Technical Sciences, 2020, 23(1), 53–67

UNIWERSYTET WARMIŃSKO-MAZURSKI W OLSZTYNIE

Quarterly peer-reviewed scientific journal
ISSN 1505-4675
e-ISSN 2083-4527
TECHNICAL SCIENCES
Homepage: www.uwm.edu.pl/techsci/

doi: 10.31648/ts.5125

THE APPLICATION OF THE RESPONSE SURFACE METHOD (RSM) TO OPTIMIZE THE CONDITIONING OF PRIMITIVE RYE GRAIN KRZYCA (*SECALE CEREALE* VAR. *MULTICAULE*) BEFORE MILLING

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Received 17 February 2020; Accepted 15 April 2020; Available online 27 April 2020.

Keywords: rye, conditioning, flour yield, ash content, falling number value, time of milling.

Abstract

The aim of the current study was to optimize the conditioning process of primitive rye, known as Krzyca in Polish, which has recently been reintroduced to farming. The experiment was conducted according to the Box-Behnken model with three independent variables: the temperature of water used for grain conditioning (10, 15, 20°C), the duration of the conditioning process (4, 10, 16 h) and the final grain moisture content (13, 14, 15%). In the obtained flours, four dependent variables were determined (time of grain milling, yield of extraction flour, ash content of flour and falling number value). The obtained polynomial equations and the response surface method point to the significance of the ranges of independent values, with the highest impact noted for flour ash and falling number values.

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Introduction

Common rye (*Secale cereale*) is an economically important crop in Poland and the European Union, mainly used for the production of bread (KONOPKA et al. 2017, WARECHOWSKA et al. 2019). Rye flour contains less gluten-type proteins, while more healthy pentosans and dietary fiber (BUKSA et al. 2012, BUSHUK 2001, GĄSIOROWSKI 1994, SZAFRAŃSKA 2011, VEJRAŽKA et al. 2012). According to Central Research Center for Crop Plants in Poland (*Lista odmian roślin*… 2019) there are 66 varieties of winter rye currently in the Polish National Register, appearing as population or hybrid plants.

In recent years, however, there has been an increase in consumer interest in traditional and regional foods. Thus, the original crop species have returned to cultivation. One of the priorities of the rural development program in Poland (Program Rozwoju Obszarów Wiejskich – PROW) for 2014-2020 is to restore, protect and strengthen ecosystems dependent on agriculture and forestry. Agri-environment-climate operations under the Package 6 PROW have been focused, among others, on the protection of endangered plant genetic resources in agriculture (PAJĄKOWSKI 2015). Among the species of local varieties of cultivated plants supported under this Package is rye krzyca (latin names *Secale cereale* var. *multicaule/Secale montanum* Guss.) (PAJĄKOWSKI 2015). Krzyca rye (other forms of dialect names are “ikrzyca” and “skrzyca”) is a genetically older, untreated form of winter rye, cultivated widely until the nineteenth century throughout Central Europe (CHRZĄSZCZ 2020, KOWALSKA-LEWICKA 2016). Due to the low grain yields (1.5-3.0 t ha^{-1}), the dark color of the grain and its easy falling out from the ear, this species was, however, displaced in the 20^{th} century by modern rye (CHRZĄSZCZ 2020).

Currently, krzyca rye is returning to cultivation, but information on the grain composition, and its technological, milling and baking quality is very scarce. In recent years, KONOPKA et al. (2017) compared the technological quality and content of selected bioactive ingredients in the grain of krzyca and common rye from organic farming. These studies have shown that both species have comparable baking value, while the grain of krzyca contains more protein, total free phenolic compounds, total phenolic acids, flavonoids, sterols, tocols and carotenoids than common rye (KONOPKA et al. 2017). A recent study of this primitive rye also showed that this grain has shorter kernels, lower thousand-kernel weight and a higher contribution of redness in surface color, while grain mechanical features during compression and specific energy of milling are intermediate between open-pollinated and hybrid rye cultivars (WARECHOWSKA et al. 2019).

