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Article

Levels of BPA in makdous, a traditional Syrian food, using solid-phase extraction followed by HPLC

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Bisphenol A (BPA), an endocrine disrupter, can migrate from packaging material into food stuff. This research was designed to measure BPA levels in makdous, a traditional Syrian food. Forty three samples of makdous stored in different plastic containers (polyethylene (PE), polypropylene (PP), and unspecified plastic containers) were analyzed every 3 months for one year beginning July 2017. Quantification of BPA was carried out by an RP-HPLC system equipped with fluorescence detector after solid phase extraction. Migration was found in PE and PP plastic containers with slight differences. Statistically significant differences in BPA levels were observed between samples assayed after two weeks of preparation and samples assayed after 12 months (mean 16.32 vs. 38.26 µg/kg, p value=0.003). According to these amounts, BPA levels were all under the specific migration limit of 0.05 mg/kg as newly referred in Regulation (EU) No 2018/213. These levels of exposure would only contribute to 2.15% and 2.75% of the EFSA t-TDI in both men and women respectively based on mean dietary exposure estimates derived from a 24-h dietary information study from 875 participants. Hence there are no concerns about potential health risks from makdous consumption.

Keywords: BPA. Makdous. Syrian traditional dish. Dietary exposure assessment. HPLC.

INTRODUCTION

Bisphenol A (2,2-*bis*(4-hydroxyphenyl) propane) is a recognized environmental chemical substance that mimics estrogen. It is used primarily as a monomer in the production of polycarbonate plastic (PC), and epoxy resins (Hoekstra, Simoneau, 2013; Vom Saal, Hughes, 2005). It is also used as a non-polymer additive in several consumer products, including internal coating of metallic cans, pipes for drinking water, thermal paper, dental fillers, cigarette filters, water pipes and tableware (Biedermann *et al.*, 2010; Huang *et al.*, 2012). Recent studies have indicated the migration of BPA traces from polycarbonate plastic packaging into food and beverages, with the amount of BPA leached depending on food or beverage composition and pH (Ehlert *et al.*, 2008; Hoekstra, Simoneau, 2013). Therefore, BPA exists widely in the environment and humans are commonly exposed to it through ingestion. Several studies, both in developed and developing countries, have reported detectable levels of BPA in urine, serum, umbilical cord blood, and breast milk (Rochester, 2013; Calafat *et al.*, 2008). BPA is now classified by the European Chemical Agency (ECHA) as an endocrine chemical disrupter affecting the reproductive system resulting in sexual impotency, fertility disturbance and abortions, together with secondary developmental defects of genital system in fetus, infants and young children (Vandenberg *et al.*, 2012; Li *et al.*, 2010; Sugiura-Ogasawara *et al.*, 2005; Bosquiazzo *et al.*, 2010; European Food Safety Agency (EFSA) 2015). Some other complications attributed to BPA are neoplastic changes, prostate and breast cancers (La Pensee *et al.*, 2010; Richter *et al.*, 2007a). These potential adverse effects of BPA on human health through beverage and food consumption have opened a fierce debate in the scientific community about the low-dose reproductive and carcinogenic effects of this contaminant. Therefore, it is essential to start by determining BPA levels in foodstuffs for both the assessment of human exposure and the control of current legislatives.

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For many Syrian families, the most important part of their kitchen is consisted of a wide variety of foods preserved naturally to be used throughout the year such as makdous (Figure 1). Makdous is a traditional Syrian dish made form baby eggplants stuffed with walnut, garlic and sun-dried red pepper then pickled in olive oil. It is a light meal usually prepared by the middle of summer to supply for winter. Makdous is usually eaten during breakfast and dinner or as a snack. Makdous is usually stacked in glass jars filled with olive oil for a period of two weeks at least in a kitchen temperature, giving it a uniquely strong taste. However, plastic containers started replacing glass jars as more practical and cheaper packaging alternatives.

The present paper reports for the first time the determination of BPA in the traditional Syrian dish, makdous, by using solid phase extraction followed by HPLC in the aim of assessing human exposure.

FIGURE 1 – Makdous, a traditional Syrian dish, made from baby eggplants stuffed with walnut, garlic and sun-dried red pepper then pickled in olive oil.

