Effect of nanoparticles of double divalent and trivalent iron oxide to protect of flax seeds from microbial spoilage

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Abstract

Introduction. The effect of nanoparticles (NPs) of double divalent and trivalent iron oxide ($FeO \times Fe_2O_3$ – nanomagnetite) on protection of flax seeds against fungal infections was studied, and the dependence of this effect on the amount of nanomagnetite nanoparticles was determined.

Materials and methods. Microscopic observation of morphological and cultural features of micromycetes, yeast *Saccharomyces cerevisiae,* and mycelial fungi, *Mucor racemosus*, grown on an agar nutrient medium was done. Experimental samples of micromycetes were obtained by sowing a standard microbial preparation in the form of a suspension (initial dilution of the microbial suspension 1:100) in Petri dishes.

Results and discussion. The ability of nanoparticles of double divalent and trivalent iron oxide to protect the flax seeds was shown: addition of 0.1%; 0.15%; 0.2% of nanomagnetite to flax seeds inhibits by 8 –20 times the development of micromycetes on them.

A decrease by 8–10 times of the cell number of micromycetes yeast *Saccharomyces cerevisiae* and mycelia fungi *Mucor racemosus* as well as diminishing of the colonies size by 10–20 times on the surface of the treated flax seeds compared to control was found.

The recommended dosage of nanoparticles of double divalent and trivalent iron oxide was determined as 0.15% from the weight of the flax seeds. The proposed mathematical model makes it possible to predict the effectiveness of NPs $FeO \times Fe_2O_3$ – nanomagnetite application to inhibit the growth of spoilage microorganisms on the flax seeds.

Conclusions. For the first time, the ability of nanoparticles of double divalent and trivalent iron oxide (NPs $FeO \times Fe_2O_3$ – nanomagnetite) to protect flax seeds from fungal spoilage was shown.

Introduction

Flaxseed (*Linum usitatissimum* L.) has high contents of protein and fiber, low contents of carbohydrate and fat, contains such useful compounds as α-linolenic acid (omega-3), phenolic acids, phytic acid, vitamins, minerals especially Ca, K, P, Mg, and are recommended to be used in preparation of functional food with potential health benefits (Stabnikova et al., 2021). The main problem of application of flaxseed, especially if it will be used for manufacturing of food products, is preservation of their high quality during the storage (Brigante et al., 2022; Bruno et al., 2020; Rollemberg et al., 2022).

Some methods for determining flax seed quality indicators, including those related to damage, were proposed but were not sufficiently developed to be used in practice (Podio et al., 2022). A serious problem in the preservation of flaxseed and the manufacture of products from it is that it is susceptible to infection by fungi, which significantly affect the nutritional properties of food and endanger the health of the consumers (Choudhry et al., 2021; Farag et al., 2021; Gu et al., 2021). This problem is described well, but real ways of solving it are not defined. Unfortunately, the undeniable harmfulness of flax seed diseases is not considered in terms of creating bacteriostatic storage conditions.

Fungal infections of flax seeds in common (Pal et al., 2022). More often the fungi of the genera *Alternaria* (from 20.0 to 42.5% of infected seed) and *Fusarium* (up to 50.0% of infected seed) caused flax seed microbial spoilage. Among others fungi that caused flax damage the representatives of the genera *Colletotrichum*, *Sydowia*, and *Septoria* are reported (Gruzdevienė et al., 2006). Six genera and eight species of fungi were found in the flax seeds (Rollemberg et al, 2022). The representatives of genus *Aspergillus* were the most abundant including *A. cibarius*, *A. appendiculatus*, and *A. amstelodami*. The second most abundant genus was *Wallemia*, with the species *W. muriae*, some strains of this genus is known can produce toxins

The norms of damage and contamination of flax seeds are described in (Asad, 2022; Soureshjani et al., 2019; Xie et al., 2019). Different ways of solving the problem were considered at the stage when the danger has already been identified (Singh et al., 2021). In our opinion, the direction of creating conditions for inhibiting flaxseed diseases at the initial stages is more promising.

