RESEARCH ARTICLE

Evaluation of the quality of nixtamalized maize flours for tortilla production with a new Mixolab protocol

Johanan Espinosa-Ramírez^a, Cristina M. Rosell^b; Sergio O. Serna-Saldivar^a, Esther Pérez-Carrillo^{a,*}

^a Escuela de Ingeniería y Ciencias, Centro de Biotecnología FEMSA. Tecnológico de Monterrey.
Av. Eugenio Garza Sada 2501 Sur, C.P. 64849 Monterrey, N. L., México.

^b Institute of Agrochemistry and Food Technology (IATA-CSIC), C/ Agustín Escardino, 7,
Paterna 46980, Valencia, Spain.

* Corresponding author.

E-mail address: perez.carrillo@tec.mx (E. Pérez-Carrillo).

Abstract

Background and objectives: Nixtamalized maize flours (NMF) are convenient products to obtain an array of nixtamalized products such as table tortillas and chips. In the present study, a Mixolab protocol was set up for the analysis of NMF and to predict mixing conditions in tortilla making. The validity of the procedure was tested with 10 commercial NMF. Water absorption (WA) and mixing times obtained from Mixolab analysis were used to produce doughs and tortillas. The correlations among the Mixolab parameters and quality features of NMF, doughs and tortillas were determined.

Findings: Mixolab curves permitted to characterize the NMF dough development time, mixing stability, starch gelatinization, starch gel stability and retrogradation. This analysis was efficient to discern among the quality of different samples. Tortillas produced with the WA and mixing time predicted with the Mixolab analysis using the optimized protocol had adequate moisture and textural characteristics. Good correlations among the Mixolab parameters and the tortilla moisture and texture confirmed the validity of the method.

Conclusion: The optimized Mixolab test proved to be a useful tool to evaluate the quality of NMF.

Significance and novelty: The new Mixolab protocol could be used to predict relevant process parameters for tortilla production.

KEYWORDS: Mixolab; nixtamalized dough rheology; nixtamalized maize flour; tortillas; water absorption.

1 INTRODUCTION

Maize tortillas are considered the most important staple food in Mexico, but are also widely consumed in Latin America and USA (Rooney & Serna-Saldivar, 2016). To produce tortillas, maize is processed through nixtamalization or lime-cooking, which is a thermal process that consists in the cooking and steeping of kernels in an alkaline water solution followed by washing of the kernels and stone-grinding to produce a nixtamalized maize dough (NMD) (recently reviewed by Santiago-Ramos et al. (2018)). Alternatively, NMD can be dried, dry-milled and sifted to produce tailor made nixtamalized maize flour (NMF) (Rooney & Serna-Saldivar, 2016). The particle size distribution of both NMD or NMF determines its application. For tortilla production finer particles are desired to obtain cohesive doughs that yield flexible tortillas, while higher proportions of coarser particles are preferred for production of both tortilla and corn chips to promote crispiness after frying (Serna-Saldivar & Chuck-Hernandez, 2019; Serna-Saldivar & Rooney, 2015).

Many new processors prefer to use NMF due to its convenience avoiding the nixtamalization process, its high shelf stability and ease of use since it only requires water and mixing (Rooney & Serna-Saldivar, 2016). However, to obtain high quality products with desired characteristics it is important to rehydrate and mix the NMF to its optimum level (Lobeira, Almeida-Dominguez, & Rooney, 1998). Limited water results in hard doughs with poor handling properties, while excessive water and/or overmixing produces sticky and slack doughs, which are difficult to handle (Flores-Farías et al., 2000; Lobeira et al., 1998). Previous studies use subjective methods to determine the water absorption (WA), which need to be performed by an experienced user and with a fix or set mixing time (Alvarez-Ramirez, Garcia-Diaz, Vernon-Carter, & Bello-Perez, 2018; Flores-Farías, Martínez-Bustos, Salinas-Moreno, & Ríos, 2002). In this sense, there is a

need to develop an objective method to predict the hydration and mixing times to produce doughs from NMF.

The Mixolab is an instrument that permits the measurement of the consistency of dough samples over time while changing the temperature (Dubat, 2010). In a typical analysis, five phases are commonly assessed in the Mixolab curve providing useful information about 1) mixing properties and dough development time, 2) weakening of proteins as a function of prolonged mechanical work and temperature, 3) starch gelatinization, 4) hot gel stability and 5) starch retrogradation in the cooling phase (Rosell, Collar, & Haros, 2007). Valuable processing parameters such as flour WA and dough development times can be obtained from this analysis. Mixolab has typically been used for the analysis of wheat flour quality (Rosell, Altamirano-Fortoul, Don, & Dubat, 2013), but also it has been used to assess rheological behavior of non-gluten blends (Matos & Rosell, 2013; Torbica, Belovi, & Tomi, 2019), impact of different ingredients (Rosell, Santos, & Collar, 2010) or additives (Morreale, Garzón, & Rosell, 2018). Yet, there are no reports of the use of Mixolab to obtain processing conditions nor to evaluate the quality of NMF. With the adequate optimization of the protocol for the analysis, this instrument could be useful to predict the hydration and mixing parameters for tortilla production using NMF. Moreover, in the same analysis information about the starch gelatinization degree and retrogradation could be obtained. These parameters have been correlated to the efficiency of the nixtamalization process and are typically included in the quality assessment of NMF (Lobeira et al., 1998; Quintanar Guzmán, Jaramillo Flores, Mora Escobedo, Chel Guerrero, & Solorza Feria, 2009). Monitoring the WA, rheological and pasting characteristics of NMF is fundamental for manufacturers to obtain good quality products (Almeida-Dominguez, Cepeda, & Rooney, 1996).

The aim of the present study was to optimize the Mixolab analysis protocol to predict processing parameters for tortilla production and to evaluate the quality of NMF. Correlations among the Mixolab parameters and the quality of NMF, NMD and tortillas evaluated by typical methods were used to validate the use of this device for predicting NMF performance in tortilla making.

2 MATERIAL AND METHODS

2.1 Materials

Nixtamalized maize flours (NMF) from seven major brands were purchased in the local market in Monterrey, Mexico. According to their labels, the commercial NMF were produced from white maize ideally suited for tortilla production (Rooney & Serna-Saldivar, 2015). These NMFs were classified as “Tortilla NMFs” (T-NMF) along this study. Additionally, three NMF for producing specialties other than tortilla were purchased in the local market. These flours were classified as “Specialty NMFs” (S-NMF) due to their particle size distribution, which make them suitable for applications such as tortilla chips and other Mexican traditional foods. The selected brands were representative of the most consumed products in Mexico.

