Acta Sci. Pol., Agricultura 11(1) 2012, 125-132

APPLICATION OF 3^{5-2} FRACTIONAL DESIGN IN EVALUATION OF THE EFFECT OF AGROTECHNICAL LEVEL ON THE BREWING QUALITY OF WINTER BARLEY GRAIN (*Hordeum vulgare* L.)

Dariusz Załuski, Anna Hłasko-Nasalska, Krystian Bepirszcz
University of Warmia and Mazury in Olsztyn

Abstract. The aim of the research was evaluation of the effect of agrotechnical level on the brewing quality of winter barley grain with the use of 3^{5-2} (IV) fractional design. Quality parameters of malting barley malt and grain were subjected to analysis. The analysis of experimental features was based on the linear model. In case of rejecting the null hypothesis (H_0), multiple comparison test SNK was applied with the significance level P = 0.05. It was proved that quality parameters of winter barley malt are typical cultivar traits, which to a slight degree may be modified with the use of a different level of agricultural techniques. The only agrotechnical factor which significantly affected quality parameters of malt obtained from winter barley grain was nitrogen fertilization. Together with the increase of nitrogen fertilization level, extractivity and Kolbach index significantly deteriorated, while value of diastatic power improved.

Key words: cultivar Corbie, grain quality, malt quality, malting barley

INTRODUCTION

Testing new technologies in crop plant cultivation requires a quick and cheap method of evaluation of studied agrotechnical factors. It results from a variable market demand for particular raw materials or plant products, as well as from the current economical conditioning. Under such conditions, it is necessary to study again the whole cultivation technology or a new evaluation of production factors [Gołaszewski and Szempliński 1998].

One of the methods of simultaneous testing of all production factors is application of s^k factorial designs, where k number of factors is tested on the s number of levels. 2^k designs are the ones most frequently used, in which k number of factors take on 2 extreme levels, denoted as {-1,1} or {0,1}. In such an approach linear relationship of the effects of quantitative factors with the level of these factors is assumed, though very

Corresponding author – Adres do korespondencji: dr inż. Anna Hłasko-Nasalska, Department of Agrotechnology and Crop Management of the University of Warmia and Mazury in Olsztyn, Oczapowskiego 8, 10-719 Olsztyn, e-mail: ahlas@uwm.edu.pl

often it is a curvilinear relationship. Thus it is justifiable to develop factorial experiments from two level designs into methodically more advanced designs with three levels of factors, 3^k designs [Załuski *et al*. 2006, Gołaszewski *et al*. 2009].

In 3^k factorial designs, with k number of factors A, B, C,…, on three levels (s = 3), there occurs a drastic increase in the number of combinations together with the increase of the number of studied factors. For example, 3^3 design requires 27 combinations, 3^4 up to 81, and 3^5 means 243 experimental variants in one replication. With such a high number of combinations, a researcher has a difficulty in planning the experiment, proper analysis of the results and in concluding. Justifiable thus becomes reduction of the number of experimental variants with simultaneous maintenance of the maximum effectiveness of the design. This may be enabled by fractional factorial designs.

Fractional designs

The essence of s^{k-p} fractional designs is based on selection for evaluation $1/2^p$ (in 2^k designs) or $1/3^p$ (in the 3^k design) parts (fractions) of combinations from all experimental variants. In case of the 3^5 design, separation of 1/3 from the set of 243 combinations reduces this set to 81 subjected to testing. Thus, 3^{5-1} design is obtained. Further reduction leads to the 3^{5-2} design, which gives 27 evaluated combinations [Box *et al*. 1978, Hinkelmann and Kempthorne 2005, Załuski and Gołaszewski 2006].

Together with the increase of the degree of reduction of combinations, the resolution degree of fractional designs decreases. Maximum decrease of objects subjected to evaluation makes it possible to test only the main effects of the design, and thus to obtain resolution III. Design of resolution V enables estimation of main effects and all first-order interactions (two-factorial combinations). There also occurs resolution IV, where apart from the main effects a selected, though incomplete, set of first-order interactions may be evaluated. The choice of resolution, and thus of the degree of reduction of objects of the design testing production factors is conditioned by the aim of such experiments. From the economical point of view, the most beneficial design of maximum reduction of combinations may be used above all for a quick evaluation of production factors. In case of evaluation of the whole technology of crop cultivation, knowledge concerning interactions between production factors is highly significant. Therefore, it is advisable to use designs of resolution V. The experimenter often faces a problem of reconciling quick and cheap evaluation of factors with a wide evaluation of cultivation technology. The solution may be here reduced fractional designs of resolution IV, in which experimenter himself decides about evaluation of some key interactions between factors at the expense of others, less significant ones from the point of view of the final product.

