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A Method for the Determination of Hop Diastatic Power – Part 2

In the first part of this work, a method for the quantification of the diastatic activity of hops using potato starch was developed and assay conditions were optimized. In this work, other substrates (corn starch, wheat starch, rice starch, maltodextrin, dextrin, and amylopectin) were tested for their suitability to serve as substrates using 17 different pelletized hop samples from crop 2020. In addition to potato starch, dextrin and maltodextrin were selected for further testing and results were compared to data from trials involving incubation of Pilsner beer with hops. Additionally, measuring hop α-amylase, β-amylase, and amyloglucosidase activities with enzymatic test kits revealed that hop β-amylase activity correlated highest with the diastatic activity as measured using potato starch ($r = 0.829$, $p < 0.0001$), followed by maltodextrin ($r = 0.655$, $p = 0.0004$) and beer ($r = 0.578$, $p = 0.015$). Potato starch results likewise showed the highest Pearson coefficient of correlation ($r = 0.853$, $p < 0.001$) and a coefficient of determination of $R^2 = 0.727$ with the release of fermentable sugars in beer. Maltodextrin results also showed a significant correlation with diastatic activity in beer, but at a lower level with $r = 0.602$, $p = 0.010$, while the dextrin correlation was insignificant at $\alpha = 0.05$. Potato starch was therefore confirmed to be the most suitable substrate. Additional trials comparing enzymatic activities in unpelletized and pelletized hop samples showed that pelletizing has no considerable effect on hop enzymatic activity. Taken together, these data suggest that determining hop diastatic activity using potato starch appears to show the best results. This method can therefore be recommended for measuring the diastatic power of hops.

Descriptors: hops, hop creep, amylases, diastatic activity, enzymes

1 Introduction

Particularly affected by the growing craft beer industry, dry-hopping techniques are now well established in the modern brewing industry. Due to the broader use of dry-hopping, new challenges arise such as the 'freshening power of hops' or 'hop creep'. Briefly, this phenomenon describes the degradation of beer dextrins by hop diastatic enzymes when dry-hopping beers. This can result in high yeast activity which, in turn, can lead to carbon dioxide and ethanol production as well as undesired fermentation by-products. *Werrie*, *Deckers*, and *Fauconnier* [1] indeed showed the effect of hop-enzyme-induced refermentation and the development of yeast-derived aromas such as higher alcohols, esters, and both diacetyl and pentanedione. With a focus on yeast, *Bruner*, *Marcus*, and *Fox* [2] examined different yeast strains with regards to their reaction to dry hopping but found no yeast-derived mitigation of hop creep. Mitigation strategies can include removal or inactivation of yeast prior to dry-hopping, or to dry-hop at low temperatures. The latter strategy may not be practical as it also affects dry-hop aroma due to changes in yeast biotransformation reactions or different transfer rates of hop volatiles at lower temperatures [3]. Different beers will very likely produce different magnitudes of hop creep when dry-hopped, and a growing list of adjuncts or non-hop ingredients added downstream to specialty beers must be accounted for. Actions to mitigate hop creep must therefore also involve an understanding of the interplay of (raw) materials used for brewing and the control and management of hop diastatic activity in the raw material. *Teraoka*, *Kanauch* and *Bamforth* [4] suggested that the phenomenon may in fact be derived from the microbiota on the surface of the hop plant, which may also open the potential for new strategies. Increasing the kilning temperatures can be effective and lower hop diastatic activity was found when kilning hops at elevated temperatures [5]. Higher temperatures during hop drying can however also have other undesired consequences such as, e.g., a change in the hop oil composition. The complexity of the factors involved in the enzymatic activity of hops was shown by *Stokholm* et al. [6] who investigated agronomic factors and found field-to-field differences in hop enzymatic power. The authors proposed further that these differences are not driven by single factors but by an interaction of weather, soil, and management coupled with influences of disease pressure and other factors.

