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Triticale Malt (x*Triticosecale* Wittmack) a Raw Material for Brewing – Using Response Surface Methodology to Optimise Malting Conditions

Response surface methodology was used to investigate the influence of three malting parameters, degree of steeping, germination time and temperature, on the quality of triticale malt. Each predictor variable was tested at three levels. Germination times were set to 5, 6, and 7 d, degrees of steeping to 42, 45, and 48 %, and germination temperatures were 15, 18 and 21 °C. The initial kilning temperature for all malts was set to 50 °C and gradually increased to 80 °C. A series of malt quality attributes were investigated including extract, viscosity, arabinoxylan, apparent attenuation limit, α- and β-amylase activity, limit dextrinase activity, α-amino nitrogen (FAN), Kolbach index, soluble N and dimethyl sulfide precursor (DMS-P). The optimum malting programme was determined with 5 d germination time, 45 % degree of steeping, 15 °C steeping and germination temperature. The amylolytic and proteolytic activity obtained, were 84.8 % extract, 78.3 % AAL, 264 U/kg limit dextrinase activity, 1055 U/g β-amylase activity, 254 U/g α-amylase activity, 155 mg/L FAN, 1.7 mg/kg dimethyl sulfide precursor (DMS-P) and 2.305 mPa × s viscosity.

Descriptors: triticale, malt, response surface methodology, alternative cereal

1 Introduction

In view of qualitative and quantitative unsatisfactory barley crops (e.g. 2006) and high raw material prices, alternatives to the traditionally used barley are investigated. Triticale can be considered as an alternative crop, furthermore due to its cereal qualities, it could be used as raw material for new types of beverages.

Triticale (x*Triticosecale* Wittmack) is the first man-made cereal. In 1875 the Scottish botanist *Wilson* [1] described infertile crosses between wheat and rye from which an intergeneric hybrid was obtained. The history of cultivation of triticale started in 1888 when *Rimpau* [2], a German breeder, found a fertile descendant. From this point on three phases of breeding are distinguishable. The first experimental cross-breeding phase took place between 1875 and late 1920s. It was followed by a short transition period (1930s to 1947) in which cytological characterisation was determined. Both phases led to systematic cross-breeding, which has been continued until today.

The decisive breakthrough in triticale breeding was reached by *Givaudon* in 1937. He took colchicin, an alkaloide from the meadow saffron, to double the chromosome number and by doing so, he enabled its fertility. The name triticale (*Triticosecale*) was formed in 1935 by *Tschermak*. It combines *Triticum aestivum* respectively *durum* with *Secale cereale* [3].

Today's triticale crossings use rye and wheat as male and female parents, respectively. The opposite was also attempted (called *secalotricum*) but did not produce the desired results. Some of the reasons were: high infertility caused by slow growing of the pollen tube and very strong straw development. Today *secalotricum* has completely lost importance.

On the other hand there are many variations of hexaploid (2 n = 6 x = 42 chromosomes) and octoploid (2 n = 8 x = 56) triticale by crossings of the tetraploid *Triticum dicoccum* (2 n = 4 x = 28) with rye (2 n = 2 x = 14) respectively crossings with the hexaploid *Triticum spelta* (2 n = 6 x = 42). Additionally, there is differentiated primary and secondary triticale, special re-crossings called substitution- as well as recombination triticale [4, 5, 6]. Generally hexaploid varieties are used because of better seed quality and higher yields. Agronomically triticale combines the insensitivity of rye with the high productivity of wheat. The grain's weight, colour and size are almost similar to those of wheat, however the spelt is more shrivel and the straw length varies between 30 and 150 cm.

Triticale Amarillo was the first approved variety in Mexico [7] and Hungary 1968 [8]. It was almost 20 years later that the hybrid was introduced to Germany. By 1999, there were over 150 approved

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Tables and figures see Appendix

varieties worldwide [8]. In 2005, approximately 2.7 million tons were cultivated in Germany, in 0.5 million hectare. The yearly worldwide production encloses about 13.5 million tons.

Triticale is commonly used for animal feed, bio ethanol and, to a lesser extent, for baking purposes. Its feed high quality is based on the favourable amino acid composition. Triticale is permitted in Germany for brewing, where the purity law is adhered to, but only for top fermenting beers.

With only few exceptions, the chemical composition of triticale is similar to wheat concerning starch ingredients and physical characteristics, protein, raw fibre, ash [9]. Compared to wheat the higher content of free sugars and pre-existent low molecular proteins results from similar enzyme composition as rye. Triticale on its own is rich in enzymes [10]. The gelatinization temperatures of unmalted as well as malted triticale are recorded a bit lower than its parents [11]. The lipid content of triticale is similar to the one of rye, however, mineral and trace elements seem to be higher than both, wheat and rye, especially Ca, Cu, Fe, P, Na, Mn and the Zn [9]. Vitamins do not significantly differ from either parent [12]. In most aspects there are enormous differences among the varieties [13].

The pentosans (here = arabinoxylans) are of high importance in triticale. Physiologically the non-starch-polysaccharides (nsp) are described as roughage, or dietary fiber, which is known to stimulate peristaltic activity, support the intestinal flora and has advantages for the removal of toxic substances from the colon. The linked ferulic acid (app. 0.2 %) has antiradical effects and shows favourable bioavailability for humans. Pentosans tend to oxidative crosslinking via ferulic acid branches. These pentosans and polymerisation products combined with synergetic effects and proteins cause the high viscosity of triticale wort and beer. The total amount of araboxylans lies around 7.6 % (1.8 % water soluble) [14]. Rye showed in these analyses 12.2 % total pentosans (3.9 % water soluble) and wheat 6.6 % (2.16 % water soluble).

2 Experimental

Unmalted triticale

Triticale variety Modus with 12.7 % moisture was used in the malting trials. The triticale samples were obtained from Saaten-Union (Nordsaat Saatzucht GmbH, Böhnshausen), grown and harvested in Germany in 2006. According to breeder's information the total N lies slightly below average.

