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Quality evaluation of emmer wheat genotypes based on rheological and Mixolab parameters

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Abstract: Mixolab has been used for rapid assessment of common wheat quality, but data about hulled wheats quality are rare. The aim of this work was to test the potential of Mixolab II in the baking quality evaluation of emmer wheat varieties. The varieties were characterised by low both – gluten content (1.7–11.0%) and Zeleny sedimentation (11.3–12.8 ml), as well as rheological properties showed lower baking quality. Significant differences in protein and starch-amylase part of Mixolab curve indicate the genotype and climatic effect. In average, emmer varieties were characterised by high protein weakening (C2 – 0.29 Nm), speed of protein weakening at the level of $\alpha = -0.05$ Nm/min, starch gelatinisation (C3 – 1.61 Nm), amylolytic activity (C4 – 1.35 Nm) and starch retrogradation (C5 – 1.98 Nm). Zeleny test positively correlated with difference C1-C2 and slope α. Falling number positively correlated with C3, C4, C5 and slope γ. Farinograph dough stability, gluten content together with Mixolab parameter C2 are the most promising characteristics to predict baking quality of emmer.

Keywords: bread making quality; Mixolab II; rheological properties; *Triticum dicoccon*

Increasing interest in hulled wheats has encouraged the studies in their nutritional benefits, biological properties of their bio-compounds, improvement of nutritional and sensory quality, development of new genotypes, etc. (Hamed & Simsek 2014). Emmer possesses interesting nutritional characteristics, is high in protein, and has unique composition of secondary components, which play a role as functional food ingredients. Gluten content with poor gluten quality limit its technological performances and its use in the food industry (Serpen *et al.* 2008; Giacintucci *et al.* 2014).

Traditional farming of hulled wheats survived in the sub-mountainous regions of the Slovak Republic on the borders with Moravia and south-eastern Poland until the last third of the twentieth century. In the western Carpathians emmer was sown as a spring crop on rocky and sandy soils on slopes, most often in unmanured plots, where other crops gave very low yields (Hajnalová *et al.* 2010). The

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search for accessions (varieties) with better adaptability to climatic conditions with desirable agronomic and quality performance is one of primary request, which can contribute to the crops diversification, diverse food production and local market development.

The bread making quality of wheats can be evaluated by various quality tests. One of the most reliable method is direct baking test, however this method is time and labour consuming. Indirect methods, more rapid and simple, such as wet and dry gluten content, sedimentation, rheological methods for the evaluation of dough and gluten strength, have been widely adopted, but it does not always differentiate wheats of medium-strong quality (Dhaka *et al.* 2012). A rheological instrument of a newer generation Mixolab has been used for rapid assessment of the wheat quality, the obtained test results explain both – the protein as well as starch characteristics – pasting behaviour of flour. Mixolab was used for the prediction of the cookie and cake quality, hydrocolloid effects on the thermo-mechanical properties of wheat, effect of antioxidants on dough mixing properties, etc. (Kahraman *et al.* 2008; Ozturk *et al.* 2008; Abdel-Samie *et al.* 2010). In addition to the importance of the protein component for the quality of the final product, more recent studies have emphasized the importance of the starch component (Torbica *et al.* 2016). However, there are not sufficient data to use Mixolab parameters for quality assessment of non-traditional cereals (Grobelnik Mlakar *et al.* 2014).

The objectives of this work were to test the potential of Mixolab II in the quality assessment of *T. dicoccon* (Schrank) varieties during several production years, to examine the relation between indirect technological quality parameters and baking test for the prediction of bread making potential of non-traditional cereal, as well as to inspect the influence of climatic factors on the quality parameters.

MATERIAL AND METHODS

Field trials and experimental design. Field experiments were established at the Experimental base of the Slovak University of Agriculture in Nitra – SUA (48°19´N, 18°07´E), as randomised – block experiment, in four replicates. The elevation of experimental area is 177–178 m a.s.l., with continental climate, belongs to warm agro-climatic region, arid subregion with predominantly mild winter with average long-term (1961–1990) annual precipitations 532.5 mm. The average long-term temperature is 9.8°C and for vegetative period it is 16.4°C. The experimental field was located on a Haplic Luvisol developed on proluvial sediments mixed with loess. Winter emmer wheat varieties were cultivated under the ecological farming conditions without fertilization and chemical treatment, within the crop rotation: common pea, emmer, spring barley. Sowing rate of emmer wheat (hulled) was 170 kg/ha.

