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# Investigations about the relative Hartong Extract at 45 °C

**The relative extract at 45 °C (VZ 45 °C) was introduced by Hartong in the 1930th. Today it is an important figure for the German barley variety trials. Investigations that were done to answer the question whether the VZ 45 °C is a proteolytic, cytolytic or amylolytic figure disproved correlations that were published in the past. The VZ 45 °C is the relation of soluble starch at 45 °C to soluble starch in the congress wort. Therefore the VZ 45 °C is an amylolytic figure. Furthermore a correlation between the VZ 45 °C and the gelatinisation temperature of the malt was found.**

Descriptors: Hartong-Kretschmer, VZ45, amylolysis, starch, gelatinisation

## 1 Introduction

The relative extract at 45 °C (VZ 45 °C) was introduced by Hartong in the 1930th (10, 11, 12) and promoted by Hartong and Kretschmer 1953 (14). It plays an important role in the evaluation of malt quality and is considered for the official German barley trials. Especially a difference of more than 3 percentage points to the Kolbach Index is associated with a lower quality (1). Even though of its great importance, the VZ 45 °C has always been under debate.

Recently barley varieties like Annabell and Pasadena were introduced which tend to give malts with a low VZ 45 °C. The barley trials of the Braugerstengemeinschaft have shown that these varieties produce malts with good brewing quality. This was evaluated by analytical and sensorial methods (2, 3).

In this paper publications about the VZ 45 °C are reviewed critically and the resulting questions about the interpretation are divided into the three major degradation procedures of the malting and brewing process. These are the cytolytic, proteolytic and amylolytic degradations. To answer these questions barley samples were analysed for the VZ 45 °C and other common figures. Physical characteristics of barley malt starch were measured using a rotation viscosimeter.

## 2 Literature review

The VZ 45 °C was developed in the 1930th by Hartong (10), as one part of a method to predict the solubility of malt. Four temperatures (25 °C, 45 °C, 65 °C and 85 °C) were chosen. At these temperatures finely grounded malt was mashed for one hour. At 25 °C the cold water extract and phosphate esterase activity was evaluated. At 45 °C the proteolytic activity was rated. The 65 °C mash covered all enzymatic activities. At 85 °C the extraction due to gelatinisation was measured. The malt extracts received from these mashes were set into relation to the extract obtained from the congress mash. The four relations were summed up and 60 subtracted. The result is called Hartong Index. Table 1 shows the interpretation of the Hartong Index (20).

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**Table 1 Hartong Index**

| Hartong Index | degreee of modification |
|---|---|
| 0–3,5 | undermodified |
| 4–4,5 | normal modified for keg beer |
| 5 | ideal modification |
| 5,5–6 | overmodified for bottle beer |
| 6,5–10 | overmodified |

In the following years the Hartong Index was under debate and further development was done. One of the essential disadvantages of the method was time consumption (16). It was in general accordance that the main focus of the Hartong Index was on carbohydrates and that the modification during mashing is influenced by all enzymes and the susceptibility of the substrate to enzymatic degradation. Susceptibility was considered as modification (12). Hartong (13) considered his index and the individual relations as figures to describe the malt quality and to reveal the causes of poor malt quality.

In 1953 Hartong and Kretschmer (14) modified the method. Instead of 25 °C they choose 20 °C and 85 °C was replaced by 80 °C. The individual relations received more attention. Especially the VZ 45 °C was of importance since its potential of evaluating the enzyme activity and the malting process. It was supposed to close a gap in malt analytics. 36 % was the standard value malt had to achieve. Malt with a VZ 45 °C below 36 % was of poor enzyme activity due to wrong treatment. This was especially applied to proteolytic enzymes.

Henceforth malts were supposed to be evaluated by fine-coarse extract difference, cold water extract, longitudinal section and the VZ 45 °C. The Hartong Index was supposed to be an additional analysis to confirm the others. Samples with a VZ 45 °C and VZ 25 °C below the standard of 36 % and 25 % are responsible for lautering, clarification and filtration problems as well as for a poor colloidal stability. These problems occur due to a low proteolytic activity because of false kilning.

