JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

X-ray Photoelectron Spectroscopy for Wheat Powders: Measurement of Surface Chemical Composition

Moustafa Saad,[†] Claire Gaiani,[§] Martine Mullet,[#] Joel Scher,[§] and Bernard Cuq*[,†]

[†]Montpellier SupAgro - INRA, UMR 1208 Joint Unit for Emerging Technology and Polymer Engineering, 2 place Viala, 34060 Montpellier Cedex 1, France

[§]LIBio, Laboratoire d'Ingénierie des Biomolécules, Nancy Université, 2 avenue de la Forêt de Haye, B.P. 172, 54505 Vandoeuvre Les Nancy Cedex, France

[#]LCPME, Laboratoire de Chimie Physique et de Microbiologie pour l'Environnement, UMR 7564 CNRS − Nancy Université, 405 rue de Vandoeuvre, 54600 Villers Lès Nancy, France

ABSTRACT: The functional properties of wheat powders depend largely on the surface characteristics of their particles. X-ray photoelectron spectroscopy (XPS) has been considered to investigate the surface composition of wheat powders. The objective of the present study is to evaluate the ability of XPS to discriminate wheat components and to calculate the surface composition of wheat powders. First, XPS surveys for the main wheat isolated components (starch, proteins, arabinoxylans, and lipids) were determined. XPS results demonstrate that it is able to distinguish wheat proteins, polysaccharides, and lipids, but it is not able to distinguish starch and arabinoxylan because of their similarity in chemical structure. The XPS analyses of simple reconstituted wheat flours based on two components (starch and protein) or three components (by adding arabinoxylan) demonstrated the ability of XPS to measure the surface composition of the wheat flours. The surface composition of native wheat flour demonstrated an overrepresentation of protein (54%) and lipids (44%) and an underrepresentation of starch (2%) compared to the bulk composition. Results are discussed with regard to difficulties in discriminating arabinoxylans and starch components.

KEYWORDS: XPS, surface chemical composition, wheat flour, protein, polysaccharides, lipids

INTRODUCTION

Wheat powders (flour and semolina) obtained by milling wheat grain are the main raw materials used to produce a large variety of cereal-based foods (e.g., bread, noodles, biscuits). The functional properties of wheat powders are classically determined according to cereal-based food applications (e.g., bread dough making properties, water absorption, biochemical composition). Methods from powder science are also used to generate relevant data (e.g., particle size distribution, densities, flow behavior) related to bulk characteristics of wheat powders (e.g., densities, bulk chemical composition, porosity).[1]

It begins to be established that the functional properties of biological powders (e.g., flowability, adsorption, rehydration, wetting properties) are greatly dependent on surface characteristics of the particles.[2] Recent studies carried out on biological powders showed that the surface chemical composition of particles is significantly different from their bulk composition[3−7] as a result of the powder manufacturing process by drying or milling. When particles are produced by milling/crushing of agricultural raw materials (e.g., wheat grains), the heterogeneous native structure and composition of the raw materials can generate particles with heterogeneous surface composition.

X-ray photoelectron spectroscopy (XPS) is a well-established method to study the surfaces of inert materials. XPS has recently been considered to investigate the surface chemical composition of biological powders obtained by spray- or freeze-drying of complex solutions.[8−11] On the other hand, only a few works investigated the application of XPS for naturally complex powders obtained directly by milling of agricultural raw materials, such as wheat powders.[12,13] The determination of the surface composition of a material by XPS is first considered at an elemental level. XPS measures the relative atomic elemental composition at the surface layer of approximately 5−10 nm thickness.[8] The elemental composition of biological powders is generally defined by considering only three main elements (carbon, oxygen, and nitrogen).[5,6,8,10,12] Other minor elements (such as phosphorus, sulfur, or minerals) are ignored as they amount to <1%,[3,6,8] except in a few papers concerning starch granules analysis.[12] From the relative elemental composition in C, N, and O, the calculation of the atomic ratios (e.g., O/C, C/N) and apparent atomic stoichiometry are used to identify the components. A reasonable agreement has been found between XPS apparent stoichiometry and those calculated from theoretical stoichiometry.[3,5,6,8,9] In addition, the peaks C_{1s}, N_{1s}, and O_{1s} obtained from the XPS survey scans can be decomposed at specific binding energies (into four, two, and three subpeaks, respectively) and assigned to well-identified chemical functions (e.g., C—C, C—O, O—C—O, C═O) that are typical for components, such as lipids, sugar derivatives, glucose polymers, and polyamino acids.[10,12,14,15]

The elemental atomic composition of isolated components is then used to calculate the surface composition of powders. The components have to be sufficiently chemically different to be

Received: June 16, 2010
Accepted: January 5, 2011
Revised: January 5, 2011
Published: February 04, 2011

distinguished by their elemental composition.[8] XPS fails to resolve similar multiple functional groups that have similar binding energies.[16] Two calculation methods have been developed to determine the surface composition of a powder.

(i) Fäld et al.[8] proposed a method in which the measured surface concentrations in atomic elements (C, N, O) are linear combinations of the elemental mole fractions of same elements, of the considered components, weighted by a degree of coverage of the components on the considered surface. By using linear relation in a matrix formula, the surface composition (i.e., degree of coverage) of the different components can be calculated. These calculations are consistent only when it is supposed that the sum of the selected atomic elements (C + N + O) is 100%.

(ii) The surface composition can also be calculated by considering the concentration of the chemical functions that are specific to components. The chemical functions are obtained from the decomposition and assignment of the XPS sub peak (C_{1s}, N_{1s}, and O_{1s}). The surface composition is calculated from the concentrations of chemical functions ratioed to total carbon in the samples, on the basis of the known composition of components.[10] This method has been supposed to refine the expression of results in terms of classes of components.[10,17,18]

XPS is considered to be a reproducible, reliable, and versatile method with a wide range of applications in analyzing surface chemical composition of biological powders. This approach has been conducted for the main components of biological powder (e.g., proteins, sugars, polysaccharides, or lipids) while ignoring the minor components (e.g., vitamins or minerals). However, sample contamination during the experiments can complicate the XPS analyses, because most abundant surface contaminants on air-exposed specimens consist only of C and O.[9] Stability issues for biological materials during XPS imaging are also important for reliable data. X-ray-induced irradiation damage and adsorption or desorption of volatile species in ultrahigh vacuum may considerably distort the data. Sample charging may further complicate data interpretation.[7]

XPS works demonstrate significant differences between the surface composition and the bulk composition for different biological powders. For instance, for dairy powders, proteins and lipids would be major components, whereas lactose and minerals would be minor components.[3,5,6,8] For wheat starch granules, proteins and lipids were thought to be largely present in the surface.[12] Only a few studies have so far investigated the surface composition of wheat flour particles.[10,13] In the case of wheat flour, the relative surface coverage in lipids and proteins was found to be much higher than the bulk contents.[10] The objective of the present study is to evaluate the XPS ability to identify the surface components and to calculate the surface composition of wheat particles. We first obtained the XPS survey scans for the wheat isolated main components (starch, proteins, arabinoxylans, and lipids). We then evaluated the XPS ability to measure the surface composition of simple reconstituted flours based on the mixing of two or three components and of different wheat flours obtained from different wheat grain varieties and milling conditions. We also investigated the impact of particle surface/volume ratio of the wheat flour particles on XPS results. To quantify the surface composition, only the main wheat components (starch, proteins, arabinoxylans, and lipids) are considered as they amount to most of the total solids of the wheat flours. The minor components, including minerals, vitamins, and trace elements, were neglected.

MATERIALS AND METHODS

Wheat Flours. Different wheat flours were selected. Reference wheat flour was prepared by milling soft wheat grain ('Impression' wheat variety, 2007 German harvest) in a pilot plant milling system in the Max Rubner Institute (Detmold, Germany) to an extraction rate of 78.8%. Another four different wheat flours with different bulk compositions were also prepared. Grains from two different soft wheat varieties ('Tiger' wheat variety, 2007 harvest; 'Crousty' wheat variety, 2007 harvest) were selected.[19] Tiger wheat grains were either peeled and milled to 100% extraction rate or pearled and milled to 75% extraction rate. Crousty wheat grains were either peeled and milled to 75% extraction rate or pearled and milled to 100% extraction rate.

Four wheat flours of different surface/volume ratios were prepared from the reference flour, by applying an additional process. The sieved wheat flours were prepared from a 500 g sample of flour that was directly sieved over a column of five metallic sieves of decreasing mesh (125, 100, 70, 50, and 25 μm). Powders remaining on sieves (100, 70, 50, and 25 μm) were collected and gave the four sieved flours with different diameters (116, 89, 52, and 27 μm, respectively) and surface/volume ratios (5.7, 7.5, 12.8, and 24.7 mm^{-1}, respectively). The ground wheat flours were prepared from a 200 g sample of flour that remained over a 125 μm sieve. This sample was ground in a laboratory grinder (ZM 200 Retsch, France) under ambient relative humidity conditions. The resulting powder was sieved over a column of five metallic sieves with decreasing mesh (125, 100, 70, 50, and 25 μm). Powders remaining on sieves (100, 70, 50, or 25 μm) were collected and gave the four ground flours with different diameters (100, 78, 57, and 24 μm, respectively) and surface/volume ratios (6.7, 8.5, 11.7, and 27.8 mm^{-1}, respectively). The flour samples were stored at 4 °C in hermetically sealed cans until experiments were carried out.

