Colloquium Biometricum 48
2018, 53−63

A METHOD FOR ANALYSIS AND INTERPRETATION OF DATA FROM FIELD AND BREWERY TRIALS

Iwona Mejza[1], Katarzyna Ambroży-Deręgowska[1], Jan Bocianowski[1], Józef Błażewicz[2], Marek Liszewski[3], Kamila Nowosad[4], Dariusz Zalewski[4]

[1] Department of Mathematical and Statistical Methods
Poznań University of Life Sciences, Poland
e-mails: iwona.mejza@up.poznan.pl, katarzyna.ambrozy@up.poznan.pl, jan.bocianowski@up.poznan.pl;
[2] Department of Fermentation and Cereals Technology
Wrocław University of Environmental and Life Sciences, Poland
e-mail: jozef.blazewicz@upwr.edu.pl;
[3] Institute of Agroecology and Plant Production
Wrocław University of Environmental and Life Sciences, Poland
e-mail: marek.liszewski@upwr.edu.pl;
[4] Department of Genetics, Plant Breeding and Seed Production
Wrocław University of Environmental and Life Sciences, Poland
e-mails: kamila.nowosad@upwr.edu.pl, dariusz.zalewski@upwr.edu.pl

Summary

In the paper a method for statistical analysis of data with brewing barley is proposed which allows to use information from designing a field experiment. The experiment was conducted during the growing seasons 2008–2010 in Agricultural Research Station in Pawłowice in Poland with two factors: methods of nitrogen fertilization and spring barley cultivars Sebastian and Mauritia. Collected barley grain for each combination of the factors and replicates was the experimental material deliveries of raw material prepared for processing in a malt-house. In the proposed method a split-block-plot design is applied, which is the combination of a split-block design (for the field experiment) and a split-plot design (for brewery experiment). The main goal of the paper is to analyze of malt in terms among others, extractivity, with full information coming up from the field experiment.

Keywords and phrases: ANOVA, HSD Tukey's test, split-block-plot design, brewing barley, extractivity, malt

Classification AMS 2010: 62K15; 62K99; 62P99

1. Introduction

Empirical studies of malt in terms of various brewery characteristics are usually carried out on grain samples coming from field experiments. The method of obtaining such samples is important in further statistical analysis. Typically, each sample is an average of data from field replicates and applies to each treatment combination. Further statistical analysis performed in the brewery on such samples does not contain information from the field experiment, i.e. the relationship between the cultivating factors (e.g. Mejza *et al*. 2019).

The aim of this paper is to present a method for statistical analysis of data with brewing barley which allows to use information from designing a field experiment. The analysis is based on a mixed linear model of observations obtained from experiment carried out in a split-block-plot design, which is a combination of a split-block design (for the field experiment) and a split-plot design (for the brewery experiment), e.g. Gomez and Gomez (1984), Federer and King (2007), Ambroży and Mejza (2006, 2012). To illustrate the method of analyzing, data from series of experiments described in Chapter 2 were used (see also Mejza *et al*. 2019).

Due to the methodological nature of the paper, the analyses were limited to empirical extractivity only. This trait is one of the most important characteristics determining the malt value, and thus the quality of the final product, which is beer (e.g. Liszewski *et al.* 2012).

2. Material and methods

Barley grain, originating from the field experiment performed in years 2008–2010 in Agricultural Research Station in Pawłowice near Wrocław prepared for processing in a malt-house, was used as the experimental material. The field experiment was conducted in the split-block design (in three blocks) with two factors: methods of nitrogen fertilization with doses of: 0, 20, 40, 60, 60-(40+20I), 60-(40+20II) (kg N·ha^{-1}) and spring barley cultivars: Sebastian and Mauritia.

Nitrogen fertilization was applied preplant and top-dressing (divided doses) in two stages of growth: I – at the end of tillering (BBCH29), II – in the stage of second node (BBCH32). Weather conditions played a big role in the analysis of the series of experiments. They were described in Liszewski *et al.* (2012).

