

OBTENTION OF PREBIOTIC OLIGOSACCHARIDES DERIVED FROM LACTULOSE USING *Kluyveromyces marxianus* CRUDE CELL EXTRACTS

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Instituto de Investigación en Ciencias de la Alimentación

Nieves Corzo^a, Beatriz Padilla^b, Carmela Belloch^b, Alejandra Cardelle-Cobas^c, Paloma Manzaneres^b, Ana I. Ruiz Matute^a

^aInstituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM) C/Nicolás Cabrera 9, Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain.

^bDepartamento de Biotecnología de Alimentos, Instituto de Agroquímica y Tecnología de Alimentos, Consejo Superior de Investigaciones Científicas (CSIC), Avenida Agustín Escardino 7, 46980 Paterna, Valencia, Spain.

^cCentro de Biotecnología e Química Fina (CBCF)/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Dr. António 18 Bernardino de Almeida, P-4200-072 Porto, Portugal.

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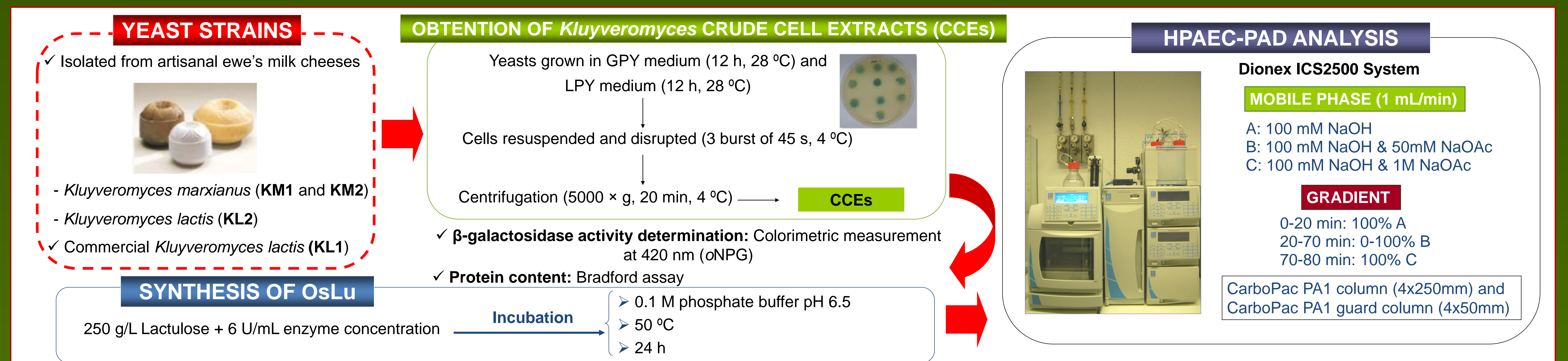
INTRODUCTION

In recent years, the synthesis of galactooligosaccharides derived from lactose (GOS) and new oligosaccharides derived from lactulose (OsLu) is receiving increasing interest since these compounds have been described to have prebiotic effects¹. The use of β -galactosidases from the genus *Kluyveromyces* for GOS synthesis has been described. Although commercial enzymatic preparations from *K. lactis* have been used for lactose and lactulose transgalactosylation^{2,3}, *K. marxianus* β -galactosidases have only been tested for the production of GOS⁴.

OBJECTIVE

The aim of this work was to evaluate β -galactosidase activity of two strains of *K. marxianus* (KM1 and KM2) isolated from artisanal cheeses to produce OsLu by hydrolysis and transgalactosylation of lactulose. Two β -galactosidases from *K. lactis* (one commercial, KL1 and other isolated from an artisanal cheese, KL2) were also introduced in this study for comparison purposes.

