Optimization of the malting process of oat (Avena sativa L.) as a raw material for fermented beverages

A. Muñoz-Insa*, M. Gastl, M. Zarnkow and T. Becker

Lehrstuhl für Brau- und Getränketechnologie. Technische Universität München Weihenstephan. Weihenstephaner Steig 20. 85354 Freising. Germany

Abstract

The influence of the three malting parameters (germination time, germination temperature, and degree of steeping) on the quality of two oat (*Avena sativa* L.) cultivars was investigated applying response surface methodology. Each predictor variable was tested at three levels, germination of 6 to 8 days; germination temperatures of 12, 15, or 18°C; and degrees of steeping of 43%, 45% or 47%. All analyses were based on methods described in EBC or MEBAK. The oats used were obtained in 2007 from Nordsaat Saatzucht GmbH, Böhnshausen, Germany. A series of malt quality attributes were evaluated including extract, apparent attenuation limit, Kolbach index, α -aminonitrogen, color, β -glucan, and viscosity. Oat malt was optimized based on barley malt specifications, which are considered appropriate in the beverage production industry for optimal processing. For cv. Ivory, the optimal malting program was achieved after 8 days of germination with moisture content of 46%, and at 17°C. Similarly, cv. Typhon required a germination period of 8 days, a moisture content of 47% and temperature of 16°C. The models showed that the value of R^2 was high and p- value was significant. Therefore it can be said that the model is highly significant. This project demonstrates that oat is an alternative cereal with potential to be used as raw material in malting and brewing purposes.

Additional key words: alternative cereal; malt, response surface methodology; wort quality.

Resumen

Optimización del malteado de avena (Avena sativa L.) como materia prima de bebidas fermentadas

Con la ayuda de la metodología de superficie de respuesta, se estudió la influencia de tres variables del malteado (tiempo y temperatura de germinación, y grado de humedad) en la calidad de dos cultivares de avena (*Avena sativa* L.). Cada variable fue evaluada a tres niveles, de 6 a 8 días de germinación; temperaturas de germinación de 12, 15, o 18°C; y grados de humedad del 43, 45 ó 47%. Todos los análisis estuvieron basados en los métodos descritos por la EBC o por la MEBAK. Los cultivares, obtenidos de Nordsaat Saatzucht GmbH, Böhnshausen, Alemania, fueron de la cosecha del 2007. Se evaluaron una serie de atributos de calidad de la malta incluyendo extracto, límite de atenuación aparente, índice de Kolbach, α -aminonitrógeno, color, β -glucano y viscosidad. La optimización del malteado de la avena se realizó siguiendo las especificaciones de la malta de cebada, consideradas las más apropiadas en la industria de producción de bebidas para un buen procesado. Para el cv. Ivory, el programa de malteado óptimo se alcanzó a los 8 días de germinación con un grado de humedad del 46% y a 17°C. De manera similar, el cv. Typhon necesitó un periodo de germinación de 8 días, un grado de humedad de 47% y una temperatura de 16°C. Los modelos mostraron valores R^2 altos y p significativos. Por lo tanto, se puede decir que el modelo es altamente significativo. Este proyecto muestra que la avena es un cereal con potencial como materia prima para el malteado y la producción de cerveza.

Palabras clave adicionales: calidad del mosto; cereal alternativo; malta; metodología de superficie de respuesta.

^{*} Corresponding author: alicia.munoz@wzw.tum.de

Received: 05-01-10; Accepted: 04-04-11.

Abbreviations used: AAL (apparent attenuation limit), CW (congress wort), EBC (European Brewing Convention), d.m. (dry matter), FAN (free amino nitrogen), MEBAK (Mitteleuropäische Brautechnische Analysenkommission), RSM (response surface methodology).

Introduction

Oat (Avena sativa L.) is currently a very popular cereal in consumer demand, but long before this, oat was the brewing cereal par excellence (Hanke *et al.*, 2005). During the Middle Ages, oat was considered among the most important of the cereals. Over the centuries, oats have lost popularity and have been replaced by other cereals to the point that their brewing properties are almost forgotten. However, oat is starting to regain the popularity once lost due to its excellent healthrelated properties: oat helps reduce the risk of coronary heart disease as well as cholesterol. For people who suffer celiac disease, oats are also of interest. Studies show that most celiac disease patients can tolerate oats (Adams, 2008), but 5% or less reject oat prolamins.

The major components of oats are carbohydrates, particularly starch. Previous studies reported starch concentrations of 536 g kg⁻¹ (Peterson, 1992), when determined on a whole grain basis, and Dendy and Dobraszczyk (2004) reported a starch content of 59-60%, which is low when compared with other cereals such as rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.) or wheat (*Triticum aestivum* L.).

Quantitatively, the next major component of oat is fiber constituting about 32% of the weight (Dendy and Dobraszczyk, 2004). Unlike most other cereals, the husk represents a large fraction of the oat grain. Due to high crude fiber contents in the husk, covered or husked oats have lower digestibility than other cereals (Reiner *et al.*, 1983) and as a result the lowest energy content that can be metabolized (Welch, 1985).

According to Mannerstedt-Fogelfors (Mannerstedt-Fogelfors, 2001), β -glucan content in oats constitutes between 3.0-6.4% of the soluble fractio. When compared to barley, oat has a lower β -glucan concentration, but a higher proportion is soluble. This could be of importance for the beverage industry since more β -glucan is obtained from oat than from barley.

Oats yield low extract levels. As early as the Middle Ages oat was combined with barley to produce easier and cheaper beer (Kunze, 2007). Beer with high oat content tends to stabilize turbidity (Zarnkow *et al.*, 2005). Using oats as brewing material can lead to an astringent and bitter taste. Beers like Oatmeal Stouts contain small amounts of rolled oats (normally no more than 30%). The flavor effect is likely to be insignificant because of the overriding presence of roasted barley (Lewis, 1995).

