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Particular Lipid Composition in Isolated Proteins of Durum Wheat

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Gas chromatographic and mass spectrometric analyses showed that two low molecular weight proteins, extracted from glutenins of two durum wheat cultivars, contained strongly bound lipids with particular composition in comparison to semolina lipids. The percentages of palmitic acid (C16), stearic acid (C18), and oleic acid (C18:1) were high; conversely, their linoleic acid (C18:2) content was lower than 5%. Thus, semolina lipids contained about 1.5% C18, while isolated proteins contained between 23 and 28%. This fatty acid composition cannot be explained by an autoxidation of lipids during the isolation procedure. Various fatty alcohols and hydrocarbons were also present in the purified proteins; the amount of dodecanol, especially in one sample, was very high.

Previous work has shown the role of DSG proteins (durum wheat sulfur-rich glutenins) on the technological quality of durum wheats (Kobrehel and Alary, 1989a; Feillet et al., 1989). These two proteins, DSG-1 and DSG-2, had low M_s (molecular masses) of 14.1 and 17.1 kDa, respectively, and they were extractable from semolina at low concentrations of sodium tetradecanoate subsequent to the extraction of albumins, globulins, and gliadins (Kobrehel et al., 1988). Their amino acid composition was similar to that of the glutenins. However, they contained a higher amount of half-cystine than other glutenins, and they were not linked to the other glutenins through S–S bonds. There were also considerable differences between the amino acid composition and the N-terminal sequences of DSG-1 and DSG-2 (Kobrehel and Alary, 1989b). On the other hand, the amino acid sequence at the N-termini of DSG-1 and DSG-2 were found to be similar to the N-termini of CM proteins CM16 and CM3, respectively, extracted from hexaploid wheat (Shewry et al., 1984; Barber et al., 1986). The CM proteins are salt-soluble proteins that can be extracted with chloroform–methanol (2:1, v/v). Results suggest that CM proteins CM1, CM2, CM3, CM16, and CM17 are components of tetrameric α-amylase inhibitors (Garcia-Olmedo et al., 1987).

Our studies showed that some specific lipids were tightly bound to DSG proteins, which may contribute to their functional properties in the technological quality of durum wheat. These results are presented in this paper.

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MATERIALS AND METHODS

Wheat Samples. Two durum wheat (*Triticum durum*, Desf.) French cultivars, Mondur and Kidur, were studied. Semolina was obtained in 70% yield on a pilot mill. For experiments on common wheat (*Triticum aestivum*, L.), French cultivars Hardi, Talent, and Fidel were milled on a pilot mill to 74% flour yield.

Isolation of DSG Proteins (DSG-1 and DSG-2). DSG proteins were isolated under the conditions described by Kobrehel and Alary (1989b). Proteins from semolina were extracted sequentially by stirring the semolina sample for 15 h at 4 °C and then centrifuging at 38000*g* for 30 min at 4 °C. Albumins plus globulins were extracted with 0.5 M NaCl and gliadins with ethanol–water (68:32, v/v), with 1 g of semolina and 10 mL of extraction solvent. Then, 3.75 mg of sodium tetradecanoate/10 mL of distilled water was added to the residues. Sodium tetradecanoate (99% pure by gas chromatography) was prepared in our laboratory as described by Kobrehel and Alary (1989a).

About 50 mg of freeze-dried extract (glutenin 1) obtained with sodium tetradecanoate was dissolved in 0.05 M acetic acid, filtered through a lipid-free folded filter, and applied to a Bio-Gel P-30 molecular sieving chromatography column (2.5 × 100 cm). The flow rate was of 25 mL/h, and the eluate was monitored at 280 nm with a single-path monitor UV (Pharmacia). Eluted fractions (5 mL) were collected with an automatic collector; all chromatographic separations were performed at room temperature.

Electrophoresis of Proteins. SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) analyses were done under the conditions described by Payne and Corfield (1979). Proteins were not reduced before electrophoresis.

Extraction of Lipids from Semolina and from Isolated DSG Proteins. Lipids from semolina were extracted in triplicate by the method of Folch et al. (1957). To 5 g of semolina was added 25 mL of chloroform–methanol (2:1, v/v); the mixture was stirred for 5 min and then filtered through a filter previously defatted with the same solvent. Five successive extrac-

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tions were performed on each sample. Organic solvents, as all chemicals used in this study, were of analytical grade.

In the case of DSG proteins, where only 1 mg of purified proteins was obtained/sample, an extraction of lipids from the protein fractions was not performed. On the other hand, such an extraction might not extract strongly bound lipids. For these reasons, the isolated protein samples were hydrolyzed (as described below) prior to the lipid analyses.