Wheat Components. The main wheat components were isolated from the reference wheat flour to get their XPS survey scans. The separation of starch and gluten proteins was conducted according to the method of Auger,[20] as reported in Figure 1. Flour was first mixed at 25 °C with water (550 g total dough mass and 1.25 flour to water ratio) in a planetary mixer bowl (P600 Brabender, Germany) at 80 rpm. The resulting dough was then diluted by adding 1000 mL of demineralized water and stirred at 28 rpm for 10 min using a K-beater blade (Brabender, Germany). The gluten−starch suspension obtained after the washing step was sieved by using a vibrating sieve (AS 200 digit Retsch, Germany) connected to tap water (water flow rate = 1500 mL. min^{-1}). The gluten was recovered on an 800 μm sieve, whereas the filtrate was collected in a separate container. The resulting gluten was then lyophilized (Alpha 2-4 LSC Martin Christ, Germany) and milled in a ball miller. To isolate starch, the previous filtrate was left overnight at 15 °C, then washed by distilled water, centrifuged for 20 min at 3000*g*, washed with ethanol 70% and then centrifuged for 20 min at 3000*g*. These steps were repeated two times. After the last centrifugation, the starch fraction was dried overnight under a hood at 25 °C and then milled. The extraction of lipids was realized by accelerated extraction using an accelerated solvent extractor (ASE 200 Dionex, USA). Five successive extraction cycles (7 min for each cycle) were carried out for the flour (at 50 °C and 100 bar, using benzine (i.e., petroleum ether) as solvent (SDS Carlo Perba, France). The volume of the solvent was 150% of the initial volume of the tested sample. The mixture benzine/oil was then evaporated under vacuum at 40 °C by using a rotating evaporator to restore the pure oil. Wheat arabinoxylans were commercially purchased from Megazym. The wheat component samples were stored at 4 °C in hermetically sealed cans until experiments were carried out.

Reconstituted Flours. Different reconstituted flours were prepared by mixing two (binary flours) or three (ternary flours) wheat components. Binary reconstituted flours were prepared by mixing starch and proteins in different starch/proteins ratios (0.90/0.10; 0.85/0.15; 0.80/0.20). Ternary reconstituted flours were prepared by mixing starch,

proteins, and arabinoxylan in different starch/arabinoxylans ratios (starch/proteins/arabinoxylans ratios = 0.835/0.150/0.015; 0.820/0.150/0.030; 0.805/0.150/0.045). Preparations were carried out manually by mixing the powdered components in a ceramic bowl. The incorporation of the isolated lipids in the reconstituted flours was not considered because of the difficulties in obtaining a homogeneous mixture with the other components due to the physical state (liquid) of the lipids. The reconstituted samples were stored at 4 °C in hermetically sealed cans until experiments were carried out.

Chemical Analysis of Wheat Flours. Starch content was determined according to the AACC 76-13 method.[21] The arabinoxylans content was determined by gas−liquid chromatography (GLC) after sulfuric acid hydrolysis and derivatization as alditol acetates. The alditol acetates obtained were injected in a DB 225 capillary column (J&W Scientific, Folsom, CA) using allose as the internal standard.[22] Total nitrogen content (TN) was determined by the Kjeldahl method, and protein content was calculated according to TN × 5.7.[23] Lipid content was determined by accelerated extraction using an accelerated solvent extractor. Chemical compositions of the selected wheat flour are presented in Table 1. Average values and standard deviation were determined from triplicates for the reference wheat flour (obtained from the Impression wheat variety). Chemical compositions of the four peeled and pearled wheat flours were determined from only one measurement. Apparent standard deviations were calculated from the relative standard deviation values measured for the reference flour.

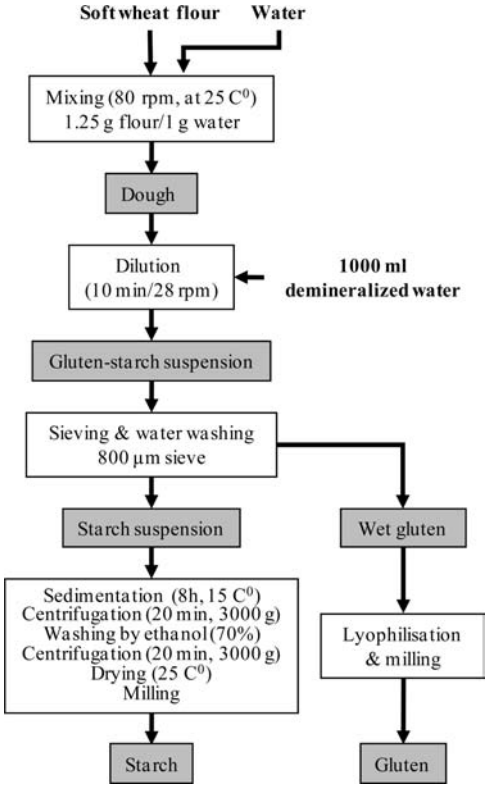

Figure 1. Process diagram for the extraction of the main components from wheat flour.

Granulometric Distribution. Particle size distributions of the wheat flours were determined using laser granulometry (Mastersizer 2000 Malvern Instruments, Worcestershire, U.K.) and described by considering the values of median diameters (d_{50}). Two measurements were carried out for each sample (Table 1).

Surface Chemical Analysis. XPS imaging was performed with a Kratos Axis Ultra Kratos Analytical (Manchester, U.K.) spectrometer using a monochromatic Al Kα source. The pressure in the working chamber during analysis was $<10^{-7}$ Torr. The takeoff angle of the photoelectrons was perpendicular to the sample. The analyzer operated with a pass energy of 65 eV. The step size was 0.1 eV, and the dwell time was 1000 ms. The powders were loosely packed in stainless steel sample holders, and the surface was leveled. The analyzed area of the powder was a region of 300 μm × 700 μm. Spectra were analyzed using Vision software from Kratos (Vision 2.2.2). A Shirley baseline was used for the subtraction of the background, and Gaussian/Lorentzian (70/30) peaks were used for spectral decomposition. Quantification was performed using the photoemission cross sections and the transmission coefficients given in the Vision package.

SEM Observations. The microstructure of particles of wheat flours and of the isolated wheat components was observed by scanning electron microscope (JSM-T2000 JEOL, Tokyo, Japan). A representative sample taken from each powder was mounted in epoxy resin and coated by gold to provide the conductivity. Three pictures from different positions and with different magnifications were taken for each sample.

RESULTS

The study of the particle surface properties of wheat powders has been conducted at first from visual analysis of microstructure. XPS analysis has been used, on the one hand, to identify the chemical functions and the components located at the surface of particles and, on the other hand, to try to quantify the surface concentrations of components.

Microstructure of Particles. The superficial microstructure of the reference wheat flour was evaluated by scanning electron microscopy (SEM) (Figure 2). Panels A and B of Figure 2 display a classical superficial microstructure for wheat flour particles. We observe a large diversity in particle size and morphology. Some isolated starch granules with almost ovoid shape and some fragments from the wheat albumen cells can be noticed (Figure 2B). The albumen fragments have irregular shape with rough surface and seem to contain starch granules partially embedded in the continuous matrix, which is supposed to be composed of proteins and arabinoxylans. Some fragments originated from the cell walls

Table 1. Bulk Chemical Composition and Particle Size Diameter of the Selected Wheat Flours

		bulk chemical composition content[a] (g/100 g dry matter)			
	diameter, d_{50} (μm)	starch	protein	arabinoxylans	lipid
reference wheat flour (Impression wheat variety)	101.7	81.3 (±1.9)	12.9 (±0.2)	3.8 (±0.2)	2.3 (±0.2)
peeled wheat flour (Crousty wheat variety)	59.7	81.9 (±1.9)	11.7 (±0.1)	5.2 (±0.3)	1.3 (±0.1)
pearled wheat flour (Crousty wheat variety)	89.6	78.7 (±1.7)	14.0 (±0.2)	4.7 (±0.2)	2.6 (±0.2)
peeled wheat flour (Tiger wheat variety)	109.4	75.0 (±1.6)	15.7 (±0.3)	6.9 (±0.4)	2.4 (±0.2)
pearled wheat flour (Tiger wheat variety)	80.1	80.9 (±1.8)	12.4 (±0.2)	5.2 (±0.3)	1.3 (±0.1)

[a] In some cases, the sums of the four components do not equal 100%, which is due to experimental error.

