Int. Agrophysics, 2007, 21, 377-383

INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Regression equation for describing gluten thermal expansion

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Received July 23, 2007; accepted October 24, 2007

A b s t r a c t. Testing of gluten ability of thermal expansion consisted in heating a small sample of freshly washed out gluten at temperatures causing the boiling of water within it. Gluten membranes extend around forming bubbles of water vapour under increase of pressure and are simultaneously being modified thermally. At certain volume of the bubbles, the membranes achieve maximum extensibility and further increasing of water vapour pressure causes their perforation. The dynamics of the expansion process was recorded by use of a digital camera.

The proposed regression equation describes the dependence of the volume increment of gluten on heating time and allows to split the thermal expansion process into hyperbolic and linear components. The hyperbolic one is determined by a three-parameter function of hyperbolic tangent. The parameters *a*, *c* and *b* characterize the half of extent and duration of the hyperbolic expansion and its rate, respectively. The linear component can be meant as viscous flow of gluten, therefore it is determined by a one-parameter linear function. The parameter *d* means the rate of linear expansion.

The volume increase of strong gluten at lowest temperature (110°C) was very slow and its character was only linear (*d*). Higher heating temperatures (above 140°C) caused considerably larger and faster hyperbolic expansion (*a* and *b*) of strong gluten than of the weak one. However, the weak gluten in whole range of used temperatures was distinguished by almost twice faster linear expansion (*d*). It may suggest the existence of significant differences between wheat cultivars in terms of extensibility of thermally modified gluten membranes.

K e y w o r d s: wheat, wet gluten, heating temperature, gluten membranes, thermal expansion

INTRODUCTION

Measurements of mechanical properties of wet gluten, freshly washed out from flour or whole meal, are a basic source of data on the baking quality of wheat. Mostly they are conducted on raw gluten, without inspection of its behaviour under heating treatment as it takes place in the baking process. High temperature treatment increases the reactivity of bread dough components, such as proteins, lipids, starch and other, and causes the occurrence of new interactions between them (Autran *et al*.,1989; Chung, 1989; Hayta and Schofield, 2004). In effect, thermally modified bread dough or gluten may radically differ – in their mechanical properties – from not modified (raw ones). This is indicated *eg* in studies of Abdelrahman and Spies (1986) who found lower values of modulus of dough elasticity (G') for flour of higher baking quality. Similar discrepancies were reported by Miś and Rusinek (2004b) who compared the results of examination of wet gluten according to the standard ICC method (gluten index) with characteristics of gluten behaviour under heating at temperature of 150°C. They observed that gluten with the lowest value of gluten index among all the examined samples underwent transformation during heating, attaining the ability to form the strongest membranes.

Therefore, the study on the influence of heat on wet gluten may provide useful knowledge in explaining the role of gluten membranes in the formation of bread quality as well as the phenomena and transformations that take place during the baking process and are still not fully understood (Dobraszczyk, 2003; Fan *et al.,* 1999; Stathopoulos *et al.,* 2006; Zhang and Datta, 2006).

Research in this field may be considerably facilitated through the application of a method for testing gluten thermal expansion, proposed by Miś and Rusinek (2004a). The method consists in heating of wet gluten in a horizontal gap between heating panels. Due to boiling of water contained in gluten, water vapour bubbles are surrounded by gluten membranes which extend as pressure inside the bubbles increases. Under constant test conditions, such as heating temperature and wet gluten moisture content, the expansion behaviour of gluten is a derivative of its primary

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mechanical properties *ie* those that the gluten had in raw state (before heating), and of the secondary mechanical properties, generated during the heating. In the course of the heating test, continuous transformation takes place, of gluten with the primary properties into gluten with the secondary, thermally modified properties. The method allows to study the rate of those transformations (thermal modifications) through continuous registration of the gluten behaviour, from the beginning of heating of the sample, through the stage of expansion, until the end of thermal setting of its structure. This kind of study, apart from the cognitive aspect, may initiate practical application of the acquired knowledge *eg* for enhancing bakery technologies and others in which the phenomenon of thermal expansion in question assumes significant importance.

The authors of the present paper applied the mentioned method to describe the thermal expansion process by means of a proposed regression equation. The experimental verification of its usefulness for study of the influence of heating temperature and gluten quality on the characteristics of expansion is the subject of the paper.