Lewis (1995) produced an experimental beer containing 30% oats, he obtained beer with unfiltered haze, grainy flavor, and an intensely astringent palatefullness. Taylor *et al.* (1998) brewed beer with alternative cereals and found in malted oat a pronounced toasted, biscuit aroma and palate, combined with a creamy and relatively intense palatefullness in beer. These flavour effects were apparent at less than 10% replacement of barley malt with malted oat (Kaukovirta-Norja *et al.*, 2004).

Other authors have studied the behavior of oat during the malting process. Peterson (1998) investigated the changes in the chemical composition of hull-less and hulled oat genotypes during malting. In another study Klose *et al.* (2009) analyzed the changes in the protein composition that take place during malting. The malting process of oat has been optimized by Hübner *et al.* (2009) with special focus on the fermentation requirements.

The objective of this study was to optimize the malting conditions of *Avena sativa* (from the processing point of view) using response surface methodology (RSM). Oat malt was optimized based on barley malt specifications, which are considered appropriate in the beverage production industry for a good processing. These specifications are viscosity, to assure processing; extract, for further fermentation with lactic acid bacteria; and nutrition of yeast, among others.

Material and methods

Raw material

Two different cultivars of *Avena sativa*, 'Ivory' and 'Typhon', were used in the malting trials. The oat cultivars showed a germinative energy (MEBAK Method 1.4.2.2) of 80.6% and 82.4% for Ivory and Typhon, respectively. Germinative energy should not be less than 95% for barley, however in preliminary tests, all oat cultivars had a germinative energy between 80-90%, which means that the germinative energy obtained for both oat cultivars lies within an acceptable range. Ivory had moisture content of 13.8%, and Typhon of 13.4%. Both cultivars were obtained from Saaten-Union (Nordsaat Saatzucht GmbH, Böhnshausen, Germany), grown and harvested in Northern Germany in 2007.

Malting procedure

Simultaneously, 25 1-kg oat batches were malted (in 1-kg micro malting system of the Brau- und Getränketechnologie chair, Freising, with 60 steeping facilities, climate chambers and kilning system) varying



Figure 1. Face-centered design (double replicates of the factorial and centre points = \bigstar). The 25 malt samples are represented with the different malting parameters of each one.

the malting conditions, germination time, germination temperature and degree of steeping (see Fig. 1). The steeping water was equilibrated by placing it 24 h prior to steeping in the temperate compartments. Steeping was done for 4 h (Ivory) and 3 h (Typhon) on the first day and, if necessary, on the second and third day final moisture contents (43, 45, and 47% degree of steeping) were reached by additional steeping according to preliminary tests. In comparison to common grain, oat has a higher ability to absorb water. Steeping and germination (6, 7, and 8 days) were done at three different temperatures, which were kept constant (12, 15, and 18°C) in temperature controlled chambers with 75% relative humidity. After kilning was done at 50°C during 16 h followed by 1 h at 60°C, 1 h at 70°C, and 5 h rest at 80°C. After kilning, rootlets were removed and the malt was allowed to rest for 7 days before mashing.

Analytical procedures

Analytical procedures were carried out in duplicate (n=2), and the means of all results were calculated. All concentrations are based on dry weight unless mentioned otherwise.

Analyses were performed according to the standard methods of the European Brewing Convention (EBC) (Eerde, 1998), Mitteleuropäische Brau- und Analysenkommision (MEBAK) (Anger, 2006) and American Society of Brewing Chemists (ASBC) (Thorn, 1992) using congress and isotherm 65°C mash programs:

— The malt extract was determined using an alcolyzer (Anton Paar, Graz, Austria) and following MEBAK method 4.1.4.2.2. — The determination of the amount of fermentable sugars so called apparent attenuation limit (AAL) was investigated following MEBAK method 4.1.4.10.

— To determine the effects of malting conditions on proteolytic activities in oat, the Kolbach index (ratio S T⁻¹) was calculated by following MEBAK method 4.1.4.5.3 from the formula:

Kolbach index =
$$\left(\frac{\text{soluble protein \%}}{\text{malt protein \%}}\right) \times 100$$

- α -amino-nitrogen, also referred to as free amino nitrogen (FAN), was determined using a Skalar working station (Skalar, Breda, Netherlands), following MEBAK method 4.1.4.5.5.

— Wort color was investigated using a CADAS 200 spectral photometer (Dr. Lange, Düsseldorf, Germany) according to MEBAK method 4.1.4.2.8.2.

 $-\beta$ -glucan from the wort obtained from congress and isotherm 65°C mash was quantified using a Skalar working station (Skalar, Breda, Netherlands), following MEBAK method 4.1.4.9.2.

— Wort viscosity (congress and isotherm 65°C mash) was measured using a falling ball viscometer, AMVn-Automated Micro Viscometer (Anton Paar, Graz, Austria), at 20°C, from the wort obtained according to MEBAK method 4.1.4.4.1.

Experimental procedure

Response surface methodology, as described by Myers and Montgomery (2002), was applied to determine the impact of three predictor factors (germination time, degree of steeping, and germination temperature) on the quality of oat malt. A face-centered cube design with double replicated factorial and three times the center point was constructed (see Fig. 1) using the software package Design Expert by StatEase (Stat-Ease Corporation, Minneapolis, USA). The objective of this experimental series was to plan the malting series in advance, so that appropriate data will be obtained to evaluate the results by statistical methods to determine optimized malting parameters. This design was chosen because the region of interest and the region of operability are nearly the same. The power at 5% α -level for effect of double standard deviation for this design is clearly above 80%.

