

The application of micro-wave treatment to reduce barley contamination

Y. Kretova*, L. Tsirulnichenko, N. Naumenko, N. Popova and I. Kalinina

South Ural State University, School of Medical Biology, Department of Food and Biotechnology, 85 Lenin Avenue, RU454080 Chelyabinsk, Russia

*Correspondence: kretova555@mail.ru

Abstract. The goal of this work is to study the applicability of ultra high frequency electromagnetic field treatment for decontaminating barley grain used in brewing while preserving its technological properties. The germination rates and/or yield of the treated sample seed were compared with those of the untreated seed germinated under normal conditions. To determine optimal treatment conditions, a two-factor analysis was carried out, taking the mycological state of the grain into account. The heating rate and the duration of electromagnetic exposure were chosen as variables; these values varied from 0.4 to 0.8 °C s⁻¹ and from 30 to 90 s, respectively. It was found that germination of the treated barley seed was increased about 10.1–15.7% compared with that of the untreated seed. The microbial load decreased up to 80%. A heating rate of 0.4 °C s⁻¹ and treatment exposure time of 30 s showed the strongest effect of decontamination while preserving the viability of the barley grain.

Key words: microwaves, barley, *Alternaria*, malt, mycotoxin, the grain viability.

INTRODUCTION

As a key global food resource, the contamination of grains with insects or microorganisms is a persistent concern for the grain industry due to irreversible damage to quality and safety characteristics and economic losses. The reason for the poor quality of barley grain is a high susceptibility of this culture to phytopathogenic microorganisms.

Alternaria fungi are one of the most common components of the grain microbiome. The most dangerous result of infecting with *Alternaria* fungi is the accumulation of a large number of mycotoxins (Logrieco et al., 1990), (Milicevic, 2009). Mycotoxins entering the human body can cause diseases associated with violation of the gene and nervous system structure, acute chronic kidney disease, and the development of cancer (Hussein & Jeffrey, 2001). Studies carried out by scientists from different countries confirm that aflatoxin is a toxic, mutagenic, and carcinogenic compound (Webley et al., 1998; Scott, 2001; Logrieco et al., 2009; Janić Hajnal et al., 2015; Wu et al., 2016)

The problem of early grain blights is still relevant as it is widespread now both in Russia (Gavrilova et al., 2016; Gavrilova et al., 2017), and abroad (Müller et al., 2002; Scott et al., 2012; Müller et al., 2015). Therefore, it is rather difficult for enterprises engaged in grain processing and storing to ensure the stable quality of plant raw materials.

To create and organize the environmentally safe production of food products, it is necessary to use fundamentally new technologies, since traditional methods cannot guarantee, a decrease in grain contamination with microorganisms and activation of its growth in malting. Some traditional methods are also very energy intensive, require expensive equipment, and have a limited field of application (Yaldagard, et al., 2008; Wilson, et al., 2016; Los, et al., 2018).

One promising method for reducing grain contamination is the resource-saving technology based on electrophysical methods, in particular, microwave heating.

Currently, microwave heating is successfully used for treating raw materials with active enzymes, wherein some treatment modes contribute both to an increase in activity and inactivation of microflora. A positive effect from microwave heating of wheat grains was observed at a power of 700 W with a treatment time of 0–60 s (Qu et al., 2017). However, analysis has shown that it is necessary to strictly regulate the treatment parameters (Yaldagard et al., 2008; Wilson, et al., 2016; Los, et al., 2018) for preserving the technological properties of barley grain used in the brewing technology.

The stimulating effect of microwave energy is caused by the excitation of active enzyme centers involved in the germination of seeds, as well as the increase in the permeability of cell membranes due to the formation of free radicals, which contributes to a better oxygen and water supply to the cells. The features of the interaction of microwave energy with food raw materials and the pronounced bactericidal effect are of particular interest (Chen, et al., 2012; Dalmoro et al., 2015; Viliche Balint, et al., 2016; Motallebi, 2016.). Usually the most useful ways to improvement of barley quality are IR-radiation; γ -beams, ultrasound, electronic ionic technologies. However some of them only allow to reduce grain contamination (Wilson et al., 2016; Los, et al., 2018), others – positively influence only process of the grain viability (Yaldagard et al., 2008). However it is difficult to reach complex effect.

