Isomerisation of lactose to lactulose using milk concentration permeate and oyster shell powder

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Milk Concentration Permeate (MCP)

- Australian dairy plants produce high volumes of MCP from the increased utilisation of ultrafiltration (UF) for pre-concentration of milk
- Mcp is a low-value by-product not suitable for human consumption
- Mcp has a high BOD and needs proper treatment before disposal

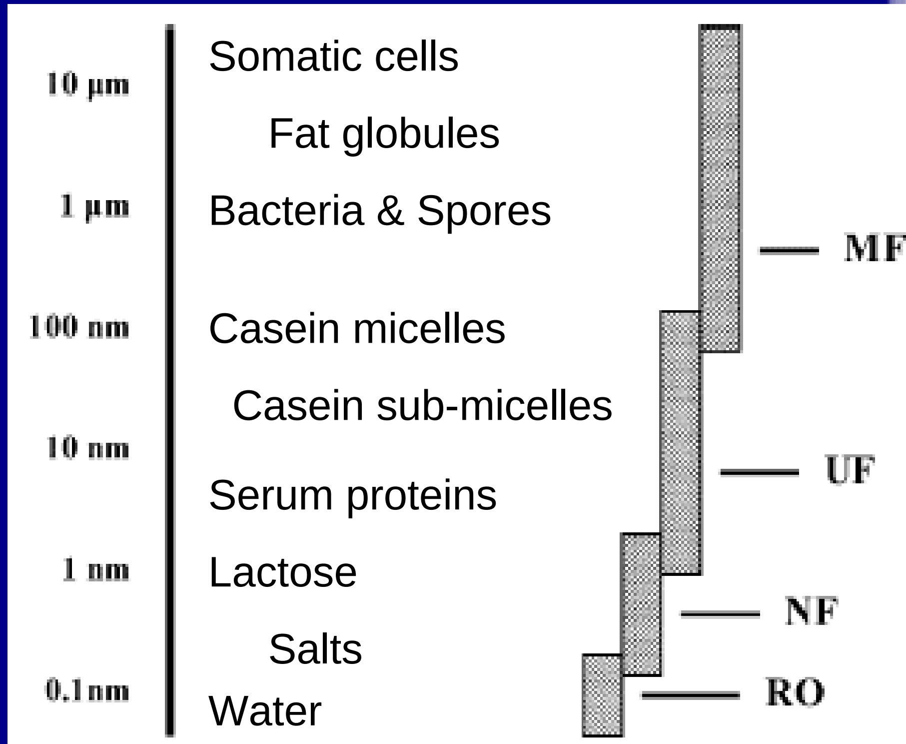


Figure 1. Component in milk: Size indication and membrane processes

Comparative composition of MCP (g/L)

Products	Protein	Lactose	Fat	Ash	Ca
Skim milk	33	48	15	7	1.2
Whey	8.5	48.5	0.75	9.2	4.6
MCP	4.2	48	1	8.5	3.3

Source: Atra et al., 2005

Utilisation opportunities for MCP

- Feeding farm animals
- Blending with other dairy liquids
- Recovering lactose for use in food formulation
- Using as a cheap lactose source to produce lactose-derivatives e.g. lactulose, lactitol and lactobionic acid

Lactulose

- A synthetic ketose disaccharide (4-*O*-β-D-galacto pyranosyl-D-fructose)
- Neither metabolised nor absorbed in small intestine
- Bacterially fermented in the colon to short chain fatty acids and gases.

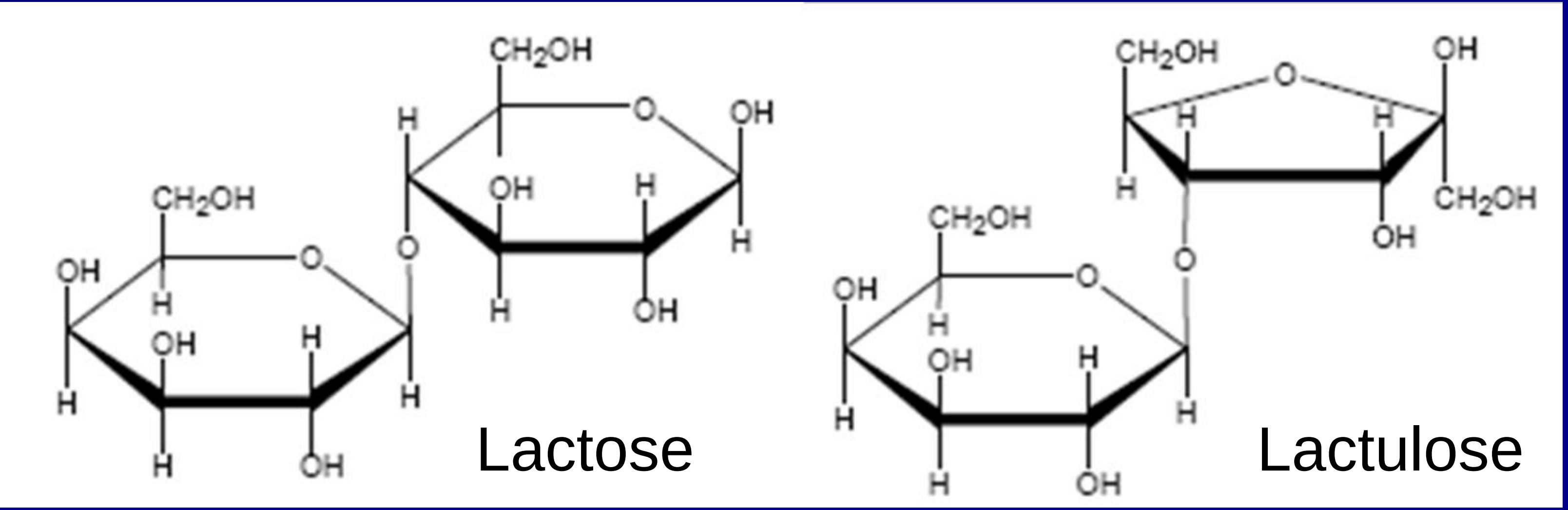


Figure 2. **Basic structure of lactose and lactulose**

Lactulose

- Used for treatment of several intestinal disorders
- Used as a prebiotic in infant formulas and health foods
- Formed in heated dairy products depending on the severity of heat treatment
- Generated via the Lobry de Bruyn-Alberda van Ekenstein isomerisation of lactose under alkaline condition (Andrews, 1989)

Previous isomerisation studies

Catalysts	Researchers
Strong organic alkaline reagents e.g. tertiary amines	Parrish, 1970
Strong alkaline reagents e.g. $Ca(OH)_2$, NaOH, KOH	Montgomery & Hudson, 1930 Nagasawa *et al*.,1974 Deya & Takahashi, 1991 Dendene *et al*., 1994 Zokaee *et al*., 2002
Complexing reagents e.g. borate and aluminate	Hicks & Parrish, 1980 Krumbholz & Dorscheid, 1991 Kozempel & Kurantz, 1994 Mahran *et al*., 1995 Carobbi *et al*., 2001 Zokaee *et al*., 2002

Catalysts employed for isomerisation

Catalysts	Researchers
❑ Strongly alkaline ion exchange resins	Martinez-Castro & Olano, 1980
❑ Alkaline earth phosphates e.g. disodium phosphate	Gasparotti, 1981
❑ Heterogeneous catalysts e.g. MgO, zeolites, sepiolites (hydrated magnesium silicate)	Carobbi *et al*., 1985 Shukula *et al*., 1985 Troyano *et al*., 1996 de la Fuente *et al*., 1999 Villamiel et al., 2002
❑ Egg shell powder	Montilla *et al*., 2005

Aims & Objectives

- To study the effectiveness of carbonate-based catalysts
- To evaluate the suitability of Oyster shell powder
- To determine parameters for maximum lactulose yield
- To use lactulose syrup as a prebiotic in dairy products
- To evaluate and compare the probiotic ability of lactulose syrup with commercially available prebiotics

Materials
&
Methods

Lactose Source

1. Lactose solution

- 4% (w/v) of α-lactose (95% pure) in distilled water
- Adjusted pH to 6.7 using 0.1 M NaOH

2. MCP

- A turbid green-yellowish liquid with 4.5% SNF (~0.5% protein & ~4% lactose) with lot-to-lot variation of 10%
- Collected from the UF unit of a local dairy plant in Victoria, Australia
- Kept by freezing and thawed before isomerisation process.

