Rheological properties of naturally gluten-free materials contrasted with gluten-free blend and wheat flour

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Abstract

In industrial bakeries and confectioneries, the rate of usage of non-traditional plant material is still increasing, so exploration of their rheological behavior may help to avoid changed machinability of sponge and fermented dough types. One of the latest apparatuses designed for such measurements is the Mixolab developed by the French company Chopin. Combining description of dough preparation and starch gelatinization in a single test, it is a unique approach approved by international standards applied in the cereal chemistry branch. The study presents the dependence of the Mixolab curves on the botanical type of the materials tested, namely flour from rice, buckwheat, and corn, plus rice-buckwheat blend (70:30 wt%). For comparison, the commercial gluten-free (GLF) blend Jizerka, based on corn starch and lupine flour, was tested in the same way, and the above-mentioned dependence was also confirmed. In this case, different pasting temperatures of both plant materials were reflected in two viscosity peaks on the curve. Wheat flour was used as a standard in terms of dough machinability as well as final bakery product volume and aging (torque points C2 and C5, respectively). In this regard, the course of corn flour pasting was the closest to the wheat flour one but the staling of corn bread could be considered as faster (C5 3.05 Nm vs. 2.68 Nm). A similar tendency was registered for the rice-buckwheat and the Jizerka blends – the torque courses partially overlapped, but starch retrogradation reached a higher extent (C5 3.57 and 3.40 Nm vs. 2.68 Nm, respectively).

Gluten-free flour, dough, rheological behavior, Mixolab, starch retrogradation

Introduction

According to recent statistics related to the health of the population within developed countries, approximately 1–2% of the population suffer from celiac disease. A systematic study of metadata published by Singh et al. (2018) mentioned the global prevalence between 1.4% and 1.7%, based on serum analysis of close to 276,000 individuals. Pooled over continents, the prevalence varies generally with sex, age, and location. This autoimmune disease is connected to gluten intolerance, namely of oligopeptide sequences, whose building blocks are repeating proline, phenylalanine, glutamine, leucine, and tyrosine, occurring naturally in common cereals except corn and rice. Such short amino acid chains interact with the small intestine and initiate a response of the immune system. Primarily, genetic mapping of susceptible individuals points to their European ancestry – owing to this, the occurrence of celiac sprue on the other continents is lower than 1% (e.g., 0.6% in Asia; Singh et al. 2018).

At minimum, such patients must restrict or even exclude gluten-based food from their daily diet, substituting wheat with rice, corn, and many other naturally gluten-free (GLF) pseudocereals or legumes. According to EU law, two type of food goods are categorized for celiac patients: without gluten and with lowered gluten content (with limits of 20 and 100 mg gluten per 1 kg of product; Regulation No. 828, 2014). In food processing plants, the manufacturing of GLF bread must be separated from other production lines due to potential cross-contamination, including air filtration. For bakery goods as alternatives to wheat bread, rolls, baguettes, etc., the main raw material is rice in the form of flour with additives and improvers, which are able to replace the functions of gluten – namely water absorption and the forming of the dough skeleton (one of the most frequent hydrocolloid hydroxypropylmethylcellulose, HPMC). The viscous and viscoelastic behavior of rice-based dough clearly differs from that of wheat flour, thus the need for testing dough

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machinability when developing a new GLF blend. By carrying out the testing, prediction of final product volume and its aging speed may be gained.