In the processing of cereal grains for baking purposes, an important element is the assessment of its milling properties, determined *inter alia* by flour extraction and its ash content (STĘPNIEWSKA, ABRAMCZYK 2011, STĘPNIEWSKA 2016). These distinguishing features depend on the participation of individual morphological parts in the grain (seed coat, aleurone layer, endosperm) and the conditions used to prepare the grain for milling, i.e. conditioning (JANKOWSKI 1981). Conditions conducive to achieving the highest flour extract (with as low ash as possible) were developed for bread cereal grains in the 1950s and 1960s (JANKOWSKI 1981). At present, they form the practical knowledge of mill workers that have adapted them into the dominant common cultivars of *Triticum aestivum* and *Secale cereale*. In turn, ancient species, currently reintroduced for agriculture and processing, due to the different physicochemical properties of grain require research, including optimizing the conditions for their conditioning before milling.

The current study analyzed the optimum conditions for conditioning krzyca grain before milling under the influence of three input parameters (independent variables): water temperature for conditioning the grain, conditioning time and grain moisture after conditioning to determine how they affect: grain milling time, flour extraction yield, ash content and flour falling value of extraction krzyca flour (dependent variables).

Materials and methods

The research material was krzyca rye grain (supplier of BIOSFERA Trade Olsztyn). Krzyca grain had an initial moisture of 10.27%, ash content 1.78% dry matter and a mass of 1,000 kernels equal to 22.4 g. The experiment was conducted according to the Box-Behnken model with the above variables at three levels, which resulted in 15 measuring points for normalized (-1, 0, +1) values of input quantities (DERRINGER, SUICH 1980). The real values of experimental factors (independent variables) were selected as: the temperature of water used to conditioning (10, 15, 20°C), the final moisture of conditioned grain (13, 14, 15%) and the time of conditioning (4, 10, 16 h). The ranges of independent variables used were selected using the classic values for traditional rye grain (JANKOWSKI 1981, GĄSIOROWSKI 1994). Real values assigned to the code (-1, 0, +1) corresponded to the sequence from the lowest to the highest factor level. Dependent variables were: time of milling [s], flour yield [%], ash content of flour [%] and falling number value of flour [s]. Grain samples of 100 g after conditioning were ground in a laboratory mill AGROMATIC AQC 109 (Laupen Schweiz) with flour sifting module with a mesh diameter of 250 μm. Main technical parameters of the mill are: number of grooves in rollers from 5 to 20/cm; dimensions of rollers – diameter 7 cm, width 3 cm; revolutions of rollers 1 and 3 – 970 rpm; revolutions of rollers 2 and 4 – 420 rpm. Gaps between rollers is factory set and diminish from 0.8 mm to 0.02 mm. The grinding time was measured using a stopwatch from the moment of opening the grain feeding slide until the last grain was milled. Moisture content of grain was determined according to PN-EN ISO 712:2012 standard, using thermal testing chamber KBC 100 (Wamed, Poland) set at 131°C. Before this assay grain was carefully ground in an A10 IKA Labortechnik mill (Staufen, Baden-Wurttemberg, Germany). Ash content of grain and obtained flours was determined according to PN-EN ISO 2171:2010 standard, using a laboratory muffle furnace (AB Utenos Elektrotechnika, Lithuania) set at 900°C. Falling number value was determined using 1600 device (Perten Instruments, Sweden) according to PN-EN ISO 3093:2010 standard. 1000-kernel weight was determined with the use of an electronic kernel counter (Kernel Counter LN S 50A, Unitra CEMI, Poland) and an electronic scale (WPE 120, Radwag, Poland).

The results of the experiments were used to develop the models of four dependent variable changes according to the 2-nd polynomial equation (without interaction effects):

$$\hat{Y}_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1^2 + b_5X_2^2 + b_6X_3^2 \quad (1)$$

where individual symbols mean:

$\hat{Y}_i$ – the estimated value of the next dependent variable (i = 1, 2, 3, 4),
b_0 – constant value in the equation,
X_1 – temperature of water used to conditioning [°C],
X_2 – final moisture of conditioned grain [%],
X_3 – time of conditioning [s],
$b_1 \div b_6$ – value of the relevant coefficient.

The response (function utility) profiles are presented in the form of contour areas of the plane illustrating the values of total usability of the response calculated for input values defined by various combinations. The response surface method (RSM) used allows for a relative distinction between the components of the "active" and "passive" of equations. At the same time, it allows users to specify stochastic models between dependent and independent variables. The suitability of individual models for predicting the variability of a given dependent variable, depending on statistically significant independent variables, was determined on the basis of a statistical scale of values R-squared (R^2) and adjusted R-square (adj.-R^2) (STANISZ 1998). Additionally, milling efficiency index (ratio of flour yield to flour ash content) was calculated. Analysis of variance (with Duncan's tests) was used to determine significant differences between tested variants.

