NMR and Electron Microscopy Studies of Soybean Seeds, Soybean Proteins and Oil for Improved Utilization in Foods and Feeds

Doina Costescu and I.C. Baianu

- AFC NMR & NIR Microspectroscopy Facility,

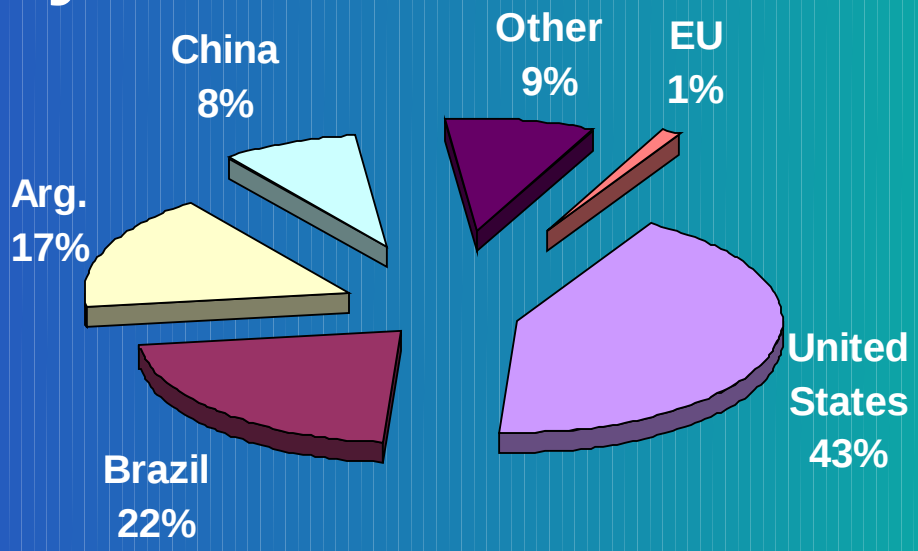
College of ACES, University of Illinois at Urbana,

URBANA, Illinois 61801, USA

Introduction

- Soybean is widely use in industry, livestock feed and human nutrition.
- Its high value composition ascertains soybean as one of the major sources of oil and protein in human consumption.

World Soybean Production 2002-2003



Background

- NMR, NIR and EM techniques are suitable methods of measuring protein and oil content in soybeans. After completion a measurement with NMR or NIR, a soybean sample could be replanted or consummated.
- NMR techniques involved in the present work are based on the response of nuclei, as nuclear spin flips, when set in a 7.05T magnetic field and excited with rf pulses of 300 MHz.
- NIR spectroscopy is based on outer electron molecular vibrations, when excited with radiation of frequency 10^{12} -$4x10^{14}$Hz, and λ=2.5 μm-750 nm.
- TEM and ESEM techniques are bulk and surface analysis techniques, respectively, working with electron beams of 15KV, and image resolution in the range of nm's. ESEM is based on the possibility of imaging with secondary emitted electrons from the samples, and TEM on the possibility to have image contrast based on the difference in electron transmission through elements of different atomic weight.

Materials and methods for NMR

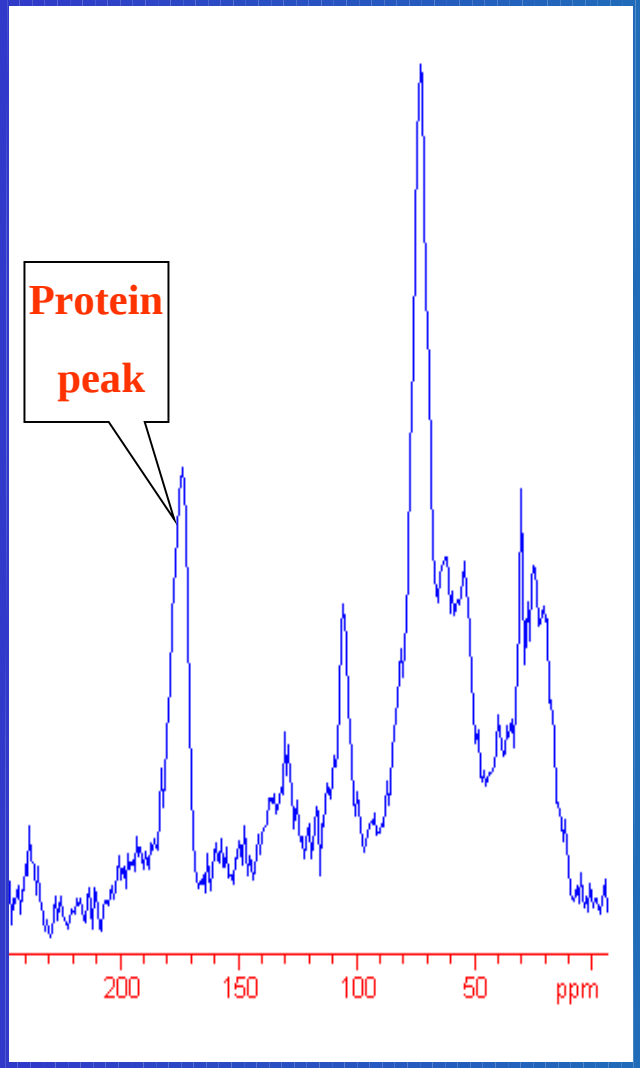
Solid State NMR measurements of oil and protein content in soybean flours were conducted on a General Electric GN300WB FT-NMR instrument, using a 7mm CPMAS probe for solids, rotated at 6.5 KHz.