While all these data and findings are helpful in understanding hop creep, there is still no internationally applied methodology for determining hop diastatic power. This would also allow a comparison of data from different studies and provide a standard

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metric for hop diastatic power. The objective of this work and the previous study [7] therefore aims to find a standardized protocol for measuring hop diastatic activity. In the first part of this study, in an adaptation of the method to determine malt diastatic power, potato starch was used as a substrate to investigate hop diastatic power, and incubation temperature, substrate concentration, and hop concentration were varied. An incubation temperature of 35 °C, a starch concentration of 3 % (w/v), and a hop concentration of 15 g/L, were found to be the optimal process parameters to maximize glucose and maltose formation as derived from starch degradation. Importantly, because hops possess amyloglucosidase activity, which can cleave one molecule of maltose to two glucose units, it is highly recommended to quantitate not solely maltose but also glucose to get a true measure of hop diastatic power. While using potato starch showed a highly significant correlation when compared to the release of fermentable sugars in a dry-hopped beer, a linear coefficient of determination of $R^2 = 0.72$ was found. In an initial search for an alternative substrate, a commercially available β-limit dextrin was also used as a substrate but yielded low, non-significant correlations. In this follow-up study, further substrates were tested for their suitability for use in this assay. Furthermore, for the first time, an optimized protocol for measuring individually α-amylase, β-amylase, and amyloglucosidase activity was applied and results were compared to results from diastatic power measurements and to results from a dry-hopped beer. Both methodologies were then applied to unpelletized and pelletized hop samples from crop 2020.

2 Materials and Methods

2.1 Chemicals

The Megazyme kits for β-amylase (K-BETA3), α-amylase (K-AMYLSD), amyloglucosidase (R-AMGR3) and limit dextrinase (K-PullG6) activity measurements were purchased from Megazyme Ltd. (Bray, Ireland). These kits already contain all required solutions and buffers. Disodium ethylenediaminetetraacetic acid (EDTA), 2-(N-morpholino)ethanesulfonic acid (MES) monohydrate, tris(hydroxymethyl)aminomethane (TRIS), sodium hydroxide, hydrochloric acid and glacial acetic acid were obtained from Sigma-Aldrich (Steinheim, Germany). Potato starch (CAS 9005-84-9) was obtained from Merck (Darmstadt, Germany), and acetonitrile from VWR international (Darmstadt, Germany). Corn starch, rice starch, wheat starch, and amylopectin were acquired from Carl Roth GmbH (Karlsruhe, Germany). Dextrin was obtained from J&K Scientific GmbH (Pforzheim, Germany). All aqueous solutions were made with double-distilled water and all chemicals were of analytical grade or higher.

2.2 Hop samples

17 different dried unpelletized and pelletized hop samples from crop 2020 were obtained from Hopsteiner, Germany. Table 1 features the different hop cultivars used. All hops were cultivated in the Hallertau region, Germany, except Tettnanger which was grown in Tettnang, Germany. The hops were stored in vacuum-sealed packaging at –18 °C. Hops were milled prior to use to give the following particle size distribution: > 0.5 mm, 3 %; 0.25 – 0.5 mm, 23 %; 0.125 – 0.25 mm, 43 %; < 0.125 mm, 31 %. To test how much fructose and glucose were brought in from hops during the assay, fructose and glucose concentrations were assessed by adding 0.15 g of the hop samples to 10 mL of double-distilled water and incubating them for 48 hours at 35 °C. Fructose, glucose, and maltose concentrations were subsequently analyzed by HPLC.

Table 1 Hop cultivars from crop 2020 used in this study. All hops were cultivated in the Hallertau region, Germany, except Tettnanger which was cultivated in Tettnang, Germany

Hop Cultivar	Abbreviation
Akoya	AKO
Ariana	ANA
Callista	CAL
Cascade	CAS
Hallertauer Mittelfrüh	HAL
Herkules	HKS
Hallertauer Magnum	HMG
Hallertauer Tradition	HTR
Mandarina Bavaria	MBA
Northern Brewer	NBR
Opal	OPL
Perle	PER
Smaragd	SGD
Saphir	SIR
Solero	SOL
Spalter Select	SSE
Tettnanger	TET

2.3 Base beer characterization

A commercially available lager beer was obtained as a base beer for determining the release of fermentable carbohydrates from beer residual dextrins. The beer was characterized according to the Mitteleuropäische Brautechnische Analysenkommission e.V. (MEBAK) methodologies [8]: Extract (2.10.3), alcohol (2.10.7), colour (2.13.2), pH

Table 2 Analytical parameters of beer used in this study

Parameter	Value
Real Extract (% w/w)	4.05
Gravity (% w/w)	11.62
Alcohol (% v/v)	4.98
Apparent Attenuation [%]	81.4
Real Attenuation [%]	66.5
pH	4.42
Color [°EBC]	6.1
Bitter Units [IBU]	28
Fructose (g/L)	0.03
Glucose (g/L)	0.09
Maltose (g/L)	1.02

(2.14), foam stability (2.19), total nitrogen (2.8.1), free amino nitrogen (2.8.4.1), total polyphenols (2.17.1), and bitter units (2.22.1). The numbers in parentheses indicate the method used. Carbohydrates were analyzed by HPLC. The beer data is presented in table 2.

