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Ethanol Bio-Production from Ricotta Cheese Whey by Several Strains of the Yeast *Kluyveromyces*

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ABSTRACT

Studies describing the ethanol bio-production from ricotta cheese whey still are few and this limits the development of an industrial bioprocess. The aim of this study was to compare the ethanol productivity by several strains of the yeast *Kluyveromyces*, from non-sterilized and sterilized ricotta cheese whey, named respectively in this study like Non Heat Treated (NHT) and Heat Treated (HT), in order to select the most suitable strain for lactose conversion of this whey into ethanol and check the possible interference of the thermal treatment from ricotta cheese whey in the ethanol productivity. The yeasts *K. marxianus* ATCC 16045, *K. marxianus* ATCC 46537, *K. marxianus* ATCC 12424, *K. marxianus* CBS 6556 and *K. lactis* ATCC 24176 were used in this study. The yeast *K. marxianus* ATCC 16045, *K. marxianus* ATCC 46537 and *K. marxianus* ATCC 12424 showed the highest efficiency of ethanol production (η) in HT ricotta cheese whey. The maximal growth rate (μ_{max}) and cell mass productivity (Q_X) values were found to be highest by *K. marxianus* ATCC 12424 from HT ricotta cheese whey, whereas the ethanol productivity (Q_P), yield coefficient for ethanol on cell mass ($Y_{P/X}$) and efficiency of ethanol production were the highest by *K. marxianus* ATCC 46537 from HT ricotta cheese whey. The yeast strains *K. marxianus* CBS 6556 and *K. lactis* ATCC 24176 from both NHT and HT ricotta cheese whey showed the lowest values for the growth kinetics determinants and conversion rate parameters for the ethanol production. In the evaluated culture conditions, the yeast *K. marxianus* ATCC 46537 from HT ricotta cheese whey showed the best results for ethanol production.

Key words: Bio-ethanol, ricotta cheese whey, thermal treatment, *Kluyveromyces marxianus*

INTRODUCTION

Cheese whey is the principal pollutant in dairy wastewaters and the most contaminated waste generated in the production of cheese (Carvalho *et al*., 2013; Rajeshwari *et al*., 2000). One of the most economical ways used by the dairies to the utilization of cheese whey generated is the

production of ricotta cheese (Pintado *et al.*, 2001). The ricotta cheese production process is based on protein precipitation of the cheese whey by heat and organic acids. However, this process generates another by-product, named ricotta cheese whey, scotta, cottage cheese whey or second cheese whey (Carvalho *et al.*, 2013; Farkye, 2004).

The ricotta cheese whey is also considered an important pollutant of the dairy, by both volume generated and the potential polluter. According to Mills (1986), to each 1 kg of ricotta cheese are needed of 15-20 L cheese whey and of 14-19 L of ricotta cheese whey are produced. Actually, part of the ricotta cheese whey is used as supplement feed for livestock. However, most are not used by dairy. The disposal of this whey remains a significant problem for the dairy industry. If is discarded prior to any treatment, causes extensive environmental damage, mainly due to its high Biological Oxygen Demand (BOD) of about 50,000 mg L^{-1} of O_2 and Chemical Oxygen Demand (COD) of about 80,000 mg L^{-1} of O_2. If the ricotta cheese whey is incorporated into these wastewater industries, increases the organic matter content of this environment, so the wastewater treatment it could be too expensive, particularly in the case of small cheese plants. Considering that lactose is the major constituent (70% of the total solids) of ricotta cheese whey, contributing significantly for the high BOD and COD, is justified the search for alternatives aimed at minimizing the environmental impacts based on the conversion of this sugar, through fermentative processes, in a value-added product, thus achieving two objectives simultaneously; reducing the pollution potential and valorization of the ricotta cheese whey (Carvalho *et al.*, 2013; Pisponen *et al.*, 2013; Sansonetti *et al.*, 2009, 2010a, b).