MATERIALS AND METHODS

Gluten isolated from grain of two Polish cultivars of spring wheat (Broma and Torka), harvested in 2004 from field experiments of the Lublin Agriculture University, was used in this study. Milling of the grain was performed using a hammer mill – Lab Mill 3100 (Falling Number AB, Sweden). Gluten was washed out from whole meal according to a standard method (ICC, 1994), by means of Glutomatic 2200 (Perten Instruments AB, Sweden). Water not absorbed by gluten was removed using Centrifuge 2015 (Perten Instruments AB, Sweden) and determined its content in freshly washed gluten (Miś, 2003). As it was stated by Miś (2005), non-absorbed water content can be a useful indicator of gluten network structure loosening. High non-absorbed water contents correspond to weak glutens whereas low contents are representative of strong (stiff) glutens. Directly after centrifugation, gluten balls of 2±0.01 g were formed by hand and dipped in a water solution of NaCl (2%) for sample temperature stabilisation (22.5+0.5°C) and to prevent loss of moisture.

Heating of the gluten sample, with registration of its expansion, was carried out by use of a measuring set presented in Fig. 1. It was made up of a source of heat (two parallel heating panels), heating temperature control system, and an image recording system. The heating panels of Glutork 2020 (Perten Instruments AB, Sweden), normally used for drying wet gluten, were set at a spacing of 5 mm to create a horizontal operating gap for heating gluten samples. The heating surfaces of the panels were coated with teflon to prevent the gluten samples from adhering to them. The bottom panel, mounted on the steel base of the apparatus, was equipped with a temperature sensor T-103 (Thermoplus, Poland). The top panel was removable and lifted up for placing gluten balls in the operating gap. After that, the top panel was secured in position to ensure constant width of the gap when heating the gluten samples. Once the heating was done, the top panel was lifted again to remove the sample. The heating elements of the panels were supplied from a single electric circuit (synchronous operation) and controlled by a temperature controller ESM3710 (Emko Elektronik A.S., Turkey). Maximum fluctuation of heating panels temperature did not exceed ±1°C of the selected value. The operating gap was uniformly illuminated to obtain brightness contrast between sample surface and the background. Acquisition of sample images was conducted at the rate of 10 fps and with spatial resolution of 2.5 pixel per mm, using a Trust USB2 HiRes Webcam WB-3500T camera.

Thermal expansion measurement procedure was as follows: after stabilization of heating panels temperature at the intended level the gluten sample was placed in the gap and image acquisition during heating was started. When the volume of gluten sample ceased to increase, the camera was stopped and the sample was removed from the gap. Gluten samples had the shape of a barrel between the heating panels (Fig. 2), its height equal to the distance between the heating panels (H). Uniform distribution of pressure inside the sample caused that side walls of the barrel became a semicircle of diameter H (Fig. 3). It was assumed that increase in the sample volume during heating caused increase in the maximum (D) and minimum diameter (D-H) of the barrel-shaped sample. Processing of the saved images consisted in the

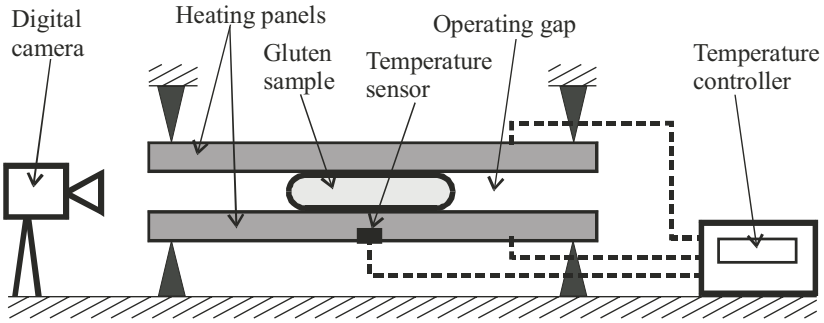

Fig. 1. Schematic diagram of the measuring set used in the study.

