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INVESTIGATION OF INITIAL PEROXIDE INDEX VALUE INFLUENCE ON AFB1, AFB2, AFG2 AND T-2 MYCOTOXINS DECOMPOSITION IN SUNFLOWER OIL

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ABSTRACT. In this paper, experiments were conducted to investigate mycotoxins homogenous photodegradation using various peroxide index (IP, caused by peroxides and hydroperoxides) in order to assess the efficiency of sunflower oil decontamination by ultravilolet light irradiation. Was studied the UV assisted peroxides activation, their contribution to decompose mycotoxins, to decrease their concentration. Was proposed a mathematical model of the process, model parameters were identified and adjusted with software environment. The photodegradation process efficiency increased differentiated using sunflower oil with higher peroxide indexes; there exist optimal values of peroxide index for each investigated mycotoxin. This way, the observed rate constants can be increased and the residual mycocotoxin concentration can be decreased. Using favorable peroxide index value in decontamination process provides an effective technology for the removal of mycotoxins from contaminated sunflower oil. The rapid degradation is related to the inherently contented peroxides transformation in reactive free radicals, this radicals reacting with mycotoxins. With the elaborated model is possible to predict the concentrations of residual mycotoxins in different operating conditions depending on initial peroxide index values.

Keywords: *Mycotoxins, Aflatoxin B1, Aflatoxin B2, Aflatoxin G2, T-2 toxin, photochemical degradation, ultraviolet light, sunflower oil, peroxide index, HPLC.*

INTRODUCTION

The term "mycotoxin" is usually reserved for toxic chemicals produced by fungi which commonly colonize crops [1]. Most plant parasitic fungi use oxygen and are found almost everywhere in small quantities, as a result of dispersion of spores. They metabolize organic substances, they develops where humidity and temperature are favorable. Species of fungi can produce different mycotoxins, and/or a mycotoxin may be produced by several species [2]. In 1971, Turner systematized 500 species of fungi and 1200 secondary metabolites. Hawksworth (1991) identified 69000 species of fungi, representing only 5% of all species, said the availability of the 1,5 million secondary metabolites.

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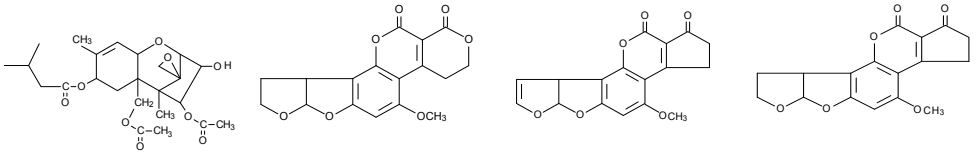
Other assessments have evaluated approximately 100000 secondary metabolites. Mycotoxins can appear in the food chain as a result of fungal infection of crops, that are consumed by humans or used as animal feeds. Even at extreme temperatures of processing, such as by boiling or roasting in the preparation process of foods (100-200 °C), mycotoxins decompose very slowly. International Commission seeks to achieve regulatory universal limit standards for mycotoxins concentrations in foods. Currently over 100 countries have adopted regulations for mycotoxins, special attention are accorded to food industry, in which 13 groups of mycotoxins are reason of concern [3]. Based on chemical structure, mycotoxins can be classified in coumarin derivatives, malonates, mevalonates, acetates and unsaturated lactones [4].

Figure 1 shows the molecular formulas of the most important representatives of the family of mycotoxins.



T-2 Toxin Aflatoxin G2 Aflatoxin B1 Aflatoxin B2

Figure 1. Chemical structure of studied mycotoxins

Table 1 shows the molecular formulas, molar masses and melting temperatures of the investigated mycotoxins.

