Researchers create formula for first synthetic sugarcane molasses with fully reproducible composition

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Ethanol production using sugarcane molasses to grow Saccharomyces cerevisiae yeast for the fermentation stage. The researchers obtained the first fully known and reproducible synthetic molasses, which can also be used in other industrial processes. Credit: Thiago Basso

Molasses, a broad term used to describe concentrated sugarcane or sugarbeet juice solutions after removal of sucrose crystals, are an industrial byproduct of the raw sugar production process. Natural molasses have variable compositions that are not entirely known. This knowledge gap is a hindrance to both scientific research and industry, where molasses are used in several processes, including production of fuel ethanol from molasses by fermentation with brewer's yeast (Saccharomyces cerevisiae).

A group of Brazilian and European scientists have now formulated synthetic molasses with a composition that can be reproduced because it is fully known. Their research was published in the journal *Scientific Reports*.

"The main aim was to find a formula for yeast that behaved as similarly as possible to the yeast used in industrial processing of molasses," said Thiago Basso, last author of the article and a professor at the University of São Paulo's Engineering School (POLI-USP) in Brazil. Basso is thesis advisor to first author Kevy Eliodório and second author Gabriel Cunha.

The researchers set out to reproduce the ethanol production process in the laboratory, including the characteristics of sugarcane molasses as yeast culture medium, so that all components and titrations could be determined. Molasses are a complex culture medium whose composition varies significantly under normal circumstances.

"We produced standardized synthetic molasses that can be tested by researchers anywhere in the world as a culture medium for the microorganisms they're studying. In addition, results obtained in different labs can more easily be compared. That's important to science," Eliodório said, adding as an example that the formulation was tested at

the Helmholtz Center for Environmental Research in Germany while he was there on a doctoral research internship.

Besides the logistical advantage afforded by enabling research centers worldwide to use it to develop bioprocesses, the laboratory culture medium can also be used to study the influence of specific components by adjusting the level of each one separately. "This is an advantage of the results obtained in our study," Basso said. "The possibility of adjusting components facilitates the study of the effects of growth inhibitors or nutritional components, for example, and the quantitative analysis of metabolic flows, among other factors."

The methodology applied was based on a combination of data reported in the literature and previous research by Basso, in which he formulated synthetic molasses without fully defining its composition, owing to the presence of peptone, a substance whose composition is neither totally controllable nor entirely known. Systematic adjustments were made using data from real samples, the literature and prior composition.

"Each adjustment was evaluated by testing yeast growth until we arrived at a composition that led to yeast behavior similar to that observed in actual molasses," Eliodório explained.

Separately analyzed elements

The components were divided into nutritional groups (salts, organic acids, vitamins, trace elements and sugars, among others) and analyzed separately. This stage entailed both developing composition and characterizing the ingredients that most influenced the yeast's behavior. Natural molasses contain large amounts of fermentable sugars and other nutrients.

Several compounds, including growth factors, macro- and

micronutrients, vary depending on the sugarcane variety, soil, climate, and processing conditions. In addition, some compounds generated during sugarcane processing can inhibit yeast performance, affecting ethanol production.

The validation stage produced highly satisfactory results. "We also demonstrated that a classical culture medium used for yeast, known as YPS [yeast extract, peptone and sucrose], doesn't permit physiological comparison because the values are very different with these characteristics, whereas our medium appropriately mimics actual molasses, offering significant advantages for both industrial use and scientific research," Basso said.

Thanks to the results of the study, the preparation of synthetic molasses has been made flexible, as the proportions of the various nutritional groups can be adjusted according to requirements. For example, the group set out to see how nitrogen influences fermentation. "It's often said that low levels of nitrogen increase ethanol production. Our experiments with adjusted nitrogen levels showed this is indeed the case," he said.

"We believe the synthetic molasses developed in this study will pave the way for researchers in various parts of the world to develop novel bioprocesses based on the use of sugarcane molasses, one of the most important raw materials in present-day industrial biotechnology."

The other Brazilian co-authors were Reinaldo Giudici, a professor at POLI-USP, and Andreas Gombert, affiliated with the School of Food Engineering at the State University of Campinas (FEA-UNICAMP). The European part of the team consisted of Morten Sommer, a professor at the Technical University of Denmark, and Felipe Lino, co-founder of German startup Nosh.bio GmbH.

More information: Kevy Pontes Eliodório et al, Physiology of Saccharomyces cerevisiae during growth on industrial sugar cane molasses can be reproduced in a tailor-made defined synthetic medium, *Scientific Reports* (2023). DOI: 10.1038/s41598-023-37618-8

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