All statistical calculations were carried out at a significance level of α = 0.05 with STATISTICA v.13.1 software (StatSoft, Inc. Poland).

Results and discussion

The list of actual real values of independent variables used in the generated Box-Behnken model (3-factor variant) and the obtained experimental results are presented in Table 1.

Table 1

Real values of independent variables of conducted experiment in Box-Behnken model and results of determined final results of experiments

Variant of the experiment	Independent variables			Dependent variables			
	temperature of water used to conditioning [°C]	final moisture of conditioned grain [%]	time of conditioning [h]	time of milling [s]	flour yield [%]	ash content of flour [%]	falling number value of flour [s]
1	10	13	10	56	42.5	0.695	190
2	20	13	10	60	42.8	0.729	219
3	10	15	10	67	33.6	0.683	246
4	20	15	10	66	34.0	0.708	227
5	10	14	4	64	41.0	0.491	203
6	20	14	4	66	38.2	0.569	206
7	10	14	16	70	44.5	0.467	237
8	20	14	16	61	39.9	0.409	254
9	15	13	4	66	41.2	0.670	250
10	15	15	4	78	37.8	0.650	241
11	15	13	16	60	40.9	0.608	246
12	15	15	16	64	39.0	0.630	236
13	15	14	10	62	37.4	0.545	244
14	15	14	10	66	38.2	0.493	242
15	15	14	10	72	39.4	0.539	237

The first analyzed feature was the time of milling krzyca grain which, depending on the conditioning conditions (variant of the experiment), varied from 56 s to 78 s for the milling of 100 g of grain sample. Table 1 shows that in variant 10 (15°C, 15%, 10 h) it was more than 40% longer than in variant 1 (10°C, 13%, 10 h). Simultaneously, the data presented in Figure 1 and Table 2 indicate that the only significant independent variable affecting this parameter (except for the constant value) was the final moisture of conditioned grain, which value of the linear dependency coefficient (b_2) was statistically significant. Generally, an increase in milling time in the range of 13-15% was observed along with the increase in the final moisture of the krzyca grain. Finally, the equation

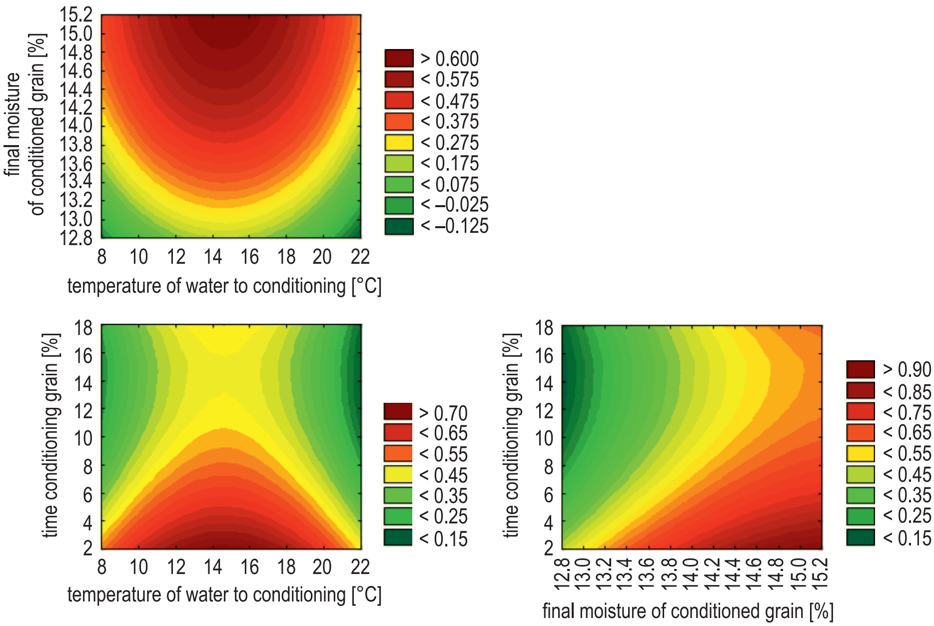

Fig. 1. Contour plot of plane areas illustrating levels of overall desired response variability of dependent variable -Y_1 (time of milling of 100 g of the sample) produced in different areas of the plane defined by values of pairs of independent variables

Table 2

The results of the assessment of the significance of the regression equation coefficients for milling time of 100 g of the sample