2.2 Nixtamalized maize flour characterization

The moisture, crude protein (Nitrogen*6.25), crude fat, ash and total starch of NMF were assessed according to the AOAC Official methods 925.10, 978.02, 920.85, 923.03 and 996.11, respectively (AOAC, 1990). Flour particle size distribution was determined by sieving 50 g of NMF through the 20, 35, 60 and 100 US Standard Testing Sieves with aperture sizes of 841 μm , 500 μm , 250 μm and 149 μm , respectively. Results were expressed as the percentage of particles retained in each mesh (overs). Analyses were performed in duplicate.

The water solubility (WSI) and water absorption (WAI) indexes were performed in triplicate according to the methodology reported by Serna-Saldivar (2012).

The pasting and apparent viscosity properties of NMF were determined with a Rapid Visco Analyzer (RVA 4500, Perten Instruments, Hägersten, Sweden) according to the AACC International Method 76-21.02 (AACC, 2017). Flours containing particles with sizes higher than 841 μm were reground before the RVA analysis using a Cyclone Mill (Udy Corporation, Model 3010- 014, Collins, CO). RVA analyses were performed in duplicate.

2.3 Mixolab protocol optimization

A protocol to analyze the mixing and pasting properties of NMF was developed in the Mixolab (Mixolab 2, Chopin Technologies, Cedex, France) using the standard Chopin+ Protocol as reference. Dough weight (75 g) and the targeted temperatures for each stage (30°C, 95°C and 50°C) were those recommended by Dubat (2010), whereas mixing speed, heating/cooling gradient (temperature rise) and optimum target torque were evaluated to adapt the methodology to NMF.

Initially, for selecting the appropriate mixing speed and temperature gradient, the NMF was hydrated according to the supplier recommendations. For the mixing speed, values ranging from 40 rpm to 200 rpm were tested and their impact on the development time and the dough stability loss was recorded at a constant temperature of 30°C. Development time was the time to reach the maximum torque (C1). The stability loss was calculated as a ratio among the maximum torque and the torque after mixing for 8 min and expressed as percentage. To select the heating/cooling gradient, values ranging from 4°C/min to 10°C/min were tested, and the peak torque during heating (C3) was used for comparison purposes.

Preliminary studies with a model NMF were carried out to determine the optimum WA based on dough machinability. For that, NMF was placed in the bowl of a domestic blender (Kitchen Aid, Model K45SS, USA) with enough water to obtain doughs with moistures ranging from 56% to 59%, and kneaded with a flat beater for 2 min at 80 rpm. The dough moisture levels were selected according to values recommended for tortilla production (Ramirez-Wong, Sweat, Torres, & Rooney, 1993; Serna-Saldivar, 2012). Tortilla discs were formed in a pilot-plant scale sheeter/former device (Villamex Model V-25 CB, Mexico), which consisted of two rotating rollers, one of which was in contact with a cutting die. Good machinability was considered when tortilla-dough discs completely detached from the rotating roller, and the amount of water added was used to calculate the optimum WA. The maximum torque during mixing of NMF dough when adding optimum WA was selected as the target torque for NMF.

Once the mixing speed, heating/cooling gradient and target torque were optimized for NMF, the Mixolab NMF protocol (Table 1) was created and used to analyze the samples. Mixing time was set as the time at which the torque was C1-10% to subject all NMFs to the same mechanical weakening. Parameters calculated from the Mixolab curve were: WA (%) or percentage of water required for the dough to reach the target torque, C1 (Nm) or the maximum consistency at phase 1 (30 °C), dough development time (min) or time to reach C1, stability (min) or time during which torque was C1 -10%, C2 (Nm) or minimum torque during phase 2 (30 °C to 60 °C), C3 (Nm) or peak torque at phase 3 (60 °C to 90 °C), the difference between C3 and C4 (Nm) or minimum torque during the heating period (phase 4, holding at 90 °C), and C5 (Nm) or maximum torque obtained in phase 5 (cooling from 90 °C to 50 °C). Mixolab analyses were performed in duplicate using distilled water.

2.4 Nixtamalized maize doughs characterization

Dough moisture was calculated with the WA in dry matter and the moisture content of NMF assessed according to the standard method 925.10 (AOAC, 1990). The subjective machinability of doughs was determined according to the methodology explained in the Section 2.3.

For the determination of textural parameters, NMFs were mixed in the Mixolab using the WA and mixing time as previously described. Two batches of doughs per NMF were produced. Doughs were immediately stored in a plastic bag, rested for 15 min and analyzed. The texture was evaluated using the TVT 6700 Texture Analyzer (Perten Instruments, Australia) according to the compression-tension test reported by Ramirez-Wong et al. (1993), with modifications. Samples were compressed to 65% of their original height with a cylinder probe (75 mm diameter, aluminum) at initial/retract speed of 1 mm/s, test speed 0.1 mm/s and trigger force of 10 g. Five replicates of each NMD were performed. The parameters evaluated were hardness (maximum force during the compression phase) and adhesiveness (area under the negative curve).

2.5 Tortilla production

Tortillas were produced using the WA and development times predicted with the optimized Mixolab protocol. NMDs were produced by mixing NMF and water using a domestic blender (Kitchen Aid, Model K45SS, USA) equipped with a flat beater at 80 rpm. Tortillas were formed in a sheeter device (Villamex Model V-25 CB, Mexico) with cutting dies to form the tortilla dough-discs. The sheeter device gap was adjusted to shape tortilla discs with approximately 24 g weight and 2 mm thick. Tortillas were baked on a three-tier gas-fired oven (Villamex Model V-25 CB, Mexico) set at 280-300 °C with a residence time of 50 s. After production, tortilla pieces were allowed to cool down at lab conditions (25 °C, ~70% relative humidity) for 20 min and placed in plastic bags for further analyses. Two batches of tortillas per NMF were produced.

