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Proc. Fla. State Hort. Soc. 100:44-46. 1987.

NONCONVENTIONAL TREATMENT OF CITRUS JUICE EXTRACTOR RESIDUE

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Abstract. **Due to the unique physico-chemical properties of citrus peel and pulp residue, utilization of this material in further processed foods offers an untapped citrus resource. Current technology involves treatment of peel and pulp with lime, pressing, and drying. Microbial and/or enzymatic production of useful and more economically valuable products is possible through solid-state fermentation. *Aspergillus niger* van Tieghem isolated from citrus pellets was used for solid-state fermentation of citrus juice finisher pulp. This organism produces pectic enzymes that can be useful for complete breakdown of the peel and pulp residue.**

Present technology for handling and processing of residues remaining after juice extraction has continued to place emphasis on the need for dewatering and production of a stable dried material. Kesterson and Braddock (10) described the basic details of a procedure for the manufacture of dried pulp as cattle feed. Briefly, the residue is comminuted, soluble and colloidal substances are precipitated by reaction with calcium oxide, liquids are separated by pressing, and the press cake is dried to near 10% moisture. This process has benefited from modern technology, automation and energy conservation; yet, it is still one of the most costly operations in citrus processing.

Besides improving traditional dewatering methods, some other processes have been proposed. Burdick et al. (5) treated liquid citrus wastes by submerged combustion and reported that scaling and fermentation difficulties common to citrus molasses production were circumvented by this process. In another process, steam stream drying, citrus residue is superheated and moisture is removed (2). The product is described as soft and fluffy, with improved digestibility. Other advantages claimed are lower energy use, no air pollution or burned particles in the product, and increased recovery of peel oils. Mannheim and Passy (14) studied processes to convert citrus peel and juice finisher pulp to dried edible products but the process generated large quantities of liquid wastes. A general review of some of these products, processes, and their utilization has been compiled by Braddock (3). Pretreatment of liquid wastes and subsequent removal of concentrated suspended colloidal and other insoluble material allows more efficient treatment of the remaining stream (11, 20).

Florida Agricultural Experiment Station Journal Series No. 8620.

The difficulty associated with complete dewatering of citrus extractor residues (4, 16) is related to the proximate composition. Dried citrus pulp is approximately 90% dry matter, 6% protein, 12% crude fiber, 63% nitrogen-free extract, 3% fat, and 7% ash (10). Sucrose, glucose, and fructose constitute the soluble carbohydrates, while pectin and cellulose dominate the insoluble carbohydrate fraction, with lesser amounts of hemicellulose and lignin.

The carbohydrate composition of soluble and insoluble polysaccharides makes this material a likely substrate for potential bioconversion to alternate products. In considering conversion, the solid nature of the material would have to be changed or modified to allow access to the nutrients. Thus, it would seem that some type of liquification of the residue would be appropriate. Marshall et al. (15) digested citrus peel wastes by pectolytic and cellulolytic enzyme treatment. They compared mass balances of the streams produced with the dry matter content resulting from the traditional liming and pressing operations for citrus processing. Maximum soluble solids removal occurred after 6 hr of incubation. Particle size reduction and combining pectinases with cellulases also increased recovery of soluble sugars and reduced the mass of the insoluble material remaining after treatment.

Solid-state fermentation (SSF) is a new name for a traditional agricultural process referred to as ensilage. Typically, SSF is used to break down agricultural waste into a more manageable material, to produce more useful products, or to extend feed supply. Since dewatering of citrus waste remains a difficult task and the abundance of citrus peel and pulp in Florida provides a readily available source, we proposed to review the previous work on SSF of citrus waste and to evaluate the potential product recovery from SSF of citrus juice pulp with a fungus isolated from rehydrated citrus pellets.

Materials and Methods

Preparation of pulp and inoculation. Mid-season Valencia orange juice finisher pulp was collected at the Citrus Research and Education Center and stored at –10°C until needed. An FMC 291 B-100 extractor was used to process fruit and an FMC Model 35 finisher with 0.02 mesh screens and 46 psi pressure was used to collect juice pulp.

