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Ekológia (Bratislava) Vol. 34, No. 1, p. 65–71, 2015
doi:10.1515/eko-2015-0007

RELATIONSHIPS BETWEEN FUNGAL CONTAMINATION AND SOME PHYSICOCHEMICAL PROPERTIES OF RAPESEEDS

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Abstract

Janda K., Markowska-Szczupak A.: Relationships between fungal contamination and some physicochemical properties of rapeseeds. Ekológia (Bratislava), Vol. 34, No. 1, p. 65–71, 2015.

The aim of this study was to evaluate some properties of rapeseed, to determine the amount of fungi including thermotolerant and xerophilic species and to specify the correlations between some physical properties of rapeseed and the number of fungi. Material was 18 samples of rapeseeds. The characteristics of seeds included volumetric weight, impurities, pH, moisture, water activity, fat content, fat acidity and critical moisture of seeds. Number of fungi were investigated on RBA, YpSs, DG18 medium at 25, 37 and 45 °C. Differences between amount of rapeseed impurities and fat acidity have been revealed. The number of fungi was diversified depending on medium and incubation temperature. The largest fungi number was isolated on DG18 at 25 °C. Correlations between physicochemical properties of seeds and number of isolated fungi have been pointed out. Our study demonstrated that low pH values and weight by volume of seeds as well as high acidity of fat can be used as indicators of contamination of rapeseeds by fungi.

Key words: rapeseed, quality of seeds, amount of fungi, correlations.

Introduction

Rape (*Brassica napus* L.) belongs to the most cultivated oilseed plants in Poland, with a crop area of around 830 thousand hectares and production of about one million tonnes of rapeseed per year, takes sixth place in the world and third in Europe (Muśnicki, 2005; Statistical Yearbook Of Agriculture, 2012). Regardless of the oilseed plants use, there is often a need for their proper storage. Stored rapeseeds quality is determined by important factors, such as its initial quality (before storage) and its microbiological contamination, particularly mycological contamination. Fungi, especially during long-term storage, contribute to decomposition of the most available chemical compound, which is a carbon source, in this case vegetable fat. In addition, the biological activity of fungi gradually creates conducive conditions for the growth of microorganisms that can tolerate the higher environment's water activity.

In the specialist literature, one can most often found works on determination of impact of diverse factors on seeds' feature, among other things on of thousand seed weights, volumetric weight, possibilities and energy of sprouting, humidity, porosity and other physical and mechanical seed properties (Razawi et al., 2009; Calisir et al., 2005; Daun et al., 1986; Broda et al., 2005; Kachel et al., 2005; Skiba et al., 2005; Kachel-Jakubowska, Szpryngiel, 2006; Sathya et al., 2006; Szwed, A., Szwed, G., 2006; Gawrysiak-Witulska et al., 2007) as well as impact of a conditions and time of storage on seed quality (Cassels et al., 2003; Song et al., 2004). Other publications relate only to quantitative and qualitative analyses of fungi inhabiting the rapeseeds (Mills, Abramson, 1986; Brazauskienė et al., 2006) and changes that occur in rapeseed mycobiota during their storage (Bielecka et al., 1995; Korniłowicz-Kowalska et al., 2000). There was usually one microbiological medium used to isolate the fungi – PDA (Potato Dextrose Agar) and one incubation temperature, mostly 25 °C. There is no data in the literature about the thermotolerant or xerophilic fungi that inhabiting the rapeseed. There is also no publications found examining correlation between selected qualitative characteristics of rapeseed and the number of filamentous fungi that inhabit them.

This study was aimed to evaluate selective qualitative characteristics of rapeseed, to determine the quantitative analysis of filamentous fungi, including thermotolerant and xerophilic species, and to specify the correlation between rapeseed's qualitative characteristics and the number of fungi that inhabit them.

