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# Study of wheat gluten plasticization with fatty acids

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## Abstract

The plasticizing effect on wheat gluten of a series of saturated fatty acids with different carbon chain length (from 6 to 10 carbons) was investigated. Plasticized materials were obtained by mixing in a counter-rotating batch mixer. The wheat gluten plasticization with the saturated fatty acids was shown to be thermodependent. Differential scanning calorimetry enabled the determination of the compatibility limits between wheat gluten and the fatty acids. Plasticizing effect, as judged from the shift of the temperature of the glass to rubbery transition, was determined by dynamic mechanical thermal analysis. As results showed a series effect on several times, it was thus possible to approximate the plasticizing effect of the different saturated fatty acids with a unique Couchman–Karasz equation. The molecular size distribution of the plasticized gluten was analyzed by size-exclusion high performance liquid chromatography (SE-HPLC). Mixing of plasticized wheat gluten induced variable and complex changes in the protein size distribution and resulted in a predominant polymerization, indicating a gluten reactivity in the presence of the fatty acids. The water vapor permeability of films obtained by pressure molding from the mixed plasticized samples showed improved barrier properties in comparison with films from wheat gluten plasticized with glycerol. The use of saturated fatty acids open thus new ways for the plasticization and the improvement of properties of gluten based biodegradable plastics.
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*Keywords:* Wheat gluten plasticization; Saturated fatty acids; Biodegradable polymer

## 1. Introduction

Some plant proteins are interesting renewable raw materials, because of their thermoplastic properties. Wheat gluten is unique among cereal and other plant proteins in its ability to form a cohesive blend with viscoelastic properties once plasticized. For these reasons, the use of wheat gluten to process edible or biodegradable films or packaging materials has been proposed [1–4].

In general, gluten-based materials require addition of plasticizer agents. Many theories have been proposed to account for the plasticization mechanism [5]. The interactions between a polypeptidic chain and a plasticizer are still not clearly understood even if they are known to be sensitive to the molecular weight, the physical structure and the chemical composition of the plasticizer. The action of a plasticizer is generally to interpose itself between the polymer chains and alter the force holding the chains together. As a result, the glass transition temperature ($T_g$) of the material is depressed. Polymer plasticization enables thus to reduce the shaping temperature of the thermoplastic process and to impart adequate flexibility to the material [3,6,7]. However, it can also greatly influence the functional properties of the material [8,9].

Plasticizing effect is thus currently estimated by the evolution of the material thermal and thermomechanical properties (characterization of the $T_g$ by differential scanning calorimetry (DSC) or dynamical mechanical thermal analysis (DMTA)) and of the material functional properties (mechanical and permeability properties for example). The effect of a plasticizer on the material $T_g$ can be modeled by different equations [10–12]. The Couchman–Karasz equation is very used to predict $T_g$ of plasticized proteins [7,13–15].

Hydrophilic compounds (water, polyols, oligosaccarides) are generally used as proteins plasticizer [13,16,17]. However, Kalichevsky et al. [14], Lai and Padua [18], Di Gioia and Guilbert [19] had also proposed the use of lipidic compounds as plasticizer of proteins such as wheat gluten, zein and corn gluten meal. In addition, several investigators

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have examined and confirmed the effectiveness of lipids (waxes, oils, fatty acids, monoglycerides), added as layers or emulsion, in reducing water vapor permeability (WVP) of composite films based on proteins or polysaccharides [20–23].

The objectives of the present study were to investigate wheat gluten plasticization with a series of lipids. Saturated fatty acids were chosen: a common hydrophilic head (carboxylic group) defines the series, while the hydrophobic zone (carbon chain) increases, thus resulting in a variation of the hydrophobicity degree. Mixing experiments were performed in order to check the ability of the different fatty acids to plasticize wheat gluten and to obtain homogeneous material. Thermal, thermomechanical and biochemical analyses were then realized to characterize the compatibility limits, the plasticizing effect and the protein size distribution, respectively. Finally, WVP measurements enabled a forecast of the water sensitivity of the materials.

## 2. Materials and methods

### 2.1. Materials

The commercial wheat gluten used in this work was graciously obtained from Amylum Aquitaine (Bordeaux, France). Its moisture content was 8.5% and its protein content, determined by the Dumas method (NA 2000, Fisons Instruments, France), was 77.3% (wb).

The plasticizers used were anhydrous glycerol (Fluka Chemie, Buchs, Switzerland) and saturated mono fatty acids (Sigma-Aldrich Chemie, Steinheim, Germany) with an even number of carbons from 6 to 18 ($C_{6:0}$: hexanoic acid, $C_{8:0}$: octanoic acid, $C_{10:0}$: decanoic acid, $C_{12:0}$: lauric acid, $C_{14:0}$: myristic acid, $C_{16:0}$: palmitic acid, $C_{18:0}$: stearic acid).

### 2.2. Mixing process

Materials were obtained by mixing in a two blade counter-rotating batch mixer turning at a 3:2 differential speed (Plasti-corder W 50, Brabender, Duisburg, Germany) connected to computer interface and controller unit (PL 2100, Brabender, Duisburg, Germany). Torque and product temperature were continuously recorded during the mixing process. Temperature of the mixing chamber was regulated using a regulation temperature unit (WTD4, Weinreich, Lüdenscheid, Germany) and water circulation in the double jacket of the mixer. Plasticizers, equilibrated at 25 °C (except decanoic acid, which need to be stabilized at 40 °C to get a liquid), were first introduced; then cold gluten (4 °C) was added in the mixer.