An unknown mold was collected and isolated from citrus pellets which had been rehydrated to known moisture levels and identified as *Aspergillus niger* using standard microbiological techniques (9). The mold was grown on potato dextrose agar (PDA) for 2 weeks at 30°C and the spores collected with 0.9% sterile saline. Four samples of 125 g each were evaluated for by-product recovery. Two

samples of Valencia juice pulp (A and C) were inoculated with 1.5 x 10^5 spores/g and 2 samples (B and D) were uninoculated controls. Pectin (Sigma Chemical Co., St. Louis, MO. Lot 34F0620) was added at the 1% level in 2 samples (C, inoculated with *A. niger,* and D, uninoculated), to evaluate the ability of the spores to produce by-products with citrus pulp as a sole substrate source.

The samples were well mixed and incubated at 30°C. At timed intervals, 5 g were aseptically removed for mold counts and 25 g were removed for preparation of enzyme extracts.

Sample preparation and assays. Twenty-five grams of pulp were homogenized (AstraMixer, Model M-100) at 4°C in 4 volumes of 0.3M NaCl, 0.25M Tris-Cl, pH 8.0. The homogenate was collected by centrifugation at 16,000 x g for 25 min at 4°C. The supernatant constituted the crude enzyme preparation. Samples were frozen at –10°C until conduction of the enzyme assays.

Protease activity was qualitatively determined by the size of the zone of clearing on casein-agarose plates (12). Polygalacturonase activity was qualitatively estimated at 30°C, pH 4.2 by viscometry (1). Mold populations were estimated using the pour plate method (6). Results reported are the average of duplicate enzyme assays and triplicate mold counts.

Results and Discussion

At time 0, all samples had a light color and typical citrus odor. At 24 hr, samples with added *A. niger* (A and C) appeared darker than samples B and D. This trend continued throughout incubation and by 96 hr, samples B and D were also considerably darker than at Day 0. Syneresis was very evident in sample B at 72 hr and in sample A at 96 hr. During the course of fermentation, the citrus-like odor was replaced by an acidic odor typical of a mixed acid fermentation. Initially, the pH of the pulp was 3.9 and decreased to pH 3.0 in samples A and C and to pH 3.2 in samples B and D at the end of the fermentation period.

The results of the total mold count are given in Table 1. Only mold counts are reported although there was evidence of other bacterial colonies and yeasts. In samples A and C, inoculated with *A. niger,* the mold count increased from about 10^5 to greater than 10^7 within 24 to 48 hr and decreased at longer times. In uninoculated samples B and D, the mold count was greatest at 48 to 72 hr and was also followed by a population decline, possibly due to a pH effect.

The results of the qualitative test for protease are given in Table 2. The zones of protease activity were greatest in samples A and C at 24, 48, and 72 hr. This activity closely coincides with the mold count and suggests that the added *A. niger* was utilizing the citrus pulp to produce the protease.

Table 1. Enumeration of molds on potato dextrose agar at the respective incubation period. Mold counts expressed in CFU/g x 10^5.

	Time (hr)					
Sample ID	0	24	48	72	96	144
A	2.1	72	66	6	$<10^{-2}$*	$<10^{-5}$*
B	$3x10^{-4}$*	0.076	70	13	0.42	$<10^{-5}$*
C	2.1	120	100	34	$<10^{-2}$*	$<10^{-5}$*
D	$3x10^{-4}$*	0.11	5.8	<0.1*	<0.04*	$<10^{-5}$*

*Estimated.

Table 2. Qualitative protease activity on casein-agarose plates at the respective incubation period.[z]

	Time (hr)					
Sample ID	0	24	48	72	96	144
A	—	+ + +	+ + +	+ + +	—	—
B	+	+	+	+	—	—
C	+	+ + +	+ + +	+ + +	—	—
D	+	+	—	—	—	—

[z]A positive test was indicated by a zone of clearing around the sample well and is denoted by "+" for protease activity or "—" for no evidence of protease activity. The size of the zone is related to the amount of protease activity and is denoted by the numbers of "+".