Plant material. The study included four winter forms of emmer varieties (*Triticum dicoccon* Schrank). Sowing material of varieties Molise sel Colli, Guardiaregia, Agnone was acquired from Regional Agency for Agriculture in Molise and University of Molise in Italy, variety Farvento from Saatbau Linz, Austria.

Data collection. Grain and flour qualitative traits were collected and analysed during four consecutive growing periods 2011/2012–2014/2015. Data for weather conditions (precipitation, temperature) during growing seasons were obtained from Department of Biometeorology and Hydrology, SUA and are summarised in Table 1. Cumulative temperature (°C) was calculated from every day average temperature over 0°C.

Quality traits. Before analyses the samples after harvest were dehulled on laboratory machine KMPP 300 (JK Machinery, Czech Republic). Indirect baking quality indicators were determined on the whole-

Table 1. Dates of sowing and harvest, meteorological conditions during research period

	2011/2012	2012/2013	2013/2014	2014/2015	Mean
Sowing date	2011/10/06	2012/10/11	2013/10/21	2014/10/14	–
Harvest date	2012/07/12	2013/07/24	2014/07/15	2015/07/16	–
Days to harvest	281	287	268	276	278
Cumulative °C to harvest	2487.7	2481.1	2627.80	2600.8	2549.4
Precipitations to harvest (mm)	260.4	522.8	357.2	322.1	365.6
Precipitations March – harvest (mm)	121.0	238.8	198.5	147.1	176.4
Cumulative °C per 1 mm of precipitation March – harvest	16.50	8.30	10.19	13.24	12.1

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meal flour. Wet gluten content (G_{tot}) was analysed by Glutomatic 2200 (Perten Inst., Sweden) according to AACC 38-12. Falling number (*FN*) was determined on Falling number 1100 (Perten Inst., Sweden) according to AACC 56-81 B. Sedimentation value (Zeleny in ml, *ZT*) was determined using Shaker – Type SDZT4 apparatus in accordance with ICC No. 116/1 (Santec, Slovakia).

The rheological properties of dough were assessed using Brabender Farinograph (Brabender Corp., Germany), to determine the water absorption capacity of flour (%), dough development time in min (DDT), dough stability in min (*DS*) and dough softening (DSo) after 12 min in farinograph unit (*FU*).

Baking test was performed according to ICC No. 131 at the water level of Farinograph absorption with some modifications. The baking formula was: flour (600 g, 14% mb), compressed yeast (9.46 g), salt (7.89 g), sugar (9.78 g). Doughs were mixed for 2 min, left to rise for 30 min in fermentation chamber. The dough was punched, molded, put into a baking pan and left for fermentation for further 70 min. After adequate proofing, doughs were baked for 20 min at 225°C. Baking tests were performed in triplicate. Bread volume was determined by the rapeseed displacement method using volumeter OBK (Mezos, Czech Republic) and specific loaf volume (SLV) was calculated.

Dough mixing and psting behaviours of three emmer varieties were analysed during 2014 and 2015 growing years using the Mixolab II (CHOPIN Technologies, France), according to the AACC International Method 54-60.01 (Dubat 2010). The parameters evaluated at Mixolab curve: Torque C1 – maximum point of the first mixing phase; Torque C2 – end point of the phase 2, protein weakening, as a function of mechanical work and temperature; Torque C3 – end point of the phase 3, maximum torque during heating stage, starch gelatinisation; Torque C4 – end point of the phase 4, minimum torque during heating, indicates amylolytic activity - hot gel stability; Torque C5 – end point of the phase 5, starch retrogradation in the cooling phase; C1-C2 difference, the protein network strength under mechanical energy input and increasing heating; C3-C4 difference, it shows amylase activity and is linked to Falling number; Slope α – between end of 30°C period and C2 – speed of the protein weakening; Slope β – between C2 and C3, indicator of pasting (gelatinisation) speed; Slope γ – between C3 and C4, enzymatic (α-amylase) degradation speed

For this study, also the torque in the 8th minute of Mixolab analyses was evaluated – Cs and the differences between Cs and C2.

