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ORIGINAL PAPER

Gelatinization properties of wheat flour as determined by empirical and fundamental rheometric method

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Abstract Certain empirical rheological methods are in extensive use in wheat and/or flour research to assess starch gelatinization and pasting behavior primarily due to easy performance and good correlation with final product quality. However, their applications are often associated with specific drawbacks that could be limiting factors for certain applications, such as poor definition of the measured parameters, time-consuming nature, difficulties in interpretation of results, large sample sizes, etc. The listed shortcomings can be overcome by application of fundamental rheological methods that are based on well-defined rheological parameters such as stress, strain, viscosity and modulus. The objective of this study was to optimize the fundamental rheological method for determination of the gelatinization properties of wheat flour that correspondents to the standard widely accepted empirical rheological method—Amylograph method and to compare them in order to determine whether they can be interchangeable depending on different analytical needs. The obtained results have shown that the application of fundamental rheometric procedure for determination of pasting properties of wheat flour provides reliable determination of the gelatinization properties of wheat flour. Moreover, substantial advantages of fundamental rheometric method over the empirical one were identified including smaller sample size, ability to set the desirable heating and shear rate, shorter test duration and better precision.

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Keywords Gelatinization properties · Wheat flour · Empirical rheological method · Fundamental rheological method · Vane geometry

Abbreviations

PCA	Principal component analysis
PC	Principal component
SDr	Repeatability standard deviation
RSD	Relative standard deviation
r	Repeatability limit
Amy	Brabender Amylograph
Slow	Slow rheometric procedure
Rapid	Rapid rheometric procedure
PV	Peak viscosity
PT	Peak temperature
PST	Pasting temperature

Introduction

Gelatinization properties of starch determine many food and non-food starch applications [1, 2]. A variety of methods for the monitoring of gelatinization of starch are available, such as different microscopic, thermoanalytical, enzymatic and rheological methods [3]. The recent trends in starch research have indicated the utilization of combined measurement techniques as reported by Li et al. [4] who coupled hot-stage light microscopy and differential scanning calorimetry (DSC) to study the dynamic changes of starch during gelatinization process. Rheological methods are based on the measurement of viscosity changes during heating and shearing of starch slurries [5]. For this purpose, different instruments are available including Brabender Visco Amylograph (BVA) and Micro Visco Amylograph (MVA), Rapid Visco Analyzer (RVA),

Ottawa starch viscometer [6, 7] and viscometer [8]. Given the variety of instruments, it is not surprising that a large number of comparative studies have been performed between certain instruments as being applied for determination of pasting properties of starch and starch-containing products [9–14]. Considering the role of starch as a functional ingredient in a diversity of food formulations, those comparative studies have been mainly performed on native or modified starches of different botanical origin [15]. Wheat flour, with 75–80 % of starch content on a dry weight basis, represents significant source of starch for the majority of population. The functional properties of starch such as gelatinization, gelation and viscosity are of great importance because they affect processing flour quality and final quality of baked products [1, 16]. The Brabender Amylograph (BA) has been in extensive use in wheat and/or flour research to assess starch gelatinization and pasting behavior primarily due to easy performance, standardized procedure and good correlation with final product quality. Taking into account the nature of flour as a testing material, it must be noted that maximum viscosity attained during the Amylograph test indicates both—the flour gelatinization behavior and the α-amylase activity present in the flour [17]. However, the use of Brabender Amylograph is characterized by certain methodological, geometrical and technical shortcomings such as complex geometry, large sample size, inability to program the temperature profiles, poor definition of the measured parameters, non-uniform shear rate within the sample, the difference in heat transfer inside the measurement cup and time-consuming nature of testing. Therefore, the development and the use of more flexible devices and tests based on well-defined rheological parameters (stress, strain, viscosity and modulus) have been initiated [5, 18]. Among them, a Rapid Visco Analyzer (RVA) has been of a particular interest among cereal researchers, due to smaller sample sizes required and similar pasting patterns it provides when compared to other similar instruments [5]. However, the RVA results are expressed as viscosity in centipoise (cP) or Rapid Visco Units (RVU), being a disadvantage compared with the fundamental rheological methods. Rosell et al. [19] reported the use of a constant stress rheometer equipped with cone-and-plate measuring geometry for determination of pasting properties of starch–hydrocolloid slurries (8.0 %, w/w). Concerning starch gelatinization in the water-limited dough system, there have been a number of examples of using rheometer to perform temperature sweep test which is especially useful in breadmaking where changes that occur during the fermentation and baking processes could be explained [20, 21]. In attempt to simulate breadmaking process (mixing and baking), where water amount for starch gelatinization is limited, Chopin Technologies developed a device called Mixolab, which in spite of the difference in heating protocol, geometry and examined system has shown good correlation with Brabender Amylograph [22].

