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Research Full Papers
Effect of food processing on aflatoxin reduction in cereals and nuts: A meta-analysis approach

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Abstract

Fungal toxins, mycotoxins such as aflatoxins, are compounds produced by different fungi during the growth and reproduction period. The most important fungal toxins that jeopardize human health are aflatoxins, which are produced by *Aspergillus* fungi and can grow in all crops. With toxic and carcinogenic effects of aflatoxins, many studies were performed using different methods to eliminate or reduce the amount of aflatoxin in cereals and nuts. On the contrary, using different methods for reducing aflatoxins in cereals and nuts make it impossible or difficult for researchers who study one or few related articles. This paper was conducted to review, investigate and do a meta-analysis on the results of the studies conducted and aimed to answer this general question as by which method can further reduce the amount of aflatoxin in cereals and nuts. Results showed that the methods of UV-irradiation, Ozone & UV irradiation and citric acid were the most important methods by 0.469, 0.441, and 0.427 of effect size respectively.

Keywords: Cereals, Food processing, Meta-analysis, Nuts.

Introduction

Fungal toxins, mycotoxins, are compounds produced by different fungi during the growth and reproduction period. Natural fungi found in human food sources, which mainly include three genera *Aspergillus*, *Fusariums*, and *Penicilliums* (Mannaa and Kim, 2017; Nešić *et al*., 2021). The most important fungal toxins that jeopardize human health are aflatoxins, which are produced by *Aspergillus* fungi and can grow in all food crops (Kumar *et al.*, 2017; SINGH *et al*., 2021). The fungi multiply rapidly in hot and humid environments, generate toxins, and very keen on nuts and grains. Aflatoxins production mostly in crops and under dehydration stress conditions, high temperature, pest and mechanical damage, rainfall and improper storage(Kumar *et al.*, 2017). With toxic and carcinogenic effects of aflatoxins , a variety of methods-- physical methods (e.g., heating, microwave, gamma, and UV, etc.,), chemical methods (e.g., the use of chlorine, ozone, sodium bisulfate, hydrogen peroxide), and mechanical methods have been studird for eliminating or reducing the amount of aflatoxin in food crops (Javanmardi *et al*., 2020; Khoori *et al*., 2020; Marshall *et al.*, 2020; Martins *et al*., 2017; Pankaj *et al.*, 2018; Patras *et al.*, 2017; Roohi *et al*., 2020; Vijayalakshmi *et al*., 2018).

Meta-analysis is a statistical method by which independent and separate research results are obtained to achieve general results about treatments. In other words, it is a concise form of previous studies, which encourages a precise estimation of indicators and explanation of incongruences in research findings (Khaneghah *et al*., 2020). Using meta-analysis, researchers can justify contradictions and discrepancies in research and arrive at conceivable, stricter and more valid conclusions. When the effect of a treatment is consistent from one study to another, a meta-analysis is useful in identifying this common effect, and when the effect changes from a study to another, meta-analysis may help

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identify the cause(s) of this change (Keikotlhaile *et al*., 2010). Meta-analysis can be considered as a review of research background which is based on a knowledge accumulation paradigm because the most important assumption of this paradigm is that knowledge is accumulated as researchers proceed with their studies following the results and findings of former researchers (Pagliai *et al., 2021)*. The statistical unit in meta-analysis is research conducted before. The meta-analysis approach is to arrive at more reliable conclusions, and useful and effective in giving prominence and adjusting existing gaps and bottlenecks in the research background of the subject under study, providing the researcher with essential insight into new approaches to study (Hoye and Elvik, 2010; Keikotlhaile *et al*., 2010).

Table 1- A summary of research on the effects of different processes on the reduction of aflatoxins in cereals and nuts