2.4 HPLC determination of fructose, glucose, and maltose concentration

HPLC analysis was performed according to the MEBAK, method 2.7.1. [8] with slight modifications and using an Agilent 1100 HPLC system equipped with an AppliChrom OTUAmino-RP (250 mm x 4.6 mm). The flow rate was 2 mL/min and the mobile phase consisted of 18 % (v/v) water and 82 % (v/v) acetonitrile. The column temperature was set to 30 °C. A five-point calibration for the carbohydrates fructose, glucose, maltose, maltotriose, and sucrose with $R^2 > 0.99$ was applied for determining the concentration in the samples.

2.5 Testing of different substrates for determining the diastatic power of hops samples

Different substrates were tested for their suitability to be used as a substrate for determining the diastatic power of hops and were compared to the release of fermentable sugars when incubating a beer with hops. An optimized measuring protocol from part 1 of this study [7] was applied and potato starch, which was originally used, was substituted with the following substrates: corn starch, wheat starch, rice starch, maltodextrin, dextrin, or amylopectin. To test how these substrates perform in the assay, a 3 % (w/v) solution was prepared for each substrate, and the assay was carried out as follows: 10 mL of a 3 % (w/v) substrate solution, or 10 mL of beer were incubated with 0.15 g of ground hop samples from 17 different hop cultivars (Table 1). Incubation was carried out for 48 hours at 35 °C. After incubation, glucose, and maltose were analyzed by HPLC. Because hops possess amyloglucosidase activity, which can cleave one molecule of maltose into two molecules of glucose, both carbohydrates must be considered for the determination of the enzymatic power of hops. The diastatic power (DP) of hops was then calculated according to equation 1:

$$DP\left[\frac{\mu Mol\ glucose\ equivalents}{hours \times g\ hops}\right] = \frac{c(glucose)\left[\frac{g}{L}\right] + 2 \times c(maltose)\left[\frac{g}{L}\right] \times 10^6}{180.156\frac{g}{mol} \times 48h \times \frac{15g}{L}} \quad \text{(Eq. 1)}$$

2.6 Determination of α-amylase, β-amylase, amyloglucosidase, and limit dextrinase activity of hops

Enzyme activities of α-amylase, β-amylase, amyloglucosidase, and limit dextrinase were analyzed according to an optimized measurement protocol which was provided by Megazyme Ltd. [9]. The extraction of enzymes in hop flour was carried out by addition of sodium maleate buffer (30 mL, 0.1 M, pH 5.5) containing bovine serum albumin (BSA, 1 mg/mL) to a sample of hop flour (0.5 g, milled to pass a 0.5 mm screen) and activated charcoal (0.5 g) in a 50 mL polypropylene tube and mixing vigorously on a vortex mixer. The enzymes were extracted by incubation for 60 minutes (with intermittent mixing) in a water bath at 22 °C. The resulting suspension was centrifuged at 13,000 rpm to remove the bulk of the suspended material, and subsequently filtered through Whatman no. 1 filter paper to clarify. The supernatant was stored on ice. Aliquots of the extract solution were analyzed using the standard procedures outlined for α-amylase, β-amylase, α-glucosidase and limit-dextrinase. All hop samples were measured in duplicate. Absorbances were recorded after 120, 180, and 240 minutes of incubation, and the enzymatic activities at the different time points were then averaged.

2.6.1 Standard procedure for the measurement of α-amylase activity in hops extracts

Aliquots (0.4 mL) of the extract solution were incubated with the Amylase SD reagent (0.1 mL) at 40 °C and reactions were terminated by addition of Tris solution (1.0 mL, 2 % w/v, pH 10.0). Reaction blanks were created by the addition of the enzyme extract (0.4 mL), followed by Tris solution (1.0 mL, 2 % w/v, pH 10.0) and then the Amylase SD reagent (0.1 mL). Absorbance was measured at 400 nm.

