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The use of response surface methodology to optimise malting conditions of quinoa (*Chenopodium quinoa* L.) as a raw material for gluten-free foods and beverages

Response surface methodology was used to investigate the influence of the three malting parameters, degree of steeping, germination time and temperature, on the quality of quinoa malt. Each predictor variable was tested at three levels. Germination times were set to 5, 6, and 7 d, degrees of steeping were set to 46, 50, and 54 %, and germination temperatures were 8, 11.5, and 15 °C. A kilning temperature of 74 °C was used for all malts. A series of malt quality attributes was investigated including extract, β-amylase activity, limit dextrinase activity, α-amino nitrogen (FAN), and dimethyl sulfide precursor (DMSP). The optimal malting programme was achieved with 5 d germination time, 46 % degree of steeping, 15 °C steeping and germination temperature. The obtained amylolytic and proteolytic attributes were 59.6 % extract, 2021 U/kg limit dextrinase activity, 20 U/g β-amylase activity, 19.1 mg/100 mL FAN, and 12.7 mg/kg dimethyl sulfide precursor (DMSP). α-amylase activity could not be proved, therefore it was not considered for the evaluation.

Descriptors: quinoa, malt, response surface technology, gluten-free, pseudo cereal

1 Introduction

Coeliac disease (CD) or in the U.S.A. celiac disease (also known as non-tropical sprue, gluten-sensitive enteropathy, celiac sprue, idiopathic steatorrhea, primary malabsorption, Gee-Herter disease, gluten-induced enteropathy, adult celiac disease) is an illness, where the person's body reacts to the gliadin fraction of wheat and the prolamins of rye (secalins), barley (hordeins), and possibly oats (avidins) [26]. Coeliac disease prevalence has been estimated to be 1 in approx. 100 people worldwide [16, 36]. Such a rate establishes Coeliac disease as one of the most common food intolerances known. This disease is caused by an immune-mediated response in the small intestine triggered by the ingestion of gluten in genetically susceptible individuals [14]. The only effective treatment for Coeliac disease is a strict adherence to a diet that avoids ingestion of cereals (wheat, spelt, triticale, rye, barley, and possibly oat) containing gluten and their products throughout the patient's lifetime [13].

Gluten is the general term used to describe an important protein fraction in wheat. Gluten proteins can be divided into two main fractions according to their solubility in aqueous alcohols: The soluble gliadins and the insoluble glutenins, commonly known as the prolamins [22]. Wheat, rye, and barley are all members of the tribus *Triticeae* in the grass family (*Poaceae*) and are taxonomically closely related. All these cereals and their prolamins are toxic to those, who suffer from Coeliac disease [19].

Cereals not containing gluten include: rice (*Oryza sativa*), maize (*Zea mais*), sorghum (*Sorghum bicolor*), and millets (e. g. *Panicum miliaceum*, *Setaria italica*, *Pennisetum glaucum*, and *Eleusine coracana*). Although belonging to the family of the Poaceae, they are members of other tribus. Other carbohydrate rich pseudocereals without gluten are buckwheat (*Fagopyrum esculentum*), amaranth (*Amaranthus* species), and quinoa (*Chenopodium quinoa*) [46]. Recent studies have focussed on the production of malt and beer from gluten-free cereals such as rice, maize, millet and pseudocereals such as buckwheat, quinoa, and amaranth [29, 39, 40, 7, 28, 41].

Quinoa is a species of *Chenopodiaceae* (goosefoot plant). It is a pseudocereal, as the principal component of the grain consists of carbohydrates. Other goosefoot plants are, amongst others, spinach or sugar-beet [32]. There are approx. 250 different quinoa species. *Chenopodium pallidicaule*, *Chenopodium berlandieri*, *Chenopodium album*, and *Chenopodium hircium* are the best known species.

Quinoa is a short day C4-plant. Its kernels have a nominal diameter of 1.0 to 2.4 mm and they are not covered with glumes. The kernel's colour can be white, brown, black or reddish. Furthermore they have a round form and a smooth surface. 1000 kernels have an approximate weight of 1.85 to 4.17 g. It has been reported that quinoa has no dormancy. [8]

The ingredients of quinoa are comparable to other cereals, in terms of nutritional physiology they are even of higher value. Principal component is starch with 57–65 %. Quinoa starch granules of the corneous perisperm are polygonal, with the size ranges from 1.0–2.5 µm (by agminated the granules about 32 µm). Quinoa starch contains 12 % amylose and 88 % amylopectin, which is

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Tables and figures see Appendix

quite different to the other grains such as sorghum and millet (sorghum 24.0 %, pearl millet 21.1 %, foxtail millet 17.5 %, kodo millet 24.0 %, and finger millet 16.0 % amylose) [1]. The high content of amylopectine can lead to a stronger gelatinisation in case of low temperatures. Free sugars were also detected in the quinoa kernel. This includes saccharose with approx. 2.7 % [2, 4, 31, 37]. The millets, e.g. *Panicum miliaceum* and types of grains have one non-starchy aleurone layer and a starchy endosperm [40]. There is no aleurone layer in quinoa [15].