A plasticizer content of 34.64% (weight/total weight) was used for all experiments. Mixer chamber (volume of 50 $cm^3$) was filled with 50 g total mass. Mixing speed was 100 rpm. The mixing chamber was regulated at 40, 60, 80, 100 or 120 °C. Usually, mixing was stopped 5 min after the time needed to reach the maximum torque.

The obtained blend was pressed to approximately 3 mm height (thanks to spacers) in a heated press at 100 °C, for 5 min, to obtain plates.

### 2.3. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine the cryofracture surface of materials. Each sample was laid on double-sided carbon adhesive tapes mounted on aluminum stubs, then coated with ionized platinum. They were observed by scanning electron microscopy (JEOL 6300 F, Tokyo, Japan) using an accelerating voltage of 7 kV.

### 2.4. DSC analysis

DSC analysis was investigated on wheat gluten plasticized samples, in order to determine the compatibility limits between wheat gluten and the plasticizers. Actually, above the compatibility limit, the fatty acid in excess did not act as a plasticizer: it is thus not in interaction with wheat gluten and its melting point can be detected. To quantify this part of plasticizer, changes in heat capacity were also measured on each fatty acid alone.

The differential scanning calorimeter used was equipped with a modulation extension device and a refrigerated cooling system (2920 Modulated DSC, TA Instruments, New Castle, USA). Nitrogen was used as a purge gas at a flow rate of 50 ml $min^{-1}$ and as a cooling gas line at a flow rate of 150 ml $min^{-1}$. The heating rate was 5 °C $min^{-1}$, and the period and the amplitude of modulation were, respectively, 60 s and $\pm 0.796$ °C. Around 1.7 mg of fatty acid or 5.0 mg of plasticized sample were packed down and hermetically sealed into aluminum DSC pans with inverted lids to achieve optimum thermal conductivity.

### 2.5. DMTA measurements

Rectangular samples of the plasticized materials ($23 \times 6 \times 3$ $mm^3$) were analyzed with a dynamic mechanical thermal analyzer (DMTA IV, Rheometric Scientific, Piscataway, USA) equipped with a cryogenic system fed with liquid nitrogen. A single cantilever bending test was performed with a temperature ramp from $-100$ to 150 °C at a heating rate of 3 °C $min^{-1}$. A variable sinusoidal mechanical stress was applied to the sample (frequency $= 1$ Hz) to produce a sinusoidal strain amplitude of 0.016%. A continuous flow of nitrogen C (Air Liquide) in the oven provides an inert atmosphere. During analysis, the storage modulus ($E'$), the loss modulus ($E''$) and tan $\delta$ ( $= E''/E'$) were recorded and plotted against temperature for further evaluation of thermal transition. Each sample was analyzed in duplicate.

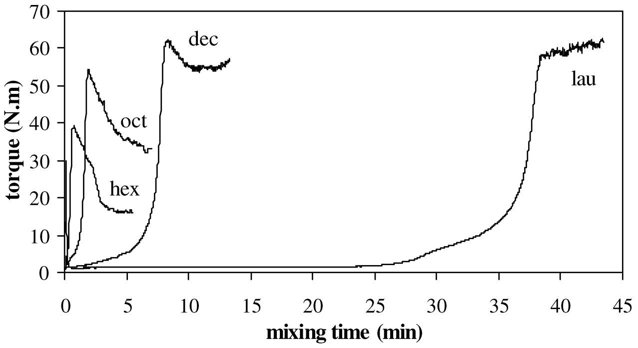

Fig. 1. Torque evolution during the mixing process of wheat gluten with hexanoic, octanoic, decanoic or lauric acids (respectively, hex, oct, dec and lau) (100 rpm, 34.64% plasticizer content, regulation temperature of 60 °C).

### 2.6. GPC analysis

The molecular size distribution of the gluten proteins was studied by size exclusion high performance liquid chromatography (SE-HPLC), as previously described by Redl et al. [4]. Briefly, samples (160 mg of ground material with starch) were stirred for 80 min at 60 °C with 20 ml of 0.1 M sodium phosphate buffer (pH 6.9) containing 1% sodium dodecyl sulfate (SDS). The SDS-soluble protein extract was recovered by centrifugation (30 min at 39,000 g and 20 °C) and 20 μl were submitted to SE-HPLC fractionation (first extract). The pellet was suspended in 5 ml SDS-phosphate buffer containing 20 mM dithioerythritol (DTE). After being vortexed and stirred for 60 min at 60 °C, the extract was sonicated for 3 min at 30% power setting. DTE reduction and sonication both allow insoluble glutenin polymers of wheat gluten to be solubilized. After centrifugation (30 min, 39,000 g, 20 °C), a part of the supernatant was mixed volume to volume with SDS-phosphate buffer containing 40 mM iodoacetamide in order to alkylate thiol groups. The reaction was carried out for 1 h in darkness, at room temperature. 20 μl of this solution was submitted to SE-HPLC fractionation (second extract).