MATERIAL AND METHODS

Samples collection

Forty three samples of makdous stored in plastic containers were collected in July 2017 from a variety of Syrian households in the coastal region. All containers were stored at room temperature. Collected samples from plastic containers were distributed as follows: 21 samples from polyethylene (PE), 8 sample from polypropylene (PP), and 14 samples from unspecified plastic containers. All samples were stored under the same conditions and were analyzed after two weeks of preparation and every 3 months during a year. After sampling 200 g of makdous for each analysis, olive oil was added to preserve the sample completely immersed as traditionally done in houses.

Olive oil used in this study was prepared by cold pressure in the coastal region of Syria and stored in glass containers. It was tested for BPA before its use for the preparation of makdous samples and results showed that they were free of BPA.

Sample pretreatment by solid phase extraction

For analysis, a sample of makdous was collected and the oil adsorbed at the surface was wiped. Then, 200 g of makdous was homogenized in a stainless steel laboratory blender. According to previously reported work by Sadeghi *et al.*, (2016), an aliquot of 200 mg of the homogenized sample was extracted at room temperature with 10 mL acetonitrile in an ultrasonic bath for 30 min at 300 rpm. The solution was centrifuged at 1000 rpm for 15 min and the supernatant was saved. The extraction was repeated twice, and all the extracts pooled. Then, the acetonitrile phase was mixed with 15 mL *n*-hexane to remove lipids and ~~was~~ shaken vigorously for 30 min. The phrase was then made up to the 100 mL mark in a volumetric flask with deionized water, and the pH of samples was adjusted to be between 6 and 7. Final solution was subjected to SPE procedure.

The SPE cartridge (OASIS HLB cartridge) was conditioned with 5 mL of methanol-2% acetic acid, 5 mL acetonitrile then with 5 mL of deionized water. 10 mL of the liquid sample was loaded after spiking with 0.5 µg of BPA (in order to increase our confidence in the accuracy and validity of the sample test results as per the quality control standards of our laboratory). The cartridge was washed by 10 mL of deionized water followed by 6 mL of deionized water:acetonitrile (60/40, v/v). BPA was eluted with 3 mL of methanol (Zhang *et al*., 2006). All the applied fractions were collected and evaporated to dryness. The residues were dissolved with 200 µL acetonitrile-water (40:60, v/v) and analyzed by HPLC. Each sample was assayed three consecutive times.

Preparation of BPA standard and blank solutions

A stock standard solution of BPA (bisphenol A >99%, Sigma-Aldrich) was prepared by dissolving 100 mg of BPA in 100 mL of methanol (Sharlau (Spain)). The standard solutions were reserved at -20 ºC in amber glass vessels. Working standard solutions ranging from 0.05-1.0 µg/mL were prepared by diluting the stock solution with acetonitrile-water (40:60, v/v). The working standard solutions were stored at 4 ºC in the refrigerator.

The blank solution was prepared using a BPA free sample from makdous stacked in glass jars.

Quantification by HPLC-fluorescence

Quantification of BPA was carried out by an RP-HPLC (SHIMADZU, LC-20AD) system equipped with fluorescence detector as described by Bendito *et al.* (2009), with little modifications. All determinations were performed at ambient temperature (25 ºC) using C18 (250×4.6 mm, 5 µm, Hypersil ODS, Thermo Fisher Scientific). The mobile phase consisted of water (A) and acetonitrile (B) at a flow-rate of 0.8 mL/min. The gradient elution program was: 60:40 (A:B) in 12 min, 50:50 (A:B) from 12-20 min then isocratic initial conditions (60:40) for 20 min to clean and stabilize the chromatographic system. The selected wavelengths were 230 nm (excitation) and 315 nm (emission). BPA eluted at 9.3 min.

The standard curve was linear over the selected points (correlation coefficient R2 = 0.996). Figure 2 shows a typical chromatogram obtained from both a standard solution of BPA and a sample.