The researches of alternative biofuels production have been quite significant in recent years, mainly due to concern with the exhaustion of fossil fuel reserves, for example, the petroleum. The most promising renewable fuels highlights the ethanol (Christensen *et al.*, 2011; Zafar *et al.*, 2005). Nowadays, most of the worldwide ethanol is obtained by fermentation of vegetable biomasses, especially cereals and sugar cane, causing problems related to soil availability and its overexploitation. It is, therefore, necessary to identify alternative renewable and non-vegetable sources for biofuels production. The development of new technologies for the biofuels production, such as ethanol, utilizing by-products of food industry contributes to increase and diversify the alternative energy sources (Sansonetti *et al.*, 2009, 2010a, b).

Several authors during the last 30 years have been studying widely the ethanol production through lactose fermentation from cheese whey. For example, Wang *et al.* (1987) studied the effects of multiple substrates in ethanol fermentations by *Kluyveromyces marxianus* CBS 397 from cheese whey containing lactose, glucose and galactose. In order to produce ethanol from cheese whey at 45°C, Banat *et al.* (1996) used a thermotolerant *K. marxianus* IMB 3 yeast strain. Already, Longhi *et al.* (2004) studied growth and ethanol production by *K. marxianus* in a batch reactor using cheese whey as the substrate and the developed mathematical model with the estimated coefficients was found to be suitable for predictions of the system behavior and process optimization. More recently, Christensen *et al.* (2011) in the search for appropriate conditions to ethanol production, tested different treatments from cheese whey, like pasteurization, cooling, freezing and storage at room temperature, checking the possible interference in the ethanol productivity. As a result of these various researches, in some countries, such as Denmark, Ireland, New Zealand and United States, there are established industrial-scale plants to produce ethanol from cheese whey (Guimaraes *et al.*, 2010).

Nevertheless, in the scientific literature, studies describing the ethanol bio-production from ricotta cheese whey still are few and this limits the development of an industrial bioprocess. The study by Sansonetti *et al.* (2009) investigated the possibility of using ricotta cheese whey as a source for ethanol bio-production. Posteriorly, the same authors evaluated the effects of different operating parameters, such as temperature, pH, agitation rate and initial lactose concentration, in the bio-conversion process of ricotta cheese whey into ethanol by batch fermentation (Sansonetti *et al.*, 2010a, b). Moreover, Saraceno *et al.* (2010) developed a kinetic model to describe batch fermentation of lactose from ricotta cheese whey in ethanol. Finally, Sansonetti *et al.* (2011) proposed a biochemically structured model to describe the conversion of lactose from ricotta cheese whey into ethanol under anaerobic conditions in batch fermentation.

In these studies, the lactose bio-conversion experiments from ricotta cheese whey in ethanol were performed always by the same yeast strain, named *K. marxianus* var. *marxianus* CBS 397. However, according to Grba *et al.* (2002), in order to achieve a good utilization of lactose from whey, it is important to choose a yeast strain with suitable physiological characteristics for the ethanol production. These authors used five different *K. marxianus* strains for alcoholic fermentation from deproteinized whey and observed that the highest ethanol yields were obtained with *K. marxianus* VST 44 and ZIM 75. Moreover, Ozmihci and Kargi (2007) compared performances of three different *K. marxianus* strains for ethanol fermentation from cheese whey powder solution in order to select the most suitable strain. On the other hand, Barba *et al.* (2001) established the kinetic modeling of *K. lactis* growth on raw cheese whey to fermentation processes carried out in batch and fed-batch mode.

So, the aim of this study was to compare the ethanol productivity by several strains of the yeast *Kluyveromyces* from non-sterilized and sterilized ricotta cheese whey, named respectively in this study like Non Heat Treated (NHT) and Heat Treated (HT), in order to select the most suitable strain for lactose conversion of this whey into ethanol and check the possible interference of the thermal treatment from ricotta cheese whey in the ethanol productivity.

