ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Behavior of Ochratoxin A (OTA) during bread making enrichment with some natural antioxidants.

Abou-Baker Salim[1], Mona E.M. Naga[2], and Mohamed T. Fouad[3].

[1]Department of Food Toxicology and Contaminants, Food Industries and Nutrition Division, National Research Centre, Dokki 12311, Giza, Egypt.
[2]Faculty of Specific Education, Minuteufiya University, Egypt.
[3]Department of Dairy Science, Food Industries and Nutrition Division, National Research Centre, Dokki 12311, Giza, Egypt.

ABSTRACT

Ochratoxin A is one of the most carcinogenic mycotoxins produced by several *Aspergillus* and *Penicillium* species as the product of secondary metabolism. It recorded as a global contamination of a wide variety of foods and feeds. Bread is one of the most important grain products where it is a staple food for most people. It is worth mentioning that bread as one of the major entry of daily intake of Ochratoxin A; mainly comes from its being in wheat grain or flour which partially eliminated through the bread making process. Therefore the objective of this investigation was to evaluate the efficiency of some natural antioxidants (curcuminute, ginger and thyme) on degradation of Ochratoxin A during bread making. This was done by using wheat flour naturally contaminated with Ochratoxin A (9.13±1.5) collected from local market. Ochratoxin A level in flour fermented kneading and bread were analyzed by high performance liquid chromatography (HPLC). Average recoveries ranged from 95% to 101% with low RSD% ranged from 2.1 to 3.2%. Considerable reduction in Ochratoxin A level ($p < 0.05$) during fermentation of the kneading with yeast (*Saccharonamyces cerevisiae*) was observed. It ranged from 46.63% to 55.63% depending on the concentration of yeast. However addition of 0.5% curcuminute, 0.5% ginger or 0.5% thyme at second proof of fermentation process highly reduced Ochratoxin A levels (83.56%, 84.11% and 86.85% respectively). The highest reduction for thyme followed by ginger and curcuminute, but no significant differences ($p > 0.05$) between them. Baking breadat different temperature/time (240°C /18 minute, 260°C /16 minute, 280°C /14 minute, 300°C /12 minute, 320°C /10 minute, 340°C /8 minute °C, 360°C/ 6minute and 380°C/4minute) showed reduction % for all baking process (fermentation + baking) ranged from % 56.63 to 63.27 % depending on the temperature and time of baking but not significant differences ($p > 0.05$).It was observed that baking stage had the least effect on reduction of Ochratoxin A. However, there was complete degradation of Ochratoxin A in bread enrichment with natural antioxidants, curcuminute, ginger and thyme baked at 380°C/4 minute;it may be then possible to introduce modifications as addition of natural antioxidants thyme, curcuminute and ginger to Commercial Manufacturing that result in a decontamination of Ochratoxin A from bread and bakery products. In conclusion: in staple food such as bread baker's yeast *Saccharomyces cerevisiae*a long with natural antioxidants curcuminute, ginger and thyme may play an important role for use as a biodegradation and detoxifying of Ochratoxin A Give us food safety and the protection of consumer health.

Keywords: Ochratoxin A, bread and bakery products, *Saccharonamy cescerevisiae* fermentation, curcuminute, ginger and thyme

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cereal crops represent global importance for human nutrition is also considered the most important group of food crops produced in the world [1, 2]. Because of the universal significance of cereals in the food and feed and their receptivity to attacked by fungi, in specific environmental situations, moreover the excretion of mycotoxins that can remain firm from the crops to the final products [3]. Ochratoxin A occurs in a variety of foodstuffs and beverages including an assortment of cereals, beans, grain-based food stuff, coffee, beer, wine, meat, cocoa, dried fruits, spices, nuts, milk, and some tissues of animal origin [4-9].Moreover, cereal products represent one of the main sources of exposure to Ochratoxin A[10].Different studies show the high presence of mycotoxins, especially Ochratoxin A, in products of high consumption like bakery products[11, 12]. One of these products is bread. Wheat bread supply more nutrients to humanness than any other single food source [13]. Bread is especially important as a source of carbohydrates, proteins and vitamins B and E [14]. Zinedine *et al*., [7] found that 48 % from analyzed bread samples were contaminated with Ochratoxin A at level in positive samples ranged from 0.00014 and 0.149 ppb/kg. The wheat, like the majority of cereals, is susceptible to be contaminated with mycotoxins [15, 16]. The European Commission has set maximum permitted levels in processed cereal products for direct human consumption in Ochratoxin A of 3 ppb in bread [17, 18].

Ochratoxin A (OTA) is a toxic secondary metabolite of several species of the genus Aspergillus such as *Aspergillus ochraceus*, *Aspergillus sulphureus*, *Aspergillus carbonarius*, *Aspergillus niger* and *Aspergillus sclerotiorum* and *Penicillium* species *Penicillium verrucosum* and *Penicillium nordicum* frequently present in food and feeds [19-21]. Ochratoxin A is a mycotoxin believed to be nephrotoxic, carcinogenic also it can cross the placenta, and as embryotoxic and teratogenic [22].In 1993, the International Agency for Research on Cancer classified Ochratoxin A as a possible human carcinogen [20]. Ochratoxin A exerts several toxicological effects such as nephrotoxic [23], hepatotoxic [24], immunotoxic [25], neurotoxic [26], carcinogenic [27] and teratogenic [28]. It has been related to Balkan endemic nephropathy [29, 30].

The certain optimum conditions such as temperature, moisture, oxygen, time and nutrient are needed to be available to production Ochratoxin A. Moreover, it is possible to detect Ochratoxin A without the presence of the producing mold since chemical processes or environmental changes can suppress the mold spores but not alter the toxin that remains in the food substance [31]. Duarte *et al*., [32] indicated that despite the conduct of many research and employing good agricultural practices (GAP) in the production, processing, and Good Manufacturing Practice (GMP) in the storage and distribution of food, the mycotoxins still represents a threat to both human and animal health. It seems that the possibility of reducing the presence of Ochratoxin A during the thermal treatment, which are food for the manufacture completely variable and dependent on processing Circumstances have been used: temperature, time, type of mycotoxin, and size of cereal product. Ochratoxin A was relatively stable at high temperature through baking [32, 33].