MATERIALS AND METHODS



RESULTS AND DISCUSSION

K. marxianus

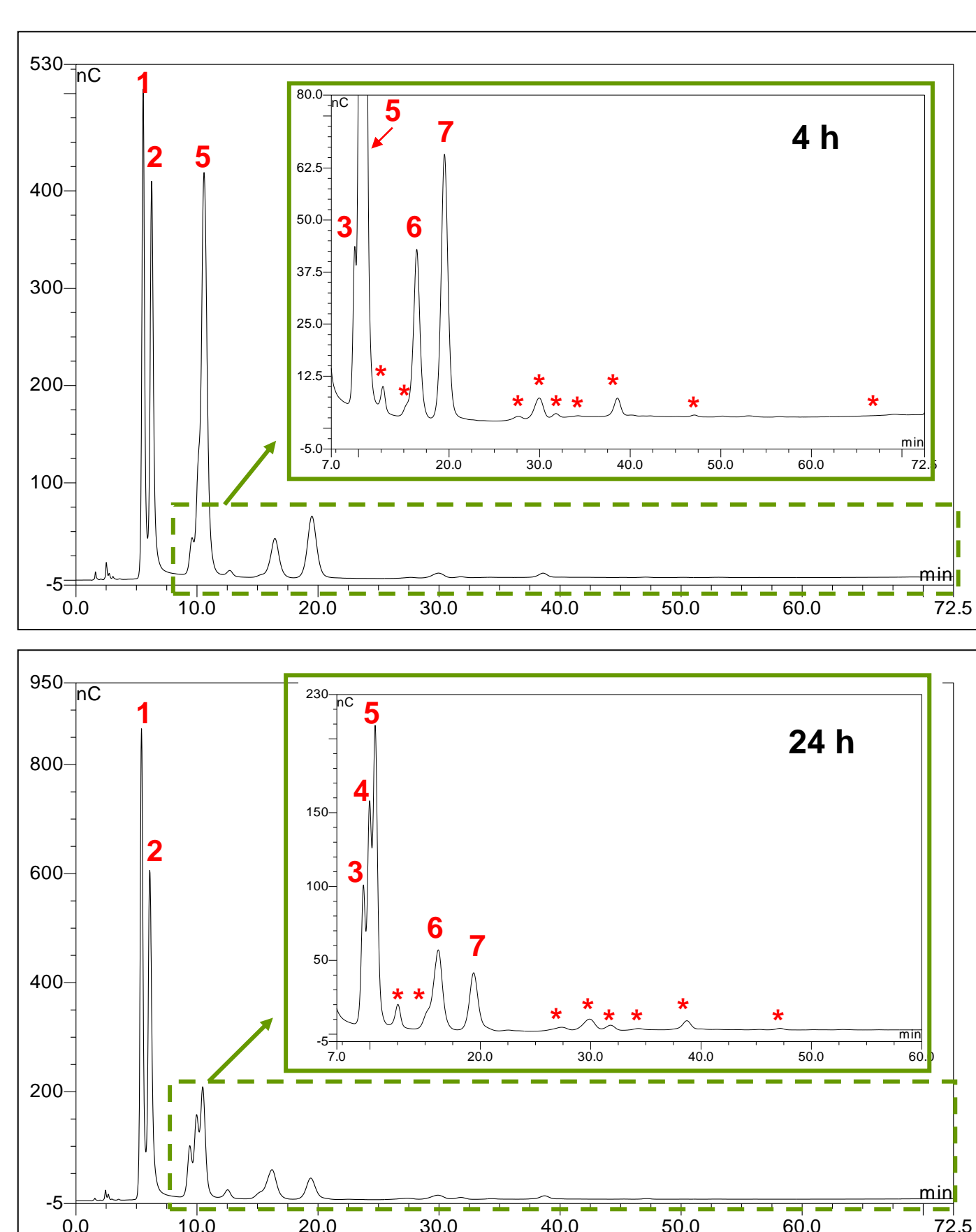


Figure 1. HPAEC-PAD profiles of carbohydrate mixtures obtained by enzymatic hydrolysis of lactulose by KM2 β -galactosidase after 4 and 24 h of incubation. Compounds: 1) galactose; 2) fructose; 3) 6-galactobiose; 4) allolactulose; 5) lactulose; 6) 6'-galactosyl-lactulose; 7) 1-galactosyl-lactulose; *) other oligosaccharides.

- All *Kluyveromyces* CCEs tested (*marxianus* and *lactis*) synthesized galactosyl derivatives of lactulose (OsLu), showing similar chromatographic profiles of OsLu production (Figure 1 and Figure 2).
- Peaks 1, 2, and 5 were assigned to galactose, fructose, and lactulose, respectively.
- All CCEs tested formed 6-galactobiose (peak 3), 6'-galactosyl-lactulose (peak 6) and 1-galactosyl-lactulose (peak 7).
- After 24 h of incubation with *K. marxianus*, allolactulose (peak 4) could be detected.
- Two unidentified di- or trisaccharides (retention times =12.5 min and 14.8 min) and other high retention time oligosaccharides (peaks marked with asterisk) were also detected.
- Under the studied conditions trisaccharide formation predominated over other oligosaccharides.

