procedure is useful for evaluating the composition of orange juice.

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BIOCONVERSION OF MUSCADINE GRAPE WASTE INTO LIVESTOCK FEED

J. BEN RODIN, O. LAMIKANRA, S. LEONG, Y. OWUSU, M. ABAZINGE, I. INYANG
Florida A&M University
Tallahassee, FL 32307

Abstract. **The United States and the world at large are facing monumental problems in the future when considering the issue of waste disposal. Alternatives to landfills must be found. In this work two waste products, pomace and vine clippings from muscadine grapes, are being examined as potential sources of animal feed. The conversion of these wastes involves three stages of operation: size reduction, where the raw pomace (residue after grapes are crushed for juice and/or wine) or the grapevine clippings are reduced in particle size to increase available surface area; chemical pretreatment, where the materials under study are modified (usually in crystal structure) to a more suitable form for biological action; and bioconversion, which involves exposure of treated waste materials to cellulolytic fungi in order to increase overall digestibility. The work discussed here was primarily directed at the chemical pretreatment step, but the other steps are also discussed. Sodium hydroxide was effective in modifying the structure of the grape pomace. This was shown by measuring crude fiber percentage (by AOAC methods) of pomace before and after processing. A decrease in crude fiber percentage indicates an increase in available nutrients from the waste material. Later, this modified material would go through a bioconversion stage. The final stage is testing the product by conducting feed trials on ruminants.**

Introduction

The problems of waste disposal in the United States are tremendous. Landfills are being rapidly filled to capacity, and new landfills are an unpopular solution to waste disposal problems. Recycling and incineration are being attempted, each with its own advantages and disadvantages. New solutions must be found to the problems of wastes. Some of the wastes being disposed of are agricultural in nature. This project involves one set of wastes, those from grape and wine production. In the United States, the grape industry produced 5.7 million tons of grapes in 1989 with an estimated market value of $16.6 billion in 1990 (USDA, 1991). From this, an estimated 2.5 million tons of pomace (which comprises the pulps and seeds left over after pressing grapes for juice and wine) were discarded at a cost of $25 million. Instead of disposing the pomace as wastes, it could have been converted into 930,000 tons of animal feed with an estimated market value of $279 million. The two materials being examined for conversion to animal feed in this study are muscadine grape pomace and grapevine clippings.

Grape pomace has been investigated previously as a potential feed for livestock (Grujic et al., 1992; Aguilera, 1987; Famuyiwa and Ough, 1990; Famuyiwa and Ough, 1982). Generally the digestibilities determined for various ruminants have been seen as approximately half that of grains. Little work has been performed on vine clippings as a source for animal feed, and no studies have been done on muscadine pomace or vine clippings. The goal of this research project is to achieve improvement in digestibility through chemical pretreatment and solid state fermentation (SSF) of the grape wastes, and later to develop a system that can be used on the farm. As muscadine grapes are common in Florida, this project has the potential to generate wide spread interest among grape growers.

Grujic et al. (1992) reported on their attempt of SSF on grape pomace using *Chaetomium cellulolyticum* as an inoculum. However, their work focused on presterilized non-muscadine pomace. Improvements in their process can be made through pH control and pretreatment optimization.

Three fundamental steps are involved in bioconversion of agricultural residues to livestock feed. The first step is size reduction. In this step, the waste materials must be processed in such a way as to maximize the surface area available for chem-

ical and biological reaction. The second step, and the part which this work focuses on, is chemical pretreatment. Here, the waste materials are exposed to chemical agents to breakdown or otherwise disrupt the crystalline structure of the cell wall. The third step is bioconversion. Here, the materials are inoculated with a filamentous fungus showing ability to break down lignocellulosic wastes.

Results

Size Reduction - Stage 1

Although no final solution to the problem of size reduction has been found, several alternatives are currently being explored. A brief description of the challenges faced in the size reduction process is mentioned below.

Two types of waste materials are being worked with, pomace and vine clippings. The goal for size reduction is to increase the surface area available for chemical/biological reaction. The parameters for solving this are economic considerations, material considerations, and process considerations. Vine clippings are highly fibrous and relatively dry materials, whereas pomace is highly wet material with hard seeds. Opening or cracking these seeds is desirable. Most milling devices are designed to process dry, nonfibrous materials. A low-cost milling device that can handle both materials and has large throughput would be optimal.

Several methods have been attempted. To this point, knife mills, chipper/shredders, and hammer mills have been tested with the materials, but with little success. Disc grinding, colloid mills, and high speed blending are currently being examined.

The laboratory shredding of materials was done by using a Vitamix shredder. Unfortunately, this method does not lend itself to large-scale continuous processing.

