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Macromolecular Interactions and Rheological Properties of Buckwheat-Based Dough Obtained from Differently Processed Grains

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The physicochemical properties of the protein and starch fractions of flour obtained from buckwheat grains that were previously dehulled or puffed after dehulling were investigated. Dehulling removed most of the nonprotein, nonstarch components of the grain, without affecting the chemical and structural features of the protein and starch components, as made evident by microstructural and spectroscopic measurements. Puffing resulted in extensive modifications of the interprotein network as well as in most of the properties of the buckwheat starch. Flours obtained from dehulled or puffed after dehulling grains were blended with 60−80% wheat flour and tested for their dough-making ability. Blends containing dehulled and puffed buckwheat flours gave dough of much lower quality than dehulled, but had water-holding properties that may be of interest for the shelf life of baked products.

KEYWORDS: Buckwheat (*Fagopyrum esculentum* Möench); protein solubility; protein surface hydrophobicity; dough rheology; scanning electron microscopy (SEM); dehulling; puffing

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Möench), along with amaranth and quinoa, is included among pseudocereals. These species are not cereals from a botanical point of view, but are called "pseudo-cereals" because their processing and their final uses resemble those of true cereals. These pseudocereals are minor crops in terms of production, but they are gaining popularity because of their potential health benefits as human food. The nutritional properties of buckwheat make this grain a suitable candidate for enhancing processing and marketing opportunities by the food industry (*1, 2*). Buckwheat intake has been associated with a lower serum cholesterol and with a higher ratio of high-density lipoprotein cholesterol to total cholesterol (*3*). Flavonoids or/and polyphenols are also found in abundance in buckwheat, especially in hulls (*4*). Buckwheat carbohydrates are digested more slowly than other carbohydrates, so that buckwheat has the potential to prevent adult-onset diabetes, as well as to improve glucose tolerance in those who have developed the disease (*5*). Buckwheat flour is also rich in K, Mg, Fe, Na, Cu, Mn, Sr, and Li, and has a higher mineral content than wheat flour (*6*).

Buckwheat is one of the best plant sources of proteins with high biological value, since their amino acidic composition is well-balanced and nutritionally superior to that of true cereal proteins (*6, 7*), and to other common sources of food proteins. The biological value of buckwheat proteins was estimated at 92.3%, a value that compares very well to 81.5% for defatted dried milk and to 62.5% for wheat. Comparison of buckwheat and wheat proteins according to aminoacid composition, electrophoresis, and immunoreactivity shows little if any similarities, since albumins and globulins represent by far the largest components in buckwheat proteins (*8*). Although buckwheat proteins have a high biological value, their digestibility is relatively modest (*9*), also because of nonprotein endogenous and exogenous factors (*10*). Moreover, as buckwheat does not contain gluten proteins, it can be used for the production of gluten-free foods.

The major protein fractions in buckwheat are globulins, representing almost half of all proteins, and albumins (25% of the total proteins, consisting mainly of low-molecular-mass single-chain polypeptide of 8−16 kDa). They are very interesting from a nutritional and technological point of view, since they have a good capacity of forming and stabilizing emulsions (*11*). Glutelin-like proteins represent only the 4% of the total seed proteins. Therefore, the production of a protein network such as the one formed by gluten proteins in wheat-based products is strongly impaired when using untreated buckwheat flour as the only (or predominant) component of dough.

The behavior of buckwheat in food processing is mainly dependent on the properties of its protein and polysaccharide fractions and on the behavior and interactions of these macromolecules during processing. The physicochemical and structural properties of proteins and polysaccharides can be greatly affected by physical treatments applied to buckwheat seeds prior to milling (*12*). Both protein and starch present in buckwheat flour

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Table 1. Chemical Composition of the Different Samples (average ± standard deviation)

	WBF	DBF	PBF	WF
moisture, %	13.6 ± 0.1	13.9 ± 0.05	7.3 ± 0.07	14.8 ± 0.1
proteins, g/100 g dm	12.7 ± 0.4	14.0 ± 0.2	13.5 ± 0.4	15.2 ± 0.1
lipids, g/100 g dm	2.7 ± 0.12	3.3 ± 0.15	3.4 ± 0.15	1.2 ± 0.2
total starch, g/100 g dm	63.0 ± 1.0	72.7 ± 0.2	72.8 ± 0.8	77.2 ± 0.1
damaged starch, g/100 g dm	6.0 ± 0.3	3.8 ± 0.3	60.4 ± 0.4	6.9 ± 0.4
damaged starch/total starch, %	9.6 ± 0.5	5.2 ± 0.2	82.9 ± 3.0	8.9 ± 0.4
amylose/total starch, %	22.0 ± 1.0	21.6 ± 0.9	24.5 ± 0.5	24.8 ± 0.9
soluble dietary fiber, g/100 g dm	1.16 ± 0.04	0.01 ± 0.0004	2.7 ± 0.12	1.2 ± 0.05
insoluble dietary fiber, g/100 g dm	20.4 ± 1.0	6.5 ± 0.3	5.9 ± 0.2	0.9 ± 0.045

may undergo structural modifications that affect the textural characteristics of the final product, but studies on process-induced changes in the physicochemical properties of buckwheat flour components and on their relationship with the functional properties of buckwheat-based foods are very limited. The formulation of buckwheat-based foods currently on the market has been defined by empirical and product-driven approaches.

A better understanding of the physicochemical properties of buckwheat proteins, as well as those of its starchy and nonstarchy fractions, can greatly enhance the potential use of buckwheat as a food ingredient. In this frame, defining suitable structural and rheological indices could be desirable for the evaluation of these materials, and could help in identifying the relationship between selected molecular parameters and performance.

The aim of this work was the investigation of the effects of physical treatments of buckwheat grain on (1) the structural organization of buckwheat proteins and starch, by means of some indices that have been shown to be useful for assessing the suitability of cereals and pseudocereals to transformation (*13–16*) , and (2) the properties of wheat-based dough containing buckwheat (up to 40%, in order to obtain blends with considerable functional value), by using some rheological indices that are known to be very useful in providing indications for pasta- or breadmaking (*17*) (*18*).

In particular in this work we investigated the role of two technological processes that may greatly influence the performance of the protein and starch fractions, namely dehulling and puffing. Dehulling results in the production of flour where nonstarch and nonprotein components are removed to a great extent. Puffing is expected to affect protein and starch structure in a way that may alter their physicochemical properties, so that dough containing flour from puffed buckwheat may have properties very different from that obtained by using flour from untreated grains.

