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RESEARCH ARTICLE

Risk of Heavy Metal Contamination in Krill Oils

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ABSTRACT

Different omega-3 sources have been widely used as a portion of supplementary food in recent years. One of the popular sources of omega-3 fatty acids is krill oil. Thus, the aim of the current study was to examine the contents of commercially available krill oils sold in the markets. For this purpose, a total of 11 different krill oil brands randomly selected from different pharmacies. The chemical analysis was carried out in a laboratory accredited by the Turkish Accreditation Agency. Our results indicated that the fatty acid contents of the commercial krill oils tested varied to quite an extent, but within the tolerable limits in 10 out of 11 samples. The peroxide content of the samples differed from 10 to 30 meqO_2/kg-oil. The mercury and cadmium levels were up to the standard limits set by Codex Alimentarius for food supplements. However, all products contained more than the tolerable limits of lead and only 1 sample had arsenic levels measured below acceptable limits. Thus, none of the krill oil samples provided the required European Union standards. It suggests that the manufacturers overlooked some issues while producing krill oils. This may pose a potential threat to public health in the long term.
Keywords: Fatty acid composition, heavy metal contamination, krill oil, peroxide levels

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Kril Yağlarında Ağır Metal Kontaminasyon Riski

ÖZ

Son yıllarda gıda takviyesi olarak farklı omega-3 kaynakları yaygın olarak kullanılmaktadır. Omega-3 yağ asitlerinin popüler kaynaklarından biri de kril yağıdır. Bu nedenle, mevcut çalışmanın amacı, piyasada bulunan kril yağlarının genel özelliklerini incelemektir. Bu amaçla farklı eczanelerden rastgele seçilen toplam 11 kril yağı markasından alınan numunelerin kimyasal analizleri Türk Akreditasyon Kurumu tarafından akredite edilmiş bir gıda analiz laboratuvarında gerçekleştirilmiştir. Elde edilen sonuçlar, test edilen ticari kril yağlarının yağ asidi içeriğinin oldukça büyük ölçüde değişse de 11 numuneden 10'unda tolere edilebilir sınırlar içinde olduğunu göstermiştir. Numunelerin peroksit içeriği, 10 ila 30 meq O_2/kg-yağ arasında bulunmuştur. Civa ve kadmiyum seviyeleri, gıda takviyeleri için Codex Alimentarius tarafından belirlenen standart limitleri içerisindeydi. Öte yandan, test edilen tüm ürünler, tolere edilebilir kurşun sınırlarından daha fazlasını içerirken, yalnızca 1 numunede kabul edilebilir sınırların altında arsenik seviyeleri görüldü. Sonuç olarak, rastgele örnekleme yoluyla toplanan kril yağlarının hiçbiri gerekli Avrupa Birliği standartlarını sağlayamadı. Bu durum üreticilerin kril yağları üretirken bazı konuları gözden kaçırdığını düşündürmektedir. Bu, uzun vadede halk sağlığı için potansiyel bir tehdit oluşturabilir.
Anahtar Kelimeler: Ağır metal kirliliği, kril yağı, peroksit seviyeleri, yağ asidi bileşimi

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INTRODUCTION

Krill" (Euphausia Superba) means "small fry of fish" in the Norwegian language. It is also a term used to describe the shellfish belonging to the Euphausiacea family (Nicol and Endo, 1997; Tou et al., 2007). Krill is a shrimp-like small crustacean that lives in the cold waters of the Antarctic Ocean (Vacchi et al. 2012), and there are about 85 species of krill. Their sizes can range from the smallest millimetric dimensions to 15 cm in length (Nicol and Endo 1997). They have opportunistic features and feed with any possible plankton. They also feed on algae to synthesize omega 3 (Eicosapentaenoic acid-EPA and Docosahexaenoic acid-DHA) for their bodies (Atkinson et al. 2004; Bettina et al. 2009).