^{13}C is present in all the significant chemical groups in fatty and amino acids of soybean, but, with its only 1% natural abundance, has low sensitivity. Moreover, solids induce severe line broadening due to chemical shift anisotropy (CSA) and dipole-dipole interaction. Consequently, high-resolution NMR implies three techniques that altogether overcome the low sensitivity and line broadening problems: cross polarization (CP), magic angle spinning (MAS) and heteronuclear decoupling.

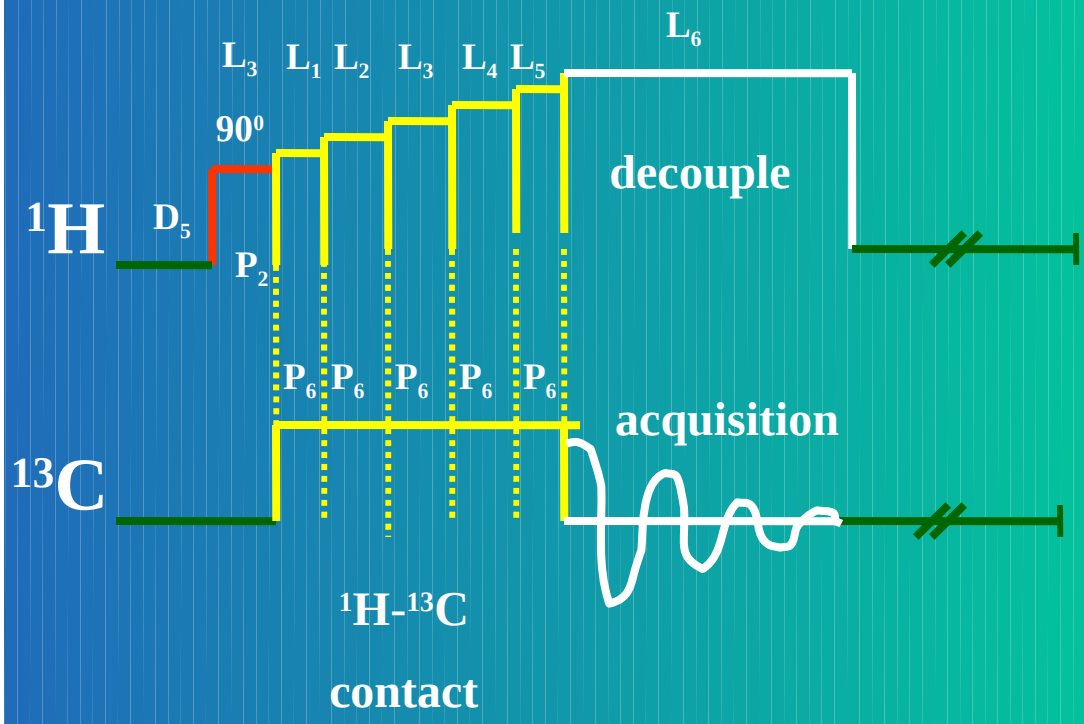
For oil content measurements we used 1PDNA composite pulse, with ^{1}H-^{13}C decoupling during acquisition and Nuclear Overhauser Effect suppression. Oil in soy flours exists in liquid phase, therefore, no cross polarization, and lower rotating speed.

For protein content measurements, VACP composite pulse was employed, with pulse of contact for nuclei cross polarization in five steps, rather than a continuous one, and time contact of 1ms. The heteronuclear decoupling was also applied, and sample rotation took place under the magic angle of 54.7^{o}.