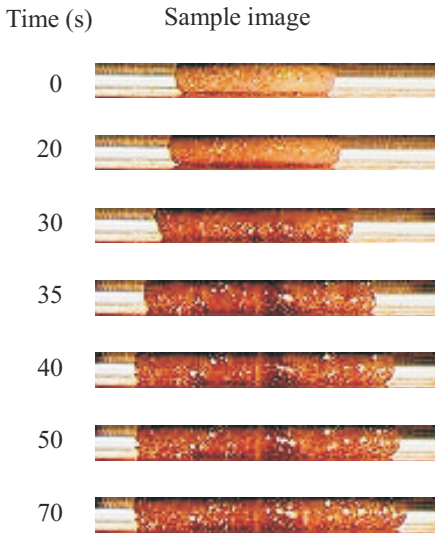

Fig. 2. Characteristic behaviour of wet gluten during heating (cv. Broma, heating temperature 160°C).

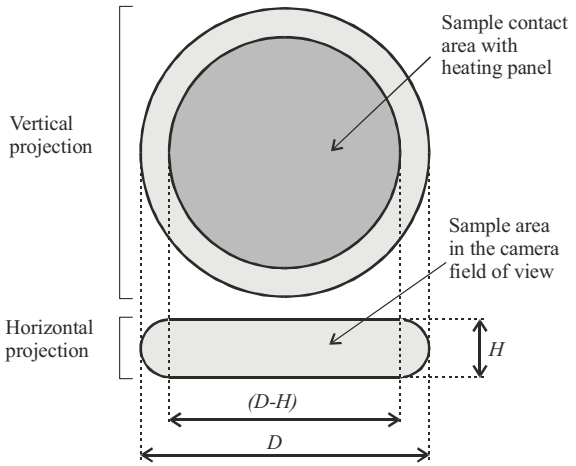

Fig. 3. Geometry of gluten sample during heating (*H* - width of operating gap, *D* -maximum diameter, *D-H* - minimum diameter).

estimation of changes in the maximum diameter (*D*). To realize this task, the middle part of the image (horizontal line of 3 pixels width) was cropped in ImageJ software (Abramoff *et al*., 2004). After binarization, the *D* value was measured. Volume of gluten sample (*V*) was calculated according to following Eq.:

$$V = 1/12\ \pi\ H\ (2D^2 + (D\text{-}H)^2)\ . \qquad (1)$$

Expansibility of gluten, defined as the ability of the wet gluten sample to increase its volume (volume expansion), was determined as the relative increment of its volume ($\Delta V/V_0$), calculated according to Eq.:

$$\Delta V/V_0 = (V - V_0)\ /\ V_0\ , \qquad (2)$$

where: V – volume of gluten sample after heating, V_0 - volume of gluten sample before heating.

Using the procedure described above, the influence of temperature of heating panels on the characteristics of volume expansion of strong (Torka) and weak (Broma) gluten was tested. The heating temperature levels applied were as follows: 110, 120, 130 and up to 190°C. The choice of the minimal temperature (110°C) resulted from procedural requirements of the method used (Miś and Rusinek 2004b), according to which the applied temperature should cause boiling of water in tested sample with an intensity necessary to produce measurable expansion, even in the most resistant gluten. Temperatures below 110°C, because of too low intensity of water boiling, proved to be inadequate for the examination of wet gluten thermal expansion. The maximum temperature (190°C) was set taking into consideration the operational limitations of the heating plates used. The presented research had a model character and its aim was the mathematical description of the phenomenon of thermal expansion of gluten. Therefore, the use of a possibly wide range of temperatures permitted a more comprehensive analysis of the phenomenon and detection of potential quality differences between tested types of gluten.

The measurements were conducted in 4 replications. Statistica v.6.0 (Statsoft) was used for statistical analysis of the results. Measurement errors were estimated using 95% confidence intervals for mean values. The method of non-linear regression analysis was used to construct a mathematical model for gluten thermal expansion in function of heating time, and to describe changes of the model parameters in relation to heating temperature.