Fatty Acid and Fatty Alcohol Analyses. Fatty acids (along with the other lipidic material) were isolated after alkaline hydrolysis (about 1 mg of protein and 2 mL of 0.5 N NaOH in methanol at boiling temperature and normal pressure) and methylated with boron trifluoride–methanol (Paquot and Hautfenne, 1987) for analysis by capillary gas–liquid chromatography. Hydrolyses were done in triplicate. For the identification of fatty acids and fatty alcohols, standards of fatty acid methyl esters, over 99.5% pure (purchased from Applied Science), and fatty alcohols, over 99.5% pure (Fluka), were also used.

Capillary Gas Chromatography (GC). A Delsi gas chromatograph (Model D 1700) equipped with a FID and a Shimadzu integrator (Model C-R4A) were used. The chromatograph was fitted with a Superox (Alltech Associates Inc., Deerfield, IL) 30-m fused silica capillary column of 0.25-mm i.d. and bound and cross-linked with poly(ethylene glycol), film thickness 0.2 μm. The analyses were performed isothermally at 180 °C with injector and detector temperatures of 210 and 220 °C, respectively. Hydrogen was used as carrier gas with a head pressure of 10 psi. Quantitation was achieved with use of the relative GC peaks areas and appropriate conversion factors. The conversion factors were 0.97, 1.02, 1.00, 1.00, and 1.01 for methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate, respectively.

Capillary Gas Chromatography/Mass Spectrometry (GC/MS). An LKB Bromma 2091 equipped with VG Digital pdp 8a computer was used for mass spectral identification of components. The gas chromatograph was operated with the same capillary column as the one used above for GC. The jet separator was maintained at 230 °C, ionizer temperature setting was 250 °C, and electron energy and multiplier voltage were 70 eV and 3500 V, respectively.

RESULTS AND DISCUSSION

Isolation of DSG Proteins. After sequential extraction of proteins from semolinas, the fractions containing albumins plus globulins and gliadins were discarded. Proteins DSG-1 and DSG-2 were obtained by the chromatographic separation of the glutenin 1, after the albumins, globulins, and gliadins were extracted. For Mondur semolina, Figure 1 illustrates the chromatographic elution curve and the SDS-PAGE electrophoretic composition of chromatographic fractions. The principal components of glutenin 1 were the DSG proteins. After the chromatographic separation, the fractions forming peak 1 were discarded and the fractions containing DSG proteins with similar composition were pooled, freeze-dried, and repurified under the conditions of the first chromatographic separation. Isolation results were similar for the cultivars Mondur and Kidur and in agreement with those previously reported (Kobrehel and Alary, 1989b). For further experiments, the purified DSG proteins showing a single band of DSG-1 or of DSG-2 on SDS-PAGE were used.

Under similar experimental conditions, no DSG-type (CM3 or CM16) proteins could have been isolated from the hexaploid wheat cultivars Hardi, Talent, and Fiedel. Contrary to durum wheats, these proteins are extracted from common wheats in the albumin–globulin fraction. The similar amino acid compositions of the N-termini suggest that they should be identical proteins, DSG-1 with CM16 and DSG-2 with CM3. However, because of the differences in their extractibility properties and since all further experiments were done on proteins isolated from durum wheats, we maintained the name DSG in this paper.

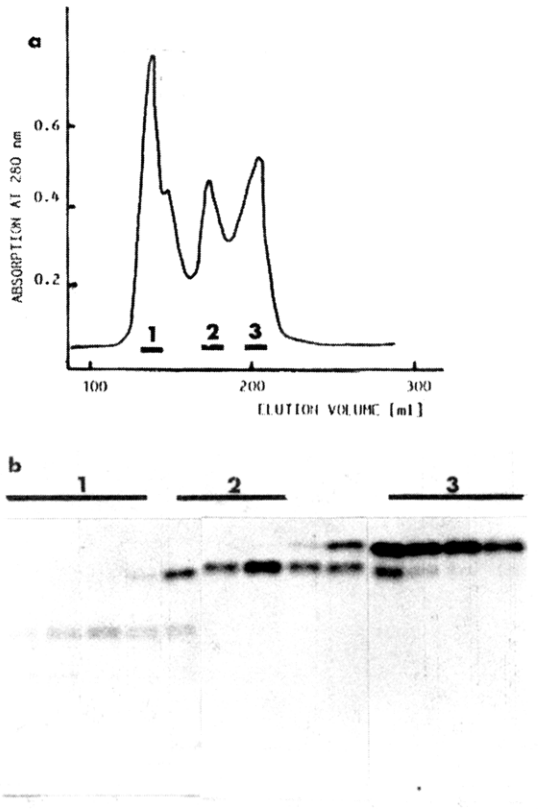

Figure 1. (a) Chromatographic elution curves of glutenins extracted with sodium tetradecanoate, cultivar Mondur. Freeze-dried samples dissolved and eluted with 0.05 M acetic acid. Column Bio-Gel P 30. (b) SDS-PAGE of glutenins, fractionated previously on Bio-Gel P 30. Composition of peaks: 1, proteins with molecular masses higher than those of DSG proteins; 2, mainly DSG-2; 3, mainly DSG-1.

