Egypt. J. Food. **Vol. 46,** pp.189-196 (2018)

Aflatoxin Contamination, Phenolic Contents Concentration in Tigernuts as Affected by Traditional Household Processes

Mohamed H. H. Roby, Samah A. Abdel Tawab and Alla Eldeen M. A. El Fakhrany
Food Science and Technology Department, Faculty of Agriculture, Fayoum University.

MOST of the physical methods used in preparing tigernuts tubers have variable effects on its phenolic content, antioxidant activities and aflatoxins (AFs) contamination. Effects of immerging in boiling water (blanching), soaking at 25± 5°C and roasting at 130 ± 5°C on the content of aflatoxin, concentration of phenolic compounds and antiradical activity of tigernuts were studied. Results showed that blanching in water showed the highest reduction on phenolic compounds than roasting and soaking. Antioxidant potency estimated by DPPH and ABTS assay demonstrated a similar pattern. The effect of blanching, roasting and soaking on reduction of AFs in artificially contaminated tigernuts were respectively, 30, 70 and 12.95%. In general, these methods reduce AFs concentrations significantly, but do not eliminate them completely.

Keywords : Aflatoxin, tigernuts, Household processes, Phenolic compounds, Herbs and Antiradical activity.

Introduction

The growing interest for natural products and discovery of its bioactive compounds in foods are needed nowadays. Every day new references published in scientific literature to present the beneficial effects of its ingredients and/or its bioactive compounds (Dada, 2015). The contamination of foods and animal feeds with mycotoxins is a worldwide problem. Mycotoxin contamination may occur in the □eld before harvest, during harvesting, or during storage and processing. certain treatments have been found to reduce speci□c mycotoxin formation in different commodities, the complete elimination of mycotoxin contaminated commodities is currently not realistically achievable (Pankaj et al., 2018).

Studies on a functional food containing underutilized crops has been encouraged. Tigernuts is an edible perennial weedy plant that primarily grows in the tropics and in the Mediterranean region.

This plant can be eaten in different forms: unprepared, soaked in water or dried and mixed with roasted ground nut (Temple, et al., 1990). Tigernuts is famous in Egypt and locally named as Hab Alaziz. Since tigernuts is not consumed raw, the effects of various preparing methods are important. Some of these preparing methods which include boiling, soaking, roasting could have some effects on their nutritional and bioactive compounds (Kapseu, et al., 1997).

So, this work aimed to establish various strategies as a preparation method to eliminate the effect of mycotoxin (aflatoxin) and improving storage conditions. Also, the effect of these preparation methods on the major phenolic compounds and the antioxidant activity of tigernuts were studied.

Materials and Methods

Tigernuts samples preparation

Tigernuts (*Cyperus esculentus* L) were purchased from local market in Fayoum province, Egypt. Tubers were separately sorted, thoroughly cleaned from other impurities and divided into four parts. One part was analyzed raw, a second part was blanched in distilled water for 45 min at 100°C until the tubers were well tender. another part was roasted into a laboratory oven set at 130 ± 5°C. Sample roasted for 30 minutes. The last part was soaked in tap water for 48 hours at room temperature (25 ± 2°C). The samples were milled with a Moulinex blender and defatted for six hours in a Soxhelt apparatus' using purified *n*-hexane. The defatted grits were then kept in plastic bags and kept at 4°C until analysis.

*Corresponding author : wael.fathi@agr.aswu.edu.eg
DOI: 10.21608/EJOH.2017.1100.1008
Email: Logy_nour87@yahoo.com

Chemicals, Solvents and Reagents

Aflatoxin standards were purchased from Sigma Chemical Co. (Supelco) 2,2-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis (3 ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, ethanol, methanol and Phenolic standard chromatographic (gallic acid, catechine, cinnamic acid, 3,4 dimethoxy cinnamic acid, naringenin, myricetin, luteoline and apigenin all with 96–99% purity) were obtained from Sigma (St. Louis, MO). A□atest immunoa-nity columns purchased from LcTech company. All other chemicals were in analytical reagent.

