RAFFAELE GUZZON[1], MATTEO PERINI[1], SILVIA PIANEZZE[1], ROB C. A. ONDERWATER[2], RUDDY WATTIEZ[3], FRANCESCA VIOLARDO[1], TOMAS ROMAN[1] AND ROBERTO LARCHER[1]

[1]*Fondazione Edmund Mach, Via Mach 1, 38098 San Michele all'Adige (TN), Italy raffaele.guzzon@fmach.it*

[2]*MateriaNova ASBL, Avenue Nicolas Copernic 3, 7000 Mons, Belgium*

[3]*Proteomics and Microbiology Department, University of Mons, Avenue du Champ de Mars 6, 7000 Mons, Belgium*

CHITOSAN IN WINE INDUSTRY

IDENTIFICATION OF ORIGIN BY A MULTIDISCIPLINARY APPROACH AND APPLICATION IN OENOLOGY

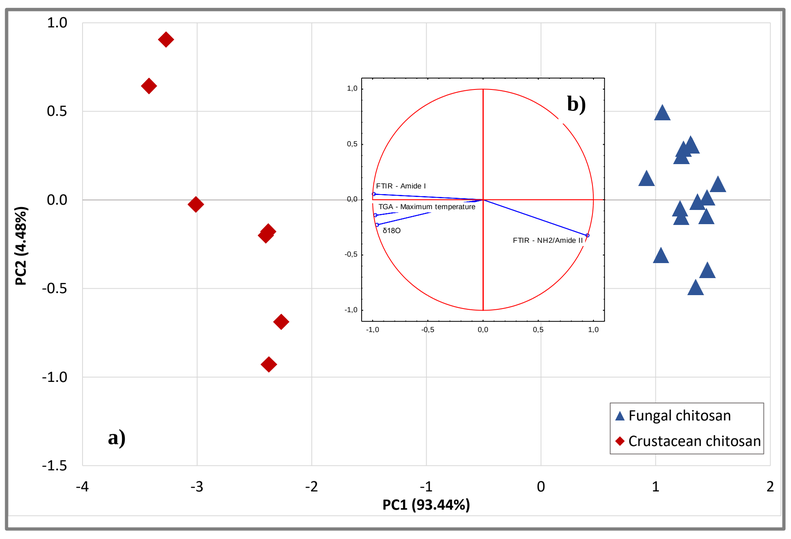
AIM OF STUDY

Chitosan is a promising antimicrobial agent, because it ensures the control of a wide range of spoilage microorganisms. To guarantee the fugal origin of chitosan, the only authorized in food industry to avoid allergenic effects, a multidisciplinary approach based on the measurement of the stable isotope ratios, Fourier transform infrared spectrometry and thermogravimetric analysis was proposed.
The activity of chitosans against food related microorganisms was evaluated by different experiments aimed to discriminate between chemical and physical action of chitosan vs. the microorganisms.

METHOD. The measurement of the stable isotope ratios (SIR) of carbon $\delta^{13}C$, nitrogen $\delta^{15}N$, oxygen $\delta^{18}O$ and hydrogen $\delta^{2}H$ of chitosan was performed to discriminate chitosan samples of different sources. The comparison between the SIR analysis and the results of Fourier-transform-infrared-spectrometry (FTIR) and thermogravimetric analysis (TGA) of chitosan allows rapid discrimination of origin, suggesting for this to be an approach suitable for limited technological laboratories. The antimicrobial activity of chitosan was tested in synthetic grape must under agitation (stirred) or static conditions, to discriminate between physical and chemical mechanism of action of chitosan against the most common wine microorganisms. The activity of the soluble portion of chitosan (< 5% of chitosan, according the OIV standards) was checked by inoculating microorganisms in synthetic grape must after chitosan removal, monitoring the fermentative activity for both alcoholic and malolactic fermentations.

COMBINATION OF SIR, FTIR AND TGA ANALYSES IN THE DEFINITION OF ORIGIN OF CHITOSAN

The combination of SIR, FTIR and TGA resulted in a robust analytical strategy for the correct identification of chitosan samples from crustaceans or fungi. Samples having $\delta^{13}C$ values above −14.2‰ and below −25.1‰ can be considered as authentic fungal chitosan without needing to analyze other parameters. On the other hand, if the $\delta^{13}C$ falls between −25.1‰ and −24.9‰, it is necessary to proceed further with the evaluation of the parameter $\delta^{15}N$, which must be above +2.7‰. Samples having $\delta^{18}O$ values lower than +25.3‰ can be considered as authentic fungal chitosan. The combination of maximum degradation temperatures (obtained using TGA) and peak areas of Amide I and NH2/Amide II (obtained using FTIR) also allows the discrimination between the two origins of the polysaccharide. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) based on TGA, FTIR and SIR data successfully distributed the tested samples into informative clusters.



