JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 1447−1452 **1447**

Molecular Determinants of the Influence of Hydrophilic Plasticizers on the Mechanical Properties of Cast Wheat Gluten Films

C. MANGAVEL,* J. BARBOT, J. GUÉGUEN, AND Y. POPINEAU

Institut National de la Recherche Agronomique, Unité de Recherche sur les Protéines Végétales et leurs Interactions, Rue de la Géraudière, B.P. 71627, 44316 Nantes Cedex 03, France

The influence of a set of hydrophilic plasticizers varying in their chain length (ethyleneglycol and longer molecules) on the tensile strength and elongation at break of cast gluten films was studied. When considered on a molar basis (moles of plasticizer per mole of amino acid), the effect of the different plasticizers depended on their respective molecular weights for plasticizer/amino acid ratios in the range from 0.10 to 0.40. However, above a ratio of 0.40−0.50 mol/mol of amino acid, these differences were abolished and both stress and strain reached a plateau value, with all plasticizers studied. In fact, when a homologous series of molecules was considered, the ability for plasticizer to decrease stress and increase strain was closely related to the number of hydrogen bonds the molecule was able to share with the protein network. Ethyleneglycol's efficiency was, however, lower than expected from its hydrogen-bonding potential; a comparison with other diols demonstrated that this was due to the small size of this molecule. The particular effect of glycerol concentration on the films' mechanical properties suggested that other molecular features of the plasticizer, such as the number and position of hydroxide groups in the molecule, were involved in the plasticization mechanism.

KEYWORDS: Wheat gluten; films; casting; plasticizer; hydrogen bonds

INTRODUCTION

Wheat gluten is an interesting source of raw material to produce renewable and biodegradable materials such as films. Many studies have previously shown their potential in terms of mechanical properties (*1*) or gas barrier properties (*2*−*4*). These studies have shown that addition of a plasticizer was necessary to obtain a flexible (not brittle) material.

A plasticizer is a small molecule of low volatility which, when added to polymeric materials, modifies the three-dimensional organization, decreases attractive intermolecular forces, and increases free volume and chain mobility (*5*, *6*). One of the consequences of these changes in molecular organization is that the addition of a plasticizer induces a decrease of the glass transition temperature (T_g) of the material. This effect on the T_g has been the object of previous studies (*7*−*9*) and may in some cases allow an easier processing of the material. Another consequence of the addition of some plasticizer in a protein blend is a modification of the mechanical properties of the material. However, if the effect of several plasticizing molecules on a weight basis on the gluten films properties was compared (*4*, *10*), the study of the molecular determinants of plasticizer efficiency has not been performed yet with this particular protein substrate.

Gluten proteins termed prolamins contain very large amounts of glutamine residues that are mostly present in repetitive domains, covering 30−85% of the polypeptide sequences (*11*). Previous studies suggested a peculiar importance of hydrogen bonding between the repeat regions of hydrated gluten in relation with their aggregation and elasticity (*12*, *13*). In the case of films, plasticizer−protein interactions are supposed to involve mainly hydrogen bonds, and this might be of particular relevance to explain the effect of plasticizers on the properties of gluten-based films.

This study compared the effects of various hydrophilic plasticizers (differing in their chain length) on the mechanical properties of cast gluten films, taking into account their molar amount in the film as well as their ability to share hydrogen bonds with the protein network. Two series of organic molecules have been chosen for this purpose: the ethyleneglycol/diethyleneglycol/triethyleneglycol/tetraethyleneglycol series, which consists of the repeat of the ethylene oxide $-CH_2-CH_2-O-$ group, and the ethyleneglycol/1,3-propanediol/1,4-butanediol series (addition of one methyl group $-CH_2-$ at each increment). In both cases, a comparison will be drawn with glycerol, a plasticizer widely used in protein films.

* Author to whom correspondence should be addressed (e-mail mangavel@nantes.inra.fr).

10.1021/jf0257704 CCC: $25.00
Published on Web 02/01/2003

Table 1. Molecular Characteristics of the Plasticizers

plasticizer	MW	N_{H-b}[a]
ethyleneglycol (EG)	62	6
diethyleneglycol (DEG)	106	8
triethyleneglycol (TEG)	150	10
tetraethyleneglycol (TEEG)	194	12
glycerol	92	9
1,3-propanediol	76	6
1,4-butanediol	90	6

[a] Number of theoretical hydrogen bonds supplied by the molecule.

