WHEAT AND LEGUMES MIXTURES INFLUENCE GRAIN QUALITY

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Abstract
This study aims to evaluate the effect of the mixture of winter wheat and legumes cultivars on grain yield and wheat rheological quality properties. The experiment was conducted in an organically certified field at Zvikov, Ceske Budejovice, to compare grain yield, baking quality, and rheological quality analyzed by Mixolab of wheat flour. Based on results, grain yield showed range from 7.03 to 8.31 t ha^{-1} and there were no significant differences under winter wheat and legumes cultivars mixtures. Productivity wheat quality was significantly different between wheat variety and legumes mixtures in terms of protein content ($P < 0.05$), wet gluten ($P < 0.01$), sedimentation value, and falling number ($P < 0.001$). There was a significant difference in rheological quality analyzed by Mixolab as stability, weakening of protein, and starch characteristics under growing winter wheat and legumes mixtures. Wheat and legumes mixtures may offer a small yield and grain quality advantage.

Key words: *baking quality, yield, Mixolab, organic farming, winter wheat.*

INTRODUCTION

Wheat (Triticum aestivum L.) is one of three main kinds of cereals consumed worldwide *(Aune et al., 2016*). Products of wheat, particularly those from organic farming, have been interesting and developing in recent years (*Mie et al., 2017*). Organic farming systems are characterized by limited soluble nitrogen. Especially, nutrient uptake and use in early spring are important in winter wheat cultivation because it affects not only growth and grain yield but also baking wheat flour quality (*Konvalina et al., 2009*). On the other hand, organic yields are often 14% lower (*Mäder et al., 2007*), 20 – 30% less, and the protein content 10 – 25% lower than conventional farming *(Konvalina et al., 2009; Osman et al., 2012*).
Organic winter wheat cultivation is limited by the low input, especially the addition of nitrogen to the soil. Additionally, wet soil and low temperatures conditions in early spring reduce microbial activity and the process of mineralization in soil affects the development of plants, particularly in the early stage. The main priorities in organic agriculture are improving grain yield, increasing grain quality, and productivity of rheological characteristics. Protein content, wet gluten, gluten index, sedimentation value (Zeleny test), falling number, and rheological quality analyzed by Mixolab are characterized for high baking quality of the organic wheat varieties. Efforts to boost grain yield and quality include breeding and selection. However, these take a long time and cost. Designing cultivation practices could be a complementary strategy in grain yield and quality improvement of grain wheat in organic farming *(Konvalina et al., 2009).*
Mixtures of wheat varieties or wheat and legumes are a viable strategy for sustainable products to help greater stability or no reduced yield, which is promoting to achieve high yields in bread quality in organic farming compared to single systems (*Kaut et al., 2009*). Mixing varieties of wheat improves baking quality (*Aart, 2006*), growing multiline cultivars and cultivar mixtures between wheat and legumes would complement properties of grain yield, grain bulk density, protein content, and also economic efficiency (*Vrtilek et al., 2016*), yield stabilization and pathogen spread in plant populations reduction (*Vidal et al., 2020*). In addition, growing mixtures of pure varieties or annual arable crop species is a promising way to improve crop productivity and complementary N accumulation, decreasing agricultural inputs, especially without chemicals use (*Borg et al., 2018; Chen et al., 2020; Dahlin et al., 2020; Gaudio et al., 2019*). The main objective of the research was to evaluate wheat yield, rheological technological quality characteristics of winter wheat flour under mixtures of winter wheat and legumes mixtures in organic farming.

MATERIALS AND METHODS

Field Experiment

The small plot experiment was carried out in the certified organic field (48.973995N, 14.612085E) at Zvikov, Ceske Budejovice, Czech Republic in vegetation season 2020. The soil texture was loamy soil. The weather condition was mild warm climate, at an altitude of 460 m. Experiments were started using the method of randomized complete block design with three replicates. Trial variants were evaluated in experiments with mixtures of winter wheat varieties (Butterfly) and different legumes (field bean – Vicia faba L., incarnate clover - Trifolium incarnatum L., spring pea Pisum sativum L. and winter pea - Pisum sativum L.).