As mentioned above, rice, corn, or buckwheat can be counted among the major foods for celiac patients as well as raw materials for manufacturing of GLF products. **Rice** (*Oryza sativa* L.) is an important tropic plant of the *Poaceae* family and a basic GLF food component. In the form of minimally processed grain, it is a staple food for the Asian civilization. The content of protein (8–12%), starch (70–80%), fat (1–2%), fibre (approx. 1.5%), and minerals and vitamins, especially E and B, depends on genetic diversity (Noomhorm et al. 1994). Within flour blends, rice is a good source of fatty acids, especially of stearic, arachidic, lignoceric, and oleic acids (contents higher compared to wheat flour). In addition, myristic or linolenic acids could be supplied by this way (Goffman et al. 2003). **Corn** or alternatively **maize** (*Zea mays* L.) is also a member of the botanical family *Poaceae*, but its origin is in Mexico and South America. Like chia and quinoa, the plant was domesticated by pre-Columbian civilizations (i.e., over 10,000 years ago). Together with wheat and rice, nowadays it forms the world's food reserves in the order of a hundred million tons each. Besides its main usage as food and a source of starch for sweeteners and alcohol production, its flour composition is comparable to that of rice (proteins 9–13%, starch 70–80%, and fat around 2%). Fibre content represents an exception, with around 5% (Zeng et al. 2011). Corn flour contains B vitamins (especially folates – B9), iron, potassium, and magnesium, as well as selenium (Gwirtz and García-Casal 2014). Finally, **buckwheat** (*Fagopyrum esculentum* Moench.) belongs to the category of pseudocereals, of the botanical family *Polygonaceae*. It was spread across the world likely from Middle Asia, and it played an important role during the colonization of the America (with wheat, oat, beans, and pea; Bogart and Thompson 1927; Oplinger et al. 1989). Its nutritional composition is similar to rice, too (starch ca. 80%, proteins ca. 12%, fat ca. 3%), but its seeds are a good source of calcium (up to 3310 mg/kg; Bhinder et al. 2020). Hulled or dehulled (alternatively unhusked or husked, respectively) seeds are consumed directly after cooking or in the form of flour, which is processed into an Asian type of thin noodles called *soba*.

The rheological apparatus Mixolab 2 (Chopin, France) is designed for analysis of the technological quality of protein and polysaccharides of flour in two consecutive steps, as an alternative to the standardly used machines Farinograph and Amylograph (Brabender, Germany). At the beginning of the new millennium, the complex method was added to the standard procedures of the AACC Company under No. 54-60.01 (or the ICC No. 173). As Dubat described (2000), the Mixolab test covers dough development, mixing, and overmixing at 30 °C, and further starch gelatinization and retrogradation during heating from 30 °C to 95 °C and cooling back to 50 °C (a simulation of processes in dough in the final product during industrial bread manufacturing). The Mixolab test is an appropriate variant to depict rheological behavior of dough prepared from GLF materials, which was unmeasurable using the above-mentioned Farinograph apparatus. Gluten-free dough does not demonstrate a sufficient extent of elasticity as well as of cohesiveness (like a playdough), thus the farinograph Z-hooks are not appropriate for such dough kneading. During the Mixolab test, torque changes are recorded as a curve 45 minutes long, and five characteristic points "C1–C5" are evaluated as the basic ones (plus water absorption corrected to flour moisture content). Additional parameters are time of occurrence of these torque points and proper temperatures as well as pair differences with technological meaning (e.g., C1 - C2 as dough consistency weakening or T3 - T2 as total time of gelatinization). The standard amount of wheat dough tested is 75.00 g, based on flour moisture content and farinograph water absorption; simplified, weight of flour and water is 50.00 g and 25.00 g, respectively (i.e., water absorption 50% at standard 14% moisture). For some GLF materials evaluation, specific protocols were designed (such as "*Rice 90 g*").

The aim of the present paper is to compare the physico-chemical behavior of pure rice flour and its two blends against that of the commercial GLF blend Jizerka as well as wheat flour, employing the modern rheological apparatus Mixolab. Based on pilot testing, its substitution by fine dehulled buckwheat flour at a level of 30 wt% resulted in an optimal dosage. To improve the nutritional score of the intended form of leavened bread, the addition of hulled hempseed wholemeal was tested, too, as a donor of healthy fatty acids and dietary fibre. The commercial blend Jizerka, based on corn starch, represented the gluten-free standard, thus also the rheological profile of fine corn flour was established. The torque profile of GLF blend designated for bread baking should be as close as possible to that of wheat, so the record of fine wheat flour represents an ideal pattern. Testing on Mixolab conjoins protein behavior during dough preparation, and that of polysaccharides during heating and cooling as a two-step simulation of the bread production process. By comparison, the technological potential of developed GLF blends could be estimated via recorded curves.