EXPERIMENTAL PROCEDURES

Materials. An industrial gluten with a protein content of 70.6% was supplied by Amylum (Aalst, Belgium). Glycerol and other chemicals were supplied by Sigma-Aldrich (St. Quentin, France).

Preparation of Protein Films by Casting. Protein films were prepared by casting as described previously (*10*)**.** Briefly, gluten (12.5% w/w) was dispersed into 0.1 N NaOH. After the addition of the plasticizer at the desired amount, the mixture was stirred again and then centrifuged (115*g*, 30 min) to remove trapped air bubbles. The solution was spread on a glass plate covered with a polyester sheet and then dried during 1 h at 70 °C in a ventilated oven. The film was peeled off from the plate and equilibrated during 72 h in a cabinet under standard conditions of temperature (20 °C) and relative humidity (60% RH, produced by a saturated solution of sodium bromide). Each film was made in duplicate.

Protein Contents. Protein content (N × 5.7) was determined after digestion of the gluten films in concentrated sulfuric acid by an automated ammonia/salicylate reaction (*14*).The method was slightly modified as follows: selenium was used as a catalyst, and the digestion was realized at 300 °C during 2 h. Two repeats were made on each film.

Plasticizer Content. The residual amount of plasticizer in the films was determined by high-performance liquid chromatography. Film samples (500 mg) were extracted twice with water (25 mL) for 60 and 30 min, and the volume of the extract was adjusted to 250 mL. The solution was applied on an ion-exchange column (Sucrex-CIL Ca^{2+}) heated at 60 °C. The plasticizer was eluted by water and detected by a differential refractometer (Jobin-Yvon). The concentration was calculated by reference to a calibration curve of the same plasticizer. The measurements were done in duplicate on each film. Taking into account the protein content in the films and assuming an average molecular weight of 121 for all amino acids, the plasticizer content was expressed in moles of plasticizer per mole of amino acid.

Residual Water. The water content in films was determined from the weight difference of the samples (500 mg) after 24 h of drying in a desiccator containing P_2O_5 under reduced pressure. Two measurements were made on each film.

Mechanical Properties. Mechanical properties were evaluated on 5A-type specimens according to the ISO-527-2 standard ($l_0 = 20$ mm). Film thickness was evaluated at five points with a micrometer. Elongation at break and tensile strength were measured in a cabinet (Eratis, Bouloc, France) at 20 °C and 60% RH with a Synergie 100 MTS Systems (Créteil, France) device, equipped with a 10 N load cell. The initial grip separation was set at 50 mm, and the cross-head speed was 20 mm·min^{-1}. Each measurement was replicated five times.

RESULTS AND DISCUSSION

Repetition of the Ethylene Oxide Group in the Plasticizer Molecule. The influence of the repetition of the ethylene oxide ($-CH_2-CH_2-O-$) group in the plasticizer molecule on the mechanical properties of films prepared by casting was studied by using polyols from the ethyleneglycol series as plasticizers. **Table 1** presents the molecular characteristics of these plasticizers. The role of the chain length can then be evaluated by plotting the mechanical properties of films versus the molar ratio of these plasticizers (**Figure 1**). A set of data concerning glycerol—previously obtained (*15*)—is also reported in **Figure 1**. For all plasticizers, the expected effect on the mechanical properties (increase of strain and decrease of stress when the amount of plasticizer increases) is observed (*4*, *10*). In this paper, we will define the relative plasticizing efficiency of a molecule (compared to another one) by taking into account their effects on a film's mechanical properties: at a given molar ratio, a plasticizer will be considered to be more efficient than another molecule if the film made with the first one presents higher strain and lower stress than the film containing the second one.

For the shortest molecules studied [ethyleneglycol (EG), diethyleneglycol (DEG), and triethyleneglycol (TEG)] and the lowest amounts of plasticizer ratio (below 0.40 mol/mol of amino acid), the size of the plasticizer seems to influence the maximal stress of the films (**Figure 1a**). In this case films made with EG present much higher stress (are less plasticized) for the same molar ratio of plasticizer than films made with DEG and TEG: there is a positive influence of the chain length on the plasticizing efficiency. This increase of plasticizer efficiency with increasing molecular weight was previously described on corn gluten meal (*16*). However, TEG and tetraethyleneglycol (TEEG) follow the same curve, thus suggesting that the chain length does not have any more influence on the maximal stress of the films above three repeats of the ethylene oxide group.