Lipid Composition of Semolinas and of Isolated Proteins. *Structure Determination of Fatty Acid Methyl Esters.* The lipid contents of semolina samples were 2.4% and 2.8% (dry basis) for Mondur and Kidur, respectively. These results are comparable to those reported by Morrison (1978) (in whole wheat kernel from 2.1 to 3.8% and up to 2.4% in endosperm) and by Chung and Pomeranz (1981), 2.81% in durum wheat. However, our values are higher than those reported by Youngs (1988), up to 1.57% in durum wheat semolina. The use of different extraction methods and varietal differences may be the explanation of the various results (Morrison, 1978).

The gas chromatograms obtained after transesterification and transmethylation of the lipids extracted from isolated DSG proteins are illustrated in Figure 2 and the mass spectra of the main peaks in Figures 3–5. The mass spectra (Figure 3) of peaks 2–4 (Figure 2) agreed with the mass spectra of the methyl esters of fatty acids. The molecular ions (M) were well-defined and corresponded to methyl myristate (M = 243), methyl palmitate (M = 271), and methyl stearate (M = 299). The loss of $-OCH_3$ gave the acylium ion (M − 31) present in the mass spectra of 14:0 and 16:0. The base fragment of the spectra was m/e 74, the ion produced by the γ-hydrogen migration to a double bond followed by β cleavage (McLafferty rearrangement). Ions of the series $CH_3OCO(CH_2)_n$ were arithmetically found at m/e (59 + 14n), i.e. m/e 87, 101, 115, 129, 143, 157, 171, 185, etc., the ion 87 being the most important because it derives its stability from the enol form (Nyberg, 1986).

The mass spectrum of peak 5 (Figure 2) was consis-

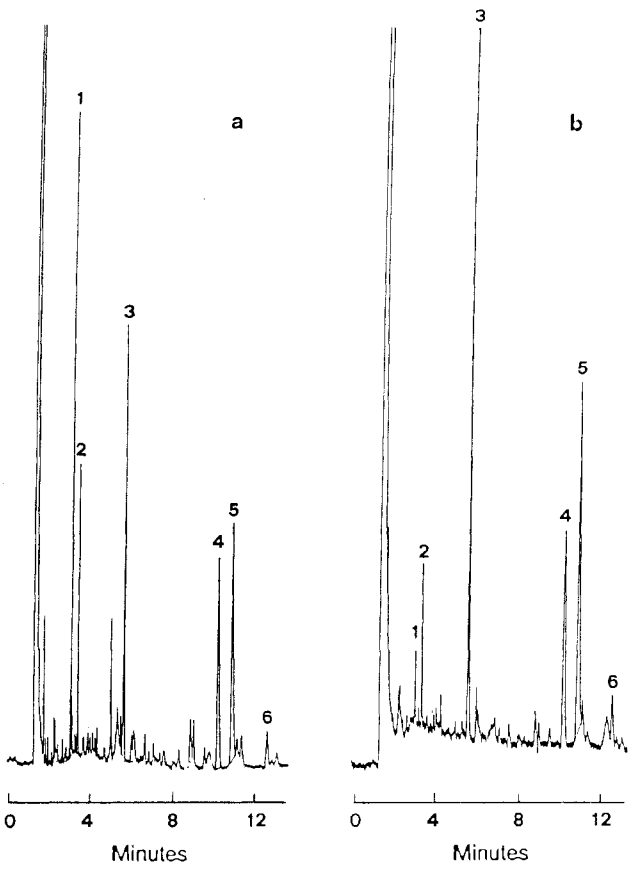

Figure 2. Gas chromatograms of transesterified and transmethylated lipids present in isolated DSG proteins: a, DSG-1, cultivar Mondur; b, DSG-2, cultivar Mondur. Major peaks: 1, dodecanol; 2, methyl myristate; 3, methyl palmitate; 4, methyl stearate; 5, methyl oleate; 6, methyl linoleate.

tent with the mass spectrum of methyl oleate (Figure 4). The molecular ion (M = 296) was absent, and the fragment at m/e 265 corresponds to an ion formed by the loss of a methoxyl radical (M - 31). The fragment at m/e 222 (M - 74) was due to an ion formed by the loss of (methoxycarbonyl)methylene, $-CH_2COOCH_3$, of mass 73 together with one hydrogen atom. This spectrum also shows ions due to hydrocarbon fragments at m/e 55 ($C_4H_7^+$), which is the base peak. In the sequence of the higher methoxycarbonyl-type fragments, the peak at a m/e 87 was the most prominent one (40% of the base peak). This is in agreement with the results reported by Hallgren et al. (1959) for the mass spectrum of methyl oleate.

The mass spectrum (Figure 4) of peak 6 (Figure 2) had a molecular peak at m/e 294, and the presence of M - 31 confirms the idea that it is methyl linoleate. The spectrum showed prominent peaks due to hydrocarbon fragments at m/e 67 (base peak), 81, and 95. They were stronger than those due to oxygen-containing fragments. However, the peak at m/e 220 (M - 74) was absent, contrary to the mass spectrum of methyl linoleate reported by Hallgren et al. (1959). Under our experimental conditions, the location and the stereochemistry of the double bond, which could be at the origin of that discrepancy, could not be determined because of the low amount of available material.

