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Compositional fingerprint of soy sauces via hydrophobic surface interaction

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ABSTRACT

In this work, the interaction of soy sauces with hydrophobic surfaces has been analyzed. Hydrophobic self-assembled monolayers on gold or silicon dioxide were used to harvest conditioning layers from soy sauce products with varying amounts of additives. The data was compared to adsorption of soy protein and glutamic acid as common ingredients. Spectral ellipsometry revealed that all tested sauces led to the formation of thin overlayers on hydrophobic surfaces. Products with less additives yielded adlayers in the same thickness range as pure soy protein. In contrast, sauces with more ingredients create distinctly thicker films. Using water contact angle goniometry, it is shown that all adlayers render the substrate more hydrophilic. Infrared spectroscopy provided a deeper insight into the adlayer chemistry and revealed that the adlayer composition is dominated by protein rich components. X-ray reflectivity on selected films provided further insight into the density profiles within the adlayers on the molecular scale.

1. Introduction

The large diversity of soy bean products, such as texturized soy proteins as substitute for animal proteins (Cassini, Marczak, & Noren, 2006) or soy sauces as an all-propose seasoning, especially in Asian countries (Chen et al., 2012), are enjoying increasing popularity. In particular, the wide spread use of soy sauce necessitates accurate quality control methods for these products. In this context the complexity of the compounds in soy sauces based on the different fermentation methods (Chen et al., 2012) is challenging. Conventional investigation methods include potentiometric and enzymatic measurements to gain information about salt concentration and lactic acid content, respectively (Stiftung Warentest, 2006). Otherwise, chromatographic methods, such as ion chromatography, HPLC or gas-liquid-chromatography, are used e.g. to determine volatile components (Aishima, 1982). Spectroscopic techniques, such as near-infrared spectroscopy, have been used to analyze soy sauces from different geographic regions in Japan and correlated to food flavouring and quality of the sauces (Ilzuka & Aishima, 1997).

Soy protein is known to readily accumulate on liquid-air interfaces, (Rodríguez Patino, Sánchez, Molina Ortiz, Rodríguez Nino, & Anón, 2004), though to our knowledge no studies regarding the interaction of soy protein with hydrophobic solid interfaces have been published. Protein adsorption assays are frequently used to characterize resistant properties of interfaces and hydrophobic monolayers are generally preferred as a non-protein resistant reference (Prime & Whitesides, 1991). In turn, unspecific adsorption after immersion into a complex medium reveals components that strongly interact with hydrophobic interfaces (Thome et al., 2014). The aim of this work is to introduce a pull-down assay which uses different surface characterization methods, such as spectral ellipsometry, water contact angle goniometry, ATR-FTIR spectroscopy, and X-ray reflectometry to distinguish between different compounds in soy sauces.

As a large variety of brands and origins are available, different soy sauces with varying amount of additives were selected. A brief overview on the different sauces used in this study is shown in Table 1. While sauces *A*, *B* and *C* were rather free of additives, sample *D* contained high amounts of additional ingredients, such as sugar, salt or wheat flour. The obtained data was compared to pure soy protein and glutamate as ingredient in additive rich soy sauces.

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Table 1
Composition of commercially available soy sauces used in this study.

Brand	
A (organic product)	Water, soy beans (33%), sea salt, wheat flour (+)
B	Water, salt, soy beans (10%), wheat (+)
C	Water, soy beans, wheat, salt (+)
D	Water, soy sauce (water, soy beans, wheat flour, salt), sugar, table salt, caramel sugar syrup, flavor enhancer: monosodium glutamate, wheat flour, acidulant: citric acid (++)

2. Materials and methods

2.1. Chemicals

All chemicals (ethanol p.a., hydrolyzed soy protein, glutamic acid and PBS buffer) were purchased from Sigma Aldrich (Germany) and used without further purification. Dodecanethiol was purchased from Prochimia (Sopot, Poland). The different soy sauce samples were obtained from local distributors. Deionized water was further purified with a Siemens Water Technologies system. As substrates, for the thiol SAMs Si wafers were used, which were coated with a 5 nm titanium adhesion layer and 100 nm of gold (Georg Albert PVD, Silz, Germany).

2.2. Surface preparation

DDT (1-Dodecanthiol) monolayers were prepared from a 1 mM solution in ethanol p.a., following previously published protocols (Thome et al., 2014). The substrates were cleaned under UV light for 90 min, ultrasonicated in ethanol p.a. for 3 min, and immersed in the alkanethiol solution. After 24 h incubation under ambient conditions the substrates were rinsed with ethanol, ultrasonicated in ethanol p.a. for 3 min, rinsed again with ethanol, and dried in a stream of nitrogen. Until further usage all samples were stored under an argon atmosphere. For X-ray reflectivity measurements, silicon wafers with a native silicondioxide layer were coated with a monolayer of octadecyl-trichlorosilane (OTS) (Mezger et al., 2006).