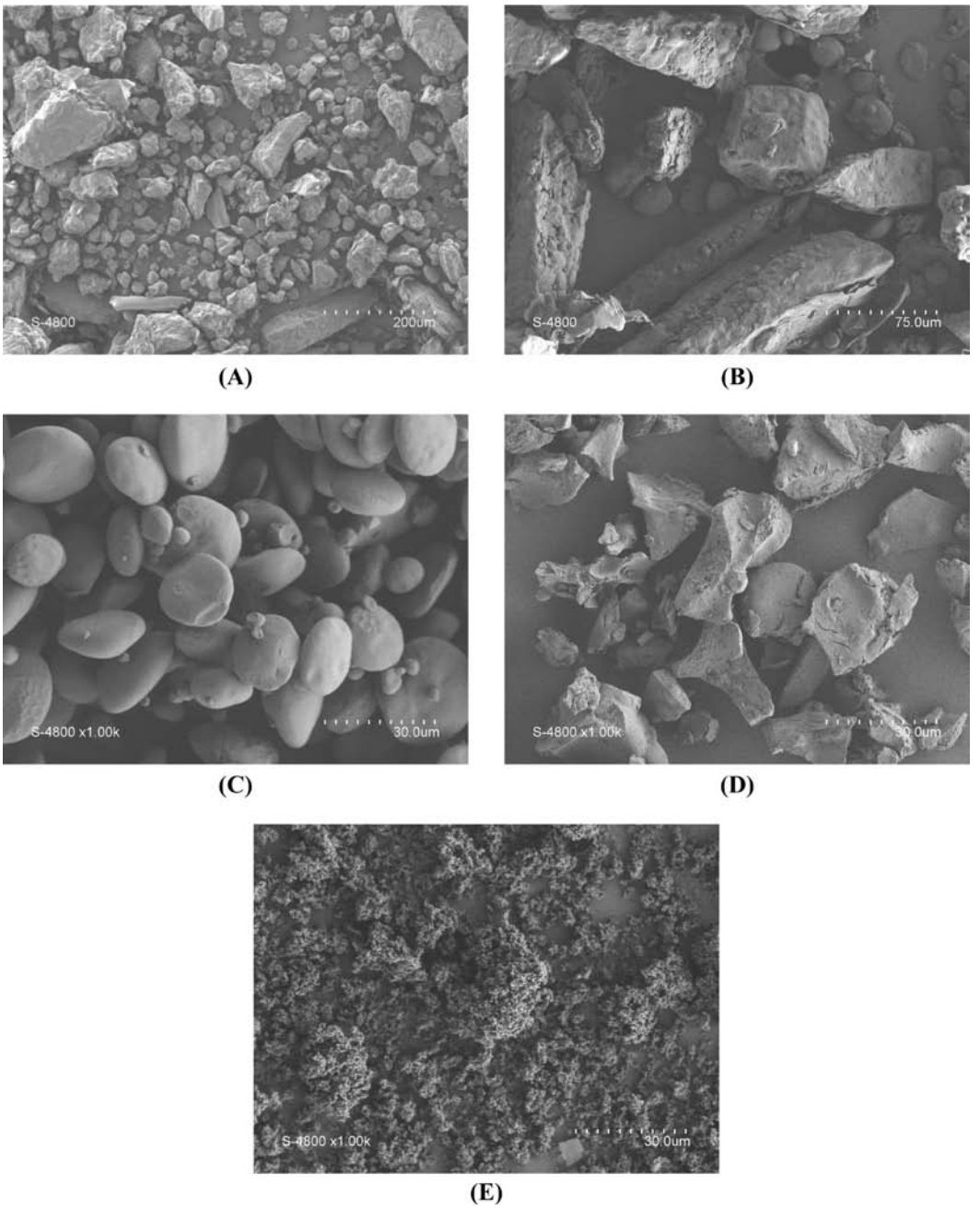

Figure 2. Scanning electron micrographs of the reference wheat flour particles (scale bar = 200 μm (A) and 75 μm (B)) and of isolated wheat flour components: starch (C), proteins (D), and arabinoxylans (E) (scale bar = 30 μm).

and are supposed to be rich in polysaccharides. Three different groups of particles according to size can be identified: small-sized particles (about 5−8 μm), medium-sized particles (about 30−40 μm), and large-sized particles (about 80−200 μm). As expected, it is not possible to identify the components existing on the surface of wheat flour particles only by means of SEM photos (Figures 2A,B). Similar SEM descriptions were obtained for the other wheat flour particles (data not shown). It is possible to claim that the heterogeneity in the surface composition is mainly due to the native highly heterogeneous internal structure of wheat grains. Thus, even if the particles were uniform by size, they would be chemically heterogeneous.

The microstructures of the wheat component particles (starch, proteins, and arabinoxylans) were also described using SEM (Figure 2). Starch granules (Figure 2C) appear to possess almost regular and homogeneous oval shape and a completely smooth surface with no pores on it. Two main particle sizes are noted for the starch granules (5−8 and 20−30 μm). Similar descriptions of the granule microstructure for wheat starch were already mentioned in the literature.[24] As the extraction process does not change the microstructure of the starch granules, the isolated granules can be considered as similar to those present in the native wheat flour. Protein particles (Figure 2D) show very irregular shapes with large diversity in shape and size. The surface of protein particles displays small flat areas, with "strong" angles. Arabinoxylan particles (Figure 2E) show light spongy apparent structure and numerous pores. Even if it is difficult to define a specific shape of the arabinoxylans particles, they seem to be

Table 2. Relative Elemental Composition Measured by XPS[a]

			atomic abundance (%) of elements at the surface of the wheat flour components			
binding energy (eV)		functions	starch	protein	arabinoxylans	lipids
101	Si		0.19			3.42
133	P					0.24
163	S			0.27		
286	C		66.4	75.8	68.4	77.7
284.6		C—C, C—H	30.2	58.8	37.8	76.1
286.1		C—O, C—N	54.6	27.4	45.6	18.3
287.5		O—C—O, C=O	12.6	11.1	13.1	2.6
288.7		O=C—OH, O=C—OR	2.6	2.7	3.5	2.9
399	N		0.7	7.4	1.3	2.0
399.7		NH, NH_2	88.5	95.4	100.0	84.6
402.4		NH_3	11.5	4.6		15.4
533	O		32.7	16.5	30.0	16.6
531		O^{-2}	1.8	40.6	7.8	4.5
532.6		C—OH	92.6	46.0	86.1	72.7
533.8		H_2O	5.6	13.3	5.2	22.7

[a] Decomposition and assignment of XPS peak components (C_{1s}, N_{1s}, and O_{1s}) for the selected wheat components.

more or less spherical, but with very irregular surfaces. Similar particle descriptions were reported in the literature on the gluten protein and the pentosans extracted from wheat flour.[24] However, the microstructure for the protein and arabinoxylan particles cannot be considered as representative of their native state in the wheat flour particles because their state was largely affected by the extraction and purification process.

XPS Analyses for the Wheat Components. The isolated main wheat components were first individually analyzed by XPS. The elemental composition of C, O, and N amounted to 99% of all detected elements (Table 2), except for lipids (only 96.3%), due to the presence of Si (3.6%). Figure 3 shows the XPS survey scans obtained for the four selected components (starch, proteins, arabinoxylans, and lipids). Atomic abundances of elements (C, N, and O) at the surface of the components samples (expressed in atomic percent (at. %)) are presented in Table 2. The XPS survey scans for the main components were analyzed to assign the peaks C_{1s}, O_{1s}, and N_{1s} to chemical functions.[10,12,14,15] As a typical example, Figure 4 shows the peak decomposition for proteins. Similar decompositions (data not shown) were obtained for starch, arabinoxylan, and lipids. Peak assignments are presented in Table 2. The C_{1s} peak was decomposed into four peaks. The peak at 284.6 eV is attributed to C making a single bond with C or H (C—C, C—H) in lipids or protein side chains. The peak at 286.1 eV is attributed to C making a single bond with O or N (C—O, C—N) in alcohol, amine, or amide functions in proteins. The peak at 287.5 eV is attributed to C making two single bonds or one double bond with O (O—C—O, O=C—N, O=C—O) in hemiacetal and acetal functions in polysaccharides) or in amide functions in proteins. The peak at 288.7 eV is attributed to C making one double or single bond with O (O=C—OH, O=C—OR) in ester and carboxyl functions in proteins and cell-wall polysaccharides. The N_{1s} peak was decomposed into two subpeaks. The peak at 399.7 eV is attributed to uncharged N in amine (C—NH_2) or in amide (O=C—NH_2, O=C—NH—C) in proteins. The peak at 402.4 eV is attributed to positively charged N (C—NH_3^+) in protonated amine or quaternary ammonium functions in proteins. The O_{1s} peak is decomposed into three subpeaks. The peak at 531 eV is attributed to O doubly bound to C (O=C—O, O=C—N, O=C—OH), due to amide, ester, and carboxyl in proteins and cell-wall polysaccharides. The peak at 532.6 eV is attributed to O making single bonds with C (C—OH) in alcohol and (C—O—C) in acetal and hemiacetal functions in polysaccharides. The peak at 533.8 eV is attributed to O singly bound to C (O=C—O—H) in ester and carboxyl functions in proteins and cell-wall polysaccharides.

Atomic Composition of Starch Particles. As expected, XPS survey scans obtained from the starch sample (Table 2) give major signals corresponding to C (66.4%) and O (32.7%) and very low signals corresponding to N (0.7%). A main peak at 532.6 eV (92.6%) is attributed to alcohol and acetal groups. The C_{1s} peaks at 286.1 eV (54.6%) and 287.5 eV (12.6%) are attributed to C—O and O—C—O in the glucose units. The calculated atomic ratio (1/4.3) of the subpeaks at 286.1 and 287.5 eV is in reasonable agreement with the expected atomic ratio (1/5) obtained from stoichiometry.[12] The relatively high binding energy (30.2%) of the C_{1s} spectrum subpeak at 284.6 eV is characteristic of C bonded only to other C and H atoms and indicates the presence of C in a nonstarch form that may arise from extraneous hydrocarbon contamination or from hydrocarbon structures in other components (e.g., lipids or proteins) naturally present at the surface of the starch granules.[12] The N_{1s} peak at 399.8 eV is attributed to amide-linked N in protein residue. Silicon at a binding energy of 101 eV was also found in starch granules at very low concentration (0.19%) and could be associated with the contamination.