MATERIALS AND METHODS

Microorganisms: Five strains of *Kluyveromyces* were used in this study. *K. marxianus* ATCC 16045, *K. marxianus* ATCC 46537, *K. marxianus* ATCC 12424 and *K. lactis* ATCC 24176 were provided by American Type Culture Collection (Manassas, VA, USA). *K. marxianus* CBS 6556 was obtained from The Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (Utrecht, The Netherlands). Previously to culture, the strains were recovered from frozen stocks in 20% (v/v) glycerol at -80°C and were kept at 4°C on Sabouraud agar (Merck, Darmstadt, Germany) plates.

Culture medium: Ricotta cheese whey kindly provided by a local dairy industry (Comercial Lac Max, Rio Grande do Sul, Brazil) was used as the culture medium. In all the ethanol production, experiments was employed the same batch of ricotta cheese whey. It was determined the average composition of this ricotta cheese whey. For each strain was evaluated both the non-sterilized (named like Non Heat Treated (NHT)) ricotta cheese whey, in other words, not subjected to any other pre-treatment but those normally carried out in the production plant and sterilized (named like Heat Treated (HT)) ricotta cheese whey by autoclaving at 121°C for 15 min. The ricotta cheese whey contained 47.65 g L^{-1} of initial lactose. The initial pH of ricotta cheese whey medium was adjusted to 4.5.

Inoculum preparation: Inoculum were prepared in 250 mL Erlenmeyer flasks filled with 50 mL of Sabouraud Broth (Merck, Darmstadt, Germany) and inoculated with a single colony from a stock culture. Strains were cultivated in an orbital shaker at 100 rpm, 30°C and grown to Optical Density (OD) of 1.0 at 600 nm (approximately 18 h). This culture procedure was then used as the standard inoculum preparation for all experiments.

Cultivation procedures: Batch cultivations were performed in 250 mL Erlenmeyer flasks containing 50 mL of ricotta cheese whey medium and inoculated with 5 mL of the standard inoculum. Flasks were incubated in an orbital shaker at 150 rpm, 34°C for 52 h. The cultivation runs were monitored through periodic sampling in order to determine the cell growth, lactose consumption, ethanol production and pH value. All experiments were carried out in duplicates and mean values were given.

Analytical determinations: The cultivated medium samples were centrifuged at 3,500 g for 20 min at 4°C. The cell-free supernatant of the culture medium was used for lactose, ethanol and pH determinations and the remaining solid was used for analysis of cells mass. Lactose concentration was determined by the dinitrosalicyclic acid (DNS) method for reducing sugars as described by Miller (1959). Ethanol concentration was estimated using a Biochemistry Analyzer YSI Model 2700 Select (YSI Incorporated, Ohio, USA). The pH of the samples was measured using a Mettler-Toledo pH meter (Mettler-Toledo International Inc., Greifensee, Switzerland). The cells mass concentration was determined as dry cell weight. Cell pellet was washed twice with cold distilled water and cells were dried in pre-weighed plastic tubes at 80°C to a constant weight in vacuum ovens.

Twenty different batches of ricotta cheese whey, including the employed in this study, from the same local dairy industry (Comercial Lac Max, Rio Grande do Sul, Brazil), were analyzed in relation to lactose concentration, according to Miller (1959), moisture, protein, fat, ash and titratable acidity contents, according to Latimer (2012), pH in a Mettler-Toledo pH meter (Mettler-Toledo International Inc., Greifensee, Switzerland) and COD according to procedure of APHA, AWWA and WEF (2012).

All analyzes were performed in triplicate.