Moreover, it was noticed that for each plasticizer there is a threshold amount above which the maximal stress is no more affected by increasing quantities of plasticizer present in the film. Over these threshold amounts, all plasticizers show similar efficiencies and the stress is then at its minimum (∼1.5 MPa). This suggests that a saturation of the protein network by the plasticizer molecules occurred beyond these critical amounts. The occurrence of these critical points was related to the plasticizer molecular weight: 0.40 mol/mol of amino acid for EG, 0.35 mol/mol of amino acid for DEG, and 0.28 mol/mol of amino acid for TEG and TEEG. This saturation amount is then reached sooner for higher molecular weight plasticizer, probably because of some steric hindrance phenomenom (EG molecules are smaller and are then supposed to have an easier insertion in the tridimensional protein network). As gluten proteins are known to roughly contain 40% of glutamine residues in their sequences, this plasticizing threshold value (between 0.28 and 0.40 mol of plasticizer/mol of amino acid) roughly corresponds to a ratio of one plasticizer molecule per one to two glutamine residues. In hydrated gluten, the glutamine residues are supposed to play a specific role in prolamine−prolamine interactions by supplying hydrogen bonds through their side chains (*12*). In that case, we could suggest a similar role of glutamine in plasticizer−protein interactions, thus explaining part of the plasticization mechanism.

These results were compared with those obtained when glycerol (MW between EG and DEG) was used as a plasticizer. The general trend of the plasticizing effect is the same for glycerol, that is, a decrease of stress when the amount of plasticizer increases. Below the threshold value (occurring at 0.60 mol of glycerol/mol of amino acid), the plasticizing efficiency of glycerol falls between those of EG and DEG, thus being consistent with the ranges of molecular weights of these molecules (**Table 1**). However, the plateau value of stress is reached for a higher amount of plasticizer than with the previous series (i.e., ∼0.60 mol of glycerol/mol of amino acid) and the stress value at the plateau is also lower, ∼0.8 MPa. Above the plateau value, glycerol is then more efficient than the other plasticizers. This example shows that the notion of efficiency, as defined previously, cannot be applied without caution to all

