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THE ROLE OF MILL MACHINERY IN REDUCING DON-TOXIN

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Abstract: In recent years the fusarium infection and accompanying DON–toxin contamination caused serious losses in the wheat growing and processing. Besides decrease of average yield it was serious problem that not a small part of the growned and harvested crop was unsuitable for food and forage production or the usage was limited. This phenomena caused problem in many countries all over the world according to data of scientific literature. There is a statement in the literature also that DON-toxin content can be decreased efficiently and safely only during the cultivation of plant and the possibilities for that are very limited during processing. We wanted to prove with our experiment that it is possible to decrease DON-toxin level of wheat items by application modern equipment and machineries, wherewith the losses and the food safety risks can be minimized. During our experiment we studied the effect of two milling machine on DON toxin content. (Sortex Z color sorter and Scule Verticone intensive surface cleaner). Both modern machine can be built-in the cleaning process in the mills. In our investigation we took samples from harvested wheat items during processing in the year of the experiment. The data were subjected to complex investigation and we evaluated them with different statistical methods. On this basis we can unequivocally state both machines are effective in reducing toxin level of basic material. In the year of the experiment the regression analysis showed that the Schule surface cleaner was more effective than the color sorting machine.

Keywords: effectivity of color sorting, effectivity of grain-surface cleaning, milling wheat, effectivity of toxin reduction

1. Introduction

Among the various fungal diseases that occur during the rainy season in wheat crops the Fusarium spp. fungal species are very significant, the infection of which appear the fusariotoxins [1], [2]. The fungus attacks among others the wheatear and the toxins produced by these microorganisms pose a serious food safety risk [3]. DON-toxin is one of the most significant representative of fusariotoxins [4].

Trichotercles - DON-toxin is the member of this group - are strong cellular poisons that have cytostatic and protein synthesis inhibitory effects, they damage the nervous system and also have an immunotoxic effect [5]. Acute DON toxicity may cause headache, dizziness, nausea, vomiting and diarrhea in humans. DON initially inhibits protein synthesis, upsets the cytokine regulation, the end result of which may be cell death. Presumably the digestive system complaints and also the immune inhibitory effect are due to this mechanism [6]. Indicates their danger that these compounds may be allowed in foodstuffs for human consumption up to 1 mg/kg while the maximum concentration in feedstuffs may be 5- 10 mg/kg [7]. In the case of unprocessed durum wheat used as food raw material the maximum allowable DON-toxin content is 1,75 ppm and in unprocessed edible wheat or otherwise in aestivum wheat this value is 1,25 ppm [8].

DON-toxin is water-soluble, but it is very stable during storage and processing of infected cereals. It is resistant to production processes and does not decomposes on heating [9], so it can go through the entire food chain. Mézes's [10] studies showed that after more than one year of storage of grains when temperature and humidity conditions allowed, owing to bacterial processes some detoxification was observable.

Degree of wheat grain formation disturbances depends on the time of infection. If the weather is favourable for the infection of fusarium fungi after fertilization, at the beginning of grain development, then the proportion of grains in which the fungus is present in the core (endosperm) is higher in the given wheat item. The color of the grains changes in this case. It may be lighter, it may be mainly greyish white, but light lilac or pink discoloration may occur. Color change is of great importance to my experiments. In early infection, some of the eyes, besides the above mentioned, may be smaller, softer, with lighter weight. If the weather becomes wet in the advanced state of the wheat grains, that is at the beginning of the whole ripening, then the fungus infection reaches the nucleus only in a small percentage, the infection centre develops in the layers of the grain shell. In such a case, the color change of the grains is less typical and the size of the grain will not be significantly smaller. But the healthy grains will be softer than the infected ones. At the end of full ripening diseased grains barely deviate from the healthy ones, the core interior remains intact. Occasionally there may be mycelium or mild discoloration on the surface of wheat, which may indicate infection, but in fact it is difficult to distinguish these grains from the healthy ones. The phenomenon described above has been proved by experimentation. Veres [11] did not find close correlation between the external and internal contamination of the grain, that is he proved, that they were developed due to independent phenomena.