The fatty acid compositions of the samples are shown in Table I. Lipids extracted from the semolinas of cultivars Mondur and Kidur had similar compositions of fatty acids, and these results were comparable to those reported in the literature (Laignelet, 1983; Youngs, 1988).

The compositions of fatty acids found in the DSG protein fractions were considerably different from the composition of fatty acids of the lipids extracted from semolina. In the total lipids extracted from semolina the major fatty acid was linoleic acid (C18:2), amounting to over 50% of the total fatty acid content. In purified DSG proteins its relative concentration was only about 4%. The concentration of other fatty acids also differed between semolina lipids and DSG proteins. In the fatty acids of semolina lipids, 1.4% and 1.6% stearic acid (C18:0) were found in cultivars Mondur and Kidur, respectively, and between 27% and 28% in the fatty acids bound to DSG proteins (with the exception of DSG-2 isolated from Mondur where it amounted to 23%). The concentrations of palmitic acid (C16) and oleic acid (C18:1) were considerably higher in the DSG proteins than in lipids of semolina; differences were also found between the isolated protein samples. Conversely, no linolenic acid (C18:3) was found in the isolated protein fractions, though its concentration was close to 3% in the lipids of semolina. The presence of myristic acid (C14) in the purified DSG protein fractions might be due to the extraction procedure used, although traces of C14 were found in the lipids of semolina too.

It is important to note that the fatty acid compositions of DSG-1 and DSG-2 could not be caused by autoxidation because the ratios of C14, C16, and C18 saturates and C18:1 are very different from total semolina lipids.

It is also interesting that the differences between the fatty acid composition of strongly bound and of easily extractible lipids of semolina, as reported by Rech (1968), have many similarities with the differences that we found between the fatty acid compositions of lipids in the isolated DSG proteins and of semolina lipids.

Results of several investigations have shown the existence of various wheat proteins having strong affinity for lipids: low-MW gliadin-like proteins (Salcedo et al., 1978), ligolin (Frazier et al., 1981), S proteins (Zawistowska et al., 1985, 1986), and purothionins (Redman and Fisher, 1968; Nimmo et al., 1974). All these proteins are of low molecular weight like DSG proteins, but they differ in amino acid composition (Kobrehel and Alary, 1989b). The CM proteins have also affinity for lipids (Garcia-Olmedo and Carbonero, 1970; Redman and Ewart, 1973; Rodriguez-Loperena et al., 1975); however, no results have been reported on the fatty acid composition of lipids that would specifically bind to these proteins.

Interestingly, most of the results of the above-mentioned studies suggest an intrinsic affinity of specific proteins for specific lipids. Thus, ligolin was strongly complexed with triglycerides on a 1:1 molar basis (Frazier et al., 1981) and S proteins with polar lipids (Zawistowska et al., 1986). However, the fatty acid composition of the lipids bound to these various proteins were not reported; hence, the comparison of these results with ours is limited.

Fatty Alcohols in Isolated DSG Protein Fractions. One of the most interesting findings was the presence of fatty alcohols in the purified DSG protein fractions. Three of them were identified by GC/MS analyses: dodecanol ($C_{12}H_{26}O$), tetradecanol ($C_{14}H_{30}O$), and pentadecanol ($C_{15}H_{32}O$). While the latter two were present only in traces in all DSG proteins, the concentration of dodecanol was much higher (Figure 2, peak 1) and showed considerable variations between the samples.

The mass spectrum (Figure 5) of peak 1 (Figure 2) showed hydrocarbon ions formed by simple cleavage and rearrangement processes; the most prominent were m/e