The C/O stoichiometry for starch (1/0.733) was calculated using only the sum of peak areas due to O—C—O and C—O as a measure of C content, to extract the peak due to extraneous

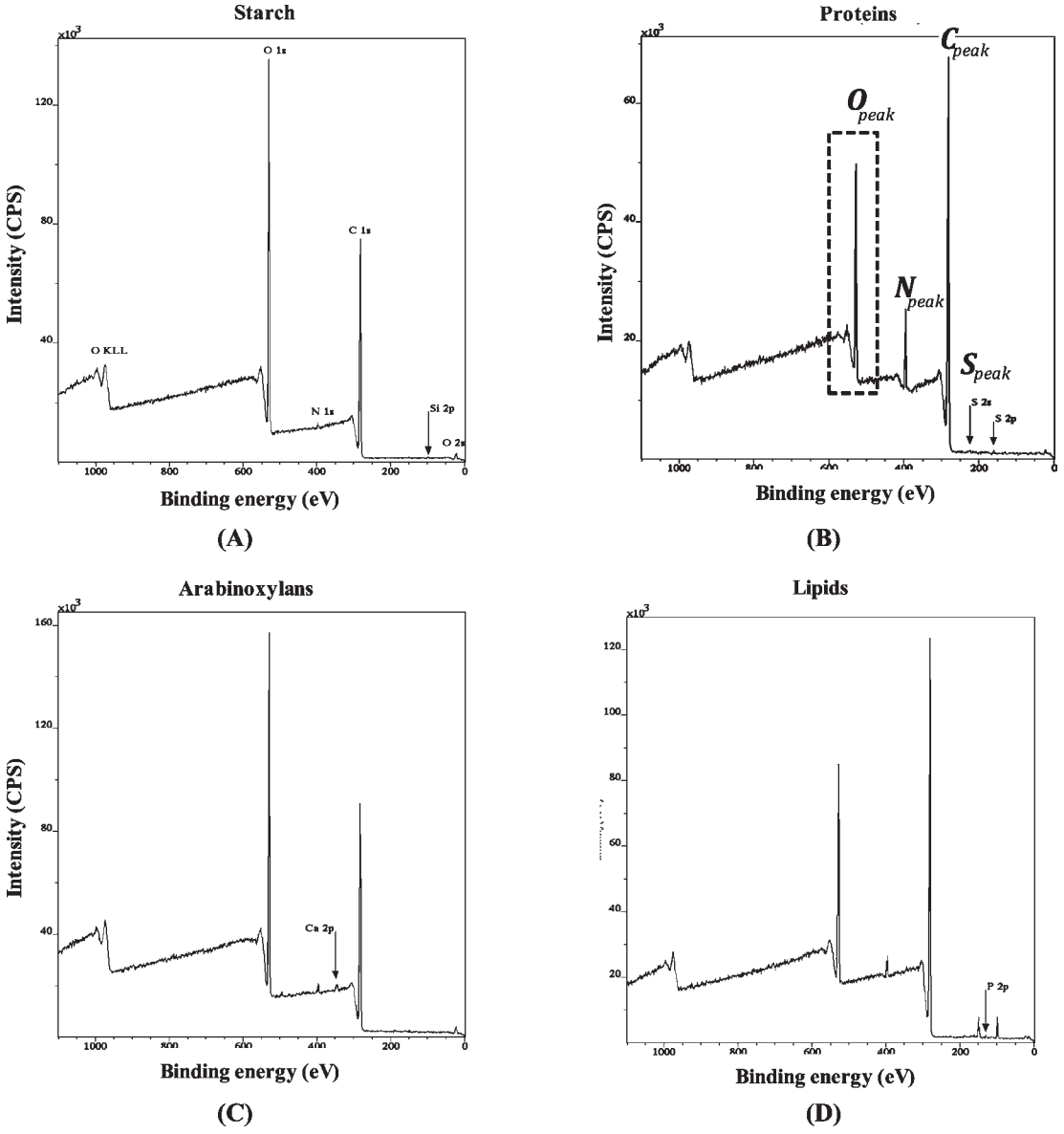

Figure 3. Survey scans obtained from XPS analyses for the four isolated components of wheat flour: starch (A), proteins (B), arabinoxylans (C), and lipids (D).

hydrocarbon components.[12] This value is relatively high compared to the theoretical value for anhydroglucose (1/0.833), when the theoretical chemical composition ($C_6H_{10}O_5$) for starch is taken into consideration. Possible surface components of the granule other than glucose polymers must be present at a rather low level.[12] Consistent with this, nitrogen was observed in the native starch at a C/N stoichiometry of only 1/0.011, which could suggest the possible presence of protein at the starch granule surface.[12]

Atomic Composition of Arabinoxylan Particles. Because arabinoxylans are polysaccharides with a chemical structure close to that of starch, the XPS signal obtained for arabinoxylan is almost similar to that obtained for starch granules (Table 2), with major intensities corresponding to C (68.4%) and O (30.0%) and very low signal corresponding to N (1.3%). The O_{1s} peak at 532.6 eV (86.1%) is attributed to alcohol and acetal groups. The peak at 531 eV (7.8%) is attributed to O bonded to C (O═C—O, O═C—N, O═C—OH) and is typical of cell-wall polysaccharides. The C_{1s} peaks at 286.1 eV (45.6%) and 287.5 eV (13.1%) are attributed to C—O and O—C—O in the arabinose and pentose residues of arabinoxylans, respectively. The C_{1s} peak at 284.6 eV (30.2%) is a characteristic of the C bonded only to other C and H atoms and indicates the presence of C in a nonarabinoxylan form. The N_{1s} peak (1.3%) at 399.8 eV peak is attributed to amide-linked N, due to protein residues. The C/O stoichiometry for arabinoxylans was calculated using the sum of peak areas due to O—C—O and C—O as a measure of C content, to extract the peak due to extraneous hydrocarbon components. The calculated value of the C/O stoichiometry for arabinoxylan particles (1/0.748) is very close to the theoretical value (1/0.800) when the $C_5H_8O_4$ formula for arabinoxylans is taken into consideration. Consistent with this, nitrogen was observed in the arabinoxylans at a C/N stoichiometry of only 1/0.019, which could suggest the possible presence of protein at the surface.[12]

Atomic Composition of Protein Particles. XPS imaging on the protein samples (Table 2) gives major signals corresponding

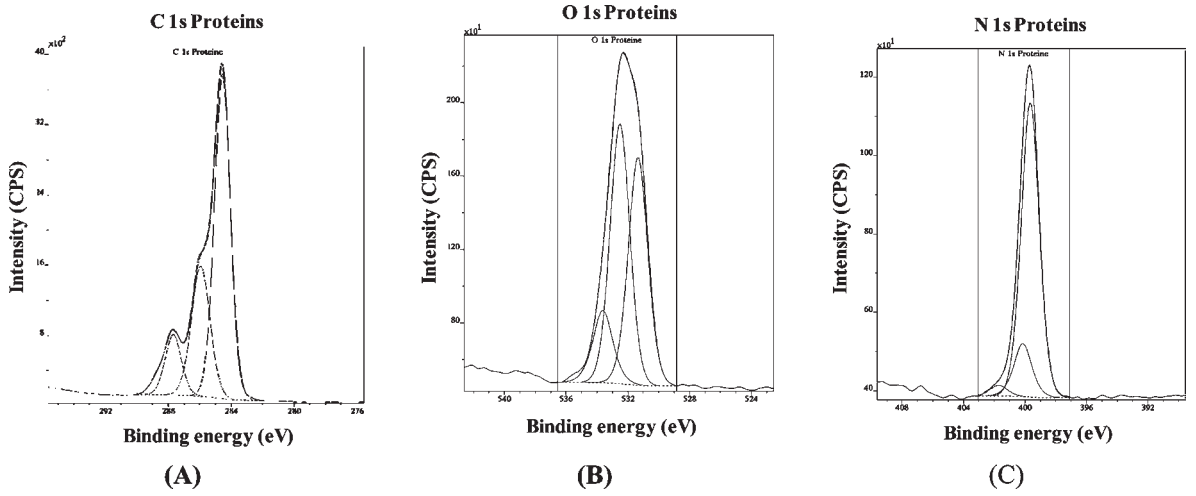

Figure 4. Decomposition of C_{1s} (A), N_{1s} (B), and O_{1s}(C) subpeaks in the case of proteins.

to C (75.8%), lower signal corresponding to O (16.5%), and significant signal corresponding to N (7.4%). The N_{1s} peak at 399.8 eV (81.9%) is attributed to amide-linked N and typifies the proteins structure. The C_{1s} peak at 284.6 eV (58.8%) is a characteristic of C bonded only to other C and H atoms and indicates the presence of aliphatic lateral chains of amino acids in the wheat protein structure or contamination by lipids. The peak at 531 eV (40.6%) is attributed to O bonded to C (O═C—O, O═C—N, O═C—OH), due to amide or carboxyl function of amino acids in the wheat proteins. The decreased content of both N and O in proteins found, according to the XPS data (C/O = 1/0.218 and C/N = 1.098) compared to the theroitcial chemical composition $C_{3.3}H_{5.9}O_{1.06}N_1S_{0.033}$ (C/O = 1/0.321 and C/N = 1.303)[10] would indicate the presence of lipid traces within the surface layers of protein particles.