Materials and methods

The basic raw material, i.e., fine rice flour (RF), was supplied by Lyckeby Culinar Company (Horažďovice, Czech Republic; milled in Belgium in year 2018). In the same year, fine corn flour (CF) and dehulled buckwheat flour (BF) were produced by the Czech industrial mills Corn mill Mrzkovice (Světlá nad Sázavou) and Mill Perner (Svijany), respectively. Whole hemp seeds of cultivar Finola were rendered by the company Hempoint (Jihlava, Czech Republic). The raw materials were ground to a granulation close to semi-fine flour in batches (25 g, 3.0 min) using the blade grinder Concept KM 5001 (Elko Valenta, Czech Republic). For this wholemeal, the abbreviation HSW was selected. The commercial GLF blend Jizerka (code JIZ) is offered by the industrial bakery Jizerské pekárny (Česká Lípa, Czech Republic); it is based on corn and potato starch, modified corn and manihot starch plus lupine flour. The blend RF30BF was prepared by mixing rice and buckwheat flour in a ratio of 70:30 (wt%); to this bi-composite blend (as well as into the Jizerka blend), HSW was added at a level of 5 wt% (codes RF30BF+5HSW and JIZ+5HSW, respectively).

Methods

For the majority of leavened pastry, fine wheat flour is commonly used; due to this, granulation of tested flour samples and the commercial product JIZ was tested on sieves 257 and 162 mm (per the definition of fine wheat flour in Annex 2, Decree of the Ministry of Agriculture of the Czech Republic No. 18/2020 Coll.). The sieves and the rotational sieving machine KM 1000 were produced by Retsch Company (Arzberg, Germany) and analysis was performed according to the Czech state norm (CSN) No. 56 0512-5. Briefly, the method is designed for flour dosage 50.00 g with sieving supported by chains at 100 rpm for 5.0 min.

As mentioned previously, the standard protocol for the Mixolab measurement is the *Chopin+* one, operating with 75.00 g dough – such protocol was directly applicable for determining the properties of the flour samples BF, CF, and blends JIZ and JIZ+5HSW. For samples RF30BF70, RF30BF70+5HSW, the Chopin+ protocol was used with dough weight correction to 90.00 g. Finally, the RF sample was evaluated according to its specific predefined protocol called *Rice 90 g*. Regardless to the time-consuming measurement with "non-standard" (non-wheat) materials, each sample was measured three times and the best-fitting curve (usually the last one) is presented. The repeatability of the Mixolab method was previously determined using an independent sample of wheat flour; standard deviations calculated on base of ten measurement are appointed into data tables.

The gathered data was statistically described in terms of one-way analysis of variance, using the General Linear Modules, namely Tukey's HSD test at likelihood 95% within the Statistica 13.0 software (TIBCO Software Inc., Palo Alto, USA). A considered factor was *Flour base*, for which partial grouping (pooling over) of samples occurred for items RF30BF and BF30BF+5HSW (the factor level *RF-BF*) as well as for triple CF, JIZ, and JIZ+5HSW (the factor level *CF/Cstarch*). Technological similarity among both tested samples and the Mixolab quality parameters was explored by the Principal Component Analysis (PCA).

Results

Granulation of GLF materials tested

Within the Decree 18/2020 Coll., fine wheat flour is defined by using sieves 257 mm and 162 mm as underflow 96% and 75% at least, respectively. With the exception of CF, all tested materials fulfilled that demand. The sample CF contains a higher proportion of coarse and medium-sized particles (Plate IV, Fig. 1), which could likely affect water absorption and prolonged dough time development (depending also on present protein character).

Conversely, the commercial blend JIZ mixed from several types of starch exhibited a higher ratio of the fine fraction – it could support volume rise of dough pieces during bread leavening phase, owing to better accessibility to hydrolytic enzymes.