2.6.2 Standard procedure for the measurement of β-amylase activity in hops extracts

Aliquots (0.2 mL) of the extract solution were incubated with the Betamyl-3 reagent (0.2 mL) at 40 °C and reactions were terminated by addition of Tris solution (1.0 mL, 2 % w/v, pH 10.0). Reaction blanks were created by the addition of the enzyme extract (0.2 mL), followed by Tris solution (1.0 mL, 2 % w/v, pH 10.0) and then the Betamyl-3 reagent (0.2 mL). Absorbance was measured at 400 nm.

2.6.3 Standard procedure for the measurement of α-glucosidase activity in hops extracts

Aliquots (0.2 mL) of the extract solution were incubated with the amyloglucosidase assay reagent (0.2 mL) at 40 °C and reactions were terminated by addition of Tris solution (1.0 mL, 2 % w/v, pH 10.0). Reaction blanks were created by the addition of the enzyme extract (0.2 mL), followed by Tris solution (1.0 mL, 2 % w/v, pH 10.0) and then the amyloglucosidase assay reagent (0.2 mL). Absorbance was measured at 400 nm.

2.6.4 Standard procedure for the measurement of Limit Dextrinase activity in hops extracts

Aliquots (0.1 mL) of the extract solution were incubated with the PullG6 reagent (0.1 mL) at 40 °C and reactions were terminated by addition of Tris solution (0.75 mL, 2 % w/v, pH 10.0). Reaction blanks were created by the addition of the enzyme extract (0.1 mL), followed by Tris solution (0.75 mL, 2 % w/v, pH 10.0) and then the PullG6 reagent (0.1 mL). Absorbance was measured at 400 nm.

The individual enzymatic activities per gram of hop sample were calculated as follows:

$$\frac{Units}{g} = \frac{\Delta A_{400}}{T} \times \frac{V_1}{V_2} \times \frac{1}{18.1} \times \frac{30}{w} \quad \text{(Eq. 2)}$$

where:

Table 3 **Diastatic power of pelletized hop samples using dextrin, maltodextrin, or potato starch, and beer as substrates and individual enzyme activities. For diastatic power, mean values +/– 1 standard deviation of a duplicate determination are presented. For enzyme activities, absorbances were measured after 120, 180, and 240 minutes of incubation and averaged enzyme activities at the three time points +/– 1 standard deviation are given. For abbreviations of hops, see table 1**