Material and methods

Eighteen seed samples were taken from polish producers and commercial traders in Szczecin, with potentially different number of fungi. The characteristics of seeds included volumetric weight (species-specific weight, weight per hectolitre) (PN-ISO 7971-2), amount of mineral and organic impurities including the seeds of other species and undeveloped and damaged seeds (Krełowska-Kułas, 1993) – the exact content of each impurity was not determined, treating them all as impurities in general, pH of seeds using waterproof pH meter type CPC-401 made by Elmetron; seeds moisture content (Krełowska-Kułas, 1993), seeds water activity (a_w) using Decagon Inc. apparatus (DE 202 Aqua Lite) with an accuracy of ±0.003 at 25 ± 1.5 °C (Gondek, Lewicki, 2005), seed fat content using Soxleht method (Krełowska-Kułas, 1993), fat acidity (PN – ISO 729:1999) and critical moisture of seeds (Tys, 2005).

Visual evaluation of seeds showed no signs of moulding. In order to determine a quantitative amount of fungi inhabited in seeds, dilution methods were performed (PN-EN ISO 6887-1:2000). Tenfold dilution were made by using the sterile solution with the following formulation: 8.5 g NaCl, 1 g peptone, 1000 cm^3 distilled water (Samson et al., 2000). To determine the number of fungi with the highest possible accuracy, three solid culture media and three incubation temperatures were used: (1) RBA (Rose Bengal Agar) pH 7.2 (Merck 2006) at 25 °C, (2) YpSs (Yeast Powder Soluble Starch Agar) pH 7.0 at 37 °C to isolate mesophilic fungi and at 45 °C to isolate thermophilic and thermotolerant fungi (Del Fratte, Caretta, 1990), (3) Dichloran Glicerol (DG18) Agar pH 5.6 (Merck, 2006) at 25 °C to isolate xerophilic fungi. Chloramphenicol was added to all media (100 mg/1000 cm^3) (Samson et al., 2000) and they were sterilised in an autoclave (121 °C; 20 min). The procedure was repeated three times.

Obtained results were statistically analysed using Excel spread sheet and Statistica 8.0 (statSoft) program, in Windows environment. Statistical significance of differences was determined with a significance level of $p \leq 0.05$

Results

Table 1 shows physicochemical properties of rapeseed.

T a b l e 1. Physicochemical properties of rapeseeds.

Seed features	**Average (n = 18)**	**Minimum**	**Maximum**	**Coefficient of variation**
Volumetric weight (kg•hl⁻)	64.09	59.21	70.62	0.07
Impurities (%)	5.76	1.34	10.65	0.59
pH	5.52	5.00	6.02	0.08
Humidity (%)	7.34	6.80	9.72	0.12
Water activity	0.60	0.52	0.75	0.12
Fat content (%)	38.79	35.50	41.38	0.04
Fat acidity* (%)	0.80	0.21	2.88	1.0

Notes: * – percentage value of oleic acid.

The lowest coefficient of variation was found in the fat content, volumetric weight and pH of seeds (respectively, 0.04, 0.07 and 0.08) and the highest in the amount of impurities and fat acidity (respectively, 0.59 and 1.0).

Table 2 shows a statistically significant correlation between the physicochemical properties of rapeseed.

T a b l e 2. Statistically significant correlations between the physicochemical properties of rapeseeds.

Lp.	**Investigated features**	**Correlation coefficient**	**Regression equation**	**Coefficient of determination**
1.	Volumetric weight – impurities	–0.52	$y = -4.753x + 21.634$	0.27
2.	Volumetric weight – seed humidity	0.66	$y = 1.020x - 2.138$	0.43
3.	Volumetric weight – a_w seeds	0.80	$y = 0.5467x - 1.814$	0.64
4.	Volumetric weight – fat acidity	–0.60	$y = -3.253x + 14.118$	0.36
5.	Volumetric weight – seeds pH	0.88	$y = 0.9173x - 1.956$	0.77
6.	Impurities – seed humidity	–0.56	$y = 0.093x + 2.281$	0,31
7.	Impurities – a_w seeds	–0.52	$y = -0.038x + 0.537$	0.27
8.	Seed humidity – a_w seeds	0.89	$y = 0.3921x - 0.361$	0.79
9.	Seed humidity – seeds pH	0.62	$y = 0.415x + 0.994$	0.38
10.	a_w seeds – seeds pH	0.78	$y = 1.191x + 1.312$	0.61
11.	Fat content – critical humidity	–1.00	$y = -0.577x + 4.414$	1.00

