

Black Seed (*Nigella sativa*) Reduced Release of Phthalates Esters from Packing Material to Prepared Cheese

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ABSTRACT

Background: Phthalate esters are chemicals that are widely used as additives to plastic products that are used in wrapped foods. It exerts a negative impact on health, especially the risk of hypertension, diabetes, CVD, endocrine disorders and fertility. *Nigella sativa* (black seed) is known to be rich in thymoquinone that exerts different biological activities. This study detected ten phthalate esters in different types of cheeses in local markets and laboratory-prepared cheese with black seed or with curd. **Materials and Methods:** 18 cheese samples (3 samples from 6 types) (mozzarella, qashquan, cheddar, sliced, Hallom and vita) were collected from local markets packaged in plastic containers. In addition, 3 samples of cheese were prepared in in different conditions (under vacuum or with black seed or with curd) wrapped in parchment paper, and stored for one month at 4°C. Phthalate esters were extracted, purified by SPE column and then determined by GC/MS. The phthalates were identified according to their retention time compared with standard. **Results:** Data obtained showed that phthalate esters were found in most samples with higher levels compared with prepared ones ($p < 0.01$). It was found that the prepared cheese under vacuum-packed showed only four phthalate compounds namely DBP, DIDP, DEHA and DNOP at very low concentrations (0.02, 0.05, 0.01 and 0.07 mg/kg (respectively). While the rest of the compounds were not detected in the sample, either less than the detection limit as BBP, DMP, or not present. While prepared and immersed in curd, it showed the presence of DEHA (0.01 mg/kg) and the other compounds were not detected. However, cheese prepared and wrapped in parchment paper under a vacuum showed the presence of DBP; 0.02, DIDP; 0.05, DEHA; 0.01 and DNOP; 0.07 which reflect reduction of phthalates levels compared with local ones. **Conclusion:** Wrapping products in parchment paper and the addition of *Nigella sativa* avoid the release of these compounds to the cheese samples and prevent health problems which reflects the role of parchment paper and *Nigella sativa* as a scavenger for these esters.

Keywords: Phthalates esters, Cheese, *Nigella sativa*, Packings.

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INTRODUCTION

Phthalate esters are plasticizer that gives elasticity and flexibility to plastics and increases transparency, which helps to see the packaged products and increase the shelf life of food products.^[1] Phthalate esters are not bound chemically in plastic products, so they are transferred to most of the materials adjacent to them, especially fat-containing foodstuffs. Due to the widespread use of plastics in different fields on a daily basis, humans are exposed to these materials continuously through eating food wrapped or preserved inside plastic containers, and through inhalation or skin contact with plastic used in many products.^[2] The dairy products packaging was found to be 52% carton packaging, 8% metal cans and 40% plastic packaging.^[3] Previous studies

reported that phthalate esters exceed safe levels causing serious health effects as endocrine,^[4] liver, kidneys, lungs and heart.^[5] Ministry of Health and Food and Drug Authority established rules and warnings for the use of plastic in the packaging of food products and encouraged follow-up analysis to ensure the presence of these harmful substances within safe levels^[6] which reduces the incidence of health problems and reflects the economy. In addition, the storage conditions affect the release of these compounds from packing materials as high temperature increased the chance of release versus low temperature. Also, an acidic medium increased the chance of release versus a basic medium.

Black seed (*Nigella sativa*) has been known to possess potent biological activity against many diseases for tens of decades. It was used as an anti-inflammatory, GIT diseases, diabetes and some tumours.^[7] Due to its high content of Thymoquinone (TQ), which is the most bioactive compound that suppresses cell cycles in some cancers via kinase inhibitors. It is used as an additive in different foods to protect against many diseases.



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The health risks will be evaluated, and food consumption of products packaged in plastic containers.^[8] This will help in awareness (frequency and percentage) and also the percentage. Expected consumption of cheese by subjects, percentage of use of food products packaged in plastic containers.^[9] This study evaluated the phthalates ester content of local cheeses in markets that were wrapped in plastic containers and evaluated the risk of consuming food products contaminated with phthalate esters and prepared cheese with the addition of black seeds in the laboratory and wrapping it in parchment paper as a protective insulator and evaluating its ability to reduce the release of phthalate esters to the product.^[10]

MATERIALS AND METHODS

Samples collection

18 samples of different types of cheeses (white feta, double cream, cheddar, sliced, Edem) were collected from local market and packaged in plastic containers produced by Saudi factories. The samples were stored at 4°C. In addition, 3 types of cheese samples were prepared in laboratory and stored under vacuum or with black seed and with curd. Then wrapping it in parchment paper and storing it wrapped in plastic to evaluate the effectiveness of the protective wrap in reducing the phthalate esters release to cheese.