The objective of this study was to optimize the fundamental rheological method for determination of the gelatinization properties of wheat flour that correspondents to the standard, widely accepted, Brabender Amylograph test and to compare them in order to determine whether they can be interchangeable depending on different analytical needs. In order to avoid different constraints associated with concentric cylinder measuring geometry, such as starch granules sedimentation [23] and/or wall slippage effects [24], a two-bladed shaped rotor was used which represents a type of vane geometry.

Materials and methods

Samples

A total of 20 wheat samples were collected in 2008 from the wheat-growing localities in Serbia. Wheat samples were milled to straight-grade flour using Bühler MLU-202 laboratory mill (Bühler, Switzerland) according to AACC method 26–31 [25]. The samples for the study were chosen on the basis of peak viscosities obtained by Brabender Amylograph in order to cover as much variability as possible (Table 1).

Table 1 The characteristics of selected sample set in terms of peak viscosity as measured by Brabender Amylograp (Amy)

Sample	Amy (BU)	Sample	Amy (BU)
S1	90.0^{a}	S11	877.5^{k}
S2	182.5^{b}	S12	922.5^{l}
S3	247.5^{c}	S13	1,005.0^{m}
S4	320.0^{d}	S14	1,022.5^{mn}
S5	422.5^{e}	S15	1,042.5^{n}
S6	555.0^{f}	S16	1,280.0^{o}
S7	650.0^{g}	S17	1,325.0^{p}
S8	672.5^{h}	S18	1,590.0^{q}
S9	707.5^{i}	S19	1,640.0^{r}
S10	835.0^{j}	S20	1,725.0^{s}
Mean	855.6		
SD	485.2		
Min	90.0		
Max	1,725.0		
CV	0.6		

Figures followed by the different letters are significantly different ($p < 0.05$)

SD Standard deviation, *CV* coefficient of variation

Amylograph tests

The Amylograph tests were performed according to ICC standard No. 126/1 [17], which involved heating the samples from 30 to 95 °C at a constant rate of 1.5 °C min^{-1}. The peak viscosity and the peak temperature were determined from the Amylogram. Three parameters were recorded in duplicate: pasting temperature, peak viscosity and peak temperature.

Fundamental rheometric tests

The flour suspension for fundamental rheological tests was prepared with 12.4 g (calculated on 14 % moisture basis) of flour and 70 ml of distilled water in order to obtain the same flour–water ratio as in the standard Amylograph method. Fundamental rheometric measurements were taken using the HAAKE MARS—Modular Advanced Rheometer Systems (Thermo Scientific, Germany). The measuring geometry consisted of measuring cup Z40 (43.4 mm diameter, 8 mm gap) and FL2B paddle-shaped rotor with 2 blades. In order to prevent moisture evaporation from the sample, the solvent trap Z40 DIN was used. Two methods for determination of pasting properties of wheat flour were developed: so-called *slow* and *rapid* procedure. *Slow* procedure comprised the complete simulation of Amylograph test with a heating rate of 1.5 °C min^{-1} from 30 to 95 °C and maintaining the temperature of 95 °C for a period of 600 s. A shear rate of 10 s^{-1} was applied, which corresponded to 95 rpm. The *rapid* procedure comprised the tempering of flour suspension at 50 °C for 180 s, heating from 50 to 95 °C at a heating rate of 3 °C min^{-1} and subsequent maintenance of temperature of 95 °C for 300 s to ensure starch gelatinization. Three parameters were recorded in duplicate: pasting temperature, peak viscosity and peak temperature.