The grain was fractionated by means of Vogel screens as well as deprived of contaminations and damaged grains. After a period of dormancy, grain with fractions of >2.5 mm was used to produce 3-, 4-, 5- and 6-day Pilsner type malts under laboratory conditions (the third factor). From malts congress worts were obtained. Results of analysis were compared with standard values recommended by European Brewing Convention. Evaluation of agrotechnic influence was carried with use of Kolbach index as synthetic factor of protein compounds conversion in grain, malts and worts (see Błażewicz and Liszewski 2003; Błażewicz *et al.* 2007, 2011, 2013; Liszewski *et al.* 2012).

3. Statistical analysis and results

Assume for simplicity that we have three factors: nitrogen fertilization (A) with six levels, cultivars (B) with two levels and germination days (C) with four levels. They were studied, inter alia, their influence on the practical extractivity of malt. The statistical analyses such as analysis of variance, HSD Tukey's test for comparisons pairs of means were performed in the research years separately and over years according to the model of the experiment designed as split-block-plot design on samples of grain coming directly from the field experiments. All calculations were mainly carried out using STATISTICA 13 software package and own procedures.

In Table 1 results of three-year analysis for malt extractivity are presented. The analysis was performed under mixed linear model for observations obtained in the experiment designed in the split-block-plot design with random block effects, fixed year as well as all other factor effects. Results of annual analyses of variance for malt extractivity are shown in Table 2.

In turn, in Tables 3-5 and in the Figures 1 and 2 we described chosen particular analyses based on HSD Tukey's test, presenting grouping of means of the extractivity for some effects in the years of research. Taking into account the results of all statistical analyzes carried out, the most important conclusions can be drawn.

Annual extractivity

The results presented in Table 1 indicate highly significant differences between effects of three years of research in terms of the mean of malt extractivity, regardless of other factors. Can be shown that the lowest malt extractivity mean (73.53% d.m.) was in 2010 (coefficient of variation cv=3.30%). Significantly higher mean extractivity (82.50% d.m.) was in 2009 (cv=0.57%). Both extreme means differ significantly from the malt extractivity mean (78.31% d.m.) in 2008 (cv=2.32%).

Table 1. Results of ANOVA for the years-A×B-C type SBP design

Source of variation	Extractivity (2008-2010)			
	df	mean squares (MS)	*F*-Statistic	*p*
Years (D)	2	2901.31	603.16**	0.0000
Error 1 – Blocks (Years)	6	4.81		
A - *Cultivars*	1	0.18	0.10	0.7625
D × A	2	9.91	5.39*	0.0457
Error 2	6	1.84		
B – *Nitrogen fertilization*	5	1.80	1.36	0.2671
D × B	10	1.33	1.01	0.4579
Error 3	30	1.32		
A × B	5	1.00	1.12	0.3710
D × A × B	10	5.19	5.83**	0.0001
Error 4	30	0.89		
C - *Germination days*	3	72.91	32.70**	0.0000
D × C	6	46.18	20.71**	0.0000
A × C	3	3.25	1.46	0.2265
B × C	15	1.76	0.79	0.6873
D × A × C	6	2.38	1.07	0.3820
D × B × C	30	2.37	1.06	0.3851
A × B × C	15	1.14	0.51	0.9329
D × A × B × C	30	1.17	0.52	0.9815
Error 5	216	2.23		
Total	431			

** - significant at $p<0.01$, * - significant at $p<0.05$

Cultivars extractivity

Both, in the analysis of the series of experiments (Table 1) and in the annual analyses (Table 2) there were no significant differences in terms of the extractivity means between effects of cultivars, regardless of the years of research and other factors. However, there is a significant interaction effects of the cultivars and the years (Table 1). In addition, no significant influence of applied nitrogen fertilization method effects on the tested trait has been indicated, both in the series of years (Table 1) and in each year separately (Table 2).