The correlation with the highest negative correlation coefficient was found between the fat content and the critical moisture (–1.0). The highest positive correlation was observed between the seed moisture – a_w and the volumetric weight – pH (0.89 and 0.88, respectively). In other cases, although the correlation was statistically significant, the regression equation corresponds, depending on the type of correlation, to only 23 to 64% of the data.

Samples of rapeseed differed in count of fungi (Table 3).

T a b l e 3. Number of fungi isolated from rapeseed depending on the medium and the incubation temperature.

Medium and the temperature of the incubation	Average (n = 18)	Minimum	Maximum	Coefficient of variation
	colony forming units•100 g^{-1}			
RBA (25 °C)	2.4×10^6	6.5×10^3	2.3×10^7	2.59
DG18 (25 °C)	6.7×10^7	8.5×10^3	6.0×10^8	2.89
YpSs (37 °C)	4.4×10^4	5.0×10^2	3.5×10^5	2.06
YpSs (45 °C)	8.9×10^6	0.0×10^0	1.0×10^6	2.87

These differences were most likely determined by humidity and temperature conditions existing during their transport and storage at the retailers. The large differences were observed regardless of the used medium or the incubation temperature. The largest seed diversification was discovered in number of xerophilic fungi on DG 18 (from 8.5×10^3 to 6.0 $\times \cdot 10^8$ colony forming units 100 g^{-1}) and in terms of thermophilic fungi on YpSs at 45 °C (from samples with no isolated fungi to 1.0 $\times \cdot 10^6$ colony forming units 100 g^{-1}). The samples with no isolated fungi on YpSs at 45 °C made 23.5%. The smallest seed's diversification was observed in number of mesophilic fungi on YpSs at 37 °C (from 5.0$\cdot \times 10^2$ to 3.5 $\times \cdot 10^5$ colony forming units 100 g^{-1}). The highest number of fungi (6.7 $\times \cdot 10^7$ colony forming units 100 g^{-1}) was isolated on DG18 at 25 °C, and they were xerophilic species.

Table 4 shows the correlations between physicochemical properties of rapeseed and the number of fungi evaluated on different media.

T a b l e 4. The correlation coefficients between the number of fungi isolated on different media and physicochemical properties of rapeseeds.

Seeds' features	Medium and the temperature of the incubation			
	RBA (25 °C)	DG18 (25 °C)	YpSs (37 °C)	YpSs (45 °C)
Volumetric weight	–0.55*	–0.58*	–0.89*	0.18
Impurities	–0.16	–0.29	0.24	–0.56*
pH	–0.66*	–0.63*	–0.81*	0.31
Humidity	–0.29	–0.23	–0.66*	0.25
Water activity	–0.41	–0.38	–0.80*	0.31
Fat content	0.23	0.20	0.16	–0.06
Fat acidity	0.76*	0.90*	0.78*	0.41

Notes: * – Correlations statistically significant at the significance level of $p < 0.0500$.

A statistically significant negative correlation was found between the count of mesophilic and xerophilic fungi (on, respectively, RBA and DG18 at 25 °C) and mesophilic (on YpSs at 37 °C) and volumetric weight as well as a pH value of the seeds. The relationship indicates that, the lower the volumetric weight and pH of seeds, the greater count of fungi grown on those media. Statistically significant, but positive correlations were between the number of fungi and the fat acidity. These correlations indicate that the greater the acidity of the fat, the greater the number of fungi. There was also a statistically significant negative correlation between moisture content and water activity of seeds and the number of mesophilic fungi on YpSs at 37 °C.

