DIPLOMA THESIS

STUDIES ON THE FATE OF HOP COMPONENTS APPLIED IN MAIZE STARCH PRODUCTION

aimed degree

Master of Science (Mag. rer. nat.)

Author:	Cornelia Egartner
Matriculation number:	0104091
Discipline of study:	Nutritional Sciences
Supervisor:	Univ.Prof. Dipl.-Ing. Dr. Emmerich Berghofer

Vienna, 13/09/2008

ACKNOWLEDGEMENT – DANKSAGUNG

Für die Übernahme des Themas und die Korrektur meiner Diplomarbeit danke ich Herrn Prof. Emmerich Berghofer vom Institut für Lebensmitteltechnologie der Universität für Bodenkultur Wien. Besonders danken möchte ich Herrn Dr. Hein für die Betreuung vor Ort, in der Zuckerforschung Tulln und seine zahlreichen wissenschaftlichen Ratschläge und Anregungen.

Mein herzlichster Dank gilt Frau Ing. Sabine Schön, dass ich auf „ihren heiligen Anlagen" arbeiten konnte und sie mir immer mit Rat und Tat zur Seite stand; Herrn DI Florian Emerstorfer, für zahllose hilfreiche Hinweise und Korrekturen und die Motivation meine Arbeit in englischer Sprache zu verfassen; sowie Herrn (Ing. in spe) Christoph Odwody, für seine Geduld und Erklärungen sowie die hilfreichen Tipps während meiner gesamten Diplomarbeitszeit.

Außerdem danke ich allen „guten Geistern" in der Zuckerforschung, die meine Arbeit in Tulln durch ihre Hilfe und freundschaftliche Aufnahme sehr angenehm verlaufen haben lassen.

Ich möchte mich auch bei Herrn Dr. Markus Posch vom Wellcome Trust Biocentre der Dundee University in Schottland für die Durchsicht meiner englischen Arbeit bedanken.

Meinen beiden Studienkolleginnen, Frau Martina Galli und Frau Christine Huber danke ich für die vielen konstruktiven Gespräche während meiner Studienzeit und die gemeinsame Durchführung „unserer" Diplomarbeit in der Zuckerforschung Tulln.

Mein größter Dank gebührt meinen Eltern, Herrn Franz Egartner und Frau Carmen Egartner, die mir herausragende Vorbilder waren und sind. Auch dafür, dass sie mich immer, nicht nur bei meiner Ausbildung, unterstützt und motiviert haben.
Last but not least: Ein unbeschreibliches Dankeschön gilt meinem besten Freund, Herrn Roman Valent, der in beispielloser Freundschaft und Geduld mich und das Zustandekommen meiner Arbeit unterstützt hat.

TABLE OF CONTENTS

1 INTRODUCTION 3

1.1 Starch 4
1.1.1 General overview of starch processing 4
1.1.2 Microbial troubles and remedies in starch processing 5

1.2 Hops 6
1.2.1 The role of hop acids in the brewing industry 6
1.2.2 Application of hop extracts outside the brewing industry 7
1.2.2.1 Application in the sugar industry 7
1.2.2.2 Application in the ethanol production 8
1.2.3 Manufacture of hop extracts 9
1.2.3.1 Hop extraction with ethanol 9
1.2.3.2 Hop extraction with carbon dioxide 10
1.2.3.3 Isomerised hop extract 11
1.2.3.4 Tetrahydroiso-extract 12
1.2.4 Chemistry of hop acids 13
1.2.4.1 α-acids (humulones) 13
1.2.4.2 β-acids (lupulones) 14
1.2.4.3 Iso-α-acids (IA) 15
1.2.4.4 Tetrahydroiso-α-acids (THIA) 15
1.2.5 Hop analytics 17
1.2.5.1 Extraction methods 17
1.2.5.2 Chromatographic conditions 18
1.2.5.3 HPLC-analysis of THIA, IA and β-acids 19

1.3 Pilot plant and starch production in ZFT 20

2 OBJECTIVES 26

3 MATERIALS AND METHODS 27

3.1 Raw materials 27
3.1.1 Raw materials from Aschach 27
3.1.2 Raw materials produced in the pilot plant of ZFT 27

3.2 Hop products and standards 27

3.3 HPLC equipment 29

3.4 Sample pre-treatment methods 30
3.4.1 Soxhlet extraction 30
3.4.1.1 Equipment and utensils: 30
3.4.1.2 Reagents and solutions: 31
3.4.1.3 Sample preparation: 31
3.4.2 Shaking out with pH-adjustment and single solvent 32
3.4.2.1 Equipment and utensils: 32
3.4.2.2 Reagents and solutions: 32
3.4.2.3 Sample preparation: 33
3.4.3 Shaking out with solvent mixture 34
3.4.3.1 Equipment and utensils: 34
3.4.3.2 Reagents and solutions: 34
3.4.3.3 Sample preparation: 35
3.4.4 Liquid – liquid – extraction 36
3.4.4.1 Equipment and utensils: 36

3.4.4.2 Reagents and solutions: 36
3.4.4.3 Sample preparation: 37
3.4.5 Solid phase extraction 38
3.4.5.1 Equipment and utensils: 38
3.4.5.2 Reagents and solutions: 38
3.4.5.3 Sample preparation: 39
3.4.6 Extract by stirring 40
3.4.6.1 Equipment and utensils: 40
3.4.6.2 Reagents and solutions: 40
3.4.6.3 Sample preparation: 41

3.5 Chromatographic methods 42
3.5.1 Standard preparation and addition 42
3.5.1.1 Equipment and utensils: 42
3.5.1.2 Reagents: 42
3.5.1.3 Preparation: 43
3.5.1.4 Addition: 43
3.5.2 Preparation of the mobile phase 44
3.5.2.1 Equipment and utensils: 44
3.5.2.2 Solvent reagents: 44
3.5.2.3 Preparation: 44
3.5.3 Chromatographic conditions 45
3.5.3.1 Standard application for THIA (isocratic elution) 45
3.5.3.2 Special application for THIA (gradient elution) 45
3.5.3.3 Standard application for IA (isocratic elution) 46
3.5.3.4 Standard application for β-acids (isocratic elution): 46
3.5.4 Calibration 46

4 TEST PROCEDURE 47

4.1 Preliminary trials 47
4.1.1 Standard addition trials with THIA 47
4.1.1.1 Determination of active ingredients of THIA 47
4.1.1.2 Starch 48
4.1.1.3 Maize feed 49
4.1.1.4 Gluten 50
4.1.1.5 Germs 50
4.1.1.6 Steep water 51
4.1.2 Standard addition trials with β-acids 52
4.1.3 Preliminary trials with IA 53

4.2 Determination of LOD and LOQ 54

4.3 Main trials with pilot plant products 55
4.3.1 Overview of pilot trials 55
4.3.2 Analysis of pilot plant products 55

5 RESULTS AND DISCUSSION 57

5.1 Preliminary trials 58
5.1.1 Standard addition trials with THIA 58
5.1.1.1 Starch 58
5.1.1.2 Maize feed 61
5.1.1.3 Gluten 64
5.1.1.4 Germs 66
5.1.1.5 Steep water 68
5.1.2 Standard addition trials with β-acids 70
5.1.2.1 Starch 70

5.2 LOD and LOQ of THIA-standard method 71

5.3 Main trials ____ 73
5.4 Balancing ____ 76
6 CONCLUSIONS ____ 78
7 SUMMARY ____ 79
8 KURZZUSAMMENFASSUNG ____ 80
9 REFERENCES ____ 81
10 APPENDIX ____ 86

INDEX OF TABLES

Table 1: Extraction trials with starch, using THIA ____ 49
Table 2: Extraction trials with maize feed, using THIA ____ 49
Table 3: Extraction trials with gluten, using THIA ____ 50
Table 4: Extraction trials with germs, using THIA ____ 50
Table 5: Extraction trials with steep water, using THIA ____ 51
Table 6: Extraction trials with starch, using β-acids ____ 52
Table 7: Pilot trials - overview ____ 55
Table 8: Results - Soxhlet No. 1 (starch) ____ 58
Table 9: Results - stirring without pH adjustment (starch) ____ 59
Table 10: Results - stirring with pH-adjustment (starch) ____ 59
Table 11: Results - Soxhlet No. 2 (starch) ____ 60
Table 12: Results - Soxhlet No. 1 (maize feed) ____ 61
Table 13: Results - stirring (maize feed) ____ 62
Table 14: Results - stirring with solvent mixture (gluten) ____ 66
Table 15: Results - stirring with B (germs) ____ 67
Table 16: Results - shaking out at pH 3 (steep water) ____ 69
Table 17: Calculation of LOD and LOQ for standard THIA-application ____ 72
Table 18: Summary of all pilot trial-results ____ 73
Table 19: Mass balance – pilot plant (maize) ____ 76

INDEX OF FIGURES

Fig. 1: Pressure temperature equilibria for carbon dioxide (BENITEZ et al., EBC Manual of Good Practice, 1997) 9

Fig. 2: Liquid carbon dioxide extraction with two extractors (BENITEZ et al., EBC Manual of Good Practice, 1997) 10

Fig. 3: The three main α-acids (GOLDSTEIN and TING in EBC Hop Monograph XXII, 1994) 13

Fig. 4: The three main β-acids (GOLDSTEIN and TING in EBC Hop Monograph XXII, 1994) 14

Fig. 5: The six main iso-α-acids (VANHOENACKER et al., 2004) 15

Fig. 6: Isomerisation of α-acids into iso-α-acids (HOFFMAN and JARVIS, 2005) 15

Fig. 7: The six main tetrahydroiso-α-acids (VANHOENACKER et al., 2004) 16

Fig. 8: Flow sheet of the maize starch pilot plant (ZFT, 2007) 20

Fig. 9: Steep tank (ZFT, 2007) 21

Fig. 10: Attrition mill (ZFT, 2007) 22

Fig. 11: Cyclone (ZFT, 2007) 22

Fig. 12: Pin mill (ZFT, 2007) 23

Fig. 13: Centrisieve (ZFT, 2007) 23

Fig. 14: Bend screen (ZFT, 2007) 23

Fig. 15: Hydro-cyclone plant (ZFT, 2007) 24

Fig. 16: Centrifuge (ZFT, 2007) 25

Fig. 17: Dryer (ZFT, 2007) 25

Fig. 18: Heatable extraction plant during Soxhlet extraction 31

Fig. 19: Comparison of ICE-2 standard with β-acids extract 48

Fig. 20: Representative chromatogram of iso-α-acids (IA) 52

Fig. 21: Chromatogram of THIA and ICS-tetra-standard 53

Fig. 22: Representative chromatogram of THIA 57

Fig. 23: Representative chromatogram of stirring at pH 4 (starch) 59

Fig. 24: Representative chromatogram of Soxhlet No. 2 (starch) 60

Fig. 25: Representative chromatogram of Soxhlet No. 1 (maize feed) 61

Fig. 26: Chromatogram comparison - stirring with solvent mixture (maize feed) 63

Fig. 27: Representative chromatogram - stirring with solvent mixture (maize feed) 63

Fig. 28: Representative chromatogram - Soxhlet No. 1 (gluten) 64
Fig. 29: Representative chromatogram - SPE (gluten) 65
Fig. 30: Representative chromatogram - stirring with solvent mixture (gluten) 65
Fig. 31: Representative chromatogram - Soxhlet No. 1 with D (germs) 67
Fig. 32: Representative chromatogram - stirring with B (germs) 67
Fig. 33: Representative chromatogram - shaking out with H, pH adjusted (steep water) 68
Fig. 34: Representative chromatogram - stirring with hexane (β-acids) 70
Fig. 35: Pilot trial: 25 ppm on starch - compared to standard 0.25 ppm 74
Fig. 36: Pilot trial: 20 ppm on starch - compared to standard 0.25 ppm 74
Fig. 37: Pilot trial: 5 ppm on starch - compared to standard 2.5 ppm 74
Fig. 38: Pilot trial: 10 ppm on starch after 24 hrs. of steeping time - compared to standard 2.5 ppm 75
Fig. 39: Pilot trial: 20 ppm on maize feed - compared to standard 2.5 ppm 75
Fig. 40: Mass balance diagram of maize processing in the pilot plant of ZFT 77
Fig. 41: Representative chromatogram of Soxhlet No. 1 with H (starch) 86
Fig. 42: Representative chromatogram of Soxhlet No. 1 with P (starch) 86
Fig. 43: Representative chromatogram of stirring with D (starch) 87
Fig. 44: Representative chromatogram of Soxhlet No. 1 with H (maize feed) 87
Fig. 45: Representative chromatogram of stirring with solvent mixture (maize feed) 87
Fig. 46: Representative chromatogram of Soxhlet No. 1 with H (gluten) 88
Fig. 47: Representative chromatogram of Soxhlet No. 1 with D (gluten) 88
Fig. 48: Representative chromatogram of Soxhlet No. 1 with P (gluten) 88
Fig. 49: Representative chromatogram of stirring with solvent mixture (gluten) 89
Fig. 50: Representative chromatogram - Soxhlet No. 1 with P (germs) 89
Fig. 51: Representative chromatogram - Soxhlet No. 1 with H (germs) 89
Fig. 52: Representative chromatogram - Soxhlet No. 1 with D, dissolved in acetonitrile (germs) 90
Fig. 53: Representative chromatogram - shaking out with P, pH adjusted (steep water) 90
Fig. 54: Representative chromatogram - Liquid-liquid-extraction with D (steep water) 90

PREFACE

The present work shares a threepart project; implemented in ZFT (Zuckerforschung Tulln G.m.b.H.; Research and Development enterprise of the AGRANA AG) from March 2006 – September 2007, in cooperation with BETATEC hop products G.m.b.H.)

Part 1 – working title:
The influence of hop acids on the lactic acid formation during wet maize storage
(ZINSBERGER, master thesis, publication: 2008)

Part 2 – working title:
The effects of hop acids on the lactic acid formation in maize steeping
(HUBER, master thesis in process, implemented 2007)

Part 3 – title:
The fate of hop components applied in maize starch production
(Topic of the present work)

Based on the application of hop extracts in the sugar and bio-ethanol industry, an employment in the field of starch processing was considered worth examining. The usage aims at an extension of storage time of wet maize and microbial control of the fermentation in maize steeping. The key requirement concerning the application of new products is an unchanged starch quality. Therefore, material (starch and by-products) of part 1 and 2 was investigated. This summarises the main objectives of the project (ZFT and BETATEC 2007).

ABBREVIATIONS

B = butanol
CO_2 = carbon dioxide
D = dichloromethane
H = hexane
P = petroleum benzene
DCHA = dicyclohexylamine

THIA = tetrahydroiso-α-acids
IA = iso-α-acids

CSL = corn steep liquor
LSL = light steep liquor
SW = steep water
TW = tap water
PT = pilot trial
DM = dry matter
S = substance

HPLC = high performance liquid chromatography
RP = reversed phase
DAD = diode array detector
MS = mass spectrometry
ICS = international calibration standard
LLE = liquid-liquid-extraction
SPE = solid phase extraction

CV = coefficient of variance
SD = standard deviation
LOD = limit of detection
LOQ = limit of quantification

hrs = hours
min = minute(s)
m*A*U = milli Ampere Units
mbar = milli bar
MΩ = Mega Ohm
nm = nano meter(s)
μm = micro meter(s)
ppm = parts per million
rpm = rotations per minute

ZFT = Zuckerforschung Tulln

1 INTRODUCTION

In the 1990´s it was observed that hop components could help to antagonise and control thermophilic bacteria in sugar factories. Certainly, the most relevant application of hops has been in the brewing industry and that already for ages. Today hops are estimated as an essential raw material in brewing all over the world. Two properties of hops are of outstanding importance. These are, on the one hand the bittering quality and on the other hand its preservative effect on microorganisms. The hop resins consist of humulones and lupulones which account for the antiseptic action. Moreover, diverse hop derivatives are used by now for their ability in providing bitterness, flavour and foam stability.

In the meantime, appropriate solutions based on hop acids were developed. They were soon applied, not only in the sugar industry but also in the field of bio ethanol and baker's yeast production.

Based on these findings, an employment of various hop components in maize starch processing was considered. In the course of maize processing, sulphur dioxide is conventionally used to control the lactic acid formation. The hop acids should serve as additional or alternative possibility; especially in the production of organic starch where sulphur dioxide is not allowed.

Preliminary work was done in the area of wet maize storage and maize steeping, in laboratory and pilot scale. This should give information on which hop acids should be applied in which concentrations.

The aim of this work was to find out how the applied hop acids distribute on starch and by-products. This should be examined via HPLC-analytics and set-up of a mass balance sheet.

The current work is prefixed by a literature review about the field of starch processing, hop components and its analytics, as well as a short overview of the construction and procedures in the pilot plant used in ZFT.

1.1 Starch

1.1.1 General overview of starch processing

After cleaning of the grain, the important processing step of maize steeping follows, as otherwise dried maize kernels cannot be separated into their technological components. Therefore maize is steeped in water for about 30-50 hours at 48-50°C with addition of sulphur dioxide. The main objectives of maize steeping are the softening of the kernel and cleaving of disulfide bonds, to achieve an effective separation of the endosperm from germs and hulls, as well as of starch and gluten (TEGGE, 2004; BISS and COGAN, 1996).

The steeping water, which is separated from the maize after the steeping process, is concentrated to syrup-like consistency by evaporation and is then called corn steep liquor, abbreviated CSL (FRANK, 1992).

Maize steeping is followed by coarse grinding with attrition mills, which operate in such a way that germs stay as intact as possible. Afterwards the germs are separated by means of hydro cyclones. The germs are dehydrated, dried and utilised for oil production. The maize suspension is fine-ground by means of impact mills in such a way as to disrupt the cells, so that the starch residue can be separated.

After starch separation, the hulls are mixed with CSL and then dried to obtain the so called maize feed.

Prior to gluten separation the starch suspension has to pass a de-sanding cyclone, to remove sand which could damage any following machines. Separation of starch and gluten is done with separators and multi-cyclones following the principle of their different specific weights. The gluten gets dehydrated and dried afterwards.

The starch is refined in a multi-step cyclone plant to remove the remaining protein. The starch slurry gets dehydrated in centrifuges and dried. Drying is usually done by means of pneumatic dryers (TEGGE, 2004).

1.1.2 Microbial troubles and remedies in starch processing

In several processing steps of the starch production unwanted microbial pollutions occur. WATSON (1984) summarised that small populations of bacteria, yeasts and molds, which are colonising raw maize are able to multiply rapidly in aqueous systems. Also during wet maize storage enterobacteria, yeasts and molds can appear. Further information about the microbiology in wet maize storage is provided in the first part of this project (ZINSBERGER, diploma thesis in process, 2008).

Moreover, prevention of uncontrollable degradation processes during maize steeping is needed. For these purposes sulphur dioxide, which fulfils a chemical and a microbial function, is applied as a steeping agent. Presumably, it was first used to prevent growth of putrefactive organisms (BERGTHALLER and TEGGE, 1972; WATSON, 1984).

Sulphur dioxide, used to disintegrate the protein matrix by cleaving disulfide bonds, also acts to restrict unfavourable fermentation during steeping (SINGH and ECKHOFF, 1996; BISS and COGAN, 1996; BOLLING et al., 1973).

As a matter of fact a certain amount of lactic acid is considered beneficial for maize steeping. Several researchers found that lactic acid facilitates the starch-protein separation by obvious softening action and also leads to higher starch yields. Lactic acid also acts as a cell toxin for competitive and unwanted bacteria, but its excessive formation may cause difficulties in further processing steps, e.g. with separation of starch and gluten or saccharification. A further unwanted side-effect of higher lactic acid levels is that fermentable carbohydrates are lost; this concerns in particular the usage of fermentable starch within the bio-ethanol production (HAROS et al., 2004 and WATSON, 1984).

WILHELM and KEMPF (1980) raised the issue of further adequate steeping agents (e.g. formaldehyde, sodium benzoate and halogen compounds) in association with a study on the reduction of sulfur dioxide residues in maize starches. However, these substances hardly ever became important in praxis.

1.2 Hops

1.2.1 The role of hop acids in the brewing industry

The various compounds of hops contribute in a considerable manner to the taste, as well as the sensory characteristics of beer (WEISS et al., 2002).

Before times of pasteurisation started, hops were applied for their antimicrobial and preservative properties. The antiseptic effects of hops on microorganisms were examined by a multitude of researchers for ages. For example, already in 1937, SHIMWELL could appoint the great inhibitory effect of hop-β-acids.

SCHMALRECK et al. (1975) found out, that hop resins target the lipophilic cell membrane regions and SIMPSONS´ PhD. thesis (1993) described the mechanism of the inhibition of lactic acid bacteria by hop acids. He found out that hop acids dissipate the transmembrane pH gradient in such a way as to inhibit uptake of metabolisable sugar into bacteria cells. This happens due to intracellular fall and extracellular rise of pH.

Today, the full extent of the conserving properties of hops still remains the purpose of scientifically researches. Hops are added to beer, not only but especially because of their bittering flavour. This flavour is a result of wort boiling, where the hop α-acids are transformed into iso-α-acids. However, beer bittering can also be done by using pre-isomerised hop extracts, thus avoiding wort boiling. The role of the second group of hop acids, the β-acids is not afflicted that positive. The β-acids and their oxidation products are of minor importance and undesired in wort and beer as they can cause off-tastes and gushing problems (VERZELE and DE KEUKELEIRE, 1991). Beside the pre-isomerised hop extracts, reduced hop extracts, namely rho-iso-α-acids, tetrahydroiso-α-acids and hexahydroiso-α-acids are used in breweries all over the world. This is due to their improving of light and foam stability.

However, they are not allowed within the "German purity law", which states that only natural hop components are allowed in the brewing process (VANHOENACKER et al., 2004). Although hop is undoubtedly associated with the brewing industry, new application fields for hop derived extracts emerged in the recent years.

1.2.2 Application of hop extracts outside the brewing industry

Beside their long lasting use as bittering and conserving agents in the brewing industry, hop acids and their derivatives were found to be effective bacterial remedies in several application fields, like baker's yeasts or tooth pastes. Due to extensive research activities in the scopes of sugar and bio-ethanol production, these two application fields are discussed in more detail.

1.2.2.1 Application in the sugar industry

In 1991, the use of the conventional disinfectant formalin was abdicated in Agrana sugar factories, due to image reasons. In the following years the challenge turned up, to find an alternative substance to limit bacterial activity. In trials with new culture media, it was found that hop acids had influence on the growth of lactic acid bacteria (POLLACH et al., 1996 and HEIN et al., 2006).

From that time on, intensive investigations started to elucidate the full potential of hop constituents for the sugar industry. At the beginning, a so-called base extract, a by-product of the isoextract manufacture was used. Later on, the application of β-acids in the sugar industry was applied for a patent and an improved solution, labelled BetaStab® 10 A, with a β-acid content of about 10 % was commercially produced. This was the starting shot for the application of hop β-acids in the sugar industry. Today, they are on second place after formalin in their application worldwide.

In a further study published by HEIN and POLLACH (1997) the issue of the fate of hop extracts in sugar and by-products was discussed. Repeated analytical investigations proved that only uncritical traces of the applied products could be found in sugar and molasses.

In subsequently carried out trials in South American cane sugar factories, it turned out that β-acids could possibly complete the effect of the locally applied sulphur dioxide or be a helpful backup if SO_2 cannot be employed. As a matter of completeness: in cane sugar factories an application of sulphur dioxide is still allowed, whereas the standard disinfectant in beet sugar factories is formalin. Although sulphur dioxide demonstrates a more cost-efficient mean to control

microbial infections, hop β-acids have the advantage to avoid corrosions as well as problems with the environment and safety (SAMARAWEERA et al., 2003).
Summing up, it can be declared that natural antibacterials derived from hops represent a valuable alternative, when conventional disinfectants are abandoned.

1.2.2.2 Application in the ethanol production

In the field of ethanol production RÜCKLE (2005) reported about the potential of hop acids to reduce bacterial contaminations which are primarily responsible for yield and quality losses.
For an economical production process it is essential that uncontrolled bacterial growth during fermentation is restricted. Otherwise the yeast is not able to convert the existing sugar into alcohol, which leads to processing troubles and reduced yields. Conventionally applied antibiotics (e.g. penicillin), have raised the issue of multiple resistances; hence it was important to find adequate alternatives, especially as by the end of 2005 the European Union demanded antibiotic exemption of fermentation by-products used as animal feed, e.g. DDGS (distillers dried grains with solubles).
Hop acids work selectively against lactic acid bacteria which cause troubles of capital importance during the fermentation and do not harm yeasts. In this study, there were controlled process conditions. In carbon dioxide atmosphere, temperatures of about 40°C and low pH values provided optimal growth conditions for lactic acid bacteria.
Furthermore RÜCKLE and SENN (2006) published a study aimed at the investigation of the inhibitory potential of hop acids against bacterial contaminations. Applying hop products containing iso-α-acids and tetrahydroiso-α-acids (IsoStab™ and LactoStab™) they found out, that their effectiveness is dependent on the their solubility as well as on the pH value, initial bacterial counts and solids contained in the mash. Although bad processing conditions were simulated, bacterial growth could be inhibited and ethanol yield improved.