Constant value/ Independent variables	Value of the coefficient	Value of the t-test	Standard error for the coefficient	Probability value
Constant value	64.833	48.589	1.3343	0.0000
X_1 – temperature of water used to conditioning [°C] (L)	-0.500	-0.3059	1.6341	0.7674
X_1 – temperature of water used to conditioning [°C] (Q)	1.541	1.281	1.2027	0.2358
X_2 – final moisture of conditioned grain [%] (L)	4.125	2.524	1.6341	0.0355
X_2 – final moisture of conditioned grain [%] (Q)	0.666	0.554	1.2027	0.5945
X_3 – time of conditioning [s] (L)	-2.375	-1.453	1.6341	0.1842
X_3 – time of conditioning [s] (Q)	-0.833	-0.692	1.2027	0.5080

effect evaluation: variable – time of milling [s]; R^2 = 0.5815, adj.-R^2 = 0.2676, mean square MS = 21.36458.
L – linear effect, Q – quadratic effect.

presents the mathematical relationship between the milling time of the sample and statistically significant independent variables (2):

$$\hat{Y}_1 = 64.833 + 4.125\,X_2 \tag{2}$$

However, the values of the coefficient of determination (R^2 = 0.5815) and adjusted coefficient of determination (adj.-R^2 = 0.2676) for this equation indicate the relatively small impact of other independent variables and poor practical possibilities to use this model to predict the value of the dependent variable in question (STANISZ 1998). In the presented experiment, the shortest, and thus the most energy-efficient milling time was found in variant 1, which was characterized by the lowest temperature of water used for conditioning, the lowest final moisture of conditioned grain and average time of conditioning (10°C, 13%, 10 h).

Grain milling is one of the most energy-consuming processes in cereal grain processing. In the industrial processing, about 60-75% of the total energy is associated with this technological operation stage (DANCIU et al. 2009). Extending the milling time of a grain batch leads to an increase in the energy input for milling. Rationalization of energy consumption in the plant is a conscious action aimed at reducing it without reducing the quality and volume of production. It is estimated that in industrial plants, a 20% reduction in electricity consumption results in a 15% reduction in the fee paid (KIRYLUK et al. 2010). Energy expenditure in the milling process depends on the type of mill used, mill settings and the physicochemical properties of the grain and the degree of grinding (DZIKI, LASKOWSKI 2002, DZIKI 2008, WIERCIOCH, NIEMIEC 2006, WIERCIOCH et al. 2008). Most research in this field concerns the impact of grain moisture and hardness on energy consumption (WARECHOWSKA 2014). Numerous researchers have noted that as grain moisture increases, energy consumption in grinding increases (more details in WARECHOWSKA 2014). Moist grains become less brittle and more plastic, which results in less susceptibility of the endosperm to comminution into flour particles (JANKOWSKI 1981).

The next measured feature was the flour extraction yield, which varied from 33.6% to 44.5%, respectively in variants 3 (10°C, 15%, 10 h) and 7 (10°C, 14%, 16 h) of the experiment (Tab. 1). This indicates that in variant of the experiment 7 it was approx. 33% higher compared to variant 3. The data presented in Figure 2 and Table 3 also indicate that the only significant independent variable (except for the constant value) affecting this parameter was the final moisture of conditioned grain, whose value of the coefficient (b_2) was statistically significant. Along with an increase in the moisture content of krzyca grain in the range of 13-15%, a linear decrease in flour extraction yield was observed. The equation describing the yield of flour from the krzyca grain as a function of statistically significant parameters (3) has the following form:

