Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2024, 30 (2), 203-208

Journal of Agroalimentary Processes and Technologies

Contributions on the use of grape pomace as an antifungal and antimycotoxin agent in wheat for bakery industry

Bota (Sicoe) Voichita Viorica[1], Sumalan Renata Maria[2], Alexa Ersilia[1]*

[1]University of Life Sciences "King Mihai I" from Timisoara, Faculty of Food Engineering, Calea Aradului, No. 119, 300645, Timisoara Romania
[2] University of Life Sciences "King Mihai I" from Timisoara, Faculty of Engineering and Applied Technology Calea Aradului, No. 119, 300645, Timisoara Romania

**Corresponding author: ersiliaalexa@usvt.ro*

Abstract
The purpose of this study is to test and analyze the antifungal and anti-toxic effects of treatments with grape pomace extracts as natural antifungal agent for wheat protection. This research aims to provide a natural solution for the prevention, control or reduction of fungal contamination in the agricultural sector, the food industry and in the storage, milling and processing sub-units of cereals, but also to encourage sustainable food systems, oriented towards innovative strategies for the recovery of by-products and waste from manufacturing processes, resulting in large amounts of waste from the fruit processing industry globally. According to the data obtained, in order to ensure optimal antifungal control of wheat in warehouses, it is recommended to use a 10% GPE treatment, which significantly reduces the contamination of wheat with *Fusarium* and DON, the effect being reported only after 14 days after administration.
Keywords: deoxinivalenol, seed contamination index, fungal frequency

1. Introduction

The study on the prevention of pathogen contamination with the help of natural grape pomace extracts is of interest in finding a viable strategy to reduce the risks associated with mycotoxin contamination of cereals and their products, in the context of the legislative requirement to reduce the use of chemicals both in the environment and in the food industry.

Grape pomace is a product resulting from the pressing of grapes and the winemaking process, treated as waste, being mostly thrown into landfills. The high humidity and organic content of the decomposing tissue pose a significant risk to the environment through the production of greenhouse gases, but also due to the high potential for contamination of water sources.

In the wine industry, after grape processing, 20% to 25% of the grapes pressed are pomace, which consists of bunches (~2%), seeds (~47%), nuts and pulp (~51%). Grape pomace is found in polyphenolic compounds responsible for antioxidant, anticancer, antimutagenic, anti-inflammatory and antimicrobial activity [1-3].

Mendoza L. *et al.* (2013) analyzed the phenolic compounds present in grape pomace extracts from a mixture of grape varieties (Cabernet Sauvignon, Carmenere, and Syrah) [4]. The results of this study indicated the presence of the flavonoid quercetin, a powerful natural antioxidant, in almost all samples taken. Also, the in vitro antifungal activity of these extracts was evaluated, reaching the conclusion that grape pomace is a good, low-cost source for obtaining antifungal extracts [5].

Deoxynivalenol (DON) belongs to the group of trichothecenes, which are mycotoxins produced by fungi of the genus Fusarium. This compound is commonly found in plant products, especially grains. More than 150

DOI: 10.59463/JAPT.2024.2.15

types of trichothecenes are known, and the most common toxins are deoxynivalenol, 3-acetyl- and 15-acetyl-deoxynivalenol [6]. The concentrations of these toxins in wheat, corn or rice are often in the order of ppm. Due to their strong cytotoxic and immunosuppressive properties, these toxins pose a major risk to human and animal health [7].

The current trend of consumers to choose safe and healthy food has led to an increase in interest in organic farming. In recent years, organic products have registered a considerable growth in the European market, covering almost one million hectares. According to the Institute for Research in Organic Agriculture (FIBL), Spain has the largest organic agricultural area (1.3 million hectares), followed by Italy (1.1 million hectares) and Germany (0.95 million hectares), Italy being the main exporter of organic products in Europe [8].

In order to practice organic agriculture for fungal and antimycotoxicogenic control, natural solutions are sought to inhibit the development of fungi and mycotoxin contamination.

In this regard, the present paper proposes the use of grape pomace extract as an antifungal and antimycotoxicogenic agent against the development of fungi and mycotoxin DON in wheat applied in storage spaces.

2. Materials and method

2.2.1. The obtaining of grape pomace extract

In order to test the antifungal effect associated with the treatment with natural extracts based on grape pomace (GPE), the product from the processing of grapes of the Romanian variety Tămâiosa, a grape variety grown in 2019, was processed within the winery in Cramele Recaș in western Romania.