2.1 Malting Procedure

Malting was carried out in 1 kg batches, 24 malts were produced simultaneously varying the malting conditions (Fig. 1). The steeping water was equilibrated by placing it 24 h prior to steeping into the temperature controlled compartments. Steeping was done for 5 hours during the first day and 4 hours during the second. In certain cases, final moisture (42, 45 or 48 % degree of steeping) was reached on the third day by additional steeping. Steeping and germination (vegetation time: 5, 6, and 7 d) were done at three different temperatures, which were kept constant (15, 18 and 21 °C) in temperature controlled chambers with 95 % RH. Kilning was done at 50 °C for 16 hours followed by an hour rest at 60 °C, one hour at 70 °C and finally four hour rest at 80 °C. After kilning, rootlets were removed and the malt was allowed to rest for 7 days before mashing.

2.2 Analytical Procedures

Analytical procedures were carried out in duplicate (n = 2). All concentrations are based on dry weight unless otherwise mentioned. Other than amylolytic activities which were determined using commercial analysing kits from Megazyme, Bray, Ireland. All analyses were carried out according to the standard methods of the European Brewing Convention (EBC) [15], Mitteleuropäische Brau- und Analysenkommision (MEBAK) [16, 17] and American Society of Brewing Chemists (ASBC) [18] using a congress mash programme with 100 % triticale.

Extract

The malt extract was determined by using an Anton Paar Alcolyzer (Anton Paar, Graz, Austria) following MEBAK method 3.1.4.2.2 [17].

Apparent Attenuation Limit

Apparent attenuation limit (AAL) was determined following MEBAK method 4.1.4.10 [19].

Gelatinisation Temperature

Gelatinisation temperature (GT) was measured with a rapid visco analyser RVA Super 4 (Newport Scientific, Warriewood, Australia) as reported elsewhere [20] and the MEBAK method 2.7 [17]. The weigh-in was 7.5 g malt and 15 g dist. H_2O.

α-Amylase Activity

ICC standard method 303 [21] by means of a Megazyme enzyme kit (Megazyme, Wicklow, Ireland) was used to measure the level of α-amylase activity. Deviating from the ICC-method, the weigh-in was 1 g instead.

β-Amylase Activity

β-amylase activity was determined using the Megazyme kit (Megazyme, Wicklow, Ireland) [22, 23].

Limit Dextrinase Activity

Limit dextrinase activity was determined using the Megazyme kit (LDZ 7/98).

α-Amino-Nitrogen (FAN)

α-Amino-nitrogen determination was based on MEBAK method 3.1.4.5.5.1 [17] using a Skalar working station (Skalar, Breda, Netherlands).

Kolbach index (ratio S/T)

The effects of malting conditions on proteolytic activities in triticale was determined using the Kolbach index, which was calculated from the formula, (% soluble protein/% malt protein) × 100, by following MEBAK method 4.1.4.5.3 [24].

Dimethyl Sulfide Precursor (DMS-P)

DMS-P determination was based on MEBAK method, 3.1.4.17 [17].

Viscosity

Wort viscosity was measured using a rolling ball viscosimeter AMVn-Automated Micro Viscometer (Anton Paar, Graz, Austria) at 20 °C according to MEBAK method 4.1.4.4.1 [25].

Total (AX) and Water-Extractable (WEAX) Arabinoxylan

The analyses of AX and WEAX have been carried out similar to the method presented by *Houben et al.* [26], with slight modifications. Due to a better separation of the monosaccharides glucose and xylose by the CarboPack 10 column, the treatment with glucose-oxidase was not necessary in the modified method.

For the determination of the total AX content the samples were milled with a laboratory hammer mill (Laboratory Mill 3100, Danfoss). Then 0.1 g of the sample was dissolved in 4.0 mL of distilled water and 4.0 mL of 4 M HCl and hydrolysed for 60 minutes in boiling water in a normal laboratory screw-cap test tube. The samples were cooled to room temperature and 4.0 mL of 4.0 M NaOH were added to neutralise the samples. Subsequently the concentration of monosaccharides was measured by HPAEC/PAD.

The amount of water-extractable arabinoxylan was determined by adding 1.5 mL of water to 0.1 g of milled sample. After stirring for approximately 60 minutes at 65 °C and subsequently centrifuging, 1.0 mL of the supernatant was removed, hydrolysed and neutralised as described above [27].

Prior to analysis by HPAEC/PAD the chloride ions were removed using OnGuard II Ag cartridges from Dionex. The contents of AX and WEAX were calculated as the sum of the pentose sugars, arabinose and xylose, multiplied by 0.88 to correct for anhydro monosaccharides [26]. The coefficient of variation was less than 2 %.

2.1 Experimental Procedure

A response surface methodology study as described by *Montgomery* [28] was conducted to determine the relative contributions of three predictor variables (degree of steeping, germination time and temperature) on the quality of triticale malt. Using the software package StatEase (Stat-Ease Corporation, Minneapolis, USA), a face-centred cube with double replicated factorial and center point was constructed (Fig. 1). This design was chosen because the region of interest and the region of operability are nearly the same. The power at 5 % alpha level for effect of double standard deviation for this design is clearly above 80 %. Maximum and minimum predictor levels were defined by carrying out preliminary malting tests. Three levels for each predictor were incorporated into the design.

Response variables measuring malt quality attributes were extract, AAL, gelatinisation temperature, α- and β-amylase activity, limit dextrinase activity, FAN, soluble N, Kolbach index, DMS-P, viscosity and AX and WEAX. After analysing the characteristic ratios, the calculated statistic models were analysed and evaluated with the help of different indices. The most important statistic indices are: (a) R^2 values quantifying the stability index of the regression model, (b) p-values which show the significance, (c) F-values describe the influences on the model, as well as (d) lack of fit, which describes the scatter of the data around the formed model. Afterwards the optimum malting parameters were calculated.