Table 1. Molecular formulas, molar masses and melting temperatures of the investigated mycotoxins

Mycotoxin	Molecular formula	Molar mass, g/mol	Melting temperature, (ºC)
AFB1	$C_{17}H_{12}O_6$	312	268-269
AFB2	$C_{17}H_{14}O_6$	314	286-289
AFG2	$C_{17}H_{14}O_7$	330	237-240
T-2	$C_{24}H_{34}O_9$	466	282

Mathematical model

The inactivation of mycotoxins by ultraviolet light involves two stages. In the first stage, the mycotoxins have to difuse to the radiation exposed area. If the mycotoxin molecule reaches a sufficiently small distance (λ) of surface irradiance, the photons are absorbed mainly by mycotoxins and peroxides, peroxide molecules generates free radicals, free radicals reacts with excited state mycotoxins, this way mycotoxins are inactivated. Distance λ is the penetration depth of radiation, where the photon flux is 10% of the initial flow at the surface radiation I_0.

Under UV decontamination process, unsaturated fatty acids specific autoxidation reactions not take place due to lack of contact with atmospheric oxygen and dissolved oxygen in sunflower oil, preliminary was removed the dissolved oxygen by vacuum degassing. Instead, must cosider hydroperoxides and peroxides which are inherently present in sunflower oil due to storage and manufacturing process steps and the peroxides and hydroperoxides possible decomposition reactions by the action of UV radiation.

Parameters and refining stages can decisively influence the effectiveness of UV treatment (UV absorbance, peroxide index). The peroxide index is usually expressed in meq/kg, also the peroxide index can be expressed in SI units as milimoles per kilogram. The value expressed in milimoles/kg is half of that expressed in meq/kg. The method is based on the properties of peroxides and hydroperoxides to free molecular iodine in the acidic environment from potassium iodide. Released iodine is very reactive and can be linked by double bonds of unsaturated fatty acids, which will lead to an error in determining the peroxide index. To block this reaction is carried out in chloroform and glacial acetic acid (2:1). High acidity environment determinine virtually blocking of the double bonds of unsaturated fatty acids. According to Daneshvar et al. studies [5], high concentrations of peroxides can lead reactions between peroxides and generated free radicals, decreasing the decontamination capacity. Therefore, high concentrations of peroxides and hydroperoxides does not offer benefits, on the other hand are not even allowed in the finished product (CMA 3.0 mEq/kg). 253,7 nm wavelength is most effective in terms of excitation of mycotoxins, since photons are efficiently absorbed by mycotoxins at this specific wavelength. UV light with wavelengths below 230 nm is most effective for the dissociation of peroxides and hydroperoxides [6].

In limited area of concentrations of investigated mycotoxins can be performed the degradation kinetics linearization, especially if it seeks to minimize changes of essential fatty acids, which logically require a relatively short irradiation times. We can write the equation:

$$-\frac{d[MT]}{dt} = k_{apMT}[MT] \tag{1}$$

For each mycotoxin, $[MT]$ represent the actual concentration of mycotoxin, k_{apMT} is the pseudo-first order transformation constant, *t* is the time of irradiation [7]. According to the analytical determinations carried out, peroxides and hydroperoxides (which concentrations are expressed by peroxide index) decomposes under the action of UV radiation, their decomposition generating free radicals that contribute to the degradation of mycotoxins. The relation between k_{apMT} and peroxide index (*IP*) can be modeled by linear regression. The model can be defined by considering ineffective the inhibitory

reactions caused by the direct irradiation of the mycotoxins. This account is very plausible, because in an inert environment, even if mycotoxins are irradiated, they decomposing just very slowly. In conclusion, k_{apMT} can be expressed as:

$$k_{apMT} = \frac{a[IP]_0 / [MT]_0}{1 + b([IP]_0 / [MT]_0) + c([IP]_0 / [MT]_0)^2} \quad (2)$$

where k_{apMT} is the dependent variable, *a*, *b* and *c* are model parameters, and $[IP]_0$ is the initial peroxide index value is the reaction mass. The calculated values of Reynolds criteria for the flow regimes clearly indicate the laminar flow in the UV reactor. Therefore, it might be considered as operating with an ideal tubular reactor [8]. For a PFR (tubular) reactor, the equation that describes the operation can be described as follows:

$$-\frac{d[MT]}{dV} = -\frac{r_{MT}}{\nu_0}, \quad (3)$$

$$\text{where } V = \frac{\pi}{4}\left(d_0^2 - d_i^2\right)l, \text{ and } -r_{AMT} = -\frac{d[MT]}{dt} \quad (4)$$

In previous relations *V*, ν_0 ,d_0, d_i and *l* are the volume, volume flow, inner diameter, outer diameter and the length of photochemical reactor [9].