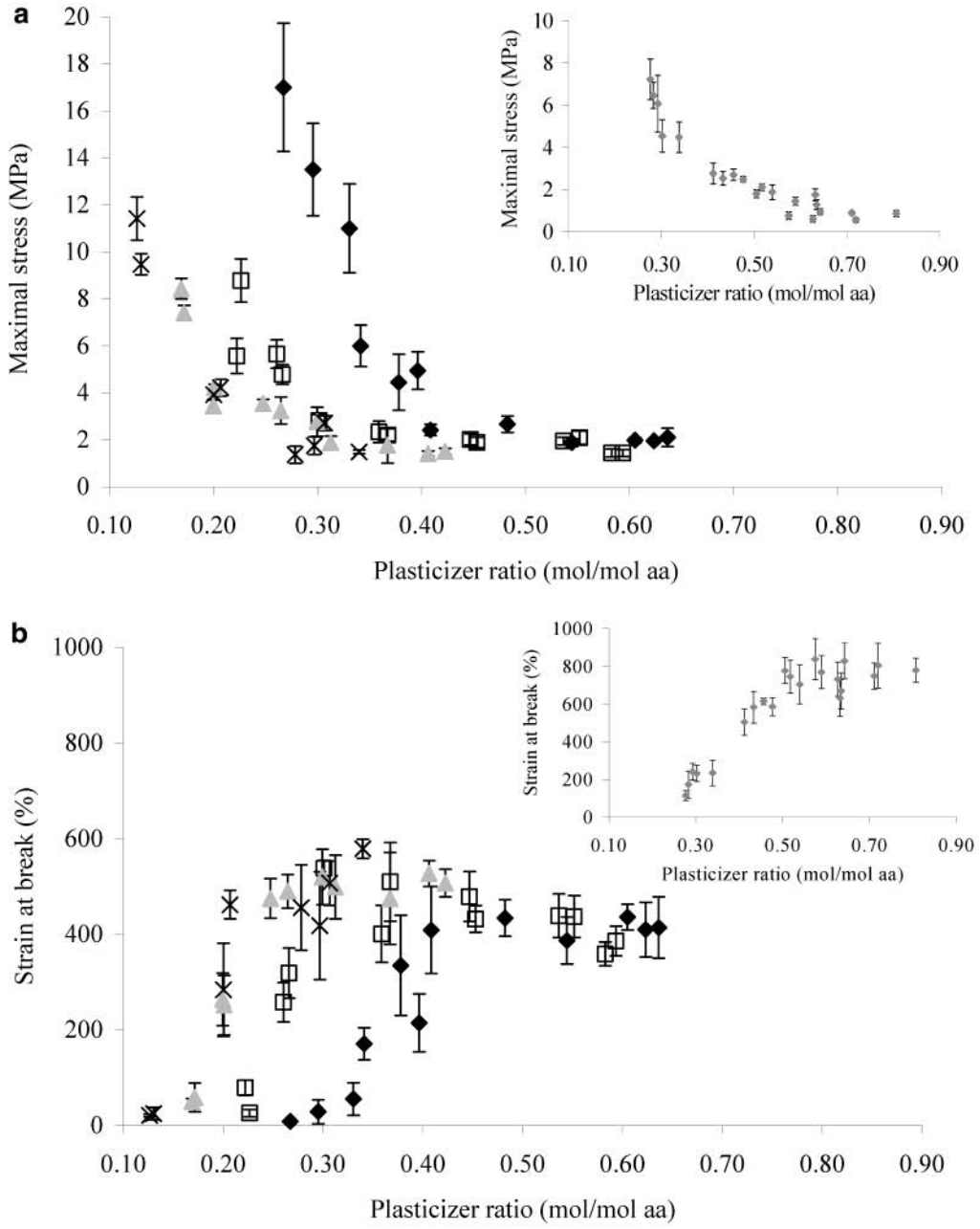

Figure 1. Evolution of maximal stress (a) and strain at break (b) for cast gluten films versus the molar amount of various plasticizers [(◆) EG, (□) DEG, (▲) TEG, (×) TEEG, and (■) glycerol in insert].

of the plasticizers, because the conclusions regarding the glycerol efficiency in comparison with that of the ethyleneglycol series depend on the plasticizer amounts considered. These differences could be due to the fact that glycerol has a molecular structure different from that of the homologous series and contains a third lateral hydroxide group. Thus, molecular features other than the molecular weight may also influence the plasticizer efficiency.

The lower reproducibility of the determination of the strain at break prevents such a fine analysis of the results (**Figure 1b**). However, it is clear that below the threshold ratio EG, DEG, and TEG have different effects, whereas TEEG could not be distinguished from TEG. These results are in agreement with those obtained on film stress: there is a positive influence of the molecular weight on plasticizer efficiency at least until a few groups' length; above a critical molecular weight (corresponding to that of TEG), plasticizer efficiency remains unaffected by the size of the molecule. For each plasticizer, the critical saturation amount is the same as that noticed on stress curves. The maximum of strain values lies between 400 and 600% for ethyleneglycol and its derivatives, whereas it reaches up to 900% with glycerol. This effect of glycerol on films strain is expected from the results obtained on stress values. This confirms that some particular feature of the molecule other than its length is involved in its plasticizing efficiency.

The same series of plasticizers had been studied previously on gliadin films (*10*). In that case, it was claimed that TEEG was the most efficient plasticizer of the series on a weight basis (films plasticized with TEEG presented the higher strain and the lower stress values for a given plasticizer weight ratio). In our case, we demonstrated a lower efficiency on a molar basis of plasticizers with the shortest chains, provided the plasticizer amount remains lower than a critical value. Beyond a six-carbon chain, no effect of the size of the molecule (no differences between TEG and TEEG) on a film's mechanical properties could be seen. Moreover, our results show that the comparison