Maximum and minimum predictor levels were defined by preliminary malting tests. Each predictor variable was tested at three levels. Figure 1 shows the value range for each component and the combination of these levels used in the face-centered cube. This implies that 25 micromaltings were done by varying the degree of steeping, germination time and germination temperature (independent variables). Germination time was set to 6, 7, and 8 days, degree of steeping to 43, 45, and 47% and germination temperature was of 12, 15, and 18°C. Response variables measuring malt quality attributes (dependent output variables) were extract, apparent attenuation limit, Kolbach index, α -amino-nitrogen, wort color, β -glucan, and viscosity were chosen as response variables. It is known that oat has high lipid content. However this was not the focus of this study therefore lipid evaluation was not done.

After analyzing the characteristic ratios, the calculated statistic models were analyzed and evaluated with the aid of different indexes. The most important statistic indexes are R^2 values, which are stability indexes of the regression model; the *p*-values, which show the significance; F-values, which describe the influence on the model; and the Lack of Fit, which describes the scatter of the data around the formed model. Subsequently, the optimal malting parameters were calculated taking in consideration that high extract level and AAL are desired for posterior fermentation requirements. Also, low viscosity and β -glucan content are required because deleterious effects during the process could be exerted. High molecular weight β -glucan is responsible for difficulties in beer filtration, precipitate formation (Grimm and Krüger, 1994), haze formation in beer and possibly reduced extraction efficiency in the brewing industry. Afterwards malt was produced under these optimal conditions and the optimized malt was analyzed in the same manner as the preliminary trials.

Results

Table 1 shows the measures as well as the calculated minimum and maximum values of the analyzed attributes. Measured and predicted values show good correlation for both cultivars. The main findings of the statistical analyses of the influence of varied germination

 Table 1. Measured and calculated minimum and maximum values of Ivory and Typhon oat malts. The values in parenthesis mean the malting conditions: germination time/degree of steeping/germination temperature

	Ivory				Typhon			
Attributes ¹	Calculated ²		Measured		Calculated ²		Measured	
-	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Extract, %	71.8	75.0	72.5	75.0	70.5	72.7	70.9	72.4
	(6/47/12) ²	(8/47/12)	(6/47/12)	(8/47/17)	(6/47/12)	(8/47/12)	(6/45/18)	(8/43/16)
AAL, %	49.8	83.8	54.2	83.7	47.3	77.1	49.8	77.1
	(6/47/12)	(8/47/18)	(6/47/12)	(7/47/18)	(6/43/12)	(8/47/18)	(6/43/12)	(8/47/16)
Kolbach index, %	26.1	41.0	26.7	40.0	25.3	34.4	26.0	34.2
	(6/43/12)	(8/47/18)	(6/45/12)	(8/47/18)	(6/47/12)	(8/47/18)	(6/47/12)	(8/47/17)
FAN, mg/100 mL	97	154	103	154	90	219	90	153
	(6/43/12)	(8/47/18)	(6/43/12)	(8/47/18)	(6/43/12)	(6/47/18)	(6/43/12)	(8/47/18)
Color, EBC	6.9	28.8	6.1	23.0	7.1	13.7	6.9	13.4
	(7/47/15)	(6/47/12)	(7/46/17)	(6/43/12)	(7/47/15)	(6/43/12)	(8/46/17)	(6/43/12)
β -glucan (CW), mg L ⁻¹	16	746	11	680	56	1,214	51	1,053
	(8/47/18)	(6/43/12)	(8/47/17)	(6/47/12)	(6/47/18)	(6/43/12)	(7/45/17)	(6/43/12)
β -glucan (65°C), mg L ⁻¹	138	847	103	781	265	1,022	268	1,030)
	(8/44/18)	(6/43/12)	(8/47/18)	(6/47/12)	(7/47/18)	(6/43/12)	(8/47/18)	(6/43/12)
Viscosity (CW), mPa×s	1.467	1.589	1.471	1.577	1.484	1.670	1.484	1.627
	(7/47/15)	(6/47/12)	(6/47/18)	(6/47/12)	(7/45/15)	(6/43/12)	(8/45/15)	(6/43/12)
Viscosity (65°C), mPa×s	1.493	1.685	1.451	1.715	1.501	1.801	1.474	1.869
	(8/47/16)	(6/47/12)	(8/47/18)	(6/47/12)	(7/47/17)	(6/43/12)	(8/45/15)	(6/43/12)

¹ AAL: apparent attenuation limit. FAN: free amino nitrogen. CW: congress wort. ² The calculated values are rounded since time patterns with decimals especially the germination time would not be practicable; in addition the calculated values are determined by topping or minimizing this value without considering the influence of other features.

Parameters	Extract	AAL ¹	Kolbach index	FAN ²	Wort color	β-glucan (CW) ³	β-glucan (65°C)	Viscosity (CW)	Viscosity (65°C)
Ivory									
Germination time	< 0.0001	< 0.0001	< 0.0001	0.0002	0.0006	< 0.0001	< 0.0001	> 0.05	< 0.0001
Degree of steeping	0.0486	0.0387	< 0.0001	< 0.0001	0.0054	> 0.05	0.006	> 0.05	>0.05
Germination temperature	> 0.05	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Interactive	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Lack of fit	0.1149	0.3781	0.9389	0.0720	0.2136	0.1542	0.1444	0.3710	0.1559
Model fit R^2	0.8345	0.9386	0.9249	0.8940	0.8582	0.9727	0.9634	0.9105	0.9205
Typhon									
Germination time	0.0082	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001	< 0.0001	0.0012	< 0.0001
Degree of steeping	0.1928	0.0003	< 0.0001	0.0003	0.0024	0.0235	< 0.0001	> 0.05	0.0427
Germination temperature	0.4159	< 0.0001	< 0.0001	0.1590	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Interactive	0.0122	< 0.0001	< 0.0001	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Lack of fit	0.1168	0.1425	0.5279	0.7350	0.0304	0.1241	0.0306	0.5850	0.5028
Model fit R^2	0.7341	0.9493	0.9021	0.8359	0.9330	0.9512	0.9883	0.8892	0.9536

Table 2. Model fits (R^2) as well as *p*-values for the influence of germination parameters of the mathematical models

¹ AAL: apparent attenuation limit. ² FAN: free amino nitrogen. ³ CW: congress wort.

parameters, as well as the respective model fits, are displayed in Table 2. If *p*-values are lower than 0.05 it means that the germination parameter had a significant effect on the response. The model showed high R^2 values and p < 0.05. Since most of the values were significant, the model was significant. The formulas of the behavior of the different attributes can be found in Table 3. All the attributes showed a quadratic model.

