Mustard seed cultivation processing and quality assessment: preliminary research in Croatia

Dorada sjemena gorušice i ocjena kvalitete: preliminarno istraživanje u Hrvatskoj

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ABSTRACT

Mustard, an annual crop from the Brassicaceae family, includes important species such as yellow (*Sinapis alba*), brown (*Brassica juncea*) and black mustard (*Brassica nigra*). It is mainly grown in North America and some parts of Europe and thrives in various climates with short growing seasons and low rainfall. Nowadays, when the global supply chain is uncertain, additional research on mustard cultivation in other geographical areas is needed. This paper investigates the qualitative and energetic characteristics of mustard seeds and biomass grown in Croatia, finding significant differences in moisture content and the effects of cleaning and drying on seed quality. The varietal analysis shows the variability of oil content and the correlation between moisture, calorific value and impurities. These results improve the understanding of agricultural and industrial applications of mustard and provide information for better cultivation and post-harvest practices.

Keywords: mustard, cultivation, seed, drying, biomass

SAŽETAK

Gorušica, jednogodišnja kultura iz obitelji Brassicaceae, uključuje važne vrste kao što su žuta (*Sinapis alba*), smeđa (*Brassica juncea*) i crna gorušica (*Brassica nigra*). Uglavnom se uzgaja u Sjevernoj Americi i nekim dijelovima Europe, a uspijeva u različitim klimatskim zonama s kratkim sezonama rasta i malom količinom oborina. U današnjem vremenu globalne nesigurnosti dobavnih lanaca, potrebna su dodatna istraživanja uzgoja gorušice sa drugih zemljopisnih područja. Stoga se u ovom radu istražuju se kvalitativna i energetska svojstva sjemena gorušice i biomase uzgojene u Hrvatskoj, pokazujući značajne razlike u sadržaju vlage i utjecaje čišćenja i sušenja na kvalitetu sjemena. Sortna analiza pokazuje varijabilnost u sadržaju ulja i korelacije između vlage, kalorijske vrijednosti i nečistoća. Ova otkrića poboljšavaju razumijevanje poljoprivredne i industrijske primjene gorušice i pružaju informacije za bolji uzgoj i praksu nakon žetve.

Ključne riječi: gorušica, uzgoj, sjeme, sušenje, biomasa

INTRODUCTION

Mustard is an annual plant from the Brassicaceae family, which includes many genera and species (Raza et. al., 2020; Sharma et al., 2024). For commercial use, the most important are the yellow or white mustard (*Brassica alba*), the brown or eastern mustard (*Brassica juncea*) and the black or dark brown mustard (*Brassica nigra*) (Kayacetin et al., 2020; Kinay and Kayacetin, 2023).

It is cultivated worldwide, with the most important growing countries on two continents North America (Canada and the USA) and Europe (Russia, Ukraina, the Czech Republic, Romania, Slovakia, Germany, France and the United Kingdom). Mustard is generally grown in cold climates but is well adapted to a short growing season (Boomiraj et al., 2010). It can also be grown in tropical and subtropical areas where it can tolerate temperatures of up to 27 °C, although it thrives best in well-aerated soils and is suitable for cultivation in areas with less rainfall due to its low water requirements. Pre-sowing tillage is primarily adapted to local conditions and practices. Common cultivation systems include conventional, reduced and no-till (Singh et al., 2021). Sowing time is also crucial for achieving good yields. Late sowing often leads to poor plant growth and low yields (Shekhawat et al, 2012).

Mustard seeds are small and should be sown in moist and shallow soil so that they germinate faster. Depending on the variety, the sowing rate is 8 to 12 kg/ha (Afroz et al., 2011). In some parts of the world, especially in India, mustard plants are harvested before the seeds are fully ripe to avoid cracking. Typically, the entire plant is uprooted, tied into small bundles and dried in the sun for 4-10 days to reduce the moisture content of the harvested mustard seeds to below 9% so that they can be stored safely (Nanduri and Dakheel, 2015). In Europe, on the other hand, mustard seeds are generally harvested in a single-stage process using harvesters, with the moisture content of the seeds being between 12 and 13%. According to Sondhiya et al. (2019), the yield of mustard seed with mineral fertilizer, micronutrients and vermicompost can be up to 1 to 2 t/ha. Mustard remains viable after harvest and shows various signs of spoilage, including browning, dehydration and loss of chemical components. Therefore, the use of post-harvest processes such as drying and storage, which extend the post-harvest shelf life and maintain the health benefits of mustard, is important for its post-harvest preservation.

