

# **Effect of long-term storage conditions on wheat flour and bread baking properties**

Eloïse Lancelot, Joran Fontaine, Joëlle Grua-Priol, Alain Le-Bail

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storage at ambient temperature led to significant modifications of flour parameters and baking

performances, whereas storage at low temperature preserved the initial quality of the flour. A

practical recommendation is to favour storage at low temperature in a sealed container to

#### **Highlights**

• The quality of wheat flour has been studied over 30 months in selected condition

prevent interaction with oxygen and moisture uptake.

*<sup>\*</sup>* Corresponding author: Alain Le-Bail alain.lebail@oniris-nantes.fr



Storing wheat grains for several months is common practice, whereas flour milling is done progressively between two harvests. Conversely, flours are usually stored for shorter durations. Indeed, milling destroys the cell walls so that the different constituents of the flour are in direct contact with the air and microorganisms, resulting in modifications of flour parameters. It has often been reported that the quality of flour is improved after several weeks of storage under good conditions (Baik & Donelson, 2018; Hrušková & Machová, 2002; Wang & Flores, 1999). Nevertheless, these benefits are short-term, and the prolonged storage of flours eventually done in unsatisfactory storage conditions, can eventually cause spoilage (Pyler & Gorton, 1973; Wang & Flores, 1999). Flour spoilage can have several origins: i) alteration of chemical origin, like protein denaturation, starch degradation, non-enzymatic oxidations; ii) alteration of enzymatic origin; iii) alteration of mechanical or physical origin, mainly due to shock, leading to cracks or breakage which favour other causes of alteration like iv) the invasion of microorganisms (Hackenberg et al., 2017). Factors influencing the evolution of flour include moisture, temperature, the presence of oxygen and/or carbon dioxide and storage time (Wang & Flores, 1999; Warwick et al., 1979). The relative humidity of the ambient air can, under certain conditions, increase the water activity, resulting in the deterioration of organoleptic qualities, and even in mycotoxin production. Moisture can also contribute to the oxidative deterioration of lipids, resulting in a typical rancid odour and an increase in the content of free fatty acids. The variation of moisture can be caused by: i) poor storage conditions, with high air humidity; ii) considerable variations in temperature; iii) proximity with products that may give off moisture; and iv) pre-harvest weather conditions, which may increase the moisture content of the product. Air temperature and air humidity during flour storage are therefore important parameters. Higher air temperature may yield higher specific humidity (kg Water / kg dry air) and thus more available water, whereas at low temperature, the contrary is observed. In addition, an increase in temperature accelerates the rate of deterioration reactions (chemical, biological and microbiological) (Vanhanen & 60 Savage, 2006). Oxygen and / or  $CO<sub>2</sub>$  may also affect the nature of the aerobic and anaerobic metabolism of microorganisms (Navarro, 2012), and the level of non-enzymatic oxidation and certain enzymatic reactions (Zia-Ur-Rehman, 2006). Finally, storage time is a factor that amplifies the alteration phenomenon (Wang & Flores, 1999). Several researchers have examined the effects of ageing on different flours (Bell et al., 1979; Brandolini et al., 2010; Hrušková & Machová, 2002; Mis, 2003; Sopiwnyk et al., 2020; Vanhanen & Savage, 2006; Warwick et al., 1979; Zarzycki & Sobota, 2015). In these studies, the packaging, storage conditions, duration and the flour parameters analysed were different each time. Most of them were limited to one year of storage or less. Hrušková and Machová (2002) analysed the moisture, wet gluten, acidity, falling number and alveograph results of two commercial wheat flours stored in jute bags for three months. They showed that the chemical characteristics and viscoelastic properties changed very rapidly during this short storage period. Mis (2003) studied the physical properties of gluten for 32 weeks and observed a gradual diminution of the quantity of washed out gluten as well as an increase in the Gluten Index value. Zarzycki and Sobota (2015) considered a storage period of 6 weeks. They focused on the falling number and apparent viscosity of wheat flour gruels stored in tightly closed containers at 76 temperatures of 20  $\degree$ C, 4  $\degree$ C and -20  $\degree$ C. They found that the falling number values increased for flours stored at 20°C and decreased for flours stored at -20°C, and that the viscosity of wheat flour increased over the 6-week storage period. Brandolini et al. (2010) studied alpha-amylase activity, falling number, pasting properties and SDS sedimentation volume in whole meal and white flours of einkorn and soft wheat over a period of 374 days, considering 81 storage at temperatures ranging between 30°C and -20°C. They showed that alpha-amylase activity decreased, while falling number, viscosity and SDS sedimentation increased with 83 ageing. The changes were drastic at high temperatures (38 and 30 °C), but were reduced or 84 negligible at medium and low temperatures (20, 5 and -20 °C). An effect of the storage temperature of wheat flours on the falling number and viscosity was also shown. However, in the case of a PhD project, ideally, flour should be kept for at least three years in order to ensure good repeatability of the results and to prevent them from being influenced by a change of batch. In this particular case, maintaining flour quality during storage is very crucial. Only a few studies have been performed for durations longer than three years. Warwick et al. (1979) focused on changes in the lipid fraction of flour during 5 years of storage. The most comprehensive study was carried out by Bell et al (1979). They focused on the lipolysis rate of flours milled from weak, medium, and strong wheats at three temperatures 93 (12, 25, and -20  $^{\circ}$ C in an inert atmosphere) for up to 66 months. They confirmed that conclusions regarding the baking quality of stored flours must be made with caution, since the results obtained are highly dependent on the nature of the baking test used. As chemical analyses were not carried out systematically at regular intervals, some information was