The aim of the research was evaluation of the effect of agrotechnical level on the brewing quality of winter barley grain with the use of 3^{5-2}(IV) fractional design.

MATERIAL AND METHODS

The results for analysis were obtained from the field experiment with winter barley cultivar Corbie. The experiment was set up and carried out at the Experimental Station Bałcyny Sp. z o.o. near Ostróda (53°90’ N; 19°50’ E) in the years 2006-2008. 3^{5-2}(IV) fractional design was applied in two replications with 27 objects each, in which at the same time 5 factors were tested (A, B, C, D, E) on three levels (0, 1, 2) (Table 1 and 2).

Table 1. Experimental factors and their levels
Tabela 1. Czynniki doświadczalne i ich poziomy

	Agrotechnical factor Czynnik agrotechniczny	Level 0 Poziom 0	Level 1 Poziom 1	Level 2 Poziom 2
A	sowing date – termin siewu	September 9-11 9-11 września	+ 7 days – dni	+ 14 days – dni
B	sowing density – gęstość siewu	250 grains·m^{-2} ziarniaków·m^{-2}	350 grains·m^{-2} ziarniaków·m^{-2}	450 grains·m^{-2} ziarniaków·m^{-2}
C	nitrogen fertilization in spring nawożenie azotem wiosną	40 kg·ha^{-1}	60 kg·ha^{-1}	80 kg·ha^{-1}
D	protection against diseases ochrona przed chorobami	dressing – zaprawa	dressing + 1 treatment zaprawa + 1 zabieg	dressing + 2 treatments zaprawa + 2 zabiegi
E	growth regulator regulator wzrostu	0	Moddus 250 EC – 0,6 dm^3·ha^{-1}	Retar 480 SL – 1,5 dm^3·ha^{-1}

Table 2. 3^{5-2} factorial design plan
Tabela 2. Plan układu czynnikowego typu 3^{5-2}

1	0	1	2	2	0	0	0	1	2	2	0	2	0	2
1	1	1	1	0	0	1	0	0	0	2	1	2	2	0
1	2	1	0	1	0	2	0	2	1	2	2	2	1	1
0	0	1	1	0	2	0	0	0	0	1	0	2	2	0
0	1	1	0	1	2	1	0	2	1	1	1	2	1	1
0	2	1	2	2	2	2	0	1	2	1	2	2	0	2
2	0	1	0	1	1	0	0	2	1	0	0	2	1	1
2	1	1	2	2	1	1	0	1	2	0	1	2	0	2
2	2	1	1	0	1	2	0	0	0	0	2	2	2	0

10122 is factor A on level 1, B on level 0, C on level 1, D on level 2 and E on level 2 – 10122 to czynnik A na poziomie 1, B na poziomie 0, C na poziomie 1, D na poziomie 2 i E na poziomie 2

Experimental features, which were subjected to analysis, are the quality parameters of malt and malting barley grain, such as: germination energy, grain filling, protein content in grain, total protein content, soluble protein content, extractivity, Kolbach index, degree of final fermentation, wort viscosity, diastatic power, malt productivity, malt crispness, time of wort filtration as well as the content of β-glucans in the wort. Some of these features are a basis for qualifying cultivars for a particular quality group according to the classification used by the European Brewery Convention [Packa *et al.* 2001].

Analysis of the experimental features in the 3^{5-2}(IV) design was based on the linear model, in which all main effects and chosen first-order interactions were estimated, whereas high-order interactions were included in the experimental error ε_{ijklm} (formula 1):

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_m + ab_{ij} + ac_{ik} + bc_{jk} + be_{jm} + cd_{kl} + ce_{km} + \varepsilon_{ijklm} \quad (1)$$

where μ denotes general mean, i, j, k, l, m are the combination levels (0, 1 or 2) for five factors, a_i, b_j, c_k, d_l, e_m are the main effects, ab_{ij}, ac_{ik}, bc_{jk}, be_{jm}, cd_{kl}, ce_{km} are chosen first-order interactions, however ε_{ijklm} is the experimental error with N(0, σ^2).