The criteria to optimize the malt were to enhance the extract level, and the apparent attenuation limit, and to minimize the β -glucan and viscosity by congress and isothermal mash. Based on the criteria and the obtained data, the optimal malting program for Ivory was achieved at 8 d germination time, 46% degree of steeping, and 17°C steeping and germination temperature and for Typhon 8 d germination time, 45% degree of steeping, and 16°C temperature. All parameters had a strong impact on the measured attributes. The malting behavior was similar for both cultivars and the optimal malting regimes slightly deviate from each other.

Amylolytic specifications

Extract

Compared to barley, which has a potential extract higher than 80%, both oat cultivars had lower extract yield. For Ivory, the potential extract ranged between 72 and 75% d.m. The calculated model reached its maximum after 8 days of germination at 47% moisture and a temperature of 18°C (see Fig. 2a). It is not possible to determine if the maximum extract is reached within this model. Longer germination time and higher degree of steeping could lead to a higher extract yield. According to the values, short germination periods and low moisture contents yields little extract. There is evidence that the extract level is directly proportional to the germination time and degree of steeping. On the other hand, extract content is directly proportional to germination temperature by low degree of steeping (figures not shown).

Like Ivory, the potential extract for Typhon malts varied at low levels of 67.6 and 72.7%. The maximum, in the investigated range, was achieved after 8 days of germination at 47% moisture (like Ivory) and a temperature of 15°C.

Apparent attenuation limit

Both cultivars had maximum AAL calculated values under 7.5/47%/18°C. With increasing time, temperature and moisture content, AAL increased. Ivory AAL values were recorded as being between 49.8 and 83.9%. Figure 2b shows the impact of germination temperature and time on AAL with a degree of steeping of 47%. Between 6.5 and 8 germination days the AAL decreased slightly.

Typhon AAL values fell in the range of 47.3 and 77.1%. AAL noticeably increased with the germination temperature (see Fig. 3).

Ivory	
Extract	$= -105.41822 - (2.93460*A) + (9.07638*B) - (2.60353*C) + (0.10532*A*B) - (0.043133*A*C) + (0.079900*B*C) - (0.033786*A^2) - (0.12095*B^2) - (0.023198*C^2)$
Sqrt [β-Glucan (CW)	$ [] = +630.33823 - (1,302.930^{*}A) - (18.51294^{*}B) - (5.54946^{*}C) + (0.16264^{*}A^{*}B) + (0.25961^{*}A^{*}C) - (0.086672^{*}B^{*}C) + (1.53656^{*}A^{2}) + (0.20621^{*}B^{2}) + (0.18624^{*}C^{2}) $
β-Glucan (65°C)	$= +24,960.17538 - (1,292.88018*A) - (710.43535*B) - (364.90947*C) - (3.64062*A*B) + (19.13542*A*C) + (0.47396*B*C) + (71.99281*A^2) + (7.87320*B^2) + (5.13809*C^2)$
Wort color	$= +231.01898 - (53.92166*A) + (1.76746*B) - (4.25228*C) + (0.17656*A*B) + (0.14479*A*C) - (0.17552*B*C) + (2.93921*A^2) - (0.015198*B^2) + (0.32936*C^2)$
Kolbach index	$= +650.66903 - (6.13448*A) - (27.18335*B) - (2.42866*C) + (0.44844*A*B) - (0.038542*A*C) + (0.19740*B*C) - (0.79586*A^2) + (0.24478*B^2) - (0.18565*C^2)$
FAN	$= +1,255.73513 + (141.70237*A) - (70.46102*B) - (26.94525*C) - (0.21685*A*B) - (0.37627*A*C) + (0.91603*B*C) - (8.43192*A^2) + (0.70452*B^2) - (0.29799*C^2)$
Viscosity (CW)	$= +3.93089 - (0.40758*A) - (0.011525*B) - (0.095073*C) + (2.98438E-3*A*B) + (5.42708E-3*A*C) - (9.01042E-4*B*C) + (0.013275*A^2) + (6.87950E-5*B^2) + (2.91946E-3*C^2)$
Viscosity (65°C)	$= +8.13837 - (0.26413*A) - (0.20281*B) - (0.11290*C) - (2.68750E-3*A*B) + (8.62500E-3*A*C) - (1.64583E-3*B*C) + (0.015405*A^2) + (2.72617E-3*B^2) + (3.51719E-3*C^2)$
AAL	$= +48.42942 + (81.89108*A) - (13.79690*B) + (0.11375*C) - (0.42344*A*B) - (0.57396*A*C) + (0.50156*B*C) - (3.53129*A^2) + (0.11093*B^2) - (0.54514*C^2)$
Typhon	
Extract	$= +211.73924 + (2.29438*A) - (7.13375*B) + (1.72146*C) + (0.028372*A*B) + (0.077916*A*C) - (2.16557E-3*B*C) - (0.31809*A^2) + (0.076727*B^2) - (0.071455*C^2)$
β-Glucan (CW)	$= +1,060.61797 - (39.03631*A) - (35.06267*B) - (9.72409*C) + (0.27057*A*B) + (0.84544*A*C) - (0.075624*B*C) + (0.68359*A^2) + (0.37271*B^2) + (0.17518*C^2)$
β-Glucan (65°C)	$= +23,609.09599 - (715.17860*A) - (577.41013*B) - (785.11995*C) + (1.30000*A*B) + (24.38125*A*C) + (2.33437*B*C) + (12.26241*A^2) + (5.45935*B^2) + (14.86527*C^2)$
Wort color	$= +112.18806 - (9.54188*A) - (0.25712*B) - (6.90262*C) + (0.083281*A*B) + (0.28844*A*C) - (0.029740*B*C) + (0.025468*A^2) - (1.75809E-3*B^2) + (0.19255*C^2)$
Kolbach index	$= +128.64747 - (26.63566*A) - (1.70802*B) + (1.60252*C) + (0.47969*A*B) + (0.067708*A*C) + (0.090104*B*C) + (0.36871*A^2) - (0.026574*B^2) - (0.18959*C^2)$
FAN	$= -185.68673 - (209.30969*A) + (19.99983*B) + (55.27989*C) + (3.55588*A*B) - (0.27525*A*C) - (0.22696*B*C) + (4.65641*A^2) - (0.39840*B^2) - (1.39929*C^2)$
Viscosity (CW)	$= +7.82721 - (0.23745*A) - (0.18068*B) - (0.16481*C) + (3.28125E-4*A*B) + (0.012073*A*C) - (8.59375E-4*B*C) + (1.62770E-3*A^2) + (2.09442E-3*B^2) + (3.68086E-3*C^2)$
Viscosity (65°C)	$= +7.98967 - (0.59455*A) - (0.017540*B) - (0.45839*C) + (9.21875E-4*A*B) + (0.021031*A*C) + (1.01562E-3*B*C) + (0.012471*A^2) - (1.32194E-4*B^2) + (8.02458E-3*C^2)$
AAL	$= -5.24822 + (0.30055*A) - (4.09256*B) + (13.89585*C) + (0.43594*A*B) - (0.37812*A*C) + (0.20365*B*C) - (0.68363*A^2) - (8.40827E-3*B^2) - (0.60929*C^2)$