missing. This review of past publications shows that none of these studies took into account the effect of prolonged storage on all the parameters of wheat flour by associating different storage temperatures and packaging conditions.

This study aims at filling this gap by carrying out a more complete investigation on the effect of storage time and packaging conditions on soft wheat flour over a period of 30 months for the purpose of determining whether one storage method is more appropriate than another. Data on chemical composition (water, protein, ash, damaged starch, oleic acidity, Hagberg falling number and TOTOX), analytical profiles (farinograph, alveograph and Solvent Retention Capacity) and Near-infrared spectra were collected after 3 months, 6 months, 12 months, 21 months and 30 months of storage for flours stored in two different types of 107 packaging and two different conditions (paper bag or watertight container at 20 and -20 °C). It is well-known that Near-Infrared Spectroscopy (NIRS) can predict wheat composition parameters such as moisture, protein, ash and wet gluten contents with a high degree of accuracy and it appeared that this technique could also model certain rheological parameters (farinograph, extensograph, alveograph) (Jirsa et al., 2007). The results presented in this study also confirmed the interest of Near-Infrared spectroscopy for quantifying most flour parameters in a rapid and non-destructive way.

**2. Materials and methods** 

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116 2.1. Materials
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Commercial type 55 wheat flour was supplied by Moulins Soufflet SA (France). The flour came from wheat harvested in the 2015/2016 season and contained no additives. A sandwich bread dough recipe was used for bread-making trials at the beginning of the experiment and after 30 months. The ingredients used in the recipe were: 100 g wheat flour, 53.5 g tap water, 4.5 g vegetable oil, 4.5 g sugar, 1.8 g salt and 0.31 g improver. The temperature of the dough at the end of mixing was controlled to be around 27°C by adjusting the sum of the room, flour and water temperatures which was equal to 55°C.