Phenolic compound extraction

Tigernuts samples were defatted, 10 g of each defatted tigernuts powdered sample were extracted individually at room temperature (~25 ºC) with 100 mL methanol: water (80:20) Hakkinen, et al., (1998). The extracts were filtered through filter paper Whatman No.1, the solvents were evaporated using rotary evaporator at 40 ± 5º C, and the dried matter was dissolved in HPLC mobile phase prior injection.

Phenolic compounds identification by HPLC

Phenolic compounds were identified using HPLC Agilent 1260 Infinity series system, equipped with on-line degasser (G 1322A), quatpump (G 1311C), auto sampler (G 1329B), column heater (G 1316A), and variable wave length detector (G 1314F). Instrument control and data analysis was carried out using Agilent HPLC ChemStation 10.1 edition through Windows 7. Column Zorbax C18 (5 µm, 4.6 mm × 150 mm, Agilent) was used. Mobil Phase consisting of A (70/30% Methanol: Water) B Methanol 100% and the injection volume was 50µL. Detection of the phenolic compounds were done by comparing with the retention time of external standard.

Determination of antiradical activity

Antioxidant capacity of tigernuts samples were determined by radical scavenging ability using stable DPPH$^{\bullet}$ radical method as described by (Akowuah et al. 2005) and ABTS method as described by (Re et al. 1999) using concentration samples (50, 100, 150 and 200 μ_g/mL).

The percent inhibition of the tested samples was evaluated by comparison with a control. Each sample was measured in triplicate, and an average value was calculated. Antioxidant activity was expressed as a percentage of inhibition compared to control as follows:

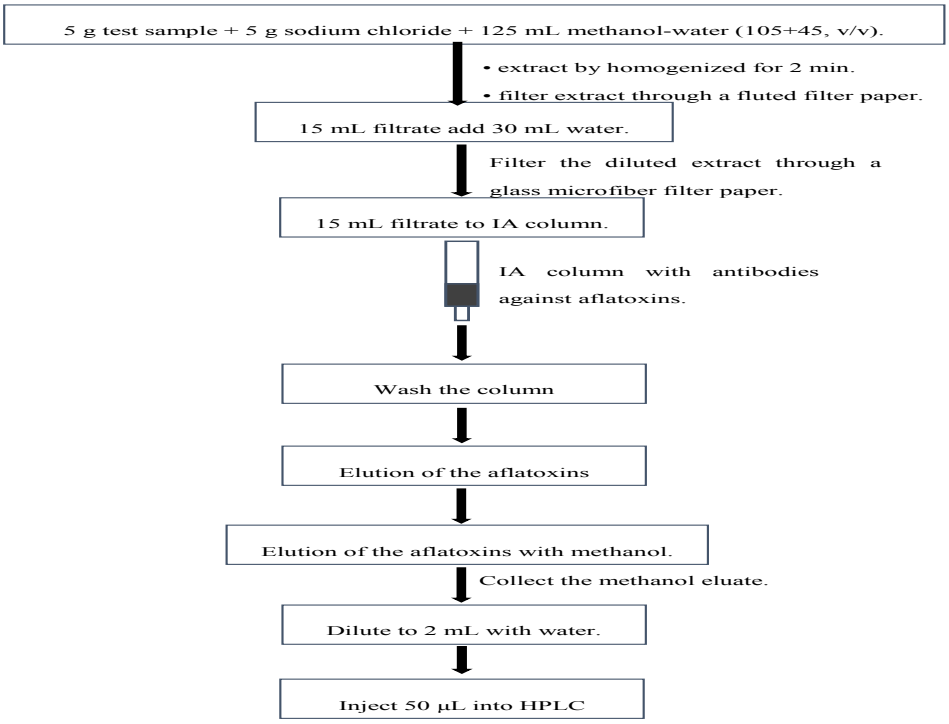
$$\% \text{ inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where A is the absorbance at 515nm in DPPH method and at 734nm in ABTS method.