Atomic Composition of Lipid Component. XPS imaging on the lipid sample (Table 2) gives major signals corresponding to C (77.7%), low signals corresponding to O (16.6%), and very low signals corresponding to N (2.0%). The C_{1s} peak at 284.6 eV (76.1%) is a characteristic of C bonded only to other C and H atoms and indicates the presence of aliphatic chains in fatty acids. The N_{1s} peak (2%) at 399.8 eV is attributed to amide-linked N, due to possible protein residues.

The decomposition and the assignment of peaks allowed XPS profiles to be defined. We observe large differences in XPS traces between proteins, lipids, and polysaccharides. As expected, only slight differences in XPS traces are found between starch and arabinoxylans, which are both polysaccharides. We can suppose that the XPS method would have some difficulties in distinguishing starch and arabinoxylans during the analysis of wheat flours.

XPS Analysis of Reconstituted Flours. XPS results for the binary and ternary reconstituted flours are presented in Table 3. Due to experimental constraints, it was not possible to duplicate experimental data. It is thus not really possible to say without any statistics whether or not the calculated values are significantly different. In addition, the observed irregularities are very likely to result from experimental errors of the XPS data and/or calculations. For instance, for the binary reconstituted flours, it can be seen that for the C_{1s} peak decomposition for all four binding energy values (284.6, 286.1, 287.5, and 288.7 eV), XPS measured values change not monotonically in going from 90/10 to 85/15 to 80/20 starch/protein content. Without any statistics, the predictive power of XPS measurements cannot be stated. Table 3 may only be used as an example of XPS data on such wheat flour systems (binary and ternary flours) as well as of deconvolution of XPS peaks and assignments of peak components.

For the binary flours, we could only notice the decrease in the C/N ratio (from 1/0.020 to 1/0.284), which is consistent with the changes in starch/protein ratio from 90/10 to 85/15 to 80/20. On the other hand, for the ternary flours, it is not possible to identify any relationships between signal intensities and changes in starch/arabinoxylan ratio, as starch and arabinoxylans are both polysaccharides.

XPS Analysis of Wheat Flours. XPS analyses (Table 4) demonstrate a major signal corresponding to C (77.0−79.2%), low signals corresponding to O (16.5−18.2%) and N (3.7−4.4%), and only traces of S (0.2−0.3%) and P (0.2−0.3%). Slight differences in intensity values after peak decomposition are observed between the flours. As expected, the energy values corresponding to C, O, and N peak decomposition for the wheat flours (Table 4) are found to range between values previously determined for the wheat components (Table 2). The differences in wheat grain types (Crousty, Tiger, or Impression variety) and in milling conditions (with peeling or pearling) seem thus to generate differences in surface composition of the flour particles.

Impact of Surface/Volume Ratio on XPS Analysis of Flours. Different samples of wheat flours were prepared to modify the ratio between the X-ray-exposed surface of the particles and their volume by using two different processes.

(i)The grinding procedure allows one to generate particles with different surface/volume ratios, with almost the same bulk chemical composition (although the damaged starch content may increase with grinding extend). As the grinding progresses, an increase in surface/volume ratio induces some noticeable decrease (Table 4) in signals corresponding to C (from 77.4 to 74.8%) and noticeable increases in signals corresponding to O (from 17.9 to 19.6%) and N (from 4.52 to 5.22%). From the peak decomposition (Table 3), we also observe decreases (from 88.4 to 85.3%) in C—OH peaks at 532.6 eV. The changes in surface/volume ratio at almost constant bulk composition seem to induce noticeable changes in the surface composition of the wheat particles.

(ii) The sieving procedure allows one to separate particles not only with different surface/volume ratios but also with possible changes in bulk composition, because particles of the isolated components such as small starch granules or large cell-wall fragments display different diameters. Sieving generates some diversity in XPS values (Table 4). However, it is not possible to

Table 3. Relative Elemental Composition Measured by XPS[a]

		atomic abundance (%) of elements at the surface					
		binary reconstituted flours			ternary reconstituted flours		
binding energy (eV)	functions	90% starch/ 10% proteins	85% starch/ 15% proteins	80% starch/ 20% proteins	83.5% starch/ 15% proteins/ 1.5% arabinoxylans	82% starch/ 15% proteins/ 2% arabinoxylans	80.5% starch/15% proteins/4.5% arabinoxylans
101	Si				0.17		
133	P		0.07				
163	S	0.08	0.12		0.11	traces	traces
286	C	67.8	67.9	68.8	68.0	67.8	67.2
284.6	C—C, C—H	36.3	33.3	38.7	34.7	33.5	31.7
286.1	C—O, C—N	48.4	51.4	46.8	49.6	51.0	52.4
287.5	O—C—O, C=O	12.5	12.7	11.6	12.8	12.3	13.0
288.7	O=C—OH, O=C—OR	2.79	2.49	2.85	2.88	3.13	2.85
399	N	1.38	1.61	1.96	1.66	1.38	1.29
399.7	NH, NH_2	100.0	95.9	93.7	95.4	100	100
402.4	NH_3		4.11	6.3	4.60		
533	O	30.7	30.3	29.2	30.1	30.9	31.5
531	O^{-2}	3.97	6.83	6.78	6.26	4.63	3.40
532.6	C-OH	88.4	76.5	85.3	75.0	88.0	86.4
533.8	H_2O	7.65	16.8	7.87	18.7	7.3	10.2

[a] Decomposition and assignment of XPS peak components (C_{1s}, N_{1s}, and O_{1s}) for the reconstituted flours.

identify specific relationships to describe the impact of surface/volume ratio (after sieving) on surface composition.

Calculations of Surface Composition. We calculated the apparent surface composition of flour particles from the XPS data obtained for wheat components (Table 2) and by using different sets of linear relations in a matrix formula.[8] The number of equations and the number of atomic elements in the formula matrix had to be adjusted to the number of components that were considered for calculations.[25] Calculations were made using different matrix formulas, based on those presented in eqs 1−234 in the case of four components.

$$I^{C}_{flour} = I^{C}_{proteins}\gamma_{proteins} + I^{C}_{starch}\gamma_{starch} + I^{C}_{arabinoxylans}\gamma_{arabinoxylans} + I^{C}_{lipids}\gamma_{lipids} \quad (1)$$

$$I^{N}_{flour} = I^{N}_{proteins}\gamma_{proteins} + I^{N}_{starch}\gamma_{starch} + I^{N}_{arabinoxylans}\gamma_{arabinoxylans} + I^{N}_{lipids}\gamma_{lipids} \quad (2)$$

$$I^{O}_{flour} = I^{O}_{proteins}\gamma_{proteins} + I^{O}_{starch}\gamma_{starch} + I^{O}_{arabinoxylans}\gamma_{arabinoxylans} + I^{O}_{lipids}\gamma_{lipids} \quad (3)$$

$$I^{S}_{flour} = I^{S}_{proteins}\gamma_{proteins} + I^{S}_{starch}\gamma_{starch} + I^{S}_{arabinoxylans}\gamma_{arabinoxylans} + I^{S}_{lipids}\gamma_{lipids} \quad (4)$$

where $I^{C}_{proteins}$, $I^{N}_{proteins}$, $I^{O}_{proteins}$, $I^{S}_{proteins}$, I^{C}_{starch}, I^{N}_{starch}, I^{O}_{starch}, I^{S}_{starch}, $I^{C}_{arabinoxylans}$, $I^{N}_{arabinoxylans}$, $I^{O}_{arabinoxylans}$, $I^{S}_{arabinoxylans}$, I^{C}_{lipids}, I^{N}_{lipids}, I^{O}_{lipids}, and I^{S}_{lipids} are the relative contents of atomic elements (C, N, O, S) measured on the surface of the isolated components (Table 2). I^{C}_{flour}, I^{N}_{flour}, I^{O}_{flour}, and I^{S}_{flour} are the relative contents of elements (C, N, O, S) on the surface of the samples (Tables 3 and 4). The parameters $\gamma_{proteins}$, γ_{starch}, $\gamma_{arabinoxylans}$, and γ_{lipids} are the calculated values of the component surface contents (Tables 5−7).

Surface Composition of Reconstituted Flours. Because simple reconstituted flours are mixed in a powder form, we could suppose that there is no molecular masking effect between the components. In these conditions, the surface composition of the reconstituted samples exposed to X-rays should be similar to the theoretical bulk composition of the added components.

For the binary reconstituted flours with different starch/proteins ratios, surface composition was calculated by considering only two components: starch and proteins (Table 5). Whatever the atomic elements (C, O; C, N; or N, O) considered in the matrix formula, the calculated surface compositions are close to the bulk composition. The XPS seems thus able to determine the surface composition and to identify specifically starch and proteins in binary reconstituted flours.