Table 3. Predicted and measured amylolytic, proteolytic and cytolytic attributes for the optimized malting regimes

AAL: apparent attenuation limit. FAN: free amino nitrogen. CW: congress wort. A: germination time. B: degree of steeping. C: germination temperature.

Proteolytic specifications

Kolbach index

The Kolbach index increased with increasing the germination parameters. Germination time sho-

wed the strongest correlation with the Kolbach index.

The Kolbach index of Ivory ranged from 26.1 to 40.1% and that of Typhon from 26.5 to 34%. As it can be seen, Typhon had lower Kolbach indexes than Ivory.



Figure 2. a) Influence of moisture content and germination time on extract with 18°C of germination temperature on Ivory. b) Influence of germination temperature and germination time on apparent attenuation limit (AAL) with 47% moisture content during germination on Ivory.

Free amino nitrogen

Ivory FAN values were recorded between 96.5 and 154 mg/100 g. Germination time did not have a significant influence on the FAN content. However, FAN content was directly related to temperature and degree of steeping since high temperatures (18°C) and moisture contents (47%) yielded more FAN content.

Typhon had considerably higher FAN values (90-219 mg/100 g) than Ivory. Unlike Ivory, Typhon FAN was directly proportional to germination time and temperature.

Wort color

The color of Ivory was recorded as being between 6.9 and 26 EBC units. Color decreased with eleva-



Figure 3. Influence of germination temperature and moisture content on β -glucan content of congress wort (CW) with 6 days of germination on Typhon.

ted germination temperature and time. The moisture content did not reveal a significant impact on the color. Some of the samples with the highest color presented also turbidity. Wort color of Typhon was found in the range of 7.2 and 14 EBC units. Again, some of the samples with the highest values presented turbidity.

The results of this study revealed that the germination time and temperature have a significant impact on the wort color. The longer the germination period and the higher the temperature, the lower the color. The color was independent of the moisture content.

Cytolytic specifications

β -glucan by congress mash

Both cultivars have an immense potential in β -glucan (Ivory: 746 mg L⁻¹ and Typhon: 1,214 mg L⁻¹) with low temperatures and short germination periods. Variation of the malting regime is a proper approach to influence the β -glucan content. Minimum β -glucan content was achieved in both cultivars at the highest temperature (18°C) and longest germination time (8 d). Both cultivars showed a similar behavior, time and temperature were inversely correlated to β -glucan content (see Fig. 4a); however, the β -glucan content of Typhon was always higher than that of Ivory. Moisture content seemed to have no influence on the β -glucan content of Ivory. However, decreasing the degree of steeping of Typhon more β -glucan would be yielded.



Figure 4. Influence of germination temperature and germination time during germination on Ivory on: a) β -glucan content of congress wort (CW) with 45% moisture content; b) β -glucan content (65°C) with 43% moisture content.

β -glucan by isothermal mash 65°C

By isothermal mashing, Ivory showed the same behavior as in congress mashing (see Fig. 4b). Additionally, Ivory recorded higher values by isotherm mashing 65° C than by congress mashing (103-867 mg L⁻¹).

Isothermal mashing seemed to have similar effects on the β -glucan content of Typhon (268-1,330.5 mg L⁻¹) as congress mashing does. Also the β -glucan content of Typhon were higher by isothermal mashing 65°C than congress mashing which could be possible due to the presence of a β -glucan solubilase. Similar to β -glucan content by congress mashing, the β -glucan content of Typhon was always higher than that of Ivory.

Viscosity by congress mash

In the present study viscosity showed the same behavior as β -glucan content. The influence of the germination temperature was also more significant than the

germination time. The degree of steeping played a rather minor role.