Catalysts

1. Limestone (pure $CaCO_3$)

- A white fine powder

(Analytical-reagent grade, BDH Chemicals, Australia Pty. Ltd.)

2. Egg shell powder

- A cream-coloured powder with an average particle size of 117 µm

[~94% $CaCO_3$, 1% $MgCO_3$, 1% $Ca_3(PO_4)_2$ and 4% organic matters]

3. Oyster shell powder

- An off-white fine powder

(~ 96% $CaCO_3$, ~ 0.696% SiO_2, 0.649% MgO, 0.984% Na_2O and 0.724% SO_3)

Preparation of Catalyst Powders

- **Egg shell powders (ESP)**
  - Removing shell membrane and washing off the residual albumen with tap water
  - Drying at 102°C overnight
  - Grinding in a micro hammer mill (Glen Mills Inc., USA) (600 *rpm,* 5 min) and sieving through a 120 mesh screen

- **Oyster shell powder (OSP)**
  - Cleaning and drying at 102°C overnight
  - Breaking into small pieces using mortar, grinding in a hammer mill and sieving through a 120 mesh screen

Isomerisation Process

MCP or Lactose solution

Catalysts *(12 mg/mL)* → **Mix**

Heat to 96°C

Reflux 96°C/150 min

Cool in ice bath

Centrifuge
(3600*g* / 10 min)

Supernatant

Analyses

1. **pH measurement** (Bench-top pH-meter, model 520A, Orion Research Inc., Boston, USA)

2. **Colour measurement**
   - Chromameter: (model CR-121, Minolta Camera Co., Ltd, Japan) with transmission cuvettes,
   - Spectrophotometer: Shimadzu model UV-1601 (Shimadzu Cooperation, Australia)

3. HPLC analyses

HPLC System

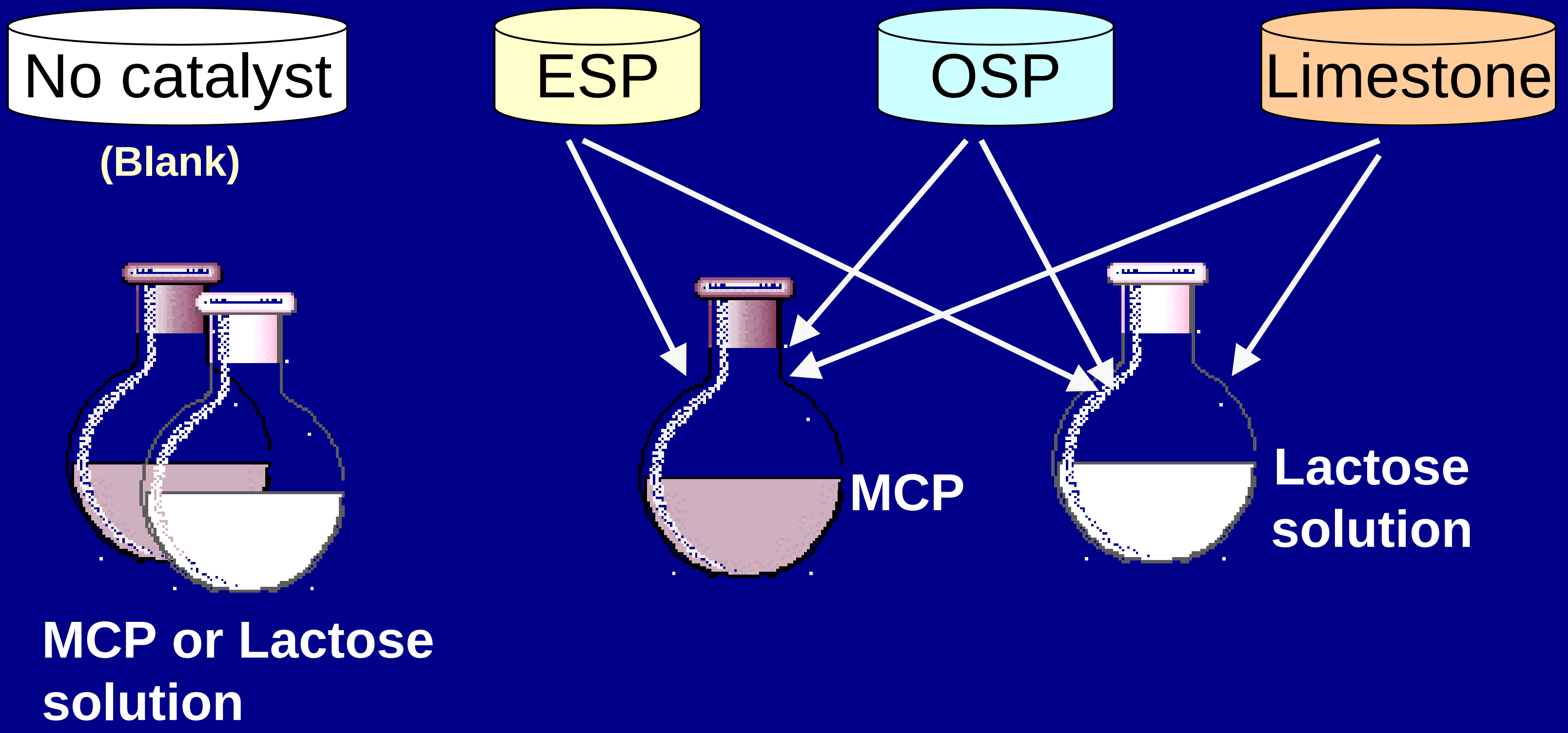
- Waters Model 6000A pump module (Waters associates Inc., Milford, MA, USA)
- A 20-ml injection loop (Rheodyne®, Cotati, CA, USA)
- A 3.9×300 mm stainless steel carbohydrate analysis column (Waters Co., USA)
- A Waters Model 41 refractive index detector (RID)

HPLC Conditions

- Mobile phase: degassed and filtered 80:20 (v/v) acetonitrile-water
- Flow rate: 0.8 mL/min
- Column temperature: ambient

Experimental Design

- Comparing the lactose isomerisation ability of different catalysts
- To establish the optimum conditions for obtaining maximum lactulose yield



Results
&
Discussion

Effect of pH on lactulose formation

- pH was measured every 30 min during heating
- At the initial pH (6.6-6.7) no lactulose formed in MCP or lactose solution
- Upon the addition of catalysts:
  - Significant pH rise (>9) in lactose solution
    - → lactulose was formed within 30 min of heating
  - Only slight pH rise (~6.8) in MCP
    - → no lactulose form within 30 min