In case of rejecting the null hypothesis (H_0), which assumes lack of significant differences between means of particular levels of main effects and their interaction, multiple comparison test SNK (Student-Newman-Keuls) was used with the significance level P = 0.05. To extend background of the result analysis, quality classes have been given in the 9° scale of quality parameter evaluation of the brewer's barley malt, where 9 denotes the best class, and 1 the worst [Klockiewicz-Kamińska 2005].

All statistical calculations were carried out with the use of STATISTICA®8.0 package.

RESULTS

The conducted analysis of variance (ANOVA) proved that the factor which significantly differentiates mean values of brewing quality parameters of winter barley grain was nitrogen fertilization in spring (factor C). Diversified levels of this fertilization significantly affected the protein content in grain and all basic quality parameters of malt (Table 3).

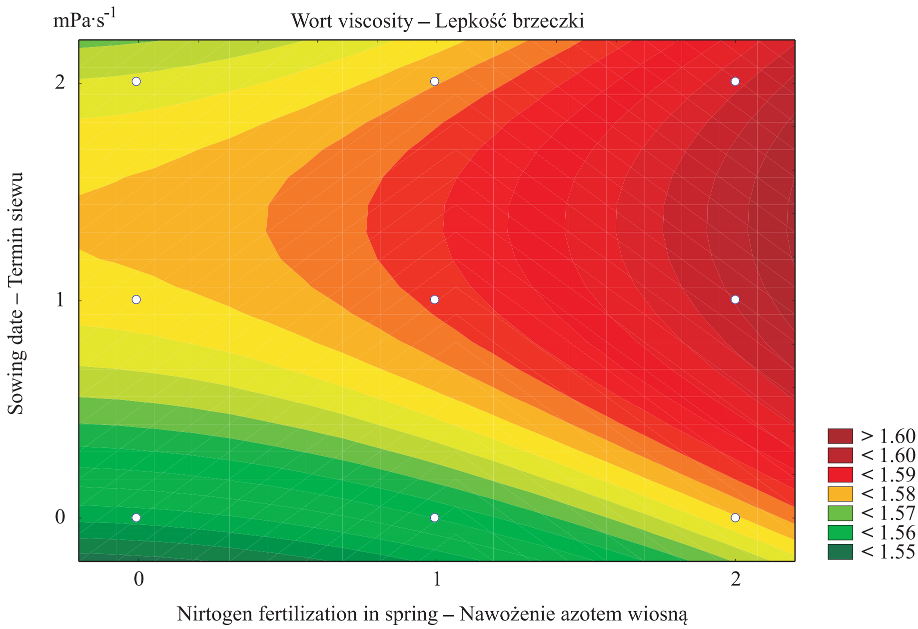
Table 3. Basic quality parameters of barley grain and malt
Tabela 3. Podstawowe parametry jakościowe ziarna jęczmienia i słodu