MATERIALS AND METHODS

Materials. All the samples (common buckwheat, *Fagopyrum esculentum* Möench) were supplied by an Italian company (Ipiesse s.r.l., Poggibonsi, Italy). Buckwheat seeds were processed by the same Italian company in their production plant. Wholemeal Buckwheat Flour (WBF) was prepared by cleaning and laboratory milling (IKA-Universalmühle M20, Janke & Kunkel GmbH & Co, IKA Labortecnic, Staufen, Germany) the untreated grains. The particle size of the various types of milled flour was always ≤200 μm.

Introduction of a dehulling step prior to milling gave a Dehulled Buckwheat Flour (DBF). For puffing of dehulled buckwheat seeds, grains were fed into a cooker and heated to a temperature between 105 and 115 °C for approximately 90 s. Subsequently they were conveyed to a second feeder, which regulates the quantity of grain to be puffed in each cycle. The puffing process took place in an expansion chamber, where the precooked grain came into contact with steam (1.3−1.5 MPa) and was kept at 200−220 °C for 75−85 s. The opening of the chamber at the end of the pre-established time causes a sudden fall in pressure, which led to the instantaneous evaporation of the water contained in the kernels and to a sudden increase in volume of the grains. After puffing, the product was conveyed into a drying tunnel (maintained at 50 °C by introducing dry air) to avoid condensation of the residual steam and to stabilize the product, which was finally milled to give a Puffed Buckwheat Flour (PBF).

A commercial Wheat Flour (WF) of high breadmaking quality was used for the preparation of blends. A total of 6 mixtures were prepared by adding 20%, 30%, and 40% DBF (or PBF) to WF. Fresh compressed baker's yeast (DSM Bakery Ingredients Italy S.p.A., Casteggio, Pavia, Italy) and common salt were also included in the blends used for the rheofermentographic test.

Scanning Electron Microscopy. Dehulled and puffed buckwheat grains were observed after freeze-drying (FD3 Heto, Allerod, Denmark), as whole kernels or as sections made along the transverse axis by dry-fracturing. Samples were mounted on aluminum stubs and sputter-coated with gold. Their ultrastructure was imaged in a scanning electron microscope (SEM, LEO438 VP, LEO Electron Microscopy Ltd., Cambridge, UK), under high vacuum conditions (10^{-4} Pa) at an accelerating voltage of 20 kV.

Grain Composition. The chemical composition of the different flours is reported in **Table 1**. Moisture was determined according to approved methods (AAAC (1983), Approved Method 44−15A) (*19*), as were total nitrogen (AOAC, (1995), Official Method 920−87) (*20*), and lipids (ICC, (1999) Standard Method 136) (*21*). The protein content was calculated by using a conversion factor of 6.25 for buckwheat and of 5.70 for wheat. Total (TDF), soluble (SDF), and insoluble (IDF) dietary fiber levels were evaluated according to the method of Prosky et al. (*22*). Total starch, damaged starch, and amylose were determined by using the Total Starch Assay Kit, the Starch Damage Assay Kit, and the Amylose/Amylopectin Assay kit, all from Megazyme (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co., Wicklow, Ireland). All the determinations were made at least in triplicate, with the only exception of lipids, which were assessed in duplicate.

Protein Solubility and Thiol Accessibility. Protein solubility under native and denaturing conditions was determined by suspending 1 g of flour in 20 mL of buffer (50 mM phosphate, 0.1 M NaCl, pH 7.0), containing also 8 M urea and 10 mM dithiothreitol (DTT) when required, as described elsewhere (*14, 23*). The amount of protein dissolved after stirring the suspensions for 20 min at 25 °C was determined by a colorimetric method (*24*) on the supernatant of low-speed centrifugation of the suspension. Results are expressed as mg solubilized proteins/g d.m.

SDS-PAGE was performed according to Iametti et al. (*14*). A fixed volume of the supernatant obtained after urea/DTT treatment of the various flours was diluted 1/1 (v/v) with SDS-PAGE denaturing buffer (containing 1% 2-mercaptoethanol, v/v) and denatured by boiling at 100 °C for 5 min. SDS-PAGE was carried out on a fixed porosity gel (12% monomer), using a MiniProtein apparatus (BioRad, Richmond, VA).

Accessible thiols were measured directly on flour suspensions as described by Iametti et al. (*14*). An aliquot (100 mg) of whole flour was suspended in 5 mL of buffer (50 mM phosphate, 0.1 M NaCl, pH 7.0), containing 0.2 mM 5,5′-dithiobis-(2-nitrobenzoate) (DTNB) (*25*). The suspension was incubated at 25 °C for 30 min then centrifuged, and the absorbance of the clear supernatant was read at 412 nm. Total

thiols were measured by the same method, but in the presence of denaturants in the buffer. Appropriate blanks were prepared by performing the same treatments in the absence of DTNB.

Protein Surface Hydrophobicity. Front-face fluorescence measurements were carried out at room temperature in a LS-50 B Perkin-Elmer instrument according to procedures described by Bonomi et al. (*13*). The standard front-face fluorescence cell provided by Perkin-Elmer was loaded with ~100 mg of each sample. Emission spectra of 1,8-anilinonaphthalene sulfonate (ANS) were taken from 400 to 600 nm, with excitation at 390 nm. Emission and excitation bandwidths were set at 5 nm. Titration with ANS was performed by adding enough water and ANS (from a stock 20 mM solution) to individual samples (2.5 g each) to give a final water content of 50%, and ANS concentrations covering the 0−0.5 mM range in appropriate increments. Individual samples were prepared for measurement at each ANS content. The added liquid was dispersed by careful manual mixing of the wet flour with a glass rod for 3 min. The resulting stiff mass was cut into lentil-size pieces, and a single piece was placed behind the quartz window in the measuring cell. The cell was closed tightly enough to cause spreading of the sample to a diameter of ~1.5 cm. Appropriate control runs confirmed that neither sample thickness nor sample amount affected the fluorimeter readings. Also, no major changes in fluorescence intensity or in spectral shape were evident for manual mixing times longer than 2 min, indicating that equilibrium solvation was achieved. Titration results were analyzed by standard binding algorithms (*13*), which allowed the estimate of the overall binding capacity of the flour proteins for the probe (given as fluorescence at saturating ANS, Fmax) and the apparent dissociation constant of the protein−ANS complex (K_d(app)). The overall binding capacity (Fmax) was then corrected for the total protein content of each sample (Fmax/p). A Protein Surface Hydrophobicity index (PSH) was calculated as [Fmax/p] × [K_d(app)]$^{-1}$ (*26, 27*).