Data analysis

All samples were analyzed in duplicate except for method precision determination, where eight replicates were performed. The obtained results were analyzed by descriptive statistics (mean, standard deviation, minimum and maximum, coefficient of variation), Pearson's correlation coefficients, one-way analysis of variance (ANOVA) and Duncan's new multiple range tests to determine the statistical significance of differences, using software Statistica 10. The principal component analysis (PCA) was carried out using the Software XLSTAT, version (2012.2.02). Determination of the method repeatability comprised determination of repeatability standard deviation (SDr), relative standard deviation (*RSD*) and repeatability limit ($r = 2.8$*SDr) [26].

Results and discussion

Determination of pasting and peak temperature

Pasting temperature indicates temperature at which the viscosity begins to increase during the heating process [27]. Pasting temperatures of selected wheat flours differed with flour sample, instrument type and heating rate as previously indicated by Suh and Jane [14]. The pasting temperature variation between the samples and observed method is shown in Fig. 1a. The pasting temperatures as determined

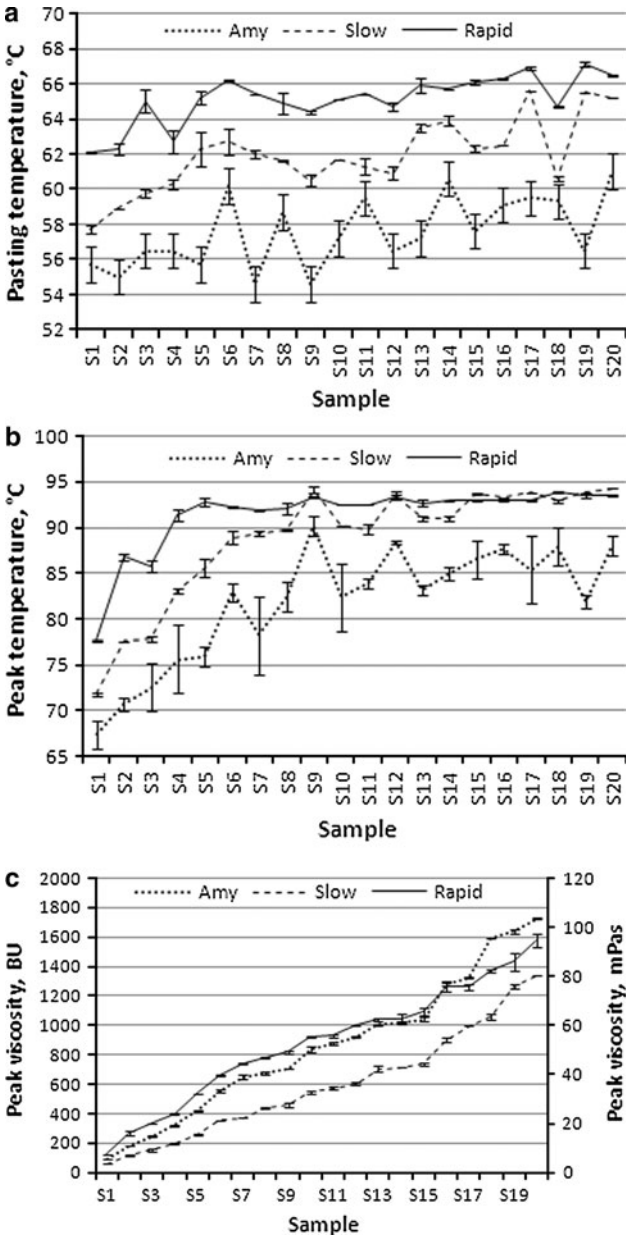

Fig. 1 Pasting temperature (**a**), peak temperature (**b**) and peak viscosity (**c**) as measured by Brabender Amylograph, *slow* and *rapid* rheometric procedure

by Amylograph varied between 54.6 and 61.0 °C, with the highest value being observed for sample S20, while the pasting temperature of selected wheat flour samples as determined by slow rheological method varied between 57.7 and 65.6 °C with the highest value being observed for sample S17. The pasting temperatures as determined by Amylograph were significantly lower than those obtained within the *slow* rheometric method ($p < 0.05$). Although these two methods were characterized by the same heating rate, it must be noted that other test conditions such as measuring geometry were different [9].