For the ternary reconstituted flours (starch, protein, arabinoxylans) with different starch/arabinoxylans ratios, surface composition was first calculated by considering only two components (starch and proteins) (Table 5). For the ternary flour at the lowest arabinoxylan content (0.835 starch/0.150 protein/0.015 arabinoxylans), good agreement is found between surface composition and bulk composition in starch and proteins. When the starch/arabinoxylan ratio increases at constant protein content, we obtained unexpected values of surface content for starch and proteins. The calculation of the surface contents for starch and proteins is anomalously affected by changes in arabinoxylan

Table 4. Relative Elemental Composition Measured by XPS[a]

			atomic abundance (%) of elements at the surface												
			Impression wheat variety	Crousty wheat variety		Tiger wheat variety		sieved flour				ground flour			
binding energy (eV)		functions	reference wheat flour	peeled wheat flour	pearled wheat flour	peeled wheat flour	pearled wheat flour	surface/ volume = 5.7 mm^{-1}	surface/ volume = 7.5 mm^{-1}	surface/ volume = 12.8 mm^{-1}	surface/ volume = 24.7 mm^{-1}	surface/ volume = 6.7 mm^{-1}	surface/ volume = 8.5 mm^{-1}	surface/ volume = 11.7 mm^{-1}	surface/ volume = 27.8 mm^{-1}
101	Si														
133	P		0.21	0.22	0.18	0.26	0.26								
163	S		0.24	0.26	0.22	0.20	0.31	0.21	0.24	0.29	0.28	0.21	0.23	0.26	0.28
286	C		77.0	77.0	78.2	79.2	77.9	77.2	78.8	77.0	76.4	77.4	76.0	75.6	74.8
284.6		C—C, C—H	57.5	57.3	60.9	59.3	57.3	56.6	57.9	58.5	57.7	54.7	55.3	55.7	57.4
286.1		C—O, C—N	32.5	34.1	30.5	31.4	33.3	31.6	31.1	30.2	31.4	34.6	33.6	32.8	30.0
287.5		O—C—O, C═O	7.0	6.17	5.85	6.73	6.66	8.83	7.65	8.32	8.16	7.60	8.19	8.46	9.89
288.7		O═C—OH, O═C—OR	3.0	2.71	2.77	2.65	2.76	2.92	3.26	2.92	2.63	3.04	2.90	3.06	2.73
399	N		4.39	4.44	4.07	3.75	4.22	4.57	4.17	5.06	4.69	4.52	5.00	5.29	5.22
399.7		NH, NH_2	91.7	92.6	94.2	--	91.4	100	100	100	100	100	100	100	100
402.4		NH_3	8.3	7.40	5.80		8.60								
533	O		18.2	18.1	17.3	16.5	17.3	18.1	16.8	16.0	18.6	17.9	18.7	18.9	19.6
531		O^{-2}	23.1	20.9	20.5	23.7	22.1	22.1	22.9	22.7	23.2	19.9	21.3	24.3	23.0
532.6		C-OH	65.2	69.8	63.4	68.3	70.5	66.4	67.5	65.0	66.5	69.1	71.9	69.7	69.1
533.8		H_2O	11.7	9.25	16.0	7.96	7.44	11.5	9.58	12.3	10.3	11.0	6.77	5.99	7.86

[a] Decomposition and assignment of XPS peak components (C_{1s}, N_{1s}, and O_{1s}) for the selected flours.

dx.doi.org/10.1021/jf102315h |J. Agric. Food Chem. 2011, 59, 1527–1540

Table 5. Calculated Values of Surface Composition from XPS Measurements for the Reconstituted Wheat Flours (Sums for either Two- or Three-Components Results Do Not Equal Unity Owing to the Calculation Method Used)

			surface composition					
			binary reconstituted flours			ternary reconstituted flours		
	elements	components	90% starch/ 10% proteins	85% starch/ 15% proteins	80% starch/ 20% proteins	83.5% starch/ 15% proteins/ 1.5% arabinoxylans	82% starch/ 15% proteins/ 2% arabinoxylans	80.5% starch/ 15% proteins/ 4.5% arabinoxylans
two components	C, O	starch	0.874	0.851	0.780	0.838	0.885	0.925
		protein	0.129	0.151	0.225	0.163	0.120	0.077
	C, N	starch	0.906	0.868	0.823	0.861	0.906	0.911
		protein	0.101	0.135	0.187	0.143	0.101	0.088
	N, O	starch	0.887	0.858	0.797	0.848	0.894	0.919
		protein	0.103	0.136	0.189	0.144	0.102	0.087
three components	C, N, O	starch				0.931	0.973	0.870
		protein				−0.076	−0.072	0.044
		arabinoxylan				0.150	0.107	0.084

content in reconstituted flour, which induce an overestimation of starch and an underestimation of protein surface contents, due to disturbances during XPS measurements and/or surface composition calculations.

Surface composition for the ternary reconstituted flours was also calculated by considering three components (starch, proteins, and arabinoxylans). The calculated values appear as significantly different from the bulk composition (Table 5), with overestimated starch surface content and underestimated proteins and arabinoxylans surface contents. We also observe some "absurd" negative values. As previously discussed, due to the fact that both starch and arabinoxylan are polysaccharides with almost similar chemical composition, XPS is not able to distinguish them specifically in ternary mixtures.

Surface Composition for Wheat Flours. When compared to the reconstituted flours, the native wheat flours are more complex materials due to the large diversity in chemical compositions and the heterogeneous distribution of components. It could be supposed that the surface composition is not a simple transposition of the bulk composition, due to possible molecular masking effects between the components. Surface compositions were first calculated for the selected wheat flours by considering only two components (starch and proteins). The calculated values of surface contents are found very different from the bulk composition (Table 6). In addition, the choices of atomic elements (C, O or C, N or N, O) used for calculations lead to different values of surface composition. The higher values of protein surface contents and the lower values of starch surface contents were obtained when using the (C, O) pair of elements. These differences, which were not observed in the case of the reconstituted flours (Table 5), could be explained by possible molecular masking effects between the components in wheat flour.

The surface compositions for the wheat flours were also calculated by considering three components (starch, proteins, and arabinoxylans) (Table 6). The results are not reliable, because "aberrant" values of starch surface contents (up to 100%) and negative values for proteins surface contents (−1.53 to −2.44) are found, due to close chemical composition of starch and arabinoxylans and possible molecular masking effect between the components.

When considering three other components (starch, proteins, and lipids) (Table 6), the calculated surface composition is found to be significantly different from the corresponding bulk composition (Table 1). Calculated values thus indicate a much higher content in lipids and proteins at the surface of the wheat flour particles compared to the bulk composition of the particles.

The surface composition for the wheat flours was also calculated by considering four components (starch, proteins, arabinoxylans, and lipids). As expected, the calculated surface composition (Table 6) does not seem reliable, because negative values for lipids (0.007 to −0.637) and arabinoxylans (−0.213 to −0.345) and high values for starch (1.521) surface contents were obtained whatever the examined wheat flour was.

Impact of Surface/Volume Ratio on Surface Composition. When particles are produced using the sieving procedure, we observe a great diversity in values of surface composition (Table 7). We do not observe any relationship between surface/volume ratio and surface contents. This could be related to the sieving procedure, which is supposed to generate heterogeneous changes in bulk composition of the flour samples.

When particles were produced using the grinding procedure, an increase in surface/volume ratio generates significant monotonous changes in surface composition. When calculations are made by considering two components (starch and proteins) (Table 7), an increase in surface/volume ratio (from 0.060 to 0.247) induces a slight decrease in starch surface contents and a slight increase in protein surface contents. When calculations are made by considering three components (starch, proteins, and lipids), an increase in surface/volume ratio (from 0.060 to 0.247) induces a slight increase in starch and lipid surface contents and a slight decrease in protein surface contents. It could be stated that changes in surface/volume ratio (at almost constant bulk composition) induce significant changes in surface composition of the wheat particles. The XPS seems thus able to give information about the chemical surface composition of wheat particles and to identify some changes induced by process conditions. However, it clearly appears that the results are affected by the considered calculation method (e.g., conflicting results are found for protein surface contents) because XPS is not

Table 6. Calculated Values of Surface Composition from XPS Measurements for the Selected Native Wheat Flours (Sums for Two-, Three-, or Four-Component Results Do Not Equal Unity Owing to the Calculation Method Used)

			surface composition				
	elements	component	reference wheat flour (Impression wheat variety)	peeled wheat flour (Crousty wheat variety)	pearled wheat flour (Crousty wheat variety)	peeled wheat flour (Tiger wheat variety)	pearled wheat flour (Tiger wheat variety)
two components	C, O	starch	0.079	0.073	0.015	-0.041	0.019
		protein	0.947	0.952	1.018	1.080	1.011
	C, N	starch	0.541	0.532	0.616	0.689	0.585
		protein	0.542	0.550	0.492	0.442	0.515
	N, O	starch	0.270	0.260	0.264	0.261	0.253
		protein	0.568	0.575	0.525	0.482	0.546
three components	C, N, O	starch	0.017	0.012	−0.065	−0.138	−0.057
		protein	0.542	0.538	0.705	0.856	0.665
		lipids	0.445	0.453	0.366	0.289	0.396
	C, N, O	starch	1.978	1.960	2.487	2.958	2.349
		protein	−1.546	−1.535	−2.012	−2.440	−1.896
		arabinoxylan	0.678	0.684	0.668	0.656	0.681
fourcomponents	C, N, O, S	starch	0.995	1.114	0.941	0.891	1.521
		protein	0.608	0.637	0.533	0.460	0.659
		arabinoxylan	-0.213	−0.241	−0.220	−0.225	−0.345
		lipids	-0.264	−0.370	−0.125	0.007	−0.637