Aflatoxin Determination

Determination of aflatoxins in the studied samples was done according to the ISO 16050:2003 method.

Extraction procedure



Schema 1. Schematic methodology of aflatoxin extraction procedure.

HPLC Apparatus and Conditions

The aflatoxin content was quantified using reverse phase Agilent 1260 Infinity series HPLC system equipped with on-line degasser (G 1322A), quatpump (G 1311C), auto sampler (G 1329B), column heater (G 1316A), and variable wave length detector (G 1314F). Instrument control and data analysis was carried out using Agilent HPLC ChemStation 10.1 edition throμgh Windows 7. Column Zorbax C18 (5 μm, 4.6 mm × 150 mm, Agilent) was used. The flow rate of the mobile phase was 1 mL/min. Mobile phase A was water phase B was methanol and phase C was acetonitrile (3:1:3 v/v/v). The temperature of column was controlled at 30°C. Injection volume was 50 μL. The detection were set at: excitation wave length to 365 nm and emission wave length 435 nm.

The concentration of the Aflatoxin calculated from the prepared calibration curve using the Aflatoxin standard solutions. These solutions cover the range of 0.6 ng/mL to 15 ng/mL for Aflatoxin.

Statistical analyses

Statistical analysis was carried out in using ANOVA procedures GenStat statistical package (version oxford, 11) (VSN International Ltd, UK). Difference between means signi□cant was compared using least difference test (LSD) at 5% level (p = 0.05).

Results and Discussion

Changes in the phenolic compounds of tigernuts during boiling water blanching for 45 minutes, roasting at 130 ± 5°C for 30 minutes and soaking in water for 48 hours at 25± 5°C) were studied. the results are presented in Table 1. The results showed that, compared to control sample all three treatments negatively affected on all phenolic compounds content and the rate of reduction affected by type of treatment. Boiling water blanching showed the highest reduction on phenolic compounds catechine, cinnamic acid and naringenin (100 %), 3,4 dimethoxy cinnamic acid (83.17 %). and luteoline (88.76%) followed by roasting followed by soaking. Roasting treatment was the highest reduction on gallic acid (27.79 %) followed by boiling water blanching (5.89 %) followed by soaking (1.48 %). The loss on these phenolic compounds in case of boiling water blanching and soaking treatments may be attributed to the leaching of these compounds in blanching and soaking water especially at elevator temperatures. Meanwhile the loss in case of roasting may be attributed to the thermal treatment applied, Xu and Chang (2008). Therefore, a thermal process can be affected both on the nutritional and bioactive characteristics of foods (Mazzeo et al., 2011). In the same trend Lemos et al., (2012) found that the roasting process caused a reduction on phenolic content of baru nuts.

TABLE 1. Effect of different preparation methods on phenolic compounds content of tiger nuts (*Cyperus esculentus* L).

Phenolic Compounds	Control Sample (without treatment)	Boiling Water Blanching (45 min)		Roasting (130° ± 5°C/30 min.)		Soaking (48 h. / 25 ± 5°C)	
	Conc. m_g / mL	Conc. m_g / mL	% of Reduction	Conc. m_g / mL	% of Reduction	Conc. m_g / mL	% of Reduction
Gallic acid	43.2±2.4	40.6±0.5	5.9	31.2±0.9	27.8	42.2±2.5	1.5
Catechine	1.5±0.2	0.00	100.0	0.7±0.1	55.3	1.3±0.2	11.8
3,4 dimethoxy cinnamic acid	9.2±1.2	1.5±0.06	83.2	3.2±0.07	65.1	7.7±0.6	16.4
Cinnamic acid	2.4±0.4	0.00	100.0	0.4±0.06	82.6	1.2±0.3	49.8
Quercetin	12.5±1.1	10.6±0.7	15.8	6.7±0.8	46.5	8.5±0.7	32.5
Naringenin	5.9±0.8	0.00	100.0	0.9±0.1	83.9	2.2±0.5	63.5
Luteoline	5.0±1.1	0.7±0.08	88.8	1.7±0.2	66.5	3.3±0.7	33.5
Apigenin	30.1±2.5	29.8±1.3	1.0	22.1±1.2	26.5	24.9±1.2	17.3

