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Research Article

Investigation of possible heavy metals and antibiotic residues in commercial collagen

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ABSTRACT

The current study investigated whether commercial collagens were within physiologically acceptable limits to ensure their safer use. For this purpose, 10 of the 25 most popular collagen from fish and farm animals (FA) sold on the internet were randomly selected and purchased from a pharmacy. The zinc, lead, cadmium, mercury, and arsenic levels in these commercial products were then analyzed by ICP-OES. Streptomycin, sulfonamide, tetracycline, and chloramphenicol levels in the samples were determined by HPLC. No lead, mercury or arsenic residues were found in any of the tested samples. The mean cadmium levels in fish and FA collagen samples were not significant between the groups (P=0.2548). The lowest cadmium level in fish collagen samples was 0.152 mg/kg and the highest cadmium level was 0.288 mg/kg. Cadmium levels detected in FA collagen samples ranged from 0.183 mg/kg to 2.78 mg/kg. The mean zinc levels in fish and FA collagen were not significant (P=0.2644). The lowest zinc level in fish collagen was 1.368 mg/kg and the highest was 2673 mg/kg. The lowest and highest zinc levels in FA collagen were 1.750 mg/kg and 1528 mg/kg, respectively. According to the current results, no streptomycin, sulfonamide, and tetracycline residues were found in any of the collagen samples evaluated. Chloramphenicol was only in two fish collagen samples, but these values were below the lower detection limits. The results indicated that there is no or very low risk of heavy metal and antibiotic residues in commercial collagens sold in our country.

INTRODUCTION

The word collagen originates from Greek: "cola" means chewing gum and "gene" means to produce. Collagen is one of the most produced proteins in humans and many other living organisms. This special molecule is the main structural fibrillar protein found in connective tissues of the skin, tendons, joints, and bones. Thanks to its fibrillar properties, it is responsible for the stability and strength of body tissues by forming support networks between cellular structures (Nimni, 1980). Collagen fibers may be damaged due to aging and may lead to loss of function in many tissues where collagen support is provided. Therefore, it has been extensively investigated as a polymer for use in many biomedical products such as cosmetics and pharmaceuticals (Meena et al., 1999; Sionkowska et al., 2020). The cosmetic industry is making great efforts to incorporate this biomolecule into many existing products for use against skin aging, and there is a great demand for collagen in the food industry, as it has a high protein content and good functional properties such as water absorption capacity and emulsifying ability (Lafarga and Hayes, 2014; Schmidt et al., 2016).

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. In science, heavy metals are metallic elements that are toxic and have a high density, specific gravity, or atomic weight. The criteria used and whether to include metalloids vary by author and context. In metallurgy, for example, a heavy metal may be defined on the basis of density, in physics, the distinguishing criterion may be the atomic number, while a chemist would probably be more interested in chemical behavior. Nowadays, the term is commonly used in a slightly different sense, referring to any metal that can cause health problems or environmental damage. A commonly used criterion for heavy metals is a density greater than 5 g/cm^3. Examples of heavy metals include mercury, cadmium, arsenic, zinc, and lead (Pourret et al., 2021).

Antibiotics have been used since time immemorial. There is also good historical evidence that ancient civilizations used a variety of naturally available treatments for infections, such as herbs, honey, and even animal feces (Keyes et al., 2003). The term "antibiosis", meaning "against life", was coined by the French bacteriologist Jean Paul Vuillemin as a descriptive name for the phenomenon exhibited by these first antibacterial drugs (Foster and Raoult, 1974). Antibiosis was first described in bacteria in 1877 when Louis Pasteur and Robert Koch observed that an airborne bacillus inhibited the growth of Bacillus anthracis (Saxena, 2015). Since then, the efficacy and easy access to antibiotics has led to their overuse (Laxminarayan et al., 2013) and some bacteria have developed resistance to them (Gualerzi et al., 2013). The World Health Organization has classified antimicrobial resistance as a widespread serious threat. Global deaths attributable to antimicrobial resistance totaled 1.27 million in 2019 (Murray et al., 2022).

