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Malt volatile compounds (Part I)

The measurement of the malt volatiles targets Maillard-products, Strecker-aldehydes, higher alcohols and compounds of the lipid metabolism of barley. These substances act as indicators for a multiplicity of technological factors, like proteolysis or thermal load during kilning. The effects of using malts with different loads of volatile compounds for beer production will be discussed in Part II of this publication series. This paper shows the improved method of analysis for these substances and the influence of various malting parameters on the formation of the volatile compounds using a statistically planed experimental design termed Response Surface Methodology. The differences between the formation of the volatiles within typical maltings parameters is very large and concentrations thus differ up to a factor 26. If one considers only malts within brewing specifications a factor of up to 7 still remains. Green malt moisture and germination temperature are the main influencing factors; high germination temperatures are suited to produce malt with low volatile concentrations if higher malting losses are acquiesced.

Descriptors: malt, malting, flavour stability, Maillard-products, Strecker-aldehydes, higher alcohols, lipid metabolism, analysis, gaschromatography, analytical method, volatile compounds, statistics, statistical methods, response surface methodology, green malt moisture, germination time, germination temperature.

1 Introduction

The measurement of the malt volatiles targets Maillard-products, Strecker-aldehydes, higher alcohols and compounds of the lipid metabolism of barley. These substances act as indicators for a multiplicity of technological factors, like proteolysis or thermal load during kilning [1, 2, 3, 4]. The effects of using malts with different loads of volatile compounds for beer production will be discussed in Part II of this paper.

This paper shows the method of analysis and results obtained from experimental maltings under various conditions and their effects on the malt volatile compounds.

2 Analysis

The analysis is based on the various water vapour distillation methods published by *Mebak* [5].

Principle

The sample's volatile compounds are expelled by water vapour distillation. The ethanolic distillate is alkalized and furthermore being saturated with NaCl. The volatile compounds are then extracted via Dichloromethane; the volume of the organic phase is further on reduced by a nitrogen flow.

The addition of ammoniac is used to separate organic acids as they are often accountable for coelutions with relevant substances.

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Tables and figures see Appendix

2.1 Instruments and Materials

Instruments

Hewlett Packard HP 5890 with Split- /Splitless-injector, 2 capillary columns (HP Innowax (Polyethylene Glycol) 60 m * 0.20 mm * 0.40 µm; HP Ultra 2 (5 % Ph.- 95 % Me-Si) 60 m * 0.20 mm * 0.33 µm) and 2 flame ionization detectors.

Hewlett Packard HP 7673 A Automatic Sampler

Heraeus-centrifuge with cooling: Varifuge RF

Turbula-shaker

Distillation unit: Büchi K-314

Chemicals

All chemicals used were of GC- or p.a. quality. Suppliers: Sigma-Aldrich, Roth, Riedel-de Haën, Merck.

Auxiliary materials

Glycerinmonostearate (ICN-Biochemicals No. 195334) as Anti-foam

Dichloromethane p.a. (Riedel-de Haën, No. 3222), redistilled NaCl p.a. (Merck 6404.5000)

Ammonia 25 % w/w p.a. (Merck 5432.5000)

Nitrogen 5.0 (Linde)

Internal Standard

The internal standard solution consists of butanoic acid methyl ester (Sigma-Aldrich, 14.8 mg/l) and heptanoic acid methyl ester (Sigma-Aldrich, 10.9 mg/l) in Ethanol p.a.

2.2 Sample Preparation

Cold extraction of the sample

50 g freshly milled grist is weighed into a beaker and 200 ml H_2O dest. at 20 °C is added. The mash is then stirred for 30 min. at

250 U/min^{-1} on a magnetic stirrer. The whole mash is then being transferred into a tumbler and centrifuged for 20 min. at 20 °C with 9000 U/min^{-1}. The supernatant is decanted into a 150 ml volumetric flask.

Water vapour distillation

6.5 ml Ethanol p.a. and 1 ml ISTD are added to the volumetric flask. A spatula's tip of antifoam is provided in a distillation tumbler; the content of the volumetric flask is completely poured into this distillation tumbler and distilled afterwards. 100 ml of the distillate is gathered in an ice-cooled volumetric flask. After thorough homogenization 20 ml of the distillate are removed (20 ml volumetric pipette).