The viscosity values of Ivory (8.6% wort) measured ranged from 1.467 to 1.589 mPa \times s. Figure 5a shows how viscosity was inversely proportional to germination temperature. At low temperatures, the viscosity increased with increasing moisture content, whereas at high temperatures the viscosity decreased with increasing the moisture content.

The viscosity of Typhon (8.6% wort) was found to be in the range of 1.484 to 1.670 mPa \times s. As for Ivory, Typhon showed an inverse relation between viscosity and germination time and temperature (but in this case for all the temperature range), but the moisture content simply showed a slight correlation.

Viscosity by isothermal mash at 65°C

Most of the values obtained in both oat cultivars were extremely high when compared to barley (maxi-



Figure 5. Influence of germination time and germination temperature on: a) viscosity of congress wort (CW) with 47% moisture content on Ivory; b) viscosity (65°C) with 43% of moisture content during germination on Typhon.

b)

b)

mal viscosity 1.6 mPa \times s). The viscosity of Ivory ranged between 1.451 and 1.715 mPa \times s. Increased viscosity was achieved by lower germination temperature and time, and higher moisture content.

The viscosity of Typhon was found between 1.475 and $1.870 \text{ mPa} \times \text{s}$. As Figure 5b shows, at lower temperatures, with increasing germination time, viscosity values were lower. Viscosity was not influenced by the moisture content.

As by β -glucan, the viscosity by isothermal mashing 65°C was higher for most of the samples than that by congress mashing.

Optimal malt

The predicted values for the quality attributes were 74.9% extract, 80.7% AAL, 38.0% Kolbach index, 149 mg/100 mL FAN, 6.9 EBC units color, 12 mg L⁻¹ β -glucan by congress mash, 107 mg L⁻¹ β -glucan by isotherm 65°C mash, 1.494 mPa×s viscosity by congress mash, and 1.447 mPa×s viscosity by isotherm 65°C mash for Ivory, as seen in Table 4. For Typhon, the predicted responses were 71.6% extract, 77.1% AAL, 33.8% Kolbach index, 151 mg 100 mL⁻¹ FAN, 14.2 EBC units color, 72 mg L⁻¹ β -glucan by congress mash, 240 mg L⁻¹ β -glucan by isotherm 65°C mash, 1.493 mPa×s viscosity by congress mash, and 1.486 mPa×s viscosity by isotherm 65°C mash, as seen in Table 4. The measured values slightly differ from the calculated ones. For both cultivars, temperature and time had the widest influence on the evaluated malt attributes. The degree of steeping seems to have no influence on the malt quality, except for the extract level of Typhon where the moisture content had the broadest influence on the model.

Discussion

In previous research (Phiarais *et al.*, 2006; Zarnkow *et al.*, 2007a,b, 2008, 2009), RSM was used to evaluate the effect of different parameters on multiple product attributes with other cereals such as Proso millet (*Panicum milaceum* L.), quinoa (*Chenopodium quinoa* L.), teff [*Eragotis teff* (Zucc.) Trotter] and triticale (×*Triticosecale Wittmack*). In the studies conducted, the obtained values by experimentation agreed with the predicted RSM values as the values of the oat malts did (see Table 4).

Based on the results of these studies and using the software package Design Expert, it was concluded that the optimal malt for Ivory is achieved after 8 days of germination with moisture content of 46%, and at 17°C. Similarly, Typhon required an extended germination period of 8 days, a slightly higher moisture content (47%) and lower temperature (16°C).

The models showed high R^2 values and almost all the *p*-values were lower than 0.05 which means that the germination parameters had a significant effect on the response. Since most of the values were significant, the model was significant.

	Iv	ory	Typhon		
Attributes	Predicted	Measured	Predicted	Measured	
Germination time, d		8	8		
Moisture, %	۷	16	45		
Germination temperature, °C	1	17		16	
Extract, % dm	74.9	74.8	72.0	71.5	
AAL, %	80.5	81.8	77.1	77.1	
Kolbach index, %	37.1	38.5	30.9	33.0	
FAN, mg/100 g	149	154	151	157	
Wort color, EBC	6.9	7.8	7.1	10.2	
β -glucan (CW), mg L ⁻¹	8	16	56	66	
β -glucan (65°C), mg L ⁻¹	129	113	291	268	
Viscosity (CW), mPa \times s	1.488	1.494	1.496	1.520	
Vicosity (65°C), mPa×s	1.445	1.451	1.462	1.475	

 Table 4. Predicted and measured amylolytic, proteolytic and cytolytic attributes for the optimized malting regimes

AAL: apparent attenuation limit. FAN: free amino nitrogen. CW: congress wort.

Amylolytic specifications

Extract

The extract yield is one of the most important malt quality attributes (Narziß, 1992). Fermentable extract generated during mashing is essential for a successful fermentation. Extract yield indicates the amount of solids that is in solution and the volume of liquid in which the solids are dispersed. Specific gravity is used among others, as a measure to express the concentration of wort. The higher the specific gravity, the more concentrated the solution of wort solids. However, different countries use different scales. European brewers use Plato degrees, which gives the specific gravities in air at 20°C/20°C related to the cane sugar weight percentages (Briggs et al., 2004). The extract allows drawing direct conclusions about the content of mostly soluble sugar substances. If there is a normal amylolytic enzymatic activity, the potential extract (maximum extract level obtained during mashing) indicates the sugar, protein and soluble β -glucan content and therefore the later alcohol percentage (Zarnkow et al., 2007a). Increased proteolytic solution increases the starch availability and could also produce, under given circumstances, higher extract values. Briggs et al. (2004) establish that by mashing under favored conditions for the enzyme activity, some 53-68% more of the malt solids are brought into solutions as the result of enzyme-catalyzed reaction.