According to Piratzky 1959 (24) the VZ 45 °C, the strongest figure in the Hartong Index, was primarily influenced by enzyme activity and secondarily by the degree of modification.

In 1963 Scriban (27) concluded upon his research that the extract of the 45 °C mash and the pH of the same wort are sufficient to describe the malt quality, in particular the α-amylase activity.

Kretschmer (17) disagreed; he estimated the α-amylase using the VZ 80 °C.

1967 Kretschmer (17) estimated a correlation between a low VZ 45 °C and poor yeast propagation which influences the clarification, aroma and stability negatively. He suggested a VZ 45 °C of at least 36 % and for a good colloidal stability higher than 40 %.

Lie and Rasch (18) found a correlation between the VZ 45 °C and the free amino nitrogen (FAN) content of the wort. Bellmer (4) reported that a VZ 45 °C of 38–40 % guarantees a sufficient supply of assimilable nitrogen for the yeast.

The VZ 45 °C as well as some other malt specifications are genetically fixed, therefore they are a variety characteristic (21).

Across Europe the VZ 45 °C is only of minor interest. In the annually EBC Barley Trials it was an optional figure until 1978 and later it was not considered anymore (8, 9).

It can be concluded, that most of the cited authors consider the VZ 45 °C as a figure that describes the modification or degradation of the kernel. Especially the proteolytical modification and the FAN content are supposed to be predicted by the VZ 45 °C. In malting and brewing the modification and degradation can be enzymatical or physical. Influencing parameters are therefore enzyme activities, physical variables (e.g. temperature) or substrate characteristics. Figures describing degradation procedures include always more than one factor.

The question to be answered in this publication is whether the VZ 45 °C is a proteolytic, cytolytic or amylolytic figure. Therefore research was done to discuss the following statements:

As a proteolytic figure the VZ 45 °C must correlate either with a known proteolytic malt specification or with a figure describing wort or beer quality that can be related to proteolytic activity.

As a cytolytic figure the VZ 45 °C must correlate either with a known cytolytic malt specification or with a figure describing wort or beer quality that can be related to cytolytic activity.

As an amylolytic figure the VZ 45 °C has to correlate with an amylolytic activity or with a figure describing an amylolytic substrate or product. The most important activities are the one of α-amylase and β-amylase. Products of the amylolysis are dextrins and fermentable sugars. The substrate is starch.

## 3 Physical characteristics of starch and its degradation

For a better understanding of the results and the discussion an excursion about the physical characteristics of starch and its degradation during malting and congress mashing is necessary.

Starch is the major constituent of barley. It makes up 55 – 65 % of the dry matter. The endosperm contains two different types of starch granules. Large granules (A-type) have a diameter of 10 to 25 µm. Small granules (B-type) have a diameter of 1 – 5 µm. Normal barley starch consist of 20 – 30 % amylose and 70 – 80 % amylopectin. The gelatinisation temperature is 60 – 62 °C for the large granules and 75 – 80 °C for the small granules (22).

The structure of the granules (chain length distribution and the degree of branching of amylopectin) influences the physical characteristics and the gelatinisation temperature, respectively (29). The gelatinisation temperature varies depending on the method used for determination (25).

Usual temperatures of germination are below gelatinisation temperature. The enzymatic degradation of starch is very slow, respectively (5, 21, 22). During congress mashing below the gelatinisation temperature only enzymatical or physical damaged starch is degraded by amylolytic enzymes (6, 7, 22, 28). In congress mashing a 30 minute rest is held at 45 °C (MEBAK I 4.1.4.2.2 (23)). When the congress mash is heated to 70 °C only a minor concentration of high molecular starch is left (5, 21). Due to the elevated gelatinisation temperature of the small starch granules, they are not degraded and get into the wort (22).

## 4 Material and Methods

### 4.1 Material

All barley samples are established varieties grown across Germany in 2003 and 2004. From 2003 (group I) four varieties from four areas were used. From 2004 (group II) six varieties from eight areas were investigated.

### 4.2 Malting

Micro malting was carried out in 1 kg scale according to MEBAK I 2.5.3.1 (23). Barley samples had a germinitive energy of at least 95 %.