The pasting temperature of selected wheat flour samples as determined by *rapid* rheometric method varied between 62.1 and 67.1 °C with the highest value being observed for sample S19, being significantly higher than those obtained within the *slow* procedure ($p < 0.05$). The differences in the pasting temperatures obtained within the *slow* and *rapid* procedure could be attributed to the differences in starch granules swelling rates affected by variable heating rates. At slower heating rate, starch granules were allowed to longer swelling which resulted in lower pasting temperature [14, 28]. There is usually a difference in the gelatinization temperature range between the starch and flour, in that is shifted toward higher temperatures in the wheat flour suspension compared with the starch–water suspension [29].

The peak temperature is the temperature at which swollen starch granules in water dispersion under shear force tend to disintegrate from the crystalline state to a gel [5]. The peak temperatures differed with flour sample, instrument type and heating rate as indicated by Suh and Jane [14]. The peak temperature variation between the samples and observed method is shown in Fig. 1b. The peak temperature as determined by Amylograph varied between 67.4 and 90.2 °C, with the highest value being observed for sample S9. The peak temperature of selected wheat flour samples as determined by *slow* rheometric method varied between 71.8 and 94.3 °C with the highest value being observed for sample S20. The same heating rate applied within those two methods, resulted in significantly lower peak temperatures as determined by Amylograph ($p < 0.05$). By increasing the heating rate within the *rapid* procedure, the peak temperature increased and varied between 77.6 and 93.9 °C with the highest value being observed for sample S18.

The observed pasting and peak temperatures variation between standard Brabender Amylograph and *slow* rheometric method might be attributed to the differences in the temperature measuring system between these two devices. The temperature sensor with Brabender Amylograph is located in one point inside the measuring bowl, thus allowing measuring actual sample temperature. Hazelton and Walker [30] previously proved that measured temperature values were not affected by the position of temperature sensor within the measuring cup. Conversely, the MARS's temperature measuring system is based on the feedback from the block temperature, but not from the temperature of the sample. The registered higher pasting temperatures within the *slow* procedure could be attributed to the existing lag behind that of the heating block [30]. Also, the temperature throughout the sample of the larger size in the Brabender Amylograph (cca. 530 g) probably changed at a slower rate in comparison with the 82.4-g sample within the *slow* and *rapid* rheometric procedure.

Determination of peak viscosity

The peak viscosity is the highest viscosity reached during starch gelatinization when the balance between granules swelling and granules breakage supported by stirring and heating is reached shortly before their physical breakdown and leaching of amylose [3]. The selected sample set covered wide range (from 90 to 1,725 BU) and uniform distribution of samples with significantly different peak viscosities ($p < 0.05$) (Table 1). The peak viscosity measured by the *slow* and *rapid* fundamental rheometric procedures ranged from 3.64 to 80.35 Pa s and from 7.54 to 94.52 Pa s, respectively (Fig. 1c). The principal component analysis was used to visualize the variability of selected flour samples with regard to their gelatinization properties, where each sample is presented as a point in a two-dimensional plane—the PCA score plot (Fig. 2a). Samples with distinctive features appeared completely separated from each other, while samples with similar gelatinization properties appeared close to each other. Moreover, samples that appeared close to the origin were characterized by such gelatinization properties that were similar to those of their mean values [31]. In order to visualize the correlation between the selected gelatinization properties of wheat flour as determined by Brabender Amylograph, *slow* and *rapid* rheometric procedure, as well as the underlying structure in experimental data and relationships between data and samples, the PCA loading plot is presented (Fig. 2b). The first principal component (PC1), accounted for 76.12 % of the total variability in the data set, correlated highly with the peak viscosity as determined by *rapid*, Brabender Amylograph and *slow* rheometric method, and peak temperature as determined by *slow* rheometric method (PC1 > 0.90). The second and third PCs (PC2 and PC3) accounted for 9.09 and 6.29 % of the total variance in the data, respectively. PC2 correlated highly with pasting temperature as determined by Brabender Amylograph method.