Hops	α-amylase activity	β-amylase activity	Amylo-glucosidase activity	Diastatic power using the substrate [µMol glucose equivalents per hour and g of hops]			
	[mU/g]	[mU/g]	[mU/g]	maltodextrin	dextrin	potato starch	beer
AKO	3.9 +/– 0.1	32.3 +/– 3.2	60.8 +/– 1.9	84.8 +/– 1.3	32.6 +/– 1.4	41.9 +/– 1.1	45.5 +/– 1.3
ANA	7.6 +/– 0.8	26.9 +/– 0.7	54.1 +/– 5.4	101.5 +/– 8.2	23.6 +/– 6.3	18.8 +/– 1.5	26.8 +/– 2.1
CAL	8.0 +/– 0.3	29.8 +/– 2.3	60.3 +/– 0.7	62.1 +/– 2.3	28.4 +/– 4.1	34.9 +/– 1.6	26.0 +/– 0.4
CAS	7.5 +/– 0.5	39.2 +/– 1.5	63.2 +/– 1.4	108.1 +/– 1.9	32.6 +/– 2.1	34.7 +/– 0.1	35.0 +/– 2.1
HAL	4.7 +/– 0.1	33.6 +/– 1.7	66.2 +/– 0.8	60.3 +/– 4.1	47.5 +/– 10.5	32.7 +/– 1.1	27.6 +/– 4.0
HKS	5.5 +/– 0.2	26.7 +/– 2.2	60.5 +/– 1.4	60.4 +/– 3.0	25.2 +/– 3.8	17.3 +/– 1.6	24.9 +/– 8.0
HMG	7.0 +/– 0.6	47.7 +/– 2.7	75.4 +/– 0.6	122.0 +/– 0.4	37.3 +/– 3.6	61.8 +/– 2.6	35.8 +/– 3.7
HTR	4.3 +/– 0.5	26.5 +/– 2.8	62.4 +/– 0.9	90.8 +/– 3.2	21.8 +/– 0.0	23.1 +/– 0.4	35.2 +/– 0.8
MBA	5.1 +/– 1.4	31.2 +/– 1.0	57.7 +/– 2.3	51.6 +/– 17.9	28.9 +/– 0.8	39.9 +/– 0.5	37.0 +/– 1.6
NBR	6.8 +/– 0.3	24.4 +/– 3.8	63.8 +/– 0.4	54.2 +/– 2.7	37.1 +/– 16.4	23.9 +/– 0.3	24.0 +/– 2.1
OPL	5.7 +/– 0.2	53.6 +/– 0.2	62.2 +/– 1.4	114.6 +/– 14.6	34.1 +/– 14.7	65.9 +/– 4.4	58.7 +/– 0.4
PER	7.4 +/– 0.2	25.9 +/– 0.2	62.3 +/– 4.0	58.4 +/– 0.5	25.5 +/– 0.2	26.9 +/– 1.6	38.7 +/– 2.8
SGD	5.6 +/– 0.1	30.2 +/– 1.6	59.9 +/– 0.2	96.9 +/– 1.5	24.4 +/– 3.0	26.8 +/– 0.4	31.4 +/– 0.4
SIR	6.8 +/– 0.3	33.9 +/– 0.8	64.8 +/– 1.1	59.4 +/– 2.5	79.1 +/– 5.3	26.2 +/– 0.9	37.9 +/– 4.8
SOL	8.9 +/– 0.6	35.2 +/– 2.9	70.8 +/– 7.3	92.1 +/– 0.6	31.2 +/– 0.1	35.9 +/– 0.5	40.6 +/– 2.0
SSE	10.7 +/– 0.3	14.4 +/– 6.3	63.9 +/– 0.2	63.5 +/– 0.1	22.8 +/– 0.1	25.1 +/– 2.3	40.5 +/– 3.4
TET	7.3 +/– 0.3	31.2 +/– 3.9	61.9 +/– 4.4	54.3 +/– 2.8	29.1 +/– 3.7	23.6 +/– 1.4	29.0 +/– 3.5

Table 4 **Pearson correlation coefficients between α-amylase, β-amylase, amyloglucosidase activity and diastatic power (DP) using dextrin, maltodextrin, potato starch, or beer as substrates**

Variablen	α-amylase	β-amylase	Amyloglucosi-dase	DP potato starch	DP dextrin	DP maltodextrin	DP beer
α-amylase	**1**	– 0.273	0.215	– 0.151	– 0.098	– 0.039	– 0.010
β-amylase	– 0.273	**1**	0.397	**0.841**	0.295	**0.639**	**0.662**
Amyloglucosidase	0.215	0.397	**1**	0.451	0.331	0.265	**0.523**
DP potato starch	– 0.151	**0.841**	0.451	**1**	0.116	**0.561**	**0.853**
DP dextrin	– 0.098	0.295	0.331	0.116	**1**	– 0.163	0.112
DP maltodextrin	– 0.039	**0.639**	0.265	**0.561**	– 0.163	**1**	**0.602**
DP beer	– 0.010	**0.662**	**0.523**	**0.853**	0.112	**0.602**	**1**

Values in bold are significantly different at α = 0.05.

Table 5 **P-values of Pearson correlation between α-amylase, β-amylase, amyloglucosidase activity and diastatic power (DP) using dextrin, maltodextrin, potato starch, or beer as substrates**

Variable	α-amylase	β-amylase	Amyloglucosi-dase	DP potato starch	DP dextrin	DP maltodextrin	DP beer
α-amylase	**0**	0.288	0.408	0.564	0.709	0.883	0.970
β-amylase	0.288	**0**	0.114	**< 0.0001**	0.250	**0.006**	**0.004**
Amyloglucosidase	0.408	0.114	**0**	0.069	0.194	0.304	**0.031**
DP potato starch	0.564	**< 0.0001**	0.069	**0**	0.658	**0.019**	**< 0.0001**
DP dextrin	0.709	0.250	0.194	0.658	**0**	0.532	0.669
DP maltodextrin	0.883	**0.006**	0.304	**0.019**	0.532	**0**	**0.010**
DP beer	0.970	**0.004**	**0.031**	**< 0.0001**	0.669	**0.010**	**0**

Values in bold are significantly different at α = 0.05.