### 2.7. Water vapor permeability measurements

To obtain films, discs (diameter = 24 mm) were cut in each plasticized material and pressed with a Teflon frame (250 μm thickness) at 100 °C, under 150 bars, for 10 min. A hand-held micrometer (Braive Instruments, Checy, France) was used for measuring film thickness. Five micrometer readings were taken for each film. The standard deviation of film thickness was ≤4%.

WVP was determined gravimetrically at 25 °C, using the modified ASTM procedure described by Gontard et al. [1]. Each test film was sealed to an individual glass permeation cell (internal diameter = 34 mm) containing distilled water (100% RH). The cells are placed at 25 °C in a ventilated desiccator containing silica gel. The water vapor transferred through the film and absorbed by the desiccant was determined from the weight loss of the cells. WVP was calculated as follows:

$$\mathrm{WVP}\ (\mathrm{mol\ m^{-1}\ s^{-1}\ Pa^{-1}}) = \frac{wx}{At(p_2 - p_1)}$$

where $w$ is the weight loss (converted in mole of water) of the cell during time $t$ (s), $x$ (m) the film thickness, $A$ (m$^2$) the film surface exposed and $p_2 - p_1$ (3166 Pa) is the partial water vapor pressure difference on either side of the film.

## 3. Results and discussion

### 3.1. Mixing data

Fig. 1 shows the torque evolution in the batch mixer within time for gluten plasticized with four different fatty acids, at a given regulation temperature (60 °C). Torque curves depend strongly on the fatty acid. For the shortest carbon chain lengths (hexanoic and octanoic acids), the torque increases immediately to a maximum and then decreases rapidly to a stable value. The torque development is associated with a change in consistency from a powder/water dispersion to a cohesive and elastic material [4]. Occurrence of rubbery-like properties after the torque peak indicates without any doubt that gluten is fully plasticized. With increasing carbon chain length of the fatty acids, a lag phase appears before the torque increase (Fig. 1). This lag time can be even more important (lack of torque developed with myristic acid after 2 h of mixing at the regulation temperature of 60 °C). The time needed to develop a torque may depend on the ability of the constituents to interact together. It may therefore be related to wettability and diffusion characteristics of plasticizers. For a given procedure, the peak characteristics (time to peak, intensity of the maximum torque, and temperature at peak) are reproducible: they are therefore good parameters to study the gluten plasticization as a function of the fatty acids and of the regulation temperature.

The time to peak, as a function of the carbon number of the plasticizers, shows an exponential increase for a given regulation temperature. This type of relationship is observed for the five tested regulation temperatures as shown by the semi-log plot in Fig. 2a. Consequently, the different saturated fatty acids display a series behavior. As the carbon chain length of the fatty acid is connected to its hydrophobicity degree and to a steric hindrance, these parameters should play a key role in plasticization. For a given fatty acid, the time to peak decreases as the regulation temperature of the mixing chamber increases. This phenomenon was already observed by Di Gioia and Guilbert [19] for octanoic acid as plasticizer of corn gluten meal. As the molecular mobility increases with the temperature, higher regulation temperatures facilitate diffusion and interactions. In addition, higher temperatures are in favor of weakening intramolecular hydrogen bonds, what results

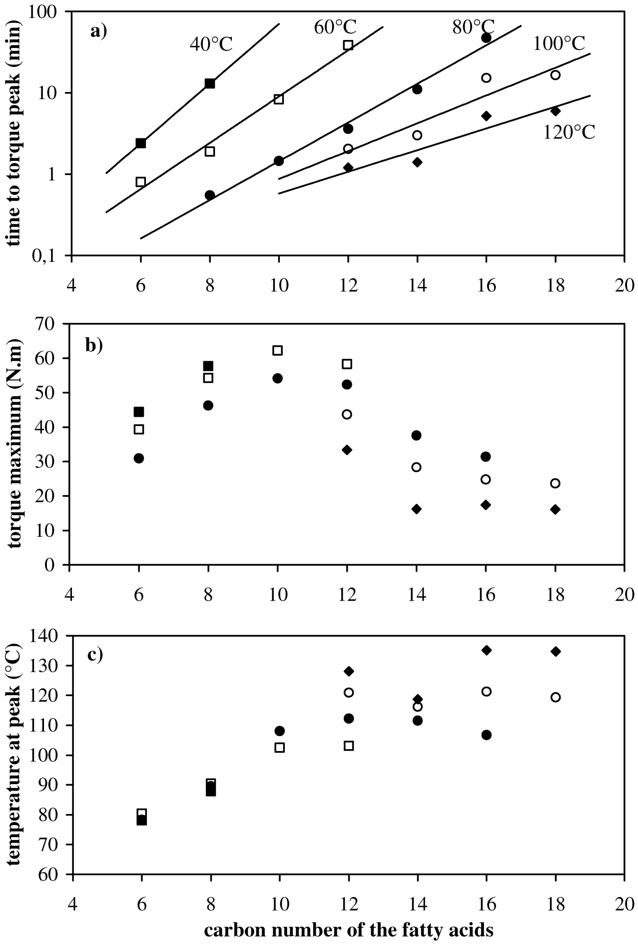

Fig. 2. Evolution of the time to torque peak (a), of the torque maximum (b) and of the temperature at torque peak (c) with the carbon number of the fatty acid used as plasticizer, for different regulation temperatures (■ 40 °C, □ 60 °C, ● 80 °C, ○ 100 °C, ◆ 120 °C) (100 rpm, 34.64% plasticizer content).

in an unfold of wheat gluten proteins, thus exposing hydrophobic sites and enabling a greater reactivity of long chain fatty acids which display a greater hydrophobic part.

