final concentration was slightly higher than expected (mean level of 5.6 and 9.0 ng/g for maize and peanut respectively). Still, this was the appropriate range for experimental design. UV-C treatments against AFB1 have been performed with the sample size set at 30 ± 1 g and according to the treatment protocol described in Chapter 2.2. All treatments were done in three replications.

2.4.2. AFB1 extraction

The homogenized samples (25 g) were measured into 250 mL Erlenmeyer flasks and 100 mL of extraction solution (methanol/water 60/40, v/v) was added. After 1 h of mixing on a gyratory shaker, samples were filtered in the sample jars through qualitative filter paper using a funnel. The extracts were diluted with PBS (pH 7.4) until the content of methanol was not higher than 20% (v/v), e.g., 4 mL of the extract and 8 mL of the PBS. The diluted extracts were applied and allowed to pass Immunoaffinity Columns. After the diluted extracts completely passed through, the columns were washed with 2x10 mL of PBS. The first portion of the wash solution was used to rinse the container. After the washing step, AFB1 was eluted from columns with 2 mL of HPLC grade methanol (applied to the columns in several small portions). After filtration through a 45 µm nylon filters eluates were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.4.3. LC-MS/MS conditions

For chromatographic separation, an Agilent 1260 (Agilent Technologies, USA) HPLC system with a binary pump was used. It was equipped with a reversed-phase C18 analytical column of 50×4.6 mm; 1.8 µm particle size (Zorbax Eclipse XDB-C18, Agilent Technologies, USA). For elution, a mobile phase consisting of eluent A (0.1% formic acid in water) and eluent B (0.1% formic acid in methanol) in gradient mode, was used. The initial mobile phase composition was 0% B and remained constant for 2 min, increasing to 50% B in 5 min, and then a further increase to 98% B in 14 min and held for 2 min. The flow was set at 0.4 mL/min, the column temperature was set at 35 °C, and the injection volume of 10 µL was used. Stop time was set at 16 min, with a post time of 3.5 min. The retention time for AFB1 was 9.33 min. For the mass spectrometric analysis, an Agilent 6460 Triple-Quad LC/MS system was used, and the Agilent MassHunter B.06.00 software was used for data acquisition and quantification. The analysis was performed in the

positive (ESI⁺) mode. For increasing sensitivity of mass spectrometer multiple reaction monitoring mode (MRM) was transferred into time segmented method, dynamic multiple reaction monitoring mode (dMRM). Instrument parameters were as follows: capillary voltage 3.5 kV, gas temperature 200 °C, drying gas flow 8 mL/min, sheath gas temperature 250 °C sheath gas flow 11 mL/min, nebuliser pressure 40 psi, fragmentation energy 100 V, collision energy 35 V, transition for quantitative ion was 313 → 269, and for qualitative 313 → 241.

2.4.4. Method validation

Recovery values, for both maize and peanut matrixes, were determined by spiking blank samples at three concentration levels (1.0, 2.0 and 10.0 ng/g) and in six replicates for each level. Intraday precision (expressed as relative standard deviation, %RSDr) was calculated for all three spiking levels, whilst inter-day precision was calculated at the level of 10.0 ng/g (expressed as relative standard deviation, %RSDr). Method limits of detection (LOD) for both matrixes were calculated by the calculator "Calculate signal-to-noise" (Quantitative Mass Hunter Software B.06.00 program) using a signal-to-noise (S/N) ratio greater than three for the lowest matrix-matched calibration level. Method limits of quantification (LOQ) for both matrixes were set at the lowest matrix-matched calibration levels. Linearity for the quantification of AFB1 in maize and peanut was performed in the mobile phase and sample matrixes at seven concentration levels (0.5, 1.0, 2.5, 5.0, 10.0, 20.0 and 40.0 ng/mL).

Recovery, precision, LOD, LOQ, and linearity data for LC/MS-MS determination of AFB1 are shown in Table 1.

All validation parameters were in line with EU regulation regarding analytical procedures for the determination of mycotoxins in foodstuffs (European Commission, 2006a).

2.5. Sensory and physical testing

2.5.1. Sample preparation

The peanut samples were subjected to the UV-C treatments at the levels of 31 W/m² and 18 W/m² of UV-C irradiance during 15, 30 or 45 min with the sample size set at 30 ± 1 g. The treated samples were packed in plastic pouches under 100% N₂ and stored in dark at ambient temperature (≈22 °C) for six months. Control samples were prepared without UV-C treatment. Two replicated batches of the peanut samples were prepared to perform two replicated measurements. Separate sample units were prepared for sensory and physical testing. The peanuts were sampled after 0 (within five days after packing), 3, and 6 months of storage.

2.5.2. Descriptive sensory analysis

Eight sensory attributes (Table 2) were selected to examine the changes in the sensory profile of the peanut samples. The sensory panel was comprised of eight assessors selected from the University staff.