3 Results and Discussion

The impact, on the malt quality, of different moisture contents (42, 45, 48 %), germination temperatures (15, 18, 21 °C) and germination times (5, 6, 7 d) were evaluated. The responses measured were extract, attenuation limit, gelatinisation temperature, α- and β-amylase activity, limit dextrinase activity, α-amino nitrogen (FAN), Kolbach index, soluble N and dimethyl sulfide precursor (DMS-P), viscosity and arabinoxylans.

In Table 1 the measured as well as the calculated minimum and maximum values of the study are listed. The measured and calculated values show good correlation. Triticale, like rye, has the ability to absorb water quickly contrary to wheat which absorbs water slowly due to its protein composition and probably also because of the lower enzyme levels.

3.1 Amylolytic Attributes

Extract

A potential extract of more than 80 % is normal for barley malt used for brewing purposes. The extract values for triticale, determined using the congress mashing system, ranged at high levels between 84.6 and 86.7 % dm. The extract yield was high for all vegetation conditions, however in average, the highest extract values were obtained from 15 °C malt (Fig. 2). Higher temperatures led to more intensive root and leaf growth, and consequently lower extract levels. Within the individual temperature groups only slight differences could be detected. Highest extract values are linked to maximum moisture and minimum vegetation time (enhanced proteolysis) and diametrical opposite at minimum moisture and maximum time (enhanced cytolysis with low malting loss). Other conditions reduced the extract because of under-modification or malting loss. In general the vegetation time factor has had slightly more influence on extract yield than grain moisture.

Attenuation limit

In contrast to favourable high extract yield, unsatisfying final attenuation as well as viscosity are the predominant problems in triticale. In previous research, attenuation limit values were of 74.3 % in 1988–91 crops [29] and 75.9 % for the 2002 crop [30]. The values normally obtained for barley malts range between 81 and 86 %. In the Modus variety surprisingly high values of 75.8 up to 78.0 % were measured. Low attenuation grades give rise to fatty tasting beers with bad drinkability and corresponding microbiological risk.

The attenuation limit shows vaulted tunnel forms in all three temperature grades (Fig. 3). The comb is placed along the 45-%-moisture-line. Lowest attenuation limits are found in, both, under- and over-modified malt. In general, maximum values are found at 15 °C, minimum at 18 °C.

Protein solubilisation is correlated to attenuation grades. *Sacher* reported that in wheat varieties, which tend to a strong proteolysis, lower attenuation limits were found [31]. Further on no obvious correlation to amylolytic enzymes (esp. β-amylase) could be detected.

Gelatinisation Temperature

The range of malting parameters was not wide enough to have an impact on the gelatinisation temperature (65.3 °C for the optimal malt). Parallel studies with other cereals showed that the gelatinisation temperature may probably be influenced by the malting time rather than the malting temperature. One example would be black rice, where the germination time was 12 days [32].

α-Amylase Activity

α-Amylase activity is an important attribute, when malting quality of a cereal is considered. The enzymatic activity normally measured in barley ranges from 200–250 U/kg. The activity in triticale malt reached 162–332 U/kg (38–77 ASBC). The broad value range suggests high sensitivity towards varying malting parameters.

The impact of the varying malting parameters is shown in figure 4. The highest average α-amylase activity is reached at 15 °C and the lowest at 21 °C. At lower temperatures increasing enzyme induction occurs in barley, to make sugar available for the growth of the embryo.

Generally the lowest α-amylase activity level is obtained in under-modified malt (5 d with 42 %), and highest for over-modified malt (7 d with 48 %). At lower temperatures, the influence of vegetation time increases whereas the impact of the moisture content decreases.

β-Amylase Activity

β-Amylase is an exo-enzyme that acts on the non-reducing end of amylase and high-molecular weight dextrines releasing maltose unit [33]. This enzyme plays an important role during the brewing process since it is the enzyme which releases the fermentable carbohydrates. Triticale is well known for high enzyme levels, particularly β-amylase, which makes it suitable for industrial ethanol production. The determined activity levels of malted triticale are in the range of 917–1231 U/g (372–480 °WK). This is well known and compared to barley and wheat a high β-amylase activity [34, 35].

The impact of temperature and degree of steeping on the β-amylase activity is depicted in figure 5. At all temperatures, the graph shows the lowest values along the 45-%-moisture-line. With higher or lower moisture contents β-amylase activity increased rapidly. The origin is similar to the Kolbach index and soluble N one as shown below. In barley similar results have been observed, it would appear that the linked β-amylase form is cleaved from protein Z [36, 37]. The temperature sensitivity of β-amylase seems to be high with a maximum activity at steeping and germination temperatures of 18 °C and a minimum at 21 °C. The tendencies observed for β-amylase seem to be related to the proteolytic activity. A reason could be that the latent (bound) β-amylase can be liberated, or converted, into free soluble β-amylase by limited proteolysis (38).

Limit Dextrinase Activity

The limit dextrinase is a debranching enzyme, which splits the α-1,6-glycoside linkages of amylopectin. It is another important enzyme for starch degradation in mashes [39]. In raw triticale a limit dextrinase activity was measured in the range of 76 to 217 U/kg. The limit dextrinase activity reported in barley is of 400 U/kg [40]. Depending on the malting conditions, the limit dextrinase content can tripled. Basically higher germination temperatures led to lowered average values (Fig. 6). For all malts germinated during a 5 day period (15, 18 and 21°C with 42 %) the lowest concentrations were recorded. As expected, the longer the germination time the higher the limit dextrinase content. Grain moisture and time contribute equally to the increasing values.

The similarity between the limit dextrinase and α-amylase graphs suggest that limit dextrinase in triticale behaves different from the one in barley. *Kessler* states that in barley the enzyme is activated by proteolysis like β-amylase [41]. This is due to the fact that limit dextrinase appears in barley malt in both freely soluble and bound forms [39]. The same holds for wheat where the bound limit dextrinase is attached to insoluble proteins via disulphide linkages [42]. From the obtained data, limit dextrinase shows no obvious correlation to soluble N or Kolbach index.