1.2.3 Manufacture of hop extracts

In general, solvent extracts can be produced using hexane, ethanol as well as liquid and supercritical carbon dioxide. They can be non-isomerised, isomerised and isomerised reduced hop extracts, e.g. extracts containing tetrahydroiso-α-acids. Furthermore miscellaneous products are employed, like base hop fraction or purified beta fraction (BRIGGS et al., 2004).

Within the scope of this work, products containing β-acids, iso-α-acids and tetrahydroiso-α-acids were investigated. To produce a hop extract, several processing steps are necessary: milling, pelleting and re-milling of hops. Afterwards a solvent is conducted through a packed column and eventually the solvent is evaporated to gain a pure extract (MAGALHAES et al., 2007).

1.2.3.1 Hop extraction with ethanol

At the beginning of the last century, ethanol was an important mean to extract aromatic and bitter components from hops. It is a strong solvent, but with poor selectivity, since all the lupulin components, plant pigments, cuticular waxes, water as well as water-soluble materials are extracted. It is a continuous process where hops are extracted with 90% ethanol. The enriched ethanol solution, containing the hop components is then evaporated in a vacuum evaporating plant. The raw extract is separated into resin extract and hot water extract, which is rich in undesired substances like nitrates (KUNZE, 1999 and FORSTER, 1994).

According to MAGALHAES et al. (2007) and MOIR (2000) ethanolic hop extracts are becoming more and more marginal (percentage of 35% around 1990), due to high costs for solvent elimination and the risk to lose volatile components.

1.2.3.2 Hop extraction with carbon dioxide

Nowadays carbon dioxide (CO_2) is the most commonly used solvent for the production of hop extracts. The critical pressure of the gaseous component is at 73 bars, the critical temperature is at 31°C.

The advantages of this method are a high ratio of humulones to the less bitter lupulones, little hard resins and minor traces of triglycerides, waxes and inorganic salts.

Liquid CO_2 (below 73 bar and 31°C) as well as supercritical (fluid) CO_2 (above 73 bar and 31°C) can be used for hop extraction, as the phase diagram in Fig. 1 points out.

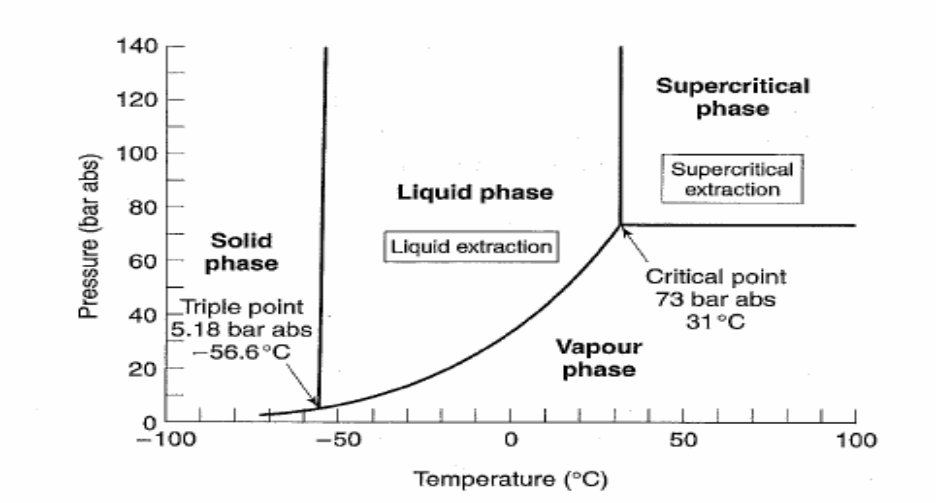

Fig. 1: Pressure temperature equilibria for carbon dioxide (BENITEZ et al., EBC Manual of Good Practice, 1997)

According to BRIGGS et al. (2004) the composition of the two extracts is not as fundamentally different as formerly assumed.

Compared to ethanol extracts, both CO_2 extracts represent fewer risks concerning unwanted substances like nitrates and pesticide residuals (MAGALHAES et al., 2007 and BRIGGS et al., 2004).

The liquid CO_2 extract (manufacturing conditions: 5-15°C and 60-65 bar, rather mild and sensitive solvent) is pale yellow, containing hop oils and soft resins, whereas the supercritical CO_2 extract (manufacturing conditions: 40-60°C and 200-250 bar,

a stronger solvent than liquid CO_2) is rather yellowish to green in colour and implies a wider spectrum of hop components (MOIR, 2000).

Fig. 2 shows the processing in a liquid CO_2-extraction plant.

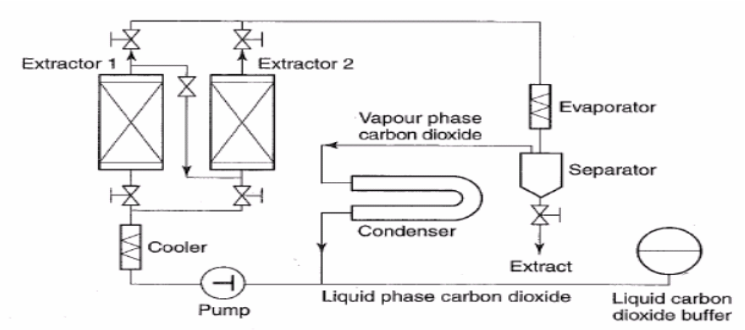

Fig. 2: Liquid carbon dioxide extraction with two extractors (BENITEZ et al., EBC Manual of Good Practice, 1997)

Both extraction modes are batch processes, only the cooler is replaced with a heat exchanger, when extracting with supercritical CO_2. In general the carbon dioxide passes through an extractor loaded with milled hop pellets. The carbon dioxide is then evaporated, condensed and returned to the extraction cycle, while the extract is collected in the separator. With two extractors a semi-continual operation is possible, where several extractors can be switched parallel or in series (FORSTER, 1994 and BRIGGS et al., 2004).

1.2.3.3 Isomerised hop extract

The first commercial production of isomerised hop extracts was carried out by Carlton United Breweries (Australia) in 1965, which produced an iso-α-acid-extract by heating the water-soluble calcium or magnesium salts of the α-acids. MOIR (2000) reported about attempts by 1980 to isomerise α-acid in CO_2 extracts directly in alkaline solution. By means of several pH-adjustments the β-acids and unisomerised α-acids can be precipitated and a pure iso-extract results.

The production of isomerised hop extract preferably starts with either a liquid or supercritical CO_2 extract that is heated and emulsified with degassed water.

These extracts have adequate purity to be directly isomerised without separation of the α-acids. During controlled isomerisation, the magnesium-ion serves as basic catalyst, where waxes and soft resins of no further use are removed. The cooled solution is adjusted to pH 7-8. At this pH the β-acids precipitate and can be removed afterwards. A further variation in pH leads to separation of unisomerised α-acids. Eventually, the isomerised α-acids are precipitated by pH reduction to 2, which are stored and formed to their potassium salts shortly before delivery (KUNZE, 1999).
FORSTER (1994) gives an account of the long term stability of isomerised extracts, pointing out that in storage trials (50°C for ten weeks) isomerised extracts only slightly differ from CO_2 extracts, produced from the same batch.
In summary can be said that isomerised hop extracts present the great advantage and comfort for brewers that they can control the beer bitterness in the final product.

1.2.3.4 Tetrahydroiso-extract

As already mentioned, there are two possibilities for the preparation of tetrahydroiso-extract from hop α- or β-acids acids. Therefore several patents have been filed concerning this topic.
Firstly HAY and HOMISKI (1991) proposed the preparation via hydrogenation of the β-acids to 4-desoxytetrahydro-α-acids, followed by oxidation to tetrahydro-α-acids and isomerisation to tetrahydroiso-α-acids.
Secondly TING et al. (2007) extracted hop acids in a homogenous solution system with low weight alcohols like ethanol, which are able to extract α- and iso-α-acids, respectively. In this work residues from two consecutive extractions were united. Best results were obtained, using a 95 % ethanolic solution. As further treatment ion exchange and alkali metal or metal ion precipitation were considered most suitable.

1.2.4 Chemistry of hop acids

Numerous studies provide a huge amount of information on hop and hop acids. Out of that some should be discussed in more detail, due to the appearance of some hop acids and their derivatives in the present work.

According to BRIGGS et al. (2004) non-specific fractions and specific compounds of hop resins can be distinguished. From the non-specific fraction, the total resins, differentiate into hard resins (insoluble in hexane) and soft resins (soluble in hexane, mainly α-acids and β-acids and uncharacterized soft resins). Enumerating all the specific compounds would go beyond the scope of this work. Hence, regarding the application of iso-α-acids (IA), tetrahydroiso-α-acids (THIA) and β-acids, their corresponding chemical structures will be discussed. Humulones represent the hop fraction, which precipitates after addition of lead(II) acetate in methanol.

The α-acids are a mixture of several homologues which are shown in Fig. 3.

1.2.4.1 α-acids (humulones)

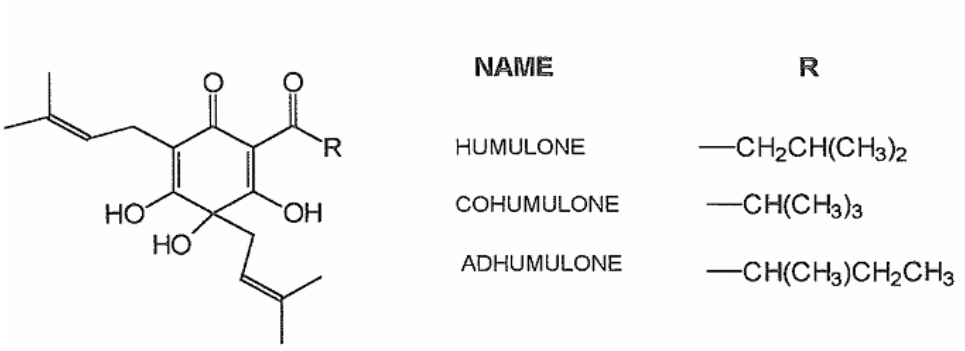

Fig. 3: The three main α-acids (GOLDSTEIN and TING in EBC Hop Monograph XXII, 1994)

This group contains three main α-acids (humulone, cohumulone and adhumulone) and two minor ones (pre- and posthumulone).

The constant percentage of 10-15 % of adhumulone faces a rather variable amount of n- (normal) humulone and cohumulone (20-70 %) which is dependent on the hop variety (VERZELE and DE KEUKELEIRE, 1991).

MOIR (2000) reviewing on hop chemistry, described the discovery of the final structure of humulone by De Keukeleire and Verzele in 1970. Furthermore he has terminated the NMR spectroscopy as most suitable demonstration media for the primary structure of humulone.

1.2.4.2 β-acids (lupulones)

For isolation of lupulones a hop extract has to be dissolved in hexane first. After extraction with disodium carbonate 0.3 N and sodium hydroxide 1 N, the β-acids are extracted with the stronger base, due to their less acidic character. Following an acidification and iso-octane extraction the solvent is evaporated. This leads to a crystalline powder, which contains a mixture of the β-acids. Fig. 4 reveals the three main beta acids (lupulone, colupulone and adlupulone), which represent over 95 % of all lupulones. The ratio of lupulone to co-lupulone is about 1 (VERZELE and DE KEUKELEIRE, 1991).

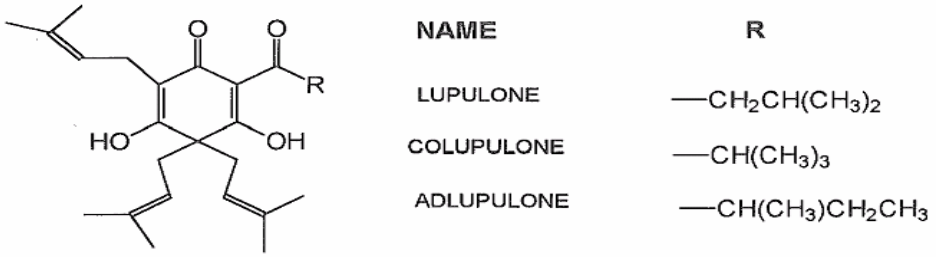

Fig. 4: The three main β-acids (GOLDSTEIN and TING in EBC Hop Monograph XXII, 1994)

1.2.4.3 Iso-α-acids (IA)

These derivatives of the α-acids, play an utmost important role as bittering agents in the brewing industry.

Each humulone leads to two epimeric isohumulones, resulting in six main isohumulones, cis- and trans-configurated, according to the three alpha acids (co-, n- and adhumulone); shown in Fig. 5. The key difference is that the iso-α-acids are much more water soluble than the precursing α-acids (HOFFMAN and JARVIS, 2005).

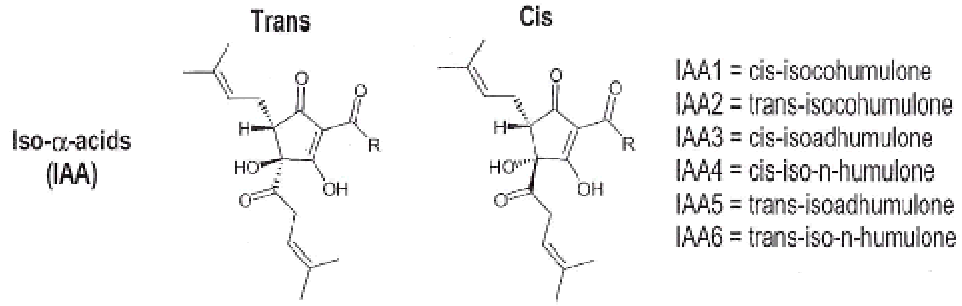

Fig. 5: The six main iso-α-acids (VANHOENACKER et al., 2004)

Principally they are formed during wort boiling by thermal isomerisation via an acyloin-type ring contraction, which means a 6-C ring is transformed into a 5-C ring. The pattern is shown in Fig. 6.

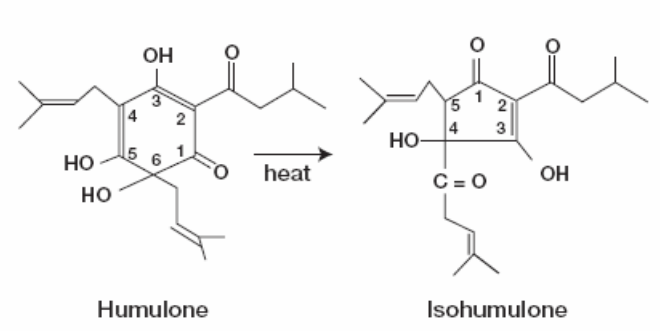

Fig. 6: Isomerisation of α-acids into iso-α-acids (HOFFMAN and JARVIS, 2005)

1.2.4.4 Tetrahydroiso-α-acids (THIA)

There are two pathways for preparing the cis- and trans-tetrahydroiso-α-acids. The first one is via isomerisation of tetrahydrohumulone and the following separation of the tetrahydroiso-α-acids. Then they can be obtained by hydrogenolysis of co-lupulone with following oxidation and isomerisation. All six major forms of THIA (cis-n-humulone, cis-cohumulone, cis-adhumulone and trans-n-humulone, trans-cohumulone, trans-adhumulone) are depicted in Fig. 7.

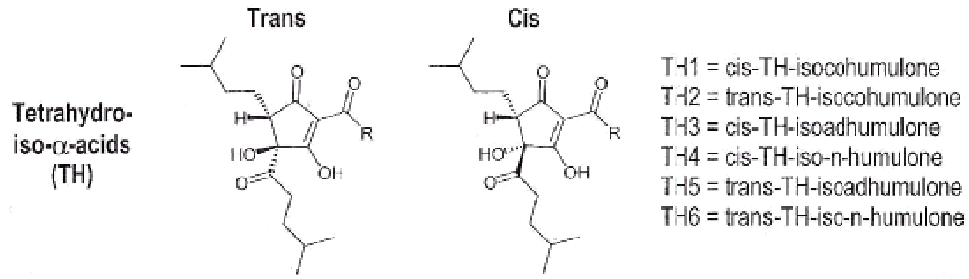

Fig. 7: The six main tetrahydroiso-α-acids (VANHOENACKER et al., 2004)

The THIA are featured by a more distinctive bitterness and bacteriostatic activity, but their key advantage is their light stability which disables formation of a skunky, sunstruck flavour caused by 3-methyl-2-butenyl mercaptan (VERZELE and DE KEUKELEIRE, 1991 and BRIGGS et al., 2004).

1.2.5 Hop analytics

Generally, it can be said that hop analytics is aimed at the requirements of brewers and the brewing industry, respectively. Institutions like the European Brewery Convention (EBC) and the American Society of Brewing Chemists (ASBC) have been working continually, making a lot of efforts to improve the methods for hop analysis. At the moment, high performance liquid chromatography (HPLC) is the method of choice. The state-of-the art HPLC methods were largely developed by VERZELE and DE KEUKELEIRE (1991).

As primary points, sample pre-treatment by means of several extractions techniques and HPLC related topics, like chromatographic conditions and separation of the applied hop acids, are discussed in more detail.

1.2.5.1 Extraction methods

For sample pre-treatment, extraction techniques like LLE (liquid-liquid extraction), SPE (solid-phase extraction) and solid-liquid extraction were proposed and discussed by several researchers (JASKULA et al., 2007 and HARMS and NITZSCHE, 2001). A rather new method named stir bar sorptive extraction (SBSE) was mentioned by VANHOENACKER et al. in 2004. This solventless extraction technique uses stir bars coated with polydimethylsiloxane (PDMS) as a sorbent. This method involves the advantage of very low detection limits and the saving of the conventional huge amounts of solvents (BALTUSSEN et al., 1999). WEISS et al. (2002) as well as JASKULA et al. (2007) reported that LLE and SPE can be successfully applied for extraction of hop acids prior to HPLC-analysis. However, the latter found out, that LLE affords clearly higher recoveries, especially when using H_3PO_4 for sample acidification. This applies in particular for the iso-α-acids, where trace metal activity can be prevented by metal complexation by means of H_3PO_4.

Although direct injection of beer has already been done (VANHOENACKER et al., 2004), it is in many cases advisable to extract the analysing hop compounds. This is due to the avoidance of disturbing matrices and the thus resulting elongation of the column life.

1.2.5.2 Chromatographic conditions

Today, HPLC is intensively used for analysing α-acids and β-acids, as well as iso-α-acids and their reduced derivatives. It is commonly accomplished in the reverse-phase mode (RP-HPLC) with nonpolar stationary phases. In DEWAELE and VERZELE´s opinion (1980) normal-phase HPLC is not the method of choice for analyses of hop acids, due to its polar character. This causes problems with trace materials. Furthermore, keeping retention times and selectivity constant is difficult.

Depending on particular separation requirements, isocratic elution (same composition of eluent for the whole run time) or gradient elution (the polarity of the eluent is changed during the analysis to cope with challenging separation problems), can be accomplished. For stationary phases C_8 or C_{18} columns can be used, like the Nucleosil C_{18} Hop or Nucleodur C_{18} end-capped (means selective placement of a functional group at the chain end) both from Machery-Nagel. After testing several columns, CLARK et al. (1998) deemed that the end-capped products can avoid chromatographic deficiencies, like peak tailing.

The mobile phases in RP-HPLC are mostly mixtures of water or buffer and acetonitrile or methanol. For advanced separation of hop acids, acidic, neutral or alkaline buffers can be used. Acidic buffers are e.g. phosphates, proposed by VERZELE and DE KEUKELEIRE (1991) and JASKULA et al. (2007), neutral ones may be e.g. citrates proposed by CLARK et al. (1998) or alkaline e.g. ammonium acetate pH-adjusted to 9.95, by means of ammonia, proposed by VANHOENACKER et al. (2004), in order to enhance peak shape and efficiency as well as to obtain a method compatible with MS detection.

Detection of separated peaks can be done by means of diverse sorts of detectors, like the UV-VIS detector. Further combination possibilities are HPLC-DAD (diode array detector, which provides the advantage to obtain the whole spectrum of the analysed substance) or HPLC-MS (mass spectrometry, which proves enhanced sensitivity and selectivity) (VANHOENACKER et al., 2004).

1.2.5.3 HPLC-analysis of THIA, IA and β-acids

From the applied hop acids in the maize starch project, the THIA are discussed most detailed, as about 90 % of all HPLC-analyses in the present work were accomplished detecting them. A large number of studies have already been devoted to analysis of THIA by means of RP-HPLC. CLARK et al. (1998), for example, worked on their analysis by using a gradient elution, enabling them to quantify IA and THIA in beer. They examined the recovery of their method by spiking their samples with THIA-standard solution. In 2001, HARMS and NITZSCHE were the first to manage the complete separation of all six isomers of IA, using isocratic elution in beer. Detection of THIA has already been done at 270-275 nm as well as 254-256 nm. For α-acids and β-acids 330 nm as well as 314 nm were proposed (VANHOENACKER et al., 2004 and ANALYTICA-EBC, 2005).

Recently, BOLIVAR et al. (2006) introduced a new rapid and low-cost method, for wort and beer analysis of IA and THIA, by direct injection of the sample and use of a new column technology (Chromolith RP, C_{18} end-capped, 100 x 4.6 with a Guard Cartridge C_{18} end-capped from Merck). For calculation of the recovery of spiked samples the standard addition method was used. The developed method proved to be very suitable, because time saving. In breweries, where time is restricted during beer filtration, beer bitter acids have to be measured fast, before and after dosing.

In 2007, TING et al. published a HPLC-method, using a short C_{18} column (100 x 4.6 mm; Hypersil) and an eluent consisting of an aqueous methanol buffer. The developed method is able to rapidly resolve analogues and stereoisomers of isomerised α-acid derivatives. These recent achievements outperform the current EBC- and ASBC-method in resolution of stereoisomers. As buffer solutions sodium or ammonium salts of different organic acids can be used. That achieves the separation and resolve of iso-α-acids and its derivatives within 20 min under isocratic conditions. The buffer solutions are not only applicable to the aforesaid Hypersil column, but also to Nucleosil and comparable ones. The detection and identification respectively were carried out with a DAD and electrospray- MS-detector. In summary, a method was provided, which is able to assess bitter acids in quality and quantity, using the combination of a C_{18} column, an aqueous buffer and MS-detection.

1.3 Pilot plant and starch production in ZFT

The ZFT operates a very well equipped pilot plant. The devices are described and shown below, according to the direction of the production. Processing steps from the raw material maize to the separated starch are shown in the following flow sheet. Fig. 8 depicts a schematic illustration of the pilot plant used for maize starch extraction.

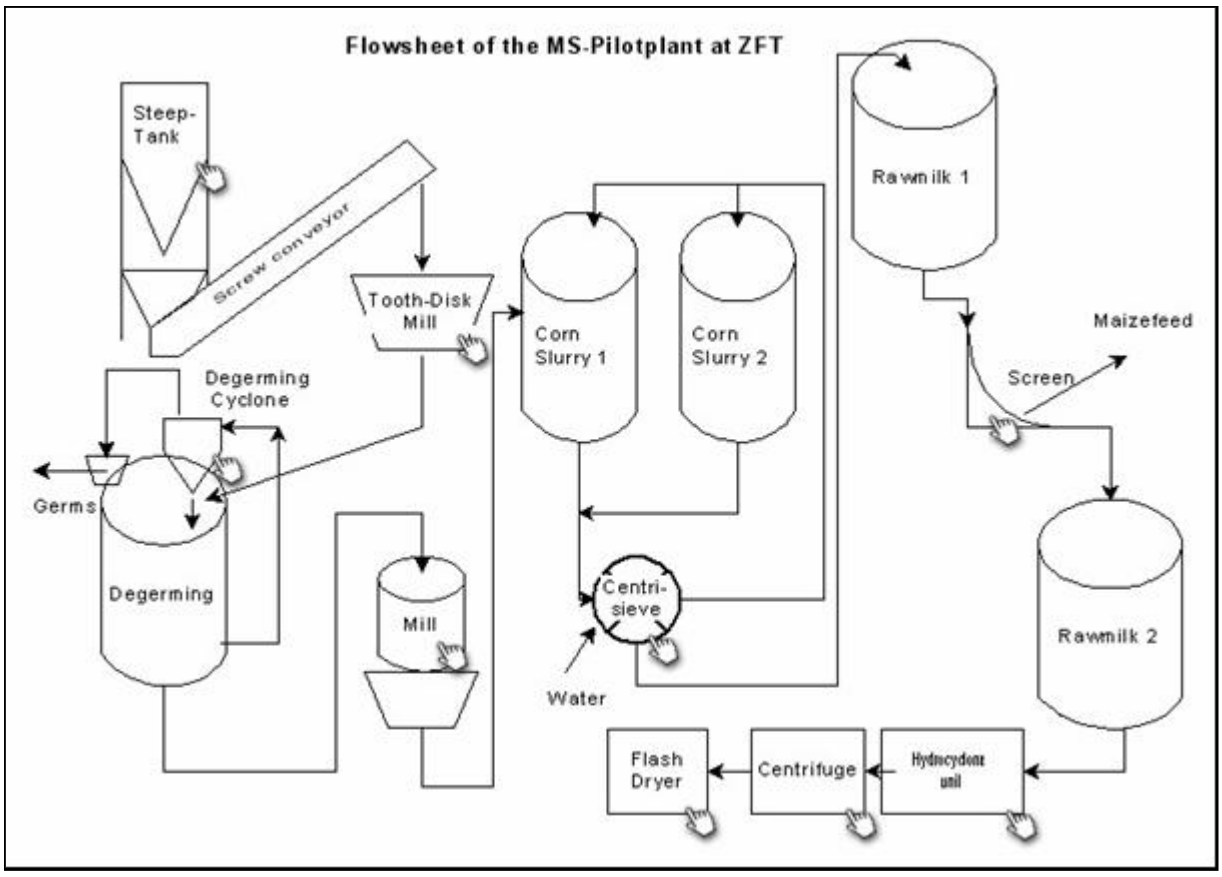

Fig. 8: Flow sheet of the maize starch pilot plant (ZFT, 2007)

This plant is located on two floors. The process starts on the upper left side of the image and then proceeds to bottom right. The pilot plant has a capacity of about 100-600 kg maize for each trial.

