Wheat Gluten Phenolic Acids: Occurrence and Fate upon Mixing

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Ferulic acid (FA, 4.9−17.7 μg/100 mg), sinapic acid (SA, 1.4−3.5 μg/100 mg), and traces of *p*-coumaric acid and vanillic acid were detected after saponification of six wheat glutens from industrial and pilot-scale origins. FA and SA occurred mostly as soluble-bound and insoluble-bound forms according to their extractability by acetone/methanol/water (7:7:6, v/v/v). The major part of FA (50−95%) was found in the unextractable fraction, whereas SA was mostly extractable (64−85%). The carbohydrate contents of the glutens were determined also after acid hydrolysis. The highest levels of glucose, arabinoxylan, and FA were obtained from the unextractable fractions of the pilot-scale extracted glutens, probably in relation with a lower efficiency of washing during extraction compared to industrial processes. On the other hand, SA compounds were in similar concentrations in all samples, suggesting their involvement in specific interactions during gluten protein agglomeration. Saponification of the soluble-bound phenolic acids released mainly glucose, whereas a β-glucosidase treatment had no effect. FA and SA extractability, especially that of soluble-bound ones, decreased strongly in overmixed gluten/water doughs. These low molecular weight conjugates of phenolic acids could be involved in the dough breakdown phenomenon.

Keywords: *Ferulic acid; sinapic acid; carbohydrate; wheat gluten; mixing*

INTRODUCTION

The gluten proteins are largely responsible for the viscoelastic properties of wheat doughs and therefore contribute to flour's potential for bread-making (Wall, 1979). The procedure for gluten isolation consists of washing a wheat flour dough. Such a process leads to glutens that contain, besides proteins, all of the other flour components (lipids, minerals, and carbohydrates) in minor amounts, varying according to the isolation procedure. Glucose is the main carbohydrate of gluten hydrolysates, originating from starch, but some nonstarch polysaccharides composed of arabinose, galactose, and xylose are also found (Roels et al., 1998; Saulnier et al., 1997). Saulnier et al. (1997) reported a positive correlation between starch and arabinoxylans (AX) content of gluten, meaning that the presence of polysaccharides is mainly related to the washing efficiency during gluten preparation. Roels et al. (1998) have shown that commercial glutens contain fewer carbohydrates than glutens isolated from a pilot-scale process, probably due to a greater efficiency of the washing in the industrial process. These carbohydrates may affect gluten functionality by interacting with the gluten proteins and participate in the dough properties.

Phenolic compounds are largely present in wheat flours, ferulic acid (FA) being predominant. Free FA, low molecular weight (LMW) conjugates, and FA covalently bound to macromolecules have been found in wheat flours and glutens (Sosulki et al., 1982; Jackson and Hoseney, 1986; Labat et al., 2000a). The macromolecules correspond to AX to which FA is ester-linked. LMW conjugates are much less documented. The presence of FA esters of stanol and stenol (Seitz, 1989) and of FA esters of glucose (Herrmann, 1989) has been reported in wheat flours. It has been suggested that during mixing of a wheat flour dough, FA, especially in soluble-bound form (LMW conjugates), could react with protein sulfhydryl groups (Jackson and Hoseney, 1986) or increase the rate of protein sulfhydryl−disulfide interchanges (Labat et al., 2000b) to cause the dough breakdown phenomenon. However, other phenolic acids have been found in wheat flours and glutens, namely, syringic, vanillic, coumaric, caffeic, and sinapic acids (Gallus and Jennings, 1971; Sosulki et al., 1982). No data are available on the contribution of the different phenolic acids in gluten mixing properties.

The objective of this study was therefore to characterize the major phenolic acids of several glutens and follow their behavior during gluten/water mixing.

MATERIALS AND METHODS

Glutens. Six vital wheat glutens were used in this study. Amylum Aquitaine (Bordeaux, France) provided commercial wheat glutens (lots 1−4). The Institut Technique des Céréales et des Fourrages (Boigneville, France) provided glutens from wheat cv. Scipion and Soissons harvested in 1997 at various locations in France, isolated from a pilot-scale extracter and collected from 250 and 400 μm sieves.