ΔA_{400} = Absorbance (reaction) – Absorbance (blank)

T = Incubation time in minutes

V_1 = Total volume in reaction in mL

V_2 = Sample volume in reaction in mL

18.1 = E_{mM} of p-nitrophenol in 2 % Tris pH 10

30 = Extraction volume in mL

w = weight of sample in extract in g

2.7 Statistical evaluation

Pearson correlation coefficients with a significance level of $\alpha = 0.05$, coefficients of determination (R^2) were calculated using XLSTAT software (Addinsoft, Andernach, Germany).

3 Results and Discussion

When testing different substrates, except for dextrin, maltodextrin, or potato starch, no or very low concentrations of maltose were found after 48 h of incubation (data not shown). As maltose is the main carbohydrate formed in beer when incubated with hops, amylopectin, corn starch, rice starch, and wheat starch were therefore not investigated further. Based on these observations, potato starch, maltodextrin, and dextrin were selected for further testing. In addition, the individual enzyme activities of all pelletized hop samples were examined (Table 3).

Next, all data from the diastatic power measurements using dextrin, maltodextrin, potato starch, beer, and the individual enzyme activity measurements were compared to test for correlation (Table 4 and 5). In pelletized hop samples, β-amylase activity in hop samples correlated highest with diastatic power value from potato starch ($r = 0.841$, $p < 0.0001$), followed by that of maltodextrin ($r = 0.639$, $p = 0.006$), beer ($r = 0.662$, $p = 0.004$) and dextrin ($r = 0.295$, not significant at $\alpha = 0.05$). Amyloglucosidase activity correlated significantly with beer diastatic power with $r = 0.523$, $p = 0.031$ while α-amylase activity did not correlate significantly with any of the substrates tested. This is in good agreement with the diastatic power of malt where the diastatic power is also stated to be primarily dependent on β-amylase activity [10]. It is also noteworthy to mention here that hop β-amylase seems to be the most important enzyme in terms of the release of fermentable sugars and therefore apparently the main source of 'hop creep'.

In terms of 'hop creep', it is also of interest to associate the behaviour of an individual hop sample with the hop-induced dextrin degradation and concomitant fermentable sugar release in beer during dry-hopping. Comparing results of selected substrates revealed the highest Pearson coefficient of correlation for potato starch ($r = 0.853$, $p < 0.001$). Maltodextrin also showed a significant correlation with beer but at a lower level with $r = 0.602$, $p = 0.010$. Dextrin values did not correlate with beer values ($\alpha = 0.05$). It becomes evident from this data that even though β-amylase seems to be the most important enzyme in terms of maltose release, the sum and interplay of all diastatic enzymes is still meaningful and informative with regards to 'hop creep'. These data suggest that potato starch is the best substrate and is also superior to the quantitative determination of the individual enzyme activities.

An 'ideal' method would utilize a standard substrate and would forecast accurately the hop-induced liberation of fermentable sugars by a particular hop variety during dry-hopping of beer of any kind. While significant Pearson correlation coefficients can tell if two variables follow each other, the coefficient of determination demonstrates how well one variable predicts another variable, i.e., how good the diastatic power can predict the liberation of fermentable sugars when dry-hopping a beer. Plotting the diastatic power as determined in beer over the diastatic power as determined using dextrin, maltodextrin, or potato starch, revealed coefficients of determination of $R^2 = 0.013$, $R^2 = 0.363$, and $R^2 = 0.727$ respectively (Table 6). This demonstrates that potato starch is clearly superior to dextrin or maltodextrin but still 27.3 % of the 'beer data' are not explained by the diastatic power metrics. The release of fermentable sugars, however, is supposed to depend to a great extent on the type of beer used and the dextrin composition of the beer and finding a standardizable substrate (which is a criterion for a standardized and reproducible method) that shows a high coefficient of determination with all types of beers seems to be unlikely. In sum, because of the highest correlation with β-amylase activity in hops and the significant correlation with beer, potato starch can be recommended as a substrate for determining the diastatic power of hops.