The following listing shows at each point, firstly technical information with pictures and secondly a description of the application.

A pre-cleaning of the maize kernels was not necessary, as they contained neglectable amounts of any fine material and cobs.

1. Steeping:

▫ Steep tank:

Fig. 9 shows a heat-insulated stainless steel tank with a volumetric capacity of 500 L, specially assembled for the steeping of maize. It is equipped with a valve flap (Ø 20 cm) and a discharge pipe used for discharging steep water and forwarding it to a heat exchanger.

Fig. 9: Steep tank (ZFT, 2007)

▫ Application:

This first step is very important for good starch quality and high yield. The aim is on the one hand to leach soluble matters (proteins and soluble carbohydrates) from the maize kernels, on the other hand to soften the kernels, to achieve good separation of germ, perikarp and endosperm. This is carried out in the steep tank at 50°C at pH 4 (adjusted by addition of hydrochloric acid). Additionally the steep water is treated with sulphur dioxide to guarantee optimal water absorption of the maize kernel, controlled fermentation by lactic acid bacteria and loosening of the protein matrix. This lactic acid bacteria milieu suppresses unwanted microorganisms such as yeasts, molds and others.

During the steeping process, the size of the kernels nearly doubles and the water content increases from 15 % to 45 %.

In the pilot plant batch wise steeping is conducted. To simulate the counter current conditions of the production plant in Aschach the steep water from the last steeping tank is used and diluted during steeping.

This step lasts for about 48 hours after which the maize is forwarded by means of a screw conveyor into a mill for coarse grinding.

2. Coarse grinding and degermination:

▫ Mill for coarse grinding:

An attrition mill is shown in Fig. 10. It is used for the coarse grinding of maize. The milling disks are made of stainless steel and have a diameter of 300 mm. The distance between the milling disks as well as the rotation speed can be adjusted.

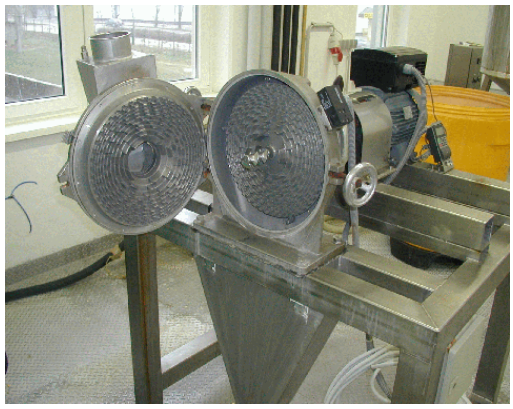

Fig. 10: Attrition mill (ZFT, 2007)

▫ Cyclone for germ removal:

The cyclone for germ removal is shown in Fig. 11. It is made of polyurethane with an inner diameter of 80 mm and an installation height of 550 mm, which is operated with a throughput of approx. 5 m³ per hour.

Fig. 11: Cyclone (ZFT, 2007)

▫ Application:

After the steeping it should be easy to break the maize kernels with a fingernail; this is important for the following degermination step. To ensure a continuous passage of the kernels the removed steeping water is added here. The grinding has to be conducted with care to avoid oil leakage out of germs, which would decrease the starch quality. Separation of the germs is conducted with a special hydro-cyclone. The grinding step takes about one hour for 100 kg of maize.

3. Fine grinding and extraction:

▫ Mill for fine grinding:

The pin mill (shown in Fig. 12) which operates with several rows of cylindrical grinding pins for the fine grinding of germ-free maize mash. The milling disk diameter is 150 mm.

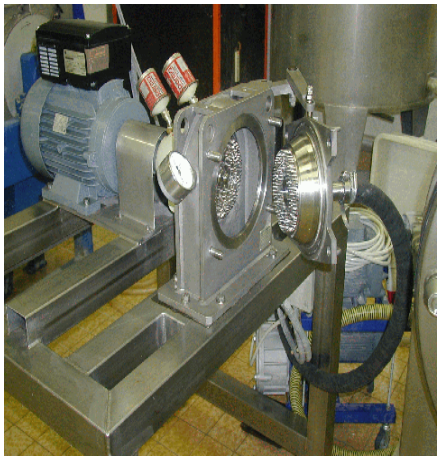

Fig. 12: Pin mill (ZFT, 2007)

▫ Centrisieve:

The centrisieve is used for the separation of fibres from the starch. It is depicted in Fig. 13. It consists of a rotating screen basket which is covered with a 120 µm sieve. While the starch suspension passes through the sieve, the fibres remain on it.

Fig. 13: Centrisieve (ZFT, 2007)

▫ Bend screen:

The bend screen (Fig. 14) is used for the separation of fine fibres from the starch slurry. Highly pressurized starch milk is applied to a 50 µm sieve where only the starch granules pass through and the fibres remain it.

Fig. 14: Bend screen (ZFT, 2007)

▫ Application:

The fine grinding in an impact mill completely disrupts the cells of the endosperm and releases the starch granules. This lasts for about 45 min.

4. Gluten separation and starch refining:

▫ Hydro-cyclone plant:

The hydro-cyclone plant (obvious from Fig. 15) is used for protein and fibre separation and the concentration of starch. It is assembled of 11 series-connected multi-cyclone units which are operated by counter flow principle.

Fig. 15: Hydro-cyclone plant (ZFT, 2007)

▫ Application:

The starch slurry, which still contains approximately 2 % of protein and fibres after separation, is then refined in a multi-step cyclone plant. The last stage of the multi-step cyclone plant is the one and only step of the wet milling process where fresh water is added. By optimal construction and adjustment of the plant it is possible to reduce the protein content in the starch to approximately 0.3 % on dry matter.

The gluten separation, concentration and refining steps last for about 3.5 hours.

5. Dehydration and drying:

▫ Centrifuge:

The centrifuge in the pilot plant station is utilized for the dewatering of starch suspensions (shown in Fig. 16).

Fig. 16: Centrifuge (ZFT, 2007)

▫ Dryer:

The flash dryer (shown in Fig. 17) is used for the drying of starch. In the drying chamber the starch cake is mixed with hot air. Dried starch is discharged from the drying chamber by means of a hot air flow and subsequently separated by means of a cyclone.

Fig. 17: Dryer (ZFT, 2007)

▫ Application:

Dehydration and drying: The refined starch slurry, which has a water content of approx. 65 %, is dehydrated in a centrifuge to a residual water content of about 40 %. Pure starch is finally dried by means of a flash dryer. For optimal shelf life residual moisture must not exceed 14 %. Duration: approx. 3 hours.

2 OBJECTIVES

During the storage of wet maize, unwanted losses of extractable starch can occur. Thus high lactic acid concentrations get involved into subsequent processes (e.g. maize steeping). The main objective of the application of hop acids is to check the lactic acid formation during wet maize storage and maize steeping. The idea is to employ the hop extracts alternatively or additionally to sulphur dioxide, the conventional processing aid. Beyond that, they could demonstrate an alternative within the production of organic starch.

The aims of this work can be summarised as following:

1. Adaptation and optimisation of the sample preparation and HPLC-method.

2. Standard addition trials using hop extracts containing THIA, IA and β-acids. The hop acids were added to starch and by-products as methanolic standard solutions.

3. Subsequent analysis of starch and by-products (previously produced in pilot-scale using the aforesaid hop extracts) on hop acids by means of the optimised extraction and HPLC-separation methods.

4. Set-up of a balance sheet, using the HPLC-results and recorded mass flow rates of the pilot-scale trials.

The information on the distribution of the products should serve as a basis for first full scale trials, in order to obtain specification data for customers. Special focus should be put on starch to ensure, that no unwanted, quality decreasing properties of starch and by-products occur.

3 MATERIALS AND METHODS

3.1 Raw materials

3.1.1 Raw materials from Aschach

The raw materials used for pre-tests, namely starch, maize feed, gluten, germs and CSL were provided by the starch factory Aschach, Austria.
The samples were stored in closable bin in the cold room of ZFT until analysing. These raw materials were used for standard addition trials to optimize the sample preparation techniques.

3.1.2 Raw materials produced in the pilot plant of ZFT

After the preliminary trials (standard addition trials), starch and maize feed (wet fibres) produced in the pilot plant in the ZFT in Tulln were used for analysis.
Note the difference between maize feed collected from the factory and self-produced in the pilot plant of ZFT. In industrial processing, maize feed is produced by spraying CSL onto the dried fibres. The maize feed produced in pilot-scale is just wet fibres.

3.2 Hop products and standards

For standard addition the products LactoStab™, IsoStab™ and BetaStab® 10A (Betatec, Germany) were used. Pilot-scale trials were carried out using LactoStab™ and BetaStab® 10 A to control the formation of lactic acid.

- LactoStab™ is an aqueous, alkaline solution of the potassium salt of tetrahydro-iso-α-acid. It is produced from a CO_2 extract of hops. It has a concentration of 8.6 - 9.4 % (w/w). It should be stored at 15°- 25°C (safety data sheets, BETATEC, 2006).

- IsoStab™ is an aqueous, alkaline solution of the potassium salt of iso-α-acid. It is produced from a CO_2 extract of hops. It has a concentration of 29.5 - 30.5 % (w/w). It should be stored at 2°- 8°C (safety data sheets, BETATEC, 2006).

- BetaStab® 10 A is an aqueous, alkaline solution that contains a mixture of natural resins and resin acids extracted of hops with liquid or supercritical CO_2. The major components are hop β-acids at a concentration of 9 - 11 % (w/w) (safety data sheets, BETATEC, 2005).

The peaks obtained by the hop products were verified by International Calibration Standards (ICS) purchased from Labor Veritas, Switzerland.
For LactoStab™ (tetrahydro-iso-α-acids, THIA) *Tetra ICS-T2* was taken. For IsoStab™ (iso-α-acids, IA) *DCHA-Iso ICS-I2* and for BetaStab® 10A (β-acids) *ICE-2* was used.

Tetra ICS-T2: Is a purified preparation containing both cis- and trans-isomers of tetrahydroisocohumulones, tetrahydroisohumulones and tetrahydroisoadhumulones.
With respect to these six isomers it is deemed to have a total tetrahydroiso-α-acids content of 99.4% (w/w).

DCHA-Iso ICS-I2: Is a purified preparation of the dicyclohexylamine salts of trans-iso-α-acids. It is deemed to have a total iso-α-acids content of 64.3% (w/w), though only the major forms of the iso-α-acids that are present, are taken into account: trans-isocohumulone, trans-isohumulone, and trans-isoadhumulone.

ICE-2: Is a hop extract containing a specified concentration of α- and β-acids. Additionally, the standard contains uncharacterized soft resins, and small amounts of waxes and residual essential oils. ICE-2 has the following composition:

Co-humulone: 14.45 %
Ad-humulone + humulone: 34.94 % } Total α-acids: 49.39 %

Co-lupulone: 12.92 %
Ad-lupulone + lupulone: 12.02 % } Total β-acids: 24.94 %

The calibration standard bottles were stored in a freezer set below -20 °C in a plastic container, covered with aluminium foil referring to the user's guide of Labor Veritas (WILSON, 2005).

3.3 HPLC equipment

1. HPLC-system Ultimate 3000 (Dionex) consisting of:
   Low-pressure-gradient-pump (LPG) Ultimate 3000 pump:
   Dionex pump series, programmable; eluent mixed low-pressure-sided
2. Autosampler: Ultimate 3000, (Dionex)
   Temperature and injection volume optionally adjustable
3. Ultimate 3000 column compartment, (Dionex)
4. Column: Sunfire™ C18 (100 mm length x 4.6 mm inner diameter, 3.5 μm particle size), (Waters, Ireland)
5. Detector: Ultimate 3000 variable wavelength detector, (Dionex)
6. Software: Chromeleon®, (Dionex)
7. HPLC – sample preparation:
   Clear glass vials 1.5 mL (VWR)
   Caps 11 mm and cap crimper (VWR)
   Disposable syringes 2 mL (Omnifix, Braun)
   Disposable hyperdermic needles Ø 0.8 x 40 mm (Sterican, Braun)
   Disposable syringe filters 0.45 μm (Pall)

3.4 Sample pre-treatment methods

Extraction methods were adopted following methods already applied in the ZFT during studies on the fate of resin and hop components in sugar and by-products (HEIN and POLLACH 1997; POLLACH et al. 1999; SIX 2002).

3.4.1 Soxhlet extraction

3.4.1.1 Equipment and utensils:

1. Soxhlet extractor (150 mL), DIN NS 45/40 (Lenz lab glass)
2. Round-bottom flask (250 mL), (Lenz lab glass)
3. Dimroth condenser, DIN NS 45/40, (Lenz lab glass)
4. Heatable extraction plant (under the hood, as obvious from Fig. 18)
5. Extraction thimbles 33.100 mm, cellulose (10.350.243) (Whatman)
6. Absorbent cotton (Rauscher)
7. Artery clamps
8. Graduated cylinders (glass) 25 mL, 100 mL, 500 mL, 1000 mL
9. Analytical balance accurate to 0.1 mg (LE 324 S, Sartorius)
10. Weighing spoon and spatula
11. Micropipets (electronic) 5-100 μL, 50-1000 μL and 100-5000 μL (Proline Biohit)
12. Pipet tips, 5-100 μL (C), 50-1000 μL (E), and 100-5000 μL (J) (Proline Biohit)
13. One-way tubes 35 mL, PP, 115x23.5 (58.537) (Sarstedt)
14. One-way push caps, 115x23.5 (65.790) (Sarstedt)
15. Rotary evaporator: vacuum controller V-800, rotavapor R-200, heating bath (Büchi, Switzerland)
16. Cork rings, time switch
17. Gloves latex, chemical resistant (VWR)
18. Refrigerator (at ~5°C) for sample storage prior to transferring into vials for HPLC-analysis (Liebherr Premium)

3.4.1.2 Reagents and solutions:

1. Dichloromethane for synthesis (8.22271.2500) (Merck, Germany)
2. N-hexane extra pure p.a. (1.04374.2500) (Merck, Germany)
3. Petroleum benzene boiling range 40 - 60°C (1.00909.5000) (Merck, Germany)
4. Methanol gradient grade (1.06007.2500) (Merck, Germany)
5. Ultra-pure water MilliQ gradient grade 18.2 $M\Omega*cm^{-1}$ (Millipore)
6. Standard stock solution (preparation see chapter 3.4.1.3 and 3.4.1.4)

3.4.1.3 Sample preparation:

The sample material is weighed into an extraction thimble (~ 20-30 g) and the hop standard (200-300 µL) is pipeted onto it. The sample is covered with some absorbent cotton to prevent it from leaching out during the extraction. Then the thimble is put into the Soxhlet extractor and a round-bottom flask (250 mL) with 150 mL of solvent is prepared. After that the round-bottom flask and the Soxhlet extractor are assembled and the extracting system is fixed. The heating and the cooling water are turned on. The extraction is carried out over night and stopped by means of a time switch to ensure enough time for the added standard to get eluted. After the extraction has finished, the solvent is evaporated to dryness and the residues are dissolved in methanol and stored in a refrigerator (about 5°C) in closable one-way tubes prior to HPLC-analysis.

Fig. 18: Heatable extraction plant during Soxhlet extraction

3.4.2 Shaking out with pH-adjustment and single solvent

3.4.2.1 Equipment and utensils:

1. Centrifuge screw-bottles 250 mL (Nalgene)
2. Glass beakers 250 mL
3. Transfer pipets, glass 10 mL
4. Pipet ball (Howorka)
5. Pasteur pipets plastic 1 mL (α-Lab Ltd.)
6. Lab pH-meter (WTW)
7. Magnetic stirrer and magnetic stir bars with retriever (IKA)
8. Bench top laboratory shaker (IKA)
9. Centrifuge Model J-6B, application: 10 min at 3000 rpm (Beckman)
10. Same as 9. - 19. chapter 3.6.1.1

3.4.2.2 Reagents and solutions:

1. N-hexane extra pure p.a. (1.04374.2500) (Merck, Germany)
2. Petroleum benzene boiling range 40 - 60°C (1.00909.5000) (Merck, Germany)
3. Methanol gradient grade (1.06007.2500) (Merck, Germany)
4. Ultra-pure water MilliQ gradient grade 18.2 $M\Omega*cm^{-1}$ (Millipore)
5. Hydrochloric acid for analysis 25 % (1.00316.1011) (Merck, Germany)
6. Titriplex ampoule: hydrochloric acid and sodium hydroxide 0.1 M (Titrisol)
7. Sodium hydroxide 50 % (J.T. Baker, Fischer Scientific)
8. Standard stock solution (preparation and addition see chapter 3.4.1.3 and 3.4.1.4)

3.4.2.3 Sample preparation:

Steep water:

100 g of the liquid sample is weighed into a beaker. The pH-electrode is dipped into the beaker and the desired pH is adjusted by adding pH buffer solution stepwise with a Pasteur pipet, while the sample is stirred on the magnetic stirrer. Then the sample is transferred quantitatively into a centrifuge screw bottle and 1 mL of hop standard and 50 mL of solvent are added. The bottles are shaken for 15 min on a lab shaker and then centrifuged at 3000 rpm for 10 min. The supernatant is drawn off by means of a glass transfer pipet and a Howorka ball. This extraction is repeated three times and the three supernatants are united for evaporation in a round-bottom flask. The solvent is evaporated to dryness and the residues are dissolved in methanol and stored in a refrigerator at 5°C in closable one-way tubes prior to HPLC-analysis.

Starch:

25 g starch and 80 mL ultra-pure water are weighed into centrifuge screw bottles and pH is adjusted in the same way as with steep water while the slurry is stirring on a magnetic stirrer for about 15 min. After that 250 μL of hop standard is pipeted to it and then 100 mL of solvent is added. The bottles are shaken also for 15 min on a lab shaker and then centrifuged at 3000 rpm for 10 min. The extraction is also performed three times and the supernatants are drawn off and poured over a folded filter into a round-bottom flask and evaporated to dryness. Resolving procedure is the same as above.

3.4.3 Shaking out with solvent mixture

3.4.3.1 Equipment and utensils:

1. Centrifuge screw-bottles 250 mL (Nalgene)
2. Bench top laboratory shaker (IKA)
3. Centrifuge Model J-6B, application: 10 min at 3000 rpm (Beckman)
4. Reagent and centrifuge tube with screw cap 50 mL (Sarstedt)
5. Deep freezer (- 30 °C) (Liebherr Premium)
6. Folded filters 595 ½, Ø 125 mm (Schleicher & Schuell)
7. Plastic funnels, 85 mm
8. Same as 9. - 19. chapter 3.6.1.1

3.4.3.2 Reagents and solutions:

1. Acetonitrile isocratic grade (1.14291.2500) (Merck, Germany)
2. 2-propanol p.a. (1.09634.1011) (Merck, Germany)
3. 1-ethanol p.a. (1.00983.1000) (Merck, Germany)
4. 1-butanol p.a. (1.01990.2500) (Merck, Germany)
5. Methanol gradient grade (1.06007.2500) (Merck, Germany)
6. Ultra-pure water MilliQ gradient grade 18.2 $M\Omega*cm^{-1}$ (Millipore)
7. Standard stock solution (preparation and addition see chapter 3.4.1.3 and 3.4.1.4)

3.4.3.3 Sample preparation:

5 g of raw material and 10 g of anhydrous sodium sulphate are weighed into a centrifuge screw tube and 50 µL of hop standard is added. The hop acids are extracted with 25 mL of a solvent mixture. The method was modified from MATISSEK and STEINER (2006). Solvent mixture 1 was adopted. Solvent mixture 2 was modified as follows:

Solvent mixture 1: acetonitrile/2-propanol/ethanol = 50/25/25 (v/v)

Solvent mixture 2: methanol/2-propanol/butanol = 50/25/25 (v/v)

The bottles are shaken on a bench top shaker for 15 min and centrifuged for 10 min at 3000 rpm. The solvent supernatant is filtrated into a reagent tube and the residual aqueous phase is extracted again in the same way. The tubes containing the united extracts are placed into a deep freezer for 2 hours (at - 30 °C) to freeze out fat and fat accompanying substances. After that the solvents are filtered again over a folded filter into a round-bottom flask. Fat lumps, which later would disturb the analysis of the hop acids via HPLC, remain in the filter. The solvents are evaporated to dryness and the residues are dissolved in methanol and stored in one-way tubes in the refrigerator.

3.4.4 Liquid – liquid – extraction

3.4.4.1 Equipment and utensils:

1. Extractor (perforator) for specific heavier solvents (250 mL) (Lenz lab glass)
2. Round-bottom flask (250 mL) (Lenz lab glass)
3. Dimroth condenser, DIN NS 45/40 (Lenz lab glass)
4. Heatable extraction plant (in the hood)
5. Plastic funnels (for sample transfer into extractor)
6. Pasteur pipets plastic 1mL (α- Lab Ltd.)
7. Lab pH-meter (WTW)
8. Magnetic stirrer and magnetic stir bars with retriever (lab equipment IKA)
9. Time switch
10. Same as 9. – 19. chapter 3.6.1.1

3.4.4.2 Reagents and solutions:

1. Dichloromethane for synthesis (8.22271.2500) (Merck, Germany)
2. Petroleum benzene boiling range 40 - 60°C (1.00909.5000) (Merck, Germany)
3. Methanol gradient grade (1.06007.2500) (Merck, Germany)
4. Ultra-pure water MilliQ gradient grade 18.2 $M\Omega*cm^{-1}$ (Millipore)
5. Hydrochloric acid for analysis 25 % (1.00316.1011) (Merck, Germany)
6. Titriplex ampoule: hydrochloric acid and sodium hydroxide 0.1 M (Titrisol)
7. Sodium hydroxide 50 % (J.T. Baker, Fischer Scientific)
8. Standard stock solution (preparation and addition see chapter 3.4.1.3 and 3.4.1.4)

3.4.4.3 Sample preparation:

Approximately 100 g of steep water is weighed into a beaker. The pH-electrode is dipped into it and the pH is adjusted by adding pH buffer solution in drops with a Pasteur pipet, while the sample is stirring on the magnetic stirrer. 100 µL of hop standard is added and the extractor is affixed at the extraction plant, slipped on a round-bottom flask and about 150 mL of solvent are filled into the extractor. When the liquid sample is added, the solvent below is drained back into the bottom flask through the siphon device. It is of particular importance to fill in solvent first, as otherwise the extracting liquid is overflows into the bottom flask. The beaker gets rinsed with solvent to assure all extractable hop compounds reach the extractor. After affixing the dimroth condenser, the cooling water and the heating are turned on. The extraction was carried out over night to assure enough time for elution and stopped by means of a time switch.

The enriched solvent was evaporated to dryness and the remnant was dissolved in defined volume of methanol.

3.4.5 Solid phase extraction

3.4.5.1 Equipment and utensils:

1. SPE-tubes 500 mg/3 mL silica based, (8B-S008-HBJ) (STRATA SAX)
2. Luer lock syringes 30 mL (Terumo)
3. Luer adaptors (Terumo)
4. Round-bottom flasks 50 mL and 500 mL (Lenz lab glass)
5. Filter circles, MN 206, Ø 240 mm (Machery-Nagel)
6. Pasteur pipets plastic 1 mL (α-Lab Ltd.)
7. Laboratory bottles with screw caps 500 mL (Schott)
8. Magnetic stirrer and magnetic stir bars with retriever (lab equipment IKA)
9. Same as 9. – 19. chapter 3.6.1.1

3.4.5.2 Reagents and solutions:

1. Methanol gradient grade (1.06007.2500) (Merck, Germany)
2. Methanol 40 % (methanol and water 40:60 v/v)
3. Ultra-pure water MilliQ gradient grade 18.2 $M\Omega*cm^{-1}$ (Millipore)
4. Standard stock solution (preparation and addition see chapter 3.4.1.3 and 3.4.1.4)

3.4.5.3 Sample preparation:

30 g of raw material is weighed into a 500 mL Schott bottle and spiked with 300 µL of hop standard. Then 200 mL methanol is added and stirred over night. The bottle content is filtered over a folded filter circle into a round-bottom flask by rinsing the bottle several times with methanol (100 %). The solvent is concentrated to dryness and dissolved in methanol again. The defined volumes are 5 and 10 mL. They are applied to a SPE cartridge, 500 mg/3 mL. This cartridge is previously conditioned with 10 mL of methanol and 10 mL of water.

After that, the cartridge containing the sample is washed with 40 % of methanol. Dilute methanol with water, 40:60 (v/v). The hop acids are eluted into a 50 mL round-bottom flask with 5 and 10 mL of methanol (100 %) according to previous sample feeding onto the SPE cartridge. The eluates are concentrated to dryness and the residues are dissolved each in 2 mL of methanol.