From the last two equations we get:

$$-\frac{d[MT]}{dl} = \frac{\pi/4\left(d_0^2 - d_i^2\right)}{\nu_0}\left(-\frac{d[MT]}{dt}\right) \quad (5)$$

With the additional use of previous equations, the obtained model is:

$$-\frac{d[MT]}{dl} = \frac{\pi/4\left(d_0^2 - d_i^2\right)}{\nu_0}\left(\frac{a[IP]_0 / [MT]_0}{1 + b([IP]_0 / [MT]_0) + c([IP]_0 / [MT]_0)^2}\right)[MT] \quad (6)$$

EXPERIMENTAL SECTION

Materials

AFB1, AFB2, AFG2 and T-2 toxin standards were purchased from Makor Chemicals Ltd. (Jerusalem, Israel), sunflower oil was purchased from SC Expur SA, methanol (HPLC grade) was purchased from Sigma-Aldrich (Redox Lab Supplies Com SRL), and carbon dioxide was purchased from Linde Gaz România SRL.

Reactor system

The degradation experiments were conducted in reactor system, 100 ml plug-flow photoreactor was serial connected with a jacketed vessel, used for temperature control and stirring. The scheme of the used experimental treatment system is presented in figure 2.

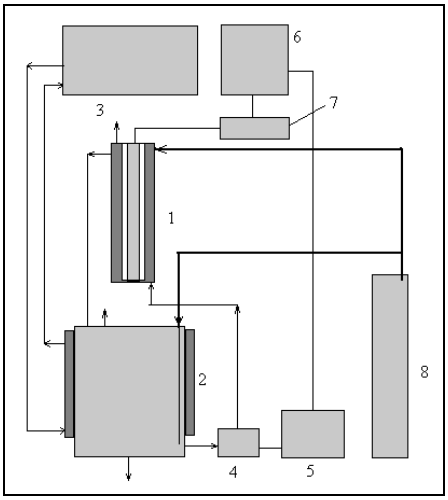


Figure 2. The used experimental system

1- PFR photoreactor;
2- storage vessel;
3- ultrathermostat;
4- adjustable flow pump;
5- pump control unit;
6- stabilized power supply;
7- UV power source control unit;
8- CO_2 cylinder.

In the UV activation experiment, a 20 W Philips-LPMHO (Low Pressure Mercury High Output) was used as the light source. UV source measured emission power was 29,8 mW/cm^2 at 5 cm distance, volume flow was 185 ml/min, system temperature was 20 °C. All experiments were carried out in same conditions. Carbon dioxide gas adding was used to remove of atmospheric oxygen.

Analysis

The degradation of mycotoxins was monitored by HPLC. The reaction mass samples were processed using Hettich Micro 20 centrifuge (13000 rot/min, 5 minutes), was split the higher density oil phase, which was pipetted into

an extraction bottle, it was determined the mass, after this was performed methanol extraction of mycotoxins in 8 steps, obtained mixtures were separated with Hettich Universal 32 centrifuge (3400rot./min, 5 minutes), alcoholic liquid phases were pipetted and unified. Since resulted alcoholic mixtures were cloudy, a new set of centrifugations were carried out to realise the perfect separation. Obtained methanolic extracts were subjected to evaporation, and portions of extracts was injected in Varian Pro Star HPLC, using fluorescent and UV detector, Supelcosil LC 18 column, flow rate was 0,9 ml/min, without derivatization. HPLC equipment was controlled with Varian Star Chromatography Workstation Version 6.00 software. The eluent was water-methanol-acetonitrile mixture, with 130:70:40 ratio. Fluorescence detector was set for excitation at 365 nm, and the emission at 435 nm. Based on HPLC chromatograms provided by the HPLC, we can evaluate the concentration of MT in the samples, which is proportional to the area under the signal curve offered by that component.On the basis of changes in decreasing concentrations of mycotoxins, taking into account the fact that the concentration of mycotoxins is much smaller than the concentration of peroxidies who are sensitive to UV irradiation, as previously we found a 12-27% reduction in the amount of IP in the time of UV treatment, we assumed that the determining factor of decomposition speed may be the initial peroxide (IP_0) of sunflower oil under photochemical decontamination.