Direct comparison of the peak viscosities obtained by the Amylograph and fundamental rheometric procedures was difficult due to the difference in the measurement units

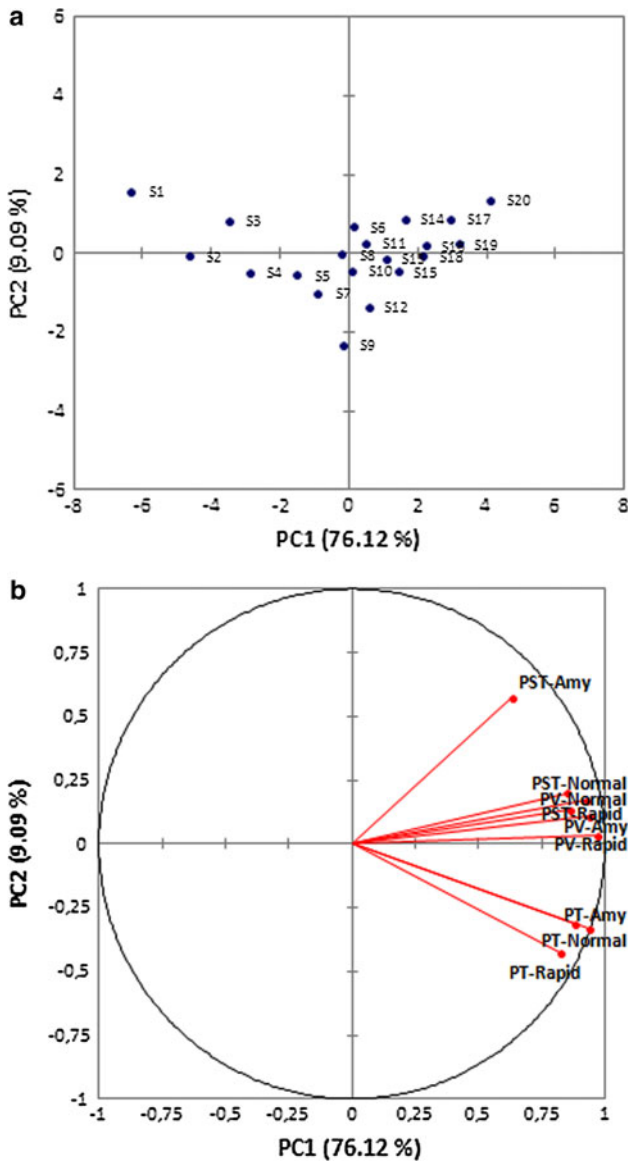

Fig. 2 PCA score plot of PC1 versus PC2 of the sample set selected for the study (**a**) and PCA loading plot of PC1 versus PC2 of analyzed gelatinization parameters (*PST* pasting temperature, *PT* peak temperature and *PV* peak viscosity) as determined by Brabender Amylograph (Amy), slow (Slow) and rapid rheometric procedure (Rapid)

used to express viscosity [14]. Although based on the same measurement principle (recording the change in viscosity of flour–water suspension during a uniform temperature increase under constant stirring), the peak viscosity obtained by Amylograph is expressed as torque in the arbitrary Brabender Unit (BU), whereas the viscosity obtained by fundamental rheometric methods is expressed in the SI units (Pa for elastic modulus, Pa s for viscosity) [32, 33]. When comparing the *slow* and *rapid* procedure, the increase in the heating rate influenced the increase in the peak viscosity, due to enhancement of starch granule swelling and amylose solubility which resulted in increased peak viscosity [14, 28, 34, 35].