Evaluation of Qualitative Parameters

Grain wheat was harvested from treatments without legumes seeds. The wheat flour samples were milled by PSY 20 (Mezos, Hradec Kralove, Czech Republic) and Quadrumat Junior machine (Brabender, Duisburg, Germany). Protein content (PC) was estimated by Kjeltec 1002 System (Tecator AB, Hoganas, Sweden), based on N * 5.7 (in dry matter). Wet gluten (WG) was measured by Glutomatic 2200 and Centrifuge 2015 (Perten Instruments, Hägersten, Sweden), according to ICC Standard No. 137/1. Falling number (FN) was determined on FN 1100 (Perten Inst., Sweden) according to AACC/No. 56-81B, ICC/No. 107/1, ISO/No. 3093. Sedimentation value (Zeleny test) (ZSV) was measured by using SDZT4 apparatus according to the ICC standard No. 116/1.

Rheological properties of wheat flour such as dough stability or weakening during mixing, as well as the quality of starch and protein were assessed by Mixolab (CHOPIN Technologies, France) according to the ICC standard method No. 173 - ICC 2006. Mixolab curves made from wheat flour. Amplitude: Elasticity of the dough. Higher the value, the more elastic the flour; Stability: Resistance to dough kneading. The longer the duration, the stronger the flour; C1: Dough development; Torque C2: Attenuation of protein due to mechanical work and temperature; Torque C3: The gelatinization of starch; Torque C4: Stability of hot gel; Torque C5: Measured retrogradation of starch in the cooling phase; Slope α: Attenuating rate of protein in warming; Slope β: starch gelatinization rate; Slope γ: enzymatic degradation rate.

Statistical Analysis

For the analysis of measured data, the STATISTICA program (version 13.2, StatSoft, Inc., California, USA) was used. One-way ANOVA was used for variance analysis. Tukey's honest significant difference (HSD) was used to identify significantly different mean values, $P < 0.05$; $P < 0.01$; and $P < 0.001$, probability level.

RESULTS AND DISCUSSION

Grain Yield

Productivity is an important indicator to evaluate manufacturing efficiency. Wheat grain production is influenced by agronomic features, cultivation practices, and environmental conditions. The addition of legumes in our experiment aimed to increase soil nutrients, resulting in increased wheat output and quality. Fig. 1 shows that grain yield did not differ statistically between combinations of Butterfly variety and legumes cultivars. The grain yield varied from 7.03 to 8.31 t ha^{-1}. The mixtures of winter wheat and legumes were not effective on yield. On the other hand, although there is no difference between the treatments when compared with the results of other studies, it shows the potential and achieves higher yield as *Tran et al., (2020)* found that grain yield ranged from 4.07 t ha^{-1} to 4.28 t ha^{-1} lower, compared to 7.78 t ha^{-1} in our experiment. *Lacko-Bartošová et al., (2021)* and *Jablonskytė-Raščė et al., (2013)* reported in their paper the grain yield of 6.7 t ha^{-1} and 4.95 t ha^{-1}, respectively, which are lower than our study. *Buraczyńska et al., (2011)* found that winter wheat mixed legumes yielded higher yields than mono cultivar cereals. Hence, cultivating mixtures of winter wheat and legumes positively impacted, potentially improving grain yield in low-input agricultural systems.

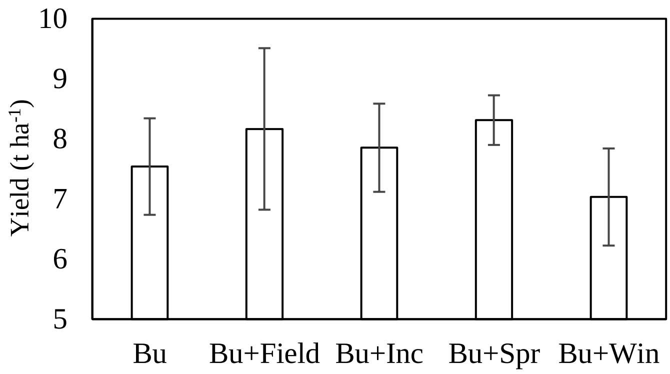

Fig. 1 Grain yield under mixtures of winter wheat variety and legumes (Bu: Butterfly, Field: Field beans, Inc: Incarnate clover, Spr: Spring pea, Win: Winter pea). Means ± standard deviation (SD), $P < 0.05$, N = 24

