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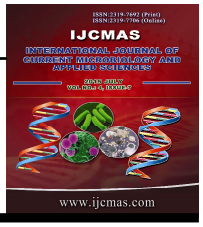

Original Research Article

Utilization of Whey as One Dairy Industrial Waste in the Production of Alcohol

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A B S T R A C T

Production of ethanol from whey and milk permeate by *Kluyveromyces marxianus* was investigated. Utilized sugar percentages in whey and milk permeate were 3.94 and 5.17 % after 48-h fermentation period. Ethanol yield revealed that the best substrate for ethanol production is whey (85.81%) followed by milk permeate (67.85%) and this parallel with the biomass products.

Introduction

Whey is a by-product of cheese making and casein production. It has a high 5-day Biochemical Oxygen Demand (BOD 5) ranges from 35,000 to 60,000 mg/l (Gardner, 1989) which is caused by its high organic matter content; protein and carbohydrate. Whey creates a problem when disposed as wastewater (Fonseca *et al.*, 2008). Of concern too is the fact that a great deal of milk permeates (MP) is now derived as by product from UF processing counted to the problem (Ali and Roushdy, 1998). About one-half of the total whey produced worldwide is disposed of as waste effluent, dumped onto the land or into water systems (Zall, 1984). Ethanol has several applications in chemistry, pharmaceutical and food industries as a form of raw materials, solvents and fuel. Ethanol production worldwide in 2011 reached 23.4 billion U.S. Gallons, where 80% of that are produced by fermentation (Ariyanti and Hadiyanto, 2013). The main objective of this investigation was to study ethanol production from cheese whey and milk permeate by the yeast strain, *Kluyveromyces marxianus*.

Materials and Methods

Yeast strain studied

The yeast strain used in this study *Kluyveromyces marxianus* ATCC 36142 American type culture collection were obtained from Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources center (Cairo Micrcen) Faculty of agriculture, Ain Shams University, these strains were activated

individually on slant agar followed activation on broth potato dextrose agar according to American Public Health Association APHA (1994).

Cheese whey

Karish cheese whey 0.5 % salt was obtained from Dairy of milk Food Technology Institute, Agriculture Research Center, Ministry of Agriculture.

Milk permeate was obtained from Animal Production Institute, Agriculture Research Center, Ministry of Agriculture.

Chemical analysis

Both cheese whey and milk permeate were analyzed to Xinmin *et al.* (2008). Also, analyzed for pH value, acidity, total nitrogen (T.N.) and water soluble nitrogen (W.S.N) according to Ling (1963). Total solids (T.S.) and ash were analyzed according to A.O.A.C. (2000). Total sugar was determined according to Miller, (1972). Ethanol was carried out by high Performance liquid chromatography (HPLC), using an ion exclusion column Aminex HPX-87 (Bio-Rad), Kept at 60 °C. The eluant for separation was 5 mM H_2SO_4, applied at an elution rate of 0.7 ml/min^{-1}. The column was coupled to the refractive index detector HP 1047 A.

Microbiological method

Biomass determination

The cells from a known volume of cheese whey and milk permeate fermentation media were separated by centrifugation at 5000 rpm for 20 minutes carefully, the clear broth was discarded and the cell paste was scraped from the centrifuge tube into a Whatman paper (weighing pan). The centrifuge tube was rinsed with a few ml of distilled water. The rinse water was poured into the weighing pan, as well. The cell paste was dried in an oven test at 50 °C for 2hr. The weigh of the pan plus the cell paste was measured periodically until there is no further decrease in the dry weight. The difference in the weight was calculated and its dry weight was expressed in percent.

Preparation of whey and milk permeat substrate media

Whey and milk permeate contained lactose at 1.26 and 3.23 %, respectively (Table 1). Both substrates are supplemented with glucose to reach sugar content of 15 % (Dosuky, 2009). Also, ammonium sulfate and yeast extract (0.45 %, 0.1 %) were added (Dessy and Hadiyanto, 2013).

The substrates were subjected to heat treatment at 110°C for 10 min, cooled to 32°C, inoculated with *Kluyveromyces marxianus* at 5 % and incubated at 32°C for 3 days (Abd-allah, 2014).

Results and Discussion

Composition

Table 1 presents the properties of whey and milk permeate substrates. pH and acidity (%) of whey valued 3.7 and 0.93 %, whereas the corresponding values for milk permeate were 6.5 and 0.09 %. Ash content and total protein (T.P., %) of whey were 4.7 and 1.9 times of these values for milk permeate. Also, soluble protein and lactose in milk permeate counted 15 and 2.5 times of those values of whey.

Table 2 and figure 1 show the changes in pH value and acidity (%) on the whey and milk permeate during fermentation period. On whey samples, pH value declined from 3.76 to 3.68; while in milk permeate decreased from 6.03 to 4.60 at the end of fermentation

period. On the other hand, the acidity percent in whey increased from 0.90 to 1.25 % and in milk permeate from 0.090 to 0.44% at the end of the fermentation. These results also explain that the acidifying capacity of whey could provide suitable environmental medium for growth of acidophilic yeast. These results are in accordance with those obtained by Gupte and Nair (2010).