Mainly derived from farm animals and seafood, the use of

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collagen today has become attractive for both cosmetic and medical use. However, global pollution poses a risk to collagen sources. Therefore, while consuming collagen for a more conscious diet and a better life, it is possible to unknowingly be exposed to heavy metal and/or antibiotic residues. Research on heavy metal and antibiotic residues in commercially available collagen products have only recently begun. Studies on the safety of collagen products in terms of heavy metal and antibiotic residues are insufficient and the safe production process has yet to be completed. Therefore, the current study aimed to investigate some heavy metal and antibiotic residues that are likely to be found in commercial collagens on sale in Turkey.

MATERIAL and METHODS

The current study was supported by Mehmet Akif Ersoy University, Scientific Research Projects Coordination Office with project number 0767-YL-21.

Collection and Storage of Samples

A total of 4 most popular online shopping site in Turkiye was chosen according to the 2021 values. From these online sites, one was randomly selected by drawing method. On this online site, collagen brands from farm animals (FA) and fish without mineral additives were listed separately using a popularity filter. Within this list, 10 of the fish and FA collagen brands in the top 25 were selected randomly by drawing method. These brands were purchased from a local pharmacy in the Burdur city center. The collagens were kept at -5 °C until the analysis was performed.

Analysis of Heavy Metal and Antibiotic Levels in Samples

Heavy metal and antibiotic analyses of the samples were carried out in the laboratories of Mehmet Akif Ersoy University, Scientific and Technology Application and Research Center. Zinc, lead, cadmium, mercury, and arsenic levels in the samples were analyzed using an inductively coupled plasma optical emission spectrometer (Perkin Elmer ICP-OES Optima 8000). The limit of detection (LOD), wavelength, and R2 values are given in Table 1.

Table 1. Limit of detection (LOD), wavelength and R2 values used in ICP-OES analysis.

	Heavy Metals				
	Zn	Pb	Cd	Hg	As
LOD (µg/L)	5	5	5	1	5
Wavelength (nm)	213.85	220.353	226.502	253.652	188.9
R^2	0.999	0.999	0.999	0.999	0.999

Zn=Zinc, Pb=Lead, Cd=Cadmium, Hg=Mercury, As=Arsenic.

The chromatogram values of the samples tested for antibiotics are in Table 2. Shimadzu Prominence Brand HPLC instrument was used for antibiotic measurements. Antibiotic standards used for the analyses were purchased from Sigma-Aldrich (St. Louis, MO, USA) as Vetranal™ analytical grade standards (certified purity >95%). Methanol (MeOH), ammonium formate, acetonitrile (ACN), and formic acid (HPLC grade >95%) were purchased from Merck (Darmstadt, Germany). Disodium ethylenediaminetetraacetate (Na2EDTA) was obtained from Sigma Aldrich (St. Louis, MO, USA).

Table 2. Limit of detection (LOD), wavelength and calibration function values used in HPLC analysis.

	Antibiotics			
	Streptomycin	Sulfonamide	Tetracycline	Chloramphenicol
LOD (ppb)	1.21	3.11	1.48	2.13
RT	1.9	4.5	2.6	3.4
Wavelength (nm)	190	190	270	280
Calibration function	$Y=1.11*10^{-6}x+7.43*10^{-5}$	$Y=2.13*10^{-6}x+3.18*10^{-5}$	$Y=3.14*10^{-6}x+2.22*10^{-4}$	$Y=4.33*10^{-6}x+3.12*10^{-5}$

Statistics

T Test procedure of SAS statistical program was used for statistical evaluation where available.

RESULTS

The amounts of heavy metals tested from the samples are shown in Table 3. No lead, mercury or arsenic residues were found in any of the analyzed samples. The lowest cadmium level in fish collagen samples was 0.152 mg/kg and the highest cadmium level was 0.288 mg/kg (mean 0.2495±0.0393). The levels detected in collagen samples of farm animal origin (FA) were also low and ranged from 0.183 mg/kg to 2.78 mg/kg (mean 0.2311±0.0301). The mean cadmium levels in fish and FA collagen samples were not significant between groups (P=0.2548).

The distribution of zinc levels in the samples showed very

Table 3. Heavy metal concentrations in the samples (mg/kg).