The literature generally assumes that during the processing there is no or very limited and uncertain possibility of reducing the degree of mycotoxin, that is the level of food safety risk for each wheat item. [4], [12], [13]. Sándor and workers [14] and Frank [15] carried out model experiments to reduce DON-toxin, in which they studied equipment developed and constructed in laboratories, similar to surface cleaning procedures used in mill processing. They started out from the assumption most of the toxins are concentrated in the germ and shell of wheat grains. They evaluated a relatively small number of samples taken from a given wheat item. The results are important from the point of view that they have demonstrated certain surface cleaning methods can be applied to reduce the toxin content of wheat item, although here the results are not always convincing. But we must not ignore that the degree and nature of the fusarium infection depends on the fungus in which phenophase attacks the wheat. This determines that only the seed, the germ, or the endosperm of the wheat is affected by the infection. It should not be ignored also under mill processing conditions, cleaning and its efficiency can show other results than under laboratory conditions due to the mixing of raw material items, the nature and modernity of the process as well as many other factors. From the point view of of food safety, however, it is important not only to obtain correct information on the theoretical possibilities but also on the effectiveness of the actual process.

From the point of view of our experiment the pre-grinding operations have an important role in the milling process. According to our assumption in the case of infected wheat items the degree of toxin reduction depends on what kind of equipment and technical tools are operated during the production process. The first phase of these operations is the cluster cleaning followed by surface cleaning. During the cluster cleaning in several stages equipment select those components that are unsuitable for milling or wastes from wheat. During surface cleaning the outer surface of wheat grains is removed together with contaminations [16], [17].

Sortex Z+ color sorter was not designed to reduce DON-toxin content. In the food sector and outside also there are very many areas where it is possible to use this machine. In mill processing during cluster cleaning it provides a new, more precise, faster and less wasteful cleaning option.

It offers an opportunity to select components from cereal items the size of which is the same as intact, healthy seeds, but optically different. This is relevant to our experiment. The previously used sorting equipment could not efficiently isolate the quality detrimental components. From the wheat basically the physical contaminations and broken, weird seeds are selected by the machine due to their different color properties [17], [18]. In our experiment, we wanted to prove that the selection of seeds infected by fusarium and resulting in discoloration in the early stage of crop formation becomes possible with the use of this modern machine. As a result, the concentration of DON-toxin in the raw material decreases.

The Schule Verticone VPC 480 is an intensive surface cleaning machine used to shell cereals, legumes and spices. (Figure 1.) In the milling industry, the primary reason for the application is to clean the outer surface of wheat grains from contaminated pollutants and microorganisms [19].

By using it the final product quality will be better and it has a positive effect on the capacity of the mill will also. During the experiment we wanted to demonstrate that the toxin level of seeds infected at a later stage of the crop formation period can be reduced by using this machine and thus achieving a reduction in the toxin concentration of the raw material.

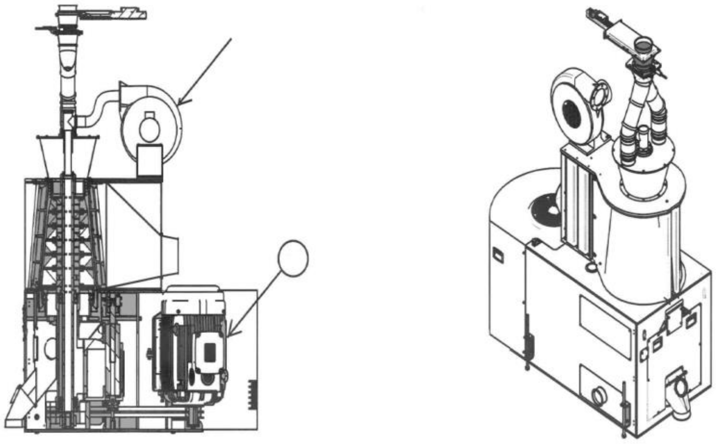

Figure 1. Schule Verticone VPC 480

2. Materials and Methods

2.1. The place of experiment, method of investigation

During our research work we investigated, whether high DON-toxin content can be decreased in the milling process by application adequate technological conditions. The goal is to enable a part of wheat items which was excluded from the food industrial or forage production to get back into the food chain, and it do should'not mean risk. It is important question also, how can the rate of toxin reduction.

For the sake of the practical usability of results it was important to set the experiment in production circumstances. Research in the case of investigation of food safety technological conditions that is reduction of risks should be achieved in production circumstances in order to prove it is possible to decrease the DON toxin content of wheat and to reduce food safety risks.