Protein properties in GLF materials tested

Comparing Mixolab curves for pure flour RF, BF, and CF against the WF one, there is an obvious dependence of the course of the curve on the botanical origin of the tested sample – it reflected protein content as well as their structure. Nevertheless, dough forming of GLF materials also had a connection to granules size and physical properties of present starch as the main polysaccharide. The Mixolab profiles course just of rice flour and buckwheat flour was closest to that of wheat (Torbica et al. 2008). For our own basic RF specimen tested, the longest dough development time owing to the second consistency maximum was recorded. The finding confirmed results published in the Mixolab Application Handbook (Anon 2012). Although the specimen CF did not reached pre-described consistency 1.10 ± 0.05 Nm (Plate V, Fig. 2a), it demonstrated a similar long dough time development. For corn starch only, very long dough stability was determined by Torbica et al. (2008), too – the C1 and the C2 torque points were recorded in times of 0.60 and 19.68 min. Comparably to our own presented results, dough-mixing part for that corn starch was also flat, fluctuating around 0.25 Nm. In addition, the Mixolab test for extruded corn flour produced a similar profile (e.g., dough development time 7.86 min against 5.07 min for wheat control; Sun et al. 2019). In case of the blends RF30BF and RF30BF5HSW (both under acronym RF-BF in Table 1), rheological properties reflected rice protein properties, as expected according to the mixing ratio used. Further, enhancement of the blend RF30BF by 5 wt% of hemp seed wholemeal did not change the dough preparing phase significantly. As it is drawn on Fig. 2b, profiles of RF30BF and RF30BF5HSW sample overlaid one each other. According to parameter C2, the tested sample cannot be significantly differentiated – in practice, likely comparable bread volumes should be determined.

Table 1. Protein properties during cool (1st) phase of the Mixolab test

Flour base	Water absorption (%)	C1 (Nm)	Cs (Nm)	Stability (min)	C2 (Nm)
RF	57.8 b	1.21 ab	1.18 b	9.4 c	0.56 a
BF	50.0 a	1.40 b	1.05 ab	0.4 a	0.50 a
RF-BF	59.0 b	1.47 b	1.21 b	0.8 a	0.63 ab
CF / Cstarch*	62.9 c	0.82 a	0.59 a	2.9 b	0.48 a
WF	61.5 bc	1.08 ab	1.03 ab	10.8 c	0.58 ab
Repeatability	0.2	0.01	0.03	0.2	0.05

Flour base	D2 (°C)	C1 - C2 (Nm)	Slope a (Nm.min-1)	(Nm)
RF	60.9 b	0.65 a	-0.064 a	0.62 bc
BF	62.9 c	0.90 ab	-0.070 a	0.55 b
RF-BF	63.9 c	0.84 ab	-0.062 ab	0.58 b
CF / Cstarch*	60.8 b	0.34 a	-0.023 b	0.11 a
WF	52.2 a	0.50 a	-0.094 a	0.45 b
Repeatability	0.2	0.10	0.005	0.05

C1, C2 – torque points of the Mixolab curve during cool phase; Cs – torque in the 8th minute of the Mixolab test (i.e., between points C1 and C2); D2 – temperature of dough immanent to the point C32

Slope α – torque gradient of protein weakening (between points Cs and C2)

RF, BF, CF, WF – rice, buckwheat, corn and wheat flour, respectively; flour base RF-BF corresponds to samples RF30BF and RF30BF+5HSW (hemp seed wholemeal)

* Corn starch is the main component of the commercial gluten-free blend Jizerka; the group also includes the sample JIZ+5HSW

A bi-composite GLF identical to RF30BF was tested by Torbica et al. (2010) – they concluded water absorption was lowered about approximately 2 percent points, shortening of dough development time to one-quarter and dough stability rather comparable in contrast to their rice flour standard. On the other hand, they measured clearly higher values in torque point C2 – 0.72 Nm for rice-dehulled buckwheat blend and 0.81 Nm for the control rice flour. Except for twice the dough development time and dough stability shortened about one-tenth, the "protein section" of the Mixolab profile for rice-hulled buckwheat mixture was comparable to the mentioned rice-dehulled buckwheat counterpart.