Sample	Zn	Pb	Cd	Hg	As
F1	2.118	ÖA	0.152	UDL	UDL
F2	3.424	UDL	0.248	UDL	UDL
F3	1.546	UDL	0.277	UDL	UDL
F4	1.368	UDL	0.261	UDL	UDL
F5	5.831	UDL	0.222	UDL	UDL
F6	1935.0	UDL	0.268	UDL	UDL
F7	910.50	UDL	0.288	UDL	UDL
F8	4.169	UDL	0.276	UDL	UDL
F9	1.973	UDL	0.242	UDL	UDL
F10	2673.0	UDL	0.261	UDL	UDL
FA1	4.480	UDL	0.257	UDL	UDL
FA2	2.204	UDL	0.278	UDL	UDL
FA3	3.133	UDL	0.240	UDL	UDL
FA4	1.770	UDL	0.246	UDL	UDL
FA5	1528.0	UDL	0.214	UDL	UDL
FA6	2.938	UDL	0.190	UDL	UDL
FA7	5.200	UDL	0.237	UDL	UDL
FA8	4.059	UDL	0.183	UDL	UDL
FA9	2.654	UDL	0.215	UDL	UDL
FA10	4.718	UDL	0.251	UDL	UDL

F1-F10=Fish collagen samples, FA1-FA10, Farm animal collagen samples, UDL=Under detection limits, Zn=Zinc; Pb=Lead, Cd=Cadmium, Hg= Mercury, As=Arsenic.

Table 4. Antibiotic residue amounts found in the samples (mg/kg).

Sample	Streptomycin	Sulfonamide	Tetracycline	Chloramphenicol
F1	UDL	UDL	UDL	UDL
F2	UDL	UDL	UDL	UDL
F3	UDL	UDL	UDL	UDL
F4	UDL	UDL	UDL	UDL
F5	UDL	UDL	UDL	UDL
F6	UDL	UDL	UDL	UDL
F7	UDL	UDL	UDL	UDL
F8	UDL	UDL	UDL	UDL
F9	UDL	UDL	UDL	0.001
F10	UDL	UDL	UDL	0.001
FA1	UDL	UDL	UDL	UDL
FA2	UDL	UDL	UDL	UDL
FA3	UDL	UDL	UDL	UDL
FA4	UDL	UDL	UDL	UDL
FA5	UDL	UDL	UDL	UDL
FA6	UDL	UDL	UDL	UDL
FA7	UDL	UDL	UDL	UDL
FA8	UDL	UDL	UDL	UDL
FA9	UDL	UDL	UDL	UDL
FA10	UDL	UDL	UDL	UDL

F1-F10=Fish collagen samples, FA1-FA10, Farm animal collagen samples, UDL=Under detection limits.

different levels of variation among the samples. The lowest zinc level in fish collagens was 1.368 mg/kg and the highest was 2673 mg/kg with a group mean of 553.89 (±498.53). In FA collagen, the lowest and highest zinc levels were 1.750 mg/kg and 1528 mg/kg, respectively, and the group means was 155.92 (±148.49). There is a significant fluctuation in zinc levels between both fish and FA collagen samples. In addition, statistically, the average zinc content in fish and FA collagen was found to be insignificant (P=0.2644).

The results of antibiotic residues measured in fish and FA collagen are in Table 4. According to the results, no streptomycin, sulfonamide, or tetracycline residues were found in any of the collagen samples evaluated. Chloramphenicol was detected in only two fish collagen samples and these values were below the lower detection limit.

DISCUSSION

According to a study from Nutrition Business Journal, the international collagen market was worth approximately $1 billion in 2019 and is estimated to have a yearly growth rate of 7.7%. Worldwide, the market could reach $6.5 billion by 2025 as collagen continues to be incorporated into more foods and beverages, topical products, and even used for patient treatment (Watrous, 2020). The major factors driving the progression of the collagen market include the increasing demand for dietary supplements, the growing adoption of collagen in the food and beverage industry, and the growing trend of consumers toward healthy and protein-rich diets. In essence, the growth of the protein powders and collagen market is further driven by the consumer perspective that food is medicine and medicine is food.