SSNMR-VACP technique measurement of protein content of soybean flours



- Variable Amplitude Cross Polarization pulse sequence



SSNMR-1PDNA technique measurement of oil content of soybean flours

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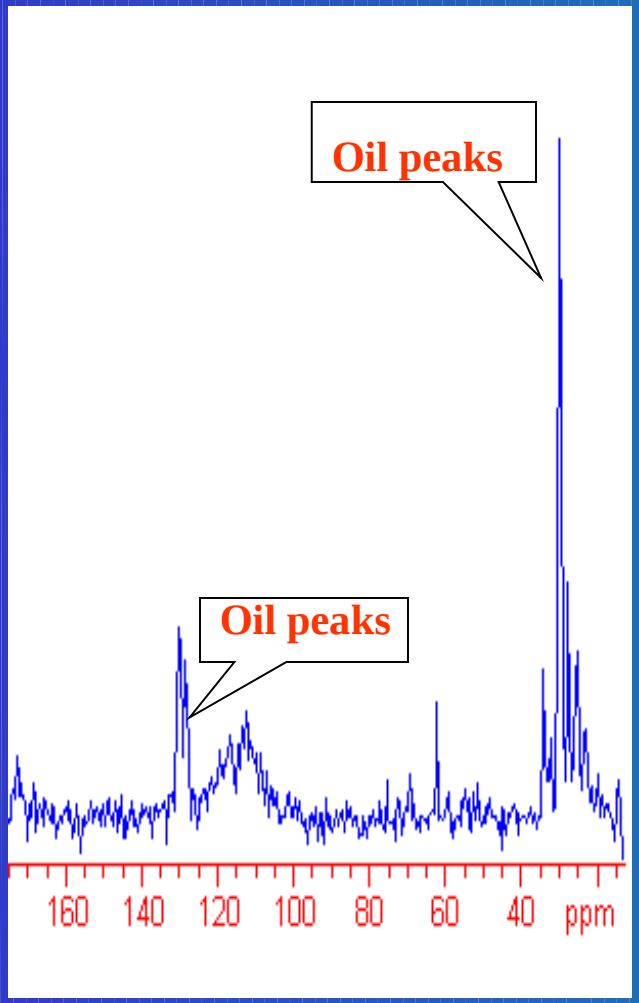
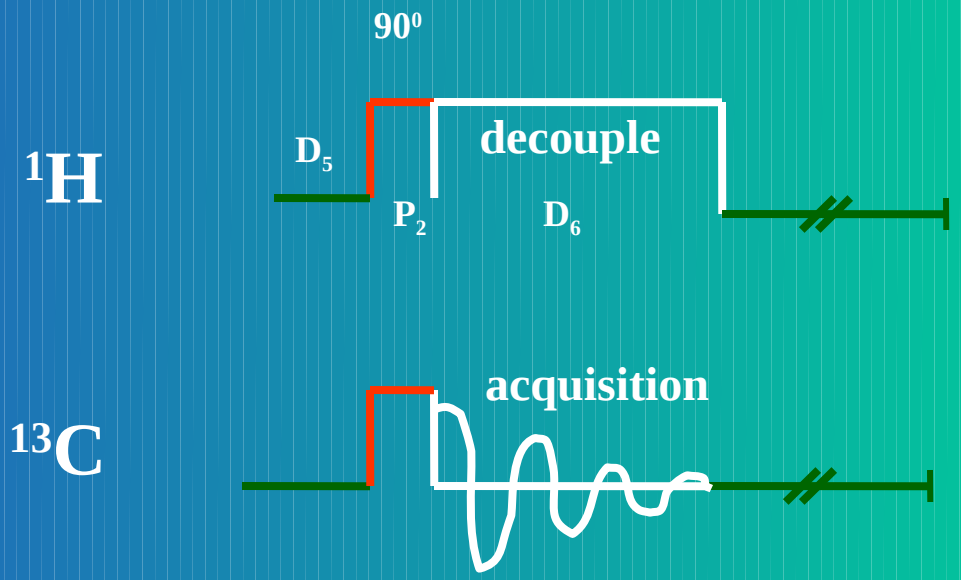


- 1 Pulse Decoupling oN during Acquisition pulse sequence



NMR oil and protein content of soybean flours results, and their correlation with NIR results

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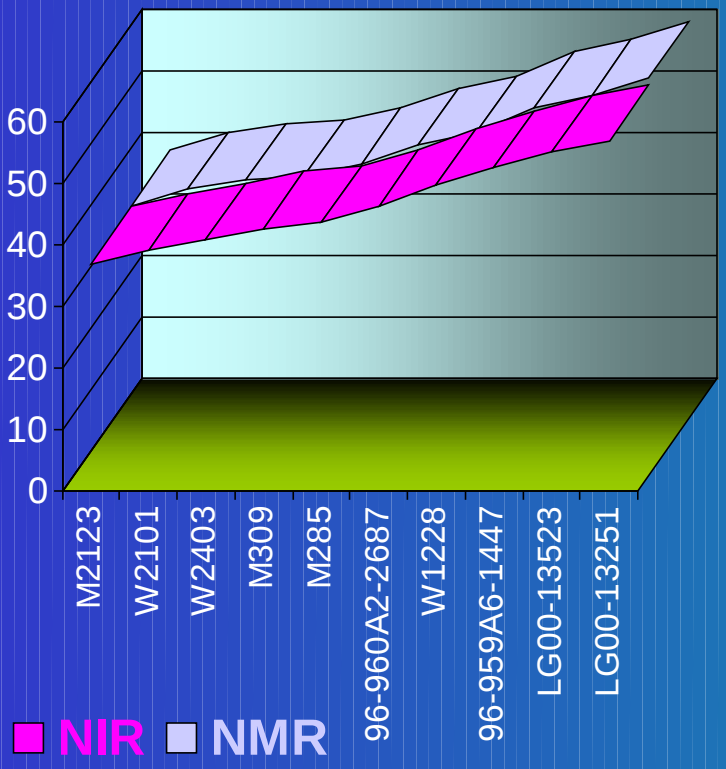
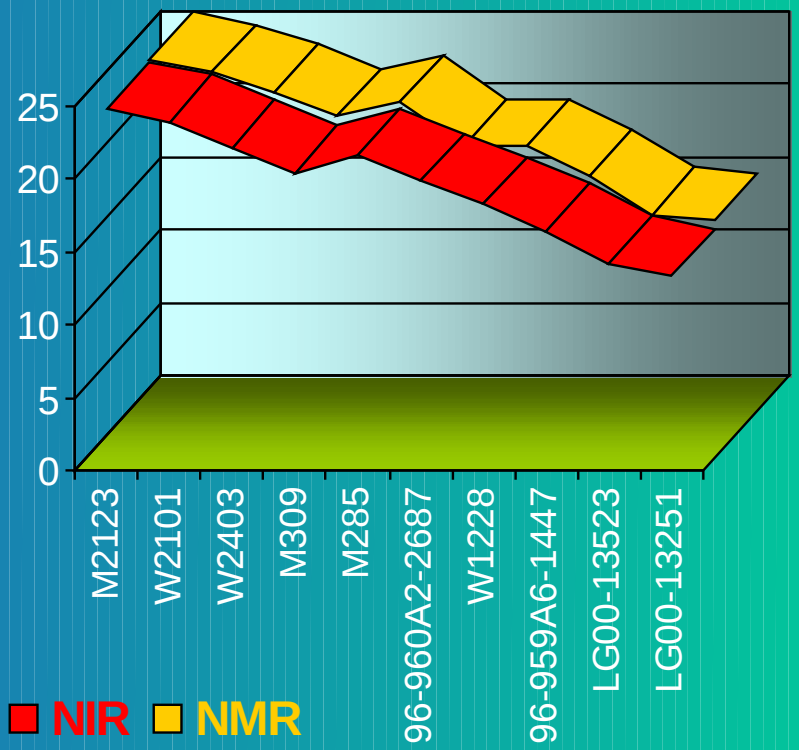