$$\hat{Y}_2 = 64.833 + 4.125X_2 \tag{3}$$

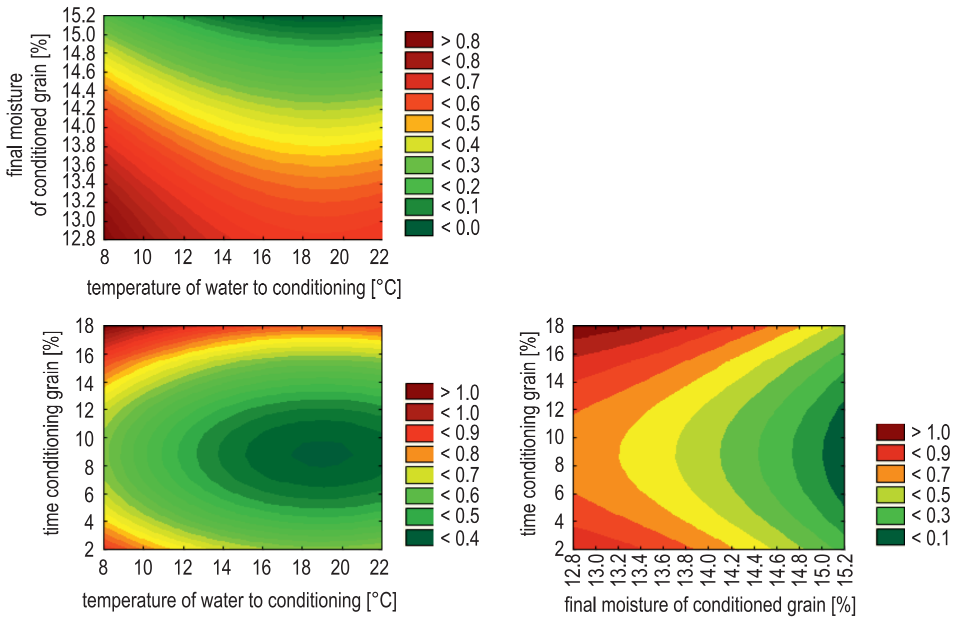

Fig. 2. Contour plot of plane areas illustrating levels of overall desired response variability of dependent variable -Y_2 (flour yield) produced in different areas of the plane defined by values of pairs of independent variables

Table 3

The results of the assessment of the significance of the regression equation coefficients for flour yield

Constant value / Independent variables	Value of the coefficient	Value of the t-test	Standard error for the coefficient	Probability value
Constant value	39.625	68.075	0.5820	0.0000
X_1 – temperature of water used to conditioning [°C] (*L*)	-0.855	-1.199	0.7129	0.2647
X_1 – temperature of water used to conditioning [°C] (*Q*)	-0.268	-0.512	0.5246	0.6220
X_2 – final moisture of conditioned grain [%] (*L*)	-2.873	-4.031	0.7129	0.0037
X_2 – final moisture of conditioned grain [%] (*Q*)	0.319	0.609	0,5246	0.5591
X_3 – time of conditioning [s] (*L*)	0.761	1.067	0.7129	0.3167
X_3 – time of conditioning [s] (*Q*)	-1.010	-1.925	0.5246	0.0903

effect evaluation: variable – flour yield [%], $R^2 = 0.7443$, adj.-$R^2 = 0.5527$, mean square MS = 4.065927.
L – linear effect, *Q* – quadratic effect.

The determination coefficient (R^2 = 0.7443) value and adjusted coefficient of determination (adj.-R^2 = 0.5527) for this equation are determined on a statistical scale as high, which indicates significant possibilities of using this model to predict the value of the dependent variable in question.

The extraction flour yield from cereal grains is determined by the share of the main morphological parts of the grain. Rye flour extract is lower than wheat flour and usually does not exceed 64-65% for modern rye (ROSENTRATER, EVERS 2018). The main reason is the fact, that it is difficult to separate rye endosperm from the seed coat in grain with a high content of non-starch polysaccharides (WARECHOWSKA et al. 2019). The highest flour extract can be obtained from grain with a high proportion of starch endosperm (GĄSIOROWSKI 1994) and the share of starch endosperm is positively correlated with a mass of 1,000 kernels (SCHULER et al. 1995). However, the grain of krzyca is relatively small and has a significantly lower mass of 1000 kernels (by about 25%) than traditional rye (own research and WARECHOWSKA et al. 2019). This indicates that the flour extract obtained in variant 7 of the experiment (44.5%) is probably close to the maximum, although a higher yield of extraction flour of this primitive rye flour (49.8%) was obtained by WARECHOWSKA et al. (2019). In the cited work, the extraction rate of flour of krzyca grain was similar to that of hybrid rye (48.9%) and was significantly lower than the extraction rate of flour from open-pollinated rye (53.5%).

The ash content of rye grain can range widely from 1.43% to 2.42% of dry matter (STĘPNIEWSKA et al. 2018, 2019). For the krzyca grain in this study, it was 1.78% of dry matter. The ash content of the obtained extraction flours varied from 0.409% to 0.729% of dry matter (Tab. 1). These values corresponded to the relatively low extraction rates of obtained flours. The data analysis presented in Figure 3 and Table 4 shows that the variability of ash content in krzyca extraction flours is significantly affected by: a constant value, the final moisture of conditioned grain (quadratic effect) and the time of conditioning (linear and quadratic effects). Finally, the functional relationship between ash content and statistically significant independent variables can be written as:

$$\hat{Y}_3 = 0.609 - 0.083X_2^2 - 0.033X_3 + 0.026X_3^2 \quad (4)$$

and very high values of the coefficient of determination (R^2 = 0.9390) and adjusted coefficient of determination (adj.-R^2 = 0.8933) for this equation indicate its suitability for predicting the discussed variable value.