Solvation studies were performed by adding to individual flour samples (2.5 g each) appropriate volumes of water and 20 mM ANS to reach a final water content covering the 20−60% range, in appropriate increments, while maintaining the ANS concentration at 0.2 mM (*13, 15*). Individual samples were prepared for measurements at each given water content. The water amount corresponding to the midpoint solvation ($W_{0.5}$) was also measured.

Starch Viscoamylographic Properties. The pasting properties of the samples were measured with a RVA-4 Rapid Visco Analyzer (Newport Scientific, Warriewood, Australia), controlled by means of proper software (Thermocline for WindowsTM, 2.2; Newport Scientific, Warriewood, Australia). The flour sample (3.5 g) was weighed into an aluminum canister and water was added (25 mL, taking into account the 14% moisture in the starting flour). The sample was stirred with the paddle for few seconds to prevent formation of lumps; then the Standard Method 2 (Newport Scientific, Warriewood, Australia) was assumed for determination, by activating the program. The test started from 50 °C for 1 min, followed by ramping the temperature linearly at 6 deg/min to 95 °C, holding at 95 °C for 5 min, then cooling the system at 6 deg/min to 50 °C, and ending the process in 23 min. The following indices can be taken from an amylographic profile: pasting temperature (°C), peak viscosity (cP), viscosity at 95 °C (cP), viscosity at 95 °C holding (cP), viscosity at 50 °C (cP), breakdown (BD, cP; peak viscosity minus viscosity at 95 °C holding), and setback (SB, cP; viscosity at 50 °C minus viscosity at 95 °C holding).

Farinographic Test. The dough mixing properties of the different samples were examined with the Brabender SEW Farinograph (Brabender OHG, Duisburg, Germany), according to the official standard method (ICC (1995), No. 115/1) (*28*).

Alveographic Test. The resistance of dough to a three-dimensional extension was measured by means of the Chopin MA 82 Alveograph (Chopin SA, Villeneuve-La-Garenne, France), according to the official standard method (AACC (1983), Approved Methods, 54−30A) (*29*).

Rheofermentographic Test. The dough development during leavening and the gas volume from the yeast activity were measured with the Chopin F3 Rheofermentometer (Chopin SA, Villeneuve-La-Garenne, France). A suitable method was set up in our laboratory for the evaluation of mixtures containing materials other than wheat (*16*). This procedure differs from the reference method in the way the dough is prepared (300 g of sample instead of 250; water according to the farinographic water absorption index instead of the alveographic P; the farinograph mixer instead of the alveograph mixer), in the stress weight applied to the dough during the test (no weight instead of 2 kg), and in the temperature at which the test is performed (30 °C instead of 28.5 °C). The test was performed for 3 h at 30 °C on a 315 g portion of the dough. The following indices can be taken from the rheofermentographic curves: Hm (mm; dough maximum development during the test), *h* (mm; dough height at the end of the test), Tx (min; time of dough porosity appearance), $CO_{2\ TOT}$ (mL; total gas production during the test), $CO_{2\ RET}$ (mL; CO_2 retained by the dough during the test), $CO_{2\ REL}$ (mL; CO_2 released by the dough during the test).

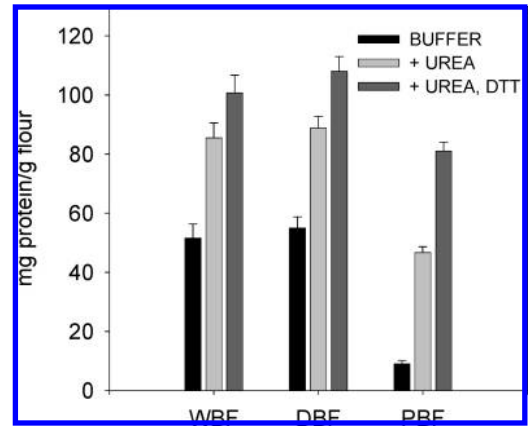

Figure 1. Solubility of buckwheat proteins in various media, as a function of the physical treatments of the seeds.

RESULTS AND DISCUSSION

Interprotein Network in Buckwheat Flour According to the Physical Treatments of the Seeds. The solubility of proteins in solvent systems with different dissociating ability can be used to discriminate among different cereals (*14, 23*) and to describe the effects of technological treatments in cereal-based foods (*30, 31*). This approach also allows a rough correlation between the physicochemical properties of proteins, as inferred from their aggregation state, and their behavior during food processing (*32*).

The amount of proteins solubilized in various media is shown in **Figure 1**. WBF and DBF presented quite similar protein solubility in plain buffer, indicating that the presence of hulls did not affect protein solubility. On the contrary, puffing of buckwheat seeds strongly decreased protein solubility in plain buffer, indicating the formation of a tight interprotein network. The role of hydrophobic interactions and disulfide bonds in the stabilization of the protein aggregates in PBF is made evident by the solubility data in the presence of denaturing and reducing agents (urea and DTT). SDS-PAGE analysis of the proteins solubilized in various media (*not shown*) indicated the absence of specific protein components involved in the formation of insoluble aggregates upon puffing.

Interprotein disulfides in protein aggregates in PBF did not derive from oxidation phenomena, since PBF had an accessible thiol content (3.1 ± 0.08 μmol/g flour) very close to that measured on DBF (3.2 ± 0.11 μmol/g flour). These values were independent of the addition of urea or SDS to the flour, indicating that residual protein thiols in either flour were promptly accessed to the reagent and were not buried into the structure of proteins or of protein aggregates. Our results, obtained after heat treatment of the whole grain, are in substantial agreement with recent studies on the temperature sensitivity of buckwheat globulins in solution (*11*).