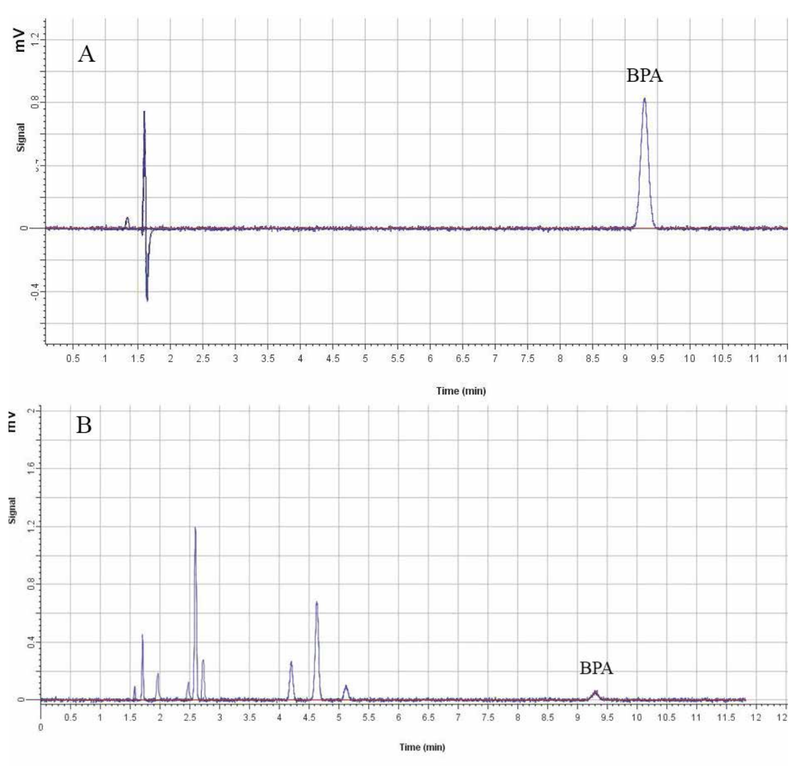

FIGURE 2 – Typical chromatograms obtained from a standard solution of BPA (0.5 µg/mL) (A) and a sample of makdous (B).

Statistical analysis

Descriptive statistics were used to summarize the study variables. Statistical analyses were conducted using the SPSS statistics 21 software. For comparison of means, a repeated measure ANOVA test was used and P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

The obtained BPA quantitative results for the tested makdous samples during the whole year are summarized in Table I. BPA levels were all under the specific migration limit (SML) of 0.05 mg/kg as newly referred in Regulation (EU) No 2018/213 (Commission Regulation (EU) 2018/213, 12 February 2018).

Statistically significant differences in BPA levels were observed between samples assayed after two weeks of preparation and samples assayed after 12 months (mean 16.32 vs. 38.26 µg/kg). This could be explained by the increase of polymer degradation over the storage time (Vandenberg *et al*., 2007). Same results were found by Abou Omar *et al*. (2017), who estimated the exposure to BPA from olive oil in Lebanon and found that BPA levels after a year of storage was significantly higher compared with samples stored for less than a year. Tawfik (2005) had previously proved that global migration of different types of plastic food packaging materials into different type of vegetable oils was temperature dependent and slightly higher into oil contained high amount of short-chain and unsaturated fatty acids. These increased levels could also be due to the addition of new quantities of olive oil continuously to the stacked makdous as a normal procedure to keep it immersed all the time which is traditionally done in houses. Kang *et al*. (2003) previously proved that levels of BPA migration for cans containing vegetable oils were significantly higher than those for cans containing water. Several previous experiments showed the same results especially in PE and PP containers, showing an increase in the migration of monomers and oligomers from plastic containers as the fat content of the food and storage temperature increased (Gao *et al*., 2011; Arvanitoyannis, Bosnea, 2004).

TABLE I – BPA levels in the makdous samples from different types of packaging during a whole year

Types of packaging	Number of samples	BPA ± SD (µg/kg)										F levels
		After two weeks of preparation		After 3 months		After 6 months		After 9 months		After 12 months		
		range	mean	Range	mean	range	mean	range	mean	range	mean	
PE	21	08-28.5	14.3±3.1	10.5-32	22.3±2.2	13-32	25.4±3.1	22.5-35	29.5±3.2	26.5-42	35.2±5.1	F(4,80)=5.20, p<0.05.
PP	8	12.5-22	16.5±4.1	15-36.5	26.3±3.5	18.1-35.1	27.3±2.2	22-36.5	31.3±4.6	28.5-45	38.1±6.2	F(4,28)=6.59, p<0.05.
UP	14	11.5-26	18.1±2.1	20.5-38	30.7±5.3	25-39.6	34.5±4.2	28.5-42	36.3±5.1	28-46.5	41.5±7.2	F(4,52)=5.85, p<0.05.