Wheat Quality

The highly significant statistical difference in the productivity of wheat quality in terms of protein content ($P < 0.05$), wet gluten ($P < 0.001$), sedimentation value ($P < 0.001$), falling number ($P < 0.001$). Protein content ranged from 9.83 to 10.61%. The highest number of protein content under Butterfly and field bean mixtures, differed significantly compared to Butterfly single by 0.76%. There was no difference between Butterfly and other combinations. *Krejčířová et al., (2008)* showed that the protein content was higher than in conventional farming (11.04%) compared to organic farming (9.48%). The number of protein content in their paper under organic farming cultivar is close to our results (10.09%). *Buraczyńska et al., (2011)* found that total N content in winter wheat grain of grown single crop was lower than crop mixtures (winter wheat and legumes).

Tab. 1 Baking quality, rheological parameters evaluation by Mixolab of winter wheat and legumes mixtures

Treatment	Protein (%)	Wet gluten (%)	ZSV (mL)	FN (s)
Bu	9.85 ± 0.25^{b}	16.66 ± 1.14^{c}	28.42 ± 0.80^{b}	310.00 ± 6.08^{b}
Bu+Field	10.61 ± 0.43^{a}	18.83 ± 0.01^{ab}	27.38 ± 0.38^{bc}	304.67 ± 6.43^{b}
Bu+Inc	9.83 ± 0.27^{b}	17.43 ± 0.08^{bc}	27.00 ± 0.00^{c}	309.00 ± 4.36^{b}
Bu+Spr	9.97 ± 0.10^{ab}	18.99 ± 0.30^{a}	30.00 ± 0.00^{a}	297.33 ± 2.52^{b}
Bu+Win	10.26 ± 0.19^{ab}	19.06 ± 0.16^{a}	26.50 ± 0.00^{c}	335.00 ± 3.00^{a}
P	*	**	***	***
Treatment	Torque C2 (Nm)	Torque C3 (Nm)	Torque C4 (Nm)	Torque C5 (Nm)
Bu	0.42 ± 0.00^{ab}	1.55 ± 0.01^{b}	0.74 ± 0.52	1.57 ± 0.02^{b}
Bu+Field	0.42 ± 0.01^{ab}	1.57 ± 0.01^{b}	0.89 ± 0.01	1.50 ± 0.03^{b}
Bu+Inc	0.45 ± 0.02^{a}	1.69 ± 0.02^{a}	1.02 ± 0.04	1.75 ± 0.08^{a}
Bu+Spr	0.41 ± 0.01^{b}	1.48 ± 0.02^{c}	0.95 ± 0.09	1.51 ± 0.03^{b}
Bu+Win	0.43 ± 0.01^{ab}	1.54 ± 0.01^{b}	0.93 ± 0.03	1.55 ± 0.00^{b}
P	**	***	*NS*	***
Treatment	Alfa	Beta	Gamma	Stability (min)
Bu	-0.07 ± 0.00	0.44 ± 0.02^{b}	-0.13 ± 0.06	6.20 ± 0.20^{c}
Bu+Field	-0.08 ± 0.01	0.47 ± 0.02^{b}	-0.10 ± 0.02	5.80 ± 0.10^{c}
Bu+Inc	-0.08 ± 0.00	0.54 ± 0.03^{a}	-0.08 ± 0.03	8.53 ± 0.06^{a}
Bu+Spr	-0.08 ± 0.01	0.45 ± 0.01^{b}	-0.12 ± 0.02	5.27 ± 0.06^{d}
Bu+Win	-0.08 ± 0.01	0.48 ± 0.03^{ab}	-0.07 ± 0.02	6.77 ± 0.32^{b}
P	*NS*	**	*NS*	***

Means ± standard deviation (SD), Tukey HSD test, influence of mixtures of winter wheat and legumes provable at *$P < 0.05$, ** < 0.01, *** < 0.001, NS – Non significant; different letters within the column shown statistically significant difference at $P < 0.05$. Bu: Butterfly, Field: Field beans, Inc: Incarnate clover, Spr: Spring pea, Win: Winter pea, FN: Falling number, ZSV: Sedimentation value (Zeleny test).