The purpose of this paper is to develop effective methods for decontaminating barley grain used in brewing while preserving its technological properties by using ultrahigh frequency electromagnetic field treatment.

MATERIALS AND METHODS

Sample collection and preparation

At the first stage, was studied the quality of raw grain materials and their applicability in the production of malt used in brewing, which grown in Russia in different areas of the Chelyabinsk, Kurgan, Sverdlovsk, and Tyumen regions (batches 1, 2, 3, 4 respectively). 100 samples were selected from each batch; the experiments were repeated at least 15 times. The control variables were grain contamination with *Alternaria* fungi and grain viability. Indicators were evaluated taking the conditions of growing barley grain into account. The coefficient of variation (v,%) was calculated using the mathematical statistics methods.

The test samples of grain were treated in the electromagnetic field – microwave treatment at a frequency of 2,450 MHz (Microwave oven SAMSUNG GE83KRS-3, Malaysia). The heating rate varied from 0.4 to 0.8 °C s⁻¹ and the duration of the electromagnetic exposure – from 30 to 90 s.

100 g weighed grain portions were selected for each treatment option. Further, the samples of barley grain were placed in specially folded standard paper bags and treated in set conditions.

Design of the experiment

After microwave treatment, was measured the heating temperature of the experimental batches of barley with a thermometer (Espada TA-288, Russia). Then, was analyzed grain contamination and grain viability.

Grain contamination with fungi was determined by the biological method based on stimulating the development and growth of microorganisms in contaminated grains. For this purpose, the grain was germinated in a wet chamber (thermostat TSO-1M, Russia) at a constant temperature (22–24 °C). Four working samples of 50 grains were used for analysis. The contamination results were analyzed four days after the grain was placed in the wet chamber.

The intervals of change for these variables were selected because at the chosen values of these variables, other things being equal, we obtain barley grain that is suitable for the production of malt. The main criterion for assessing the influence of each factor on the disinfection of barley grain was heating temperature. The minimum value depended on the environment, and the maximum value was limited to barley grain quality indicators.

At the next stage of the study, was evaluated the effect of the electromagnetic influence on the grain viability. In the process of barley grain decontamination and finding effective modes of microwave field influence thereon, our main task was to preserve grain viability. This quality index is the main indicator of the physiological usefulness of barley and characterizes the suitability of barley grain for the production of malt.

The grain viability was determined by germinating the pre-wet grains. For this purpose, an average sample with the weight of 50 ± 1 g of grains was first formed, from which two analytical samples of 500 whole grains were taken. The grain viability was determined 120 hours after the beginning of the experiment by visual evaluation.

Statistical data analysis

The experimental data was processed using traditional variation statistics methods and expressed as an arithmetic mean (m) and standard error (m). To determine the statistically significant differences between the test and control groups was used the Mann-Whitney test (U). Results were expressed as an arithmetic mean and its standard deviation. The differences were considered significant at $p < 0.05$. Statistical interrelations were studied using the nonparametric correlation analysis by calculating the Spearman correlation coefficients (PC).

RESULTS AND DISCUSSION

Mycological analysis of the barley grain contamination revealed the presence of various species of fungi belonging to *Alternaria*, *Aspergillus*, *Fusarium*, *Bipolaris*, *Cladosporium*, *Penicillium*, *Mucor*, and other genera in the microbiota. The most

widespread among the detected micromycetes were the representatives of *Alternaria* – from 50 to 78% depending on the batch (Fig. 1).

