Effect of Bisphenol A on the Quality Characteristics of Meat in a Chicken Embryo Model

1Parisa Sadighara, 1Gholamreza Jahed Khaniki, 1Elham Baseri, 2Mohammad Hadi Dehghani, 3Abbas Barin and 4Ramin Mazaheri Nezhad Fard
1Department of Environmental Health, Food Safety Division, 2Department of Environmental Health, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran 3Department of Clinical Pathology, 4Rastegar Reference Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

ABSTRACT
Background: Carcass compositions affect the meat quality significantly. This study reported whether bisphenol A (BPA) affects the characteristics of chicken meat. Methods: BPA were injected in 50, 100 and 200 ppm at the day 4 of incubation. The muscle samples were collected from the chickens. Lipid peroxidation, vitamin E, total carotenoids, total protein and total fat contents of muscles were measured. Results: Significant variation in vitamin E, lipid peroxidation and protein contents was observed (p<0.05). The toxin decreased the yield of protein and vitamin E. The mean concentration of protein was significantly lower in 100 and 200 ppm than in 50 ppm. Furthermore, BPA could induce lipid peroxidation significantly. The values were not significantly different for the carotenoid and total fat contents. Conclusion: These results demonstrated that BPA contamination changes the meat composition and this may also have adverse effects on the human health.

Key words: Meat quality, bisphenol A, food contamination


INTRODUCTION
The residual will enter to meat food products. They don't break down when the meat is cooked. The contaminated food can lead to cause chronic problem in consumers. The poisons persist in animals for varying times of their life. In animal body, these poisons affect various functions of the organs such as liver, kidney, nervous system and immune system. It is also possible that toxin affects the muscle. Therefore, meat quality could be affected by toxins.

Bisphenol A (BPA), 2, 2-bis (4-hydroxyphenyl) propane, is an endocrine disruptor and exhibits estrogenic activity. BPA has been reported to interact with α and β-estradiol receptors and increases pituitary prolactin secretion. BPA may also induce the estrogen-sensitive breast cancer. In utero exposure to BPA increased the incidence of terminal ducts, terminal end buds and alveolar buds in 6-month-old mice which is associated with mammary gland tumors in mice. BPA is used to produce epoxy resins and polycarbonate plastics. The epoxy resins are used in food packages. BPA can transfer from cans and polycarbonate plastic bottles to foods and drinks. BPA can be found in wastewater from factories that produce it. This wastewater can contaminate aquatic environment. BPA has been traced in freshwater and detected in marine and freshwater fish. A study reported that levels of BPA in fish varied from 2-75 ng g⁻¹ dry weight in the liver and from 1-11 ng g⁻¹ dry weight in the muscle, meanwhile levels of BPA in their water ranged from 0.01-0.33 ng mL⁻¹. Therefore, animals may be exposed to BPA through their food and water supplies. The food contaminated with animal origin is the considerable route of contamination for humans. The aim of the present study was to address the possible effects of BPA on the quality characteristic of meat. In addition to the residual problem, the results of this study suggest whatever BPA can change the quality of food contaminated with animal origin.
MATERIALS AND METHODS
Toxicity tests
Chicken embryotoxicity study: The protocol used for the study is a sensitive, inexpensive and rapid toxicity test, providing information on embryonic toxicity, lethality, teratogenicity, growth retardation and metabolism. Studies have shown that a positive correlation exists between the magnitude of effective concentration in chick embryo toxicity test and those in other systems including mammal tests. Fertile leghorn eggs collected from a breeding farm (Iran Dairy Farm, Tehran) two days after lay. Infertile and damaged eggs were discarded. The eggs were candled on the day 4 and injected with BPA. Following sterilization with ethanol, BPA was injected in 50, 100 and 200 ppm concentrations into the egg yolk. The injection sites were closed with paraffin and the eggs were incubated at 37.5±0.1˚C and 50-60% relative humidity. The eggs were candled the day after injection and thereafter every 48 h for checking dead embryos. The experiment was terminated on the day 20 of incubation. Then, embryo thigh muscle samples were collected for the meat quality analysis.