Apart from the difference in measurement units and homogeneity of temperature, there are other distinctions between those two methods, with regard to measurement geometry, shear rate, definition of measured parameters, the time required for testing and sample size. Brabender Amylograph is a rotational viscometer that consists of rotational bowl rotating at 75 rpm with built-in pins and a stationary upper spindle with sensing pins [1, 17]. Although the role of the pins is to prevent the sedimentation of particles present in the dispersion, they make Brabender Amylograph measurement geometry very complex. Hence, the shear modulus is too complicated to calculate, and the flow field within the Amylograph measuring bowl is too difficult to describe [1]. Concerning the fundamental rheological devices applied to characterize the pasting properties of starch and starch-containing products, and the rotational rheometer equipped with different geometries (concentric cylinder, cone-and-plate, parallel plate) has been the most common choice [1, 23]. Despite the numerous advantages their applications offer, it must be noted that a relatively narrow gap with cone-and-plate geometry could contribute to capillary suction of the paraffin oil from the edge of the geometry into the tested dispersion, applied to minimize moisture loss. On the other hand, with the application of a concentric cylinder, there is a possibility of starch granules sedimentation in the early stages of heating [23]. Applicability of the various forms of vane geometry for measuring rheological properties of food and non-food systems, especially for coarse and dispersed systems, has been reported [23, 36]. However, the vane-in-cup geometry has been mostly used to obtain yield stress of food systems and to measure flow properties as it acts as an effective cylinder. By given review of Barnes and Nguyen [36] regarding the reported uses of vane geometry for food and non-food systems, there is no reported application of vane geometry for flour systems. One example of the application of vane geometry to the starch system was reported by Tucker et al. [37] who used it for the measurement of viscoelasticity and apparent viscosity of modified starch systems. Genovese and Rao [38] reported the use of vane geometry for the measurement of yield stress of cross-linked starch dispersions of different botanical origin at different rotational speeds. The chosen vane geometry consisted of paddle-shaped rotor with two blades with slits (Fig. 3).

Suh and Jane [14] reported the differences in the peak viscosity obtained by RVA and MVA that could had been attributed to the different spindle geometry between those two devices. Paddle-shaped spindle, due to the larger surface area, affected increased breakdown and consequently

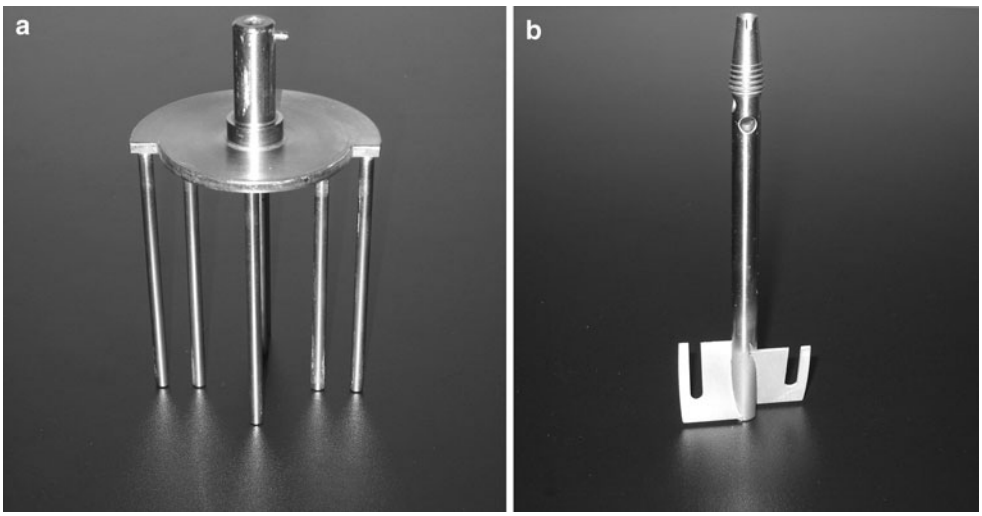

Fig. 3 Brabender Amylograph geometry (**a**) and two-bladed shaped rotor (**b**)

the increased shear force with regard to hollow-structured spindle [14, 39].

Another important distinction between the Amylograph and *slow* and *rapid* rheometric method is a sample size. The sample size which is used with Brabender Amylograph has been a limiting factor for certain applications, such as breeding programs, where small amounts of samples are normally available [28, 32]. To overcome this drawback, the attempts to record the viscosity profile using a reduced amount of sample have been reported and, new instruments based on similar operating principles, such as Rapid Visco Analyzer (RVA), introduced [14, 28, 35]. It was shown that obtained viscograms were comparable with those obtained by Brabender Amylograph. Moreover, the reduction in amount of sample had not disturbed the possibility to deduce the α-amylase activity, being in agreement with that of Brabender Amylograph [28].

