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The Chemical Isomerization of Whey Lactose to Lactulose by Using Column Reactor

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ABSTRACT
The isomerization of whey lactose to lactulose was carried out using column reactors of 1, 2 and 3M sodium aluminates at 30 and 70^o C. The effect of different sodium aluminates molarities, temperature and flow rates on the formation of lactulose and overall degradation have been studied. There was a proportional relationship between lactulose formation and sodium aluminates molarities, the same as with the isomerization temperature, 67 and 80% lactulose were respectively obtained at 30 and 70^o C by using 3M sodium aluminates. A reverse relationship was found between lactulose formation and flow rates. The previous relationships were true with overall degradations.

Keywords: Whey Lactose, column isomerization, lactulose.

1. INTRODUCTION
The disaccharide lactulose is an artificial sugar with a significant impact on human digestion. It can be generated by either alkaline isomerization of lactose via the Lobry de Bruyn - Alberda van Ekenstein rearrangement or by enzyme-catalyzed synthesis. Based on the first reaction, different process schemes for the preparation of lactulose have been developed. The enzymatic synthesis of lactulose can be carried out using different pathways with the trans galactosylation reaction being the most promising[1].
Lactulose is recognized as a prebiotic and it is used inthe form of syrup for the treatment of some intestinaldisorders. It is slightly sweeter than lactose and can be used as a partial sucrose substitute in some food products[2]. Lactulose can also be used as a food supplement in pediatric diets for the development of functional foods and in geriatric medicine for some targeted populations with severe constipation syndrome[3]. Lactulose is also widely used as a statement in hepatic encephalopathy.
Lactose is an expensive raw material for lactulose synthesis. Applying milk whey, which contains 4.5–5% (w/v) lactose, is much more cost effective. Whey is a by-product of cheese and casein production and an inexpensive raw material containing a high amount of lactose. Much of the whey produced by the dairy industry is used as animal feed, fertilizer and as a food ingredient or additive[4].Nevertheless, about 30% of the annual world whey production (177 million tons in 2006) remains underutilized[5]. Whey is considered an environmentally high-strength wastewater pollutant due to its high biological oxygen demand and chemical oxygen demand[6]. Its cost-effective disposal and utilization has become increasingly important to the dairy industry. Additionally, environmental and economic concerns demand that whey should be converted to value-added products.
The aim of the present work was to study the effect of different molarities of sodium aluminates, temperature degrees (30 and 70˚C) for different periods of time on the isomerization of whey lactose to lactulose.

2. MATERIALS AND METHODS
27, 54 or 81 g of cutting aluminum wires (99.9% purity) were stirred with 1L of 1, 2 or 3M sodium hydroxide to prepare 1, 2 and 3M sodium aluminates. The mixture was allowed to cool at room temperature and filtered through Whitman paper No. 54. The precipitated sodium aluminate was washed with distilled water to get rid of access base. Purified sand (250-300 µm diameter)can be used as supporting material, instead of silica gel to hold (by adsorption) sodium aluminates molecules. 3 double jacket glass columns (77 cm height, 3 cm inner diameter, 5 cm outer diameter and 395.64 cm^3 reactor bed) were used in the present study. The sweet whey was heated for 15 min. at boiling point to separate the whey proteins. The proteins free whey was filtered by using Buchner funnel through Whitman filter paper No. 1, to get rid of whey protein residues. The obtained solution was stored under refrigeration in a sealed container for the subsequent analysis and isomerization processes[7].
To 500 mL 1M, 2M or 3M sodium aluminates solution 500 g of silica gel (100-200 mesh) or 500 g sand (250-300 µm) was added. The mixture was stirred for 3h at room temperature using magnetic stirrer to fix the sodium aluminates on silica or sand surfaces. The mixture was transferred to a separating column with a continuous vibration to prevent gaps formation and discard air bubbles. The packed column has been washed several times with deionized water to reduce the pH value to 9.5-10.5.
For each isomerization run, a 100 mL of clarified sweet whey (4.6 % lactose) was added on the top of column with different flow rates (1, 2 and 3 mL/min), at 30 and 70^{o}C. The pH value of isomerized solution was adjusted to 7.0 by using 0.5% phosphoric acid. The aluminum phosphate precipitation was separated by centrifuge at 4,000 rpm for 10 min.; the supernatants were collected and stored at refrigeration. High performance liquid chromatography (HPLC) method was used to determine the degree of lactose isomerization into lactulose[8]withanion exchange shimpack A1-