Feature Cecha	Factor level Poziom czynnika	Factor – Czynnik*				
		A	B	C	D	E
Germination energy, % Energia kiełkowania, %	0	93.60	93.33	92.99	93.52	93.32
	1	93.54	92.71	93.38	93.27	93.49
	2	93.03	94.13	93.81	93.39	93.37
Protein content in grain, % dry weight Zawartość białka w ziarnie, % s.m.	0	9.74	9.77	9.12 b (9)	9.72	9.79
	1	9.75	9.73	9.97 ab (9)	9.72	9.79
	2	9.70	9.69	10.10 a (9)	9.75	9.61
Extractivity, % dry weight Ekstraktywność, % s.m.	0	81.57	81.52	82.09 a (3)	81.65	81.52
	1	81.52	81.64	81.49 b (1)	81.61	81.56
	2	81.73	81.66	81.24 b (1)	81.57	81.74
Kolbach index, % Liczba Kolbacha, %	0	44.11	43.44	44.34 a (7)	43.34	43.28
	1	43.19	43.65	43.57 a (7)	43.28	43.69
	2	43.31	43.51	42.69 b (6)	43.98	43.63
Degree of final fermentation, % Stopień ostatecznego odfermentowania, %	0	78.86	78.56	78.58 ab (1)	78.53	78.44
	1	78.41	78.55	79.06 a (1)	78.60	79.05
	2	78.54	78.70	78.17 b (1)	78.68	78.32
Wort viscosity, $mPa \cdot s^{-1}$ Lepkość brzeczki, $mPa \cdot s^{-1}$	0	1.55 b (5)	1.57	1.56 b (5)	1.57	1.57
	1	1.57 a (5)	1.57	1.56 b (5)	1.56	1.56
	2	1.57 a (5)	1.56	1.58 a (5)	1.57	1.57
Diastatic power, u. W-K Siła diastatyczna, j.W-K	0	266.67	269.72	244.44 b (3)	266.67	264.17
	1	266.39	265.83	284.17 a (5)	267.22	270.56
	2	266.39	263.89	270.83 a (4)	265.56	264.72

* denotations as in Table 1 – oznaczenia jak w tabeli 1
a, b,… homogenous groups of SNK test (P < 0.05) – grupy jednorodne testu SNK (P < 0,05)
(9) – quality class in a 9° evaluation scale of quality parameters of brewer's barley malt – klasa jakościowa w 9° skali oceny parametrów jakościowych słodu jęczmienia browarnego

Together with the increase of nitrogen fertilization in spring, quality parameters of the brewer's barley malt significantly deteriorated. These differences in some cases even determined different evaluation scale of a given quality parameter. For example, a very low estimate of extractivity of cultivar Corbie was analogical with higher rates of nitrogen fertilization. However, application of the lowest rate (40 kg $N \cdot ha^{-1}$) caused occurrence of significantly higher values of this feature, which led them to be classified as a higher quality class, i.e. from the first to the third class. Similar dependences were observed in case of Kolbach index, and the opposite ones in the diastatic power values. In case of the evaluation of wort viscosity, another factor which is the sowing time (factor A), revealed its significant effect on this quality parameter of malt (Table 1).

Area of response in the form of a contour graph presented in Figure 1 shows that the most desirable value of this feature was obtained in case of the earliest sowing time (level 0). It is worth emphasizing that together with delaying the sowing time and increasing nitrogen rate, parameter value of wort viscosity increased, which is an unfavourable tendency from the point of view of barley malt quality. Other experimental factors, both in the form of main effects and interactions, did not significantly affect the discussed features.



denotations as in Table 1 – oznaczenia jak w tabeli 1

Fig. 1. Reaction area of interaction between nitrogen fertilization and sowing date
Rys. 1. Powierzchnia odpowiedzi interakcji nawożenia azotem z terminem siewu

Additional quality parameters of grain and malt were not significantly diversified by agrotechnological level (Table 4). The results show that cultivar Corbie was characterized by a good grain homogeneity (grain filling), by the protein level favourable for beer production, both total protein and protein dissolved in malt, whose crispness oscillating between 77.54-83.97% reaches the quality class range from 6 to 9. However, high values of β-glucan content were observed in wort (250.78-329.00 mg·dm^{-3}).

Table 4. Additional quality parameters of grain and malt
Tabela 4. Dodatkowe parametry jakościowe ziarna i słodu