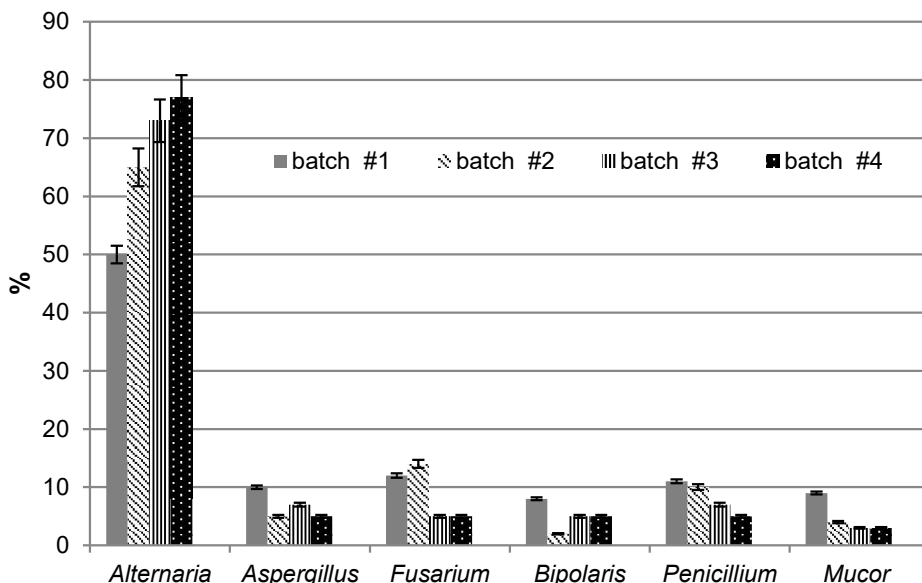


Figure 1. Percentage of fungi grain contamination in control batches from different areas of the Chelyabinsk, Kurgan, Sverdlovsk, and Tyumen regions of Russia.

The control batches of brewing barley had the grain viability equal to $80 \pm 2\%$ (Fig. 2), consequently, such barley grain is not suitable for malt production, since normally germinated barley has the grain viability should be not less than 95% (for barley of class I) and 90% (for barley of class II).

To determine optimal treatment conditions, a two-factor analysis was carried out. The heating rate (τ) and the duration V_t of the electromagnetic exposure were selected as variables. Monitored parameter: grain contamination and viability (Figs 3, 4). Was established that the type of electro physical exposure used at the heating rate of $0.6\text{--}0.8\text{ }^\circ\text{C s}^{-1}$ with a treatment exposure of 60–90 s leads to the disinfection of almost all types of fungal infection, including *Alternaria* fungi (Fig. 3).



Figure 2. The grain viability of control sample.

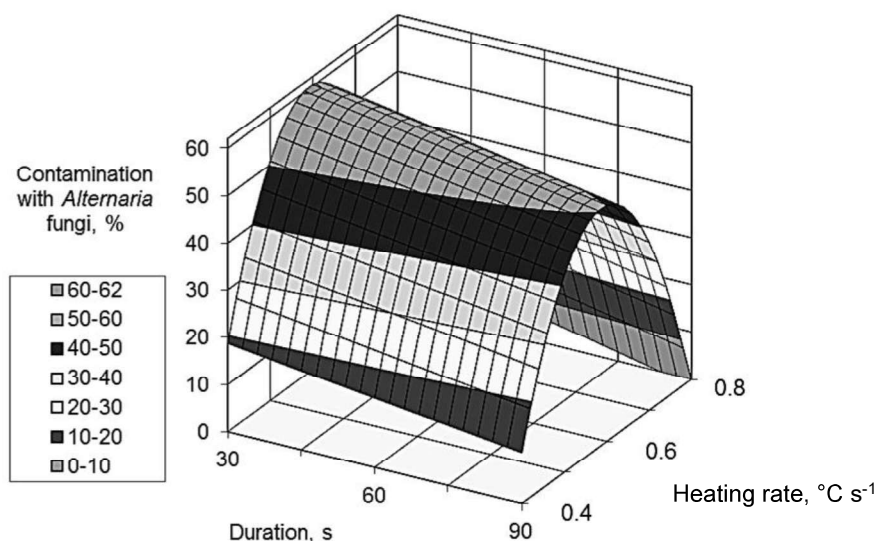


Figure 3. Influence of the microwave energy on the *Alternaria* fungi contamination of barley grain used for brewing.