Different methods were developed to counteract the toxicity of contaminated product and improve food safety [34].However, the detoxification process is associated with a loss of palatability and nutritional values; it may then be possible to develop modifications to industrial processes that result in a big reduction of mycotoxin content in the retail product. One of the nutritional approaches to decrease mycotoxin toxicity is addition of nutrients with protective properties to contaminated foodstuffs [35].A new procedure under study is the use of antioxidants, such as vanillic acid or 4-hydroxybenzoic acid [36], and essential oils extracted from plants, such as *Thymus vulgaris*, *Aframomumdanielli* [37, 38], cinnamon, and clove leaf [39], which inhibits mold growth and Ochratoxin A production. Therefore the objective of this investigation was to evaluate the efficiency of some antioxidants from natural herbal (curcuminute, ginger and thyme) on biodegradation of Ochratoxin A during bread making.

MATERIALS AND METHODS

Ochratoxin Acontaminated flours

Twenty kilograms were collected from the local market. A representative sample of about 2kg was examined for Ochratoxin A. It was found naturally contaminated with Ochratoxin A at mean level of 9.13±1.5ppb.

Chemicals and standard

Chemicals and solvents were of HPLC grade or equivalent. All water used was deionized and for HPLC, obtained from a Milli-Q purification system (Millipore, London, United Kingdom). Acetonitrile, methanol, sodium acetate and acetic acid used for mobile phases were of HPLC grade and provided by Fisher Scientifics (Fisher chemicals HPLC, United States).Ochratoxin A (powder; CAS No. 303-47-9; purity of ≥ 98%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The standard of Ochratoxin A was dissolved in methanol at a concentration of 5.0 mg/ml and stored at 4 °C in a sealed vial until use. Working standard solutions were prepared by appropriate dilution of known volumes of the stock solution with the HPLC mobile phase and used to obtain calibration curves in the appropriated chromatographic system.

Plant materials

Ginger (*Zingiberofficinale Rosc*.), *Curcuma longa* L (turmeric) and Thyme (*Thymus vulgaris)* dried were obtained from Saudi Arabian market. All plants were in high quality, clean and free from any mycotoxins and fungi.

Kneading preparation and bread making

The kneading made from wheat flour, with different formula (1% baker's yeast; 1.5% yeast; 0.5% curcuminute+ 1.5% yeast; 0.5% ginger+ 1.5% yeast and 0.5% thyme+ 1.5% yeast), 1 % sodium chloride, 1% sugar and water as need. Kneading ingredients were mixed and manually kneaded until join together with a non-sticky, sleek and satiny semblance and optimum handling properties. It was covered with a wet cloth and left for fermentation at 30°C for 1hr. After fermentation, the kneading was divided into 130-150 g pieces. In this stage of fermentation the natural antioxidants were added because their additions in the first stage of fermentation lead to inhibition of the activity of yeast. Each segment was molded on a plastic boards prior covered with a thin layer of bran and left to ferment about 20minute at 30°C. The fermented kneading pieces were flattened and left for final proofing for 40 minute then baked at eight temperature levels (240°C /18 minute, 260°C /16 minute, 280°C /14 minute, 300°C /12 minute, 320°C /10 minute, 340°C /8 minute °C, 360°C/ 6minute and 380°C/4minute). Samples were taken from fermented kneading and bread loaves and stored at -20 °C until Ochratoxin A analysis.

Ochratoxin A extraction, clean-up and analysis

Samples were extracted and clean up according to Vicam instruction manual with some modifications. Briefly, 10 g of ground samples were extracted with 30 ml of extraction solution (60% acetonitrile, 40% water) in high speed blender for 5 minute. The extract was filtered through filter paper and all filtrate was collected and centrifuged at 6000 rpm for 6 minute at 4 °C (Hermale Z 200A, Germany). Supernatant (10 ml) was diluted with 40 ml of Phosphate buffer saline (PBS) solution and drained through the Immunoaffinity chromatography column (IAC). After this, the column was washed with 20 ml of PBS and Ochratoxin A was eluted by applying 1.5ml of methanol grade HPLC (three times back flushing) and 1.5 mlofmilli-Q water, consecutively. The purified extract was dried under nitrogen stream. Each dried sample was re-suspended with acetonitrile: water: acetic acid (57:41:2). Analysis was performed using an HPLC instrument consisting of Water Binary Pump model 1525, a model Waters 1500 Rheodyne manual injector; Water 2475 multi wave length fluorescence detector and a data workstation with software Breeze. A phenomoex C18 (250 X4. 6 mm I,d), particle size 5 µm from Waters Cooperation USA. Water was purified through a Milli- Q treatment system (Millipore, London, U.K.) and Phosphate Buffered Saline (PBS) was prepared as per Vacuum (NaCl 8 g/ l, KCl 0.2 g/ l, Na2HPO4 1.15 g/ l, KH2PO4 0.2 g/ l; pH 7.4).For recovery calculation, Ochratoxin A free samples spiked at 2 ng /g were extracted as it described previously.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and computing using the SAS General Linear Model producer[40].Significance was set at ($p<0.05$).

ISSN: 0975-8585

RESULTS AND DISCUSION

The mean Ochratoxin Aextraction recoveries are presented in Table (1). Recoveries at 2 ng/g spiking level were 98 ± 3.1, 96 ± 3.5 and 101± 2.4 for wheat flour, fermented kneading and bread with low RSD% 3.2, 3.64 and 2.38 respectively such recoveries were found to be fully compliant with EC directives[17].

Table 1: Percentage Mean recovery of OTA for Flour, Fermented kneading and bread at 2ng/g spiking level.