Because IP_0 have determinant influence on the kinetics of photo-degradation process, we performed a series of experimental determinations with contaminated sunflower oil, starting with different initial concentrations of peroxides, peroxide index, respectively. Initial, the sunflower oil IP value was IP_0 = 0,89 and for the next experimental determinations became necessary preparation of sunflower oil with higher IP values. To this, in sunflower oil was introduced dry air until was reached a higher peroxide index, after that was applied preparation of mixtures between the original sunflower oil and artificially increased IP oil [10], were prepared samples with required peroxide indexes (1-10) for further determinations.

RESULTS AND DISCUSSION

Variations of mycotoxins HPLC areas (A_{MT}) by the starting IP (IP_0) are shown in table 2, mycotoxins concentrations variation (dc_{AFB1}, dc_{AFB2}) and apparent photodegradation constant variation (k_{apAFB1}, k_{apAFB2}) according on ratio between initial peroxide index value (IP_0) and initial mycotoxin concentration ($[MT]_0$) are shown in table 3 and 4.

Variations in concentrations ($c/c_{0\ MT}$) of the studied mycotoxins by the starting IP (IP_0) of the reaction mass (with equal times of irradiation) are shown in figure 3.

Table 2. Variations of mycotoxins HPLC areas (A_{MT}) according on initial peroxide index (IP_0) of sunflower oil

IP_0	A_{AFB1}	A_{AFB2}	A_{AFG2}	A_{T2}
1	147994	96304	98251	132562.00
2	146805	95859	97528	131734.00
3	145794	95347	96734	130763.00
4	144758	94985	96045	130095.00
5	142894	94438	95264	129264.00
6	141634	93985	94467	128321.00
7	140421	93589	93793	127563.00
8	139452	93245	93049	126635.00
9	138253	92675	92330	125865.00
10	137045	92236	91524	125058.00

Table 3. AFB1, AFB2 concentrations variation (c_{AFB1}, c_{AFB2}) according on ratio between initial peroxide index value (IP_0) and initial mycotoxin concentration ($[MT]_0$)

c_{AFB1}	c_{AFB2}	$IP_0/[MT]_0$
2.00000	2.00000	0.5
1.98393	1.99076	1.0
1.97027	1.98013	1.5
1.95627	1.97261	2.0
1.93108	1.96125	2.5
1.91405	1.95184	3.0
1.89766	1.94362	3.5
1.88456	1.93647	4.0
1.86836	1.92463	4.5
1.85203	1.91552	5.0

Table 4. AFG2, T-2 concentrations variation (c_{AFB1}, c_{AFB2}) according on ratio between initial peroxide index value (IP_0) and initial mycotoxin concentration ($[MT]_0$)

c_{AFG2}	c_{T2}	$IP_0/[MT]_0$
2.00000	2.00000	0.5
1.98528	1.98751	1.0
1.96912	1.97286	1.5
1.95509	1.96278	2.0
1.93920	1.95024	2.5
1.92297	1.93601	3.0
1.90925	1.92458	3.5
1.89411	1.91058	4.0
1.87947	1.89896	4.5
1.86307	1.88679	5.0