The protein content ranges between 14 and 21 %. 48 % of the amino acids contained are essential for the human nutrition. Therefore quinoa has a considerably high protein quality and quantity compared to other grain sorts. This applies, first of all, to the content of methionine (3.7 g/100 g of protein) and lysine (5.6 g/100 g of protein) [32]. All amino acids required for the yeast nutrition are also available in sufficient amount. On that score, it is assumed that quinoa is well qualified for malt and wort production or for an alcoholic fermentation. Quinoa has also a high fat content (approximate 5.3 %) and additionally up to 90 % of unsaturated fatty acid. By comparison, wheat and barley contain only 1–2 % of fat. Particularly the content of unsaturated fatty acids is remarkably high, what keeps increasing the nutritional-physiological importance of quinoa. Fats divide themselves in oleic acid with 48 %, linoleic acid with 50.7 %, palmitic acid with 10 %, lecithin with 1.8 %, and linolenic acid with 0.8 % [8, 30, 33]. The fruit also exhibits a high concentration of minerals. First of all, potassium (0.9 %), magnesium (0.5 %), and calcium (0.1 %) are to be mentioned here. Regarding vital trace elements, quinoa contains iron (6.2 mg/100 g), manganese (42.7 ppm), and zinc (7.9 ppm). The iron content of 6.2 mg/100 g is essentially higher than in grain sorts. Concerning vitamins, quinoa contains the important antioxidants vitamin E (8.4 mg/100 g) and vitamin C (16.4 mg/100 g). Especially the vitamin C1 content of 6.4 mg/100 g is remarkably higher than in conventional grain sorts. The B-vitamins thiamine (B1), riboflavine (B2), niacine, and folic acid are also worth mentioning [8, 11, 21, 33]. Besides, quinoa contains approx. 4.9 % dietary fibres. The β-glucane content of 0.03 % is very low, considering that oats may contain up to 4 % more β-glucane [5, 8].

However, quinoa also comprises substances, which may have negative nutritional physiological effects. These include primarily the saponins. The seeds have a bitter outer coat with higher levels of saponins. That hulls have to be removed by washing before consumption to avoid a bitter final taste in the product. Saponins are antinutritional glycosides that cannot be digested and can sometimes cause intestinal damage or reduce intestinal absorption of nutrients. But there are also quinoa cereals with a very low content of saponins. They are referred to as sweet, such as Chewecca, Kanccolla, Sajama, Cochabamba, Apelawa, and Tango. Saponins serve as defensive substances of the plant against bacteria, fungi, bird damages, and insects. In turn, the positive characteristics of saponins in small quantities are also being discussed. Saponins are to have a complexing effect on cholesterol, they are, besides, diuretic, anti-inflammatory, hormone-stimulating, anti-hypertensive, and could prevent against bowel cancer because of the repressive effect on the cell division. [15, 8, 5] Furthermore, 1 % phytine acid (complexing agent with minerals) and 0.5 % tannine (antioxidant) were detected in quinoa. The antioxidative capacity of quinoa is comparable to cereals [5].

Quinoa is well adapted to low rainfall, high temperatures, high saline soils and, in comparison to barley and wheat, to many soil conditions [17, 32]. It is assumed that *Ch. quinoa* was domesticated and first cultivated in the Andes region of South America, where it has been the primary crop before barley and wheat was established. The first provable plant cultivation began approx. 3500 B. C [32]. The first cultivation attempts in Germany took place during World War 1; during World War 2 the Japanese also initiated different attempts. *Ch. quinoa* is widely spread in arid and semiarid areas. Today about 46000 tons are harvested world wide by 66000 ha cultivation area, most of it originates from Peru, Bolivia and Ecuador.

There are numerous malting and brewing studies for sorghum, pearl and finger millet [10], but none for quinoa. Quinoa is used as raw material for the production of Chicha, a traditional maize beer in the Andes, whereas Lopez-Garcia encouraged to further applications [9].

The objective of this study was to optimise the malting conditions for *Ch. quinoa* using response surface methodology (RSM). The degree of steeping, germination temperature and time were investigated. The range for these parameters were chosen on the basis of preliminary trials reported previously [41].

2 Experimental

Unmalted Quinoa

Ch. quinoa of 14.1 % moisture was used in the malting trials. The quinoa samples were grown near Lima in Peru. The name of the quinoa variety is Real. Naturkost Übelhör GmbH & Co. KG, Leutkirch, Germany made 2 × 25 kg of this white quinoa cereal available as basic material. The exporter was Exportaciones Sierra Y Selva from Lima, Peru.