Extraction

22.5 g NaCl are weighed into a screw top tumbler. The remaining 80 ml of distillate, 4 ml 25 % w/w Ammonia and 1 ml Dichloromethane are added; the tumbler is tested for tightness. The tumbler is being shaked for 30 min. in the Turbula-shaker and subsequently centrifuged for 15 min. at 0 °C and 2400 /min^{-1}.

After siphoning off some parts of the aqueous phase with a water jet pump, the organic phase (in the form of Dichloromethane-bead) is transferred into a 1 ml vial with the aid of a Pasteur-pipette. The organic phase is then reduced to ~150 µl by a nitrogen flow and transferred into a conus vial.

2.3 Gaschromatographtic Conditions

Table 1 shows the gaschromatographic conditions.

2.4 Calibration

The calibration is done by addition of the reference substances in six different concentrations and reporting of the relative peak areas. The added concentrations are plotted over the corresponding relative peak areas. Evaluation is done by linear regression analysis. The slope of the regression graph then denotes the calibration factor of each respective substance.

2.5 Reproducibility

Table 2 shows the reproducibility of the analysis as coefficients of variation (CoV) with n = 10.

3 Malt volatile compounds in dependency of the malting parameters

Two authentic charges of each a spring and winter barley were put on trial under various malting conditions [6] using the Response Surface Methodology as provided by the DesignExpert™ Software (see 3.1) and analysed for the resulting malt volatile contents.

3.1 Experimental Design and Analysis

The following malting parameters were varied within the noted ranges: germination temperature (12 to 18 °C), germination time (5 to 7 days) and green malt moisture (42 to 48 %). Using these limits the experimental design was created with DesignExpert™ Software Version 6.0.11 (StatEase Ltd, Minneapolis, MN, USA). A Central Composite Design (CCD) for the three numeric factors resulted in 24 individual maltings (see Table 3) for the 2 barley varieties each.

Each individual resulting response (malt volatile compounds content) was analysed statistically using the DesignExpert™ Software. This was done according to the rules of the Handbook for Experimenters provided by StatEase Ltd [7]. The process was well presented by *Brown* and *Hammond* [8].

The following graphs do not show the analytical results but rather the values given by the mathematical models. The models are calculated by multidimensional regression analysis/ANOVA. The models are then verified by various diagnostic tools that they satisfy the assumptions of the ANOVA (e.g. outliers, autocorrelation, normal distribution of residues). The models have been verified by further maltings using randomly selected malting parameters within the range given in Table 3; the analytical results of this maltings do not deviate more than 10 % from the mathematical prediction.

3.2 Results

For each the Maillard-products, Strecker-aldehydes, higher alcohols and the compounds of the lipid metabolism typically the most strongly concentrated substance of those groups has been chosen to depict the results of the malting experiments, unless there were significant differences within these groups.

Figures 1 and 2 show the variation of the 3-Methylbutanal, representing the Strecker-aldehydes for barley variety A in dependency of the maltings conditions.

Both the germination temperature and the green malt moisture influence the formation of this substance greatly. Higher green malt moisture significantly increase the formation, whilst high germination temperatures lower the resulting contents. Longer germination times only slightly decrease the aldhyde content. It may be assumed that the effects of both high germination temperature and longer times can be explained by the increased growth of the rootlets and the sprout under these conditions. All other Strecker-aldehydes behave similarly to 3-Methylbutanal.

Variety B shows an identical behavior regarding Strecker-aldehydes, however reaches higher values (see Table 4). The effect of germination time is even less distinct in variety B than in variety A.

Figure 3 shows the dependency of 3-Methylbutanol, representing the higher alcohols in the malt, of barley variety A in dependency of the germination temperature and green malt moisture. Germination time has no influence on any of the measured alcohols within the given range.

The interdependencies between the formation of higher alcohols and both the germination temperature and the green malt moisture are similar to those of the Strecker-aldehydes; the damping effect of higher germination temperatures is, however, less distinct.

Variety B again displays similar correlations between the malting parameters and the higher alcohol content, however also reaches higher concentrations in this substance group.

In unison with the substance groups of the higher alcohols and Strecker-aldehydes the Maillard-products, here represented by 2-Furfural, demonstrate the same dependencies in variety B.

Again higher values of the green malt moisture result in increased amounts, whilst higher temperatures suppress the formation. The germination time has only little effect (see Figure 4).