Wet gluten results were also significantly different, with the highest wet gluten under intercropping with field beans (18.83%), spring pea (18.99%), and winter pea (19.06%), higher than sowing only Butterfly seed variety (16.66%), and no difference between winter wheat and incarnate clover (17.43%). A lower number on wet gluten of 6.34% compared to *Jablonskytė-Raščė et al., (2013)* results and similar to *Krejčířová et al., (2008)*, gluten content stood at 18.59%. There is a correlation positively between sedimentation value with protein content and loaf volume, with the higher sedimentation value, the greater the baked bread volume. The evaluation of sedimentation value characteristics indicated a significant difference, sedimentation value was highest when growing winter wheat with spring beans (30.00 mL), however, incarnate clover (27.00 mL) and winter pea (26.50 mL) were lower than single grown Butterfly (28.42 mL), field bean mixes (27.38 mL) were not statistically different from single Butterfly.

Testing of falling number is used to evaluate the amount of sprout damage wheat and correlates negatively with alpha-amylase (an enzyme found in sprout-damaged wheat), with a large increase in this enzyme if the germination occurs. The decreasing number test will be reduced when the amount of alpha-amylase in the wheat increases. A high falling number or the longer indicates that the wheat is more suitable for most baking quality, with an ideal falling number range of 250 - 280 s. There was a considerable variation between winter wheat and legume cultivar mixes. Winter wheat and winter pea mixes produced a greater result (335.00 s) than the other treatments, which ranged from 297.33 to 310.00 s. Our falling number is higher than an ideal falling number, however, it is 140 s slower than the results of *Lacko-Bartošová et al., (2021)*. The baking quality of winter wheat flour was improved by combining winter wheat and legumes.

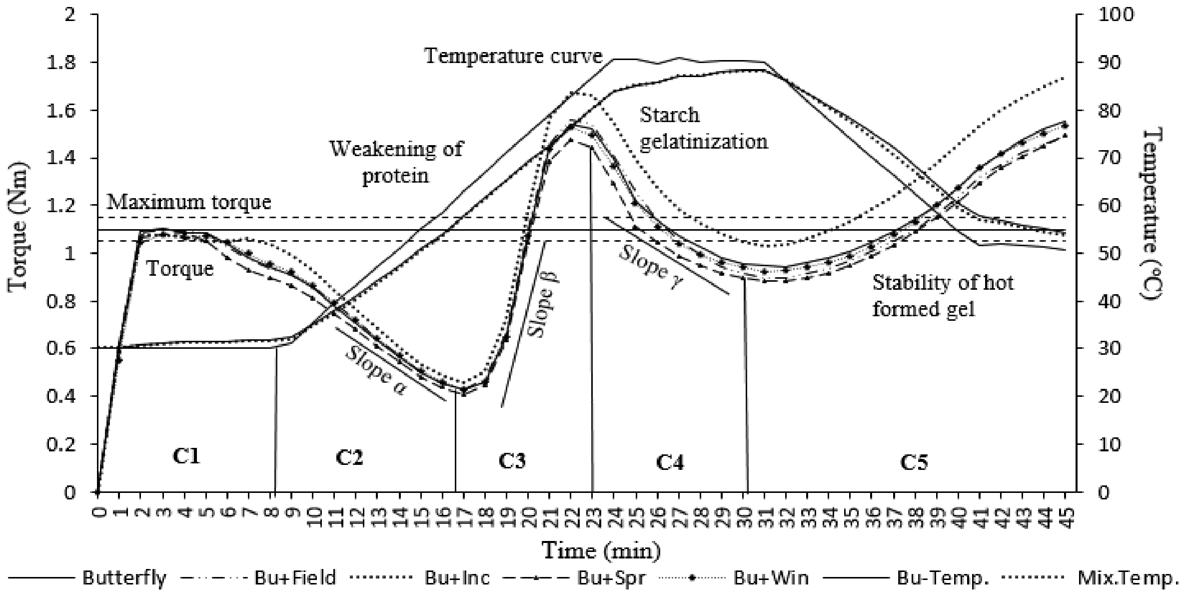

Fig. 2 Mixolab curve of flour milled under mixtures of winter wheat variety and legumes (Bu-Temp.: Butterfly dough temperature, Mix-Temp.: Mixtures of Butterfly and legumes dough temperature, Bu: Butterfly, Field: Field beans, Inc: Incarnate clover, Spr: Spring pea, Win: Winter pea)