Malting Procedure

Malting was carried out in 300 g batches. The steeping water was equilibrated by placing it 24 h prior to steeping into the compartments. Steeping was done at day 1 for 5 hours. At the second day final moisture (46, 50, or 54 % degree of steeping) were reached by additional steeping if necessary. In comparison to common grain, quinoa has a higher ability to absorb water. Steeping and germination (vegetation time: 5, 6, and 7 d) were done at the same temperatures (8, 11.5, and 15 °C) in temperature controlled chambers with 95 % rh. Kilning was done at 50 °C for 16 hours followed by a 1 hour rest at 60 °C and 5 hours rest at 74 °C.

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.

All analyses were carried out according to the standard methods of the European Brewing Convention (EBC) [12], Mitteleuropäische Brau- und Analysenkommision (MEBAK) [24, 3], and American Society of Brewing Chemists (ASBC) [38] using a mash program under congress mash conditions (rootlets were removed before mashing). Differing is the time-temperature regime with 20 minutes rests at 40, 50, 60, and 70 °C, to avoid a raised gel building effect at stadiums of higher temperature.

Extract

By using an Anton Paar Alcolyzer (Anton Paar, Graz, Austria) and following the MEBAK method 3.1.4.2.2 [3], the malt extract was determined.

Gelatinisation Temperature

Gelatinisation temperature (GT) was measured with a rapid visco analyser RVA Super 4 (Newport Scientific, Warriewood, Australia) as reported elsewhere [20] and as new MEBAK method 2.7 [3]. The weigh-in was 7.5 g malt (5 % moisture) and 15 g dist. H_2O.

α-Amylase Activity

ICC standard method 303 [18] by means of a Megazyme enzyme kit (Megazyme, Wicklow, Ireland) was used to measure the level of α-Amylase activity. The weigh-in was 1 g in difference to the ICC-method.

β-Amylase Activity

β-amylase activity was determined using the Megazyme kits (Megazyme, Wicklow, Ireland) [23, 34].

Limit Dextrinase Activity

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

α-Amino-Nitrogen (FAN)

α-amino-nitrogen determination was based on MEBAK method 3.1.4.5.5.1 [3] using a Skalar working station (Skalar, Breda, Netherlands)

Dimethyl Sulfide Precursor (DMSP)

DMSP determination was based on MEBAK method, 3.1.4.17 [3].

2.1 Experimental Procedure

A response surface methodology study as described by Montgomery [25] was conducted to determine the relative contributions of three predictors (degree of steeping, germination time and temperature) to the quality of quinoa malt. A face-centred cube with double replicated factorial and center point was constructed (see Figure 1) using the software package StatEase (Stat-Ease Corporation, Minneapolis, USA). 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 of each predictor were incorporated into the design.

Response variables measuring malt quality attributes were extract, β-amylase activity, limit dextrinase activity, alpha amino nitrogen, dimethyl sulfide precursor, and soluble nitrogen. After analysing the characteristic ratios, the calculated statistic models were analysed and evaluated with the help of different indexes. The most important statistic indexes are, for example, R^2 values (stability index of the regression model), the p-values, which show the significance, the F-values, which describe the influences on the model, and the Lack of Fit, which describes the scatter of the data around the formed model. Afterwards the optimal malting parameters were calculated, and malt was produced under these conditions. On the basis of the characteristic ratios of the underlying calculation, this malt was again analyzed on extract, β-amylase activity, limit dextrinase activity, alpha amino nitrogen, dimethyl sulfide precursor and soluble nitrogen.

3 Results and Discussion

The impact of three different steeping moisture contents (46, 50, 54 %), three different germination temperatures (8, 11.5, 15 °C), and three different germination times (5, 6, 7 d) were evaluated. The responses measured were β-amylase, limit dextrinase activity, extract, alpha amino nitrogen, and dimethyl sulfide precursor.

In Table 1 the measured as well as the calculated results of the investigations are listed. The measured and calculated values show a good correlation.

3.1 Amylolytic Specifications

α-Amylase Activity

α-amylase activity, a feature important for the amylolysis of barley malt, could not be detected in quinoa malt and will therefore not be considered in the evaluation. In quinoa malt the measured activity of α-amylase ranked at a maximum of 3 U/g. It is important to mention that the activity in the barley malt ranges between 200 and 250 U/g. Therefore the α-amylase activity was not considered for the statistical evaluation and was not incorporated in the optimal malting conditions. Perhaps quinoa has another strategy to decompose the energy and reserve substance starch during germination.

β-Amylase Activity

β-amylase is an exo-enzyme that acts on the non-reducing end of amylase and high-molecular weight dextrins releasing maltose unit. [27] The activity is very important in order to make a statement about the starch degradation, as these enzymes provoke the development of fermentable sugar. This sugar is immediately transformed into alcohol by yeasts. The determined β-amylase activity levels of malted quinoa are in the range of 17-99 U/g, of unmalted quinoa approximately 58 U/g. The impact of temperature and the degree of steeping on the β-amylase activity is depicted in Figure 2. The optimum to achieve a maximum β-amylase activity is at the lowest temperature (8°C) and lowest degree of steeping (46 %). It becomes clear that under conditions of low germination intensity the β-amylase activity increases in respect to the unmalted material, but it decreases again when the germination intensity raises. The effect of germination time is not shown in Figure 2. The modification of germination time does not change the model. Therefore the model was demonstrated in a period of 6 days. This means that the influence of germination time is minor. This significant influence of temperature is not only visually demonstrated by the model, but also proved by statistical ratios. This model brought very high F-values for the temperature. The R^2-values of the model were also significant and a marginal diffusion of the analyzed values could be certifiable, a fact that amplifies the significance of the model.