Krill is one of the most abundant animal species due to its existence in oceans around the world. As a result, the focus has been on the use of Krill in aquaculture, sport fishing and as a commercial product in the form of bait in the aquariums (Tou et al. 2007; Vacchi et al. 2012). Although widely known as whale food, it is also a food source for seals, seabirds, fish, and, to a lesser extent, humans. Of the different species of krill, only two species of Antarctic (Euphausia Superba) and Pacific (Euphausia Pacifica) Krill are collected as commercial products (Nicol and Endo, 1997). Commercial krill products for human consumption are mostly in the form of frozen raw krill, boiled krill, and peeled krill meat. The use of krill as a food source for humans is expected to increase with the development of new products and technological advances (Tou et al. 2007).

Krill oil for human consumption is a newer product compared to fish oils. Krill oil was started to be used all over the world as a food supplement in the early 21st century (Schiermeier 2010). Krill oil has a better nutritional value in terms of the chemical composition of fatty acids and other antioxidant content than fish oils. There are basically four ingredients that make krill oil superior to other oils: The krill oil is in the form of phospholipids, its ORAC (oxygen radical absorption capacity) value is high, it has a high omega-3 and astaxanthin content (Farooqui and Farooqui 2009; Schuchardt et al. 2011). Thus, the use of krill oils as food and antioxidant supplements to support the physiological processes becoming more popular around the world. However, as with fish oils, heavy metal contamination is one of the biggest doubts about the safe use of krill oils.

The habitat of krill is mostly in the cold oceans of southern Antarctica and Antarctic Krills do not face heavy metals and pollutants like other fish because they live away from industrial areas (Tou et al. 2007). Therefore, it is accepted that krill oil should not contain heavy metals, considering that it is obtained from environments where there are no heavy metal contaminations and toxic wastes. The companies state that they generally obtain krill oil from Antarctic krill, but do not provide any assurance for the heavy metal content in the pharmaceutical products they sell. In this case, it is important to determine the safety and suitability of the conditions of the commercial krill oil products for sale. Thus, the main subject and scope of this research were to investigate whether commercially available krill oils were contaminated with heavy metals and toxic residues.

MATERIAL and METHODS

Commercial krill oils from 11 brands with the largest market share were selected. The selected brands are either directly sold in the European Union (EU) or packaged in Turkey from the krill oils directly acquired from the EU. These commercial krill oil samples were purchased randomly from the pharmacies, and these samples were analysed in the current study. Production dates and storage conditions of the krill oil samples were checked prior to the purchase. The samples were stored in appropriate conditions (refrigerated at +4 ºC) until the analysis. Each sample was measured twice for security purposes. Measurements were carried out at Ege University, Pharmaceutical Development Pharmacokinetics Research and Application Centre, Environment and Food Analysis Laboratory (ARGEFAR). The laboratory has been accredited by the Turkish Accreditation Agency, according to the TS EN ISO/IEC 17025: 2017 standards. The accredited certificate of the institution continues to be valid until March 14, 2023. The acidity values, peroxide levels, fatty acid contents, and heavy metal concentrations were determined according to TS EN ISO 660, TS EN ISO 3960, TS EN ISO 12966-4 and TS EN ISO 12966-2, and TS EN ISO EC 17025 AB-0040-T analyses, respectively.

RESULTS

From the 11 commercial brands tested, total of 25 fatty acids were tested. The types and amounts of tested fatty acids were in Table 1. A total of 12 saturated and 5 omega-3 fatty acids were found in the krill oil samples. Among the saturated fatty acids, Palmitic acid was the highest fatty acid present in 10 out of 11 samples. Among the 11 different brands of krill samples, EPA and DHA were the highest omega-3 fatty acids except for sample X10. Alarmingly, in the 10th sample, EPA and DHA levels were lower compared to the expected levels in krill oils, while the highest omega-3 fatty acid was Linolenic acid (Table 1).