The concentration of 5 and 10 mL, respectively to 2 mL roughly corresponds to a concentration factor of 2.5 and 5. The samples are stored in one-way tubes in the refrigerator until the analyses are carried out.

3.4.6 Extract by stirring

3.4.6.1 Equipment and utensils:

1. Centrifuge screw-bottles 250 mL (Nalgene)
2. Laboratory bottles with screw caps 500 mL (Schott)
3. Plastic funnels and Pasteur pipets plastic 1 mL (α-Lab Ltd.)
4. Magnetic stirrer and magnetic stir bars with retriever (lab equipment IKA)
5. Lab pH-meter (WTW)
6. Filter circles, MN 206, Ø 240 mm (Machery-Nagel)
7. Round-bottom flasks, 250 mL (Lenz lab glass)
8. Reagent and centrifuge tube with screw cap 50 mL (Sarstedt)
9. Freezer at - 30 °C (Liebherr Premium)
10. Centrifuge Model J-6B, application: 10 min at 3000 rpm (Beckman)
11. Ultrasonic bath, transonic digital S (Elma)
12. Same as 9. - 19. chapter 3.6.1.1

3.4.6.2 Reagents and solutions:

1. Dichloromethane for synthesis (8.22271.2500) (Merck, Germany)
2. 1-butanol p.a. (1.01990.2500) (Merck, Germany)
3. 2-propanol p.a. (1.09634.1011) (Merck, Germany)
4. Methanol gradient grade (1.06007.2500) (Merck, Germany)
5. Hydrochloric acid for analysis 25 % (1.00316.1011) (Merck, Germany)
6. Titriplex ampoule: hydrochloric acid and sodium hydroxide 0.1 M (Titrisol)
7. Sodium hydroxide 50 % (J.T. Baker, Fischer Scientific)
8. Ultra-pure water MilliQ gradient grade 18.2 $M\Omega*cm^{-1}$ (Millipore)
9. Standard stock solution (preparation and addition see chapter 3.4.1.3 and 3.4.1.4)

3.4.6.3 Sample preparation:

Fat-rich raw materials (e.g. germs, gluten and maize feed):

30 g of raw material is weighed into 250 mL beaker and spiked with 300 µL of hop standard. 100 mL of solvent is added and stirred for about half an hour. Then the beaker content is filtered over a folded filter into a 250 mL round-bottom flask and flushed well. The solvent is concentrated to dryness and the residue is dissolved in methanol in a plastic reagent bottle with screw cap. The sample is deep-freezed (- 30 °C) for another 2 hours to remove disturbing fat accompanying substances. Then it is filtered into one-way tubes and stored in refrigerator before analysing.

Starch (including pH-adjustment):

30 g of starch and 300 µL of hop standard are weighed into a 500 mL Schott-bottle and 100 mL of water is added. This slurry is allowed to stir for about 15 min. Then pH is adjusted to acidic (pH 4) and alkaline pH (pH 10) by adding pH buffer solution drop wise with a Pasteur pipet while the slurry is stirring for another 60 min. After adding 100 mL of extracting agent (dichloromethane) the mixture is stirred thoroughly over night. Then it is transferred into a centrifuge screw-bottle quantitatively and centrifuged for 10 min. at 3000 rpm. The overlaying aqueous phase is decanted and the starch-dichloromethane phase is mixed by means of an ultrasonic bath for 20 min. Afterwards it is filtered through a folded filter into a round-bottom flask. It is rinsed well and evaporated to dryness. The remnants are dissolved in methanol. The samples are stored in one-way tubes in the refrigerator until the HPLC-analyses are carried out.

3.5 Chromatographic methods

Detailed information on HPLC-instruments and disposals was given in chapter 3.3. According to ANALYTICA-EBC methods 7.8 (iso-α-, α- and β-acids in hop and isomerised hop extracts by HPLC, 2005) and 7.9 (iso-α-acids and reduced iso-α-acids in hop products by HPLC, 2004) chromatographic separations were accomplished by default. Hop compounds were separated at best with isocratic elution referring also to the user's guide of Labor Veritas (WILSON, 2005). For more difficult separation tasks special programs were developed and mobile phases were modified, according to the analysed hop acids. Detailed information is given with corresponding analyses.

3.5.1 Standard preparation and addition

3.5.1.1 Equipment and utensils:

1. Analytical balance accurate to 0.1 mg, LE 324 S (Sartorius)
2. Micropipets electronic 5-100 μL, 50-1000 μL and 100-5000 μL (Proline Biohit)
3. Pipet tips, 5-100 μL (C), 50-1000 μL (E), and 100-5000 μL (J) (Proline Biohit)
4. Laboratory bottles with screw caps 50 mL (Schott)
5. Measuring flasks: 20, 25, 50 and 100 mL and stoppers
6. Gloves latex, chemical resistant (VWR)

3.5.1.2 Reagents:

1. Hop product containing THIA (Betatec, Germany)
2. Hop product containing β-acids (Betatec, Germany)
3. Methanol gradient grade (1.06007.2500) (Merck, Germany)

3.5.1.3 Preparation:

All standards were diluted with MeOH gravimetrically. Hop product containing THIA 10 mg was dissolved by 100 % methanol in a 100 mL measuring flask. This is the stock solution or standard "1". For generation of a calibration curve a dilution series was made. For standard "2" stock solution 1 mL was dissolved by 100 % methanol in a 50 mL measuring flask, for standard "3" stock solution 1 mL was dissolved by 100 % methanol in a 25 mL measuring flask and for standard "4" stock solution 1 mL was dissolved by 100 % methanol in a 20 mL measuring flask. Weights were recorded and used for calculations of the standard concentrations. Standards were decanted into screwable Schott-bottles in order to facilitate the pipeting. They were stored in a refrigerator (at 5 °C) prior to standard addition and HPLC-analyses, respectively.

3.5.1.4 Addition:

Standard addition was always carried out with standard "1", the stock solution. The concentration was about 1 ppm on substance with THIA and 1 - 1.5 ppm with β-acids.

3.5.2 Preparation of the mobile phase

3.5.2.1 Equipment and utensils:

1. Graduated cylinder (glass) 100, 500, 1000 mL
2. Laboratory bottles with screw caps 500, 1000, 2000 mL (Schott)
3. Micropipets electronic 100-5000 μL (Proline Biohit)
4. Pipet tips, 100-5000 μL (Proline Biohit)
5. Ultrasonic bath, transonic digital S (Elma)
6. Gloves latex, chemical resistant (VWR)

3.5.2.2 Solvent reagents:

1. Methanol gradient grade (1.06007.2500) (Merck, Germany)
2. Ortho-phosphoric acid (H_3PO_4) 85 % p.a. (1.00573.1000), (Merck, Germany)
3. Ultra-pure water MilliQ gradient 18.2 $M\Omega*cm^{-1}$ (Millipore)

Note: when water was required for analyses, ultra-pure water was always used.

3.5.2.3 Preparation:

The mobile phase was prepared by mixing 750 mL methanol, 240 mL water and 10 mL ortho-phosphoric acid (85 %) in a 1000 mL Schott-bottle (ANALYTICA-EBC 7.9, 2004). Before using as an eluent, it was degassed by means of an ultrasonic bath for 20 min.

3.5.3 Chromatographic conditions

3.5.3.1 Standard application for THIA (isocratic elution)

- Standard eluent: 750 mL methanol, 240 mL water and 10 mL H_3PO_4 (85 %) (Addition of EDTA is meant to sharpen the peaks in some HPLC-systems by preventing trace metal activities of the iso-α-acids)
- Flow: 1 mL*min^{-1} (100 % eluent A using the standard eluent)
- Column: Sunfire C_{18} 100 x 4.6 mm (recommended by the manufacturer Waters as current model)
- Injection volume: 20 μL
- Column temperature: 35°C
- Pump pressure limits: 5-200 bar
- UV-wavelength: 270 nm

3.5.3.2 Special application for THIA (gradient elution)

- Cleaning program for fat-rich samples
- Eluent A: 100 % methanol
- Eluent B: 50 % methanol, 48 % water and 2 % H_3PO_4 (85 %)
- Programme:

- 3 - 23 min	50 % B
23.1 - 30 min	20 % B
30.1 - 40 min	50 % B

→ 3 min equilibration of the column to separation conditions of the α-acids (75 % percentage absolute of methanol) before start of the analysis.

→ 23 min run of the analysis (75 % MeOH = standard eluent composition)

→ 7 min cleaning program (90 % MeOH, in order to remove fat matrix)

→ 10 min column re-equilibration

The remaining applications, like HPLC-apparatus, column, runtime etc. were identical with standard applications above.

3.5.3.3 Standard application for IA (isocratic elution)

- Eluent A: 100 % methanol
- Eluent B: 50 % methanol, 49 % water and 1 % H_3PO_4 (85 %)
- Flow: 1 mL*min^{-1} (40 % A and 60 % B → 70 % organic percentage)
- Column: Sunfire C_{18} 100 x 4.6 mm
- Injection volume: 20 µL
- Column temperature: 35°C
- Pump pressure limits: 5-200 bar
- UV-wavelength: 270 nm

3.5.3.4 Standard application for β-acids (isocratic elution):

- Eluent A: 100 % methanol
- Eluent B: 50 % methanol, 49 % water and 1 % H_3PO_4 (85 %)
- Flow: 1 mL*min^{-1} (60 % A and 40 % B → 80 % organic percentage)
- UV-wavelength: 340 nm
- Column: Sunfire C_{18} 100 x 4.6 mm
- Same injection volume, column temperature and pump pressure limits

3.5.4 Calibration

Standards were always injected at the beginning and at the end of a sequence. The standards used for calibration were products containing THIA, IA and β-acids. The peaks of these products were always verified, using ICS-standards from Labor Veritas. Calibration series for each THIA, IA and β-acids were made. Four-point calibration graphs in the range of 2.5 - 120 mg/L were obtained by analysis of methanolic standard solutions of THIA, IA and β-acids, as external standards. Whereby, the highest concentrated standard was injected first. These standards were used for calculation of recoveries of THIA in spiked raw material samples by comparing peak areas as a percentage. The preparation or extraction method respectively, which obtained the highest recoveries in preliminary trials were used for HPLC analyses of starch and by-products, produced in pilot-scale.

4 TEST PROCEDURE

The primary focus of the preliminary trials was the adaptation and optimization of the extraction methods. Furthermore their objective was to find suitable HPLC-methods for the analysis of the hop acids. Additionally the active ingredients of the hop product/extract containing THIA were determined.
During wet maize storage and steeping trials, the tetrahydro-iso-α-acids (THIA) proved to be most effective in reducing lactic acid formation. Therefore it was decided to carry out pilot-scale trials with THIA and β-acids. For this reason, the main focus of HPLC-analyses was put on them. However, preliminary trials, were also carried out with β-acids and IA. Extraction methods, which resulted in the highest and most reliable recoveries, were later applied to examination of starch and by-products processed in the pilot plant.

4.1 Preliminary trials

4.1.1 Standard addition trials with THIA

4.1.1.1 Determination of active ingredients of THIA

The peaks of the THIA extract and the ICS-tetra-standard were compared, to calculate and verify the manufacturer's data. The standard specification document reads 8.6 - 9.4 %; the calculated result was exactly 9.08 %. From the ICS-tetra-standard a dilution series was made, containing 10, 25 and 50 ppm of THIA; the THIA extract contained about 31 ppm of active ingredient. The chromatogram given by the THIA extract is shown as the green curve in Fig. 19. As apparent, the peaks obtained by the extract and the three standard peaks are matching entirely satisfactory.

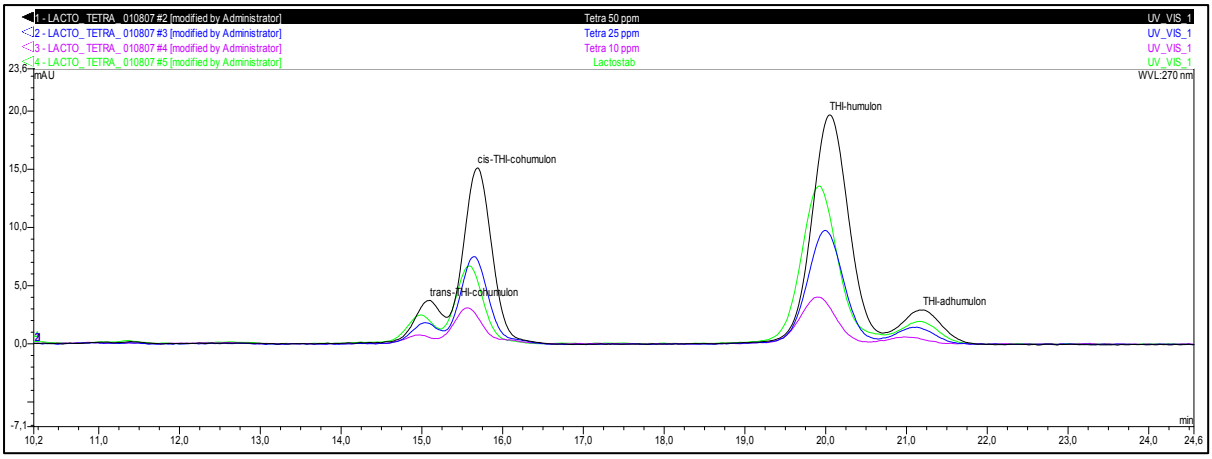

Fig. 19: Chromatogram of THIA and ICS-tetra-standard

The capital letter-abbreviations for the solvents mean:

H = n-hexane,

D = dichloromethane

P = petroleum benzene

B = butanol

All abbreviations can be found at the beginning of this work. HPLC applications and extraction techniques have already been introduced in chapter 3.

4.1.1.2 Starch

Table 1 depicts the extraction trials, which were carried out with starch using the standard addition method with THIA. This implies that samples were spiked with THIA. Using a four-point calibration recoveries were calculated by comparing amounts of THIA in samples and standards. Soxhlet extraction without absorbent cotton was applied, further on denoted as "Soxhlet No. 1". Extract by stirring was performed with and without pH-adjustment using dichloromethane as a solvent. Moreover, Soxhlet extraction with absorbent cotton was done, further on denoted as "Soxhlet No. 2".

Table 1: Extraction trials with starch, using THIA

Trial	Extraction method	Solvent(s)	HPLC application
1	Soxhlet No. 1*	H, D, P	Standard THIA
2	Stirring (without pH-adjustment)	D	Standard THIA
3	Stirring, pH-adjusted to 4 and 10	D	Standard THIA
4	Soxhlet No. 2**	D	Standard THIA

* without absorbent cotton ** including absorbent cotton

4.1.1.3 Maize feed

Table 2 shows the extraction trials, which were carried out with maize feed, using the standard addition method with THIA. Attempts were made with Soxhlet No. 1, by means of stirring and afterfiltration with butanol and solvent mixture 2. Solvent mixture 2: methanol/2-propanol/butanol = 50/25/25 (v/v).

Table 2: Extraction trials with maize feed, using THIA

Trial	Extraction method	Solvent(s)	HPLC application
1	Soxhlet No. 1*	H, D, P	Standard THIA
2	Stirring and Filtration	B	Special THIA
3	Stirring and Filtration	Solvent mixture 2	Special THIA

* without absorbent cotton

4.1.1.4 Gluten

In Table 3 the extraction trials, which were carried out with gluten, are presented. The first tests were done with Soxhlet No. 1 and solid phase extraction (SPE) with methanol. Extract by stirring was carried out with butanol and afterfiltration. Moreover extract by stirrings with solvent mixture 2 was performed. Solvent mixture 2 contains: methanol/2-propanol/butanol = 50/25/25 (v/v).

Table 3: Extraction trials with gluten, using THIA

Trial	Extraction method	Solvent(s)	HPLC application
1	Soxhlet No. 1*	H, D, P	Standard THIA
2	SPE	MeOH	Standard THIA
3	Stirring and Filtration	Solvent mixture 2	Special THIA
4	Stirring and Filtration	B	Special THIA

* without absorbent cotton

4.1.1.5 Germs

As apparent from Table 4, the extraction trials with starch were carried out with Soxhlet No. 1. Then extract by stirring with butanol, filtration over folded filter, followed by Soxhlet No. 2 with dichloromethane was tried. At last just stirring with butanol and filtration afterwards, was done. Trials 2 and 3 always included freezing out of samples, in order to remove fat remnants.

Table 4: Extraction trials with germs, using THIA

Trial	Extraction method	Solvent(s)	HPLC application
1	Soxhlet No. 1*	H, D, P	Standard THIA
2	Stirring and Soxhlet No. 2**	B and D	Special THIA
3	Stirring and Filtration	B	Special THIA

* without absorbent cotton ** including absorbent cotton

4.1.1.6 Steep water

All trials conducted with steep water are listed in Table 5. Firstly, experiments were done by means of shaking out with n-hexane and petroleum benzene. Steep water was initially pH-adjusted to acid, neutral and alkaline pH-values. Secondly, trials used the same method varying the pH-value in acidic milieu. Then LLE (liquid-liquid-extraction) with dichloromethane was tested by means of half-continuous working extractors. Shaking out was performed by means of a separating funnel, using n-hexane.

Table 5: Extraction trials with steep water, using THIA

Trial	Extraction method	Solvent(s)	HPLC application
1	Shaking + pH-adjustment to pH 3, 7, 10	H, P	Standard THIA
2	Shaking + pH-adjustment to pH 2, 3, 4	H	Standard THIA
3	LLE (extractor) and Separating funnel	D, H	Standard THIA

4.1.2 Standard addition trials with β-acids

First it was determined, whether the β-acids are detectable with the Sunfire-column and the standard eluent (75 % MeOH, 24 % H_2O and 1 % H_3PO_4 [85 %]). Then the obtained peaks were compared with the ICE-2 standard. The β-acids separate into two minor peaks (co-lupulone and a second combined peak of lupulone and ad-lupulone), as apparent from Fig. 20 (black curve = ICE-standard). All trials conducted with β-acids are represented in Table 6.

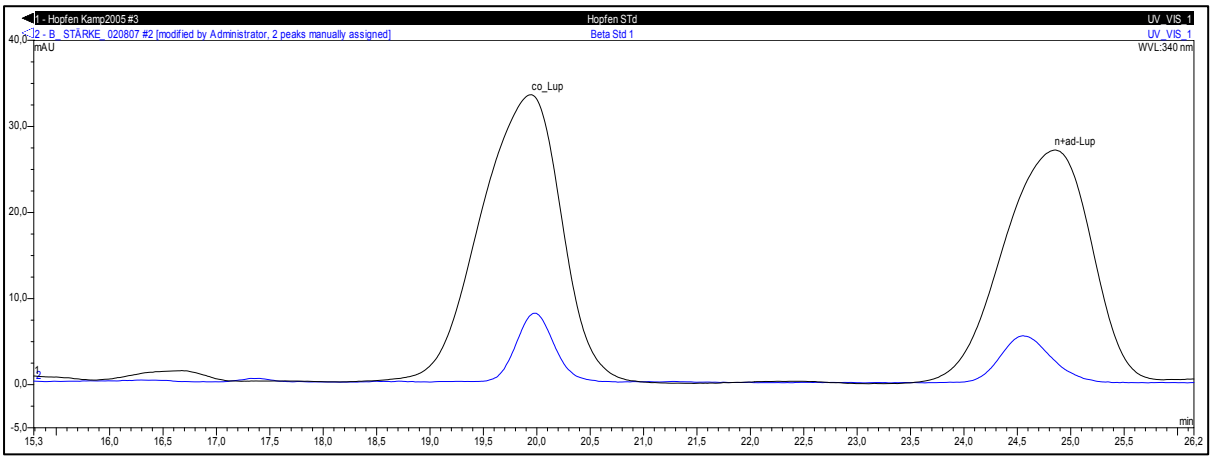

Fig. 20: Comparison of ICE-2 standard with β-acids extract

Table 6: Extraction trials with starch, using β-acids

Trials	Extraction method	Solvent(s)	HPLC application
1	Soxhlet No. 2*	H, D	Standard Beta
2	Hydrolysis, Stirring + pH-adjustment	H	Standard Beta
3	Stirring without pH-adjustment	H, B, H+B (1:1)	Standard Beta

* including absorbent cotton

The starch-hydrolysis was carried out as follows: a starch-slurry was cooked with hydrochloric acid at 105°C in a water quench over a magnetic stirrer. The pH was adjusted to 8.5; the standard was added and allowed to stir with hexane for 2 hours. Afterwards the starch was filtered and evaporated to dryness. Special attention has to be paid when the hexane is evaporated to dryness, as β-acids do not

withstand harsh evaporating conditions. Therefore a temperature of 40 °C at 335 mbar is recommended.

4.1.3 Preliminary trials with IA

The chromatogram in Fig. 21 shows the obtained peaks, when analysing the IA, using IA standard application. For the IA standard application see chapter 3.5.3.3 As apparent isocohumulone, isohumulone and isoadhumulone are detectable. The chromatogram is compatible with the chromatogram provided by Labor Veritas for DCHA-Iso, ICS-I2 (MULQUEEN, 2004). However, it remained with preliminary trials for IA, as THIA and β-acids performed better in restricting of lactic acid formation in the steeping process. After a meeting of BETATEC and ZFT (2007), it was decided to put the main focus of the pilot trials on THIA.

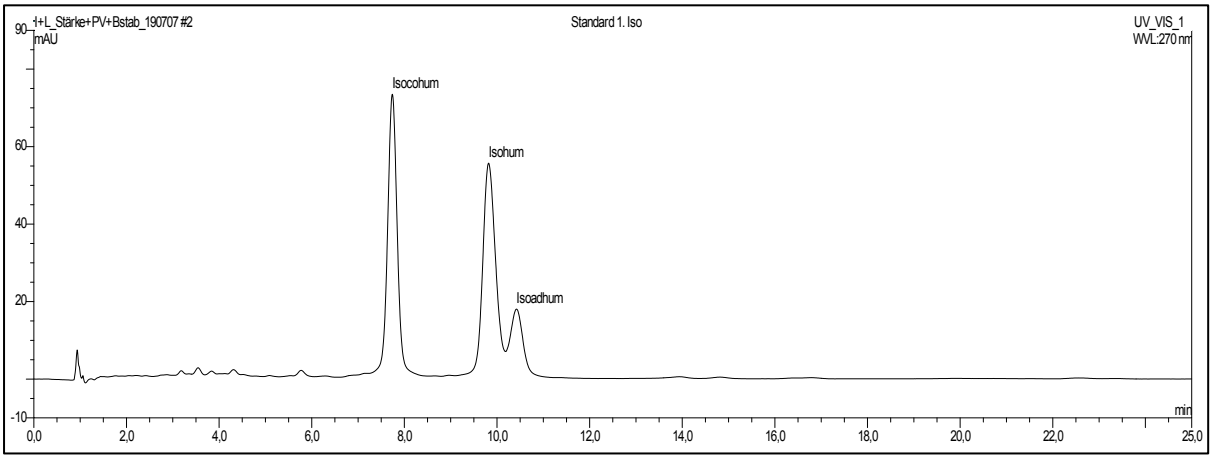

Fig. 21: Representative chromatogram of iso-α-acids (IA)

4.2 Determination of LOD and LOQ

The LOD (limit of detection) can be defined as three times the noise, where it is possible to determine amounts qualitatively.
The LOQ (limit of quantification) on the other hand side is the lowest concentration at which a quantitative detection is possible. The LOQ is the concentration where quantitative results can be reported with a high degree of confidence. It can furthermore be defined as approximately three times the LOD.

As the method for the calculation of the LOD and LOQ, the signal to noise ratio-method was used. It was applied on the THIA-standard method for analysis of starch from pilot trials.
For the calculations, mean values of initial and resulting weights of the preliminary trials with starch were drawn on.

4.3 Main trials with pilot plant products

4.3.1 Overview of pilot trials

Table 7 summarises the conducted pilot trials including detailed information on addition amounts of the conventional sodium bisulfite and alternative hop products. Furthermore the pH-values, as well as the steep water to tap water ratio are denoted. The pilot plant trials should help to assess the processability of maize, treated with hop extracts and to confirm the results obtained by preliminary maize steeping experiments. The results of these trials are discussed in detail in part 2 (HUBER, 2007).