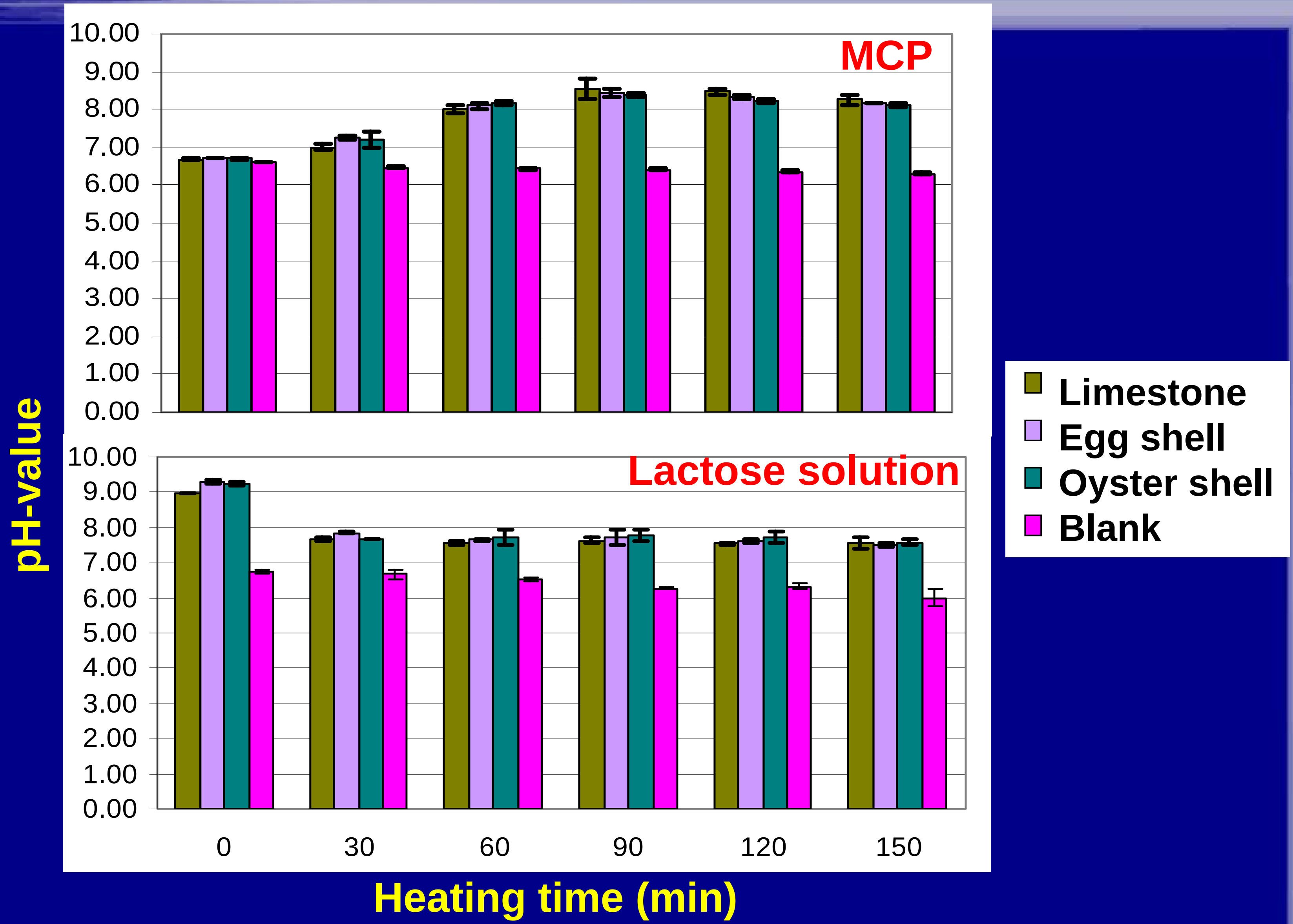


Figure 3. Changes in pH of MCP and lactose solution during isomerisation using different catalysts

- **Buffering effect of proteins in MCP may be the cause of pH stability in MCP**
- **The subsequent rise in pH of MCP to >8 after 60 min may be due to proteins denaturation and loss of their buffering capacity**
- **Prolonged heating caused lactulose degradation and depressed the pH in both solutions, indicating the formation of organic acids i.e. isosaccharinic and formic acids (Berg & van Boekel, 1994)**
- **pH was used as the end point indicator**