Analysis of a relationship between physicochemical characteristics of rapeseed and the number of fungi inhabiting them shows that the most significant correlations were observed between these characteristics and number of mesophilic fungi on YpSs at 37 °C. An inversely proportional correlation was found between the volumetric weight and the count of xerophilic and mesophilic fungi grown on DG18 at 25 °C, RBA at 25 °C and YpSs at 37 °C, respectively.

Discussion

The study proved that moisture of rapeseed was between 6.80 and 9.72%, whereas fat content inside 35.5–41.38%. Similar results were found by Sujak and Kachel-Jakubowska (2012). The object of their study was different rapeseed from Poland too. Their study proved that moisture of rapeseed was located between 6.98 and 7.70% and fat content inside 42.2–42.43%. Results obtained by Daun et al. (1986) indicated that oil content of rapeseed varied between 39.5 and 46%. In our study, fat acidity varied between 0.21 and 2.88%. Similar results was found by Daun et al. (1986) – fat acidity oscillated between 0.1 and 1.8%. More often than not to isolate fungi from rapeseed one medium and one incubation temperature were used, like Martin's medium (Korniłowicz-Kowalska et al., 2000) or PDA (Brazauskienė et al., 2006). In this study, to determine the number of fungi, three solid culture media and three incubation temperatures were used. Samples of rapeseed differed in count of fungi. These differences were most likely determined by humidity and temperature conditions existing during their transport and storage at the retailers. The large differences were observed regardless of the used medium or the incubation temperature. The highest number of fungi ($6.7 \times \cdot 10^7$ cfu $\cdot 100$ g^{-1}) was isolated on DG18. This result verifies that DG 18 is the best medium to isolate fungi from low humidity environments products (Samson et al., 2000). Volumetric weight is an indicator of the plumpness of the seeds – if the seeds are plumper (better), their volumetric weight is higher. Reduced volumetric weight value can be caused by impurities (organic and mineral), higher humidity of the seeds and a presence of poorly developed or damaged seeds. On the other hand, those factors contribute to the growth of microorganisms, including the filamentous fungi. It can therefore be assumed that the lower volumetric weight the more favourable factors for a growth of fungi. Obtained results confirmed that thesis. Negative correlation between volumetric weight and count of fungi (the greater the number of fungi, the lower the volumetric weight) indicated direct relationship between the quality of seeds and their mycological contamination. Another fact that indicates a relationship between the number of fungi present in the seeds on their quality is the positive correlation between the number of xerophilic fungi on DG18 at 25 °C and mesophilic fungi isolated at 25 and 37 °C (on, respectively, RBA and YpSs) and the fat acidity. This correlation indicates that the greater the number of fungi, the higher the acidity of the oil, and therefore – the lower its quality. This could indicate that fungi, present in the seed, have ability for the biosynthesis of lipolytic exoenzymes. There were no statistically significant correlations between the amount of seed's impurities and the number of xerophilic fungi grown at 25 °C on DG18 and mesophilic fungi isolated on RBA and YpSs (respectively at 25 and 37 °C). The correlation was however observed for the number of thermophilic fungi grown at 45 °C – it was negative. Based on these results, it appears that there was no relationship between the amount of impurities (mineral and organic) in rapeseeds and the number of inhabited fungi.

The information about correlation between some physical properties of rapeseed and the number of fungi present in them has not been found in the available literature.

Conclusion

Rapeseeds are rich source of moulds, including xerophilic species. Statistical analysis showed that a low pH value and volumetric weight of the seed as well as high fat acidity may indicate the high filamentous fungal contamination of seeds. This could indicate that fungi isolated from rapeseed may have ability to fats biodegradation.

Acknowledgements

This work was supported by grant from the Ministry of Science and Higher Education of Republic of Poland, Project No. 2P06R 025 30.

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