RESULTS

Properties of wet gluten (unheated)

A summary of the physical properties of wet gluten 'in raw state' is given in Table 1. As can be seen, cv. Broma, as compared to Torka, had gluten of more loose structure, which is expressed by 11-fold higher value of non-absorbed water content. Gluten of cv. Broma had lower mechanical strength than gluten of cv. Torka, which was expressed by its value of the gluten index, twice lower than that of Torka. This may serve as a basis for the classification of the two studied cultivars in two different classes: of low (Broma) and high baking quality (Torka). No significant differences were noted in the density and moisture of gluten of the two cultivars. This means that the heating treatment was applied to samples of gluten of the same volume and water content, which gave the assurance that the tested samples had identical area of contact with the source of heat and – potentially - were able to produce equal amounts of water

T a b l e 1. Gluten characteristics of studied wheat cultivars

Wheat cultivars	Freshly washed out gluten		Centrifuged gluten	
	Non-absorbed water content[1]	Gluten index[2]	Moisture content[3]	Gluten density[4]
	(%)	(%)	(%)	(g cm^{-3})
Broma	17.0	37.4	65.8	1.08
Torka	1.5	96.8	65.7	1.08
LSD[5]	0.7	4.5	0.8	0.02

1 – water removable by centrifugation (Miś, 2003) 2 – determined according to standard (ICC, 1994), 3 – determined using Glutork 2020 (150°C for 4 min), 4 – determined by means of a syringe, 5 – least signifficant differences.

vapour under heating. This is of particular importance as the amount of water vapour is the driving force that causes the expansion of gluten membranes, which provides the basis for the estimation of their mechanical properties.

Proposed regression equation of gluten thermal expansion

An overview of wet gluten volume changes during heating at temperatures causing the boiling of water contained in it is shown in Fig. 2, and Fig. 4 presents a graph of the relation of gluten volume increase ($\Delta V(t)/V_0$) to the heating time (t). Measured values of $\Delta V(t)/V_0$ were described by the regression Eq:

$$\Delta V(t)/V_0 = a\,(1+tanh\,(b\,(t\text{-}c))) + d\,t\,, \qquad (3)$$

where: a, b, c, d – equation parameters, expressed in [-], [s-1], [s], and [s-1], respectively, tanh(b (t-c)) – hyperbolic tangent function, a – a half of sample volume increment described by hyperbolic tangent, also called the half of extend of hyperbolic expansion; b – volume expansion rate, also called the hyperbolic expansion rate; c – moment in the time of heating, when the maximum volume expansion rate of the sample occurs (point of inflection of curve on graph), also called the half of duration of hyperbolic expansion; d – volume expansion rate of the sample described by linear function, also called the linear expansion rate.

The components of this regression model were well-chosen because the degree of fitting of predicted to experimental data was very high. Determination coefficients (R^2) exceeded the value of 0.99, while estimated equation parameters (a, b, c and d) had low errors. Facility of interpretation of the parameters and their physical meaning are the main practical advantages of the expansion model.

The characteristic shape of expansion curve, shown in Fig. 4, makes possible to distinguish, during heating, three processes: (I) heat absorption, (II) expansion, and (III) thermal setting of gluten. The process of heat absorption begins after a sample of gluten is placed in the operating gap and rapid heat transfer form the heating panels to the sample takes place. In this time volume of the sample increases slowly in only linear way, and extent of this kind of gluten expansion is characterized by parameter d. When the temperature of the sample achieves the boiling point of water (its content in wet gluten is very high, about 65% w.b.), the expansion process intensifies. The boiling water causes the formation of bubbles of water vapour surrounded with impermeable and extensible gluten membranes. Evaporating water absorbs excess heat, preventing the overheating of the sample, as a result of which the maximum temperature inside the sample does not exceed the water boiling temperature. Gluten membranes enforce heat transfer in the form of water vapour flux within one bubble first, and then, after the first one breaks, in the surrounding bubbles. As a result of heat absorption, water vapour pressure inside the bubbles increases, causing rapid enlargement of their diameter, which leads to multiplication of the total volume of tested sample. This kind of expansion is characterized by parameters of

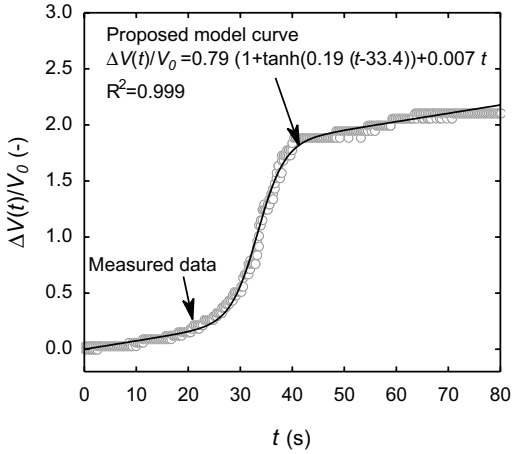

Fig. 4. Graph of gluten volume increase ($\Delta V(t)/V_0$) in relation to heating time (t) (cv. Broma, heating temperature 160°C).