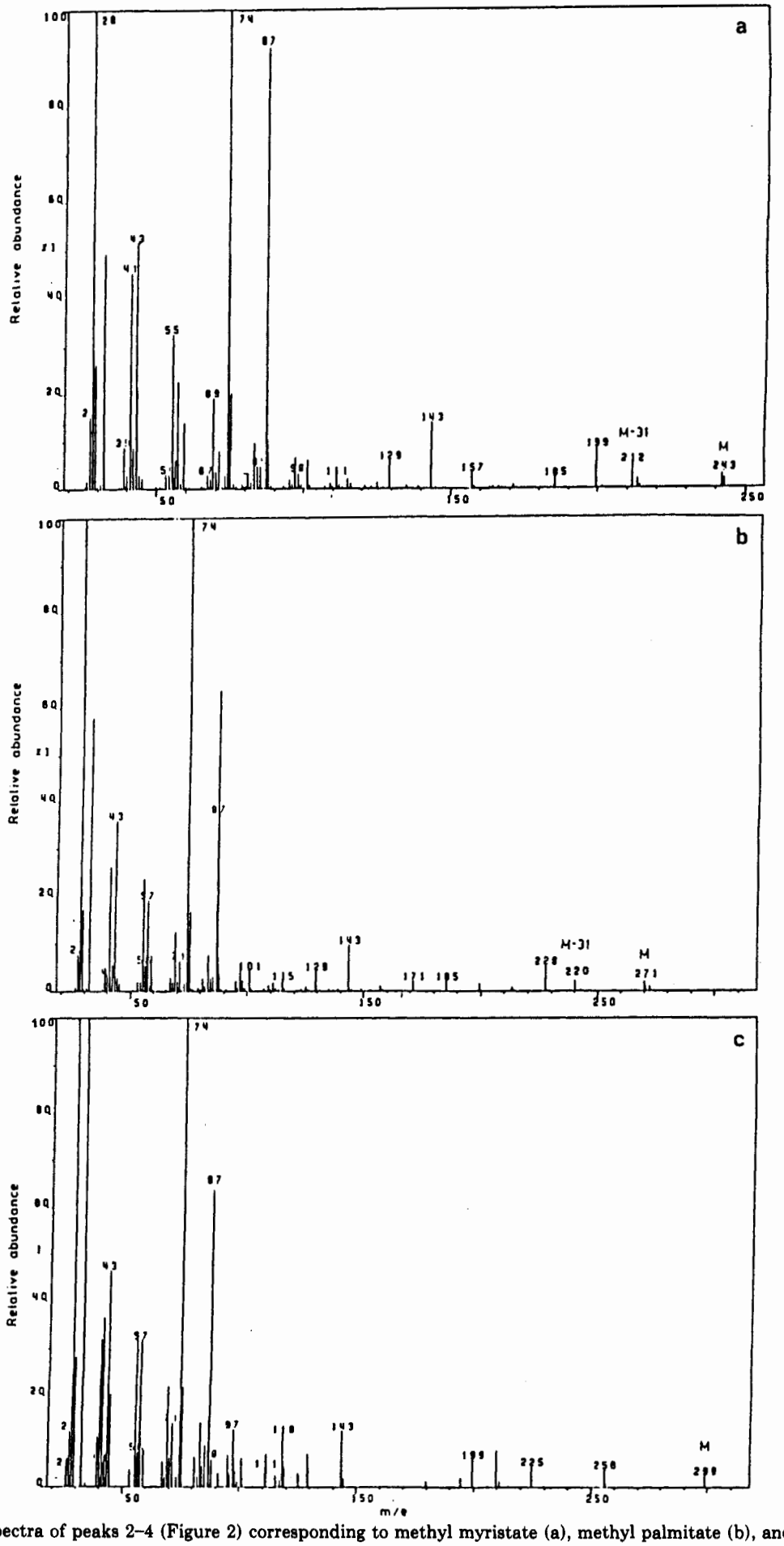

Figure 3. Mass spectra of peaks 2–4 (Figure 2) corresponding to methyl myristate (a), methyl palmitate (b), and methyl stearate (c), respectively.