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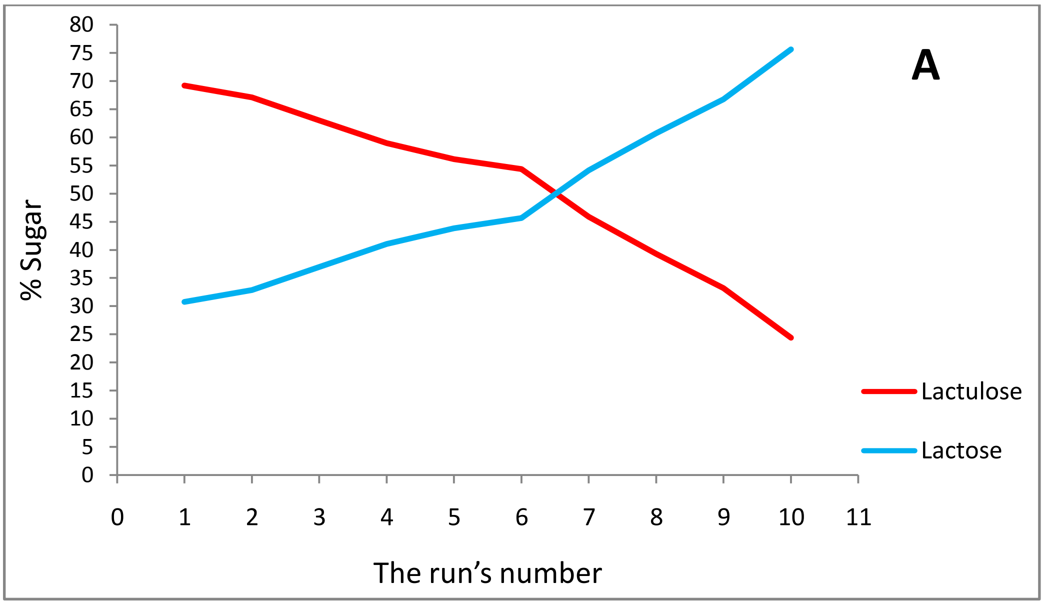
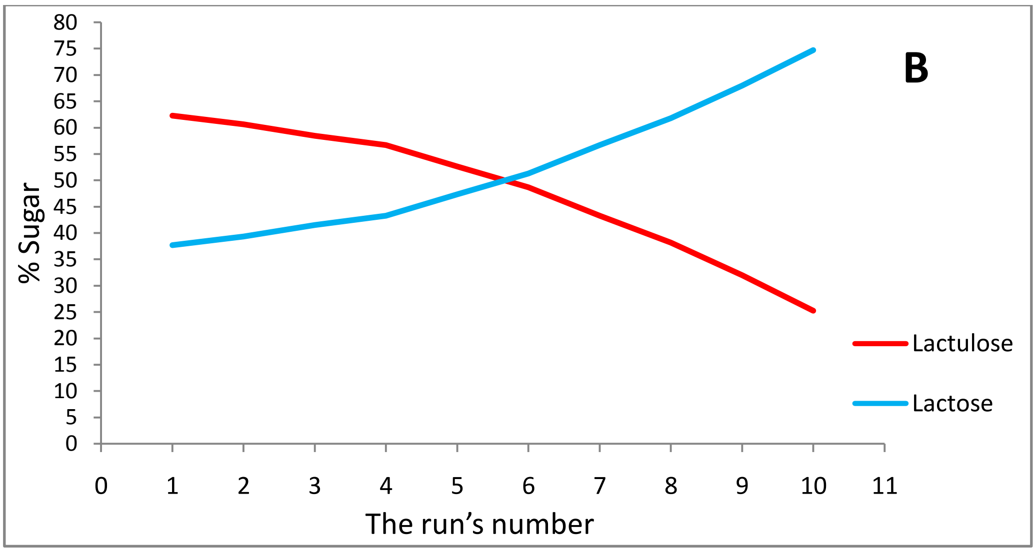
column (50 x 4.6 mm, 3µm particle size).The mobile phase was a mixture of 15 mM NaOH spiked with freshly prepared 1mM barium acetate, the flow rate was 1.5 mL/min, the detector was Refractive index detector, the column temperature was controlled at 25 º C and the injection size was controlled at 20µl.