2.6 Tortilla characterization

Tortillas were characterized in terms of moisture content, size ratio and texture. The moisture was determined according to the AOAC Official gravimetric method 925.10 (AOAC, 1990). To determine the size ratio, the minor and major axes of 5 tortillas from each batch were measured and a ratio of the two measurements was calculated. A value of 1 indicated a circular tortilla and lower values indicated oval tortillas. The texture of fresh (day-0) and 1-day stored tortillas was evaluated in a TA.XT2 texture analyzer (Stable Micro Systems Ltd, Surrey, UK) equipped with the Tortilla Burst Rig platform (HDP/TPB) and a 25 mm spherical probe. Tortilla samples were extended at a test speed of 1 mm/s to a penetration of 5 mm to determine the firmness (force needed to extend 5 mm the tortilla sample), and 40 mm to determine force to break and extensibility (maximum extension before breakage). Five tortillas from each NMF and batch were tested.

2.7 Statistical analysis

Analysis of variance (ANOVA) was used for the statistical analysis of the results. Means were compared using Tukey's test to detect significant differences ($p < 0.05$). Pearson correlations were performed to establish relationships among Mixolab parameters and the flour/dough/tortilla features. All statistical analyses were performed using the Minitab 18 statistical software and 95% confidence.

3 RESULTS AND DISCUSSION

3.1 Nixtamalized maize flour characterization

Nixtamalized maize flours (NMF) were characterized using the common quality parameters evaluated in this type of flours. The classification of NMF into tortilla flours and other applications depends mainly of their particle size distribution. Flours used for table tortillas contain higher proportions of particles $<250\text{ }\mu\text{m}$ (60 US mesh) and does not contain particles higher than $841\text{ }\mu\text{m}$ (20 US mesh) whereas NMF used for fried products such as tortilla/maize chips contain a high proportion of particles $>250\text{ }\mu\text{m}$ (Gomez, Waniska, & Rooney, 1991; Lobeira et al., 1998). Accordingly, the flours labeled as T-NMF fulfilled the particle size distribution requirements for table tortilla production (Table 2). In the same way, the S-NMF2 and S-NMF3 met the common standards for fried products. The S-NMF1 had an intermediate particle size distribution that could be useful for other traditional products like tamales and corn chips (Serna-Saldivar & Chuck-Hernandez, 2019). The moisture of NMF differed among samples and ranged from 7.93% to 10.64% (Table 2). In general, T-NMF contained higher moisture contents in comparison to S-NMF, with exception of S-NMF1. Likely that high moisture was due to differences in the milling processes and particle size distribution. The protein and starch contents did not show significant differences among samples whereas the crude fat contents of S-NMF were lower compared to T-NMF. Sahai et al. (2001) found lower fat contents in fractions with higher particle size. The ash content varied among NMF samples (Table 2). The ash content is mainly related to the absorption of calcium and other minerals during the nixtamalization process and the losses of pericarp tissue during the critical steps of lime cooking, steeping and nixtamal washing (Palacios-Fonseca, Vazquez-Ramos, & Rodríguez-García, 2009; Serna-Saldivar & Chuck-Hernandez, 2019; Serna-Saldivar & Rooney, 2015). Thus, the differences observed in ash contents were associated to variations in the industrial processes among commercial brands. The chemical composition found for the NMF used herein

were within those reported before for commercial NMF (Flores-Farías et al., 2002; Palacios-Fonseca et al., 2009; Sahai et al., 2001).

The WAI and WSI were evaluated to determine the affinity and interactions between NMF and water. WAI is positively related with the water absorption of NMF (Calderón-Peralta et al., 2017). The WAI of the NMFs evaluated herein differed regardless the type of flour and ranged from 2.43 to 3.11 g/g (Table 2). These values were within those previously reported by Almeida-Dominguez et al. (1996) in commercial NMFs. The differences in the WA capacity of NMF has been related to their particle size distribution, the extent of starch gelatinization mainly affected by the lime-cooking, starch damage, kernel endosperm texture and to the presence of hydrocolloids (Flores-Farías et al., 2000; Lobeira et al., 1998; Rooney & Serna-Saldivar, 2015; Serna-Saldivar & Rooney, 2015). According to Flores-Farías et al. (2000) the WAI of NMFs is one of the factors that better predict the quality of NMD and tortillas. High water absorption values are required in NMFs to produce tortillas with acceptable flexibility and reheating ability (Almeida-Dominguez et al., 1996). Among the samples evaluated herein, T-NMF4, T-NMF6 and S-NMF3 presented the lower WAI values. On the other hand, the WSI of samples ranged from 3.34% to 4.25% (Table 2). WSI reflects the quantity of soluble solids in water and the cooking degree of kernels during the NMF production (Flores-Farías et al., 2000). Also, the WSI of NMFs may be influenced by the presence of intrinsic gums and deliberately added hydrocolloids, other grain flours and additives (Almeida-Dominguez et al., 1996; Calderón-Peralta et al., 2017). WSI values were lower for S-NMFs, which was associated to their higher particle sizes. The differences found among WSI values of T-NMFs were associated to their production process.

The RVA analysis is a common practice to characterize the quality of NMF by relating their results with the efficiency of the maize cooking (Quintanar Guzmán et al., 2009). In general,

tortilla flours analyzed presented higher peak viscosities than those prepared for fried products (Fig. 1A, Table 3), which agree with previous reports (Almeida-Dominguez et al., 1996). The differences in the RVA parameters among flour samples have been attributed to different degrees of gelatinization caused by variations in heating and cooling profiles during the cooking and drying processes, to the shearing conditions during the milling process and also to the presence of additives such as hydrocolloids (Almeida-Dominguez et al., 1996; Calderón-Peralta et al., 2017).

3.2 Mixolab protocol optimization

Parameters of the Mixolab protocol were optimized for the NMF characterization. Higher values of maximum consistency and stability loss were observed at higher mixing speed (Supplementary data, Figure S1). Development time was not affected by mixing speed, thus 80 rpm was selected because it gave an intermediate stress to the dough (Supplementary data, Figure S1), and it is the one recommended in the standard method (Dubat, 2010).

Since the NMF contain partially gelatinized starch, the heating/cooling gradients were optimized to obtain higher and narrower gelatinization peaks and therefore enhance differences among samples. When different heating/cooling rates were tested, the same curve profile was observed for all cases and only slight increments were observed in the gelatinization peak (C3) (Supplementary data, Figure S2). Moreover, at high cooling rates (8 °C/min and 10 °C/min), non-continuous gradient was obtained. Therefore, the selected heating/cooling rate for the NMF protocol was 6 °C/min since it reduced the analysis time compared to the standard 4°C/min and showed a good behavior in the block and dough temperatures.