The process of obtaining the pomace powder consisted of drying the grape pomace in the oven at a temperature of 103°C for 24 hours. After drying, the product was ground with a laboratory mill (Gindomix Retsch GM 2000).

The extract was obtained from 20 g of ground pomace grapes, diluted with 70% ethanol, then 3 different concentrations 5% GPE, 6.5% GPE and 10% GPE were prepared to pulverize the wheat samples taken in the study.

2.2. Determination of mycoflora of wheat seeds

To test the protective effect associated with treatment with natural grape pomace extracts, 200 g of wheat seeds with a known concentration of DON were used.

The incidence of DON mycotoxin in cereals was determined in this study as a result of contamination of wheat grains with species of *Fusarium, Alternaria, Cladosporium, Dreschlera* and *Saccharomyces*. The average DON content (ppm) initially determined in the wheat samples was 6,45 ppm and the moisture content of the sample was 12,41 % and aw 0,9.

The incidence of wheat seed microflora was determined at the beginning of the experiment and then monitored at 7 days and 14 days. Every 10 seeds of each working variant were sterilized with 1:9 hypochlorite, washed with double-distilled water, dried on sterile paper in one place and transferred to DRBC (pink dichloromethane chloramphenicol).

Figure 1. Grape pomace

The wheat seed samples were each treated with natural grape pomace extracts obtained with 70% ethanol in 3 different concentrations (5%, 6.5% and 10%). Distilled water was added, according to the water adsorption curve to wheat, to obtain a water action of aw = 0.995. For ethanol evaporation, the samples were placed at 25°C for 2 hours and periodically mixed, then incubated at 25°C for 28 days and stored at aw - 0,85 by periodic weighing of the samples and spraying with water.

After 7 and 14 days, the samples were analyzed to determine the wheat seed contamination index (SCI) and fungal frequency (FR), and DON procedure using the enzyme-linked immunosorbent method (ELISA). During the experiment, the samples were stored in the incubator at 25 ± 2°C [9].

$$\text{SCI (\%)} = \frac{\text{contaminated seed}}{\text{total seed}} \cdot 100$$

$$\text{FR (\%)} = \frac{\text{number of genra occurrences}}{\text{total number of fungi in sample}} \cdot 100$$

2.3. Determination of DON

The method used to determine DON mycotoxin in wheat seed was enzyme-linked immunosorbent assay (ELISA) and sample preparation was performed following the in manufacturer's instructions for DON analysis cereals (R-Biopharm).

The ground sample (5 g) was extracted with 100 ml of distilled water and homogenized using a 20-minute stirrer for extraction. The extract was filtered and 1 mL of filtrate was used directly for enzymatic analysis. Standard solutions and samples (50 µL) are mixed with 50 µL of enzyme conjugate in individual cells in the ELISA kit plates. Add 50 µl of antibody solution and incubate for 10 minutes at room temperature. The cells were washed three times with 250 µl of distilled water, then the substrate (100 µl) was added and incubated for another 5 minutes at room temperature. The stopping solution (100 µl) was added to each cell and the yellow color intensity was measured at 450 nm using an ELISA reader 96 (PR-1100, laboratoarele Bio-Rad, SUA) [10].

3. Results and discussion

The wheat seed colonization index (SCI) after 1 week of application of grape pomace extract (GPE) extract treatments is shown in Figure 2.

