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Optimized analytical parameters for the viscometric determination of pasting temperatures of barley malt

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ABSTRACT

Starch pasting is the prerequisite for subsequent enzymatic hydrolysis, required in numerous fermentation based industrial processes. Thermal pasting is induced by exceeding a raw material specific pasting temperature which can be determined viscometrically. The analytical parameters for this indirect analysis must however be adapted to raw material characteristics. For unmalted cereals several viscometric procedures were developed and approved. Although barley malt represents a major starch and enzyme source for the production of drinking alcohol, the analytical parameters were not adapted to the modifications induced by germination.

The aim of this study was to investigate and evaluate parameters enabling a precise and reproducible determination of the pasting temperature of barley malt on basis of the commonly used rotational viscometer *Rapid-Visco-Analyser*.

An optimal sample concentration (1:4) was determined by performing dilution series. The comparison of different evaluation criteria resulted in a slope threshold (5 mPa s/6 s) providing most accurate and reproducible results. Enzymatic inhibition with silver nitrate showed no alteration of the resulting pasting temperatures, proving that the grains intrinsic pasting temperature is independent of its amylolytic activity.

The analytical parameters were evaluated intern- and externally by the analysis of heterogeneous samples. The resulting pasting temperatures ranged from 61.5 to 65.9 °C featuring a satisfying repeatability of 0.24 °C and reproducibility of 0.34 °C. The results were thereby independent of the laboratory or the used RVA model.

Therefore, the newly developed procedure proved its applicability to determine the intrinsic pasting temperature of germinated barley and thus supplements existing procedures of unmalted grains. The method might be transferred to other germinated cereals in the future.

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1. Introduction

The determination of pasting properties is a common application of viscometry in order to simulate food processing and predict product qualities of starch based foods (Copeland, Blazek, Salman, & Tang, 2009; Crosbie & Ross, 2007). The pasting temperature (PT) characterizes the specific temperature at which starch granules in an aqueous suspension begin to swell and lose their native structure (BeMiller & Whistler, 2009). In the brewing industry the PT of barley malt is of high interest because starch pasting is the prerequisite for the subsequent amylolytic hydrolysis of starch into fermentable sugars. The pasting properties of cereals are however inconsistent and depend on intrinsic factors such as the starch composition (Izydorczyk & MacGregor, 2001; MacGregor & Ballance, 1980; Naguleswaran, Vasanthan, Hoover, & Bressler, 2013), (Schirmer, Höchstötter, Jekle, Arendt, & Becker, 2013), proteins (Jekle, Muehlberger, & Becker, 2016; Mohamed & Rayas-Duarte, 2003), lipids (Salman & Copeland, 2010; Yoo & Jane, 2002), and extrinsic factors like growth conditions (Noda et al., 2004; Tester, 1997). In order to detect these variations, a reliable analyzing method is indispensable.

The pasting temperature of cereals is commonly determined by the use of rotational viscometers such as the *Rapid-Visco-Analyser (RVA, Perten Instruments, a PerkinElmer Company, USA)* or the

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Amylograph® (Brabender GmbH & Co. KG, Germany). The basic principle is to heat up a starch suspension while continuously recording the torque applied to the paddle rotating in the fluid. By using calibration factors this torque can be used to determine the viscosity (Crosbie & Ross, 2007). The general set-up of the analysis can be divided into 5 successive phases (comp. Fig. 1): Mixing of the sample (I), equilibration and water uptake (II), heating (III), holding (IV) and cooling (V) (Crosbie & Ross, 2007). The exceed of a specific temperature during the heating phase leads to the swelling of starch granules (Steeneken, 1989) which induces -in combination with the leaching of amylose (Eliasson, 1986)—an abrupt rise of viscosity, illustrated in Fig. 1. Therefore this specific temperature equates the pasting temperature and is viscometrically defined as "*temperature at which the viscosity trace first rises above the baseline during the initial heating phase*" (Crosbie & Ross, 2007). This instant of time is determined by the RVA Software *Thermocline®* by calculating the slope of the viscosity progression during starch pasting. A slope threshold which has to be exceeded can be specified, by default this value is set to 24 mPa over an interval of 6 s.

Previous investigations have demonstrated that the rising viscosity and its slope are correlated to the amount of swollen starch granules in the sample (Almeida-Dominguez, Suhendro, & Rooney, 1997; Steeneken, 1989). Up to a saturation concentration, higher solid concentrations cause steeper slopes and consequently lead to a decrease in pasting temperatures ascertained by the automatic software determination (Acosta-Osorio et al., 2011; Bao, 2008). Depending on the analyzed sample material, reduced slope thresholds proved to be more sensitive regarding the accurate determination of the onset of starch pasting (Bao, 2008; Zhou & Mendham, 2005). This slope optimization exhibits a lower limit due to the circumstance that too flat slopes lead to misinterpretations caused by unavoidable viscosity fluctuations prior to starch pasting and therefore unrealistic low pasting temperatures (Bao, 2008).