Methods

Phthalates esters were extracted from all cheese samples and purified by Solid Phase Extract (SPE) column and identified by GC/MS (Agilent) according to the method of Sorensen.^[7]

RESULTS

Data obtained in Table 1 showed variation in the incidence of phthalates esters in 6 types of local cheeses in markets. DEHP, DBP, BBP are detected in significant levels in chider samples while DINP, DIDP, DEP, DIBP, DEHA are not detected and DNOP was detected in very low level. This variation may be due to many factors such as materials packing, distance to cheese, temperature, and storage conditions. In mozzarella samples the DINP, DMP, DEP, and DIBP were not detected while other types were detected. The DBP, DINP, DIDP, DEP, DIEP were detected in burger slices and other compounds found at low level. In Hallom and qashquan samples showed the lowest levels of DEHP, DBP, DEHA and DONP only and other compounds were not detected. Previous studies reported that DEHP level in fruity yoghurt is 15-37 µg/kg, and DBP, BBP, DINP and DIDP are below the detectable limit. In a similar study, yoghurt, DEHP was 24-122 µg/kg, DBP was 46-229 µg/kg, and BBP was 22-63 µg/kg.^[17] Lipid content affects the migrations of phthalates. The phthalate esters in foods depend on phthalate concentration in packaging material, storage time and temperature, fat content of the food and contact surface. Table 2 shows the results of

the manufactured sample to which black seed was added as an antioxidant and an inhibitor of the release of phthalate ester compounds. The data showed the presence of DMP only at a very low concentration (0.03 mg/kg) and none of the rest of the compounds were identified. It was found that the prepared and vacuum-packed cheese sample showed only four phthalate compounds, namely DBP, DIDP, DEHA, and DNOP, out of ten compounds, as shown in the Table 2, at low concentrations of 0.02, 0.05, 0.01, and 0.07, respectively. The rest of the compounds were not detected in the sample, either because it was less than the permissible detection limit, such as BBP, DMP, or not present, such as. As for the sample prepared and immersed in the whey, it showed the presence of DEHA only at a very low concentration, which is the permissible limit of detection (0.01), and the rest of the compounds were not detected.

DISCUSSION

Commercial foods are available to consumers after have been packaged to preserve, protect and extend their shelf life. Plastic products are the most widely used in packaging. Some agents are added to plastic to give it some desirable properties, such as increasing durability, flexibility, transparency etc.^[11] These additives are not chemically bound to the plastic, and therefore they are transferred from packaging materials to foods over time, and their transfer is increased by a number of factors, especially increasing temperature and frequent operations. Trading of products, fat, protein and moisture content of foods.^[12,13] The presence of these compounds in foods, if they are not within safe limits, may affect the physical properties and sensory evaluation of food products, and result in imbalances and disturbances in some physiological functions, endocrine functions, and hormones.^[14]

Three samples of cheese were prepared, packaged, and stored in different ways (a vacuum sample, a vacuum sample with black seed added as an antioxidant and a sample immersed in whey). The samples were kept in the refrigerator at 4°C for a month, and the phthalate ester compounds were determined after a month of manufacturing. Comparing these results with samples collected from the markets, a noticeable decrease in this value was found. This is due to several reasons, namely packaging methods-packaging materials-storage period in the factory and markets-preservation methods-humidity-temperature. From the results and their comparison with market samples, there is a statistically significant difference in the level of phthalate esters in samples manufactured at home and wrapped in parchment paper, which reduces the possibility of the release of phthalate esters from packaging materials into cheese samples. Also, adding some additives from natural materials such as *Nigella sativa* reduces the chances of these compounds being transferred to the cheese samples, which reflects the role of *Nigella sativa* as a scavenger for these esters. Knowing the percentage of phthalate ester compounds in Saudi cheeses packed in plastic containers, and

Table 1: Phthalate esters in different cheese samples from local markets (Mean±SD).

