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# X-ray photoelectron spectroscopic analysis of rice kernels and flours: Measurement of surface chemical composition

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ABSTRACT

The objectives of this study were to evaluate the ability of X-ray photoelectron spectroscopy (XPS) to differentiate rice macromolecules and to calculate the surface composition of rice kernels and flours. The uncooked kernels and flours surface composition of the two selected rice varieties, Thadokkham-11 (TDK11) and Doongara (DG) demonstrated an over-expression of lipids and proteins and an under-expression of starch compared to the bulk composition. The results of the study showed that XPS was able to differentiate rice polysaccharides (mainly starch), proteins and lipids in uncooked rice kernels and flours. Nevertheless, it was unable to distinguish components in cooked rice samples possibly due to complex interactions between gelatinized starch, denatured proteins and lipids. High resolution imaging methods (Scanning Electron Microscopy and Confocal Laser Scanning Microscopy) were employed to obtain complementary information about the properties and location of starch, proteins and lipids in rice kernels and flours.



## 1. Introduction

Rice kernels and flour are used to produce a large variety of cereal-based foods, including semolina, gluten free bread, noodles and biscuits. The functional properties (e.g., water absorption, pasting properties etc.) and biochemical composition of rice affect the overall quality of the processed foods (Matos & Rosell, 2013). Rice can be classified into waxy and non-waxy varieties based on the native starch type present in the endosperm. Waxy rice contains branched amylopectin and becomes very sticky after cooking. However, non-waxy rice contains straight chain amylose and is less sticky (Nawaz, Fukai, & Bhandari, 2015).

A scientific understanding has been established that the functional properties of complex biological materials are greatly dependant on the surface characteristics (Rouxhet et al., 2008). Therefore, the space distribution of components is a key to understanding complex biological systems (Saad et al., 2009). Recent studies carried out on biological powders has showed that the surface chemical composition of particles is significantly different from their bulk composition (Baer & Engelhard, 2010; Shrestha, Howes, Adhikari, Wood, & Bhandari, 2007; Zhao et al., 2015).

X-ray photoelectron spectroscopy (XPS) has become a well-established technique to study the nature of many different types of surfaces (Gaiani et al., 2011). XPS has been extensively used to investigate the surface composition of biological powders (mainly milk powders) obtained by spray or freeze drying of complex biological solutions (Kim, Chen, & Pearce, 2002; Zhao et al., 2011). On the other hand, there has been very limited research focused on investigating the application of XPS for natural biological powders obtained by the milling of agricultural produce (Russel, Gough, Greenwell, Fowler, & Munro, 1987; Saad, Gaiani, Mullet, Scher, & Cuq, 2011; Saad et al., 2009; Zhao et al., 2015). The surface composition of rice flour and/or kernels has received relatively little research attention, despite the importance of these factors in providing a better understanding of some of functional properties of cooked rice. The inter-particulate interactions (such as stickiness in the case of rice) and exposure to external environment that may cause chemical changes (such as oxidation of fat/oil) are depended on the surface composition and properties of these surface materials.

The determination of the surface composition of a material by XPS is first considered at an elemental level. XPS provides the relative atomic elemental composition of approximately 5–10 nm of the surface layer (Rensmo & Siegbahn, 2015). Generally, the elemental composition of biological material is defined by considering only the three main elements, carbon, oxygen, and nitrogen.

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Usually minor elements (such as phosphorus, sulphur, silicon, boron, manganese or other minerals) are ignored, they account for as little as 1% the bulk composition (Gaiani et al., 2006; Nijdam & Langrish, 2006; Rensmo & Siegbahn, 2015; Rouxhet et al., 2008). The relative elemental composition (carbon, oxygen, and nitrogen) is used to identify components such as proteins, lipids and polysaccharides. For milk powders, the XPS apparent atomic stoichiometry has been found to have reasonable agreement with theoretical stoichiometry based calculations (Nikolova et al., 2015). In addition, the $C_{1s}$, $N_{1s}$, and $O_{1s}$ peaks obtained from the XPS survey scans can be decomposed at specific binding energies into various sub-peaks and assigned to well-identified chemical functions (e.g., C—C(H), C—O, C—N, C=O, O—C=O etc.) that are typical for specific components, such as lipids, sugar derivatives, glucose polymers, and poly-amino acids (Rouxhet & Genet, 2011).

The surface composition of biological materials can be estimated by using the relative elemental composition of isolated components. Fäldt, Berenstahl, and Carlson (1993) developed a method by quantifying the relative atomic concentrations of carbon, oxygen, and nitrogen, and by using a matrix formula related to the surface content of the different compounds (i.e., polysaccharides, proteins and lipids) that make up the sample. These calculations have been found to be reliable only when significant differences among C, O, and N are present in the various components (Saad et al., 2011). XPS has its limitations when it comes to differentiation of the multiple functional groups that have similar percentages of atoms. Therefore, isolated components should be significantly different in elemental composition to be able to be differentiated by XPS. As an example, it appears to not be possible to differentiate whey proteins and caseins in milk from the C, O, and N signatures, as these two protein categories are present in the same atomic percentages (Gaiani et al., 2011).

