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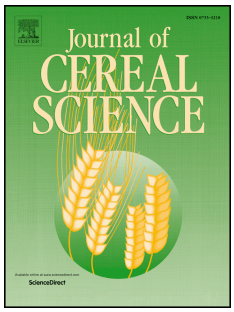

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Changes in wheat (*Triticum aestivum* L.) flour pasting characteristics as a result of storage and their underlying mechanisms

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ABSTRACT

White wheat flour of a high and a low protein cultivar were stored at 25 °C for 84 days to study age-related changes in flour components and pasting properties. Both cultivars showed similar increases in free fatty acid contents and slight but significant decreases in extractable thiol contents. Rapid Visco Analyser peak, minimal and final viscosities as well as breakdown and setback readings of the high protein flour increased rather gradually along the storage period, while the low protein flour showed more pronounced increases of pasting profiles during the first 21 days of storage. Flour functionality is co-determined by changes in extractable thiol contents during flour storage pointing to their oxidation and the release of free fatty acids which results in additional formation of amylose-lipid complexes and additional substrate for lipoxygenase action which can lead to more extensive co-oxidation of gluten, thereby influencing rheological properties.

KEY WORDS

Wheat flour aging, storage, thiol content, amylose-lipid complex, free fatty acid

ABBREVIATIONS

5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB); amylose-lipid (AM-L); cultivar (cv.); diacyl glycerol (DAG); differential scanning calorimetry (DSC); dithiotreitol (DTT); dry matter (dm); ethylene diamine tetra-acetate (EDTA); free fatty acids **(**FFAs); free oxidized glutathione (GSSG); free reduced glutathione (GSH); high molecular weight glutenin subunit (HMW-GS); moisture contents (mc); monoacyl glycerol (MAG); protein-

39 glutathione mixed disulfides (PSSG); Rapid Visco Analyser (RVA); room temperature
40 (RT); thiol (SH); triacyl glycerol (TAG).

INTRODUCTION

Post-milling maturation of wheat flour is well-known to millers and bakers. Freshly milled flour often requires a resting period in order to acquire its optimal bread making quality (Yoneyama *et al.*, 1970; Chen & Schofield, 1996; Wang & Flores, 1999). Conversely, prolonged storage can cause adverse effects on processing and end product characteristics (Tsen & Dempster, 1963; Wang & Flores, 1999).

As thiols are determining factors in the development of the gluten network during dough mixing (Wieser, 2007), changes in thiol (SH) contents during flour storage may explain the above mentioned effects. However, contradictory results have been described for the changes in thiol content during flour storage. Some authors note a decrease in SH content (Tsen & Dempster, 1963; Yoneyama *et al.*, 1970; Ewart, 1988; Chen & Schofield, 1996), while others report an increase (Mann *et al.*, 2000; Tomic *et al.*, 2013). Bellenger and Godon (1972) noted a drastic increase in total SH content during the first two weeks of flour storage, after which a 2 week period of constant SH levels was followed by a decrease in the next 9 weeks of storage.

Lipids can also contribute to the changes in quality of aging flour. Clayton and Morrison (1972) suggested the involvement of several enzymes in the hydrolysis of flour lipids during flour storage. Although lipids are minor constituents of flour, they are important determinants of bread making quality. During dough mixing, the majority of flour lipids becomes bound to the gluten network (Gerits *et al.*, 2013). Polar lipids are believed to stabilize the gluten network through hydrophilic interactions with gliadin and hydrophobic interactions with glutenin (Hoseney *et al.*, 1970).

Although much research has been done concerning flour aging and its significance in bread making, the (bio)chemical processes causing the changes in flour are still ill-understood as are their implications for the properties of batters such as e.g. in pancake systems. Batter models can be studied using the Rapid Visco Analyser (RVA). In the instrument, the flour suspension is subjected to a temperature–time profile (a controlled heating phase to 95°C – a holding phase at 95°C – a controlled cooling phase to 50°C) during which the viscosity of the system is monitored. Due to starch gelatinization and swelling of the granules, flour suspensions reach a peak viscosity, followed by a viscosity decrease caused by polymer alignment as well as shear-induced disintegration of the swollen granules. “Breakdown” is the difference between the peak viscosity and the minimum or hot paste viscosity at 95°C. During the cooling phase, leached amylose molecules form a continuous three-dimensional network. This increases the viscosity up to what is referred to as “cold paste viscosity”. The difference between the cold paste and minimum viscosity at 95°C is defined as “setback” (Delcour & Hoseney, 2010).

We here set up a flour aging experiment taking into account different aspects related to flour quality with a main focus on changes in proteins and lipids. Both a high protein [cultivar (cv.) Akteur; 14.6% protein in flour dry matter (dm)] and a low protein (cv. Apache; 9.9% protein, dm base) wheat were used to study differences in aging behavior of flour and its implications for flour pasting properties.