Mixing Conditions and Sample Preparation. Gluten and water were mixed in a Brabender counter-rotating batch mixer (Brabender O.H.G., Duisburg, Germany) at 100 rpm. The mixing was monitored and recorded by the software PL2000 (Brabender). The mixing chamber (thermostated at 25 °C) was filled with 46.0 g total mass with a water absorption level of 130% (w/v). Doughs were sampled after two peak times (overmixing conditions) and immediately frozen in liquid nitrogen, freeze-dried, and ground in an IKA laboratory mill (IKA, Janke & Kunkel, Staufen, Germany) to pass a 0.5 mm sieve.

Analysis of Phenolic Acids. The procedure for phenolic acid extraction was based on the method described by Sosulki

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Table 1. Total, Insoluble-Bound, Soluble-Bound, and Free Forms of Phenolic Acids in Different Vital Wheat Glutens

	sinapic acid			ferulic acid[a]			
	total[b]	insoluble[c]	soluble[c]	total[b]	insoluble[c]	soluble[c]	free[c]
lot 1	2.7	15	85	7.7	50	45	5
lot 2	1.4	36	64	5.2	58	40	2
lot 3	2.3	26	74	4.9	52	46	2
lot 4	3.5	23	77	16.8	83	14	3
cv. Scipion	1.8	21	79	17.7	95	4	1
cv. Soissons	1.8	17	83	17.4	89	9	2

[a] Sum of cis and trans FA. [b] Expressed in μg/100 mg of total gluten. [c] Expressed as percent of total.

et al. (1982) with the following modifications: 500 mg of freeze-dried and ground gluten dough was extracted three times with 10.0 mL of methanol/acetone/water (7:7:6, v/v/v) at room temperature. The pooled extracts were evaporated to 9.0 mL volume, then adjusted to pH 2.0 with 4.0 N HCl, and extracted with diethyl ether at a solvent to water phase ratio of 1:1 (free phenolic acids). The phenolic esters remaining in the aqueous phase were hydrolyzed (2 h, 35 °C) with 2.0 N NaOH and extracted twice with 5.0 mL of diethyl ether (soluble-bound form). Alkaline hydrolysis (2.0 N NaOH) of the unextractable residue (insoluble-bound form) took place (2 h, 35 °C) before extraction twice with 5.0 mL of diethyl ether. The diethyl ether extracts were evaporated to dryness and then resolubilized in 1.0 mL of aqueous methanol (50%) before injection in a HPLC. Phenolic acid determinations were performed as described in Figueroa-Espinoza and Rouau (1998). Concentrations in total phenolic acids are expressed in micrograms per 100 mg of gluten (dry basis). A treatment by a β-glucosidase from almonds (Sigma, St. Louis, MO) of the extracts remaining in water after acetone/methanol evaporation was performed. Digestion was carried out after adjustment to pH 5.0 with 0.1 M sodium acetate buffer, during 2 h at 25 °C.

Determination of Carbohydrates. The carbohydrate contents of glutens and soluble extracts were determined by gas−liquid chromatography of alditol acetates as described by Figueroa-Espinoza et al. (1998). The carbohydrates from the acetone/methanol/water extracts were also determined by HPLC, directly or after saponification (2.0 N NaOH, 2 h, 35 °C). Filtered samples (0.45 μm) were injected on a Polyspher CHCA column (300 × 6.5 mm) (Merck, Chelles, France), using water at 0.5 mL/min as eluent at 85 °C. Sugars were monitored by refractometry and quantified using inositol as internal standard. Concentrations in carbohydrates are expressed in milligrams per 100 mg of gluten (dry basis).

Repeatability. Relative standard deviations for phenolic acid and sugar determinations were <6%. Results are expressed as mean values of duplicate analyses.

RESULTS AND DISCUSSION

Gluten Phenolic Compounds. Wheat glutens contained various phenolic acids including FA, SA, *p*-coumaric acid, vanillic acid, and phenolic acid dimers. FA was the main phenolic acid present in all of the studied glutens (4.9−17.7 μg/100 mg of gluten) followed by SA (1.4−3.5 μg/100 mg) (Table 1). Only traces of the other phenolic compounds (monomers or dehydrodimers) were present. The presence and content of these phenolic acids have already been reported in wheat flours (Sosulki et al., 1982) but were not well documented in wheat gluten. In 1971, Gallus and Jennings evoked the presence of phenolic compounds in rather small amounts in washed gluten. Recently, Hilhorst et al. (1999) found 8.0 μg of FA/100 mg of gluten obtained by hand washing from Kolibri flour dough.