In the Mixolab analysis with the standard Chopin+ Protocol for wheat, the WA is defined as the amount of water to form a dough with a consistency of 1.1 ± 0.07 Nm, which is the target torque. In the case of NMF, the target torque was selected based on the dough machinability. Since one of the most valued quality characteristic in NMD is dough machinability and it varies according to moisture (Lobeira et al., 1998; Serna-Saldivar, 2012), this was the parameter selected to define the optimum WA. When the subjective machinability of the doughs was tested, only the dough with 57% moisture showed good machinability. Higher moistures (58% and 59%) led to sticky doughs that did not fully detached from the shaping roll of the conventional sheeting device whereas a lower moisture (56%) led to a dough that was not cohesive enough to form a continuous sheet. When the dough with 57% moisture was analyzed in the Mixolab (30°C and 80 rpm), it yielded a maximum torque of 0.990. Therefore, the target torque for the NMF protocol was set in 1.000.

3.3 Mixolab analysis

The Mixolab parameters obtained after the analysis of the samples with the optimized NMF protocol are summarized in Table 3. NMFs were hydrated to reach a maximum torque during mixing (C1) within 1.000 ± 0.015 Nm, that was set as the target torque. The WA predicted by Mixolab differed up to 29% in comparison to that suggested by the supplier (data not shown). No differences were observed in the WA among the two types of NMF. Higher WA values were obtained with doughs of T-NMF7 and S-NMF1. The T-NMF7 was the only NMF tested herein containing an added hydrocolloid, which was carboxymethyl cellulose (CMC). Calderón-Peralta et al. (2017) also found that the addition of hydrocolloids such as CMC increased the WA of NMF due to their high interaction with water molecules. However, the S-NMF1 had a similar WA and without adding hydrocolloids. It has been reported that excessive heating during lime

cooking or milling for flour production causes the loss of the structure of starch granules leading to higher WA (Chel-Guerrero, Parra-Pérez, Betancur-Ancona, Castellanos-Ruelas, & Solorza-Feria, 2014). Therefore, the higher WA observed in S-NMF1 might be associated to a more severe nixtamalization process. In contrast, low WA values were related to incomplete modification of the endosperm due to short cooking periods during nixtamalization or to coarser grinding (Gomez, Waniska, & Rooney, 1990).

The consistency drop to C2 has been related to the weakening of proteins when the temperature increase (Rosell et al., 2007). S-NMF samples presented higher C2 values (Table 3). Likely, the higher granulation of these flours improved the stabilization of proteins during heating.

Significant differences were observed in C3 parameter being T-NMF7 the sample with the lowest value (Table 3). In general, the differences in C3 could be associated to a different extent of starch gelatinization during the nixtamalization and milling processes. However, the lower peak viscosity observed for T-NMF7 was related to the presence of CMC. According to Shi and BeMiller (2002), the presence of negatively charged hydrocolloids such as CMC decreased the pasting peak viscosity of potato starch suspensions. This is consistent with Calderón-Peralta et al. (2017) who also found lower peak viscosity values when NMFs containing CMC were evaluated. No significant differences, with exception of S-NMF2, were observed in the difference between C3 and C4, which has been related to the stability of the starch gel (Rosell et al., 2007). Concerning the starch retrogradation occurring during the cooling phase, T-NMF7 dough that contained hydrocolloids, showed the lowest C5 value. The lower starch retrogradation due to addition of hydrocolloids has been related to the ability of these compounds to form networks with good water holding capacity retarding the aggregation and recrystallization of

starch chains (Alvarez-Ramirez et al., 2018). Also, association among maize amylose and CMC molecules could be the reason of the minor retrogradation (Shi & BeMiller, 2002).

It was not possible to determine the C2 to C4 parameters for T-NMF4 with the NMF protocol (Table 3) due to dough separation into two parts during heating, which caused a collapse of the Mixolab curve (Fig. 1B). Probably, due to the fine particle size distribution of T-NMF4 (Table 2) and its low WA (Table 3), the dough produced was hard and compact, leading to its separation during mixing and to the torque collapse. However, T-NMF4 was the only flour that presented this behavior, thus it was not considered representative of NMFs (Fig. 1B). The complete Mixolab profile was obtained for T-NMF4 by increasing the amount of dough to 90 g, but data were not included because tests with different dough-weights cannot be compared.

3.4 Nixtamalized maize dough characterization

One of the most important aspects of the production of maize tortillas is the texture of the NMD since it affects the molding into flattened round discs and is critical for good machinability (Ramirez-Wong et al., 1993; Ramirez-Wong, Sweat, Torres, & Rooney, 1994). To assess textural parameters of NMD the subjective machinability was evaluated and also the instrumental texture, which was determined in doughs after reaching the development time and at the so-called stability time (C1-10%) (Table 4). The moisture of doughs ranged from 57.1% to 60.9% (Table 4). When the subjective machinability of NMD was tested, only two samples (T-NMF7 and S-NMF1) did not detach from the shaping roll (Table 4). These NMF samples had the higher WA (Table 3) or higher moistures (Table 4). Ramirez-Wong et al. (1994) found that adhesiveness increased with higher NMD moisture. Yet, the higher adhesiveness could also be caused by higher damage of starch during production of NMF (Chel-Guerrero et al., 2014).

All T-NMF and S-NMF1 had lower development and stability times compared to S-NMF2 and S-NMF3. The hardness and adhesiveness of NMD presented significant differences among samples when mixed using development time or stability times, with exception of T-NMF1, S-NMF1 and S-NMF2 (Table 4). With longer mixing (stability times), dough hardness decreased, with exception of T-NMF1 that kept its texture. Softening of doughs over the time was expected since the consistency of doughs decrease over time (Fig. 1B). In contrast, the adhesiveness of doughs kneaded at stability times was higher compared to its counterpart kneaded at development times, although no significant change was observed in S-NMF1 and S-NMF2. These results were consistent with previous reports that related overmixing of NMD with stickier doughs (Lobeira et al., 1998). The highest adhesiveness value was observed for S-NMF1 dough, which was expected owing its bad machinability (Table 4). However, the adhesiveness of the T-NMF7 dough, which also presented bad machinability, was lower to that of S-NMF1 and similar to doughs with good machinability. Therefore, the bad machinability in T-NMF7 dough could be ascribed to its higher WA conferred by the added hydrocolloids and not to the increase in its stickiness. This could explain the adequate adhesiveness values during the dough texture analysis of T-NMF7, while in S-NMF1 the adhesiveness might be related to damaged or gelatinized starch leading to a stickier and more adhesive dough even with short kneading times. On the other hand, S-NMF2 yielded stable doughs in terms of adhesiveness, which was also reflected in its highest stability time (Table 4). This flour contained the highest content of coarse particles ($>841\ \mu\text{m}$) (Table 2). Lobeira et al. (1998) also found higher time to stickiness when a coarser NMF was evaluated.

