Commentary Article

The Estrogenic Contaminants in Food and Its Detection Methods: A Systematic Review

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ABSTRACT

Background: Many compounds are known as estrogen contaminants. Estrogenic components may enter the body through food consumption.

Objectives: This systematic review aimed to determine various estrogens contaminants, the foods that are primarily contaminated with these compounds, and their common detection methods.

Methods: The relevant studies with the keywords "estrogen," "detection," and "food" were systematically searched in PubMed and Scopus databases. Science Direct and Google Scholar were also searched.

Results: A total of 221 studies were initially found regardless of publication time. The preliminary screening was based on the exclusion and inclusion criteria. Then, the qualitative evaluation of the articles was done, and finally, 9 articles were selected. Among different foods, most estrogenic compounds were identified in seafood. This finding indicates that estrogenic compounds have entered the waters. The most reported compound was bisphenol A. Cell culture was used for bioassay evaluation, and liquid chromatography methods were used for analysis.

Conclusion: Both analytical and bioassay methods were used to evaluate estrogenic compounds. Most studies found that the bioassay method was also valid.

Keywords: Bioassay, Detection, Estrogen, Food, Analytical methods

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Introduction

Estrogens in the body act through alpha and beta receptors. They demonstrate beneficial effects, like protecting the cardiovascular system (Gurrala et al., 2021) and sustaining the metabolic and natural processes of the body. This hormone affects calcium absorption (Qaid & Abdoun, 2022). However, Endocrine disturbance chemicals can interfere with the release, synthesis, activity, and metabolism of endogenous hormones (Law et al., 2012). One of the toxins that disrupt the endocrine system is environmental estrogens. Environmental pollution of these components is widespread (Capriotti et al., 2013), so the probability of their entering the food chain is very high. About 90% of human exposure to environmental estrogen is through contaminated food (Adeel et al., 2017; Law et al., 2012). Hormones act in small amounts, so if similar compounds enter the body, even in small quantities, they can significantly create serious side effects. These compounds negatively affect human and plant health (Cheraghi et al., 2021). In fish, these compounds cause fish feminism (Van Nuijs et al., 2011). Fish is a food source that is considered to be contaminated with estrogenic compounds (Rahmati et al., 2022). Environmental studies have shown that low amounts of estrogens in water disrupt the reproduction of fish and amphibians (Ojoghoro et al., 2021).

Wastes from pharmaceutical and plastic factories and pesticides with estrogenic activity enter the waters (Dey, 2022). These components affect both sexes. Increased estrogen in females leads to various cancers in the uterus, colon, pituitary, and breast, as well as blood clots and cardiovascular diseases (Adeel et al., 2017; Watson et al., 2011). The estrogenic chemicals reduce sperm and increase prostate cancer in men (Adeel et al., 2017). Estrogenic compounds are found in pesticides, PVC, food packaging materials, PET (polyethylene terephthalate) bottles, and various industrial materials (Inoue et al., 2002; Wagner & Oehlmann, 2009). According to previous studies, the mineral water in PET and Tetra Pak packages has more estrogenic compounds than the mineral water in glass bottles (Wagner & Oehlmann, 2009). Foods of animal origin can contain significant amounts of these estrogens (Chighizola & Meroni, 2012). Sometimes, synthetic hormones with these effects are used in livestock breeding (Qaid & Abdoun, 2022). In addition, soybean, used in animal feed and provides the protein needed by livestock (Messina, 2022), contains active components like isoflavones, called phytoestrogens (Qaid & Abdoun, 2022).

Analytical methods, such as Gas chromatography (GC) and high-performance liquid chromatography (HPLC), are used to identify estrogens (Giese, 2003). Furthermore, these compounds bind to estrogen receptors or transcription factors (Watson et al., 2011). They can bind to estrogen receptors at very low doses and lead to cellular changes such as the proliferation of apoptosis and metastasis (Qie et al., 2021). Bioassay methods identify active compounds in different matrices (Amoli et al., 2009). One of the bioassay methods is E-screen, which is usually used to evaluate estrogenic activity in food (Sadighara et al., 2022). In addition to threatening human health, these compounds endanger water resources, soil, and plants (Adeel et al., 2017).

Therefore, their identification method is essential. This review discusses the type of foods, estrogenic contaminants, and the diagnostic methods of these compounds in foods.

