https://doi.org/10.3311/PPch.19019
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Periodica Polytechnica Chemical Engineering, 66(3), pp. 437–447, 2022

Investigation of the Role of Arabinoxylan on Dough Mixing Properties in Native and Model Wheat Dough Systems

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Received: 03 July 2021, Accepted: 23 February 2022, Published online: 08 March 2022

Abstract

The aim of this work was to investigate and compare the effect of arabinoxylan (AX) addition and incorporation on the mixing properties of native and model doughs of different wheat types, to get more insight into the role of AXs in dough formation. In the experiments, flour samples of a wheat variety (normal starch type) and two wheat lines (waxy and high amylose) were used. Model doughs were composed by fractionating flours into starch and gluten followed by subsequent reconstitution according to their original gluten to starch ratio. AX isolate was dosed in 1% and 3% to the native and model doughs. Incorporation of AX was performed by reduction and re-oxidation of wheat dough with dithiothreitol (DTT) and KIO_3, respectively.

Model doughs behaved similarly to native doughs thus were found appropriate for the model experiments. In general, higher AX level resulted higher dough consistency in every dough system compared to the corresponding base dough, however, the extent of the growth was different. In case of assumed AX incorporation only small differences were found in the mixing properties compared to AX addition. Based on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis, some minor but clear changes were observed in the protein subunit profile of AX containing doughs compared to base doughs, but no difference was identified between doughs made by AX addition and AX incorporation. However, the characterization of the gluten-AX interactions requires more detailed investigation, in which a pure gluten-starch-AX model system can offer a valuable, well-defined matrix.

Keywords

arabinoxylan, gluten network, model dough, addition, incorporation

1 Introduction

Wheat is one of the oldest cereals [1] and is the most important raw material of bread and other baked goods due to its unique ability to form a viscoelastic dough when hydrated and mixed, which can trap carbon dioxide during fermentation and form a stable porous structure when baked.

In the structure formation of dough, storage proteins of wheat, termed as gluten proteins (80–85% of the total wheat proteins), play a key role by creating a continuous protein network, which interact with the other flour constituents (starch, non-starch polysaccharides, lipids) and with the added ingredients (salt, sugar, etc.). This macromolecular complex is stabilized by covalent disulfide bond and non-covalent forces (hydrogen bonds, hydrophobic bonds, ionic bonds) [2–5].

Starch is the most abundant component of wheat flour, and its functional properties depend on several factors, such as granule size, composition (lipids, minerals), molecular structure of amylose and amylopectin (degree of polymerization, branching) and their ratio. Starch properties can be influenced by technological parameters (e.g., level of starch damage) and are also in relation to the activity of hydrolytic enzymes. Starch contributes greatly to the formation of dough properties and the stabilization of crumb structure and affects the staling process of bread [6–8].

The third most important flour constituents are the non-starch polysaccharides (NSPs), which have several health benefits as dietary fibers as well. The major non-starch polysaccharides of wheat are pentosans, especially arabinoxylans (AXs). AXs consist of linear

Cite this article as: Németh, R., Fekete-Papp, R., Orosz, K., Jaksics, E., Juhászné Szentmiklóssy, M. K., Török, K., Tömösközi, S. "Investigation of the Role of Arabinoxylan on Dough Mixing Properties in Native and Model Wheat Dough Systems", Periodica Polytechnica Chemical Engineering, 66(3), pp. 437–447, 2022. https://doi.org/10.3311/PPch.19019

(1,4)-β-D-xylopyranosyl-chains, which can be substituted at the O-2 and/or O-3-positions with α-L-arabinofuranosil side chains. An important minor component of AXs is ferulate bound to arabinose as an ester at the O-5-position, which allows the formation of covalent cross-links of the AX molecules with each other and/or with proteins [9]. The physicochemical properties of AXs highly depend on their molecular weight, A/X ratio, and the number of di-ferulate crosslinks [10–13]. Generally, water extractable AXs (WE-AXs) are considered to be beneficial to techno-functional and nutritional properties, while water unextractable AXs (WU-AXs) usually have detrimental effect on dough and end-product properties [2]. However, there is still a polemic about the type of interactions between AX molecules and gluten proteins both in wheat and rye doughs. Most studies concluded that AX is affecting the protein network formation by competing for water and by forming physical barriers causing interference in protein interactions [14–16]. Some studies presumed covalent cross-links between proteins and AX [9, 17, 18], while others refuted this [16, 19]. There is also only a few information about protein-AX-starch interactions [20, 21] therefore, further investigations are needed in this field.

Different approaches exist for investigating the contributions of individual flour components to techno-functional properties. Firstly, there can be used native or reconstituted flours as matrices. In the first case, flour constituents can be studied in their native form, however, the ratio of the flour constituent changes, and other matrix components might interfere also. The other approach means the separation of flour into fractions or components and then reconstitute the flour/dough creating a simpler model system, which allows the analysis of the effect of a specific component without the influence of further ingredients. In this case the main problem might be the possible modification of the constituents during the isolation process [22, 23]. The foundation of wheat flour fractionation and reconstitution was laid by Finney [24]. Since then, reconstitutional studies and the use of model doughs are important tools to investigate the role of flour constituents [15, 22, 25–30].

