Bioactive metabolites in fermented whole wheat flour and fruit extracts: NMR analysis and microbiological test

Roberto Consonni, Laura Ruth Cagliani, Antonella Caterina Boccia

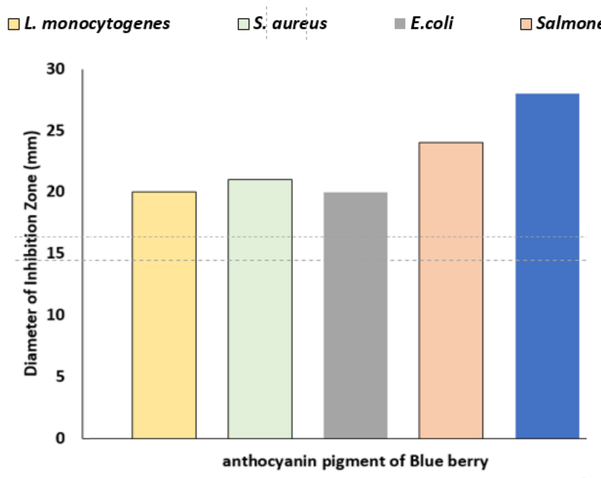
Istituto di Scienze e Tecnologie Chimiche - SCITEC "G. Natta" - Consiglio Nazionale delle Ricerche - CNR, via A. Corti, 12, 20133 Milano, Italia

roberto.consonni@scitec.cnr.it, lauraruth.cagliani@scitec.cnr.it, antonella.boccia@scitec.cnr.it

Background: NMR metabolomics studies allowed to evaluate the content of bioactive compounds in extracts, as well as the details on their chemical structure. In the present studies, the NMR approach have been adopted to evaluate the metabolite profiles of fermented whole wheat flours, which provide an increased content of bioactive molecules and on extracts of lyophilized *myrtus communis* berries, lemon and baby preakly pears of *Opuntia ficus-indica.*
Lyophilized *myrtus communis* berries are a rich source of natural antioxidants, polyphenols, and anthocyanins, bioactive compounds known for their potent antioxidant and antimicrobial properties. To harness these functional benefits, lyophilized berry powder was incorporated into starch-based hydrogels and cryogels, which were subsequently evaluated for use in active food packaging applications and tested for active food packaging applications. Previous studies have demonstrated the capacity of these starch-based materials to enable the sustained release of active compounds over time, making them promising candidates for enhancing food preservation and safety.

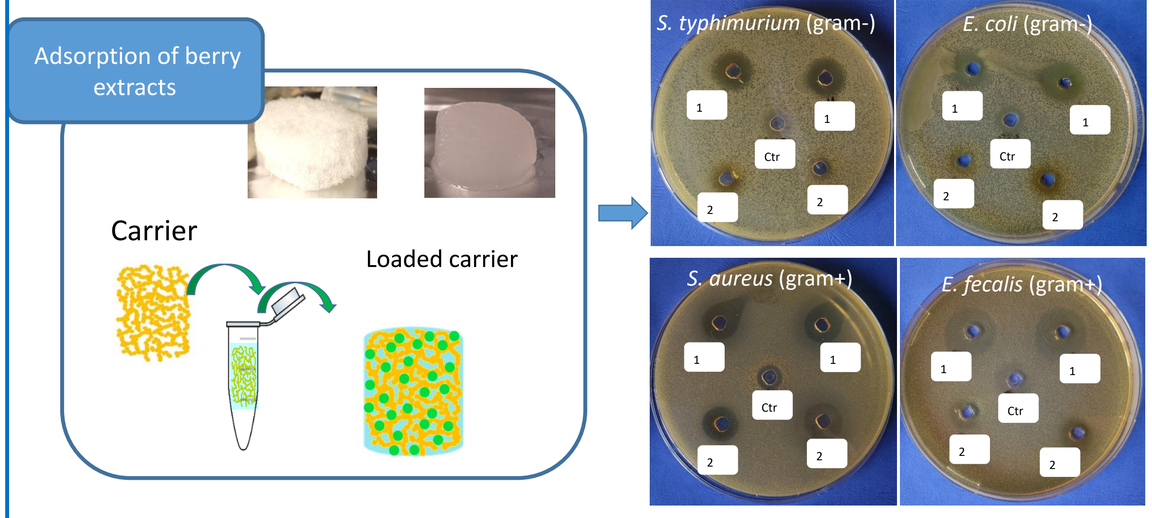
Methodology: All NMR spectra were recorded on Bruker AVANCE NEO 600 spectrometer (Bruker Biospin, GmbH Rheinstetten, Karlsruhe, Germany, with Bruker Bio-spin software TOPSPIN 4.1.4 version), operating at 14.07 T, equipped with a 5 mm reverse Z gradient cryoprobe Prodigy, and thermostated autosampler. Monodimensional modified Nuclear Overhauser Effect Spectroscopy sequence (1D-NOESY) was employed in a quantitative mode, including a solvent presaturation scheme. Bidimensional ^{1}H-^{13}C Heteronuclear Single Quantum Coherence (HSQC) and ^{1}H-^{1}H Total Correlation Spectroscopy (TOCSY) spectra have been recorded with the same spectral width parameters of the monodimensional spectra.
Dried fruit extracts. About 50 mg of dried extract of *myrtus communis*, lemon and *Opuntia ficus indica* were dissolved in 600 µL buffered deuterated methanol/water solution (4:1) at pH=7.5.
Lyophilized myrtus berries. 10.5 mg were suspended in 1mL of milliQ water; furthermore, 40mL of this solution were placed into the Petri as control, as well as used to load the hydrogels and cryogels and tested against Gram-positive and Gram-negative bacterial strains, and the unicellular fungus *Candida albicans.*
Fermented and non-fermented whole wheat flour. A total of 900 µL of methanol-d_4 and 100 µL of D_2O were added to 100 mg of lyophilized flour. After vortexing for 1 min, the mixture was allowed to stand for 10 min in an ice–water bath, followed by centrifugation at 4 °C for 20 min at 3500 rpm. A 600 µL aliquot of the supernatant was collected for the NMR analysis.