Before this process, the mustard seeds must be cleaned of impurities (soil, stones, weeds). Drying is widely used in the food industry to remove moisture content from grains, vegetables and fruits (Inyang et al., 2017). Many studies have shown that some properties such as flavor, aroma and nutrient content can be altered by drying technology (Guiné, 2018; Oliveira et al, 2016; Omolola et al, 2017; Matin et al, 2019; Matin et al, 2024). It can also lead to a significant reduction in weight and volume, minimizing packaging, storage and transport costs and allowing the product to be stored at room temperature (Owolarafe et al., 2007). The most common method of drying mustard seeds is convection drying, but the choice of drying temperature is very important and it is desirable that the temperature is as low as possible to preserve nutritional values and allow evaporation of excess water. According to Srinivasakannan (2008), a temperature range of 60 to 80 °C should be used for drying mustard seeds, but according to Damian (2014), the temperature should not exceed 65 or 45 °C. When using mustard seeds for mustard production, microbiological composition must be checked and, if necessary, a short-term sterilization must be carried out to minimize microbial risks and ensure suitability for human consumption.

Post-drying storage conditions and durations are determined by the specific requirements of the intended use or processing method. Due to their hygroscopic properties, mustard seeds can form carbon dioxide and water during storage, which can lead to a deterioration in seed quality. These effects are amplified by factors such as moisture, temperature and relative humidity. Temperature plays an important role in the post-harvest storage of fruit, vegetables and seeds (He et al., 2017; Cao et al., 2019). To minimize these risks, mustard seeds should be stored at a low moisture content of 8% and a temperature of 25 °C (Sharma et al., 2021).

However, too low temperatures can lead to cold damage to the stored products during post-harvest handling and storage (Ahmad et al., 2015).

Mustard is a highly versatile crop, with all parts of the plant offering various practical applications. The seeds are primarily used for mustard paste or condiment production in the food industry, but they also serve as a valuable raw material in several industries, including the production of soap, fertilizers, pharmaceuticals, and biofuels (Sharma et al., 2024). Beyond the seeds, mustard is also cultivated as a nectar-rich plant beneficial for beekeeping, and its leaves have demonstrated notable antioxidant properties (Rodgers et al., 2008; Rahman et al., 2018). Mustard is also the third most important oilseed to produce edible oil in the world, following soybeans and palm oil (Waseem et al., 2017). Mustard seeds have a high oil content of 23 %-47%, of which erucic acid dominates (26.5%-36.5%), but depending on the species, the proportion of other fatty acids varies, so white mustard oil has a high content of oleic acid (22%), while in brown and black mustard oil, linoleic acid is most common (19.5 %-22%) (Grygier, 2023).

According to Ammar (2012) it also contains protein (24.9%), crude fiber (14.7%), carbohydrates (34.94%), sugar (6.89%), but according to protein (24.9%), crude fiber (14.7%), carbohydrates (34.94%), sugar (6.89%) Youssef et al. (2014) mustard seeds contain protein (26.08%), crude fiber (12.2%), carbohydrates (28.09%), oil (33.63%).

In the conditions of disruptions to main supply chains (the war in Ukraine) and climate change, this study aims to comprehensively evaluate the potential of mustard as a multipurpose crop, focusing not only on the cultivation and yield of seeds but also on the utilization and processing of the entire plant biomass. This also includes evaluating the suitability of different parts of the plant for various applications in the food, energy and industrial sectors.