Pilot-scale trials in July:

PT 1: Trial with reduced sodium bisulfite addition ($NaHSO_3$; 40 %)

PT 2: Trial with reduced sodium bisulfite and 20 ppm THIA addition

PT 3: Trial with conventional sodium bisulfite addition

Pilot-scale trials in August:

PT 4: Trial with addition of β-acids: 100 ppm

PT 5: Trial with addition of THIA: 5 ppm

PT 6: Trial with addition of THIA: 10 ppm after 24 hrs. steeping time

Table 7: Pilot trials - overview

BetaTec pilot trials July/August 2007								
PT	Steeping time	$NaHSO_3$ (40 %) / 82 kg grain maize***	Hop products	pH 0 h	pH 24 h	pH 48 h	HCl add. [mL] / 100 kg Maize°°+100 L Steep water + 150 L TW** with NaHSO3 (40 %)	SW*/TW** [mL]
1	7.-9.7.	1kg	-	4	4	4	0	40/60
2	9.-11.7.	1kg	20 ppm THIA	4,2	4,1	4,1	400	40/60
3	21.-23.7.	2kg	-	4,4	4,3	4,1	900	40/60
4	6.8.-8.8.	1kg	100 ppm β-acids	4,8	4,4	4,2	1400	28/72
5	18.-20.8.	1kg	5 ppm THIA	4	4,25	4	500	28/72
6	20.-22.8.	1kg	10 ppm THIA 24h	4,8	4	4,2	1300	28/72

*SW = steep water **TW = tap water PT = Pilot trial

***100 kg grain maize with 82 % dry matter= 82 kg maize absolute °° Substance values not related to dry matter

4.3.2 Analysis of pilot plant products

Six starch samples were analysed for evaluation of the method. They were taken from a pilot trial, carried out in January 2007 in the pilot plant of ZFT (further on, referred to as PT "J"). 25 ppm (25 mg active ingredient per kg maize) was sprayed onto the maize. The data of this trial is not listed in Table 7. Soxhlet No. 2 (including absorbent cotton, in order to prevent leaching out of sample material) was carried out, using dichloromethane and standard HPLC conditions for THIA.

Then, the starches produced in July and August, were determined for residual THIA amounts. In detail, starch samples from pilot trials PT2 (addition of 20 ppm THIA) and PT 6 (addition of 10 ppm THIA after 24 hours of steeping time) were analysed six times. Furthermore, starch samples from PT5 (addition of 5 ppm THIA) were analysed three times.

Maize feed from PT 2 (addition of 20 ppm THIA) was analysed three times, taking a 5 g sample each. Adding always 10 g of sodium sulfate, samples were shaken out twice, with 25 mL of solvent mixture 2. It contains of methanol/2-propanol/butanol = 50/25/25 (v/v).

The bottles were shaken on a bench top shaker for 15 min and centrifuged for 10 min at 3000 rpm. The supernatants were filtered and the united extracts were placed into a deep freezer for 2 hours (at -30 °C) in order to freeze out fat and fat accompanying substances. After that the solvent was filtered again over a folded filter into a round-bottom flask. The solvent was evaporated to dryness and the residues were dissolved in methanol. The special THIA HPLC-application was used (chapter 3.6.7.1).

Finally, a balance sheet was created using analysed and calculated values to show the distribution of the THIA in starch and by-products.

5 RESULTS AND DISCUSSION

At the beginning, the THIA peaks shown in the following chromatograms and their naming should be explained. The THIA (tetrahydroiso-α-acids) peaks are referred to as α1 – α4. The peaks of the THIA product consist approximately as follows:

→ α1 = trans-THI-cohumulone
→ α2 = cis-THI-cohumulone } → α1 + α2: 29.6 % of total THIA peak area
→ α3 = THI-humulone → 61.1 % of total THIA peak area
→ α4 = THI-adhumulone → 8.7 % of total THIA peak area

For the calculations of recoveries, the α3-peak (THI-humulone) was usually drawn on. The chromatographic separation of THIA is shown in Fig. 22.

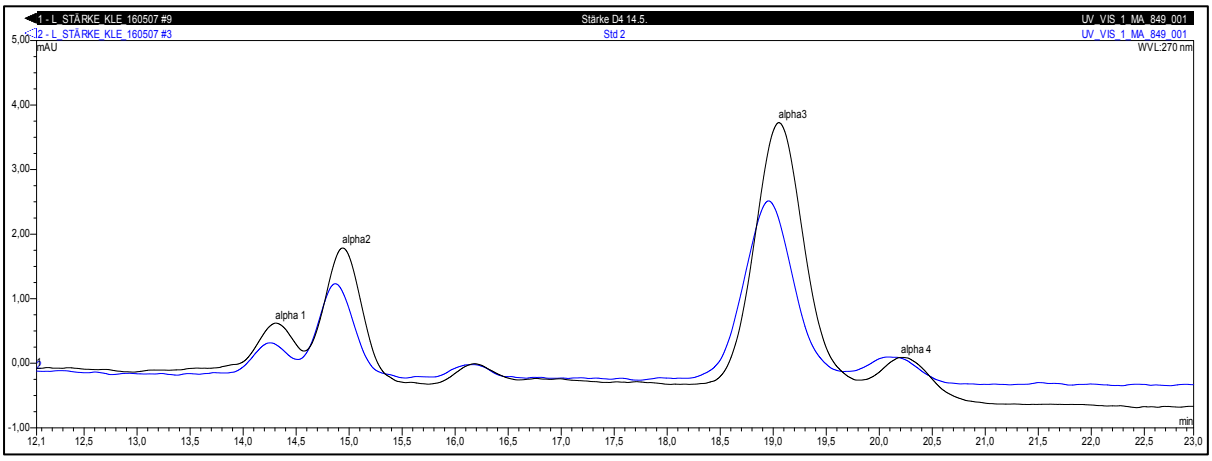

Fig. 22: Representative chromatogram of THIA

The identification of the THIA compounds was performed at 270 nm (UV-detector), by comparing the retention times of spiked samples and standards. The most promising results (highest recoveries) were validated by calculation of standard deviation (SD) or relative standard deviation, respectively. As percentage indication it is referred to as coefficient of variance (CV) and is expressed in % in the present tables. For the main trials, the most reproducible method was chosen.

5.1 Preliminary trials

5.1.1 Standard addition trials with THIA

5.1.1.1 Starch

Table 8 reveals the results obtained by Soxhlet extraction ("Soxhlet No. 1": without absorbent cotton; "Soxhlet No. 2": with absorbent cotton) using several solvents (H, D and P). As obvious, dichloromethane yielded the highest recoveries and was consequently attempted further. Recoveries, which are obvious from Table 8, were calculated in relation to initial weight of stock solution (with what the starch and by-products were spiked) and to resulting weight of methanol (in what the evaporated THIA where dissolved).

Table 8: Results - Soxhlet No. 1 (starch)

Trials	Solvent	Amount THIA [mg/kg]	Recoveries [%]
1	H	7.63	64.12
2	H	5.95	52.44
3	D	7.54	84.30
4	D	8.65	82.91
5	P	6.14	23.42
6	P	6.00	33.17

Methods:
Trials 1-6: Soxhlet 1

Further experiments were carried out by means of extract by stirring. The results are shown in Table 9. First trials were performed without pH-adjustment. In order to obtain better results, the pH was adjusted to an acidic (pH 4) and alkaline value (pH 10). The adjustment to pH 4 did yield in better results and was therefore investigated further. These results can be seen in Table 10. A corresponding chromatogram is depicted in Fig. 23. The extraction was done six times. Due to better results the SD (standard deviation) and CV (coefficient of variance in %) were calculated.

Table 9: Results - stirring (starch)

Trials	Solvent	Amount THIA [mg/kg]	Recoveries [%]
1	D	8.90	63.42
2	D	5.35	61.99
3	D	5.05	58.77
4	D	1.12	12.49

Methods:
Trials 1-2: Stirring without pH-adjustment
Trial 3: Stirring, pH adjusted to pH 4
Trial 4: Stirring, pH adjusted to pH 10

Table 10: Results - stirring with pH-adjustment (starch)

Trials	Solvent	Amount THIA [mg/kg]	Recoveries [%]
1	D	2.25	76.45
2	D	2.33	76.19
3	D	2.58	82.85
4	D	2.73	88.74
5	D	2.61	84.75
6	D	2.64	86.99
Mean value		2.52	82.66
SD		0.19	5.30
CV (%)		7.50	6.41

Methods:
Trial 1-6: Stirring, pH adjusted to pH 4

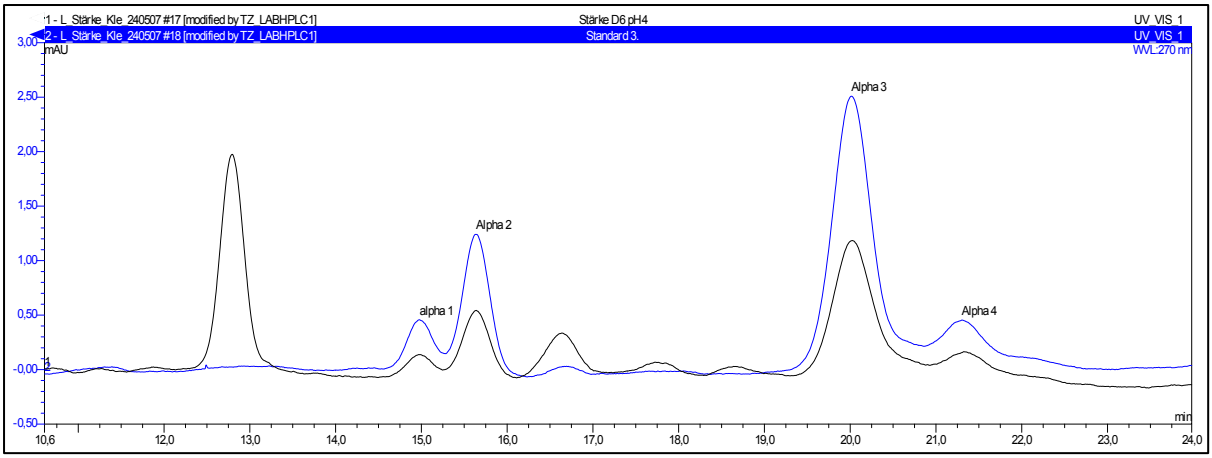

Fig. 23: Representative chromatogram of stirring at pH 4 (starch)

Eventually, Soxhlet No. 2 with dichloromethane was carried out. Compared to method 1, recoveries could be improved about 5 %. These results are apparent from Table 11. A corresponding chromatogram is shown in Fig. 24.

Table 11: Results - Soxhlet No. 2 (starch)

Trials	Solvent	Amount THIA [mg/kg]	Recoveries [%]
1	H	3.05	88.37
2	H	3.09	89.53
3	D	3.05	87.19
4	D	3.09	88.30
5	P	3.07	91.15
6	P	3.10	88.17
Mean value		3.08	88.79
SD		0.02	1.38
CV (%)		0.69	1.55
Methods: Trials 1-6: Soxhlet 2			

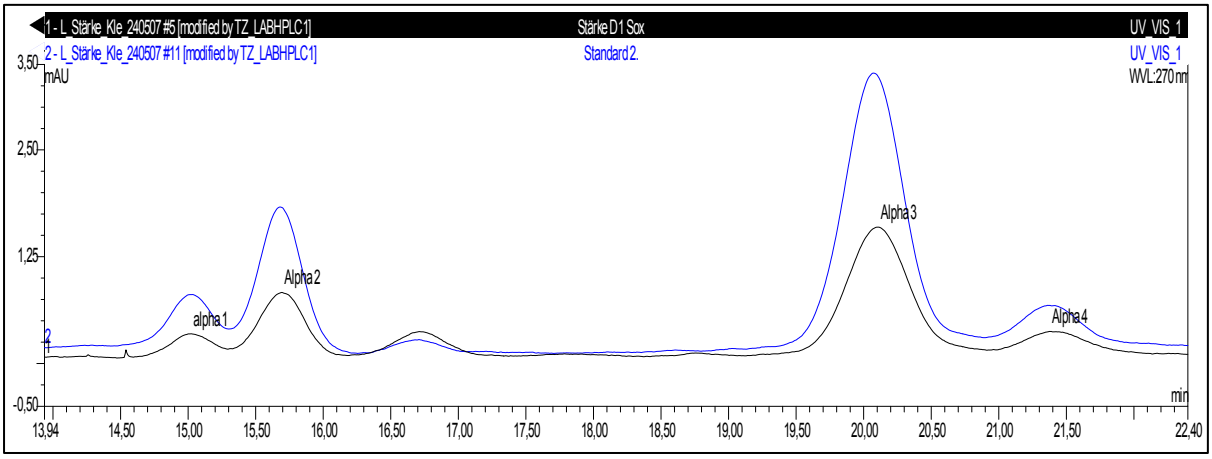

Fig. 24: Representative chromatogram of Soxhlet No. 2 (starch)

Comparing CV values of extract by stirring (Table 10) and Soxhlet No. 2 (Table 11), it can be said that the Soxhlet method performed better, due to a lower coefficient of variance. Hence, the Soxhlet No. 2-method was applied in the main trails, when analysing starch produced in pilot-scale.

5.1.1.2 Maize feed

Table 12 summarises the results, obtained by Soxhlet No. 1, using H, D and P as solvents. With THIA extraction from maize feed, it could also be observed that dichloromethane reached reliable results. The recovery of 100 %, with n-hexane was considered as an outlier, as the second trial did only yield 40 %. However, problems occurred, when analysing the maize feed samples by HPLC. The chromatogram in Fig. 25 makes obvious, that finding an adequate solvent was not sufficient, as matrix components (fat) were disturbing the separation.

Table 12: Results - Soxhlet No. 1 (maize feed)

Trials	Solvent	Amount THIA [mg/kg]	Recoveries [%]
1	H	3.47	40.13
2	H	8.60	100.02
3	D	4.60	52.31
4	D	6.01	46.82
5	P	5.52	41.93
6	P	4.80	35.81

Methods:
Trials 1-6: Soxhlet 1

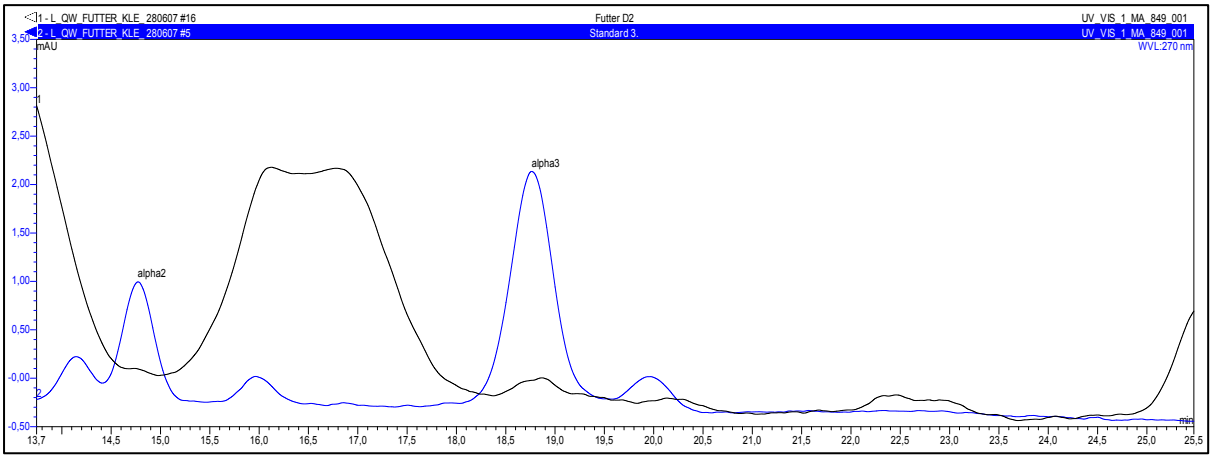

Fig. 25: Representative chromatogram of Soxhlet No. 1 (maize feed)

Therefore, a method or solvent was needed, that could help minimise the fat contamination. The data shown in Table 13, reveal further attempts of extract by stirring, using just butanol and a solvent mixture containing butanol, plus 2-propanol and methanol in a ratio of 1:1:1 (v/v). Table 13 includes the mean and CV values, which prove that extract by stirring with the solvent mixture was more reliable.

Table 13: Results - stirring (maize feed)

Trials	Solvent	Amount THIA [mg/kg]	Recoveries [%]
1	B	2.98	54.04
2	B	2.53	67.16
3	Solvent mixture 2	3.36	69.07
4	Solvent mixture 2	2.30	71.61
Mean value (trial 1+2): 60.60		SD: 9.28	CV (%): 15.31
Mean value (trial 3+4): 70.34		SD: 1.80	CV (%): 2.55
Methods:			
Trials 1-4: Stirring and Filtration			

Fig. 26 represents the comparison of two chromatograms, which very well demonstrates the troubles that occurred, when injecting several samples in a row. The blue curve shows the peaks of the first injection, the black one of the sixth injection. As it shows up, the latter chromatogram has already been fairly overlaid by fat matrix. Therefore the standard THIA HPLC-program was modified. After the usual run time of about 23 min, a seven-minute cleaning step was inserted, followed by a ten-minute column re-equilibration.

Using the modified HPLC-program (special HPLC-application) and extract by stirring with solvent mixture 2 (methanol/2-propanol/butanol = 50/25/25 (v/v), best results were obtained. These proved to be satisfactory to apply the method in main trials (on maize feed produced in pilot scale, using THIA).

A corresponding chromatogram is shown in Fig. 27.

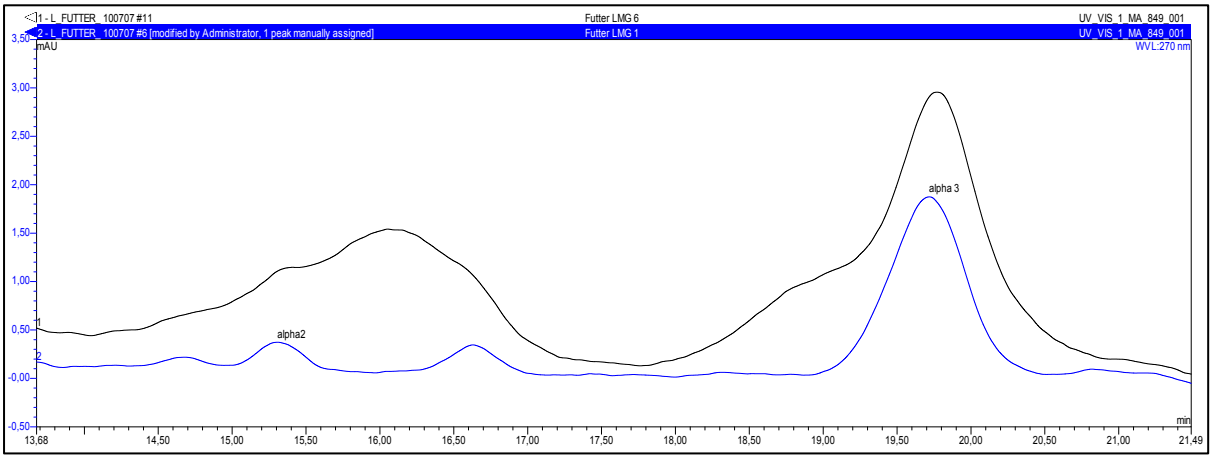

Fig. 26: Chromatogram comparison - stirring with solvent mixture (maize feed)

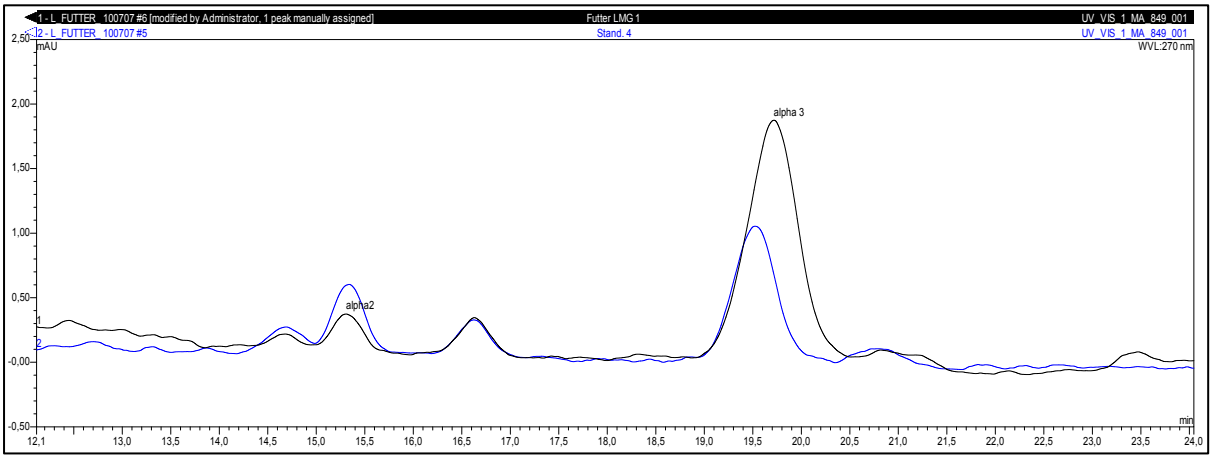

Fig. 27: Representative chromatogram - stirring with solvent mixture (maize feed)

5.1.1.3 Gluten

This was also a matter more difficult to deal with. Trials with Soxhlet No. 1 and 2 did not led to assignable peaks. The attempts with dichloromethane are shown in the chromatogram in Fig. 28. It is furthermore obvious that the THIA-peaks were overlaid by a fat matrix.

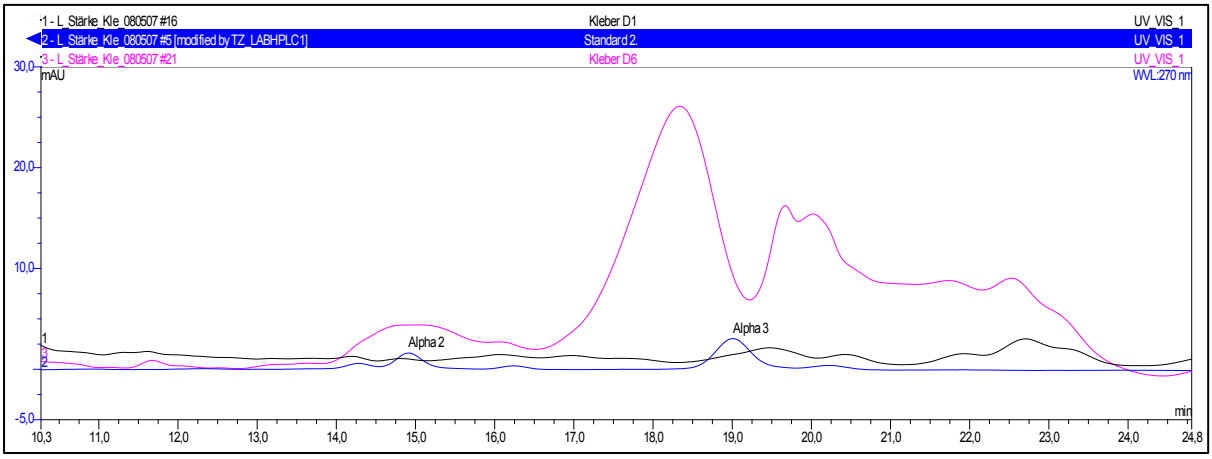

Fig. 28: Representative chromatogram - Soxhlet No. 1 (gluten)

Referring to the literature solid phase extraction using methanol was tested, which inexplicably did not yield any assignable peaks, either. However, due to time constraints, it was not possible to repeat trials, as the main focus at the end of the practical work was on the main trials. A chromatogram of SPE-extraction can be seen in Fig. 29. As absolutely no THIA-peaks are obvious in the chromatograms, an assumption which suggests itself would be that errors have already occurred during sample preparation. After studying the literature SPE is, despite negative results in the present work considered an adequate sample preparation-method prior to HPLC-analysis.

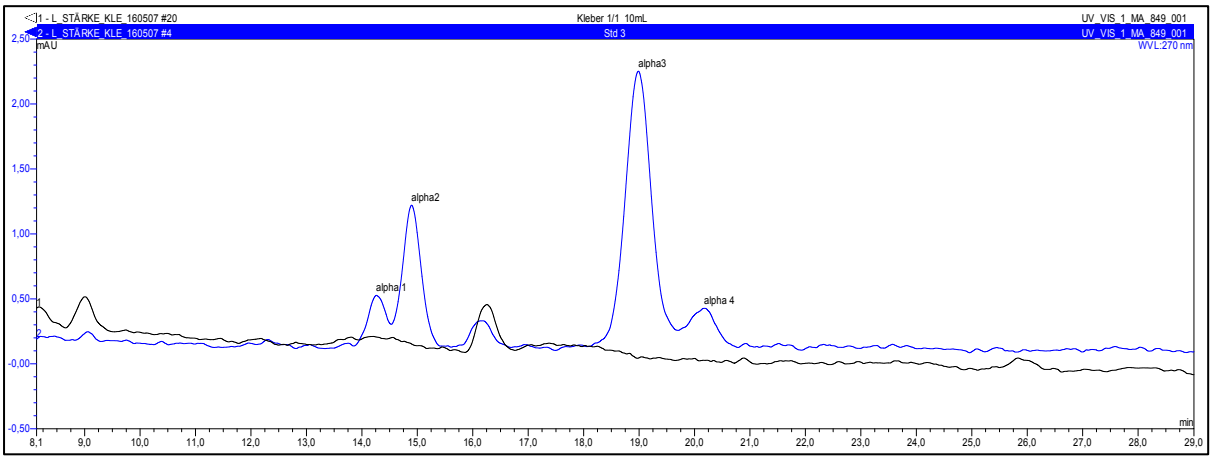

Fig. 29: Representative chromatogram - SPE (gluten)

Further attempts were started using stirring and filtration afterwards. As solvents, butanol and a mixture of methanol, 2-propanol and butanol in a ratio of 1:1:1 (v/v) were used. Since the peaks were also matrix-overlaid, the special HPLC-program was also applied in the present trials. A chromatogram of THIA-separation is obvious from Fig. 30. By modifying the method and HPLC-program the recoveries could be increased. The results (recoveries between 50 - 77 %) are shown in Table 14. Definitely, further attempts are to be conducted, in order to obtain more reliable data.