Colour Change During Isomerisation

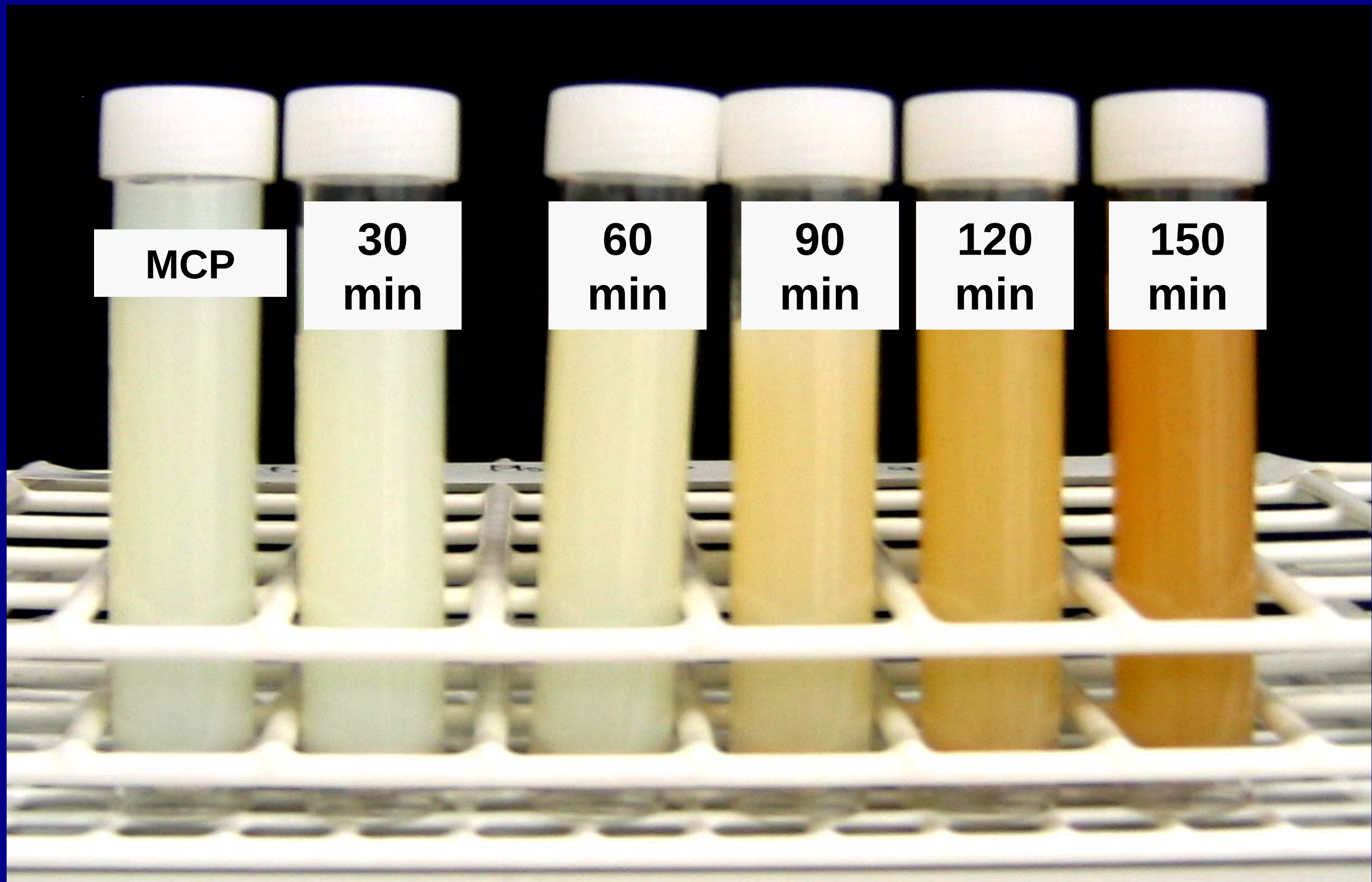


Figure 4. MCP Colour change at 96°C with 12 mg/mL catalyst

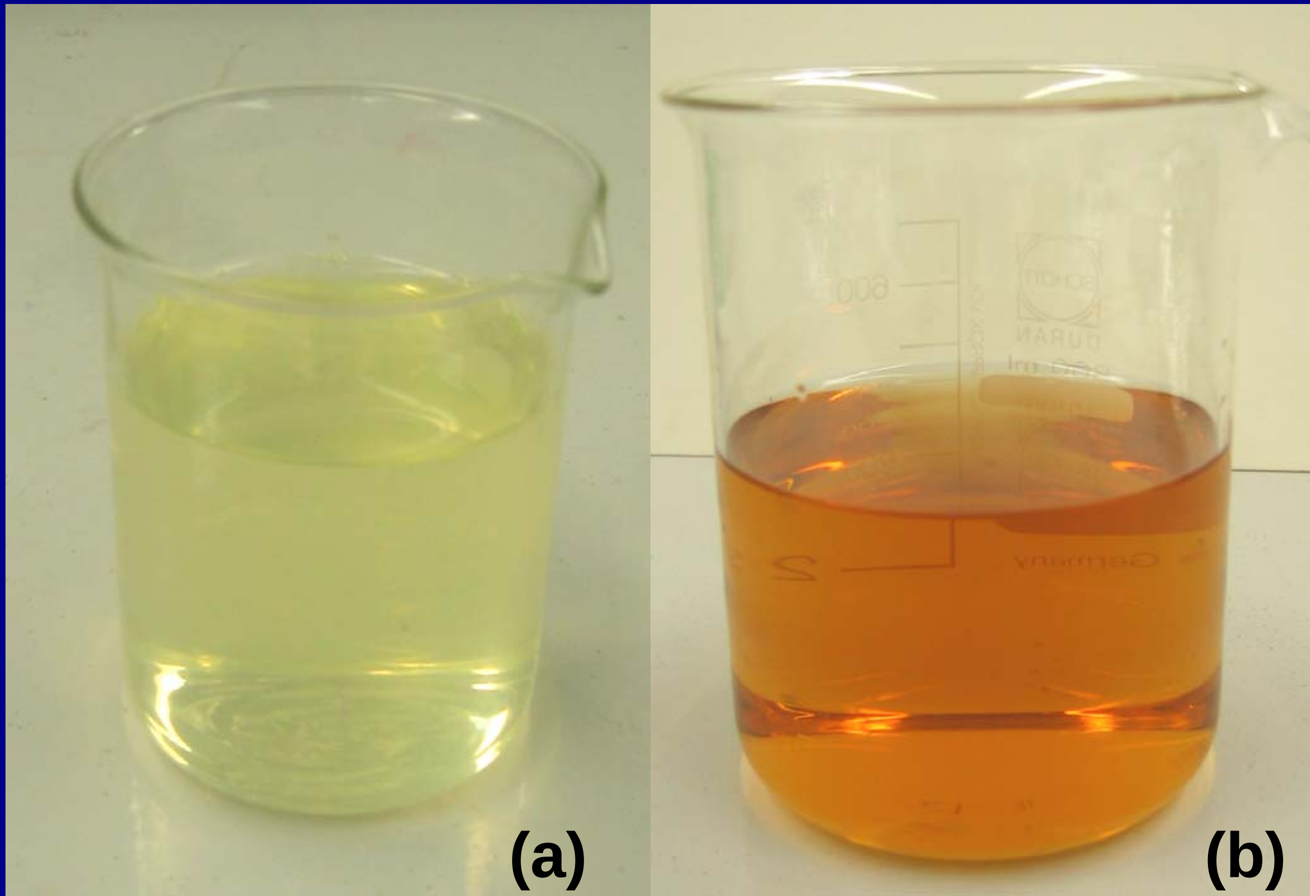


Figure 5. **De-proteinated MCP (a) before and (b) after isomerisation at 96°C for 120 min with 12 mg/mL egg shell powder**

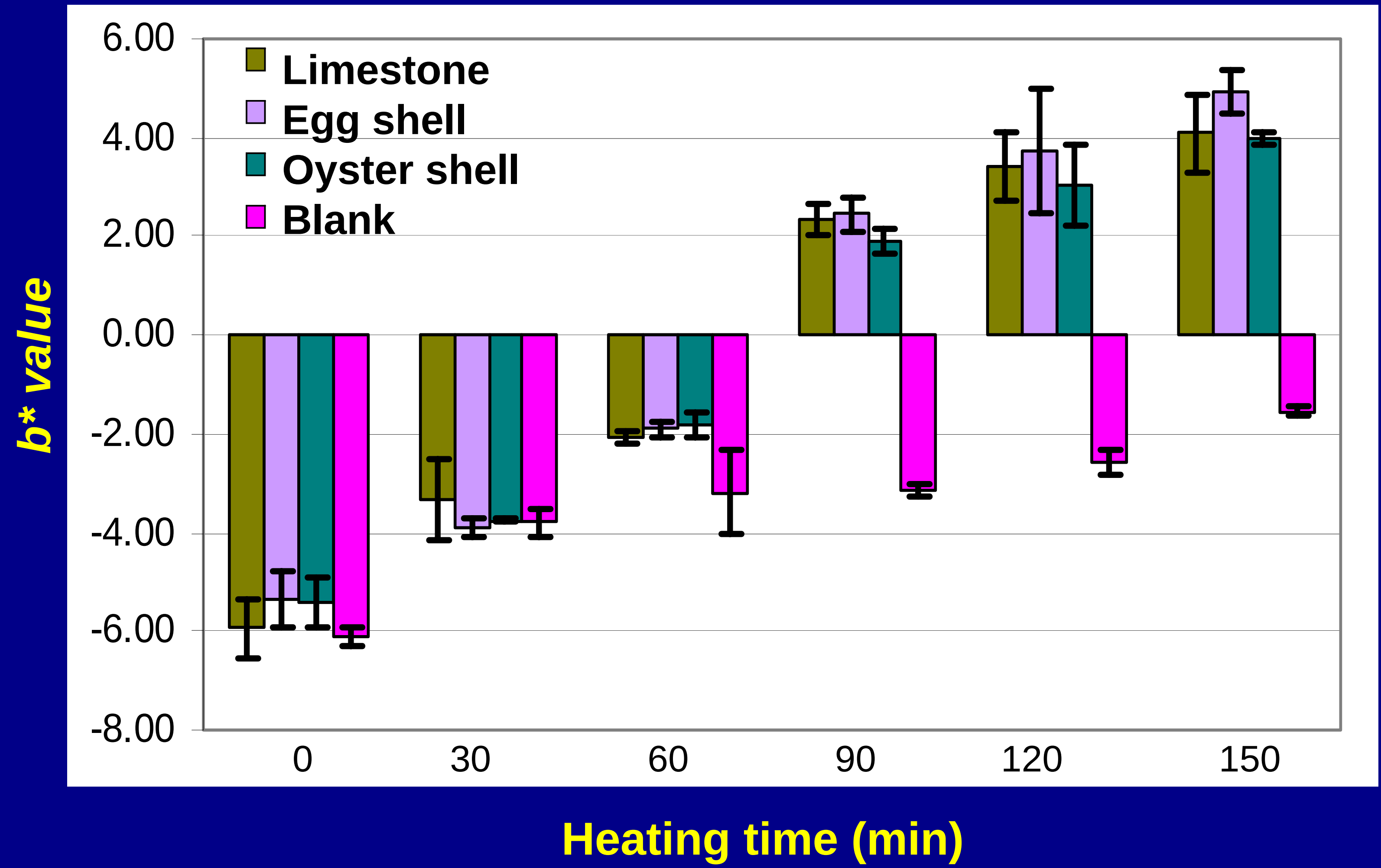


Figure 6. Increase of *b** values during isomerisation of MCP using different catalysts