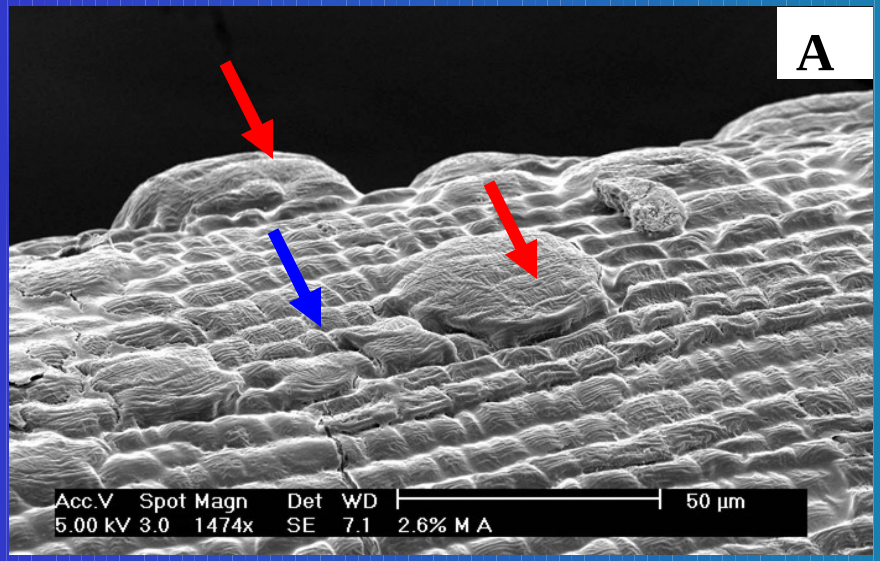
Protein content %



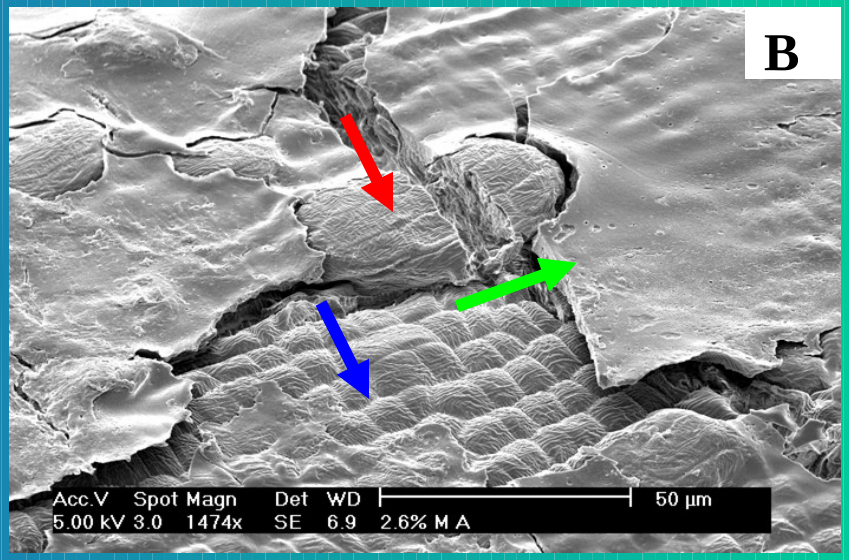
Oil content %

SCANNING ELECTRON MICROGRAPHS

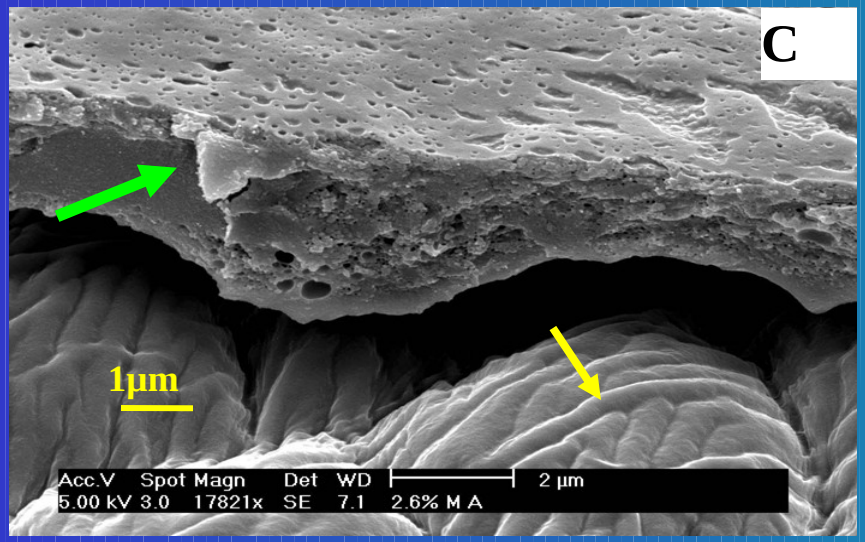
A. Red arrows point oil floccules of size ~40µm in soy seed.
Blue arrows point single oil droplets of size ~12µm in soy seed.