No studies on heavy metal contamination in collagens were found in the current literature. However, Consumer Reports (2010) purchased 15 of the best-selling protein powders in the US and tested them for heavy metals. While the results revealed that levels of heavy metals did not pose a threat to human health, the levels detected in a few products were at alarming levels. Consuming three servings a day of some products was found to be enough to cause daily exposure to arsenic, cadmium, or lead in excess of recommended limits. The study concluded that cadmium is particularly important since it accumulates in different organs and causes damage specifically in the liver and kidneys. Also in 2018, the Clean Label Project, a national non-governmental organization, conducted a study of 134 protein powder products of animal origin and screened these products for heavy metals, bisphenols, and pesticides. The study found that 70% of the best-selling protein powders in the US had lead levels up to 123.5 ppm and 74% had cadmium levels up to 306 ppm (Organic Consumer Reports, 2020).

In a recent study, some heavy metals were also detected in krill oils, one of the popular food supplements today (Kızılırmak et al., 2022). Although cadmium (1.0 mg/kg) and mercury (0.1 mg/kg) levels of the tested samples of 11 randomly selected krill oil brands were found to comply with the standard limits, all of the tested krill oils contained lead above the tolerable lead limits (0.08 mg/kg) specified by Codex Alimentarius for food supplements. Furthermore, only 1 sample had arsenic levels below acceptable limits (0.1 mg/kg). The results showed that krill oils in Turkiye may pose a potential threat to public health in the long term (Kızılırmak et al., 2022).

The same concerns apply to collagen since it is produced from animal sources. In a study conducted by the Clean Label Project in the USA, 30 collagen products were tested for arsenic, cadmium, lead, and mercury. It was reported that the amount of mercury was below the detectable level in 66% of the supplements tested, and although trace amounts of mercury were detected in the remaining 34%, these values were lower than 8ppm and did not pose public health. Moreover, arsenic, cadmium, and lead residues were not detected in 36%, 83%, and 63% of the samples tested. Although 64% of the collagen samples contained measurable concentrations of arsenic ranging from 0.09 to 4.7 µg within the recommended daily allowance, these values were below the State of California level of 10 µg. Cadmium was found in 17% of the collagen products tested, ranging from 0.23 to 9.17 µg in the recommended daily allowance of collagen. This 4.1 µg dose was more than twice the daily limit for the state of California. Lead was measured in 37% of the products tested at levels ranging from 0.09 to 1.57 µg in the recommended daily allowance of collagen. The amount of lead found in the 4 products investigated was 2 to 3 times higher than the maximum acceptable level of 0.5 µg. One of the products tested failed to meet the safety criteria in both the cadmium and lead categories. In addition, four out of a total of 30 products (13%) failed to meet the standards for maximum limits, which the Collagen Stewardship Alliance characterizes as "clearly unacceptable" (Clean Label Project, 2021).

In contrast to these studies, it is pleasing to note that none of the samples in our study had detectable levels of mercury, lead, and arsenic, while cadmium was found at minimal levels. Mercury is a naturally occurring metal in the environment. However, mercury can be found in different parts of the world due to agricultural processes, industrial applications, manufacturing, and pollution (Gworek et al., 2020). Lead is also a toxic heavy metal. It has many industrial applications and was commonly used in paint and water pipes before modern trends were established. Like other toxins, lead can leach into water and soil and contaminate food. Like other toxins, lead can leach into water and soil and contaminate food (Bouchard et al., 2009). Arsenic is a naturally occurring element on the earth's surface. However, it is also used in industrial settings such as smelting and mining operations. Arsenic is very toxic to animals and humans. It can leach into soil and water and can be absorbed by plants. Aquatic animals, red and white meat, dairy products such as milk and yogurt, and grains are the main sources of dietary arsenic. In areas where arsenic is naturally present at high levels, foods such as rice prepared with high-arsenic water and food crops irrigated with contaminated water also contribute to the total daily intake (Grund et al., 2008). As a toxic natural element found worldwide cadmium can be found in our food chain through industrial pollution of water sources and topsoil. Thus, grains, vegetables, fruits meats, seafood, and protein drinks can be contaminated by cadmium (EFSA, 2012). In this context, the absence of these heavy metals in the collagen products tested in our study shows that these products sold in Turkey do not pose a health threat in terms of heavy metals.