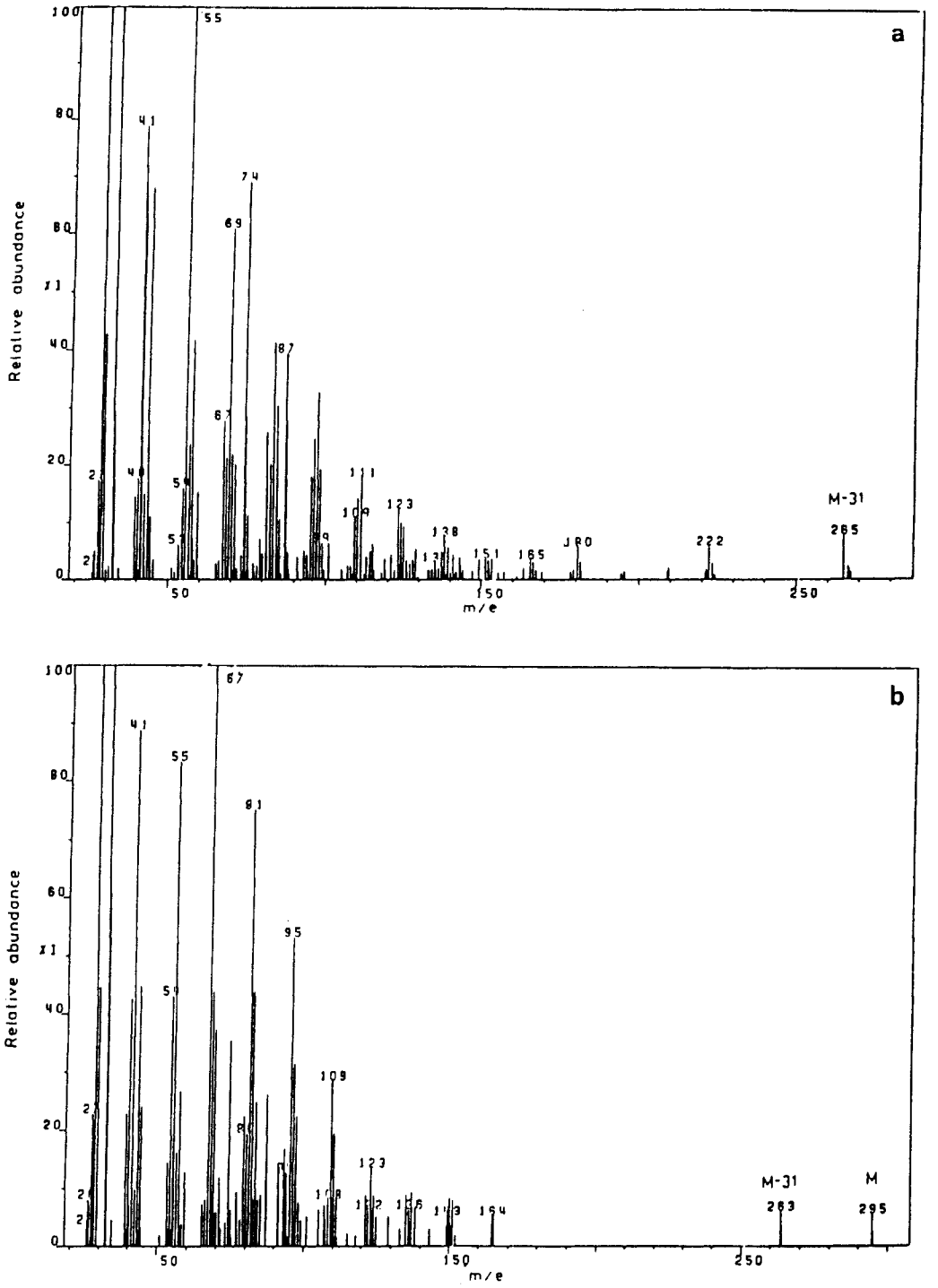

Figure 4. Mass spectra of peaks 5 and 6 (Figure 2) corresponding to methyl oleate (a) and methyl linoleate (b), respectively.

28, 43, 55, 69, 83, etc. In spite of the absence of a molecular ion (M = 186), we find M − 18 (M = 168) due to the loss of water as well as M − 18 − 28 (M = 140). Moreover, the identification of this component as dodecanol was confirmed by comparison of the mass spectrum obtained by coinjection of commercial dodecanol.

In Kidur 1.1 and 1.7% of dodecanol were found with DSG-1 and DSG-2, respectively, while in Mondur 19.4 and 2.1% with DSG-1 and DSG-2, respectively. These percentages were calculated as the ratio between the area of the peak of dodecanol versus the sum of the areas of the fatty acids determined. Only the peaks of the major fatty acids were taken into account, omitting minor peaks (Table II). The very high relative concentration of dodecanol bound to DSG-1 of Mondur is especially noteworthy. It can be stressed that results were similar on three, separately isolated DSG proteins. Under our experimental conditions, except dodecanol, present in traces (Table II), fatty alcohols could not be identified in the lipid extracts of semolina and they were not present in the sodium tetradecanoate used for the extraction of the glutenin fraction containing DSG proteins.

Various type of alcohols are known to be present in cereals: glycerol, sterols, and tocols. Short-chain diols (ethane-, propane-, butane-, and pentanediols) have been found in wheat and maize (Morrison, 1983). Long-chain alcohols with straight or branched chains (from C20 to C36) esterified with fatty acids have been reported in rice bran oil (Ito et al., 1981) and in sorghum (Sauvaire et al., 1986). To our knowledge no long-chain alcohols have been reported in wheat.