3.2 Proteolytic Properties

FAN

The FAN content in malted triticale has been recorded to be in the range of 103 and 206 mg/100mL as shown in figure 7. Low molecular nitrogen compounds play a central role in the colour and flavour development of malt following the *Strecker* reaction. The FAN content influences the yeast growth as well as the fermentation process and the formation of fermentation by-products. Thus, the amino acid concentration and composition are important factors to obtain a desirable flavour profile in beer. Particular attention should be paid during kilning and wort boiling.

The temperature response seems to be distinct, e.g. lowering the vegetation temperature from 18 to 15 °C induces maximum

FAN content. The higher the temperature the lower the achieved FAN level, because higher temperatures enable faster metabolic nutrient consumption for metabolism. The lowest contents were found for all temperatures at five days germination time trial. The highest values were obtained with long germinated malt. In general, an increase in moisture content had higher impact on the FAN content than time did.

Soluble N and Kolbach index

Soluble nitrogen and Kolbach index followed similar trends; the data are shown in figure 8. Both range at high levels between 930–1204 mg/100 g dm (soluble N) and 43.4–59.7 % (Kolbach index). In triticale the proteolytic activity is higher than the values reported for barley [33]. The nitrogen content determines foam, taste, haze and microbiological stability of beer. An all over difference of higher than 250 mg/100 g dm soluble N depicts a good malting influence within the chosen malting parameters as reported before [43, 44].

Similar to β-amylase the soluble N and Kolbach index graphs show lowest values along the 45 %-moisture-line for all temperatures. The highest levels were obtained with 18 °C and the lowest with 15 °C. Extreme moisture and vegetation times generally led to unfavourably high amounts. After 6 to 6.5 days the maximum values for both attributes were reached. This trend could be explained by complex proteolysis degradation of albumine, globuline, prolamine and gluteline in the endosperm and the growth of the germ, as a result of multiple modifications of these four fractions [45]. In barley the quantities and relative amounts of the solubility fractions change substantially during malting. Nitrogenous substances are lost by leaching during steeping, but there are no gains or appreciable losses from the whole grain during the other stages [39].

DMS-P

The DMS-precursor (DMS-P) or S-methylmethionine (SMM) is an amino acid, which occurs in malt. It is considered to be one of the most important characteristics for malt quality. In the Modus variety, the obtained values were between 0.4 and 1.7 ppm suggesting that DMS-P is not a problem in triticale malt brewing (normal specification < 7 ppm). Compared to wheat the SMM amount was 25 to 30 % less. A considerable proportion of SMM, which is located in rootlets and leaflet is removed during cleaning of the malt. [33]

3.3 Cytolytic Attributes

Viscosity

A remarkable feature of triticale is its high wort and beer viscosity. For 8.6 % wort, levels between 2.134 and 2.472 mPa × s were found. At all temperatures, similar graphs were obtained. With higher temperatures, the average viscosity levels tend to increase (Fig. 9). As described earlier, minimum viscosity was obtained in the 5 day malt (42 % moisture) and maximum for longer malting periods. However moisture content rather than time is the determining factor, since higher wort viscosity was obtained from the 5 day germinated malt at 48 % rather than from the 7 day germinated malt with 42 % moisture.

Normally, increasing modification supports the degradation of viscous substances into short chained β-glucan dextrines. For instance, β-glucans in barley are solubilised by β-glucansolubilase from the endosperm and degraded by endo-β-1,3- and 1,4-glucanases into insignificant molecular weights < 10 kDa. For this publication β-glucan was not measured because of the reported low content [34]. Also, in barley, increasing moisture content, prolonged vegetation time and temperatures between 13 and 15 °C support this degradation. This process leads to over-modified malts, which result in a very low viscosity. Moreover, vegetation time normally has greater influence on cytolysis than moisture. However, as explained above, triticale was found to differ from barley in both cases. Wheat behaves in the same way like triticale where over-modification with increasing temperatures from 14 to 20 °C, high moisture content (much more than 42 %) and 7 days germination time do not necessarily lead to viscosities less than 1.9 mPa × s [46].

Total Arabinoxylan (AX) and Water-Extractable Arabinoxylan (WEAX)

Another possibility for viscouse substances in cereal based worts is arabinoxylan. There was only a slight difference between the samples in total arabinoxylan. Content values between 4.60 and 5.52 g/100 g dm were measured. Different water-extractable arabinoxylans were found (1.47–2.12 g/100 g dm.). However for AX and WEAX no correlation could be established between the malting parameters and the resulting wort viscosity. Preceding authors referred no correlation between viscosity and pentosan contents (absolute or water soluble), pentosanase activity, protein content or oxygen pick-up with tenable significance. They ascertained that arabinoxylans from different cereals have different gelling potentials, rye and barley extracts gave stronger gels than AX from wheat and triticale [47, 48].

4 Conclusion

In this study response surface methodology was used to investigate the influence of three malting parameters on the quality of triticale malt. For each variable, three different values were assigned. The chosen parameters and their values were steeping degree (42, 45 and 48 %), vegetation temperature (15, 18 and 21 °C) and vegetation time (5, 6 and 7 days). In order to study the impact of these variables on triticale malt quality, the best applicable model was determined and applied to all samples. The measured quality attributes were extract, apparent attenuation limit, gelatinisation temperature, α- and β-amylase activity, limit dextrinase activity, Kolbach index, α-amino-nitrogen, DMS-P, viscosity and arabinoxylan. Based on the results of these studies, it was concluded that the optimal malt is achieved after the 5th germination day with moisture content of 45 % and a set temperature of 15 °C for steeping and germination. The predicted values for the quality attributes (Table 3) were 84.9 % extract, 78.3 % AAL, 254 U/g α-amylase activity, 1055 U/g β-amylase activity, 264 U/kg limit dextrinase activity, 45.8 % Kolbach index, 155 mg/100 mL FAN, 1.7 µg/L DMS-P and 2.305 mPa × s viscosity. The measured values slightly differ from the calculated ones. The obtained attribute values were 83.2 % extract, 78.8 % AAL, 276 U/g α-amylase activity, 1024 U/g β-amylase activity, 293 U/kg limit dextrinase activity, 46.0 % Kolbach index, 155 mg/100 mL FAN, 3.3 µg/L DMS-P and 2.575 mPa × s viscosity. Both results correlate well.