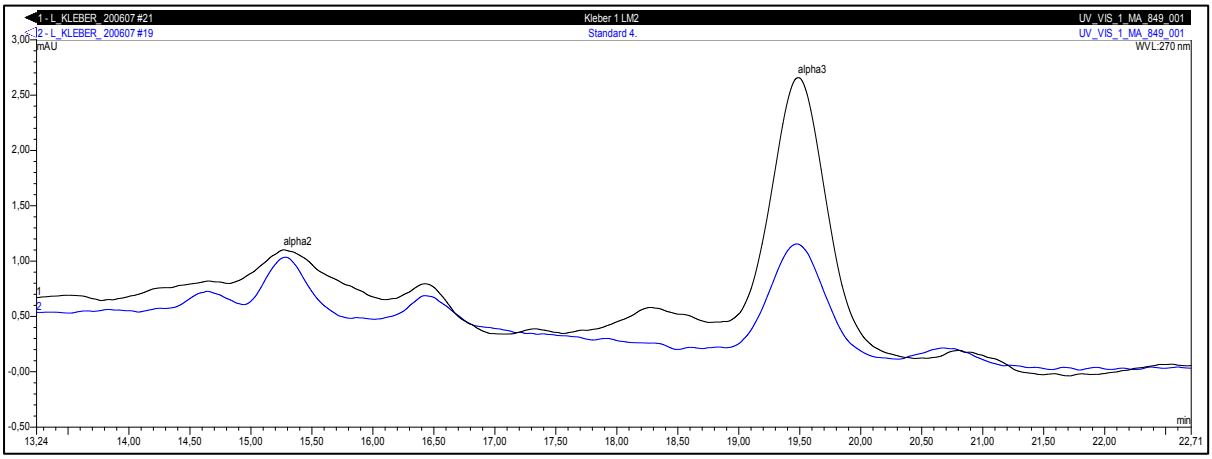

Fig. 30: Representative chromatogram - stirring with solvent mixture (gluten)

Table 14: Results - stirring with solvent mixture (gluten)

Trials	Solvent	Amount THIA [mg/kg]	Recoveries [%]
1	Solvent mixture	1.98	51.37
2	Solvent mixture	1.46	57.57
3	Solvent mixture	3.20	76.09
4	Solvent mixture	2.73	77.94

Methods:
Trials 1-4: Stirring and Filtration

5.1.1.4 Germs

At the beginning, the extraction of THIA was tested with Soxhlet No. 1. Instead of dissolving in methanol acetonitrile was also tested as a solvent, but without appreciable results. The corresponding chromatogram is shown in Fig. 31. As maize feed and gluten were overlaid by fat matrix, new methods and programs had to be developed. Although germs, as well as gluten were not analysed in main trials, sundry ideas were tried and varied. Moreover, extract by stirring with butanol was accomplished, in order to remove the fat at least superficially from the germs. Afterwards the degreased germs should be used for Soxhlet extraction with dichloromethane. It was found out that the THIA are removed before by stirring with butanol. The chromatogram in Fig. 32 depicts the separation very well. It ought to be stated that an absolutely essential step with extracting THIA from germs, and also gluten or maize feed, is the freezing out of fat remnants, as otherwise it is nearly impossible to get the peaks separated properly. On the other hand side, it must be said that just this step is a potential source of errors. It cannot be assured that not also THIA are removed during freezing out. It is also recommended to inject methanol after every sample injection (especially with germs) to rinse and clean the column. The final results can be found in Table 15 and clarify that the very low recoveries necessitate a lot of progressing tests, before more satisfying results can be achieved.

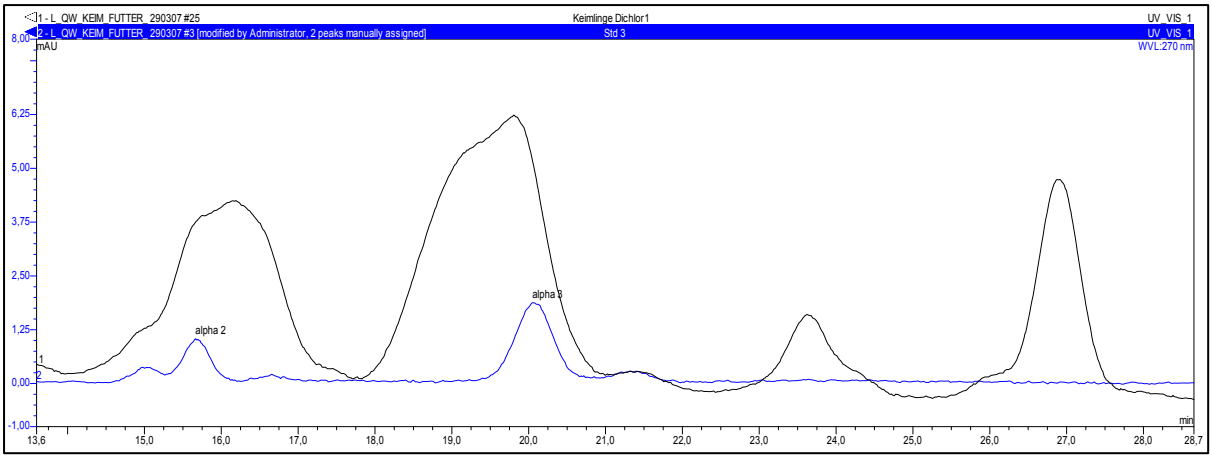

Fig. 31: Representative chromatogram - Soxhlet No. 1 with D (germs)

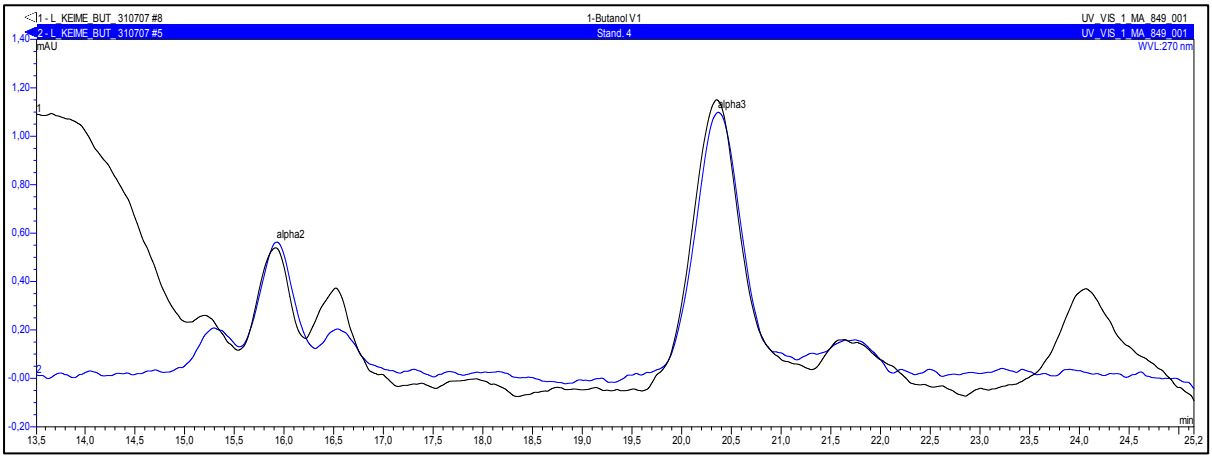

Fig. 32: Representative chromatogram - stirring with B (germs)

Table 15: Results - stirring with B (germs)

Trials	Solvent	Amount THIA [mg/kg]	Recoveries [%]
1	B	0.85	40.08
2	B	0.69	35.98
3	B	0.60	26.91
4	B	0.99	32.71

Methods:
Trials 1-4: Stirring and Filtration

5.1.1.5 Steep water

At the beginning of the extraction experiments, it was demanded to find out at what pH-value the THIA are extracted best. Attempts were made at pH 3, 7 and 10 with H, P and D. The extraction method of first choice was shaking out, as formerly applied in ZFT with hop acid-extraction from pressed sugar beet pulp. The corresponding chromatogram is pictured in Fig. 33.

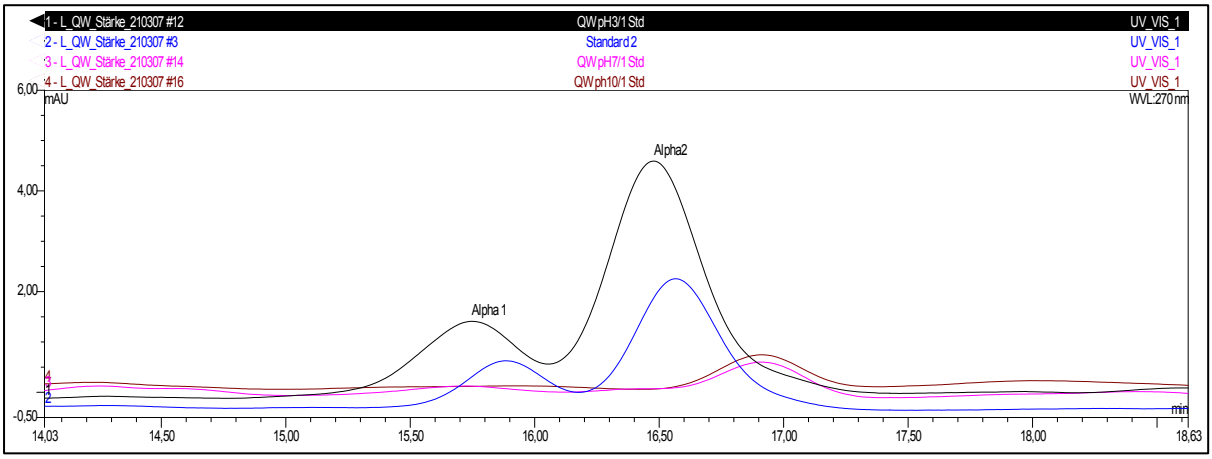

Fig. 33: Representative chromatogram - shaking out with H, pH adjusted (steep water)

The comparative peaks make clear that the extraction at pH-value 3 yields the highest peaks and the highest recoveries. For this reason the extractions were always attempted in acidic milieu. Results are shown in Table 16. Further modifications in acidic pH-values (pH 2, 3 and 4) did not result in better separability or higher recoveries. For THIA separation extracted from steep water, the standard THIA HPLC-program was sufficient.

Table 16: Results - shaking out at pH 3 (steep water)

Trials	Solvent	Amount THIA [mg/kg]	Recoveries [%]
1	H	7.28	81.14
2	H	9.9	74.14
3	H	11.45	73.58
4	H	8.5	77.84

Methods:
Trials 1-4: Shaking out at pH 3

The most suitable method was shaking out with n-hexane and previous pH-adjustment to pH 2-3. Further attempts using LLE (with extractor overnight or separating funnel) did not yield assignable peaks. Using LLE-extractors with D (as specific heavier solvent) did not work well enough to extract all the THIA, even though allowing extraction last for about 12 hours. The problem with shaking by means of a separating funnel was extreme foam formation. It did not disappear despite several hours of waiting for phase separation.

5.1.2 Standard addition trials with β-acids

5.1.2.1 Starch

The first trials with β-acids and starch were performed, using Soxhlet No. 2 with H and D. The standard beta HPLC-program was used for all separations. Moreover, extract by stirring was carried out. The first trials were performed, using n-hexane and pH adjustment to pH 8.5, as these conditions have already been successfully applied in ZFT, with extraction of β-acids from sugar and molasses. The starch was hydrolysed by cooking with hydrochloric acid at 105°C, pH adjusted to 8.5 and stirred for 2 hours. These efforts did not lead to satisfying recoveries of β-acids. Therefore extract by stirring was modified using again n-hexane and butanol, as well as a mixture of both solvents in a ratio of 1:1. However, extract by stirring with n-hexane (without pH adjustment) only yielded recoveries of about 40 %. The chromatogram is shown in Fig. 34.

The β-acids are recommended to be evaporated at gentle conditions (at a temperature of 40 °C and a pressure of 335 mbar). In any case, further experiments with β-acids will be necessary to obtain higher recoveries and reproducible results.

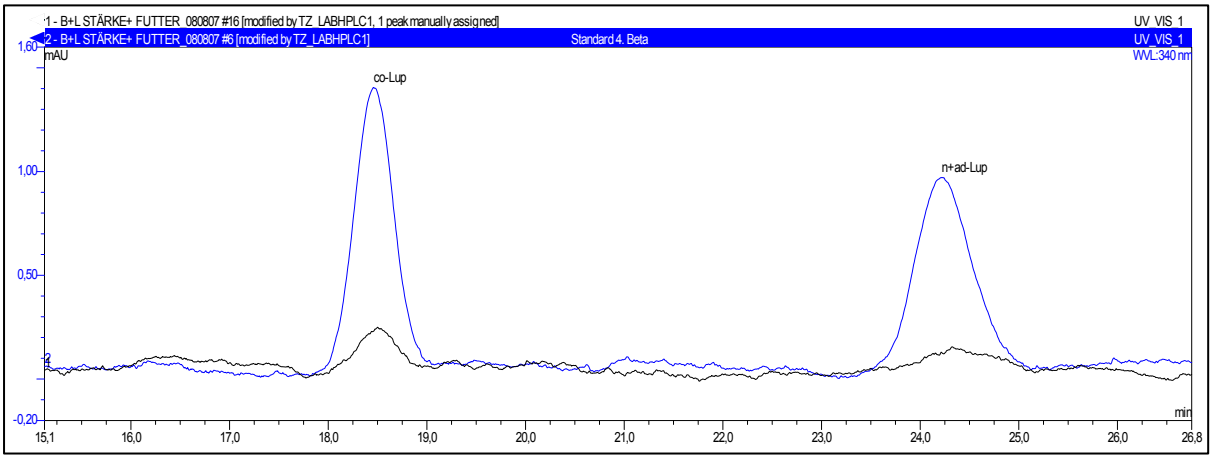

Fig. 34: Representative chromatogram - stirring with hexane (β-acids)

5.2 LOD and LOQ of THIA-standard method

The LOD and LOQ values of the THIA-standard method (sample preparation via Soxhlet extraction) for analysis of starch were considered most important, as starch was the most treated product with THIA.

Table 17 represents firstly, the calculation of the LOD via the amount of noise (determined directly from a chromatogram, as difference of height from peak top to peak bottom); the LOD was calculated by multiplying the noise level by 3 and moreover the LOQ results out it (three times the LOD).

Secondly, the analysis results directly from the Chromeleon®-Software, in this case the height and amount of a humulone- (α3) peak of a standard were taken to calculate the amount of THIA detected in solution. The calculation is represented via a final account. This LOD-value of THIA in solution was about 1.47 ppm. This value was then calculated on starch (with the mean initial weight of starch and the resulting weight of methanol).

The result was rounded 3904 g (then converted to mg) of starch in one kg of methanol. Supposing 3,903,904 mg as 100 %, 1.4734 ppm in solution correlate with $3.7742*10^{-5}$ % in starch. The percentage value was multiplied by 10,000 to obtain ppm. This step was done directly and the LOD-result of 0.3774 ppm of THIA (calculated on starch) as well as the LOQ of 1.1347 ppm can be seen below.

Table 17: Calculation of LOD and LOQ for standard THIA-application

Calculation LOD:

Peak top		0.311	[m*A*U]
Peak bottom	-	0.161	[m*A*U]
Δ		0.15	[m*A*U]
0.15 * 3 =		0.45	[m*A*U]

Calculation LOQ:

3*LOD	1.35	[m*A*U]

Examplary calculation starch: 26 g starch/ 6.66 g MeOH

height	1.909	0.45 (LOD)
amount	6.2507	x
	x = 1.47 ppm (mg/kg) in solution	

Calculated on starch:

	26 g of starch	in 6.66 g of MeOH
	x	in 1000 g of MeOH
	x = 3903.90 g of starch/ kg MeOH	

3903.904 g = 3903904 mg	390390	100%
	1.4734	x
	x = 0.000037742	

0.000037742 % = 0.37742	ppm	0.38	LOD
	ppm	1.13	LOQ (LOD*3)

5.3 Main trials

Table 18 summarises all the results achieved by HPLC-analysis of products produced in pilot scale. There is information on which samples (materials) were examined, how much THIA were sprayed onto the maize (before processed in pilot-scale) and also on the amount of THIA quantified by HPLC-analyses. The absolute amount of THIA was calculated (in mg per kg or ppm, respectively) related to the initial weight of starch or maize feed and the resulting weight in methanol prior to HPLC-analysis. Thereupon mean values and standard deviations were calculated except for starch pilot trial no. 5, where just one peak was assignable what led to just one result (signed as single value). Fig. 35-39 represent the chromatograms obtained by analysing starch and maize feed produced in pilot-scale. All additions result in residues below 0.05 ppm in starch. However, it is obvious that addition of 20 ppm of THIA resulted in mean amount of 0.02 ppm, whereas addition of only 10 ppm of THIA gave results of about 0.04 ppm residues of THIA. This fact might be due to the latter addition of the 10 ppm amount (after 24 hours of steeping time). It might be that active ingredients have degraded in that time.

Table 18: Summary of all pilot trial-results

Sample Name	PT	THIA-addition (Pilot trials) [mg/kg]	Amount THIA (HPLC) [mg/kg]	Amount THIA (calculated) [mg/kg]
Starch	J	25		
Mean (n=6)			0.14	0.04
SD			0.03	0.01
Starch	2	20		
Mean (n=6)			0.09	0.02
SD			0.03	0.01
Maize feed	2	20		
Mean (n=3)			2.24	4.9
SD			0.19	0.79
Starch	5	5		
Single value			0.13	0.04
Starch	6	10		
Mean (n=6)			0.21	0.04
SD			0.08	0.02

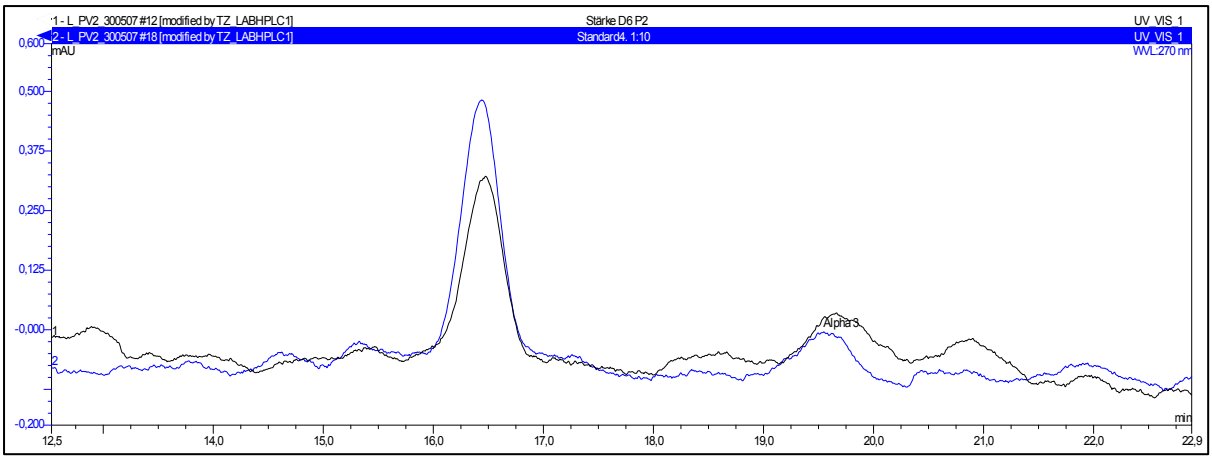

Fig. 35: Pilot trial: 25 ppm on starch - compared to standard 0.25 ppm

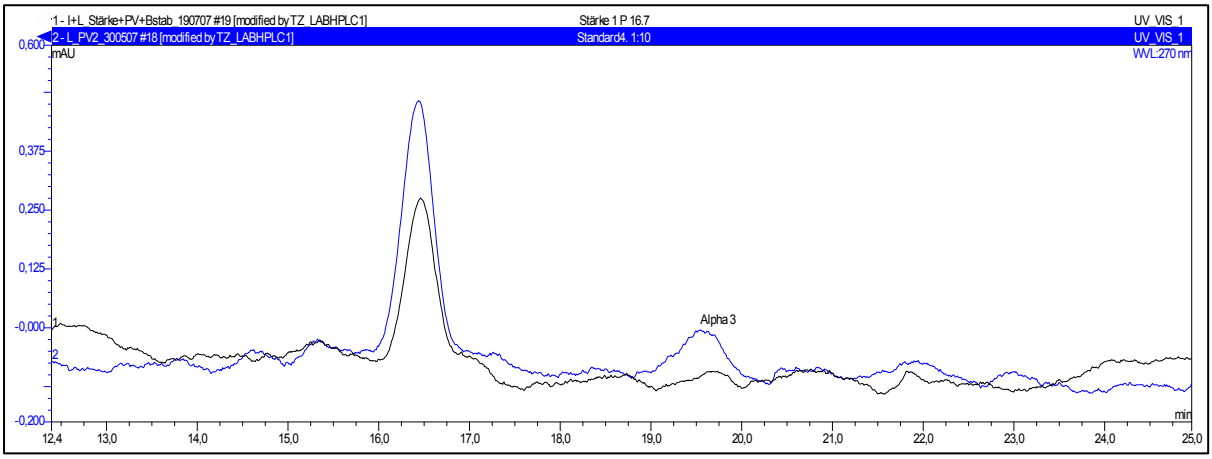

Fig. 36: Pilot trial: 20 ppm on starch - compared to standard 0.25 ppm

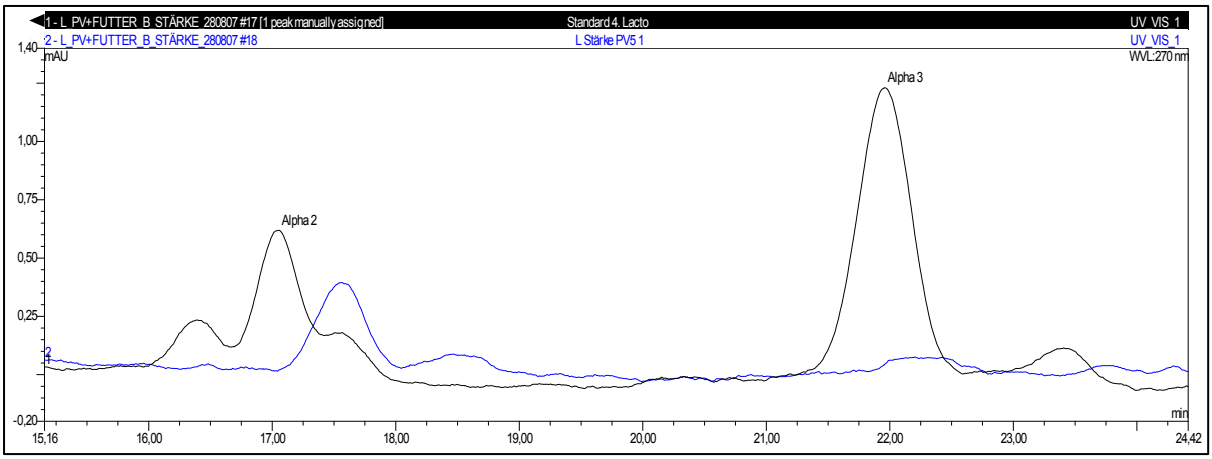

Fig. 37: Pilot trial: 5 ppm on starch - compared to standard 2.5 ppm

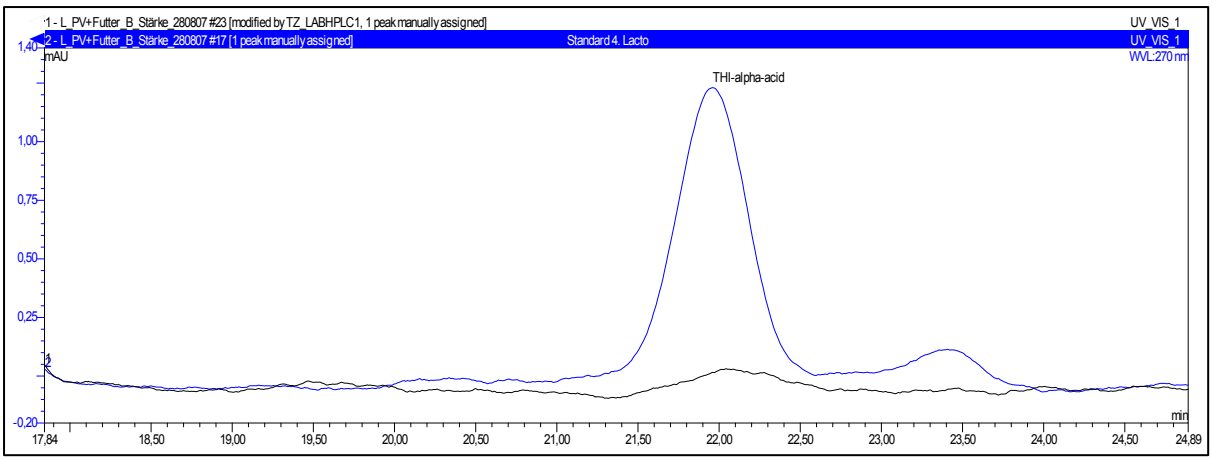

Fig. 38: Pilot trial: 10 ppm on starch after 24 hrs. of steeping time - compared to standard 2.5 ppm

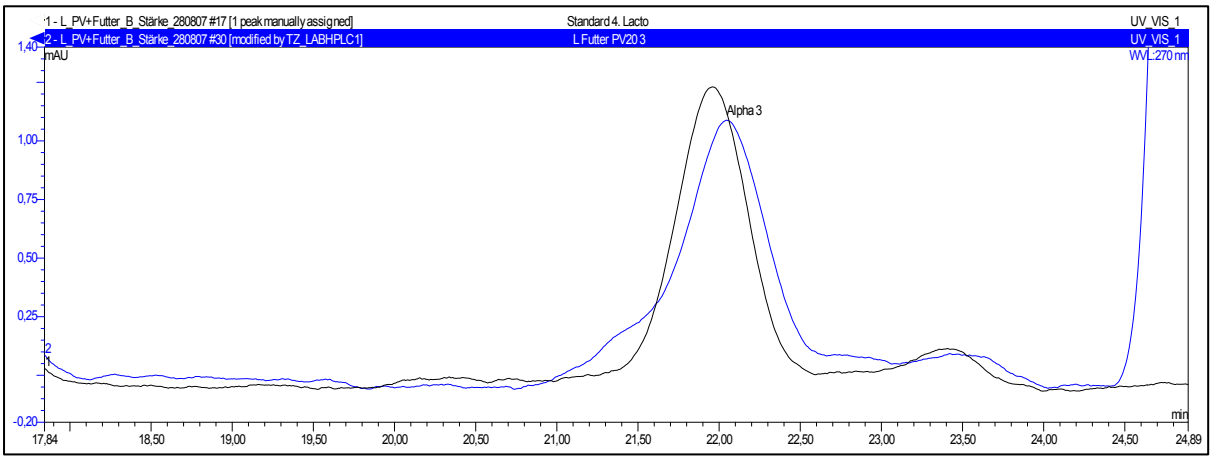

Fig. 39: Pilot trial: 20 ppm on maize feed - compared to standard 2.5 ppm

5.4 Balancing

Accounting the data taken from the pilot trials a mass balance sheet of THIA was drawn up. In Table 19, the entering materials (maize and steep water) as well as the leaving products (starch, maize feed, gluten, germs and LSL, light steep liquor) are listed. Sums are calculated and moreover converted on matter. Moreover, the data is depicted in Fig. 40. Note: maize feed wet is declared as peelings.