3. RESULTS AND DISCUSSION

In the present study, we have used the sweet whey separated from the cheese curd when the starter cultures and rennet are applied to milk during the manufacture of cheese[9].Fig. 1 shows the effect of 1, 2 and 3 mL/min flow rates on the formation of lactulose from 4.6% whey lactose when a column reactor of 1M sodium aluminates was used as an alkaline catalyst at 30° C. The highest conversions to lactulose were observed during the first run (100 mL), 69.22, 62.31 and 57.28 % using 1, 2 and 3 mL/min flow rates respectively. The column efficiency decreased throughout the following runs, with 1 mL/min the percentage of lactulose decreased from 69.22% to 24.38% upon the run number 10.After each run the column was washing with deionized water and kept wet to the next run (another 100 mL). At the end of each run the pH of the isomerized mixture has been determined. The decreasing of lactulose conversion due to the dropping in pH values throughout the following runs caused by the exhausting sodium aluminates. So, we need at the end of each run to precipitate the sodium aluminates as aluminum phosphate which it is white slurry separated by centrifugation process. There was a negative correlation between lactulose formation and the flow rate. No detectable degradation was noticed at these conditions**;** the by-products formation always accompanied high molarity alkaline and high temperature degree[10, 11].By collecting the volumes of the 10 runs (1000 mL), the conversion to lactulose will be 51.16, 47.83 and 42.66% when 1, 2 and 3 mL/min flow rates were used. Thus we have gained 3.18, 2.87 and 2.63 g lactulose (of 4.6% lactose) by using 1, 2 and 3 mL/min flow rates. Therefore, and for economical reasons, the use of 3 mL/min is better than 2 ml/ min and 1 mL/min flow rates respectively.





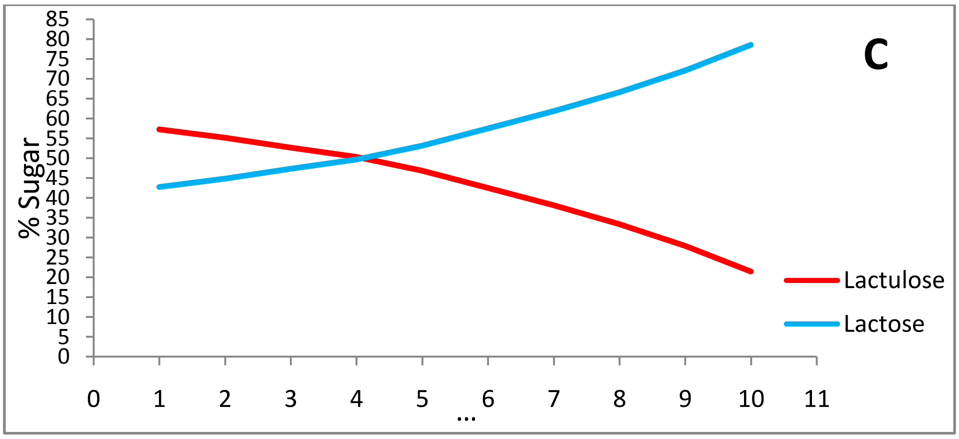
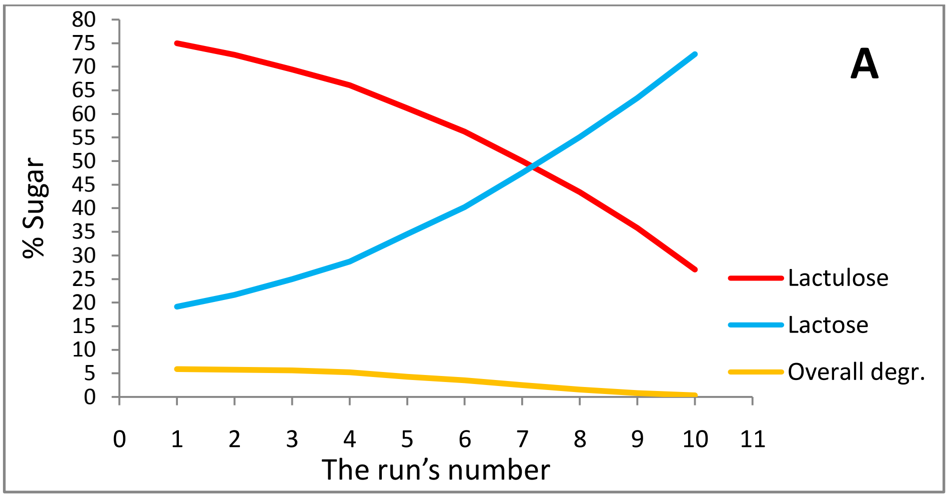
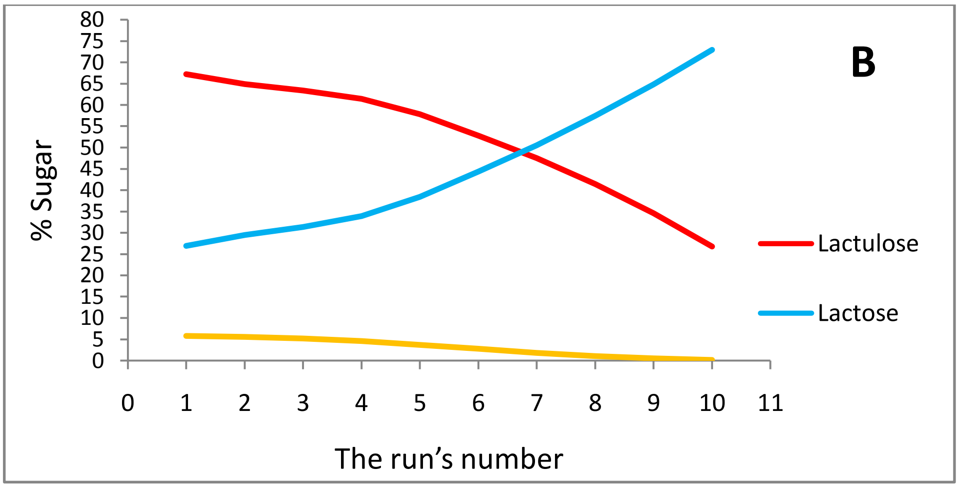