hiperbolic function (*a*, *b* and *c*) in the proposed Eq. (3). When the pressure in the particular bubbles exceeds the mechanical strength of the encasing membranes, the membranes break and at this point the expansion rate drops down, and the expansion ceases altogether when the membranes lose their viscoelastic properties due to thermal denaturation. Further heating causes the setting of shape and hardening of structure of the membranes. Characteristic spongy structure of thermally set gluten is presented in Fig. 5.

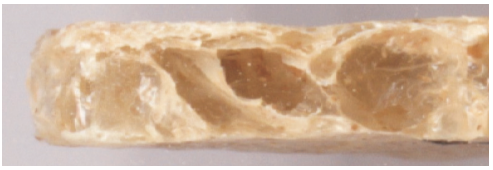

Fig. 5. Structure of gluten membranes thermally set – cross section of gluten sample.

Influence of heating temperature on equation parameters

Differentiation of parameters *a*, *b*, *c* and *d* of the proposed regression Eq. (3) of gluten thermal expansion is shown in Fig. 6. To facilitate interpretation of the results, some general information about differences in the behaviour of wet gluten of two examined cultivars during heating at 110°C should be taken into consideration. At this temperature, the volume increase of strong gluten (cv. Torka) was very slow and was of linear character. That was the reason why it was impossible to calculate the values of parameters *a*, *b* and *c* (characteristic for hyperbolic expansion) for the lowest temperature. The regression equation in this case contained only one parameter, *d*, which indicated linearity of gluten expansion. For all other experimental combinations, the course of gluten expansion was compliant with the proposed model.

The above facts suggest that the phenomenon of expansion of the hyperbolic type occurs when heating temperature is considerably higher than the boiling point of water

