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# POSSIBILITIES OF USING PROPIONIC BACTERIA IN THE PRODUCTION OF ACETIC ACID FROM WHEY

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Key words: acetic acid, lactose, propionic bacteria, propionic acid, whey.

Acetic acid was obtained directly from lactose contained in whey with the use of microorganisms. A *Propionibacterium* strain was selected which converted about 90% of the lactose into acetic acid and 10% into propionic acid. Applying the presented method of lactose transformation into acetic acid by propionic fermentation it is possible to obtain about 60 kg of pure acetic acid from every 100 kg of lactose contained in whey.

## INTRODUCTION

Acetic acid is the biologically produced organic acid that is most widely used in the food industry, either as seasoning or as preservative. This acid may be obtained both by chemical synthesis (for technological purposes) and through fermentation. The product for actual consumption is vinegar, i.e. a water solution of acetic acid obtained by acetic fermentation of ethyl alcohol with the use of *Acetobacter* bacteria. This is justified nutritionally since naturally obtained acetic acid, thanks to trace amounts of fermentation side-products it contains, has a characteristic bouquet as well as vitamin traces and mineral salts.

The existing method of acetic acid production by fermentation involves two different biological processes. The first stage consists in the production of ethyl alcohol by fermentation of carbohydrates with *Saccharomyces cerevisiae* yeasts. After being distilled from the medium the obtained alcohol goes through the anaeorobic acetic fermentation proper performed with a suitable *Acetobacter* strain.

The performed studies involved attempts to obtain acetic acid directly from lactose in whey by anaerobic propionic fermentation by a selected

*Propionibacterium* strain with the view to producing the greatest possible quantities of this acid.

## MATERIAL

The experiments were performed on laboratory scale with acid whey and available strains of propionic bacteria. The acid whey was produced in industrial conditions at the Dairy Plant in Warsaw. The medium was prepared by deproteinizing the whey by its neutralization with a 25% solution of ammonia water with pH = 6.8, at temperature of 95-98°C maintained for 30 min. The deproteinized whey was filtered through a funnel with cotton wool to obtain a clear filtrate. The whey thus prepared served as fermentation medium in all the experiments reported in this paper.

The biological material was a selected strain of propionic bacteria, but in the first stage of research a total of 11 *Propionibacterium* strains, obtained from the collection of pure cultures of the Department of Industrial Microbiology of the Agricultural-Technical Academy in Olsztyn, were used:

1. *P. freudenreichii subsp. shermanii* T-107
2. *P. jensenii* T-112
3. *P. theoni* T-114
4. *P. theoni* T-117
5. *P. jensenii* T-118
6. *P. theoni* T-120
7. *P. acidipropionici* T-122
8. *P. jensenii* T-124
9. *P. acidipropionici* T-126
10. *P. jensenii* T-127
11. *P. jensenii* T-128

## METHODS

### I. TECHNOLOGY

The technological process consisted of three stages:

1. Selection of one strain from among 11 propionic bacteria strains capable of inducing fermentation producing acetic acid that were investigated. The fermentation potential of each *Propionibacterium* strain was assessed in whey medium. To this end the obtained bacterial cultures were first grown on standard propionic bacteria medium, and then fermentation with all 11 strains was repeated twice. The fermentation medium was de-

proteinized whey neutralized with 25% $NH_4OH$ solution to pH = 6.8. Fermentation was carried out in anaerobic conditions under a fermentation bung at 28°C; the produced acids were neutralized daily with ammonia water.

2. Obtaining of postfermentation fluid by fermentation with the selected *Propionibacterium* strain in whey medium. Fermentation was done in two repetitions by the semicontinuous method using a 50% inoculum; 2.5 $dm^3$ samples were fermented at 30°C, five days being allowed for each propagation stage. The acids produced during fermentation were neutralized daily with limewater to pH = 6.8. The fermentation was maintained until the sugars in the medium were completely fermented.