MATERIALS AND METHODS

Materials

Wheat from cvs. Akteur and Apache was from Dossche Mills (Deinze, Belgium) and Ceres (Brussels, Belgium), respectively. Both wheat cvs. were stored at room temperature (RT) and milled 140 days after harvest. All reagents, solvents and chemicals were of analytical grade and obtained from Sigma-Aldrich (Diegem, Belgium) unless indicated otherwise.

Standard Analyses

Moisture contents (mc) of flour samples were determined according to Approved Method 44-19.01 (AACC International, 1999). Those of wheat samples were determined by drying whole wheat kernels (2.00 g) at 130 °C for 16 h. Ash contents were determined according to Approved Method 08-01.01 (AACC International, 1999). Protein contents (N x 5.7) of the samples were determined using an adaptation of Approved Method 46-30.01 (AACC International, 1999) to an automated Dumas protein analysis system (EAS VarioMax N/CN, Elt, Gouda, The Netherlands).

Wheat milling and flour storage

Prior to milling, wheat samples were conditioned to 16.0% mc for 24 h at RT. Additional water was added 60 min before milling to reach a final mc of 16.5%. The samples were milled at 100 g/min on a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill with a diagram as in Delcour et al. (1989) and according to Approved Method 26-21.02 (AACC International, 1999). The gaps between rolls were set to 0.50 mm (B1), 0.10 mm (left side of B2) and 0.08 mm (right side of B3) for the break rolls and 0.07 mm (left side of C1) and 0.03 mm (right side of C3) for the reduction rolls. Bran and shorts are discarded. The six flour streams (three break and three reduction) are blended and called "flour". Freshly milled flour was put in airtight plastic containers. A portion was stored at -20 °C to serve as reference samples (day 0) for all analyses. The remaining containers were stored at 25 °C for 7, 14, 21, 28, 42, 56 and 84 days and then kept at -20 °C until analysis.

Free fatty acid content

Free fatty acids **(**FFAs) were extracted from flour with n-hexane using an ASE 200 device (Dionex, Amsterdam, The Netherlands). Prior to extraction, flour (8.00 g) was mixed with 14.0 g of pure sand and put into a 22 mL ASE extraction cell. Extraction proceeded at 69 bar and 40 °C and included a heating step of 5 min followed by 10 min static extraction, a cycle that was repeated three times before the ASE cell was purged. Hexane was then evaporated under nitrogen and the lipids were redissolved in 4.00 mL isooctane. FFA contents were determined using an adaptation of the copper soap assay of Kwon and Rhee (1986). An aliquot (1.00 mL) of 5.0% (w/v) cupric acetate adjusted to

121 pH 6.1 with pyridine was added to the extracts. After vigorous shaking for 1 min to allow
122 the FFAs to complex with the copper ions, the mixtures were allowed to separate into two
123 layers. The extinction of the upper layer (isooctane with copper soaps) was read at
124 715 nm. A standard curve of oleic acid in isooctane (0.5–5.0 mM) was constructed.
125
126 **Extractable and available thiol content**
127 Extractable and available thiol contents of flour samples were analyzed essentially as in
128 Veraverbeke et al (2000) and determined colorimetrically after reaction with 5,5'-dithio-
129 bis(2-nitrobenzoic acid) (DTNB) at RT. An adaptation of the method consisted of the use
130 of ethylene diamine tetra-acetate [Na_2EDTA (Stevens *et al.*, 1983)] to prevent oxidation
131 by oxygen during analysis. Its use reduced day-to-day variation of the extinctions of
132 standard solutions to less than 2%. Under such conditions, there was no significant
133 difference between the measurements performed in air or under nitrogen atmosphere
134 (data not shown).
135 For extractable thiol contents, samples (0.80 g flour) were shaken (150 times/min) for
136 20 min at RT in 6.00 mL 50 mM sodium phosphate buffer (pH 8.0) containing 1.0 mM
137 Na_2EDTA (hereafter referred to as sample buffer). After centrifugation (4100 g, 15 min,
138 20 °C) and filtration (0.45 µm), 1.00 mL of the extract was mixed with 100 µl 0.1% (w/v)
139 DTNB in sample buffer (further referred to as DTNB reagent). Exactly 70 min later, the
140 extinction at 412 nm was determined. Extinction values were corrected for readings with
141 appropriate control samples and converted to thiol contents using a calibration curve of
142 reduced glutathione (0.01-0.10 mM) in sample buffer.

Available thiol contents were measured on flour suspensions. DTNB reagent (100 µl) was added to flour samples (0.80 g) suspended in 6.00 mL sample buffer. The suspension was shaken (150 spm) for 20 min at RT. After centrifugation (4100 g, 15 min, 20 °C) and filtration (0.45 µm), the extinction at 412 nm of the supernatant was measured exactly 70 min after addition of DTNB reagent. A calibration curve of reduced glutathione was used as described above.