Fig.1: The effect of 1M sodium aluminates on the formation of lactulose byusing sweet whey of 4.6 % lactose at 30˚C , (A) 1mL/min ,(B) 2 mL/minand(C) 3mL /min.

Fig. 2 shows the effect of 1, 2 and 3 mL/min flow rates on the formation of lactulose from 4.6% whey lactose when a column reactor of 1M sodium aluminates was used as an alkaline catalyst at 70°C. The overall degradation percentages of the isomerization are higher in the first run as compared with 30^{o} C, but in general, the overall degradation by using column reactor was less comparing with batch reaction. The by-products formation always accompanied the high alkaline molarities and high temperature degrees. There was a reverse relationship between by-products formation and the runs number[1], the same as between lactulose formation and the flow rate. The conversions to lactulose are dropped from 74.99, 67.26 and 60.76% within the first run by using 1, 2 and 3 mL/min flow rates to 26.99, 26.80 and 22.93% respectively during the run number 10. We had gained 3.75, 3.36 and 3.04 g of lactulose by using 1, 2 and 3 mL/min. Therefore, and for economical reasons, the use of 3 mL/min flow rate is better than 2 and 1 mL/min.





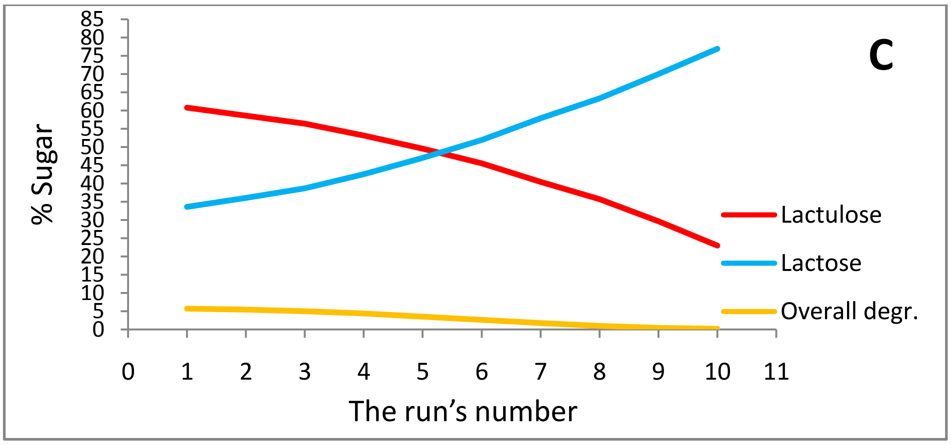
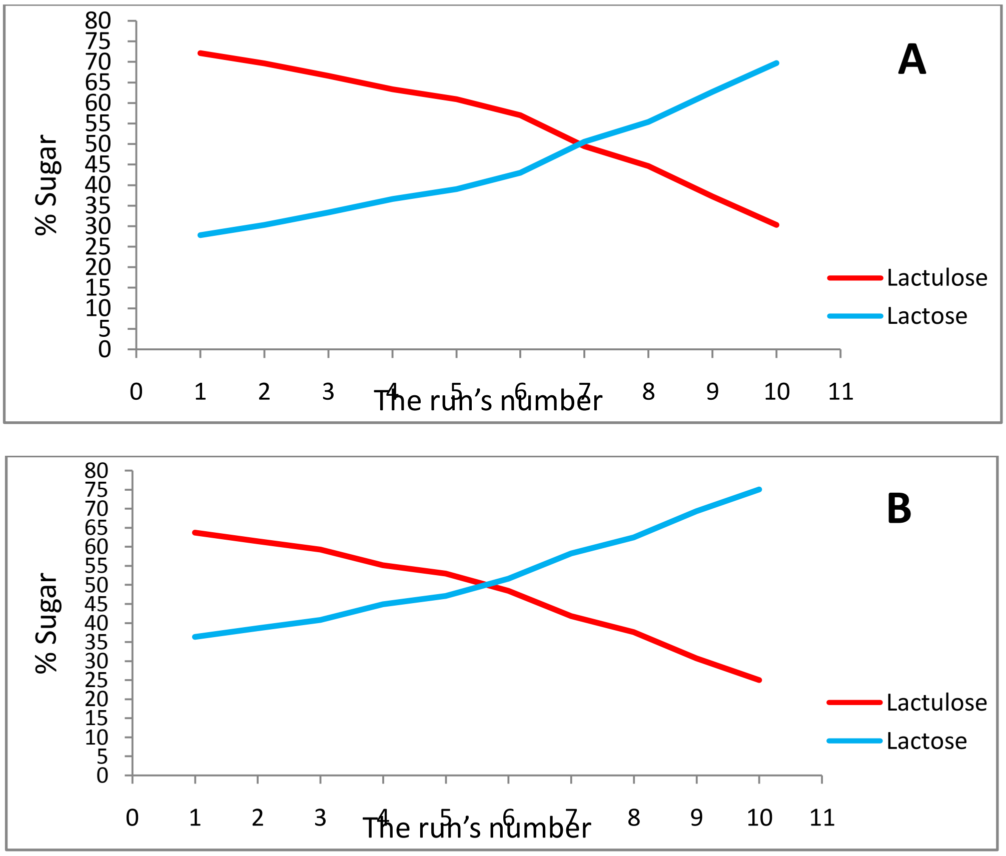