Feature Cecha	Factor level Poziom czynnika	Factor – Czynnik*				
		A	B	C	D	E
Grain filling, % Celność ziarna, %	0	94.60	95.88	95.78	95.18	96.07
	1	95.59	95.36	95.73	95.79	95.73
	2	96.95	95.91	95.63	96.17	95.34
Total protein content in malt, % dry weight Zawartość białka ogólnego w słodzie, % s.m.	0	9.37	9.45	8.85	9.44	9.47
	1	9.43	9.37	9.53	9.43	9.48
	2	9.44	9.41	9.86	9.37	9.29
Soluble protein content in malt, % dry weight Zawartość białka rozpuszczalnego w słodzie, % s.m.	0	4.13	4.10	3.92	4.08	4.09
	1	4.07	4.08	4.14	4.07	4.13
	2	4.07	4.09	4.21	4.12	4.05
Malt yield, % dry weight Wydajność słodu, % s.m.	0	93.29	93.52	93.54	93.54	93.46
	1	93.62	93.58	93.45	93.58	93.44
	2	93.65	93.45	93.57	93.44	93.65
Malt crispness, % Kruchość słodu, %	0	80.30	80.34	83.97	80.25	80.58
	1	80.28	80.51	80.87	81.05	81.64
	2	81.80	81.54	77.54	81.08	80.16
Time of wort filtration, min. Czas sączenia brzeczki, min.	0	65.00	69.72	70.83	59.72	61.11
	1	65.28	65.00	62.50	72.50	72.22
	2	70.00	65.56	66.94	68.06	66.94
β-glucan content in wort, mg·dm^{-3} Zawartość β-glukanów w brzeczce, mg·dm^{-3}	0	286.72	281.83	250.78	281.50	284.67
	1	277.94	279.56	255.67	277.78	272.67
	2	270.78	274.06	329.00	276.17	278.11

* denotations as in Table 1 – oznaczenia jak w tabeli 1

DISCUSSION

The use of 3^{5-2}(IV) fractional design enabled a complex evaluation of the effect of agrotechnical level on the brewing quality of winter barley grain. Simultaneous testing of all experimental factors brings the experimenter closer to the actual production conditions of a given technology, which cannot be obtained in classical one- or two--factor experiments. Thanks to such an approach a more effective critical analysis may be carried out to the applied production technology [Załuski *et al*. 2006].

Obtained results show that to a slight degree brewing quality of winter barley grain cultivar Corbie was affected by different agrotechnical levels. From the conducted experiment it follows that mainly nitrogen fertilization may significantly change the value of many quality parameters of malt. It should be emphasized that lower rates are preferable, because together with the increase of the amount of the fertilizer, brewing quality of barley grain significantly decreases. In the experiment the lowest nitrogen rate applied in spring, i.e. 40 kg·ha^{-1}, guaranteed such a high level of extractivity quality that class 3 quality could be obtained from the quality class 1.

From the three-year research it followed that early sowing time may significantly affect beneficial viscosity value of wort, which is one of the measures of starch looseness, indicating how advanced the enzymatic processes were, induced mainly by cytolytic enzymes at the time of malting [Klockiewicz-Kamińska 2005]. According to Zembold-Guła and Błażewicz [2007], wort viscosity depends on the activity of mentioned enzymes, and also on the content of non-starch polysaccharides, mainly β-glucans.

High values of β-glucan content in wort observed in the research are an undesired phenomenon, because they unfavourably affect the production process and storage of beer, causing decrease in the speed of wort filtration [Klockiewicz-Kamińska 2005].

It is difficult to find here a direct dependence between the time of sowing and enzymatic activity or the content of β-glucans. However, some interpretation may be done here on basis of the chain of intermediate events, concerning physiological processes closely connected with grain ripening. In own research, it concerns germinating energy, affecting a particular enzymatic activity, significant from the point of view of fast starch looseness, that is wort viscosity.

Presented results confirm literature data, in which it is emphasized that quality parameters of malt are typical cultivar features [Piasecki *et al*. 1997, Packa *et al*. 2001, Klockiewicz-Kamińska 2005]. It means that the predominant role is played here by genetics and strictly defined breeding progress, and to a lesser degree by agrotechnical level. However, skillful application of production factors together with the effective selection of their level will be the key to a complete utilization of the potential of a given malting barley cultivar.

CONCLUSIONS

1. Application of 3^{5-2} experimental fractional design allowed for the evaluation of the effect of agrotechnical level, time and density of sowing, nitrogen fertilization, protection against diseases, and application of growth regulator on the brewing quality of winter barley grain, and for selection of factors modifying quality parameters of malt. It was proved that only nitrogen fertilization had a significant effect on basic quality parameters of malt obtained from winter barley grain.

2. Increase in the level of nitrogen fertilization from 40 to 60 and 80 kg·ha^{-1} N significantly deteriorated extractivity and Kolbach index, while it did not have a diversifying effect on the degree of final fermentation, wort viscosity, though it improved values of diastatic power.