The obtained results showed that the lowest level of ash content in krzyca extraction flour can be obtained by milling the grain at ca 14% of the final moisture and a relatively long time of conditioning is preferable (above 16 h). These conditions lead to making the coat of krzyca grain more plastic, which has become less susceptible to crushing during milling (JANKOWSKI 1981, GĄSIOROWSKI 1994). Currently preferable in the diet are wholemeal flours, but there is still

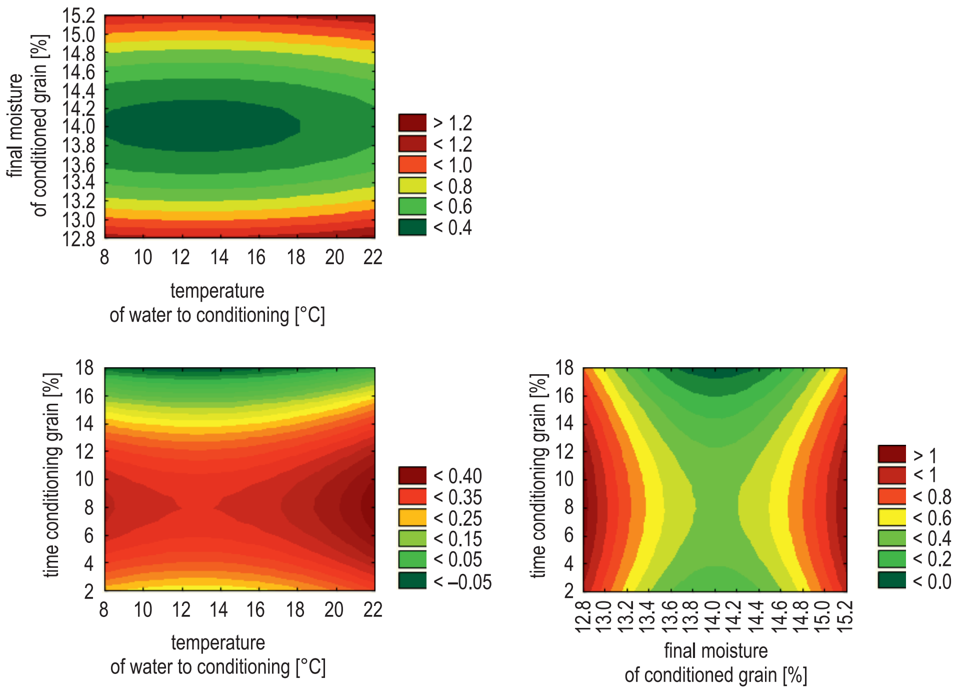

Fig. 3. Contour plot of plane areas illustrating levels of overall desired response variability of dependent variable Y_3 (ash content of flour) produced in different areas of the plane defined by values of pairs of independent variables

Table 4

The results of the assessment of the significance of the regression equation coefficients for ash content of flour

Constant value / Independent variables	Value of the coefficient	Value of the *t*-test	Standard error for the coefficient	Probability value
Constant value	0.609	65.317	0.0093	0.0000
X_1 – temperature of water used to conditioning [°C] (*L*)	0.010	0.864	0.0114	0.4124
X_1 – temperature of water used to conditioning [°C] (*Q*)	-0.006	-0.671	0.0084	0.5207
X_2 – final moisture of conditioned grain [%] (*L*)	-0.004	-0.339	0.0114	0.7431
X_2 – final moisture of conditioned grain [%] (*Q*)	-0.083	-9.921	0.0084	0.0000
X_3 – time of conditioning [s] (*L*)	-0.033	-2.911	0.0114	0.0195
X_3 – time of conditioning [s] (*Q*)	0.026	3.150	0.0084	0.0135

effect evaluation: variable – ash content of flour [%], R^2 = 0.9390, adj.-R^2 = 0.8933, mean square MS = 0.00104.
L – linear effect, *Q* – quadratic effect.

a need to produce relatively pure, refined baking flours for special baking and culinary needs. In this context, extraction flour of krzyca rye exhibits better pro-healthy properties than traditional rye grain (KONOPKA et al. 2017).