Table 1
Validation data for LC/MS-MS method.

Product	AFB1 level (ng/g)	Recovery (%)	% RSDr	% RSDr*	LOD/LOQ	Correlation coefficients (R ²)
Maize	1.0	102.4	15.2	5.0		R ² = 0.998
	2.0	94.8	6.4		0.28/0.5	
	10.0	100.0	2.6			
Peanut	1.0	105.7	7.4	3.1		R ² = 0.997
	2.0	96.8	4.5		0.25/0.5	
	10.0	100.1	3.8			

%RSDr – Relative standard deviation (intraday precision); %RSDR – Relative standard deviation (inter-day precision); LOD - Limit of Detection, LOQ - Limit of Quantification.

Table 2

Sensory attributes and reference standards used in descriptive analysis.

Attribute ^a	Definition with terminal anchors	Reference standards	Intensity
Appearance			
Brown colour	The colour associated with dark roasted peanuts ('white' to 'brown').	White typing paper Raw peanuts (see 'raw/bean') Dark roasted peanuts (see 'burnt')	0 3.0 8.0
Flavour/Aromatics			
Raw/bean	The flavour associated with raw peanuts.	Raw peanuts Runner-type (used for the samples preparation)	5.5
Roasted peanutty	The flavour associated with medium roasted peanuts.	Roasted unsalted peanuts ('Jumbo', Fun & Fit, Serbia)	7.5
Burnt	The flavour associated with dark roasted peanuts.	Dark roasted peanuts ('Lucky', Z.T.R. Srecko, Serbia)	5.5
Cardboard	The flavour associated with oxidized fats and reminiscent of cardboard	Wet cardboard	8.5
Painty	The flavour associated with oil-based paint.	Oil-based paint solution in peanut oil: 0.09 g of paint (Helios, Serbia) in 100 g of oil (Olitalia, Italy)	7.5
Rancid	The flavour associated with oxidized fats and oils.	Peanut oil (Olitalia, Italy) heated at 110 °C for 5 h	2.5
Texture			
Crunchiness	The force needed and amount of sound generated from chewing a sample with the molar teeth ('none' to 'crunchy').	Roasted unsalted peanuts ('Jumbo', Fun & Fit, Serbia)	7.0

^a Selection of the attributes and reference standards was done with the help of published literature (Abegaz, Kerr, & Koehler, 2006; Plemmons & Resurreccion, 1998).

Panel training and attribute selection were done according to the protocol described for classical descriptive analysis (Heymann, King, & Hopfer, 2014). Line 15 cm structured scales, anchored by the reference standards (Table 2), were used for the attributes intensity measurements.

2.5.3. Colour measurement

RGB colour space was examined using a Lutron RGB-1002 colour analyser (Lutron Electronic). The peanut samples were homogenized in a ceramic mortar with ceramic pestle and transferred into plastic Petri dishes until forming a flat layer of ca. 5 mm in height. One replication was represented by the arithmetic mean of eight different measurements over the surface of the homogenized and flattened peanut sample. Two replications were performed in total. The obtained RGB colour space data were not transferred into more common CIELAB coordinates since the results showed no statistically significant differences neither in one colour dimension among the examined samples.

2.5.4. Texture analysis

Mechanical properties of the peanut kernel halves were analysed using CT3™ Texture Analyzer (AMETEC, Brookfield). Hardness and cohesiveness, as parameters of the texture profile analysis mode that performs two compression cycles on the sample, were measured using an acrylic cylindrical probe with a flat base of 50.8 mm in diameter. The test speed was 0.4 mm/s, maximum load 1000 g, with 8% deformation. Hardness was expressed as the peak load of the first compression cycle (Hardness 1). Cohesiveness was expressed as the ratio between the area

on the chart under the compression stroke of the second cycle and the area under the compression stroke of the first cycle. A cutting-share test (a single compression of the sample) was performed using a clear acrylic knife-edge probe 60 mm wide. The test speed was 1.0 mm/s, with a maximum load of 1000 g. The parameters recorded were peak load (the maximum measured load during the test) and deformation at peak (the distance to which the sample was compressed when the peak load occurred).

To prevent undesirable kernel breaking during testing, the bases of kernel halves were slightly flattened by sanding off the base edges. One replication was represented by the arithmetic mean of eight individual measurements on the peanut samples. Two replications were performed in total.

2.6. Statistical analysis

Basic descriptive statistical processing was performed for the model analysis defining optional vibrational parameters and assessing equal irradiation. Fungal count and AFB1 concentration data were subjected to different ANOVA models to explore the influence of the following experimental factors: UV-C intensity and UV-C treatment time. Also, different ANOVA models were applied to sensory and physical data to test the influence of the factors: UV-C intensity, UV-C treatment time, and storage period. All models set for sensory data included 'assessors' and 'replications' as random factors. Tukey's HSD posthoc test was used for multiple comparisons among sample means in all cases. The software used was SPSS Statistics 17.0.