Biomass production

The initial biomass production (Table 3) on whey was 6.85 g/l and increased gradually up to 48 h, while it was 5.85 g/l in the milk permeate and decreased with the progress of fermentation time up to the previous hours. The results indicate whey superiority as substrate for the production of yeast biomass. These results are in agreement with those obtained by Grba *et al.* (2002).

Sugar utilization

Table 3 presents the utilization of sugar by *Kluyveromyces marxianus* during the fermentation period. On whey substrate, sugar content decline from 12.59 to 8.65 % after 72- h fermentation period and the utilized sugar represented 3.94 %. In the case of milk permeate; the utilized sugar valued 5.17 %. The differences in sugar utilization of *Kluyveromyces marxianus* in whey and milk permeate could reflect the compositional variation and properties of the substrates (Table 1).

Table.1 Whey and milk permeate properties

Properties	Whey	Milk permeate
pH	3.7	6.5
Acidity (%)	0.93	0.09
T.S. (%)	6.82	5.97
Ash (%)	1.632	0.343
T.P. (%)	5.17	2.67
S.P. (%)	0.016	0.25
Lactose (%)	1.26	3.23

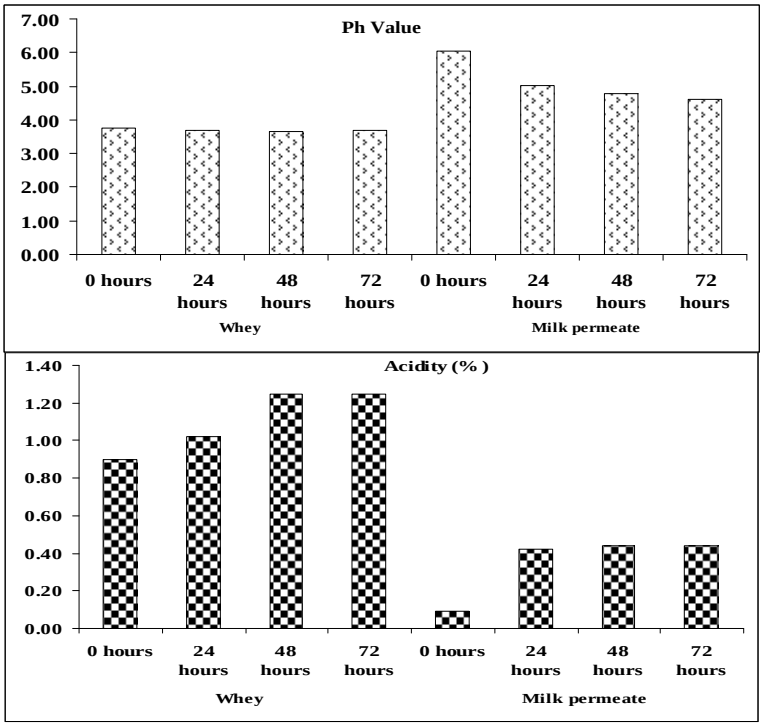
Table.2 Changes in PH values and acidity (%) of whey and milk permeate during fermentation period

Substrate	**Fermentation time (h)**	**pH Value**	**Acidity (%)**
Whey	0	3.76	0.90
	24	3.68	1.02
	48	3.65	1.25
	72	3.68	1.25
Milk permeate	0	6.03	0.09
	24	5.03	0.42
	48	4.79	0.44
	72	4.60	0.44

Table.3 Ethanol production by *Kluyveromyces marxianus* using whey and milk permeate

Substrate	Fermentation time (h)	Biomass g/l	Sugar content (%)	Utilized sugar (g/100 ml)	Ethanol kinetics production		Growth rate
					ml/100	Yield (%)	
Whey	0	6.85	12.59	-	0.05	-	-
	24	7.25	11.52	1.07	1.32	12.35	0.06
	48	7.60	9.76	2.83	2.33	82.40	0.08
	72	6.35	8.65	3.94	3.38	85.81	0.07
Milk permeate	0	5.85	12.77	-	0.04	-	-
	24	3.15	10.44	3.33	0.90	38.71	0.09
	48	2.00	9.23	3.54	1.72	48.61	0.05
	72	2.80	7.60	5.17	3.51	67.83	0.05

Fig.1 Changes in pH values and acidity percent of whey and milk permeate during fermentation period



Ethanol production

Ethanol production (Table 3) was determined by comparing batch cultures of *Kluyveromyces marxianus* grown on whey and milk permeate. On whey as a substrate, the utilized sugar (%) was 31.29 %, while on milk permeate utilized sugar was 40.48 after 72- h fermentation period. Concerning the Ethanol kinetics production, Ethanol yield on whey and milk permeate was 85.81 and 67.85%, respectively.

Growth rate

Furthermore, Table 2 indicates that growth rate as determined by biomass production to utilized sugar of *Kluyveromyces marxianus* in whey and milk permeate is strongly related to the production of ethanol yield (Dessy and Hadiyanto, 2013).

From these results, it can clearly stated that whey as a substrate was favorable for ethanol production than milk permeate (Grba *et al.,* 2002). It is possible to use whey as a substrate for the production of ethanol by *Kluyveromyces marxianus* than milk permeate.

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