Samples	Mean recovery% ±SD	RSD%
Flour	98±3.1	3.2
Fermented kneading	96±3.5	3.64
Bread	101±2.4	2.38

Results are the mean ± standard deviations of three replicates

Despite the fact that amount of free water in dry flour causing inhibit to mold activity or Ochratoxin A secretion, wheat flour used in this study was naturally contaminated with mean level 9.13±1.5ppb which is much higher than the European guideline level (3ppb) [41]. This may be due to bad storage of flour which support mold growth and mycotoxin production. Storage, condition at the farm, on the manufacturing building or in the grocery store, is one of the most sensitive post-harvest phases in food handling. Inadequate environmental conditions, incorrect packaging or bad foodstuffs maybe this reasons lead to mycotoxin contamination through this period [42, 43]. Cengiz*et al*., [44] determinates Ochratoxin A levels in 132 various Kinds of flour and bread (34 white flour, 14 whole meal flour, 10 corn flour, 36 white bread, 28 whole meal bread and 10 corn breads in Turkey. greatest average concentricity was appeared in whole meal flour samples (9.30 ppb). Ochratoxin A has been found in 110 samples (83%). In 92 (70 %) of the samples the toxin levels were overridden maximum acceptable limits (3 ppb). Also, Riba*et al*., [45] showed that flour samples in North Africa included a more repetition of ochratoxigenic species of *Aspergillus*. In addition, Hashemi-Karouei*et al*., [46] found that wheat flour contaminated with 48.8 ppb Ochratoxin A. Several studies have considered bread one of the major gates of daily intake of Ochratoxin A, [46-48].The existence of Ochratoxin A in baking fundamentally arrive from the wheat meal used for its making duo to being in cereal or in flour is slightly reduced through the bread making operations [49].These results suggest the possibility that the health of consumers at risk as a result of contamination of flour and bread.

Effect of yeast (*Saccharomyces cerevisiae*) on Ochratoxin A reduction during kneading fermentation.

The results in Figure 2 illustrated the effect of baker's yeast *Saccharomyces cerevisiae* on biodegradation of Ochratoxin A after fermentation stage (2 hrs.) during bread making. There was a significant reduced ($p < 0.05$) in Ochratoxin A level by 46.64% and 55.62% for 1% and 1.5% yeast respectively, according to the quantity of yeast utilized to contaminated flour. This result was higher than obtained by Valle-Algarra*et al*.[50] who found that through kneading fermentation (30 minute) resulted a considerable reduction ($p < 0.05$) of the Ochratoxin A quantity which ranged from 29.8% to 33.5%Ochratoxin A reduction after fermentation for 1hr. This is may be due to the different in fermentation time. likewise found that Ochratoxin A reduction through fermentation due to yeasts is Relies on the specific strain take part in fermentation process [51-53].Track biodegradation is probable for *S. cerevisiae* in addition to the Ochratoxin A adsorption happening[54].The adsorption of Ochratoxin A to yeast cells walls (composed of β-glucans and mannan oligosaccharides) was reported by Huwing *et al.*[55]and Nunez *et al.* [56], as mentioned by Ringot *et al*. [57], yeast cells may be considered a good stock of adsorbent substances, because of the outer membrane consist from some special large molecules, like manno proteins and beta glucans. Abrunhosa, *et al*., [58] reported that several types of bacteria, yeast and filamentous fungal are capable to biodegrade Ochratoxin A by the hydrolysis of the amide bond that links the L-β-phenylalanine molecule to the Ochratoxin alpha (Ochratoxin α; where the phenylalanine moiety is missing) (Figure1). Whereas Ochratoxin α and L-β-phenylalanine are practically non-toxic, this technicality can be helpful to removing toxic from Ochratoxin A.

ISSN: 0975-8585

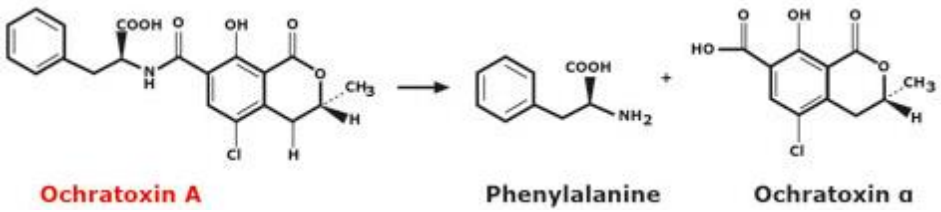


Figure1: Biotransformation of Ochratoxin A into a detoxified form (Ochratoxin alpha)

Efficiency of addition natural antioxidants on destruction of Ochratoxin A during kneading fermentation

Data in Figure (3) demonstrated that addition of 0.5% curcuminute, 0.5% ginger or 0.5% thyme at second proof of fermentation process using 1.5% yeast / 2hr significantly reduced ($p < 0.05$) Ochratoxin A levels (83.56%, 84.11% and 86.85% respectively).The highest reduction for thyme followed by ginger and curcuminute, but no significant differences ($p > 0.05$) between them.Plant substances proven ability to inhibit fungal growth and Ochratoxin A production. 1% oils of thyme and anise and 2% oil of cinnamon Achieved a full stop to the secretion of Ochratoxin A in wheat [59].Pereira et al., [60], showed that wild thyme essential oil reduced Ochratoxin A production by 75 % and 100 %. Also, Sokolić-Mihalak, *et al*., [61] reported that thyme and total phenol extracted from wild thyme showed Ochratoxin A inhibition in ranging from 58 % to 77 %. In addition, Jeršek *et al*., [62] found that after 21 days of incubation, the extreme inhibitory effect on ochratoxin production (inhibition was 96.9 %) was achieved with thyme at 0.25 MIC (0.0313 mg/mL). The antioxidant, antibacterial, and antifungal activities of thyme are related to its phenolic compounds, particularly thyme, carvacrol and *p*-cymene.