Gluten FA and SA were distributed in the fractions described by Sosulki et al. (1982) for wheat flour phenolics, according to their extractability in acetone/methanol/water (7:7:6, v/v/v). They can be classified into free, soluble-bound, and insoluble-bound forms. The solvent used allowed separation of LMW compounds from macromolecules. The proportions of the different forms varied according to the gluten considered. Free FA was in very low amounts in glutens, and no free SA could be detected. Insoluble-bound FA and SA ranged from 50 to 95% of total FA and from 15 to 36% of total SA, respectively, according to the studied gluten. The majority of FA was esterified to insoluble material. Insoluble FA may be largely ester-linked to AX trapped in gluten.

Soluble-bound FA accounted for 4−46% of total FA present in gluten. For SA, the soluble-bound form contributed 64−85% of the total SA. Hence, although total FA was in much larger amounts than SA, LMW conjugates (soluble-bound forms) of FA an SA were found in similar proportions in glutens from different origins. Sosulki et al. (1982) found 38 μg/100 mg of soluble esters of FA in wheat flour and only trace of SA esters. Because the gluten represents ∼10% of a wheat flour, the amount of soluble esters of FA present in gluten occurred in the same proportion as in flour. On the contrary, soluble esters of SA were largely concentrated in gluten. This result suggests the existence of a specific interaction between SA and gluten proteins during the preparation procedure. In canola meal, Rubino et al. (1996) reported that free SA and canola protein were weakly bound through electrostatic interactions or hydrophobic associations, at pH 4.5 and 7.0 or 8.5, respectively.

Gluten Carbohydrates. The total carbohydrate content of the glutens ranged from 8.1 to 29.6 mg/100 mg (Table 2). Glucose (6.7−25.8 mg/100 mg) was the main sugar obtained after hydrolysis. The high content in glucose probably originated mainly from starch contamination. It was in higher concentration in the glutens isolated from pilot-scale process (cv. Scipion and Soissons) than from industrial process. This is in agreement with the conclusions of Saulnier et al. (1997) and Roels et al. (1998). The galactose content was also quite high (0.8−1.2 mg/100 mg). As pointed out by Roels et al. (1998), galactose from gluten can originate from both arabinogalactan and galactolipids. Arabinose (0.2−0.9 mg/100 mg) and xylose (0.2−1.2 mg/100 mg) were found also in appreciable amounts. The AX content (Table 3), calculated as the sum of anhydroarabinose and anhydroxylose, after correction for the arabinose associated with arabinogalatan (Roels et al., 1998), ranged from 0.2 to 1.8 mg/100 mg. The AX content was higher in glutens isolated from pilot-scale process. A correlation was then found between starch and AX (Figure 1) in agreement with Saulnier et al. (1997). The glutens with the highest concentration in glucose and AX had also the highest FA contents. Thus, AX associated with gluten was in low proportion and exhibited a relatively low arabinose-to-xylose (A/X) ratio (0.57−0.78). These data agree with the results of Saulnier et

Table 2. Total and Acetone/Methanol/Water-Soluble Sugars[a] Obtained after Hydrolysis (2.0 N H_2SO_4, 2 h, 100 °C) of Different Glutens

	glucose		galactose		arabinose		xylose		mannose		rhamnose		ribose		sum of sugars	
	total	soluble	total	soluble	total	soluble	total	soluble	total	soluble	total	soluble	total	soluble	total	soluble
lot 1	13.9	1.18	1.2	0.36	0.6	0.06	0.6	0.07	0.4	0.09	0.1	0.2	0.0	0.0	16.7	2.0
lot 2	7.8	0.65	1.1	0.26	0.3	0.03	0.3	0.04	0.3	0.08	0.0	0.01	0.2	0.0	10.0	1.1
lot 3	6.7	1.32	0.8	0.31	0.2	0.03	0.2	0.03	0.2	0.12	0.0	0.02	0.0	0.0	8.1	1.8
lot 4	14.0	0.53	1.2	0.22	0.6	0.03	0.8	0.11	0.4	0.07	0.1	0.06	0.2	0.0	17.3	1.0
cv. Scipion	23.2	0.78	1.1	0.14	0.7	0.02	1.0	0.02	0.2	0.06	0.1	0.01	0.1	0.0	26.5	1.0
cv. Soissons	25.8	0.35	1.2	0.21	0.9	0.01	1.2	0.11	0.3	0.04	0.1	0.02	0.1	0.0	29.6	0.7

[a] Expressed in mg/100 mg of total gluten.