The scavenging activity of tigernuts extracts against DPPH• was dependent on concentration as presented in (Figure 1). Significant ($P < 0.05$) differences between extracts were observed, but the results clearly indicate that all extracts exhibited antioxidant activity. The extracts that showed relatively high antioxidant activity (those with soaking and roasting), as strong as that of control, contained the highest amount of total phenolic content (Table 1).

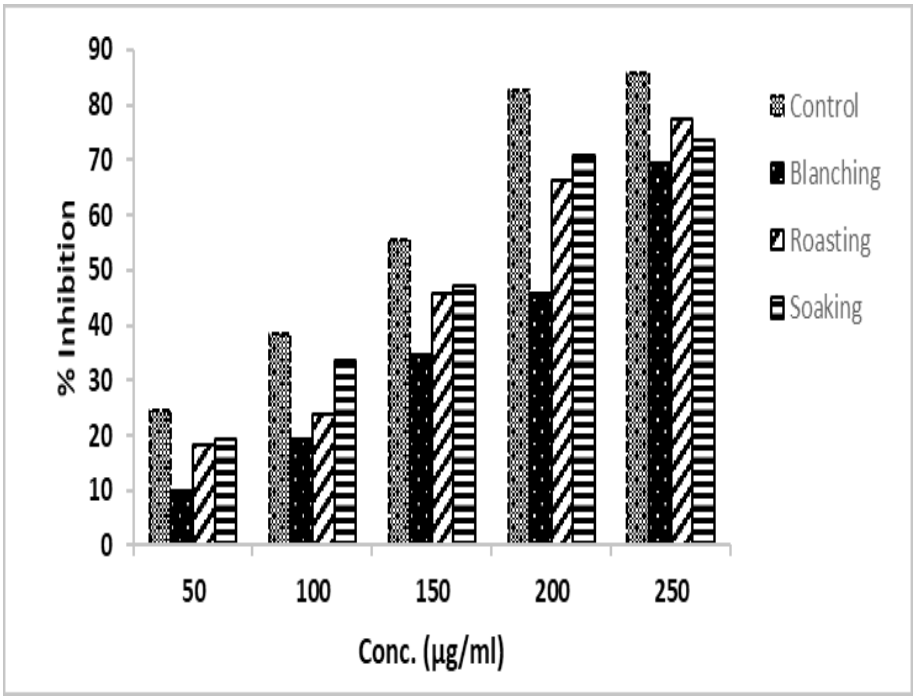


Fig. 1. Processing Effects on the Antioxidant Activities of tigernuts using DPPH assay.

Having consider the advantages and disadvantages of DPPH$^{\bullet}$ technique, it was necessary to further another assay for antioxidant activity detect (Dorman et al., 2004). ABTS$^{\bullet+}$, a protonated radical, has a characteristic absorbance maximum at 734 nm that decreases with the scavenging of proton radicals. The extracts demonstrated a wide range of ABTS$^{\bullet+}$ scavenging activities from 10 to 79% (Figure 2).

