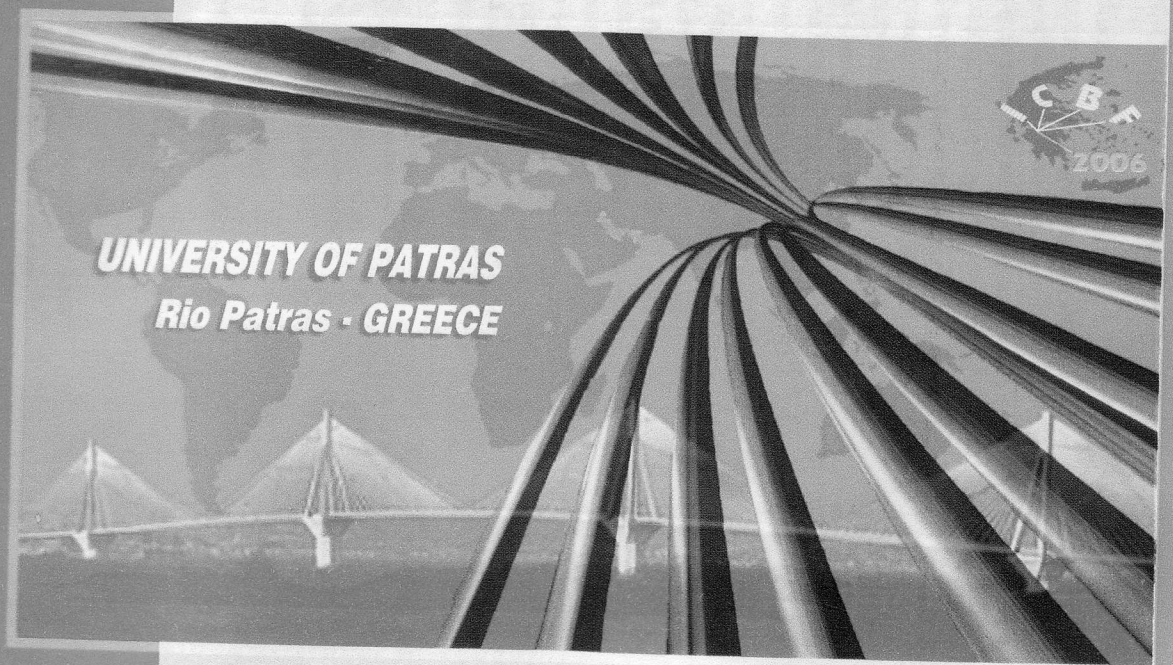
2nd INTERNATIONAL CONGRESS ON BIOPROCESSES IN FOOD INDUSTRIES

ICBF - 2006



ICBF Forum

ICBF-Forum

University of Patras

University of Ioannina

18 - 21 June 2006

CONGRESS PROCEEDINGS

PATRAS 2006

16.00- 16.30 **Papagianni, M.**
Aristotle University of Thessaloniki, Greece
Engineering the Metabolism of Lactococcus lactis by Cloning Key Fungal Genes from Aspergillus niger

16.30- 17.00 **Weinberg, Z.G**
The volcani center, Israel
The Safety and Hygiene of Sewage Irrigated Forage Crops in Israel

17.00- 17.30 **Rao, L.V.**
Osmania University, India
Biotechnological Production of Xylitol and its Application in Food Industry

17.30- 18.00 **Thakur, I.S.**
Jawaharlal Nehru University, India
Bioremediation and Detoxification of Dioxin- Like Compounds in Food by Products by Serratia marcescens.

Session I, Room **I** - 12

Chairmen: Pandiella, S. and Berger, R.G

Fermentation technologies

Invited Lectures

15.30 -16.00 **Singh- Nigam, P.**
University of Ulster, Northern Ireland, UK
Comparative Study of Encapsulated and Entrapped Yeast for Fermentation Purposes

16.00- 16.30 **Ferreira- Dias, S.**
Instituto Superior de Agronomia, Portugal
On the Use of Immobilized Lipases in the Food Industry- Factors and Facts

Short Lectures

16.30- 16.45 **Binod, P.**
Regional Research Laboratory (CSIR), India
Purification of Chitinase Isoenzymes from Fungal Strains

16.45- 17.00 **Babitha, S.**
Regional Research Laboratory (CSIR), India
Effect of Stress on Growth and Pigment Production in Monascus purpureus under Solid State fermentation

17.00- 17.15 **Drichoutis, P.**
National and Kapodistrian University of Athens, Greece
Studies of the Mechanical and Fermentation Behaviour of Double Layer Alginate-Chitosan Beads Produced Using Saccharomyces cerevisiae Entrapped Cells.