Variety A also displays the usual interdependencies at a germination temperature of 12 °C (see Figure 5). The results at 18 °C however deviate considerably, especially as they show a maximum at a green malt moisture of 42 %. It is currently not clear why this single model differs that much.

Volatile fatty acid products on the other hand show a slightly different behaviour within these malting parameters than the other three substance groups. In both varieties some fatty acid degradation products, namely tr-2,cis-6-Nonadienale and gamma-nonalactone, do have a minimum at a green malt moisture of roughly 45 % (see Figure 6), whilst others, e.g. Hexanal (see graph 7) and tr-2-Nonenal, behave exactly like the Strecker-aldehydes.

Within the malting parameters the variation of the volatile compounds has been very large (see Table 4). 3-Methylbutanal e.g. differed by a factor of 26 within the given settings; the differences in the concentrations of the other substance groups were also very high and typically they showed a factor of 7–10.

Variety B typically achieved higher volatile compounds. This however does not mean that this variety has a higher formation potential. Due to its obviously higher enzymatic potential the germination and thus the formation of the volatile compounds precursors proceeded faster than variety A. On the contrary, variety B had lower volatile compounds at a comparable Kolbach-index (see Table 5).

3.3 Summary

It is clear, that a comparison between just two malts is not representative and thus a longer study, taking also different harvests into account, would be preferable. The variation of the resulting volatile compound concentrations is however very distinct; clear results in a further study are therefore to be expected.

The malt volatile compounds directly contribute to the concentration of wort volatile compounds in the kettle-full wort (Pfanne-Voll-Würze) and consequently to the wort quality and to some degree to flavour stability. Results obtained with the newly developed analysis for malt volatile compounds allows following conclusions:

- The colour of the malt does not correlate with the content of the malt volatile compounds in pale malts (data not presented); colour is therefore not a good indicator for the measured substance groups.
- With similar Kolbach-indices there are relatively large differences in the malt volatile contents; the Kolbach-Index is thus only a weak indicator for the measured substance groups.
- Since the content of the substance groups varies with a factor of up to 26 (5–7 if only malts are considered which are within brewing specifications) within the given malting parameters, it is clear that the malting conditions can be used to control the content of this substances.
- Strecker-aldehydes and higher alcohols show mostly a strong dependancy on the green malt moisture.
- High germination temperatures can reduce the content of all measured substance groups; this would however lead to higher malting losses. Higher germination temperature could thus be considered for “premium” malts with low volatile content.
- The germination time has no or very little effect on the measured substance groups. Shortening the germination time even further is expected to be not negligible.

4 Perspective

The presented method of analysis will contribute to the further enhancement of flavour stability in beer, as the results in Part II will show.

The results from the malting however will allow to create premium malts with low concentrations of Strecker-aldehydes, Maillard-products and fatty acid degradation products content, if one takes higher malting losses into account.

5 Literature

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Appendix

Table 1 Gaschromatographic conditions

		Temperatures	Flow rates
Injector	Pressure: 190 kPa	250 °C	
Injection volume	4 µl		
Carrier gas	Hydrogenium 5.0		1.9 ml/min.
Septum-Purge			1.0 ml/min.
Split	01:07		
Capillary column I	HP Innowax (Polyethylene Glycol) 60 m * 0.20 mm * 0.40 µm	4 min.: 50 °C 4 °C/min. to 210 °C 36 min.: 210 °C	
Capillary column II	HP Ultra 2 (5 % Ph.- 95 % Me-Si) 60 m * 0.20 mm * 0.33 µm	4 min.: 50 °C 4 °C/min. to 210 °C 36 min.: 210 °C	
Detector	2 x FID	250 °C	
Detector gases	Hydrogenium 5.0 Synthetic Air Nitrogenium 5.0 (Make-up-Gas)		31 ml/min. 300 ml/min. 40 ml/min.
Reporting	Area modus with ISTD		