Statistical analysis. Collected data were subjected to multifactorial analysis of variance (ANOVA), with varieties, years, replicates and their interactions as a source of variation. For all ANOVA analysis, significant differences between factors were determined by F-test at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ probability levels. When significant, it was followed by Fisher's least significant difference test at $^{*}P < 0.05$, to identify significantly different means. Correlation analyses were applied in order to evaluate the strength of the correlation between the parameters under study. Correlations were considered significant when the correlation coefficient achieved a significance level of $^{**}P < 0.01$. The statistical analyses were performed with the software STATISTICA version 10.0 (StatSoft Inc., USA)

RESULTS AND DISCUSSION

Technological parameters of quality. The basic indirect characteristics used for evaluation of the quality and baking value of flour is gluten content and its quality. Total gluten content (G_{tot}), *ZT*, *FN* mean values of emmer genotypes were 1.7–11.0%, 11.3–12.8 ml, 363–428 s, respectively (Table 2), indicating significant differences between varieties, growing years and their interaction. Strong influence of climate conditions was recorded in 2012, with the lowest precipitations and highest cumulative °C per 1 mm of rainfall. Similar results were obtained by Mondini *et al*. (2014) comparing winter and spring emmer wheats, who reported sedimentation values from 11.5 ml to 17.0 ml for winter emmer. Farinograph rheological properties such as DDT, *DS* and DSo were 0.49–1.41 min, 0.57–2.05 min, and 134–191 *FU*, respectively. The significantly lowest G_{tot}, DDT, *DS* and reversely the highest DSo was determined for variety Farvento. Higher values of DSo indicate that dough will not be able to sustain long mechanical processing treatments (overmixing). Results confirmed low to medium indirect baking quality characteristics, with high influence of environmental conditions and tested genotypes. Previous work of Marconi and Cubadda (2004) reported a longer development time (mean 1.70 min) for Farvento and Molise varieties. In the study of Rao *et al*. (2010) DDT ranged from 2.60 min to 7.00 min in the emmer-derived genotypes.

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Table 2. Quality and farinograph parameters of *T. dicoccon* varieties

Variety/Year	G_{tot} (%)	*ZT* (ml)	*FN* (s)	DDT (min)	*DS* (min)	DSo (FU)	SLV (cm^3/100g)
Variety means							
Farvento	1.7^a	12.3^c	402^b	0.49^a	0.57^a	191^c	300^a
Guardiaregia	3.8^b	12.8^d	363^a	1.41^d	2.05^d	119^a	344^c
Molise Colli	11.0^c	11.8^b	409^b	0.99^c	1.71^c	134^b	343^c
Agnone	4.7^b	11.3^a	428^c	0.75^b	1.49^b	134^b	328^b
Mean	5.3 ± 8.0	12.0 ± 3.3	401 ± 43	0.91 ± 0.61	1.46 ± 0.79	145 ± 35	329 ± 20
P	***	***	***	***	***	***	***
Annual means							
2012	15.2^b	16.8^c	444^b	1.43^d	1.90^c	149^c	–
2013	1.7^a	9.8^a	392^b	0.72^b	1.06^a	160^d	327
2014	2.1^a	9.5^a	383^a	0.41^a	1.11^a	130^a	327
2015	2.2^a	12.0^b	384^a	1.09^c	1.75^b	139^b	332
P year	***	***	***	***	***	***	ns
P variety × year	***	***	***	**	***	***	***

DDT – dough development time; DS – dough stability; DSo – dough softening; G_{tot} – gluten content; *ZT* – Zeleny test; *FN* – falling number; SLV – specific loaf volume; values in column followed by different letters are significantly different at $P \leq 0.05$; mean values ± standard deviations; **, ***influence of factors variety or harvest year provable at $P < 0.01$ and 0.001, respectively

Emmer varieties used in this study varied in flour and dough properties, therefore bread produced also differed in the SLV, with the lowest volume for variety Farvento.