Determination of kinetic parameters: The cultivation parameters of growing and ethanol production by strains of the yeast *Kluyveromyces* from NHT and HT ricotta cheese whey were calculated at the time course of the experiments. The specific growth rate (μ_{max}, h^{-1}) was calculated as slope of ln X (X is cell mass, g L^{-1}) verses time of cultivation. The cell mass productivity (Q_X, g cells/L.h) was defined as the ratio between cell mass concentration (g L^{-1}) and cultivation time (h) (Eq. 1). The substrate consumed (Q_S, g consumed lactose/L.h) was defined as the ratio between consumed lactose concentration (g L^{-1}) and cultivation time (h) (Eq. 2). The ethanol productivity (Q_P, g ethanol/L.h) was defined as the ratio between ethanol concentration (g L^{-1}) and cultivation time (h) (Eq. 3). The ethanol yield factor ($Y_{P/S}$, g ethanol/g consumed lactose) was defined as the ratio between the ethanol concentration (g L^{-1}) and consumed lactose (g L^{-1}) (Eq. 4). The cell mass yield factor ($Y_{X/S}$, g cell mass/g consumed lactose) was defined as the ratio between the cell mass concentration (g L^{-1}) and consumed lactose concentration (g L^{-1}) (Eq. 5). The ethanol yield per cell mass ($Y_{P/X}$, g ethanol/g cell mass) was defined as the ratio between ethanol and cell mass concentrations (g L^{-1}) (Eq. 6). The efficiency of ethanol production (η, %) was defined

as the ratio between the ethanol concentration (g L^{-1}) and the maximum theoretical ethanol concentration (g L^{-1}) that could be achieved considering the theoretical value of 0.538 g ethanol per g consumed lactose:

$$Q_X = \frac{(X_f - X_i)}{h} \quad (1)$$

$$Q_S = \frac{(L_f - L_i)}{h} \quad (2)$$

$$Q_P = \frac{(E_f - E_i)}{h} \quad (3)$$

$$Y_{P/S} = -\frac{(E_f - E_i)/(L_f - L_i)}{h} \quad (4)$$

$$Y_{X/S} = -\frac{(X_f - X_i)/(L_f - L_i)}{h} \quad (5)$$

$$Y_{P/X} = \frac{(E_f - E_i)/(X_f - X_i)}{h} \quad (6)$$

where, X_f is the final cell mass concentration (g L^{-1}), X_i is the initial cell mass concentration (g L^{-1}), L_f is the final lactose concentration (g L^{-1}), L_i is the initial lactose concentration (g L^{-1}), E_f is the final ethanol concentration (g L^{-1}), E_i is the initial ethanol concentration (g L^{-1}) and h is the cultivation time (h).

RESULTS AND DISCUSSION

Table 1 shows the average composition of the twenty different batches of ricotta cheese whey analyzed in this study. The ricotta cheese whey employed in all the ethanol production experiments of this study showed the following composition, moisture 94.50% w/v, lactose 47.65 g L^{-1}, proteins 0.45% w/v, ash 0.48% w/v, titratable acidity 1.39% w/v lactic acid, pH 5.23 and DQO 50,420 mg L^{-1}. The fat content of all ricotta cheese whey samples was zero.

Pisponen *et al.* (2013), in order to study ricotta cheese whey as a raw material for the lactose production, determined the composition of 15 samples of this whey. The results of moisture (94.57%) and protein (0.50%) contents were similar (Table 1) but the fat content (0.26%) and pH (5.51) were

Table 1: Average composition of the ricotta cheese whey

Components	Results*
Moisture (% w/v)	94.850±0.89
Lactose (g L^{-1})	44.760±7.31
Proteins (% w/v)	0.490±0.17
Ash (% w/v)	0.510±0.11
Titratable acidity (% w/v lactic acid)	2.030±1.08
pH	5.100±0.49
COD (mg O_2 L^{-1})	51.948±12.426

*Results are the mean±standard deviation of twenty samples, COD: Chemical oxygen demand

higher than those obtained in the present study (Table 1). The fat content of the ricotta cheese whey is influenced by milk added to cheese whey during ricotta production, such as the ratio of milk to cheese whey and the characteristics of milk. The pH value of the ricotta cheese whey depends of both amount and type of the organic acid employed in the ricotta production (Pintado *et al.*, 2001).