Fig.2: The effect of 1M sodium aluminates on the formation of lactulose by using sweet whey of 4.6 % lactose at 70˚C , (A) 1mL/min ,(B) 2mL/min and (C) 3mL/min.

Fig.3 and 4 refer to the effect of 2M sodium aluminates and 1, 2 and 3 mL/min flow rates on the formation of lactulose using sweet whey of 4.6 % lactose at 30 and 70˚C respectively. There was a negative correlation between lactulose formation and the run number. The decreasing of lactulose conversion, due to the dropping of pH values which is associated with sodium aluminates exhausting. These results are agreed with[12],who found, that the maintenance of high pH favors lactulose formation. There was a negative correlation between lactulose formation and the flow rate.

As shown in Fig.3,no detectable degradation was noticed at 30°C which refers that the by-products formation always accompanied high temperature degree[1]. The conversions to lactulose are dropped from 72.16, 63.68 and 57.19% within the first run using 1, 2 and 3mL/min flow rates to 30.30, 24.99 and 19.89% respectively during run number 10 (for reasons mentioned before).

Fig. 4 shows the effect of 1, 2 and 3 mL/min flow rates on the lactulose formation by using 2M sodium aluminates at 70°C. The overall degradation percentages are high in the first runs comparing with 30° C and with 1Msodium aluminates column reactor at the same temperature degree. 78.06 % of the maximum lactulose was formed at the first run by using 1mL/min flow rate. The percentage of lactulose was dropped from 78.06 % within the first run to 31.71 % during run number 10 at the same conditions. There was a negative correlation between lactulose formation and the run number. The decreasing in lactulose percentages were due to the dropping of pH values and the sodium aluminates exhausting. These results are agreed with[13], who showed that higher temperatures cause a decrease in the formation rate of lactulose; this can probably be due to higher carbohydrate degradation.



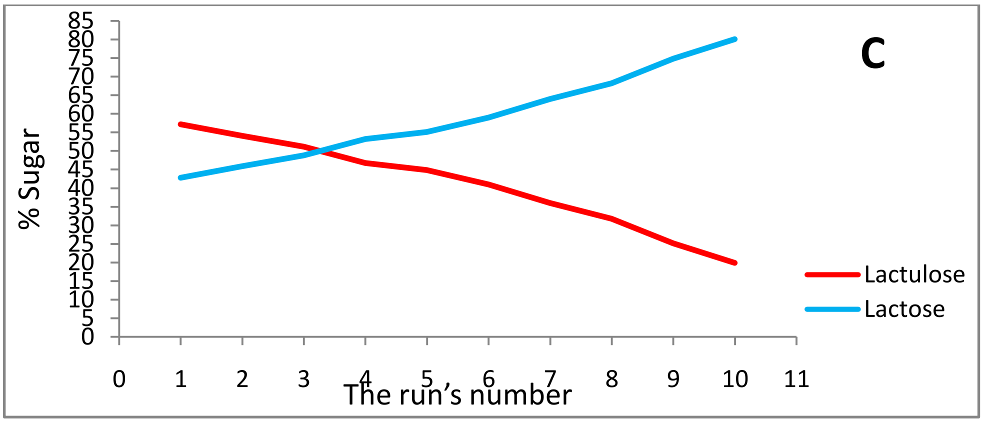


Fig.3 : The effect of 2M sodium aluminates on the formation of lactulose by using sweet whey of 4.6 % lactose at 30˚C , (A) 1mL/min ,(B) 2 mL/min and (C) 3m L/min.