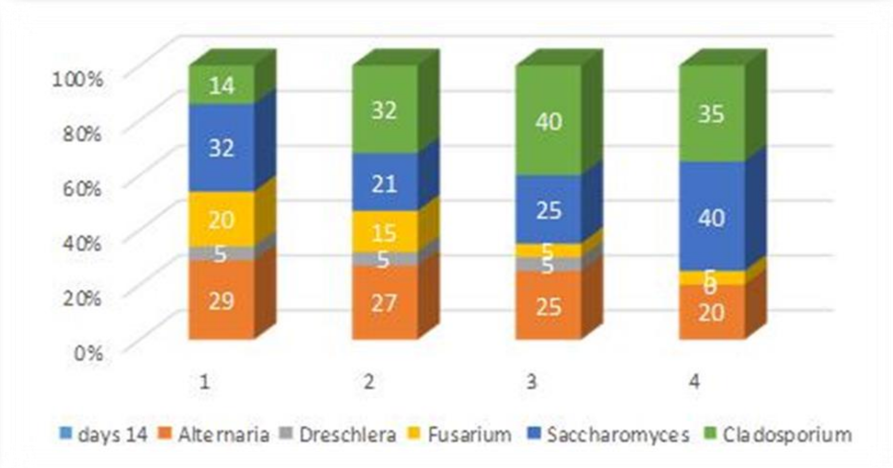

Figure 2. Seed Colonization Index (SCI) after 14 days of treatment with GPE in different concentrations 1 (5%), 2 (6.5%), 3 (10%) and 4 (Control)

Figure 2 shows that the *Alternaria* content increased after 7 days of treatment when using the extract by 5% from 28% at the control to 33%, with a 15% decrease in contamination when using the extract by 6.5%. The application of the 10% treatment leads to the total inhibition of the development of *Alternaria*. *Alternaria* developed in the treated samples compared to the control, and the initial concurrent microflora was determined in the treated samples in a smaller percentage.

The application of the 10% treatment leads to the total inhibition of the development of *Alternaria*. *Alternaria* developed in the treated samples compared to the control, and the initial concurrent microflora was determined in the treated samples in a smaller percentage.

On *Dreschlera* and *Cladosporium* fungi, the extract does not show antimicrobial effects and there is an increase in the incidence compared to the control for all concentrations used. The development of *Fusarium* is inhibited after 7 days of treatment compared to the control (25%) when extracts in concentrations of 5%, 6.5 and 10% (incidence 20%) are used.

The same behaviour is observed for *Saccharomyces* yeasts, where a concentration of 5 % extract has a maximum inhibiting effect (20 % contaminated seeds) compared to the control (30 % contaminated seeds).

After 14 days of treatment, SCI with *Alternaria* is lower when extracts are applied compared to control, regardless of the concentration applied.

In the case of *Dreschlera*, treatment at 5 and 6.5% does not lead to a decrease in SCI, while the 10% extract totally inhibits the development of this fungus.

Grape pomace extract has the effect of inhibiting *Fusarium* after 14 days of treatment, the inhibition increasing with the administered concentration.

In the case of *Saccharomyces* yeasts, the treatment with grape extract reduces contamination when concentrations of 5 and 6.5% were used, but increases with the

application of the concentration (10%).
Chladosporium increases compared to control for all applied concentrations. The differences can be explained by the competitive effects of mushrooms, the development of some leading to the reduction of others.
The results compared for the antifungal effects observed over time, i.e. after 7 and 14 days of treatment, indicate that the control, except for *Dreschlera,* which has not changed over time, has seen an increase in the number of contaminated seeds.
Grape extract 10% leads to increased inhibition in *Fusarium,* the incidence being reduced after 14 days of treatment to 5% compared to 33% of contaminated seeds after 7 days.
The results obtained regarding the antifungal effect of grape pomace on *Fusarium* developed on wheat seed indicate that at lower concentrations (5 to 6.5%) the inhibiting effect is observed even after 7 days of treatment and increases over time.
If a higher grape pomace concentrate (10%) is used, it is observed, intact, after 7 days, that fungal growth is observed compared to the control, but decreases over time, after 14 days after administration. In this regard, it is recommended to ensure optimal monitoring of wheat in warehouses, the use of the treatment with a concentration of 5 to 6.5 % for a short period (7 days) will lead to a partial reduction in contamination, while the addition of the 10 % extract concentration will significantly reduce the contamination of wheat with *Fusarium*, but the effect is only noticeable after 14 days after administration.
In this study, we considered the inclusion of previous data on cereal contamination with mycotoxins and the incidence of these toxic compounds in the western part of Romania [11]. In this regard, the effect of GPE treatment has been shown to inhibit ochratoxin synthesis (OTA), even at a minimum dose of 500 ppm. OTA content drops from 12.93 ppb to 8.89 ppb after 28 days of treatment [8]. Previous studies show that adding 500 ppm of natural resveratrol extracted from grapes is more effective in controlling OTA synthesis compared to essential oils [12-13].
The addition of GPE in concentrations of 1000 and 2500 ppm leads to similar results, so at the industrial level, the use of high concentrations for OTA control is not recommended [14].
Recently, the use of agricultural waste as mycotoxin bioabsorbents has been investigated because they contain carboxyl and hydroxyl groups, which may be involved in mycotoxin binding mechanisms, grape pomace, artichoke waste and almond shells have been selected as the best bioabsorbents, being effective in adsorption. in the case of AFB1, ZEA and OTA mycotoxins [15-16].