Table 6 Coefficients of determination (R^2) between α-amylase, β-amylase, amyloglucosidase activity and diastatic power (DP) using dextrin, maltodextrin, potato starch, or beer as substrates

Variablen	α-amylase	β-amylase	Amyloglucosidase	DP potato starch	DP dextrin	DP maltodextrin	DP beer
α-amylase	1	0.075	0.046	0.023	0.010	0.001	0.000
β-amylase	0.075	1	0.158	0.708	0.087	0.408	0.439
Amyloglucosidase	0.046	0.158	1	0.203	0.110	0.070	0.273
DP potato starch	0.023	0.708	0.203	1	0.013	0.315	0.727
DP dextrin	0.010	0.087	0.110	0.013	1	0.027	0.013
DP maltodextrin	0.001	0.408	0.070	0.315	0.027	1	0.363
DP beer	0.000	0.439	0.273	0.727	0.013	0.363	1

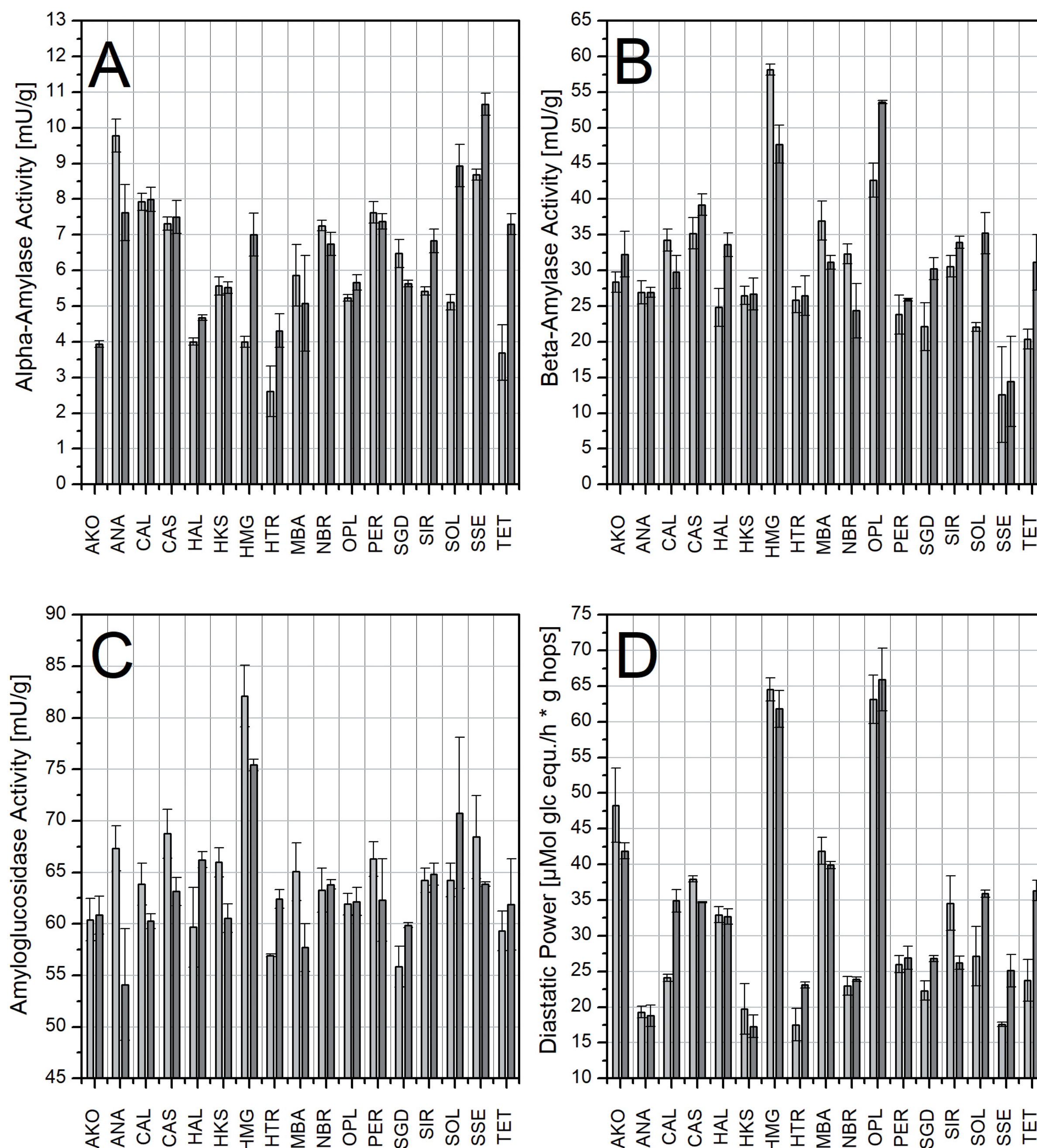

Fig. 1 α-amylase activity (A), β-amylase activity (B), amyloglucosidase activity (C), and diastatic power (D) in unpelletized (dark grey) and pelletized (light grey) hop samples. For hop abbreviations, see table 1. Enzyme activities were determined after 120, 180, and 240 minutes of incubation and mean values +/– 1 standard deviation are shown. Diastatic power measurement was carried out as described in the materials and methods section using potato starch as substrate. Mean values of duplicate determination +/– 1 standard deviation are shown.

The results from measuring α-amylase, β-amylase, and amyloglucosidase activity in pelletized and unpelletized hops is shown in figure 1. Limit dextrinase was also measured according to the optimized methodology provided by Megazyme Ltd. [9] and was not detected in any of the samples measured. This observation was also confirmed from Megazyme Ltd. [9] and contradicts to some degree findings from *Kirkpatrick* and *Shellhammer* [11] who were in fact able to detect limit dextrinase activities in American hop samples, yet only at low activity. The hop variety Akoya showed no α-amylase activity in unpelletized hops which was confirmed in two additional separate analysis runs but did show β-amylase and amyloglucosidase activity. It could not be determined why there was no α-amylase activity found. For all other hops, enzyme activities of unpelletized hops ranged from 2.6 to 9.8, 12.6 to 58.2, and 55.8 to 82.1 mU/g for α-amylase, β-amylase, and amyloglucosidase activity, respectively. Enzymatic activities of pelletized hops ranged from 3.9 to 10.7, 14.4 to 53.6, and 54.1 to 75.4 mU/g for α-amylase, β-amylase, and amyloglucosidase activity, respectively. For most samples, enzyme activities tended to be slightly higher in pelletized than in unpelletized hop samples, particularly for Hallertauer Magnum (HMG), Solero (SOL), and Tettnanger (TET). On average, activities were ~20 % and ~12 % higher in pelletized hops with regards to α-amylase and β-amylase activity, respectively, while activities were alike with regards to amyloglu-