We carried out research work at Júlia Malom Ltd. The company started the production and the distribution of mill products in 2005. Later it performed developments in several steps and nowadays it operates three milling production lines. The site is in Kiskunszállás, which is 18 km from Kecskemét.

Owing to modern technology milling of the products happens in a closed system, starting from the mill-loading-hopper to the storage of flour in silo and packaging.

When we selected the place for research it was important aspect that the experiments should be completed in controlled circumstances, the whole mechanism of the process and its surroundings should be transparent, mappable and measurable. Besides the conditions, experimental settings should be changeable and repeatable [20].

2.2. Sampling method

During our research work the subject of investigation was the analysis of wheat items harvested in different years. In this article we summarized the evaluation results of samples of wheat items harvested in the year of the experiment. The sampling system was elaborated according to the technological phases because of nonstop operation. The goal is to follow up the change of toxin content of wheat items during the production process. We took five samples from a given wheat item on different spots. First three sampling spot was before Sortex color sorting machine (fraction V_1) and after the machine. After color sorting there were two fractions: from the purified milling wheat (fraction V_2), and from the remaining, selected so called by-products (fraction V_3). We created two sampling spots before Schule machine (fraction V_4) and after the machine (fraction V_5) (Figure 2.).

The samples of fractions V_4 and V_5 can be taken 12,5 hours the color sorting. The time lag was determined by the duration of technological phases. We sampled one wheat item on one day, and we took four sub-samples from each sampling spot. That is we tested DON toxin content of 20 wheat sub-sample from one wheat item. Sub-samples were taken in equal intervals, that is in every 10 minutes. (We started 0. minute and finished 30. minutes)

Figure 2. The place of sampling (Fraction V_5)

20 wheat items were investigated from the wheat harvested in the year of the experiment. That means we tested DON-toxin content of 400 sub-sample.

2.3. Statistical methods to evaluate datas

During experiment we would like to examine how change the toxin content the color sorting machine respectively the surface cleaner as well as joint use of two machines. We adjust the statistical analysis to this, and we compare samples as follows:

1. before Sortex color sorting (fraction V_1) and after (fraction V_2),
2. before Schule machine (fraction V_4) and after (fraction V_5), and
3. before Sortex color sorting (fraction V_1) and after Schule machine (fraction V_5).

The statistical method was elaborated that away we could make analysis with statistically really related samples. Thus we had to determine a given item and the related samples which best describes the changes taking place during the process. That we could reach by averaging four sub-sample which was taken on the same day from the given sampling spot. Statistical analysis was performed with averages.

As a first step we examine the distribution of samples. Generally known, in case of samples with normal or approximately normal distribution the range of statistical procedures available is wider and more accurate results can be obtained than with using non-normal distribution samples. We help approximate to normality a bit by averaging the sub-samples also based on the central boundary distribution thesis. Although in our case the thesis is not fulfilled completely, still usage of averages gives us a wider opportunity.

We used two of the fit testing procedures to determine normality: Kolmogorov–Szirnov-test amended version proposed by Stephens [21], [22] and Wilk–Shapiro-test [23]. The Kolmogorov–Szirnov-test with Stephens's amendment is user-friendly, because the same critical value can be used for different sample sizes, but it can not be said to be too strong test. Opposite Wilk–Shapiro-test, which is one of the strongest normality tests. We submit our data to both tests so we can create a clear picture whether the more permissive or stricter conditions are fulfilled. After the normality test the DON-toxin level change is investigated.

For this a comparison of four related sample (V_1; V_2; V_4; V_5) is performed. In addition to normality in this experiment we have to examine the sphericity of samples also, that is, whether the standard deviation of related samples is equal, which will be checked with Mauchly-test [24].

In case if the distribution of data is not normal or the sphericity is not met, then for a related samples the Friedman test is the most frequently used nonparametric statistical procedure. However, if normality and at the same time sphericity are met then repeated measurement variance analysis should be used for full data analysis. For variance analysis, we consider that statistical literature qualifies as a robust process, which means that if the conditions are not fully met, the procedure can be safely applied [25].

If we do not want to make a complete data test, we only want to investigate two related samples, then we need to use a difference-sample created from sample pairs. The parametric procedure is the even t-test, which precondition is normality also, in turn the nonparametric equivalent is Wilcoxon signed rank test. This test can be used for symmetric distribution difference-samples. That is this procedure has also preconditional. The presence of symmetry can be checked by a Pearson index.