According to the Table 1, the protein (0.49%) and ash (0.51%) contents obtained in this study were higher and lower, respectively, 0.15-0.22% and 1.0-1.3%, than in ricotta cheese whey used by Sansonetti *et al.* (2009) to produce ethanol employing the yeast *K. marxianus* var. *marxianus* CBS 397. The protein content depends on process parameters of protein precipitation in ricotta manufacture, such as time and temperature coagulation and type and amount of the organic acid employed in the ricotta cheese production process (Mahran *et al.*, 1999). Adding of salt during ricotta production increases the salt content and therefore of ash, of the ricotta cheese whey (Pisponen *et al.*, 2013).

The lactose content (47.65 g L^{-1}) of ricotta cheese whey, used in this study to ethanol bio-production is similar to value of 48-50 g L^{-1} of scotta employed by Sansonetti *et al.* (2010a), to bio-conversion process in ethanol by the yeast *K. marxianus* var. *marxianus* CBS 397.

The average lactose content (44.76 g L^{-1}) (Table 1) of ricotta cheese whey is similar to those reported for cheese whey. Christensen *et al.* (2011), aiming to find the best way of utilizing whey and to design a process for conversion of organic whey into bioethanol by fermentation using *K. marxianus*, determined the chemical composition of raw whey taken from the cheese manufacturing process and stored cold (2-5°C) and verified a lactose content of 46.8 g L^{-1}. Yorgun *et al.* (2008) characterized the curd cheese whey in order to investigate the treatment of this whey by using different membrane processes namely ultrafiltration, nanofiltration and reverse osmosis to produce cleaner discharge and determined a lactose content of 43.92 g L^{-1}. According to Carvalho *et al.* (2013), in spite of during the process of the second flocculation, carried out to obtain the ricotta cheese, occur the formation of agglomerates that trap suspended solids, proteins and fats, the ricotta cheese whey still keeps a high lactose concentration similar to that observed in cheese whey.

The COD average result (51, 948 mg O_2 L^{-1}) (Table 1) is similar to verified by Janczukowicz *et al.* (2008). These authors studied the wastewater originated in selected sections of the dairy processing line and found COD of 58,550 mg O_2 L^{-1} to second cheese whey.

The kinetics of batch cultivation of ethanol production by five strains of the yeast *Kluyveromyces* from both Non Heat Treated (NHT) and Heat Treated (HT) ricotta cheese whey were studied in detail. The growth kinetics determinants and conversion rate parameters for the ethanol production by strains of the yeast *K. marxianus* ATCC 16045, *K. marxianus* ATCC 46537, *K. marxianus* ATCC 12424, *K. marxianus* CBS 6556 and *K. lactis* ATCC 24176 from both NHT and HT ricotta cheese whey at the time course of the batch cultivation process are presented in Table 2.

At the exponential phase the cells have adjusted to the new environment and the net specific growth of *K. marxianus* ATCC 46537 and ATCC 12424 reached the maximum value of 0.35 and 0.37 h^{-1}, respectively, during the exponential phase in HT ricotta cheese whey. The maximal growth rate (μ_{max}) and cell mass productivity (Q_X) values were found to be highest by *K. marxianus* ATCC 12424 from HT ricotta cheese whey, whereas the ethanol productivity (Q_P), yield coefficient for ethanol on cell mass ($Y_{P/X}$) and efficiency of ethanol production (η) were the highest by *K. marxianus* ATCC 46537 from HT ricotta cheese whey. The yeast strains *K. marxianus* CBS 6556

Table 2: Cultivation parameters of growing and ethanol production by strains of the yeast *Kluyveromyces* from both Non Heat Treated (NHT) and Heat Treated (HT) Ricotta Cheese Whey (RCW)