Recent advances in plant and food protection have often been linked to nanotechnology and nanomaterials (Fursik at el., 2019). In particular, the effect of iron oxides is considered positive (Tsykhanovska et al., 2020). These studies considered the bacteriostatic properties of nanomaterials for dairy products subject to fungal infections. Considering the research data on the danger of contamination of flax seeds with fungal infections, we consider it relevant to check the effect on these products. Tsykhanovska with co-authors (2021) describe the use of magnetite nanoparticles with bactericidal and bacteriostatic properties in the food industry. These results make it possible to predict a positive effect in the protection of flax seeds from fungal spoilage.

We propose the creation of preventive methods that act at the initial stages of the occurrence of infection diseases.

Analysis of publications on flaxseed diseases showed the following:

1. The main problem for flax seeds is fungal infections, the effectiveness of combating those remains low.
2. Methods of combating fungal infections involve the treatment of infected products, it is desirable to develop preventive methods.
3. Preliminary studies of magnetic nanomaterials testify their potential bacteriostatic properties. It is desirable to study these properties towards flax seeds.

The aim of this work is to investigate the effect of magnetite nanoparticles on the resistance of flax seeds to fungal diseases at the stage of their development.

Materials and methods

The method of determining harmful fungi is based on seeding the product or product homogenate and (or) their dilutions in nutrient media, determining the affiliation of isolated microorganisms to fungi by the characteristic growth on nutrient media and cell morphology.

The method is designed to establish the compliance of microbiological indicators of food quality to the requirements of regulatory and technical documentation, to clarify the causes of product defects (Chow et al., 2019; te Giffel and Zwietering, 2009).

Sowings of the product or its corresponding dilutions are carried out on Petri dishes, spend two parallel determinations. At the bottom of a sterile Petri dish make 1 cm^3 of product or its dilution and sterile pour 15–20 cm^3 of nutrient medium Saburo, or agar medium to identify harmful fungi. Petri dishes with crops are placed to solidify on a horizontal surface, then the Petri dishes are turned upside down and placed in a thermostat with a temperature of (24±1) °C for 5 days or at a temperature of (30±1) °C for 3–5 days. The crops are incubated at a temperature of (24±1) °C for 5 days (Chow et al., 2019; te Giffel and Zwietering, 2009). After 3 days, a preliminary count of typical colonies or the appearance of characteristic signs of growth on liquid nutrient media.

If in crops on dense media there are flour, very fast-growing fungi, the removal of preliminary results should be carried out very carefully, not allowing the spores of these fungi to crumble and give rise to secondary colonies. In 5 days the final account of results of thermostating of crops is carried out. Mushroom colonies are divided visually.

Colonies of fungi on flax seeds are shown in Figure 1.

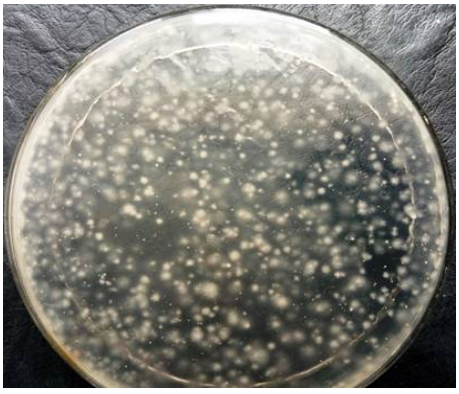

Figure 2. Colonies of fungi on flax seeds

The development of harmful fungi on nutrient media is accompanied by the appearance of mycelia of different colours.

For quantitative counting, cups are selected on which 5 to 50 colonies of harmful fungi have grown.

If it is necessary, microscopic studies are carried out to separate colonies of harmful fungi. For this aim, preparations are prepared from individual colonies or from cultures on a liquid medium using the crushed drop method. A drop of sterile tap water is applied to the

slide. Then a part of the colony is introduced into this drop with a heated needle or a drop of culture liquid is applied with a loop. The resulting suspension is covered with a cover glass.