Table 3. Calculated Values of Arabinoxylan Content[a] in Glutens

	xylose	arabinose	A + X[b]	A/X[c] ratio
lot 1	0.6	0.3	0.9	0.58
lot 2	0.2	0.1	0.4	0.65
lot 3	0.1	0.1	0.2	0.78
lot 4	0.7	0.4	1.2	0.58
cv. Scipion	1.0	0.6	1.5	0.57
cv. Soissons	1.1	0.7	1.8	0.57

[a] Expressed in mg/100 mg of total gluten calculated from the equations gave by Roels et al. (1998). [b] A + X, arabinoxylan. [c] A/X, arabinose-to-xylose ratio.

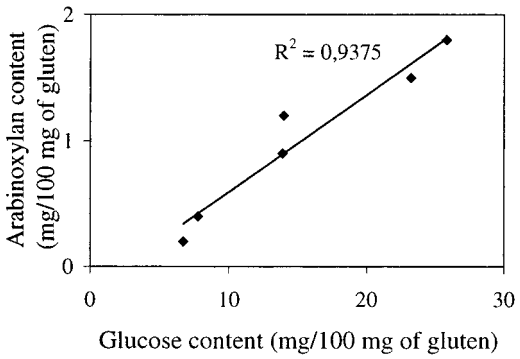

Figure 1. Relationship between glucose and arabinoxylan contents of glutens.

al. (1997) and Roels et al. (1998). Gluten AX were largely unextractable in acetone/methanol/water. Rhamnose, ribose, and mannose were also detected in low amounts.

The acetone/methanol/water extraction led to the separation of LMW carbohydrates, that is, sugars and oligomers, from polysaccharides which remained precipitated. The hydrolyzed extracts contained mainly glucose, in the range 0.35−1.32 mg/100 mg of gluten, corresponding to a low percentage of the initial amount of glucose (<20%). A high proportion of galactose (up to 38%) was also extracted, ranging from 0.14 to 0.36 mg/100 mg, in agreement with the presence of galactolipids. The other sugars were present in trace amounts only. These results suggest that most of the gluten carbohydrates were polymers with a low proportion of monomers and oligomers.

The carbohydrates of the acetone/methanol/water gluten extracts were analyzed by HPLC before and after the saponification that led to the liberation of the phenolic acids. Only glucose monomer was identified in chromatograms with other peaks corresponding to unidentified compounds. No maltose, maltotriose, or maltotretraose was detected. The amount of glucose monomer increased in all glutens after saponification, especially in commercial glutens (Table 4). These results suggest that, at least part of, the phenolic compounds occurred as esters of glucose in gluten. Treating the

Table 4. Glucose Content[a] Determined without and with Alkaline Hydrolysis of Acetone/Methanol/Water Gluten Extracts

	without saponification	with saponification	% of liberated glucose[b]
lot 1	0.05	0.22	14.4
lot 2	0.06	0.26	30.8
lot 3	0.06	0.11	3.8
lot 4	0.14	0.29	28.3
cv. Scipion	0.08	0.09	1.3
cv. Soissons	0.07	0.08	2.8

[a] Expressed in mg/100 mg of total gluten. [b] Expressed in percent of total glucose.

extracts with a β-glucosidase did not liberate any phenolics (data not shown). The presence of β-glucoside of phenolic acids, described in other plants (Herrmann, 1989), is not likely in this fraction.

Effect of Gluten Mixing on Phenolic Acids. The mixing of gluten/water (20:26, w/v) was characterized by a consistency peak corresponding to the optimal mixing time. Glutens from pilot-scale isolation needed more water than the others to reach the same consistency. Because their contamination with carbohydrates (starch and AX) was higher, their hydration properties could be due to a lesser amount of protein or to the hydrophilic properties of the contaminants. Upon overmixing, after the consistency peak, a decrease in consistency was observed (data not shown). This corresponds to the breakdown phenomenon, largely described for wheat flour doughs but not expected with pure glutens (Hoseney, 1985). Jackson and Hoseney (1986) suggested that the dough breakdown was provoked by the reaction of FA, especially the soluble-bound form, with thiyl radicals formed by disruption of interchain protein disulfide bonds during mixing; disulfide bridge re-formation would therefore be hindered.

No relationship was observed between the initial concentration of the glutens in FA and SA and their mixing behavior. However, it is generally accepted that the mixing behavior depends essentially on protein content. After overmixing of the glutens (two peak times), the amount of total phenolic acids decreased (up to 46% of the total amount). This resulted from a decrease in FA and SA monomers, but no formation of FA dehydrodimers or SA dehydrodimers could be observed (Figure 2).