3.5 Tortilla characterization

The physical and textural characteristics of the tortillas produced by applying the development time and WA obtained from the Mixolab are summarized in Table 5. The moisture of tortillas ranged from 43.56% to 51.70% and no differences were observed among table maize tortillas and tortillas for frying, even though it has been reported that table maize tortillas have moisture contents around 46-52%, whereas maize tortillas for frying have lower moistures (25 to 46%) (Brooker, 2015). The tortilla moisture depends on the baking conditions and the NMD moisture (Sahai et al., 2001). Therefore, it could be concluded that the target torque set for the Mixolab protocol developed herein, was adequate to predict the WA to produce table tortillas despite the particle size distribution of NMF. In general, the tortillas had size ratios higher than 0.96 indicating that the shape was circular. T-NMF7 and S-NMF1 produced tortillas with the lowest size ratios, 0.931 and 0.913, respectively. The lower size ratios of tortillas produced with these flours were associated to their bad dough machinability (Table 4), because a detachment device was used to separate the discs from the shaping roll causing a deformation in their shape and oval-shaped tortillas.

The texture of tortillas is one of the most important quality attributes and is affected by differences in the nixtamalization process (Martínez-Bustos et al., 2001). The textural properties of tortillas were assessed in fresh (day 0) and after 1 day (Table 5). The force to break correlates with the hardness of tortillas detected in the mouth and their resistance to tearing by hand (Reyes-Vega, Peralta-Rodríguez, Anzaldúa-Morales, Figueroa-Cárdenas, & Martínez-Bustos, 1998), whereas the extensibility is related to tortilla rollability and flexibility (Suhendro, Almeida-Dominguez, Rooney, Waniska, & Moreira, 1999). The force to break of tortillas ranged from 2.55 N to 3.97 N. These values were within those reported for commercial maize tortillas (Reyes-Vega et al., 1998). After 24 h storage, tortillas behaved differently regarding the force to

break, either augmenting in some samples and decreasing in others. The increase was associated to the tendency of aged tortillas to become hard and brittle (Suhendro et al., 1999), but staling is also dependent on the tortilla making process, particularly moisture content. The tortilla samples presented similar extensibility values in day 0, only significantly lower in T-NMF4, but after 1-day storage, extensibility values decreased up to 29%. Suhendro et al. (1999) also found shorter distances of extension before rupture in aged tortillas compared to their fresh counterparts. Significant differences were observed in the tortilla firmness in day 0, which was expected considering the differences in the production process and functional features of commercial NMF (Table 5). Firmness values greatly increased after 1-day storage, which were up to 81% higher in comparison to firmness of fresh tortillas (day 0). Firmness increase has been related to the staling of tortillas, which involves the retrogradation of the starch fraction (Alvarez-Ramirez et al., 2018; Limanond, Castell-Perez, & Moreira, 2002). Interestingly, tortillas produced with T-NMF4 presented the worst textural features (low extensibility and high firmness) in day 0. During the Mixolab analysis of this flour, it was not possible to obtain its complete profile due to a curve collapse (Fig. 1B). Therefore, the observed collapsing behavior during Mixolab analysis using the protocol presented in this study could be indicative that NMF samples would yield tortillas with poor textural characteristics.

3.6 Pearson correlations

A correlation matrix was constructed using the parameters obtained from the Mixolab analysis and the features of NMF, doughs and tortillas (Table 6). The WA was positively correlated with the WAI of NMF. Thus, even when the WAI methodology could not be used to predict the WA to produce tortilla doughs, it could be useful to compare the WA capacity of NMFs samples. Positive correlation was also found between WA and particle size, specifically the NMF fraction

that absorbed more water was that with particle size $250\ \mu\text{m} > X > 149\ \mu\text{m}$. Evidently, WA had a positive correlation with moistures of both doughs and tortillas. The WA of NMF was negatively correlated with the dough hardness both at development (tD) and stability times (tS). At high dough moistures, water acts as a plasticizer and lubricant yielding a softer dough texture (Lobeira et al., 1998). WA was also negatively correlated with the texture of tortillas in terms of force to break and firmness, both at day 0 and day 1. Therefore, NMFs with higher WA yield softer tortillas.

The development and stability times had a strong positive correlation with the proportion of particle sizes higher than $500\ \mu\text{m}$ in NMF, and presented negative correlations with flour moisture, WSI and flours with high amounts of particles $<149\ \mu\text{m}$. This result indicated that the presence of finer and more soluble particles leads to shorter kneading times and coarser NMF need longer mixing times. Also, less soluble and coarser NMF are more stable to kneading. The presence of finer particles eased the development of dough cohesiveness to reach the target consistency in shorter times but also led to quicker weakening. The stability time showed a moderate negative correlation with the final viscosity and setback values obtained with the RVA. The lower stability times were observed in the finer T-NMF. According to previous studies, starch in finer nixtamalized particles was more damaged and retrograded (Gomez et al., 1991; Gomez et al., 1990) which would result in higher final viscosities and setback values. It is worth to mention that these were the only RVA parameters that were correlated with the Mixolab ones, even when it was expected more similarities in phases C3 to C5 related to starch gelatinization and retrogradation. Nevertheless, it must consider that RVA analysis of NMF contained lower solid concentrations in comparison to Mixolab analysis, but those could not be increased $>27\%$ because it leads to out of scale values (Lobeira et al., 1998). On the other hand, coarse NMF

generates RVA curves without a distinct breakdown making difficult to detect peak and through viscosities (Sahai et al., 2001). Therefore, NMF samples with coarse particles were reground herein before the analysis to improve their RVA profile, which could cause additional starch damage (Sahai et al., 2001). The solids concentration during analysis and regrinding could be the reasons of the lack of correlations among RVA and Mixolab results. In this sense, Mixolab allowed to analyze the NMF features during mixing, heating and cooling at dough moisture hydrations and without re-milling coarse flours (Fig. 1B). Mixolab analysis was sensitive to differentiate both the gelatinization profile of NMF and their behavior during the kneading step yielding significant discrimination among the curves of diverse samples (Fig. 1B).