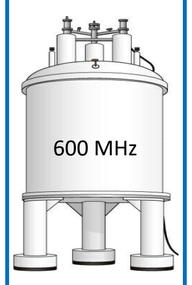
Antimicrobial activity of berry fruit extracts, applied individually or integrated within a delivery system, and NMR analysis



Antimicrobial activity.
The antimicrobial activity of blue berry extracts was assessed against Gram-positive strains (*Listeria monocytogenes* ATCC 19155 and *Staphylococcus aureus* ATCC 43300), Gram-negative strains (*Escherichia coli* ATCC 8739 and *Salmonella* sp. ATCC 14028), and the unicellular fungus *Candida albicans* ATCC 10231 using the agar well diffusion technique.

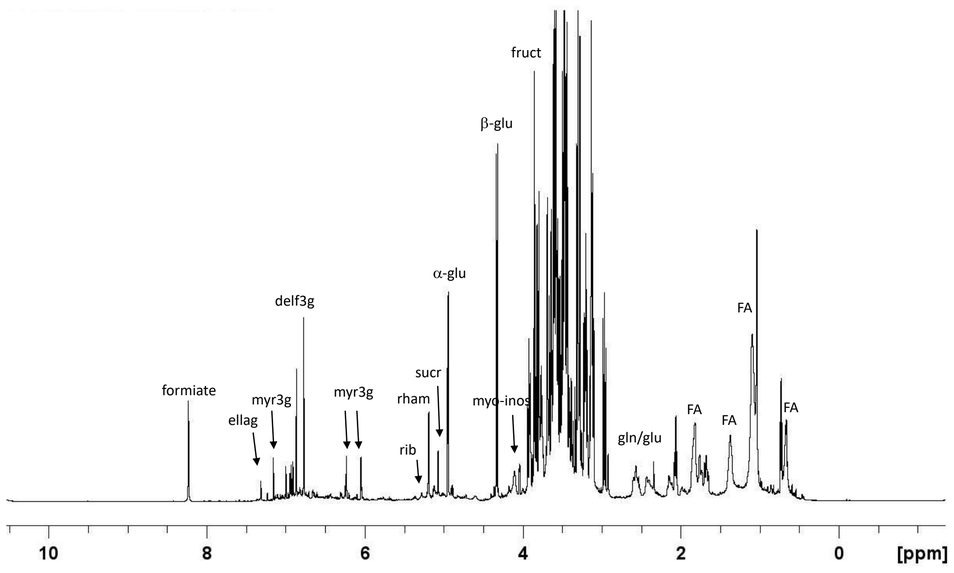
Results. These results highlight the broad-spectrum antimicrobial efficacy of blueberry extracts due to high level of anthocyanins, with pronounced activity against *C. albicans* and *Salmonella sp.*, supporting their potential application in natural antimicrobial formulations.