Extract yields were found at predominantly high levels. The extract includes not only starch but also soluble proteins and non-starch-polysaccharides, e.g. pentosans. This soluble but unfermentable "extract" could be responsible for the opposite behaviour between Kolbach index as well as soluble N and attenuation limit. The values associated with proteolytic activity are soluble N and Kolbach index. They clearly show the large range of activities depending on the enzymes regarding proteolytic characteristics during malting. In addition to the proteolytic enzymes amylolytic enzyme levels are considerably high, particularly β-amylase.

High wort viscosity is a remarkable feature of triticale worts. However, no correlation could be established between the total AX or water-extractable arabinoxylan content of the wort.

This study was able to show that triticale is a suitable raw material for the malting and brewing process. Beers brewed from triticale are described as being soft in taste as well as having good foam and palatefullness. Additionally, triticale beers have a very stable turbidity similar to that recommended for Bavarian wheat beers. Lautering or filtration problems caused by proteins or pentosans could be compensated by using exogenous enzymes or using lower grist load ratio.

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Appendix

Table 1 **Measured and calculated minimum and maximum values of the analysed features in comparison to the values of the triticale raw material**

			triticale malt			
		triticale	measured		predicted	
attributes	**unit**	**raw material**	**min**	**max**	**min**	**max**
extract	%, dm	n.a.	84.2 (5/48/21)	86.2 (7/42/21)	84.4 (5/42/21)	86.0 (7/42/21)
AAL	%	n.a.	75.8 (5/48/15)	78.0 (7/45/18)	75.9 (7/48/18)	78.3 (5/45/15)
α-amylase activity	U/g	276	163 (5/42/21)	319 (7/48/15)	167 (5/42/21)	328 (7/48/16)
β-amylase activity	U/g	505	917 (5/48/21)	1231 (7/48/15)	967 (5/46/21)	1245 (6/48/17)
limit dextrinase activity	U/kg	293	76 (5/42/21)	215 (7/48/15)	119 (5/42/21)	216 (7/48/15)
FAN	mg/100 mL	n.a.	103 (5/42/21)	204 (7/48/15)	104 (5/42/21)	206 (7/48/15)
Kolbach Index	%	n.a.	43.3 (5/45/18)	58.9 (7/48/21)	45.6 (5/45/15)	58.8 (6/48/18)
DMS-P	µg/L	n.a.	0.4 (7/42/21)	1.6 (7/48/15)	0.4 (7/42/21)	1.6 (7/48/15)
viscosity (8.6%)	mPa × s	n.a.	2.134 (7/48/21)	2.472 (7/48/15)	2.214 (5/42/15)	2.421 (7/48/21)

The values in parenthesis mean the malting conditions: germination time/degree of steeping/germination temperature; n.a. = not applicable; 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 minimising this value without considering the influence of other features.

Table 2 **Predicted and measured values with the optimal malting regime**

attributes	**unit**	**predicted**	**achieved**
extract	%, dm	84.8	83.2
AAL	%	78.3	78.8
α-amylase activity	U/g	254	276
β-amylase activity	U/g	1055	1024
limit dextrinase activity	U/kg	264	293
FAN	mg/100 mL	155	155
Kolbach Index	%	45.8	46.0
DMS-P	µg/L	1.7	3.3
viscosity (8.6%)	mPa × s	2.305	2.575

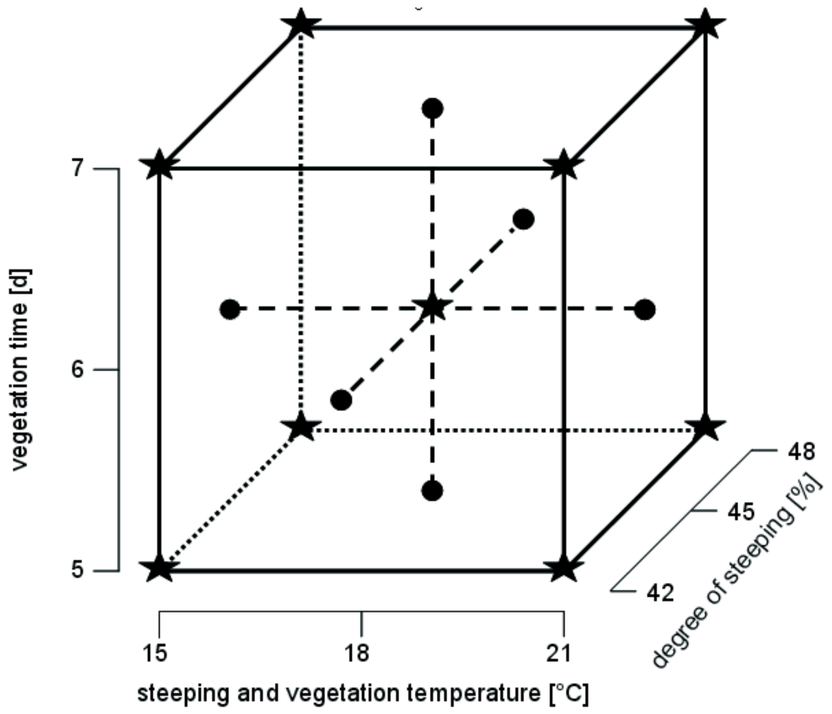

Fig. 1 Face-centred design (double replicates of the factorial and centre points = ★)