3.2. The effect of grape pomace against DON development

Figure 3 shows the level of DON contamination after 7 and 14 days of treatment.
As for the concentration of DON, the antimycotoxin effect can be observed, the level of DON was reduced after treatment with GPE, in all experimental variants tested, the decrease being more pronounced after 7 days of treatment, 14 days and in the case of a higher concentration of grape extract.

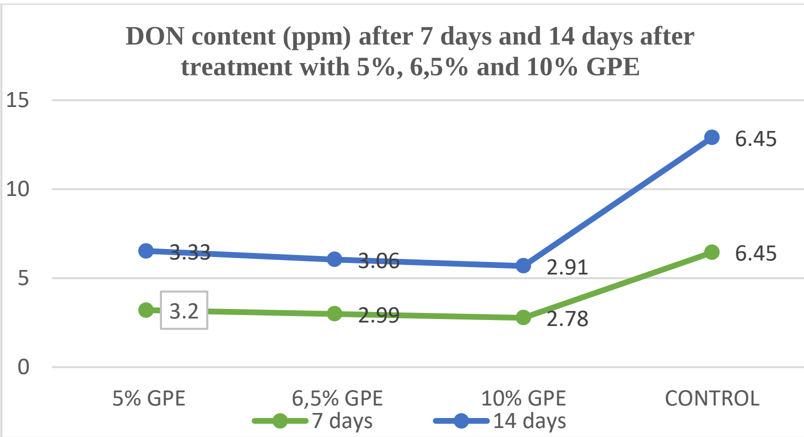

Figure 3. Development of Don content (ppm) after 7 days and 14 days of treatment with GPE (5%, 6.5% and 10%)

The greatest antimycotoxin potential was demonstrated by GPE at a concentration of 10%, after 7 days of treatment, the inhibitory effect of DON treatment decreased after 14 days.

In this direction of research, these viable alternatives based on natural extracts from grape pomace (GPE) and grape seeds (GSE) have been previously studied within our group compared to a synthetic product, antioxidant-butyl hydroxytoluene (BHT), to prevent the development of fungal populations and ochratoxins from naturally contaminated wheat, the results obtained showed the efficacy of these natural preparations that led to a significant reduction in populations and a decrease in mycotoxin content. The results of the study suggested that the natural extracts GPE and GSE are able to provide fungicidal and fungistatic protection against OTA accumulation in wheat, at least as the synthetic BHT [9].

The effect of trans-resveratrol and natural products obtained from the by-products of the winemaking process in the development of *Fusarium* species and the production of mycotoxins has also been studied by Marin and al. (2006), no differences in the action of synthetic resveratrol and natural products were reported [17].

Our results are in agreement with previous studies and suggest that, in terms of the antimycotoxigenic effect, DON levels decrease after treatment with extracts based on grape extract concentration in all experimental variants tested, with a greater decrease after 7 days after treatment compared to at 14 days.

Conclusions

According to the data obtained, in order to ensure optimal antifungal control of wheat in warehouses, it is recommended to use a 10% GPE treatment, which significantly reduces the contamination of wheat with *Fusarium*, the effect being reported only after 14 days after administration.

Regarding the antimycotoxin effect of GPE, the DON level decreases with the concentration of GPE in all experimental variants tested, with a greater decrease after 7 days compared to 14 days after treatment.

Due to its high bio-active potential and absence of toxicity, grape pomace can be used as a natural antifungal and antimycotoxigenic agent in grain protection in the context of the circular economy as an alternative to synthetic products.

The resulting data leave the possibility of implementing these strategies based on natural, environmentally safe, biodegradable and low toxicity products, in the prevention and control or reduction of fungal contamination, in the agricultural sector, in the food industry and in the and in subunits. storage, grinding and processing of cereals.