cosidase activity. These individual enzymatic activities were also reflected when measuring the diastatic power of the unpelletized and pelletized hop samples and, with a few exceptions, only minor differences were found (Fig. 1, D).

Comparing again the diastatic data and the enzymatic data from unpelletized hop samples revealed a highly significant correlation between β-amylase activity and diastatic power ($r = 0.758$, $p < 0.001$) which confirms again the findings from the pelletized hop samples (cp. Table 3). This is also proof that hop β-amylase activity seems to be the most important enzyme in terms of sugar liberation from starch (and from beer).

4 Conclusion

Testing different substrates proved that potato starch is the most suitable substrate for the determination of the diastatic power of hops. The data correlates significantly with β-amylase activity in hops and shows a highly significant Pearson correlation coefficient with the hop-induced sugar liberation in beer. The method presented can therefore be recommended for the measurement of the diastatic activity of hop samples. This now allows numerous applications and will help determine how different growing conditions, crop years, the processing of hops, etc. affect the diastatic power of hops. Compared with other substrates tested, potato starch as substrate also showed the highest coefficient of determination of $R^2 = 0.727$ with beer. While there is a strong positive correlation between the diastatic power of hops and liberation of sugars in beer, suggesting a clear and close association, the predictive power of the method with regards to the liberation of fermentable sugars in beer is restricted. This can however also not be the goal of this method as every beer may behave differently due to its varying dextrin composition. It seems to be clear that one ‘standardized’ substrate cannot serve as a predictor for all individual beers. This, in fact, would require incubating the ‘in-house’ beer with the hops or hop mixture to be used during dry-hopping, as e.g. suggested by Stockholm et al. [12].

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