3. Obtaining of acetic acid from the postfermentation fluid. A series of physico-chemical operations were performed to liberate acetic acid from its salts contained in the postfermentation fluid:

a. Centrifugation of the postfermentation fluid intended to separate bacterial cell biomass and calcium carbonate (10 000 r.p.m. for 10 min).

b. Decolourization of the postfermentation fluid with an addition of active carbon amounting to 3% of the entire sample's volume.

c. Ca. 10-fold concentration of salts in the water solution (rotary vacuum evaporator, temperature about 55°C).

d. Crystallization of the concentrated solution for 24 h at room temperature (ca. 20°C).

e. Centrifugation and separation of the crystals (6000 r.p.m. for 5 min).

f. Preparation of water solutions.

g. Determination of calcium content in the obtained solution by the ASA method.

h. Hydrolysis of the obtained salts with a concentrated $H_2SO_4$ solution accompanied by acetic acid liberation — addition of steichiometrically calculated amount of $H_2SO_4$ (d = 1.84).

i. Centrifugation separating the emergent gypsum (6000 r.p.m. for 5 min).

j. Preparation of acetic acid solution (vinegar) of desired concentration.

## II. ANALYSES

The determinations in whey, postfermentation fluid and the produced acetic acid included:

— total sugars content by Soczyński's method [6],
— active acidity measured with a pH-meter,
— titrable acidity,
— volatile acidity by the method of distillation with water vapour,
— calcium content by the ASA method [5],
— acetic-to-propionic acid ratio by the gas chromatography method [7].

The conformity of physico-chemical and organoleptic properties of the

obtained acetic acid with the Polish Norm for distilled vinegar [4] was also assessed.

The following determinations were made in the biomass obtained by centrifuging the postfermentation fluid:

— dry mass content by the drier method,

— total protein content by the Kjeldahl method,

— vitamin $B_{12}$ content by the microbiological plate method using the test mutant *E. coli* 113-3 [2, 3].

## RESULTS AND DISCUSSION

Various *Propionibacterium* strains produce different quantities of total volatile acids, depending on the composition of the medium and on culture conditions (pH, temperature, content of metals, growth-promoting substances and other components). The proportion between the produced amounts of propionic and acetic acids also varies, and the presence of simple or complex nitrogen compounds in the medium is seen as being largely responsible for this [1].

In the first stage of research the fermentation abilities of the various propionic bacteria strains in whey medium were assessed. This was done by determining in all the samples after fermentation the total volatile acids content and by separating these acids by gas chromatography. The obtained results are presented in the Figure: the bars illustrate the volatile acids contents obtained with the respective strains (in g/100 $cm^3$) and the various amounts of propionic and acetic acids in the different samples.

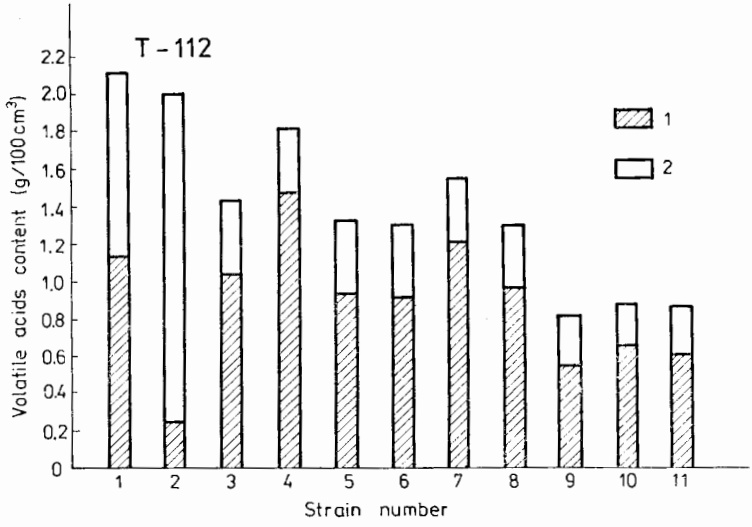


Fig. The ability to produce propionic and acetic acids (expressed as total volatile acids) of investigated *Propionibacterium* strains fermenting whey medium

The greatest amounts of volatile acids were produced by propionic bacteria strains No. 1 — T-107 (2.15 g/100 $cm^3$), No. 2 — T-112 (2.01 g/100 $cm^3$) and No. 4 — T-117 (1.79 g/100 $cm^3$), but the propionic-to-acetic acids ratio in these total acids contents differed.