Of all the tested mixtures, the Jizerka sample showed the highest water binding capacity, which was comparable to wheat flour. Secondly, it had the greatest resistance to overmixing (C2 equal to 0.66 Nm; mean of the CF / Cstarch group 0.59 Nm; Table 1), i.e., its proteins had a higher mechanical-thermal resistance. The addition of HSW to the commercial blend did not influenced dough stability, but slightly reduced the resistance – depreciation reached approximately 9%. In summary, bread from this blend could reach proper volume during fermentation as well as after baking (correlation between C2 and specific bread volume $r = 0.63$, P = 95%; Hrušková et al. 2013).

As individual species of the botanical genus *Triticum* (e.g., *T. aestivum*, *T. durum, T. spelta*, and *T. diccoccum*) differ in their protein properties (Rachoń et al. 2016), thereby more diverse were tested GLF plant materials and blends in further Mixolab parameters (temperature D2, differences C1 - C2 and Cs - C2, or alternatively slope α; Table 1). There was noted somewhat better distinguishing in the temperature D2 and the torque difference Cs - C2, signalizing the important role of starch in the former case (at the beginning of gelatinization). Temperature immanent to the torque point C2 is on the border between proteins and polysaccharides behavior evaluation.

Polysaccharides properties in GLF materials tested

Dependence of starch pasting condition on its granules size and shape, i.e., on botanical origin of the biopolymer has been known for a long time. A difference could be noticed over 10 °C between WF and BF samples (Table 1). The value for the control RF was statistically comparable to the one for the CF/Cstarch group. However, rice flour tested by Torbica et al. (2008), demonstrated a significantly lower pasting temperature close to WF – 50.4 and 52.2°C vs. 60.9 °C, respectively. Conversely, the average temperature D2 for the CF/Cstarch group (i.e., samples CF, JIZ and JIZ+5HSW equal to 60.8 °C; Table 1) is verifiably lower than onset temperature determined for native corn starch during the Differential Scanning Calorimetry analysis (66.7 and 65.5 °C, Wang et al. 2018 and Zhang et al. 2018, respectively). Compared to this, the duration of starch pasting was determined in a close interval 6.8–9.2 min, signifying that starch is more susceptible to gelatinize and vice versa for the samples BF and RF, respectively.

The course of the pasting part of the readings were obviously dissimilar from each other (Plate V, Fig. 2a, 2b, and 2c). The Mixolab procedure had the potential to distinguish botanically different samples, especially in the stability of the hot-formed gel and the rate of starch retrogradation during the cooling period (points C4 and C5, respectively). From a statistical point of view, these basic points shown greater data scatter (variance *a-c* and *a-d*, Tab. 1). According to Codina et al. (2010) and Szafranska (2014), the rate of starch gelatinization C3 depends on ash and dietary fibre contents. These parameters could be altered by slopes β and γ, which were significantly correlated to dough stability ($r = 0.764$, P = 95 %) and to water absorption ($r = -0.898$, P = 95%) within the tested set (data not shown). Both relationships of starch gelatinization speed and enzyme degradation speed found could be explained on the basis of the accessible amount of water (free water) in dough. Both for our own blend RF30BF and the commercial one JIZ, almost one and half times higher extent of retrogradation should be partially lowered for prolongation of bread

Table 2. Polysaccharides properties during heating (2nd) and cooling (3rd) phase of the Mixolab test

Flour base	C3 (Nm)	D3 (°C)	T3 - T2 (min)	C4 (Nm)
RF	3.04 b	86.8 b	9.2 b	2.97 c
BF	1.97 ab	84.8 ab	6.8 a	2.13 b
RF-BF	2.36 b	87.9 b	9.3 b	2.29 b
CF / Cstarch*	1.31 a	75.6 a	7.6 ab	1.54 a
WF	2.12 ab	79.2 a	7.8 ab	1.90 ab
Repeatability	0.10	0.20	0.20	0.03

Flour base	C5 (Nm)	C3 - C4 (Nm)	Slope b (Nm.min-1)	Slope g (Nm.min^{-1})
RF	4.36 d	0.06 a	0.734 c	-0.060 ab
BF	3.75c	-0.16 a	0.066 a	0.134 c
RF-BF	3.48 bc	0.07 a	0.147 a	-0.006 a
CF/Cstarch*	2.68 a	-0.23 a	0.028 a	-0.159 bc
WF	3.34 b	0.23 a	0.352 b	-0.022 c
Repeatability	0.08	0.20	0.005	0.005