### 4.3 Enzyme activities

α-amylase activity was measured according to the ICC method 303 (15). Units per gramme were transformed into ASBC units by formula 1.

Formula 1: Dextrinising units (ASBC) = 0,23 × ceralpha units + 0,61

β-amylase activity was measured by using the method of McCleary (19). Units per gramme were transformed using formula 2 (26).

Formula 2: DP (°WK) = 0,365 × betamyl units + 65,7

### 4.4 Mashing

The congress mash was produced according to MEBAK I 4.1.4.2.2 (23) and the measurement of the VZ 45 °C was carried out according to MEBAK I 4.1.4.11 (23). All analysed figures of the resulting worts, respectively for congress wort, were in accordance to MEBAK I (23).

The extract development of the congress mash and the isothermal 45 °C mash was investigated. For that reason the congress mashing was interrupted by cooling the mash down to 20 °C at the following

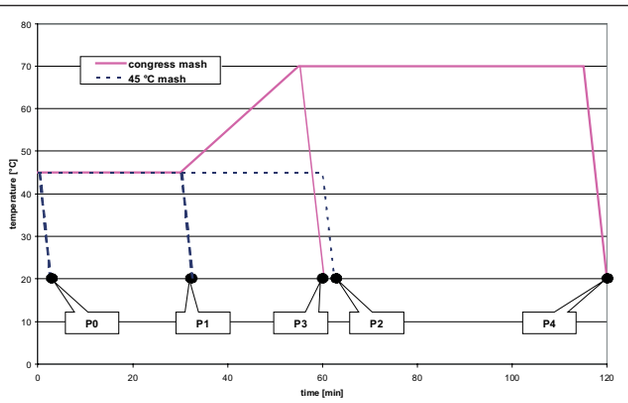


**Fig. 1 Sample chart for extract development**

stages: At mashing-in (P0), after 30 minutes (P1) and after heating up to 70 °C (P3). In figure 1 the complete sample overview is presented. P2 is the isothermal 45 °C and P4 is the congress mash. After cooling the mashes were balanced to 450 g and filtered. The resulting wort was analysed for extract and nitrogen content.

Soluble nitrogen was determined using the Kjeldahl method as described by MEBAK I 4.1.4.5.2.1 (23). Soluble protein was calculated by formula 3.

Formula 3: soluble protein (g/100 g) = soluble nitrogen (mg/100 g) × 6,25 × $10^{-3}$

The protein free extract ($extract_{pf}$) was calculated by formula 4.

Formula 4: $extract_{pf}$ (g/100g) = extract (g/100 g) - soluble protein (g/100 g)

VZ 45 °C protein free (VZ 45 °$C_{pf}$) results from the relation of the protein free extracts of P2 and P4 (formula 5).

Formula 5: VZ 45 °$C_{pf}$ (%) = ($extract_{pf}$ P2 / $extract_{pf}$ P4) × 100

### 4.5 Starch analysis

Gelatinisation temperature was measured by using the rapid-viscoanalyser RVA-4 from Newport Scientific. Analyses were carried out according to ICC standard No. 162 (15) using the manufactures profile STD 1 (figure 2). 15,0 g of distilled water were added to 7,5 g of finely grounded malt (5 % moisture). Data collection was done in 0,5 s intervals instead of 4,0 s. Malt was grounded using the Laboratory Mill 3100 (0,8 mm sieve, 16.000 rpm) from PERTEN INSTRUMENTS GMBH, Hamburg, Germany.

## 5 Results

To answer the question whether the VZ 45 °C is a proteolytic, cytolytic or amylolytic figure a great number of investigations were done. The results needed for the discussion are presented in this chapter.

### 5.1 Extract development during the mashing process

The extract development of barely malt samples during laboratory mashing was determined. Table 2 shows the mean, minimum and maximum values of 16 barley malt samples (group I). At mashing-in 19,7 % of the extract is already solubilised. The extract increases to 28,5 % after 30 min (P1) and to 32,7 % after 60 min at 45 °C (P2). When the congress mash is heated to 70 °C (P3) 81,5 % of the extract is solubilised and the final congress wort (P4) has 83,0 %. The range between maximum and minimum is 7,3 % for P1, 7,9 % for P2, 36 % for P3 and 4,4 % P4.