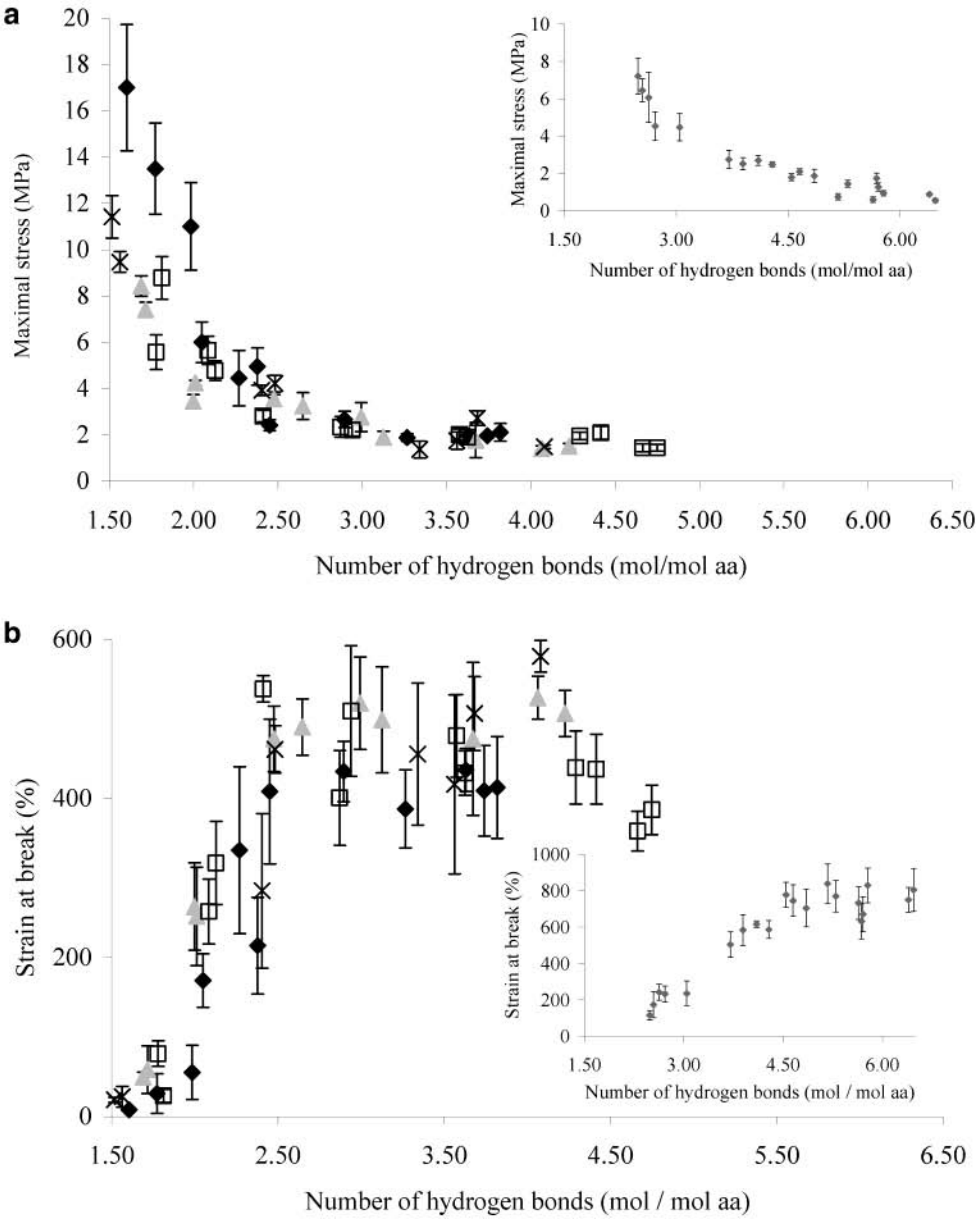

Figure 2. Evolution of maximal stress (a) and strain break (b) for cast gluten films versus the number of hydrogen bonds potentially supplied by various plasticizers [(◆) EG, (□) DEG, (▲) TEG, (×) TEEG, and (■) glycerol in insert].

between different plasticizers is also dependent on the molecular amounts of these plasticizers in the films: above a critical ratio, all plasticizers in the same series present similar efficiencies, whereas below this critical ratio an influence of the molecular parameters on the stress and strain values is revealed. We also emphasized the specific behavior of films plasticized with glycerol, in relation with the features of this molecule, most probably the presence of a third hydroxide group.

Other authors (*17*) reported that a set of hydrophilic plasticizers (glycerol, sorbitol, and sucrose) presented similar efficiencies to plasticize fish myofibrillar proteins films, when considered on a molecular basis. However, the authors noticed that glycerol had a slightly higher plasticizing effect than sucrose or sorbitol for the highest plasticizer contents; they suggested that this was due to a better efficiency of the glycerol small molecule for inserting and positioning inside the protein network. In our case, we also found a higher efficiency of glycerol above a molecular ratio of 0.60 mol/mol of amino acid; however, if this may reveal a better insertion of glycerol in the protein network, the small size of the molecule may not be the sole parameter involved, as ethyleneglycol—which is smaller—is revealed to be less efficient for these higher amounts. Moreover, when the plasticizers belong to the same structural series, we found a lower efficiency of the shorter molecules at the lowest plasticizer amounts. However, the series of plasticizers studied in both works were not the same. Because we showed the involvement of molecular features other than the molecular weight (or size), both results may be reconcilable.