The grain quality parameters of *T. dicoccon* confirm the dependency of different metabolites production or quantities of metabolite production and their variation on both variety and environmental conditions. For improved quality of products from hulled wheats, sourdough technology of prolonged fermentation with lactic acid bacteria can be a suitable method (Hamed & Simsek 2014).

Mixolab test results. Mixolab data of flour samples are presented in Tables 3 and 4, and in Figure 1. Initial part of the Mixolab curve characterizes the behaviour of the flour protein complex affected by thermomechanical changes. At the beginning of heating, proteins underwent the thermal weakening and additional decrease in dough consistency (Rosell *et al.* 2007). Mixolab values for dough development time, stability during

Table 3. Mixolab parameters in relation to protein characteristics

Variety/Year	C2 (Nm)	α (Nm/min)	C1-C2 (Nm)	Cs (Nm)	Cs-C2 (Nm)
Variety means					
Farvento	0.23^a	–0.04^a	0.85^b	0.54^a	0.31^a
Guardiaregia	0.32^b	–0.05^b	0.79^{ab}	0.71^b	0.40^c
Molise Colli	0.34^b	–0.05^b	0.76^a	0.68^b	0.35^b
Mean	0.29 ± 0.06	–0.05 ± 0.01	0.81 ± 0.06	0.64 ± 0.11	0.35 ± 0.05
P	**	*	*	***	***
Annual means					
2014	0.31	–0.06^b	0.79	0.69^b	0.38^b
2015	0.27	–0.05^a	0.82	0.59^a	0.32^a
P year	ns	**	ns	**	***

C2 – protein weakening; α – speed of protein weakening; C1-C2 – difference; Cs – torque in 8th min of the test; Cs-C2 – difference; values in column followed by different letters are significantly different at $P \leq 0.05$; mean values and standard deviations shown; *, **, ***influence of factors variety or harvest year provable at $P < 0.05$, 0.01 and 0.001, respectively; ns – non-significant

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Table 4. Mixolab parameters in relation to starch characteristics and α-amylase activity

Variety/Year	C3	C4	C3-C4	C5	β	γ
	(Nm)				(Nm/min)	
Variety means						
Farvento	1.68^{b}	1.51^{b}	0.18^{a}	2.24^{b}	0.37^{a}	-0.04^{a}
Guardiaregia	1.46^{a}	0.98^{a}	0.48^{b}	1.40^{a}	0.47^{b}	-0.07^{b}
Molise Colli	1.69^{b}	1.55^{b}	0.14^{a}	2.31^{b}	0.41^{ab}	-0.02^{a}
Mean	1.61 ± 0.20	1.35 ± 0.34	0.26 ± 0.17	1.98 ± 0.59	0.42 ± 0.08	–0.04 ± 0.03
P variety	**	***	***	***	**	***
Annual means						
2014	1.75^{b}	1.53^{b}	0.22^{a}	2.26^{b}	0.39^{a}	-0.03^{a}
2015	1.47^{a}	1.16^{a}	0.31^{b}	1.70^{a}	0.44^{a}	-0.05^{b}
P year	***	***	*	**	ns	*

C3 – starch gelatinization; C4 – amylolytic activity; C5 – starch retrogradation; β – gelatinisation speed; γ – α-amylase degradation speed; C3-C4 difference; values in column followed by different letters are significantly different at $P \leq 0.05$; mean values and standard deviations shown; $^{*, **, ***}$influence of factors variety or harvest year provable at $P < 0.05$, 0.01 and 0.001, respectively; ns – non-significant