At the minimum microwave field loads, there is a significant reduction in grain contamination with these fungi compared to other ways of processing grain (IR, cold plasma) (Wilson et al., 2016; Los et al., 2018), even at a low heating temperature (30 °C).

A further increase in the microwave field load (up to 0.6 °C s⁻¹), regardless of the treatment exposure time, leads to the active development of microorganisms; grain contamination by pathogens of these fungi reaches 50–60%. Under such conditions, the fungus mycelium develops abundantly, which can cause the darkening of the grain shell. Increase in the dissemination of fungi of *Alternaria* occurs not only due to the development of fungi already present on the grain and under its membrane (in the endosperm), but also because of the transition of spores to healthy grains, since sprouts of mycelial fungi freely penetrate to the embryo and grain tissues (Justé et al., 2011).

Provided that the heating rate is high (0.8 °C s⁻¹), and the exposure reaches 30–90 s, the barley grain contamination with *Alternaria* fungi disappears completely or falls to the permissible level.

The combination of these parameters of the electromagnetic field effect positively influences the cytology of the mycelial fungal cell, thereby reducing the number of mycotoxins produced.

The treatment mode of a heating rate of 0.4 °C s⁻¹ and an exposure time of 30–90 s, as well as the mode with a heating rate of 0.8 °C s⁻¹ and exposure time of 30–90 s can be considered effective for this genus of pathogens; the microbial load decreases up to 80%. Use of IR-radiation makes it possible to lower the microbial load of grain to 42% with a treatment duration of 180 s and a radiation intensity of 5.55 kW m⁻¹ (Wilson et al., 2016). Disinfection to the permissible levels can also be achieved by using high-voltage closed processing by atmospheric cold plasma – in this case, processing time is 20 minutes with a voltage of 80 kV (Los et al., 2018).

The results of the microwave effect influence on the grain viability of barley grain are shown in Fig. 4, Table. 1.

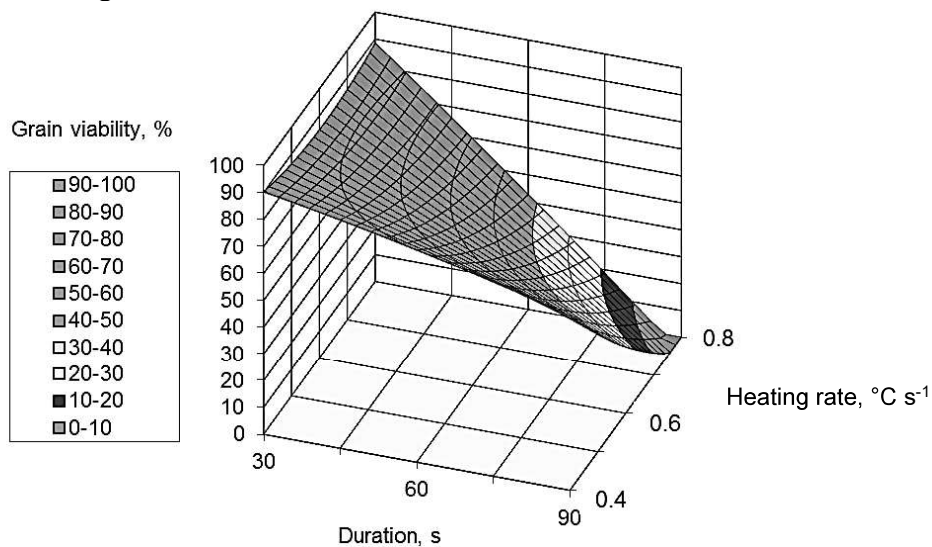


Figure 4. Influence of microwave heating on the grain viability.