3. Results and discussion

3.1. Effect of UV-C treatment on the fungal growth

The growth of *A. flavus* on maize and peanut samples is shown in Table 3. Two-way ANOVA showed that both investigated experimental factors (UV-C intensity and UV-C treatment time) significantly influenced ($p < 0.05$) *A. flavus* count in both maize and peanut samples and for both incubation periods. Greater values for the irradiance or treatment time gave rise to a greater reduction of *A. flavus*.

The maize samples treated with 18 or 31 W/m² of irradiance during

Table 3

Enumeration data for *A. flavus* artificially inoculated on maize and peanut treated with UV-C light at different irradiances (12, 18, or 31 W/m²) and different exposure time (15, 30, or 45 min), after 5 days (A) and 10 days (B) of incubation.

UV-C irradiance/ treatment time combination	Fungal count (log CFU/g)			
	Maize		Peanut	
	5 days of incubation	10 days of incubation	5 days of incubation	10 days of incubation
31 W/m ² /45min	6.44 ± 0.05 ^a	5.79 ± 0.13 ^a	7.03 ± 0.25 ^a	6.93 ± 0.14 ^a
18 W/m ² /45min	7.02 ± 0.34 ^a	8.01 ± 0.03 ^b	7.06 ± 0.11 ^a	7.37 ± 0.14 ^a
12 W/m ² /45min	8.00 ± 0.19 ^b	8.41 ± 0.07 ^b	7.79 ± 0.35 ^a	7.92 ± 0.69 ^{a, b}
31 W/m ² /30min	7.02 ± 0.04 ^a	7.80 ± 0.27 ^b	7.16 ± 0.08 ^a	7.39 ± 0.16 ^a
18 W/m ² /30min	7.57 ± 0.39 ^b	8.37 ± 0.07 ^b	7.27 ± 0.09 ^a	8.54 ± 0.26 ^b
12 W/m ² /30min	8.40 ± 0.08 ^c	8.59 ± 0.36 ^b	9.55 ± 0.06 ^c	9.40 ± 0.35 ^c
31 W/m ² /15min	8.15 ± 0.08 ^{b, c}	9.19 ± 0.33 ^c	8.69 ± 0.72 ^b	8.01 ± 0.49 ^b
18 W/m ² /15min	8.39 ± 0.27 ^c	9.49 ± 0.46 ^c	8.37 ± 0.11 ^b	9.04 ± 0.54 ^c
12 W/m ² /15min	8.45 ± 0.06 ^c	10.14 ± 0.07 ^{c, d}	10.02 ± 0.16 ^c	10.05 ± 0.08 ^c
Control	8.92 ± 0.40 ^c	10.21 ± 0.12 ^d	9.26 ± 0.07 ^{b, c}	10.06 ± 0.08 ^c

Presented as mean log CFU/g ± SD; The mean values under the same column denoted with the same letter are not statistically different ($\alpha = 0.05$).

both 30 and 45 min (UV-C dose 3240–8370 mJ/cm²) after five days of incubation showed significant ($p < 0.05$) reduction of *A. flavus* count (1.3–2.5 log CFU/g). Although the drop of *A. flavus* count in the 45 min - 12 W/m² sample was also statically significant, the obtained reduction was below 1 log CFU/g. Only the most intensive treatment (45 min - 31 W/m², 8370 mJ/cm²) achieved *A. flavus* reduction greater than 2 log CFU/g. This treatment level resulted in *A. flavus* reduction greater than even 4 log CFU/g after ten days of incubation upon the treatment. Considering the rest of the maize samples, after ten days of incubation, again greater antifungal effect against *A. flavus* was achieved with the longer exposure time, 30 or 45 min (1.6–2.4 log CFU/g of reduction), as compared to 15 min of the UV-C treatments, regardless the irradiance level applied.

The UV-C treatments applied to the peanut samples showed similar effects toward *A. flavus* reduction as it was observed in the maize samples. After five days of incubation, longer exposure time (30 and 45 min) with higher levels of irradiance (18 and 31 W/m²; UV-C dose 3240–8370 mJ/cm²) resulted in 2.0–2.2 log CFU/g of *A. flavus* reduction in peanut samples. Again, the effect of the 45 min - 12 W/m² treatment showed statistical significance compared to the control ($p < 0.05$) with the reduction level of 1.5 log CFU/g, but this time it was not significantly different from the effects of the more intensive treatments ($p > 0.05$). The absence of significant difference between several treatment levels indicates that the use of the higher intensities and longer exposure times may not be necessary to obtain a certain *A. flavus* reduction effect. This will foremost have an impact on the lower power consumption of UV-C treatments and the possible effects of UV-C on the production of free radicals and the oxidation of unsaturated fatty acids (X.-Z. Shen et al., 2014). Very similar results regarding *A. flavus* reduction caused by the UV-C treatments, with a slightly higher reduction levels (1.5–3.1 log CFU/g), were obtained in peanut samples after ten days of incubation upon the treatment.