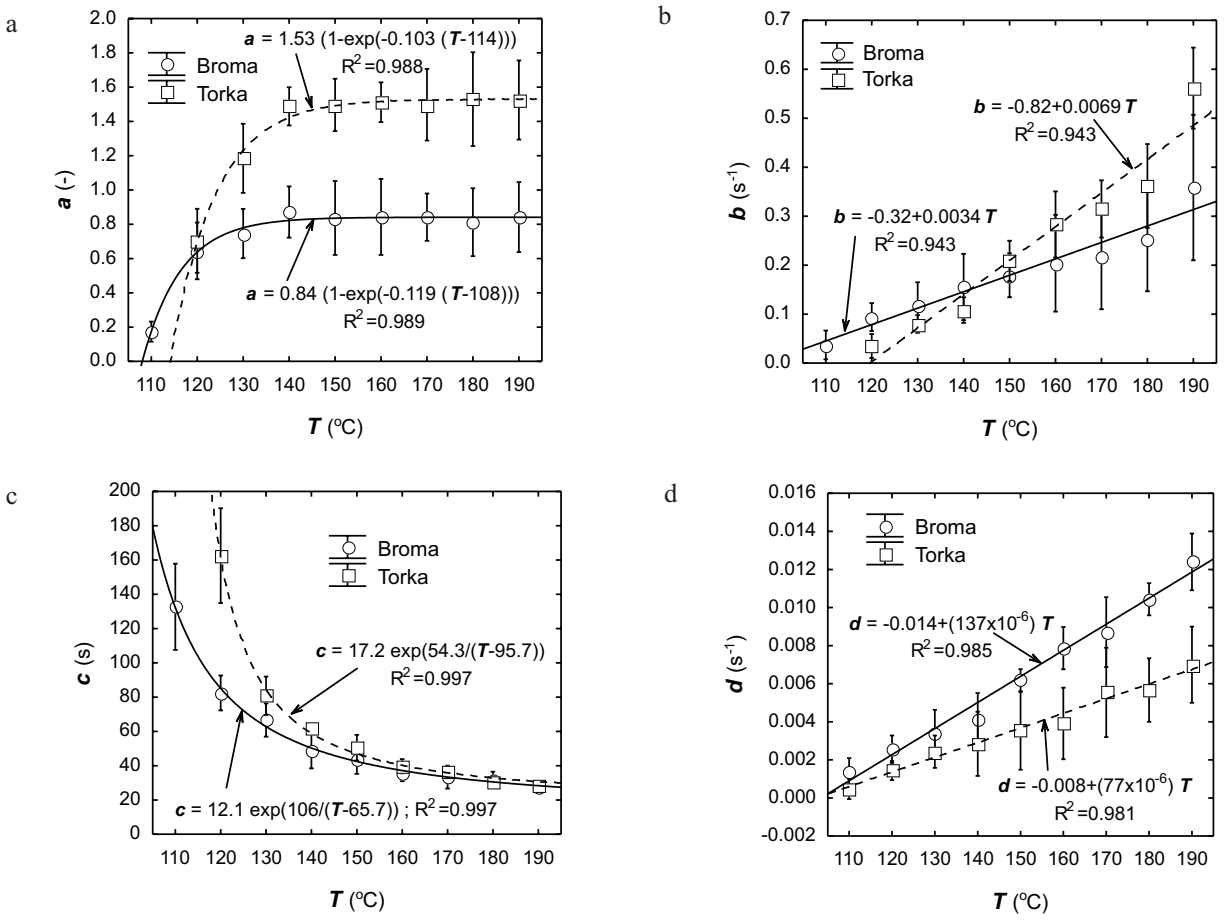

Fig. 6. Influence of heating temperature - *T* on Eq. (3) parameters: *a* (a), *b* (b), *c* (c), *d* (d), (mean values with 95% confidence intervals are marked).

within a gluten sample. This can be observed on the graph illustrating changes of parameter *a* in the function of heating temperature (Fig. 6a). The regression equations in this figu- re contain values of temperature which causes the zero hyperbolic expansion. The temperature was 114°C for the strong gluten (cv. Torka) and significantly lower for the weak gluten of cv. Broma (108°C). Therefore, the temperature of initiation of hyperbolic expansion can be a valuable indicator of quality differences between strong and weak gluten.

With temperature rise, values of the half of extent of hyperbolic expansion (parameter *a*) significantly increased from 0.17 to 0.87 (-) and from 0.70 to 1.53 (-) for the weak (cv. Broma) and strong (cv. Torka) gluten, respectively. It shows that the strong gluten was nearly twice as expansible as the weak one. The heating temperatures above 140°C did not cause further increase in gluten expansion.

Values of the second analysed parameter, *b*, ranged from 0.037 to 0.359 (s-1) and from 0.035 to 0.562 (s-1) for the weak and strong gluten, respectively. Changes in the hyperbolic expansion rate in relation to the heating temperature were described by linear functions (Fig. 6b). Slopes of the lines showed that increase of the temperature caused a two-fold higher expansion rate of the strong gluten in comparison to the weak one. However, the mentioned lines crossed at the point of 140°C. That indicates that the weak gluten (cv. Broma) at lower temperatures (110-140°C) expanded at a higher rate than that strong one (cv. Torka). Furthermore, at this temperature range the weak gluten needed a significantly shorter time of heating (parameter *c*) to reach the maximum expansion rate (Fig. 6c). Halves of duration of hyperbolic expansion for Broma and Torka cultivars were 82 and 163 s, respectively, at 120°C. These differences tended to disappear with increasing temperature.

The values of parameter *d* were comprised within the range from 14 to $124x10^{-4}$ $s^{-1}$and from 5 to $70x10^{-4}$ s^{-1} for the weak and strong gluten, respectively. Analysis of them proved that weak gluten, in the whole range of applied temperatures, was characterized by significantly higher linear expansion rate than strong gluten (Fig. 6d). This means that, in the course of heating, the loose structure of weak gluten was subject to a higher extent of non-elastic deformation *eg* resulting from viscous flow. Therefore it is justified to include also the parameter *d* in the proposed model describing the thermal expansion of gluten.