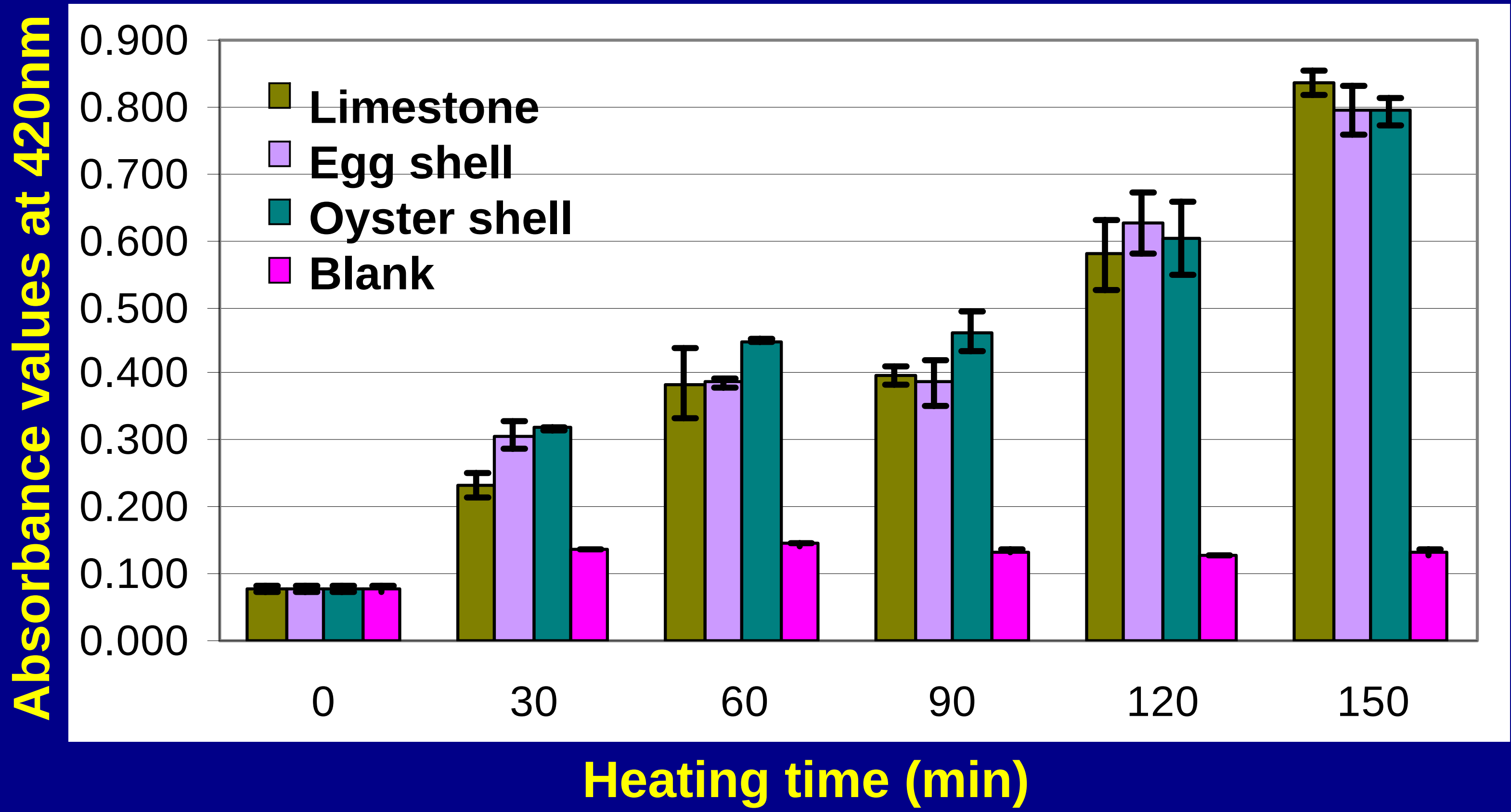


Figure 7. Increase of absorbance values during isomerisation of lactose solution using different catalysts

HPLC determination of lactulose and lactose content

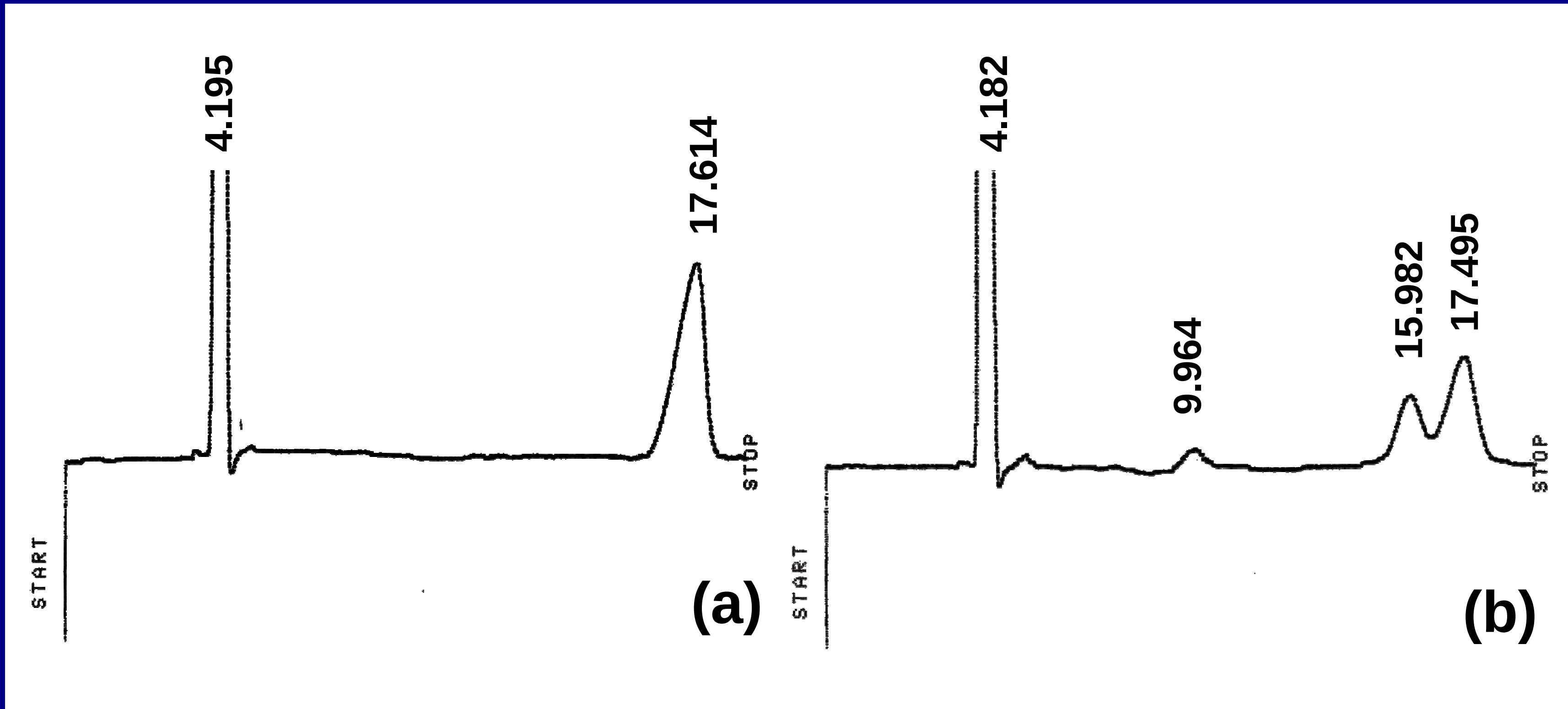


Figure 8. **HPLC-RID chromatogram of MCP (a) before and (b) after isomerisation at 96°C for 120 min**

Solvent (4.20 & 4.18), lactose (17.61 & 17.50), galactose (9.96) and lactulose (15.98)

- Lactose content dropped in favour of lactulose formation
- The presence of galactose peak (Fig.8b) indicates the decomposition of lactose or lactulose
- In the absence of catalyst the conversion level was very low and only 3% lactulose formed after 120-150 min isomerisation (Fig. 9 & 10)
- In the presence of catalyst, significantly higher level of conversion was observed and in the earlier stages of heating