The minimum torque (C2) was correlated with the particle size distribution of flours, WSI and crude fat (Table 6). Therefore, the initial heating affected less to the flours with coarser and less soluble particles yielding higher C2 values. The negative correlation between C2 and fat could be associated to fat melting due to increment of temperature leading to a lower dough consistency. C2 showed a positive correlation with the firmness of tortillas in day 0 and a negative correlation to their extensibility, likely this effect was also related to the fat content. Therefore, the processing of NMF with lower C2 values would be expected to yield softer and more extensible tortillas.

The gelatinization peak (C3) was negatively correlated to the WAI of flours and the moisture of dough and tortillas. Lower values of C3 was related to higher fraction of pregelatinized starch in NMF. In contrast to native starch, the partially gelatinized starch can rapidly absorb water at room temperature (Gomez et al., 1990; Majzoobi, Kaveh, Blanchard, & Farahnaky, 2015). Therefore, higher proportion of native starch represented by higher C3 values, led to lower WA, and consequently lower WAI and dough/tortilla moistures. The NMF with higher values of C3

produced harder doughs and tortillas (Table 6). Again, this was associated to their lower proportion of pregelatinized starch, which caused lower water uptake during dough production. Higher hydrations have been related to softer NMD (Ramirez-Wong et al., 1993) and also softer tortillas (Gomez et al., 1991).

The particle size distribution affected the stability of the starch gel or breakdown (C3-C4) (Table 6). The starch gel presented higher stability (lower C3-C4) in NMF with higher proportion of particles $>500\text{ }\mu\text{m}$, and lower stability in those rich in particles $250\text{ }\mu\text{m} > X > 149\text{ }\mu\text{m}$. The higher stability of starch gel (lower C3-C4) led to firmer tortillas in days 0 and 1, and with lower extensibility after 24 h.

Most of the correlations observed for C3 were also found for C5 (Table 6). Based on these correlations, it was concluded that lower content of pregelatinized starch (high C3) in NMF samples led to higher retrogradation (high C5). Alvarez-Ramirez et al. (2018) found that adding completely gelatinized NMF retarded starch retrogradation. According to these authors, the gelatinized starch acts as self-hydrocolloid due to its binding water capacity. In this sense, the negative correlations among C5 and WAI, particle size $250\text{ }\mu\text{m} > X > 149\text{ }\mu\text{m}$, moisture of dough/tortillas and dough hardness were attributed to the content of gelatinized starch present in NMF samples which affected the water binding capacity. As expected, flours with higher C5 values yielded tortillas with higher firmness values in days 0 and 1. The positive correlation between firmness and C5 was associated to the staling process which involves changes in the starch fraction through the phenomena of retrogradation (Limanond et al., 2002).

4 CONCLUSIONS

A Mixolab protocol was set up for characterizing NMF, which was useful to determine the WA and mixing time to produce NMF doughs for tortillas production. High WA could be indicative of NMF containing hydrocolloids or excessive damaged starch that would lead to non-machinable and sticky doughs. Therefore, prediction of WA of samples containing hydrocolloids should be taken with care especially when sheeting/former devices will be used to form tortilla dough discs since detachment from the rotating rollers would be needed. Maize tortillas obtained taking into consideration the Mixolab process parameters (WA and mixing time) showed good quality features. The Mixolab parameters obtained with the NMF protocol showed high correlation with the quality features of NMF, doughs and tortillas. Moreover, collapsing of Mixolab curves was related to NMF that yielded tortillas with poor textural features. When the characterization of this type of NMF is required, increase of the dough weight could lead to achieve the complete profile although it is not comparable to curves obtained with 75 g. Hence, the Mixolab showed good potential as a tool to assess and differentiate the quality of NMF and to predict the quality of nixtamalized doughs and tortillas.

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Table 1. Mixolab protocol for nixtamalized maize flours (NMF)

NMF protocol	
Mixing speed	80 rpm
Target torque (for C1)	1.00 Nm
Dough weight	75 g
Tank temperature	30 °C
Temperature 1 st step	30 °C
1 st gradient	At C1 -x%
Value of x	10%
Temperature 2 nd step	90 °C
1 st temperature gradient	6 °C/min
Duration 2 nd step	5 min
2 nd temperature gradient	-6 °C/min
Temperature 3 rd step	50 °C
Duration 3 rd step	5 min

Table 2. Physical and chemical characterization of nixtamalized maize flours (NMF)^a

Sample	Moisture (%)	Chemical composition (% dm)				US mesh particle size distribution ^b (% overs)					WAI (g/g)	WSI (%)
		Protein	Crude fat	Ash	Starch	+20	+35	+60	+100	-100		
T-NMF1	8.98 ^d	8.29	4.77 ^a	1.39 ^{bc}	70.99	0.0 ^c	0.2 ^c	25.6 ^{bc}	27.3 ^{b-d}	46.9 ^b	2.82 ^b	4.09 ^{ab}
T-NMF2	10.58 ^{ab}	8.55	4.57 ^{bc}	1.30 ^c	72.62	0.0 ^c	0.1 ^c	16.9 ^{ef}	34.5 ^{ab}	48.4 ^{ab}	2.96 ^{ab}	3.80 ^{bc}
T-NMF3	10.64 ^a	9.07	4.38 ^{cd}	1.37 ^{bc}	71.74	0.0 ^c	0.6 ^c	27.9 ^b	26.0 ^{c-e}	45.6 ^b	3.03 ^a	3.89 ^b
T-NMF4	10.17 ^b	8.55	4.40 ^{cd}	1.68 ^a	68.36	0.0 ^c	2.9 ^c	21.8 ^{c-e}	21.8 ^{d-f}	53.6 ^a	2.43 ^d	4.25 ^a
T-NMF5	8.62 ^d	8.45	4.61 ^{ab}	1.50 ^b	71.08	0.0 ^c	1.8 ^c	29.0 ^b	21.1 ^{d-f}	48.2 ^{ab}	2.82 ^b	4.24 ^a
T-NMF6	9.46 ^c	8.21	4.68 ^{ab}	1.47 ^b	72.40	0.0 ^c	2.8 ^c	35.7 ^a	26.8 ^{cd}	34.7 ^c	2.65 ^c	4.08 ^{ab}
T-NMF7	9.62 ^c	8.96	4.38 ^d	1.51 ^b	71.13	0.0 ^c	2.4 ^c	22.4 ^{cd}	29.9 ^{a-c}	45.4 ^b	2.97 ^a	4.08 ^{ab}
S-NMF1	10.15 ^b	8.99	4.07 ^{ef}	1.31 ^c	72.98	0.0 ^c	8.1 ^b	29.3 ^b	36.6 ^a	26.0 ^d	3.11 ^a	3.54 ^{cd}
S-NMF2	8.18 ^e	8.34	4.23 ^{de}	1.37 ^{bc}	72.10	17.3 ^a	22.0 ^a	13.7 ^f	18.8 ^{ef}	28.3 ^d	3.07 ^a	3.45 ^d
S-NMF3	7.93 ^e	8.86	3.95 ^f	1.39 ^{bc}	71.61	11.8 ^b	22.1 ^a	19.0 ^{de}	16.4 ^f	30.7 ^{cd}	2.50 ^{cd}	3.34 ^d