The samples X2 and X5 had the highest level of saturated fatty acids (41.207% and 43.039%, respectively). The lowest saturated fatty acid was in sample X7 (7.547%). Accordingly, the highest EPA and DHA content were in sample X7 (42.454% and 26.993%, respectively). The lowest EPA and DHA content were in sample X10 (4.102% and 3.577%, respectively), and these levels of EPA and DHA were very low for standard krill oils. The highest level of omega 6 fatty acids was Linoleic acid in all samples tested except for sample X7. Furthermore, the Linoleic acid content of sample X10 was very high (41.722%; Table 1).

The peroxide levels were in Table 2. The overall peroxide levels for the samples tested were high. The lowest levels of peroxide were 10 mEq O2/kg, and the levels raised up to 30 mEq O2/kg in samples X7 and X10.

The heavy metal contents of commercial krill oils tested in the current study were in Table 2. Overall, except for one sample (X7), the arsenic levels tended to be high and varied between 0.028 and 9.824 mg/kg among the tested samples. The mercury levels were highest in X1 (0.041 mg/kg), whereas no traces of mercury were detected in X10. In general, the cadmium levels were low and ranged between 0 to 0.012 mg/kg. The lead levels were very high in all samples tested. The lowest levels of lead were in X8 (0.157 mg/kg), while X4 had the highest levels of lead (0.435 mg/kg).

Table 1. Fatty acid (FA) contents of the commercial krill oils tested in the current experiment.

Types of FA	Names of FA	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
Saturated FA (%)	Caprylic acid (C8:0)	-	-	-	0.319	-	-	0.328	-	0.438	0.004	0.007
	Capric acid (Cl0:0)	-	-	-	0.277	-	-	0.282	-	0.403	0.007	0.004
	Lauric acid (Cl2:0)	0.193	0.248	0.231	0.239	0.277	0.223	0.009	0.116	0.264	0.040	0.102
	Tridecanoic acid (C13:0)	0.050	0.083	0.079	0.056	0.067	0.078	0.002	0.032	0.092	0.014	0.029
	Myristic acid (Cl4:0)	8.875	13.517	12.101	12.842	14.466	11.926	0.268	6.917	12.787	2.256	5.818
	Pentadecanoic acid (C15:0)	0.397	0.558	0.471	0.446	0.516	0.518	0.030	0.279	0.481	0.178	0.294
	Palmitic acid (C16:0)	26.05	27.69	26.72	26.87	26.71	27.07	3.350	18.99	26.32	19.72	18.24
	Heptadecanoic acid (Cl7:0)	-	-	-	-	0.179	-	0.352	0.829	0.245	-	-
	Stearic acid (C18:0)	-	-	-	-	1.920	-	4.197	3.913	-	-	-
	Arachidic acid (C20:0)	0.032	0.031	0.026	0.026	0.109	0.024	0.964	0.351	-	0.339	0.426
	Behenic acid (C22:0)	0.035	0.042	0.048	0.021	0.047	0.041	0.078	0.170	0.062	0.035	0.135
	Tricosanoic acid (C23:0)	-	-	-	-	0.580	-	-	-	0.319	-	-
Total SFA %		34.929	41.207	38.825	39.715	43.093	38.994	7.547	29.821	39.104	21.972	24.054
Omega-3 FA (%)	Linolenic acid (C18:3n-3)	3.479	2.227	2.109	1.990	1.256	2.761	1.132	2.053	2.918	6.165	2.317
	Trans-Linolenic acid (C18:3n6)	0.192	0.235	0.218	0.237	0.189	0.215	0.221	0.307	0.252	0.069	0.323
	cis-8,11,14-Eicosatrienoic acid (C20:3)	0.090	0.090	0.122	0.097	0.132	0.085	0.393	0.192	0.119	0.049	0.283
	cis-11,14,17-Eicosatrienoic acid (C20:3)	0.404	0.379	0.327	0.267	0.243	0.361	0.621	0.257	0.371	0.066	0.298
	Eicosapentaenoic acid (C20:5n3)	28.413	18.532	22.300	21.461	15.734	21.941	42.451	22.181	20.685	4.102	23.379
	Docosahecsaenoic acid (C22:6n3)	13.419	8.524	9.796	8.651	7.157	11.195	26.993	12.387	11.206	3.577	14.601
Omega 3 %		45.311	29.283	34.205	32.102	24.147	35.897	70.576	36.621	34.809	13.844	40.297