According to the solubility data discussed above, hydrophobic

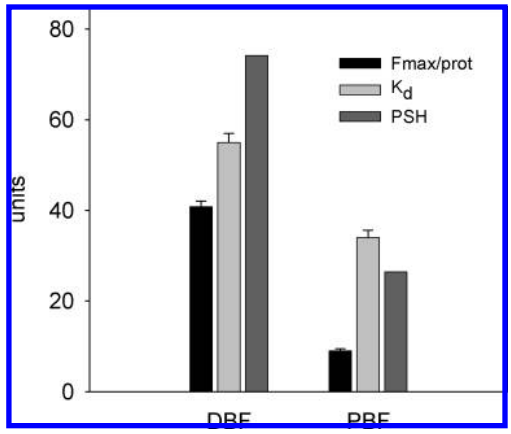

Figure 2. Surface hydrophobicity of buckwheat proteins as a function of the physical treatments of the seeds. Units are as follows: Fmax/p, maximum fluorescence at saturating probe, corrected for protein concentration; K_d app, mM (scale expanded 100-fold); PSH, (Fmax/p)/K_d app.

interactions are relevant to the associative behavior of buckwheat proteins and to its modification upon treatment. To assess more specifically the nature of these changes, we used a spectrofluorimetric approach based on the binding of a fluorescent hydrophobic probe (ANS), which has been extensively used to monitor process-induced structural changes in food proteins (*13, 14, 33*). In this case, we used solid-state spectrofluorimetry to carry out these probe-binding studies, since this approach provides information about the structural changes occurring in the system without resorting to denaturing or dissociating extraction procedures.

Application of these methodologies was not possible on WBF, because colored compounds in the residual buckwheat hull interfered heavily with the spectrofluorimetric measurements by quenching the probe fluorescence. WBF was also not used for the physical studies presented in the next section for various reasons, mostly related to the fact that consumers usually prefer the light buckwheat flour to WBF. WBF is characterized by strong sensorial properties (color and appearance) that are retained in products containing WBF. DBF does not present these sensorial drawbacks, since the dark-colored hull was mechanically removed.

Figure 2presents the results of ANS titration studies carried out on dehulled buckwheat, before (DBF) and after puffing (PBF), and makes it evident that the hydrothermal treatment associated with puffing modified to a great extent the surface hydrophobicity of buckwheat proteins. The surface available for binding of the probe (as expressed from Fmax/p) was greatly decreased by puffing (from 41 in DBF to 9 in PBF), with a concomitant increase in the average affinity toward the probe (as indicated by the decrease in K_d(app) from 0.55 mM in DBF to 0.34 mM in PBF). This has been interpreted (*34, 35*) as a consequence of the aggregation of heat denatured proteins as discussed above. Formation of hydrophobically stabilized aggregates implies an increased mutual interaction between hydrophobic surfaces that are no longer available for binding of the probe. On the other hand, those hydrophobic surfaces remaining on the denatured protein are more compact, and thus display a higher affinity toward the probe (*35*). The balance between these two events was expressed by the overall hydrophobicity index (PSH), which in puffed buckwheat was less than 50% of that measured in flours from dehulled, non-heat-treated grains.

The same solid-state spectrofluorimetric approach was used to assess solvent-induced structural changes in the various starting materials. In fact, structural changes in grain proteins

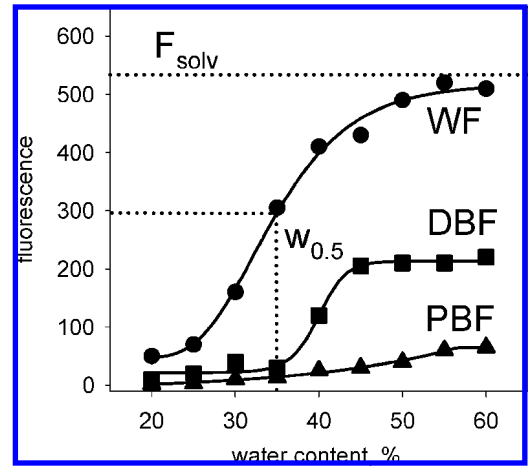

Figure 3. Solvation-induced changes in the ANS binding properties of proteins in buckwheat flours. Thin dotted lines exemplify the criteria used for estimating ANS fluorescence at complete solvation (F_{solv}), and the water amount required for midpoint solvation as measured here ($W_{0.5}$).

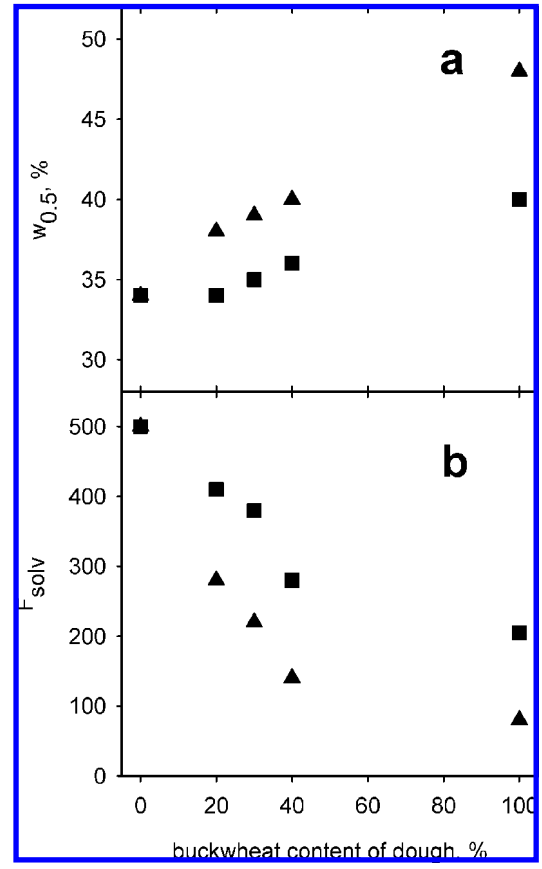

Figure 4. Solvation parameters for various mixtures of wheat and buckwheat flours from differently processed seeds. Squares, DBF; triangles, PBF. Data are taken from solvation experiments in the presence of 0.2 mM ANS similar to those reported in **Figure 3.**

are strongly dependent—even in the absence of other treatments—on their solvation (*13, 15*), as expected for a system where the protein environment changes dramatically as a consequence of the different water content (typically, from 10−15% in the seed to 40−60% in wheat-based bread dough). By carrying out solid-state spectrofluorimetric studies at fixed ANS and variable water concentration, it was therefore possible to monitor the progressive and solvent-dependent exposure of surface hydrophobic sites, as exemplified in **Figure 3** for flours obtained from various grains and/or various treatments. The use of subsaturating ANS