Cheese type	# samples	Di-2 ethyl hexyl phthalates (DEHP)	Di-n-butylphthalates (DBP)	Diisononyl phthalate (DINP)	Diisodecyl phthalate (DIDP)	Dimethyl phthalates (DMP)	Diethyl phthalated DEP	Diisobutyl phthalates DIBP	Di-(2-Ethylhexyl) Adipate. DEHA	Di-n-Octylphthalate (DNOP)
Cheeder	I	0.114	0.22	ND	LOQ	0.017	ND	ND	ND	0.032
	II	0.132	LOQ	ND	LOQ	0.019	ND	ND	ND	ND
	III	0.110	0.10	ND	LOQ	LOQ	ND	ND	ND	0.011
	Mean ±SE	0.118±0.01	0.106±0.01	-----	-----	0.012±0.001	-----	-----	-----	0.014±0.001
Mozarella	I	4.30	0.27	ND	0.018	ND	ND	ND	1.91	1.01
	II	3.55	0.29	ND	0.02	ND	ND	ND	2.1	0.78
	III	3.10	ND	<LOQ	0.011	ND	ND	ND	1.5	0.54
	Mean ±SE	3.65±0.46	0.186±0.05	-----	0.016±0.001	-----	-----	-----	1.83±0.23	0.77±0.06
Burger Slices	I	1.7	ND	ND	ND	0.043	ND	ND	0.56	ND
	II	2.1	ND	ND	ND	0.030	LOQ	ND	LOQ	0.033
	III	LOQ	ND	ND	ND	LOQ	LOQ	<LOQ	ND	<LOQ
	Mean±SE	1.26±0.15	-----	-----	---	0.024±0.02	-----	-----	0.18±0.02	0.011±0.01
Haloom	I	4.78	0.25	ND	ND	LOQ	LOQ	ND	1.71	1.23
	II	4.11	0.24	ND	ND	LOQ	LOQ	ND	1.52	<LOQ
	III	3.99	ND	<LOQ	ND	LOQ	LOQ	ND	1.32	1.11
	Mean ±SE	4.29±0.51	0.16±0.012	-----	-----	-----	-----	-----	1.51±0.022	0.78±0.06
Qashquan	I	1.42	ND	ND	ND	0.022	ND	ND	0.59	ND
	II	2.11	ND	ND	ND	0.035	ND	<LOQ	0.45	<LOQ
	III	1.87	ND	ND	ND	LOQ	ND	ND	0.71	ND
	Mean ±SE	1.8±0.23	-----	-----	-----	0.019±0.001	-----	-----	0.583±0.065	-----
Vita	I	LOQ	0.31	ND	0.022	0.06	ND	ND	LOQ	0.023
	II	2.11	ND	<LOQ	0.020	0.051	ND	ND	LOQ	0.013
	III	1.56	0.22	ND	LOQ	LOQ	LOQ	ND	LOQ	0.01
	Mean±SE	1.22±0.143	0.17±0.02	-----	0.014±0.001	0.037±0.03	-----	-----	-----	0.015±0.01

Table 2: Phthalates esters levels in prepared cheese sample (vacuum dry, vacuum dry with black seed and soaked in Curd.

Cheese prepared	Di-2 ethyl hexyl phthalates (DEHP)	Di-n-butylphthalates (DBP)	Butylbenzyl phthalate (BBP)	Diisononyl phthalate (DINP)	Diisodecyl phthalate (DIDP)	Dimethyl phthalates (DMP)	Diethyl phthalated DEP	Diisobutyl phthalates DIBP	Di-(2-Ethylhexyl) Adipate (DEHA)	Di-n-Octylphthalate (DNOP)
Dry	ND	0.02	LOQ	ND	0.05	LOQ	ND	ND	0.01	0.07
Dry+Nigella	LOQ	ND	ND	ND	ND	0.03	ND	ND	LOQ	ND
Soaked in Curd	ND	ND	ND	ND	ND	ND	ND	ND	0.01	ND
Mean ±SE	---	0.02±000	---	---	0.05±000	0.01±000	---	---	0.01±000	0.07±000

Concentration as (mg/kg).LOQ: Limit of quantification: 0.01 mg/kg.ND: Not detected.

comparing the percentages present with the global percentages allowed by international health and regulatory bodies to ensure the safety of food products of phthalate ester compounds in local cheese products,^[15] which may have an impact in setting requirements from regulatory authorities for food safety and quality. Phthalate esters are plastic additives that transfer to packaged foods or packaged in plastic containers, especially foods containing fats, as the inner layer of polyethylene or polyvinyl chloride that touches the foods contains high levels of phthalate ester compounds

The levels of phthalate esters released in plastic-wrapped foods by the time and temperature at which the packaged or packaged foods are kept, the frequency of thermal processes such as reheating foods in the microwave.^[16] It was found that people who consume foods wrapped in cling film, especially those containing a high percentage of fat, accumulated high levels of phthalate esters, which indicates the transfer of these chemicals from packaging materials to foods and increases the health risks for consumers,^[17] while foods containing a high percentage of moisture and low in fat content have a low level of transfer of phthalate esters into foods.^[18] There were differences in the percentage of phthalate esters between types of cheese, and that transferred depended on the duration, fat, moisture content, and texture of the cheese samples. Avoid storing them inside containers made of plastic that are not intended for them. For a long period of time, do not put hot foods in plastic dishes, including those made of melamine, to avoid interactions between them. It is preferable to use kitchen utensils, porcelain or glass for this purpose. Avoid using plastic containers in which not stable or are affected by acids, oils, and heat when storing the food that is placed in it. It is better to add some natural antioxidant agents for scavenger any toxicity.

CONCLUSION

It was concluded that, addition of black seeds during cheese processing prevent release of phthalates esters from packing materials that affect health status and avoid complications.

ETHICS APPROVAL

The ethical approval according to King Abdulaziz University.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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