Table 1 (Cont). Fatty acid (FA) contents of the commercial krill oils tested in the current experiment.

Types of FA	Names of FA	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
Omega-5 FA (%)	Myristoleic acid (Cl4:1)	0.157	0.208	0.210	0.195	0.203	0.190	0.005	0.101	0.210	0.014	0.076
Omega-6 FA (%)	Linoleic acid (C18:2n6)	2.838	3.777	3.003	3.126	3.922	3.494	1.520	3.332	3.298	41.722	4.169
	Arachidonic acid (C20:4n6)	0.393	0.408	0.411	0.393	-	0.381	3.284	1.400	-	0.273	1.762
Omega-7 FA (%)	Palmitoleic acid (C16:1n7)	4.439	7.774	7.727	8.293	9.546	5.904	1.350	6.219	6.271	2.456	5.748
Omega-9 FA (%)	Oleic acid (C18:1n9)	9.821	14.720	12.451	13.280	15.637	12.832	8.054	17.281	12.282	18.513	18.471
	Gondoic acid (C20:1n9)	0.668	0.898	0.897	0.886	1.114	0.745	4.119	2.692	0.860	0.286	3.199
	Nervonic acid (C24:1)	0.051	0.055	0.048	0.025	-	0.017	-	-	0.121	0.122	0.324

Table 2. Peroxide content and heavy metal levels of the commercial krill oils tested in the current experiment.

Parameters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
Peroxide (meq O_2/kg-oil)	10.0	10.0	10.0	10.0	10.0	10.0	30.0	10.0	10.0	30.0	10.0
Arsenic (As-mg/kg)	3.348	3.492	1.126	3.509	2.703	9.824	0.028	2.017	7.694	0.572	1.451
Mercury (Hg-mg/kg)	0.041	0.027	0.015	0,010	0.005	0.009	0.002	0.026	0.003	0.000	0.001
Cadmium (Cd-mg/kg)	0.003	0.005	0.001	0.001	0.004	0.012	0.000	0.005	0.003	0.001	0.002
Lead (Pb-mg/kg)	0.364	0.287	0.358	0.435	0.229	0.216	0.191	0.157	0.254	0.184	0.187

DISCUSSION

Today, the characteristic Western diet includes mass amounts of saturated fat, trans-fatty acids, and increased omega-6: omega-3 fatty acid ratio. It has been estimated that the diet of industrialized societies is deficient in omega-3 fatty acids. Moreover, the omega-6:omega-3 ratio is about 15-20:1, instead of 1:1 compared with the diet on which human beings used to have (Simopoulos, 2008). As a result, omega-3 sources such as fish and krill oils are suggested as a supplement for human diets and placed in the market for human use. The current experiment showed that 10 out of 11 commercial krill oil products had EPA and DHA content comparable to the Codex standard for krill oil (Codex Standards 2017; Xie et al. 2019).