The correlation coefficients between the gelatinization properties of wheat flour as measured by Brabender Amylograph, *slow* and *rapid* rheometric procedure is presented in Table 2. The low and not significant values of correlation coefficient (r) were observed between Amylograph pasting temperature and pasting temperature as determined by *slow* and *rapid* rheometric procedure ($r = 0.53$ and $r = 0.54$, respectively). However, pasting temperature as determined by *slow* and *rapid* rheometric procedure exhibited strong mutual relationship ($r = 0.89$). Moreover, the significant coefficient of correlation (r) was observed between peak temperature as determined by empirical and fundamental rheometric procedures, being the highest between Amylograph and *slow* procedure ($r = 0.95$).

Thiewes and Steeneken [10] previously reported the poor correlation coefficients between peak viscosity and pasting temperature for modified starches, while Limpisut and Jindal [13] observed the same phenomenon for rice flour. Despite the listed differences between Brabender Amylograph and *slow* and *rapid* rheometric procedure regarding the sample size, applied heating rates and measurement geometry, peak viscosity as measured by Brabender Amylograph showed significant positive correlations with the peak viscosity measured by *slow* and *rapid* rheometric method ($r = 0.99$, $p < 0.01$). The relationship between the peak viscosities obtained by *slow* and *rapid* rheometric method is described by significant positive coefficient of correlation ($r = 0.98$, $p < 0.01$) (Table 2).

These observations confirmed the universal nature of rheological measurements which should be independent of size, shape and how they are measured. Moreover, the obtained results confirmed the objective of rheology to determine properties reproducibly in a manner that allows

Table 2 Correlation coefficients (r) between the peak viscosity (PV), peak (PT) and pasting (PST) temperature of wheat flour as determined by Brabender Amylograph (Amy), *slow* and *rapid* rheometric procedure

Variable	PST-Amy	PST-Slow	PST-Rapid
PST-Amy	**1**		
PST-Slow	0.53	**1**	
PST-Rapid	0.54	**0.89**	**1**
	PT-Amy	PT-Slow	PT-Rapid
PT-Amy	**1**		
PT-Slow	**0.95**	**1**	
PT-Rapid	**0.82**	**0.91**	**1**
	PV-Amy	PV-Slow	PV-Rapid
PV-Amy	**1**		
PV-Slow	**0.99**	**1**	
PV-Rapid	**0.99**	**0.98**	**1**

Values in bold are significant at $p < 0.01$

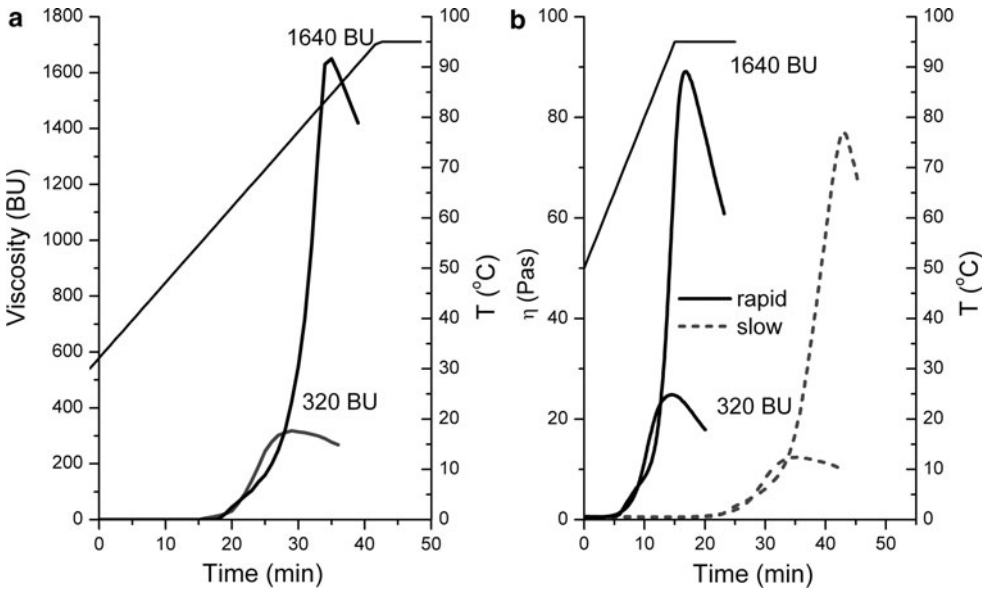

Fig. 4 Variability in viscosity curves obtained by the Brabender Amylograph (**a**) and *slow* and *rapid* fundamental rheological procedure (**b**) of selected samples

comparison between different samples, test sizes and shapes and test methods [18].