Thereafter, we are also looking for whether it can be predicted based on the measured data how much toxin reduction can be achieved upon completion of the cleaning (fraction V_1 and V_5). For this we perform regression analysis [26]. In case of normal distribution from regression functions the linear gives the best estimate

3. Results and discussion

First we sought statistical verification to determine data analysis and evaluation methods. That is, we checked whether the averaging samples could be considered as normal distributions. During which the four samples were tested (V_1, V_2, V_4, és V_5) with version of Kolmogorov–Szirnov test recommended and with Stephens and Wilk–Shapiro method. Our H0 hypothesis in this case is that the sample is derived from a normal distribution. Using the modified version of the Kolmogorov-Smirnov test, recommended by Stephens, the test statistic and the critical value were as follows:

V_1	0,96	
V_2	0,85	critical value α=0,05: 0,895
V_4	0,84	
V_5	0,69	

The data show that, with the exception of the V_1 sample, our samples have normal distribution according to this statistical procedure.

When the Wilk–Shapiro-test was applied the test statistic and the critical values were as follows:

V_1	0,82	
V_2	0,84	critical value α=0,05: 0,98
V_4	0,85	
V_5	0,93	

In this case, the strongest normality test rejects normality for all four samples.

The two normality tests had different results. Therefore, in order to reach an exact conclusion, we also subject our data to the evaluation of both the statistical methods assuming normality and statistical methods that do not require normality. If we are strict in terms of normality, the four related samples should be examined using the Friedman-probe. The null hypothesis H_0 of the Friedman-probe is that the distribution of all four samples is the same. For our related samples the value of the probe is 54.78, the critical value at the significance level $\alpha = 0.05$ is 7.815. Since the test statistic is much higher than the critical value, we reject the null hypothesis, distribution of our samples is different. That is the reduction in toxin content of starting wheat items can be attributed to the cleaning process.

If we are less strict about the normality test then based on the Kolmogorov-Smirnov test it can be said that normality was only damaged at one sample. As discussed earlier, sphericity should be investigated in this case as well. On the basis of the corrected empirical deviations, the deviations were the same except for the last sample.

V_1	0,27
V_2	0,27
V_4	0,27
V_5	0,21

Although this is convincing at first sight, but for the sake of statistical accuracy we used the Mauchly-test, which has the H_0 null hypothesis that the standard deviations are the same. The value of test statistic in this case is 3.12 $*10^{-6}$. Here, the critical value for the significance level $\alpha = 0.05$ is 0.38. We accept H_0 null hypothesis if the test statistic value is lower than the critical value. The results clearly indicate that we can consider the deviations as the same, so the sphericity was fulfilled.

Because of the damage to normality, we applied variance analysis referring to robustness. The null hypothesis of the variance analysis is same as the null hypothesis of the Friedman-probe, but in this case the test conditions are stricter. So, due to the conditions, this null hypothesis is actually equivalent to the correspondence of the expected values. If these conditions - normality and sphericity - are fulfilled only the expected values should correspond to distributions. The test statistic in our case was 63.66 for related samples. The critical value for the significance level $\alpha = 0{,}05$ is 2,766. Since the test statistic is also much higher than the critical value, we reject the null hypothesis again, distribution of our samples is different. The test confirmed the previous finding that the direct cleaning process prior to milling has an effect on reducing the DON-toxin content of the raw material. So both statistical analysis show that the distribution of DON-toxin levels varies during selecting and cleaning, and based on variance analysis, we can say that the average value of this level has changed.

Then we were wondering if there was any difference in efficiency between the two cleaning processes. For this we have tested the sample pairs. In this case we also followed the principle that we tested the data with both the statistical methods assuming normality and statistical methods that do not require normality.