Materials and Methods

This systematic review was conducted using the PRISMA (preferred reporting items for systematic reviews and meta-analyses) checklist. Two authors performed all study stages, including evaluation of inclusion and exclusion criteria and data extraction to prevent bias (P.S and S.M).

Search strategy

The articles in the English language were searched in PubMed and Scopus on 14 May 2021 without time limitation. The research team compiled the keywords based on the prepared protocol. Possible synonyms were also checked for words that did not have a specific case. The keywords for searching were as follows: “Estrogenic,” “detection,” and “food.” In addition to selected databases with compiled keywords, Science Direct and Google Scholar were searched to complete the search process.

Inclusion and exclusion criteria

Two reviewers (P.S and S.M) independently searched the keywords in databases. The inclusion criteria for this systematic review included original articles that measured xenoestrogen levels by valid methods. At first, the title and abstract of the papers were read. Papers without our objectives and research questions were excluded from the study. Two authors screened the articles. In case of disagreement, the opinions of the corresponding author were obtained.

Data extraction and data item

The name of the first author, study time, type of food and estrogen, country, and method of measuring xenoestrogens were extracted. Furthermore, the types of estrogen in three classifications of natural, industrial, and synthetic estrogens, with relevant examples, were extracted from the articles. The data were extracted by two reviews independently. If the articles' full text was unavailable, the authors of the article were emailed to provide the full text.

Results

Study selection

After searching PubMed, Scopus, Science Direct, and Google Scholar, we extracted 221 potential articles. Sixty-seven duplicate articles were initially excluded from the study. The abstracts of the remaining articles were carefully screened, and 121 articles were excluded from the study at this stage. Also, 5 articles were excluded due to conference abstracts, 54 animal studies articles, and 25 biomonitoring articles, detection in water, feed, packages, sediments, and wastewater. In addition, 37 articles were review articles and were excluded from the study. Next, the full text of 33 papers was carefully studied. Ultimately, 9 articles were selected based on the inclusion criteria of the proposals, and their quality assessments were performed. To evaluate the quality of the articles, 5 factors were considered. Papers that received a score of 3 or more were included in this study. This step was also performed independently by the two authors. This systematic review used the PRISMA flow diagram (Figure 1).

Classification of estrogenic compounds in food

Table 1 presents the estrogenic compounds identified in food. Most of the compounds in this table are produced due to human activities and industrial pollution. In foods of animal origin, the hormonal compounds of 17α estradiol (αE2), 17β estradiol (βE2), estrone (E1), and estriol (E3) were found whose estrogenic power is higher than plant and fungal estrogens (Capriotti et al., 2013). A possible source of nonylphenol (NP) is its use in car washes (Fenlon et al., 2010). Furthermore, humans can be exposed to NP of medical PVC devices (Inoue et al., 2002). Some pesticides, such as Methiocarb, have estrogenic properties (Sinha et al., 2021). In addition, most organochlorine insecticides have estrogenic properties (Sharma et al., 2021).

Discussion

Environmental estrogens enter the body through food. Estrogen affects the metabolic system in very low doses, so tracking and determining their amount in food is necessary. Zearalenone (ZEN) is a known xenoestrogen among fungal metabolites with a very high estrogen receptor affinity (Braun et al., 2020a). Zearalenone passes through the placenta and enters the fetal body, leading to estrogenic effects (Braun et al., 2020b). ZEN significantly affects the reproductive system and even infertility. This mycotoxin is produced by Fusarium culmorum and Fusarium graminearum (Videmann et al., 2009). Two studies were conducted on the evaluation of ZEN in breast milk. In both studies, amounts of ZEN were not measured (Braun et al., 2020a). In both studies, the LC-MS/MS (liquid chromatography with tandem mass spectrometry) method was employed for ZEN detection. This method is reliable and sensitive.