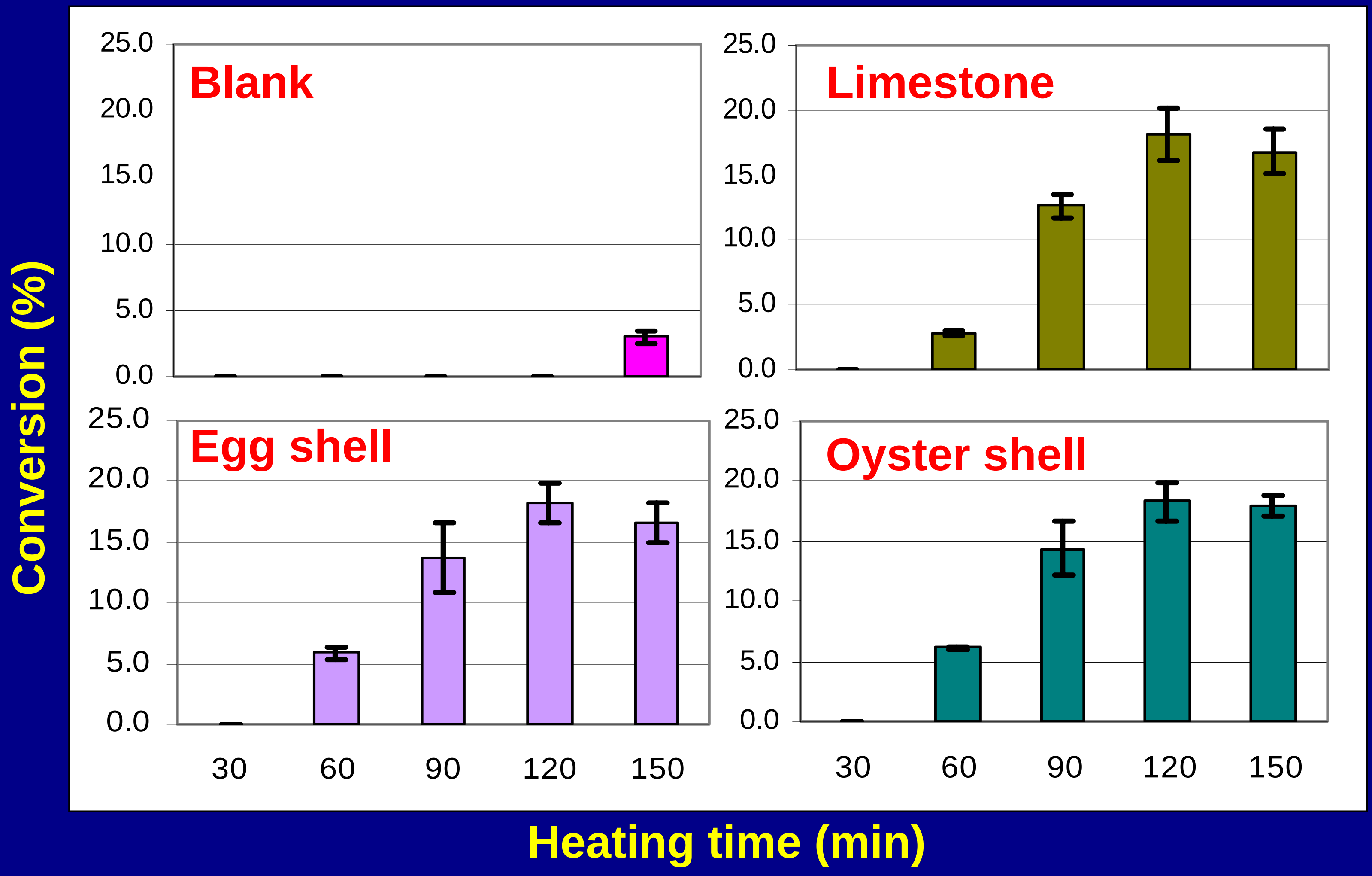


Figure 9. **Effects of catalyst type on the conversion of lactose to lactulose in MCP**

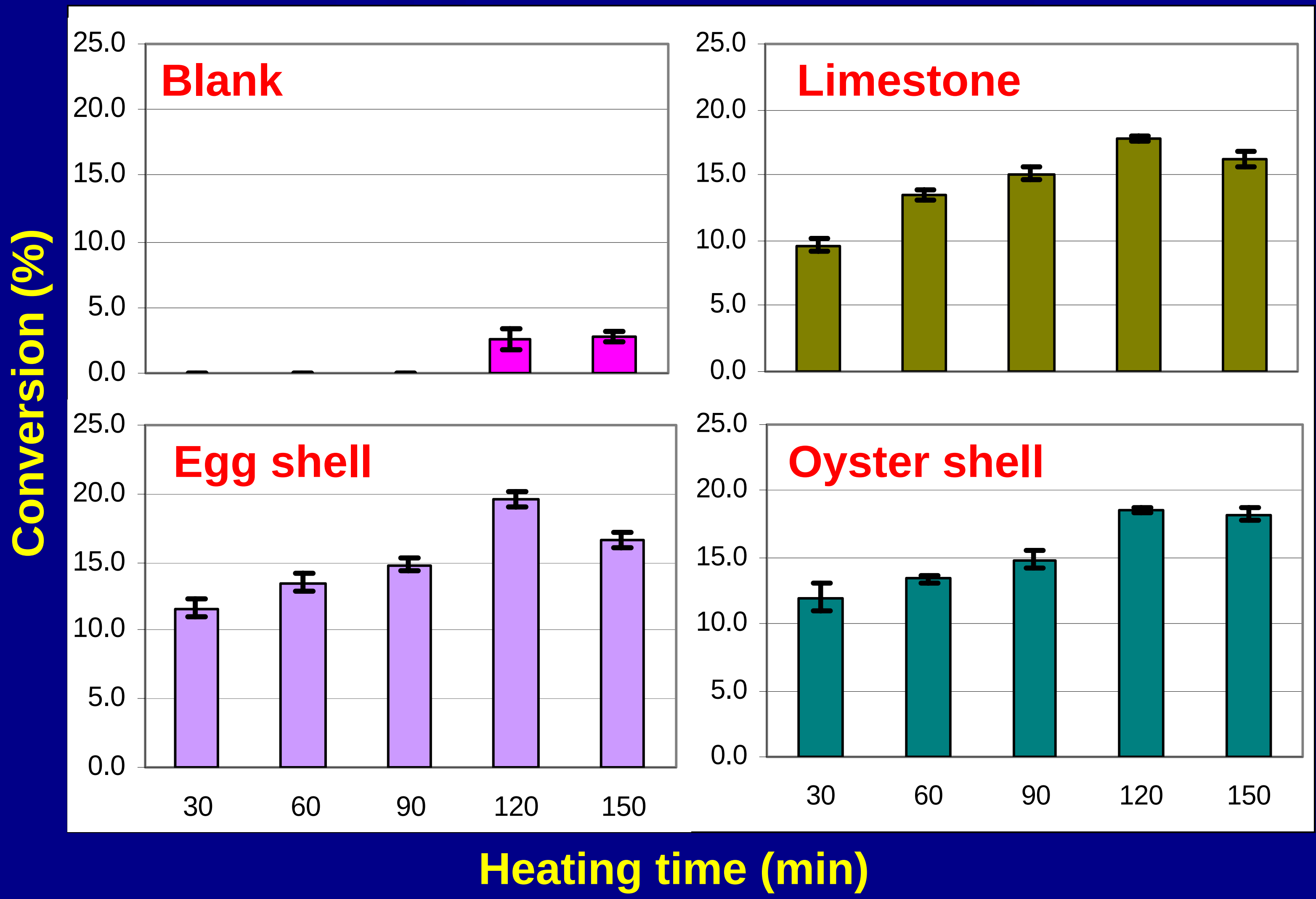


Figure 10. Effects of catalyst type on the conversion of lactose to lactulose in lactose solution

- In the earlier stages of heating, lower level of lactulose formed in MCP than in lactose solution due to:
  - The buffering effect of proteins and peptides
  - The reduction of available lactose and/or lactulose through the formation of lactosyl- and/or lactulosyl-amino compounds
- Changing catalyst type had only a small effect on the conversion rate of lactulose
  - Limestone in MCP achieved:
    - 3% less conversion than shell powders after 60 min,
    - 2% less in lactose solution after 30 min
  - The effectiveness of all catalysts was similar between 90 and 150 min in both solutions.