Wilcoxon's Signal rank-probe can be used independently of normality. Its condition is symmetry which was determined by the Pearson index. The index for difference samples derived from sample-pairs are as follows:

V_1 and V_2 difference sample	-0,63
V_4 and V_5 difference sample	0,12
V_1 and V_5 difference sample	0,55

If this skew indicator number is in absolute value greater than 1, then we are talking about a strong asymmetry of distribution, otherwise the distribution is considered almost symmetric. This means that symmetry can be assumed for all three samples, so the test was applicable. The null hypothesis of Wilcoxon's sign-on rank-test is that the distributions are the same. The statistic values and the critical value of the test are as follows:

V_1 and V_2 difference sample	3,92	
V_4 and V_5 difference sample	3,92	critical value α=0,05: 1,64
V_1 and V_5 difference sample	3,92	

Since the test statistic value is greater than the critical value, therefore we reject the null hypothesis, thus statistically it can be demonstrated that DON-toxin concentration decreased for each sample pair. Hereinafter we used even t-test supposing normality. The H_0 null hypothesis of the even t-test is that the mean of the difference sample formed from two samples is zero. The test statistic values and critical value are as follows:

V_1 and V_2 difference sample	8,46	
V_4 and V_5 difference sample	6,01	critical value α=0,05: 1,73
V_1 and V_5 difference sample	11,29	

As the test statistic values are higher than the critical value, we also rejected the null hypothesis, which means that all three sample pairs cause change. That is to say, with the even t-test we could prove the efficiency of cleaning of the two machines to reduce the concentration of DON-toxin in wheat crops.

Finally, we've been looking for the answer whether the decrease of DON-toxin level of the raw material can be deduced from the knowing the starting value. The practical significance of this is that the mixing rates of known toxin-contaminated lots can be determined by milling experts in such a way so that the mixture can not cause a food safety problem. In the case of each sample pair regression analysis was used to estimate the rate of reduction of DON-toxin level examining the color sorting separately, then after the surface cleaning and the combined effect of sorting and cleaning. First we determined the regression analysis of the samples taken before and after the Sortex color sorting. The equation of the line that matches the V_1 and V_2 pairs:

$$y=-0,04+0,96x,$$

The function demonstrates that the output DON-toxin level can be estimated based on the initial toxin content by taking its 0.96 portion and then reducing the resulting value by 0.04. The explanatory value of the model is $r^2 = 0,98$, which means what proportion of variance we can give of the variable y with the estimated value y, that is with the model. The standard error of the match is 0.04. The practical expert so exactly can determine to what extent the cleaning process can reduce the toxin concentration of the starting raw material. The margin of error we will also get an answer how much this value can be within that range. Knowing the function by mixing the wheat lots, the starting toxin level can be set precisely which makes it possible to produce a safe end product even in the most infected years.

We have also done the regression analysis for samples taken at the Schule machine. The equation of the line that matches the V_4 and V_5 pairs:

$$y=0,76x,$$

The output DON-toxin level can also be calculated based on the function. The explanatory value of the model is $r^2= 0,91$. Standard error of matching 0,06.

We examined the results of the joint operation of the color sorting and the surface cleaner machines. To this we have determined the equation the line that matches the V_1 and V_5 pairs:

$$y=-0,07+0,76x,$$

The explanatory value of the model is $r^2= 0,93$. Standard error of matching 0,04. In accordance with the description detailed above for the V_1 and V_2 sample pairs, in order to minimize losses and achieve maximum yield, milling experts can estimate the DON-toxin content after cleaning of wheat items based on these values.

4. Conclusions

Summing it up it can be stated that by statistical analysis of the sample pairs we have proved that using the Sortex and Schule machines the DON-toxin level decreased as a result of the cleaning process. When comparing the three regression functions, it can be seen that in this year the Schule machine could produce a greater proportion of the decrease in DON-toxin levels and, in essence, resulted in a total decrease. All this means that our research goal is fulfilled. It is verifiable that with the use of modern equipment we can achieve a significant reduction in the level of toxin in the mill processing technology, which is also indicated by r values. This is extremely important in rainy and heavily fusarium infected years. Due to the experimental results, we can minimize serious economic damage, since by using the calculations described above the mixing ratios can be set appropriately and it may also be possible to use wheat crops that were previously considered to be higher risk and excluded from production. We also presented a basis for milling experts who can determine, optimize the mixing rate of wheat lots and can plan the purchasing even more deliberately. The results of the regression analysis can be used to estimate the toxin level of wheat to be milled based on the DON-toxin content of the starting lot.

Other side approaching this issue, the use of the results is also important for the health protection of the population. It is necessary to encourage the milling enterprises to develop technology and to apply modern equipment in the production process in order to keep the toxin contamination as low as possible even in wheat crops within the allowable limit. As wheat flour is a raw material of basic foodstuffs, this is of great importance for the health protection of the population.

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