K. lactis

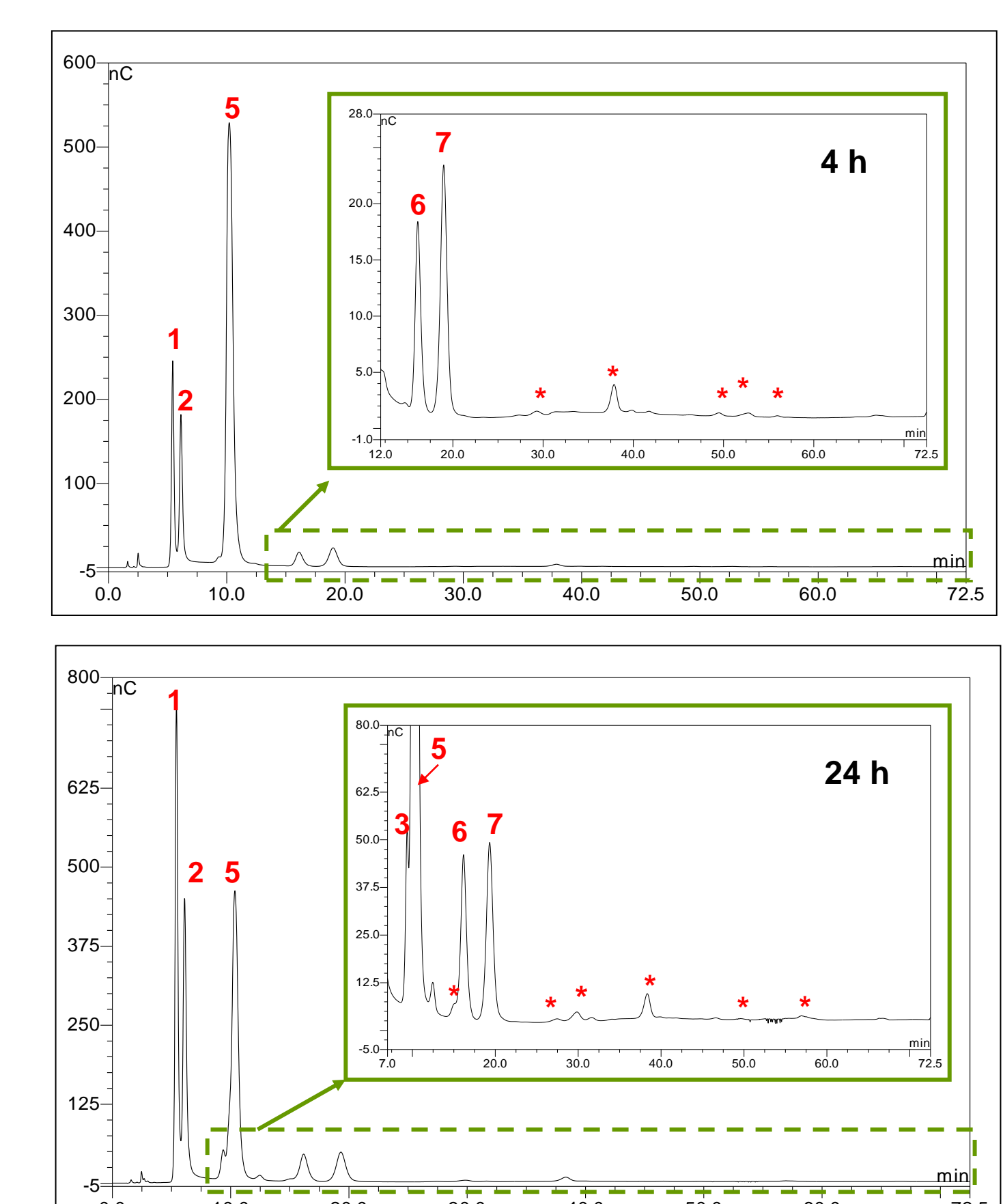


Figure 2. HPAEC-PAD profiles of carbohydrate mixtures obtained by enzymatic hydrolysis of lactulose by KL1 β -galactosidase after 4 and 24 h of incubation. Compounds: 1) galactose; 2) fructose; 3) 6-galactobiose; 5) lactulose; 6) 6'-galactosyl-lactulose; 7) 1-galactosyl-lactulose; *) other oligosaccharides.