The rhizomc of *Curcuma longa* L (turmeric) has been by a large degree known as a condiment and coloring agent in varyfoods. The effective components of turmeric are the flavonoid, curcuminute (diferuloylmethane), phenolic compounds, terpenoids and various volatile oils, including tumerone, atlantone and zingiberone. Their main yellow bioactive materials of raw tumeric are curcuminute and two related demethoxy components, demethoxycurcuminute and bisdemethoxy curcuminute [63, 64]. The plant extract from curcuminuteoids has strong antifungal activity [65], and antioxidative[63, 66, 67]. Also, Ginger, the dried rhizome of the plant (*Zingiberofficinale Roscoe, Zingiberacae)*, is one of the most widely spices used everywhere in the world and is a common seasoning for a vary types of foods and beverages [68, 69].Recently, ginger has drawn more attention because of its significant antioxidant activity [70-73]. Its roots and the obtained extracts contain volatile essential oils include zingeberene, curcumene and farnesene[74]and nonvolatile bioactive polyphenol compounds (gingerol,shogaol and theirderivatives), which have a high antioxidant activity. This was attributed to the presence of α, β-unsaturated ketones moieties of (6)–shogaol, and the presence of short carbon chains of (6)–gingerol and (6)–shogaol, which made their antioxidants more potent than the other four long carbon chain compounds [73]. It was indicated that the inhibitory effects for spices and herbs has been due to, phenolic compounds, such as coumarins and flavonoids [75]. Certainly, flavonoids, including rutin, quercetin, and caffeic acid established that prevented *A. carbonarius* growth and secretion of Ochratoxin A [76]. furthermore, alkaloids produced by Piper longum, and ingredients of sesame oil and turmeric have also been found to inhibit both fungal growth and ochratoxin production in a number of Ochratoxin A-producing aspergilla [77, 78].

Heat stability of Ochratoxin A during baking at different temperature/time

After fermentation, the bread was breaded at various series from temperature/time(240°C /18 minute, 260°C /16 minute, 280°C /14 minute, 300°C /12 minute, 320°C /10 minute, 340°C /8 minute °C, 360°C/ 6minute and 380°C/minute). The results in Figure 4 indicated that baking stage had the least effect on reduction of Ochratoxin A. The reduction % for all baking process (fermentation+baking) ranged from % 56.63 to 63.27 % depending on the temperature and time of baking but not significant differences ($p > 0.05$). The lowest reduction was for baking at 240 ºC/18minute and the highest reduction at 360 ºC/6 minute. It was reported that Ochratoxin A is a thermo-stable molecule [79, 80] where not totally removed through the food processing and, so that, it arrive in the end products [81]. Scudamore *et al*., [82] illustrated that scouring removed up to 44% of Ochratoxin A but only a small further lose occurred in the bread-making process. In addition, Vidal et al., [32] confirmed the high stability of Ochratoxin A as no significant change in its content

could be observed as a result of the bread-making process. On the other hand, Hashemi-Karoueiet *al*., [46] proved that reduction in the amount of Ochratoxin A in samples during the heating process was significant and longer duration of heating was more effective, than raising the temperature, on Ochratoxin A reduction.

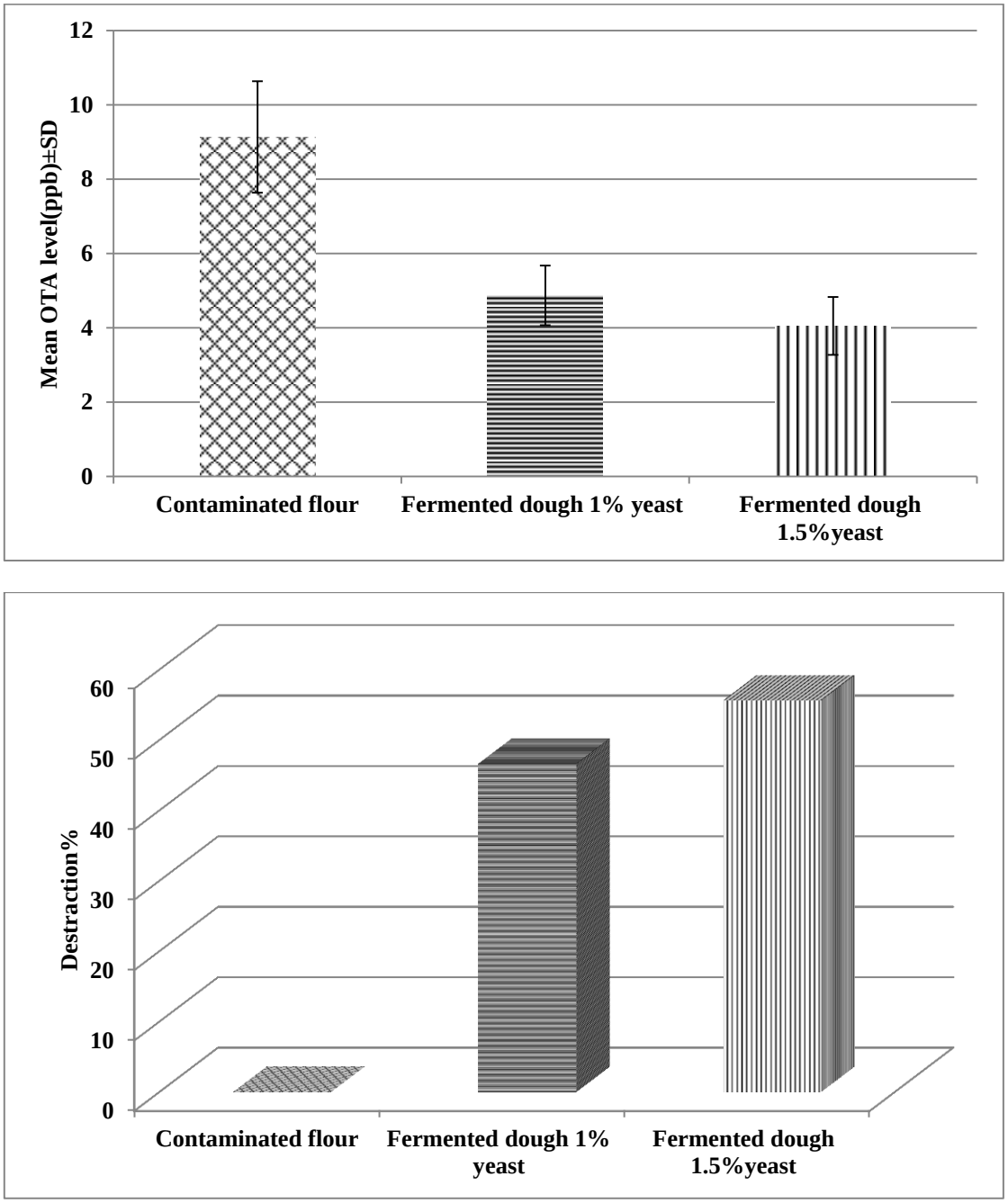
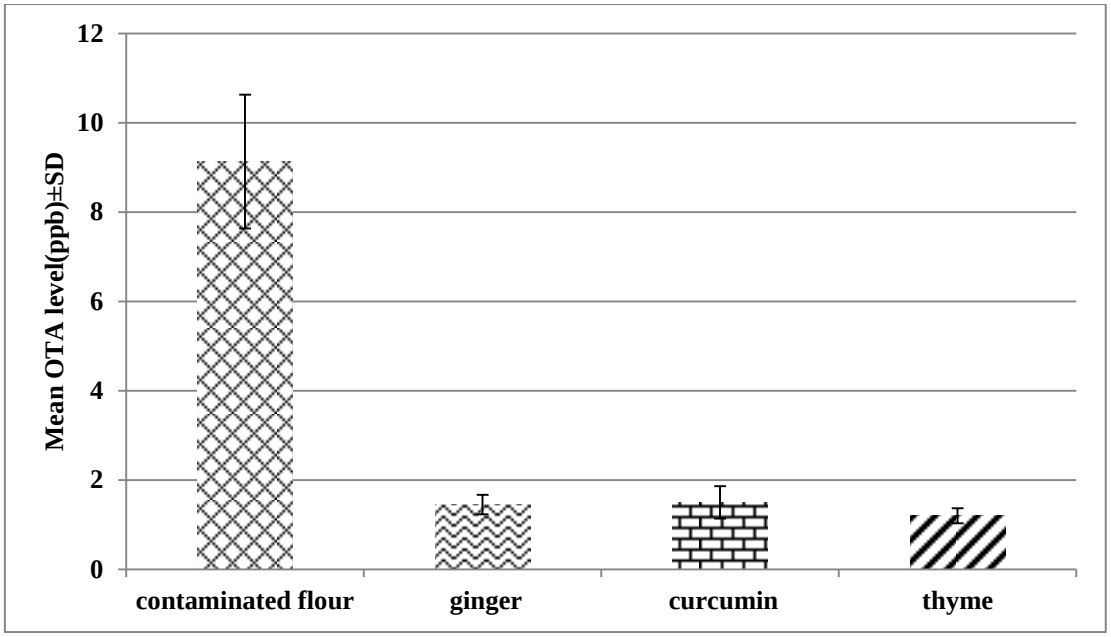