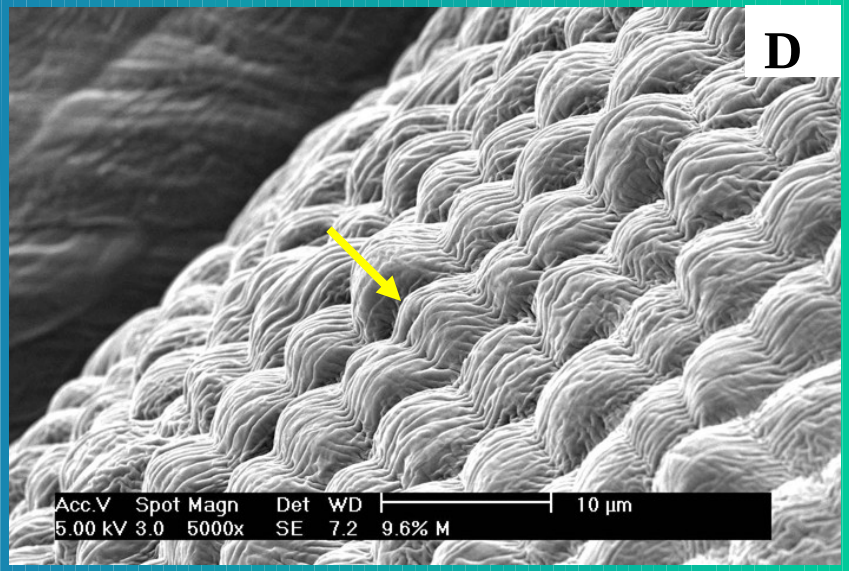
B. Green arrows point soy seed coating.



C. Yellow arrows point protein ribbons of ~1µm width of in soy seed.



D. Protein strands network adherent to the surface oil droplets in soy seed.



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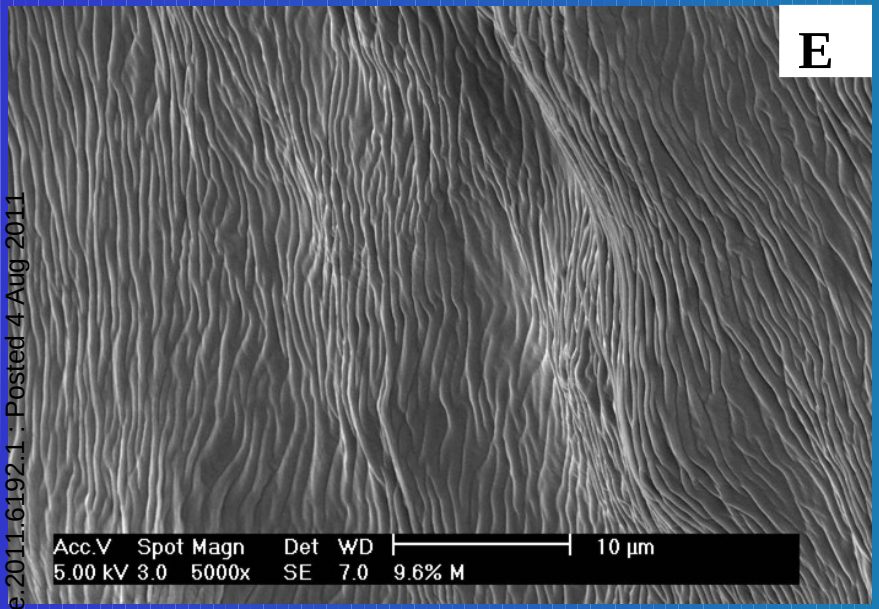
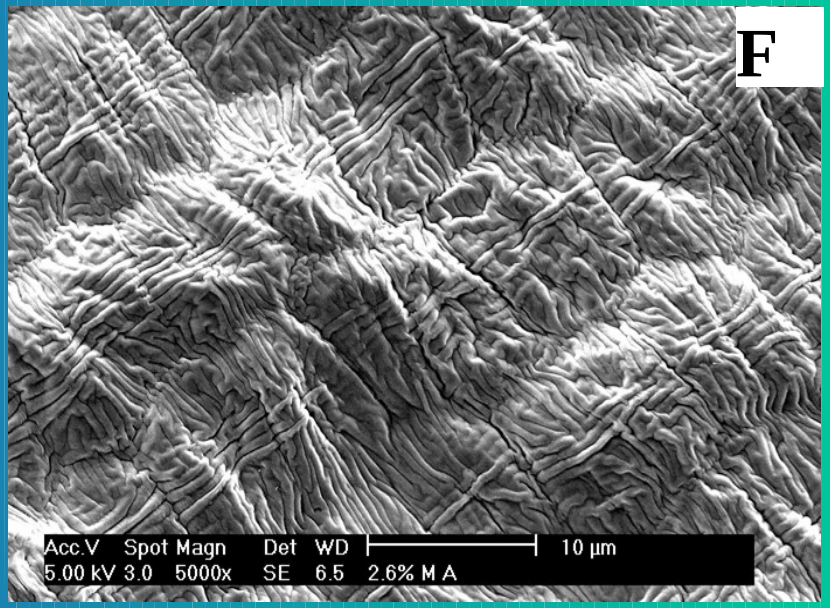
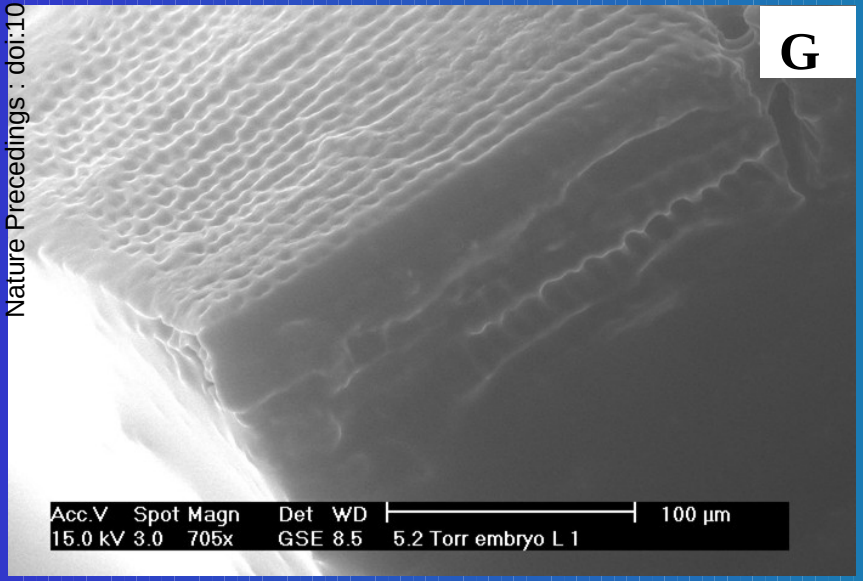
E. ***Aligned protein fibers on uncoated soy seed surface.***