The β-amylase activity of barley is increased during malting fourfold, when a germination time of four days is applied. It is well known that germination time has an impact. During the kilning the barley β-amylase activity decreases to about 500 U/g, which is similar to the starting level. Hence, if malt is kilned, the technological treatment during malting on the impact of β-amylase activity is very low [27]. The total amount of barley β-amylase is pre-existing and only activated during germination. In case of quinoa the β-amylase activity has a different behaviour. It is obvious that intensive malting procedures significantly lowers the amount of β-amylase activity which may be explained by the hydrolyses of the enzyme protein itself. However, the measured activities are very low in relation to barley malt (average activities of approx.

250 U/g). This allows the conclusion that in quinoa grain, starch is decomposed by enzymes, which are not identified by this method or that it synthesizes and needs much smaller amounts of β-amylase for metabolism.

Limit Dextrinase Activity

The activity of raw material was about 2968 U/kg and was higher than in malted quinoa (1400-2400 U/kg). A deviation of 568 U/kg between grain and malt is a very clear difference. Figure 3 shows the influences of germination temperature and degree of steeping on the limit dextrinase activity with a germination time of 5 days. The effect of the germination temperature is also higher than the degree of steeping and the germination time. Therefore the expected model was shown at germination time of 6 days, so that the impacts could be better demonstrated. Also in case of the evaluation of the statistical ratios temperature had the widest influence on the model. The degree of steeping and vegetation time play a rather minor role. The lack of fit of each feature is higher than in case of the β-amylase activity, the R^2-values are also very good here.

The maximum enzyme activity of malted quinoa occurs at 10 °C germination temperature and 46 % degree of steeping. This is comparable to the behaviour of the β-amylase activity in Figure 2. The activity of the enzyme also decreases here while germination intensity increases. This also allows the conclusion that intensive malting procedures significantly lowers the amount of limit dextrinase activity which may be explained by the hydrolyses of the enzyme protein itself. As the activity is the highest in the grain, it is assumed that the grain features the maximum of limit dextrinase even before the beginning of the germination and lives on these reserves during the whole germination process. In this case a loss of activity is also possible because of the heat impact during kilning. Compared to a barley malt, which has on average 400 U/kg limit dextrinase activity, the enzymatic power in the quinoa grain is very high. It seems that quinoa primarily pursues the starch degradation during germination with these enzymes, which are measured by this limit dextrinase measurement kit.

Extract

The extract yield is one of the most important quality attributes of malt. Extract generated during mashing is essential for a successful fermentation. The extract allows drawing direct conclusions about the content of soluble substances. In case of a normal enzymatic activity, the potential extract indicates the sugar content and therefore the later alcohol percentage. An altered mashing programme was applied for the extract modification, because no sufficient saccharification took place during the congress mashing programme (standard procedure for barley) and the wort was not filterable. The potential extract ranged at a low level between 40 and 65 %. A potential extract of more than 80 % is normal for barley malt used for brewing purposes. However, the low potential extract is not surprising, considering that the largest part of the quinoa starch was, despite a changed mashing programme, gelatinized but not hydrolysed due to a lack of enzyme supply and could therefore not be modified. The lacking enzyme energy of the malt could be balanced by adding exogenous enzymes or applying gluten free malt, richer in enzyme, from another grain (e. g. panicle millet). Figure 4 shows the impact of germination temperature and the degree of steeping on the extract with a germination time of 6 days. A very similar behaviour could be observed with a germination time of 5 and 7 days. It can be noticed that an increase of temperature at a low degree of steeping causes a strong increase of the extract. When the degree of steeping increases, an extract minimum occurs at approximately 10 °C. However, an optimisation of the extract yield is only possible, when the temperature (15 °C) is maximised and the degree of steeping (46 %) minimised. The influence of temperature is much higher than the influence of moisture content. The influence of germination time is insignificant, which is proven by the statistical ratios. The R^2-values of this model range between 0.3 and 0.5, the regression and the significance are clearly worsened. In return, the diffusion of each feature is lower, having a positive influence on the model.