C3, C4, C5 – torque points of the Mixolab curve during heating and cooling phase; D3 – temperature of dough immanent to the point C3

Slope β – torque gradient of starch gelatinization (between points C2 and C3)

Slope γ – torque gradient of enzyme degradation of starch gel (between points C3 and C4)

RF, BF, CF, WF – rice, buckwheat, corn, and wheat flour, respectively; flour base RF-BF corresponds to samples RF30BF and RF30BF+5HSW (hemp seed wholemeal)

* Corn starch is the main component of the commercial gluten-free blend Jizerka; the group also includes the sample JIZ+5HSW

shelf life. For such purpose, the addition of fat or hydrocolloid such as xanthan gum could help to reach the goal.

In summary, the rheological properties of our own blend RF30BF could be considered almost comparable to ones of the commercial blend JIZ – from the Mixolab records, partial points to be improved further are:

- To shorten stability of rice-buckwheat dough moderately to maintain usual technological times of dough fermentation;
- To support thermo-mechanical resistance of rice-buckwheat protein complex partially to ensure sufficient bread volume (point C2), as well as increase total portion of protein due to its general lack within diet of celiac patients;
- To elevate amylases activity for the development of desired bread porosity as well as volume (difference C3-C4);
- To decelerate speed of starch retrogradation to prolong the shelf life of bread.

Statistical analysis of data

Principal Component Analysis of 8 samples and 17 Mixolab parameters pointed to both known and less known inter-relations between proteins and polysaccharides as the major components of plant materials. In summary, the analysis explained 75% of data variability by the first two principal components (PC), namely 44% by PC1 and 31% by PC2 (Fig. 3a).

For the GLF materials, dependence of dough consistency both on protein and polysaccharide properties was confirmed (triple C1, Cs, and C3 in the IV quadrant); in this regard, the contribution of non-starch polysaccharides able to absorb water at lower temperature should be considered. As expected, the higher the water absorption, likely the weaker the dough – the water absorption feature is in opposite position to torque points C1 and Cs. Within the tested set, thermal stability of proteins described by differences C1 - C2 and Cs - C2 and hot gel stability C4 reached a similar extent (Plate VI, I quadrant, Fig. 3a). Further deduction lead to confirmation of a tight bond between initial gelatinization temperature D2 and the length of this process (interval T3 - T2). On the other hand, closeness of the features of protein weakening C2 and amylases activity estimated by difference C3 - C4 seems like an agreement in data measured.

On a score plot, qualitative similarity of tested samples quality discussed above is presented visually (Plate VI, Fig. 3b). Approved technological potential of rice and buckwheat flour in terms of raw materials suitable for leavened bread production was confirmed. Distance of this four-member cluster RF – BF – RF30BF – RF30BF+5HSW to wheat flour is similar to one of pair JIZ – JIZ+5HSW, which was based on corn starch. In case of preparation of bread from these materials, clearly distinct water amount should be added and partially different processing conditions should be arranged to obtain a final product of desired quality.

Conclusions

Rheological properties of flour have a direct influence on leavened dough machinability and quality of the final product. From old ages, the role of wheat and wheat flour gradually increased and finally dominated over other crops and raw materials for food production within the milling-baking industry. With the development of civilization, some health disorders also developed, and nowadays, there is a challenge to produce non-wheat goods with quality comparable to the traditional wheat variants. Aiming at celiac patients' nutrition, among others, rice, buckwheat, corn flour, or starches were found to be safe; but technological machinability of dough from these raw materials dramatically differs from that of wheat. For the developed gluten-free blend based on 70 wt% of rice and 30 wt% of buckwheat flour, technological potential was predicted by using the Mixolab apparatus and the predefined Chopin+ protocol. Contrasted with a commercial corn starch blend, quality of our own product should be strengthen in terms of support of protein resistance against overmixing, support of amylases activity to ensure sufficient bread porosity and volume, and in reduction of starch retrogradation extent to prolong bread shelf life. In this regard, addition of 5 wt% of hemp seed wholemeal was unsuccessful, but a slight nutritional benefit in fatty acids and dietary fibre content could be considered at the same time. Further rice-buckwheat blend enhancements have to include also protein-rich sources, both plant and animal, e.g., legume flour or egg white.