Basing on the results of these studies the *Propionibacterium jensenii* strain T-112 [1] was selected for use in further experiments. This strain produced in whey medium 20 g/$dm^3$ total volatile acids, 11.5% of which was propionic acid, and 88.5% — acetic acid (0.13 : 1 proportion).

In stage two of the studies the whey medium was fermented with the use of the T-112 strain according to the method described above. The experiments yielded a combined amount of 5.200 $cm^3$ of postfermentation fluid containing 2.76% volatile acids, with acetic acid accounting for 68.05% and propionic acid for 31.95% of this amount. The subsequent physico-chemical operations were then performed to liberate acetic acid from the postfermentation fluid. In order to check the correctness of the applied technological scheme, the successive stages were first carried out with a model solution of pure propionic and acetic acid solutions prepared on the basis of the known composition of the postfermentation fluid. In this way acetic acid was obtained from calcium acetate contained in the postfermentation fluid.

The experiments with acetic acid production directly from lactose showed that as a result of propionic fermentation 95.1 g of acetic acid was obtained from 5 $dm^3$ of whey containing 3.1% lactose, which means that 61 kg of pure acetic acid may be produced in this way from every 100 kg of lactose. The efficiency of acetic acid extraction from the postfermentation fluid was 97%. The figures concerning the effectiveness of the process of acetic acid extraction from the postfermentation fluid are collected in Table 1.

Once the acetic acid was obtained it was tested as to its conformity with the Polish Standard specifying the requirements for distilled vinegar.

Table 1. Yield of the process of obtaining acetic acid from postfermentation fluid

| Determination | Postfermentation fluid | Obtained vinegar |
|---|---|---|
| Volume ($cm^3$) | 5200 | 1650 |
| Content of volatile acids as $CH_3COOH$ (%) | 2.76 | 6.00 |
| Per cent composition of volatile acids: | | |
| acetic acid | 68.05 | 96.05 |
| propionic acid | 31.95 | 3.95 |
| Acetic acid content | 97.67 | 95.10 |

Efficiency of the process — 97%

The results of this analysis are given in Table 2. As can be seen in this Table, in two cases the obtained vinegar failed to satisfy these requirements. Firstly, it was found to contain sulphuric acid; the content of this compound decreased in the course of storage of the vinegar. Since sulphuric acid was added to the acetate solution with a 10% excess (in agreement with stoichiometric calculations) its presence in the vinegar could be due to inadequate time (10 min) allowed for the reaction with calcium acetate.

Table 2. Comparison of properties of the obtained vinegar with requirements of the Polish Standard for distilled vinegar

| Properties | 6% distilled vinegar | Studied 6% vinegar |
|---|---|---|
| I: Organoleptic: | | |
| Clarity | transparent | transparent |
| Colour | colourless | colourless |
| Odour | characteristic | characteristic |
| Taste | specific for vinegar | specific for vinegar |
| Presence of vinegar eels | inadmissible | none |
| II. Physico-chemical: | | |
| Vinegar strength (g/100 $cm^3$) | 6±2 | 6 |
| Contents (mg/$dm^3$) of: | | |
| free $SO_2$ | 40 | 1.28 |
| total $SO_2$ | 100 | 1.28 |
| Inorganic acids | inadmissible | present |
| Contents (mg/$dm^3$) of: | | |
| lead | 0.4 | 0.70 |
| copper | 2.0 | 0.28 |
| zinc | 5.0 | 0.76 |
| iron | 20.0 | 0.10 |

The second incompatibility with the norm was an excessive content of lead — 0.7 mg/1000 $cm^3$, the admissible level being only 0.4 mg/$dm^3$. The presence of lead was probably due to impurities in the CaO used to make limewater which in its turn was used to neutralize acids produced during whey fermentation (a black sediment appeared in the solution). The obtained vinegar contained about 4% of propionic acid which enhanced its effectiveness as a preservative.