#### **2.2. Storage conditions of wheat flour**

The flour was conserved using two different types of packaging: either in 25 kg paper bags (permeable to oxygen and humidity) or in 26 L HDPE watertight containers (non-permeable to oxygen and humidity). As shown in Figure 1, sealed flour bags and containers were stored 131 at controlled temperature (at either 20  $^{\circ}$ C or cold storage room at -20  $^{\circ}$ C). The chemical composition and analytical profiles of the flours (packed in two different types of packaging, and stored in two different conditions) were determined after 3 months, 6 months, 12 months, 21 months and 30 months of storage,

**2.1. Flour analyses** 

Chemical analyses were performed and analytical profiles (alveograph, farinograph and 139 Solvent Retention Capacity) obtained by Upscience Labs Solution (St-Nolff, France) at t=0, 140  $t=3$  months,  $t=6$  months,  $t=12$  months,  $t=20$  months and  $t=30$  months. The principles and standards of the various analyses performed are summarized in the Supplementary data (Table 142 ). 



pre-mixing step was performed at 100 rpm for 330 s to uniformly disperse the raw materials. Then, kneading was performed at 200 rpm until the optimum mixing time, the so-called "time 155 to peak" or "t<sub>PEAK</sub>", which is set to the time needed to reach the maximum power 156 consumption of the mixer. After a rest period of 3 minutes, dough pieces of  $280 \pm 5$  g were cut from the dough mass. Each dough piece was rolled out by hand and left to rest for a further 10 minutes. They were then shaped by hand and placed in moulds. The moulds were placed in a Bi-Tronic Control fermentation chamber (Hengel, France) at a relative humidity of

160 80% and a temperature of 40 °C, until they reached a growth corresponding to 200 % of the initial volume. The time needed to reach this volume was about 50 minutes.

Baking was carried out in an MIWE deck oven (Arnstein, Germany). After loading, 300 mL of water was sprayed onto the loaves. Baking time was programmed for 20 minutes at a 164 temperature of 220  $\degree$ C. At the end of baking, the loaves were removed from the moulds and left to sweat for at least 2 hours at room temperature.

#### **2.3. Evaluation of bread quality**

Six breads were analysed using the following method for each storage condition. The breads were cut into 15 mm thick slices along their entire length using a professional multifunction slicer (Sofraca, France). Approximately seven slices were obtained for each bread. Then, the bread slices were scanned one by one against a black background in an Epson scanner (Epson Perfection V37, Japan). The scan window was set to 120 x 120 mm corresponding to a size of 1889 x 1889 pixels. Each image was processed using Fiji (ImageJ). First, they were converted into 8-bits and thresholded, then the area and the diameter of each crumb cell were calculated. The height of the bread slices was estimated by measuring the length of a vertical line drawn in the middle of the slices.

#### **2.4. Near-infrared Spectroscopy**

181 Near-Infrared (NIR) spectra of flour at t=3 months, t=6 months, t=12 months, t=20 months and t=30 months were recorded using an ANTARIS MII FT-NIR Analyzer (Thermo Scientific, France). About 10g of flour was placed in a quartz sample cup spinner and measured using the integrating sphere module for diffuse reflectance sampling. Triplicates 185 were acquired over the 4000-10 000 cm<sup>-1</sup> spectral range with a spectral resolution of 8 cm<sup>-1</sup>. Each spectrum was the average of 16 scans and represented as log (1/R) values as a function of wavelength (nm). A Standard Normal Variate (SNV) transformation was applied to the data set to remove the particle size effect and scattering offset. Principal Component Analysis (PCA) of the NIR data was performed using MATLAB R2019b software (The MathWorks, Inc). This technique extracts the main sources of variability of a set of spectra with no prior knowledge of the chemical composition of the samples. PCA reduces the dimensionality of the data while retaining most of the variance in the data set (Dale et al., 2012), by identifying directions, called principal components, along which the variance of the data is maximal. Thus, each sample can be represented by a few numbers instead of thousands of variables. The observation of the distribution of the samples in the PC subspace allows analysing their common features and/or their grouping.