Rapid Visco Analysis

The rheological behavior of flour was studied with a Rapid Visco Analyzer (RVA-4D; Newport Scientific, Sydney, Australia). It converts the current required to maintain constant mixing speed (160 rpm) of a paddle into a dynamic viscosity value in centiPoise (1 cP = 0.1 kg m^{-1} s^{-1}). Flour suspensions [25.00 g, 12.0% dm (w/w)] were prepared in 100 mM Tris-HCl buffer (pH 7.0). The samples were mixed at 900 rpm for 20 s at RT to homogenize the suspension and then submitted to a temperature profile consisting of a holding step at 50 °C (1 min), a linear temperature increase to 95 °C at 7.5 °C/min, a holding step at 95 °C (7 min), a cooling step with a linear temperature decrease of 7.5 °C/min to 50 °C, and a final holding step at 50 °C (10 min). 'Bump' area was calculated using a linear baseline with Origin 6.0 (OriginLab Corporation, Northampton, MA, USA).

α-Amylase activity

α-Amylase activity was assayed using the Amylazyme Kit (Megazyme, Bray, Ireland) as in Approved Method 22-05.01 (AACC International, 1999).

166

167 **Differential scanning calorimetry**

168 Differential scanning calorimetry (DSC) measurements were performed with a Q1000
169 DSC (TA Instruments, New Castle, DE, USA). Flour was accurately weighed (3.0-4.5
170 mg) in aluminum pans and deionized water added in a ratio of 1:3 (w/w) dm flour:water.
171 The pans were hermetically sealed and equilibrated at 0 °C before heating from 0 to 140
172 °C at 3 °C/min). The system was calibrated with indium and an empty pan was used as
173 reference for the measurements. The peak (Tp) temperatures and the endotherm
174 enthalpies (ΔH; expressed in J/g dm flour) of phase transitions were determined using TA
175 Universal Analysis software.

176

177 **Statistical analysis**

178 Significant differences ($\alpha<0.05$) based on at least three individual measurements were
179 determined with the ANOVA procedure using the Statistical Analysis System
180 software 9.2 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Flour yields were 76.3% (cv. Akteur) and 73.8% (cv. Apache) with respective ash, protein and moisture contents of 0.48% and 0.43%, and 14.6% and 9.9%, and 14.3% and 14.6% respectively.

Free fatty acid contents

Flour from both cultivars showed strong increases in free fatty acid (FFA) contents upon storage (Fig. 1). Both freshly milled flour samples had similar initial levels of FFAs. The increase during the full storage period was more pronounced for cv. Akteur (1.73 to 4.80 µmol FFA/g dm flour) than for cv. Apache (1.92 to 3.67 µmol FFA/g dm flour).

These results are in line with Maraschin et al (2008) who found FFA level increases from ca. 2 to ca. 6 µmol/g dm flour when flour (14.0% mc) was stored for 16 weeks at 22 °C. In their study, lower flour mcs and storage temperatures resulted in less pronounced increases. Clayton and Morrison (1972) reported that FFA contents of flours at least doubled when high- and low-grade flour was stored for 4 months at 25 °C and for 3 months at 37 °C, respectively.

During storage, FFAs are presumably released by lipase action. In wheat kernels, lipases are unlikely to cause extensive hydrolysis as the enzyme is mainly present in the bran (O'Connor & Harwood, 1992) while its substrate, triacyl glycerol (TAG), is primarily located in germ and aleurone (Hargin & Morrison, 1980). Milling redistributes wheat components and results in lipases in flour to come into contact with TAG. Some flour lipids can bind to different components of gluten during processing (McCann *et al.*,

2009). The release of FFAs upon storage of flour evidently changes the lipid composition.

Thiol contents

Extractable thiol contents comprise low molecular weight thiol compounds such as glutathione or cysteine. Flour from both cultivars had comparable initial levels of extractable thiols (146 and 143 nmol/g dm flour for cv. Akteur and cv. Apache, respectively). A slight but significant decrease was observed after 14 days (cv. Akteur) or 21 days (cv. Apache) of storage (Fig. 2A). A slower but significant decrease was observed during the next 70 days of storage for cv. Akteur.

Available thiol contents were determined on flour suspensions. They consist of both extractable thiol groups and thiol groups located at the outer side of unextractable proteins (mainly gluten proteins). Upon flour storage, no change in available thiol contents is observed for cv. Apache, while for cv. Akteur a slight but significant decrease is measured (Fig 2B). This decrease, however, can totally be ascribed to the decrease is extractable thiols (Fig 2A). Furthermore, there was a remarkable difference between the available thiol contents of both cultivars. For Apache flour, the available thiol contents were hardly higher than the extractable thiol contents, suggesting that only a very limited portion of thiols is located on the outside of unextractable proteins in this flour. In Akteur flour, on the contrary, approximately 50% more available thiol groups occur than extractable.