Other Minor Lipid Components in the Isolated DSG

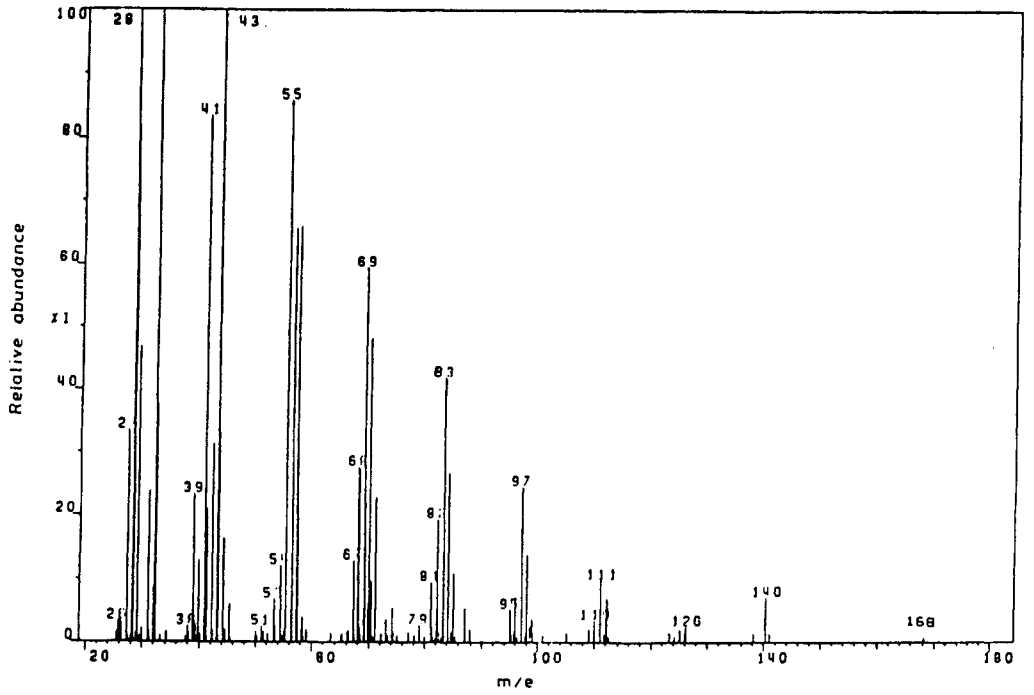

Figure 5. Mass spectrum of peak 1 (Figure 2) corresponding to dodecanol.

Table I. Fatty Acid Composition of Lipids Extracted from Semolina and from Isolated DSG Proteins

fatty acid	Mondur DSG-1	Mondur DSG-2	Mondur semolina lipid	Kidur DSG-1	Kidur DSG-2	Kidur semolina lipid
C14	9.6	4.5	trace	7.9	10.3	trace
C16	25.1	34.7	17.9	31.6	28.1	22.2
C18:0	28.1	23.0	1.6	27.3	27.2	1.4
C18:1	32.5	33.6	20.1	28.3	30.5	21.4
C18:2	4.5	4.1	57.1	4.8	3.7	51.5
C18:3	0.0	0.0	2.8	0.0	0.0	2.9

Protein Fractions. Beside the major fatty acids, several other fatty acids could have been identified in the DSG protein fractions by GC/MS analyses. There were dodecanoic (C12:0), pentadecanoic (C15:0), heptadecanoic (C17:0), and eicosanoic acid (C20:0). All these fatty acids were present only in traces.

Traces of hydrocarbons $C_{17}H_{36}$, $C_{18}H_{38}$, and $C_{24}H_{50}$ were also found in the isolated DSG proteins. According to Youngs and Gilles (1970), both in durum and in bread wheat, hydrocarbons are found throughout the kernel, as determined by analyzing different flour mill streams. They detected all the *n*-hydrocarbons between C9 and C33. In durum wheat semolina, 47% of the hydrocarbons were between C16 and C31, (58% of them were odd-numbered); however, the most abundantly occurring hydrocarbons were C10 and C11. Among the *n*-hydrocarbons with longer chains C17 was the major one, which is also present with DSG proteins.

GENERAL DISCUSSION

The solubilization of wheat glutenins in the presence of sodium salts of fatty acids is supposed to occur through hydrophobic interactions between the hydrophobic chains of the soaps and the apolar side chains of the proteins' amino acids (Kobrehel and Bushuk, 1977; Wasik et al., 1979). Because of the fact that, in the presence of low amounts of sodium tetradecanoate, DSG proteins are much more easily extractable than the other glutenins, it seems that, beside the apolar side chains of the amino acids, some fatty acids tightly bound to DSG proteins were able to interact readily with sodium tetradecanoate.

Table II. Ratio of Dodecanol to Fatty Acids in Lipids of Semolina and in Lipids Bound to DSG Proteins

Mondur	
DSG-1	19.4
DSG-2	2.0
semolina	trace
Kidur	
DSG-1	1.1
DSG-2	1.7
semolina	trace

Under the experimental conditions used, however, the fatty acid nature of the components, extracted after hydrolysis from the freeze-dried proteins and then separated by gas chromatography, could not be determined with certainty. For this reason, in order to identify fatty acids with certainty, mass spectrometric analyses were necessary. These analyses confirmed the fatty acid nature of the major gas chromatographic peaks and allowed the identification of some other lipid components, fatty alcohols and hydrocarbons, present in the isolated protein fractions.

Results revealed the existence of strong lipid–protein interactions; some fatty acids remained tightly bound to DSG proteins throughout the isolation procedure. Moreover, the fact that the compositions of the fatty acids found with the isolated DSG proteins were very different from that found in the lipids of semolina suggests that the interactions between fatty acids and DSG proteins are specific and not at random. These results, on the other hand, are in agreement with that previously reported on the composition of fatty acids strongly bound to semolina (Rech, 1968) and on proteins with specific affinity to specific lipids (Frazier et al., 1981; Zawistowska et al., 1986), as discussed above.