17.15- 17.30 **Tataridis, P.**
TEI of Athens, Greece
Ethanol Production via Solid State Fermentation of Grape Pomace: Combined Effect of Fermentation Parameters on Ethanol Yield

Ethanol Production via Solid State Fermentation of Grape Pomace: Combined Effect of Fermentation Parameters on Ethanol Yield

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Summary: Grape pomace is a by-product of the wine making industry. Today, if not used as compost material or discarded as waste, grape pomace is fermented to produce ethanol, after addition of water. This technique has several disadvantages like excessive use of water, high waste production and high-energy consumption for distillation. In the present study grape pomace was fermented via solid state fermentation in order to minimize the above mentioned disadvantages. Grape pomace was recovered after pressing and fermented with or without the use of inoculum. The pomace humidity was 71,8%, pH 3,46, the reducing sugar concentration was 509 g/Kg, and the total acidity (as tartaric acid) was 13,1 g/Kg. Additionally, the measured volatile acidity was 1,83 g/Kg and the ammonium nitrogen was 198 mg/Kg while the a-amino nitrogen was 387 mg/Kg. All measurements were expressed in dry weight. The potential of ethanol production from grape pomace under different fermentation conditions was studied. The optimum conditions for high ethanol yield (40%) and productivity 5,07 g/Kg/h were: pH 4.5, fermentation temperature 30ºC, inoculum 20.10^7 cells/gr of dry weight, moisture 70%. No nitrogen or nutrient addition was necessary. The efficiency of the ethanol recovery was between 87% and 94%.

Keywords: Solid state fermentation, grape pomace, ethanol production.

Introduction

Grape pomace is the residue left after the pressing of grapes for must extraction and constitutes about 11% to 16% of the grape (Rice, 1976). According to the Greek Ministry of

Rural Development and Food (2001), approximately 350.000 tons of grapes are produced annually in Greece. Grape pomace is rich in carbohydrates, but its protein content is rather low (Rice, 1976). At present, most pomace is just buried in the ground or spread out in the vineyard as a crude fertilizer, making essentially no use of a resource that could have high value, despite the increasing disposal problems and the efforts for the by-product utilization.

The direct incorporation of grape marc into agricultural land, a common practice, has caused serious problems since degradation products can inhibit root growth (phenols, tannins) (Inbar *et al.*, 1991). An alternative to overcome such disadvantages and to recycle wastes, is composting (Diaz *et al.*, 2002). Grape pomace is also used for tartrate recovery (Braga *et al.*, 2002). Results indicate values ranging from 50 to 75 kg/t (grape pomace) with respect to the potential production of calcium tartrate. Grape pigment concentration is about 9 kg/t for red grape pomace (Leber, 2004). Other uses include recovery of food ingredients (Hang, 1988, Rice, 1976) and feedstuff production (Vaccarino *et al.*, 1992).

After the sugar extraction of the pomace, the sugar medium can be used as a submerged fermentation broth. Lo Curto and Tripodo (2001) used this technique to produce single cell protein (SCP), and Tataridis et al., (2006) for bacterial cellulose production. Ethanol production with this technique has low yield and ethanol recovery is unattractive.

Solid state fermentation involves the growth of microorganisms on wet solid supports in the absence (or near absence) of free water. New interest in this technology derives from the fact that it is considered to be an appropriate approach for processes including the bioremediation or the biodegradation of toxic compounds, the detoxification of agricultural wastes, the biotransformation of crops and biopulping, etc. Moreover, SSF has been successfully applied in the production of new high value products, such as secondary metabolites, organic acids, pesticides, aromatic compounds, fuels and enzymes (Bensoussan *et al.*, 1997). Grape pomace has been used in the past for the production of ethanol (Hang *et al.*, 1986), citric acid (Hang and Woodams, 1985), gluconic acid (Buzzini and Gobbetti, 1993), carotenoids (Buzzini and Martini, 2000), xanthan gum (Stredonsky and Conti, 1999) ethanol (Hang *et al.*, 1986). The advantages of SSF in comparison to traditional submerged fermentation are: better yields,

easier recovery of products, absence of foam formation and smaller reactor volumes. Moreover, contamination risks are significantly reduced due to the low water contents. The objective of this study was to examine the potential of ethanol production from grape pomace, under different fermentation conditions.