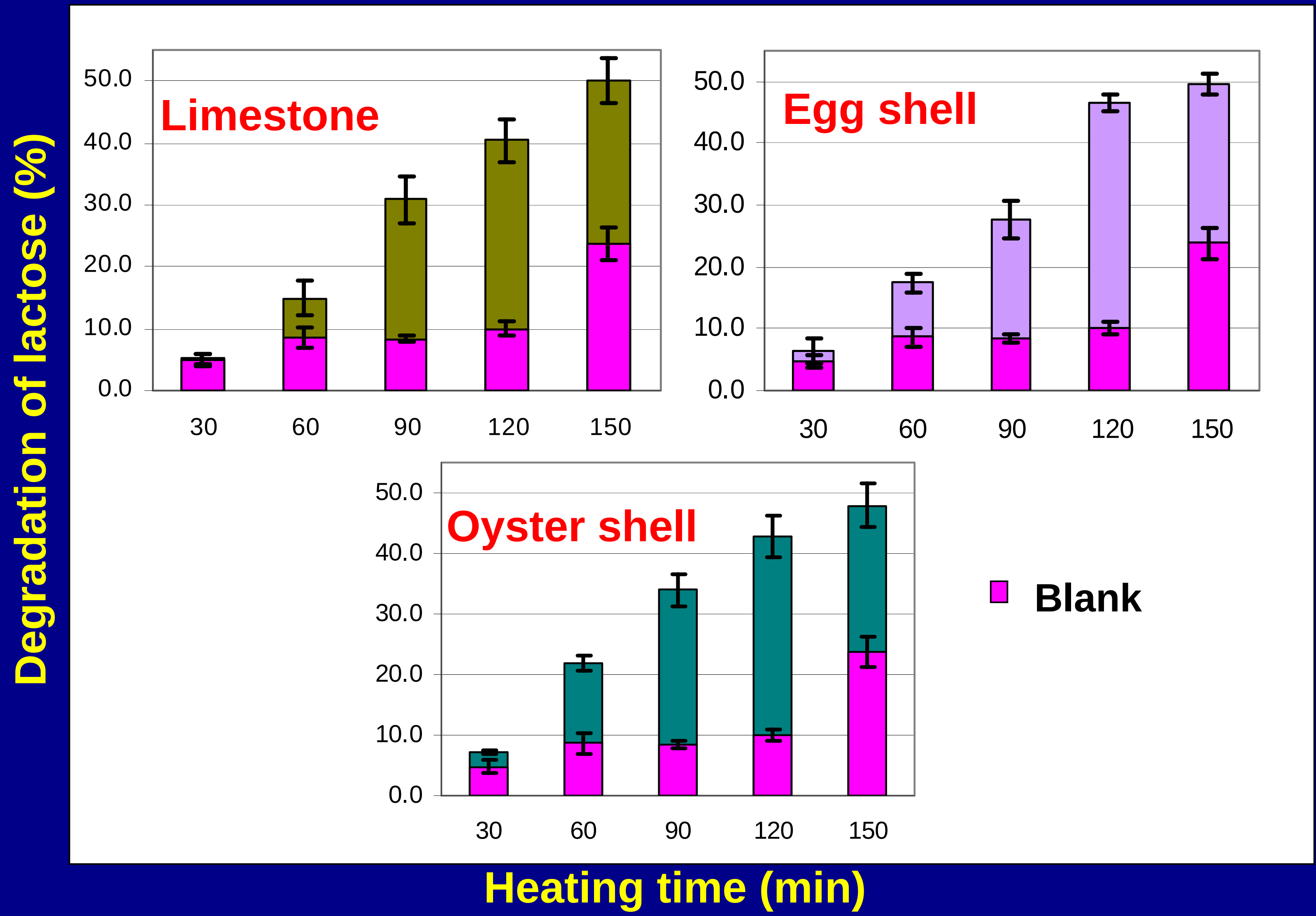


Figure 11. Effects of catalyst type on the degradation of lactose in MCP

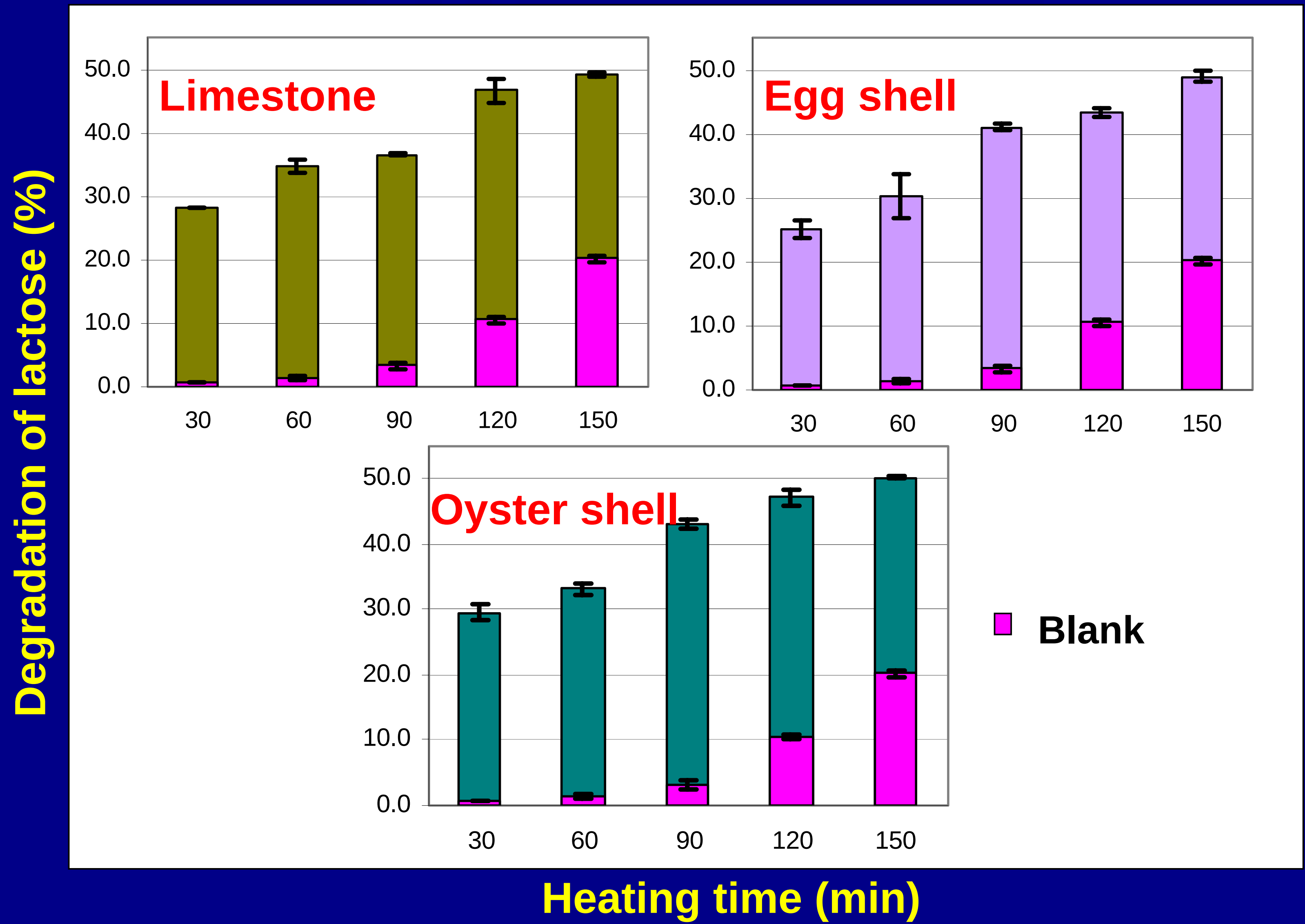


Figure 12. Effects of different catalysts on the degradation of lactose in lactose solution