TQ Analyst 9.5 Software (Thermo Scientific, France) was used to develop prediction models. The samples were divided into a calibration set of 42 samples, and a validation set of 20 samples. Calibration was carried out by Partial Least Square (PLS) regression. PLS calculates new variables by selecting the dimensions that explain the maximum variance in both the spectral range and the dataset. It therefore ensures that the factors derived are correlated to the reference data (Dale et al., 2012).

#### **3. Results and discussion**

- **3.1. Flour properties**
- **3.1.1. Chemical analyses**

Protein, damaged starch and ash contents remained quite stable during the entire storage period for all types of packaging (Figure 2 a, c and d). The water content was subject to more

210 drastic variation in the case of packaging in paper bags. An increase of about 1.5 % in water 211 content was observed from the first three months of the storage of bagged flours at -20 °C. 212 The increase continued during the first year up to about 4% and then stabilized.



Conversely, after three months of storage in a paper bag at room temperature, the water 235 content gradually decreased to more than 7% after thirty months of storage. Less than 1% variation was observed for the flours stored in watertight containers (Figure 2 b).

The Hagberg falling number tended to increase for flours stored at room temperature while it 238 remained constant at -20  $^{\circ}$ C (Figure 2 d). This trend had already been observed in the study of Brandolini et al. (2010). This means that amylolytic activity decreases during the extended storage of flours at room temperature.

Oleic acidity increased drastically for flour stored at ambient temperature (Figure 2 f), meaning that the resulting dough would be weaker with reduced strength and increased plasticity. The hydrolysis of glycerides by wheat and microbial lipases could increase the 244 amounts of free fatty acids in the flours stored at  $20^{\circ}$ C.

TOTOX is the total oxidation measurement, taking into account the contribution of the peroxide value, an expression of primary oxidation, and the anisidine value, an expression of secondary oxidation. The TOTOX value was used here to obtain overall information on oxidative degradation during flour storage. The oxidation peak occurred at about one year of storage before a decrease that could have been due to the better resistance of saturated fats toward oxidation. This oxidation peak was more pronounced for flours stored at room 251 temperature (Figure 2 h). Samples stored at  $-20$  °C appeared to have better oxidation stability, and less tendency to develop rancidity.

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#### **3.1.2. Alveograph analyses**

The initial P/L ratio was around 1 which is ideal for bread baking. It remained quite stable for 257 the flours stored at -20  $^{\circ}$ C, whereas after 6 months, it increased considerably for the flours stored at room temperature (Figure 3 a). This means that the dough did not have the desired degree of extensibility. Baking strength fluctuated noticeably but on average centred around  $250.10^4$  J regardless of the storage condition (Figure 3 b). Logically, the Swelling index decreased after 6 months for the flour stored at room temperature (Figure 3 c). Conversely, 262 the height at 20 mm increased for the flours stored at ambient temperature (Figure 3 d).



276 *Figure 3* : Alveograph analyses of flours stored in paper bags at 20 °C (B+  $\rightarrow$  ), paper bags at -20 °C (B- $\rightarrow$  ), watertight containers at 20 °C (C+  $\blacksquare$ ) and watertight containers at -20 °C (C-  $\spadesuit$ ) over 30 months a) P/L ratio, b) Baking strength, c) Swelling index, d) Height at 20 mm

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### 278 **3.1.3. Farinograph Analyses**

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# 280 Although the water content was quite stable in the watertight container at room temperature, 281 water absorption increased by more than 2.6% during the 30 months of storage (Figure 4 a). 282 An increase of about 1.8% was also observed for the flour bagged at ambient temperature. For 283 the flour stored at -20  $^{\circ}$ C, a small increase was observed at the beginning of storage but

284 finally it quickly returned to its original value. In the same way, stability decreased for the 285 flours stored at room temperature but was more stable for those stored at -20  $^{\circ}$ C (Figure 4 c). 286 The development time remained quite stable over the storage period except for the flour 287 stored at -20  $^{\circ}$ C in a sealed container at one year of storage (Figure 4 b).