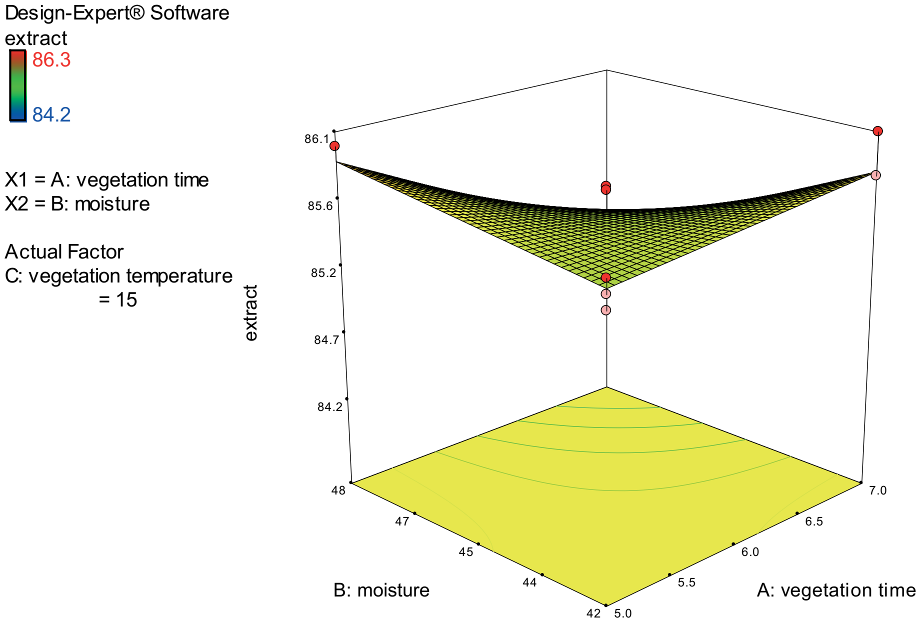

Fig. 2 The influence of moisture content and vegetation time on extract with 15 °C vegetation temperature

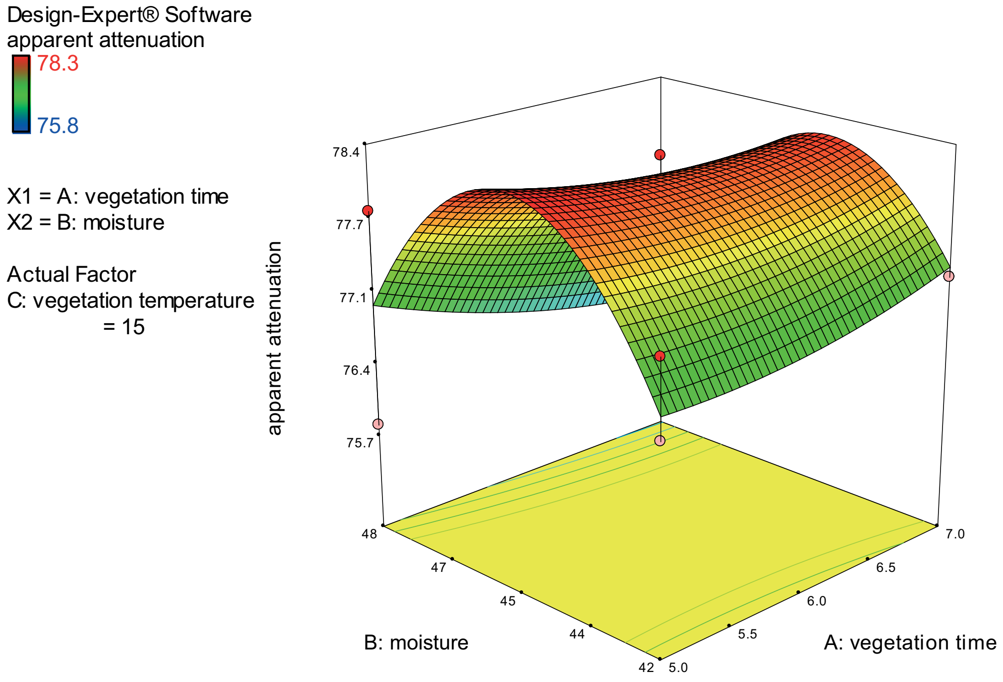

Fig. 3 The influence of moisture content and vegetation time on the AAL with 15 °C vegetation temperature

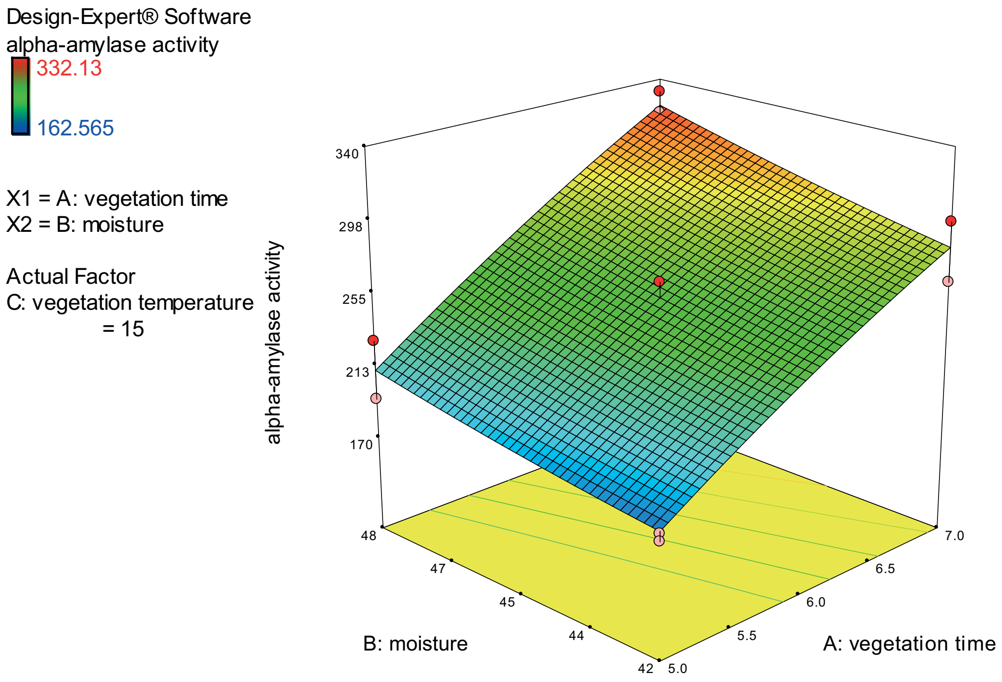

Fig. 4 The influence of moisture content and vegetation time α-amylase activity with 15 °C vegetation temperature