Table 7. Calculated Values of Surface Composition from XPS Measurements for the Sieved Flours and for the Ground Flours (Sums for either Two- or Three-Components Results Do Not Equal Unity Owing to the Calculation Method Used)

			surface composition							
			sieved flour				ground flour			
	elements	components	surface/ volume = 5.7 mm^{-1}	surface/ volume = 7.5 mm^{-1}	surface/ volume = 12.8 mm^{-1}	surface/ volume = 24.7 mm^{-1}	(surface/ volume = 6.7 mm^{-1}	surface/ volume = 8.5 mm^{-1}	surface/ volume = 11.7 mm^{-1}	surface/ volume = 27.8 mm^{-1}
two components	C, N	starch	0.513	0.609	0.425	0.479	0.525	0.418	0.362	0.360
		protein	0.569	0.506	0.644	0.588	0.561	0.636	0.681	0.671
	N, O	starch	0.254	0.241	0.151	0.261	0.251	0.243	0.228	0.256
		protein	0.594	0.541	0.669	0.609	0.587	0.653	0.693	0.681
three components	C, N, O	starch	0.012	−0.103	−0.104	0.058	−0.005	0.078	0.104	0.158
		protein	0.519	0.738	0.548	0.435	0.548	0.352	0.267	0.209
		lipids	0.467	0.374	0.546	0.511	0.463	0.573	0.633	0.634

able to accurately identify the main wheat components. XPS results should thus be considered with caution.

DISCUSSION

In the present study, we use XPS to investigate the surface composition of wheat flour particles. XPS has first been used to identify the wheat main components from their specific chemical function. The results demonstrated that XPS is able to distinguish proteins, polysaccharides, and lipids from their specific chemical functions. As expected, XPS is not able to distinguish starch and arabinoxylan because of the similarity in their chemical structures. The use of XPS for wheat flours has thus been confronted with the large diversity of the chemical functions that are present on the four main wheat components (starch, protein arabinoxylan, and lipids). However, it is clear that it is very difficult to differentiate using XPS the starch and arabinoxylans molecules, because they belong to the same molecular family, with close chemical functions. The differences between these macromolecules are mainly located at the level of the macromolecular chains scale. A more systematic experimental XPS (with purified wheat components from several types of wheats) to get a better characterization of the macromolecular structures of the wheat components seems still necessary. In comparison, the use of XPS for dairy powders relies on the description of molecules containing very different chemical functions (proteins, lipids,

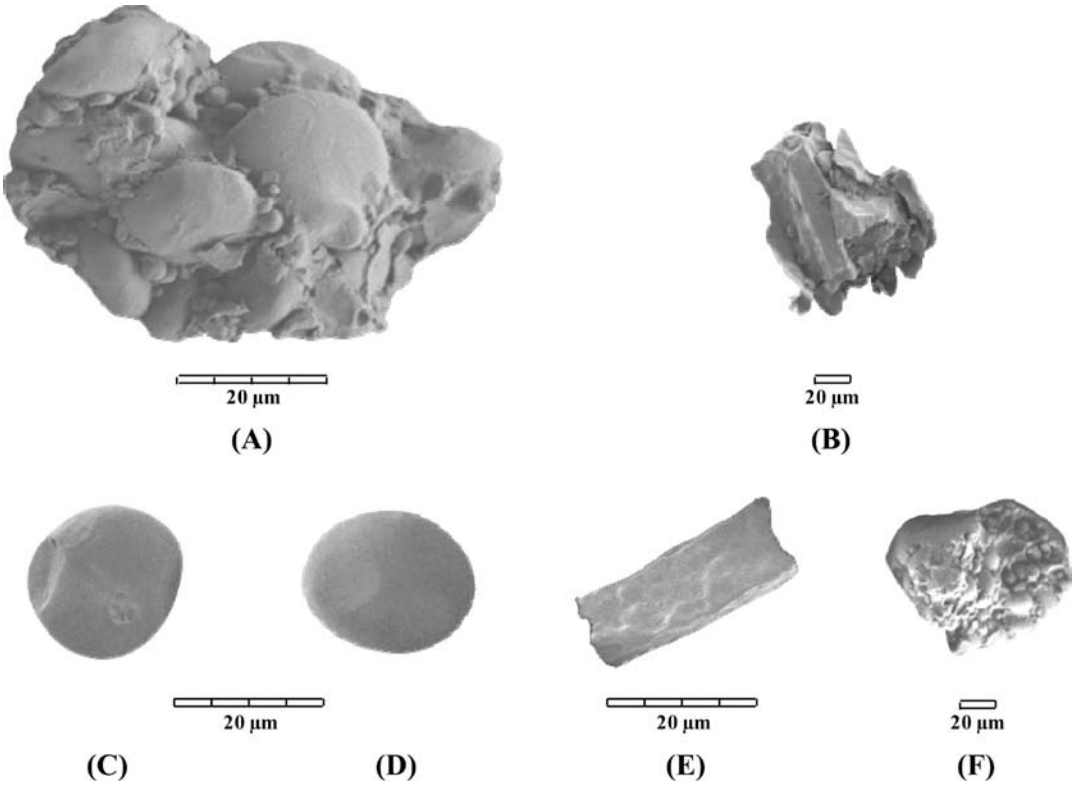

Figure 5. Scanning electron microscopy images taken for the main individual particles (at different magnification scales) found in wheat flour, with whole endosperm cells (A), wedges of protein (B), damaged starch granules (C), native starch granules (D), pieces of bran (E), and protein and starch clusters (F).

and lactose) and is more adapted to discriminate between them.[4,5,11]

From the XPS analysis of the chemical functions of the wheat components, we have tried to calculate the particle surface composition of the wheat powders, in particular, the respective contents in proteins, starch, lipids, and arabinoxylans. The calculations carried out for the simple reconstituted flours with known surface characteristics (based on protein and starch) demonstrated the relevance of our calculation approach and the coherence of the calculated values of surface composition. As expected, the incorporation of arabinoxylans in reconstituted flours troubled our calculation approach and led to "absurd" values of surface concentrations.

The use of XPS for the description of native flours allowed the surface composition of particles to be determined. When investigations are carried out on proteins, starch, and lipids, XPS is able to monitor the surface composition of flour particles and to describe changes induced by grinding process. Surface composition of wheat flours is found to be significantly different from the bulk composition, with an overrepresentation of protein and lipids and an underrepresentation of polysaccharides. These results are consistent with data already mentioned in the literature.[10] Overrepresentation of lipids and proteins at the surface of wheat particles could be related to the presence of a thin layer of lipids and proteins that was directly identified on the surface of starch granules.[12] Rouxhet et al.[10] considered values with caution as some approximations were made for calculations, even if the lipid surface content reaches a level that exceeds what may be attributed to the adventitious contamination usually observed on polar solids.

XPS results can be related to technical knowledge available about wheat grain milling and flour production.[26] Wheat components

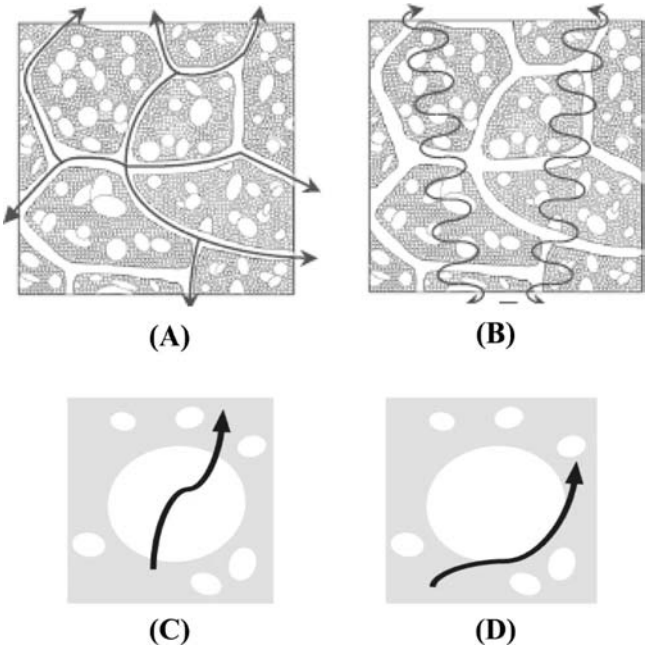

Figure 6. Structural hypothesis to describe wheat grain fractionation, based on a cross-sectional diagram in wheat kernel. Grain breaking is hypothesized to take place at the edge of endosperm cells (A), through the endosperm cell (B), through the starch granule (C), or at the edge of the starch granule (D).