The evolution of the maximum torque as a function of the carbon chain length of the fatty acids is shown in Fig. 2b. First, the maximum torque increases with the carbon chain length of the fatty acids until 10 carbons, then it decreases to reach 20 N m in average. As the plasticizer content was constant in weight, its mole content and the added volume, respectively decreases and increases with the molecular mass of the fatty acid. The fatty acid mole content drops from 0.5 (hexanoic acid) to 0.2 mol/100 g dry gluten (stearic acid). This variation is much more important than the volume increase (from 18.7 to 20.5 ml) and can be related to the initial torque increase with fatty acid length (Fig. 2b): the lower the plasticizer mole content, the higher the apparent viscosity of the material. The decrease observed when the fatty acid carbon chain length exceeds 10 carbons may be due to a lubricant effect rather than to a volumetric dilution effect, since a part of the long chain fatty acids is exuded from the material as it was demonstrated by DSC (see below).

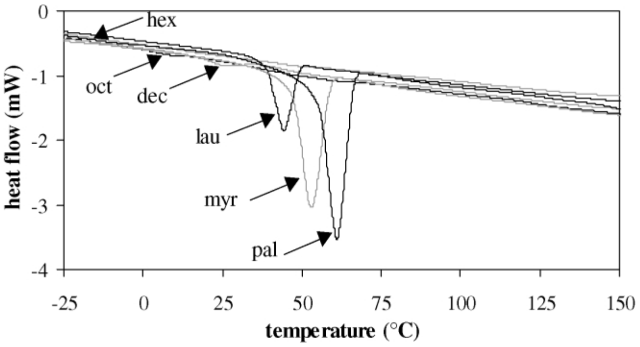

Fig. 3. DSC thermograms of wheat gluten plasticized with 34.64% hexanoic (hex), octanoic (oct), decanoic (dec), lauric (lau), myristic (myr) or palmitic (pal) acids.

Even though the time to peak and the maximum torque vary with the regulation temperature for a given fatty acid, a unique temperature is reached at the torque peak for each fatty acid until a chain length of 10 carbons (Fig. 2c). Redl et al. [4] already observed this phenomenon for glycerol as plasticizer (the temperature was in the range of 50–55 °C) and correlated it with important rheological and molecular changes observed by other authors in this range of temperature. Determining changes in conformation of the polypeptide chains seem to appear at a specific temperature, and may coincide with its complete plasticization. Consequently, the threshold temperatures (about 80, 90 and 105 °C for the hexanoic, octanoic and decanoic acids, respectively) indicate a thermodependence of wheat gluten plasticization with fatty acids. As the temperature at torque peak is specific to each plasticizer, determining changes must be inherent to a given blend rather than to wheat protein only. No precise temperature can be determined for the blends with fatty acid of more than 10 carbons (Fig. 2c). As the temperature at peak cannot be inferior to the regulation temperature, if a threshold temperature exists for these blends, it cannot be evidenced since high regulation temperatures were used in order to shorten the times to peak.

### 3.2. Thermal properties

DSC and DMTA measurements were carried out with the series of samples obtained by mixing at a regulation temperature of 80 °C because it was the mixing conditions that enabled to test most of the fatty acids in the same mixing conditions (from hexanoic to palmitic acid). The analyses were also realized for a sample plasticized with glycerol.

The DSC curves are shown in Fig. 3. There is no clear evidence of a change in heat capacity for the materials plasticized with the fatty acids from 6 to 10 carbons, indicating that these plasticizers are mobilized in interactions with wheat gluten, then fatty acid fusion is hardly

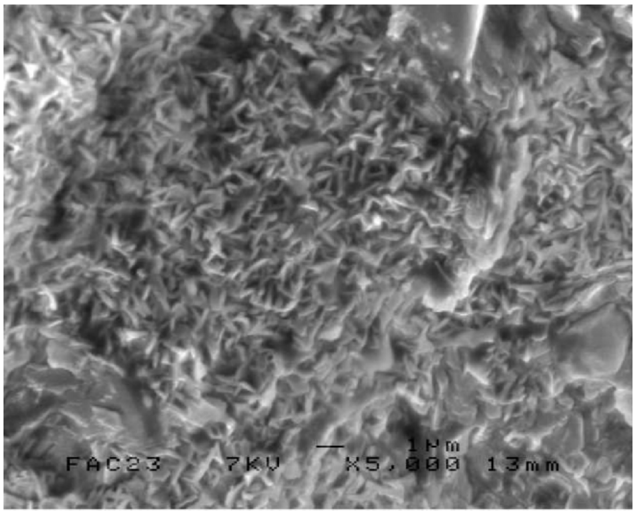

Fig. 4. Fatty acid crystals observed by SEM on the cryofracture surface of a wheat gluten based material plasticized with palmitic acid.