Evidence of the Role of the Hydrogen Bonds. As the role of the hydrogen bonds is supposed to be of primary importance in protein material plasticization (*18*) and in gluten network organization (*12*, *13*), the maximal stress of the films was plotted versus the number of hydrogen bonds theoretically supplied by each plasticizer (*19*, *20*) (**Table 1**). In this case, the differences between plasticizers are almost completely abolished (**Figure 2a**) except for EG, which provided a lower decrease of the stress below 2 mol of hydrogen bonds/mol of amino acid. The small size of EG could have a negative influence on its plasticizing role. However, as EG presents a sligthly different chemical structure (no C−O−C ether bond) from the other plasticizers

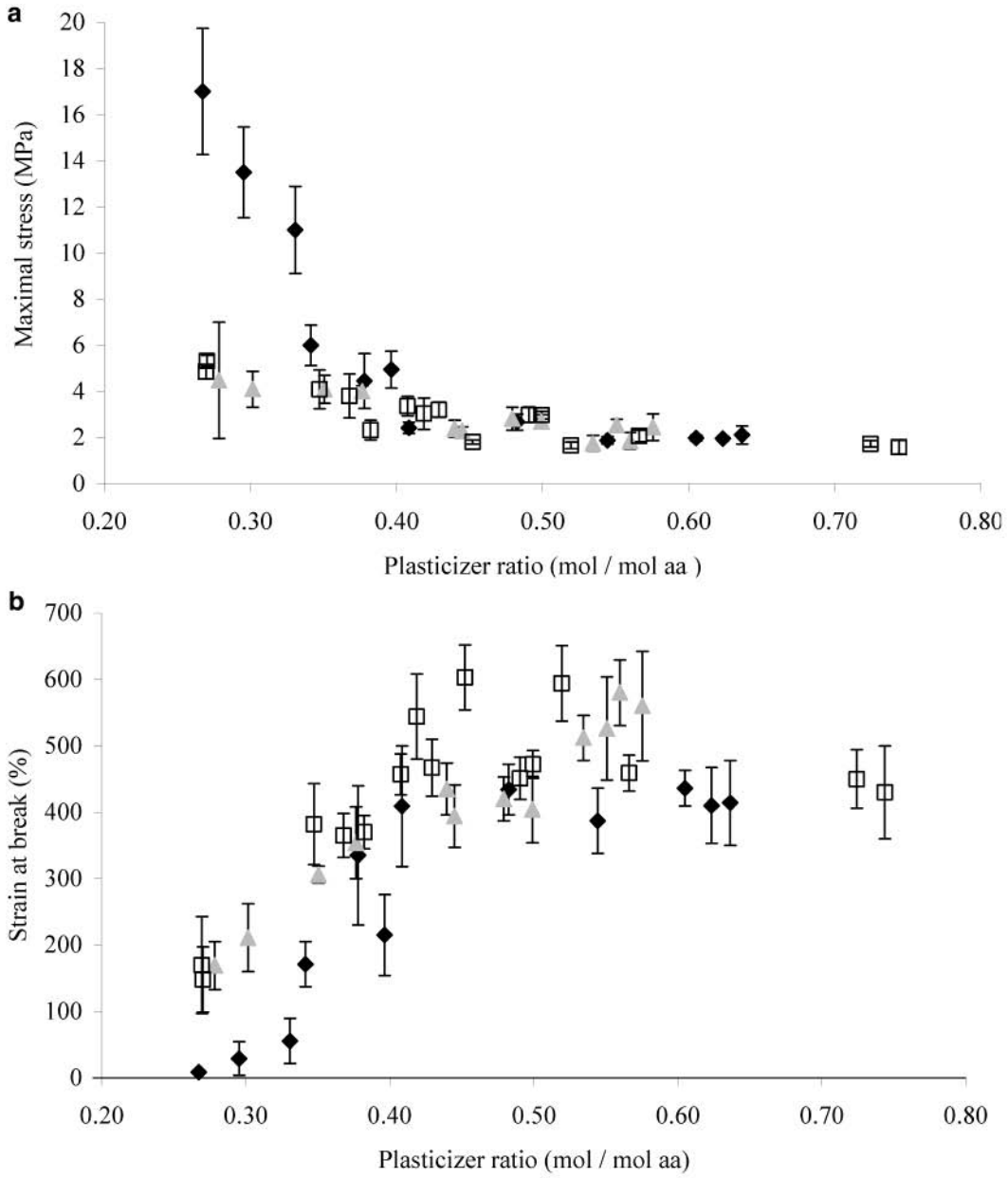

Figure 3. Evolution of maximal stress (a) and strain break (b) for cast gluten films versus the molar amount of various plasticizers [(◆) EG, (▲) 1,3-propanediol, and (□) 1,4-butanediol).

of the series, this hypothesis should be viewed with caution. Results drawn from the strain values curves (**Figure 2b**) are similar and in agreement with results from stress values. They suggested that >2.5−3 mol of hydrogen bonds/mol of amino acid, additional plasticizer has no effect on a film's mechanical properties. Considering hydrogen-bonding potential, another important feature is that the effect of glycerol on a film's mechanical properties was again not the same as that of the molecules from the ethyleneglycol series. Below a critical amount corresponding to 3.5 mol of hydrogen bonds/mol of amino acid, glycerol efficiency is lower than that of all the other plasticizers of the series. Above this amount, the phenomenom reverses and glycerol can be considered to be the most efficient plasticizer. Thus, the higher potential of glycerol to supply hydrogen bonds is not alone sufficient to explain its behavior over the whole range of plasticizer amounts studied.

A last point to take into account is that all of the curves (stress and strain) cannot be extrapolated beyond the limits of the present experiments for the different plasticizers: in other words, it is clear that for each plasticizer of the series, the limits of a film's feasability (i.e., handling) were reached at both ends of the curves. Thus, it is not possible to reach with TEEG stress values as high as those obtained with EG, because TEEG amounts <1.50 mol/mol of amino acid did not give rise to a self-supporting film. For each of the plasticizers considered, we therefore described on these curves the whole range of the molar amount (per mole of amino acid) that allows to obtain a self-standing film.