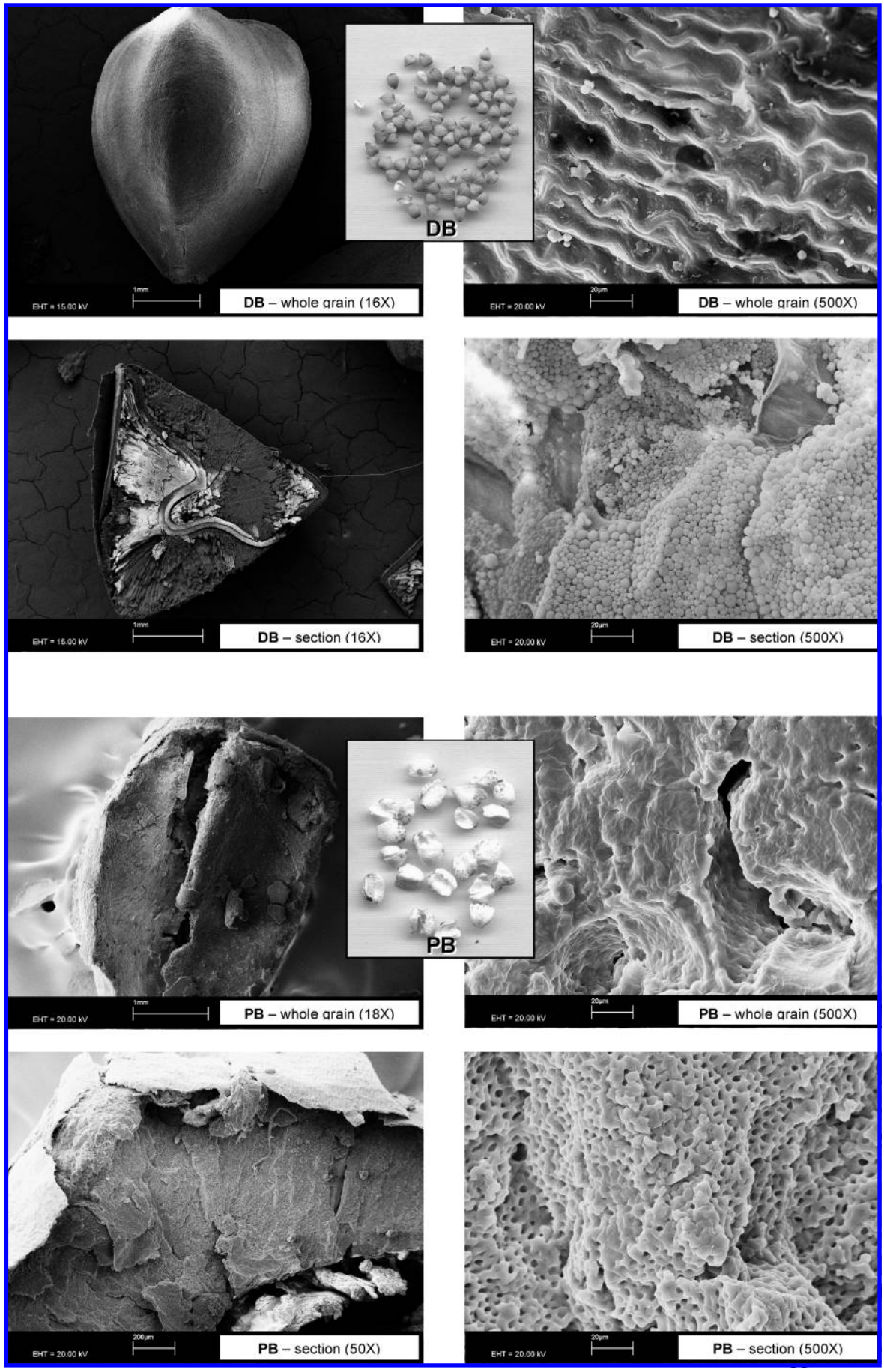

Figure 5. SEM images of dehulled (DB) and puffed (PB) buckwheat seeds.

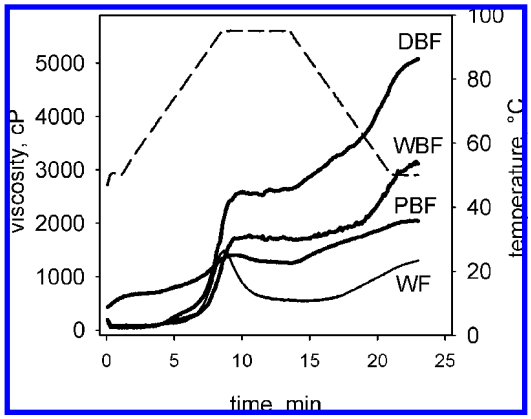

Figure 6. Pasting properties of buckwheat flours obtained from differently processed seeds. The WF profile is also shown for comparison.

concentrations (around 0.2 mM) allowed the estimation of changes in either the number of surface hydrophobic sites or in their affinity toward the probe (*34*) simultaneously.

This approach allowed the estimation of two phenomenological parameters that may be helpful in assessing how solvation is relevant to structural changes. One of these parameters was the overall fluorescence change related to increased binding of ANS as hydrophobic regions become available/accessible to the probe as a consequence of solvation (F_{solv}). The other one ($W_{0.5}$) estimated the amount of water required to elicit 50% of the overall exposure of hydrophobic sites, and is related to the solvation requirements of the proteins in the system. **Figure 3** shows that buckwheat proteins had a much lower content in hydrophobic surfaces with respect to proteins in WF, and that puffing further lowered the number of surface hydrophobic sites (see also **Figure 2**), with a concomitant lower sensitivity to solvation-induced structural rearrangements.

The same parameters were monitored on mixtures of wheat and buckwheat flours of varying composition. As reported in the two panels of **Figure 4**, $W_{0.5}$ increased almost linearly as a function of the PBF content in the mixture, indicating that proteins in PBF and WF behaved as independent systems The same trend was observed in mixtures containing DBF. The changes in F_{solv} (**Figure 4b**) confirm the presence in these mixtures of independent systems.

From a practical standpoint, these studies indicate that the addition of 30% DBF to WF does not induce coarse changes in the solvation behavior of wheat proteins, so that this can be considered a convenient threshold for formulation. The data also make it evident that the protein aggregates present in PBF do not interact with proteins in WF, and therefore the use of PBF in formulations is not advisable, at least from a molecular-based standpoint.