**Table 2 Extract development of 16 samples**

| sample | malt extract [%; d.m.] | | | |
|---|---|---|---|---|
| | mean | minimum | maximum | range |
| P0 | 19,7 | 18,3 | 21,7 | 3,4 |
| P1 | 28,5 | 25,0 | 32,3 | 7,3 |
| P2 | 32,7 | 28,6 | 36,5 | 7,9 |
| P3 | 81,5 | 79,9 | 83,4 | 3,6 |
| P4 | 83,0 | 81,5 | 85,9 | 4,4 |

**Table 3 Coefficient of correlation of 16 samples**

| | P1 | P2 | P3 | P4 |
|---|---|---|---|---|
| P0 | 0,71 | 0,63 | 0,04 | -0,13 |
| P1 | | **0,96** | -0,06 | 0,02 |
| P2 | | | -0,05 | 0,02 |
| P3 | | | | **0,93** |

In table 3 the coefficients of correlation for the samples P0 through P4 are shown. The highest correlation is between P1 and P2 and the second highest is between P3 und P4.

The linear correlations indicate that the extract increases by the same rate for all samples. In figure 3 the extract development of three samples is presented. Parallel extract development can be noted between P1 and P2 and between P3 and P4, respectively.

### 5.2 Solubilisation of protein during mashing

The samples of group I were analysed for soluble protein. In table 4 the mean, minimum and maximum values of the soluble protein are presented. At mashing-in already 3,6 % protein is solubilised.

After 30 min (P1) the soluble protein increases to 4,2 % and after 60 min at 45 °C (P2) to 4,4 %. When the congress mash is heated to 70 °C (P3) 4,3 % of the protein is solubilised and in the final congress wort (P4) 4,6 % of the protein. The range between maximum and minimum is between 0,9 and 1,1 percentage points for P0 through P3. The range for P4 is 1,4 percentage points.

As shown in table 5 there is a high correlation of the content of soluble protein between all stages of the mashing process.

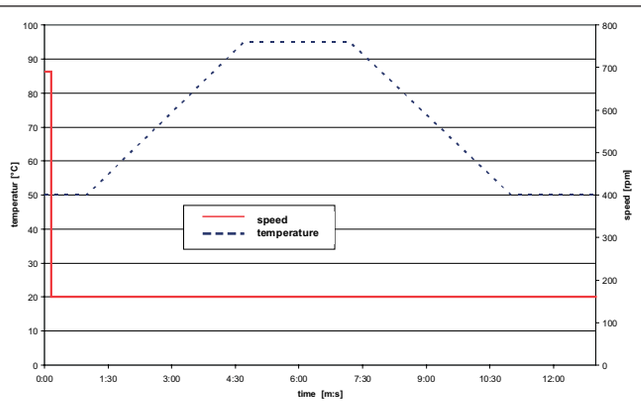


**Fig. 2 Temperature time profile of the RVA**

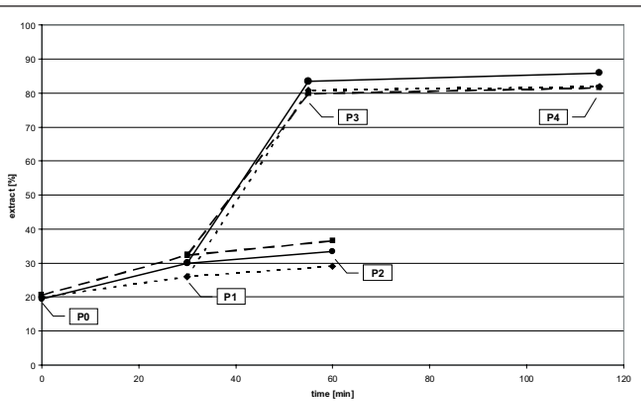


**Fig. 3 Extract development of three samples**

**Table 4 Development of soluble protein of 16 samples**

| sample | soluble protein [%; d.m.] | | | |
|---|---|---|---|---|
| | mean | minimum | maximum | range |
| P0 | 3,6 | 3,2 | 4,2 | 1,0 |
| P1 | 4,2 | 3,7 | 4,7 | 0,9 |
| P2 | 4,4 | 3,9 | 4,9 | 1,0 |
| P3 | 4,3 | 3,8 | 4,9 | 1,1 |
| P4 | 4,6 | 3,9 | 5,2 | 1,4 |