Based on flour yield and its ash content milling efficiency index (MEI) was calculated and presented on Figure 4 (to compare the values was used variance analysis ANOVA). In general, the higher MEI value the better the milling value (DZIKI et al. 2012). The highest MEI values (95.3 and 97.6) were determined for variants 7 and 8, respectively, characterized by medium final moisture of conditioned grain (14%) and the longest time of conditioning (16 h). In contrast, the lowest MEI values (49.2 and 48.0) were found for variants 3 and 4, respectively, characterized by the highest final moisture of conditioned grain (15%) and the medium time of conditioning (10 h). Temperature of water used to conditioning had no significant effect on MEI values.

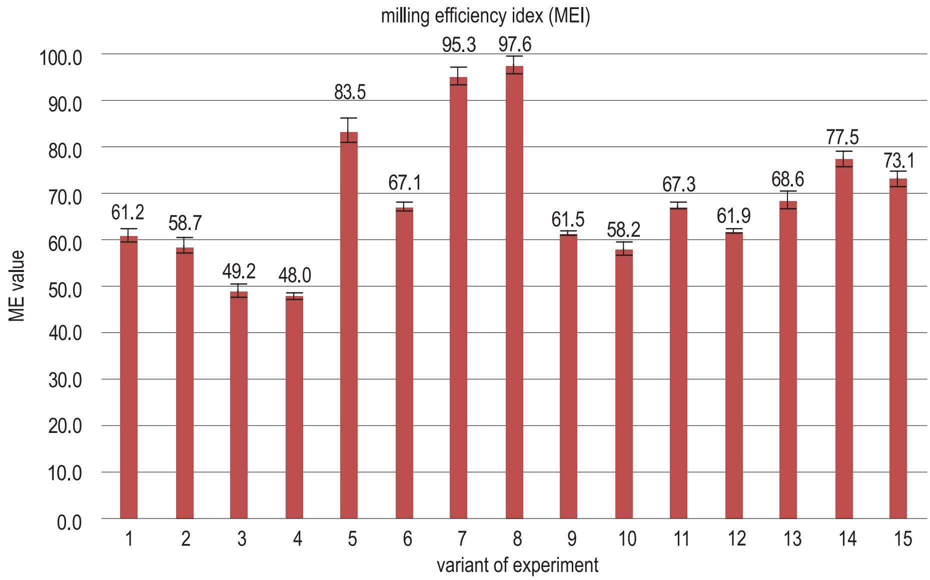

Fig. 4. Milling efficiency index (MEI) of obtained flours from tested variants of experiment – the values marked in superscript with different letters are significantly different (α = 0.05)

The last analyzed feature of krzyca extraction flours was the falling number value. This feature indirectly measures the viscosity of gelatinized flour sample and is related to α-amylase activity in grain (GĄSIOROWSKI 1994, STĘPNIEWSKA et al. 2018, WARECHOWSKA et al. 2019). This factor was analyzed to detect any possible undesirable impact of conditioning parameters on flour quality. Rye flour of good baking quality has falling number values in the range of 125-200 s (GĄSIOROWSKI 1994). Flours with falling number values above 250 s need additional sources of enhanced starch degrading activity.

The results of falling number values in krzyca flours are presented in Table 1. They varied from 190s in variant 1 (10°C, 13%, 10 h) to 254 s in variant 8 (20°C, 14%, 16 h). These results show the relatively good baking values of all produced extraction flours. The values of coefficients of an equation describing the relationship of the falling number value in relation to used independent variables are presented in Table 5. The values of this dependent variable (Y_4) are highly determined by: constant value, the temperature of water used for conditioning (quadratic effect), the final moisture of conditioned (linear effect) and the time of conditioning (linear effect). The general (stochastic) model of this relationship is represented by the equation (5):

$$\hat{Y} = 229.583 + 9.688X_1^2 + 9.083X_2 + 12.917X_3 \quad (5)$$

The very high values of R-squared (R^2 = 0.9949) and adjusted R-square (adj.-R^2 = 0.9641) for this equation they show that the predictors determined largely explain the variability values of the dependent variable in question.