Chemical Processing - Stage 2

Several treatment processes have been attempted on the pomace, and, to a lesser extent, the grapevine clippings. Room temperature processes have been the preferred operation, as heating costs would add to the overall process cost. Most of the work performed has been concentrated on sodium hydroxide treatments of the materials. The method used to determine extent of alkali digestion has been AOAC crude fiber percentage (of dry mass) (AOAC, 1990). It has been assumed that drops in crude fiber content are directly related to increases in digestibility of the material, and, in turn, susceptibility of the material to biological reaction.

The first measurements taken are the crude fiber percentage of the raw, untreated (but shredded) vine clippings and pomace. The vine clipping samples were shredded with a Glen Mills P-15 mill. The crude fiber percentage for the vine samples was 52.5%. The pomace, Vitamix shredded, (untreated, but shredded) showed a crude fiber percentage of 37.9%.

The pomace treatments will be discussed first. As the initial step, the dry mass percentage of pomace was determined to be approximately 15% (15.2%). This was determined by drying large samples of pomace overnight at 120°C.

One set of studies involved a thoroughly shredded sample where 700 g of pomace was added to 300 ml of deionized water prior to vitamixing. The water was added to enhance the pomace shredding. Upon thorough shredding of several pomace/water aliquots, 4-1 kg samples were taken to which

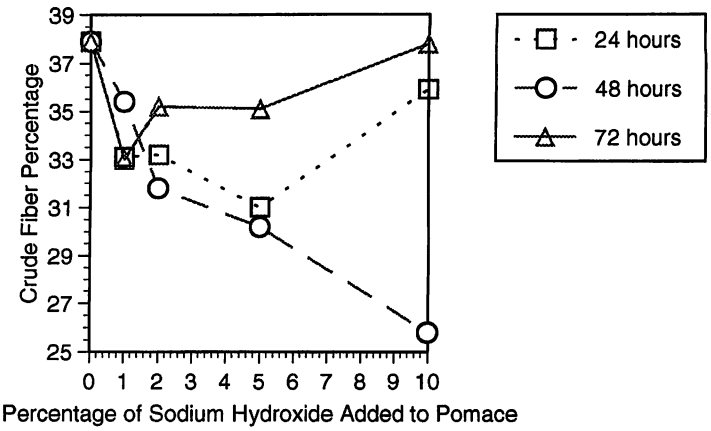

Figure 1. Effect of percentage sodium hydroxide treatment on crude fiber percentage. Legend indicates exposure time of pomace to the treatment.

1.05 g, 2.10 g,. 5.25 g, and 10.5 g of sodium hydroxide chips were added (based on 15% dry mass percentage, this would correspond to 1%, 2%, 5%, and 10% sodium hydroxide per mass treatments respectively). These experiments were all performed at room temperature. At 24 hour intervals, a large aliquot was removed and immediately put in a drying oven at 120°C overnight. The dried samples were then used for crude fiber analysis (AOAC methods), where 2 g samples were processed. The results of the crude fiber analysis are shown in Figs. 1 and 2. Each point on the plots represents an average of between 4 and 6 measurements. Fig. 1 shows crude fiber percentage as a function of sodium hydroxide concentration after 24 hour, 48 hour and 72 hour processing times, respectively. The 48 hour results were as expected, where decreases are seen with increasing sodium hydroxide concentration (the 0 concentration point represents the raw pomace crude fiber percentage). The 24 and 72 hour results were surprising in that the 10% point was the highest crude fiber seen, and, at 72 hours, the 1% treatment is apparently the most effective. Fig. 2 shows crude fiber vs. time plots at different concentrations and shows evidence of unusual behavior, especially in the case of 10% treatment. More investigation into this behavior is warranted. Generally, though it would seem that 2-5% 48 hour treatment should be the recommended pretreatment. Ammonium hydroxide pretreatment was also tested on pomace samples with discouraging results. Preliminary results have shown that, even with addition of 32 ml of 5% am-

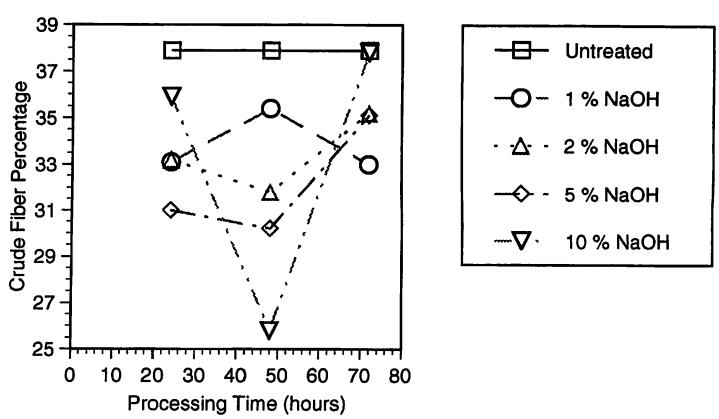

Figure 2. Effect of processing time on crude fiber percentage. Legend indicates treatment level based on percentage of dry mass.

monium hydroxide solution to 6 g of dried pomace, crude fiber results showed values around 50% for pomace (a net decrease in digestibility over the raw values).