MATERIALS AND METHODS

Materials

To investigate cultivation, the qualitative characteristics of the seeds and biomass of mustard, analysis were carried out using standard methods in triplicate. The study was conducted on six mustard varieties (Gracja, Attack, Zlata, Lyra, Andromeda and Solisa). The samples were grown at the experimental site of the University of Zagreb Faculty of Agriculture in Šašinovec, Croatia (45°50'59.3" N 16°11'26.2" E), where the agroclimatic conditions were determined. The soil at the experimental site in Šašinovec was classified as Fluvisol, with a pH value in KCl of 7.23, humus content of 2.09%, available phosphorus (P₂O₅) of 20.7 mg/100 g, and available potassium (K2O) of 21 mg/100 g, provided by data of the University of Zagreb Faculty of Agriculture. The soil texture is determined as powdery clay loam. Mustard was sown on March 22, 2024, at a sowing rate of 20 kg/ha. Before sowing, fertilization of 500 kg/ha of NPK 7-20-30 was conducted. During the vegetation period, one nitrogen application was applied using 100 kg/ha of KAN. Weed control was achieved with the application of Butisan, and pest control with Karate Zeon. Harvest was conducted on July 15, 2024, at full seed maturity. During the vegetation period, air and ground temperature as well as ground moisture content, were monitored as shown in Figure 1. After harvesting, the seeds were processed and then analyzed. Sampling was carried out in accordance with the Regulation on the methods of sampling and testing of seed quality (Official Gazette 99/2008, Ministry of Agriculture, 2008). After sampling, the samples were cleaned, i.e. separated according to size and shape, using a funnel, a sieve and an aspirator.

Thermal treatment

Due to the increased moisture content after harvesting, the seeds were subjected to convection drying at a temperature of 50 °C and an air flow of 1 m/s for 30 minutes. This temperature and drying time were chosen to preserve the quality and chemical composition of the seeds.

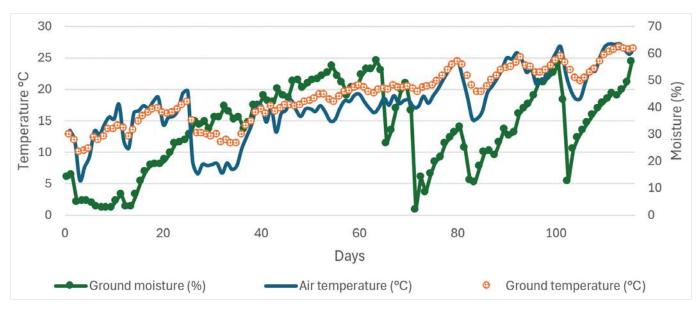


Figure 1. Agroclimatic conditions during the vegetation period

Analytical research

The analytical research was carried out in the Laboratory for Agricultural Biomass Research (ABR) of the Department of Sustainable Technologies and Renewable Energy Sources at the University of Zagreb Faculty of Agriculture.

From the qualitative properties of the seeds, the water content (HRN EN ISO 6540:2021) was determined in a laboratory furnace (Memmert UF55+, Germany), the ash content according to protocol (HRN EN ISO 18122:2015) in a muffle furnace (Nabertherm B170, Lilienthal, Germany) and the oil content (HRN EN ISO 3961:2019) with a Soxhlet extractor R 304 (Behr Labortechnik GmbH, Germany).

From the energy value of the biomass, the moisture content (HRN EN ISO 18134-3:2015) was determined in a laboratory furnace (Memmert UF55+, Germany) and the calorific value according to the method (HRN EN ISO 18125:2017) with an IKA 6000 oxygen bomb calorimeter (IKA Analysentechnik GmbH, Heitersheim, Germany) as the most important characteristics. The calorific value is given in MJ/kg on a dry basis.

Statistical analysis

Statistical data processing was performed using analysis of variance (ANOVA) to determine significant differences between the tested mustard varieties with respect to various physicochemical properties. Tukey's HSD test (P<0.05) was used for post-hoc analysis to determine statistically significant differences between individual groups. Principal component analysis (PCA) was performed to visualize the variability between varieties and determine the dominant factors influencing their differentiation. Correlations between the tested variables were analyzed using Pearson's correlation coefficient, and the results are presented in the form of a correlation heat map. All statistical calculations and visualizations were performed using the software packages R and SPSS.

RESULTS AND DISCUSSION

The results in terms of seed moisture content and biomass are shown in Table 1. Depending on the variety, the diagram of the moisture content (MC) of the raw sample and after the drying process at 50 °C for 30 minutes is shown in Figure 1, the diagram of ash content, impurities and percentage of impurities after the cleaning process of mustard seeds is shown in Figure 2 and the oil content is shown in Figure 3.