Furthermore, the effect of a specific constituent can be investigated by simple addition or by incorporation [31]. In the case of addition, no integration of the component can be assumed in contrast to incorporation. Several research confirmed that when wheat dough is taken through a carefully controlled reduction/re-oxidation cycle, its behavior is similar to that of the untreated original dough [24, 32]. This phenomenon was utilized first by Békés et al. [33] for the external incorporation of high molecular weight glutenin subunits into wheat dough using 2g-Mixograph as measuring tool. In the study dithiothreitol (DTT) and KIO_3 were found as the most appropriate redox agents. DTT prevents the formation of the glutenin polymer, while KIO_3 serves as a fast-acting oxidant resulting the formation of disulfide bonds [34]. Since that, several studies have been published about successful incorporation of protein subunits into wheat dough [31, 35] or even rice dough [36, 37] using dough reduction/re-oxidation. However, this method has not been applied in non-starch polysaccharides related studies yet.

In most of the previously mentioned studies, the effect of AXs was investigated by simple addition into native or reconstituted model doughs, and usually only one variety was involved in the experiments.

The aim of our work was to investigate the effect of AX on dough mixing properties by incorporation applying reduction/re-oxidation of the dough and to compare it with simple AX addition. Three wheat flour samples of different gluten content and amylose to amylopectin ratio were used in the experiments to provide different technological properties. Incorporation and addition studies were carried out both in native dough and reconstituted model dough systems. This study serves as the first steps of a more extensive fundamental research study.

2 Materials and methods

2.1 Flour samples

White flours of three wheat samples provided by Agricultural Institute, Centre for Agricultural Research (Martonvásár, Hungary) were used for the experiments: MV HOMBÁR, 999-22/LONA//LONA/3/LONA/4/-999-22/LONA//-LONA/3/LONA (abbr. LONA), KOLO/NX02Y4481 (abbr. KOLO). MV HOMBÁR is a Hungarian winter wheat cultivar representing an average wheat quality and normal starch composition (amylose content: 23%). Therefore, it was hereinafter referred to as "normal wheat". "LONA" and "KOLO" are wheat lines with special starch composition. "LONA" is a high-amylose line (amylose content: 34%, indicated as "high-amylose wheat"), while "KOLO" is a waxy type (amylose content: 15.5%, indicated as "waxy wheat"). The flour samples were produced with a CD1 laboratory mill (Chopin, Villeneuve-la-Garenne, France) according to ISO 27971:2015 standard [38].

2.2 Preparation of model flours

Gluten and starch were extracted from the flours by using a gluten washer (GluStar System®, developed by BME and

Lab-Intern Ltd., Hungary) equipped with a filter module for separating starch from the effluent as follows. Firstly 10.0 g flour was kneaded into a dough ball with 5.05 ml 2% NaCl solution. After 1 min resting time, a dough ball was kneaded for 30 s and was put into the washing chamber of the gluten washer. The program settings of the instrument were as follows: pre-mixing time: 5 s, washing time: 8 min. The gluten retaining in the washing chamber was rinsed manually with distilled water for 1 min. For starch separation, qualitative filter paper (125 mm, 12–15 µm particle retention) was applied. After filtration, starch was rinsed three-times with distilled water and removed from the filter paper. The gluten and starch samples were freeze-dried for 24 h and pulverized in a mortar. The model flours were prepared by mixing the isolated flour fractions based on the original gluten to starch ratio of the native flours just before the analysis of mixing properties as described in Section 2.4.

2.3 Compositional analysis

Moisture and ash content of native flour samples and the isolated starch and gluten fractions were determined by oven drying method according to ICC Standard Method Nr. 110/1 [39] and by muffle furnace method (ISO 2171:2007) [40], respectively. Determination of protein content was carried out by Dumas method (ISO 16634-2:2016) [41] using a FP-528 instrument (Leco, Saint Joseph, USA). To calculate protein content, conversion factor of 5.70 was applied.

Wet and dry gluten content of the flours were determined according to ISO 21415-2:2015 [42], and ISO 21415-4:2006 [43] methods, respectively, using Glutomatic 2200 and Glutork 2020 (Perten, Höganas, Sweden) instruments.

Total arabinoxylan (TOT-AX) and water-extractable arabinoxylan (WE-AX) content of flours and isolated starch and gluten fractions were determined by gas chromatography method described by Gebruers et al [44]. For the analysis an Elite-17 column (SN: 455178; 60 m × 0.25 mm × 0.25 µm, Perkin Elmer, USA) and Clarus 500 Gas Chromatograph (Perkin Elmer, USA) was used, equipped with an auto-sampler and injection port (injection volume: 1 µl, split: 1:8; speed: normal, sample pumps: 6 temperature: 300 °C). Analytes were separated at 250 °C (hold: 14 min) and detected by Flame Ionization Detector (attenuation: −1, offset: 5.0 mV, range: 1) at 300 °C (carrier gas: He, 1 ml/min). The used auxiliary gasses were H_2 (45 ml/min) and air (450 ml/min). Other settings were as follows: bunching factor (pts): 1, noise threshold (µV): 100. The evaluation of the chromatograms was carried out by TotalChrom Navigator software (Perkin Elmer, USA). The compositional measurements were carried out in duplicates.