PE: polyethylene, PP: polypropylene, UP: unspecified plastic.

As indicated in Table I, the global migration was slightly higher in PP plastic container compared with PE plastic containers which is in accordance with the results of Tawfik that had previously found that overall migration from plastics was found to be lower in PET and PVC compared to PS and PP (Tawfik, 2005). Another study showed that olive oil stored in PP containers for more than 10 days at 44 ºC contained PP molecules in amounts close to 10 mg/dm^2 (Wang, Storm, 2006).

The tolerable daily intake (TDI) level established by EFSA in 2015 is 4 µg/kg BW/day. This level is twelve and a half times lower than the previous level established by the U.S. Environmental Protection Agency (Vom Saal, Hughes, 2005); however, this new TDI level is considered as temporary (t-TDI) pending the anticipated outcome of a long-term toxicity study on BPA in rodents being undertaken by the National Toxicology Program/Food and Drug Administration (NTP/FDA) in the United States of America (USA) (Commission Regulation (EU) 2018/213, 12 February 2018).

The mean dietary exposure estimates derived by combining mean BPA levels from the current study with 24-h dietary information from 875 participants are summarized in Table II. The estimated daily ingestion of BPA from makdous is 0.086 and 0.11 µg/kg BW/day for men and women respectively. These levels of exposure would still only contribute to 2.15% and 2.75% of the EFSA t-TDI in both men and women respectively.

TABLE II – Mean dietary exposure estimates for BPA from the traditional Syrian food, makdous

Total number of participants (TNP)	**875**
Age range men & percentage of TNP women & percentage of TNP	18-62 y 310 (35.42%) 565 (64.57%)
Mean body weight of men	87.12 kg
Mean body weight of women	69.15 kg
Mean BPA level in makdous samples	28.46 µg/kg
Dietary consummation of makdous per day	150-400 g Mean: 265.13 g
Mean dietary exposure for BPA	7.55 µg/day

It should be taken in consideration that makdous is only one of several foods ingested daily that may contain BPA such as canned foods. Additionally, endocrine disrupting effects could be due to several other food constituents such as isoflavones and alkyl phenols (Kiely *et al*., 2003); and BPA was considered to contribute to about 35% of all oestrogenicity from food (Thomson *et al*., 2003). Moreover, it should be kept in mind that children are more susceptible to the adverse effects of BPA contamination based on their higher intake relative to their low body weight (Schecter *et al*., 2010). Based on different studies, urinary BPA levels in children seem to be higher in comparison with adults (Bushnik *et al*., 2010; Frederiksen *et al*., 2013). It should also be noticed that makdous is generally stacked in reusable plastic containers which could release high levels of BPA due to the dual interaction between oils and plastic packages that have the ability to absorb volatile and non-volatile substances from olive oil (Kiritsakis *et al*., 2002). Finally, it is also hard to evaluate the safety of makdous intake in pregnant women even if the BPA levels were small based on our results; since some receptors in placenta specifically accumulate BPS (Birnbaum *et al*., 2013). Previous studies detected BPA and BPA-GA in fetuses and amniotic fluid (Kubwabo *et al*., 2009). *In vivo* studies on animals exposed to BPA during gestation or lactation have revealed that doses between 2 and 20 µg/kg/day could be associated with higher risk of reduced birth weight, neurological effects, as well as higher risk of breast and prostate cancer (Richter *et al*., 2007 b; Ho *et al*., 2006; Komada *et al*., 2012). Recently, Ohtani *et al.* confirmed that offspring are more vulnerable to exposure to BPA in late pregnancy but not during early pregnancy (Ohtani *et al*., 2018).

CONCLUSION

BPA levels detected in makdous samples during a year of storage in different plastic containers were below the current EFSA t-TDI, hence there are no concerns about potential health risks from makdous consumption.

However and based on evidences indicating that BPA may accumulate in human body leading to toxic effects, we recommend the use of glass containers instead of plastic containers or avoid long-term storage of makdous in plastic containers. Moreover, the dietary exposure estimates in this study were limited to adults and further consumption information for other population is needed.

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