Properties of Buckwheat Starch As Related to the Physical Treatments of the Seeds. The properties and the structural organization of buckwheat starch and proteins were influenced by the different processes, as made evident by SEM (**Figure 5**). Before puffing the achene had a compact and relatively homogeneous external and internal structure. The embryo, with the characteristic sinusoidal shape, extended throughout the whole cross-section of the grain. The starch granules of buckwheat, in the untreated seeds, were very small (1−7 μm), regular in shape, and grouped in clusters.

Puffing radically changed the ultrastructure of the seeds. As reported in a previous study on puffed cereals (*12*), the very compact structure evident at low magnification proved to be made up of a large number of small and regular cavities (about

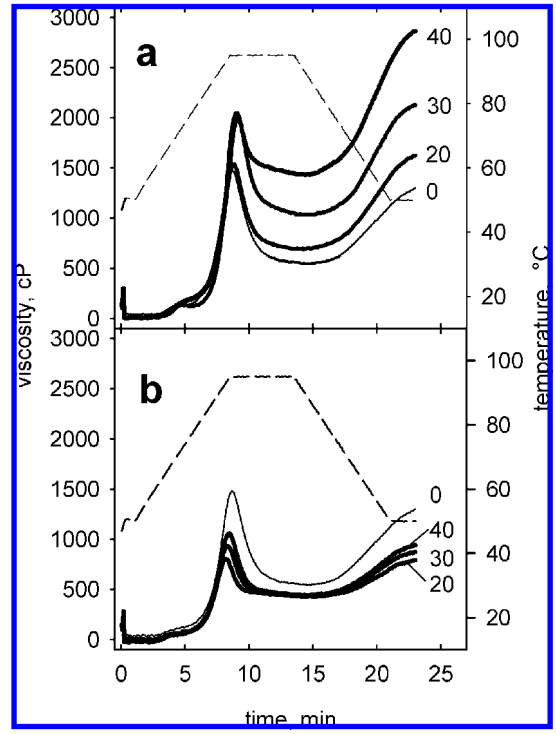

Figure 7. Pasting profiles of blends obtained by adding from 20% to 40% DBF **(a)** or PBF **(b)** to WF. The pasting profile of WF alone (0%) is given for comparison.

2 μm) visible only at a high magnification and regularly distributed in the solid mass, where individual starch granules were no longer recognizable. These ultrastructural changes strongly affected the chemical-physical properties of buckwheat starch (**Table 1** and **Figure 6**).

The data in **Table 1** indicate that the amylose/total starch ratio in PBF was 24.5%, that is, much higher than that in DBF (21.6%). We attribute this increase to the structural breakdown of amylopectin induced by the hydrothermal treatment, as reported previously (*36*).The starch damage level, which is an index of the extent of modification induced by the technological process, was higher than 60% d.b. in PBF, indicating that more than the 80% of the total starch was damaged by the puffing treatment, which induced starch gelatinization and disruption.

The highest TDF content was found in WBF (where it occurred almost completely as IDF), and the lowest in DBF. The decrease in fiber content that occurred with refinement was evident, since dietary fiber is mainly present in the outer layers of the seed (*37*). The puffing treatment caused a minor decrease in IDF and an appreciable increase in SDF. This is of nutritional relevance, because SDF slows gastric emptying and increases transit time in the small intestine. SDF, and to a lesser extent IDF, is also fermented by the microflora in the digestive tract producing short chain fatty acids and gases, the local and systemic of which may explain the correlations observed between SDF uptake and low levels of blood cholesterol, and low chances of developing colon cancer (*38*).

Figure 6 shows the viscosity profiles of the different buckwheat flour samples. The curves for the unheated buckwheat flours (WBF and DBF) had a peculiar shape: neither a sharp peak nor breakdown was observed during heating. Rather, there was a continuous increase in viscosity on holding the temperature at 95 °C. The viscosity at 95 °C was higher than that of wheat starch, and rapidly increased concomitantly to the temperature drop due to cooling, suggesting that buckwheat starch has a good water absorption ability during gelatinization,