Our results suggest that octadecanoic acid (C18:0), for instance, whose concentration is incomparably higher in the isolated protein fractions than in the lipids of semolina is specifically bound to DSG proteins. This can be supposed also for C16:0 and for C18:1. Conversely, the very low concentration of C18:2 in the isolated protein fractions, in comparison to that in the lipids of semo-

lina, may suggest that it was but a contaminant in the isolated proteins. This can be the case of C14:0 too. Only traces of it were found in the lipids of semolina, but its sodium salts were used to extract the glutenin fraction containing mostly DSG proteins. Thus, C14:0 could be bound at that stage to the DSG proteins through hydrophobic interactions. The presence of both C14:0 and C18:2, in relatively low concentrations, might had been the result of secondary interactions established randomly between these fatty acids and either the apolar residues of the DSG proteins or the fatty acids bound to these proteins.

The existence of fatty acid binding proteins in serum is well-known (Glatz and Veerkamp, 1985), and the occurrence of posttranslational addition of lipid-derived hydrophobic moieties to proteins is widespread (Magee and Hanley, 1988). Direct attachment of long-chain fatty acids to proteins is known to occur in eukaryotic cells (Kaplan et al., 1988). It is not known whether similar proteins exist in wheat or, in general, in any plants.

The CM proteins from various cereals were found to be members of the great trypsin/α-amylase inhibitor family (Garcia-Olmedo et al., 1987). Although their affinity for lipids is known, the nature of their interactions with lipids has not been investigated; neither is it known whether lipids have any role in the physiological activity of these proteins. Some of the CM proteins seem to have one molecule of carbohydrate per subunit, which might be important for their inhibitory activity (Silano et al., 1977; Petrucci et al., 1978). The possible physiological role of lipids bound to CM (DSG) proteins is yet to be determined. Moreover, the different solubility properties of CM proteins of common wheats may suggest that they have different types of interactions with lipids than the corresponding DSG proteins in durum wheats and, consequently, different physiological roles.

From the technological point of view, besides the relatively high content in -SH plus S-S groups (Kobrehel and Alary, 1989a), the very tightly bound fatty acids and alcohols should also participate in the functional role of DSG proteins during technological processes.

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Registry No. Linolenic acid, 463-40-1; linoleic acid, 60-33-3; stearic acid, 57-11-4; dodecanol, 112-53-8; tetradecanol, 112-72-1; pentadecanol, 629-76-5; heptadecane, 629-78-7; octadecane, 593-45-3; tetracosane, 646-31-1.

Approaches to Protein Hydration and Water Activity in Food Proteins by Nuclear Magnetic Relaxation and Vapor Pressure Equilibration Techniques

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^1H and ^{17}O NMR relaxation rates for two proteins, lysozyme and corn zein, were treated by employing nonlinear regression analysis and a protein activity model. The dependence of the NMR transverse relaxation rates on protein concentration was markedly nonlinear due to interactions between protein molecules. Using a virial expansion, protein activities were calculated for the entire concentration range. From the protein activities, theoretical water activity (a_w) curves were derived with a simple hydration model. Comparison of the a_w values as a function of protein concentration to the published sorption isotherms showed significant differences between the expected RVP and sorption isotherms, especially at protein concentrations >0.2 g/mL. This difference is likely to be caused by the fact that sorption "equilibration" depends on the (relatively slow) translational diffusion of water molecules whereas NMR relaxation monitors primarily the fast motions of water.

1. INTRODUCTION

Recently, there has been an increasing interest in the study of protein hydration (Kumosinski and Pessen, 1982; Kumosinski et al., 1987, 1988; Lioutas et al., 1986, 1987; Myers-Betts and Baianu, 1990; Kakalis and Baianu, 1988). The importance of investigating the hydration properties of proteins in food systems has its basis in practical applications. For example, the functionality of many food proteins depends, in part, on their hydration characteristics. In muscle products, the interactions between myofibrillar proteins, solute, and water will determine the functional behavior of the food, such as heat gelling, hardening, texture changes, and binding of water (Woeff, 1982). In addition to these functional implications, deteriorative reactions in foods depend greatly on the characteristics of the water in the food system. Microbial growth and undesirable chemical reactions (e.g., Maillard browning, lipid oxidation) can have an adverse effect on the stability and shelf life of the food product in addition to affecting the organoleptic properties. Therefore, it is important to investigate the dynamics of protein hydration and to determine the range of water perturbations induced by proteins.

Traditionally, sorption isotherm "equilibrium" methods have been used to investigate the retention of water by proteins (Bull, 1944), and several different models have been proposed to interpret the data in terms of the "water activity", often identified with the relative vapor pressure (RVP) (Brunauer et al., 1938; D'Arcy and Watt, 1970). (Quotes are employed throughout the text to indicate a generic use of the terms in quotes for foods that differs significantly from the original, precise definitions that are strictly valid only for simpler systems such as electrolytes solutions. The terms in quotes are, therefore, often misused in the context of food systems.) At thermodynamic equilibrium, the "water activity", a_w, is related to the equilibrium relative humidity (ERH) by

$$a_w = (p/p^0) = \text{ERH}\,(\%)/100 \quad (1)$$

where p and p^0 are the water vapor pressures in the food

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