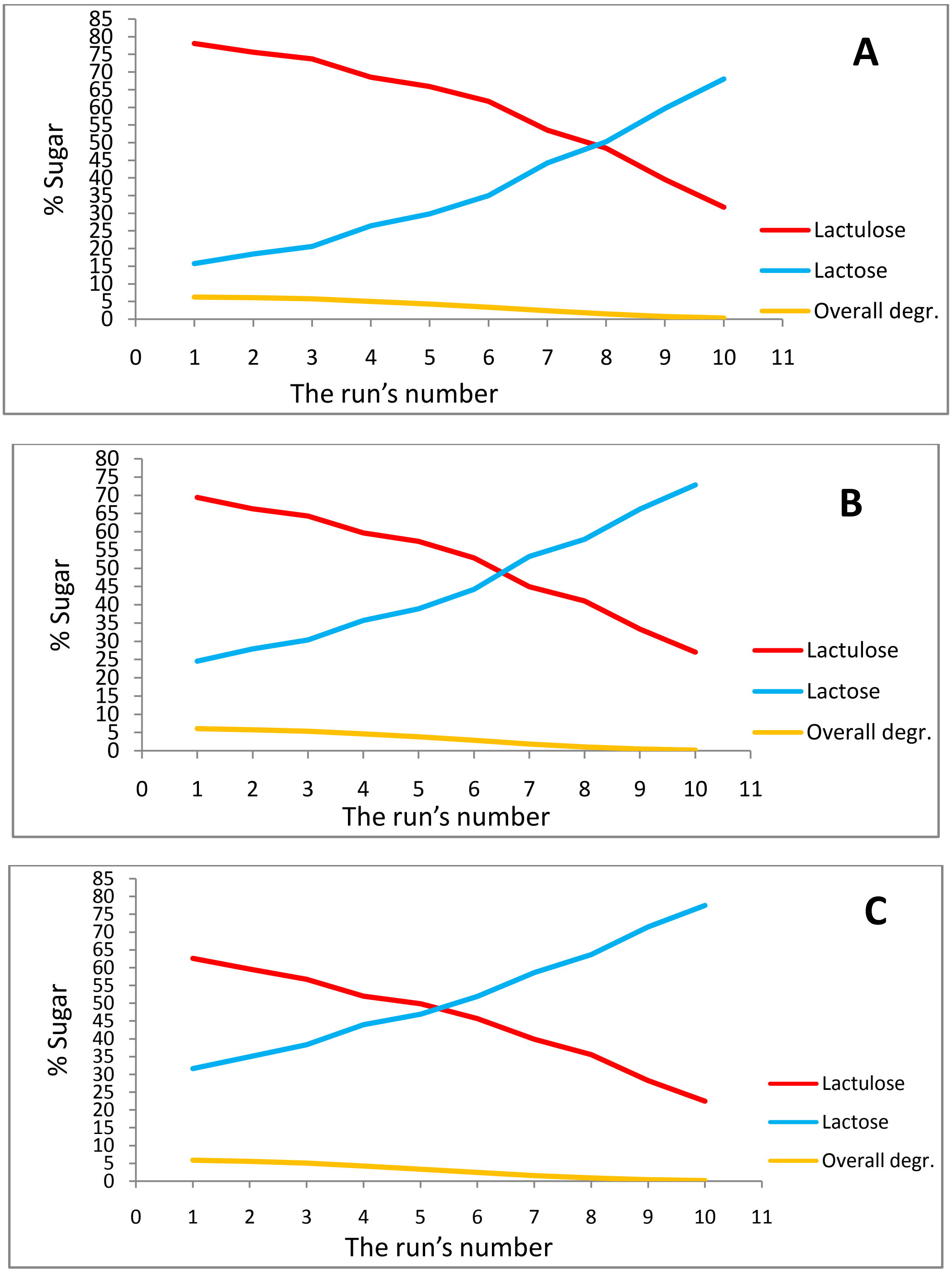


Fig.4: The effect of 2M sodium aluminates on the formation of lactulose by using sweet whey of 4.6 % lactose at 70˚C , (A) 1mL/min ,(B) 2 mL/min and (C) 3m L/min.

