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SOLID STATE ALCOHOLIC FERMENTATION OF APPLE POMACE

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Solid state fermentation of apple pomace for the production of ethanol using the B-4 strain of *Saccharomyces cerevisiae* baker's yeast and natural microflora is described. Ethanol yield ranged from 78 to 90% of the theoretical yield. Natural fermentation is slower than fermentation with yeast, and the obtained raw ethanol contains more impurities.

In the period 1981-85 the average annual global apple crop was 38 million tons, with Poland providing about 4.1% of the world production [6]. Considering the current amount of apples processed into juice concentrate and wine, it is forecast that in the coming few years upwards of one million tons of apples will be processed in Poland. In juice production, the amount of apple pomace varies from 20 to 25%, depending on the kind of press that is used [9]. In some plants the pomace is dried and later used to produce pectin. Fresh pomace is sometimes purchased by farmers for use as fodder component, mainly for feeding ruminants. The newly built apple juice producing facilities are very efficient, processing 400-1000 tons of apples a day, and it is hard to dispose of all fresh pomace as fodder. Accordingly, the search for alternative methods of utilizing this by-product remains an urgent matter. In many countries, fruit pomace is used to produce ethanol. The possibility of processing apple pomace in Polish rural distilleries was investigated by Rycerska, Górný and Wróblewski [3, 10], who found that water-diluted and fermented apple pomace yielded an average of 4.4 dm³ of pure ethanol out of every 100 kg of fresh raw material. The pomace must be processed in the distillery immediately after it comes off the press since it loses practically all its sugar content already after 24 h of storage at 18-24°C. The sugar content remained almost unchanged for 48 h only at temperatures ranging from 2 to 8°C [10].

The dilution of pomace with water prior to fermentation causes a number of problems, and it is thus advisable to investigate possibilities of alcoholic fermentation of natural pomace, straight out of the press, which is a loose material with a 25-30% dry mass content.

Solid state alcoholic fermentation of various raw materials, such as mangel, is the subject of numerous studies [1, 2]. Hang, Lee and Woodams investigated solid state alcoholic fermentation of apple and grape pomace [4, 5]. They obtained their apple pomace from an experimental station and stored them prior to experiments at -23°C . Fermentation was either with or without wine yeast. The authors found that natural microflora did not cause alcoholic fermentation of the pomace, while the addition of yeast led to an ethanol yield of 70-94% of the theoretical figure. The natural microflora of grape pomace does cause alcoholic fermentation in solid state. The studied grape pomace sample came from an industrial plant; it contained 13.7% of sugar, and dry mass was 35-36%. One kilogram of the pomace yielded 53 g of ethanol (82% of theoretical yield).

MATERIAL AND METHODS

Apple pomace was obtained from three industrial plants: in Milejów, Płudy and Chrzczonowice. Two of these plants are equipped with Bucher-Guyer apple juice presses, and one with POK-200 layer presses. The samples were collected on September 26, October 17, and October 23, 1986, chemically analysed immediately thereafter, and subjected to fermentation. When storage of samples was necessary, they were kept in refrigerators at -4°C .

Dry mass was determined by drying ca 5-g samples to constant weight at 105°C . Saccharides content was determined in water extracts colorimetrically using DNS [7].

500-g portions of apple pomace were fermented in 1-l glass conical flasks stopped with fermentation tubes at room temperature ($20-23^{\circ}\text{C}$) and at 30°C . Yeast was added to the pomace in either of two quantities: 0.5 and 1.0% (dry mass) of pomace. Two kinds of yeast were used: strain B-4 of *S. cerevisiae* from the collection of the Institute of Fermentation Industry, and pressed baker's yeast from the Józefów yeast-producing plant. Pomace was also fermented without yeast additions, basing on the natural microflora of the raw material. Fermentation time was 72 h.