REFERENCES

Box G.E.P., Hunter W.G., Hunter J.S., 1978. Statistics for Experimenters. An Introduction to Design, Data Analysis and Model Building. Willey New York.

Gołaszewski J., Szempliński W., 1998. Doświadczenie czynnikowe ułamkowe jako narzędzie badawcze w opracowaniu technologii uprawy roślin rolniczych [Factorial fractional experiment as a research tool in developing crop cultivation technologies]. Rocz. Nauk Rol. 133 A(1-2), 77-93 [in Polish].

Gołaszewski J., Załuski D., Stawiana-Kosiorek A., Sulima P., 2009. The usefulness of some soil properties and plant traits for the estimation of spatial variation in the 3^5 field experiment with pea (*Pisum sativum* L. *sensu lato*). EJPAU 12(2), #01, http://www.ejpau.media.pl/volume12/issue2/art-01.html
Hinkelmann K., Kempthorne O., 2005. Design and Analysis of Experiments. Advanced Experimetnal Design. Wiley New Jersey.
Klockiewicz-Kamińska E., 2005. Metoda oceny wartości browarnej i klasyfikacja jakościowa odmian jęczmienia [Evaluation method of brewing quality and quality classification of barley cultivars]. Wiad. Odmianozn. 80, COBORU Słupia Wielka [in Polish].
Packa D., Kuraczyk A., Tworkowski J., 2001. Podstawy hodowli jęczmienia [Principles of barley cultivation]. http://www.eurequa.pl/pl/Ru_I.htm (zweryfikowano 13.10.2009) [in Polish].
Piasecki M, Kwiatkowski P., Gąsiorowski H., 1997. Zarys biochemii i technologii słodowania. Jęczmień chemia i technologia [Outline of biochemistry and malting technology]. PWRiL Poznań [in Polish].
Załuski D., Gołaszewski J., 2006. Efficiency of 3^{5-p} fractional factorial design determined using additional information on the spatial variability of the experimental field. J. Agron. Crop. Sci. 192, 303-309.
Załuski D., Gołaszewski J., Stawiana-Kosiorek A., Zaręba A., 2006. Układy czynnikowe pełne i frakcyjne w praktyce doświadczalnictwa polowego [Full factorial and fractional designs in field experimentation practice]. Post. Nauk Rol. 4, 39-47 [in Polish].
Zembold-Guła A., Błażewicz J., 2007. Wpływ modyfikacji czasu słodowania ziarna jęczmienia na cechy brzeczek otrzymanych z udziałem grysu kukurydzianego [The effect of barley grain malting time modification on selected properties of worts obtained with maize grits addition]. Żywność. Nauka. Technologia. Jakość 5(54), 77-83 [in Polish].

ZASTOSOWANIE UKŁADU FRAKCYJNEGO TYPU 3^{5-2} DO OCENY WPŁYW POZIOMU AGROTECHNIKI NA JAKOŚĆ BROWARNĄ ZIARNA JĘCZMIENIA OZIMEGO (*Hordeum vulgare* L.)

Streszczenie. Celem badań była ocena wpływu poziomu agrotechniki na jakość browarną ziarna jęczmienia ozimego z wykorzystaniem układu frakcyjnego typu 3^{5-2}(IV). Poddano analizie parametry jakościowe słodu i ziarna jęczmienia browarnego. Analizę cech doświadczalnych oparto o model liniowy. W przypadku odrzucenia hipotezy zerowej (H_0) zastosowano test wielokrotnych porównań SNK przy poziomie istotności P = 0,05. Wykazano, iż parametry jakościowe słodu z jęczmienia ozimego to cechy typowo odmianowe, które w niewielkim stopniu modyfikować można za pomocą różnego poziomu agrotechniki. Jedynym czynnikiem agrotechnicznym, który istotnie wpływał na parametry jakościowe słodu uzyskanego z ziarna jęczmienia ozimego, było nawożenie azotem. Wraz ze wzrostem poziomu nawożenia azotowego istotnie pogarszała się ekstraktywność i liczba Kolbacha, a poprawiała wartość siły diastatycznej.

Słowa kluczowe: jakość słodu, jakość ziarna, jęczmień browarny, odmiana Corbie

Accepted for print – Zaakceptowano do druku: 05.03.2011