The experiments also provided a certain quantity of bacterial biomass centrifuged from the postfermentation fluid; this is an additional advantage of the applied method of acetic acid production. The biomass contained 21% dry substance and about 45% total protein in the dry substance. It was moreover found that the biomass contains about 10 mg of vitamin $B_{12}$ in every 100 g of dry bacteria mass.

## CONCLUSIONS

1. The experiments demonstrated the possibility of obtaining acetic acid by direct transformation by propionic bacteria of lactose contained in whey.

2. Fermentation with the selected *Propionibacterium* strain (T-112) was carried out in anaerobic conditions at about 30°C and lasted five days.

3. The efficiency of the process was close to 95%, considering the transformation of sugars in the medium and the recovery of acetic acid from the postfermentation fluid.

4. The process provides 3-5 g dry substance of cell biomass per 1 $dm^3$ of whey. The biomass of propionic bacteria may be a source of protein and of vitamin $B_{12}$ (ca. 10 mg of the vitamin per 100 g biomass).

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## MOŻLIWOŚCI WYKORZYSTANIA BAKTERII PROPIONOWYCH DO PRODUKCJI KWASU OCTOWEGO I SERWATKI

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Streszczenie

Przeprowadzono badania mające na celu otrzymanie kwasu octowego bezpośrednio z laktozy zawartej w serwatce metodą mikrobiologiczną, prowadzoną z zastosowaniem wybranego szczepu bakterii z rodzaju *Propionibacterium*. W wyniku dokonanej selekcji spośród 11 szczepów bakterii priopionowych wybrano jeden należący do gatunku *Propionibacterium freudenreichii subsp. shermanii* T-112, który zastosowano w dalszej części doświadczeń. Szczep ten przekształcał laktozę zawartą w serwatce w ok. 90% w kwas octowy i w ok. 10% w kwas propionowy, co przedstawiono na rysunku.

W kolejnym etapie przeprowadzono fermentację przy użyciu szczepu T-112 na podłożu serwatkowym. Proces przebiegał w warunkach beztlenowych w temp. 30°C w ciągu 5 dni. W ten sposób otrzymano płyn pofermentacyjny, z którego bezpośrednio w wyniku przeprowadzenia wielu operacji fizykochemicznych otrzymano kwas octowy (zaplanowany proces technologiczny sprawdzono najpierw na roztworach czystych — wzorcowych, tj. wodnych roztworach kwasu octowego i propionowego).

Wytwarzane w czasie fermentacji kwasy lotne były wiązane w sole wapniowe (octan i propionian wapnia), co pozwoliło na ich wydzielenie z płynów pofermentacyjnych przez krystalizację. Kwas octowy uwolniony był z kryształów przez rozszczepienie jego soli po dodaniu stechiometrycznych ilości kwasu siarkowego. I tak uzyskany kwas octowy — ocet zawierał również kwas propionowy w ilości ok. 4%.

Otrzymany kwas octowy poddano badaniom mającym na celu porównanie właściwości uzyskanego produktu z niektórymi wymaganiami, jakie stawia Polska Norma dla octu spirytusowego, a dane są przedstawione w tab. 2.

Określono również wydajność procesu pozyskiwania kwasu octowego, biorąc pod uwagę przemianę cukrów zawartych w podłożu oraz odzysk kwasu octowego z płynów pofermentacyjnych, co zilustrowano w tab. 1.

Otrzymane wyniki z tej pracy upoważniają nas do stwierdzenia, że metodą bezpośredniej przemiany (beztlenowej) z cukrów zawartych w serwatce można otrzymać kwas octowy spożywczy.