observed. On the contrary, lauric, myristic and palmitic acids melting points can clearly be identified (Fig. 3), suggesting that the corresponding materials are heterogeneous, containing plasticized gluten matrix and a fatty acid phase partially crystallized at ambient temperature. The heterogeneity of these blends was confirmed by SEM: fatty acid crystals are clearly visible on samples cryofractured surface as shown in Fig. 4 with the material plasticized with palmitic acid. It can be concluded that the plasticizer quantity is in this case too important, thus leading to a partial exudation of the fatty acid. As a result, the compatibility limit is reached, and the molar percent of exuded plasticizer can be calculated by comparison between the melting heat capacities obtained for the fatty acid alone and for the plasticized sample.

The melting temperature of fatty acids, corresponding enthalpy and molar percent of exuded plasticizer are reported in Table 1. These results enable the calculation of the effective fatty acid mole content that plasticized wheat gluten (called the fatty acid plasticizing mole content). These plasticizing mole contents correspond to the compatibility limits between wheat gluten and each fatty acid. A phase diagram can thus be established between the different fatty acids (defined by their carbon number) and their corresponding maximum mole content that is compatible with wheat gluten. As shown in Fig. 5, the compatibility limit decreases as the carbon chain length increases, in accordance with an exponential law. Beyond this limit, a fatty acid exudation is observed on the material. The plasticizer is then no more compatible with wheat gluten and the corresponding material will be heterogeneous.

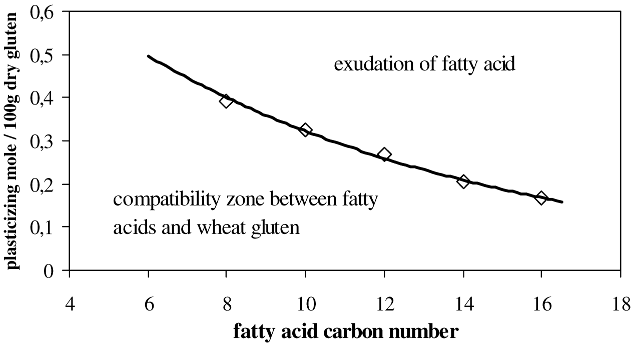

Fig. 5. Phase diagram for a wheat gluten blend mixed with different saturated fatty acid ($y = 0.95\ e^{-0.11x}$ with $R^2 = 0.996$).

As DMTA is very sensitive to the changes in molecular motions and interactions occurring over the transition region, this technique was used to study the glass transition of the series of sample. Typical DMTA scans of wheat gluten plasticized with different fatty acids are shown in Fig. 6. After a linear domain, a sudden decrease in the storage modulus $E'$ (from about $10^9$ to $5 \times 10^6$ Pa) and a pronounced peak in tan $\delta$ indicate drastic changes in the sample physical state (Fig. 6). This major event corresponds to the transition zone from glass-like to rubber-like consistency of the sample and is attributed to the α-relaxation of the material [24]. As the α-relaxation temperature is measured at 1 Hz, it can be assimilated with the glass transition temperature of the materials [25].

The glass transition temperature ($T_g$) was identified to the temperature of the maximum of the tan $\delta$ peak. The tan $\delta$ peak temperatures are reported in Table 1. For the samples plasticized with lauric, myristic and palmitic acid, a smaller tan $\delta$ peak is detected before the major peak related to glass transition, as shown in Fig. 6b with palmitic acid.

Table 1
Characteristics of the thermal and thermomechanical events observed for the different plasticizers

| Plasticizer | DSC results | | | DMTA results |
|---|---|---|---|---|
| | Melting temperature (°C) | $\Delta H$ (J g$^{-1}$) | Molar percent of exuded plasticizer (%) | tan $\delta$ peak temperature (°C) |
| Glycerol | – | – | – | 15 |
| Hexanoic acid | – | – | 0 | 58 |
| Octanoic acid | 13.96 | 0.37 | 2.2 | 72 |
| Decanoic acid | 25.46 | 2.38 | 3.4 | 82 |
| Lauric acid | 44.26 | 16.18 | 7.2 | 97 |
| Myristic acid | 52.95 | 38.94 | 19.4 | 110 |
| Palmitic acid | 61.02 | 53.62 | 25.7 | 106 |