Table 2 Coefficients of variation

Substance	CoV [%]	Substance	CoV [%]
3-Methylbutanal	3.5	Methional	6.6
2-Methylbutanal	3.9	Hexanal	6.2
2-Pentanone	n.a.	Benzaldehyd	9.8
Pentanal	3.5	trans-2,cis-6-Nonadienal	8.8
3-Methylbutanol	4.3	trans-2-Nonenal	10
2-Methylbutanol	3.5	Pentanol	3.0
2-Acetylfuran	n.a.	Octen-3-ol	4.3
Phenylethanal	6.4	Octanol	5.1
γ-Nonalacton	5.5	Isobutyraldehyde	8.1
2-Furfural	3.9	Phenylethanol	11
Heptanal	4.1		

n.a. = not analyseable

Table 3 Malting conditions

Green malt moisture [%]	Germination temperature [°C]	Germination time [d]	Green malt moisture [%]	Germination temperature [°C]	Germination time [d]
48	12	7	48	15	6
48	12	7	45	12	6
48	18	7	45	15	6
45	15	7	48	18	5
42	18	7	42	12	5
42	12	7	48	12	5
42	18	7	48	18	5
48	18	7	42	12	5
42	12	7	42	18	5
45	15	6	45	15	5
45	18	6	42	18	5
42	15	6	48	12	5

Table 4 Minimum/Maximum values of selected compounds

	A		B	
[µg/kg]	min	max	min	max
3-Methylbutanal	93	2662	501	3462
2-Furfural	27	509	46	504
3-Methylbutanol	133	768	390	1218
trans-2,cis-6-Nonadienal	15	116	17	79

Table 5 Volatile compounds content at a comparable Kolbach-Index (42 %)

at comparable Kolbach-index [µg/kg]	A	B
3-Methylbutanal	2585	1741
2-Furfural	477	185
3-Methylbutanol	768	696
trans-2,cis-6-Nonadienal	116	53

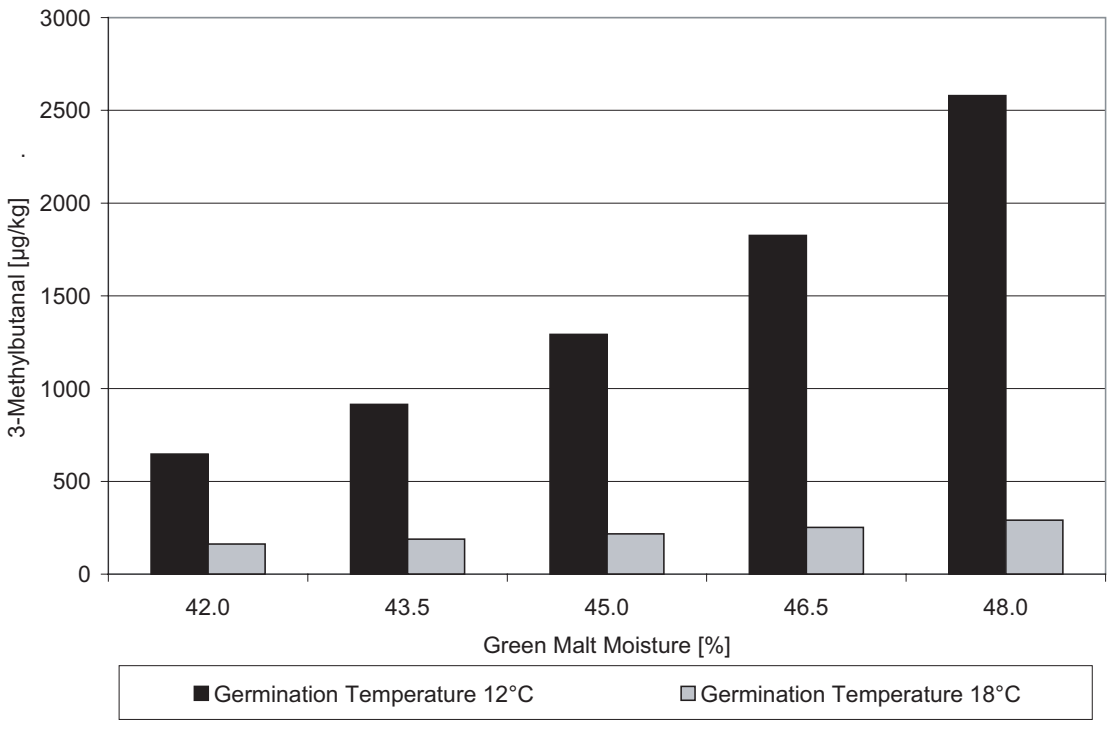

Fig. 1 3-Methylbutanal, Variety A, Parameters: green malt moisture and germination temperature, 6 days germination time