Another estrogenic compound that was identified was bisphenol A. It is one of the monomers of polycarbonate and is used as an additive in many plastics, including polystyrene resins (Ocharoen et al., 2018; Vivacqua et al., 2003). Bisphenol A impairs the reproductive system and can cause cancer and diabetes (Ocharoen et al., 2018). Bisphenol A is released from lacquer-coated cans. One of the significant sources of bisphenol A is canned foods. Exposure also occurs through water stored in polycarbonate bottles (Le et al., 2008). Bisphenol A contamination is also found in rivers and groundwater (Pignotti et al., 2017). In the study of Vivacqua et al., the level of contamination with bisphe-

Table 1. The various types of estrogen compounds found in the food

Industrial Estrogen	Natural Estrogen			Synthetic Estrogen		
	Mycoestrogen	Phytoestrogen	Mammalian Estrogens	Hormonal Agent	Alkyl Phenol	Pesticide
2,3,7,8-tetrachlood-ibenzo-p-dioxin (TCDD), polychlo-rinated biphenyls (PCBs)	Zearalenone (ZON)	Isoflavones Polyphenols lignans	17α-estradiol (αE2), 17β-estradiol (βE2), estrone (E1), and estriol (E3)	Diethylstilbestrol (DES) 17-α-Ethynylestradiol	Bisphenol A (BPA), octylphenol (OP), nonylphenol (NP),	Chlorinat-ed insecti-cides

Table 2. The type of food xenoestrogen and the detection method according to the published data

Authors/Year	Country	Type of Xenoestrogen	Type of Food	Analysis Method	Bioassay	Statements
Braun et al., 2020	Austria	Zearalenone (ZEN)	Breast milk	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)	-	< Limit of quantification (LOQ)
Braun et al., 2020	Austria	Zearalenone (ZEN)	Breast milk	LC-MS/MS	-	All samples were free of ZEN and its metabolites.
Brotons et al., 1995	Spain	Bisphenol-A	Canned food: peas, artichokes, green beans, mixed vegetables, corn, mushrooms, asparagus, palm, hearts, peppers	LC-MS/MS	E-screen test (Cell proliferation in MCF-7 cells)	Different levels of bisphenol A were found in the range of 22.9 to ND (µg/can) The highest amount of bisphenol A was found in canned peas A direct relationship was observed between analytical and bioassay methods
Garritano et al., 2006	Italy	Total polychlorinated biphenyls (PCBs)	Fish	Gas chromatography-electron capture detector (GC-ECD)	In vitro yeast reporter gene assay	Among PCBs studied, PC180, PC153, PC101 and PC28 had the highest estrogenic activity
Law et al., 2012	Hong Kong	Residual hormonal	Meat, fish, chicken	-	Luciferase reporter assays	Residual concentrations of hormones in fish and poultry skins are higher than elsewhere.
Lu et al., 2012	USA	Alkylphenols, bisphenol A, estrone, 17α-estradiol, 17β-estradiol, 17α-ethynylestradiol	Lettuce, tomato, potato, citrus	GC/MS	-	Bisphenol A and 17β-estradiol were detected in all samples In none of the samples, 17α-estradiol and 17α-ethynylestradiol was not detected
Ocharoen et al., 2018	Thailand	Bisphenol-A 17β-estradiol	Green mussel	HPLC	-	The detection range for bisphenol is 15.3–109.97 ng/g, and for 17β-estradiol: 12.96–152.8 ng/g
Teh & Morlock, 2015	Germany	Phytoestrogen	Cold-pressed hemp, flax, and canola seed oil	-	Estrogen-sensitive yeast cells *S. cerevisiae* (pYES assay)	These oils have estrogenic activity
Vivacqua et al., 2003	Italy	Bisphenol-A 4-nonylphenol	Apple, cherry, courgette, cucumber, eggplant, fennel, green bean, lettuce, medlar, orange, peach, pepper strawberry, tomato	GC/MS	Proliferation assay transfection assays	The detection range for bisphenol is 0.25-1.11 mg/kg, and for 4-nonylphenol: 0.12-1.2

ND: Not determined.