CARBOHYDRATE YIELD DURING LACTULOSE HYDROLYSIS

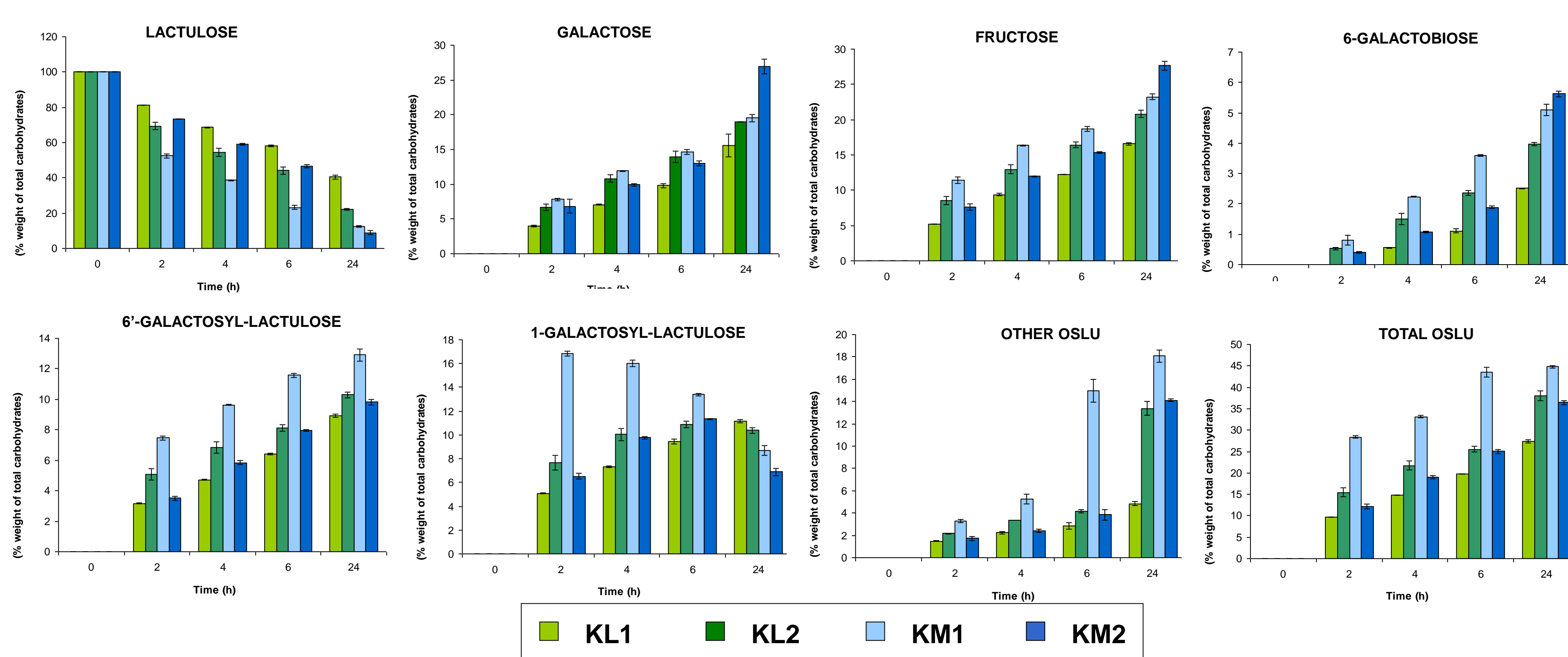


Figure 3. Carbohydrate yields during lactulose hydrolysis by yeast β -galactosidases. KL1: commercial *K. lactis*; KL2: *K. lactis* CEE isolated from artisanal cheese; KM1 and KM2: *K. marxianus* CCEs isolated from artisanal cheese. Error bars indicate standard deviations.

- KL1 hydrolyzed lactulose to galactose and fructose to a lesser extent (60 % hydrolysis) than the rest of assayed CCEs (80-90 % hydrolysis).
- Synthesis of 6-galactobiose, 6'-galactosyl-lactulose and other OsLu increased with time, reaching a maximum after 4 h of reaction.
- Production of 1-galactosyl-lactulose increased gradually and attained a maximum value after 2 h reaction in case of CEE KM1 and after 6 h reaction for CEE KM2.
- In contrast, 1-galactosyl-lactulose increased through the reaction time in the case of KL1 and KL2.
- CEE KM1 produced the highest yields of total OsLu after 6 h of reaction, reaching a 45% which corresponded to 87% of lactulose hydrolysis.
- The yield of synthesized OsLu for both *K. marxianus* was higher than that obtained using *K. lactis*.

CONCLUSIONS

- ✓ The present study shows the feasibility of β -galactosidases from *K. marxianus* strains isolated from cheese to hydrolyze and transglycosylate lactulose producing high yields (45%) of prebiotic oligosaccharides.
- ✓ *K. marxianus* is considered as a thermophilic microorganism which suggests the possibility of developing transgalactosyl reactions at higher temperatures than 50°C, with the benefit of using higher substrate concentrations for OSLu yield improvement.