Number of $-CH_2-$ Groups: Specific Behavior of Ethyleneglycol. To elucidate the lower plasticizing efficiency of EG, we prepared films using three diol molecules as plasticizers: EG, 1,3-propanediol, and 1,4-butanediol. In this series one methyl $-CH_2-$ group was added at each step, but in that case, all three plasticizers present the same potential to supply hydrogen bonds (**Table 1**), that is, 6 mol of hydrogen bonds per amino acid; their effects were then compared on a molar basis (**Figure 3**). Under the threshold value of 0.40 mol/mol of amino acid, EG has a lower plasticizing efficiency than both

other diols, whereas 1,3-propanediol and 1,4-butanediol presented similar efficiencies (for both stress and strain). This confirms that hydrogen bonding is primarily involved in the plasticizing efficiency and suggests that the lower efficiency of EG is not related to the absence of the ether bond, but rather to its small size. Thus, one can conclude that the carbon chain length of the plasticizer has no influence on the mechanical properties of the film above a "critical carbon chain length" of three atoms.

Conclusions. The effect of different hydrophilic plasticizers (EG, DEG, TEG, TEEG, propanediol, and butanediol) on gluten films' mechanical properties were compared in this study. When plasticizer efficiencies were considered on a molar basis, there appeared to be a clear influence of their molecular weights on the mechanical properties of cast films below a molar ratio specific to each molecule. A finer analysis revealed that these differences of efficiency were partly related to the ability of the plasticizers to supply hydrogen bonds to the proteins.

The effect of glycerol was somewhat different from that of the other plasticizers examined here: the threshold value occurred at a higher plasticizer amount but led to lower stress and higher strain for films; this was probably due to the specific chemical structure of this molecule. In terms of applications, glycerol is able to provide cast films presenting a wider range of variations of elongation than the other plasticizers examined here.

This study underscores the fact that gluten protein plasticization is a complex phenomenom that can be primarily explained by the ability of the hydrophilic plasticizer molecules to share hydrogen bonds with the protein network.

ABBREVIATIONS USED

EG, ethyleneglycol; DEG, diethyleneglycol; MW, molecular weight; RH, relative humidity; TEEG, tetraethyleneglycol; TEG, triethyleneglycol.