3.2 Proteolytic specifications

FAN

The FAN content in malted quinoa has been found in a range of 12 to 23 mg/100 mL. Low molecular nitrogen compounds, especially amino acids in wort, influence the fermentation performance and the development of fermentation by-products. Thus the concentration and composition of amino acids are important factors for a desirable flavour profile of beer. They also play an important role as part of Maillard reactions, especially during kilning and wort boiling. Maillard reaction products like melanoidines influence the reductive potential, colour and flavour of beer. Figure 5 shows the impact of germination temperature and degree of steeping on the FAN content with a germination time of 6 days. FAN parallels moisture content and germination temperature. The linear coherences become clear in Figure 5. As in the case of the previous models the impact of temperature is the widest. A modification of the vegetation time causes only little changes on the model. The influence of the degree of steeping is considerably wider for the FAN than at the previous models. The maximum FAN content is achieved through a maximum degree of steeping of 54 % and a maximum temperature of 15 °C. This can be ascribed to the high germination intensity, which provokes more protein hydrolysis, which is needed for metabolism by the germ bud itself and is predominantly used for its cell structure. A FAN of 19.1 mg/100 mL was calculated for optimal malt (Table 2). This is a high value, which suggests that the proteins are well modified and promises sufficient nitrogen supply. This is a good value for an alcoholic fermentation (for 8.6 % barley malt congress wort 135 to 155 mg/100 mL), as many free amino acids are available for the yeast. The high content of FAN is not surprising considering the high protein amount described in literature. The statistical ratios show a very broad distribution of the data (R^2-values 0.8 and 0.7).

DMSP

DMSP is one of the most important responses to characterise the quality of malt. The DMS-precursor (DMSP) or S-methylmethionine (SMM) is an amino acid, which occurs in malt. Dimethylsulfide is developed from DMSP beyond a temperature of approx. 70 °C, which means in case of all thermal treatment steps in the course of malt and beer production. An increased DMS in the finished beer can lead to a vegetable-like flavour. The sensorial limit for free DMS in beer is specified with approx. 100 µg/L [6]. Depending on the boiling intensity the content of DMSP in malt should not exceed 7 mg/kg. The curing temperature and time allow the most effective influence during malt production.

In malted quinoa the content of DMSP is about 7.7-22 mg/kg and much higher than the DMSP content in barley malt. The values of all produced malting courses lie above the maximum values tolerated for barley malt. This can be ascribed to the low curing temperature of 74 °C. The curing temperature of barley malt ranges at a minimum of 80 °C. The influence of the model (Fig. 6) is similar to the previous attributes. The degree of steeping and

the germination temperature have the main influence. The germination time has not noticeable influence on the DMSP content. The influence of the degree of steeping is slightly higher than the influence of temperature. This effect only occurred with this model. The statistical ratios showed bad, nonrelevant R^2-values (0.6 and 0.5) and a very high diffusion of the data. Therefore the significance of this model is very minor.

4 Conclusion

In this study the impact of three different steeping moisture contents (46, 50, 54 %), three different germination temperatures (8, 11.5, 15 °C), and three different germination times (5, 6, 7 d) on quinoa were evaluated. The responses measured were extract, β-amylase activity, limit dextrinase activity, alpha amino nitrogen, and dimethyl sulfide precursor. The best applicable model was determined to the measured data. This optimal malting programme was achieved with 5 d germination time, 46 % degree of steeping, and 15 °C steeping and germination temperature. The predicted responses were 59.6 % extract, 12.7 mg/kg DMSP, 20 U/g β-amylase activity, 2021 U/g limit drextrinase activity, and 19.1 mg/100 mL FAN, as seen in Table 2 which also shows the obtained measured amylolytic and proteolytic attributes with 54.7 % extract, 49 U/g β-amylase activity, 11.1 mg/kg DMSP, 2245 U/g limit dextrinase activity, 920 mg/L soluble nitrogen and 17.1 mg/100 mL FAN. Except for the feature DMSP, the germination temperature had the widest influence. In case of DMSP the degree of steeping had the broadest influence on the model. Changes in the vegetation time showed only a slight influence on the calculated models.

During the realisation of the congress mashing system a gel building effect could be ascertained, although the programme was adapted. Cytolytic features were not recorded, as the modified congress mashing programme produced only little liquified wort. Apparently the mash gelatinised (gelatinisation temperature between 62.9 and 64.9 °C) beyond the optimal temperature of the amylolytic enzymes, which showed comparable low activities too. Besides this effect decreased the extract content, because the largest quantity could not be solubilised. Further research is required here in order to optimise the use of enzymes of the quinoa grain for wort production and to adjust the mashing programme according to it. The DMSP in the finished malt (11.1 mg/kg) was also very high. This had to be balanced by changing the kilning programme. In turn, the solubility capacity of the proteins was very high, improving the malt quality to a large extent.

In spite of the partially not so favourable statistical ratios (FAN, DMSP, extract), the Response Surface Methology contributed to calculate optimal malting conditions for quinoa and to verify them through the production of an optimal malt.

Therefore it is absolutely possible to produce quinoa malt, which can provide a basis for gluten-free food, beverages and, of course, beer.