**Table 5 Coefficient of correlation of 16 samples of soluble protein**

| | P1 | P2 | P3 | P4 |
|---|---|---|---|---|
| P0 | 0,92 | 0,85 | 0,79 | 0,74 |
| P1 | | 0,96 | 0,91 | 0,9 |
| P2 | | | 0,96 | 0,96 |
| P3 | | | | 0,96 |

Figure 4 shows the correlation between the FAN content of the congress wort and the VZ 45 °C. According to Pearson there is no linear correlation.

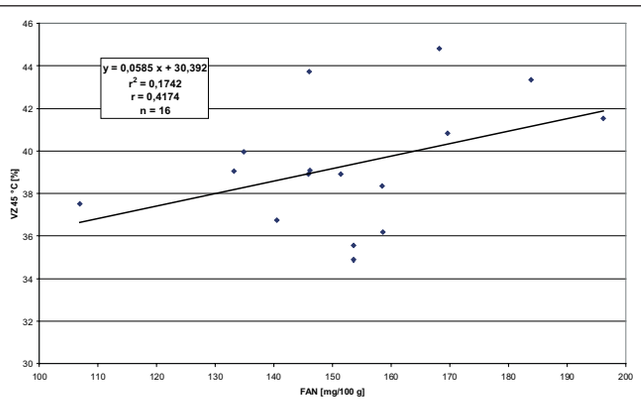


**Fig. 4 FAN of the congress wort against VZ 45 °C**

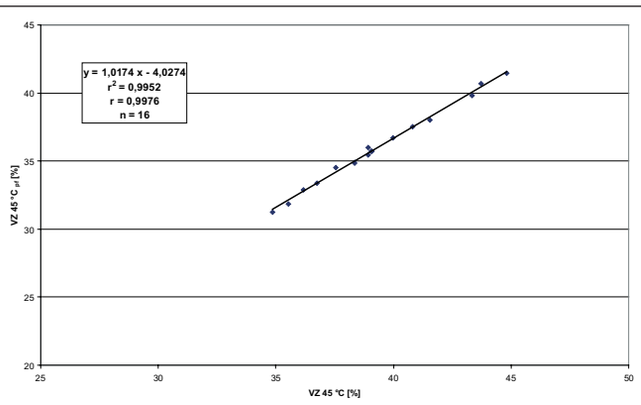


**Fig. 5 Correlation between VZ 45 °C and VZ 45 °C$_{pf}$**

### 5.3 Relative extracts

As shown in figure 5 a highly significant correlation exists between VZ 45 °C and VZ 45 °C$_{pf}$.

### 5.4 Influence of amylolytic activities on the VZ 45 °C

The samples of group II were analysed for amylolytic activities. In figure 6 the correlation between the α-amylase activity and the VZ 45 °C is shown. In figure 7 the correlation between the diastatic power and the VZ 45 °C is shown. In both cases a significant correlation does not exist.

### 5.5 Correlation between amylolytic products and VZ 45 °C

A correlation exists between the apparent attenuation limit (AAL) and the VZ 45 °C. As shown in figure 8 the correlation coefficient is r = 0,27. According to Pearson, this is a correlation for a = 0,10. This has to be considered as a tendency.