Next to the lethal effect of UV irradiation, which limits the size of the fungal population and its dispersion, exposures to sublethal doses of UV radiation can reduce conidial germination speed and virulence (Braga, Rangel, Fernandes, Flint, & Roberts, 2015). UV irradiation is known to cause several different lesions in DNA (Shama, 2007). Pyrimidine bases, especially thymine, are particularly sensitive to UV, inducing the formation of covalently linked dimers (Bolton, 2001). These thymine dimers inhibit the correct replication of DNA during the cell's reproduction. When DNA polymerase encounters a lesion, such as a pyrimidine dimer, the sliding clamp presses the holoenzyme down to the DNA molecule so that DNA polymerase continues synthesis opposite to the lesion, while 3' → 5' exonuclease cannot remove a mismatching nucleotide which results in a frameshift mutation (Grebneva, 2014). The frequency of these UV induced dimers was directly related to the delay of spore germination (Nascimento, Da Silva, Dos Reis Marques, Roberts, & Braga, 2010). In addition to DNA damage UV irradiation damage proteins and lipids (Trautinger, Kindas-Mügge, Knobler, & Hönigsmann, 1996).

Apart from a reduced number of viable cells, another basic presumption of the effective fungal decontamination method implies an absence or at least a decrease of mycotoxins production during storage. A study by Basaran et al. (2009) on the *A. parasiticus* in hazelnuts indicates that reduced fungi growth will lead to a lower AFs production, while Hamed et al. (2013) reported a change in mycotoxin production pattern of irradiated fungi (increase for *A. parasiticus* and decrease for *A. flavus*).

Literature research has shown a limited number of studies on the efficiency of UV-C irradiation on *Aspergillus* spp. Hameed, Ayesh, Razik, and Mawla (2013) reported a 22% (less than 1 log reduction) survival rate of *A. flavus* and *A. parasiticus* conidia in Petri dishes after 6 h of UV-C exposure delivering 2,160 mJ/cm². Begum et al. (2009) reported 1, 3 and 4 log reduction of *A. flavus* conidia in the aqueous medium after 1, 2 and 3 min of UV-C exposure delivering a dose of 4644 J/m²/min (464.4 mJ/cm²/min), respectively. In the same study, after UV-C exposure of

A. flavus on the surface of the agar plates, the initial conidial number was reduced to 19% of the initial load after 15 s with no viable count after 1 min of UV-C exposure (Begum et al., 2009). Green, Scarpino, Jensen, Jensen, and Gibbs (2004) reported that the UV germicidal irradiation dose necessary to inactivate 90% of the *A. flavus* and *A. fumigatus* was 35 and 54 mJ/cm², respectively.

Compared to the in vitro studies, studies on the UV-C effect on *Aspergillus* spp. on contaminated products have shown that higher applied dosages were needed to obtain a similar effect. Hidaka et al. (2006) reported a 90% reduction (1 log) of the natural load of *Aspergillus* spp. on wheat with UV-C dose per batch of 1222 mJ/cm² (irradiance of 97 W/m² for real irradiation time of 126 s-based on conveyor belt system). Jubeen, Bhatti, Khan, Zahoor-Ul, and Shahid (2012) reported <1 to 1.2, 1.3 to 3.2 and 2 to 4.2 log reduction of *A. flavus* fungal count in walnut, and at two moisture levels (16 ± 3% and 10 ± 3%) following 15, 30 and 45 min exposure to UV-C irradiation, respectively (reported as UV radiations at 108 J/m² for 0, 15, 30 and 45 min). *A. flavus* in inoculated almond and pistachio have shown a similar level of sensitivity to UV-C, while *A. flavus* in inoculated peanut, similar to this study result, have shown higher resistance to UV-C irradiation with a maximum of reduction of 1.3 and 3.5 log at two moisture levels. Garg, Aggarwal, Javed, and Rakesh (2013) reported approximately 4 log reduction of *A. flavus* in inoculated peanut after UV-C treatment in the duration of 6 h delivering a total UV-C dose of 324 kJ/m² (32400 mJ/cm²), with no viable count of *A. flavus* after 12 h and total delivered UV-C dose of 648 kJ/m² (64800 mJ/cm²).

3.2. Effect of UV-C treatment on AFB1 content

The UV-C irradiation effect on AFB1 in maize and peanut are presented in Table 4 and Table 5. Two-way ANOVA showed that both investigated experimental factors (UV-C intensity and UV-C treatment time) significantly influenced ($p < 0.05$) AFB1 content in both maize and peanut samples.

The ranges of AFB1 decontamination levels were 17–43% and 14–51% in maize and peanut respectively. The highest levels of AFB1 reduction (about 43% and 51% in maize and peanut respectively) were obtained with the most severe UV-C treatment applied (31 W/m² - 45 min, 8370 mJ/cm²).