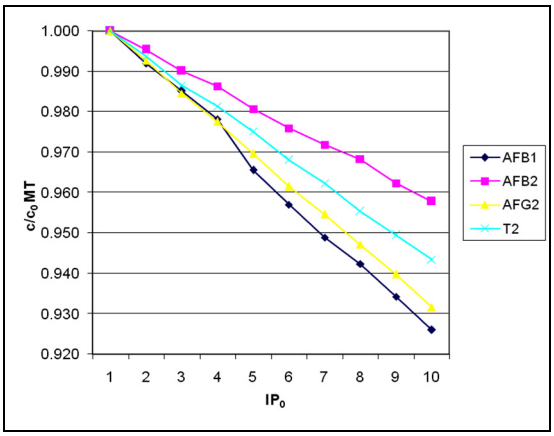


Figure 3. Variations of relative concentrations of MT (c/c_{0MT}) depending on the initial IP value of reaction mass (IP_0)

Variations of the mycotoxins (AFB1, AFB2, AFG2 and T-2) concentrations (dc_{MT}) depending on the ratio between initial peroxide index and initial mycotoxin concentration ($IP_0/[MT]_0$) are presented in figure 4.

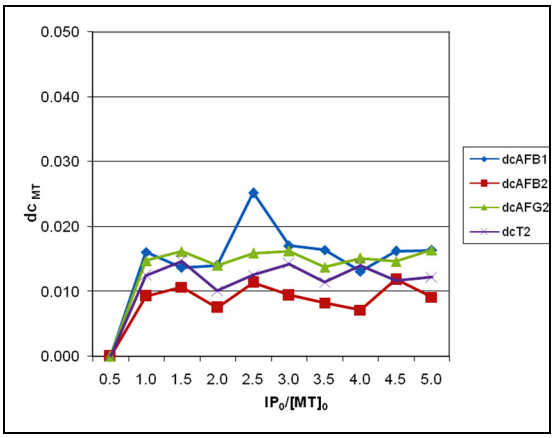


Figure 4. Variations of AFB1, AFB2, AFG2 and T-2 concentrations (dc_{MT}) depending on the ratio between initial peroxide index and initial mycotoxin concentration ($IP_0/[MT]_0$)

Variations of the studied MT photodegradation apparent coefficients (k_{apAFB1}, k_{apAFB2}, k_{apAFG2} and k_{apT2}) depending on the initial peroxide index of the reaction mass (IP_0) are presented in figure 5.

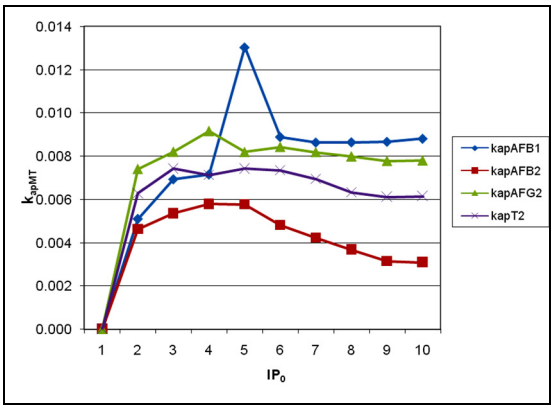


Figure 5. Variations of the studied MT photodegradation apparent coefficients (k_{apAFB1}, k_{apAFB2}, k_{apAFG2} and k_{apT2}) depending on the initial peroxide index of the reaction mass (IP_0)

To obtain the individual kinetic parameters for MT, we adjusted the experimental data to the proposed model by Polymath software environment. Using the model equation, the parameters of individual models with 95% confidence level and using the sum of the squares error method (SSE) as a function of error were obtained by nonlinear regression; results are presented in Figures 6, 7, 8 and 9.