2.4 Measurement of mixing properties using micro-doughLAB

To study mixing properties, micro-doughLAB instrument (Newport Scientific, Warriewood NSW, Australia, now Perten Instruments AB, Stockholm, Sweden) was applied, which requires only 4 g of flour at 14% moisture basis, therefore is suitable for measuring materials available in limited amount.

2.4.1 Standard protocol

Native and model flours were analyzed and compared using the Standard Flour Testing Method (mixing speed: 63 rpm; temperature: 30 °C, pre-mixing time: 1 min, analysis time: 20 min) of the instrument [45]. Pre-mixing time of 15 min was set in the software for preparing the model flours from gluten and starch fractions before starting the measurement. The measured parameters were water absorption (WA%, the amount of water in % to reach 500 ± 20 FU target consistency at 14% moisture basis); dough development time (DDT, time (min) for reaching 500 FU consistency of the middle line); stability (S, difference (min) between the time at which the top of the curve reaches the 500 FU line and the time at which the top of the curve falls below 500 FU); degree of softening (DS, difference in FU between consistency of middle line at DDT (500 FU) and at the end of analysis). The measurement was performed in triplicates.

2.4.2 Protocol for arabinoxylan addition and incorporation

For the experiments, commercial wheat arabinoxylan was applied (P-WAXYI, LOT: 120801b; insoluble from wheat flour; purity: 80%; sugar composition: 36% arabinose, 51% xylose, 6.5% glucose, 4.4% mannose, 1.6% galactose; A/X ratio= 0,71; protein content: 2.7%; Megazyme, Bray, Ireland) in 1% and 3% on flour basis.

The reduction and re-oxidation of native and model doughs with or without AX was performed based on the work of Oszvald et al. [36] with slight modifications using the micro-doughLAB.

For the partial reduction of the doughs, dithiothreitol (DTT BioChemica, A1101, LOT: 0C012275, purity: 97%, AppliChem, Darmstadt, Germany) was used and dosed to the flours or flour-AX mixtures as 1 ml aqueous solution of

10 mg/ml concentration (optimized based on pre-trials, data not shown) at the start of the measurement. To re-oxidize doughs, 250 µl aqueous solution (25 mg/ml) of KIO_3 (a.r., B-002360, Reanal, Budapest, Hungary) was applied.

Dry ingredients were pre-mixed for 15 min. Initial dough mixing was performed for 30 s to hydrate flour particles and to add DTT solution, then the dough was rested without mixing for 4 min to reduce gluten network. Then mixing was implemented for further 30 s, while KIO_3 solution was dosed to the dough, which was rested for further 6 min. The total analysis time was 30 min. During the measurements, constant water absorption levels were used as determined in the standard protocol (Section 2.4.1) for each native and model flours. The investigated curve parameters were the same as in the case of standard protocol with slight modifications: instead of 500 FU, maximum consistency was used as reference point.

"Base dough" of native and model flours was defined as dough without any treatment. AX addition was defined as AX dosage without reduction/re-oxidation (indicated as 1% AX and 3% AX), while incorporation means AX dosage with reduction/re-oxidation (indicated as 1% AX + Red-Ox and 3% AX + Red-Ox). Reduction/re-oxidation was performed also in the case of no AX dosage (indicated as Red-Ox). The measurements were performed in duplicates due to the limited amount of the isolates.

2.5 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

At the end of micro-doughLAB measurement described in Section 2.4, dough samples were collected and freeze-dried. The effect of AX addition or AX incorporation on the protein fractions were investigated by SDS–PAGE according to the method of Laemmli [46]. Both non-reduced and reduced SDS–PAGE were performed using a 12% separating gel (pH 8.8) and a 4% stacking gel (pH 6.8). Briefly, 20 mg dried dough sample was stirred in 400 µl loading buffer (pH 6.8, 0.076 M Tris–HCl, 2.5% (w/v) sodium dodecyl sulfate (SDS), 12% (v/v) glycerol, 0.05% (w/v) bromophenol blue). For reduced SDS–PAGE, the sample buffer contained 6% (v/v) β-mercaptoethanol. The dispersions were centrifuged (4000 g, 3 min) after heating in boiling water for 5 min. The supernatants (10 µL) were loaded into each lane. Electrophoresis was performed at 80 V for 10 min and 120 V for 80 min using Mini-PROTEAN Tetra System (Bio-Rad, USA).

The applied ladder comprises of 10 fragments: ~260, ~140, ~95, ~72, ~52, ~44, ~34, ~26, ~17 and ~10 kDa (Protein Marker VI (pre-stained), peqGOLD, VWR Peqlab).