Figure 4 shows the variability in viscosity profiles of the selected samples (S4 and S19) obtained by Brabender Amylograph and *slow* and *rapid* rheometric procedure using vane geometry. Their shape was similar to that obtained by the Brabender Amylograph. During the first stage of heating of flour water suspensions and during the increase in viscosity associated with starch gelatinization and α-amylase liquefaction, a negligible difference between the *slow* and *rapid* fundamental rheometric procedure was observed. The more apparent differences in viscosity profiles obtained by the *slow* and *rapid* fundamental rheometric procedure were observed in the peak viscosity being higher for *rapid* procedure. Weipert [40] indicated the significance of test duration when describing the pasting behavior of wheat starch. The starch granule requires certain time to absorb and bind the water, to swell and finally to gelatinize, so the rapid tests could result in the higher viscosity. Moreover, the differences in viscosity profiles of selected samples between slow and rapid procedure are more apparent for sample S4, which was characterized with lower peak viscosity (and higher α-amylase activity). This system was subjected to the more efficient breakdown under the longer joint influence of shearing and heating. The enzyme acts on (1,4)-bonds of starch fractions, resulting in a peak viscosity decrease. The lower the alpha amylase activity, the lower the difference in the appearance of curves was, regardless of the duration of the test due to the fact that the lower α-amylase activity generally contributes to the stability and rigidity to the swollen granule structure and less breakdown with shear [41].

Precision of methods used for determination of gelatinization properties of wheat flour

The final step of the evaluation of the method characteristics comprised the determination of method repeatability. Repeatability checks were made for each observed pasting parameter in eight replicates. As indicated in Table 3, the fundamental rheometric method (*slow* and *rapid*) was characterized by better repeatability in terms of pasting and peak temperature and peak viscosity in comparison with Brabender Amylograph method. The rapid procedure had better repeatability of pasting and peak temperature

Table 3 Repeatability of different methods for determination of gelatinization properties of wheat flour

	SDr	*r*	RSDr
Amylograph			
Pasting temperature (°C)	1.19	3.33	1.82
Peak temperature (°C)	2.25	6.30	2.64
Peak viscosity (BU)	9.04	25.31	2.10
Slow procedure			
Pasting temperature (°C)	0.29	0.81	0.48
Peak temperature (°C)	0.58	1.62	0.68
Peak viscosity (mPa s)	0.17	0.48	1.23
Rapid procedure			
Pasting temperature (°C)	0.23	0.64	0.35
Peak temperature (°C)	0.22	0.62	0.24
Peak viscosity (mPa s)	0.60	1.68	0.93

SDr Repeatability standard deviation (expressed in corresponding units), *r* repeatability limit (expressed in corresponding units), *RSDr* repeatability relative standard deviation (%)

determination in comparison with the *slow* procedure. Moreover, determination of peak viscosity by *slow* rheometric procedure appeared to be better repeatable in comparison with that of *rapid* procedure.

Conclusion

In general, the fundamental rheometric procedure corresponds to the standard Brabender Amylograph test and hence can be used to assess the gelatinization properties of wheat flour. It has been shown that the application of fundamental rheometric procedure for determination of gelatinization properties of wheat flour provides several substantial advantages over the Brabender Amylograph method including smaller sample size, ability to set the desirable heating and shear rate, shorter test duration and better precision. In particular, the gelatinization parameters of wheat flour determined by Brabender Amylograph such as peak temperature and peak viscosity were in high correlation with those determined by fundamental rheometric procedure (*slow* and *rapid*) regardless of the differences in sample size, heating and shear rate and measurement geometry. However, the pasting temperature determined by Brabender Amylograph was in poor correlation with the pasting temperature determined by *slow* and *rapid* rheometric procedure. By modifying the operational conditions of *slow* fundamental rheometric procedure to accelerate the determination of gelatinization properties of wheat, the high correlation coefficients between all gelatinization parameters were demonstrated.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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