Measurement of lipid peroxidation: The formation of thiobarbituric acid in muscle samples was assessed for the measurement of muscle lipid peroxidation according to an original method. Briefly, the supernatant of the meat homogenate was mixed with 20% trichloroacetic acid and the mixture was centrifuged. Then, thiobarbituric acid was added to the supernatant and heated. The absorbance of the supernatant was measured at 532 nm. The values were expressed in nmol of malondialdehyde, using a molar extinction coefficient of 1.56×10^5 M^-1 cm^-1.

Measurement of vitamin E content of muscle: Muscle vitamin E content was measured according to a published method. Samples were exposed to Fe³ solution, TPTZ and acetate buffer (pH 4). Then, the standard curve was prepared with appropriate vitamin E concentrations. The absorbance of samples was read at 595 nm wavelength.

Measurement of total carotenoids of muscle: Muscle total carotenoids were measured using β-carotene standard curve and spectrophotometry at 470 nm wavelength. The total carotenoid content of the samples was calculated on the basis of the standard curve of β-carotene.

Measurement of total protein content of muscle: Muscle total protein content was measured using Bradford method. Bovine Serum Albumin (BSA) was used as standard.

Measurement of total fat content of muscle: The solvent extraction method is one of commonly used for measuring total fat in food. Therefore, muscle total fat content was measured by Soxhlet extraction method using organic solvents. The tubes containing extractions was heated and the solvent evaporates. The lipids remain in the tubes. The total fat was calculated by the difference between the weight of tube containing extractions and the weight empty tube.

Statistical analysis: The data were tested by analysis of T-Test in SPSS software. The evaluation was made by comparing group. The difference more than 95% (p<0.05) was considered significant.

RESULTS
Results are given in Table 1-3. The concentration of carotenoid pigments in the extracts were calculated using the standard curve obtained by a commercial β-carotene reagent. The formula used for the calculation was as follows:

\[ y = 3240.7x + 0.1129; \quad R^2 = 0.9974 \]

No significant changes in carotenoid pigment were observed. The carotenoid content was not modified by the BPA exposure (50, 100, 200 ppm) (Table 1). Significant variations in vitamin E, lipid peroxidation and total protein contents were seen (p<0.05). The level of vitamin E was significantly different between 100 and 200 ppm concentrations compared to control (p<0.045). The level of protein was significantly different between 100 and 200 ppm concentrations compared to control. The results

Table 1: Vitamin E content in chicken muscles after the exposure to BPA

<table>
<thead>
<tr>
<th>Group (ppm)</th>
<th>Carotenoid (ppm)</th>
<th>Vitamin E (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.13±0.07</td>
<td>3.06±2.54</td>
</tr>
<tr>
<td>50</td>
<td>0.13±0.06</td>
<td>1.96±1.63</td>
</tr>
<tr>
<td>100</td>
<td>0.14±0.10</td>
<td>0.65±0.05</td>
</tr>
<tr>
<td>200</td>
<td>0.13±0.05</td>
<td>0.66±0.05</td>
</tr>
</tbody>
</table>

Table 2: Level of malondialdehyde (MDA) in chicken muscles after the exposure to BPA

<table>
<thead>
<tr>
<th>Group (ppm)</th>
<th>MDA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.43±0.39</td>
</tr>
<tr>
<td>50</td>
<td>3.98±0.40</td>
</tr>
<tr>
<td>100</td>
<td>5.62±2.20</td>
</tr>
<tr>
<td>200</td>
<td>8.33±0.81</td>
</tr>
</tbody>
</table>

*nmol/0.5 g tissue

Table 3: Total protein and fat contents in chicken muscles after the exposure to BPA