2.6 Statistical analysis

Data were evaluated using MS Excel (Microsoft 365, Microsoft Corporation, Redmond, WA, USA) and Statistica 13 software (TIBCO Software Inc., Palo Alto, California, USA). One-way ANOVA and Tukey's Honestly Significant Difference (HSD) post hoc test were used to determine if means were significantly different at 95% level of confidence.

3 Results and discussion

3.1 Chemical composition of native flours and isolated flour fractions

The chemical composition of the native flours is summarized in Table 1. The ash content of the flours, since they were produced at the same conditions, did not differ, and ranged from 1.31% to 1.48%. Among wheat types, flour of waxy wheat had the highest protein content (17.75%), while the lowest protein level belonged to normal wheat (12.36%). The highest TOT-AX content (2.19%) accompanied with the highest WE-AX content (0.72%) was measured in the case of high-amylose wheat. Normal wheat had the lowest TOT-AX (1.42%) and WE-AX (0.44%) content among the samples, however, waxy wheat had relatively lower WE-AX to TOT-AX ratio (0.27) than normal and high-amylose wheat flours (0.31 and 0.33, respectively). To reconstitute the flours according to their original gluten to starch ratio, wet and dry gluten content of the native flours were also determined. Waxy wheat can be characterized with the highest wet gluten (44.4%) and dry gluten (14.6%) content, while normal wheat and high-amylose wheat had significantly lower wet gluten (29.1% and 28.6%, respectively) and dry gluten content (9.9% and 9.4%, respectively). These were in line with the protein content of the flours.

According to the results, native flours were significantly different in technologically most important compositional parameters, thus providing different matrices for the experiments, as expected.

Flours were fractionated with a gluten washer into starch and gluten fractions as described in Section 2.2. The yields of gluten isolates at dry matter basis were 8.9% for normal wheat, 9.3% for high-amylose wheat and 14.6% for waxy wheat, while starch yields were 73.0%, 76.5% and 65.8% at dry matter basis, respectively. Purity of the isolates was determined by compositional analysis. Based on the results (Table 1), the purity of gluten isolates was higher than 80% (according to the measured protein levels), which was in agreement with the results of others [22, 30]. The ash content of isolated gluten samples was between 1.84–2.64%, which can be

Table 1 Chemical composition of native flours, isolated starch and gluten fractions (mean ± SD)

		Moisture (%)	Ash (% DM)	Crude protein (% DM)	TOT-AX (% DM)	WE-AX (% DM)
Normal wheat	native flour	13.98 ± 0.24^{A}	1.31 ± 0.20^{A}	12.36 ± 0.24^{A}	1.42 ± 0.2^{A}	0.44 ± 0.003^{A}
	starch isolate	1.92 ± 0.45^{a}	$< LOD^{a}$	1.13 ± 0.24^{a}	1.44 ± 0.12^{a}	$< LOD^{a}$
	gluten isolate	$3.87 \pm 0.11^{\alpha}$	$2.64 \pm 0.15^{\alpha}$	$80.62 \pm 0.24^{\alpha}$	$0.46 \pm 0.11^{\alpha}$	$< LOD^{\alpha}$
High-amylose wheat	native flour	14.08 ± 0.35^{A}	1.48 ± 0.17^{A}	13.28 ± 0.02^{B}	2.19 ± 0.01^{B}	0.72 ± 0.01^{B}
	starch isolate	2.22 ± 0.14^{a}	$< LOD^{a}$	1.32 ± 0.04^{b}	2.10 ± 0.01^{b}	0.12 ± 0.01^{b}
	gluten isolate	$3.55 \pm 0.00^{\alpha}$	$1.84 \pm 0.03^{\beta}$	$87.99 \pm 0.30^{\beta}$	$< LOD^{\beta}$	$< LOD^{\alpha}$
Waxy wheat	native flour	13.65 ± 0.11^{A}	1.43 ± 0.42^{A}	17.75 ± 0.07^{C}	1.81 ± 0.00^{C}	0.48 ± 0.00^{C}
	starch isolate	2.55 ± 0.04^{a}	$< LOD^{a}$	1.07 ± 0.05^{a}	1.70 ± 0.02^{c}	$< LOD^{a}$
	gluten isolate	$3.70 \pm 0.12^{\alpha}$	$2.86 \pm 0.01^{\alpha}$	$81.91 \pm 0.35^{\alpha}$	$0.67 \pm 0.03^{\gamma}$	$< LOD^{\alpha}$

Results within a column with different superscript letters indicate that the difference between wheat types within the investigated material (native flour: A, B, C; starch: a, b, c; gluten: α, β, γ) is significant at the 0.05 level.

explained by sodium chloride from the washing solution remained in the gluten network.

Based on the determined TOT-AX and WE-AX values, the removal of AXs from gluten was accomplished with high efficiency. Starch isolates contained around 1% protein, and their ash content was below limit of detection. This is in line with findings in the literature [22]. However, the TOT-AX content of the starches was 1.44–2.10% but no water extractable AX was detected from the samples, suggesting that mainly insoluble constituents remained in the starch fraction.