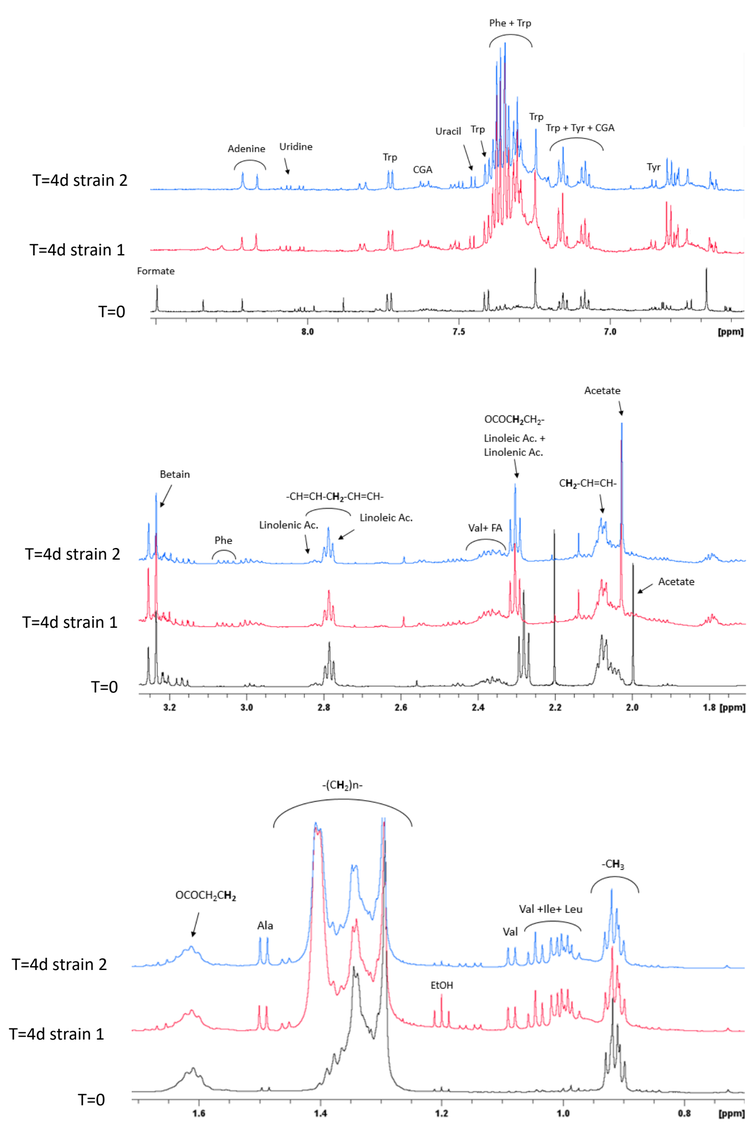


Ctr) berry as control; 1) hydrogel loaded with a berry water solution; 2) cryogel loaded with a berry water solution

^{1}H NMR spectrum of Myrtus communis berries, MeOD/D_2O pH=7.5



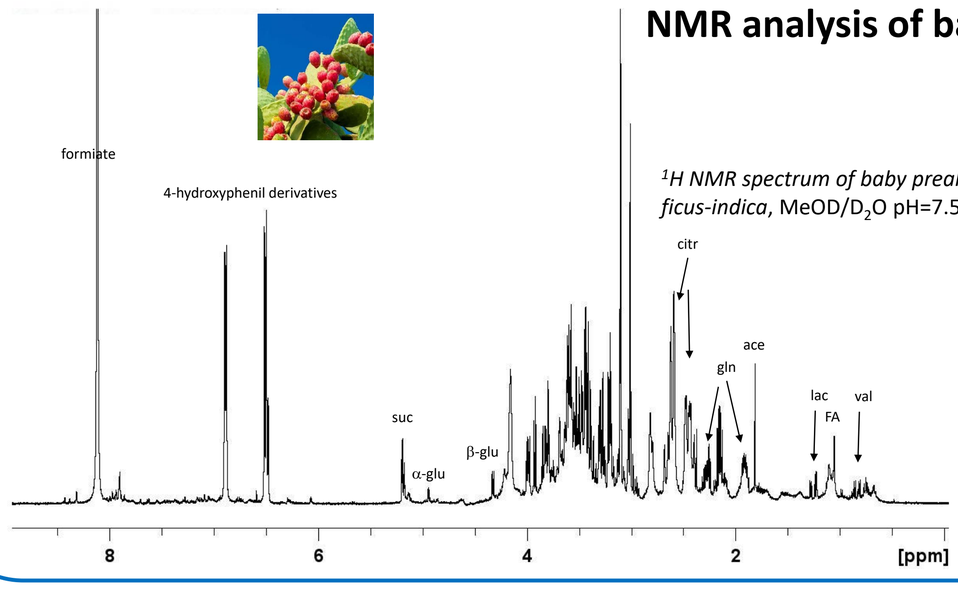
NMR analysis of fermented whole wheat flours