A possible explanation for the difference in available thiols between the two cultivars could be found in the difference in their high molecular weight glutenin subunit (HMW-

GS) composition. Cv. Apache contains subunits Ax2*, Bx7, By9, Dx3 and Dy12 while cv. Akteur contains subunits Ax1, Bx7, By9, Dx5 and Dy10 (Lagrain *et al.*, 2012). The major difference between these subunits is the difference between subunit Dx3 (cv. Apache) and Dx5 (cv. Akteur): Dx5 contains an extra cysteine residue compared to Dx3. Wieser and Zimmermann (2000) found that proportions of subunits in a given combination are strongly conserved between different cultivars and are only scarcely influenced by genotype and growing conditions. Their data allow estimating that Dx3 and Dx5 both make up the same proportions of the total amount of HMW-GS in cvs. Apache and Akteur, respectively. Thus, when assuming that the extra cysteine residue of Dx5 is not involved in a disulfide bond, this may explain the higher level of available thiols in flour samples of cv. Akteur than in those of cv. Apache.

Earlier reported results on the changes in thiol content of flour during storage have not been univocal. Tsen and Dempster (1963) noted losses in both accessible (measured in water) and total (measured in 6.0 M urea) thiol contents during the first year of storage of flour of 14.5% mc. Total thiols decreased from ca. 0.90 to 0.70 µmol/g dm flour and accessible thiols from ca. 0.68 to 0.44 µmol/g dm flour. Ewart (1988) stored flour of different protein contents (8.9, 9.4 and 14.7% protein) for 240 days at RT. A significant linear decrease, starting from the first day of storage, in accessible thiol content (measured in sodium acetate buffer at pH 4.2) was observed for all three flour samples, but the decrease in total thiols (measured in the same buffer, containing 8.0 M urea) was only significant for the high protein flour. Chen and Schofield (1996) reported decreases in contents of free reduced glutathione (GSH), free oxidized glutathione (GSSG) and protein-glutathione mixed disulfides (PSSG) during the first 10 days of storage after

249 which their levels remains constant up to 40 days of flour storage at 20 °C. However, the
250 decrease in GSH content could not be explained by oxidation to GSSG or linkage to
251 proteins to form PSSG. For flour of wheat 6 weeks post-harvest, Mann et al. (2000)
252 reported a slight significant increase in total free SH groups after 1 week of flour storage.
253 No significant changes in contents of GSH or GSSG were seen, while for PSSG a
254 significant increase was reported. Bellenger and Godon (1972) noted a slight linear
255 increase in free SH content in flour during 16 weeks of storage, while the level of SH
256 determined after sulfitolysis increased drastically during the first two weeks of flour
257 storage, after which a period of constant SH level was followed by a period of decrease of
258 9 weeks. They concluded that some disulfide bonds were already present at time of
259 milling, while additional formation occurred during the first weeks of flour storage.
260 Because this oxidation occurred without any change in the number of free SH, some SH
261 groups must have been present in an inaccessible form during quantification of free SH
262 prior to sulfitolysis. Tomic et al. (2013) reported a slight increase in the level of free SH
263 groups on washed gluten after 1 week of flour storage.
264
265 **Pasting properties**
266 Flour storage led to increases in RVA viscosities of flour from both cultivars (Fig. 3).
267 However, there were some differences between the cultivars. Peak, minimal and final
268 viscosities, and breakdown and setback readings of Akteur profiles increased rather
269 gradually over the storage period resulting in 7 to 10% higher values after 84 days
270 (Table I). Apache flour samples showed the largest increases in viscosity readings in the
271 first 21 days of storage. Moreover, the minimal viscosity increased by 18% over the total

storage period of 84 days, while final viscosity only rose by 10%, resulting in a less pronounced increase in setback (6% increase).

Since **α-amylase activities** were negligible (below detection limit) in the freshly milled flour of both cultivars, it is unlikely that the changes in RVA profiles upon flour storage are caused by such enzyme activity.

FFAs and other **lipids** (e.g. monoacyl (MAG) and diacyl glycerols (DAG), the products of TAG hydrolysis) can form inclusion complexes with amylose. As such complexes can impact viscosity of wheat wholemeal pastes (Copeland *et al.*, 2009), changes in lipid composition as a result of flour rather than wholemeal storage may also alter flour pasting characteristics. In general, higher final viscosities and setback readings are noted in RVA for starches mixed with fatty acids (FAs) and MAG than for granular (defatted) starches, while for peak viscosity both increases and decreases have been described (Deffenbaugh & Walker, 1990; Ravi *et al.*, 1999; Liang *et al.*, 2002).

Salman and Copeland (2007) also found increases in pasting profiles of stored wheat whole meal. However, the impact on final viscosity and setback in their results was higher than noted here. As the final viscosities correlated positively with fat acidity and negatively with iodine binding values of flour pastes measured after RVA analysis, they concluded that FAs released during storage form complexes with starch during gelatinization leading to changes in pasting profiles (Salman & Copeland, 2007).