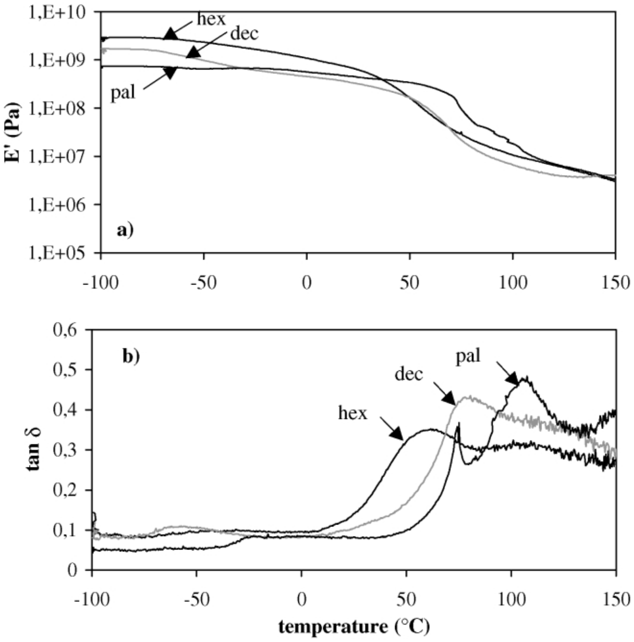

Fig. 6. DMTA thermograms of wheat gluten plasticized with 34.64% hexanoic (hex), decanoic (dec) and palmitic (pal) acids. (**a**), storage modulus ($E'$). (**b**) tan $\delta$.

These events correspond to the melting of the fatty acid and thus confirm the observations already made with DSC measurements, i.e. the heterogeneity of the materials plasticized with lauric, myristic and palmitic acid (Fig. 3).

A simple comparison of the temperatures at tan $\delta$ peak shows that the lowest $T_g$ are obtained with the smallest fatty acids (Table 1). Since the differences in plasticizing effect (i.e. reduction of wheat gluten $T_g$) can depend on the plasticizer mole content, the temperatures at tan $\delta$ peak are shown in Fig. 7 as a function of the fatty acid plasticizing mole content (estimated from DSC results). As a reference value, the $T_g$ obtained for the wheat gluten based material plasticized with glycerol is also indicated. Moreover, $T_g$ of wheat gluten based materials plasticized with water and glycerol were predicted from the Couchman–Karasz equation [11] as follows:

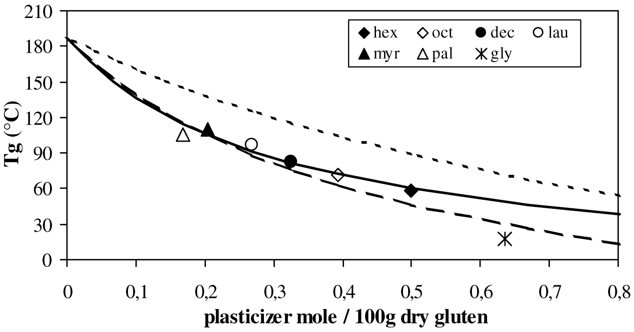

Fig. 7. Glass transition temperature ($T_g$, identified to the tan $\delta$ peak temperature) of wheat gluten plasticized with the different fatty acids or with glycerol (gly) as a function of the plasticizer mole content (data corrected from DSC results). Lines are $T_g$ predictions from the Couchman–Karasz model for water (- - -), glycerol (– –) or a hypothetical fatty acid (—). For the hypothetical fatty acid the model parameters were fitted from experimental data.

$$T_g = \frac{x_1 \Delta C_{p1} T_{g1} + x_2 \Delta C_{p2} T_{g2}}{x_1 \Delta C_{p1} + x_2 \Delta C_{p2}}$$

where the subscript '1' refers to the polymer (wheat gluten) and the subscript '2' to the plasticizer, $x$ is the mass or mol fraction of the component, and $\Delta C_p$ (J g$^{-1}$ K$^{-1}$ or J mol$^{-1}$ K$^{-1}$) the change in heat capacity observed at the glass transition temperature ($T_g$) of the component. For gluten, the thermodynamic characteristics used were those published by Pouplin et al. [17]: $\Delta C_p = 0.4$ J g$^{-1}$ K$^{-1}$ and $T_g = 460$ K. For the plasticizers, the parameters used were $\Delta C_p = 1.94$ and 0.88 J g$^{-1}$ K$^{-1}$ and $T_g = 136$ and 180 K for water and glycerol, respectively [26,27].

$T_g$ value obtained for the sample plasticized with glycerol is in good agreement with the Couchman–Karasz prediction (Fig. 7). The series behavior of the saturated fatty acids enables to consider them as a unique, hypothetical fatty acid. Although the $T_g$ data were obtained from different fatty acids, these points are considered to belong to the same curve. The plasticizing effect of the fatty acids on wheat gluten was thus modeled with the Couchman–Karasz equation, using molar fractions (Fig. 7). The unknown parameters ($\Delta C_p$ and $T_g$ of the hypothetical fatty acid) were optimized from experimental data. The good agreement between experimental data and the model ($R^2 = 0.94$) confirms that no significant difference of plasticizing efficiency among these series of plasticizers can therefore be concluded on a molar base. In addition, the plasticizing effect of the fatty acids is manifest and obviously intermediate between those of water and of glycerol in the molar range tested (Fig. 7).