As only one analysis was performed and the value returned to its original value afterwards, we can consider that this point was probably an artefact. The degree of softening fluctuated between 40 and 60 UF and increased at 70 UF for the flour stored in a watertight container at  $-20$  °C (Figure 4 d).





All these results suggested that bread quality should be affected for the flours stored at room temperature.

#### **3.1.4. Solvent Retention Capacity**

As shown in Figure 5, the SRC values remained quite stable for the flours stored at -20°C while they tended to decrease for the flours stored at room temperature. The Water Holding Capacity of these two flours was thus substantially reduced after 30 months storage (Figure 5 a). However, the Gluten Performance Index, calculated from a ratio of the lactic acid, sodium carbonate and sucrose SRC value, was stable over the storage period whatever the storage condition (Figure 6). The performance of flour glutenins should be almost the same.





*Figure 5:* Solvent Retention Capacity analyses of flours stored in paper bags at 20 °C (B+  $\triangle$ ), paper bags at  $-20$  °C (B- $\rightarrow$ ), watertight containers at 20 °C (C+ $\rightarrow$ ) and watertight containers at -20 °C (C $\rightarrow$ ) over 30 months a) Water absorbance capacity, b) Development time, c) Stability, d) Degree of softening



 *Figure 6* : Gluten Performance Index calculated for flours stored in paper bag at 20 °C (B+  $\triangleq$ ), paper bag at -20 °C  $(B - \rightarrow \rightarrow)$ , watertight container at 20 °C (C+  $\rightarrow \rightarrow$ ) and watertight container at -20 °C (C- $\rightarrow \rightarrow$ ) over 30 months

#### **3.1.5. NIR analyses**

PCA was applied to the set of samples composed of 60 spectra. Figure S1 a presents the score plots of the first and third components after SNV pre-treatment, explaining 86.6 % of the total variance. Figure S1 b and Figure S1 c present the first and third associated eigenvectors respectively. The samples stored in bags were clearly separated along the first axis while the samples stored in watertight containers did not seem to be affected according to this first component. The first eigenvector was characteristic of the water signal with an intense broad band around 1950 nm and a smaller one around 1430 nm (O-H), suggesting that the flour conserved in permeable bags at -20 °C absorbed water due to freezing during the first months 352 of storage. Conversely, the flour conserved in paper bags at  $20\degree$ C seemed to dry out progressively. As expected, the quantity of water in the flour was stable in the watertight containers. The third eigenvector indicated that the samples with the longest shelf-life regardless of packaging presented a weaker signal from the starch component with bands around 1930 nm: O-H stretching and H-O-H bending from polysaccharides, 2100 nm: C=O and C-O stretching and O-H bending from polysaccharides, 2283 nm and 2324 nm: C-H stretching and CH2 deformation from polysaccharides, 2480 nm: C-H stretching and C-C and C-O-C stretching combination from polysaccharides.

The standard errors and correlation coefficients of the PLS models are shown in Supplementary Table 2. A particularly good prediction was observed for water, protein and damaged starch content, Hagberg Falling Number, P/L, Index of swelling and all SRC parameters. An acceptable prediction accuracy was obtained for ash, oleic acidity and TOTOX. Conversely, the strength and farinograph parameters did not yield satisfactory results (data not shown).

#### **3.2. Bread quality**

Power consumption curves as a function of mixing time for each storage condition are displayed in Figure S2. During the pre-mixing step, the power consumption increased only slightly. Then, during the development time, the power consumption increased more quickly to reach a maximum.

The doughs produced from Control, C- and B- flours were very similar. They required almost the same time to reach maximum power consumption (around 230 W), approximately 8 minutes for all three. The C+ and B+ flours needed more time to reach maximum power consumption (10 minutes), with a final power lower than 200 W, indicating that these doughs were also less resistant to tool rotation. This result was consistent with the Alveograph and the Farinograph analyses.