Reference	Processing	Commodity
Pukkasorn *et al*., 2018	Ultra superheated steam	**Peanuts**
Martins *et al*., 2017	Roasting	**Peanuts**
Rastegar *et al*., 2016	Roasting	**Pistachio**
Lee *et al*., 2015	Heating process	**Soybean**
Mohamadi *et al*., 2012	Cooking	**Rice**
Arzandeh & Jinap, 2010	Heating	**Peanuts**
Tatrishrili *et al*., 2019	Microwave heating	**Peanuts**
Schmidt *et al*., 2019	Microwave heating	**Wheat**
Meenatchi *et al*., 20115	Microwave heating	**Maize**
Basaran & Akhan, 2010	Microwave heating	**Hazelnuts**
Zhang *et al*., 2020	Microwave heating	**Corn**
Patill *et al*., 2019	Microwave heating & Gamma irradiation	**Peanuts**
Li *et al*., 2019	Ozone & vu irradation	**Peanuts**
Porto *et al*., 2019	Ozone	**Corn**
El- Desouky *et al*., 2012	Ozone	**Wheat**
Bashiri *et al*., 2013	Ozone	**Pistachio**
Ferreira *et al*., 2020	Ozone	**Brazil nuts**
Chen *et al*., 2014	Ozone	**Peanuts**
Luo *et al*., 2014	Ozone	**Corn**
Abuagela *et al*., 2019	Citric acid	**Peanuts**
Jubeen *et al*., 2020	Citric acid, lactic and propionic acid	**Almond, peanut, pistachio, Walnut**
Rastegar *et al*., 2017	Citric acid	**Pistachio**
Safara *et al*., 2010	Citric acid	**Rice**
Ghanghro *et al*., 2016	UV radiation	**Wheat**
Basaran, 2009	UV radiation	**Hazelnuts**
Mazaheri, 2012	UV radiation	**Pistachio**
Jubeen *et al*., 2012	UV radiation	**Ground Nut**
Patil *et al*., 2019	Gamma irradiation	**Peanuts**
Serra *et al*., 2018	Gamma irradiation	**Corn**
Assunca *et al*., 2015	Gamma irradiation	**Nuts**

Despite many studies and the use of different methods to eliminate or reduce the amount of aflatoxin in cereals and nuts, no precise conclusion has ever been made so far about the

studies. On the contrary, using different methods for reducing aflatoxins in cereals and nuts make it impossible or difficult for researchers who study one or few related articles. Therefore, the research was conducted to review, investigate and do a meta-analysis on the results of the studies conducted and aimed to answer this general question as by which method can further reduce the amount of aflatoxin in cereals and nuts.

Materials and methods

The terms "aflatoxin reduction", "aflatoxin elimination" "processing", "cereals", "nuts", and "effects" were used as keywords in Google Scholar, Scopus, ISI WEB of knowledge, which allows for further investigations into different processes of aflatoxin reduction or elimination in cereals and nuts. Moreover, the article reference section was used for a better search. Afterward, such data as a type of process, crop, and its influence on the reduction or elimination of aflatoxin level were collected in Microsoft Excel spreadsheet (Table 1).

Meta-analysis calculations

Meta-analysis is widely used to combine the results of previous research (Hoye and Elvik, 2010). To make meta-analysis dispose of errors, we need to collect all studies conducted in a particular field, because failure to consider all studies would result in dissemination error, making results less reliable (Caird *et al.*, 2008; Thornton and Lee, 2000). Meta-analysis is divided into six consecutive stages; clear expression of a problem and hypothesis, determining criteria for including independent studies in meta-analysis, search and retrieval of resources and related studies, data coding, and statistical analysis of select studies, synopsis and report of results, and explanation of result application (Wang and Bushman, 1999). The meta-analysis is built on the size of an effect (r) (Caird *et al*., 2008).

Results and discussion

Calculating effect size

The size of an effect indicates the ratio of significance test to study the size. The concept of effect size was introduced by Cohen's studies, emphasizing its importance. Cohen contends that it is not enough to focus only on a significant level to approve or disapprove a hypothesis, but effect size has to be seriously taken into account (Kouba and Lysek, 2019). Meta-analysis invariable can calculate the size of an effect by working out mean, variance, and standard deviation values of groups or methods. But the most common statistics in this field are (r) and (d). The most important formula for calculating the effect size (ES) are those proposed by (Lipsey and Wilson, 2001) as follows;

$$d=\frac{2t}{\sqrt{df}}\quad d=\frac{2\sqrt{f}}{df}\quad d=\frac{2\,r}{\sqrt{1-r^2}} \tag{1}$$

$$r=\sqrt{\frac{x^2}{n}}\quad r=\sqrt{\frac{t^2}{t^2+df}}\quad r=\sqrt{\frac{F}{F+df}} \tag{2}$$

In addition to the above statistics, Fisher's Z index was used to make sure the research results are reliable. Therefore, both r and z indices were used in this research, which will be addressed later. For the significance test of a hypothesis in meta-studies, there is a need for at least five studies (Thompson *et al.*, 1997). Therefore, certain methods were introduced in the meta-analysis in this research, which the number of available studies was more than 5.

Dissemination bias

Before we determine significance test of hypotheses in this research, it is first necessary to examine the homogeneity and heterogeneity of effect size of each hypothesis. One of the methods used for dissemination bias is the use of a funnel plot. Examining the plot in this study showed that all graphs are symmetrical in this study, and no black dot indicating asymmetry was seen, which represents the lack of any publication bias (Fig 1).

However, meta-analysis argue that the results of funnel plots are not reliable enough, and it is better to use inferential tests to complete research results. To this end, two common inferential tests in meta-analysis

studies- correlation and regression, which are based on diagram results- were utilized.