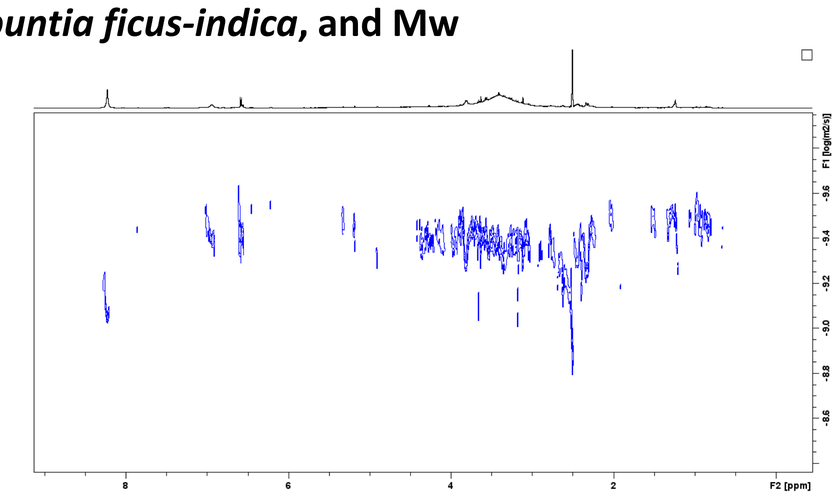
Expanded regions of ^{1}H NMR spectra of non-fermented (T=0) and fermented (T=4d) whole wheat flours by two different bacterial strain mixtures

Cereals contain a wide range of nutrients and bioactive compounds; however, many like polyphenols, are bound to the cell wall, lowering their bioavailability. In a previous study (Tomassi, 2025) has been evaluated the use of fermentation to enhance the bioavailability of functional compounds in whole-wheat flour by using different lactic acid bacteria and yeast strains. The effectiveness of targeted fermentation in improving the bioactivity and antioxidant properties of whole wheat flour was confirmed by DPPH, FRAP, ORAC, and CAA-RBC assays.
In the present study the metabolomic NMR analysis of non-fermented and fermented whole wheat flours by two different bacterial strain mixtures have been performed. The results, as reported in the figures, showed a higher content of branched-chain amino acids (valine, leucine and isoleucine) and alanine, phenylalanine along with other aromatic compounds in flours fermented by both bacterial strain mixtures investigated, compared to the non-fermented flour.

NMR analysis of baby preakly pears of *Opuntia ficus-indica*, and Mw evaluation



^{1}H NMR spectrum of baby preakly pears of Opuntia ficus-indica, MeOD/D_2O pH=7.5



Molecular weight evaluation by 2D DOSY NMR.
Mw of PPW-P-M fraction estimated are of the order of 10^3 $gmol^{-1}$

References
Tomassi, E.; Arouna, N.; Brasca, M.; Silvetti, T.; de Pascale, S.; Troise, A.D.; Scaloni, A.; Pucci, L. Fermentation of Whole-Wheat Using Different Combinations of Lactic Acid Bacteria and Yeast: Impact on in vitro and ex vivo antioxidant activity. *Foods* **2025**, *14*, 421. https://doi.org/10.3390/foods14030421

Acknowledgments
Non-fermented and fermented whole wheat flours were kindly provided by Dr. Laura Pucci (CNR-IBBA Pisa); extracts of *myrtus communis* berries, lemon and baby preakly pears of *Opuntia ficus-indica* were kindly provided by Dr. Laura Siracusa (CNR-ICB Catania).