Yeast/thermal treatment RCW	μ_{max} (h^{-1})	Q_X (g L^{-1}.h)	Q_S (g L^{-1}.h)	Q_P (g L^{-1}.h)	$Y_{P/S}$ (g g^{-1})	$Y_{X/S}$ (g g^{-1})	$Y_{P/X}$ (g g^{-1})	η (%)
***Kluyveromyces marxianus* ATCC 16045**								
NHT	0.22±0.01	0.22±0.01	4.52±0.12	0.41±0.01	0.38±0.01	0.85±0.03	3.08±0.11	42.93±2.69
HT	0.20±0.01	0.20±0.01	2.11±0.08	0.58±0.01	0.52±0.02	0.27±0.01	3.07±0.10	58.93±3.02
***Kluyveromyces marxianus* ATCC 46537**								
NHT	0.32±0.01	0.27±0.01	7.19±0.55	1.26±0.06	0.29±0.00	0.10±0.00	4.60±0.15	53.52±3.15
HT	0.35±0.02	0.29±0.01	4.75±0.31	1.31±0.03	0.40±0.01	0.17±0.00	5.99±0.23	68.92±3.34
***Kluyveromyces marxianus* ATCC 12424**								
NHT	0.35±0.01	0.33±0.01	7.54±0.29	0.74±0.04	0.30±0.01	0.10±0.00	4.16±0.19	52.48±2.12
HT	0.37±0.01	0.35±0.01	3.88±0.13	0.88±0.02	0.34±0.01	0.37±0.01	4.18±0.02	58.15±2.89
***Kluyveromyces marxianus* ATCC 6556**								
NHT	0.18±0.00	0.12±0.00	3.05±0.11	0.01±0.00	0.04±0.00	0.18±0.00	0.66±0.01	4.44±0.25
HT	0.13±0.00	0.15±0.00	2.27±0.08	0.01±0.00	0.02±0.00	0.23±0.00	0.16±0.00	4.68±0.32
***Kluyveromyces lactis* ATCC 24176**								
NHT	0.24±0.02	0.10±0.00	2.73±0.04	0.02±0.00	0.04±0.00	0.17±0.00	0.69±0.01	3.45±0.22
HT	0.19±0.01	0.08±0.00	0.77±0.02	0.01±0.00	0.01±0.00	0.35±0.01	0.03±0.00	1.71±0.09

μ_{max}: Maximal growth rate, Q_X: Cells mass productivity, Q_S: Consumed lactose, Q_P: Ethanol productivity, $Y_{P/S}$: Yield coefficient for ethanol over lactose, $Y_{X/S}$: Yield coefficient for cells mass over lactose, $Y_{P/X}$: Yield coefficient for ethanol over cells mass, yield coefficients were calculated using values of ΔX, ΔS and ΔP (difference between maximal and initial values)

and *K. lactis* ATCC 24176 from both NHT and HT ricotta cheese whey showed the lowest values for the growth kinetics determinants and conversion rate parameters for the ethanol production. According to Fonseca *et al.* (2008) and Schaffrath and Breunig (2000), some microbial strains able to convert whey to ethanol cannot tolerate high levels of alcohol and others strains cannot metabolize lactose from the whey. And besides, whey contains proteins, lipids, mineral salts and other compounds which could affect yeast growth and lactose fermentation. Therefore, it is important to select microorganisms that are not pathogenic, are able to use lactose and the others compounds from whey, produce ethanol at high rates and in short time and, at the same time, are resistant to high ethanol concentration (De Felice *et al.*, 2012).

Among the five *Kluyveromyces* yeast strains evaluated, four of them (*K. marxianus* ATCC 16045, *K. marxianus* ATCC 46537, *K. marxianus* ATCC 12424 and *K. marxianus* ATCC 6556) showed the highest efficiency of ethanol production (η) in HT ricotta cheese whey by autoclaving at 121°C for 15 min. Probably in the experiments performed with ricotta cheese whey submitted to thermal treatment, the yeast *K. marxianus* had no problem competing with the live microorganisms present in the non-sterilized ricotta cheese whey, named in this study like Non Heat Treated (NHT) ricotta cheese whey (Christensen *et al.*, 2011).

Figure 1 and 2 show growth pattern, lactose consumption profile, ethanol production and pH variation by *K. marxianus* ATCC 46537 and *K. marxianus* ATCC 12424 from both NHT and HT ricotta cheese whey containing 47.65 g L^{-1} lactose, in the temperature at 34°C, under 150 rpm and initial pH 4.5. When ricotta cheese whey as culture medium is inoculated with *K. marxianus*, the microorganism selectively take up the dissolved nutrients from the medium and convert them into cell mass and ethanol. Growth pattern of *K. marxianus* shows the lag phase, exponential growth phase and stationary phase. Lag phase occurs immediately after inoculation and it takes 4 h for the cells to adapt to a new environment, after starts the linear growth phase.