LITERATURE CITED

(1) Gontard, N.; Guilbert, S.; Cuq, J. L. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *J. Food Sci.* **1992**, *57*, 190−195, 199.

(2) Gennadios, A.; Weller, C. L.; Testin, R. F. Modifications of physical and barrier properties of edible wheat gluten-based films. *Cereal Chem.* **1993**, *70*, 426−429.

(3) Micard, V.; Belamri, R.; Morel, M. H.; Guilbert, S. Properties of chemically and physically treated wheat gluten films. *J. Agric. Food Chem.* **2000**, *48*, 2948−2953.

(4) Gontard, N.; Guilbert, S.; Cuq, J. L. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *J. Food Sci.* **1993**, *58*, 206−211.

(5) Cuq, B.; Gontard, N.; Guilbert, S. Proteins as agricultural polymers for packaging production. *Cereal Chem.* **1998**, *75*, 1−9.

(6) Budi Santosa, F. X.; Padua, G. W. Tensile properties and water absorption of zein sheets plasticized with oleic and linoleic acids. *J. Agric. Food Chem.* **1999**, *47*, 2070−2074.

(7) Gontard, N.; Ring, S. Edible wheat gluten film: influence of water content on glass transition temperature. *J. Agric. Food Chem.* **1996**, *44*, 3474−3478.

(8) Lai, Y. H.; Padua, G. W. Properties and microstructure of plasticized zein films. *Cereal Chem.* **1997**, *74*, 771−775.

(9) Pouplin, M.; Redl, A.; Gontard, N. Glass transition of wheat gluten plasticized with water, glycerol or sorbitol. *J. Agric. Food Chem.* **1999**, *47*, 538−543.

(10) Sanchez, A. C.; Popineau, Y.; Mangavel, C.; Larré, C.; Guéguen, J. Effect of different plasticizers on the mechanical and surface properties of wheat gliadin films. *J. Agric. Food Chem.* **1998**, *46*, 4539−4544.

(11) Shewry, P. R.; Miles, M. J.; Tatham, A. S. The prolamin storage proteins of wheat and related cereals. *Prog. Biophys. Mol. Biol.* **1994**, *61*, 37−59.

(12) Belton, P. S. On the elasticity of wheat gluten. *J. Cereal Sci.* **1999**, *29*, 103−107.

(13) Shewry, P. R.; Popineau, Y.; Lafiandra, D.; Belton, P. S. Wheat glutenin subunits and dough elasticity: findings of the EUROWHEAT project. *Trends Food Sci. Technol.* **2001**, *11*, 433−441.

(14) Wall, L. L.; Gehrke, C. W. An automated total protein nitrogen method. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 1221−1226.

(15) Mangavel, C.; Barbot, J.; Bervas, E.; Linossier, L.; Feys, M.; Guéguen, J.; Popineau, Y. Influence of prolamin composition on mechanical properties of cast wheat gluten films. *J. Cereal Sci.* **2002**, *36* (2), 157−166.

(16) di Gioia, L.; Cuq, B.; Guilbert, S. Effect of hydrophilic plasticizers on thermomechanical properties of corn gluten meal. *Cereal Chem.* **1998**, *75*, 514−519.

(17) Cuq, B.; Gontard, N.; Cuq, J. L.; Guilbert, S. Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. *J. Agric. Food Chem.* **1997**, *45*, 622−626.

(18) Kalichevsky, M. T.; Blanchard, J. M.; Marsh, R. D. Application of mechanical spectroscopy to the study of glassy biopolymers and related systems. In *The Glassy State in Foods*; Blanshard, J. M. V., Lillford, P. J., Eds.; Nottingham University Press: Nottingham, U.K., 1993; pp 133−156.

(19) Beck, M. I.; Tomka, I.; Waysek, E. Physico-chemical characterization of zein as a film coating polymer. A direct comparison with ethyl cellulose. *Int. J. Pharm.* **1996**, *141*, 137−150.

(20) di Gioia, L.; Guilbert, S. Corn protein-based thermoplastic resins: effect of some polar and amphiphilic plasticizers. *J. Agric. Food Chem.* **1999**, *47*, 1254−1261.

Received for review June 27, 2002. Revised manuscript received November 5, 2002. Accepted December 16, 2002. This research was funded by the European Community within the framework of "Gluten Biopolymer" (FAIR CT 96 1979).

JF0257704