Similar to observed reduction rates of *A. flavus* count, in some cases reduction rates of AFB1 content were very close for treatments with lower intensities and/or exposure times compared to most intensive ones. This confirms that in some cases it is not necessary to use higher intensities and longer exposure times to obtain a certain reduction effect.

Like with literature data on the effect of UV-C on fungi, there was limited information on the UV-C effect on AFB1, especially in solid foods. Jubeen et al. (2012) reported minimum and maximum AFB1 reduction rate of 32.7–87.8%, 50.9–96.5%, 40.8–95.3% and 27.5–96.5% after 15, 30 and 45 min UV-C treatment (reported as UV radiation at 108 J/m² for 0, 15, 30 and 45 min) in walnut, almond, pistachio and peanut, respectively and at two moisture levels (16 ± 3% and 10 ± 3%). Garg et al. (2013) reported a 60% of reduction of total

Table 4

Effect of UV-C treatment on AFB1 levels in maize.

UV-C irradiance	UV-C treatment time		
	15 min	30 min	45 min
31 W/m ²	4.50 ± 0.35 ^{a, b} /20.7	3.69 ± 0.09 ^{a, b} /34.9	3.23 ± 0.37 ^{a, b} /43.2
18 W/m ²	4.14 ± 0.41 ^{a, b} /27.1	3.83 ± 0.79 ^{a, b} /32.6	3.25 ± 0.02 ^{a, b} /42.8
12 W/m ²	4.71 ± 0.37 ^b /17.1	4.31 ± 0.75 ^{a, b} /24.1	3.93 ± 0.18 ^{a, b} /30.7

Presented as mean concentration (ng/g)±SD/% reduction.

^a Significantly different ($\alpha = 0.05$) from AFB1 concentration in the control maize sample (5.68 ± 0.81 ng/g); The mean AFB1-values within the table denoted with the same letter are not statistically different ($\alpha = 0.05$).

Table 5

Effect of UV-C treatment on AFB1 levels in peanut.

UV-C irradiance	UV-C treatment time		
	15 min	30 min	45 min
31 W/m ²	6.28 ± 0.50 ^{a, b} / 30.5	5.17 ± 0.52 ^{a, b} / 42.9	4.45 ± 0.44 ^{a, b} / 50.8
18 W/m ²	6.03 ± 1.47 ^{a, b} / 33.4	6.79 ± 0.24 ^{a, b} / 24.9	5.29 ± 0.58 ^{a, b} / 41.4
12 W/m ²	7.79 ± 0.37 ^{b, c} / 13.8	7.59 ± 0.19 ^{b, c} / 16.1	5.32 ± 0.93 ^{a, b} / 41.1

Presented as mean concentration (ng/g)±SD/% reduction.

^a Significantly different ($\alpha = 0.05$) from AFB1 concentration in the control peanut sample (9.04 ± 0.89 ng/g); The mean AFB1-values within the table denoted with the same letter are not statistically different ($\alpha = 0.05$).

AFs in peanut (in 3 cm thick layer) after UV-C treatment in the duration of 2 h delivering a total UV-C dose of 108 kJ/m² (10800 mJ/cm²), which is to some extent in line with the result obtained in this study. Results cannot be fully comparable, as this study evaluated the effect of UV-C on total AFs content using ELISA test kits for quantification. After UV-C treatment in the duration of 6 h delivering a total UV-C dose of 324 kJ/m² (32400 mJ/cm²), AFs level was reduced by 95%, with a total reduction of 99% after 12 h treatment and total delivered UV-C dose of 648 kJ/m² (64800 mJ/cm²). Two later doses were significantly higher than the doses used in this study. These variations in achieved AFs reduction are to some extent expected, as the use of UV-C irradiation, regarding AFs decontamination, can be considered as the novel method still in development. Other prospective novel physical methods have also shown similar variations in achieved AFs reduction (Sipos et al., 2021). For example, after treatment with cold plasma, reduction of AFs was in the range of 62–82% in maize (Shi, Ileleji, Strohshine, Keener, & Jensen, 2017), 23–28% in peanut (Iqdiem et al., 2020) and 21–50% in hazelnut (Basaran, Basaran-Akgul, & Oksuz, 2008; Sen, Onal-Ulusoy, & Mutlu, 2019).

Presented results indicate a potential for use of UV-C irradiation to decontaminate AFB1. However, under the conditions reported here use of UV-C irradiation as a single treatment to reduce AFB1 levels in product to the levels which are appropriate for direct consumption (European Commission, 2006b), could be limited to the products contaminated at lower levels. Using UV-C irradiation as follow up method of AFB1 decontamination after well-established mechanical methods (washing, sorting, cleaning, milling) could expand the use even on highly contaminated products, particularly if such treatment is intended for raw commodities followed by a thermal treatment.