Fig. 2 Effect of yeast (*Saccharomyces cerevisiae*) on reduction of OTA in Fermented kneading after 2 hr. /30°C.

ISSN: 0975-8585



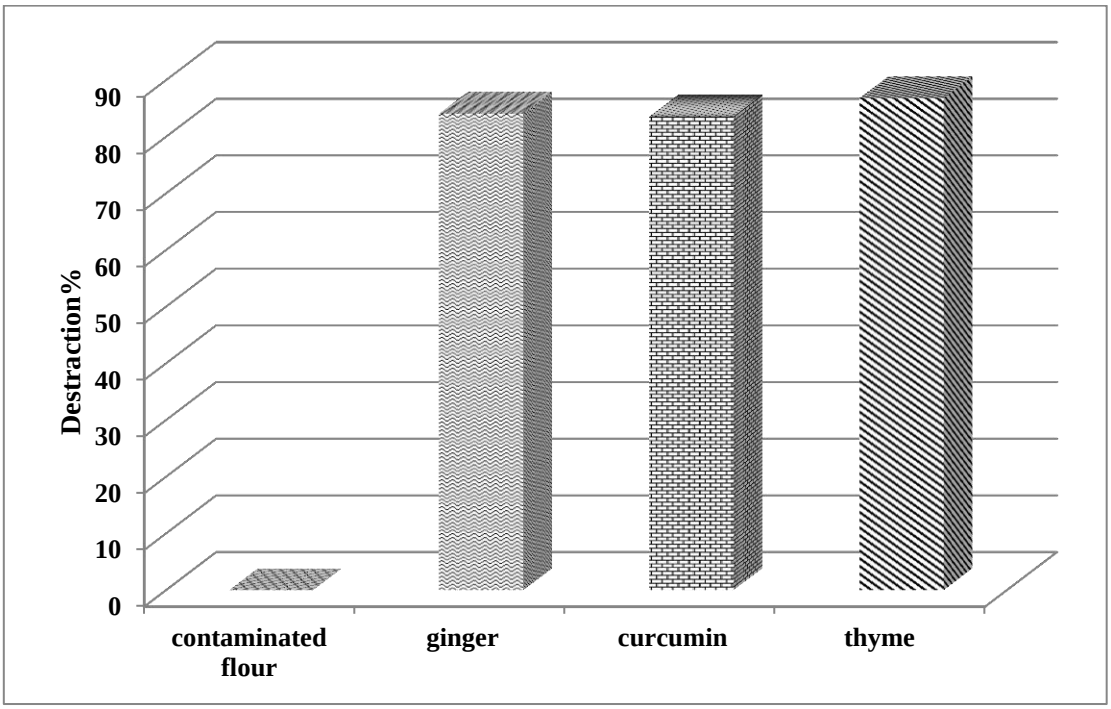
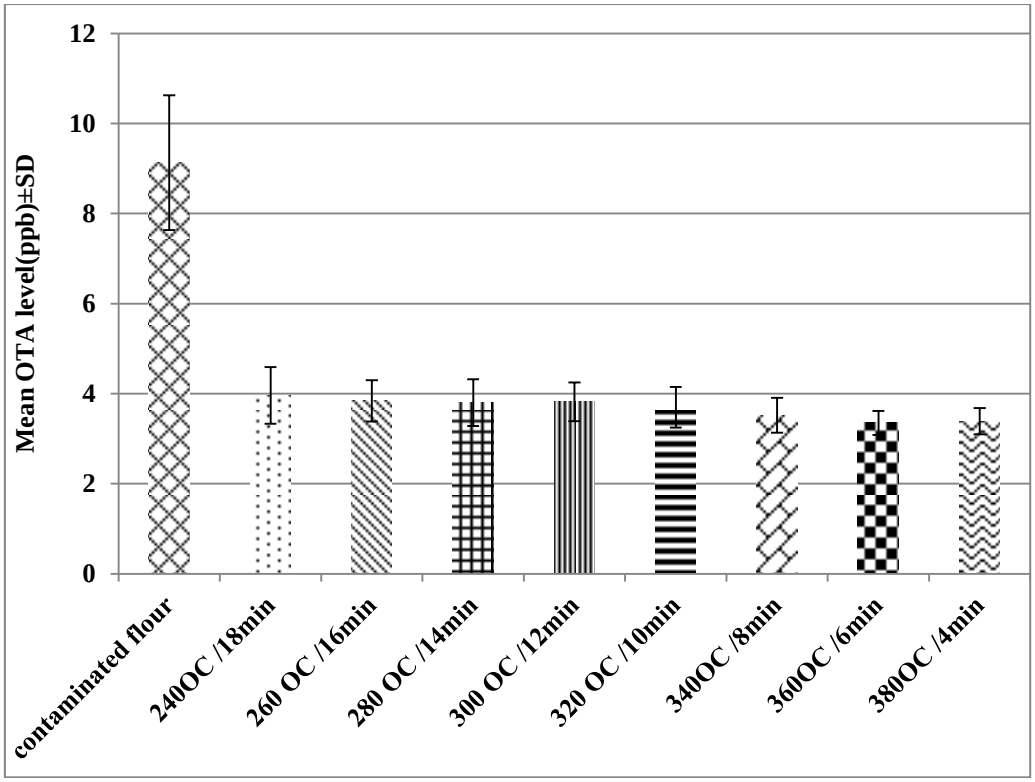