Microscopy results are evaluated using the characteristics of harmful fungi.

If the growth of harmful fungi is detected when testing the product on nutrient media and their presence is confirmed by microscopy, then a conclusion is drawn about the presence of these microorganisms in the product.

Each cup is placed upside down and counted using a magnifying glass to count the number of colonies that have grown, counting the colonies of harmful fungi separately. Each counted colony is marked at the bottom of the cup.

The following method of counting the number of harmful fungi colonies is proposed in the work:

In the graphic editor, a circle is created, the diameter of which is equal to the real diameter of the Petri dish (95 mm) and a system of squares with a size of 1×1 mm. Rows of squares are placed in mutually perpendicular directions.

The photo of the drug is placed on the same sheet, its size is adapted to the size of the circle. A system of squares is superimposed on top (Figure 2).

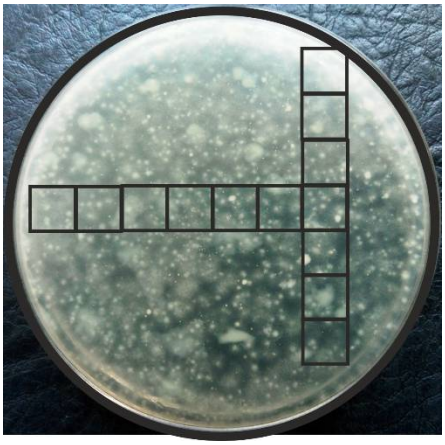

Figure 2. Example of calculation of number of colonies in graphic editor

The number of colonies of harmful fungi is counted separately in each square. The number of colonies is recalculated according to the proportionality of the area of the circle and the area of the squares:

$$N_p = \frac{n\pi r^2}{z},$$

n – number of colonies counted in all squares;
r –the radius of the Petri dish;
z – the number of squares in which the calculation was carried out (in our case, 13).

For convenience when counting, you can increase or decrease the size of the image. The number of harmful fungi N, Q/g of product was calculated according to the formula:

$$N = \frac{N_p}{m} 10^p,$$

where: m is the weight of the sample taken for sowing; p is the number of 10-fold dilutions.

If the test of the product on nutrient media revealed the growth of fungi and their presence is confirmed by microscopy, it is concluded that the presence of these microorganisms in the product.

It is possible to slow down the development of the microflora and, accordingly, to extend the shelf life of the product by introducing additives that have a bacteriostatic effect into the product.

We offer as this additive a nanomaterial in the form of double oxide of ferrous and trivalent iron (NPs $FeO \times Fe_2O_3$), described in our previous works (Tovma, 2020, Tsykhanovska at el., 2021; Riabchykov at el, 2022) .

To determine the properties of the environment where the fungi grew, nanomagnetite powder (NPs $FeO \times Fe_2O_3$) was added to the flax seeds.

Results and discussion

Effect of magnetite nanoparticles on the growth of harmful fungi

The main component of magnetite is nanoparticles of mixed oxide of divalent and trivalent iron with the general formula Fe_3O_4 or $FeO \times Fe_2O_3$ with a nanoparticle size of 30–78 nm.

Nano-objects, which include nanomagnetite (NPs $FeO \times Fe_2O_3$), have enormous potential and carry many important fundamental discoveries, new functional and technological properties and promising technological applications. It should be noted that most nanomaterials used in food products occupy an intermediate position between nano- and microstructures. Thus, the diameter of DNA is 12 nm, liposomes 30–10000 nm, amylopectin 44–200 nm, cubosomes 500 nm, nanosensors < 1000 nm (Tovma, 2020)

