

A Note – Production of Vinegar from Whey

Javier Parrondo¹, Mónica Herrero¹, Luis A. García¹ and Mario Díaz^{1,2}

ABSTRACT

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Cheese whey supplemented with lactose was employed to produce vinegar. Whey was first transformed into an alcoholic beverage via fermentation with the yeast *Kluyveromyces fragilis*, and then the alcoholic product was employed as a substrate for an acetic acid fermentation. The bacteria used for the acetic acid production process were isolated from a starter culture provided by a vinegar factory. The bacteria employed were classified as *Acetobacter pasteurianus*. The vinegar obtained had a concentration of acetic acid between 5 and 6% (v/v). Ethyl acetate and fusel alcohols (isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol) were detected in the final product. The process and the substrates employed satisfied FAO requirements that the product was acceptable for human consumption. The efficiency of biotransformation of ethanol into acetic acid was 84%.

Key words: Acetic acid, *Acetobacter pasteurianus*, lactose, vinegar, whey.

INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) establishes that vinegar is a liquid allowed for human consumption and that it must be produced from raw materials of agricultural origin, that contain starch and/or sugars, by means of two consecutive fermentations, first an alcoholic fermentation that transforms the sugars into ethanol, and then an acetic fermentation that converts ethanol to acetic acid, the main product of the vinegar.

Vinegar is an astringent product produced primarily from red wine and cider. Other vinegars are obtained from distilled alcohol, white wine, sherry, honey, malt, rice, champagne, and fruits. Vinegars so obtained can be flavoured with tarragon, basil, garlic, lemon, raspberry, etc.

Concentration of acetic acid in commercial vinegar ranges from 5 to 6 g/100 mL (acetic degrees). Vinegar is a seasoning used in vinaigrette, mayonnaise and is employed in the cooking of meat and fish and in the manufacture of canned foods.

In 1997 in the USA the production of vinegar reached 60 millions hL, and this consisted primarily of white wine and cider vinegars. In the European Union production was 4.5–5 million hL/year, of which 1/3 was red wine vinegar. The main vinegar producers are Italy, Spain and France².

¹Department of Chemical Engineering and Environmental Technology, Faculty of Chemistry, University of Oviedo, C/ Julian Clavería, 8; 33071 Oviedo, Asturias, Spain.

²Corresponding author. E-mail: mariodf@correo.uniovi.es

The aim of the present study was to evaluate the production of vinegar from cheese whey supplemented with lactose. Whey lactose was first transformed into ethanol via fermentation with the yeast *Kluyveromyces fragilis*, and then the alcoholic product obtained was employed as a substrate for an acetic acid fermentation. The acetic acid bacteria employed were *Acetobacter pasteurianus* isolated from cider vinegar. The process utilizes an abundant by-product, whey, and lactose which can be purchased at a low price. The resultant vinegar is a product suitable for human consumption.

MATERIALS AND METHODS

Fermentation medium

The medium employed in this study was sweet cheese whey supplemented with food grade lactose. The medium consisted of 78 g of whey powder (RENYLAT 1300), 100 g of lactose, and 1000 g of distilled water. Whey and lactose were provided by Reny Picot (Anleo-Navia, Asturias, Spain).

The medium had a density of 1055 kg/m³, a lactose concentration of 139 kg/m³, and a protein concentration of 9 kg/m³. It was pasteurised before fermentation by heating to 65°C for 30 minutes.

Alcoholic fermentation

Yeast strain *Kluyveromyces fragilis* CECT 1123 was provided by the Spanish Type Culture Collection and was employed for the fermentation. The strain was preserved and cultured as described previously⁶.

Erlenmeyer flasks closed with cotton bungs were employed for cell culturing in batch. Stirred and aerated alcoholic fermentations were carried out at 30°C and 200 rpm in 1000 mL flasks filled with 200 mL of whey supplemented with lactose. The pH was not controlled.

The whey fermentation medium was inoculated with a 20 mL aliquot of a culture grown for 24 h in a synthetic medium of the following composition: 2 g tryptone, 1 g yeast extract, 15 g lactose, and 100 mL distilled water. The yeast cell dry weight after inoculation was 0.7–0.8 g/L.

Cell dry weight, lactose and ethanol concentrations were measured as described previously⁶.

Isolation and classification of acetic acid bacteria

Acetic acid bacteria employed in this work were isolated from a starter culture used for the industrial production of cider vinegar. Isolation was performed using the following medium: 2 g glucose, 1 g yeast extract, 2 g agar,

and 100 mL distilled water. The colonies previously isolated were spread onto Petri dishes to obtain single colonies. This procedure was repeated eight times to ensure that the colonies were a pure culture.

Isolated bacteria were classified according to Bergey's Manual³. The following tests were performed: overoxidation of ethanol; catalase production; growth in Hoyer's medium; production of acid when growing in glucose; production of dihydroxyacetone from glycerol, sorbitol, and mannitol; production of cellulose; growth in sodium acetate, and production of γ -pyrones¹.

Acetic acid fermentation

Acetic acid fermentations were carried out in 250 mL Erlenmeyer flasks containing 33 mL of the alcoholic product, obtained from the fermentation of the whey supplemented with lactose and 17 mL of bacterial inoculum. Yeast used for the alcoholic fermentation were removed by filtration (0.45 μ m pore size) before inoculation with the acetic acid bacteria. Acetic fermentations were carried out at 30°C and 250 rpm in an orbital shaker.