<table>
<thead>
<tr>
<th>Group (ppm)</th>
<th>Total Fat*</th>
<th>Protein (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.027±0.001</td>
<td>6.3±2.20</td>
</tr>
<tr>
<td>50</td>
<td>0.025±0.002</td>
<td>6.3±2.30</td>
</tr>
<tr>
<td>100</td>
<td>0.025±0.005</td>
<td>1.5±0.98</td>
</tr>
<tr>
<td>200</td>
<td>0.025±0.001</td>
<td>1.6±1.64</td>
</tr>
</tbody>
</table>

*mg/0.5 g of muscle
were not significantly different for total fat content. In contrast, total fat was not significantly different in the studied groups. The level of malondialdehyde (MDA) in chicken embryo meat was considerable at 100 and 200 ppm concentrations of BPA. The level of lipid oxidation was significantly different in 200 ppm concentration compared to control (p≤0.003). The level of lipid oxidation was also significantly different between 50 and 200 ppm concentrations compared to control (p≤0.007).

DISCUSSION

In the current study, the major factors which can affect the meat quality contaminated with BPA were surveyed. The meat quality depends on bioactive compounds such as proteins, lipids and vitamins. The application of BPA-based polymer products is very wide, from packaging items for foods and beverages to parts in car industries. To trace BPA in food stuff, a variety of methods have been established. One of these methods is MDA method. The findings of previous studies suggest that BPA exposure will promote oxidative stress and ROS attacks polyunsaturated fatty acids and causes lipid peroxidation. MDA is a product of lipid peroxidation that has been used as an indicator in oxidative damages. Consumption of MDA contains foods has been associated with health problems. This component is a highly reactive and can cause deterioration of biological molecules such as DNA. MDA is mutagenic and tumorigenic. Furthermore, lipid peroxidation causes changes in meat quality factors such as taste, shelf life, texture and nutritional parameters. In the current study, the level of lipid oxidation was changed during the experiment. Chicken meat is rich in polyunsaturated fatty acids (PUFA); therefore, it is sensitive to oxidative damages.

The oxidative stability of muscles depends on the balance between antioxidant and oxidizing agents. In this study, vitamin E content was significantly affected by BPA. Results suggest that presence of vitamin E has an important role on the prevention of lipid oxidation. Furthermore, it prolongs the stability and shelf life of meat products. In contrast, total carotenoids of muscle seem unlikely to be influenced by BPA. Vitamin E disappears more quickly from muscles than carotenoids, meaning that muscles have a higher requirement for vitamin E to reduce oxidative stress than carotenoids. This evidence suggests that co-administration of vitamin E can suppress the toxicity of oxidative stress caused by BPA. Vitamin E attenuates lipid peroxidation and protein oxidation and increases antioxidant defense mechanisms. In the current study, vitamin E had a better bioavailability than carotenoids.

Poultry meat color is considered a major quality factor. Carotenoids, as natural antioxidant pigments, have a role in the formation of yellow color in poultry carcasses. Poultry accumulate carotenoids in muscles. Accumulated carotenoids also increase the quality of poultry meat by improving flavors. As mentioned previously, the level of total carotenoids was not decreased by the exposure to BPA.

Protein yield is an important factor in meat quality. In this study, meat protein was significantly decreased in 100 and 200 ppm BPA concentrations (p<0.05) (Table 3). A relationship between bisphenol metabolism and protein yield may exist. Therefore, growth rate can be affected due to decreased protein yield.

Fats are the main energy sources in animals and have the highest calorie amongst all nutrients. Total fat of muscles seems unlikely to be influenced by BPA. However, fats composed of unsaturated fatty acids are affected by the attack of free radicals. This causes lipid peroxidation in muscles due to high-levels of PUFA in chicken meat.

In summary, consumption of the residue of BPA throughout the food and drink can harm the consumers and result in serious health problems. Furthermore, BPA is also known to change meat quality. Therefore, tracking BPA residues in foods and drinks seems a priority.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES