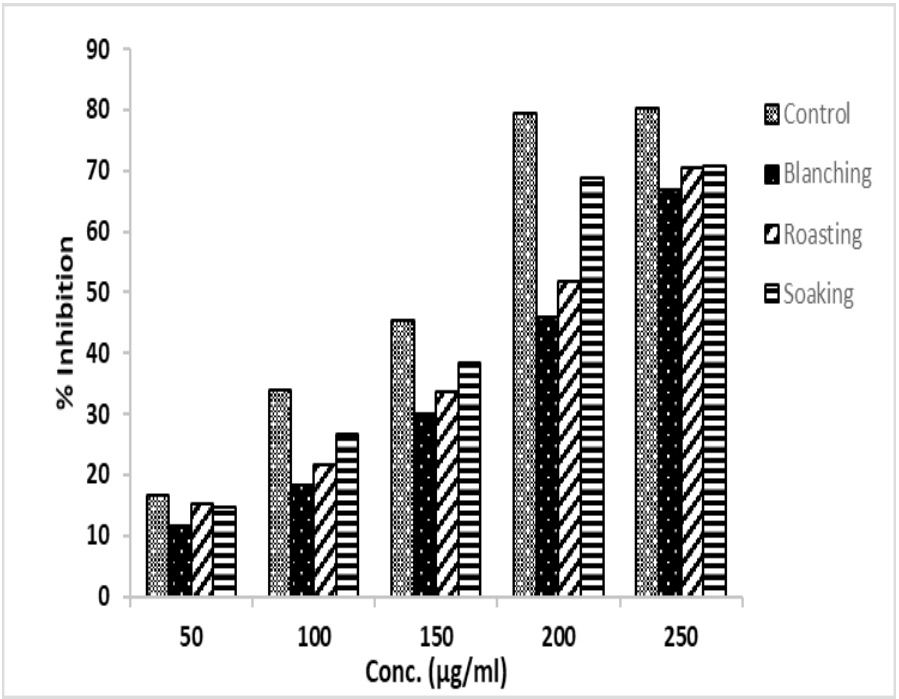


Fig. 2. Processing effects on the antioxidant activities of tigernuts using ABTS assay.

This study showed that, all tigernuts extracts presented a significant antioxidant activity. The extracts which soaking were the most efficient $ABTS^{\bullet+}$ scavengers, followed by those with roasting and blanching. In Table 2, measurement antioxidant activity by DPPH and ABTS show all treatments compared to control samples negatively affected, the higher antioxidant activity it was noticed in control samples followed by soaking followed by roasting followed by blanching. The reduce in antioxidant capacity may be attributed to the loss of phenolic compounds as a result of these treatment especially the decrease in antioxidant capacity parallel with the rate of reduction on phenolic compounds or take the same trend. Nicoli, et al., (1999) although observed in another study that the concentration of natural antioxidants decreased as a result of the heat treatment. Also, Lima, et al., (2010) observed a significant loss of phenolics content after roasting process approximately 21.6% of total phenolics in cocoa, 18 % in coffee beans and in decaffeinated coffee the loss was 5%. Studies with other foods show that even after being subjected to heat, the antioxidant capacity was unchanged (Turkmen, et al., 2005). Normally, which acid is released during acid hydrolysis of lignocellulose materials due to partial degradation of lignin (Luo, et al., 2002).

The antioxidant capacity of roasted nuts decreased significantly without change the levels of gallic acid in the baru nut with peels, while the nuts heated without peels reduced significantly the levels of gallic acid without altering the antioxidant capacity also. So, these results suggest that other phenolic compounds might be the main bioactive compounds responsible for the antioxidant activity in this nut. The catechin and ferulic acid, for example, could be the main antioxidant compounds, once contents of these compounds decrease by approximately 50% upon roasting, which is in agreement with the reduction in the antioxidant capacity of nuts with peels after roasting. Those compounds might be responsible for the protective effect against oxidative stress observed in rats supplemented with oral iron and fed a diet containing baru nuts, as described by Siqueira et al. (2012).

TABLE 2. IC_{50} and antiradical power (ARP) of Tiger nut samples.

