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STUDIES ON THE PRODUCTION OF
LACTIC ACID FROM WHEY

By
NAGWA EMAM AHMED SULTAN

THESIS

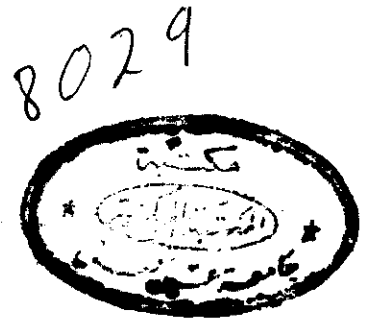
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This Thesis for the Ph. D. Degree has been approved by :

Muhammad R. Haghmoud

A. Mohamed A. G. Hassan

[Signature]

Committee in Charge

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May the Lord Rest His Soul.

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INTRODUCTION

Whey resulting from cheese making is quite a nutritious medium for lactic acid bacteria growth. Besides it contains some other components which could be ~~some~~ industrial use... eg lactose whey proteins....etc.

Moreover, approximately 9 kg of whey remain after the manufacture of each 1 kg of hard cheese. According to this information, the total production of whey from hard cheese in Egypt can be estimated by about 3258 tons per year based on the annual hard cheese production which amounts to 362 tons (Egyptian commercial Chamber, 1970).

Thus, there is a considerable volume of whey which should be made of instead of ~~disposed~~ of as waste. whey has been suggested as a substrate for use in the manufacture of yeast, alcohols, lactic acid, vitamins, acidic and alcoholic beverages, vinegar and other fermentation products. Some of the suggested uses have found substantial commercial acceptance, whereas others have remained a laboratory curiosity. Use of whey as a substrate for fermentations industry has attracted dairy scientists and dairy

products processors and others who have been faced with the problem of converting the raw material into useful products which can be manufactured and marketed on profitable basis. Production of lactic acid from fermentation of whey may be considered as an important process since a useful product (lactic acid) can be obtained from one which often is of little economic value.

The composition of whey indicates that lactose is the principle carbohydrate to be fermented. Consequently, the availability of a microorganism capable of carrying out the desired fermentation may impose a limitation on the suitability of whey as a substrate. The microorganism used must be able to utilize lactose. Also, the great hindrance to whey utilization in some fermentations is the low concentration of nutrients in whey.

Although whey contains a variety of salts, it is deficient in manganese which markedly improves the growth and activity of certain lactic acid bacteria (Demeter, 1949). In addition, whey is deficient in inorganic nitrogen compounds which must be added or furnished if certain fermentations are to be proceed satisfactorily. Growth and acid

production from whey by lactic acid bacteria are greatly affected by the level of protein present (Ritter, 1953) Addition to whey of malt sprouts or extract, various yeast derivatives, extracts of liver, feces, (Rosell, 1949) accelerates the fermentation, with these facts in view, this work was planned to study :

1) The effect of fortification of whey with some additives as well as treatments of whey with certain proteolytic enzyme on the growth and lactic acid production by *Lactobacillus bulgaricus*.

2) The recovery and properties of the resultant lactic acid.

3) Kinetic parameters of the fermentation under the forementioned conditions.

REVIEW OF LITERATURE

Lactic acid, or alpha-hydroxy propionic acid ($\text{CH}_3\text{-CHOH.COOH}$), as an unnamed component of soured milk must have been known in human experience since the days when man was first had his flocks and herds. Its true nature was discovered by Scheele, who isolated and identified it as the principal acid in sour milk in 1780.

Lactic acid was first discovered as a fermentation product by Blondeau in 1847.

It has commercially been produced in the United States for many, first by Chales E. Avery, at Littleton, Massachusetts, in 1881 (Garrett, 1930) in an attempt to make calcium lactate which was to be used as a substitute for cream of tarter in baking powder. This first venture was unsuccessful and the development of industrial applications in leather, textiles, food and solvents did not start until later. Lactic acid production was likewise developed in Europe and, for many years, much of the highest grade acid used in the United State was imported from Germany. Improved processes in purifying crude acid in the United States, however, had effectively stopped this importation of German acid well before World II.