Table 2. Results of one year ANOVA for the A×B-C type SBP design

Source of variation		Extractivity 2008		Extractivity 2009		Extractivity 2010	
	df	MS	*F*	MS	*F*	MS	*F*
Blocks	2	6.07		1.05		7.31	
A - *Cultivars*	1	9.05	1.99	0.21	3.19	10.73	11.67
Error 1	2	4.53		0.07		0.92	
B - *Nitrogen fertilization*	5	3.29	1.54	0.15	0.84	1.03	0.62
Error 2	10	2.13		0.18		1.65	
A × B	5	8.11	5.61*	0.61	4.07*	2.65	2.48
Error 3	10	1.45		0.15		1.07	
C - *Germination days*	3	24.16	11.38**	1.25	7.87**	139.85	31.74**
A × C	3	6.12	2.88*	0.05	0.29	1.86	0.42
B × C	15	5.29	2.49**	0.33	2.04*	0.89	0.20
A × B × C	15	2.26	1.07	0.14	0.89	1.07	0.24
Error 4	72	2.12		0.16		4.41	
Total	143						

** - significant at $p<0.01$, * - significant at $p<0.05$

Results of ANOVA for the series of years (Table1) also did not show any significant interaction effects between the cultivars and the methods of fertilization. However, there is a highly significant interaction effects between these two factors and the years. This means that climatic conditions, represented by years of research have influenced differently on effects of the combination of the tested cultivars and the methods of nitrogen fertilization (Table 3, Fig. 1). It can be seen that indeed the highest mean of extractivity of the combination of both factors was achieved in 2009, whereas significantly the lowest was in 2010.

Table 3. Means of extractivity for the combination of years, cultivars and nitrogen fertilization

Years	Cultivars	Nitrogen fertilization	Extractivity (% d.m.)
2008	Mauritia	0	78.28[a]
		20	79.35[a]
		40	78.12[a]
		60	77.55[a]
		60(40+20I)	77.71[a]
		60(40+20II)	77.34[a]
	Sebastian	0	79.06[a]
		20	77.76[a]
		40	78.47[a]
		60	79.51[a]
		60(40+20I)	78.58[a]
		60(40+20II)	77.98[a]
2009	Mauritia	0	82.52[b]
		20	82.27[b]
		40	82.58[b]
		60	82.66[b]
		60(40+20I)	82.65[b]
		60(40+20II)	82.55[b]
	Sebastian	0	82.74[b]
		20	82.60[b]
		40	82.40[b]
		60	82.15[b]
		60(40+20I)	82.35[b]
		60(40+20II)	82.53[b]
2010	Mauritia	0	73.57[c]
		20	73.62[c]
		40	74.06[c]
		60	74.03[c]
		60(40+20I)	73.92[c]
		60(40+20II)	73.63[c]
	Sebastian	0	73.50[c]
		20	73.95[c]
		40	72.99[c]
		60	72.53[c]
		60(40+20I)	73.55[c]
		60(40+20II)	73.01[c]

a, b, c – homogeneous groups ($\alpha = 0.01$)