Ethanol, acetic acid and other major volatile compounds were determined as previously described⁶. Butyl acetate was used as internal standard. Analyses were performed in triplicate, with coefficients of variation less than 6%.

RESULTS AND DISCUSSION

The first step in the production of whey vinegar is the alcoholic fermentation of supplemented whey with *Kluyveromyces fragilis*. Cell growth, lactose consumption, and ethanol production are shown in Fig. 1.

Cell growth stopped before total lactose consumption due to exhaustion of the nitrogen source. Lactose assimilation became slower after cell growth had ceased and ethanol production became slower as well. Final ethanol concentration was reached after 4 days of fermentation. The alcoholic beverage obtained from this fermentation with *Kluyveromyces fragilis* was used as the substrate for the acetic acid fermentation.

The acetic acid bacteria, isolated from a starter culture employed in cider vinegar manufacture, were classified as *Acetobacter pasteurianus*³. The strain was catalase-positive, did not grow in Hoyer's medium, produced acid when

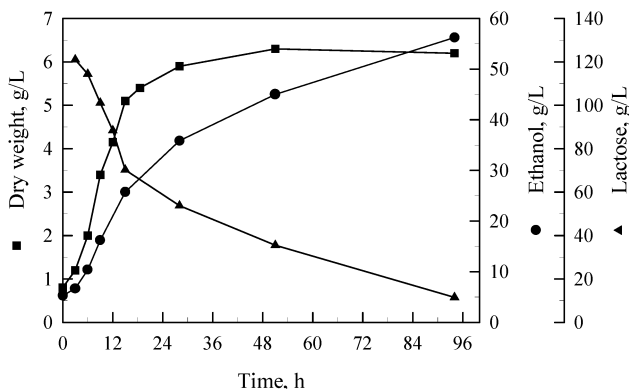


Fig. 1. Ethanol fermentation of whey supplemented with lactose at 30°C and 200 rpm. Dry weight (■), ethanol (●), lactose (▲).

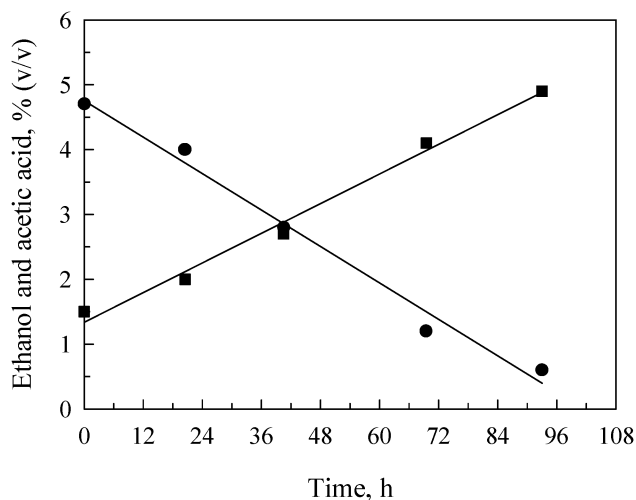


Fig. 2. Acetic acid (■) and ethanol (●) evolution during acetic acid fermentation of whey beverage at 30°C and 250 rpm.

growing in glucose, and exhibited overoxidation of ethanol. Cellulose production was observed and it did not produce γ -pyrones, nor did it produce dihydroxyacetone from glycerol, sorbitol, and mannitol, and it did not grow in sodium acetate.

The isolated strain grew adequately in the alcoholic product obtained from the fermentation of supplemented whey. A small inoculum was enough to allow acetic acid fermentation in the whey beverage. Better fermentative yields are obtained when higher inoculum loadings are used⁸.

The evolution of ethanol and acetic acid concentrations during an acetic fermentation are shown in Fig. 2. The inoculum loading proportion of 1/3 was used and ethanol was metabolised in four days. The final acetic acid concentration was 5% (v/v).

Other volatile compounds were analysed in the final vinegar. Results are shown in Table I. Ethyl acetate and fusel alcohols (isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol) were detected. Acetaldehyde, ethyl acetate and fusel alcohol concentrations in the alcoholic product from whey, under different fermentation conditions have been previously reported⁷. Acetaldehyde, although present in the alcoholic product, could not be detected in the final vinegar. Fusel alcohols are quantitatively the largest group of the flavour compounds in alcoholic beverages⁵. Past publications report that some of them maintain unaltered levels during the acetic acid transformation, but others suffer a marked decrease during the process, mainly due to the highly aerobic conditions, the oxidizing properties of acetic acid bacteria as well as ester formation.⁴

At the shake flask scale, the acetification rate is controlled by the oxygen transfer rate. The acetification rate in

Table I. Major volatile compounds (mg/L) in the final vinegar, corresponding to the mean value of three analyses \pm SD.

Ethyl acetate	2-methyl-1-propanol	2-methyl-1-butanol	3-methyl-1-butanol
13.36 \pm 0.43	33.88 \pm 0.05	8.98 \pm 0.03	40.75 \pm 0.24

the fermentations at 30°C and 250 rpm was 0.4 g/L h. The efficiency of the biotransformation of ethanol into acetic acid was 84%.

CONCLUSIONS

The production of vinegar from whey supplemented with lactose was studied. The vinegar so obtained had a concentration of acetic acid of 5.3 acetic degrees. At the shake flask scale, the efficiency of the acetic fermentation was 84%. The process employed for the production of the vinegar fulfilled the FAO requirements, and the vinegar obtained was suitable for human consumption.

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