Gerits et al. (2015) noted similar increases in RVA pasting profiles as seen here when adding lipases to the RVA samples. They concluded that the increase in end viscosity after *in situ* formation of FFA could not only be ascribed to AM-L complex formation but

294 mainly to a different behavior of the starch granule remnants during heating (Gerits *et al.*,
295 2015).
296 A remarkable difference between the profiles of the two cultivars in this study is the
297 presence of a 'bump' around 60 °C in the RVA profile during the cooling phase for
298 Apache but not for Akteur samples. This indicates formation of amylose-lipid (AM-L)
299 complexes (Xu *et al.*, 1992). Conde-Petit and Escher (1995) have suggested that *in situ*
300 formed AM-L complexes act as junction zones in a network between granules, and thus
301 induce gelation. However, the RVA profiles of flour from cv. Akteur contained no such
302 'bump' although both flour samples contained similar levels of FFAs. Putseys et al.
303 (2010a) studied the effect of the addition of glycerol monostearate to a wheat starch
304 suspension in the RVA. They reported a similar 'bump' in the profile during cooling as
305 seen here for cv. Apache (Fig. 3B). Remarkably, no such 'bump' was observed when the
306 same amount of glycerol monostearate was added in the form of pre-synthesized
307 amorphous AM-L complexes (Putseys *et al.*, 2010a) as seen here for cv. Akteur (Fig.
308 3A). Even though the complexes dissociate and set the glycerol monostearate free during
309 the heating phase (Gelders *et al.*, 2006), the liberation of the lipid does not induce a
310 viscosity increase comparable to that during the cooling phase in the presence of
311 uncomplexed glycerol monostearate. The reasons for this observation remained unclear
312 (Putseys *et al.*, 2010a).
313 It is widely assumed that some starch lipids are complexed with amylose in native starch
314 granules, although the evidence is entirely indirect (Evans, 1986; Morrison *et al.*, 1993).
315 There is also evidence supporting the view that amylose and lipids co-exist independently
316 in the wheat kernel and only form complexes under gelatinisation conditions (Morrison *et*

317 *al.*, 1993). Whether AM-L complexes can be formed depends on the lipid fatty acid chain
318 length, its degree of (un)saturation and the identity of the polar head (Putseys *et al.*,
319 2010b). Fatty acids with a chain length of at least 14 (Bhatnagar & Hanna, 1994) and
320 preferentially 16 or 18 carbon atoms more easily form AM-L complexes (Krog, 1971). In
321 wheat starch, the most common lipid fatty acids are palmitic (C16:0) and linoleic (C18:2)
322 acids (Morrison, 1988; Vasanthan & Hoover, 1992). The more unsaturated bonds a FA
323 contains, the lower its tendency to enter into the hydrophobic cavity of amylose helices.
324 The nature of these bonds (i.e. *cis* vs. *trans*) affects complex formation and the thermal
325 properties of the complex, with *cis*-unsaturated fatty acids forming the less thermally
326 stable AM-L complexes (Putseys *et al.*, 2010b). Finally, the type of lipid determines
327 whether AM-L complexes can be formed. FFA and MAG more easily form such
328 complexes than DAG, whereas TAG do not form them at all (Eliasson, 1994).
329 Based on the above it is tempting to speculate that cv. Akteur contained significantly
330 more AM-L complexes in its native kernel than cv. Apache and/or that the FFA formed
331 in cv. Akteur are less suited than those formed in cv. Apache to *in situ* form AM-L
332 complexes during the cooling phase with the corresponding impact on the viscosity
333 profile. DSC results seem to support this hypothesis. For cv. Akteur, the average
334 dissociation enthalpy of the AM-L complexes is 0.23 J/g dm flour, while for cv. Apache
335 significantly less AM-L complexes were measured (only 0.08 J/g dm flour). This
336 observation is in agreement with findings by Gerits et al. (2015). They used flour with a
337 relatively high level of AM-L complexes (approximately 0.4 J/g dm dough) and observed
338 no ‘bump’ in the corresponding RVA profile (Gerits *et al.*, 2015). The hypothesis is

339 further supported by the linear relation between the FFA content and the area of the
340 'bump' in the RVA profile of cv. Apache (Fig.4).
341 However, even though the increase in FFA contents with flour storage time (Fig. 1) is
342 similar for both cultivars, the effect on the RVA profile differs. This may indicate that
343 also factors other than lipids cause the changes in pasting characteristics of wheat flour
344 upon storage.
345 Here, changes in the proteins come to mind. The decrease of approximately 50 nmol/g
346 dm flour in extractable **thiol content** in the present study could also be partly responsible
347 for the upward shift in RVA profiles. Unfortunately, Salman and Copeland (2007) did not
348 measure the changes in thiol content during flour storage. However, the impact of low
349 molecular weight thiol compounds on RVA viscosity was studied by adding cysteine to
350 the suspension. Ravi et al (1999) noted small downward shifts in the RVA profiles of
351 wheat flour upon addition of 380 nmol cysteine/g flour. For rice flour, addition of
352 approximately 200 nmol cysteine/g flour slightly altered the peak viscosity and
353 breakdown, while the hot paste viscosity, final viscosity and setback of cooked rice paste
354 were drastically decreased in the presence of this cysteine level (Likitwattanasade &
355 Hongsprabhas, 2010). Reducing agents dithiothreitol (DTT) and β-mercaptoethanol also
356 reduced the RVA viscosity of different rice flours (Zhou *et al.*, 2003; Derycke *et al.*,
357 2005).
358 In this study, there was a remarkable difference between the available thiol contents of
359 both cultivars. For Apache flour, the available thiol contents were only slightly higher
360 than the extractable thiol contents, suggesting that only a limited portion of thiols is
361 located at the outside of unextractable proteins. Akteur flour, in contrast, contained