Materials and Methods

Medium

Grape pomace from the Greek variety "*Rhoditis*" was recovered after using pressure (1,8 bar). Pomace was dried at 60ºC and stored at 4ºC until it was used. Another batch was fermented immediately after pressing. The pomace moisture was 71,8%, the pH 3,46, the reducing sugar concentration was 509 g/Kg, and the total acidity (as tartaric acid) was 13,1 g/Kg. Additionally the measured volatile acidity was 1,83 g/Kg, and the ammonium nitrogen was 198 mg/Kg while the a-amino nitrogen was 387 mg/Kg. All measurements were expressed in dry weight. The effect of the composition on the final product and ethanol yield was studied.

Microorganisms -Culture

Commercial enological yeast *Saccharomyces cerevisiae* from Lalvin Rhone strain L 2056 was used. Dry yeasts rehydrated in minimal amount of water.

Solid state fermentation

Solid state fermentation was performed in 5 L jars equipped with fermentations locks. Dried grape pomace was rehydrated and properly mixed so that no free water was available. Each jar contained 423,3 gr of pomace dry weight (1500 gr wet pomace) and placed in incubation chambers with regulated temperature (20ºC or 30ºC). After inoculation the content of the jars was mixed 3 times per day. The initial parameters for each of the fermentations are shown in table 1.

Table 1. Initial composition of the medium.

Fermentation number	Inoculum 10^7cell/gr of dry pomace	Temperature in °C	Initial pH	Initial sugar gr/kg of dry pomace	Moisture content %
1	5	30	4,5	617	77,3
2	5	30	4,5	274,5	59
3	5	30	4	274,5	59
4	5	30	3,5	274,5	59
5	20	30	3,5	274,5	59
6	20	30	4	274,5	59
7	20	30	4,5	274,5	59
8	5	20	3,5	274,5	59
9	5	20	4	274,5	59
10	5	20	4,5	274,5	59
11	20	20	3,5	274,5	59
12	20	20	4	274,5	59
13	20	20	4,5	274,5	59

Analysis

Analysis was performed after extraction of pomace (50g of sample) with 100ml of water. Reducing sugars analysis was performed with the dinitrosalicylic acid reagent (D.N.S.) (Miller, 1959), Total acidity by titration. Ethanol, glycerin and acetic acid by enzymatic kits (R-biopharm. Ethanol UV Method Cat.No.10 176 290 035, Acetic Acid UV Method Cat No 10 248 261 035, Glycerol UV method Cat No 10 148 270 035). Final ethanol was also determined by distillation. All measurements were performed after extraction of the fermented pomace with water (1 part pomace and 2 parts of water).

Initial sugar concentration of the non-dried medium was slightly higher due to partial seed removal. Moisture content of the dried medium was lower than the initial. The sugar concentration was also lower. Grape pomace after drying could not be rehydrated to the initial moisture content probably due to partial caramelisation of sugars. For fermentation No1 the pomace was sterilized while in all the others the pomace was not sterilized.

Results and Discussion

Solid state fermentation is associated with non homogenous environmental parameter distribution. Therefore the pH was measured after mixing. In all the fermentation the final pH

was between 3,8 and 3,9 (Fig. 1). Similar results have been observed in other studies (*Hang et al., 1982*). The total acidity increased by 65 to 100% of the initial value, while the volatile acidity increased by 0,7 to 1,6 g/Kg (Fig. 2 and Fig. 3 respectively). Sugar concentration did not have any distinct effect on the produced volatile acidity (acetic acid). Glycerol production increased from 5g/Kg up to 7g/Kg.

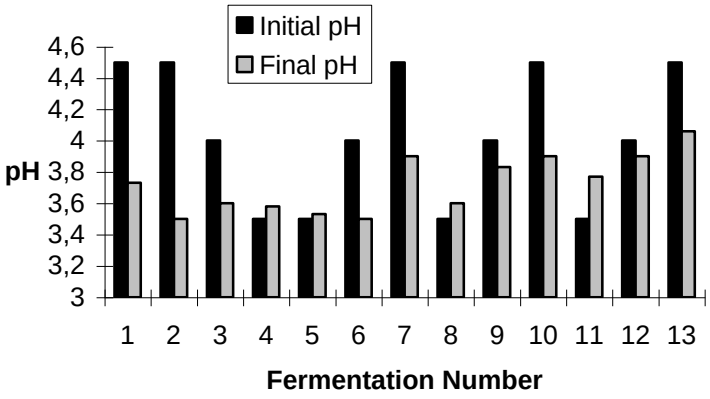

Figure 1. pH of grape pomace before and after solid state fermentation.