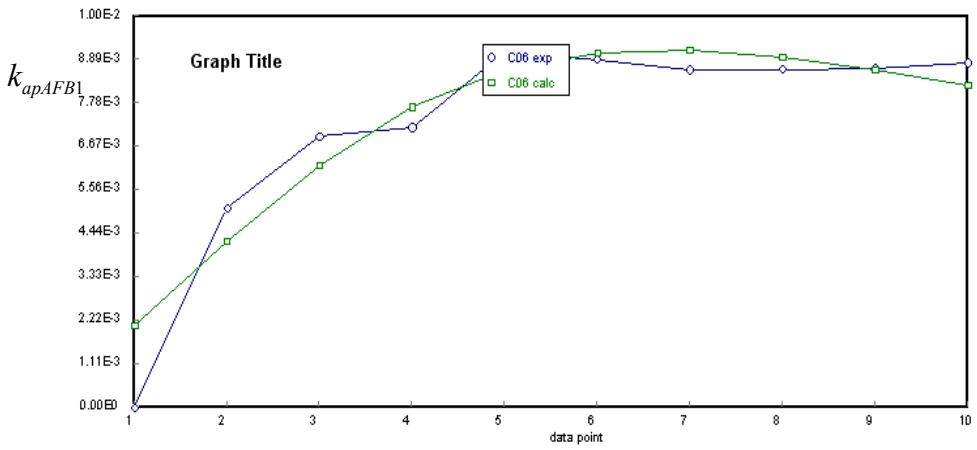


Figure 6. AFB1 experimental data adjustment

k_{apAFB2}

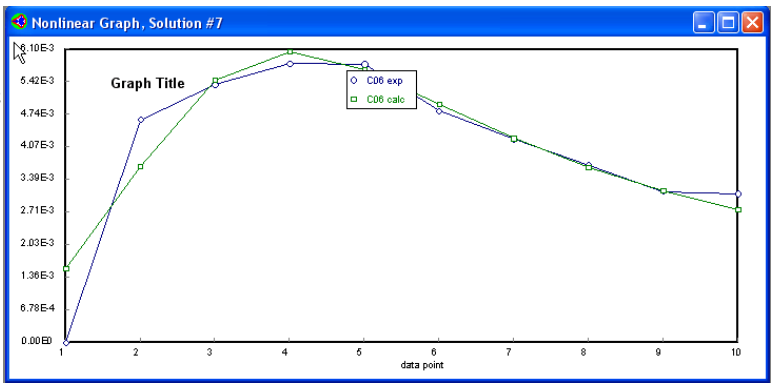


Figure 7. AFB2 experimental data adjustment

k_{apAFG2}

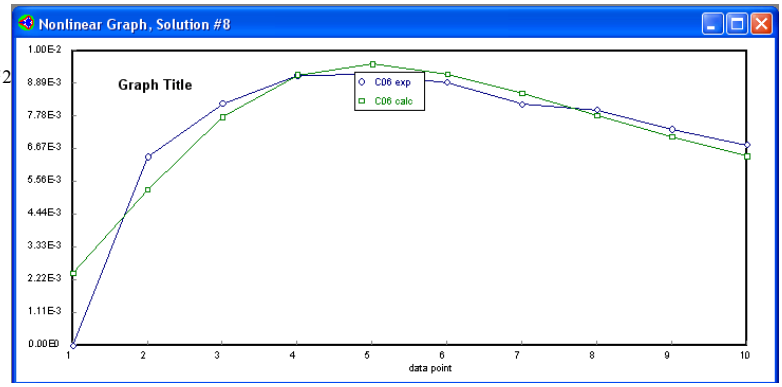


Figure 8. AFG2 experimental data adjustment

k_{apT-2}

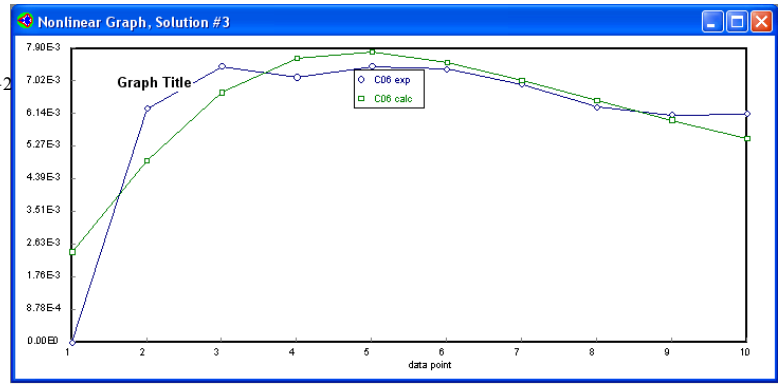


Figure 9. T-2 experimental data adjustment

Values obtained by regression of the parameters *a*, *b* and *c* is presented in Table 5.