The main goal of barley germination on malt is the synthesis and activation of enzymes, which is achieved by dissolving the mealy body of the grain. In this case, the grain structure changes, important biochemical changes occur: some enzymes pass from the inactive state to the active state, others are formed as a result of synthesis. The activity of enzymes changes the structure of endosperm cells, as a result of which the cell walls soften. Changes in the grain structure determine the degree of loosening of the grain endosperm – an important technological factor of malting. This factor is due to the action of a complex of cytolytic and proteolytic enzymes, the rate of accumulation of which determines the quality of preparation of malt. These data are consistent with those of other scientists (Woonton, et al., 2005).

The nature of metabolic processes in germinating grains can be influenced by the use of various physical factors of influence (Woonton, et al., 2005). In particular, using microwave treatment, it is possible to enhance the activity of hydrolytic enzymes in the process of barley germination to malt.










The moderate thermal effect of the microwave field (heating to 30 °C) creates a brewing condition for barley grain that coincides with the conditions for the beginning of embryo growth. This favorably influences the grain viability, which increases to 90.3%. These conditions lead to the decontamination of *Alternaria* fungi alone as well.

With a further increase in the microwave field load (heating rate up to 0.6 °C s⁻¹ and treatment exposure time of 30–60 s), the grain viability of brewing barley grain is reduced and amounts to 60–85% versus 90.3% (under the condition of ‘weak’ microwave field loads). These standard parameters of microwave heating make it possible to almost completely remove the fungal infection. When treatment exposure time is increased to 90 s, the grain viability decreases six times, and with an increase in the heating rate to 0.8 °C s⁻¹, the grain viability is equal to zero. Thus, modeoffered the strongest

decontamination effect while preserving barley grain viability: a heating rate of $0.4\text{ }^{\circ}\text{C s}^{-1}$ and treatment time of 30 s (the grain viability – 90.3%). This favorably influences the grain viability: compared to the control, this indicator increases by 10.1–15.7%. Compared to the use of ultrasound, germination of the treated barley seed was increased about 4–6% (Yaldagard et al., 2008) and this processing method doesn't provide the decontamination effect.

The Table 1 demonstrates that not all modes of processing of grain by the microwave field are effective.

Table 1. The typical examples of the microwave treatment influence on the grain viability

$(\tau = 90\text{ s}; V_t = 0.8\text{ }^{\circ}\text{C s}^{-1})$ the grain viability – $0.0 \pm 0.5\%$	$(\tau = 30\text{ s}; V_t = 0.8\text{ }^{\circ}\text{C s}^{-1})$ the grain viability – $87.7 \pm 0.7\%$	$(\tau = 90\text{ s}; V_t = 0.4\text{ }^{\circ}\text{C s}^{-1})$ the grain viability – $71.0 \pm 0.5\%$
		
$(\tau = 30\text{ s}; V_t = 0.4\text{ }^{\circ}\text{C s}^{-1})$ the grain viability – $90.3 \pm 0.6\%$	$(\tau = 90\text{ s}; V_t = 0.6\text{ }^{\circ}\text{C s}^{-1})$ the grain viability – $12.7 \pm 0.7\%$	$(\tau = 30\text{ s}; V_t = 0.6\text{ }^{\circ}\text{C s}^{-1})$ the grain viability – $86.7 \pm 0.5\%$
		
$(\tau = 60\text{ s}; V_t = 0.8\text{ }^{\circ}\text{C s}^{-1})$ the grain viability – $37.7 \pm 0.5\%$	$(\tau = 60\text{ s}; V_t = 0.4\text{ }^{\circ}\text{C s}^{-1})$ the grain viability – $73.7 \pm 0.5\%$	$(\tau = 60\text{ s}; V_t = 0.6\text{ }^{\circ}\text{C s}^{-1})$ the grain viability – $67.3 \pm 0.5\%$
		

The different reaction of barley grains to the electrophysical effect allows us to assume the mechanism of action, which is largely determined by the parameters of the effect, i.e. speed and processing time. With some parameters, activation of seeds is observed (acceleration of their germination, increase in the rate of root growth), and in others – inhibition. An important consequence of such impacts may be a change in the dynamics of water absorption. At certain frequencies, due to the vibration of individual heterogeneous parts of the grain, the microstructure of the natural grain canals becomes favorable for the transport of water and nutrients. It is also possible to accelerate the processes of denaturation and untwisting of polypeptide chains in the embryo and endosperm during the water absorption stage, resulting in the formation of cavities into which additional osmotic water rushes. As a result, good conditions are created for the life of the embryo, the release and transport of enzymes into the cells of the aleuron layer, the synthesis of enzymes necessary for the cleavage of starch in endosperm cells

and nutrition of the embryo during germination (processing time – 30 s and heating speed $0.4\text{ }^{\circ}\text{C s}^{-1}$).