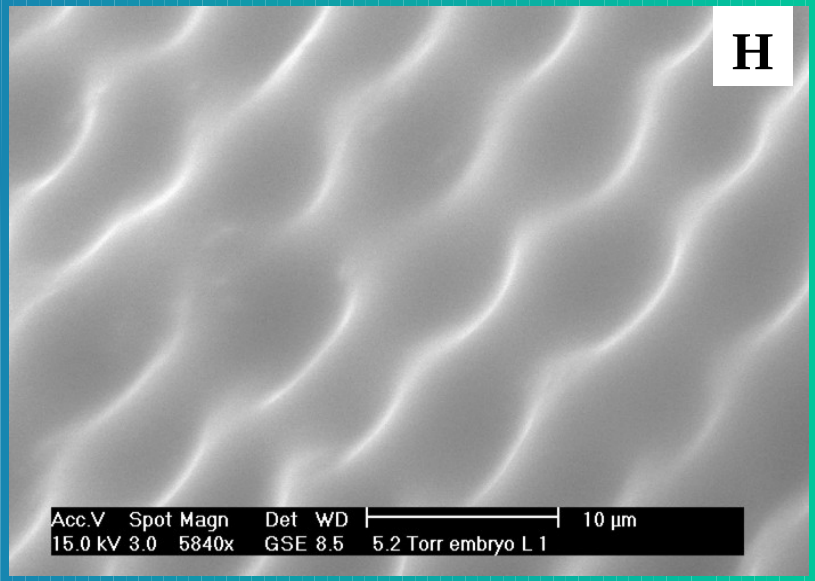
F. ***Oriented protein ribbons on uncoated soy seed surface.***



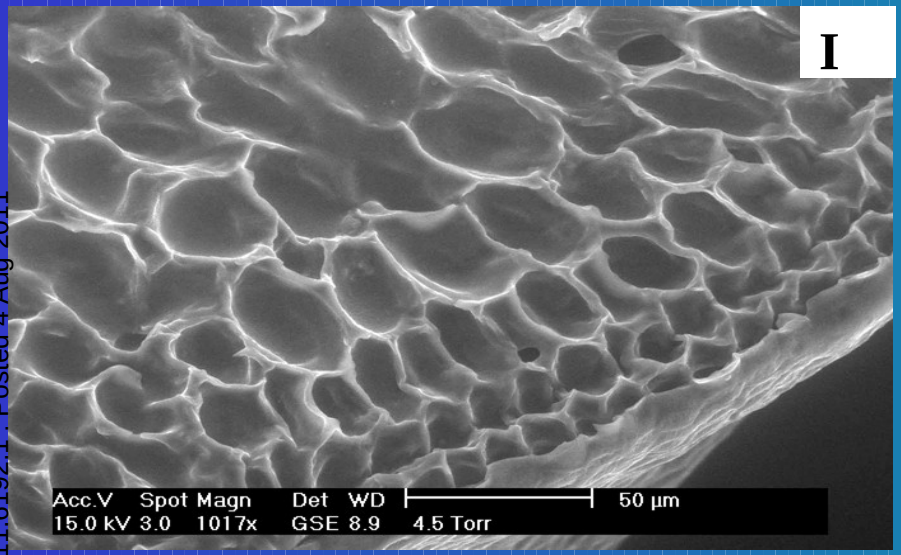
G. ***Free surface and cross section of hydrated mature soy embryo.***