Year				2024				Statistical
Month	July	June	May	April	March	February	January	significance
Air temperature (°C)	23.96±3.07	21.51±3.17	17.08±1.36	13.17±4.48	9.9±2.86	7.84±2.84	0.75±4.33	*
Air temperature (minimum) (°C)	16.77±2.76	14.6±3.22	10.22±2.21	5.13±3.4	3.46±2.98	1.58±3.9	4.13±4.43	*
Air temperature (maximum) (°C)	31.55±3.66	28.24±4.02	24.23±2.39	21.07±6.12	16.56±4.31	15.15±2.13	7.07±4.92	*
Air moisture (%)	78.97±6.21	79.52±5.99	78.33±8.43	72.32±8.69	76.76±9.23	78.68±7.18	86.53±6.85	*
Air moisture (minimum) (%)	51.78±7.23	54.77±7.87	52.35±12.57	45.97±12.27	52.75±16.21	52.76±8.84	67.35±13.8	*
Air moisture (maximum) (%)	99.38±1.01	98.74±1.37	96.59±2.97	95.52±4.43	95.05±3.27	94.29±6.86	95.6±5.42	*
Leaf temperature (°C)	24.68±3.21	22.4±3.27	18.04±1.54	14.53±4.74	10.45±3.09	7.93±2.72	0.75±4.17	*
Leaf temperature (minimum) (°C)	16.85±2.73	14.47±3.19	9.94±2.29	4.23±3.3	2.6±2.97	0.89±3.87	-4.7±4.73	*
Leaf temperature (maximum)(°C)	35.1±4.58	31.79±4.7	28.51±3.23	25.99±6.41	19.51±5.26	16.82±2.25	8.39±5.43	*
Ground temperature (°C)	24.97±1.79	22.42±1.7	18.86±1.33	14.5±2.02	10.49±1.57	6.96±1.9	2.66±1.93	*
Ground temperature (minimum) (°C)	23.04±1.82	20.48±1.77	16.91±1.35	12.3±1.7	8.75±1.38	5.53±2	2.05±1.62	*
Ground temperature (maximum) (°C)	27.09±1.98	24.51±1.92	21±1.56	16.97±2.6	12.36±1.99	8.54±1.92	3.43±2.29	*
Ground moisture (%)	41.66±21.68	29.22±14.01	47.13±6.66	25.73±12.02	8.92±5.29	6.44±0.91	7.62±1.16	*
Ground moisture (minimum) (%)	36.11±23.28	24.04±13.69	39.92±9.9	20.84±11.26	6.91±5.94	5.55±1.64	5.94±2.89	*
Ground moisture (maximum) (%)	48.81±24.63	33.01±13.08	52.78±5.59	28.98±12.08	13.11±6.42	7.48±3.74	8.56±2.51	*
Global radiation (MJ/m²)	247.26±59.93	242.27±63.57	220.19±62.54	238.87±69.9		nd		ns
Global radiation (maximum) (MJ/m²)	848.86±110.54	887.84±121.1	876.77±122.35	790.93±129.58		nd		ns
Maximum wind speed (m/s)	2.87±1.03	3.25±1.07	3.83±1.26	4.84±1.89		nd		*
Wind speed (m/s)	0.34±0.13	0.46±0.22	0.64±0.41	0.94±0.52	0.76±0.6	0.71±0.66	0.48±0.39	*
Leaf wetness (%)	44.35±12.8	41.38±12.46	46.17±24.14	44.47±21	55.29±23.02	55.52±20.31	71.59±24.44	*
Precipitation (mm)	4.28±15.31	2.14±4.21	3.48±7.52	1.39±3.17	3.39±7.43	1.27±4.03	2.52±5.18	ns

Abbreviations: nd - not determined, ns - not statistically significant. * P<0.01.

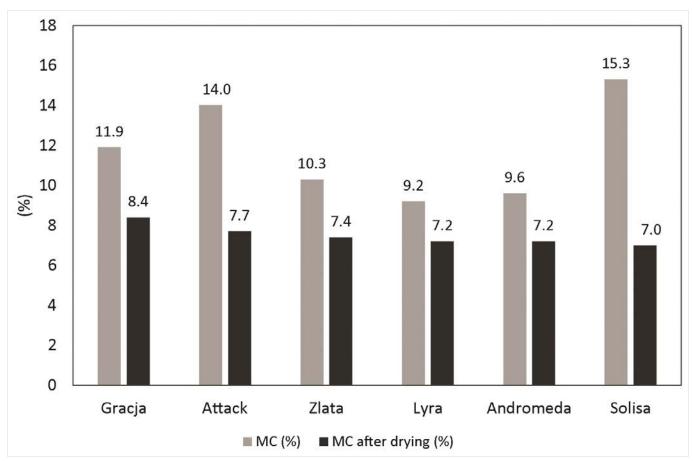


Figure 2. Diagram of the moisture content (MC) of the raw sample and after the drying process