362 approximately 50% more available than extractable thiol groups. The higher level of
363 available thiols on gluten in Akteur wheat may allow for higher degrees of thiol-disulfide
364 interchange reactions. However, this was not reflected in the RVA profiles.
365
366 **CONCLUSIONS**
367 Akteur and Apache flour samples showed similar increases in FFA contents upon storage.
368 Flour from both cultivars showed slight but significant decreases in extractable ol
369 contents, pointing to oxidation of low molecular weight thiol compounds. Furthermore,
370 storage of flour resulted in increases of RVA viscosities and thus changed flour pasting
371 properties. In summary, we believe that changes in flour properties upon aging are (at
372 least partially) related to oxidation reactions occurring either during storage or processing
373 of flour. In addition, enzymatic activity of lipases with concomitant release of FFAs is an
374 important process during flour storage. Changes in lipid composition can have a
375 significant impact when resulting in the formation of inclusion complexes with amylose
376 upon heating.

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505 **TABLES**

506 **Table I** Changes in Rapid Visco Analyzer (RVA) pasting properties of flour samples
507 from cv. Akteur (A) and cv. Apache (B) upon flour storage at 25 °C in airtight containers.

508

	cv. Akteur				cv. Apache			
Storage time (days)	**0**	**21**	**42**	**84**	**0**	**21**	**42**	**84**
Peak viscosity (cP)	3322	3392	3525	3624	2961	3273	3225	3396
Min. viscosity (cP)	1195	1233	1261	1318	1244	1372	1313	1463
Final viscosity (cP)	2874	2938	3037	3091	3236	3467	3415	3571
Breakdown (cP)	2127	2159	2265	2307	1717	1901	1912	1933
Setback (cP)	1679	1705	1777	1773	1992	2096	2102	2108

509

FIGURES

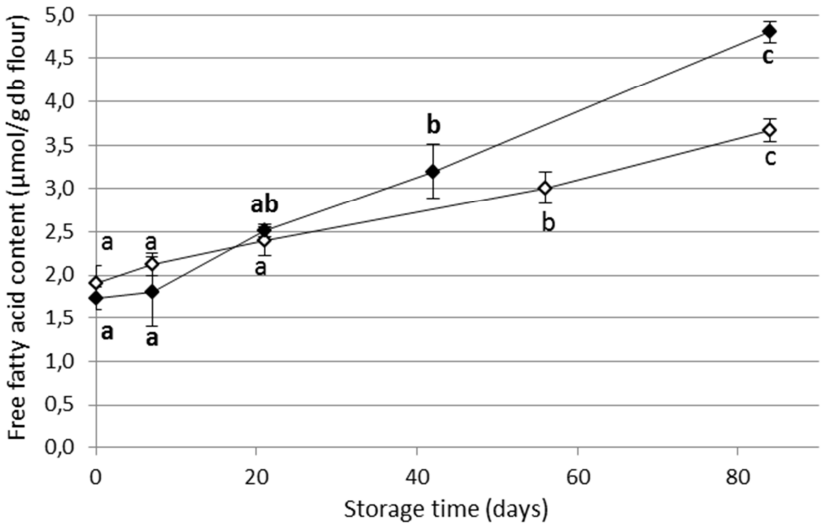
Fig. 1 Changes in free fatty acid (FFA) content of flour samples from cv. Akteur (—◆—) and cv. Apache (—◇—) upon flour storage at 25 °C in airtight containers. Within one cultivar, values of points with the same letter are not significantly different (α = 0.05).

Fig. 2 Changes in extractable (A) and available (B) thiol contents of flour samples from cv. Akteur (—◆—) and cv. Apache (—◇—) upon flour storage at 25 °C in airtight containers. Within one cultivar, values of points with the same letter are not significantly different (α = 0.05).

Fig. 3 Changes in Rapid Visco Analyzer (RVA) pasting profiles of flour samples from cv. Akteur (A) and cv. Apache (B) upon flour storage at 25 °C in airtight containers for 0 days (*i.e.* freshly milled flour; ——), 21 days (– – –), 42 days (- - - -) and 84 days (-------). The dotted line (·····) represents the applied temperature profile.