mixing are generally in agreement with Farinograph values (Köksel *et al.* 2009). Torque C2 values, indicating protein weakening, were significantly affected by emmer variety, influence of production year was not significant. The average C2 was 0.29 Nm, the lowest was recorded for Farvento, significantly higher values for Guardiaregia and Molise Colli. Values of C2 below 0.40 Nm indicates that dough from this species is less tolerant to mixing. The difference between C1 and C2 is related to gluten quality, higher values indicate weaker gluten properties. The difference C1-C2 was significant for factor variety, averaging 0.81 Nm, showing genotype effect on proteolytic activity, with the highest value for Farvento (0.85 Nm) indicating weaker gluten properties of this variety. Slope α, as an indicator of the speed of the protein network weakening due to the effects of heat, varying from –0.04 Nm per min to –0.05 Nm/min. The factors variety and years were significant. Farvento was characterized with the lowest speed of the protein weakening. Rachoń *et al.* (2016) reported the lowest values of torque C2, slope α and the highest difference C1-C2 for *T. dicoccon*, compared to *T. aestivum*, *T. durum* and *T. spelta*. The torque at point Cs as well as the difference Cs-C2 were significantly influenced by variety and year. The highest values were observed for Guardiaregia variety (Cs = 0.71 Nm; Cs-C2 = 0.40 Nm), lowest for Farvento (Cs = 0.54 Nm, Cs-C2 = 0.31 Nm) which confirmed the lowest protein quality of Farvento variety. Significant differences in production years highlighted the effect

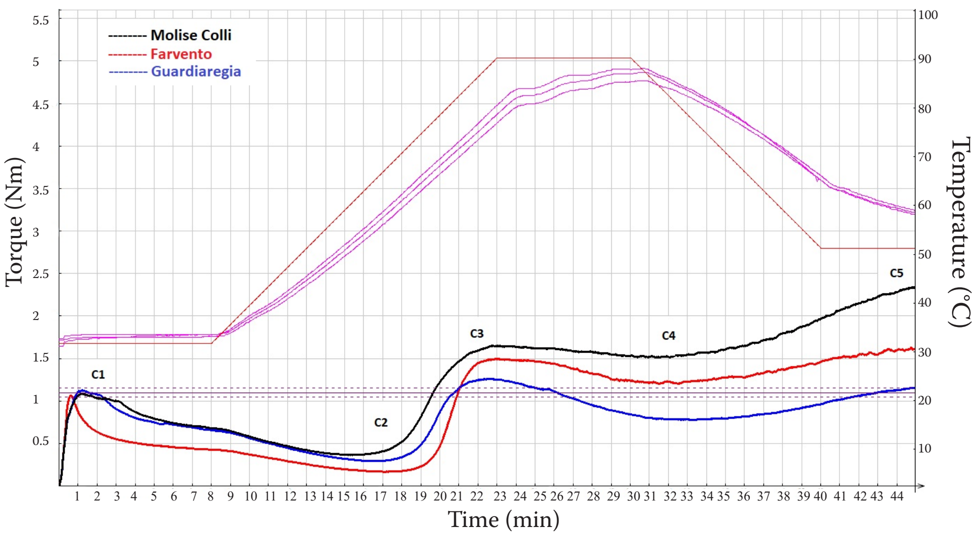

Figure 1. Example of Mixolab curves of emmer varieties. Thin red line demonstrates temperature of mixing bowl, the pink ones temperature of dough prepared from flour of three tested emmer wheat samples

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of meteorological conditions, distribution of rainfalls and temperature during vegetative period.

Starch properties, determined by Mixolab such as gelatinisation (C3), amylolytic activity (C4) and starch retrogradation (C5), were significantly influenced by both experimental factors. Maximum value of torque in the heating phase C3 averaged 1.61 Nm and was higher for Farvento and Molise Colli varieties, indicating a higher starch gelatinization temperature and higher dough viscosity. Stability of the hot paste in the test – phase C4 – when consistency decreases as a result of amylolytic activity, was significantly lowest for Guardiaregia variety (0.98 Nm) compared to Farvento and Molise Colli (1.51 and 1.55 Nm respectively). Difference between C3 and C4 indicates the stability of the starch gel when heated. Higher values, reflecting the lower stability of hot starch paste and higher level of starch disintegration, were recorded for Guardiaregia variety (significantly). Higher torque in the phase C5, a measure of retrogradation of starch molecules in the dough during cooling stage, of varieties Farvento (2.24 Nm) and Molise Colli (2.31 Nm) are indicators of lower amylolytic activities of these varieties.