Preparation method	IC50		ARP	
	DPPH	ABTS	DPPH	ABTS
Control	39.96±5	51.20±8	0.025±0.001	0.020±0.002
Blanching	63.66±7	62.91±4	0.016±0.002	0.016±0.004
Roasting	52.99±3	56.53±2	0.019±0.005	0.018±0.007
Soaking	41.85±2	53.65±2	0.024±0.002	0.019±0.003

During food processing, the time and temperature combination remains one of the most important method by which the aflatoxins content in the food can be decreased in the end product. Tables 3 and 4 show the effect of blanching, roasting and soaking on reduction of AFs in artificially contaminated tigernuts.

TABLE 3. Effect of preparation process on spiked aflatoxins concentration in tiger nuts.

Treatment	Aflatoxins conc. (µg/kg)				
	B1	B2	G1	G2	Total
Blanched	1.08 ±0.3	0.492 ±0.07	1.83 ±0.6	0.48±0.06	3.88 ± 1.3
Roasted	0.64 ±0.3	0.33 ±0.07	0.63 ±0.6	0.28 ±0.06	1.88 ±0.6
Soaked	2.01 ±0.3	0.60 ±0.07	2.08 ±0.6	0.58 ±0.06	5.27 ±0.6
Without treatment	2.14 ±0.3	0.66 ±0.07	2.12 ±0.6	0.64 ±0.06	5.56 ±0.6

TABLE 4. Reduction of aflatoxins as affected by treatments.

Treatment	Aflatoxins reduction %				
	B1	B2	G1	G2	Total
Blanched	15.89	22.42	13.68	20.63	30.22
Roasted	70.09	50.00	70.28	56.25	66.19
Soaked	6.07	9.09	1.89	9.38	5.22

In our study, the blanching in boiling water for 45 minutes was reduced the AFs content in the tigernuts by 30%; as the AFs content was reduced from 5 µg /kg to 3.855 µg /kg. In this study, all types of AFs in tigernuts showed some degradation by blanching and the highest reduction percentages were observed in AFB_2 and AFG_2 (37.58 and 36.25% respectively). The reduction in AFs content in tigernuts by blanching is attributed to the presence of water with heating that helps in opening the lactone ring in AFB_1 by the addition of a water molecule to the ring to form a terminal carboxylic acid, and the terminal carboxylic acid group thereafter undergoes heat-induced decarboxylation (Kabak et al., 2006). Our results are paralleled with the results of Stoloff and Trucksess (1981), who observed that boiling corn grits gave an average reduction of aflatoxins by 28%.

Roasting is one of the most effective physical methods to reduce AFs content in tigernuts, which reduces possible health risks from AFs to the consumers. As presented in Tables 3 and 4, roasting at 130 ± 5°C for 30 minutes, reduces the amount of AFs in artificially contaminated tigernuts from 5 µg /kg to 1.65 µg /kg; about 70% of AFs in samples were decreased. A similar reduction in the levels of AFB_1, AFB_2, AFG_1 and AFG_2 were observed, and their reduction percentages were 70.09, 72.73, 70.28 and 68.75% respectively. A similar result was recorded by Yazdanpanah et al., (2005) when roasting pistachio nuts at 90, 120 and 150° C for 30, 60 and 120 min that resulting in reduction the AFs content of nuts by 17-63%, and they added that the reduction in AFs content is being dependent on time and temperature. Furthermore, Hussain et al., (2011) reported that roasting resulted in a significant decrease in the AFs content of nuts, corn and oilseed meals. Although most mycotoxins are chemically and thermally stable and conventional food preparation with temperatures up to 100 °C have little effect on most mycotoxins, the higher temperatures used in frying, roasting, toasting, and extrusion might reduce mycotoxin contamination (Petr et al., 2016), that could be given an acceptable explanation for our results.