### *3.3. Molecular size distribution analysis*

Mixing of wheat gluten plasticized with glycerol has been shown to induce changes in the molecular size distribution of gluten protein [4]. SE-HPLC analysis of gluten protein, soluble in SDS-buffer and possibly extracted with the help of sonication, provides indications on the change in the covalent structure of the protein network. Gluten comprises polymeric protein named glutenin, and monomeric protein named gliadin, which are resolved by SE-HPLC. Glutenin eluted within fraction F1 and F2, F1 being the exclusion volume ($M_r$ superior to 660,000) and F2 including polymers from $M_r$ 660,000 to 150,000. Fractions F3 to F5 ($M_r$ 150,000 to 15,000) include the monomeric proteins. Gluten protein is partly insoluble in SDS-buffer due to its huge molecular size [28]. Thanks to DTE reduction and to the severe shear stress induced by ultrasounds, gluten polymer undergoes chain scissions that

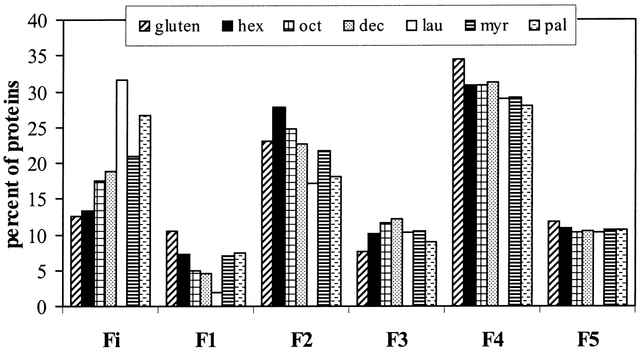

Fig. 8. Distribution of the different protein fractions obtained by SE-HPLC for wheat gluten alone (gluten) or plasticized with hexanoic (hex), octanoic (oct), decanoic (dec), lauric (lau), myristic (myr) or palmitic (pal) acids.

decrease the polymer size. It is thus possible to bring the insoluble protein fraction into solution (extract Fi).

Protein molecular weight distribution analyses were carried out with the series of samples obtained by mixing at a regulation temperature of 80 °C. The areas of the different soluble fractions (F1 to F5) and the total area obtained for the insoluble fraction (Fi), expressed in percent of total protein, are shown in Fig. 8. Mixing of plasticized wheat gluten with fatty acids induced variable and complex changes in the protein size distribution.

We observe a net increase in insoluble protein fraction, irrespective of the type of plasticizer. This phenomenon suggests that mixing induced protein aggregation, probably through the formation of covalent disulfide bonds, other types of bonds (hydrogen, hydrophobic or ionic) being strongly disrupted by SDS. Increase in the insoluble protein fraction comes from a decrease in SDS-soluble fraction but according to contrasted ways. For wheat gluten plasticized with fatty acids with a carbon number from 6 to 12, fractions F1 drop whereas fractions F3 increase. An opposite tendency is observed when the carbon number of fatty acid increases over 12.

The contrasted behavior suggests that different mechanisms occurred depending on the plasticizer structure. Decrease in fraction F1 accompanied with an increase in fraction F2 and especially F3 indicates that a partial de-polymerisation of gluten polymers had occurred during mixing. Indeed similar changes but coming with a decrease in fraction Fi were observed for mixed gluten/glycerol blend in so far as the sample temperature remained below 60 °C [4]. In opposite, decrease in F4, F3 and F2 at the benefit of fractions F1 and Fi indicates unambiguously a polymerisation mechanism. The phenomenon is typically observed when the carbon number of the plasticizer exceeds 12 carbons. De-polymerisation of gluten polymer during mixing is caused by the mechanical shear stress whereas polymerisation is promoted by temperature through disulfide bonding, once gluten is plasticized [29,30]. If we remind that the fraction Fi showed a net increase we can conclude that the polymerisation mechanism prevails in any case. Nevertheless, the balance between the two phenomena (de-polymerisation and polymerisation) depends on thermal and mechanical inputs during mixing, thereby on the fatty acid used as plasticizer (Fig. 2).

Table 2
WVP values of wheat gluten films plasticized with glycerol and different fatty acids

| Plasticizer | Film thickness (μm) | WVP ($10^{-12}$mol m$^{-1}$ s$^{-1}$ Pa$^{-1}$) |
|---|---|---|
| Glycerol | 255 | 62.0 |
| Hexanoic acid | 383 | 17.9 |
| Octanoic acid | 337 | 21.9 |
| Decanoic acid | 267 | 17.2 |
| Lauric acid | 484 | 16.4 |
| Myristic acid | 299 | 7.7 |
| Palmitic acid | 245 | 5.0 |

### *3.4. Water vapor permeability*

The tested films came from the blends obtained by mixing at a regulation temperature of 80 °C. Film thickness and WVP values are given in Table 2. The film corresponding to wheat gluten plasticized with glycerol have a relatively high WVP value ($62.0 \times 10^{-12}$ mol m$^{-1}$ s$^{-1}$ Pa$^{-1}$) in comparison with values reported in the literature (3.1, 5.3 or $6.6 \times 10^{-12}$ mol m$^{-1}$ s$^{-1}$ Pa$^{-1}$) [21,22,31]. This can be explained by two different ways. It can first be due to the high glycerol content of our films (57.9 g/100 g dry gluten contrary to 40, 20 or 23 g/100 g gluten for the films used in the cited literature), since Gontard et al. [9] found that water vapor transmission rate through plasticized gluten film increases with glycerol content. However, our high permeability value can also be due to the high thickness of our film (255 μm contrary to 127 or 50 μm for the films used in the cited literature). Indeed, in this range of thickness, it is known that the WVP value increases with the film thickness [32,33].