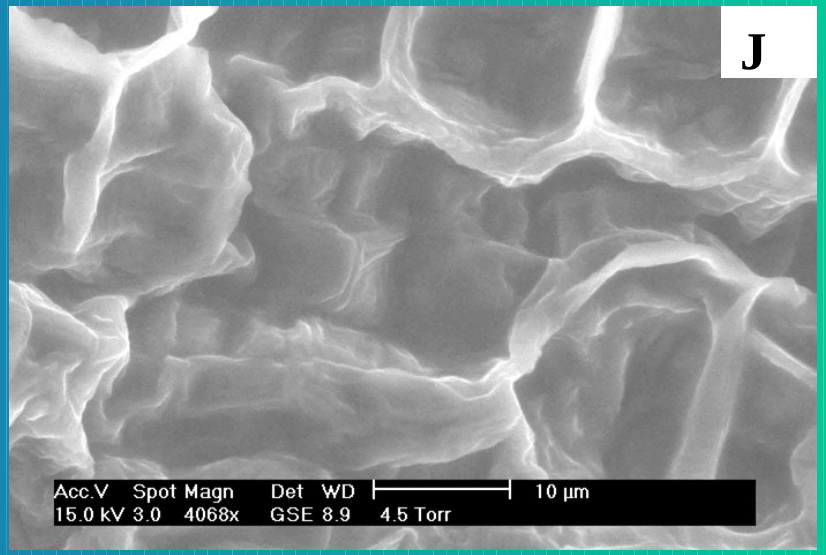
H. ***Free surface of hydrated mature soy embryo.***