The lower potential extract yield of both oat cultivars could be explained by the higher insoluble husk fraction of oat, which is approximately 30% (Pomeranz, 1987), whereas barley malt was approximately 18% (Zarnkow *et al.*, 2007b). Taylor *et al.* (1998) among others, who brewed with other cereals than barley, recorded relatively low carbohydrate content in oat, which was reflected in low potential extract.

Apparent attenuation limit

The AAL values obtained of both oat cultivars are low. It has to be noticed that low attenuation grades give rise to fatty tasting beers with bad drinkability and corresponding microbiological risk (Zarnkow *et al.*, 2009). AAL depends also on the extract yield. Oat is known to have lower extract yields than other cereals, and therefore, lower AAL.

Proteolytic specifications

Kolbach index

Kolbach index represents the amount of total nitrogen present in the malt that is soluble. The amounts of soluble nitrogen depend on the malt and the way it is mashed (Briggs et al., 2004). During germination, in the cell, storage proteins are hydrolyzed (reduction) and proteins are synthesized (assembly). The correlation between the hydrolysis of storage proteins and the synthesis of hydrolyzed proteins needed could be displaced. If a balance between reduction and assembly is reached, the Kolbach index does not alter during germination despite progressive proteolysis in the endosperm. In barley, lower germination temperatures can influence this balance by weakening the development of germ buds. A balance for barley is reached at approximately 15°C (degree of steeping 45%) (Zarnkow et al., 2007b). Variation of the protein content in the grain (available proteins) and proteolytic potential could vary among years and therefore also the Kolbach index. However it is mainly a characteristic of the cultivar. In the present study, for both oat cultivars, the balance was reached at 16.5°C.

Compared with barley and wheat, which have a Kolbach index of about 40.7 and 37.4%, respectively, both oat cultivars had low Kolbach indexes.

Warmer malting conditions lead to a displacement towards root and acrospire growth, which in turn leads to a lower Kolbach index. For Ivory, the maximum Kolbach index was reached at about 16.5°C (inflection point) which is also the temperature where the maximum soluble nitrogen was obtained. At high moisture contents, more nitrogen was solubilized as a result the Kolbach index increases.

At about 16-16.5°C the Kolbach index of Typhon presented a critical point (maximum) for all moisture contents. This is also the temperature at which the maximum soluble nitrogen was obtained.

Free amino nitrogen

The amount of low molecular weight nitrogen substances is called free amino nitrogen (FAN). Low molecular nitrogen compounds, especially amino acids in wort, influence the fermentation performance and the development of fermentation by-products (Narziß, 1992). In beer production, if the FAN concentration is high, the yeast has more nutrients available and alcohol production is higher. Because of this, FAN values must be sufficiently high to ensure that lack of nitrogenous yeast nutrients does not limit fermentation (Briggs *et al.*, 2004). Low molecular weight nitrogen compounds play a central role in the color and flavor development of malt following the Strecker reaction (Narziß, 1992). Also a high FAN concentration can develop undesired off-flavors due to the Maillard reactions.

Appropriate barley malt specifications suggest FAN content, to assure yeast nutrition, between 120 and 150 mg/100 g. According to this specification both oat cultivars presented in some cases lower levels than recommended and Typhon, in some cases, higher levels than required. Samples with lower levels could have limited fermentation, and samples with higher levels could develop undesired off-flavors.

Hübner *et al.* (2009) studied the influence of malting on oat FAN concentration. The suggested model indicated that germination temperature had a significant impact increasing the FAN concentration by increasing the germination temperature. The same behavior is found for Ivory and Typhon.

Wort color

Wort color is a consequence of the Maillard reaction where a sugar reacts with an amino acid. Depending on the step of the Maillard reaction, uncolored or colored products are formed. The color of malt and beer are mostly attributed to melanoidins, product of the final phase of the Maillard reaction (Siefker and Pollock, 1956; Nursten, 2005; Zarnkow *et al.*, 2007b). The color values obtained from Ivory are extremely high when compared to barley [2.9 EBC according to Back (2005)]. The values obtained from Tyhpon are lower than those for Ivory however they are still considerably high when compared to barley.

The reason of minimum color values at longer germination periods and higher temperatures is that low molecular weight substances are used during germination for assembling leaves and roots. Therefore, the longer the germination period, the lower the content of low molecular weight substances and the lower the color values.

According to Narziß (1992) low molecular weight nitrogen compounds play a central role in the color and flavor development of malt following the Strecker reaction. However wort color did not show the same behavior as FAN content did. A possible explanation could be that more substances than low molecular weight proteins are available for the Maillard reaction.

Cytolytic specifications

β -glucan by congress mash

 β -glucan is a linear unbranched polysaccharide composed of β -D-glucopiranose units joined by single $\beta(1-3)$ links with no single $\beta(1-4)$ or double $\beta(1-3)$ links leading to a structure of predominantly $\beta(1-3)$ linked cellotriosyl and cellotetraosyl units (Wood, 2001) in the molar ratio 2.1:1 (Welch, 1995). β-glucan has long been known to exert deleterious effects in malting and brewing (Altunkaya et al., 2001). During malting and mashing β -glucan is enzymatically cracked and solved (Grimm and Krüger, 1994). Pentosans when combined with β -glucan form hot water soluble gums. These create a highly viscous solution, as a consequence the rate of wort filtration is diminished (Altunkaya et al., 2001) and turbidity problems are encountered. Also, high molecular weight β -glucan is responsible for difficulties in beer filtration, precipitate formation (Grimm and Krüger, 1994), haze formation in beer and possibly reduced extraction efficiency in the brewing industry (Altunkaya et al., 2001). Due to the negative effects on lautering and filtration; minimum β -glucan content is desired.

Wilhelmson *et al.* (2001) recorded low β -glucan content at longer germination periods as Ivory and Typhon malt samples did.