The medium temperature of the water for conditioning, prolonged conditioning duration and the higher final moisture of conditioned grain resulted in an enhanced level of falling number values (Fig. 5). It is a rather unexpected phenomenon (the reverse trend was expected), but krzyca grain differs in chemical composition from traditional rye grain (KONOPKA et al. 2017, WARECHOWSKA et al. 2019) and this variation can affect the obtained results.

Table 5

The results of the assessment of the significance of the regression equation coefficients for falling number value of flour

Constant value / Independent variables	Value of the coefficient	Value of the t-test	Standard error for the coefficient	Probability value
Constant value	229.583	220.577	1.0408	0.0000
X_1 – temperature of water used to conditioning [°C] (L)	3.333	2.481	1.3437	0.1313
X_1 – temperature of water used to conditioning [°C] (Q)	9.688	10.326	0.9382	0.0092
X_2 – final moisture of conditioned grain [%] (L)	9.083	6.760	1.3437	0.0212
X_2 – final moisture of conditioned grain [%] (Q)	0.563	0.599	0.9382	0.6097
X_3 – time of conditioning [s] (L)	12.917	9.613	1.3437	0.0106
X_3 – time of conditioning [s] (Q)	-1.688	-1.799	0.9382	0.2139

effect evaluation: variable – falling number value of flour [s], R^2 = 0.9949, adj.-R^2 = 0.9641, mean square MS = 13.05733.
L – linear effect, Q – quadratic effect.

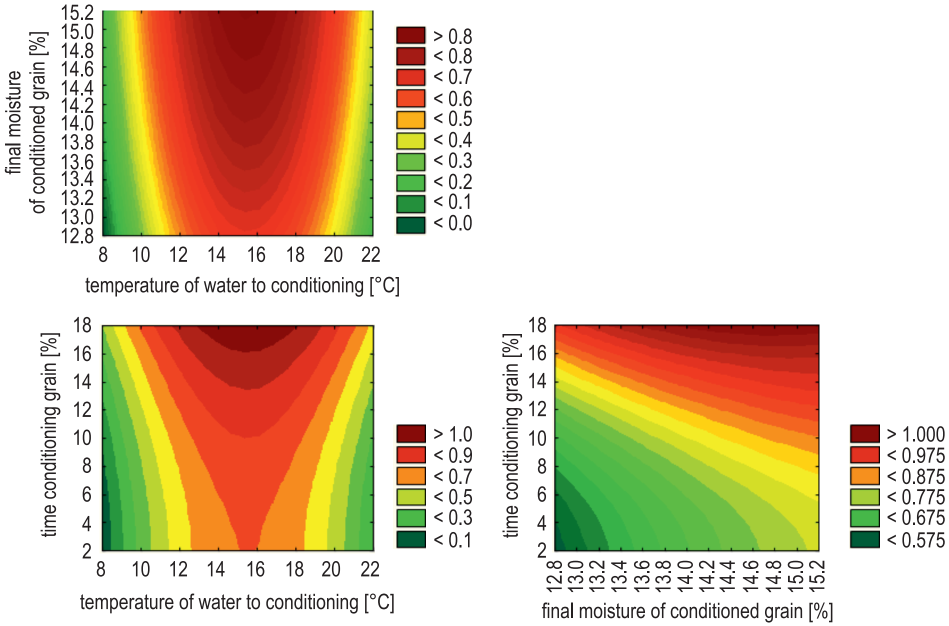

Fig. 5. Contour plot of plane areas illustrating levels of overall desired response variability of dependent variable Y_4 (falling number value of flour) produced in different areas of the plane defined by values of pairs of independent variables

Conclusions

The results of the presented study show that the milling properties of krzyca grain and the quality of obtained extraction flours can be affected by the conditions of grain conditioning. The time of grain milling increases primarily with an increase in the final moisture of conditioned grain (from range 13-15%). The rate of extraction flour is the lowest with a medium time of conditioning duration (10 h) and at an enhanced final moisture of conditioned grain (above 15%) and temperature of water used for conditioning (20°C). Flour ash content of flour seems to be minimized at the highest values of water temperature (20°C), time of conditioning (16 h) and a medium moisture of conditioned grain (14%). The highest milling efficiency indices were determined for variants characterized by medium final moisture of conditioned grain (14%) and the longest time of conditioning (16 h). A falling number value of flour is the lowest after the use of the coldest water (10°C); the lowest moisture of conditioned grain (13%) and medium time of conditioning (10 h). The highest impact of the used independent variables of condition was found for flour ash and flour falling number value.

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