The AFs concentration was reduced in tigernuts by soaking in water from 5 µg /kg to 4.84 µg /kg with reduction percent of 12.95% as shown in Tables 3 and 4. The soaking in water for 48 hours at room temperature, as physical method has the lowest effect on the reduction AFs content in tigernuts; due to the low solubility of AFs in water. On the other hand, Hwang, 2006 reported that washing of mycotoxins-contaminated wheat were reducing of AFB_1 by about 40% as the AFs are usually attached on surface of wheat, so it's possible to remove them by washing but it is very difficult to remove aflatoxins bonded or attached strongly to the inner texture of food (Fandohan, 2005). So, it is generally hard to remove AFs by soaking.

Conclusions

This work revealed that raw tigernuts had higher phenolic compounds than treated samples, but the decrease in some phenolic compounds were not observed in the soaked and roasted samples as was observed in the boiling water blanched ones. Antioxidant activity (AOA) of tigernuts is strongly affected by the type of treatment. Blanching, roasting and soaking are physical methods used to decrease aflatoxins (AFs) content in tigernuts tubers. Because roasting and blanching processes had more desirable effects on antioxidant activity and aflatoxin reduction it should be encouraged as best methods for preparing tigernuts for human consumption.

References

Akowuah, G.A., Ismail, Z., Norhayati, I. and Sadikun, A. (2005) The effects of different extraction solvents of varying polarities of polyphenols of Orthosiphon stamineus and evaluation of the free radical-scavenging activity. *Food Chemistry* **93,** 311-317.

Dada, O.S.O. and O.O. (2015) Antioxidant properties and consumer acceptability of pearl millet – tigernuts biscuits. *Nutr. Food Sci.* **45**, 818–828.

Pankaj, S.K., Shi, H., Keener, K.M. (2018) A review of novel physical and chemical decontamination technologies for aflatoxin in food. Trends Food Sci. Technol. 71, 73–83. https://doi.org/10.1016/J.TIFS.2017.11.007

Dorman, H. J., Bachmayer, O., Kosar, M., and Hiltunen, R. (2004) Antioxidant properties of aqueous extracts from selected lamiaceae species grown in Turkey. *Journal of Agricultural and Food Chemistry* **52,** 762- 770.

Fandohan P. Zoumenou D., Hounhouigan D.J., Marasas WF, Wingfield MJ, Hell K. Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. *Int. J. Food Microbiol.* 2005; **98**(4): 249–59.

Hakkinen, S. H., Karenlampi, S. O., Heinonen, M., Mykkanen, H. M., & Torronen, A. R. (1998) HPLC method for screening of flavonoids and phenolic acids in berries. *Journal of the Science of Food and Agriculture,* **77,** 543–551.

Hussain A, Ali J, Akther S, Shafqatullah, Degradation of aflatoxins by roasting in contaminated peanuts. *Pak. J. Biochem. Mol. Biol.* 2011; **44** (2): 56-59.

Hwang JH, Lee KG. Reduction of aflatoxin B1 contamination in wheat by various cooking treatments. *Food Chem*. 2006; **98** (1): 71–75.

Iso 16050 (2003) Foodstuffs Determination of aflatoxin B1, and the total content of aflatoxins B1, B2, G1 and G2 in cereals, nuts and derived products - High-performance liquid chromatographic method. (https://www.iso.org/standard).

Kabak B, Dobson ADW, Var I. Strategies to prevent mycotoxin contamination of food and animal feed: A review Crit. Rev. Food Sci. Nutr. 2006; **46** (8), 593-19.

Kapseu, C., Mbofung, C. M. F., and Kayem, G. J. (1997) Fatty acids and triglycerides of fruit oils from Cyperus esculentus and Balanites aegyptiaca. *Sciences des Aliments,* (5), 531–537.

Lemos, M. R. B., Egle, M. A. S., Sandra, F. A., and Rui, C. Z. (2012) The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts [Dipteryx alata Vog.]. *Food Research International* **48**, 592–597.

Lima, A. R., Pereira, A. F. P., Abrahão, A. S., Duarte, S. M., and Paula, F. B. A. (2010) Coffee bioactive compounds: in vitro antioxidant activity of green and roasted coffees before and after decaffeination. Química Nova, 33, 20–24.