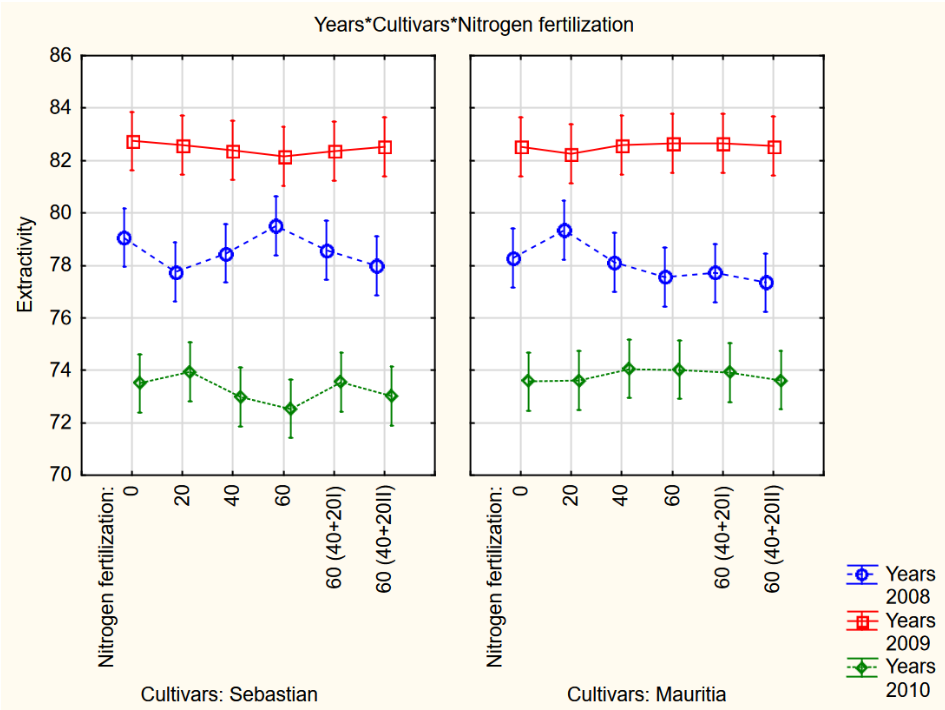

Fig. 1. Interaction chart of the D × A × B.

Bearing in mind the above conclusions, the effects of interaction of the cultivars and the methods of fertilization were checked separately in each year (Table 2). In the analyzes in 2008 and 2009 only there were also a significant interactions effects between cultivars and fertilization (α=0.05), which means that in these years Sebastian and Mauritia cultivars effects did not react equally to the changing methods of nitrogen fertilization. However, in 2010 in the analysis of the field experiment, no general hypotheses concerning main effects of the cultivars, the methods of nitrogen fertilization and connected with them interaction effects were rejected (Table 2).

The Tukey's test (Table 4) showed little differences between means of the combinations of the cultivars and methods of fertilization in 2008 and 2009. Comparing both cultivars in 2008, Mauritia achieved the lowest mean of extractivity (77.34% d.m.) at the divided nitrogen dose of 60 (40+20II) kg·ha^{-1}, while Sebastian obtained the highest average extractivity (79.51% d.m.) at the nitrogen dose 60 kg·ha^{-1}. Both means are in different groups, which means that there is a significant difference between them (α=0.05). In 2009, Tukey's test showed significant differences between the means of extractivity of Mauritia cultivar (82.66% d.m.) at the dose of 60 kg·ha^{-1}, Sebastian cultivar (82.74% d.m.)

at the dose of 0 kg·ha^{-1} (they are in the same group) and Sebastian cultivar (82.15% d.m.) at the dose of 60 kg·ha^{-1} (see also Fig. 2).

Table 4. Means of extractivity for the combination of cultivars and nitrogen fertilization in years

Cultivars	Nitrogen fertilization	Extractivity		
		2008	2009	2010
Mauritia	0	78.28^{ab}	82.52^{ab}	73.57^{a}
	20	79.35^{ab}	82.27^{ab}	73.62^{a}
	40	78.12^{ab}	82.58^{ab}	74.06^{a}
	60	77.55^{ab}	82.66^{a}	74.03^{a}
	60(40+20I)	77.71^{ab}	82.65^{ab}	73.92^{a}
	60(40+20II)	77.34^{a}	82.55^{ab}	73.63^{a}
Sebastian	0	79.06^{ab}	82.74^{a}	73.50^{a}
	20	77.76^{ab}	82.60^{ab}	73.95^{a}
	40	78.47^{ab}	82.40^{ab}	72.99^{a}
	60	79.51^{b}	82.15^{b}	72.53^{a}
	60(40+20I)	78.58^{ab}	82.35^{ab}	73.55^{a}
	60(40+20II)	77.98^{ab}	82.53^{ab}	73.01^{a}

a, b – homogeneous groups (α=0.05)