In our study, different levels of zinc were found in collagen samples. Zinc is known as an essential mineral that has been used as a dietary supplement for recent years. It can be found naturally in foods. Zinc is also used in some cold medicines and denture adhesive products. Zinc is an important mineral that has a part in cellular metabolic events. It is essential for the catalytic activity of many enzymes and plays a role in enhancing immune function. Zinc is crucial for growth and development (Ryu and Aydemir, 2020), it is essential for cell division and signaling, DNA and mRNA synthesis, and wound healing (Ryu and Aydemir, 2020; King and Cousins, 2014). Thus, the zinc values obtained from the samples analyzed did not pose a health risk in general. On the other hand, in the collagen products tested, there was no indication on the labels that they contained zinc. Therefore, the relatively high zinc levels in some samples may pose a health problem for people taking additional zinc as a food supplement. High zinc intake can cause headaches, upset stomachs, nausea, loss of appetite, and dizziness (Ryu and Aydemir, 2020; King and Cousins, 2014). Zinc consumption for longer time periods for doses of 50 mg or more (usually from overuse of zinc-containing denture adhesive products or supplements) might lower HDL cholesterol concentration hamper copper absorption and negatively affect immune function (King and Cousins, 2014; Plum et al., 2010; Ryu and Aydemir, 2020). Higher doses of zinc up to 142 mg/day could also impede magnesium absorption and disturb magnesium homeostasis (Spencer et al., 1994). Therefore, unintentional excess zinc intake can be detrimental to an individual's health and should therefore be stated on the label of food supplements such as collagen.

The use of antibiotics is essential in the prevention and treatment of animal diseases (Darwish et al., 2013). Nowadays, antibiotics are used in animals not only to treat diseases but also to improve animal production. Antibiotics improve growth and feed efficiency and reproductive performance and synchronize the reproductive cycles. Growth improvement due to antibiotics was first described in the mid-1940s and growth-promoting antibiotics became common practice in animal practice. Antibiotics are among the most important compounds involved in animal feed production. Approximately 80% of farm animals used in food production are treated with antibiotics during their lifetime (Pavlov et al., 2008). However, The World Health Organization (WHO) has called for a ban on growth-promoting antibiotics, arguing that antibiotic usage could cause a variety of health problems in individuals (Graham et al., 2007). This is because, if not used with caution, these antibiotics can cause residues to accumulate in animal products such as milk, cheese, eggs, and meat that are not allowed in foods intended for human consumption.

Antibiotics can enter the human body in different ways, either directly or indirectly through use in animals as growth stimulants, disease prevention and treatment, and contamination (Phillips et al., 2004). A recent study ranked antibiotic use in farm animals by country. China and United States ranked first and second places with 23% and 13%, respectively. The ranking of antibiotic use in farm animals by other countries was 9% for Brazil, 3% for India, and 3% for Germany (Van Boeckel et al., 2015). Antibiotic residues are metabolites present in trace amounts in any edible part of animal products after antibiotic administration. Antibiotic residues greater than the maximum tolerable limit in food animals may contribute to the development of antibiotic resistance in animals or humans. In this context, the absence of antibiotic residues in commercially available collagen is important for public health. In our literature review, no study investigating possible antibiotic residues in collagen products was found. The data obtained from our study revealed that sulfonamide, chloramphenicol, tetracycline, and streptomycin were not found in either fish or bovine collagen supplements.

CONCLUSION

The results of this study showed that commercial collagens (from livestock and fish) selected by random sampling in Turkiye do not contain heavy metals (zinc, lead, cadmium, mercury, and arsenic), and antibiotics (streptomycin, sulfonamide, tetracycline, and chloramphenicol) residues tested at a level that may threaten public health. Our study also revealed that zinc, a useful metal for mammals, were present in some samples at considerable levels. Although not at toxic levels, these levels of a metal that is not listed on the label may cause health problems in people who take zinc as a food supplement.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interests.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: DD, OYG

Data collection and analysis: DD, OYG

Drafting of the manuscript: DD, OYG

Critical review: DD, OYG

Data Availability

Data supporting the findings of this study are available fromthe corresponding author upon reasonable request.

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