Figure 3: Efficiency of 0.5% different natural antioxidants on destruction of OTA in kneading 1.5%yeast.

Effect of addition of natural antioxidant on OTA level in bread

Wheat cereals are the substances which give a maximum level to daily intake from Ochratoxin A [83]. Through the white baking making, cereal purifying by grabbing of the brain and offal fragments reduced Ochratoxin A up to 25% of the first level [82]. Related to heat process through flour and baking manufacture, they don't have influence on the Ochratoxin A levels [82, 84, 85]. Therefore, it perhaps need to insert alteration to commercial procedures that achieve a big decrease of mycotoxin levels in the retail product as shown in Table 2 complete degradation of Ochratoxin A on bread Supported by 0.5% curcuminute, 0.5% ginger or 0.5% thyme baked at 380°C/4 minute. These results indicate that natural antioxidants thyme, curcuminute and ginger is not only one of the simplest and inexpensive ways to food protection in addition to give more nutritional and organoleptic benefits to foods but also protective effect against Ochratoxin A toxicity, providing food safety and to protect consumer's health.

ISSN: 0975-8585



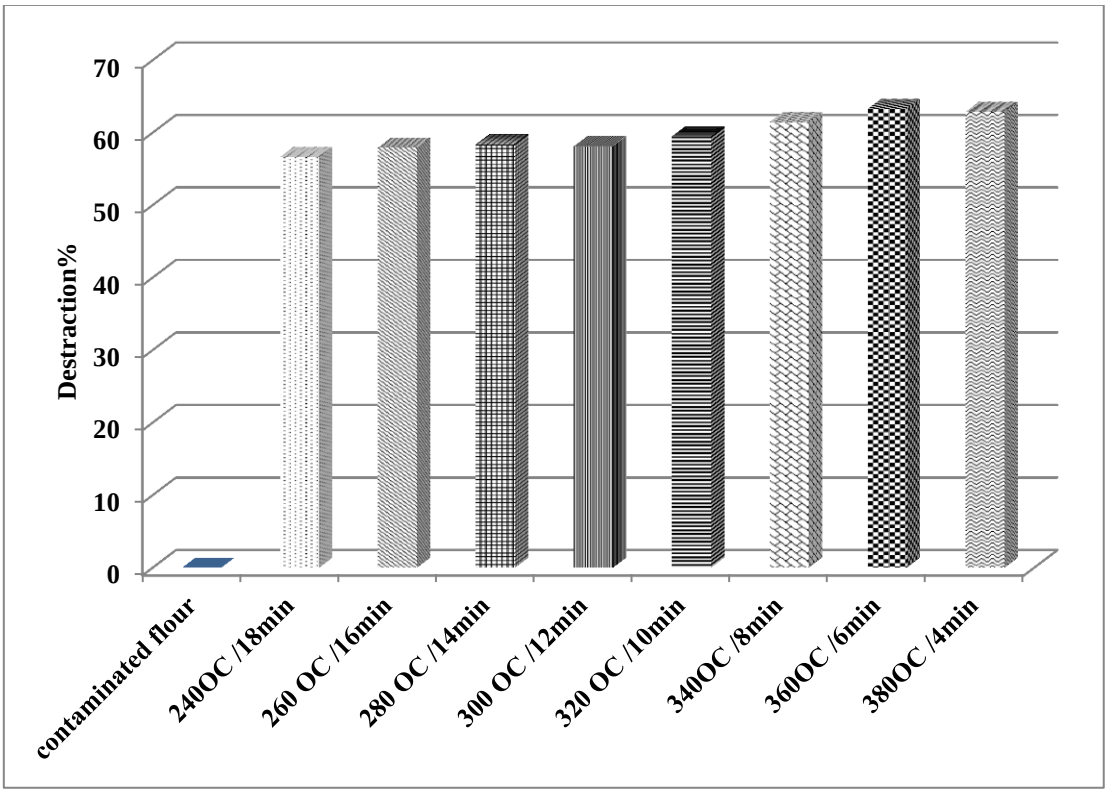


Fig.4: Stability of OTA during bread baking at different temperature / time

Table2: Behavior of OTA during bread baking Supported by natural antioxidants.

treatment	Mean OTA level(ppb)±SD	Distraction%
contaminated flour	9.13±1.5	0
0.5% Ginger	ND	100
0.5% Curcuminute	ND	100
0.5% Thyme	ND	100

Results are the mean ± standard deviations of three replicates

From the above results it could be concluded that in main food such as bread baker's yeast *Saccharomyces cerevisiae* along with natural antioxidants curcuminute, ginger and thyme play an important role for use as a bioremediation and detoxifying This kind of bioremediation could hence establish a useful strategy for partly exceed the problem of some mycotoxins and offer new methods to decrease mycotoxins (Ochratoxin A) in baking and bakery products. The most important, *S. cerevisiae* are the main yeast participatory for leavening food in equatorial nations with high content of mycotoxin contamination in their foods, and strains of *S. cerevisiae* isolated from local fermented foods can be used as starter cultures with more ability to removal mycotoxins from the food.