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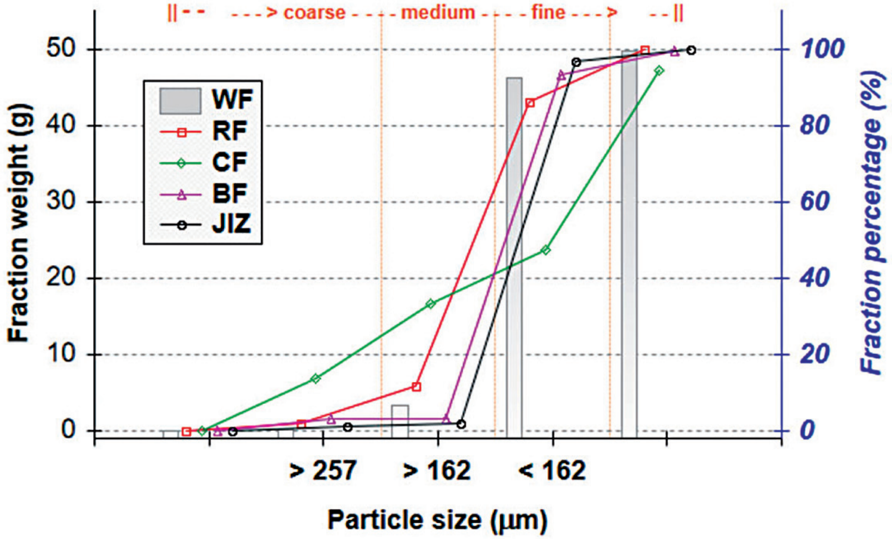

Fig. 1. Comparison of granulation of rice, corn, buckwheat flour and commercial gluten-free blend Jizerka (RF, CF, BF, JIZ, respectively) with that of wheat flour (WF). *Note: sieves with mesh 257 and 162 microns defines granulation of fine wheat flour*