So, for example, the processing time 90 s and heating speed $0.8\text{ }^{\circ}\text{C s}^{-1}$ leads to an inactivation of enzymes, the grain viability decreases to 0% what is inadmissible at the malt production. The processing parameters: the processing time 60–90 s and heating speed $0.4\text{--}0.8\text{ }^{\circ}\text{C s}^{-1}$ also lead to decreasing of the grain viability. The processing parameters: processing time 30 s and heating rate $0.4\text{--}0.8\text{ }^{\circ}\text{C s}^{-1}$ and processing time 45 s and heating rate $0.4\text{--}0.44\text{ }^{\circ}\text{C s}^{-1}$ promote increase in activity of enzymes and grain viability.

Thus, the microwave field can have a stimulating, an inhibitory, and even a sparing influence on the biological activity of barley grain. Moreover, the exposure effect depends on the value of the variables.

The treatment mode with a heating rate from $0.4\text{ }^{\circ}\text{C s}^{-1}$ and exposure time of 30 s showed the strongest decontaminating effect while preserving barley grain viability.

CONCLUSIONS

Ultra high frequency heating can be used as a stimulating factor aimed at decreasing the fungal infection of barley grain contamination and increasing the activity of enzymes.

The use of the ultra high frequency electromagnetic field energy made it possible to reduce *Alternaria* fungi infection in grain from 80 to 10%, up to complete disinfection, and to increase viability by 10.1–15.7%.

ACKNOWLEDGEMENTS. This article was written with support from the Government of the Russian Federation (Resolution №211 of 16.03.2013), Agreement №02.A03.21.0011 and subsidies for the fulfilment of a fundamental portion of a state order, project №40.8095.2017/BCh. The work was supported by Act 211 Government of the Russian Federation, contract №02.A03.21.001, Project №19.8259.2017/BCh. Agreement № 02.A03.21.0011, and subsidies for the fulfilment of a fundamental part of a state order under Project № 40.8095.2017/BCh.

REFERENCES

- Chen, L.Y., Zhang, S.Q., Li, J.F., Shi, S.L. & Huo, P.H. 2012. Effect of microwave irradiation on germination and seedling growth physiological characteristics of alfalfa seeds after storage. *Yuanzineng Kexue Jishu /Atomic Energy Science and Technology* **46**, 729–735.
- Dalmoro, A., Barba, A.A., Caputo, S., Marra, F. & Lamberti, G. 2015. Microwave technology applied in post-harvest treatments of cereals and legumes. *Chemical Engineering Transactions* **44**, 13–18.
- Gavrilova, O.P., Gannibal, Ph.B. & Gagkaeva, T.Yu. 2016. Fusarium and Alternaria fungi in grain of oats grown in the north-western Russia regarding cultivar specificity. *Sel'skokhozyaistvennaya Biologiya* **51**, 111–118.
- Gavrilova, O.P., Orina, A.S., Blinova, E.V. & Loskutov, I.G. 2017. Response of wild Avena species to fungal infection of grain. *Crop Journal* **5**, 499–508.
- Hussein, H.S. & Jeffrey, M.B. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicol* **167**, 101–104.
- Janić Hajnal, E., Orčić, D., Torbica, A., Kos, J., Mastilović, J. & Škrinjar, M. 2015. Alternaria toxins in wheat from the Autonomous Province of Vojvodina, Serbia: a preliminary survey.

Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment **32**, 361–370.