The Myristic acid (C14:0), Palmitic acid (C16:0), Palmitoleic acid (C16:1), Oleic acid (C18:1), EPA (C20:5), and DHA (C22:6) are the major fatty acids in krill oils (Phleger et al., 2002; Xie et al., 2017). The Codex standards (2017) reports the common fatty acids and their percentages in krill oil as saturated fatty acids [Myristic (5-13%) and Palmitic acids (17-24.6%)], omega 3 fatty acids [Eicosapentaenoic acid-EPA (14.3-28%) and Docosahecsaenoic acid-DHA (7.1-15.7%], omega 6 [Linoleic acid (0-3%], omega 7 [Palmitoleic acid (2.5-9%)] and omega 9 [Oleic acid (6-14.5%)]. In the current study, the fatty acid contents of the commercial krill oils tested varied to quite an extent. When compared with the Codex standards (Codex Standards 2017), the Myristic acid levels of 4 samples were not within the limits (samples X2, X5, X7, and X10). Only 3 krill oil samples had standard Palmitic acid content (samples X8, X10, and X11). The major omega 3 fatty acids in krill oils, EPA and DHA, were within the Codex standards in 9 out of 11 samples, while the amounts of EPA and DHA were higher in sample X7 and lower in sample X10 compared to the standards (Codex Standards 2017). A total of 8 krill oil samples differed in Linoleic acid contents (samples X2, X4, X5, X6, X8, X9, X10, and X11) against the standards. Nevertheless, except for two samples (samples X7 and X10), these variations were not quite prominent and within the tolerable limits. The 7th sample had very low saturated fatty acid and high omega 3 levels, whereas the 10th sample had very low omega 3 and high omega 6 content compared to standard krill oil (Codex Standards 2017).

Different studies stated that fatty acid content and concentrations differ seasonally due to factors such as sexual maturity, reproduction, water depth, and phytoplankton abundance/quality at different times of the year (Phleger et al. 2012; Reiss et al. 2015; Schmidt et al. 2014). In addition, the storage conditions, transportation processes, and pre-treatment methods of raw materials also have an influence on the lipid composition of krill oil extracted (Sun et al. 2017; Tilseth and Hostmark 2015; Yin et al. 2015). However, very high omega 6 and very low omega 3 contents in the 10th sample were unacceptable since the main reason people buy these kinds of commercial oils for their presumably high omega-3 contents.

Nowadays, most of the people in modern society visualize themselves living a long and healthy life. Therefore, there are plenty of commercially available products that offer longer and healthier life. Among them, krill oils draw attention in recent years due to high and biologically available EPA and DHA content. The positive health effects of omega-3 fatty acids, especially EPA, and DHA, have come to the attention when diets of the Greenland Eskimos were explored (Bang et al. 1971). The Eskimos had high intakes of omega-3 rich seafood diet and very low rates of inflammatory and autoimmune diseases, asthma, cardiovascular disease, and multiple sclerosis. Nowadays we know that omega-3 fatty acids have several beneficial health effects on human health such as psoriasis, cardiovascular disease, cancer, and rheumatoid arthritis (Yashodhara et al. 2009). However, the fatty acid composition in the 10th sample is alarming and suggests that better control of commercial products is needed.

High peroxide levels in oils are one of the parameters that suggest a deterioration in the products. Although peroxide levels are not the only factor to decide deterioration levels of oils, in general, the high peroxide concentrations detected in oils suggest the deterioration and rancidity in the oils. The peroxide levels should be maximum of 5 mEq O2/kg according to the Krill Codex Standards (Codex Standards 2017). However, all tested samples had higher peroxide content than the standards: 9 out of 11 samples contained peroxide levels of 10 mEq

O2/kg, and the remaining 2 samples had peroxide levels of 30 mEq O2/kg. The high peroxide content of tested samples suggested that there could be a deterioration in these products before packaging since there was no problem with the expiration dates of these products.

According to Codex Alimentarius, maximum tolerable limits for arsenic and lead in edible fats and oils are 0.1 and 0.08 mg/kg, respectively. In this case, it is alarming that almost all commercial krill oil products tested contained more than the tolerable limits of arsenic and lead. Allowable limits of mercury and cadmium in food supplements are 0.1 and 1.0 mg/kg, respectively (Codex Alimentarius 2019). All the tested samples contained below the appropriate levels of mercury and cadmium.