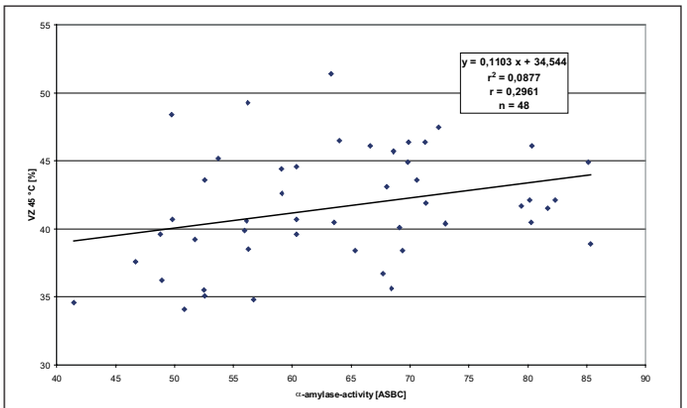


**Fig. 6 Correlation between α-amylase-activity and VZ 45**

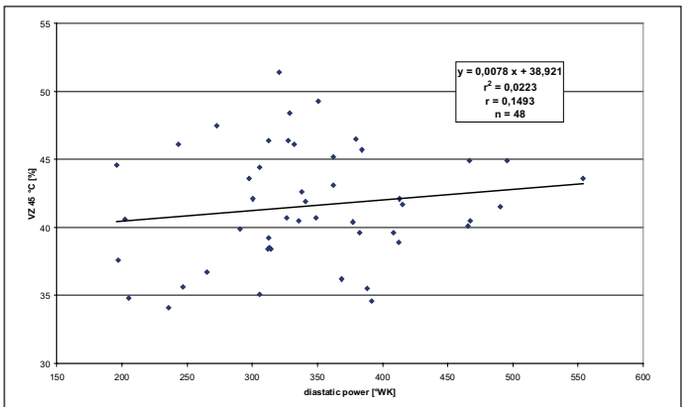


**Fig. 7 Correlation between diastatic power and VZ 45**

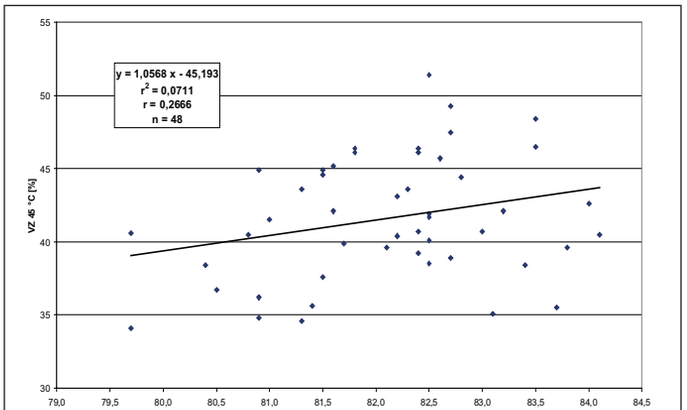


**Fig. 8 Correlation between AAL and VZ 45 °C**

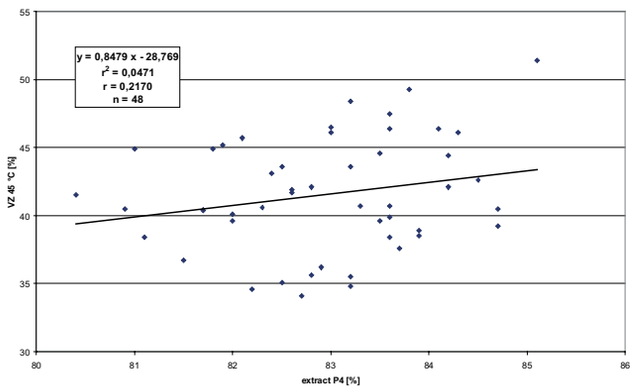


**Fig. 9 Correlation between extract of P4 and VZ 45 °C**

In figure 9 the correlation between the malt extract and the VZ 45 °C is presented. There is no significant correlation given (r = 0,22).

### 5.6 Starch analysis

The analysing profile of the manufactures software defines the gelatinisation temperature at that point where the viscosity increases by 24 mPa×s in 0,1 minutes. This method tends to misinterpret the gelatinisation temperature due to a noisy baseline. This problem was already discussed by Zhou and Mendham (30) and seemed to be solved by measurement of the time and viscosity interval (30). This method assumes that all malt samples gelatinise at the same speed. The difference of the viscosity increase for two samples over time is shown in figure 10. Instead of using the manufacturers profile the resulting curve was analysed manually.