Starch and maize feed were analysed by HPLC. On the contrary the remaining by-products are implied to the balance sheet as calculated values. As obvious from analysed THIA amounts in starch and maize feed, the hop acids distribute primarily on by-products and as assumed mostly on steep water or LSL.

Table 19: Mass balance – pilot plant (maize)

Material	[kg]/S	[%] DM		[kg]/DM	THIA	[ppm]	[mg]	results
Maize	100	85		85		20	4000	
Steep water	100	8		8				
				93				
Starch	45.1	82		37		0.002	0.1	analysed
Maize feed (wet)	65	40		26		0.49	31.9	analysed
Gluten	16	50		8				
Germs	46.7	30		14				
LSL	135	4		5.4				
Sum residues	198		Sum products	90.4	THIA	20	3968	calculated

S = substance DM = dry matter

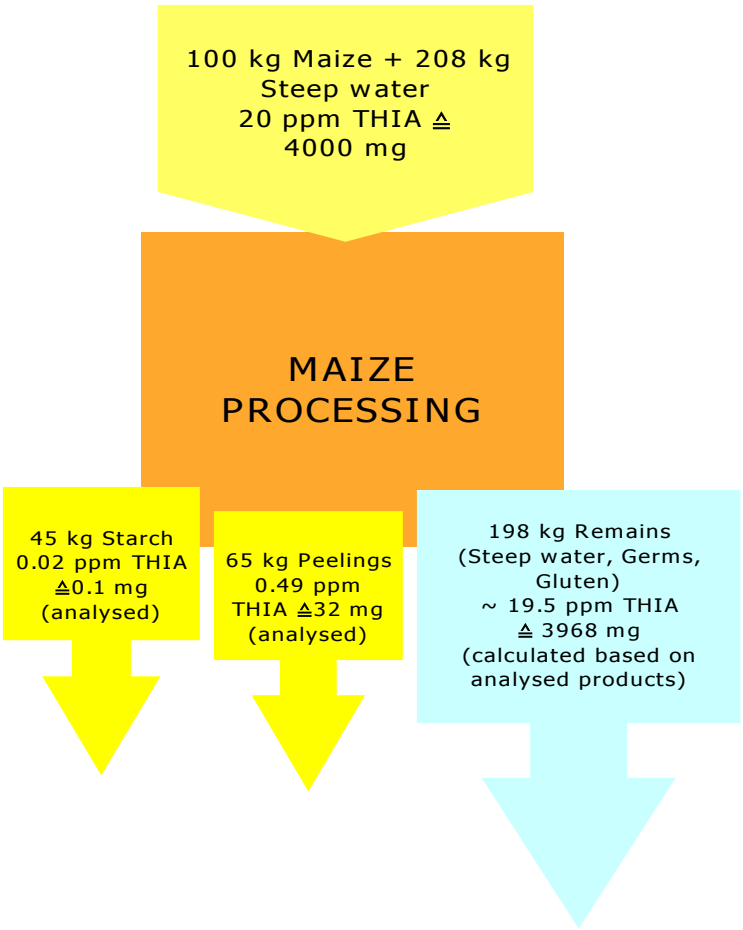

Fig. 40: Mass balance diagram of maize processing in the pilot plant of ZFT

6 CONCLUSIONS

Naturally, starch is the most important product emerging the maize processing and thus special attention in analyses was given to it. By adaptation and a lot of modifications in extraction methods and the HPLC application, it succeeded to develop adequate analytical methods for starch and maize feed and also steep water. When assaying the most fat-containing germs and gluten, it is indispensable to avoid an injection of fat matters into the HPLC-apparatus as firstly, the column gets polluted or even ruined after a short time and secondly, no assignable peaks will result, as fat matrix overlays the hop acid peaks, one wants to quantify.

As already supposed in the beginning, the tetrahydroiso-α-acids distribute primarily in the by-products. Presumably they can be found mostly in steep water and process water. They were also the most analysed hop components, due to their effectiveness to control lactic acid formation in maize processing. Further on, various approaches were performed with β-acids and iso-α-acids. It was possible to successfully match peaks of the used commercially hop products with the conventionally available ICS-standards. For the β-acids, special treatment in sample preparation was needed, as they are especially sensitive to pressure and heat. For β-acids and iso-α-acids it is necessary to perform further investigations, as the main focus in the present work was put on tetrahydroiso-α-acids.

The considerable point of starch quality will be discussed and enrolled in the second part of this project concerning maize steeping.

In summary, the results of starch were most satisfying concerning as well the analytical side as the amounts found in produced starch.

These trials were the first HPLC analyses on hop acids in maize starch and by-products. Hence, the present work can be seen as preliminary work concerning hop analytics in maize sector.

7 SUMMARY

Application of hop products in the fields of wet maize storage and in the steeping process can help to reduce extensive losses and control the growth of lactic acid bacteria. Tetrahydroiso-α-acids, which performed best in steeping trials by reducing the formation of lactic acid, were primarily examined in preliminary trials to find out the best extraction method. Standard addition trials were carried out with starch, maize feed, gluten, germs and steep water. It was possible to figure out an adequate method of analysis for starch (Soxhlet extraction) and maize feed (extract by stirring with solvent mixture containing methanol,2-propanol and butanol). The analysis of gluten and germs proved to be more difficult, because of the high fat contents. Afterwards, the findings of these pre-trials were applied to the main trials, which were carried out using starch and maize feed, produced in ZFT.

As pilot-scale trials were run with 5 ppm, 10 ppm (addition after 24 hours of steeping time) and 20 ppm tetrahydroiso-α-acids, the distribution of this substance in starch and by-products (fibers, gluten, germs and steep water) was investigated most thoroughly. HPLC-analyses were carried out with starch and maize feed. The results indicate that the major part of the tetrahydroiso-α-acids accumulates in the by-products. 5 ppm, 10 ppm and 20 ppm of tetrahydroiso-α-acids were added to starch and 20 ppm were added to maize feed.

In all trials with application of tetrahydroiso-α-acids the residual amounts in starch were below 0.05 ppm and in maize feed about 5 ppm. Additionally, pre-trials with β-acids were accomplished. Pre-trials proved to be challenging, as certain attention has to be paid on sample preparation, because β-acids seem to be sensitive to harsh evaporating conditions (recovery at 40 %). Furthermore a balancing sheet for the pilot trial with 20 ppm addition of THIA was set up. It has confirmed that bulks of these hop acids distribute onto by-products and only traces of the active ingredient are detectable in starch. Eventually, the most important and advantageous finding was, that an addition of hop products has absolutely no unfavourable effects on starch quality.

8 KURZZUSAMMENFASSUNG

Durch die Verwendung von Hopfenprodukten bei der Nassmaislagerung und Maisquellung kann das Wachstums von Milchsäurebakterien kontrolliert werden. Dadurch ist es möglich, höhere Verluste an extrahierbaren Kohlenhydraten zu reduzieren. In den Vorversuchen konzentrierten sich die Analysen auf die Tetrahydroiso-α-säuren, da diese sich in den vorangegangenen Quellversuchen, bezüglich Reduktion der Milchsäurebildung, als am wirksamsten erwiesen hatten. Standardadditionen wurden mit Stärke, Maisfutter, Gluten, Keimen und Quellwasser durchgeführt. Passende Analysenmethoden wurden für Stärke und Maisfutter ermittelt. Die Analyse anderer Nebenprodukte (Gluten, Keime) erwies sich, aufgrund der hohen Fettgehalte, als schwierig. Anschließend wurden die Erkenntnisse der Vorversuche auf die Hauptversuche angewendet. Dabei wurden Stärke und Futter (Schalen), die in der hauseigenen Pilotanlage in der Zuckerforschung Tulln hergestellt wurden, analysiert. Pilotversuche wurden mit Einsatz von 5, 10 und 20 ppm Tetrahydroiso-α-säuren gestartet, wodurch eine mehrmalige Untersuchung der Verteilung der Hopfensäuren auf Stärke und Nebenprodukte ermöglicht wurde. Hauptversuche mittels HPLC wurden mit Stärke und Maisfutter durchgeführt. Die Ergebnisse deuteten darauf hin, dass sich der Hauptteil der Tetrahydroiso-α-säuren in den Nebenprodukten ansammelt. Es erfolgte eine Zugabe von 5 ppm, 10 ppm und 20 ppm zur Stärke, sowie 20 ppm zum Maisfutter. Für alle Stärkehauptversuche kann ein Restmengengehalt unter 0.05 ppm und für Maisfutter um 5 ppm, angegeben werden. Weiters wurden Vorversuche mit β-Säuren durchgeführt. Diese erwiesen sich als schwieriger, da an die Probenvorbereitung höhere Anforderungen gestellt werden müssen (Wiederfindungen um 40 %). Außerdem wurde eine Bilanz, (Zugabe 20 ppm) mithilfe der Massenströme aus der Pilotanlage und der Analysendaten der HPLC, erstellt. Dabei wurde bestätigt, dass sich der Grossteil der Hopfensäuren auf die Nebenprodukte verteilt und lediglich Spuren davon in der Stärke auffindbar sind. Schließlich sei deutlich gemacht, dass die Zugabe von Hopfenprodukten an diversen Stellen der Maisstärkeherstellung, absolut keine negativen Auswirkungen auf die Stärke hat.

9 REFERENCES

9.1 ANALYTICA-EBC, Analysis Committee of the European Brewery Convention, Chapter 7.8: iso-α-, α- and β-acids in hop and isomerised hop extracts by HPLC. Fachverlag Hans Carl, Nürnberg (2005).

9.2 ANALYTICA-EBC, Analysis Committee of the European Brewery Convention, Chapter 7.9: iso-α-acids and reduced iso-α-acids in hop products by HPLC. Fachverlag Hans Carl, Nürnberg (2004).

9.3 BALTUSSEN, E.; P. SANDRA; F. DAVID and C. CRAMERS: Stir Bar Sorptive Extraction (SBSE), a Novel Extraction Technique for Aqueous Samples: Theory and Principles. J. Micro. Sep. 11 (1999) 737-747.

9.4 BENITEZ, J. L.; A. FORSTER; D. DE KEUKELEIRE; M. MOIR; F. R. SHARPE; L. C. VERHAGEN and K. T. WESTWOOD: European Brewery Convention. Manual of Good Practice, Hops and Hop Products. Fachverlag Hans Carl, Nürnberg (1997).

9.5 BERGTHALLER, W. und G. TEGGE: Schwefeldioxidaufnahme bei der Maisquellung. 2. Technologische Aspekte. Starch/Stärke 24 (1972) 348-353.

9.6 BETATEC Hopfenprodukte GmbH: Verfahren zur Herstellung von Stärke. Patent specification, WR/ST/060288P (2007).

9.7 BETATEC Hopfenprodukte GmbH: personal message (2007).

9.8 BETATEC Hopfenprodukte GmbH: safety data sheets (2006).

9.9 BISS, R. and U. COGAN: Sulfur Dioxide in Acid Environment Facilitates Corn Steeping. Cer. Chem. 73 (1996) 40-44.

9.10 BOLIVAR, A.; M. GASPARRI and C. ZUFALL: A Rapid and Low-Cost Method for Quantification of Reduced Iso-α-Acids in Brewing. Am. Soc. Brew. Chem. 64 (2006) 39-46.

9.11 BOLLING, H.; W. KEMPF; H. D. OCKER; W. NIERLE und A. W. EL BAYA: Einfluss von Schwefeldioxid auf die Lipide stärkehaltiger Lebensmittel: Mais. Starch/Stärke 25 (1973) 402-405.

9.12 BRIGGS, D. E.; C. A. BOULTON; and P. A. BROOKES: Brewing: Science and Practice. Woodhead, Cambridge (2004).

9.13 CLARK, J. G.; L. J. BURROUGHS and J. A. GUZINSKI: High-Performance Liquid Chromatography Analysis of Tetrahydro-iso-α-acids in Beer. Am. Soc. Brew. Chem. 56 (1998) 76-79.

9.14 DEWAELE, C. and M. VERZELE: High-Performance Liquid Chromatography of Beer Bitter Acids. J. Chrom. 197 (1980) 189-197.

9.15 FORSTER, A.: Trends in the Production of Non-Isomerised Hop Products. In: EBC-Symposium on Hops, Monograph XXII, Zoeterwoude (1994) 72-91.

9.16 GOLDSTEIN, H. and P. L. TING: Post Kettle Bittering Compounds: Analysis, Taste, Foam and Light Stability. In: EBC-Symposium on Hops, Monograph XXII, Zoeterwoude (1994) 141-164.

9.17 HARMS, D. and F. NITZSCHE: High-Performance Separation of Unmodified and Reduced Hop and Beer Bitter Compounds by a Single High-Performance Liquid Chromatographic Method. Am. Soc. Brew. Chem. 59 (2001) 28-31.

9.18 HAROS, M.; O. E. PEREZ and C. M. ROSELL: Effect of Steeping Corn with Lactic Acid on Starch Properties. Cer. Chem. 81 (2004) 10-14.

9.19 HAY, B. A.; and J. W. HOMISKI: A Simplified Method for Synthesis of Tetrahydroiso-α-acid Standards for High-Performance Liquid Chromatography. J. Am. Soc. Brew. Chem. 49:0115 (1991) 115-118.

9.20 HEIN, W., G. POLLACH and F. EMERSTORFER: 10 Years´ Experience with Natural Antibacterials within Agrana. Zuckerind. 131 (2006) 477-491.

9.21 HEIN, W. and G. POLLACH: New Findings with the Use of Hop Products in the Sugar Industry. Zuckerind. 122 (1997) 940-949.

9.22 HUBER, C.: The Influence of Hop Products on Lactic Acid Formation during the Maize Steeping Process. Part 2 of the Final Report: Application of Hop Products in Maize Starch Production (2007) 1-42.

9.23 JASKULA, B.; K. GOIRIS; G. DE ROUCK; G. AERTS and L. DE COOMAN: Enhanced Quantitative Extraction and HPLC Determination of Hop and Beer Bitter Acids. J. Inst. Brew. 113 (2007) 381-390.

9.24 KUNZE, W.: Technologie der Brauer und Mälzer. VLB Berlin (1999).

9.25 MAGALHAES, P. J.; L. F. GUIDO; J. M. CRUZ and A. A. BARROS: Analysis of Xanthohumol and Isoxanthohumol in Different Hop Products by Liquid Chromatography-Diode Array Detection-Electrospray Ionization Tandem Mass Spectrometry. J. Chrom. A 1150 (2007) 295-301.

9.26 MATISSEK, R. and G. STEINER: Lebensmittelanalytik: Grundzüge, Methoden und Anwendungen. Springer Verlag, Berlin (2006).

9.27 MOIR, M.: Hops – A millennium Review. J. Am. Soc. Brew. Chem. 58 (2000) 131-146.

9.28 POLLACH, G.; W. HEIN and F. HOLLAUS: Use of Hop Products as Bacteriostaticum in the Sugar Industry. Zuckerind. 121 (1996) 919-926.

9.29 POLLACH, G.; W. HEIN und G. RÖSNER: Neue Erkenntnisse zur Lösung mikrobieller Probleme in Zuckerfabriken. Zuckerind. 124 (1999) 622-637.

9.30 RÜCKLE, L.: Hop Acids as Natural Antibacterials in Ethanol Fermentation. Int. Sug. J. 107, 1275 (2005) 162-165.

9.31 RÜCKLE, L. and T. SENN: Hop Acids Can Efficiently Replace Antibiotics in Ethanol Production. Int. Sug. J. 108 (2006) 139-147.

9.32 SAMARAWEERA, I. S.; L. BUSCHETTE; D. L. RHEAULT and D. NOBLE: Bench Studies and Factory Trials with the Use of the Beta Hop Acid Beta Stab 10A. American Crystal Sugar Company. Paper of the joint meeting of the IIRB and ASSBT. San Antonio, (2003) 1-10.

9.33 SCHMALRECK, A. F.; M. TEUBER; W. REININGER and A. HARTL: Structural Features Determining the Antibiotic Potencies of Natural and Synthetic Hop Bitter Resins, Their Precursors and Derivatives. Can. J. Microbiol. 21 (1975) 205-212.

9.34 SHIMWELL, L. J.: On the Relation between the Staining Properties of Bacteria and Their Reaction towards Hop Antiseptic. J. Inst. Brew. 43 (1937) 111-118.

9.35 SIMPSON, W. J.: Molecular Structure and Antibacterial Function of Hop Resin Materials. Ph. D. Thesis. Council of National Academic Awards, UK (1991).

9.36 SINGH, N. and ECKHOFF, S.R.: Wet Milling of Corn – A Review of Laboratory-Scale and Pilot Plant-Scale Procedures. Cer. Chem. 73 (1996) 659-667.

9.37 SIX, B.: Studien zur Wirksamkeit und zum Verbleib von Harzkomponenten beim Einsatz als Bakteriostatikum in der Zuckerfabrikation. Diploma Thesis at the University of Natural Resources and Applied Life Sciences, Vienna (2002).

9.38 TEGGE, G.: Stärke und Stärkederivate. Behr`s Verlag, Hamburg (2004).

9.39 TING, P. L.; H. GOLDSTEIN; A. A. MURAKAMI; M. VANSANFORD; J. R. REFLING; J. R. SEABROOKS and D. S. RYDER: Preparation of Light Stable Hops. US-Patent 7,258,887 B2 (2007).

9.40 TING, P. L.; S. KAY and D. RYDER: Separation and Identification of Stereoisomers of Isomerized α-Acid Derivatives by HPLC/DAD and Electrospray HPLC/MS. Am. Soc. Brew. Chem. 65 (2007) 9-14.

9.41 VANHOENACKER, G.; D. DE KEUKELEIRE and P. SANDRA: Analysis of Iso-α-Acids and Reduced Iso-α-Acids in Beer by Direct Injection and Liquid Chromatography with Ultraviolet Absorbance Detection or with Mass Spectrometry. J. Chrom. A 1035 (2004) 53-61.

9.42 VERZELE, M. and D. DE KEUKELEIRE: Chemistry and Analysis of Hop and Beer Bitter Acids. Elsevier Science, Amsterdam (1991).

9.43 WATSON, S. A.: Corn and Sorghum Starches: Production. In: Starch: Chemistry and Technology. Ed. By R. L. Whistler, J. N Bemiller and E. F. Paschall, Academic Press: Orlando, (1984) 432-444.

9.44 WEISS, A.; C. SCHÖNBERGER; W. MITTER; M. BIENDL; W. BACK and M. KROTTENTHALER: Sensory and Analytical Characterisation of Reduced, Isomerised Hop Extracts and Their Influence and Use in Beer. J. Inst. Brew. 108 (2002) 236-242.

9.45 WILHELM, E. and KEMPF, W.: Zur Reduktion von Schwefeldioxid-Restmengen in Getreidestärken. Starch/Stärke 32 (1980) 83-90.

9.46 WILSON, R.: New International Calibration Standard (IDS-R2) for HPLC Analysis of Reduced (rho) Iso-α-Acids. Monatsschr. Brauwiss. 58 (2005) 25-26.

9.47 ZINSBERGER, S.: Einsatz von Hopfenprodukten bei der Gewinnung von Maisstärke. Diploma Thesis in progress, University of Vienna (2008).

9.48 http://www.epibio.com/posters/MicrobialGeneticsposter.pdf; accessed: 2008/03/13. HOFFMAN, L. M. and B. W. JARVIS: Molecular Analysis of Lactic Acid Bacteria in an Inhospitable Environment: Beer. Corporate Poster of Epicentre Biotechnologies (2004).

9.49 http://www.laborveritas.ch/files/en/produkte/ICS-I2/ics_i2_normal.pdf; accessed: 2007/07/29. MULQUEEN, S.: ICS-I2-Chromatogram. Labor Veritas Corporate Literature (2004).