Mixolab Analysis

The advantage of Mixolab is being able to measure cereals flour characteristics in one test as proteins, starch, and associated enzymes. The mean values of each treatment for stability, Torque C2, Torque C3, Torque C4, Torque C5, and slope alfa, beta, gamma are displayed in Tab. 1 and Fig. 2. The mixing of dough against evaluation is indicated in the first phase. Mixing resistance of dough, the longer this time is, the more the flour will be strong. The value of stability normally ranges from 4.96 to 11.42 minutes. Tab. 1 shows that the stability assessed by Mixolab under growing winter wheat Butterfly variety and different types of legumes mixtures between 5.27 and 8.53 minutes, was high significantly statistically different ($P < 0.001$). The value of stability of mixtures of Butterfly with incarnate clover, and Butterfly with winter pea, respectively, was 2.33 and 0.57 minutes longer, however, Butterfly intercropping with field beans (5.80 minutes), and Butterfly with spring pea (5.27 minutes) was shorter compared to singly Butterfly (6.20 minutes). The mixtures of winter wheat and legumes were effected on stability of winter wheat flour, intercropping with legumes systems (6.59 minutes) longer, and related to the high gluten in

mixtures compared with only winter wheat (6.20 minutes). Growing winter wheat in organic farming with fertilizer (8.83 minutes) is higher than growing winter wheat organically without fertilizer, according to *Lacko-Bartošová et al., (2021)* (3.19 minutes). Addition of organic fertilizer or mixtures with legumes positively affected the baking quality of wheat flour. Torque C2 measures protein weakening as a function of mechanical work and temperature. The weakening of protein was significantly different in growing seed mixtures of winter wheat and legumes. Despite the fact that there was a significant difference ($P < 0.01$) between the treatments, however, there was no significant difference when comparing the intercropping of each type to the control (no intercropping). Torque C2 ranged from 0.41 Nm to 0.45 Nm, the highest being a mixture of Butterfly with incarnate clove and the lowest being a mixture of Butterfly with spring pea (0.41 Nm).
Starch characteristics were significantly different in growing seed combination of winter wheat namely Butterfly with different types of legumes (except Torque C4). Torque C3 measures starch gelatinization indicated the mixtures reached highest by Butterfly intercropping with incarnate clover (1.69 Nm) and was significantly different compared to Butterfly, followed by Butterfly with field bean and winter pea stood at 1.57 Nm, 1.54 Nm, respectively. Butterfly and spring pea (1.48 Nm) were less than sown single Butterfly (1.55 Nm). Torque C5 under sowing seed Butterfly variety mixtures incarnate clover (1.75 Nm) was higher than other treatments, which ranged from 1.50 to 1.57 Nm. Torque C4, the number of measures of hot gel stability between 0.74 and 1.02 Nm, there was no difference. There was no effect under mixtures of winter wheat with legumes on slope alfa, which ranged from -0.08 to -0.07, and slope gamma from -0.13 to -0.07. On the other hand, the difference in wheat flour starch gelatinization speed was significant for factors of winter wheat varieties and legumes mixtures. The highest number of slope beta of the curve between C2 and C3 was Butterfly and incarnate clover (0.54), similar to Butterfly and winter pea, the number was higher than control (Butterfly variety only). Tab. 1 shows that almost rheological characteristics analyzed by Mixolab were higher under mixtures of Butterfly and incarnate clover compared to other mixtures or grown single Butterfly variety. However, comparison to *Lacko-Bartošová et al., (2021)* research reported torque C2, C3, C4, and C5 values of 0.51, 1.91, 1.94, and 3.82 Nm, respectively, which was higher than rheological quality characteristics of winter wheat flour in our site.

CONCLUSIONS

The current research aims to give an understanding of the effects of changing cultivation practices on grain production, wheat quality, and rheological quality features analyzed by Mixolab evaluation in organic farming. Four percent of average grain yield under mixtures of winter wheat and legumes was higher than the single winter wheat variety. Winter wheat variety and legume cultivar mixtures impacted grain wheat quality. The highest protein content was under Butterfly and field bean mixtures, while there was no difference between Butterfly and other combinations. The highest wet gluten under intercropping with field beans, spring pea, and winter pea was higher than sowing a single Butterfly variety. The rheological properties evaluation of grain wheat in Mixolab yielded the same findings. The mixtures of winter wheat and legumes were a potential method for better winter wheat quality. Therefore, improving the grain dough and baking properties under combinations of winter wheat and legumes should be additional research for adaptation to low input cultivations conditions.

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