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A newly isolated *Kluyveromyces marxianus* strain capable of producing oleic acid-rich oil for human consumption from whey

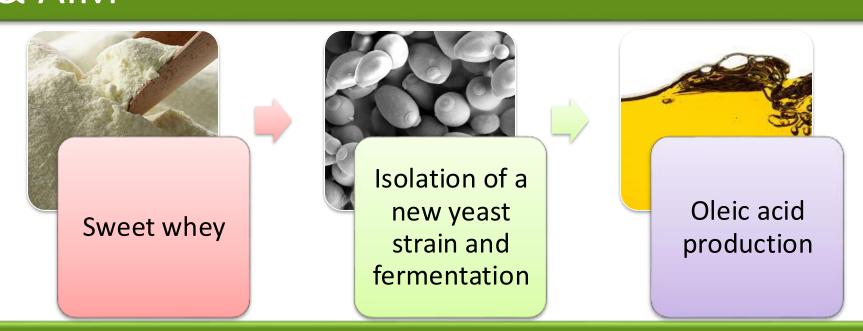
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INTRODUCTION & AIM

Most of the global production of oleic acid comes from olive oil. According to the International Olive Council (IOC), global consumption is expected to reach approximately 3 million tons by the end of 2025. This growing demand underscores the need to explore new and sustainable sources of oleic acid.

Oleaginous yeasts are a promising alternative, as they can accumulate more than 20% oil on a dry weight basis and exhibit fatty acid profiles similar to conventional oilseeds and fruits. These microorganisms offer several advantages, including using carbon sources from industrial by-products and wastes, such as whey, for their growth and for the production of value-added compounds. Furthermore, their cultivation is independent of geographic conditions and helps to prevent deforestation.

This study aimed to isolate a yeast strain with the highest oleic acid yield and productivity and to establish a biotechnological alternative to meet the increasing global demand for this fatty acid.



METHOD Part II: Kinetic evaluation of oleic acid production Colonies' growth in Separation of the Part I: Isolation of the best lipid-production yeast agar whey medium organic phase Drv weiath quantification 1 g of different Isolation in samples agar whey Lactose Enrichment culture Confocal medium Collection of concentration in whey, 120 rpm, Batch kinetic study using Ultrasonication for 90 microscopy soil samples quantification whey medium for 48 h, at 24 h, 20°C min, with chloroformobservation of 120 rpm and 25°C methanol 2:1 lipids with ROO staining -ester functional group Transesterification Molecular identification of 24-hour kinetic study for the Various lipid-producing the best lipid-producing selection of the yeast strain Yeast genus identification Identification of the lipid profile and oleic acid morphotypes with the highest lipid yield by the VITEK® system yeast strain quantification by GC-MS

RESULTS & DISCUSSION

Soil samples were obtained from different environmental sources and used to isolate oleaginous yeasts. After confocal microscopy observation with ROO staining, only five yeast isolates were able to accumulate lipids intracellularly. They were identified as non-pathogenic by the VITEK® system. The strain with the highest lipid yield (CM 9b) reached 50% w/w lipid content after 24 hours of cultivation in whey medium (Fig. 1). Molecular identification based on PCR amplification of the 16S–ITS1–5.8S–ITS2–26S rDNA region confirmed its classification as *Kluyveromyces marxianus*.

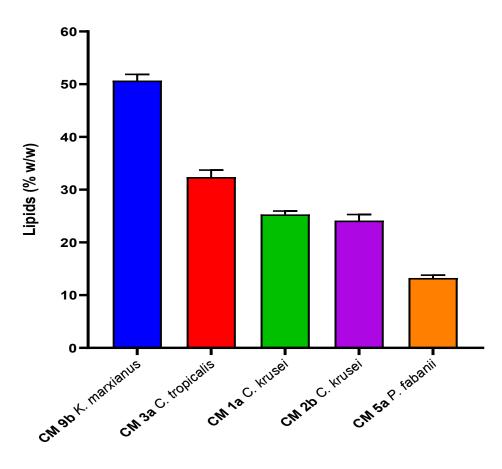


Fig. 1. Maximum lipid yield of isolated yeast strains after 24 h of cultivation using whey as a carbon source.

The kinetic growth profile of *K. marxianus* (Fig. 2a) showed maximum biomass production at 30 h (5.63 \pm 0.01 g L⁻¹). No evident lag growth phase was observed. Maximum lipid production (Fig. 2b) occurred at 24 h (2.70 \pm 0.05 g L⁻¹), then decreased after 36 h.