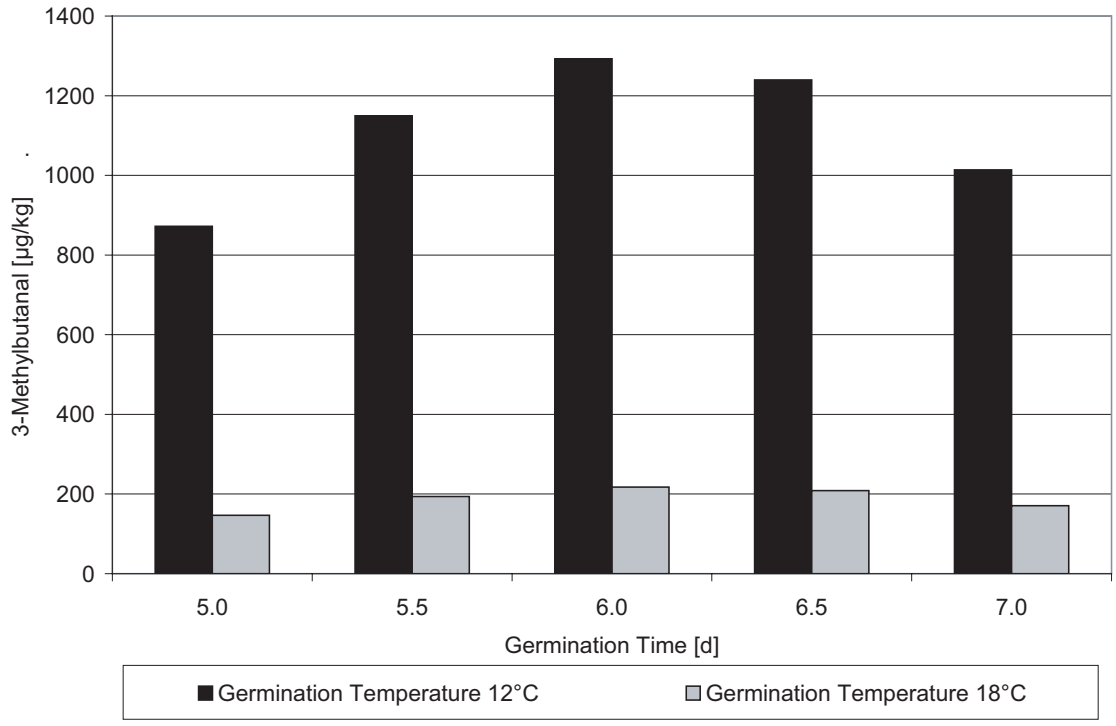

Fig. 2 **3-Methylbutanal, Variety A, Parameters: germination temperature and germination time, 45 % green malt moisture**