In conclusion, the isolation procedure using GluStar System provided flour fractions of acceptable purity, therefore the isolates were found suitable for creating model flours.

3.2 Mixing properties of native and model flours

Based on dry gluten contents of the native flours, the gluten to starch ratio of model flours was defined as follows: 0.11 for normal wheat, 0.10 for high-amylose wheat and 0.17 for waxy wheat.

The mixing curves of native and model doughs are presented in Fig. 1. High-amylose wheat can be characterized with the strongest dough showing high resistance against mixing, while normal wheat provided the weakest dough structure. Waxy wheat showed similar mixing profile to high-amylose wheat. These observations were supported by the parameter values of the curves, also in Table 2. Water absorption of the flours varied in a wide range: the lowest value belonged to normal wheat (48.91%) and the highest to high-amylose wheat (64.43%). The native flours showed significant difference from each other in DDT (1.30–5.53 min) and DS (70.0–189.9 FU) values as well. The stability of normal wheat was the lowest with 1.73 min, while high-amylose and waxy wheat flours reached very similar values (5.0 min and 5.07 min, respectively. When investigating model dough, based on the curve profiles, it was found that model systems of normal wheat and waxy wheat were highly similar with their native counterparts (Fig. 1).

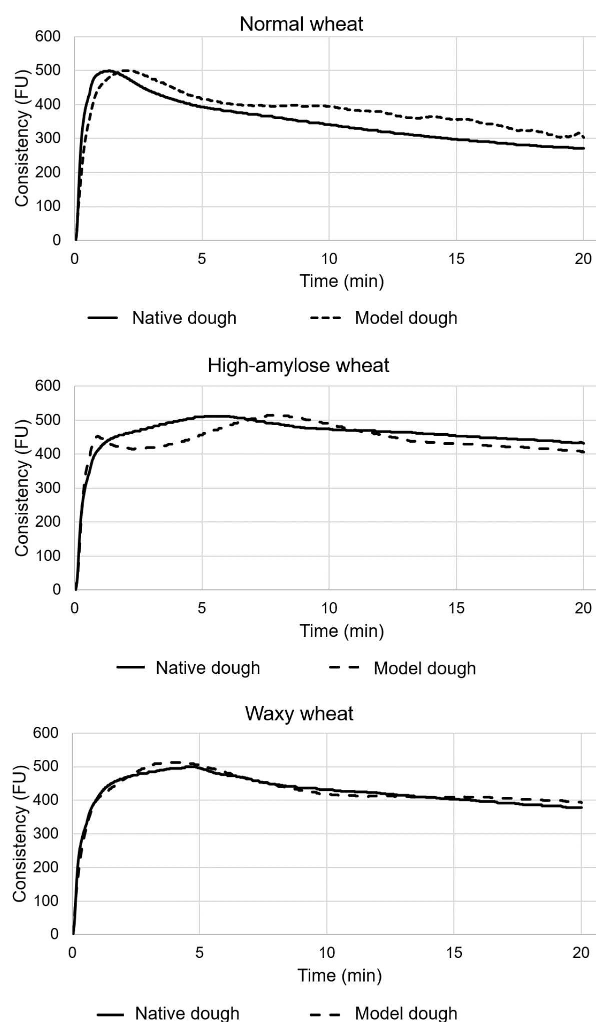

Fig. 1 Micro-doughLAB mixing curves of native and model dough systems

Table 2 Micro-doughLAB parameters of native and model dough systems measured by standard protocol (mean ± SD)

		WA (%)*	DDT (min)	S (min)	DS (FU)
Native dough	Normal wheat	48.91	1.30 ± 0.10^{Aa}	1.73 ± 0.06^{Aa}	189.90 ± 8.66^{Aa}
	High-amylose wheat	64.43	5.53 ± 0.42^{Ba}	5.00 ± 0.20^{Ba}	70.00 ± 10.00^{Ba}
	Waxy wheat	56.85	4.70 ± 0.10^{Ca}	5.07 ± 0.12^{Ba}	105.00 ± 5.00^{Ca}
Model dough	Normal wheat	47.3	2.00 ± 0.00^{Ab}	2.50 ± 0.00^{Ab}	134.93 ± 9.95^{Ab}
	High-amylose wheat	69.08	7.87 ± 0.06^{Bb}	3.80 ± 0.10^{Bb}	106.67 ± 2.89^{Bb}
	Waxy wheat	56.85	3.93 ± 0.21^{Cb}	4.07 ± 0.31^{Cb}	103.33 ± 12.58^{Ba}

*Constant value: results within a column with different superscript letters indicate that the difference is significant at the 0.05 level. A, B, C are used for indicate differences between wheat types within dough systems (native or model), while a, b, c letters refers to significant difference between dough systems within wheat types (normal, high-amylose or waxy).

Notable difference in the curve profile (slowest dough development) was observed in case of high-amylose wheat. Regarding curve parameters of native and model dough systems, significant differences were found between native and model doughs in most cases, however in tendencies, model doughs reflected the behavior of that of native doughs. Modified properties of reconstituted doughs compared to native ones were experienced by other researchers as well [22], which could be explained by the removal of soluble constituents and/or molecular changes during the fractionation. Relative to each other, model dough systems held the properties of native doughs, and based on standard deviations, they could be prepared with good reliability. Therefore, they were found suitable for further experiments.