A most important assumption of the successful decontamination treatment is that newly form residues are less toxic than the parent compound. UV treatment has been proved to degrade AFs to less toxic compounds (M.-H. Shen & Singh, 2021a), as UV irradiation destroys the C8-9 double bond in the terminal furan ring or opens the lactone ring of AFB1, which are essential for its toxic and carcinogenic activity (Diao, Li, et al., 2015). Stanley, Patras, Pendyala, Vergne, and Bansode (2020) proposed hydration and demethylation as the main degradation pathways after UV-A irradiation of AFB1 in water, with two degradation products (P1 and P2) observed. P1 was the result of hydration on the double bond of terminal furan ring of AFB1, while P2 was the result of hydration on furan ring and demethylation on the side chain of benzene. Diao et al. (2015b) reported reduced mutagenicity and toxicity of AFB1 after irradiation of peanut oil, based on Ames test and cytotoxicity of HepG2 cells test. A study on the toxicity of photodegradation products of AFB1 in water (Pw) and peanut oil (PO) on HepG2 cells have shown that cytotoxicity of Pw and PO decreased about 40 and 100%, respectively (R. Liu et al., 2012). Liu et al. (2011) reported complete loss of the mutagenic activity for the residual AFB1 in peanut oil after UV irradiation.

3.3. Sensory and physical testing

Sensory and physical testing of the peanut samples showed only minimal changes (almost none) in the evaluated characteristics caused by different levels of the UV-C treatment. The results of the descriptive sensory analysis are shown in Table 6. After six months of storage in N₂, neither one peanut sample showed perceptibly significant cardboard, painty and rancid flavour notes which indicate oxidative changes that can occur in the first place in oils due to exposure to UV light. It is known that UV irradiation initiates the formation of free radicals, such as lipid radicals, and catalyses other oxidation processes (Kolakowska, 2003) that can make oils and fats rancid (Bekbölet, 1990), but if applied at moderate levels nutritional and sensory qualities of foods can be preserved (Diao, Li, et al., 2015). The scores obtained for the three off-flavour attributes were close to zero in all experimental samples. Although statistically significant differences were found among the cardboard flavour mean scores, their absolute values ranged between 0.1 and 0.6 on a 15 cm line scale. The roasted-peanut flavour was significantly influenced ($p < 0.05$) only by the UV-C treatment time, but the maximum score-values found in the samples without storage (1.2–1.7) were far below the reference value set for the truly roasted peanuts (the reference standard) indicating a low level of the roasted flavour development. The applied UV-C treatments and period of storage did not influence the colour of peanuts. Both visual (surface observation) and instrumental (smashed and homogenized mass) colour measurements showed no statistically significant differences within the data obtained (RGB data are not shown).

Textural characteristics also appeared not to be influenced by the UV-C treatment. Only sensory crunchiness and physical hardness showed a certain level of discrimination among the samples (Fig. 2 and Table 6). Multifactor ANOVA showed that storage time was the only factor ($p < 0.05$) that influenced the changes in crunchiness. The level of crunchiness for all 6-months stored samples, including untreated Control, was significantly higher ($p < 0.05$) compared to all the rest, indicating that this change was not dependant on the UV-C treatment. Changes in hardness were influenced by the 'UV-time' and 'Storage' factors ($p < 0.05$), but according to Tukey's HSD test the samples were grouped into four homogenous subsets with heavy overlapping among each other. There was an increase in hardness after six months of

storage, but it seems that no clear conclusion can be drawn related to the influence of the UV-C treatment. The other texture parameters measured did not significantly change under the influence of the 'UV-C' and 'Storage' factors. The ranges of mean values for cohesiveness, peak load, and deformation at the peak were as follows (respectively): 0.33 ± 0.02 – 1.26 ± 0.93 ; 20.40 ± 1.58 – 27.13 ± 0.71 N; 0.6 ± 0.1 – 1.5 ± 0.8 mm.

4. Conclusion

Presented results and reported reductions of *A. flavus*, which reached over 3 log CFU/g in some cases, indicate a potential for use of UV-C irradiation as an effective post-harvest prevention method for reducing both *A. flavus* growth and possible AFB1 production. UV-C irradiation as a single treatment to reduce AFB1 levels could be limited to products contaminated at lower levels while combining UV-C irradiation with other physical methods of AFB1 decontamination/reduction could expand use even for highly contaminated products. Irradiation doses applied within this research did not influence the sensory and physical attributes examined and did not cause the appearance of rancid off-flavour notes during six months of storage under N₂-atmosphere. Further research is needed to evaluate the effect of higher UV-C doses on *A. flavus* and AFB1, mycotoxin production patterns of irradiated fungi on food, as well as the effect of such treatment on other products prone to AFB1 contamination and the sensory and nutritional properties of such treated products. Also, further efforts should be applied to the development of commercial-scale applications of presented lab-scale equipment.