^a Means with different letters in the same column are significantly different ($P < 0.05$). Means without letters in the same column did not show significant differences. T-NMF, tortilla nixtamalized maize flour; S-NMF, specialty nixtamalized maize flour; WAI, water absorption index; WSI, water solubility index; dm, dry matter.

^b The US mesh +20, +35, +60, +100 and -100 particle size distribution corresponded to particle sizes of >841 μm , >500 μm , >250 μm , >149 μm and < 149 μm , respectively.

Table 3. RVA and Mixolab parameters of nixtamalized maize flours (NMF)^a

Sample	RVA parameters			Mixolab parameters					
	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	WA ^b (% dm)	Minimum torque (C2) (Nm)	Peak torque (C3) (Nm)	C3-C4 (Nm)	C5 (Nm)
T-NMF1	1864 ^a	340 ^{ab}	2756 ^b	1232 ^{ab}	144	0.543 ^c	0.933 ^c	0.107 ^a	1.267 ^{ef}
T-NMF2	1573 ^{cd}	175 ^{c-e}	2602 ^{cd}	1204 ^{ab}	147	0.546 ^c	0.944 ^c	0.109 ^a	1.299 ^{d-f}
T-NMF3	1515 ^{de}	159 ^{c-e}	2519 ^d	1163 ^{a-c}	149	0.548 ^c	0.925 ^c	0.101 ^a	1.286 ^{d-f}
T-NMF4	1868 ^a	272 ^{a-c}	2894 ^a	1299 ^a	125	ND	ND	ND	1.589 ^b
T-NMF5	1814 ^a	364 ^a	2652 ^{bc}	1202 ^{ab}	136	0.550 ^{bc}	1.011 ^b	0.103 ^a	1.358 ^{c-e}
T-NMF6	1618 ^{bc}	225 ^{b-d}	2525 ^{cd}	1133 ^{bc}	133	0.545 ^c	1.057 ^b	0.074 ^{ab}	1.432 ^{cd}
T-NMF7	1431 ^f	225 ^{b-d}	1991 ^e	785 ^d	156	0.542 ^c	0.750 ^d	0.117 ^a	1.033 ^g
S-NMF1	1451 ^{ef}	150 ^{de}	2081 ^e	780 ^d	153	0.574 ^{ab}	0.890 ^c	0.123 ^a	1.208 ^f
S-NMF2	1221 ^g	87 ^e	1787 ^f	653 ^d	140	0.589 ^a	0.939 ^c	0.036 ^b	1.453 ^{bc}
S-NMF3	1696 ^b	245 ^{a-d}	2502 ^d	1051 ^c	116	0.579 ^a	1.277 ^a	0.067 ^{ab}	1.848 ^a

^a Means with different letters in the same column are significantly different ($P < 0.05$). T-NMF, tortilla nixtamalized maize flour; S-NMF, specialty nixtamalized maize flour; C4, minimum torque during the heating period; C5, torque obtained after cooling at 50°C.

^b WA, water absorption. Amount of water expressed on NMF basis.

ND, not determined.

Table 4. Moisture, machinability and textural parameters of doughs produced at development and stability times with nixtamalized maize flour (NMF)^a

Sample	Dough moisture (%)	Subjective machinability ^c	Development time ^b			Stability time ^b		
			Mixing time ^d (min)	Hardness (N)	Adhesiveness (N*cm)	Mixing time ^d (min)	Hardness (N)	Adhesiveness (N*cm)
T-NMF1	59.1	Good	1.73 ^{cd}	32.6 ^{c,A}	1.43 ^{b,B}	5.00 ^{ef}	31.8 ^{bc,A}	2.27 ^{ab,A}
T-NMF2	59.5	Good	1.33 ^{cd}	36.1 ^{bc,A}	1.12 ^{cd,B}	4.20 ^f	33.4 ^{b,B}	1.85 ^{bc,A}
T-NMF3	59.8	Good	1.82 ^{cd}	34.6 ^{c,A}	1.36 ^{bc,B}	4.56 ^f	31.5 ^{bc,B}	2.27 ^{ab,A}
T-NMF4	55.6	Good	1.58 ^{cd}	46.8 ^{a,A}	1.01 ^{d,B}	4.22 ^f	40.2 ^{a,B}	1.29 ^{d,A}
T-NMF5	57.7	Good	1.17 ^d	40.7 ^{b,A}	1.41 ^{b,B}	4.90 ^{ef}	31.6 ^{bc,B}	1.83 ^{bc,A}
T-NMF6	57.1	Good	2.07 ^c	39.9 ^{b,A}	1.46 ^{b,B}	6.31 ^{cd}	33.3 ^{b,B}	1.89 ^{bc,A}
T-NMF7	60.9	Bad	1.76 ^{cd}	32.3 ^{c,A}	1.36 ^{bc,B}	5.52 ^{de}	28.5 ^{c,B}	2.49 ^{a,A}
S-NMF1	60.4	Bad	1.87 ^{cd}	36.8 ^{bc,A}	1.77 ^{a,A}	6.61 ^c	32.1 ^{bc,B}	1.92 ^{bc,A}
S-NMF2	58.3	Good	6.12 ^a	39.4 ^{b,A}	1.34 ^{bc,A}	15.08 ^a	34.5 ^{b,B}	1.58 ^{cd,A}
S-NMF3	53.6	Good	4.63 ^b	48.0 ^{a,A}	1.40 ^{bc,B}	9.92 ^b	40.6 ^{a,B}	2.13 ^{ab,A}

^a Means with different lowercase letters among the same column are significantly different ($P < 0.05$). T-NMF, tortilla nixtamalized maize flour; S-NMF, specialty nixtamalized maize flour.