Nanoparticles of the multifunctional food additive of complex action magnetite (NPs $FeO \times Fe_2O_3$) have a huge potential and high bioaffinity to biopolymers, in particular, proteins, carbohydrates. Therefore, they carry many important fundamental discoveries, new functional and technological properties and promising technological applications. Noncovalent adsorption of polymer molecules, H_2O dipoles occurs on the surface of magnetic nanoparticles (NPs $FeO \times Fe_2O_3$). The process of adsorption of biopolymeric food ingredients and water is mainly determined by ionic, vanderwaals, hydrogen and hydrophobic types of interactions. These interactions occur between the surface of nanoparticles (NPs $FeO \times Fe_2O_3$) and adsorbing molecules and entail a change in the Gibbs free energy. The result is the formation of supramolecular ensembles, which significantly affect the functional and technological properties of raw components and semi-finished products, as well as the quality of finished products (Tsykhanovska at el., 2021).

In previous studies (Tovma, 2020, Tsykhanovska at el., 2021)it was established that the addition of nanomagnetite (NPs $FeO \times Fe_2O_3$) to food products leads to a comprehensive improvement of their food, consumer and technological properties. So, in particular, nanoparticles NPs $FeO \times Fe_2O_3$ have bacteriostatic, bactericidal, and antioxidant properties, promotes better digestion of protein components of food, exhibits a moisture-, fat-retaining, and fat-emulsifying effect, is a source of easily digestible iron, and has a beneficial effect on metabolic processes (Riabchykov at el, 2022).

To determine the degree of development of harmful fungi, a microscopic examination of drugs derived from fungal colonies was performed. Images of the corresponding drugs were obtained by microimaging (Figure 3–4).

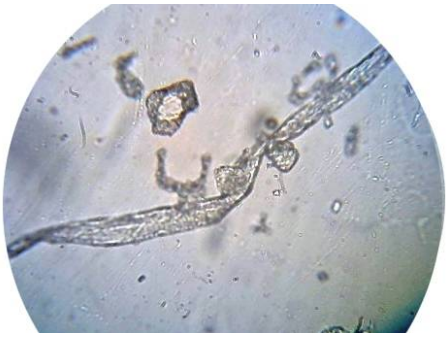

Figure 3. Micrograph of the drug from sample No 0 from a colony of fungi (×400 times magnification)

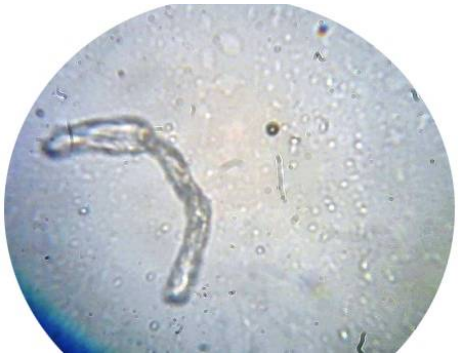

Figure 4. Micrograph of the drug from sample No 1 from a colony of fungi (×1000 times magnification)

From the given micrographs it is visible that in the sample which does not contain nanomagnetite (NPs $FeO \times Fe_2O_3$) there is an intensive development of microflora. So in a preparation which is made of a colony of fungi, even at rather small increase (400 times) the developed mycelium is visible (Figure 3).

Effect of nanomagnetite on the quantitative composition of harmful fungal colonies on flax seeds.

In a sample containing nanomagnetite (NPs $FeO \times Fe_2O_3$) in a relatively small amount (0.1%, sample №1) there is inhibition of microflora development. Only at a magnification of 1000 times in the colonies of fungi can be found small and sluggish fragments of mycelium (Figure 4).

In preparations made from sample №3 (content of nanomagnetite – NPs $FeO \times Fe_2O_3$ 0.2%) from colonies of fungi externally similar to colonies, even at 1000-fold magnification it was not possible to establish the presence of signs of mycelium.

More informative were the studies of samples conducted by a similar method after five days of exposure. Selected samples were diluted three times for seeding. The appearance of the drugs after five days of incubation is shown in Figure 5.