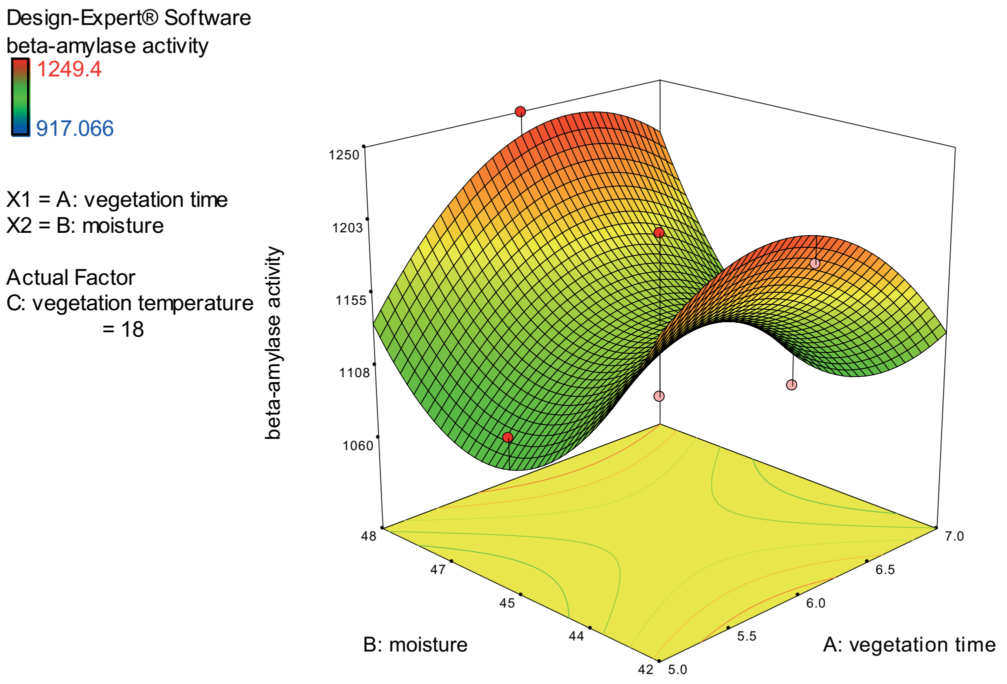

Fig. 5 The influence of moisture content and vegetation time β-amylase activity with 18 °C vegetation temperature

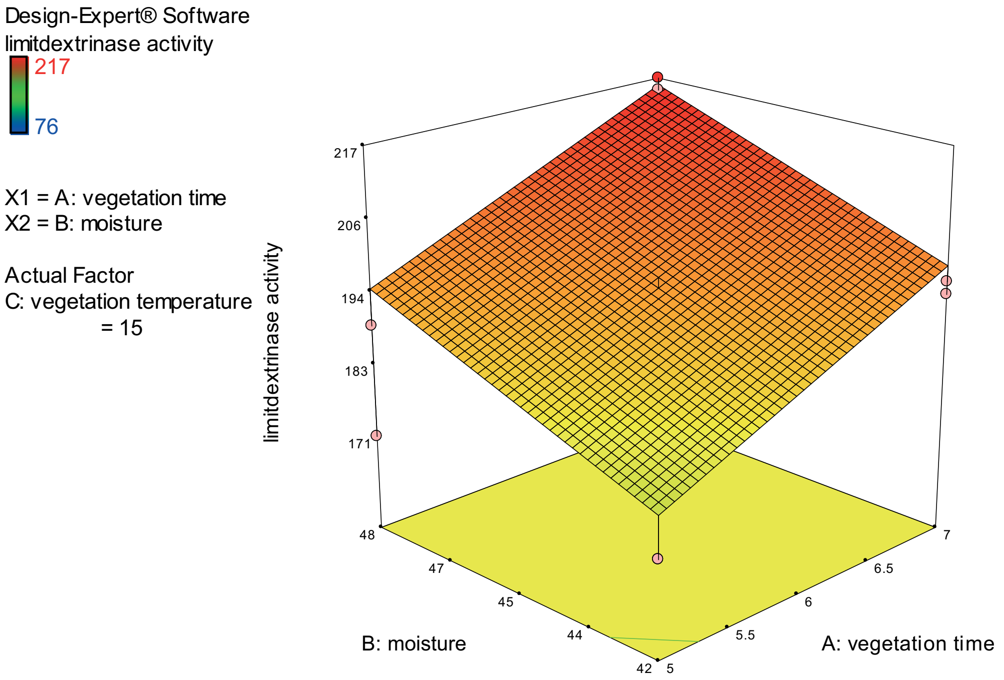

Fig. 6 The influence of moisture content and vegetation time on limit dextrinase activity with 15 °C vegetation temperature