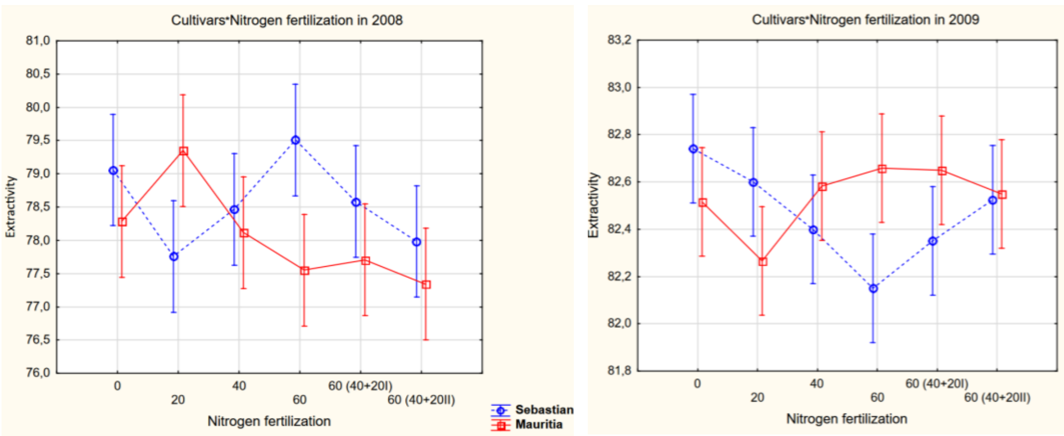

Fig. 2. Interaction charts of the A × B.

Malt extractivity

Both, the results presented in Table 1 and Table 2 indicate that the effect of the germination time of malt turned out to be highly significant for the extractivity, regardless of the years of research and other factors. It can be shown that 3-day malts (regardless of the years and other factors) had a significantly lower mean extractivity compared to other means, while 4-, 5-, 6-day malts did not differ significantly in terms of mean extractivity.

A highly significant interaction of germination time of malt and years (α=0.01) has also been found. For each time of the germination of malt separately, the mean extractivity differed significantly in each year of the study, which is the lowest in 2010 and the highest in 2009 (Table 2). On the other hand, no significant interaction effects of germination time of malt and other factors over the years is (Table 1).

In annual analyses (Table 2), germination time was highly significant (α=0.01) affecting the malt extractivity. The lowest mean extractivity was obtained after three days, and significantly the highest after 6 days of malting germination regardless of other factors (Table 5).

From Table 2 it results that only in 2008 the interaction effects of germination time of malt and cultivars turned out to be significant at the level of α=0.05. The Tukey's test showed a significant difference in mean extractivity for Mauritia cultivar between the 6th day of germination (the highest mean) and the 3rd and 5th day of germination. Table 2 shows also that in the years 2008 and 2009 there was also a significant interaction of fertilization methods and germination time, at the level of α=0.01 and α=0.05, respectively.

Table 5. Means of the extractivity for the germination days of malt in each year separately

Germination days	Extractivity		
	2008	2009	2010
3	77.52^{a}	82.44^{ab}	70.78^{a}
4	78.69^{b}	82.29^{a}	75.27^{c}
5	77.75^{a}	82.52^{ab}	74.58^{bc}
6	79.27^{b}	82.74^{b}	73.49^{b}

a, b, c – homogeneous groups (α = 0.01)

4. Discussion

In current paper we consider situation, when the barley grain prepared for processing in a malt-house was collected for each combination A × B (cultivars × methods of nitrogen fertilization) from each block in the field experiment in the years of research. The statistical analysis takes into account the fact that the field experiment was carried out in the split-block design.

Analyzing the entire three-factor experiment, a linear model of observation from the split-block-plot design experiment was used. It is a variation of the split-block design (for the field experiment) and the split-plot design with respect to the combination of the field factors (for the brewery experiment), see e.g. Gomez and Gomez (1984), Federer and King (2007), Ambroży and Mejza (2002, 2006, 2008).

We can note that the results from this analysis, concerning for the practical extractivity, obtained over years of research and in each year separately are similar to the results obtained in the analysis performed in accordance with the linear model of observation proper for an experiment laid out in a completely randomized design, when the field experiment design is not taken into account (e.g. Błażewicz *et al*., 2011; Liszewski *et al*., 2012; Mejza *et al.,* 2019).