REFERENCES

[1] FAO http://www.fao.org/docrep/x2184E/x2184-00.HTM. 1999; Retrieved June 30.
[2] Duarte SC, Pena A and Lino CM. Food Microbiol. 2010; 27(2): 187-98.
[3] Molinié A, Faucet V, Castegnaro M and Pfohl-Leszkowicz A. Food Chem., 2005; 92: 391–400.
[4] JECFA http://www.inchem.org/documents/jecfa/jecmono/v47je04.htm 2001; accessed on 27-07-2012.
[5] Gonzalez L, Juan C, Soriano JM, Moltó JC and Manes J. Int J Food Microbiol. 2006; 107: 223-227.
[6] Tjamos SE, Antoniou PP and Tjamos EC. Int J Food Microbiol.2006; 111(1): 61-66.
[7] Zinedine A, Soriano JM, Juan C, Mojemmi B, Moltó JC, Bouklouze A, Cherrah Y, Idrissi L, El Aouad R and Mañes J. Food Addit. Contam 2007; 24(3):285-299.
[8] Marin DE, Ţăranu I, Tabuc C. and Burgehelea M. Arch. Zootech. 2009; 12(1): 5-17.
[9] Haighton LA, Lynch BS, Magnuson BA and Nestman ER. Crit. Rev. Toxicol. 2012; 42: 147-168.
[10] Marin S, Ramos A J, Cano-Sancho G and Sanchis V. Food and Chemical Toxicology 2013; 60: 218–237.
[11] AnliE andAlkis I M. Journal of the Institute of Brewing 2010; 116(1):23-32.
[12] Vendl O, Crews C, Macdonald S, Krska R and Berthiller F. Food Additives and Contaminate ants Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 2010; 27(8): 1148-1152.
[13] Peña R J http://www.fao.org/ DOCREP/006/Y4011E/Y4011E00.HTM Accessed 21.01.13.2002.
[14] Pomeranz Y. Modern cereal science and technology 1987; 258-333.
[15] Samar MM, Fontán CF, Resnik S L, Pacin AM and Castillo MD. Journal of AOAC International 2003; 86(3): 551-556.
[16] Pleadin J, Vahcic N, Persi N, Sevelj D, Markov K and Frece J. Food Control 2013; 32(1: 49-54.
[17] European Commission (EC). J. Eur. Communities L 70 2006a; 12–34.
[8] European Commission (EC). Journal of the European Union L44 2010; 6: 1-127.
[19] Magan N. and Aldred D. Food Additives and Contaminants 2005; 22:10–16.
[20] Valero A, Farre JR, Sanchis V, Ramos AJ and Marin S. International Journal of Food Microbiology 2006; 110: 160–164.
[21] Cavin C, Delatour T, Marin-Kuan M, Holzhause D, Higgins L, Bezencon C, Guignard G, Junod S, Richoz-Payot J, Gremaud E., Toxicol. Sci. 2007; 96: 30–39.
[22] Reddy KRN, Salleh B, Saad B, Abbas HK, Abel CA. and Shier WT. Toxin Rev. 2010; 29:3-26.
[23] Zaied C, Bouaziz C, Azizi I, Bensassi F, Chour A, Bacha H and Abid S. Toxicol. Pathol.2011; 63: 613-618.
[24] Gagliano N, Doone ID, Torri C., Migliori M, Grizzi F, Milzani A, Filippi C, Annoni G, Colombo P, Costa F, Ceva-Grimaldi G, Bertelli AA, Giovannini L and Gioia M. Toxicology. 2006; 225: 214-224.
[25] Al-AnatiL.andPetzinger E. J. Vet. Pharmacol.Ther.2006; 29(2):79-90.
[26] Zhang X, Boesch-Saadatmandi C, Lou Y, Wolffram S, Huebbe P and Rimbach G. Genes Nutr. 2009; 4: 41-48.
[27] Schilter B1, Marin-Kuan M, Delatour T, Nestler S, Mantle P, Cavin C.Food AdditContam. 2005; 22(1):88-93.
[28] Benford D, Boyle C,Dekant W, Fuchs E,Gaylor DW, Hard G,McGregory DB Pitt JI,Plestina R,Shephard G,Solfrizzo M and Verger PJP. WHO, Geneva, WHO Food Additives Series 47, FAO Food and Nutrition2001; 47: 281-415.