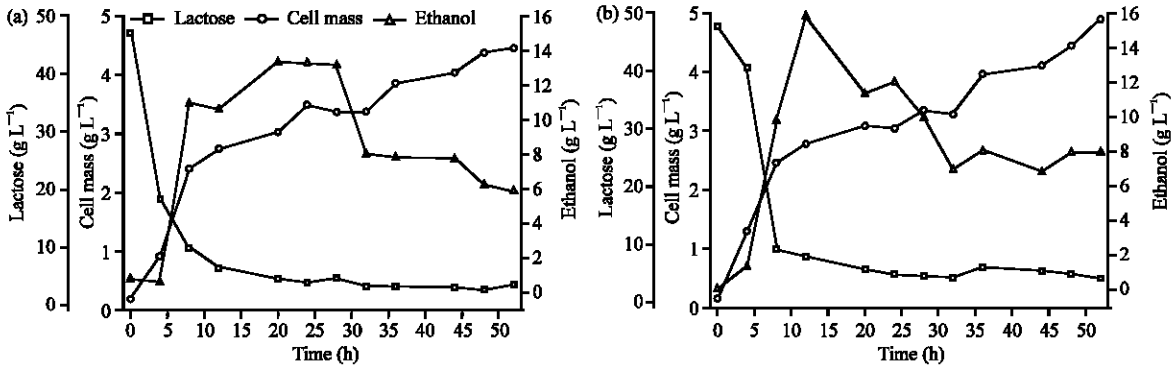

Fig. 1(a-b): Batch cultivation kinetic by strain of the yeast *K. marxianus* ATCC 46537 from both (a) Non-heat-treated (NHT) and (b) heat-treated (HT) ricotta cheese whey containing 47.65 g L^{-1} of initial lactose, in the temperature at 34°C, under 150 rpm and initial pH of 4.5

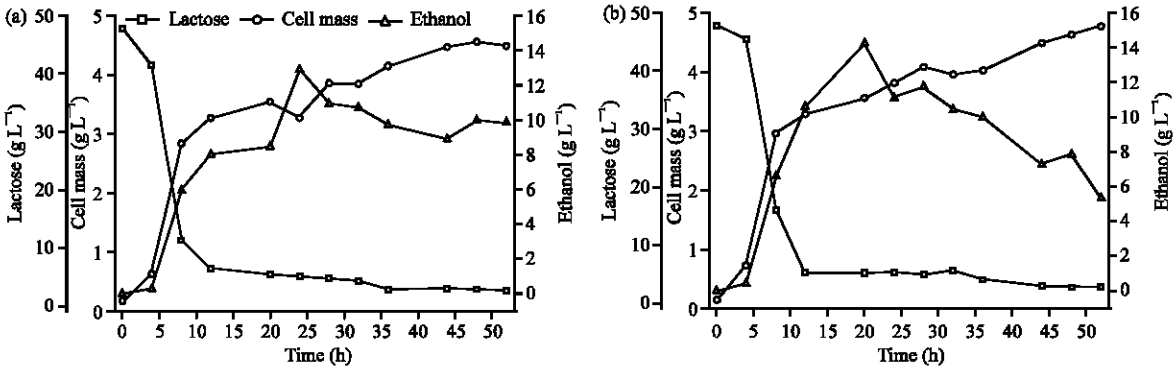

Fig. 2(a-b): Batch cultivation kinetic by strain of the yeast *K. marxianus* ATCC 12424 from both (a) Non-heat-treated (NHT) and (b) Heat-treated (HT) ricotta cheese whey containing 47.65 g L^{-1} of initial lactose, in the temperature at 34°C, under 150 rpm and initial pH of 4.5