5 Literature

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Appendix

Table 1 The measured and calculated minimum and maximum values of the analysed features in comparison to the values of the quinoa raw material*

		quinoa raw material	quinoa malt			
			measured		calculated	
attributes	**units**		**min**	**max**	**min**	**max**
extract	%	n.a.	39.9 (5/46/8)	64.0 (7/54/8)	39.9 (5/46/9)	63.8 (7/46/15)
gelatinisation temperature	°C	65.3	63.5 (5/46/8)	65.2 (5/46/15)	63.2 (5/49/8)	64.7 (6/54/12)
α-amylase activity	U/g	< 1	< 1 (5/46/8)	3 (7/54/15)	0.3 (5/46/8)	2.5 (7/54/15)
β-amylase activity	U/g	58	17 (7/54/15)	99 (5/46/8)	14 (6/52/14)	93 (5/46/8)
limit dextrinase activity	U/kg	2968	1343 (7/54/15)	2493 (5/46/8)	1277 (7/54/15)	2492 (5/46/10)
DMS-P	mg/kg	n.a.	7.7 (5/45/8)	28.8 (5/54/15)	9.4 (7/46/8)	20.3 (5/54/15)
FAN	mg/100 mL	n.a.	12.6 (7/46/8)	23.3 (7/54/15)	13.8 (5/46/8)	21.8 (7/54/15)

* 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 The calculated and achieved predicted amylolytic and cytolytic attributes for the malting regime with 15 °C germination temperature, 5 d germination time and 46 % steeping degree

	calculated	**achieved**
DMS-P (mg/kg)	12.7	11.1
β-amylase activity (U/g)	20	49
limit dextrinase activity (U/kg)	2021	2245
FAN (mg/100 mL)	19.1	17.1
extract (% dm)	59.6	54.7

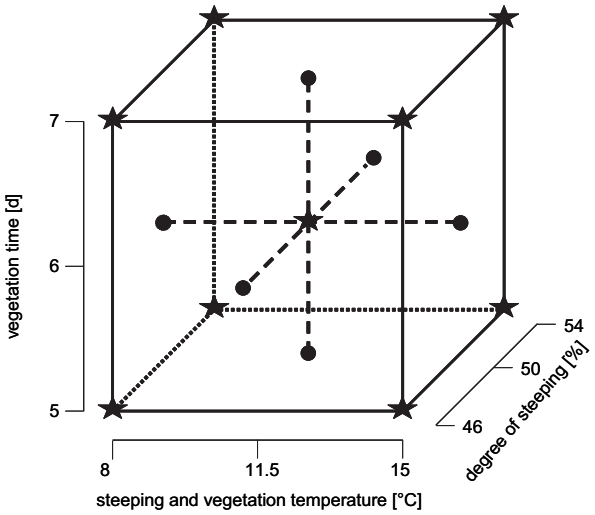

Fig. 1 Face-centred design (double replicates of the factorial and centre points =) ★

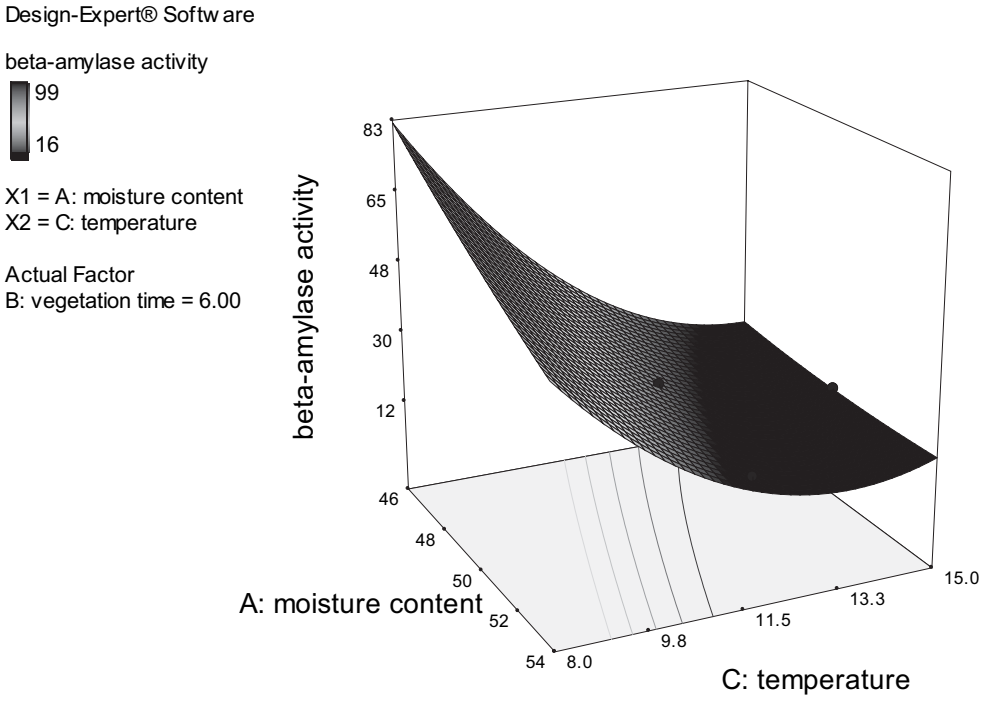

Fig. 2 The influence of moisture content and germination temperature on β-amylase activity with 6 days vegetation time