CRediT authorship contribution statement

Bozidar Udovicki: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Slavica Stankovic:** Resources, Writing – review & editing. **Nikola Tomic:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing, Visualization. **Ilija Djekic:** Validation, Formal analysis, Resources, Writing – review & editing, Visualization. **Nada Smigic:** Formal analysis, Investigation, Writing – review & editing. **Bojana Spirovic Trifunovic:** Investigation, Writing – review & editing. **Dragan**

Table 6

The results of descriptive sensory analysis applied to the UV-C treated peanut samples.

Samples ^a	Appearance ^b	Flavour/Aromatics ^b						Texture ^b
	Brown colour	Raw/beany	Roasted peanutty	Burnt	Cardboard	Painty	Rancid	Crunchiness
31 W, 45 min; 0M	3.5 ± 0.5	4.2 ± 1.1	1.7 ± 0.9 ^b	0.3 ± 0.4	0.6 ± 0.7 ^b	0.2 ± 0.3	0.2 ± 0.4	2.4 ± 0.9 ^a
31 W, 45 min; 3M	3.2 ± 0.4	5.0 ± 1.1	1.0 ± 0.8 ^{a,b}	0.2 ± 0.2	0.4 ± 0.4 ^{a,b}	0.1 ± 0.2	0.3 ± 0.4	3.8 ± 1.0 ^b
31 W, 45 min; 6M	3.6 ± 0.8	4.3 ± 1.5	1.2 ± 0.6 ^b	0.1 ± 0.2	0.4 ± 0.4 ^{a,b}	0.1 ± 0.3	0.2 ± 0.4	6.0 ± 1.1 ^c
31 W, 30 min; 0M	3.4 ± 0.5	5.0 ± 1.2	1.2 ± 0.7 ^b	0.2 ± 0.3	0.2 ± 0.3 ^{a,b}	0.1 ± 0.1	0.1 ± 0.2	2.7 ± 0.9 ^{a,b}
31 W, 30 min; 3M	3.1 ± 0.3	5.5 ± 0.9	1.0 ± 1.0 ^{a,b}	0.3 ± 0.5	0.3 ± 0.4 ^{a,b}	0.1 ± 0.3	0.2 ± 0.3	3.0 ± 0.9 ^{a,b}
31 W, 30 min; 6M	3.2 ± 0.6	4.6 ± 1.1	0.5 ± 0.5 ^a	0.2 ± 0.3	0.4 ± 0.4 ^{a,b}	0.1 ± 0.2	0.1 ± 0.2	5.7 ± 1.3 ^c
31 W, 15 min; 0M	3.4 ± 0.7	4.9 ± 0.9	0.7 ± 0.7 ^{a,b}	0.2 ± 0.3	0.1 ± 0.2 ^a	0.0 ± 0.1	0.1 ± 0.2	3.3 ± 1.1 ^{a,b}
31 W, 15 min; 3M	3.2 ± 0.5	5.3 ± 0.7	0.8 ± 0.9 ^{a,b}	0.3 ± 0.4	0.3 ± 0.4 ^{a,b}	0.1 ± 0.3	0.3 ± 0.5	3.6 ± 1.0 ^{a,b}
31 W, 15 min; 6M	3.1 ± 0.4	4.8 ± 1.2	0.8 ± 0.7 ^{a,b}	0.1 ± 0.1	0.5 ± 0.5 ^b	0.2 ± 0.3	0.2 ± 0.3	6.2 ± 1.1 ^c
18 W, 45 min; 0M	3.3 ± 0.4	5.1 ± 1.2	1.3 ± 0.7 ^b	0.3 ± 0.3	0.2 ± 0.3 ^{a,b}	0.1 ± 0.1	0.1 ± 0.2	3.1 ± 0.9 ^{a,b}
18 W, 45 min; 3M	3.2 ± 0.4	5.0 ± 0.8	0.8 ± 0.6 ^{a,b}	0.2 ± 0.3	0.3 ± 0.5 ^{a,b}	0.2 ± 0.3	0.2 ± 0.4	3.3 ± 1.2 ^{a,b}
18 W, 45 min; 6M	3.4 ± 0.8	4.6 ± 1.2	0.7 ± 0.6 ^{a,b}	0.1 ± 0.2	0.6 ± 0.7 ^b	0.2 ± 0.3	0.3 ± 0.5	5.8 ± 1.1 ^c
18 W, 30 min; 0M	3.2 ± 0.3	5.1 ± 0.9	1.0 ± 0.5 ^{a,b}	0.3 ± 0.3	0.1 ± 0.2 ^a	0.0 ± 0.1	0.1 ± 0.2	3.0 ± 1.1 ^{a,b}
18 W, 30 min; 3M	3.2 ± 0.3	5.2 ± 0.6	1.1 ± 1.0 ^{a,b}	0.2 ± 0.3	0.3 ± 0.4 ^{a,b}	0.1 ± 0.1	0.3 ± 0.6	3.4 ± 0.8 ^{a,b}
18 W, 30 min; 6M	3.0 ± 0.4	5.1 ± 1.1	0.5 ± 0.6 ^a	0.1 ± 0.3	0.4 ± 0.5 ^{a,b}	0.1 ± 0.3	0.3 ± 0.5	5.5 ± 1.1 ^c
18 W, 15 min; 0M	3.3 ± 0.5	5.1 ± 1.8	0.5 ± 0.4 ^a	0.2 ± 0.3	0.1 ± 0.3 ^a	0.1 ± 0.2	0.2 ± 0.4	2.8 ± 0.9 ^{a,b}
18 W, 15 min; 3M	3.3 ± 0.6	5.2 ± 0.8	1.2 ± 1.2 ^b	0.1 ± 0.2	0.3 ± 0.5 ^{a,b}	0.2 ± 0.3	0.3 ± 0.4	4.1 ± 1.3 ^b
18 W, 15 min; 6M	3.1 ± 0.4	4.8 ± 1.0	0.4 ± 0.6 ^a	0.1 ± 0.2	0.4 ± 0.5 ^{a,b}	0.2 ± 0.3	0.2 ± 0.3	5.2 ± 1.1 ^c
Control; 0M	3.3 ± 0.6	5.1 ± 1.0	0.3 ± 0.3 ^a	0.2 ± 0.3	0.1 ± 0.1 ^a	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 1.1 ^a
Control; 3M	3.1 ± 0.5	5.3 ± 0.7	0.9 ± 0.9 ^{a,b}	0.2 ± 0.4	0.3 ± 0.4 ^{a,b}	0.1 ± 0.2	0.2 ± 0.4	3.9 ± 1.4 ^b
Control; 6M	3.0 ± 0.5	5.0 ± 1.2	0.4 ± 0.3 ^a	0.1 ± 0.2	0.6 ± 0.6 ^b	0.1 ± 0.2	0.1 ± 0.3	5.5 ± 1.1 ^c