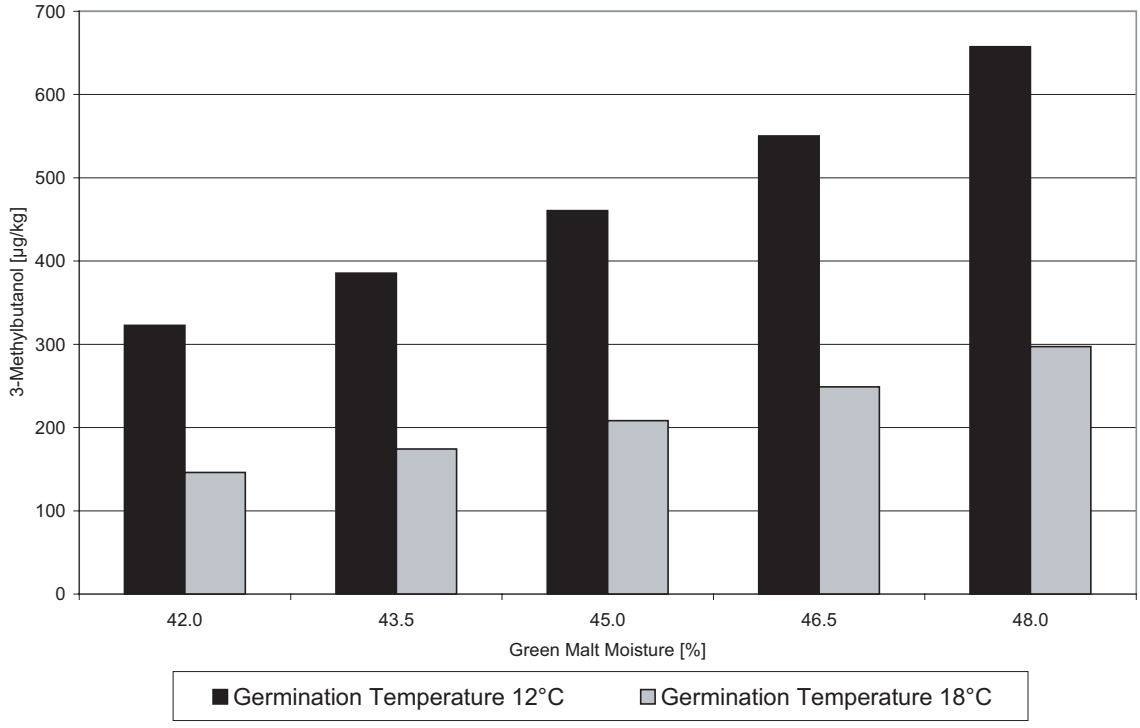

Fig. 3 **3-Methylbutanol, Variety A, Parameters: green malt moisture and germination temperature, 6 days germination time**

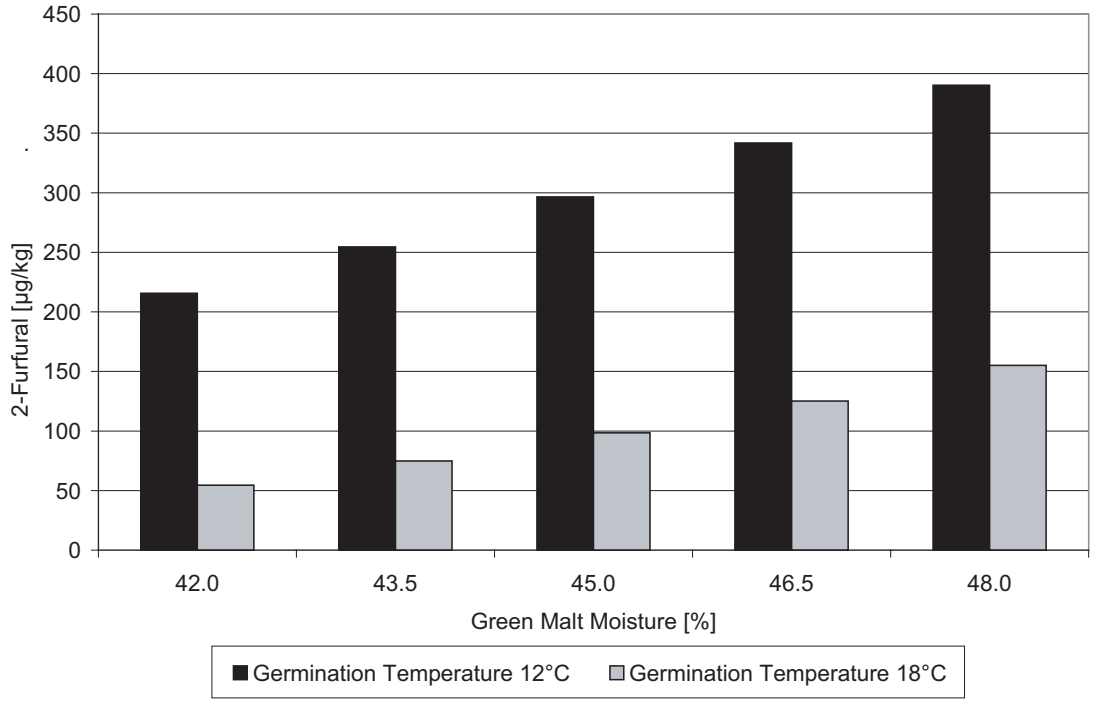

Fig. 4 **2-Furfural, Variety B, Parameters: green malt moisture and germination temperature, 6 days germination time**