The substitution of glycerol by a fatty acid greatly influences WVP of films since permeability values are lowered from 65 to 92% (Table 2). These good results confirm the interest of using hydrophobic plasticizers to lead to a different structuration of the wheat gluten network. Non-polar sites are probably more exposed in this case than in a hydrophilic plasticization (with water or glycerol for example). It can thus be expected that the wheat gluten-based materials plasticized with fatty acids have a greater water resistance than those plasticized with glycerol.

## 4. Conclusion

Wheat gluten can be plasticized with saturated fatty acids. The fatty acids tested as plasticizer of wheat gluten behave as a homogeneous series. Their plasticizing abilities were thus approximated with a unique Couchman–Karasz equation and were found to be intermediate between water and glycerol, in the molar range tested. A compatibility limit between fatty acids and wheat gluten was evidenced by DSC and described by an exponential relationship: the longer the fatty acid carbon chain, the smaller the compatible quantity with wheat gluten. The wheat gluten plasticization with the fatty acids from 6 to 10 carbons at least was shown to be thermodependent. Moreover, the gluten reactivity is enhanced in presence of fatty acid, leading to a polymerization phenomenon. Concerning the WVP measurements, the low values obtained show a significant influence of the amphiphilic plasticization on this parameter. The use of saturated fatty acids open thus new ways for the plasticization and the improvement of properties of gluten based biodegradable plastics.

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## References

[1] Gontard N, Guilbert S, Cuq JL. J Food Sci 1992;57(1):190–5, see also p. 199.
[2] Guilbert S, Cuq B, Gontard N. Food Add Contam 1997;14:741–51.
[3] Cuq B, Gontard N, Guilbert S. Cereal Chem 1998;75(1):1–9.
[4] Redl A, Morel MH, Bonicel J, Guilbert S, Vergnes B. Rheol Acta 1999;38:311–20.
[5] Sears JK, Darby JR. In: Sears JK, Darby JR, editors. The technology of plasticizers. New York: Wiley-Interscience; 1982. p. 42–164.
[6] Slade L, Levine H. In: Blanshard JMV, Lillford PJ, editors. The glassy state in foods. Loughborough, England: Nottingham University Press; 1993. p. 35–102.
[7] Gontard N, Ring S. J Agric Food Chem 1996;44:3474–8.
[8] Donhowe IG, Fennema O. J Food Process Preserv 1993;17:247–57.
[9] Gontard N, Guilbert S, Cuq JL. J Food Sci 1993;58(1):206–11.
[10] Gordon M, Taylor JS. J Appl Chem 1952;2:493–500.
[11] Couchman PR, Karasz FE. Macromolecules 1978;11:117–9.
[12] Kwei TK. J Polym Sci: Polym Lett Ed 1984;22:307–13.
[13] Kalichevsky MT, Jaroszkiewicz EM, Blanshard JMV. Int J Biol Macromol 1992;14(5):257–66.
[14] Kalichevsky MT, Jaroszkiewicz EM, Blanshard JMV. Int J Biol Macromol 1992;14(5):267–73.
[15] Cuq B, Gontard N, Guilbert S. Polymer 1997;38(10):2399–405.
[16] Cuq B, Gontard N, Cuq JL, Guilbert S. J Agric Food Chem 1997;45: 622–6.
[17] Pouplin M, Redl A, Gontard N. J Agric Food Chem 1999;47(2): 538–43.
[18] Lai HM, Padua GW. Cereal Chem 1998;75(2):194–9.
[19] Di Gioia L, Guilbert S. J Agric Food Chem 1999;47:1254–61.
[20] Kamper SL, Fennema O. J Food Sci 1984;49:1478–81, see also p. 1485.
[21] Gennadios A, Weller CL, Testin RF. Cereal Chem 1993;70(4):426–9.
[22] Gontard N, Duchez C, Cuq JL, Guilbert S. Int J Food Sci Technol 1994;29:39–50.
[23] Callegarin F, Quezada Gallo JA, Debeaufort F, Voilley A. JAOCS 1997;74(10):1183–92.
[24] Ferry JD. Viscoelastic properties of polymers. New York: Wiley; 1980.
[25] Perez J, Cavaille JY, Tatibouet J. J Chim Phys 1990;87:1923–67.
[26] Orford PD, Parker R, Ring SG, Smith AC. Int J Biol Macromol 1995; 17(2):81–5.
[27] Donth EJ. Relaxation and thermodynamics in polymers: glass transition. Berlin: Akademie; 1992.
[28] Wrigley CC. Nature 1996;381:738–9.
[29] Schofield JD, Bottomley RC, Timms MF, Booth MR. J Cereal Sci 1983;1:241–53.
[30] Weegels PL, van de Pijpekamp AM, Graveland A, Hamer RJ, Schofield JD. J Cereal Sci 1996;23:103–11.
[31] Micard V, Belamri R, Morel MH, Guilbert S. J Agric Food Chem 2000;48(7):2948–53.
[32] Park HJ, Chinnan MS. International Winter Meeting, Chicago; 1990; paper No. 90-6510.
[33] McHugh TH, Avena-Bustillos R, Krochta JM. J Food Sci 1993;58(4): 899–907.