The results of the qualitative test of polygalacturonase activity are given in Table 3. In sample A, PGase activity is greatest at 72 and 96 hr. This time coincides with a decrease in the total mold count. The PGase activity may be the result of other organisms generating PGase, but is unlikely to be due to the added *A. niger.* In sample C, PGase activity is highest at 24 hr. However, this sample was spiked with 1% pectin, suggesting that the added *A. niger* did not prefer citrus pulp over pectin as a substrate source. Surprisingly, samples B and D showed considerable PGase activity, especially at 48 to 96 hr.

Clearly, the choice of inoculum as well as substrate will influence the ability to utilize citrus waste as well as the products that are formed. Most solid-state fementation studies of citrus wastes have either used shake cultures (8, 13, 18, 19) or dried waste (7, 17, 21). Since our long term objectives are to develop a microbial protocol to generate useful products as well as increase the efficiency of the dewatering procedure of citrus waste, we elected to evaluate only wet waste without the addition of water to enhance commercial feasibility. Our proposed protocol is summarized in Fig. 1. Citrus juice extractor waste will be fermented in submerged culture. Sukan and Yasin (21) have shown that fermentation of lemon waste with mixed cultures of *Sporotrichum pulverulentum* and *A. niger* will produce cellulase as well as pectinase and increase the protein yield of the residue 5-fold. Based on the results of Marshall et al. (15) as well as the syneresis observed in this study, fermentation and generation of pectinases/cellulases will improve the dewatering process over traditional liming as well as increase the protein content of the final residue.

Recovery of the pectic enzymes and proteases could conceivably have other useful functions to those in the bev-

Table 3. Qualitative polygalacturonase activity by viscometry at the respective incubation period.[z]

	Time (hr)					
Sample ID	0	24	48	72	96	144
A	—	+	+	+ + +	+ + +	+
B	—	+	+ + + +	+ + +	+ +	+ +
C	—	+ + + +	+ +	+ +	+ +	—
D	—	+	+ +	+ +	+ +	+ +

[z]A positive test was indicated by a decrease in the time for a pectin solution to flow through a capillary viscometer at 30°C and is denoted by "+" for polygalacturonase activity or "—" for no evidence of polygalacturonase activity. The relative amount of polygalacturonase activity is denoted by the numbers of "+".

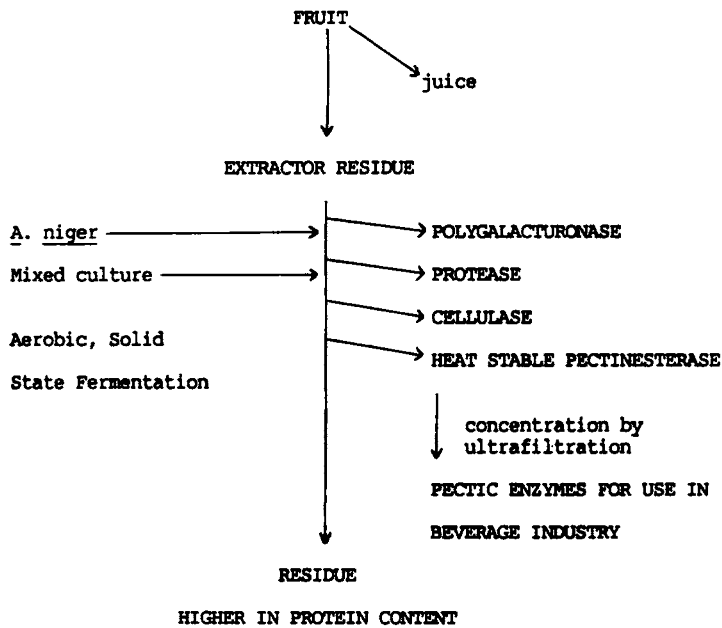

Fig. 1. Proposed protocol for solid state fermentation of citrus juice extractor residue.

erage industry involved in juice recovery and clarification. Wicker et al. (22) reported on the extraction of a heat stable pectinesterase from Valencia juice pulp that shows three times the residual activity to heat at 70°C for 2 min as the control. This heat stable PE fraction was released only after disruption of the cell wall with pectinase and cellulase. Recovery and concentration of thermostable pectic enzymes has major advantages for the beverage industry.

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