3M sodium aluminates gave higher lactulose formation at the first run as compared with 1M and 2M sodium aluminates at the same conditions**.** The overall degradation is also higher than those with 1M and 2M column reactor**.** Figs. 5 and 6 refer to the effect of 3M sodium aluminates by using 1, 2 and 3 mL/min flow rates on the formation of lactulose by using sweet whey of 4.6 % lactose at 30 and 70˚C respectively. Fig 5 shows the effect of 3M sodium aluminates with 1, 2 and 3 mL/min flow rates column reactors at 30^{o}C on the lactulose formation. The higher percentage of lactulose was observed during the first run (100 mL), 76.65% had been obtained by using 1 mL/min flow rate.The percentage of lactulose was dropped from 76.65% (1 mL/min flow rate) to 41.39 %during the run number 10. The decreasing in lactulose formation at the latest runs was due to the exhausting of sodium aluminates associated with pH dropping[12]. No detectable degradation was noticed at 2 and 3 flow rats.

Fig. 6 illustrates the effect of 3M sodium aluminates by using 1, 2 and 3 mL/min flow rates on the formation of lactulose at 70 ˚C. The first runs, by using 3M sodium aluminates are higher by 7.55, 9.73 and 12.82 % than using 1M sodium aluminates at the same flow rates. The higher conversion to lactulose (81.12%) was observed during the first run by using 1 mL/min flow rate. The percentage of lactulose formation was dropped to 43.20 % during the run number 10. The overall degradation percentage by using 3Mishigher in the first run comparing with 1Mand 2M sodium aluminates at the same temperature degree, this result agrees with[1],who showed that, the by-products formation always accompanied high molarities alkaline and high temperature degree.

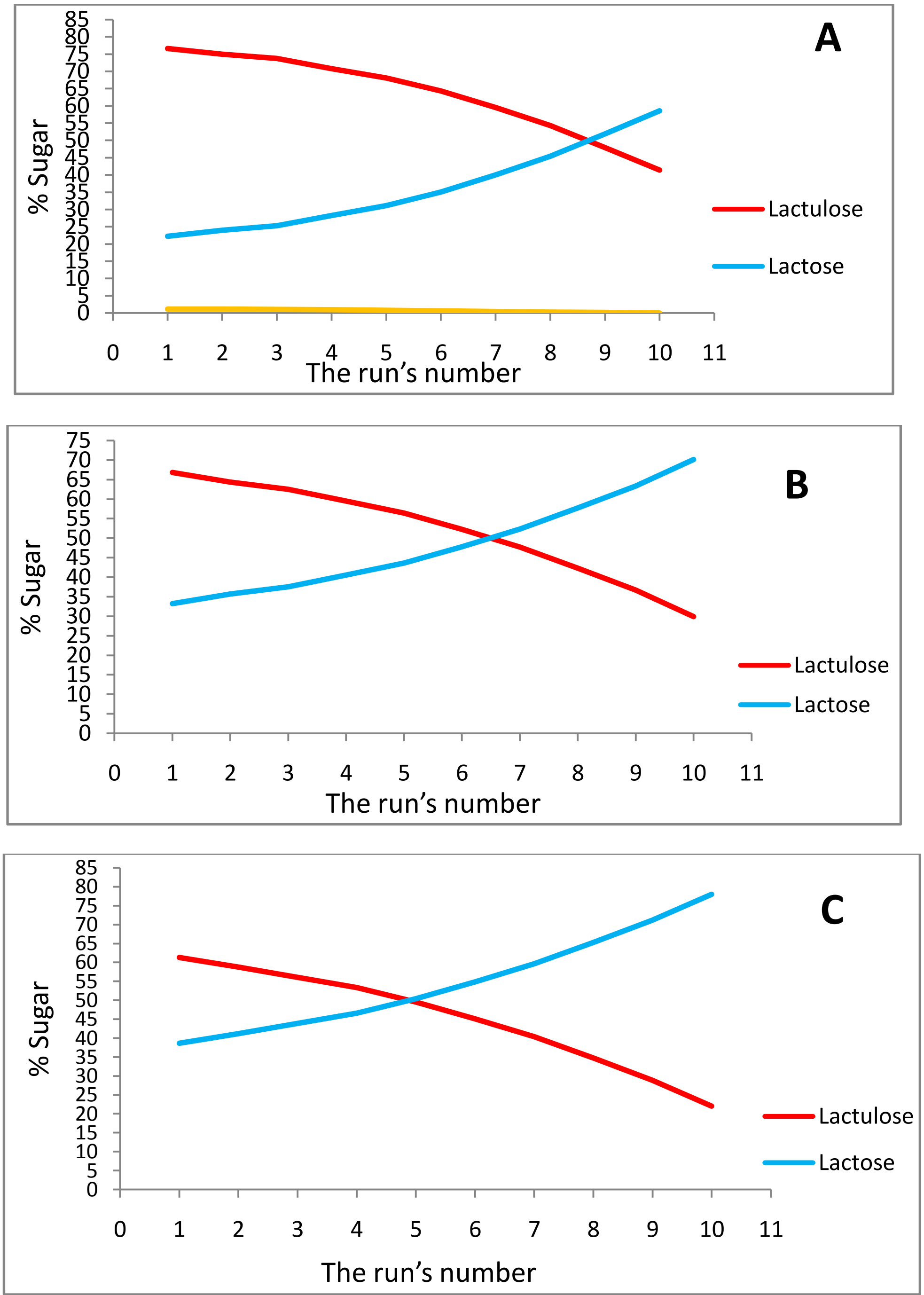


Fig.5: The effect of 3M sodium aluminates on the formation of lactulose by using sweet whey of 4.6 % lactose at 30˚C , (A) 1mL/min ,(B) 2 mL/min and (C) 3m L/min.