Probably it was influenced by various factors such as: varied climatic conditions in the years of research (e.g. Bertholdsson, 1999; Albrizio *et al.,* 2010) and additionally small diversity of varietal effects (e.g. Benetrix *et al*., 1994) and effects of applied nitrogen fertilization methods (e.g. Błażewicz *et al.,* 2011; Moll *et al.,* 1982; Oscarsson *et al.,* 1998). Weather conditions in the research years described in Liszewski *et al.* (2012) also have played a big role in the analysis of the series of experiments.

References

Albrizio R., Todorovic M., Matic T., MariaStellacci A. (2010). Comparing the interactive effects of water and nitrogen on durum wheat and barley grown in a Mediterranean environment. *Field Crops Research* 115(2), 179-190.

Ambroży K., Mejza I. (2002). Some incomplete split-block-plot designs. *Scientific Papers of Agriculture University of Poznań, Agriculture* 3, 3-12.

Ambroży K., Mejza I. (2006). *Doświadczenia trójczynnikowe z krzyżową i zagnieżdżoną strukturą poziomów czynników*. Wyd. Polskie Towarzystwo Biometryczne i PRODRUK, Poznań.

Ambroży K., Mejza I. (2008). The relative efficiency of split–plot × split–block designs and split–block–plot designs. *Biometrical Letters* 45(1), 29–43.

Ambroży K., Mejza I. (2012). Modeling data from three-factor experiments with split units set up in designs with different block structures (in Polish). *Biul. IHAR* 264, 23-31.

Benetrix F., Sarrafi A., Autran J.-C. (1994). Effects of genotype and nitrogen nutrition on protein aggregates in barley. *Cereal Chemists* 71(1), 75-82.

Bertholdsson N. O. (1999). Characterization of malting barley cultivars with more or less stable grain protein content under varying environmental conditions. *European Journal of Agronomy* 10(1), 1-8.

Błażewicz J., Liszewski M. (2003). Ziarno jęczmienia nagiego odmiany 'Rastik' jako surowiec do produkcji słodów typu pilzneńskiego. *Technologia Alimentaria* 2(1), 63-74.

Błażewicz J., Liszewski M., Zembold-Guła A. (2007). Usability of Bishop formula in evaluation of malting quality of barley grain. *Pol. J. Food Nutr. Sci.* 57 4(A), 37-40.

Błażewicz J., Liszewski M., Zembold-Guła A., Kozłowska K., Szwed Ł. (2013). Liczba Kolbacha jako ważny wskaźnik wartości przetwórczej ziarna jęczmienia browarnego. *Fragmenta Agronomica* 30(3), 46-53.

Błażewicz J., Zembold-Guła A., Żarski J., Dudek S., Kuśmierek-Tomaszewska R. (2011). Wpływ deszczowania i nawożenia azotem w technologii uprawy jęczmienia browarnego na wydajność procesu słodowania – Wstępne wyniki badań. *Infrastructure and Ecology of Rural Areas* 6/2011, 109-117.

Federer W.T., King F. (2007). *Variations on Split Plot and Split Block Experiment Designs*. Wiley. New Jersey.

Gomez K.A., Gomez A.A. (1984). *Statistical procedures for agricultural research*. Wiley, New York.

Liszewski M., Błażewicz J., Zembold-Guła A., Szwed Ł., Kozłowska K. (2012). Wpływ sposobu nawożenia azotem na ekstraktywność słodu jęczmiennego. *Fragmenta Agronomica* 29(1), 93-104.

Mejza I., Ambroży K. (2003). Modelling some experiments carried out in incomplete split-block-plot designs. Proc. of the 18th International Workshop on Statistical Modelling, Leuven, 7-11 July 2003, 293-297.

Mejza I., Ambroży-Deręgowska K., Bocianowski J., Błażewicz J., Liszewski M., Nowosad K. (2019). On modeling and analyzing barley malt data in years. *Biometrical Letters* (in press).

Moll R.H., Kamprath E. J., Jackson W. A. (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agronomy Journal* 74(3), 562-564.

Oscarsson M., Andersson R., Åman P., Olofsson S., Jonsson A. (1998). Effects of cultivar, nitrogen fertilization rate and environment on yield and grain quality of barley. *Journal of the Science of Food and Agriculture* 78(3), 359-366.