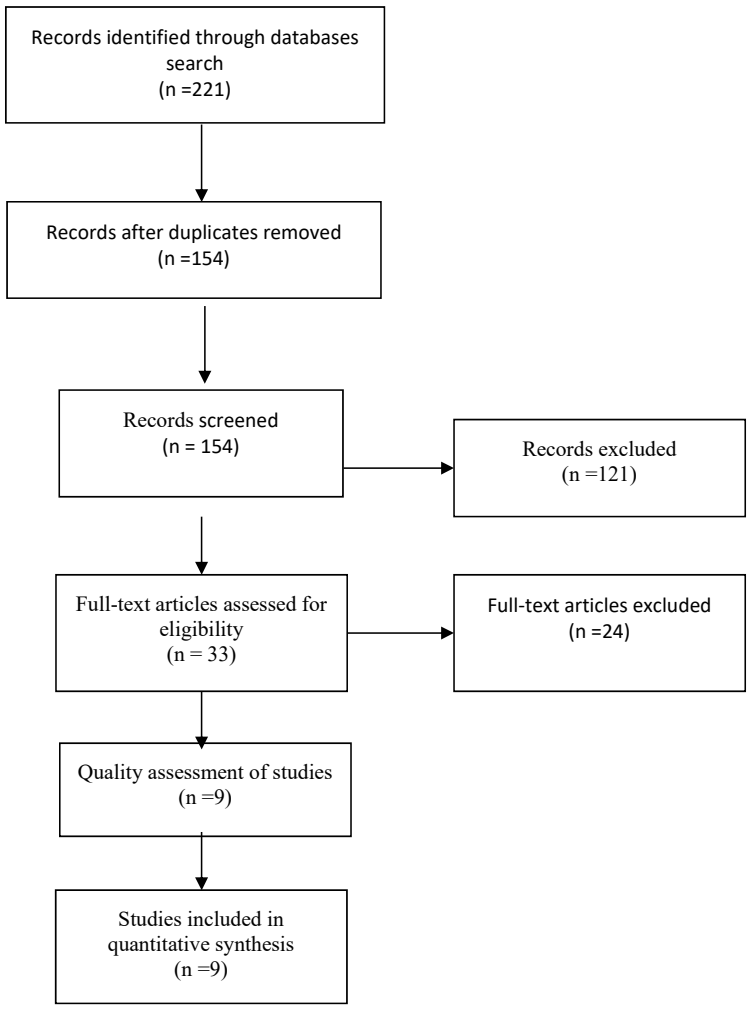

Figure 1. The diagram of systematic search of the study

nol A and 4-nonylphenol in fresh food was also observed (Vivacqua et al., 2003). In the study of Lu et al. (2012), estrogenic compounds were measured in plant-based foods. All samples had bisphenol A and 17β-estradiol. In this study, 17α-estradiol and 17α-ethynylestradiol were not detected in any of the samples. 17α-Ethynylestradiol is a synthetic estrogen that humans use. Therefore, its presence in food seems unlikely. In this study, all compounds were measured with GC/MS (gas chromatography–mass spectrometry). One of the most common methods of measuring bisphenol A is GC/MS (Adeyi & Babalola, 2019; Howdeshell et al., 2003). This method has a higher diagnostic power and is cheaper than LC/MS/MS (liquid chromatography with tandem mass spectrometry) for the detection of bisphenol A (Martín-Pozo et al., 2022).

Brotons et al. used LC-MS/MS and bioassay tests to detect bisphenol A in canned food products. The cell proliferation in MCF-7 cells was used as an E-screen test. This study observed a positive correlation between the amount of bisphenol A and estrogenic activity bioassay assessment (Brotons et al., 1995). Green mussel is a type of seafood. Ocharoen et al. (2018) found significant amounts of bisphenol A and 17β-estradiol. This study used HPLC chromatography to measure bisphenol A and 17β-estradiol. Samples were collected from near industrial centers that do not manage wastewater treatment (Ocharoen et al., 2018).

Polychlorinated biphenyls (PCBs) are estrogenic compounds that cause sperm reduction abnormalities in the reproductive system and malformation (Bagale, 2021). Furthermore, PCBs have other toxic effects, such as immunotoxicity and neurotoxicity (Garritano et al., 2006). This study investigated PCBs' presence by GC chromatography and bioassay method. A correlation was not found between the amount of PCBs and estrogenic activity (Garritano et al., 2006).

Teh and Morlock, (2015) used a planar yeast estrogen screen (pYES) assay for cold-pressed hemp, flax, and canola seed oil. This modified yeast has a human estrogen receptor. In this study, all three oils contained estrogenic activity due to phytoestrogens that are naturally present in the composition of these oils (Teh & Morlock, 2015). In previous studies, the sensitivity of this method to identify estrogenic compounds in food packaging has been confirmed (Bergmann et al., 2020). This method has also been used to identify estrogenic compounds in water. Its sensitivity and specificity have been confirmed as a screen test to determine these compounds in water (Bistan et al., 2012).