The level of free FA did not change significantly during mixing. The amount of detectable insoluble-bound FA decreased from 0.1 to 2.5 μg/100 mg in mixed gluten (up to 36% of the initial amount). This tendency to decrease concerned also the insoluble-bound SA (from 0 to 0.4 μg/100 mg). The soluble-bound form of the phenolic acids was lowered after overmixing, contributing to the decrease of the total amount. Thus, depending on the gluten, 0.5−1.6 μg/100 mg of FA disappeared

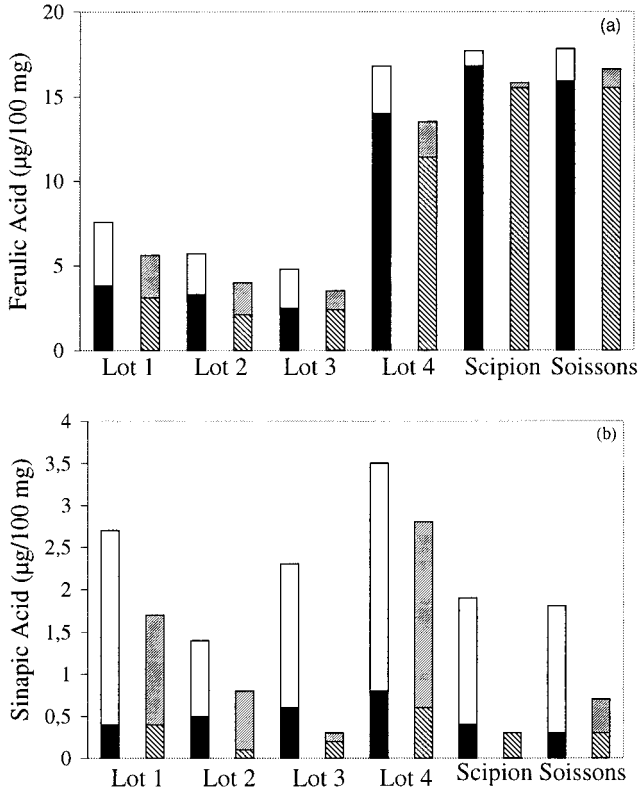

Figure 2. Evolution of ferulic acid (a) and sinapic acid (b) concentrations in insoluble-bound form (black) and soluble-bound form (white) in different glutens, in vital glutens (plain), and in overmixed gluten/water (hatching). The amounts of phenolic compounds are expressed on a microgams per 100 mg dry basis.

from this extract, that is, 21−66% of the initial soluble-bound FA. These results are in agreement with those obtained on wheat flour dough by Jackson and Hoseney (1986). Concerning SA, 0.2−1.6 μg/100 mg (22−94% of soluble-bound form) disappeared after mixing. The soluble-bound forms of SA and FA were therefore more reactive than the free or insoluble forms. This could be due to their fine structure; the nonphenolic part of the conjugates could facilitate the interactions during the gluten isolation and promote their reactivity during the mixing of the gluten. The disappearance of FA and SA could be explained by linking reactions of the phenoxyl moieties of these esters to some components in gluten, for example, thiyl radicals as suggested by Jackson and Hoseney (1986) to explain FA disappearance concomitant with wheat flour dough breakdown. Phenolic acids could therefore be no longer released by saponification.

CONCLUSION

In wheat gluten, phenolic acids, and more particularly FA and SA, occur as free and soluble- and insoluble-bound forms. The soluble-bound form of SA in gluten was found in a high proportion relative to its amount in flour. A specific association between SA conjugates and some gluten component could occur during the preparation of the gluten. Significant amounts of the conjugates are likely to be ester-linked to glucose. The fine structure of these SA compounds remains to be determined.

Losses in FA and SA during gluten mixing suggest that both may be rheologically active, especially in the form of soluble alkali labile conjugates. However, SA compounds appear to be proportionally more reactive than FA counterparts. These compounds, present at significant levels in commercial glutens, could be related to the mixing breakdown of the gluten.

Further research will be necessary to gain more insight into the interaction between phenolic compounds and cysteine residues, especially by an analysis of the amino acid composition of native and mixed glutens.

ABBREVIATIONS USED

FA, ferulic acid; SA, sinapic acid; AX, arabinoxylan; LMW, low molecular weight; A/X, arabinose-to-xylose ratio.

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