Table 1 shows the agroclimatic conditions during the first seven months of 2024 in the mustard trial, including temperature, humidity and weather parameters. Results are presented as means and standard deviations. Table 2 shows that air temperature is subject to seasonal variation, with the expected trend of higher values in the summer months, followed by corresponding changes in leaf and soil temperature. Humidity also fluctuated, with minimum and maximum values varying significantly over the months, affecting plant growth conditions. The soil shows applications in temperature and humidity. Global radiation and wind speed have an additional effect on microclimatic conditions, while rainfall and water retention in the leaves can significantly affect disease development and plant productivity.

Table 2. Moisture content in the individual parts of the mustard sample examined

Type	Analysis (n = 3)	MC (%)
Seed	1	9.93±0.31 ^b
	2	8.75±0.01 ^a
	3	11.4±0.31°
Biomass	1	13.61±0.2e
	2	16.68±0.17 ^f
	3	12.08±0.07 ^d
Statistical significance	*	*

Abbreviations: MC - Moisture Content; different letters in the column indicate statistically significant differences according to Tukey's HSD post hoc test (P<0.05). * P<0.01.

The data in Table 2 show that mustard seeds have a lower moisture content (8.75–11.4%), while mustard biomass has a higher moisture content (12.08–16.68%), which is to be expected given the greater water holding capacity of plant parts compared to seeds. These differences may be due to cultivation, seed size and storage method. The statistical analysis and the test revealed the heterogeneity of moisture content between the sample types. Similar results were obtained by Stojanović et al. (2023) in their study.

Weather conditions during the final ripening phase should be considered. As shown in Figure 1, a significant increase in soil moisture was observed in the last third of the growing period, indicating rainfall events. Combined with high air and soil temperatures, this likely increased overall evapotranspiration, but may have also prevented effective moisture release from the seed due to elevated relative humidity in the plant canopy. Consequently, a

higher initial moisture content was observed in seeds at harvest. According to the study by Stojanović et al. (2023), the moisture content in mustard seeds ranged from 5.87 to 6,27%, while in the present study, a higher average moisture content of 9.2 up to 15.3% was recorded. Depending on the variety, a diagram of the moisture content (MC) of the raw sample and after the drying process at 50 °C for 30 minutes is shown in Figure 2 and all samples showed a decrease in moisture content. The greatest decrease in moisture content was observed in the Solis sample (from 15.3% to 7.0%) and the least in the Lyra sample (from 9.2% to 7.2%). These results can be explained by the fact that the effects of temperature and drying time on individual samples can depend on the properties of the material itself, such as density, porosity, particle size or even water type, all of which can affect the drying rate and final moisture content (Bonazzi and Dumoulin, 2011).

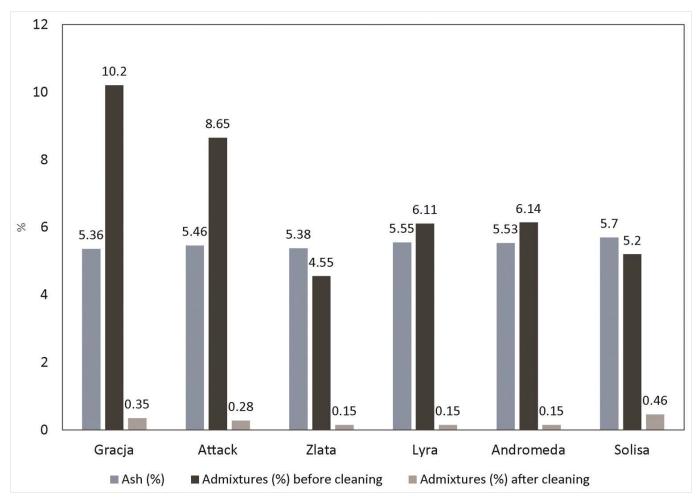


Figure 3. Diagram of ash content, impurities and percentage of impurities after the cleaning process