Correlation analysis. Correlation coefficients were calculated between Mixolab parameters, indirect flour quality characteristics and bread quality – SLV (Table 5). Significant, negative correlations between Mixolab parameters C2, C3, C4, C5 and *ZT* were found, but *ZT* positively correlated with C1-C2 and slope α (r = 0.76; r = 0.82, respectively). Correlations between C2-C1 difference, slope α and *ZT* (r = 0.62; r = –0.49 respectively) were confirmed by Köksel *et al.* (2009). Falling number significantly positively correlated with the peak viscosity during pasting (C3, r = 0.62), C4 (r = 0.81), C5 (r = 0.80), slope γ (r = 0.83) and negatively correlated with slope β, which confirm also the results of Torbica *et al.* (2016). These authors claim, that falling number is not exclusively influenced by amylolytic activity but it is the consequence of the way of packing starch granules and their size. These findings are in disagreements with results reported by Banu *et al.* (2011), who noted negative correlations between C3 and *FN* and positive between slope β and *FN*.

Farinograph DSo was found to be better correlated with Mixolab torque C2 (r = –0.85), difference C1-C2 (r = 0.67), slope α (r = 0.74), Cs-C2 (r = –0.73) than were the correlations between DS, DDT and Mixolab parameters connected with protein characteristics. Specific loaf volume showed strong correlation with C2 among Mixolab parameters, followed by farinograph *DS* (r = 0.82), DSo (–0.87) and G_{tot} (r = 0.71). Köksel *et al.* (2009) reported that these values also correlated with alveograph *G*, *P*/*L* and bread volume. In our study, no correlations between Mixolab parameters related to starch characteristics and SLV were found. Further studies are required to investigate the relationship between Mixolab C3, C4, C5 values and baking quality of non-traditional cereals.

Table 5. Correlation coefficients between Mixolab, Farinograph and baking quality characteristics

	C4	C5	C1-C2	Cs-C2	α	β	γ	DDT	*DS*	DSo	*ZT*	*FN*	G_{tot}	SLV
C2	ns	ns	–0.89	0.69	–0.91	ns	ns	ns	ns	–0.85	–0.72	ns	0.65	0.80
C3	0.95	0.91	ns	ns	ns	ns	0.77	–0.77	–0.68	ns	–0.81	0.62	ns	ns
C4		0.98	ns	ns	ns	–0.66	0.91	–0.68	–0.62	ns	–0.70	0.81	ns	ns
C5			ns	ns	ns	–0.77	0.93	ns	ns	ns	–0.69	0.86	ns	ns
C1–C2				ns	0.87	ns	ns	ns	ns	0.67	0.76	ns	–0.61	–0.64
Cs–C2					–0.83	ns	ns	ns	ns	–0.73	ns	ns	ns	ns
α						ns	ns	ns	ns	0.74	0.82	ns	ns	ns
β							–0.74	ns	ns	ns	ns	–0.87	ns	ns
γ								ns	ns	ns	–0.65	0.83	ns	ns
DDT									0.97	–0.67	ns	ns	ns	0.69
DS										–0.79	ns	ns	ns	0.82
DSo											ns	ns	ns	–0.87
ZT												ns	ns	ns
FN													ns	ns
G_{tot}														0.71

for abbreviations see Tables 1–4; correlations are significant at $^{**}P < 0.01$; ns – not significant

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CONCLUSIONS

Results of the study indicated that Mixolab could be used to predict the baking quality of emmer wheat and differentiate the genotypes and climate conditions for different quality characteristics. Clear distinction between genotypes and climatic conditions showed that these experimental factors affected the parameters related to the protein and the starch characteristics of Mixolab curves. Results indicated significant positive and negative correlations between analytical, farinograph, Mixolab and baking quality indicators. Mixolab torque points C2, C3, C4, C5 and slope γ significantly correlated with Zeleny test (negatively), positive correlations were found between difference C1-C2, slope α and Zeleny test. Falling number positively correlated with Mixolab starch gelatinisation C3, amylolytic activity C4, starch retrogradation C5 and slope γ (enzymatic degradation speed), negatively with slope β (gelatinisation speed). As expected, specific loaf volume showed strong positive correlation with protein quality and properties, namely with gluten content, Mixolab protein weakening C2, farinograph dough stability, and negative with dough softening. Specific loaf volume did not correlated with Mixolab parameters related to starch characteristics, although amylases activity depicted by Mixolab difference C3-C4 (as well as by Falling number) affects progress of the dough leavening.

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