3.3 Comparative analysis of AX addition and AX incorporation

The mixing curves for AX addition and incorporation in native and model dough systems of the three different wheat types are presented in Fig. 2, while the values of curve parameters can be found in Fig. S1.

It was found that AX levels of 1% and 3% caused higher dough consistency and maximum consistency in both native and model dough systems compared to the corresponding base doughs, but it did not influence the shape of the curves. Although, model doughs had slightly different profile, they showed similar tendencies to native dough systems in case of AX addition. As a result of reduction/oxidation, native and model doughs without AX (Red-Ox) showed similar curve profile to the corresponding base doughs indicating that the dough structures were restored in a great extent. Based on the curves, however, no prominent difference can be observed between simple AX addition and AX incorporation, overall.

The highest consistency growth (Fig. S1 A in the Supplement) was observed in case of normal wheat dough systems (both native and model) in the most cases, meaning around 100 FU and 300 FU higher consistency compared to the corresponding base doughs, in case of 1% AX and 3% AX addition, respectively. In case of the other two wheat types, the tendency of consistency growth was similar, but its extent was slightly lower than that of normal wheat. The higher consistency of AX dosed doughs at constant water absorption can be explained by the fact that AXs act as hydrocolloids binding significant amount of water [47].

Reduced and re-oxidized (Red-Ox) native and model doughs reached similar maximum consistency as the corresponding base doughs in case of all wheat types, being in accordance with the observed restoration of mixing curves. The incorporation of 1% and 3% AX (1% AX and 3% AX + Red-Ox) resulted similar consistency increase to simple AX addition, even the measured values were very close to each other. When comparing native and model dough systems with each other, lower maximum consistency of the model doughs in case of normal and high-amylose wheat was measured, while in case of waxy wheat, the values of model doughs were similar (in case of AX incorporation) or in some cases slightly higher (AX addition) than that of native doughs.

The effect of AX addition on dough development time (DDT) showed different tendencies among wheat types, and in some cases native doughs and their model counterparts behaved also different (Fig. S1 B). It was found that in native doughs of normal and waxy wheat, AX additions resulted longer dough development compared to native base dough, while in case of high-amylose wheat, the opposite was observed. When studying model doughs of the three wheat types, it can be stated that AX additions caused faster dough development compared to model base doughs in all cases.