Heavy metals in human foods and supplements can and will negatively affect consumers and human foods should be free of heavy metals for health purposes. Normally, krill oil should be extracted from the krill harvested in clean deep-water seas far from industrial developments in the Southern Atlantic Ocean. In addition, krill is near the lowest end of the food chain. Therefore, krill and krill oil should not accumulate heavy metals and other pollutants. However, previous studies showed pesticide residues in krill products (Corsolini et al. 2006; Covaci et al. 2007). Similarly, almost all samples examined in the current study had arsenic and lead contaminations that could be harmful to human health.

Heavy metal contamination of krill oil is an important risk and how these kinds of contaminations took place needs to be studied. One of the possibilities for heavy metal contamination in krill oil is the actual source of krill. The krill used for krill oil production might have caught from the oceans closer to the industrial areas rather than the South Atlantic Ocean or contaminated during the shipping process. Another possibility that explains the contamination was that the products may have been contaminated with these heavy metals during the extraction, production, and/or packaging phases of krill oils in the factories. In both cases, people buy these kinds of supplements to eat right and stay healthy. Therefore, leaving aside commercial concerns, it is inevitable to make the necessary adjustments to create a healthier production environment. The methods and techniques used to obtain oil should be checked again. Factors that may harm human health and cause contamination should be removed from the production lines of factories. A problem that may occur in any link of the food chain affects all living things. People should carefully use heavy metal residues that cannot be easily destroyed in nature, considering other animals. Wastes in production areas should be isolated in a way that does not pollute the environment. The worst scenario would be that humanity already started to pollute Antarctica and therefore started to harm even the creatures living in Antarctica. Antarctica is supposedly one of the most perfectly conserved regions on the planet (NISTAER 2014). Since there is no industrial area in or near Antarctica, it is unlikely that heavy metal residues will contaminate the krill population. Antarctica has been historically considered a remote and untouched continent. However, long-range transport of pollutants from other continents, local activities in research stations, and the Antarctic tourism industry have introduced many contaminants to the region (Bargagli 2008). Understanding the distribution of trace pollutants such as heavy metals and organic pesticides in Antarctica is considered a top research priority for the next decades (Kennicutt 2015). Paralleled to these concerns, a recent study, unfortunately, reported the presence of pesticides and mercury in mosses, topsoil, and water in Antarctica (Subhavana et al. 2019). Liu et al. (2021) also studied the concentrations of some heavy metals (such as Al, Cr, Pb, Hg, and As) in soil samples collected in East Antarctica. Although the contamination level of these heavy metals was relatively low, the elevated levels of heavy metals compared to the baseline concentrations started to be alarming for this pristine continent (Xu et al. 2020). Areas with high mercury and lead levels corresponded to the station areas that researchers or tourists live and bird colony areas. The rise in mercury and lead around bird population was attributed to the biomagnification of birds' food chain, and various types of anthropogenic activities (Li et al. 2020; Liu et al. 2021).

CONCLUSION

The presence of heavy metals in most of the samples exceeding the WHO and EU standards for oils and food additives poses a significant risk for public health. Furthermore, the varying degrees of alterations in the fatty acid composition from the codex standards for krill oils also create a handicap for food safety. Moreover, the presence of some heavy metals above the tolerable levels in some of the tested krill oil samples indicates that the risk is quite important from a public health point of view. The presence of heavy metals as well as the differences in the fatty acid composition between the statements in companies' prospectus and the values from the analysis is disturbing. These discrepancies suggest that the regulatory mechanisms are required to be more sensitive in monitoring dietary supplements. Thus, more stringent and sustainable control of the compliance of these products with WHO and EU standards is required by the authorities. All food supplements should be strictly controlled whether they comply with the values specified in the packaging and prospectus content. It can also be recommended to develop online control processes for consumers.

Conflict of interest: The authors declared that there is no conflict of interest.

Ethical Approval: : This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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