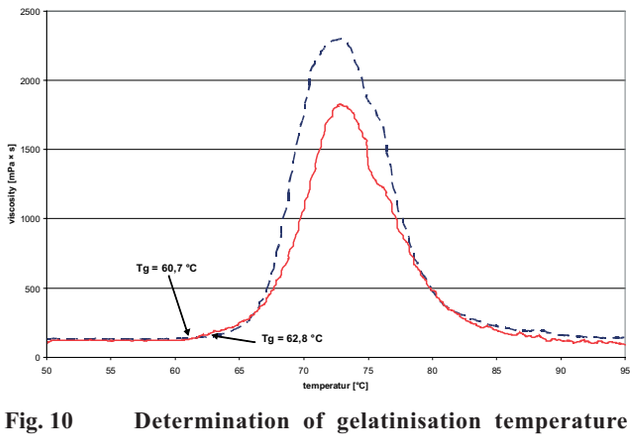


**Fig. 10 Determination of gelatinisation temperature**

The samples of group II had gelatinisation temperatures in the range of 57,6 – 65,1 °C. Figure 11 shows the correlation between the VZ 45 °C and the gelatinisation temperature. The coefficient of correlation is r*** = -0,84 for a = 0,001.

## 6 Discussion

The question to be answered is, whether the VZ 45 °C is a figure of the proteolytic, cytolytic or amylolytic degradation procedures. If it correlates significantly with a known figure the question would be answered, but the VZ 45 °C would not give any additional information about the malt. In case it does not correlate with any known figure, it has to be investigated if there is any interaction between the VZ 45 °C and any known wort or beer quality figure. Under these circumstances it would supply the brewer with additional information about the expected wort or beer quality.

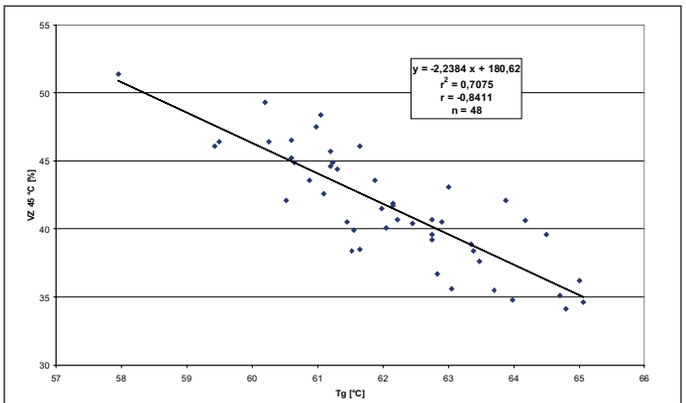


**Fig. 11 Correlation between gelatinisation temperature and VZ 45 °C**

According to the shown results the VZ 45 °C does not correlate with any known cytolytic figure. Whether the VZ 45 °C is a cytolytic figure, can be answered by considering the variety Annabell. The barley trials of the Braugerstengemeinschaft (1, 3) have proven, that the cell wall compounds of Annabell are very well degraded under standard malting conditions and that the VZ 45 °C is very low. The filterability (figure 12) is very good, respectively. It can be concluded, that the VZ 45 °C is not a cytolytic figure.

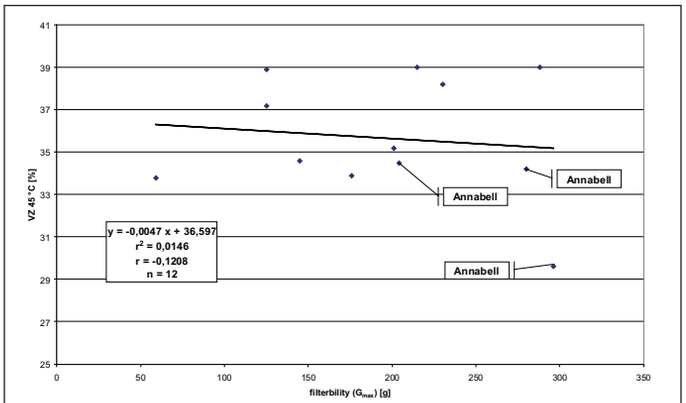


**Fig. 12 VZ 45 °C vs. filterability [3]**

The VZ 45 °C does not correlate with any proteolytic figure. The barley trials of the Braugerstengemeinschaft have proven that the VZ 45 °C does not correlate with any figure of wort and beer analytics that have been investigated. In table 6 an overview of the accomplished analyses is given including the coefficients of correlations. It can be concluded that the VZ 45 °C does not describe the quality of the proteolysis of the malt.