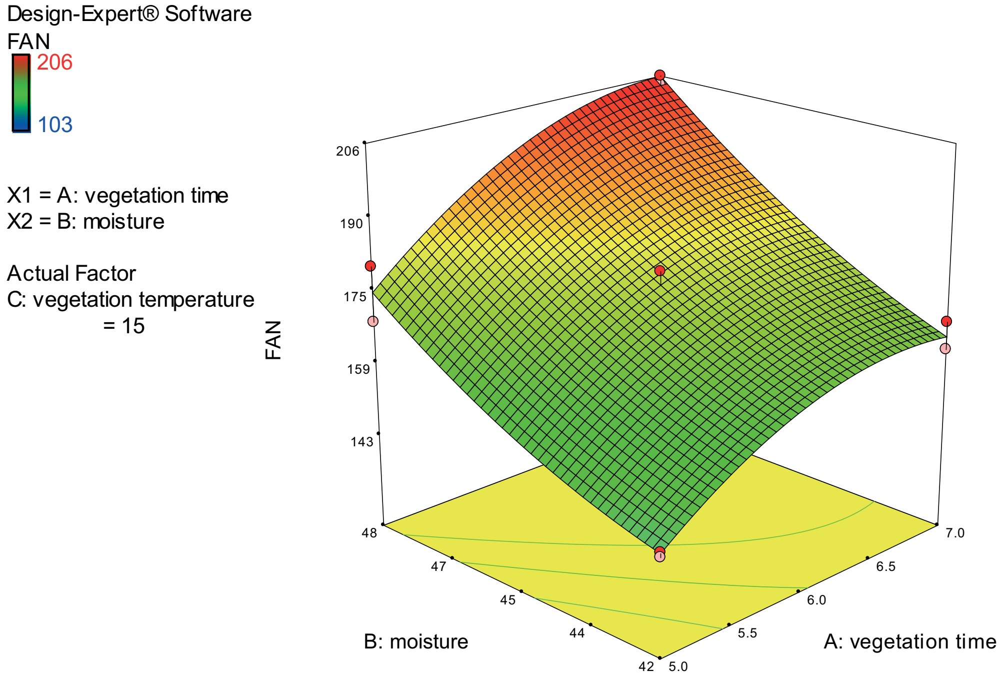

Fig. 7 The influence of moisture content and vegetation time on free amino nitrogen with 15 °C vegetation temperature

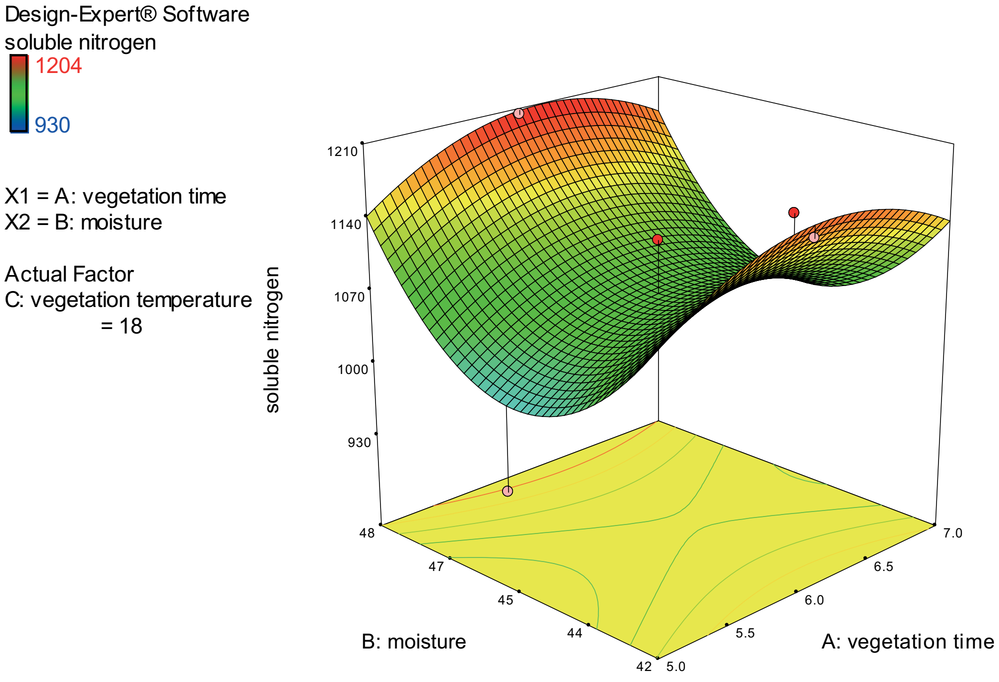

Fig. 8 The influence of moisture content and vegetation time on soluble nitrogen with 15 °C vegetation temperature

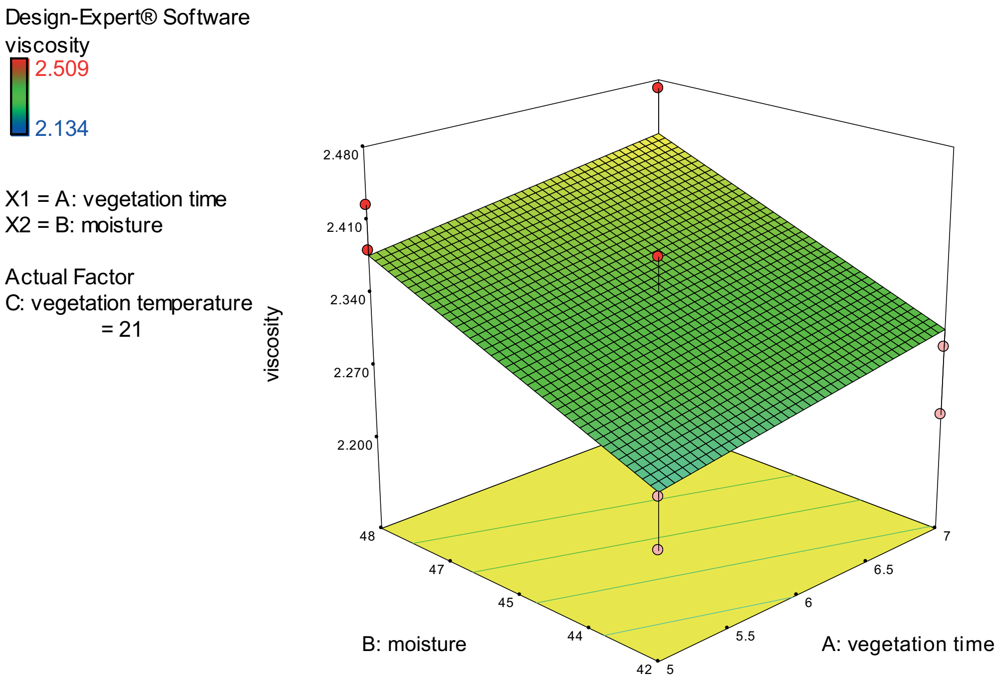

Fig. 9 The influence of moisture content and vegetation time on viscosity with 21 °C vegetation temperature