^a Abbreviations: W = W/m², min = minutes; M = months of storage.

^b The mean values under the same attribute marked with the same letter are not statistically different ($p > 0.05$). The data represent arithmetic means ± standard deviations.

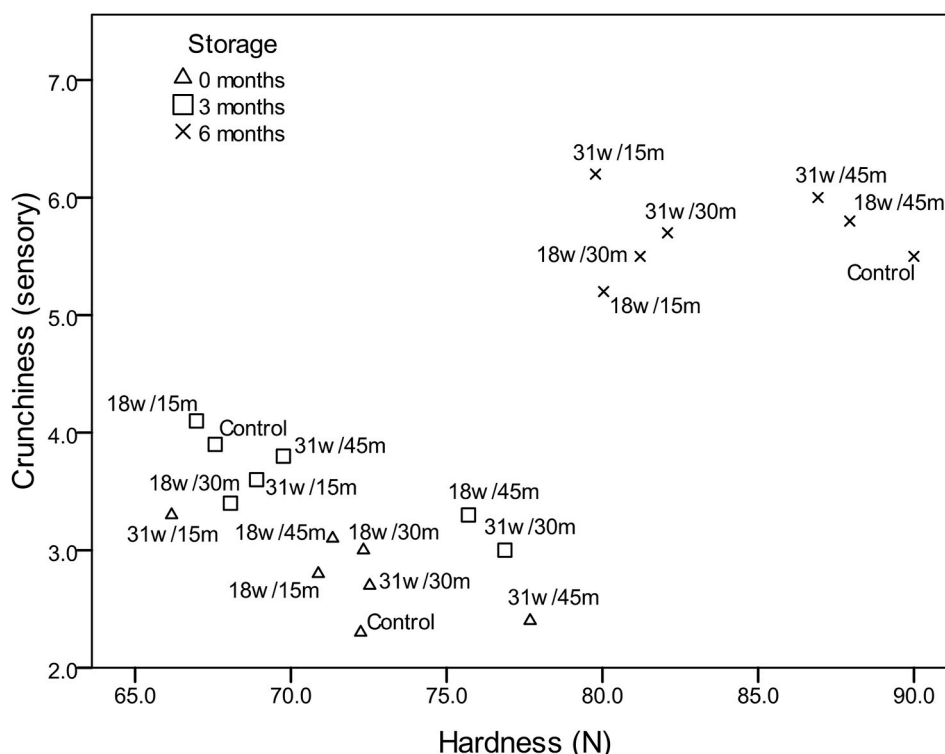


Fig. 2. Relationship between sensory crunchiness and physical hardness in the UV-C treated peanut samples. The sample labels refer to the UV-C treatment ($w = W/m^2$, $m = \text{minutes}$).

Milicevic: Writing – review & editing. **Andreja Rajkovic:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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