- Justé, A., Malfliet, S., Lenaerts, M., De Cooman, L., Aerts, G., Willems, K.A. & Lievens, B. 2011. Microflora during malting of barley: Overview and impact on malt quality. *Brewing Science* **64**, 22–31.
- Logrieco, A., Bottalico, A., Solfrizzo, M. & Mule, G. 1990. Incidence of *Alternaria* species in grains from Mediterranean countries and ability to produce mycotoxins. *Mycologia* **82**, 501–505.
- Logrieco, A., Moretti, A. & Solfrizzo, M. 2009. *Alternaria* toxins and plant diseases: An overview of origin, occurrence and risks. *World Mycotoxin Journal* **2**, 129–140.
- Los, A., Akkermans, S., Boehm, D., Cullen, P.J., Van Impe, J. & Bourke, P. 2018. Improving microbiological safety and quality characteristics of wheat and barley by high voltage atmospheric cold plasma closed processing. *Food Research International* **106**, 509–521.
- Milicevic, D. 2009. Mycotoxins in the food chain – old problems and new solution. *Tehn mesa* **50**, 99–111.
- Motallebi, A. 2016. Effect of microwave radiation on seed viability, survival of *Aspergillus Niger* van tieghem and oil quality of oilseeds crops canola, soybean and safflower. *Acta Agriculturae Slovenica* **107**, 73–80.
- Müller, M.E.H., Urban, K., Köppen, R., Siegel, D., Korn, U. & Koch, M. 2015. Mycotoxins as antagonistic or supporting agents in the interaction between phytopathogenic *Fusarium* and *Alternaria* fungi. *World Mycotoxin Journal* **8**, 311–321.
- Müller, M., Van Der Waydrink, G., Peters, M., Umann, K. & Seyfarth, W. 2002. Contamination of winter wheat with *Alternaria* mycotoxins in the state of Brandenburg. *Mycotoxin Research* **18**, 217–220.
- Qu, C., Wang, H., Liu, S., Wang, F. & Liu, C. 2017. Effects of microwave heating of wheat on its functional properties and accelerated storage. *Journal of Food Science and Technology* **54**, 3699–3706.
- Scott, P.M. 2001. Analysis of agricultural commodities and foods for *Alternaria* mycotoxins. *Journal of AOAC International* **84**, 1809–1817.
- Scott, P.M., Zhao, W., Feng, S. & Lau, B.P. 2012. *Alternaria* toxins alternariol and alternariol monomethyl ether in grain foods in Canada. *Mycotoxin research* **28**, 261–266.
- Viliche Balint, C., Surducan, V., Surducan, E. & Oroian, I.G. 2016. Plant irradiation device in microwave field with controlled environment. *Computers and Electronics in Agriculture* **121**, 48–56.
- Webley, D.J. & Jackson, K.L. 1998. Mycotoxins in cereals – a comparison between North America, Europa and Australia, Austral. *Postharvest Technical Conf.*, pp. 63–66.
- Wilson, S.A., Atungulu, G.G. & Olatunde, G. 2016. Drying and decontamination of corn using a pilot-scale continuous-flow radiant heating system. *American Society of Agricultural and Biological Engineers Annual International Meeting, ASABE 201620162016 ASABE Annual International Meeting; Disney's Coronado Springs Resort Orlando; United States; 17 July 2016 – 20 July 2016*.
- Wu, L.X., Ding, X.X., Li, P.W., Du, X.H., Zhou, H.Y., Bai, Y.Zh. & Zhang, L.X. 2016. Aflatoxin contamination of peanuts at harvest in China from 2010 to 2013 and its relationship with climatic conditions. *Food Control* **60**, 117–123.
- Yaldagard, M., Mortazavi, S.A. & Tabatabaie, F. 2008. Application of ultrasonic waves as a priming technique for accelerating and enhancing the germination of barley seed: optimization of method by the Taguchi approach. *Journal of the Institute of Brewing* **114**, 14–21.
- Woonton, B.W., Jacobsen, J.V., Sherkat, F. & Stuart, I.M. 2005. Changes in germination and malting quality during storage of barley. *Journal of the Institute of Brewing* **111**, 33–41.