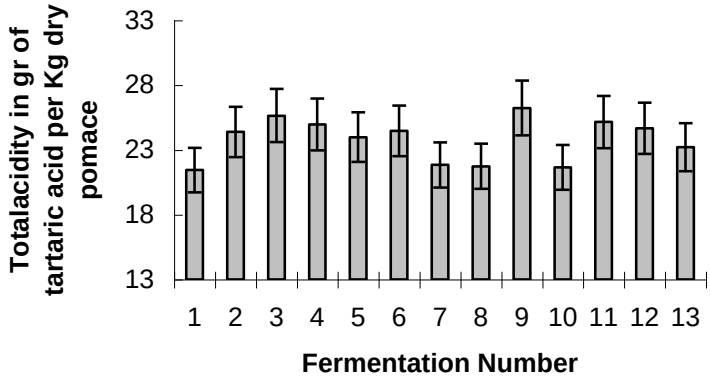

Figure 2. Changes in total acidity of grape pomace after solid state fermentation.

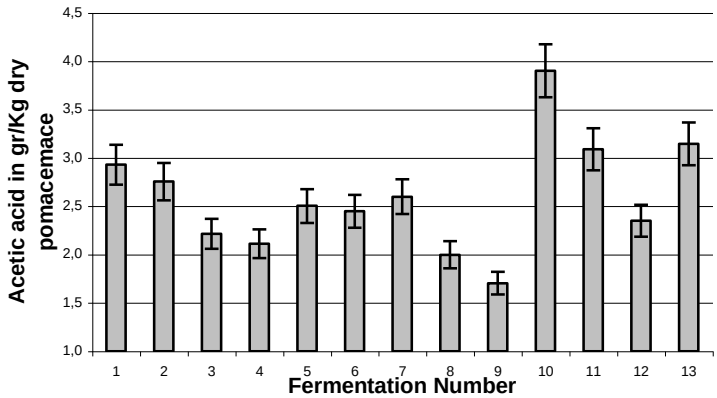

Figure 3. Volatile acidity of grape pomace after solid state fermentation.

Effect of initial pH

For fermentations with low inoculum concentration at 30°C and increased pH (4,5) had a positive effect on ethanol production and yield. At 30°C the opposite effect is observed. For fermentations with high inoculum concentration the pH had an adverse effect on ethanol production and yield (Figure 4).

Residual sugar concentration ranged from 37 to 67 gr/Kg of dry pomace for fermentation No2 to No13 and 110 gr/Kg of dry pomace for fermentation No1. Ethanol yield varied from 24% up to 40% under optimum conditions in fermentation No1. Hang *et al.*, (1986) obtained 41 % to 42 % yield from grape pomace.

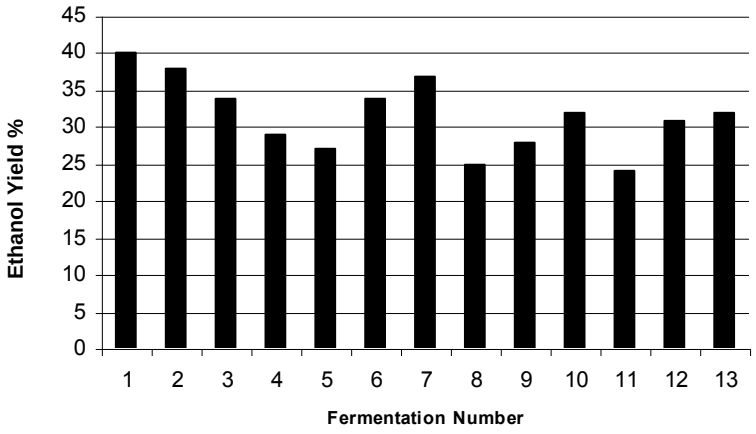

Figure 4. Comparison of ethanol yield of all fermentations.

The optimum conditions (fermentation No1) for high yield and ethanol production were: pH 4.5, fermentation temperature 30ºC, inoculum 20.10^7 cells/gr of dry weight, humidity 70%. No nitrogen or nutrient addition was necessary. The fermentation was completed after 40 hours and productivity was 5,07 g/Kg of dry weight/h.

Level of contamination and performing of the fermentations

Due to massive inoculum addition, low water content, together that the fact that volatile acidity concentration after SSF was not unreasonably high, contamination of fermentations

did not seem probable, except perhaps in fermentation No10. However a new series of fermentations was tested for signs of contamination that would pose a threat.

In the new fermentation series a fresh grape pomace was used. The initial sugar concentration was lower (310 gr/Kg of dry pomace) than fermentation No1 so as to compare them with fermentations No2 to No13 and verify optimal production conditions both sterilized and non sterilized pomace. Inoculum concentrations of 5.10^7 and 20.10^7 cells/g of dry pomace were tested. Experiments were performed both with sterilized and non sterilized fresh pomace in triplicate.