I. ***Cross section of hydrated mature soy embryo.***

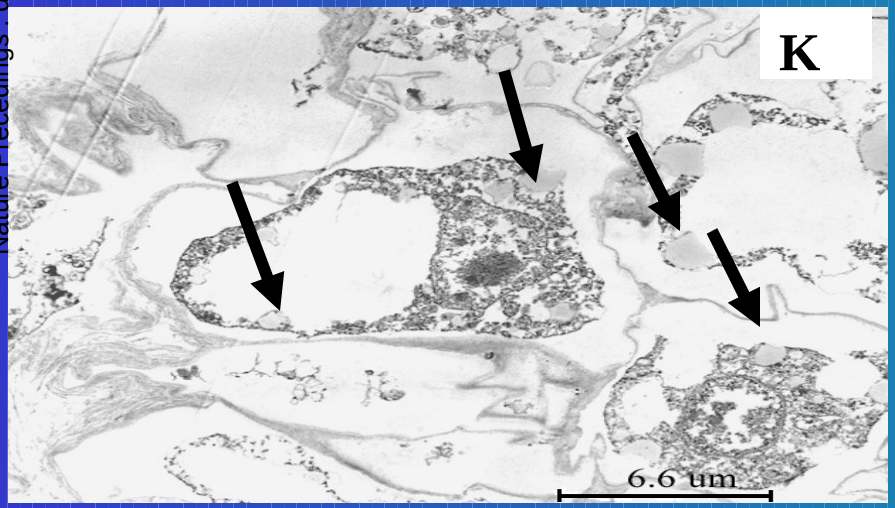


J. ***Vacuoles in hydrated mature soy embryo***

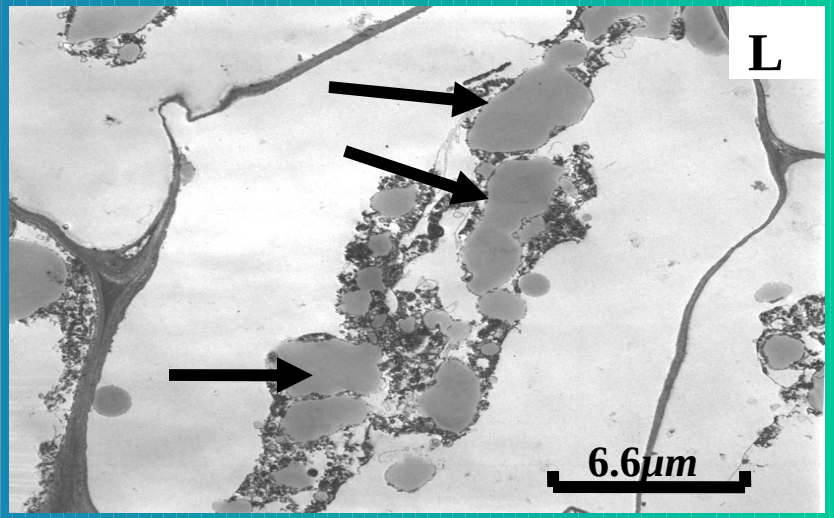


TRANSMISSION ELECTRON MICROGRAPHS

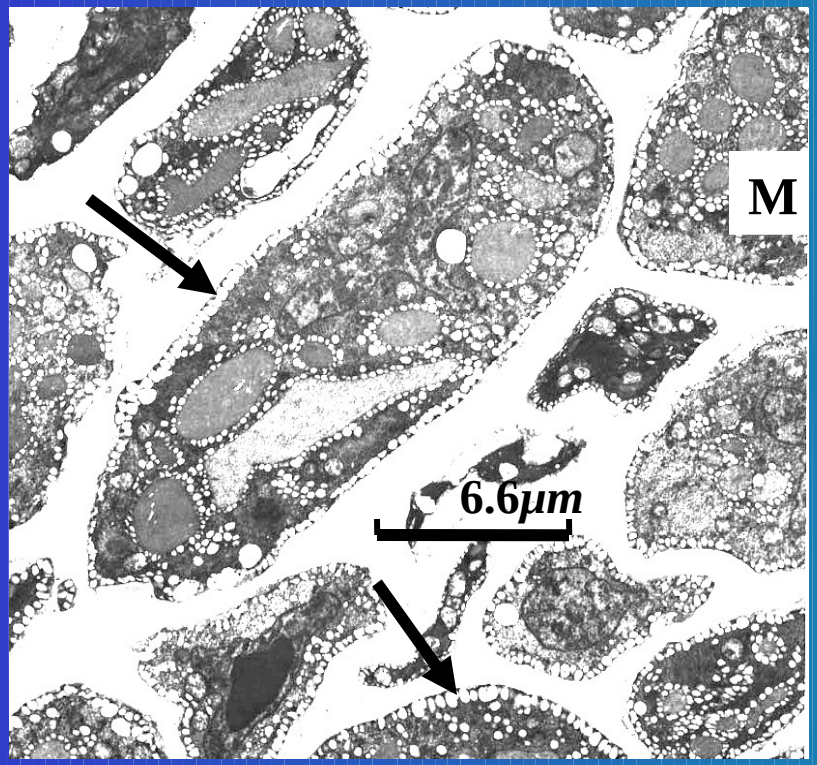
K. ***Arrows point small spherical oil droplets in immature soy embryo culture with 1.33 % oil content.***



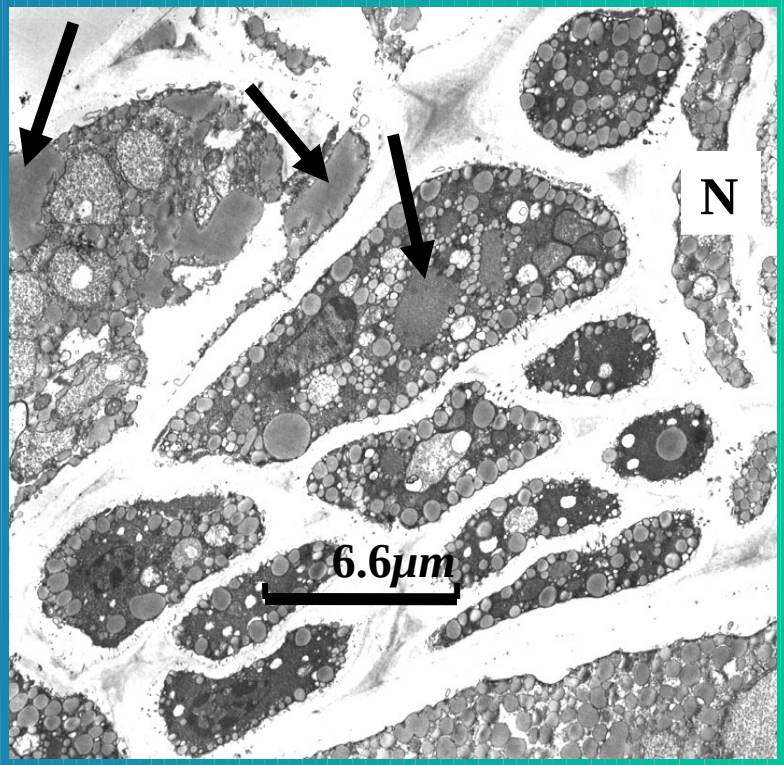
L. ***Arrows point coalesced oil droplets in immature soy embryo culture with 2.3% oil content.***



M. ***Arrows point small spherical oil droplets, near membranes, in mature embryo of soy with 13% oil content.***



N. ***Arrows point coalesced oil droplets in soy flour with 20% oil content .***



NOTE: Samples were prepared by primary fixation in Karnowski's fixative, wash in Cacodylate buffer, secondary fixation in OsO_4, dehydration, infiltration and microwave embedding with pure epoxy.

Conclusions

- The values obtained by integration of the resolved, sharp peaks present at, respectively, ~130ppm and ~172ppm in the processed spectra ^{13}C NMR spectra, were the relevant indicators of oil and protein content.
- The 90% correlation between SSNMR and corresponding DDA-NIRS oil and protein measurements, on the same samples of well defined soybean lines from the National Germplasm Collection at UIUC, suggests that both techniques are suitable for the non-destructive, practical determination of both oil and protein content of soybean flour.
- Field Emission ESEM observations were similar with previous reports on oil droplets size in gels. In soy seeds oil droplet size varies from 10 μm to 40μm. Protein network is adherent to the oil surface, and the protein strands are oriented, fiber-like formations of ~1μm width;
- TEM results show the oil aggregates in high oil content of soy embryos and flours. The oil droplet size varies from 0.2μm to 12μm in soy embryos, and 1μm to 10 μm in soy flours. The oil droplet shape is spherical for low oil content of soy samples, and coalescent, mostly in high oil content soy samples.

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