DISCUSSION

The present research confirms usefulness of the proposed regression equation for the study of significant differences between the studied wheat cultivars in gluten thermal expansibility. It has shown that increase of heating temperature caused greater expansibility of the strong gluten (Torka) as compared to the weak gluten (Broma). Higher increments of the extent (*a*) and rate (*b*) of hyperbolic expansion were observed. However, the strong gluten required higher heating temperatures to induce hyperbolic expansion. Additionally, the duration of the expansion (*c*) was longer and the rate of linear expansion (*d*) was lower.

The above disproportions between the wheat cultivars studied are attributable primarily to differences in the chemical composition of gluten membranes. As follows from a review of the available literature on the subject (Miś, 2005), stronger gluten in general has a higher contribution of glutenin (causing higher elasticity and mechanical strength) instead of gliadin (responsible for viscosity). These fractions have different ability to aggregate under heating (Pommet *et al.,* 2005). Autran *et al*. (1989) indicated that glutenins are extremely sensitive to thermal treatment, as opposed to ω-gliadins (very resistant). Heating of wet gluten intensifies also the mobility of other components. Chung (1989) reported that free lipids may produce tighter bonds with gluten proteins and provide new mechanical properties of gluten. Residual starch, which is an irremovable fraction of gluten during the washing out process, also undergoes physical disintegration under thermal treatment and modifies the mechanical features of gluten membranes, as confirmed by Dobraszczyk (2003). Even infinitesimal differences in chemical composition may significantly influence the rate of physical and chemical processes under heating, which may result in changes in the behaviour of gluten membranes in the thermal expansion process.

In the expansion process gluten membranes behaviour is a result of their mechanical properties. Usually, these properties are expressed by extensibility, strength and permeability – three independent features. Thus, to explain gluten behaviour, apart from measuring the volume rise (extensibility), changes in water vapour pressure inside gluten bubbles (strength) and water vapour permeability through gluten membranes should be measured as well. Lack of information on these features obstructs the interpretation of the effects of mild heating described by equation parameters *a* and *b* (larger and faster expansion of weak gluten). We cannot say at this stage to what extent the higher extensibility of gluten membranes was a result of their lesser strength (hence lower pressure within the sample causing its expansion) and to what extent it was due to the lesser permeability of the membranes (hence faster rate of pressure increase within the sample causing proportional increase in its volume). Similar interpretation difficulties are encountered in the attempt at explaining the effects of intensive heating when considerably higher and faster volume increase of strong gluten than of the weak one were observed. Probably one of the reasons of the differences observed was higher susceptibility of strong gluten to thermal modifications, which improved its extensibility.

The above discussion visualizes the necessity of further optimisation of measuring apparatus applied for the testing of mechanical properties of gluten membranes in the thermal expansion process. Therefore, in near future, the authors

plan to equip the measuring set presented herein (Fig. 1) with force sensors for the acquisition of data on vapour pressure changes inside gluten bubbles and on the rate of water vapour loss through gluten membranes. Only simultaneous measurements of extensibility, strength and permeability of gluten membranes in the thermal expansion process can provide data that will permit more comprehensive analysis of changes of their mechanical properties during thermal expansion.

CONCLUSIONS

1. The proposed regression equation of thermal expansion satisfactorily describes changes in volume of wet gluten in function of heating time. It enables to distinguish two types of expansion - hyperbolic and linear components which correspond to elastic and viscous nature of gluten membranes.

2. Duration of expansion of gluten membranes (equation parameter *c*) shortened exponentially with increase of heating temperature. Strong gluten (Torka) expanded in significantly longer time in comparison with weak one. Differences between two studied cultivars were the more clear while the milder heating was applied.

3. Volume of gluten as a result of hyperbolic expansion (model parameter *a*) increased gradually with increase of temperature in range of 110 to 140°C. Higher temperatures did not cause further increment of gluten volume, however they influenced the increment of dynamics of both types of expansion (model parameters *b* and *d*).

4. Studied cultivars of bread wheat varied significantly in reaction of gluten on thermal processing. Weak gluten (Broma) required less intensive heating to start the hyperbolic expansion in comparison to strong one (Torka). Additionally, strong gluten indicated higher rates of hyperbolic expansion and lower rates of linear expansion with the heating temperature increase.

5. Presented results show that the proposed thermal expansion model is a useful tool for testing changes thermally induced in gluten and their influence on the mechanical properties of gluten membranes.

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