Table 5. Values obtained by regression of the model parameters for the MT photodegradation

Parameter	AFB1	AFB2	AFG2	T-2
a	0,0029	0,0155	0,0028	0,0030
b	0,4489	0,2642	0,3454	0,4407
c	0,0498	0,0808	0,0562	0,0820

RESULTS AND DISCUSSION

On the basis of changes in decreasing concentrations of mycotoxins, taking into account the fact that the concentration of mycotoxins is much smaller than the concentration of peroxidies who are sensitive to UV irradiation, as previously we found a 12-27% reduction in the amount of IP in the time of UV treatment, we assumed that the determining factor of decomposition speed may be the initial peroxide (IP_0) of sunflower oil under photochemical decontamination. Because IP_0 have determinant influence on the kinetics of photodegradation process, we performed a series of experimental determinations with contaminated sunflower oil, starting with different initial concentrations of peroxides, peroxide index, respectively. Initial, the sunflower oil IP value was IP_0 = 0,89 and for the next experimental determinations became necessary preparation of sunflower oil with higher IP values. To this, in sunflower oil was introduced dry air until was reached a higher peroxide index, after that was applied preparation of mixtures between the original sunflower oil and artificially increased IP oil, were prepared samples with required peroxide indexes (1-10) for further determinations.

CONCLUSIONS

The proposed model for prediction of AFB1 photodegradation has a maximum for k_{apAFB1} at around 3,0 for the $[IP]_0 / [AFB1]_0$ ratio, which is in good agreement with experimental data obtained for AFB1, which resulted for k_{apAFB1} a 2,5 value of the ratio. The proposed model for prediction of AFB2 photodegradation has a maximum for k_{apAFB2} at around 2,5 for the $[IP]_0 / [AFB2]_0$ ratio, which is in good agreement with experimental data obtained for AFB2, which resulted for k_{apAFB2} a 2,0 value of the ratio. The proposed model for

prediction of AFG2 photodegradation has a maximum for k_{apAFG2} at around 2,5 for the $[IP]_0 / [AFG2]_0$ ratio, which is in good agreement with experimental data obtained for AFG2, where resulted for k_{apAFG2} a 2,0 value of the ratio. The proposed model for prediction of T-2 photodegradation has a maximum for k_{apT-2} at around 3,0 for the $[IP]_0 / [T-2]_0$ ratio, which is in good agreement with experimental data obtained for T-2, where resulted for k_{apT-2} a 2,5 value of the ratio.

The constructed models are useful to estimate the initial IP demand (IP_0) for the efficient photochemical degradation of mycotoxins in each case. On the other hand, it becomes obvious need for the sunflower oil refining such that when performing decontamination of mycotoxins, to achieve the photodegradation with low UV doses, must have IP_0 values between certain limits, depending on the nature of the present mycotoxins.

The necessary IP_0 values for decontamination at the same time require restrictions on storage conditions and storage period of sunflower seeds in silos.

After substituting the appropriate values of *a*, *b* and *c* for each mycotoxin, using d_0 and d_i, we can be obtain the mycotoxins concentrations at different lengths of the photoreactor, starting from initial concentrations of mycotoxins and from the initial value of IP, for different flow speeds through the fotoreactor. As we can see, it is possible to predict the concentrations of mycotoxins in different operating conditions depending on IP_0.

Based on obtained experimental data, on the four investigated molecular species of mycotoxins, under identical conditions, by comparing obtained data, we may notice some differences and similarities in the photochemical behavior of these mycotoxins. Highest sensitivity to the effect of peroxides and hydroperoxides decay occurs in case of AFB1, followed by T-2 toxin, AFB2, and finally, AFB1 showing the lowest sensitivity to peroxide index increase (at usual values), and at the same time it can be observed the existence for AFB1 of a favorable IP_0 for photodegradation process, which may be in correspondence with the behavior of other chemical species which are present in the matrix of sunflower oil.

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