2.3. Adsorption assay

Adsorption of the soy sauces on DDT surfaces were carried out similar to previously published protocols for protein adsorption and surface conditioning experiments (Thome et al., 2014). For each soy sauce brand, surfaces of the hydrophobic samples were immersed in the pure sauce for 20 min on a shaking table. After incubation the solution was diluted with copious amounts of deionized water. While taking the samples through the air/water interface, they were gently rinsed with MilliQ water to prevent the formation of Langmuir layers. The adsorption of soy sauce components (soy protein and glutamic acid) on DDT surfaces followed previously published protocols (Thome et al., 2014). Therefore, the hydrophobic samples were immersed into PBS buffer (pH 7.4) for 20 min before adding an equal volume of 20 mg/ml of the compound of interest in PBS buffer to obtain a final concentration of 10 mg/ml. After an additional incubation for 20 min, the solution was diluted with copious amounts of deionized water. In order to prevent the formation of Langmuir layers the samples were gently rinsed with MilliQ water while they were taken through the air/water interface.

2.4. Spectral ellipsometry

Film thicknesses were determined by spectral ellipsometry (M-2000, Woollam, USA; CompleteEASE software package). All samples were measured before assembly and modeled as a *B*-spline. The DDT SAM was modeled as organic adlayer with a wavelength depending refractive index described by a Cauchy model (A = 1.45, B = 0.01, C = 0). The adlayer formed after immersion in soy sauce or its components were modeled in the same way. All presented data represent the average of at least three replicates and four different positions on each sample. Error bars represent the standard deviation.

2.5. Contact angle goniometry

The static water contact angles (CAs) were measured using a custom-build goniometer. The droplets (MiliQ water) were applied on the sample surface and their shapes were recorded via a CCD camera. The shape analysis was accomplished by using Young's equation. The presented values were obtained on at least three replicates and three measured positions. Error bars represent the standard deviation.

2.6. ATR-FTIR

The ATR-FTIR spectra (VariGATR, Harrick, USA) were obtained with a Bruker Tensor 27 spectrometer (Ettlingen, Germany), with a liquid N_2-cooled MCT detector. Before measuring the first spectra the system was purged with nitrogen for 20 min. As background the spectrum of the Ge-ATR crystal was used.

2.7. X-ray reflectivity

The X-ray reflectivities of the wafers were measured using a Bruker AXS D8 advance diffractometer with a copper anode (wavelength $\lambda = 0.154$ nm). From the X-ray reflectivity data vertical electron density profiles of the samples were determined by refining the data with the Parratt (Parratt, 1954) algorithm, in combination with the Effective density model (Tolan, 1999).

3. Results and discussion

3.1. Thickness and wettability measurements of adsorbed adlayers

The prepared DDT SAMs were characterized via spectral ellipsometry and CA goniometry. Average film thicknesses of (1.05 ± 0.2) nm and CAs of (101 ± 4)° were determined and verified an adequate film formation (Laibinis et al., 1991). Fig. 1a shows the thicknesses of the adsorbed adlayer on the DDT SAM, after incubation in the different soy sauces or in a soy protein or glutamic acid containing solution. The highest adlayer thickness of ≈4.5 nm was observed for soy sauce *D*. Incubation in the sauces *A*, *B*, and *C* resulted in thinner adlayers, with an approximate thickness of 2 nm. These thicknesses were in a similar range as the adsorbed pure soy protein, which formed 2 nm thick layers. The adhesion of glutamic acid alone yielded only relatively thin films (0.25 nm). A comparison with the list of ingredients (Table 1) reveals that thicker films were formed from soy sauces rich in additives, while samples which contain only the pure, fermented soy sauce components formed thinner films.

Fig. 1b shows the change in wettability of the surfaces after adsorption of adlayers from soy sauce. The water contact angle of 101° of the DDT SAM is indicated by the dashed line. All samples rendered the surface more hydrophilic and contact angles between 50° and 60° were obtained. Within the error bars, no differences between the soy sauces were found. Adsorbed overlayers from soy protein solutions showed similar contact angles as the adlayers from the soy sauces. Immersion into a solution of glutamic acid ($6.7{\cdot}10^{-2}$ mM) resulted in contact angles of 98°, which were very