Lima, A., Silva, A. O., Trindade, R. A. T., Torres, R. P. T., and Mancini-Filho, J. (2007) Composição química e compostos bioativos presentes na polpa e na amêndoa do pequi (*Caryocar brasiliense, Camb.). Revista Brasileira de Fruticultura,* **29** (3), 695–698.

Luo, C., Brink, D. L., and Blanch, H. W. (2002) Identification of potential fermentation inhibitors in conversion of poplar hydrolizate to ethanol. *Biomass and Bioenergy*, **22**, 125–138.

Mazzeo, T., N'Dri, D., Chiavaro, E., Visconti, A., Fogliano, V., and Pellegrine, N. (2011) Effect of two cooking procedures on phytochemical compounds, total antioxidant capacity and color of selected frozen vegetables. *Food Chemistry,* **128,** 627–633.

Miriam Rejane B. L., Egle Machado A. S., Sandra Fernandes A., and Rui Carlos Zambiazi (2012) The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts (Dipteryx alata Vog.) *Food Research International,* **48**, 592–597.

Nicoli, M. C., Anese, M., and Parpinel, M. (1999) Influence of processing on the antioxidant properties of fruits and vegetables. *Trends in Food Science & Technology,* **10**, 94–100.

Pankaj, S.K., Shi, H., Keener, K.M. (2018) A review of novel physical and chemical decontamination technologies for aflatoxin in food. Trends Food Sci. Technol. 71, 73–83. https://doi.org/10.1016/J.TIFS.2017.11.007.

Petr K., Michele S., Franz B., Johan M., Gerhard E., Irène P., Isabelle P. O., Gerrit S., Alessandro C., Tobias R. and Pierre D. (2016) Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Res* (2016) **32**, 179–205.

Ranalli, L. Pollastri, S. Contento, E. Iannucci, L. and Lucera. (2003) Effect of olive paste kneading process time on the overall quality of virgin olive oil. *Eur J Lipid Sci Technol.* **105**, 57–67

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine,* **26** (9-10), 1231-1237.

Siqueira, E.M., Marin, A.M., Arruda, S. F., Cunha, M. S., Fustinoni, A.M., and Dourado, L.P. (2012) Consumption of baru seeds [Dipteryx alata Vog.], a Brazilian savanna nut, prevents iron-induced oxidative stress in rats. *Food Research International,* **45**, 427–433.

Stoloff, L. and Trucksess, M.W. (1981) Effect of boiling, frying, and baking on recovery of aflatoxin from naturally contaminated corn grits or cornmeal. *Journal Association of Official Analytical Chemists* **64** (3), 678–680

Temple, V. J., Ojobe, T. O., and Kapu, M. M. (1990) Chemical analysis of tigernuts (Cyperus esculentus). *Journal of the Science of Food and Agriculture,* **50** (2), 261–263.

Turkmen, N., SariI, F., and Velioglu, Y. S. (2005) The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chemistry,* **93**, 713–718.

Yazdanpanah, H., Mohammadi, T., Abouhossain, G., Cheraghali, A.M. (2005) Effect of roasting on degradation of aflatoxins in contaminated pistachio nuts. *Food and Chemical Toxicology* **43,** 1135–1139.

Xu, B., and Chang, S. K. C. (2008) Total phenolics, phenolic acids, isoflavones, and antioxidant properties of yellow and black soybeans as affected by thermal processing. *Journal of Agricultural and Food Chemistry,* **56**, 7165–7175.

Zambiazi, R. C. (1997) The role of endogenous lipid components on vegetable oil stability. These in Foods and Nutritional at the Sciences Interdepartmental Program. Manitoba, Canada: University of Manitoba Winnipeg [http://hdl.handle.net/1993/931].

(*Received*:11/12/2018;

accepted:10/01/2019)