In summary, a viscometric method to determine the pasting properties correctly must be adapted to the starch characteristics and the enzymatic activity of the analyzed grain. This includes the solid concentration of the sample as well as the optimization of the slope sensitivity for the detection of the pasting onset. Several viscometric methods for **ungerminated** grains have been developed and approved for the RVA (wheat and rye flour or starch: AACCi 76–21.01/ICC 162; rice: AACCi 61–02.01 and AACCi 61–04.01; oat: AACCi 76–22.01). However, no adapted and approved method to determine the pasting temperature in **germinated** barley, the most common raw material for the production of alcoholic beverages worldwide (Tester, 1997), has been developed yet. Due to national laws (e.g. the 'purity law'), germinated grains represent the only permitted source of starch and enzymes for the production of beer in specific countries like Germany (Verordnung zur Durchführung des Vorläufigen Biergesetzes, 1931).

In contrast to ungerminated grains, the amylolytic enzymes required for the starch hydrolysis in the brewhouse are developed naturally during the germination process and remain in the kernels after the malting process (Kuntz & Bamforth, 2007; Palmer, 1972). This autolytic benefit regarding processing, however, complicates the determination of water based starch analysis such as the determination of the gelatinization temperature by *Dynamic-Scanning-Calometry* (DSC) (Derde, Gomand, Courtin, & Delcour, 2012; Leman, Goesaert, Vandeputte, Lagrain, & Delcour, 2005; van Steertegem, Pareyt, Brijs, & Delcour, 2013). Therefore, enzymatic inhibition during the measurement or prior starch purification in combination with segregation of enzymes is necessary (Rittenauer, Kolesnik, Gastl, & Becker, 2016). Former researches investigating the impact of amylolytic enzymes on the pasting properties of barley malt mainly focused on the maximal viscosity developed during pasting (Peak Viscosity, comp. Fig.1). It was shown that increasing levels of α-amylase activity significantly reduce the maximal peak viscosity (Fox, Visser, Skov, Meijering, & Manley, 2014; Glennie-Holmes, 1995b, 1995a; Goode, Wiltschko, Ulmer, & Arendt, 2005; Leman, Bijttebier, Goesaert, Vandeputte, & Delcour, 2006) whereas exogenous β-amylase has no impact

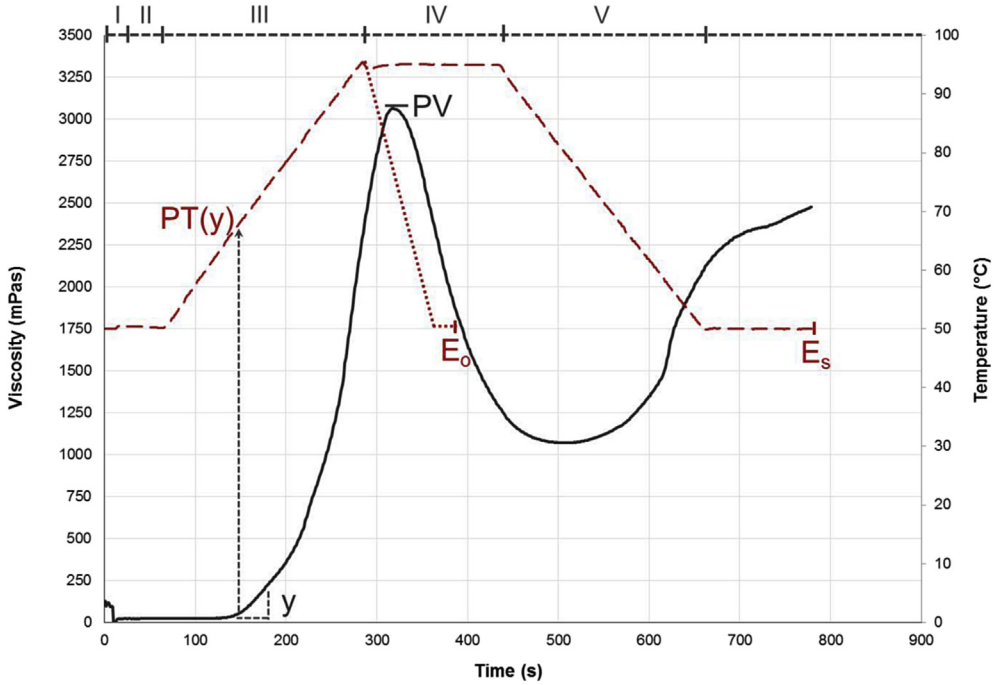

Fig. 1. Viscogram of viscometric analysis of unmalted barley performed by RVA: — Viscosity, – – – Standard temperature profile, — Optimized temperature profile; y: Slope sensitivity, PT(y): Slope dependent pasting temperature, PV: Peak viscosity, E_S: End of standard method (780 s), E_O: End of optimized method (390 s).

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