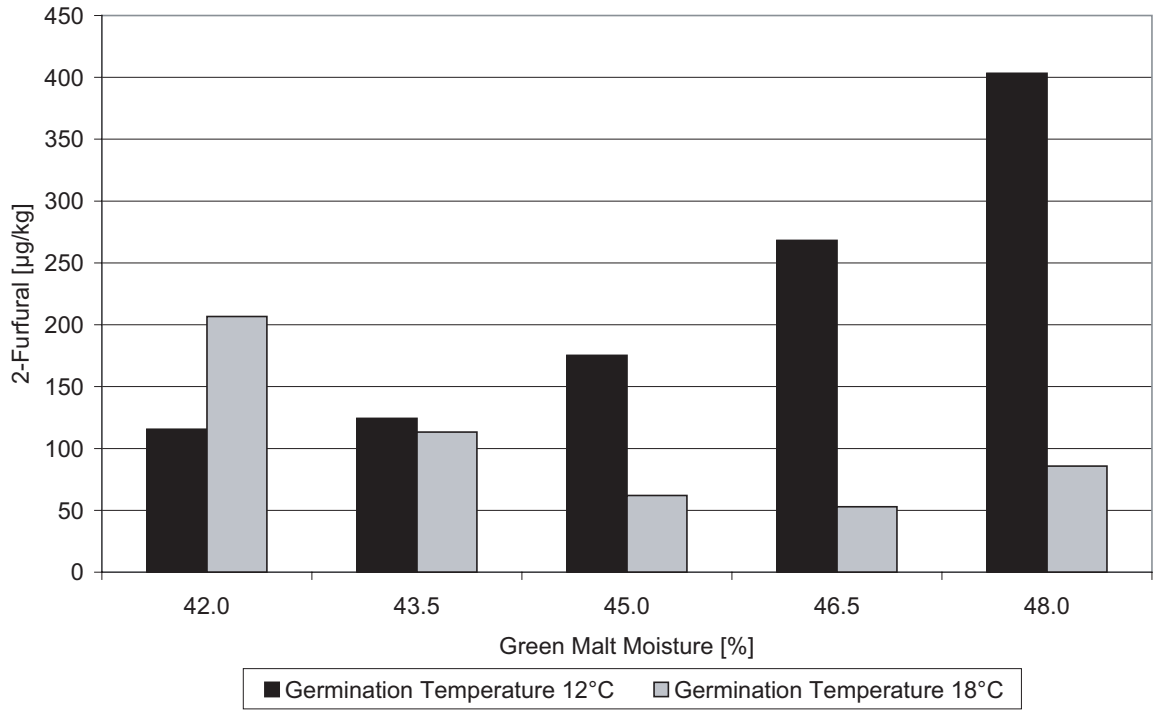

Fig. 5 **2-Furfural, Variety A, Parameters: green malt moisture and germination temperature, 6 days germination time**