Every 24 h the ethanol content was determined with gas chromatography using a Pye-104 chromatograph. The column was filled with a mixture of Carbowax 200 and 400 on celite; the carrier gas was argon, and the apparatus featured a flame ionization detector. The ethanol obtained from apple pomace was analysed according to Polish Norm PN-80/A-79528 [8].

RESULTS AND DISCUSSION

The results of physico-chemical analyses of apple pomace samples obtained in the three industrial plants are collected in Tab. 1. The pomace produced by the Bucher-Guyer presses has a higher dry mass content than pomace from the layer press. Saccharides content ranged from 7.0 to 7.9%, and was similar to that

Table 1. Physico-chemical analysis of apple pomace

Sample No.	Type of press	Dry mass	Saccharides	pH
1	Bucher-Guyer	29.0	7.9	3.5
2	Bucher-Guyer	28.5	7.7	3.5
3	POK-200	26.5	7.0	3.5

found by Rycerska [10]. The pH of a 1:1 water solution of pomace is low but adequate for alcoholic fermentation with yeast without the need to neutralize the medium.

Table 2 contains results of ethanol content determinations after solid state fermentation of pomace from plant 1 for 24, 48 and 72 h. The results are means from two experiments. Fermentation was carried out at room temperature (20-23°C) and at 30°C using the natural microflora of apple pomace (with no yeast addition) or various doses of B-4 and pressed baker's yeasts.

Table 2. Alcoholic fermentation of apple pomace (sample 1)

Specification	Ethanol (% weight)					
	fermentation temperature					
	20-23°C			30°C		
	24 h	48 h	72 h	24 h	48 h	72 h
No yeast addition	1.0	2.2	2.6	2.9	3.2	3.2
With 0.5% dry mass of B-4 yeast	2.0	2.5	2.8	3.4	3.4	3.3
With 0.5% dry mass of baker's yeast	2.1	2.7	2.9	3.4	3.4	3.4
With 1.0% dry mass of B-4 yeast	2.2	2.7	3.0	3.3	3.2	3.1
With 1.0% dry mass of baker's yeast	2.3	2.8	3.0	3.2	3.2	3.0

As expected, alcoholic fermentation was much quicker at 30°C than at room temperature. At 30°C fermentation was over after just 24 h, while after this period at room temperature (20-23°C) only 60-70% of the saccharides underwent fermentation and this only when yeast was added. In the latter conditions, i.e. 24 h at room temperature, natural microflora ferments only about 30% of the sugars.

It was found that the 0.5% yeast addition to apple pomace was enough to ensure good fermentation. We used the B-4 yeast strain relying on Górný et al. [3] who report that in apple pomace fermentation their effect is not inferior to that of wine yeasts.

In this study we established that similar results may be obtained with the generally available pressed baker's yeast.

After 72 h of fermentation at 30°C the sugars content in the pomace was 4-7 g/kg, which means that ethanol yield relative to the utilized sugar was 78-90% of

theoretical yield. In their studies, Hang et al. [4] obtained 70-94% of theoretical yield, and also found that alcoholic fermentation with natural microflora is not feasible. Our results indicate that such fermentation is possible in the case of Polish pomace, but that the process is greatly accelerated by a yeast addition.

Table 3 gives results of alcoholic fermentation of pomace from plants 2 and 3. The process was conducted either without yeast or with additions of pressed baker's yeast. The results are similar to those obtained during fermentation of pomace from plant 1, the exception being fermentation of pomace from plant 3 using natural microflora. In this latter case, saccharides utilization after 72 h, even at 30°C, did not exceed 50% of the initial content, and at room temperature it was still lower. These results indicate that in practice there may occur pomace batches containing small amounts of natural microflora capable of alcoholic fermentation.