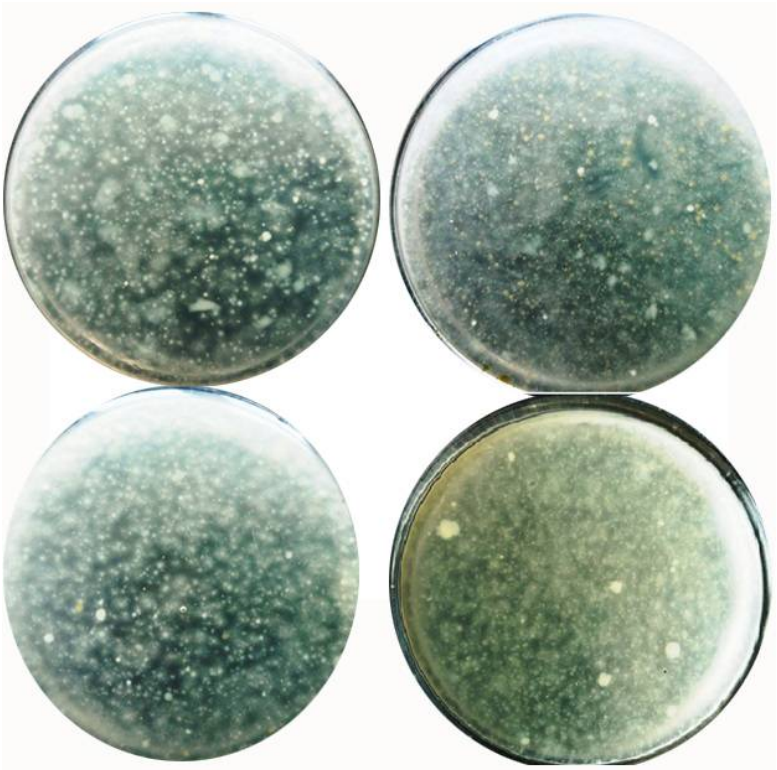

Figure 5. Micrograph of the drug from sample No 1 from a colony of fungi (×1000 times magnification)

On the photomicrograph of the preparation from the fungi colony of sample No 2 (Figure 6), weak mycelium without reproductive organs can be observed only at maximum magnification. Colonies of this sample contain a large number of cells that do not show vital activity.

Yeast colonies of sample No 3 (Figure 7) also contain non-viable cells, and in the fungal colonies of this sample, microscopy did not even reveal signs of the presence of mycelium. It is likely that the fungal colonies counted on the petri dishes were false.

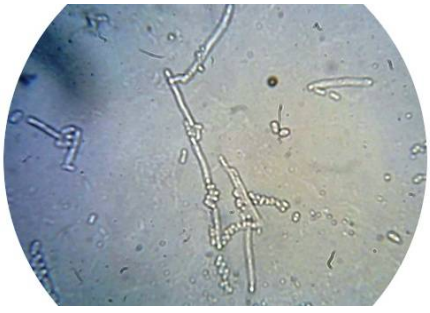

Figure 6. Photomicrograph of the preparation from sample No 2 from the fungi colony (×1000 times magnification)

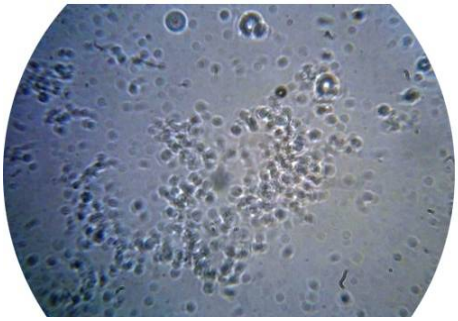

Fig 7. Micrograph of the drug from sample No 3 (×1000 times magnification)

From the obtained data it is seen that the addition of nanomagnetic powder (NPs $FeO \times Fe_2O_3$) significantly inhibits the development of microflora in flax seed samples (Table 1)

Table 1

Microbiological indicators of samples

Sample	0	1	2	3
The number of harmful fungi in 1 gram of product, Q	45×10^4	37×10^4	220×10^3	65×10^3

The addition of nanomagnetite powder (NPs $FeO \times Fe_2O_3$) has a bacteriostatic effect on harmful fungi in flax seeds (inhibits their development) and the concentration of 0.15% (sample No 2) is sufficient to implement this function.