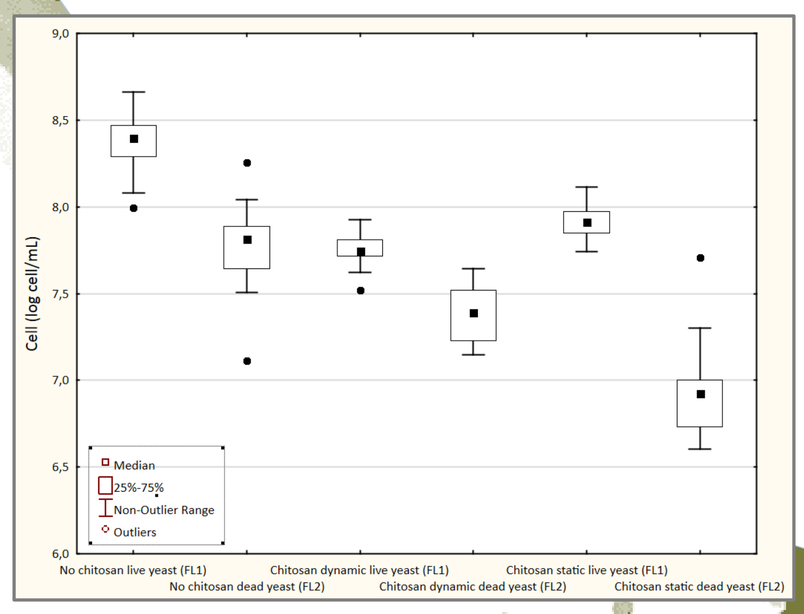
PCA scatter plot (a) and related biplots (b) of SIR ($\delta^{18}O$), TGA (DTGmax) and FTIR (Amide I, NH2/Amide II) data. Active observations are represented by blue triangles (fungal chitosan) or red losange (crustacean chitosan).

MICROBIOLOGICAL TESTS

The microorganisms of oenological interest showed a different sensitivity to chitosan, allowing selective control of spoilage agents such as *B. bruxellensis*. However, yeast and bacteria involved in wine fermentations resulted sensible to the chitosan, and the synthetic grape must treated with this molecule showed a less fermentative aptitude, despite more than the 95% of chitosan (insoluble portion) was removed before microorganism inoculum.

Effect of the addition of chitosan of fungal origin (0,1 g/L) in synthetic grape must containing microbes of oenological interest. Each microorganism had an initial concentration of 7 log cell/mL, the residual cell concentration was measured, for both microorganisms, after 10 days of incubation at 18 °C. The microorganisms showed a different sensitivity at the chitosan, in some cases the population remined stable (e.g. *S. cerevisiae*), in others cell completely disappeared, as in the case of *B. bruxellensis* the main wine spoilage microorganism.

SPECIE	DYNAMIC TEST	STATIC TEST
	Log cell(UFC)/mL (mean ± SD n=3)	
S. cerevisiae ATCC 9763	7.4 ± 6.5	8.2 ± 7.4
S. bayanus DSMZ 70547	2.7 ± 1.5	7.5 ± 6.7
B. bruxellensis ATCC 52304	Not detectable	5.6 ± 4.9
T. delbrueckii DSMZ 70526	7.6 ± 6,8	8.1 ± 7.6
O. oeni ATCC 27311	6.8 ± 5.4	5.0 ± 5.3
P. damnosus LMG 28219	6.6 ± 6.0	5.2 ± 4.8
L. Plantarum NRRL B1927	3.6 ± 3.4	3.5 ± 2.4
A. aceti ATCC 15973	6.7 ± 6.2	6.1 ± 5.1
G. oxidans NRRL B72	6.8 ±6.3	6.5 ± 5.0
S. pombe ATCC 24843	Not detectable	7.7 ± 6.7
C. stellata FEM	Not detectable	6.5 ± 6.7
P. anomala FEM	1.9 ± 2.2	8.1 ± 7.5
S. Ludwigii FEM	Not detectable	7.6 ± 7.7