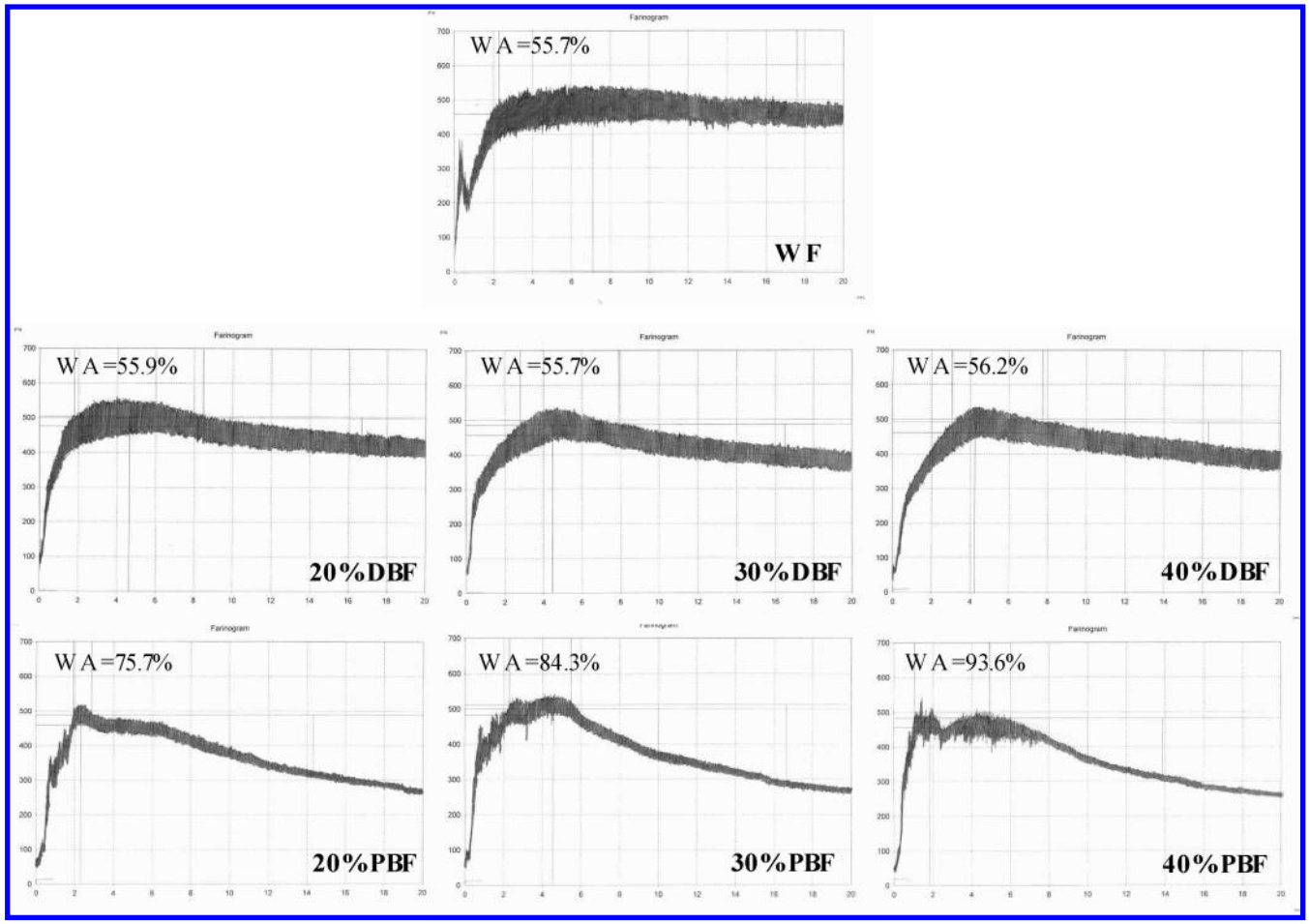

Figure 8. Farinographic indices for blends obtained by adding from 20% to 40% DBF or PBF to WF. Values for WF alone (0%) are given for comparison.

and good gelling properties upon cooling (*39*). Thus, although the starch content in buckwheat grains lies within the range reported for other cereals, the chemical composition and physicochemical properties of buckwheat starch are visibly different.

The influence of the puffing treatment on buckwheat flour properties was made evident also by the RVA test. As a consequence of the high content of damaged starch, characterized by an increased ability of absorbing water, the initial viscosity of PBF was very high compared to that of the other samples. Moreover, there was only a small increase in viscosity during the heating phase, due to the swelling and gelatinizing of the remaining native starch granules, and a reduced increase in viscosity during the cooling phase, indicating a limited retrogradation. Gelatinization of buckwheat starch results in changes in its swelling power, viscosity, ability to adsorb water, and solubility (*36*). Water absorption depends on complex starch−water−protein interactions that govern the solid-phase structure, and is increased by gelatinization and by process-induced fragmentation of amylose and amylopectin (*40*). The alveolate structure in PBF is also able to take in, and hold in stable form, high amounts of water by capillarity, thus influencing remarkably the behavior of the resulting dough.

Properties of Wheat−Buckwheat Dough According the Physical Treatment of Buckwheat Seeds. Buckwheat flour cannot be developed into a viscoelastic dough with good elasticity and plasticity because its proteins have a modest content in prolamines, and gluten-like proteins are absent (*6*), so that buckwheat flour is frequently mixed with wheat flour during food processing. In this study, the amount of buckwheat flour mixed with wheat flour was high (20%, 30%, 40%), in order to obtain blends with considerable functional value. Only DBF and PBF were used for this part of the experimental plan, and conventional rheological tests were used to evaluate the breadmaking quality of various flour mixtures, hoping to bring up the potential positive effects of physical treatments on starch properties and network-forming capabilities.

Properties of Blends during Heating and Cooling. Figure 7 shows the pasting properties of blends in comparison to that of WF. The peculiar viscosity profile of WF changed with increasing amount of DBF in the blend: the peak viscosity rose, the breakdown became less evident, and the setback became more pronounced. The high increase of the viscosity of the blends during the heating phase (the peak raised from 1480 cP for WF to 2045 cP for 40% DBF) suggested that DBF starch could play an important structuring action, mostly during the final steps of the breadmaking process, thus assuring a good consistency to the final product. On the other hand, viscosity of blends after the cooling period ranged from 1624 cP for 20% DBF to 2863 cP for 40% DBF in comparison to 1300 cP for WF, indicating a high tendency of DBF starch to retrogradation (see **Figure 5** and related comments). This could negatively affect the softness of the bread crumb during shelf life.

As for the WF−PBF blends, the presence of heat-treated buckwheat flour was detrimental to viscosity, which worsened appreciably with increasing PBF content. Peak viscosity decreased from 1480 cP for WF to 807 cP for 40% PBF, due to the increased presence of damaged starch in the slurries. Thus,

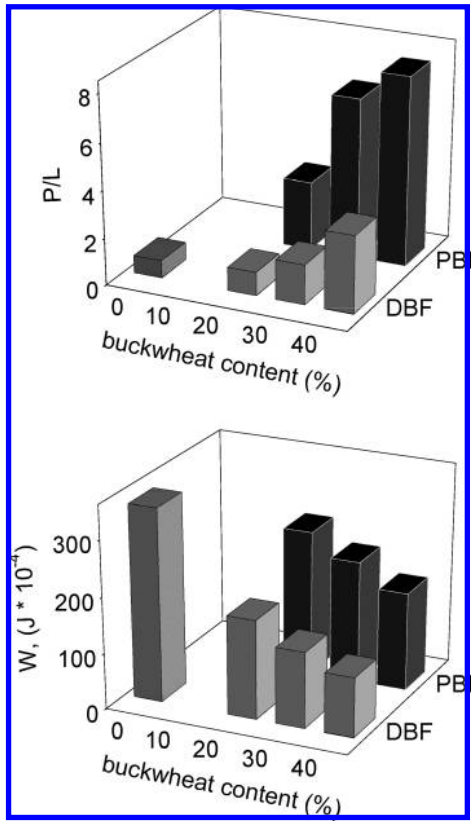

Figure 9. Alveographic tracings for blends obtained by adding from 20% to 40% DBF or PBF to WF. The tracing obtained with WF alone (0%) is given for comparison.

no structuring or thickening effect can be played by buckwheat starch during heating of PBF mixtures. On the other hand, the highly damaged starch in PBF was not affected markedly by retrogradation during cooling, and this can be a useful property if the shelf life of bread is taken into account.

Properties of Blends during Mixing. The rheological and technological properties of the different blends were evaluated by using the Farinograph (**Figure 8**) and the Alveograph (**Figure 9**) tests. The alveographic test was carried out according to the Official AACC Standard Method, both for the sake of comparison and because addition of a high amount of water (as indicated by the farinographic test) would have caused a high stickiness and a low workability of the dough during the execution of the alveographic test.