[29] Pfohl-Leszkowicz A. Arh.Hig. RadaToksikol2009; 60: 465-483.
[30] Hope JH and Hope BE. J. Environ. Public health 2012; 8:35-59.
[31] Gimeno A. http://www.engormix.com2000.
[32] Vidal A, Morales H, Sanchis V, Ramos A J and Marin S. Food Control2014a; 40: 234–242.
[33] Vidal A, Marin S, Morales H, Ramos A J and Sanchis V. Food and Chemical Toxicology 2014b; 68: 53–60.
[34] Gholampour Azizi I, Rahimi K and Shateri S. Global Veter2012; 8:519-524.
[35] Sorrenti V, Di Giacomo C, Acquaviva R, Barbagallo I, Bognanno M and Galvano F.A review Toxins 5 2013; 1742-1766.
[36] Palumbo JD,O"Keeffe TL and Mahoney NE. Mycopathologia2007;164:241–248.
[37] Aroyeun SO and Adegoke GO. *Afr*. J. Biotechnol. 2007; 6: 612–616.
[38] Nguefack J, Nguikwie SK,Fotio D, Dongmo B, AmvamZollo PH and Leth V. *J. Essent*, *Oil Res*. 2007;*19*: 581–587.
[39] Magan N and Aldred D. Int. J. Food Microbiol 2007; 119: 131–139.
[40] SAS/STAS User's Guide Food Addit. Contam.1990; 22 (1): 88-93.
[41] European Commission (EC). Official Journal of the European Union, L3642006b; 5-24.
[42] FAO/WHO/UNEP Third Joint FAO/WHO/UNEP International Conference On Mycotoxins1999;ftp://ftp.fao.org /es/esn/food/myco6c.pdf
[43] Atalla MM, Hassanein NM, El-Beih AA and Youssef YAG. Nahrung 2003; 47: 6–10.
[44] Cengiz MH, hüseyinOruc, İlknur Uzunoglu and SongülSonal Uludag Univ. J. Fac. Vet. Med. 2007; 1(2): 7-10.
[45] Riba A, Mokrane S, Mathieu F, LebrihiA and Sabaou N. Int. J. FoodMicrobiol2008; 122: 85-92.
[46] Hashemi-Karouei M, Gholampour-Azizi I, Rouhi S and Tashayyo M. J Adv Environ Health Res2014; 2(4): 209-14.
[47] Bento JMV, Pena A, Lino CM and Pereira JA, Micro. chem. J. 2009; 91: 165-169.
[48] Duarte SC, Bento JMV, Pena A and Lino CM. Food Addit.Contam2009; 26: 1411-1420.
[49] Arroyo, M., Aldred, D. and Magan, N. (2005): International Journal of Food Microbiology, 98, 223–231.
[50] Valle-Algarra FM, Mateo EM, Medina Á, Mateo F, Gimeno-Adelantado andJiménez M Food Addit.Contam.2009; 26: 896-906.
[51] Cecchini F, Morassut M, Garcia-Moruno E and Di Stefano R. Food Microbiol. 2006; 23: 411– 417.
[52] Westby A, Reilly A and Bainbridge Z. Food Control 1997; 8: 329–339.
[53] Heidari Sara,Milani JM and Borj EP Intenational J. of Agricultural and biosystems engineering 2015;2:No: 5.
[54] Shetty PH and Jespersen L. Trends Food Sci. Technol. 2006; 17: 48-55.
[55] Huwing A, Freimund S, Kappeli O and Dutler H. Toxicology Letters2001; 122: 179–188.
[56] Nunez YP, Pueyo E, Carrascosa AV and Martinez- Rodriguez AJ. Journal of Food Protection2008; 71: 1496–1499.
[57] Ringot D, Lerzy B, Chaplain K, Bonhoure J, Auclair E andLarondelle Y. Bioresource Technology2007; 98: 1812–1821.
[58] Abrunhosa L, Paterson RRM. And Venâncio A. Toxins. 2010; 2: 1078-1099.
[59] Soliman KM and Badeaa RI. Food and Chemical Toxicology 2002; 40: 1669–1675.
[60] Pereira MC, Chalfoun SM, Pimenta CJ, Angelico CL. And Maciel WPSci Res Essays 2006; 1:38-42.
[61] Sokolić-Mihalak D, Frece J, Slavica A, Delaš F, Pavlović H and Markov K. Arch. Indust. Hygiene Toxicol2012; 63:457-462.
[62] Jeršek Barbara, NatašaPoklarUlrih, Mihaela Skrt1, Neda Gavarić3, BiljanaBožin and Sonja Smole Možina Arh Hig Rada Toksikol 2014; 65:199-208.
[63] Jayapra kasha, GK, RaoL J and Sakariah KK. Food Chem2006; 98: 720-724.
[64] Pothitirat and Gritsanapan Current Science2006; 91: 1397-1400.
[65] Lee HS, ChoiK J, Cho KY and Ahn Y. J. *Agric. Chem. Biotechnol.*, 2003; 46: 25-28.
[66] Jayaprakasha G K, RaoL J and Sakariah K K. Industrial Crops and products2007; 25: 143-153
[67] Cousins M, Adelberg J, Chen Fand Rietck J. Industrial Crops and products2007; 25: 129-135.
[68] Chrubasik S, Pittler MH and Roufogalis BD. *Phytomedicin*2005; 12: 684–701.
[69] Gupta RK. Ginger. A Wonderful Spice 2008; 21: 1–10.
[70] El–Ghorab A H, Nauman M, Anjum FM, Hussain S and Nadeem M.*J. Agric. Food Chem*., 2010; 58: 8231–8237.
[71] Mesomo MC, Scheer AP, Perez E, Ndiaye PM and Corazza M LJ. Supercrit. Fluids2012; 71: 102–109.
[72] Oboh G, Akinyemi AJ and Ademiluyi AO.*Exp. Toxicol. Pathol*., 2012; 64: 31–36.

[73] Guo J, WuH, Du L, Zhang Wand Yang J. J. Agr. Sci. Tech.2014; 16: 1063-1072.
[74] Offei-Oknye R, PattersonJ, Walker LT and Martha Verghese Food and Nutrition Sciences2015; 6: 445-451.
[75] Juglal S, Govinden R and Odhav B. *J. Food Prot*., 2002; *65*: 683–687.
[76] Romero SM, Alberto MR, de Nadra MCM and Vaamonde G. *Res.*2009; *25*: 165–170.
[77] Lee SE, Park BS, Bayman P, Baker JL, Choi WS and Campbell BC. *Food Addit.Contam.*2007. *24*:391–397.
[78] VargaJános, SándorKocsubé, ZsanettPéteri, CsabaVágvölgyi and BeátaTóthb*Toxin*s 2010;*2*: 1718-1750.
[79] Boudra H, Le Bars P and Le Bars J. Applied and Environmental Microbiology 1995; 1: 1156–1158.
[80] Czerwiecki L, Czajkowska D and Witkowska-Gwiazdowska A. Food Additives and Contaminuteants, 2002; 19: 470–477.
[81] Bullerman LB and Bianchini A. Journal of Food Microbiology 2007; 119: 140–146.
[82] Scudamore KA, Banks J and MacDonald SJ 2003Food Addit. Contam.2012; 11:53-63.
[83] Miraglia M and Brera C. Reports on Tasks for Scientific Cooperation SCOOP 2002; 1–153.
[84] Osborne BG. Journal of the Science of Food and Agriculture 1979; 30:1065–1070.
[85] Scott PM. Journal of the Association of Official Analytical Chemists International 1996; 79:875–882.