Plate V

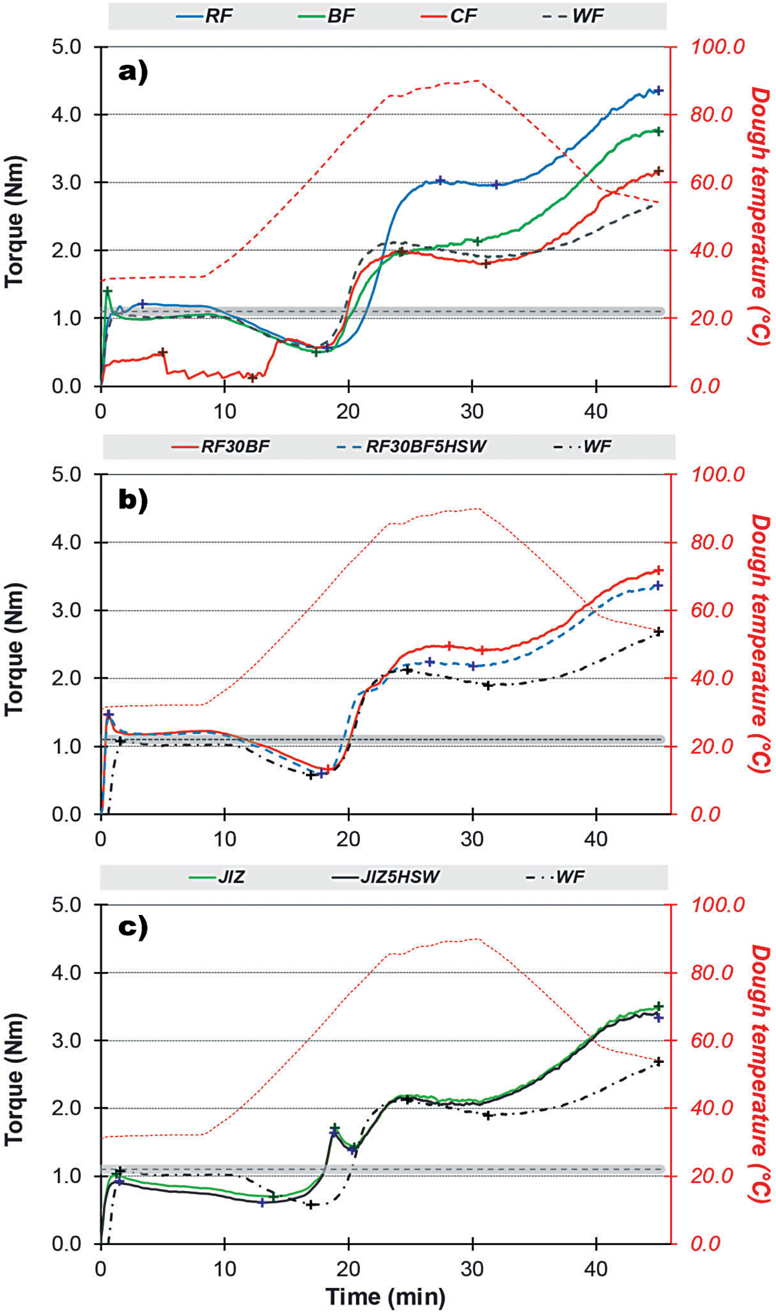

Fig. 2. Mixolab curves for gluten-free flour samples: a) RF, BF, CF, WF – rice, buckwheat, corn, and wheat flour, respectively; b) RF30BF, RF30BF5HSW, WF – rice-buckwheat blend 70:30 wt% without and with addition of hulled hempseed wholemeal and wheat flour, respectively; c) Signs “+” identify characteristic torque points C1, C2, C3, C4, and C5 (from left to right)

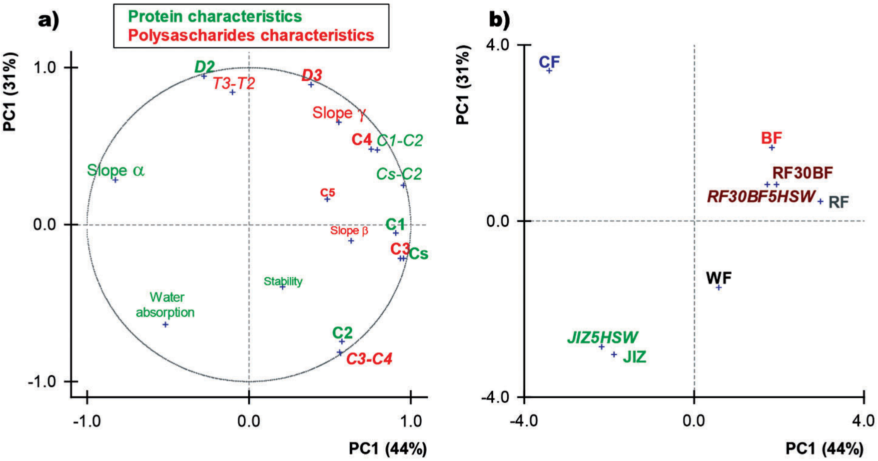

Fig. 3. Plots of the principal component (PC) analysis. a) plot of loadings (variables, i.e., Mixolab parameters): C1 – C5 – torque points (plus their differences as e.g., *C1 - C2*), D2, D3 – initial and final temperatures of gelatinization (immanent to C2 and C3); T3 - T2 – gelatinization time (interval between C2 and C3); slopes α, β, γ – curve slope (gradient) between Cs and C2, C2 and C3, and C3 and C4, respectively. b) plot of scores (samples): RF, BF, CF, WF – rice, buckwheat, corn, and wheat flour, respectively; RF30BF, JIZ, RF30BF5HSW, JIZ5HSW, – rice-buckwheat blend 70:30 wt% and commercial gluten-free blend Jizerka without and with 5% addition of hulled hempseed wholemeal