Fig. 4 Relation between free fatty acid (FFA) content and area of the 'bump', calculated from the Rapid Visco Analyzer (RVA) pasting profile for cv. Apache for flour stored at room temperature for 0, 21, 42 and 84 days.

531 **Fig. 1**



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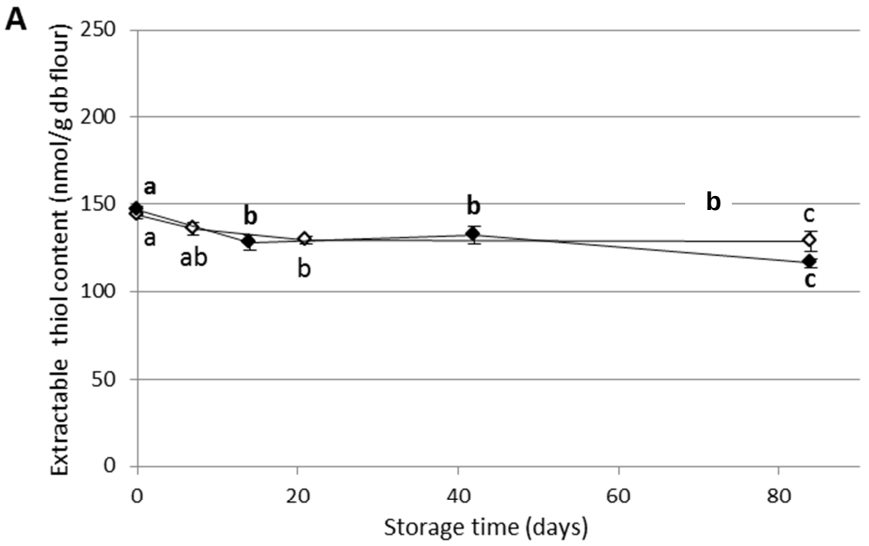
533 **Fig. 2**



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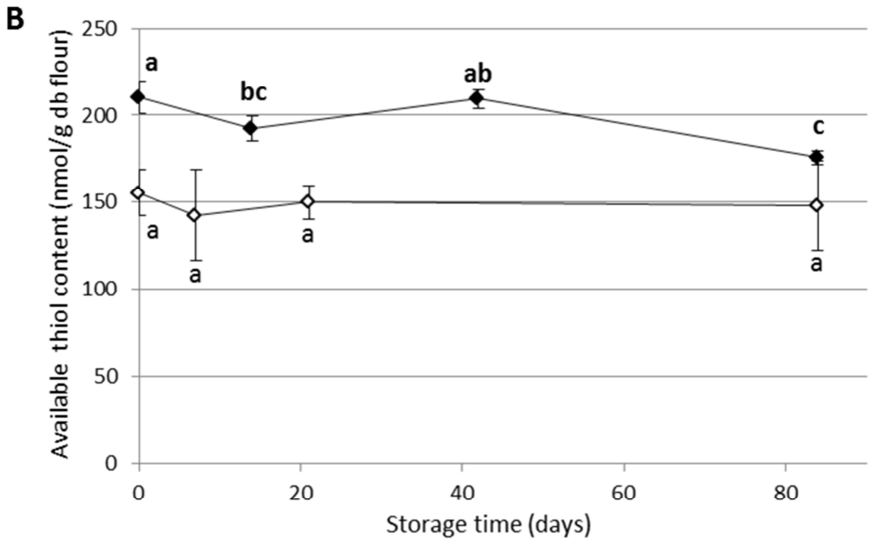

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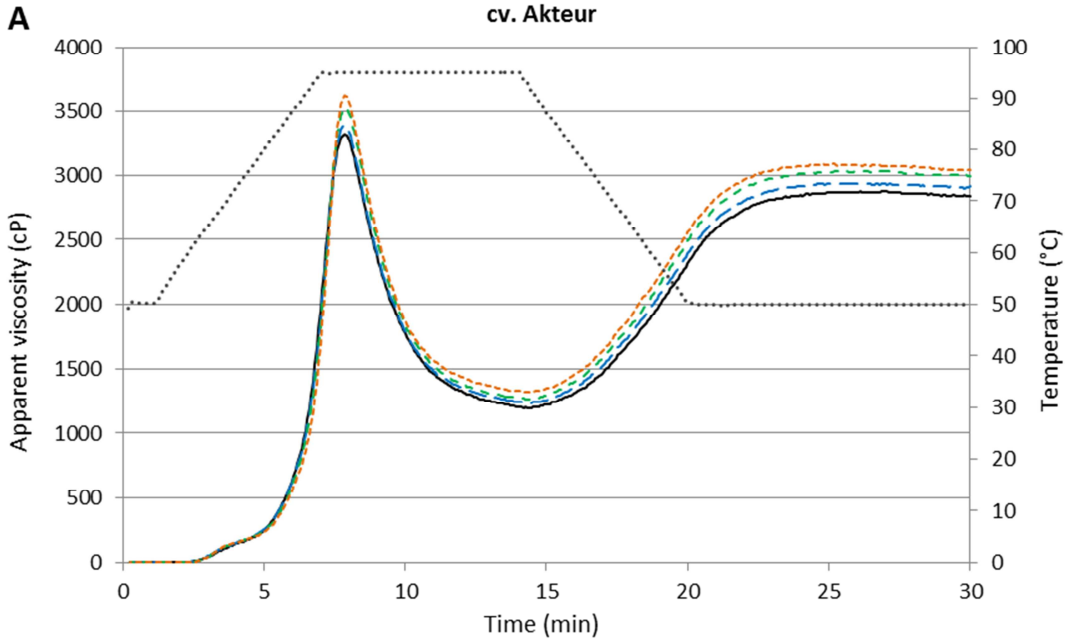
537 **Fig. 3**