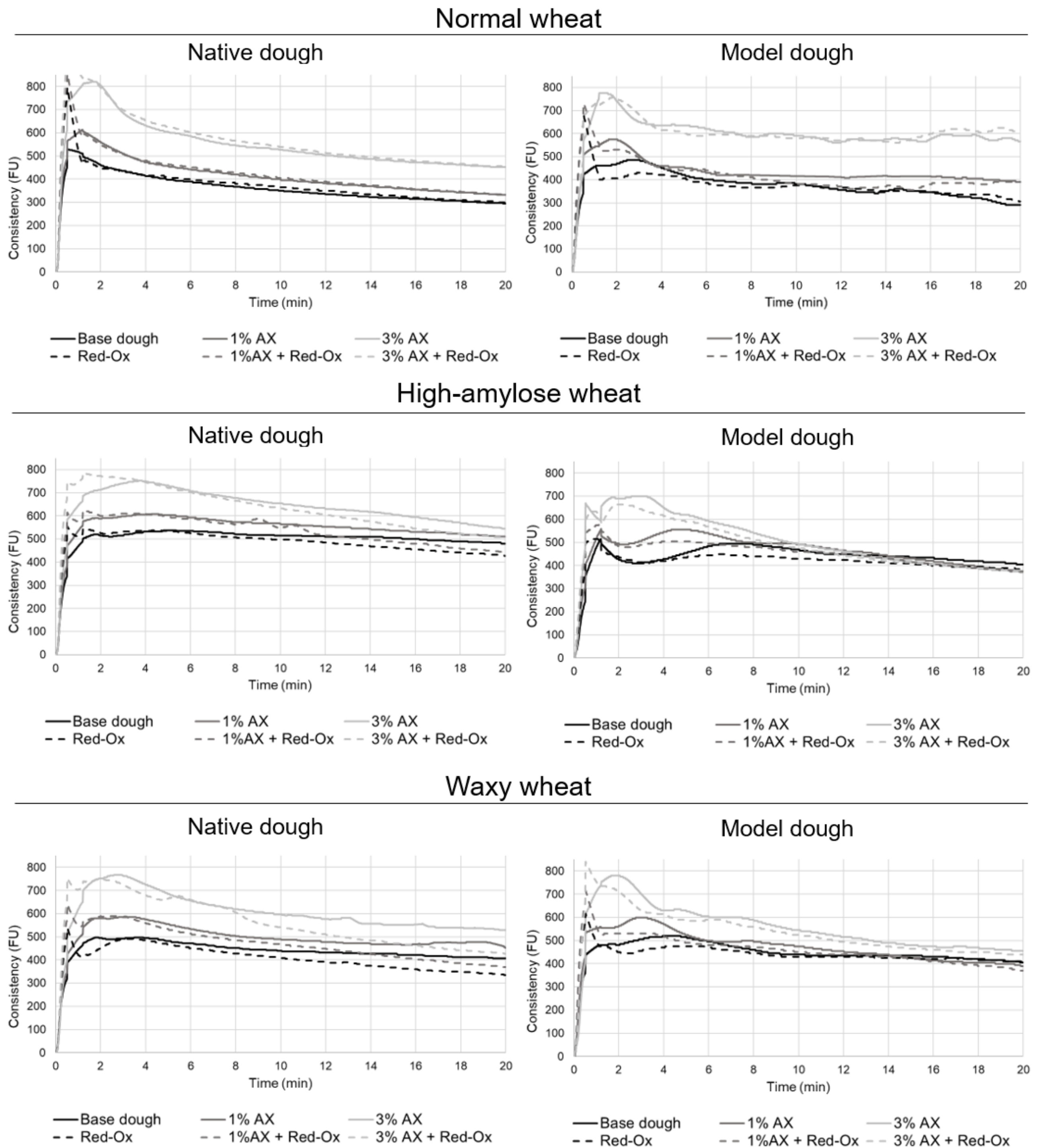

Fig. 2 Micro-doughLAB mixing curves of native and model dough systems without AX (base dough and Red-Ox), and in case of AX addition (1% and 3% AX) and AX incorporation (1% and 3% AX + Red-Ox)

Reduced DDT triggered by AX addition was described by others as well, and it might be attributed to weak secondary bonds forming between AX and gluten molecules [48, 49].

Red-Ox doughs (native and model) had longer dough development than base doughs in the most cases, which can be explained by the time required for re-oxidation of gluten network. AX incorporation had the same DDT reducing effect as simple addition with some exceptions. In case of reduced/re-oxidized native normal wheat dough systems, AX incorporation did not change DDT, but did in case of model systems.

The different tendencies found in DDT values might be explained by methodological reasons or by the compositional differences between native and model doughs

(missing soluble proteins and pentosans) resulting different interactions with AX.

In general, dough stability (Fig. S1 C) was influenced negatively by AX addition in all dough system except native normal wheat dough, where no difference was found between the value of base dough and AX added doughs. The observed dough weakening effect of AX addition was in accordance with other findings [47]. The same stability lowering effect was found in case of AX incorporation as in AX addition. The similar dough weakening effect of AX addition and AX incorporation was supported by the measured degree of softening values (Fig. S1 D) as well.

To investigate the effect of AX addition and AX incorporation on protein fractions, SDS-PAGE was also carried out in case of normal wheat as an example (Fig. 3).

Native base dough in non-reducing and reducing conditions (BD and BD*, respectively) showed similar protein profile to the flour (F and F*) but the bands were much lighter, which can be explained by the lower extractability of proteins from dough, in which glutenin macropolymer (GMP) has been formed [50].

In non-reducing conditions, AX addition (in 1% and 3%) in native and model doughs resulted a broadening in the molecular weight range of 25-36 kDa (region of *α*-*β*-*γ* gliadins in flour) as well as of 10-12 kDa (albumin/globulin region in flour) fractions compared to the base doughs. However, this effect disappeared when applying reducing sample preparation.

In case of the AX containing Red-Ox native and model doughs, the same phenomenon can be observed as in the case of simple AX addition. Although, the used AX isolate contained 2.7% protein, this amount is not that high that would explain so intensive band broadening. Furthermore, this isolate was chosen because, according to the manufacturer's specification, it was carefully extracted and purified to maintain the ferulic acid crosslinks, which are the reactive parts of AXs.