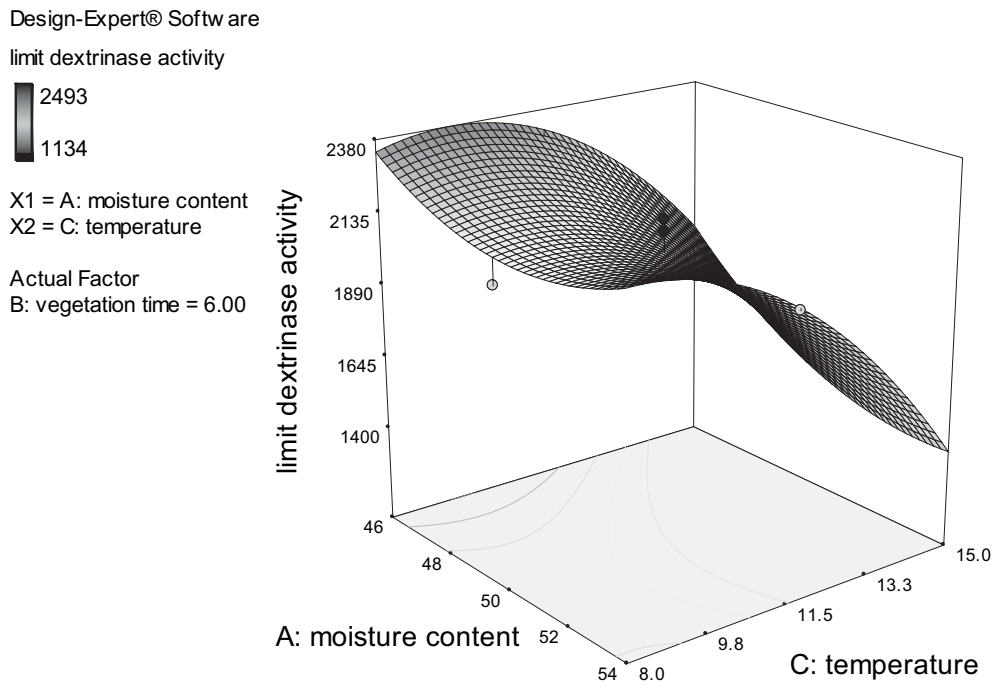

Fig. 3 The influence of moisture content and germination temperature on limit dextrinase activity with a germination time of 6 days

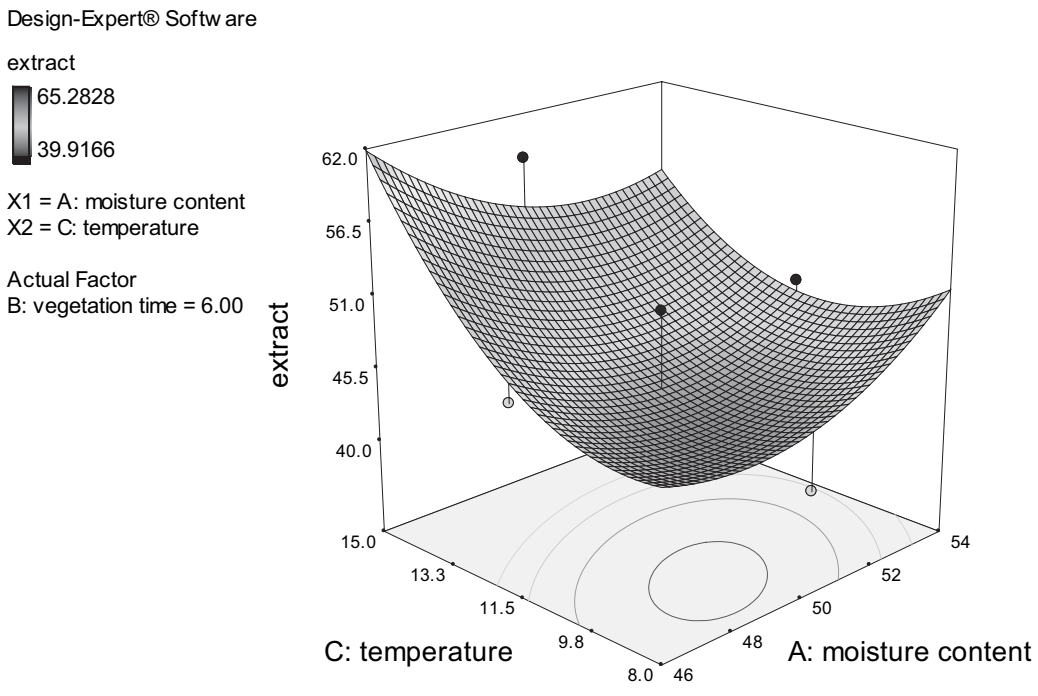

Fig. 4 The influence of steeping moisture content and germination temperature on extract with a germination time of 6 days