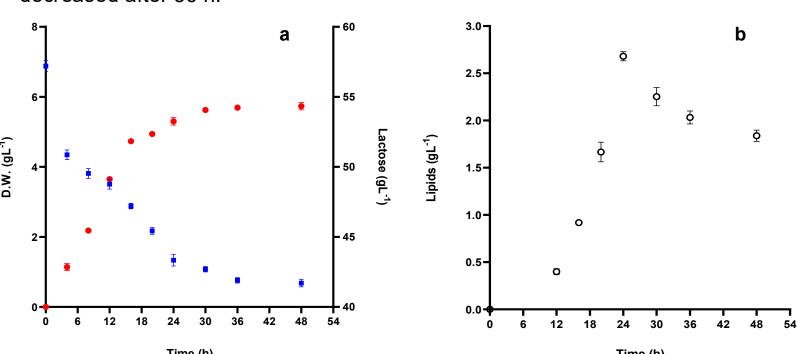


Fig. 2. Kinetic growth and substrate consumption (a), and lipid production (b) by *K. marxianus* cultivated in whey medium.

The fatty acid profile of *K. marxianus* was determined by gas chromatography–mass spectrometry (GC–MS). Only five fatty acids were identified, comprising 68.10% monounsaturated fatty acids and 31.90% saturated fatty acids, while polyunsaturated fatty acids were not detected. *K. marxianus* exhibited a high lipid yield, with more than 50% of the total fatty acids corresponding to oleic acid. In addition, the maximum oleic acid productivity (R_{fa}) was achieved at 24 h of cultivation (Table 1).

Table 1. Fatty acid profile and productivity (R_{fa}) of *K. marxianus*.

Fatty Acid	Formula	Lipidi yield (%w/w)	R _{fa} (mg L ⁻¹ h ⁻¹)
Miristic	C14:0	1.0	0.46
Palmitoleic	C16:1	16.0	6.36
Palmitic	C16:0	28.0	10.98
Oleic	C18:1	54.0	21.2
Stearic	C18:0	1.0	0.60

The fatty acid profile of *K. marxianus* cultivated in whey medium for 24 h was compared to the profiles of various vegetable oils extracted from avocado (a), olive (b), peanut (c), soybean (d), palm (e), and sunflower (f) (Fig. 3). The *K. marxianus* fatty acid profile was similar to that of avocado oil when the yeast was grown on whey as the carbon and energy source. Moreover, *K. marxianus* is a GRAS (Generally Recognized As Safe) yeast, which makes it a promising alternative for oleic acid production and, according to previous studies, potentially applicable for human

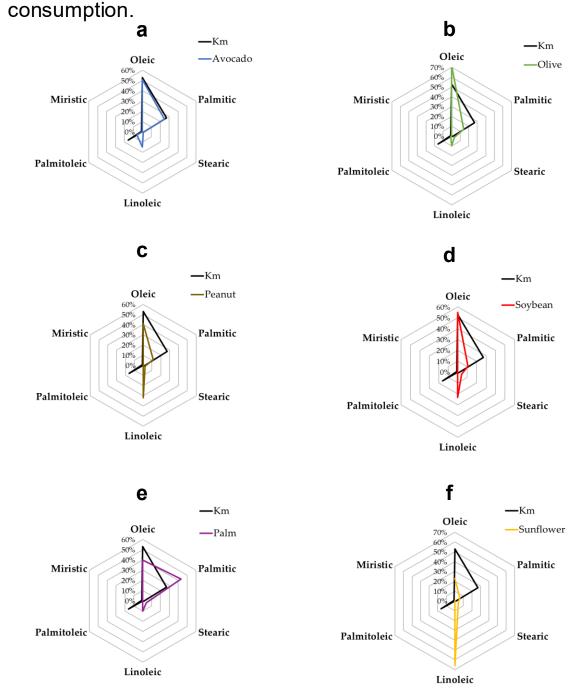


Fig. 3. Comparison of fatty acid profiles between *K. marxianus* oil and various vegetable oils extracted from avocado (a), olive (b), peanut (c), soybean (d), palm (e), and sunflower (f).

CONCLUSIONS

- The isolation of non-conventional yeasts capable of accumulating fatty acids provides a promising alternative to meet the increasing global demand for lipids.
- In this study, *Kluyveromyces marxianus* exhibited a high oleic acid yield and a fatty acid profile comparable to avocado oil, making it a viable alternative to conventional plant-based lipid sources.
- Furthermore, the GRAS status and the ability to utilize industrial wastes such as whey make *K. marxianus* a sustainable and biotechnologically relevant organism for oleic acid production intended for human consumption.

FUTURE WORK / REFERENCES

As future work, different fermentation reaction systems and bioreactors will be evaluated for the overproduction of olive acid by *K. marxianus* using whey as a carbon source. References:

- Béligon, V., Christophe, G., Fontanille, P., & Larroche, C. (2016). Microbial lipids as potential source to food supplements. *Current Opinion in Food Science*, 7, 35-42.
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 Cristiani-Urbina, E., Netzahuatl-Muñoz, A. R., Manriquez-Rojas, F. J., Juárez-Ramírez, C., Ruiz-Ordaz, N., & Galíndez-Mayer, J. (2000). Batch and fed-batch cultures for the treatment of whey with mixed yeast cultures. *Process Biochemistry*, 35(7), 649-657. https://doi.org/10.1016/S0032-9592(99)00116-8