References

1. Bursac Kovacevic D, Levaj B, Dragovic-Uzelac V: Free radical scavenging activity and phenolic content in strawberry fruit and jam. Agriculturae Conspectus Scientificus 2009, 74:155-159
2. Giovanelli G, Buratti S: Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. Food Chem, 2009, 112:903-908
3. Trust M. Pfukwa, Olaniyi A. Fawole, Marena Manley, Pieter A. Gouws, Umezuruike Linus Opara and Cletos Mapiye, Food Preservative Capabilities of Grape (*Vitis vinifera*) and Clementine Mandarin (*Citrus reticulata*) By-products Extracts in South Africa, Sustainability, 2019, 11, 1746; doi:10.3390/su11061746, 1-2
4. Mendoza Leonora, KarenYañez, Marcela Vivanco, Ricardo Melo, Milena Cotoras, Characterization of extracts from winery by-products with antifungal activity against Botrytis cinerea, Industrial Crops and Products, Volume 43, 2013, 360-364
5. Almanza-Oliveros, A.; Bautista-Hernández, I.; Castro-López, C.; Aguilar-Zárate, P.; Meza-Carranco, Z.; Rojas, R.; Michel, M.R.; Martínez-Ávila, G.C.G. Grape Pomace Advances in Its Bioactivity, Health Benefits, and Food Applications. Foods 2024, 13, 580. https://doi.org/10.3390/foods13040580
6. Pereira V., Fernandes J., Cunha S., Mycotoxins in cereals and related foodstuffs: a review on occurrence and recent methods of analysis. Trends Food Sci. Technol, 2014, 36, 96–136.
7. Peng, Z.; Chen, L.; Nüssler, A.K.; Liu, L.; Yang, W. Current Sights for Mechanisms of Deoxynivalenol-Induced Hepatotoxicity and Prospective Views for Future Scientific Research: A Mini Review: DON-Induced Hepatotoxicity and Prospective Views. J. Appl. Toxicol. 2017, 37, 518–529, doi:10.1002/jat.3428.
8. Willer H., Kilcher L., The World of Organic Agriculture. Statistics and Emerging Trends,

IFOAM, Bonn, and FiBL, Frick, 2011.

9. Alexa E., Poiana M.A. si Sumalan R. M., Mycoflora and Ochratoxin A Control in Wheat Grain Using Natural Extracts Obtained from Wine Industry By-Products, International Journal of Molecular Sciences ISSN 1422-0067, 2012, 13, 4949-4967
10. Alexa E, G Pop, RM Sumalan, I Radulov, M Poiana, C Tulcan Fusarium species and fusarium mycotoxins in cereals from West Romania: preliminary survey. Communications in Agricultural and Applied Biological Sciences 76 (4), 661-666, 2011
11. Alexa E., Cristina Adriana Dehelean, Mariana-Atena Poiana, Isidora Radulov, Anca-Maria Cimpean, Despina-Maria Bordean, Camelia Tulcan și Georgeta Pop, The occurrence of mycotoxins in wheat from western Romania and histopathological impact as effect of feed intake, Chemistry Central Journal 2013, 7:99
12. Yu, J.; Smith, I.N.; Mikiashvili, N. Reducing Ochratoxin A Content in Grape Pomace by Different Methods. Toxins 2020, 12, 424. https://doi.org/10.3390/toxins12070424
13. Mangiapelo L, Frangiamone M., Pilar Vila-Donat, Paşca D, Federica Ianni, Lina Cossignani, Lara Manyes, Grape pomace as a novel functional ingredient: Mitigating ochratoxin A bioaccessibility and unraveling cytoprotective mechanisms in vitro, Current Research in Food Science,Volume 9, 2024,100800, https://doi.org/10.1016/j.crfs.2024.100800
14. Antonić, B.; Jančíková, S.; Dordević, D.; Tremlová, B. Grape Pomace Valorization: A Systematic Review and Meta-Analysis. Foods 2020, 9, 1627.https://doi.org/10.3390/foods9111627
15. Cheli Federica, Mycotoxin Contamination Management Tools and Efficient Strategies in Feed Industry, 2020, Toxins, Volume 12, Issue 8
16. Marin D. E., Ionelia Ţăranu, Gina Cecilia Pistol, Metode de decontaminare a micotoxinelor, 2014, 20-47, 46-63, 90-108
17. Marin, S.; Ramos, A.J.; Cuevas, D.; Sanchis, V. Fusarium verticillioides and Fusarium graminearum infection and fumonisin B1 and zearalenone accumulation in resveratrol treated corn. Food Sci. Techn. Int. 2006, 12, 353-359