9.50 www.zuckerforschung.at; accessed: 2007/07/28

9.51 www.laborveritas.ch; accessed: 2007/0

10 APPENDIX

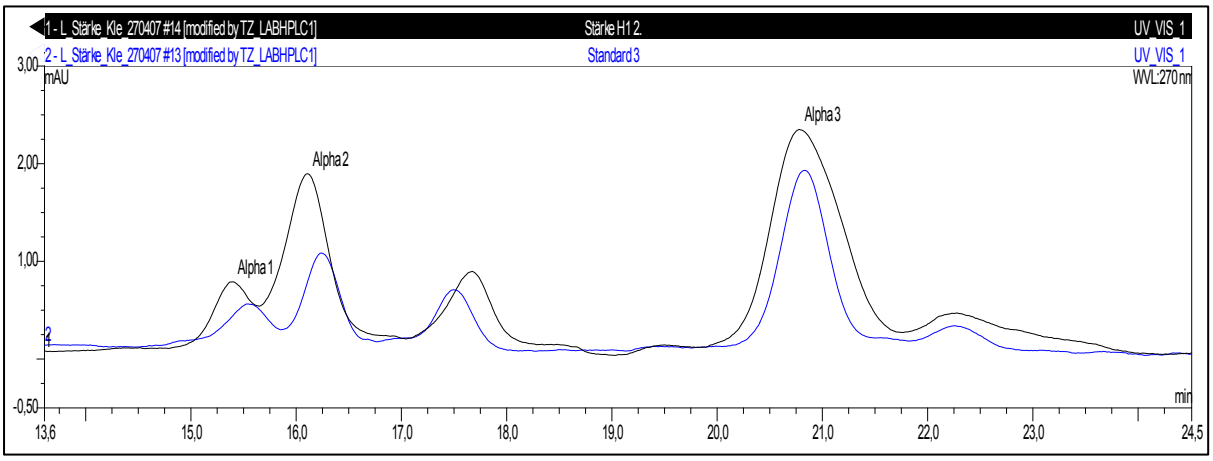

Fig. 41: Representative chromatogram of Soxhlet No. 1 with H (starch)

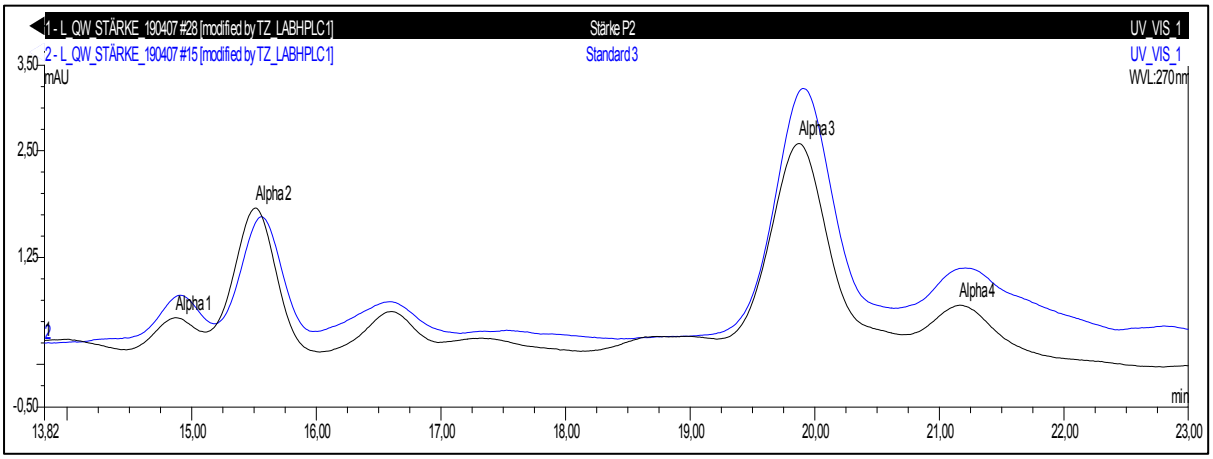

Fig. 42: Representative chromatogram of Soxhlet No. 1 with P (starch)

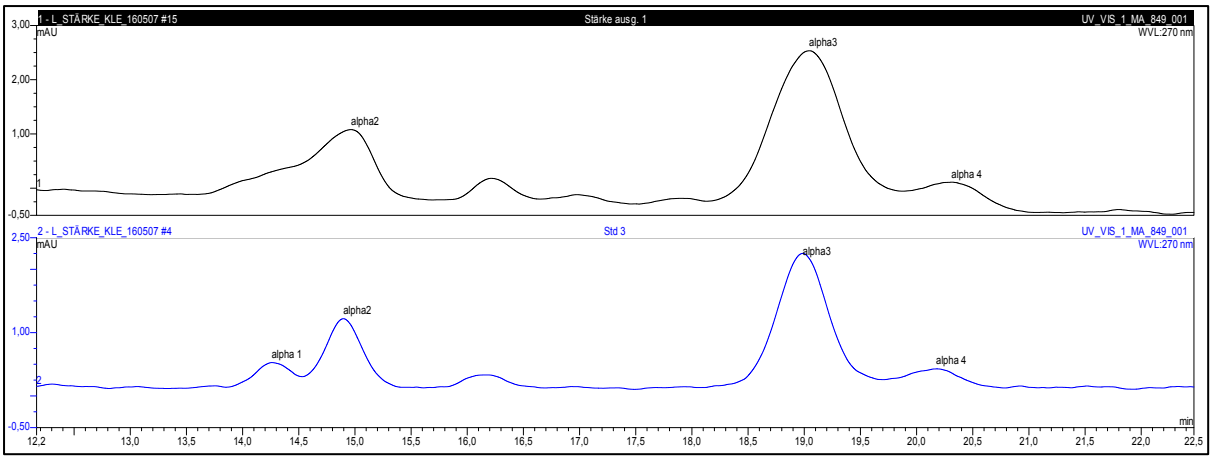

Fig. 43: Representative chromatogram of stirring with D (starch)

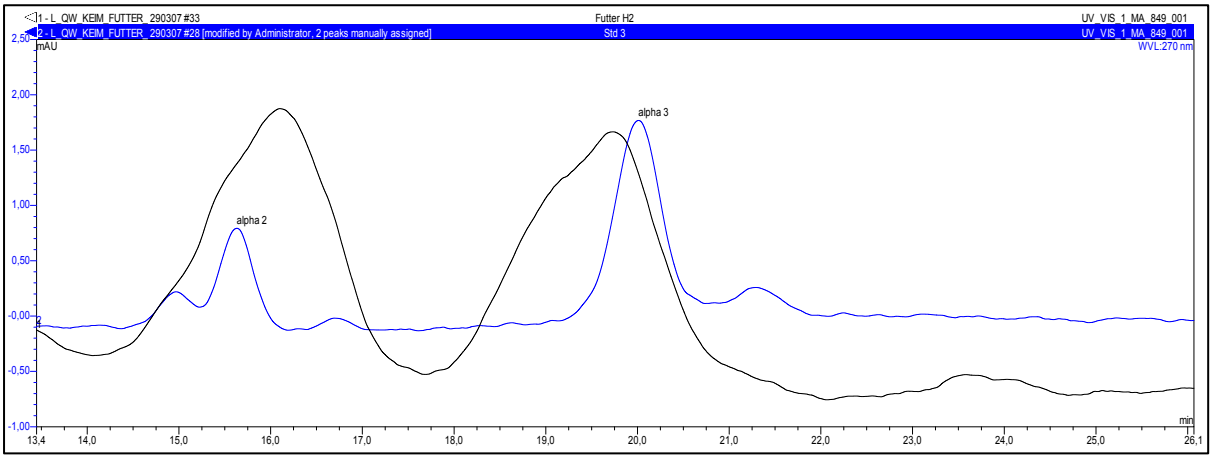

Fig. 44: Representative chromatogram of Soxhlet No. 1 with H (maize feed)

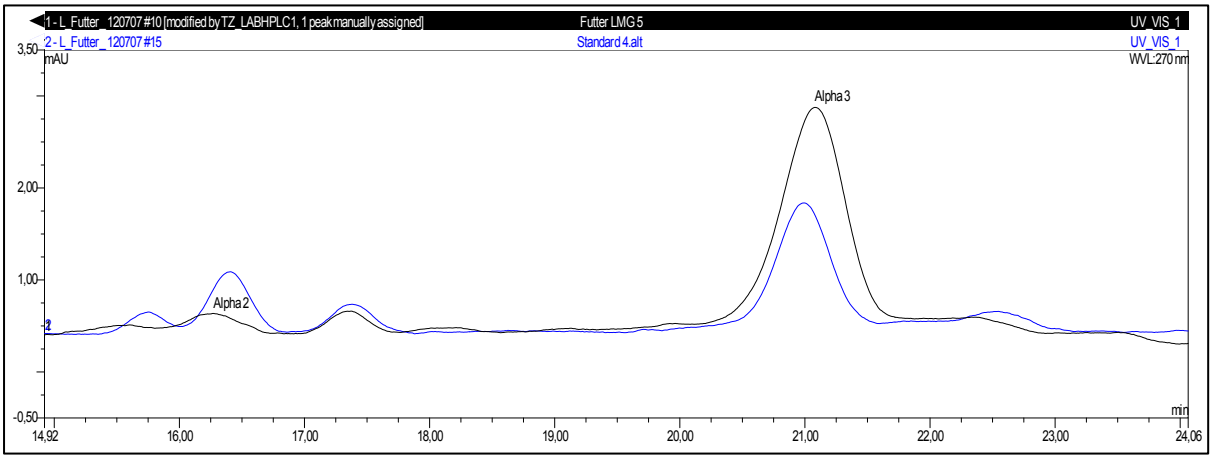

Fig. 45: Representative chromatogram of stirring with solvent mixture (maize feed)

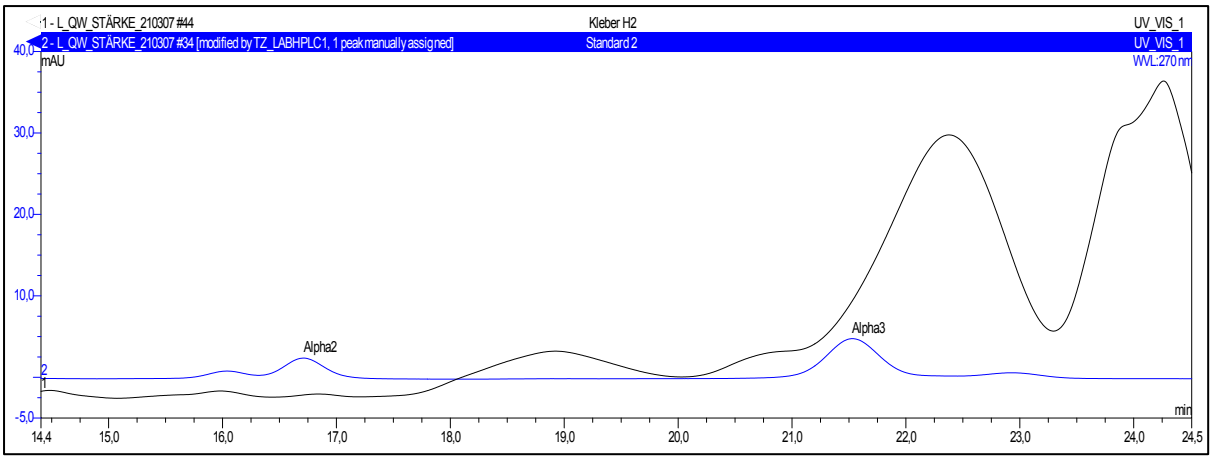

Fig. 46: Representative chromatogram of Soxhlet No. 1 with H (gluten)

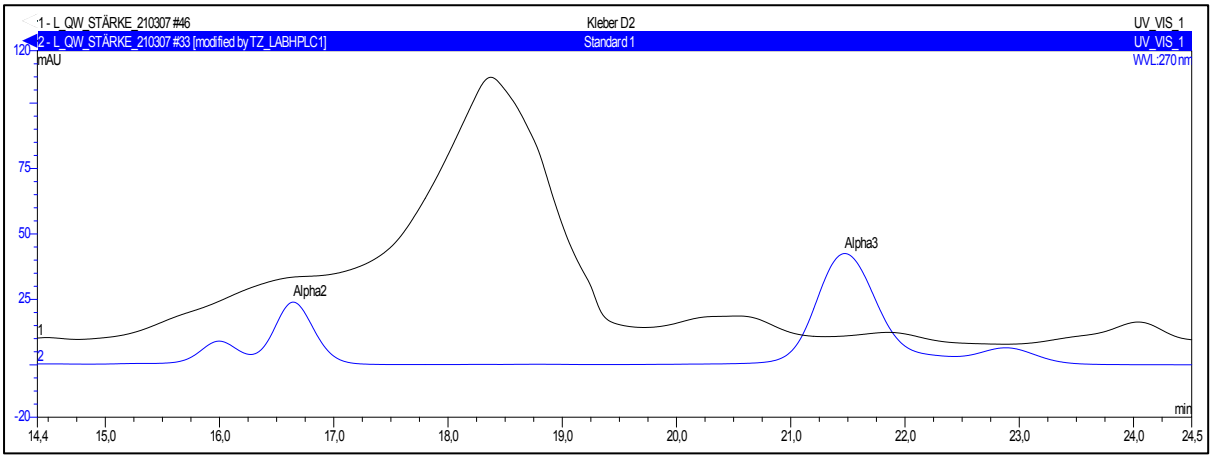

Fig. 47: Representative chromatogram of Soxhlet No. 1 with D (gluten)

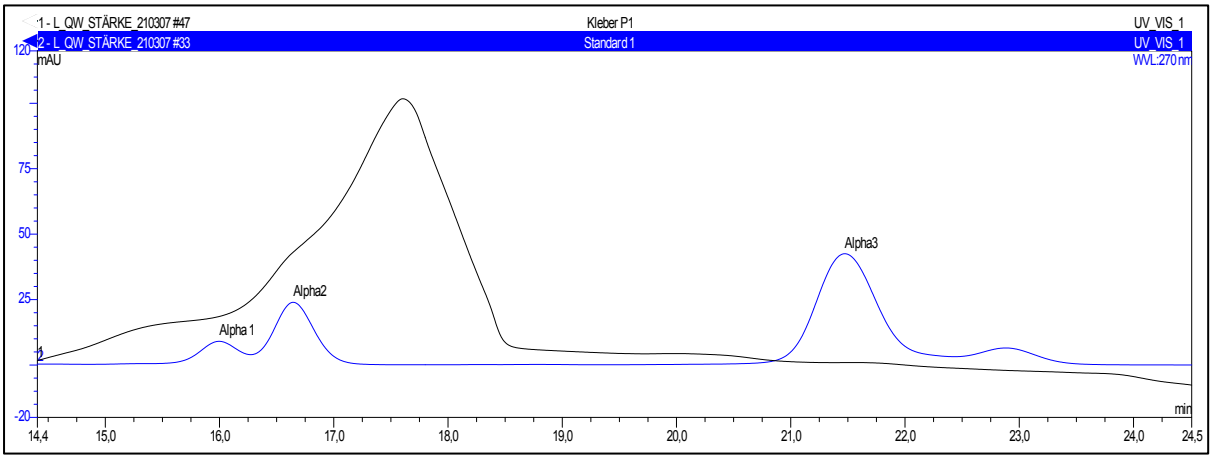

Fig. 48: Representative chromatogram of Soxhlet No. 1 with P (gluten)

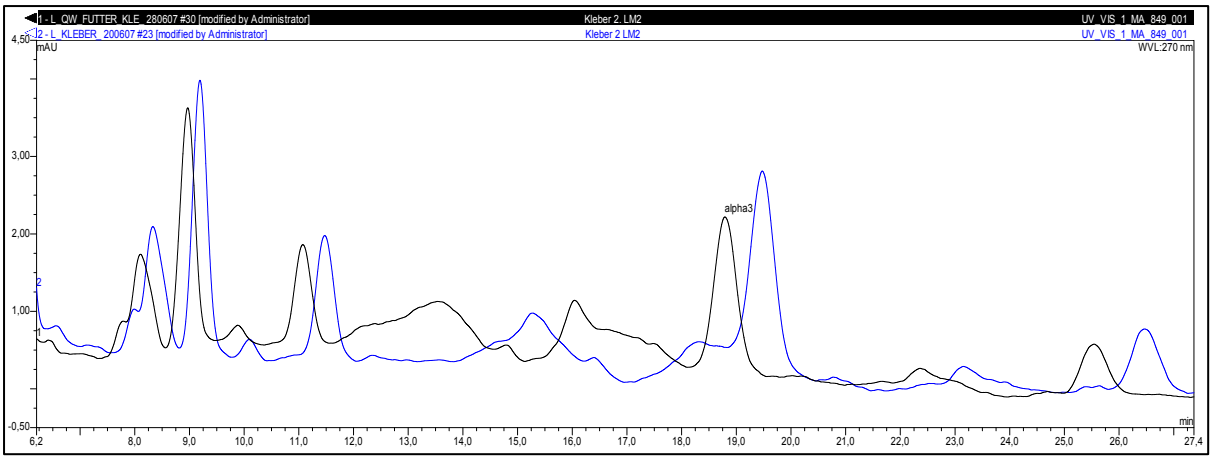

Fig. 49: Representative chromatogram of stirring with solvent mixture (gluten)

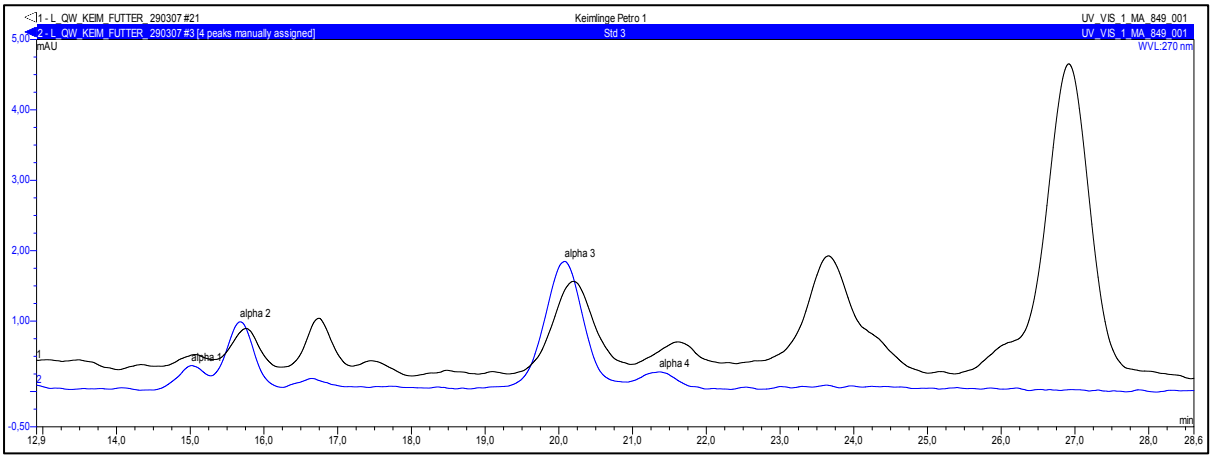

Fig. 50: Representative chromatogram - Soxhlet No. 1 with P (germs)

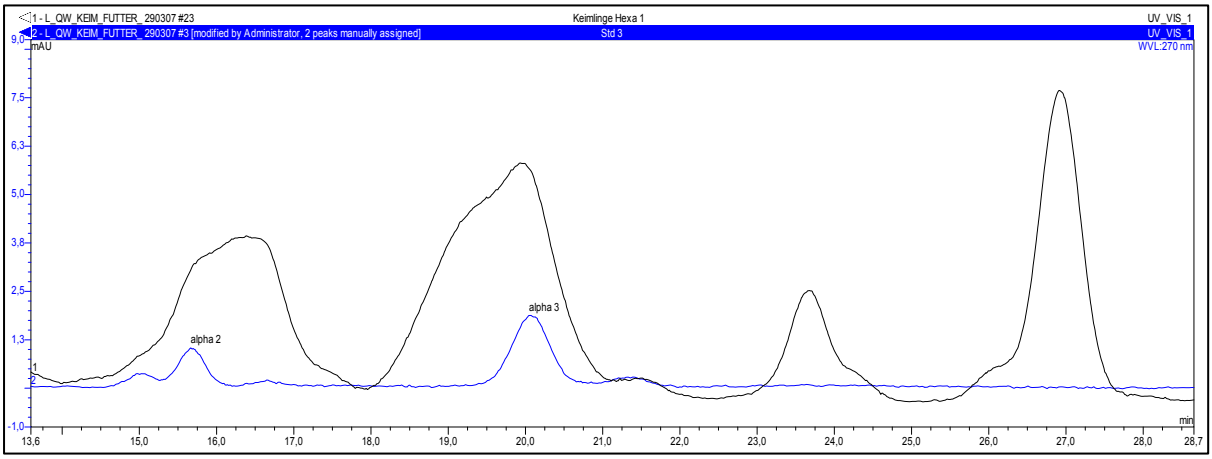

Fig. 51: Representative chromatogram - Soxhlet No. 1 with H (germs)

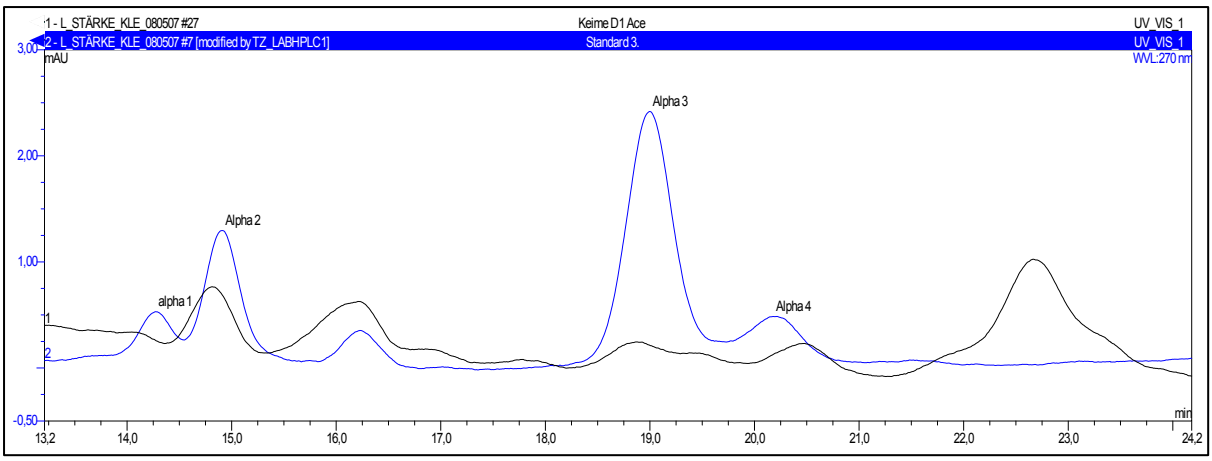

Fig. 52: Representative chromatogram - Soxhlet No. 1 with D, dissolved in acetonitrile (germs)

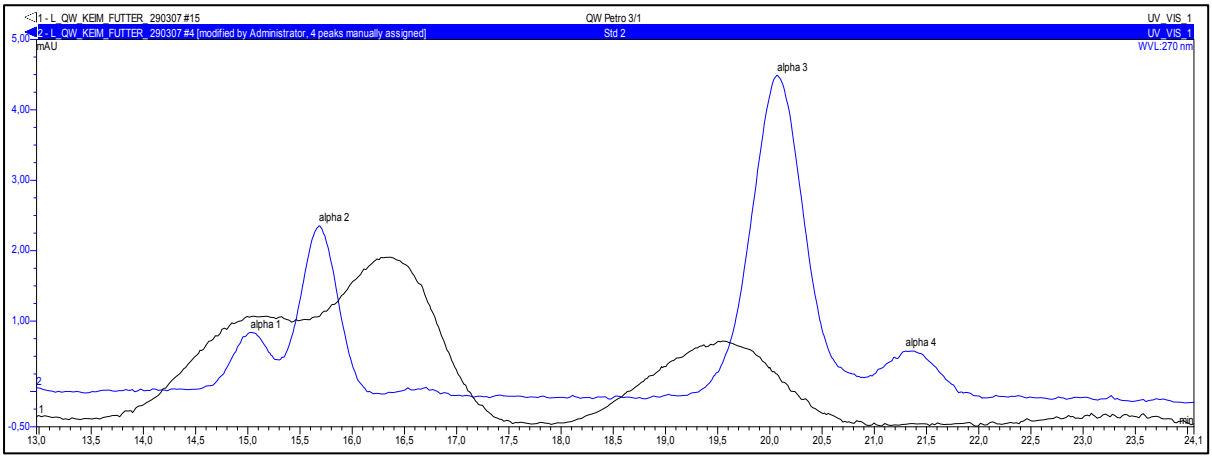

Fig. 53: Representative chromatogram - shaking out with P, pH adjusted (steep water)

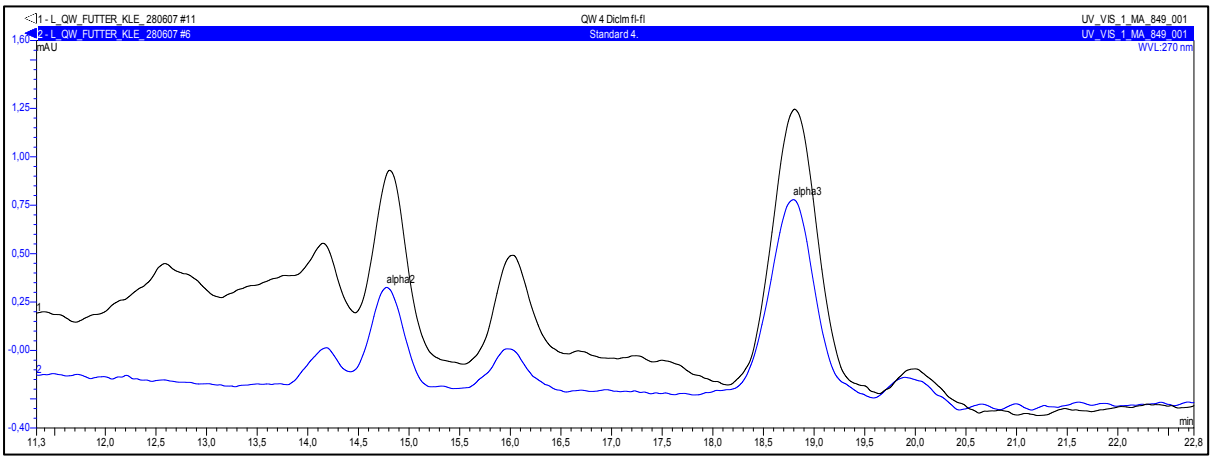

Fig. 54: Representative chromatogram - Liquid-liquid-extraction with D (steep water)

Lebenslauf - Curriculum Vitae

Persönliche Daten

Egartner Cornelia
Duelerweg
3
A-9232 Rosegg
0043/69919822809
egartner.cornelia@gmx.at

Geboren in Villach	28.09.1982
ledig	
Staatsangehörigkeit: Österreich	

Schulausbildung

Volksschule, Rosegg	1989 - 1993
Gymnasium St. Martin, Villach	1993 - 2001
Matura	2001

Hochschulausbildung

Studium der Ernährungswissenschaften an der Universität Wien	2001 - 2008
Beginn des Studium Lebensmittel- und Biotechnologie an der Universität für Bodenkultur Wien	2003

Fachpraktika

- Kurbad Althofen, Fitnessarena, Treibach Althofen, Praktikantin	Sommer 2004
- Vonwiller Mühle Schwechat, Labor-Praktikantin	Sommer 2006
- Agrana Zuckerfabrik, Leopoldsdorf, Kampagnechemikerin	Kampagne 2006/2007
- Agrana Zuckerfabrik, Leopoldsdorf, Kampagnechemikerin	Kampagne 2007/2008
- Agrana Bioethanol, Pischelsdorf, Labor-Praktikantin	Sommer 2008

Sonstige Kenntnisse

Sprachen:
Englisch (sehr gut in Wort und Schrift)
Italienisch (ausbaufähige Grundkenntnisse)

Computerkenntnisse:
Vertiefte Kenntnisse in MS Word, Power Point und Excel