Appropriate barley malt specifications suggest β -glucan content, to assure lautern and filtration, lower than 300 mg L⁻¹. However, according to Jin *et al.* (2004), as long as the β -glucan concentration do not exceed 800 mg L⁻¹, β -glucan is not the main component responsible for high viscosity in wort. Typhon had higher β -glucan values (56.5-1,213.0 mg L⁻¹) than Ivory (16-746 mg L⁻¹) and some of the samples exceeded the concentration, therefore, for Typhon, filtration problems could be expected.

β -glucan by isothermal mash 65°C

 β -glucan by isothermal mashing was measured to monitor the influence of β -glucan solubilase in oat. β -glucan solubilase is a cytolytic enzyme present in cereal endosperm which converts insoluble cell walls to soluble β -glucans. This heat stable enzyme shows optimum activity in barley at 60°C and is still active at 73°C (Kühbeck *et al.*, 2005). Once β -glucans are soluble, it would be further degraded mainly by β -glucanases (Klein, 2009) which shows optimum activity between 40 and 50°C (depending on the enzyme) and are inactivated at 55°C (heat sensitive) (Narziß, 1999).

Due to the difference between optimum and inactivation temperature between β -glucan solubilase and β -glucanases of barley, by measuring the β -glucan content by isothermal mash at 65°C, the presence of β -glucan solubilase could be demonstrated. According to the temperatures of barley enzymes, if no β -glucan solubilase is present the β -glucan content by isothermal mashing should be the same as by congress mashing.

The higher β -glucan values of Ivory and Typhon by isotherm mashing 65°C than by congress mashing besides the same behavior by both mashing programs could be explained by the presence of a β -glucan solubilase that solubilizes the β -glucan present in the cell walls. Even so, it is worth noting that no information about a β -glucan solubilase in oat was found in the literature.

Peterson (1998) analyzed the effect of malting on different samples of hull-less and hulled oat genotypes. The malting regime was $16^{\circ}C/45^{\circ}/6d$. The β -glucan content obtained ranged at low levels between 30 and 390 mg L⁻¹. Degree of steeping of 45% leads also to Ivory and Typhon to a minimum point for all the germination temperatures and times. But both oat cultivars showed higher β -glucan content than the oat cultivars malted by Peterson (1998).

Viscosity by congress mash

Wort and beer viscosity is influenced by the macromolecules present, Sadosky *et al.* (2002) found that arabinoxylan, β -glucans and dextrins all increased the viscosity of model solutions with the dextrins having the largest effect (Briggs *et al.*, 2004). Generally a low viscosity is considered advantageous for the filtration process.

Taylor *et al.* (1998) found that the only partially ground oat grains do not achieve full starch breakdown and thus caused considerable increase in wort viscosity and lauter runoff rate.

Viscosity showed the same behavior as β -glucan content did, therefore based on these results β -glucan

could be the main component which increases viscosity. Also the viscosity values of Typhon are slightly higher than those of Ivory, which confirms that β -glucan is the main substance responsible for the high viscosity values.

When compared to barley, oat has a lower β -glucan concentration, but a higher proportion is soluble.

Viscosity by isothermal mash at $65^{\circ}C$

According to Narziß *et al.* (1999), the optimum temperature of β -glucan solubilase for barley is 65°C. By isotherma 65°C (see β -glucan by isothermal mashing 65°C) mashing the activity of β -glucan solubilase in barley is measured. There was no information in the literature about the presence of β -glucan solubilase in oat, but if the viscosity at 65°C is higher than the viscosity by congress mash, the presence of a β -glucan solubilase can be considered as described before.

Viscosity values obtained in both oat cultivars were extremely high when compared to barley (maximal viscosity 1.6 mPa×s). As explained before, the main substance responsible for the high viscosity values is β -glucan. When compared to barley, oat has a lower β -glucan concentration, but a higher proportion is soluble giving higher viscosity values than barley malt.

Hübner *et al.* (2009) reported highest values in mashes prepared from malts germinated for short times. However, no minimum content was in malt germinated for a minimum time at low temperature. The same behavior was found for Ivory and Typhon.

The higher viscosity values found in both oat cultivars by isothermal mashing 65°C confirm, as β -glucan content does, the presence of a β -glucan solubilase that solubilizes the β -glucan present in the cell walls.

Optimal malt

The optimal malting regimes for both cultivars (Ivory: 8 d/46%/17°C; Typhon 8 d/45%/16°C) slightly deviate from each other, as it can be seen in Table 4. Comparing both oat cultivars Ivory had better malt attributes than Typhon. For example β -glucan content and viscosity values were lower for Ivory than for Typhon which would probably lead to better filterability and lautering.

Both optimal malts have low extract content compare with barley malt standard values. The Kolbach index and the AAL of Ivory optimal malt are appropriate for good quality malt, but those of Typhon are lower than desirable. However the β -glucan and viscosity values of Typhon are higher than those of Ivory, both malts attributes are in the range to assure good processing in further beverage production.

It is worth noting that the optimal calculated germination time and germination temperature (in the case of Ivory) are on the limits of the analyzed range. Probably an optimal malting point would exist outside this range (>8 d, > 17°C) but when malting under higher temperatures and degree of steeping, the seeds could get moldy or drown.

In conclusion, this study shows that oat is a cereal with raw material potential for malting purposes. Additionally, it is possible to use this cereal in «normal» barley malt houses under commonly used malting conditions for a further innovative beverage production. Therefore it is possible to produce oat malt which can provide a basis for alternative food, beverages, and beer production. This would also help expand the food uses of oat for human consumption and nutrition.

Acknowledgment

The authors would like to thank Saaten-Union, D-30916 Isernhagen HB for their assistance and Dr. Steffen Beuch, Nordsaat Saatzucht, Zuchtstation Granskevitz, D-18569 Granskevitz for supplying both oat cultivars.

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