After baking, the breads obtained from each flour storage condition were somewhat different in height and crumb fineness (Figure S3). The breads obtained from the flour stored in 381 watertight containers at -20  $^{\circ}$ C were the closest to the control bread with an average number 382 of bubbles around 1,900 and an equivalent bubble size close to 1.40 mm<sup>2</sup>, which fell within

383 the standard deviation. The breads obtained from the flour stored in paper bags at -20  $\degree$ C had 384 fewer bubbles per slice but with diameters around  $1.70 \text{ mm}^2$ . On the other hand, the breads obtained from flours stored at ambient temperature presented more than 2 000 bubbles per 386 slice with diameters smaller than  $1.30 \text{ mm}^2$ .

Except for the breads obtained with flour stored in watertight containers at ambient temperature, the height of the bread was almost unchanged for all the other storage conditions and was consistent with those obtained for the control bread (Figure S4). These results should be compared with those obtained with the farinograph, which showed that the flour stored in the watertight container at room temperature had a high level of water absorption, a higher degree of softening and poorer stability.

Images of the bread slices are shown in Figure S5 in order to visually compare the fineness and size of the bubbles for the different storage conditions. Coarser gas cells were observed in control and B- slices, which exhibited the greatest similarity.

#### **4. Conclusion**

In this study, flours were stored for 30 months to assess the impact of storage conditions on flour quality. A major outcome was that the flour stored at room temperature exhibited many 401 more changes than the flour stored at  $-20$  °C. Most of the analyses remained very stable over 402 the storage period for the flours stored at  $-20$  °C, except for the water content in the flour stored in permeable bags. Indeed, the water content in the flour progressively stabilized with atmospheric humidity, with an increase of water content of about 4% over 30 months. The water permeability of the bag was also a problem at room temperature where water loss was estimated to be close to 7%. The NIR results were consistent with these trends. The first PCA component classified the samples according to their water content in the same way. The potential of the NIR technique as known from the literature and experience was confirmed for the prediction of chemical components and certain rheological parameters like P/L and the Swelling Index. Solvent Retention Capacity was also predicted with accuracy. Moreover, the results presented in this study showed that storage at room temperature greatly modifies other chemical wheat flour components. In both the paper bag and watertight container, it appeared that the flours stored at room temperature presented increased acidity and Hagberg Falling Number, strong peak oxidation during the first year of storage and a considerable decrease in Solvent Retention Capacity. This separation between the two storage conditions was also confirmed with the Farinograph and Alveograph results. Both analyses indicated poorer performance for flour stored at ambient temperature and suggested that bread quality was poorer. This point was noticeable from the mixing step. Indeed, the flours stored at room 419 temperature required more time to reach t<sub>PEAK</sub>, with lower power. After baking, it appeared 420 that only the flour stored in the watertight container at  $-20$  °C retained similar bread-making properties to that of a loaf made with a recently milled flour.

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- **5. Recommendations**
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The choice of suitable packaging impermeable to both oxygen and moisture, and stored in a cold room, is of upmost importance when planning long duration flour storage. It can therefore be recommended to store flour at negative temperature in sealed container in the case of storage of wheat flour 6 months. A more convenient alternative would be to store the flour at -20°C in paper bags, which allows also a good preservation of the initial quality of wheat flour except a partial oxidation of the flour lipids. This can be useful in the case of long term research projects for which a reference flour is needed.



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## 488 **Figure caption**

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507 paper bags at -20 °C (B-), watertight containers at 20 °C (C+) and watertight containers at -20 508 °C (C-) over 30 months a) Water absorbance capacity, b) Development time, c) Stability, d) 509 Degree of softening.

510

- 511 Figure 6 : Gluten Performance Index calculated for flours stored in paper bag at 20 °C (B+),
- 512 paper bag at -20 °C (B-), watertight container at 20 °C (C+) and watertight container at -20 °C
- 513 (C-) over 30 months.