**Table 6 Coefficients of correlation of several wort and beer quality figures [2]**

| n = 32 | wort analysis | | | beer analysis | | |
|---|---|---|---|---|---|---|
| | soluble nitrogen | high-molecular nitrogen | FAN | foam (NIBEM) | filterability ($G_{max}$) | viscosity |
| r | [mg/100 ml] | [mg/100 ml] | [mg/100 ml] | [s] | [g] | [mPa × s] |
| VZ 45 °C | 0,08 | -0,08 | -0,02 | 0,01 | 0,23 | 0,14 |

The fact that the VZ 45 °C correlates significantly with the VZ 45 °$C_{pf}$ as shown in figure 5 proves once again that it is not a figure that describes the proteolytic degradation.

Wort extract consists of 91 % carbohydrates and 6 % protein. The remaining 3 % are minerals and some other minor components. The carbohydrates originate by 99,5 % from starch (22). If the proteins, the minerals and the other minor components are neglected, the VZ 45 °C is a relation of starch degradation products. Therefore it can be concluded that the VZ 45 °C is an amylolytic figure. As an amylolytic figure the VZ 45 °C has to be in correlation with amylolytic activities, with products of the amylolysis or with substrates of the amylolysis. In figure 6 the VZ 45 °C against the α-amylase activities is plotted. The coefficient of determination (r2) is 0,09 which indicates an influence of 9 % of the α-amylase activity on the VZ 45 °C. This is rather a minor correlation. No correlation is given between the VZ 45 °C and the diastatic power. As shown in figure 7 there is no significant correlation (r = 0,15).

The AAL and the extract are figures that give information about the starch degradation. Therefore they describe the products of the amylolysis. Even though there is a significant correlation (r* = 0,27) between the VZ 45 °C and the AAL, the coefficient of determination is only r2 = 7,1 %. For the extract it is even lower (r2 = 4,7 %). It can be concluded that the VZ 45 °C is not a figure of the amylolytic activities or the product of the amylolysis. Thus the VZ 45 °C is a figure of the substrate of the amylolysis: Starch.

Native starch granules are hydrolysed below the gelatinisation temperature very slow (7, 28, 29). The gelatinisation temperatures of the investigated samples are between 57,1 °C and 65,1 °C therefore above 45 °C. The starch that is solubilised during the mashing at 45 °C must consist of granules which are enzymatical attacked during germination or damaged during milling.

Starch from barley with an elevated VZ 45 °C is more susceptible to enzymatic hydrolysis than samples with a low VZ 45 °C. A higher susceptibility is not attributed to a higher amylolytic activity (figure 6 and figure 7). This agrees with Slack and Wainwright (28).

On the basis of the 48 investigated samples (group I, six varieties × eight areas) a correlation is found between the gelatinisation temperature and the VZ 45 °C (figure 11). The coefficient of correlation indicates a highly significant correlation (r*** = -0,84). This is a conformation that the VZ 45 °C is an amylolytic figure. The literature review did not indicate an interaction between a lower gelatinisation temperature and an enhanced enzymatic susceptibility. The explanation for that correlation remains open.

## 7 Conclusion

It was shown that the relative Hartong Extract at 45 °C (VZ 45 °C) is an amylolytic figure and is not a cytolytic or proteolytic, respectively. Starch degradation during mashing starts above the gelatinisation temperature which is between 57,6 °C and 65,1 °C for barley malt. Since todays mashing procedures have excessive temperatures rests at temperatures between 60 °C and 65 °C the relative extract difference of the VZ 45 °C is negligible.

The correlations of the VZ 45 °C and other figures that were presented in the reviewed literature were disproved. Therefore the observance of the VZ 45 °C for the evaluation of barley variety trials in Germany seems to be critical.

The technological meaning of the correlation between the VZ 45 °C and the gelatinisation temperature needs further investigation.

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