Results in both cases were similar. However fermentations with inoculum concentrations of 20.10^7 cells/g of dry pomace at 30 ºC were faster. For these fermentations, final sugar concentration was 16,7 gr/Kg of dry pomace, final ethanol concentration was 117,2, gr/Kg of dry pomace and acetic acid concentration was 2,6 gr/Kg of dry pomace. Fermentation duration was 40 to 45 hours (Fig. 5).

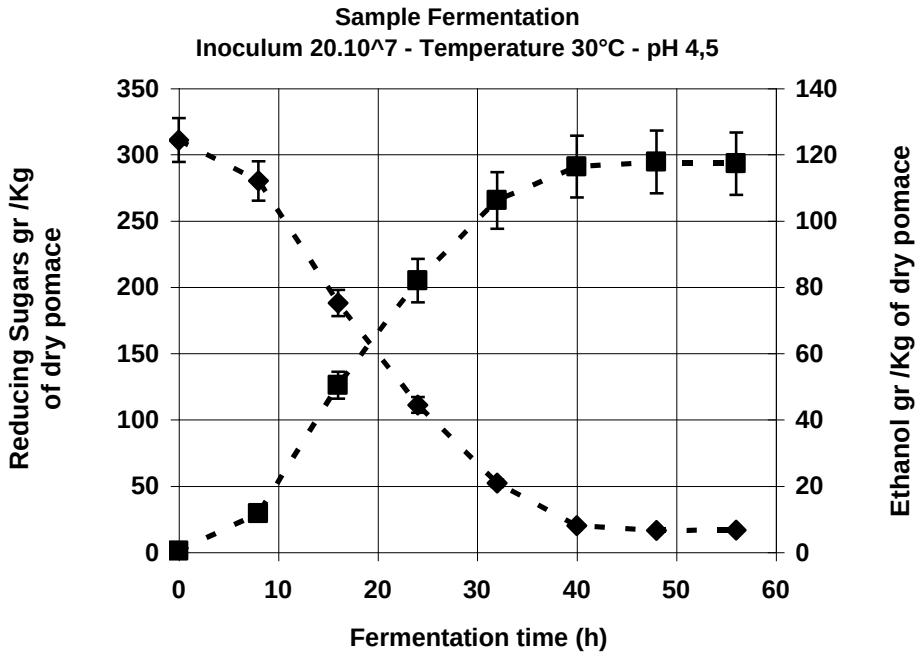

Figure 5. Evolution of sugar consumption and ethanol production under optimal SSF of grape pomace.

Effect of fermentation temperature

Although that for the fermentation series with the rehydrated pomace temperature of (30ºC had a negative effect on ethanol production and yield compared to 20ºC, for the fresh pomace it had a positive effect. The later is in accordance with results observed by Roukas (1994) for carobs, Hang *et al.* (1982) for apple pomace and Hang *et al.* (1986) for grape pomace. In this case however differences could be due to the yeast strain that was used, fermentation volume, sampling procedures and regular agitation may have also affected ethanol concentration.

Effect of inoculum concentration

Inoculum concentration did not have a significant effect on ethanol yield. In the experiments with fresh pomace inoculum of 20.10^7 gr of yeast/g of dry pomace resulted in better ethanol yield of only 2%. Results from experiments of solid state fermentation on other media indicate that optimal inoculum for sweet sorghum was at 1.10^7 gr of yeast/g of dry pomace (Kargi *et al.,* 1985), 1.10^8 gr of yeast/g of dry pomace for fodder beets (Gibbons and Westby, 1986), 5.10^8 gr of yeast/g of dry pomace for sugar-beet particles (Amin, 1992) and 5.10^8 gr of yeast/g of carob pulp (Roukas, 1994).

The efficiency of the ethanol recovery by distillation ranged from 87% to 94%. By using a rotary vacuum evaporator Hang *et al.,* (1982) obtained between 92 % to 99 % of the ethanol produced in apple pomace solid state fermentations.

Conclusions

Solid state fermentation is an efficient process for the production of ethanol from grape pomace. In our experiments fresh non sterilized grape pomace gave similar results with sterilized pomace. Drying and storage have an adverse effect on ethanol production and yield. Duration is under 40 hours for fermentation of 300 gr of sugar / Kg of pomace (in dry weight) and yield reaches 40%. Not only solid state fermentation was faster than submerged fermentation but it was advantageous regarding water usage. Wastewater minimization is also

achieved. Scale up trials are however necessary for the verification of the results. This method can also be used for bioethanol production using different agricultural wastes.

References

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