Table 3. Alcoholic fermentation of apple pomace (samples 2 and 3)

Specification	Ethanol (% weight)					
	fermentation temperature					
	20-23°C			30°C		
	24 h	48 h	72 h	24 h	48 h	72 h
Sample 2 with no yeast addition	0.8	1.9	2.5	2.7	3.0	3.2
Sample 3 with no yeast addition	0.2	0.6	1.0	0.3	0.8	1.5
Sample 2 with 0.5% dry mass of baker's yeast	2.2	2.8	3.1	3.4	3.4	3.4
Sample 3 with 0.5% dry mass of baker's yeast	1.1	2.3	2.7	2.9	3.0	3.0

In order to determine the quality of ethanol obtained from apple pomace, we performed solid state fermentation of pomace from plant 2. Five-kilogram samples of the raw material were fermented by natural microflora alone and with 0.5% additions of baker's yeast dry mass.

Fermentation was carried out at 21°C. After a dozen or so hours the temperature inside the pomace mass rose to 28-29°C. After 48 h the alcohol together with by-products were extracted with water, following which about 85% of the alcohol contained in the fermented raw material was recovered. Ethanol was distilled off with a glass distiller, and distilled again with a Goldetz dephlegmator. Results of physico-chemical analyses of the obtained distillate are given in Table 4.

The spirit produced from apple pomace is characterized by a fairly high methanol content (over 1%). The content of higher alcohols (fusel oil) is higher in the spirit obtained after fermentation with yeast, while the spirit produced by fermentation using natural microflora contains large amounts of aldehydes and esters, several times more than the spirit made from pomace with a yeast addition. The further processing of the spirit from natural microflora-induced fermentation would be difficult, and so, wishing to obtain good-quality spirit, we should ferment pomace with a yeast addition.

Table 4. Physico-chemical analysis of apple pomace distillates

Specification	Proof (% vol at 20°C)	Methanol (in %, converted to 100% ethanol)	Aldehydes Esters Acids Fusels (g/dm ³ of pure spirit)			
Spirit from fermentation with no yeast addition	55.0	1.2	4.0	6.2	0.42	3.2
Spirit from fermentation with 0.5% dry mass of baker's yeast	58.0	1.3	0.36	0.82	0.31	5.0

The research into solid state alcoholic fermentation of apple pomace is continued. The studied issues include optimum conditions of ethanol extraction from fermented pomace.

CONCLUSIONS

1. Apple pomace may be subjected to alcoholic fermentation in solid state.
2. Alcoholic fermentation is induced by the natural microflora of pomace, but a yeast addition accelerates the process.
3. In order to achieve an ethanol yield relative to the utilized saccharides amounting to about 80% of theoretical yield, it is sufficient to add 0.5% yeast dry mass to the pomace.
4. The respective ethanol yields of saccharides fermented with the B-4 strain of *S. cerevisiae* and with pressed baker's yeast are similar.
5. The ethanol distillate obtained from pomace fermented with the use of natural microflora contains several times more aldehydes and esters than such a distillate from pomace fermented with a yeast addition.

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FERMENTACJA ALKOHOLOWA WYTŁOKÓW JABŁKOWYCH W STAŁEJ FAZIE

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Streszczenie

Przeprowadzono fermentację alkoholową w stałej fazie 3 próbek wytłoków jabłkowych pobranych w zakładach przemysłowych. Skład chemiczny wytłoków podano w tab. 1. Fermentację prowadzono wykorzystując naturalną mikroflorę oraz dodając 0,5 i 1,0% suchej masy drożdży *Saccharomyces cerevisiae* rasy B-4 oraz drożdży piekarskich w stosunku do masy fermentowanych wytłoków. Proces prowadzono w temp. 20-23 i 30°C.

W temp. 30°C fermentacja alkoholowa jest praktycznie zakończona po 24 h. Otrzymano 78 do 90% etanolu w stosunku do wydajności teoretycznej. Oznacza to, że z 1 t wytłoków jabłkowych można otrzymać ok. 40 dm³ 100° spirytusu. Fermentacja przy wykorzystaniu naturalnej mikroflory wytłoków przebiega znacznie wolniej w porównaniu z fermentacją z dodatkiem drożdży, a otrzymany spirytus zawiera kilkakrotnie więcej aldehydów i estrów (tab. 4).