The first method for checking publication bias was the use of Begg and Mazumdar's rating correlation, as its formula is as follows (Sahebi *et al.*, 2021).

$$t^*_i = \frac{t_i - t'}{\sqrt{V_i}} \quad (3)$$

The second test was regression, which was calculated by the following formula if it was above (Lin and Chu, 2018):

$$Z_i = \beta_0 + \beta_1 \frac{1}{\sqrt{V_i}} + \varepsilon_i \quad (4)$$

The statistical results of regression and correlation indicate that the null hypotheses in $\alpha = 0.05$ cannot be excluded in either of the two methods, so the funnel plot is symmetric, and the evaluation is free from dissemination error (table 2).

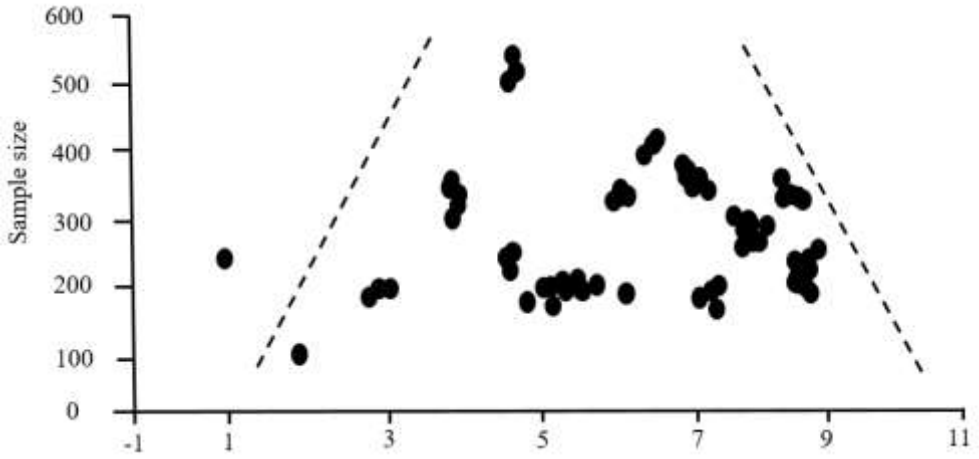

Fig. 1. The logarithm of the odds ratio in the studies used

Table 2- Results of examining dissemination error

Method	Regression		Correlation test	
UV-irradiation	**z**	**P-value**	**t**	**P-value**
ozone	-0.692	0.152	0.124	0.15
Gamma irradiation	-0.862	0.325	-0.251	0.152
microwave heating	-0.782	0.142	-0.214	0.174
citric acid	-1.062	0.257	-0.551	0.324
Ozone & uv irradiation	-0.699	0.324	-0.141	0. 264
conventional thermal	-0.771	0.152	-0.201	0.324
Microwave heating & Water	-0.855	0.103	-0.238	0.157

Q and τ^2 heterogeneity tests

Following the examination of dissemination bias in studies, there is a need for examining homogeneity tests or the heterogeneity of effect size for each method. In doing so, Cochrane (Q) and τ^2 are used. Q test follows χ^2 dissemination with g^{-1} degree of (Shadish and Haddock, 2009):

$$Q = \sum_{i=1}^{g} w_i y_i^2 - \left[\left(\sum_{i=1}^{g} w_i y_i \right) \Big/ \sum_{i=1}^{g} w_i \right] \quad (5)$$

To derive τ^2, we can use the following equation (Pigott, 2012):

$$\tau^2 = \frac{Q - (g-1)}{C} \quad (6)$$

C is an estimator, which is derived from the following equation;

$$C = \sum_{i=1}^{g} w_i - \left[\sum_{i=1}^{g} w_i^2 \Big/ \sum_{i=1}^{g} w_i \right] \quad (7)$$

If the effect sizes are homogenous, a fixed effect model is used, if they are heterogeneous, random effects are used for testing each method used in the research. The results of Q and τ2 were significant for all methods and processes, indicating heterogeneity of effect size; in which case, we should study the research hypotheses by using the random effect model (Table 3).