Figure 8 shows the effects of the presence of increasing levels of DBF and PBF on the farinographic behavior of the blends. In the case of WF, the dough development time was higher than 7 min, the dough stability was more than 15 min, and the degree of softening was very low suggesting a great resistance to prolonged mixing time and a high capacity to sustain stresses occurring during the bread making process.

The presence of buckwheat had different effects on these properties. Increasing contents of DBF (up to 30%) had no influence on the water absorption of the blends, which were close to that of WF, in accordance with the probe-binding studies presented above. On the contrary, this index was strongly affected by the presence of PBF, increasing to more than 90% for blends containing 40% PBF. This phenomenon can be easily explained taking into account that ~80% of the total starch in PBF has been damaged by the puffing treatment and that PBF is able to take in and hold high amounts of water by capillarity.

The presence of DBF did not worsen wheat dough rheology to a great extent even at the highest DBF content. The elasticity of blends (represented by the width of the band) was good, suggesting an interesting resistance to mixing and a good capacity to withstand stresses. The good behavior of DBF-enriched dough could also be related to the properties of its protein. As discussed above, proteins in DBF were much more prone to establish hydrophobic interactions and less aggregated than those in PBF.

In contrast, the high amount of water required to reach the optimum dough consistency (500 BU) in blends at high PBF content led to a gluten network diluted both by aggregated buckwheat proteins—not available for further interactions—and by water. In particular, the alveolate structure and the peculiar structure of starch in PBF allowed the water to be soaked up without playing an active role in the formation of a useful network. Therefore, dough stability decreased, and the extent of softening increased (up to 146 BU in blends at 40% PBF).

Figure 9 shows the effects of the presence of increasing amounts of DBF and PBF on wheat dough alveographic indices. As the amount of DBF increased the *W* value decreased and the *P*/*L* ratio became more unbalanced and equal to 3.29 for the 40% DBF blend. The tenacity (*P*) of the resulting dough was similar to that of WF, but the extensibility (*L*) was much lower, indicating a poor breadmaking quality. This was much more evident for PBF-containing blends, which showed quite good *W* values, although this was essentially due to an anomalous increase in dough tenacity and to a severe loss of dough extensibility, resulting in *P*/*L* ratios as high as 8.02.

Properties of Blends during Leavening. Since high PBF contents in flour blends gave a hughly hydrated and sticky dough difficult to handle and to manipulate, it was decided to conduct the rheofermentographic test on these samples with a 60% hydration level, more suitable for predicting the behavior of these blends during the baking process. A rheofermentometer could be used to monitor changes in dough rise, gas formation, and retention as related to bread quality and to detect differences in flour quality due to protein content, flour treatment, and/or dough treatment. The results of this test are reported in **Figure 10**. The presence of up to 30% DBF (or up to 20% PBF) did not strongly decrease the maximum dough development during leavening (Hm), whereas the presence of >20% PBF was quite detrimental. As discussed above, this could probably be related to the lower availability in PBF of the hydrophobic regions that can take part to interprotein network formation in the gluten matrix.

The blends also showed a shorter time of dough porosity appearance (Tx) in comparison to WF, and a higher volume of released CO_2 ($CO_{2\ REL}$), after 3 h of test, indicating a requirement for shorter leavening periods for buckwheat—wheat blends to obtain satisfactory development of the dough. At the end of the proofing phase the dough must contain a large volume of gas and yet have sufficient gas retention in reserve for oven rise (*41*).

The structural features of proteins and starch in buckwheat are important factors for assessing the textural characteristics of buckwheat products, and are quite sensitive to various physical treatments. Dehulling had little, if any, influence on these properties, whereas hydrothermal treatments made the structure of buckwheat proteins too compact, because of aggregation. This impaired their ability to undergo further solvation-related structural changes, and made it impossible for them to take part in further interprotein interactions, since protein hydrophobic surfaces in treated grains were buried at the interface between individual polypeptides in aggregates.

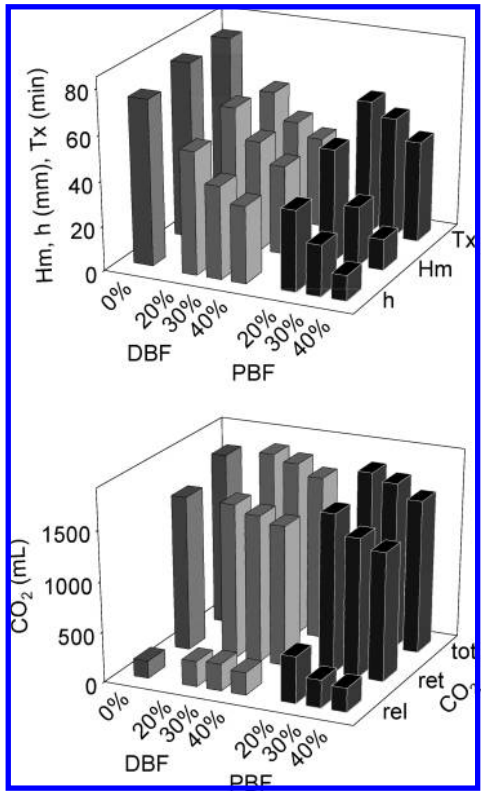

Figure 10. Leavening behavior of blends obtained by adding from 20% to 40% DBF or PBF to WF. Values for WF alone (0%) are given for comparison.

Also the starch fraction underwent remarkable changes upon heat treatment. These modifications strongly affected the rheological properties and the workability of wheat dough enriched with PBF. The use of this flour at high percentage (>40%) should thus be avoided for the production of bread, while it could be very useful for the production of nutritionally enriched, nonleavened food (i.e., buckwheat biscuits).

Given the absence of protein denaturation and the retention of the starch structure, DBF did not affect to a great extent the rheological properties of wheat flour when added in various amounts (up to 40%). Thus, DBF can be used in buckwheat-enriched products, including leavened ones, although the starch in DBF-containing foods may show a tendency to retrogradation. This is less likely for PBF-containing foods, as a consequence of the water-binding and water-holding properties of the biopolymers in PBF.

Thus, the use of a properly balanced mixture of both dehulled and puffed buckwheat flours may be studied for obtaining a buckwheat-enriched bread of good quality. Another aspect that deserves further investigation is the assessment of whether the interprotein interactions (and the starch structural modifications) induced by various hydrothermal treatments of dehulled buckwheat flours may be exploited for using buckwheat as a "structural" ingredient in gluten-free foods.

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