In the study of Law et al. (2012), receptor-mediated responses by estrogen and androgen hormones, glucocorticoid-like, progesterone-like, and dioxin-like components in food of animal and marine origin were investigated for the detection of hormonal residues. The luciferase reporter assay test was used in this study. The concentration of these compounds was higher in the skin of fish and chickens. Luciferase assay is a bioassay with high sensitivity in detecting estrogenic compounds in food. A study observed that this assay can detect low amounts of bisphenol A in food (Ishida et al., 2023). Furthermore, this method is cost-effective for assessing estrogenic compounds in foods for risk assessment (Law et al., 2012).

Table 2 presents that the LC-MS/MS is used more than other chromatographic methods. In previous studies, this method has been emphasized (Van Nuijs et al., 2011). Chromatographic methods are expensive methods.The GC-MS method for detecting of most estrogenic components requires derivation (Van Nuijs et al., 2011). In this systematic review, the sensitivity of bioassay tests was identified in some studies. Therefore, these bioassay methods can be used for regular food monitoring.

One of the limitations of this study is that in some studies, both methods were not investigated together. More research studies are needed so that both tests (bioassay and chromatography) are used together to evaluate these compounds.

Conclusion

In this study, the estrogen contaminants in food were identified. These contaminants are divided into three categories: Industrial estrogens, natural estrogens, and synthetic estrogens. Measurement of bisphenol A is more prominent than other estrogenic contaminants among the selected studies. Bisphenol A is produced through human and industrial activities, so we need further processing for wastewater treatment. The analytical methods are costly. Therefore, screen tests can be applied to identify these components in foods. According to some published manuscripts, bioassay methods are valid to detect these toxic compounds in food. In future studies, it is recommended that the correlation between analytical and bioassay methods and the sensitivity of these methods be measured to detect different estrogenic compounds in various food matrices.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Study design and search the databases: Parisa Sadighara; Data extraction: Sarah Mohamedi; Drafting the manuscript: Naiema Vakili Saatloo, Intissar Limam, Melina Sadighara and Tayebeh Zeinali; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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مقاله مروری کوتاه

آلاینده‌های استروژنیک در غذا و روش‌های شناسایی: مروری نظام‌مند

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چکیده

زمینه مطالعه: بسیاری از ترکیبات به‌عنوان آلاینده‌های استروژن شناخته می‌شوند. اجزای استروژنی ممکن است از طریق غذا وارد بدن شوند.

هدف: هدف از این مطالعه، بررسی نظام‌مند تعیین انواع آلاینده‌های استروژن و غذاهایی که عمدتاً به این ترکیبات آلوده هستند و همچنین روش‌های تشخیص رایج آن‌ها بود.

روش کار: مطالعات پژوهشی با کلیدواژه‌های استروژن، شناسایی و غذا به‌طور نظام‌مند در پایگاه‌های اطلاعاتی پابمد و اسکوپوس جست‌وجو شدند. علاوه‌براین در پایگاه‌هایی چون گوگل‌اسکالر و ساینس‌دایرکت هم مورد جست‌وجو قرار گرفتند.

نتایج: درمجموع ۲۲۱ مطالعه بدون توجه به زمان انتشار به دست آمد. در ابتدا غربالگری اولیه صورت گرفت و سپس ارزیابی کیفی مقاله انجام شد. درنهایت تنها ۹ مقاله براساس معیارهای خروج و ورود انتخاب شدند. در بین غذاهای مختلف، بیشتر ترکیبات استروژنیک در غذاهای دریایی شناسایی شد. این موضوع مؤید آن است که ترکیبات استروژنی در حال ورود به آب‌ها هستند. بیشترین ترکیب گزارش‌شده هم بیسفنول A بود. برای ارزیابی زیست‌سنجی از کشت سلولی و برای تحلیل از روش کروماتوگرافی مایع استفاده شد.

نتیجه‌گیری نهایی: در ارزیابی این ترکیبات از روش تحلیلی و زیست‌سنجی استفاده می‌شود. اکثر مطالعات نشان دادند که روش سنجش زیستی نیز معتبر است.

کلیدواژه‌ها: استروژن، سنجش زیستی، شناسایی، غذا، روش‌های دستگاهی

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