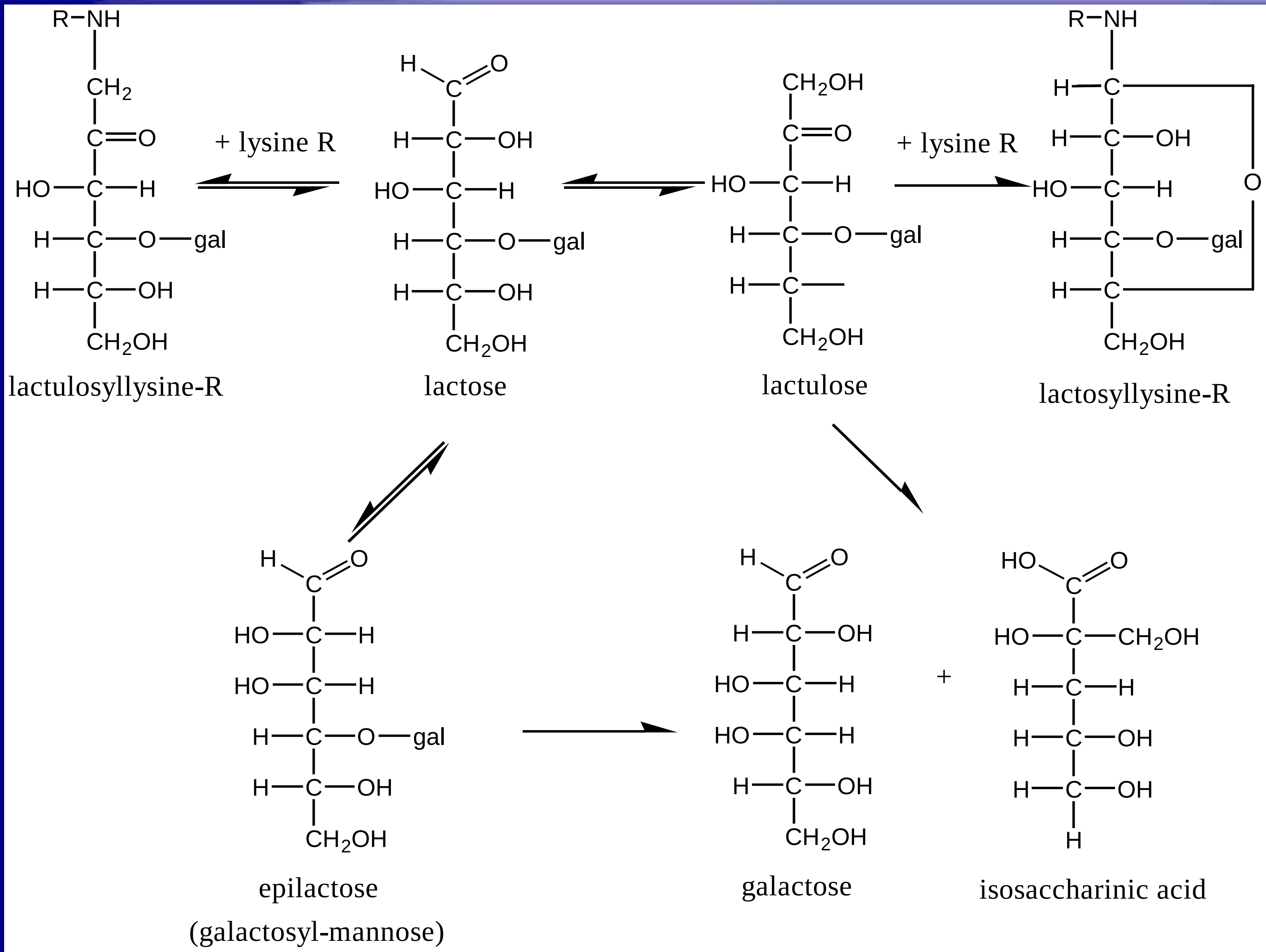


Figure 13. Scheme for isomerisation and degradation of lactose

(Adapted from Berg & van Boekel, 1994)

Removal of Protein

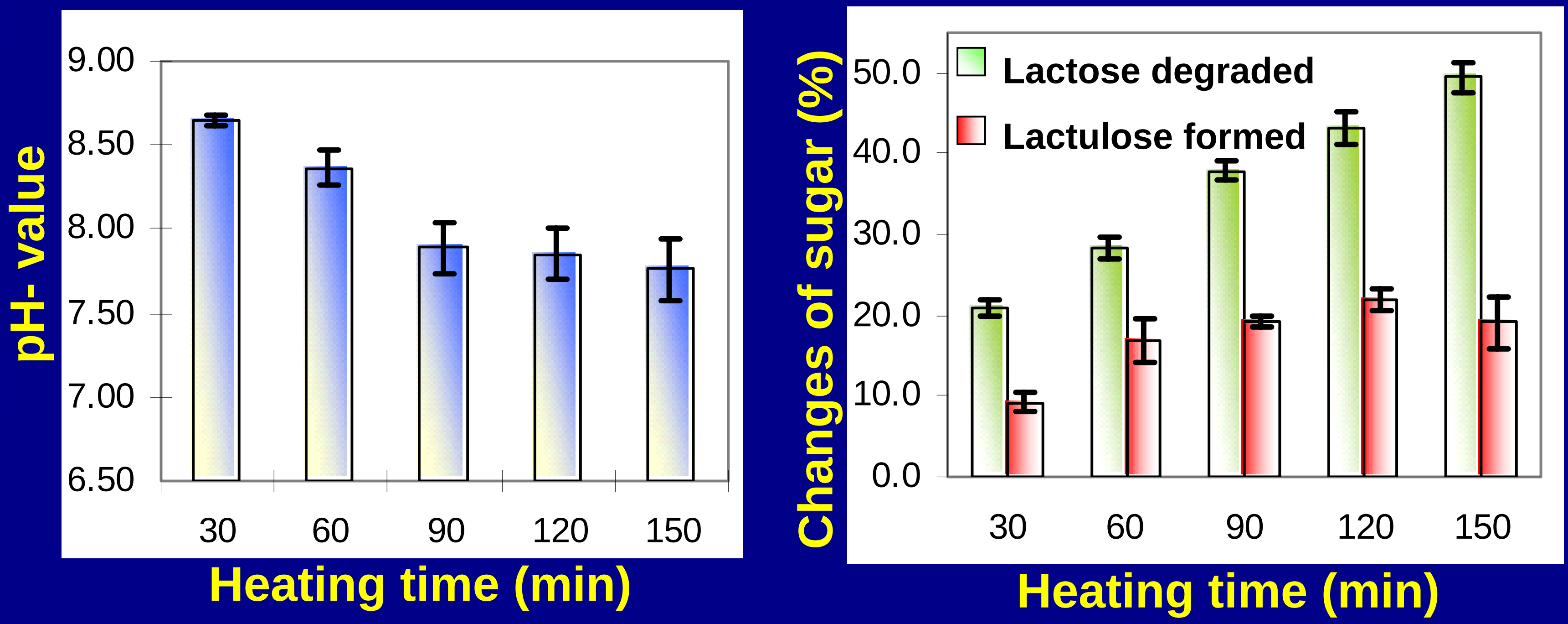


Figure 14. Effect of de-proteination of MCP on lactose isomerisation

Future works

- Concentration of the resulting syrup to 40ºB
- Removal of residual minerals from concentrated syrup
- Investigation of the bifidogenic property of lactulose syrup in probiotic milk model

Conclusions:

- MCP can be used as convertible source of lactose
- Residual proteins in MCP may adversely affect the yield of lactulose
- Oyster shell powder gave comparable yield of lactulose with egg shell powder and limestone
- With the optimum conditions of 120 min at 96°C, a maximum yield of 18-21% lactulose was achievable to the net cost of <37% of original lactose degradation
- The resulting syrup can be directly added to food formulations as a food-grade prebiotic.

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Спасибо

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