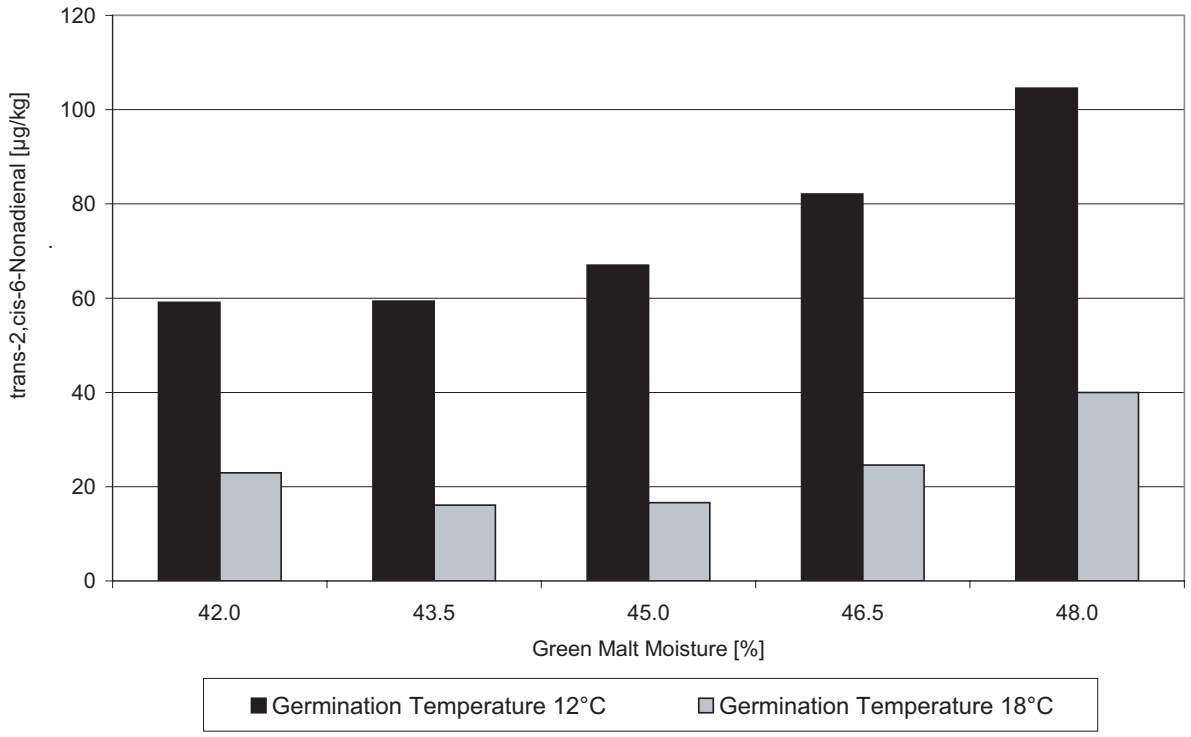

Fig. 6 **tr-2,cis-6-Nonadienale, Variety A, Parameters: green malt moisture and germination temperature, 6 days germination time**

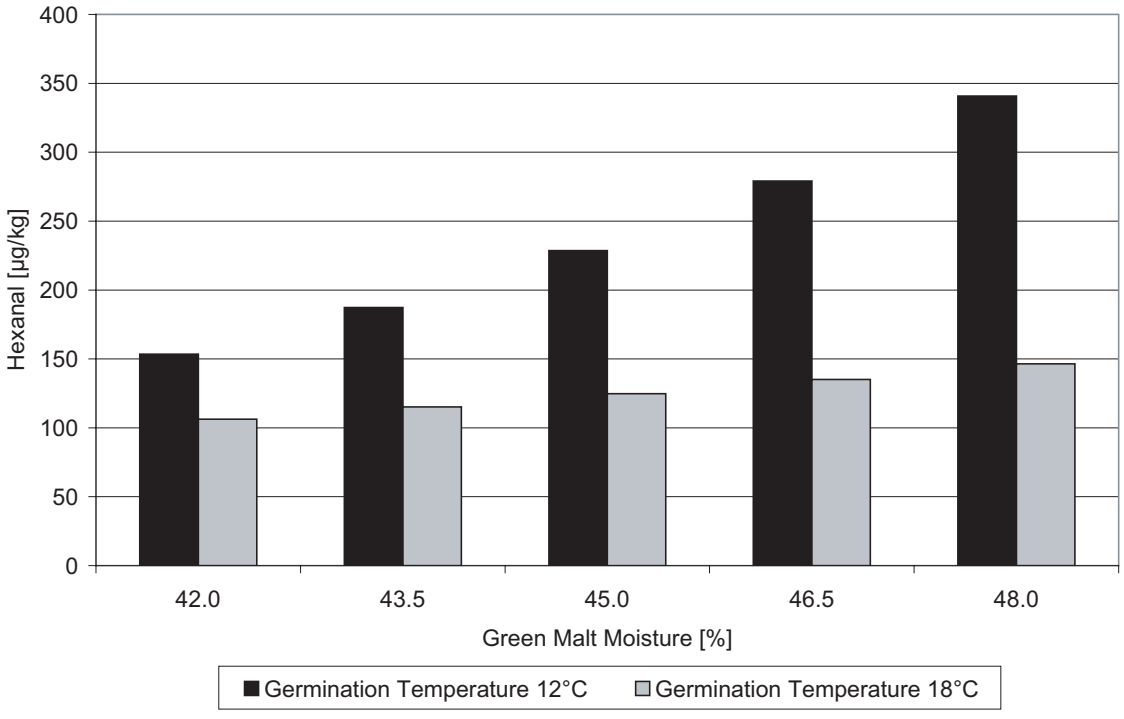

Fig. 7 **Hexanal, Variety A, Parameters: green malt moisture and germination temperature, 6 days germination time**