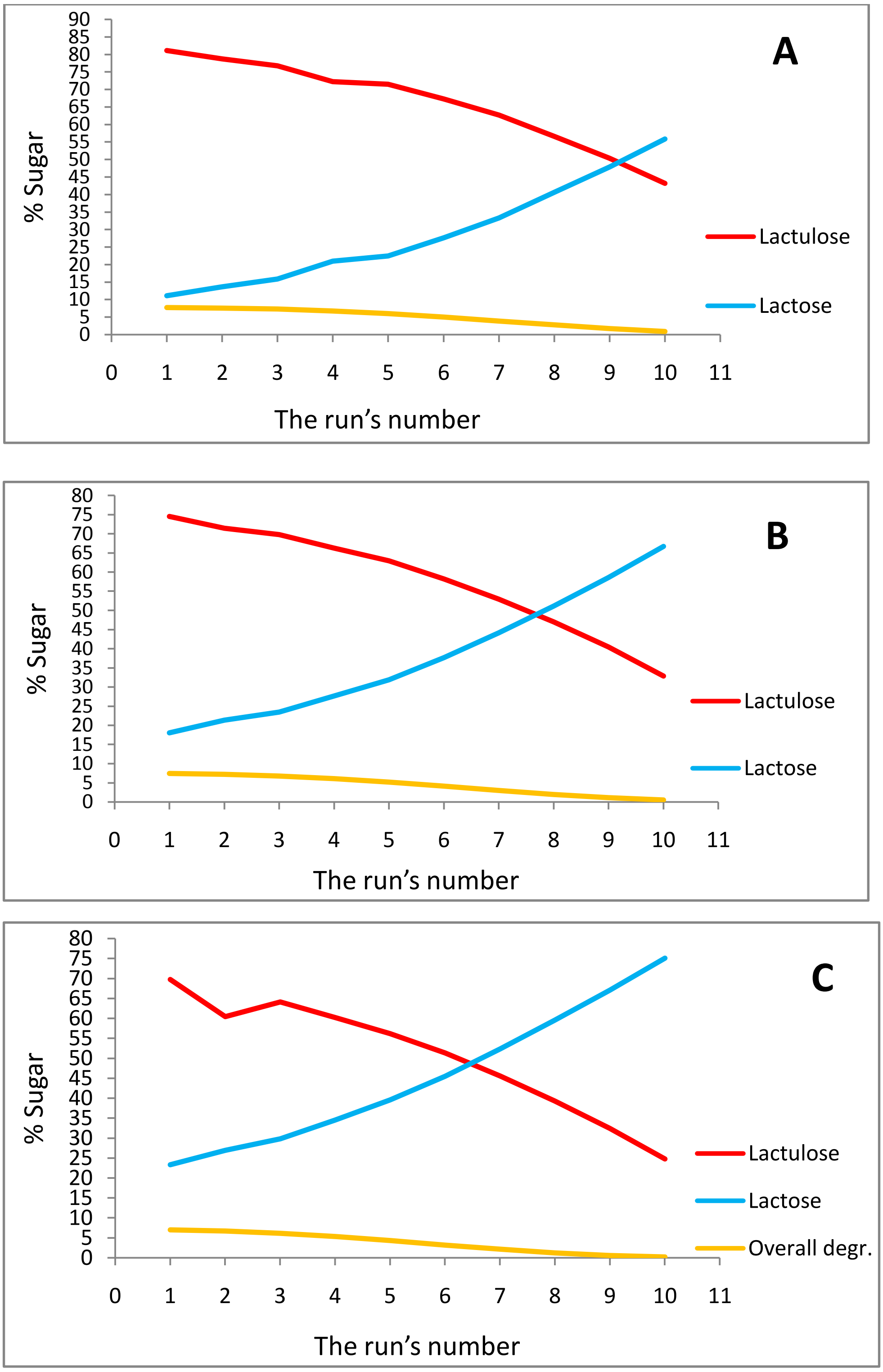


Fig.6 : The effect of 3M sodium aluminates on the formation of lactulose by using sweet whey of 4.6 % lactose at 70˚C , (A) 1mL/min ,(B)2 mL/min and (C) 3m L/min.

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