Figure 3 shows the ash and impurity content before and after the cleaning process of the mustard seeds, with all samples showing a significant reduction in impurities after cleaning. The highest initial impurity content was recorded for the Gracja variety (10.2 %), while the value dropped to 0.35% after cleaning (Sadeghi and Balali-Mood, 2015). Similar trends can also be observed in the ash content, with a range from 5.36 to 5.70% for the Solisa variety, which is similar to Sharif et al. (2017) and Stojanović et al. (2023). The reason for the high content of impurities and ash in mustard seeds is a combination of untreated post-harvest residues, foreign matter from the field and the natural mineral composition of the plant. The cleaning process removes most of these impurities, improving the quality of the seeds for further use. Therefore, understanding post-harvest processes is crucial for minimizing impurities and moisture-related degradation during storage.

The data in Figure 4 shows that Andromeda has the highest proportion of oil (36.15%) because it probably has favorable genetic traits and makes better use of environmental resources. On the other hand, Lyra has the lowest oil content (33.72%), which may be a result of its genetic characteristics or unfavorable growing conditions. The amount of oil can depend on the soil, temperature, humidity and climate conditions under which the plant is grown. Varieties that are better adapted to certain conditions can produce more oil (Rathore et al., 2018). According to Abul-Fadl et al. (2011), the oil content in brown mustard seed flour was 36.22%, while in yellow (white) mustard it was 31.78%. These values are comparable to those obtained in our study. The first principal component explains 52.08% of the variability, while the second covers 29.70%, which is sufficient for interpretation (Figure 5).

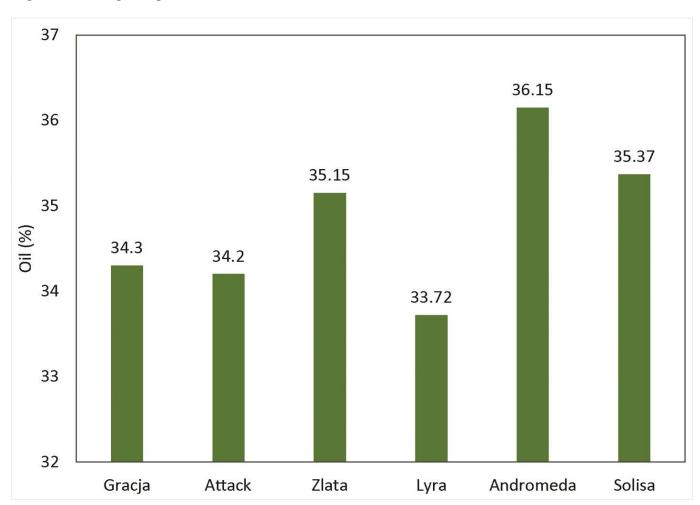


Figure 4. Oil content according to individual mustard seed varieties

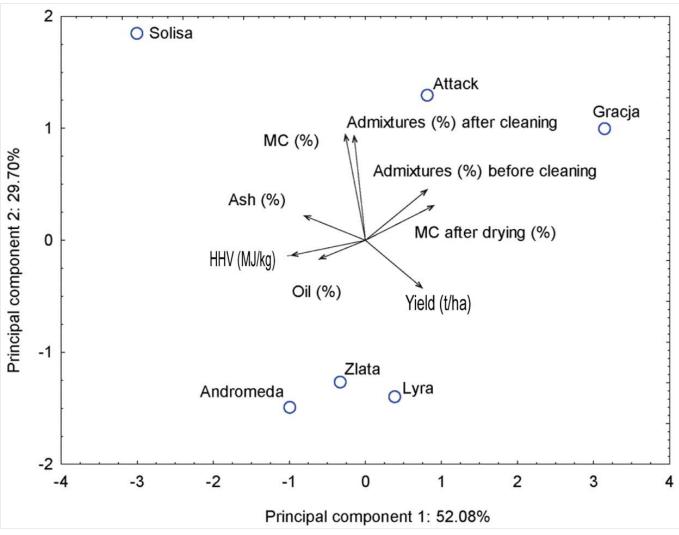


Figure 5. Principal component analysis (PCA) for different mustard varieties

Among the genotypes, Zlata and Lyra were positioned on the positive side of the first principal component (PC1), which was predominantly influenced by variables such as oil content, yield, and higher heating value (HHV). This positioning indicates that these two cultivars exhibit superior energetic and productive properties, making them promising candidates for both bioenergy production and oil extraction purposes.