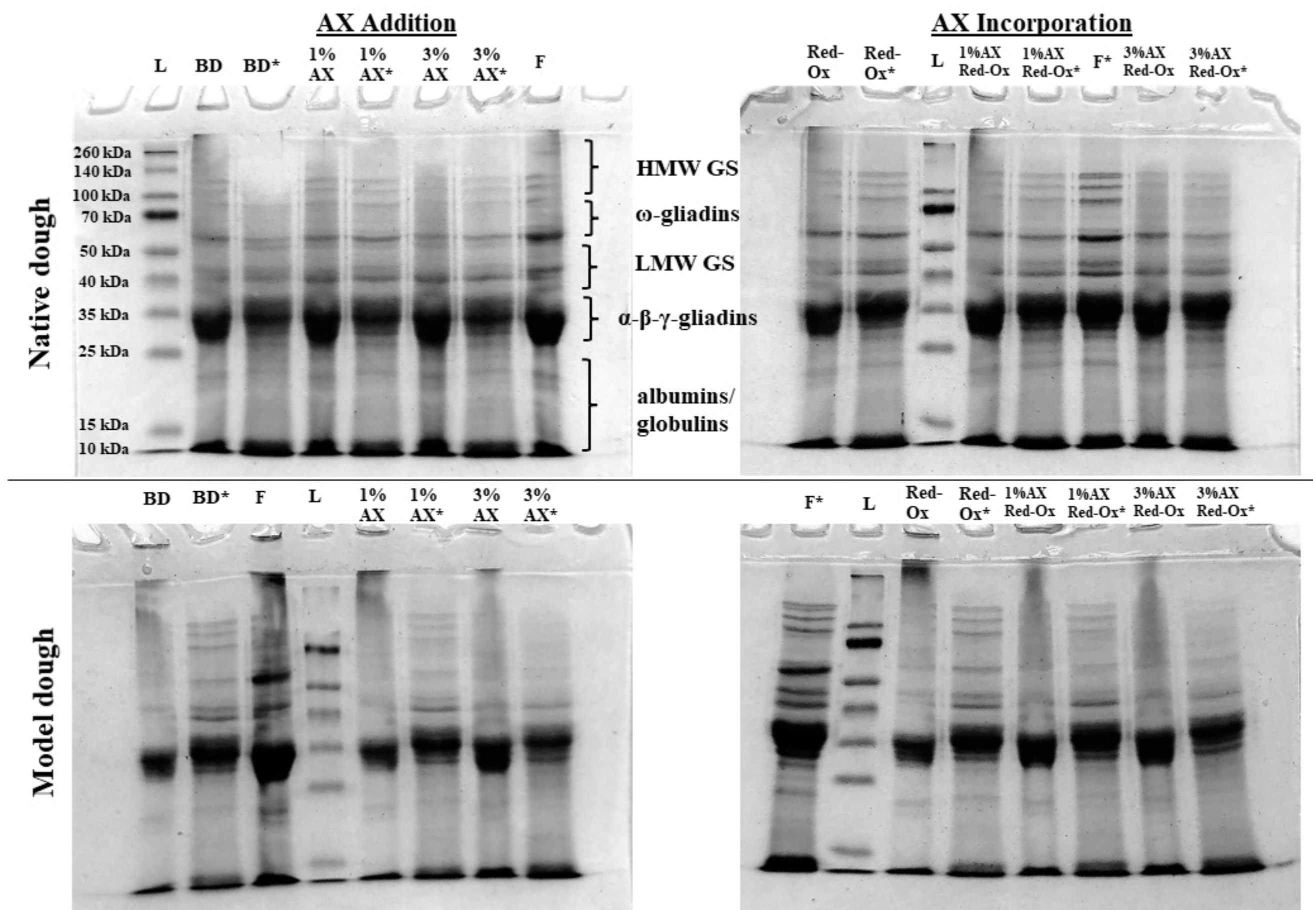

Fig. 3 Electropherograms of native and model doughs of normal wheat in case of AX addition and AX incorporation. BD: base dough, F: flour, L: ladder, HMW/LMW GS: high/low molecular weight glutenin subunit.
*Reducing sample preparation

These findings might strengthen the hypothesis about AX-protein interactions, however, revealing the exact mechanism needs further investigations.

4 Conclusion

Our hypothesis was that experiments in model doughs with a simplified gluten-starch composition compared to complex flour addition experiments might allow the identification of macromolecular interactions (AX-protein) better than the widely applied addition procedure. Incorporation using reduction and re-oxidation of wheat dough components was expected to identify complex forming ability derived from ferulic acid side chains. Changes in the rheological properties of the dough in the case of addition and incorporation could provide indirect information on the molecular changes and their effects. The relatively small differences observed between simple addition and incorporation systems suggested that such processes that significantly affect the rheological behavior do not take place, or their effect on the mixing properties is not significant. This was partially confirmed by SDS-PAGE. However, changes in molecular weight distribution of specific protein fractions (25–36 kDa, 10 kDa) refers to the formation of AX-protein interactions.

This is the first time when incorporation was applied as research tool for investigating the role and the mechanism of non-starch polysaccharides in dough formation and structure. Our aim was primarily to develop the methodology of AX incorporation in a simple model dough system and to investigate its applicability. Based on the results, model doughs seemed to be suitable for studying the effect of different treatments, however, reproducibility of the methodology should be studied further.

In the continuation of this work, possible changes of macromolecules during treatments, as well as molecular interactions (AX-protein, AX-starch and AX-starch-protein) will be investigated in more detail. In our further experiments, the involvement of other AX isolates and non-starch polysaccharides (e. g. β-glucans), as well as the study of other dough properties (thermomechanical and baking properties) are part of our future plans.

Acknowledgement

The project presented in this article was supported by applied research project titled "New aspects in wheat breeding: improvement of the bioactive component composition and its effects" (Hungarian Scientific Research Fund (OTKA) K112179 and K112169). The work is connected to the fulfil of scientific goals of Thematic Excellence Program (BUTE Biotechnology thematic program, TKP 2021 – BME-EGA 2, NRDI Office), and the National Talent Program of Ministry of Human Resources (NTP-NFTÖ-20-B-0272). Special thanks for Erika Szűcsné Makay for her kind help in compositional analyses.

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