The application of XPS to biopolymers appears to be very reliable, and versatile with wide range of applicability (Kelly et al., 2015). However, natural surface contamination during experiment can complicate XPS analysis by overexpression of carbon, because most abundant surface contaminants on air-exposed samples consist of carbonated particles (McArthur, Mishra, & Easton, 2014). Moreover, some biological specimens (such as wood pulps) have shown instability during XPS-scanning due to X-ray induced irradiation damage and adsorption or desorption of volatiles in ultrahigh vacuum conditions during the experimentation. This damage can distort the data and further complicate the data interpretation (Zhou, Baumann, Brumer, & Teeri, 2006).

As noted above, over the past ten years XPS has been extensively used to evaluate the surface composition of dairy powders. However, only a limited number of studies have been undertaken to investigate natural agricultural products, with most research to date focused on wheat flours (Rouxhet et al., 2008; Saad et al., 2009, 2011). The objective of the present study was to evaluate the ability of XPS to identify the surface composition of rice kernels and the flour of waxy and non-waxy rice varieties. First, XPS survey scans of pure rice components (starch, proteins, and lipids) were obtained. Then an assessment was made of the surface composition of rice kernels and rice flours. To quantify the surface composition, only macro-nutrients (starch, protein, and lipid) were taken into account.

## 2. Materials and methods

Two rice varieties, Thadokkham-11 (TDK11) (glutinous) and Doongara (DG) (non-glutinous) were used in this study. The rice grains were provided by Rice Research Australia Pty Ltd. (RRAPL). Powdered rice starch (Sigma S7260, Castle Hill, NSW, Australia), commercially available rice protein (Bulk Nutrients, TAS, Australia) and pure rice bran oil (Coles, QLD, Australia) were used to estimate the relative elemental composition of pure rice components. The flow chart of the experimental design is presented in Supplementary section Fig. S1.

### 2.1. Milling of paddy

The effect of the degree of milling (DOM) on the surface composition of rice kernels was analysed for TDK11 only. Paddy rice was milled to brown rice by using rice husker (Satake, Japan). The brown rice was milled to white rice using an abrasive polisher (Satake, Japan). Three different DOMs, 0% or brown rice, 9%, and 16%, were used in the study. DOM was calculated using the following Eq. (1) as described by Marshall (1992):

$$DOM(\%) = 1 - (WWR/WBR) \times 100 \quad (1)$$

*WWR* and *WBR* are the weight of white rice and brown rice in grams, respectively.

### 2.2. Grinding of rice kernels

The milled white TDK11 and DG rice grains were ground to flour using a hammer mill (Good Friends of the Guangzhou Machinery Co. Ltd., Guangzhou, China) equipped with a plate of 500 μm size.

### 2.3. Chemical analysis of milled white rice

The starch content of the milled rice flours was determined according to the AACC 76-13.01 method (AACC, 1999a, 1999b). Total nitrogen content (TN) was determined by the Kjeldahl method, and crude protein content was calculated as TN × 5.95. Lipid content was determined by using the Soxhlet extraction method according to AACC 30-25.01 (AACC, 1999a, 1999b). The apparent amylose content (AAC) was determined by the iodine colorimetric method (Hoover & Ratnayake, 2005).

### 2.4. Sample preparation of defatted rice kernels and flours

The milled rice kernels and flour of TDK11 and DG were defatted using solvent extraction. Kernels/flour (10 ± 1 g) samples were taken in cellulose thimbles and treated with petroleum spirit at 70 °C for two hours. After two hours of reflux, the petroleum spirit was separated from sample using rotary evaporator (RV 10, IKA® Werke GmbH & Co. KG, Germany). The defatted samples were left in a fume hood overnight to fully evaporate the petroleum spirit.

### 2.5. Sample preparation of cooked rice kernels

To five gram samples of milled rice kernels, 15 mL of deionised water (rice to water ratio 1:3) was added in a glass beaker. The samples were then cooked at 95 ± 1 °C in a water bath, after which they were held overnight in a freezer. The frozen cooked rice samples were then freeze dried using an Alpha 1-4 LSC Freeze Dryer (John Morris Scientific, Australia). The moisture content of the samples was reduced to 10% to ensure sample stability in ultrahigh vacuum conditions (base pressure as low as $1.33 \times 10^{-7}$ to $1.33 \times 10^{-6}$) during XPS imaging.

### 2.6. Surface chemical analysis

The surface chemical analysis of pure rice components (starch, proteins, and lipids), rice kernels with three different DOM (0%, 9%, and 16%) (TDK11 only), uncooked rice kernels (control and defatted), freeze dried cooked rice kernels, and rice flour (control and defatted) were analysed by using a Kratos AXIS Ultra Kratos