Similar vine experiments are currently being performed. Some preliminary experiments have shown that sodium hydroxide treatments are not particularly effective (crude fiber percentage actually increased). Ammonium hydroxide treatment will be further investigated.

Biological Processing - Stage 3

Some preliminary growth studies of *Chaetomium cellulolyticum* on a pomace base was attempted, but with little success. It was difficult to initiate a starter culture, as an aerobic environment appears to be the environmental choice of most of the fungi being evaluated as possible bioconversion agents. Aerobic experiments are difficult (except for surface studies), and a test bioreactor is in the process of being modified for our studies. Unfortunately, air flow through concentrated pomace solutions is difficult, and mixing will be required. Additionally, due to the aerobic requirements, each possible culture must be tested in the bioreactor. Seven days of operation, according to literature sources [2], would be the minimum period required to test the feasibility of a given feed/culture combination. Therefore, only a limited number of cultures can be feasibly tested; one or two strains of *T. reesei*, one of *C. cellulolyticum*; and perhaps one of *S. roseum*. Chemically pretreated feed, along with raw feed, will also be tested.

Discussion

As the chemical pretreatment step has been optimized for pomace conversion to livestock feed, the pomace conversion project is now in the phase of developing the actual bioconversion process. Vine clippings are also currently being examined in terms of pretreatment and biological treatment. Again, the overall goal of this project is development of an on-farm, relatively simple, inexpensive process for grape farmers and/or wine producers to make profit off of their wastes. Simple additions of agents, such as mixing with sodium hydroxide and, later, fungal cultures, is the preferred method of operation.

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COMPOSITIONS OF CELLULOSE COATINGS AFFECT POPULATIONS OF YEASTS IN THE LIQUID FORMULATION AND ON COATED GRAPEFRUITS

RAYMOND G. MCGUIRE AND ELIZABETH A. BALDWIN
U.S. Department of Agriculture, ARS
13601 Old Cutler Road
Miami, FL 33158.

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Abstract. **The surfaces of citrus fruits support populations of many species of bacteria, yeasts, and other fungi while fruits are developing in the grove, but most are reduced or lost during fruit processing. Restoring surface populations of beneficial microorganisms can provide an opportunity for biological control of postharvest decay pathogens. If the biocontrol organism is incorporated into the coating, no additional processing steps are required during fruit packing. One biocontrol candidate, the yeast *Candida oleophila* Montrocher, has proven to prolong the storage life of grapefruits (*Citrus paradisi* Macf.), but its growth on the fruit is dependent upon the coating composition. Films based upon cellulose can support very high populations of this species. The yeasts *C. oleophila*, *Rhodotorula mucilaginosa* (Jorgensen) Harrison, and *Cryptococcus albidus* (Saito) Skinner survive many weeks in the refrigerated bottled coating, although addition of the preservative, potassium sorbate at 0.15%, will kill these yeasts within 2 weeks. Application of a coating with 0.15% potassium sorbate does not hinder development of fruit surface populations of *C. oleophila*, however.**

A wide variety of bacteria, yeasts, and other fungi typically resides on the surfaces of leaves and on fruits before they are picked from the tree and undergo normal processing (Andrews, 1992). Although some of these microorganisms may be pathogens, most are harmless epiphytes, living and dying with little or no effect on the plant itself. These epiphytes can have a considerable effect on one another, however, as they compete for the nutrients that accumulate on the fruit surface. This competition, as well as more vigorous forms of aggression, offers a convenient method of biological control for species of *Penicillium* on grapefruits (Wilson et al., 1991).

Green mold and blue mold, caused by *P. digitatum* Sacc. and *P. italicum* Wehmer, respectively, can seriously reduce the value of citrus fruits during storage. These fungi are less of a threat when populations of a native yeast, *Candida guilliermondii* (Castelani) Langeron and Guerra, originally isolated in Florida from a lemon fruit, are artificially elevated (Wilson and Chalutz, 1989). The yeast can be added to citrus coatings that are typically applied to fruits to reduce moisture loss and increase luster (McGuire, 1994). Shellac and wax coatings can be toxic to the yeast, however, due to the addition of alcohols and bases such as KOH, NH_4OH, and morpholine that