Mathematical model of the influence of the composition of nanomagnetite on the growth efficiency of harmful fungi in flax seeds

Analysis of the dependence of the growth of the number of harmful fungi on the composition of nanomagnetite allows us to determine the following properties.

1. The general dependence demonstrates a continuous decrease in the number of harmful elements with an increase in nanomagnetite
2. At the initial stage, with a small amount of magnetite, the bacteriostatic efficiency decreases slightly, which is mathematically proven by the zero value of the derivative.
3. With a significant composition of magnetite, the bacteriostatic efficiency significantly decreases compared to the initial stage and gradually turns into an asymptotic dependence.

A function with a negative exponent whose argument has the form of a power function can correspond to similar properties.

If we determine the number of harmful fungi N, the composition of nanomagnetite u, the proposed function can look like this

$$N = N_0 e^{\left(\frac{u}{a}\right)^2}.$$

The unknown coefficients of this dependence can be determined by the method of least squares. For our case, the searched dependency looks like this:

$$N = 4.5 \cdot 10^5 e^{\left(\frac{u}{0.192}\right)^2}$$

The graph of this dependence is shown in Figure 8.

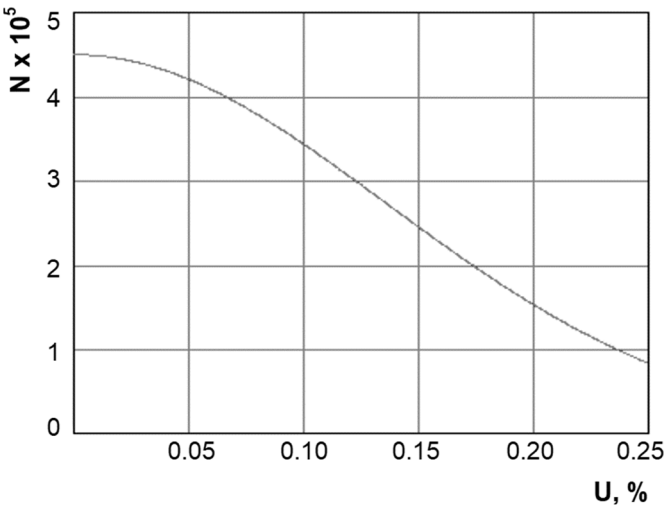

Figure 8. Dependence of the number of cells of harmful fungi on the amount of nanomagnetite.

The proposed dependence makes it possible to predict the effectiveness of the growth of the number of harmful fungi depending on the content of nanomagnetite and to determine the amount of nanomagnetite necessary to ensure bacteriostatic properties.

Conclusion

The main harmful substances in the storage of flax seeds intended for food use are harmful fungal microorganisms. The use of nanomagnetic powder (NPs $FeO \times Fe_2O_3$) containing of double oxide of ferrous and trivalent iron (NPs $FeO \times Fe_2O_3$) significantly reduces the growth rate of such microorganisms on the seeds of flax. These results allow us to recommend the addition of nanoparticles of magnetic powders during storage of flax seeds.

1. The addition of nanomagnetite to flax seeds infected with harmful fungi significantly reduces their ability to grow. The size of harmful fungi is reduced by 3–5 times, with a higher concentration, the size of harmful fungi is reduced by 10–20 times.
2. The number of colonies of harmful fungi decreases by 1.5–2 times, by 8–10 times with the addition of 0.2% nanomagnetite
3. The proposed mathematical function allows predicting the number and size of harmful fungi when nanomagnetite is added. The composition of nanomagnetite 0.15% is sufficient to significantly suppress the development of harmful fungi

4. The proposed methods of protecting flax seeds with the help of nanomagnetite allow to increase significantly their protective properties and significantly slow down their development even at the stage of infection.

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