(starch, proteins, arabinoxylans, and lipids) are not evenly distributed within flour particles due to the heterogeneous

structure of wheat grains endosperm and due to the complexity of the milling process. When wheat endosperm is fragmented by grinding, it is usually reduced to a mixture of particles, differing in size and composition (Figure 5). Some whole endosperm cells (Figure 5A), protein wedges (<20 μm) (Figure 5B), damaged starch granules (Figure 5C), native starch granule (7−30 μm) (Figure 5D), bran pieces (Figure 5E), and clusters of starch granules in a protein matrix (about 20 μm) (Figure 5F) are present in flour. In addition, wheat flour components are not evenly distributed over the particles. Particles of segment endosperm cell fraction have a protein content similar to that of the native endosperm. Large- and medium-sized starch granules have protein contents of half to two-thirds that of the native endosperm. Small chips of protein and detached small starch granules have protein contents approximately twice the content of the native endosperm. Figure 6 shows that, depending on applied milling conditions, wheat kernel could be crushed in two ways. (i) Crushing may take place at the edge of the endosperm cell walls (Figure 6A) and thus generate whole endosperm cells in the flour. Consequently, XPS would mainly detect components on the outer layer of the fragmented cell walls covering the endosperm cells. (ii) Crushing may take place through endosperm cells (Figure 6B) and thus may cause the starch granules to be damaged (Figure 6C). XPS then detects the protein network and cell-wall components besides starch exhibited on the damaged surfaces. In this way, crushing may also happen at the edge of the starch granules (Figure 6D), and XPS then detects the components on the surface of the native starch particles besides the protein network and the cell-wall components.

In addition, some characteristics of the wheat flour particles could contribute to disturb the analysis of XPS results. Particle roughness (Figure 2B) could affect measurement of the surface chemical composition. A rough surface may shadow some parts of the sample from the XPS beam, and some parts might be hidden and not detected by XPS. A large distribution of particle size (Figure 2B) is also a factor that could affect XPS measurements, because small particles might be hidden under large particles and then would not be detected by X-rays during measurements.

For the characterization of wheat flours, it thus remains impossible to distinguish starch and arabinoxylans and to calculate their respective concentrations on the surface. A better understanding of the potential applications of XPS method to describe the surface composition of wheat flour powder thus requires additional experimental data. It may be necessary to continue investigating XPS signals for the isolated main wheat components by considering components obtained by different extractions and from different wheat grain types. The reconstitution process of simple flours could also be extended from simple to different complex mixtures, by considering powder mixing or molecular mixing of components, or by modifying the exposed surface.

AUTHOR INFORMATION

Corresponding Author

*Phone: 0033499612892. Fax: 0033499613076. E-mail: cuq@supagro.inra.fr.

Funding Sources

We acknowledge the financial support provided by the Ministry of Higher Education and Scientific Research in Syria and by the Agence National de la Recherche (ANR) in France (Reactive-Powder program).

ACKNOWLEDGMENT

We are grateful to Aurelian Renard (LCPME, Laboratoire de Chimie Physique et de Microbiologie pour l'Environnement, Nancy, France), Thérèse-Marie Lasserrd, Aurélie Putois (INRA Montpellier, France) for providing technical support, and for Prof. Xavier Rouau (UMR-IATE 1208 Joint Research Unit for Agropolymers Engineering and Emerging Technology Montpellier, France) for providing some wheat flour samples.

REFERENCES

(1) Landillon, V.; Cassan, D.; Morel, M. H.; Cuq, B. Flowability, cohesive, and granulation properties of wheat powders. *J. Food Eng.* **2008**, *86*, 178–193.

(2) Fitzpatrick, J. J.; Ahrné, L. Food powder handling and processing: industry problems, knowledge barriers and research opportunities. *Chem. Eng. Process.* **2004**, *44*, 209–214.

(3) Kim, E. H. J.; Chen, X. D.; Pearce, D. Surface characterization of four industrial spray-dried dairy powders in relation to chemical composition, structure and wetting property. *Colloids Surf. B* **2002**, *26*, 197–212.

(4) Kim, E. H. J.; Chen, X. D.; Pearce, D. Melting characteristics of fat present on the surface of industrial spray-dried dairy powders. *Colloids Surf. B* **2005**, *42*, 1–8.

(5) Gaiani, C.; Ehrhardt, J. J.; Scher, J.; Hardy, J.; Desobry, S.; Banon, S. Surface composition of dairy powders observed by X-ray photoelectron spectroscopy and effects on their rehydration properties. *Colloids Surf. B* **2006**, *49*, 71–78.

(6) Nijdam, J. J.; Langrish, T. A. G. The effect of surface composition on the functional properties of milk powders. *J. Food Eng.* **2006**, *77*, 919–925.

(7) Zhou, Q.; Baumann, M. J.; Brumer, H.; Teeri, T. T. The influence of surface chemical composition on the adsorption of xyloglucan to chemical and mechanical pulps. *Carbohydr. Polym.* **2003**, *63*, 449–458.

(8) Fäldt, P.; Bergenstahl, B.; Carlson, G. The surface coverage of lipids on food powders analyzed by ESCA (electron spectroscopy for chemical analysis). *Food Struct.* **1993**, *12*, 225–234.

(9) Bosquillon, C.; Rouxhet, P. G.; Ahimou, F.; Simon, D.; Culot, C.; Preat, V.; Vanbever, R. Aerolization properties, surface composition and physical state of spray-dried protein powders. *J. Controlled Release* **2004**, *99*, 357–367.

(10) Rouxhet, P. G.; Misselyn-Bauduin, A. M.; Ahimou, F.; Genet, M. J.; Adriaensen, Y.; Desille, T.; Bodson, P.; Deroanne, C. XPS analysis of food products: toward chemical functions and molecular components. *Surf. Interface Anal.* **2008**, *40*, 718–724.

(11) Kim, E. H. J.; Chen, X. D.; Pearce, D. Surface composition of industrial spray-dried milk powders: effects of spray drying conditions on the surface composition. *J. Food Eng.* **2009**, *94*, 169–181.

(12) Russel, P. L.; Gough, B. M.; Greenwell, P.; Fowler, A.; Munro, H. S. A study by ESCA of the surface of native and chlorine-treated wheat starch granules: the effects of various surface treatments. *J. Cereal Sci.* **1987**, *5*, 83–100.

(13) Saad, M. M.; Gaiani, C.; Scher, J.; Cuq, B.; Ehrhardt, J. J.; Desobry, S. Impact of re-grinding on hydration properties and surface composition of wheat flour. *J. Cereal Sci.* **2009**, *49* (1), 134–140.

(14) Rouxhet, P. G.; Mozes, N.; Dengis, P. B.; Dufrêne, Y. F.; Gerin, P. A.; Genet, M. J. Application of X-ray photoelectron spectroscopy to microorganisms. *Colloids Surf. B* **1994**, *2*, 347–369.

(15) Dengis, P. B.; Gerin, P. A.; Rouxhet, P. G. X-ray photoelectron spectroscopy analysis of biosurfaces: examination of performances with yeast cells and related model components. *Colloids Surf.B* **1995**, *4*, 199–211.

(16) Yip, J.; Chan, K.; Sin, K. M.; Lau, K. S. Study on the surface chemical properties of UV excimer laser irradiated polyamide by XPS, ToF-SIMS, and CFM. *Appl. Surf. Sci.* **2003**, *205*, 151–159.

(17) Koljonen, K.; Osterberg, M.; Johansson, L. S.; Stenius, P. Surface chemistry and morphology of different mechanical pulps determined by ESCA and AFM. *Colloids Surf. A* **2003**, *228*, 143–158.

(18) Aguie-Beghin, V.; Adriaensen, Y.; Périn, N.; Valade, M.; Rouxhet, P.; Douillard, R. Structure and chemical composition of layers adsorbed at interfaces with champagne. *J. Agric. Food Chem.* **2009**, *57* (21), 10399–10407.

(19) Hemery, Y.; Rouau, X.; Lullien-Pellerin, V.; Baron, C.; Abecassis, J. Dry processes to develop wheat fractions and products with enhanced nutritional quality. *J. Cereal Sci.* **2007**, *46*, 327–347.

(20) Auger, F. Etude des mécanismes d'agglomération du gluten au cours du pétrissage des suspensions farine-eau. Ph.D. thesisMontpellier SupAgro, Montpellier, France, 2008.

(21) American Association of Cereal Chemists. *Official Methods of the AACC*, 10th ed.; Method 44-15A first approval Oct 30, 1975; reapproval Nov 3, 1999; Method 76-13 first approval Nov 8, 1995; reapproval Nov 3, 1999; St. Paul, MN, 2000.

(22) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113* (2), 291–299.

(23) NF V 03-050. Directives générales pour le dosage de l'azote avec minéralisation selon la méthode de Kjeldahl, 1970.

(24) Roman-Gutierrez, A. D.; Guilbert, S.; Cuq, B. Description of microstructural changes in wheat flour and flour components during hydration by using environmental scanning electron microscopy. *Lebensm.-Wiss. Technol.* **2003**, *35* (8), 730–740.

(25) Fäldt, P. Surface composition of spray dried emulsions. Ph.D. thesis, Lund University, Stockholm, Sweden, 1995.

(26) Feillet, P. Protéines. *Le Grain de Blé: Composition et Utilisation*; INRA: Paris, France, 2000; pp 57−60.