A. Microorganisms used :

Since lactose is the carbohydrate to be fermented in the whey, the microorganisms used must be able to utilize lactose. Several workers (Burton, 1937, Johnson et al. 1937, Campbell, 1953 and Foster et al. 1961) reported that the culture best suited for production of lactic acid from whey is Lactobacillus bulgaricus. It is homo-fermentative and is able to convert over 90% of lactose to DL-lactic acid. This culture grows rapidly, particularly at 45° to 50°C, and can ferment all the lactose in whey to lactic acid in about 40 hours.

The growth of many cultures of L. bulgaricus is enhanced by associative growth of Mycoderma sp., a film-forming yeast, (Nilsson, 1948 and Swaby 1945).

Stock cultures of these micro-organisms are usually carried as mixtures of the two types. There are large differences in the suitability of various strains for this fermentation.

Therefore, strain having the ability to give the highest yield of lactic acid in shortest time should be

selected. Also, the strains can be selected to accomplish this without associative growth of the film-forming yeast.

However, other organisms suggested for this fermentation are L. acidophilus (Havletko and Knez 1959; Maxova and Maxa 1958; Siman and Mergl 1961); a strain of L. delb-rueckii which was adapted to ferment lactose (Rosell, 1949), a starter consisting of 3 strains of L. lactis, 3 strains of L. bulgaricus and one strain of Streptococcus thermophilus (Surazynski et al., 1967) and, a mixture of L. lactis and L. bulgaricus (Poznanski et al., 1973).

Mention should be made that there are varieties of micro-organisms used for production of lactic acid from other raw materials. For example, production of lactic acid from starch by L. thermophilus (Kitahara and Ishida (1949, 1952), wood sugar by L. pentoaceticus (Allgeier, 1929; Fred and Peterson, 1921), Sulfite waste-liquor by L. pentosus (Fries, 1949) and from dextrose solution containing MgSO_4 , KH_2PO_4 , CaCO_3 and urea by Rhizopus oryzae (Ward et al. 1933, Bernhauer et al. 1950).

B. Production of Lactic acid from whey

The whey which is the by-product of cheese industry contains about 50% of the milk solids (Van Slyke and Price, 1952). However, the composition of whey is variable because cheese making procedures and milk composition are not constant. Whey resulting from the manufacture of cottage or cream cheese contains more lactic acid and correspondingly less lactose than does whey from Cheddar or Swiss cheese manufacture (Marth, 1970). Moreover, Foster et al. (1961), reported that the nutrients present in whey in addition to the lactose usually are sufficient to supply the nitrogen, vitamins and other growth requirements of the selected culture for fermentation. Therefore, whey is considered a potentially important raw material for lactic acid fermentation. This was also based on the quantity of whey produced from cheese industry which can be estimated by about 75-90% of the amount of milk used in this industry. Furthermore, Cable (1971) reported that production of lactic acid from whey may be economically feasible as a means of whey utilization in Australian cheese and casein factories, provided an adequate market is developed and cheaper methods of purification are applied. Marshall (1970) estimated that a daily

alkali, such as calcium hydroxide, at a high temperature and pressure to produce lactic acid. Another interesting method for the synthesis of lactic acid is the heating of carbon monoxide and acetaldehyde in the presence of sulfuric acid at 130° to 200°C under 900 atm. pressure (Loder 1942). In addition to these novel methods for preparing lactic acid, there are several classical methods such as 1) from acetaldehyde and hydrocyanic acid, 2) by action of nitrous acid on alanine, 3) by oxidation of propylene glycol and others.

C. Recovery of lactic acid :

The recovery of lactic acid depends greatly upon the extraction and purification techniques used. The first step in recovering the lactic (i.e. calcium lactate) is to adjust the fermented whey to pH. 7. The whey is then heated to coagulate the whey proteins; the precipitate is filtered off. Marth (1970) reported two methods for the recovery of lactic acid. In the first, calcium lactate is immediately converted to lactic acid by addition of sulphuric acid, CaSO_4 (which was formed) is removed by filtration, and the liquid is concentrated in a vacuum pan to the desired strength of