For the strain *K. marxianus* ATCC 46537 in NHT ricotta cheese whey (Fig. 1a), the most of the initial lactose (47.65 g L^{-1}) was metabolized by the yeast within 20 h and produced 13.28 g L^{-1} of ethanol and 4.44 g L^{-1} of cell mass. For the same yeast in HT ricotta cheese whey (Fig. 1b) after 12 h of cultivation the most of the initial lactose was consumed, maximum ethanol and cell mass concentrations achieved 15.75 g L^{-1} (68.92% theoretical yield) and 4.87 g L^{-1}, respectively. The strain *K. marxianus* ATCC 12424 in NHT ricotta cheese whey (Fig. 2a) metabolized the most of the initial lactose during first 24 h cultivation and produced 12.85 g L^{-1} ethanol and 4.55 g L^{-1} cell mass. Already in HT ricotta cheese whey (Fig. 2b) the same yeast consumed the most of initial lactose after 20 h of cultivation, both ethanol and cell mass concentrations reached 14.20 (58.15% theoretical yield) and 4.76 g L^{-1}, respectively. These findings show that the heat treatment of ricotta cheese whey by autoclaving at 121°C for 15 min resulted in a higher ethanol concentration in a shorter cultivation time, probably due to the absence of competitors microorganisms. The highest ethanol concentration obtained (15.75 g L^{-1}) is 7% higher than that

observed by Sansonetti *et al.* (2010a) from ricotta cheese whey by *K. marxianus* var. *marxianus* CBS 397, on batch cultivation performed at 37°C, pH controlled in 5, under 300 rpm and 47.16 g L^{-1} of initial lactose.

Studies cited in the literature evaluating the ethanol production by the yeast *K. marxianus* from cheese whey in batch cultivation processes verified results of cell mass concentration of about two times higher than the maximum values shown in Fig. 1 and 2. However, maximum ethanol production results of these same studies were 3-8 times lower than those obtained in this study (Fig. 1 and 2) from ricotta cheese whey. The *Kluyveromyces marxianus* strain MTCC 1288 from crude cheese whey containing 35 g L^{-1} of lactose at 34°C and pH maintained at 4.5 produced 2.10 g L^{-1} of ethanol and 8.9 g L^{-1} of cell mass (Zafar and Owais, 2006). The ethanol production from medium containing 50 g L^{-1} of lactose by the same strain was 3.98 g L^{-1} and cell mass reached 10.34 g L^{-1}, in the same temperature and pH conditions (Zafar *et al.*, 2005). Probably in these conditions, the *K. marxianus* utilized the lactose consumed to cell growth and in this study from ricotta cheese whey the lactose consumed was employed to ethanol production. According to Zafar and Owais (2006), the overall lactose consumption is employed for different cellular functions, including growth and for the production of ethanol and other products.

CONCLUSION

Among the five strains of *Kluyveromyces* (*K. marxianus* ATCC 16045, *K. marxianus* ATCC 46537, *K. marxianus* ATCC 12424, *K. marxianus* CBS 6556 and *K. lactis* ATCC 24176) evaluated in this study to ethanol production from ricotta cheese whey, the yeast *K. marxianus* ATCC 46537 in heat-treated ricotta cheese whey showed the best results for ethanol volumetric production, ethanol productivity (Q_P) and efficiency of ethanol production (η). The yeast strains *K. marxianus* CBS 6556 and *K. lactis* ATCC 24176 from both non-heat-treated and heat-treated ricotta cheese whey showed the lowest values for the growth kinetics determinants and conversion rate parameters for the ethanol production. The others three yeast strains evaluated (*K. marxianus* ATCC 16045, *K. marxianus* ATCC 46537 and *K. marxianus* ATCC 12424) showed the highest efficiency of ethanol production (η) in heat-treated ricotta cheese whey by autoclaving at 121°C for 15 min. In the evaluated culture conditions, the yeast *K. marxianus* ATCC 46537 from heat-treated ricotta cheese whey showed the best results for ethanol production.

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