Table 3- Results of Q and I^2 heterogeneity

Methods	Q	Sig	Result	τ^2	Result
UV-irradiation	329.841	0.000	Heterogeneous	98.74	Heterogeneous
ozone	238.61	0.000	Heterogeneous	95.63	Heterogeneous
Gamma irradiation	52.63	0.000	Heterogeneous	91.63	Heterogeneous
microwave heating	263.84	0.000	Heterogeneous	97.34	Heterogeneous
citric acid	85.652	0.000	Heterogeneous	92.33	Heterogeneous
Ozone & uv irradiation	297.63	0.000	Heterogeneous	99.52	Heterogeneous
conventional thermal	152.36	0.000	Heterogeneous	94.68	Heterogeneous
Microwave heating & Water	138.76	0.000	Heterogeneous	93.55	Heterogeneous

In the random effect model, it is postulated that the effect size of a random variable has a normal distribution with θ mean and τ^2 variance (Pigott, 2012). Therefore, the random effect model is composed of two parts sampling variance (V_i) and between-studies variance (τ^2), which is obtained by the following equation:

$$v_i^* = \tau^2 + v_i \quad (8)$$

Finally, the statistical weight of the effect sizes is obtained in the random effect model by the following equation;

$$w_i^* = \frac{1}{v_i^*} \quad (9)$$

Calculating the statistical weight obtained either by fixed or random effect model, the mean weight of the effect is obtained based on the sum of effect sizes from the following equation;

$$\bar{y}_{\bullet} = \exp\left(\sum_{i=1}^{g} w_i y_i \Big/ \sum_{i=1}^{g} w_i \right) \quad (10)$$

To estimate the mean weight of an effect within 95% reliability range, the following equation is used;

$$95\%CI = \exp\left[\left(\sum_{i=1}^{g} w_i y_i \Big/ \sum_{i=1}^{g} w_i \right) \pm 1.96 \Big/ \sqrt{\sum_{i=1}^{g} w_i} \right] \quad (11)$$

Moreover, for integrating effect sizes according to the weighting method (Stouffer), the following formula are used (Wj is the same sample size in each method).

$$Z_r = 0.5 \log_e \left[\frac{1+r}{1-r} \right] \quad (12)$$

$$Fisher\ Z = \frac{\sum w_j Z_r}{\sum w_j} \quad (13)$$

The results of Table 4 indicate that the methods of UV-irradiation, Ozone & UV irradiation and citric acid were the most important methods by 0.469, 0.441, and 0.427 respectively.

Conclusion

The meta-analysis method was used to summarize the effect of different food processes on the reduction and elimination of aflatoxin in cereals and nuts. This method determines which food process is more capable

of reducing aflatoxin levels in cereals and nuts. The results of this study showed that among the methods used to reduce or eliminate aflatoxin in cereals and nuts, UV-irradiation, Ozone & UV irradiation and citric acid were the most effective.

Table 4- The results of the significance test of effect size for each method

Methods	Model	Number of studies	R^2	95% CI	Z	Sig	Results
UV-irradiation	Random	10	0.462	0.234-0.722	4.869	0.000	Confirm
Ozone & uv irradiation	Random	8	0.441	0.266-0.634	3.527	0.000	Confirm
Citric acid	Random	8	0.427	0.317-0.544	2.658	0.015	Confirm
Conventional thermal	Random	6	0.367	0.248-0.588	4.025	0.004	Confirm
Microwave heating	Random	8	0.328	0.185-0.511	3.698	0.002	Confirm
Microwave heating & Water	Random	7	0.246	0.098-0.421	4.297	0.001	Confirm
Ozone	Random	7	0.204	0.145-0.285	2.367	0.004	Confirm
Gamma irradiation	Random	7	0.178	0.105-0.261	3.597	0.005	Confirm

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چکیده

مایکوتوکسین‌ها (سموم قارچی) مانند آفلاتوکسین ترکیباتی هستند که توسط قارچ‌های مختلف در طول دوره رشد و تولیدمثل تولید می شود. با توجه به اثرات سمی و سرطان‌زا بودن آفلاتوکسین، از روش‌های مختلف برای کاهش یا از بین بردن مقدار آفلاتوکسین در غلات‌ها و مغزها استفاده شده است. برای مقایسه روش‌های مختلف که برای کاهش یا حذف میزان آفلاتوکسین استفاده می‌شود چون در هر مقاله از یک یا چند روش استفاده شده است بنابراین مقایسه کارایی روش‌های مختلف امکان‌پذیر نیست بنابراین در این پژوهش با استفاده از روش فراتحلیل، روش‌های مختلف که برای کاهش یا حذف میزان آفلاتوکسین در غلات و مغزها استفاده شده است با هم مقایسه شد. نتایج نشان داد که روش‌های استفاده از اشعه فرابنفش، ترکیب روش ازن- فرابنفش و استفاده از اسید سیتریک با اثر اندازه ۰/۴۶۹، ۰/۴۴۱ و ۰/۴۲۷ دارای بیشترین کارایی در کاهش میزان آفلاتوکسین در غلات و مغزها داشتند.

واژه‌های کلیدی: غلات، فرایندها، فراتحلیل، مغزها

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