^b ‘Development time’ is the mixing time to reach the target torque (C1); ‘Stability time’ is the time at which torque was C1-10%.

^c ‘Good’ or ‘Bad’ machinability meant that dough tortilla discs were: ‘completely detached’ or ‘not detached’ from the shaping rollers, respectively.

^d Mixing times at the two points were determined in the Mixolab using the NMF protocol. Means with different uppercase letters within a row are significantly different ($P < 0.05$).

Table 5. Physical and textural characterization of tortillas produced with nixtamalized maize flours (NMF)^a

Sample	Moisture (%)	Size ratio	Texture, Day 0		Texture, Day 1			
			Force to break (N)	Extensibility (mm)	Firmness (N)	Force to break (N)	Extensibility (mm)	Firmness (N)
T-NMF1	47.53 ^{b-d}	0.984 ^{ab}	3.09 ^{bc}	11.8 ^a	0.575 ^d	3.27 ^{ab}	9.0 ^a	0.913 ^d
T-NMF2	46.69 ^{cd}	0.991 ^a	2.91 ^{bc}	10.9 ^{ab}	0.639 ^{cd}	3.13 ^{ab}	8.3 ^{a-c}	1.027 ^{cd}
T-NMF3	48.01 ^{bc}	0.966 ^b	2.55 ^c	10.7 ^{ab}	0.582 ^d	2.62 ^b	8.5 ^{a-c}	0.873 ^d
T-NMF4	43.76 ^e	0.988 ^{ab}	3.14 ^{bc}	9.7 ^b	0.890 ^a	3.20 ^{ab}	7.2 ^d	1.556 ^a
T-NMF5	48.49 ^{bc}	0.977 ^{ab}	3.08 ^{bc}	11.0 ^a	0.693 ^{b-d}	3.09 ^{ab}	8.8 ^{ab}	0.962 ^d
T-NMF6	45.64 ^{de}	0.980 ^{ab}	3.27 ^{ab}	11.1 ^a	0.687 ^{b-d}	3.46 ^a	8.3 ^{a-c}	1.243 ^{bc}
T-NMF7	51.70 ^a	0.931 ^{cd}	2.78 ^{bc}	11.5 ^a	0.624 ^d	2.48 ^b	8.1 ^{bc}	0.984 ^{cd}
S-NMF1	49.72 ^{ab}	0.913 ^d	2.70 ^{bc}	10.9 ^{ab}	0.662 ^{cd}	2.94 ^{ab}	8.6 ^{a-c}	0.915 ^d
S-NMF2	48.75 ^{bc}	0.938 ^c	3.02 ^{bc}	10.6 ^{ab}	0.770 ^{a-c}	2.79 ^{ab}	7.8 ^{cd}	1.154 ^{b-d}
S-NMF3	43.56 ^e	0.971 ^{ab}	3.97 ^a	10.7 ^{ab}	0.808 ^{ab}	3.34 ^{ab}	7.8 ^{cd}	1.434 ^{ab}

^a Means with different letters in the same column are significantly different ($P < 0.05$). T-NMF, tortilla nixtamalized maize flour; S-NMF, specialty nixtamalized maize flour.

Table 6. Pearson correlation matrix among Mixolab parameters and quality features of nixtamalized maize flours/doughs/tortillas.

	WA	Development time	Stability time	Minimum torque (C2)	Peak torque (C3)	C3-C4	C5
<i>Flour parameters</i>							
Flour moisture		-0.684*	-0.689*				
WAI	0.866***				-0.817**		-0.736*
WSI		-0.747*	-0.721*	-0.884**			
PS > 841 µm		0.987***	0.959***	0.843**		-0.871**	
PS > 500 µm		0.943***	0.917***	0.928***		-0.760*	
PS > 149 µm	0.769**					0.756*	-0.738*
PS < 149 µm		-0.673*	-0.739*	-0.840**			
Crude fat				-0.801**			
<i>RVA parameters</i>							
Final viscosity			-0.670*				
Setback			-0.695*				
<i>Dough parameters</i>							
Moisture	0.999***				-0.967***		-0.966***
Hardness, tD	-0.926***				0.906***		0.914***
Hardness, tS	-0.884***				0.909***		0.932***
<i>Tortilla parameters</i>							
Moisture	0.870***				-0.920***		-0.872***
Force to break, d0	-0.890***				0.882**		0.848**
Extensibility, d0				-0.670*			
Firmness, d0	-0.787**			0.779*	0.675*	-0.743*	0.773**

Force to break, d1	-0.686*	0.733*		
Extensibility, d1			0.680*	
Firmness, d1	-0.856**	0.785*	-0.710*	0.815**

Correlations indicated by the coefficient values. $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$. WA, water absorption; WAI, water absorption index; WSI, water solubility index; C4, minimum torque during the heating period; C5, torque obtained after cooling at 50°C; PS, particle size; tD, development time; tS, stability time; d0, day 0; d1, day 1.

Fig 1. RVA (a) and Mixolab (b) profiles of nixtamalized maize flours (NMF). T-NMF, tortilla nixtamalized maize flour; S-NMF, specialty nixtamalized maize flour. Each curve is the average of two RVA or Mixolab analyses. [Color figure is available in the online version].

Fig S1. Influence of mixing speed in the development time and stability loss of nixtamalized maize dough. Analyses were performed at fixed hydration according to the supplier recommendations. Each curve is the average of two Mixolab analyses.

Temperature rise	C3 (Nm)
4°C/min	0.898 b
6°C/min	0.915 ab
8°C/min	0.933 a
10°C/min	0.932 a

Fig. S2. Influence of the heating/cooling rate in the Mixolab profile. Continuous line represents the nixtamalized maize flour curve, dashed line is the block temperature and the dotted line is the dough temperature. Analyses were performed at fixed hydration according to the supplier recommendations. Each curve is the average of two Mixolab analyses.