In contrast, Attack and Gracja were grouped on the upper right side of the plot, aligning more closely with variables such as MC before and after drying, and admixtures. This suggests that these varieties had a higher initial impurity load and retained more MC even after processing, which could be a limiting factor in terms of storage stability and processing efficiency. Solisa was distinctly separated along the second principal component

(PC2), primarily due to its elevated ash content and MC. This might indicate a higher proportion of non-combustible content in the material, which can negatively impact its energy potential. Andromeda appeared on the negative side of both PC1 and PC2, indicating lower values across most measured parameters. Its lower oil content, HHV, and yield suggest limited adaptability for further applications.

Figure 6 shows a correlation heatmap indicating the significance of the relationship (Pearson correlation) between the variables studied. A negative correlation was observed between MC after drying and heating value (HHV (MJ/kg)). The yield (t/ha) also correlated negatively with the MC (%) (-0.68). Ash content shows a strong positive correlation with HHV (0.80), while impurities before cleaning have a negative effect on HHV (-0.85).

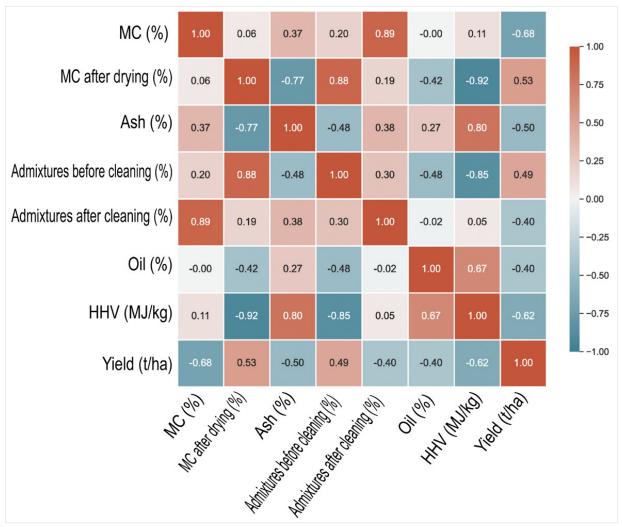


Figure 6. Correlation heatmap of the investigated variables for different mustard varieties

Oil content is strongly positively correlated with HHV. Although oil content was determined on seed material and the HHV was measured on the whole biomass, a moderate positive correlation (r = 0.67) was observed. While these represent different fractions of the plant, it is possible that genotypes with higher lipid biosynthesis in seeds also possess overall higher energy density in above-ground biomass. This could be due to underlying metabolic traits or biomass composition. The correlation between seed composition (especially oil content and ash) and the higher heating value (HHV) indicates the potential of using mustard seed residues or by-products (after oil extraction) as solid biofuels. Variants with lower ash content and higher HHV can improve the combustion efficiency and sustainability of biomass-based energy solutions in rural areas.

CONCLUSION

In summary, the research results point to the importance of post-harvest treatment and confirm the potential of mustard as a valuable crop for multiple sectors. The findings indicate that moisture variability between seeds and biomass, as well as the reduction in impurities after cleaning and drying, underscore the need for careful post-harvest handling to preserve quality and minimize losses. The obtained results may serve as a foundation for improving both cultivation and processing strategies, to enhance overall efficiency and product consistency. In addition to its value as a food and bioenergy crop, mustard also shows potential for applications in the pharmaceutical and cosmetic industries due to its unique biochemical profile, further supporting its role as a multifunctional crop. For farmers

and processors, these findings highlight the need to adapt processing technology and cultivation strategies to each genotype to maximize economic return and ensure consistent product quality.

By understanding and applying these properties, growers can improve the yield, quality and economic viability of cultivation, contributing to sustainable agriculture and food security in Croatia and similar agroecological regions. However, to draw broader conclusions and define clear recommendations, further research over multiple growing seasons and locations would be necessary. This study should therefore be considered preliminary, providing an initial insight into the variability of seed quality and yield characteristics among selected genotypes under specific environmental conditions. Future research should aim to investigate physiological and biochemical mechanisms responsible for oil accumulation and seed drying behavior, especially in relation to environmental stress factors such as air temperature and soil moisture content during seed maturation.

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