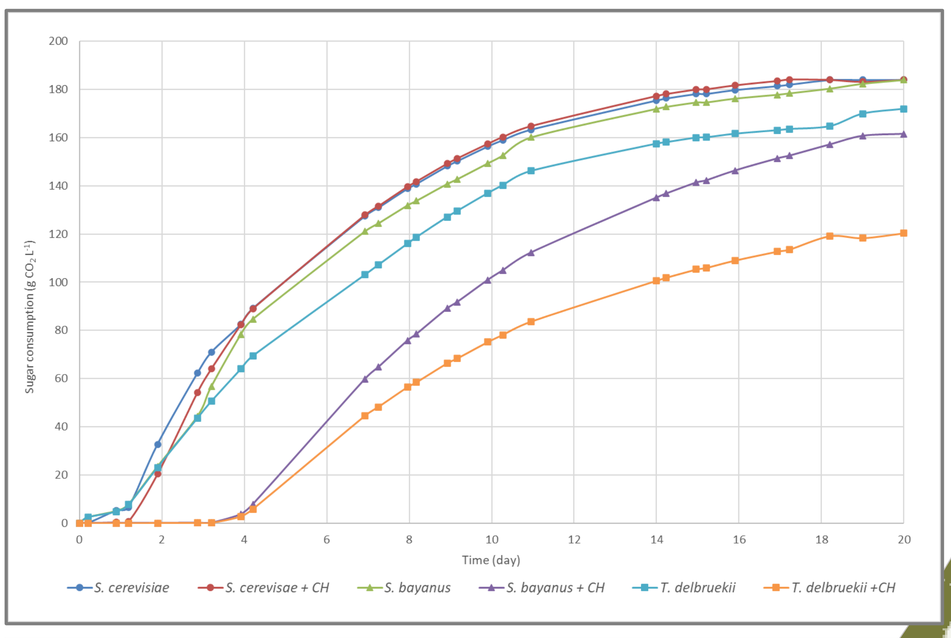


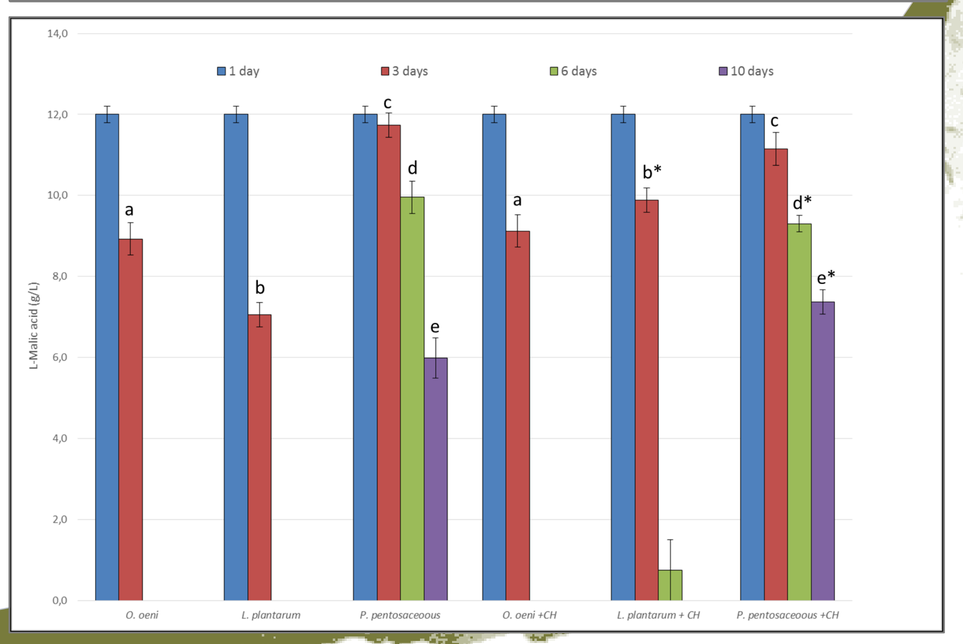
Box plot of the yeast concentration measured by flow cytometry of different active dry yeast (wine yeast for alcoholic fermentation) purchased on the Italian's market (n=16). After rehydration yeasts were inoculated in synthetic grape must supplemented by a chitosan of fungal origin (0,1 g/L). Measure of cell concentration (Live and dead cells, according to Guzzon & Larcher, 2015) was performed 24 h after the chitosan addition. Despite the presumed tolerance of *S. cerevisiae* to chitosans, a general increase in cell mortality is observed in the presence of this molecule.

Alcoholic (upper figure) and malolactic (lower figure) fermentation performed in synthetic grape must using the 3 main specie of yeasts or lactic bacteria involved wine production.

In alcoholic fermentation data are expressed as weight loss due to CO_2 evolution (mean n= 3), instead malolactic fermentation was followed by measure of L-malic acid concertation.

Before fermentation, the medium was treated by chitosan of fungal origin, removed after 24 hours by centrifugation. Despite chitosan removal the soluble portion that remained in the media caused a delay in alcoholic and malolactic fermentation (CH tests) confirming the generalized sensitivity of oenological microorganisms to chitosan.





CONCLUSION

A robust analytical strategy for the correct identification of chitosan samples from crustaceans or fungi was presented, based on the observation that diverse biosynthetic pathways during the formation of the chitin influenced the isotopic composition of chitosan. Results of toxicity tests suggest that chitosan is a promising tool in fermented beverage production, but an in-depth study of the biochemical interaction between chitosan and food microorganisms is necessary.

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