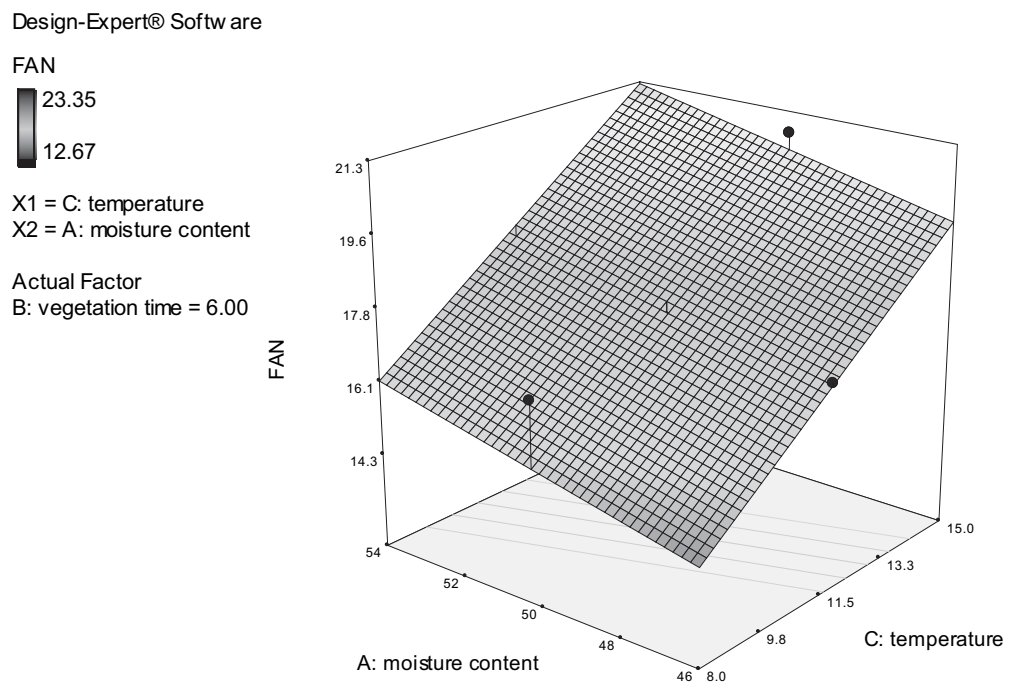

Fig. 5 The influence of moisture content and germination temperature on FAN with a germination time of 6 days

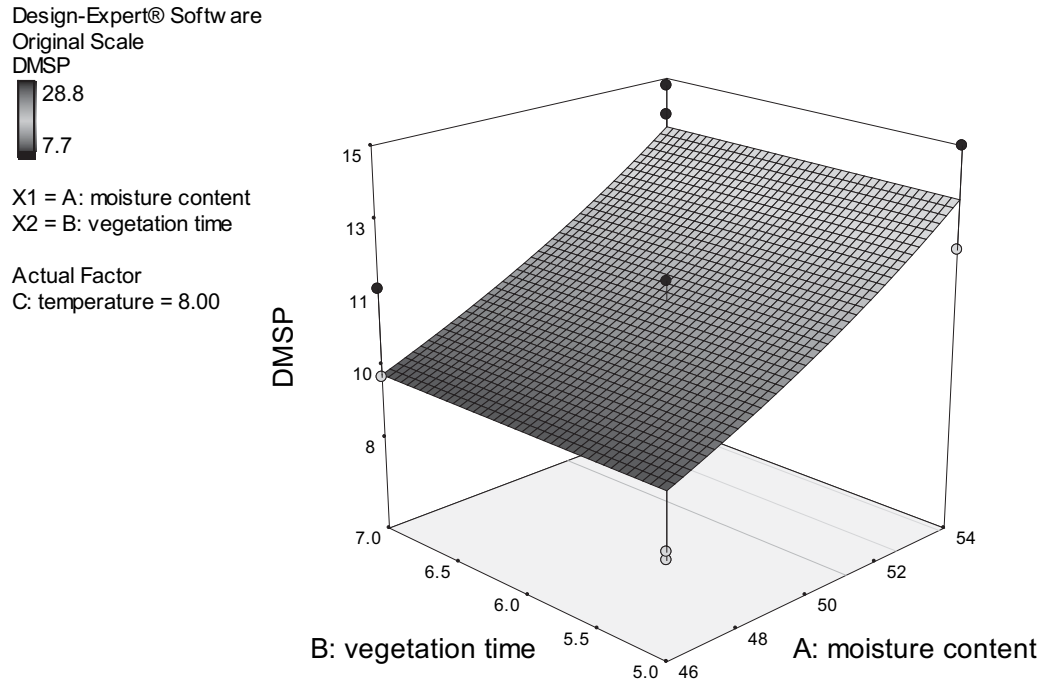

Fig. 6 The influence of moisture content and vegetation time on DMSP with a germination temperature of 8 °C