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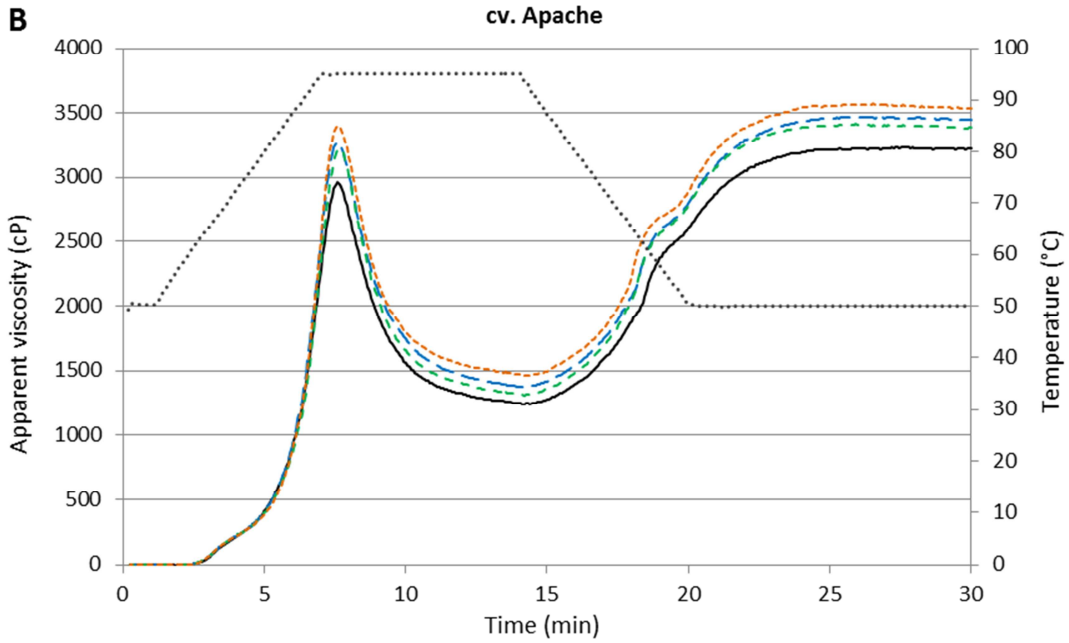

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543 Fig. 4
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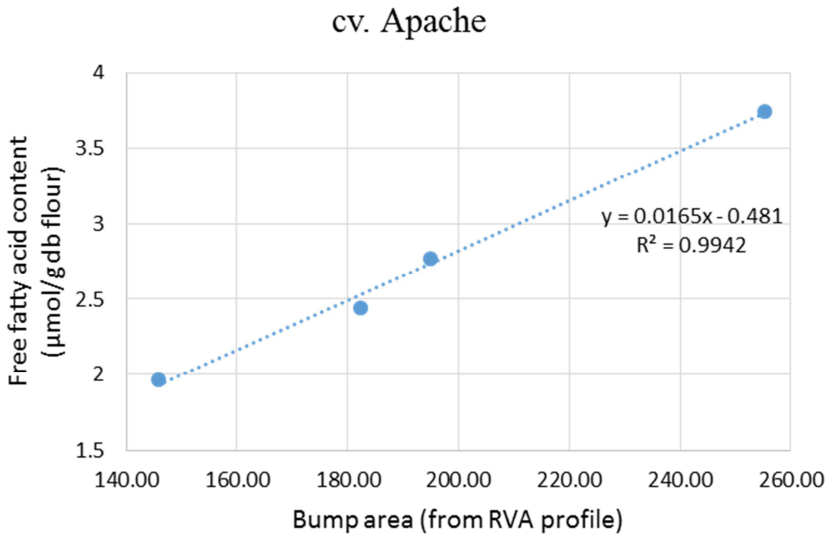

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HIGHLIGHTS

- Free fatty acid content increases during flour storage
- Extractable thiol content decreases during flour storage
- Rapid Visco Analyser viscosity profiles shift upwards during flour storage
- Flour aging impacts flour pasting properties