Microbiological and Chemical Changes in Poi Stored at 20 °C

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Microbiological and chemical changes of poi (cooked taro paste) stored at 20 °C were quantitatively measured. Commercial poi samples were stored in a constant-temperature chamber and sampled periodically. Both the pH and bacterial counts changed drastically in the first 2–3 days but thereafter remained at 4.0–4.5 and $\log_{10}$ 5.8 CFU/g, respectively. *Lactococcus lactis* was identified to be the predominant bacterial species in sour poi. Sucrose is the main carbon source for bacterial metabolism at the early stage of the souring process. Lactic acid and acetic acid accumulated in sour poi to 92 and 45 mg/100 g of fresh weight, respectively, while oxalic acid and succinic acid contents decreased substantially.

INTRODUCTION

Poi, a purplish to grayish paste made mainly of ground taro (*Colocasia esculenta*), is a traditional staple food in Hawaii. In ancient times, poi comprised a large portion of the Hawaiian diet, and today it is still sold commercially in Hawaii and parts of California. When fresh, poi tastes rather bland. Most people prefer to eat poi with other food or to leave it in a cool place for 2–3 days before consumption, so that the poi can develop a strong sour taste. The only studies on the souring of poi were reported more than half a century ago. Allen and Allen (1933) studied the poi-souring process and concluded that it was caused by acid-producing bacteria such as *Lactobacillus* and *Streptococcus*. Almost simultaneously, Bilger and Young (1935) investigated the changes in reducing sugars and organic acids in sour poi. They identified lactic acid, acetic acid, formic acid, alcohol, and acetaldehyde in the fermented product. However, because of available technology, these authors were unable to determine the predominant acid-producing bacteria species and to quantify individual sugars and organic acids.

Poi is manufactured from cooked taro corms in which starch is the main component. The souring process is a natural fermentation proceeding without inoculated starter cultures. Although the tools used in producing poi have changed through the years, the method has essentially remained the same. The corms are cooked, washed, peeled, trimmed, ground, strained, and blended with some water to produce the final product (Moy and Nip, 1983). The purpose of this study was to re-examine the poi-souring process and compare the results with previous findings. Current methodologies were used to determine the predominant bacteria responsible for souring poi and to quantitatively measure the changes of organic acids and simple sugars in sour poi. A further purpose of this study was to provide basis for experiments on manufacturing sour poi by nonmicrobial means.

MATERIALS AND METHODS

Sample Preparation. Poi samples were obtained directly from a poi factory located in Honolulu, HI, on six different days approximately 3 weeks apart. The poi samples were collected fresh on the process line by the researchers and were immediately brought back to the University of Hawaii campus, also located in Honolulu. The poi samples were stored at 20 ± 0.5 °C in a constant-temperature chamber (Revco RI-12-1060, Asheville, NC). This storage temperature was chosen because poi is displayed in retail stores at around this temperature, according to our prior investigations. At periodic intervals, representative fractions of poi samples were collected for microbial enumeration and chemical analysis of sugar and organic acid profiles. The moisture content of samples were determined in a convection oven at 100 °C following the standard AOAC (1990) procedure. The samples were weighed repeatedly until a constant weight was reached. The pH value of each sample was measured using an electronic pH meter. The samples were diluted five times in deionized distilled water and mixed prior to pH measurement.

Microbiological Study. Poi samples were evaluated for aerobic, acid-producing, *Lactobacillus*, mold/yeast and coliform bacterial numbers (Diliello, 1982). At selected intervals, three bags each of poi were mixed by hand kneading; then 10-g subsamples were aseptically transferred in a clean air hood to chemically sterilized blenders. The portions (10 g) were homogenized with distilled sterile water (90 g) to achieve a 1:10 dilution. This was followed by appropriate serial dilutions in 0.1% peptone water. The pour plate method was used to enumerate presumptive coliform bacterial numbers. The procedure involved pouring duplicate plates of appropriate dilutions with 20–25 mL of violet red bile agar (VRBA) (Difco), allowing the basal medium to solidify while being rotated gently, and adding a 4 mL overlay of VRBA. The plates were incubated for 24 h at 35 °C.

The spread plate method of pipetting 0.1 mL in duplicate of appropriate dilutions was used to estimate plate counts in other media (Diliello, 1982). The media used for aerobic, acid-producing, and mold/yeast plate counts were plate count agar (PCA) (Difco), PCA with bromcresol purple (BCP) (Difco), and potato dextrose agar (PDA) (BBL), respectively. The PCA and BCP plates were incubated for 48 h at 35 °C. The pH of the PDA medium was adjusted to 3.7 after sterilization by means of a sterile 10% tartaric acid. The PDA plates were incubated at 25 °C for 3–5 days. Counts of lactobacilli were made on *Lactobacillus*-selective agar (Merck). The plates were incubated aerobically for up to 5 days at 30 °C.

The predominant bacteria were isolated by streaking onto fresh PCA media and then cultured onto slants. Isolates were initially characterized as to morphology and Gram-stain reaction and further identified using the API Rapid STREP (API System, Plainfield, NY) according to the protocol given by *Bergey's Manual of Systematic Bacteriology* (Sneath, 1984).

Analysis of Sugar Profile. The sugars in poi were extracted in triplicate using the standard AOAC method (982.14C, 1990). Four grams (weighed to 0.01 g) of freeze-dried poi powder was extracted in 100 mL of ethanol/water (1 + 1). The extract was treated with a C_{18} Sep-Pak cartridge using a mobile phase of acetonitrile/water (82/18 v/v). The second pass of the eluate

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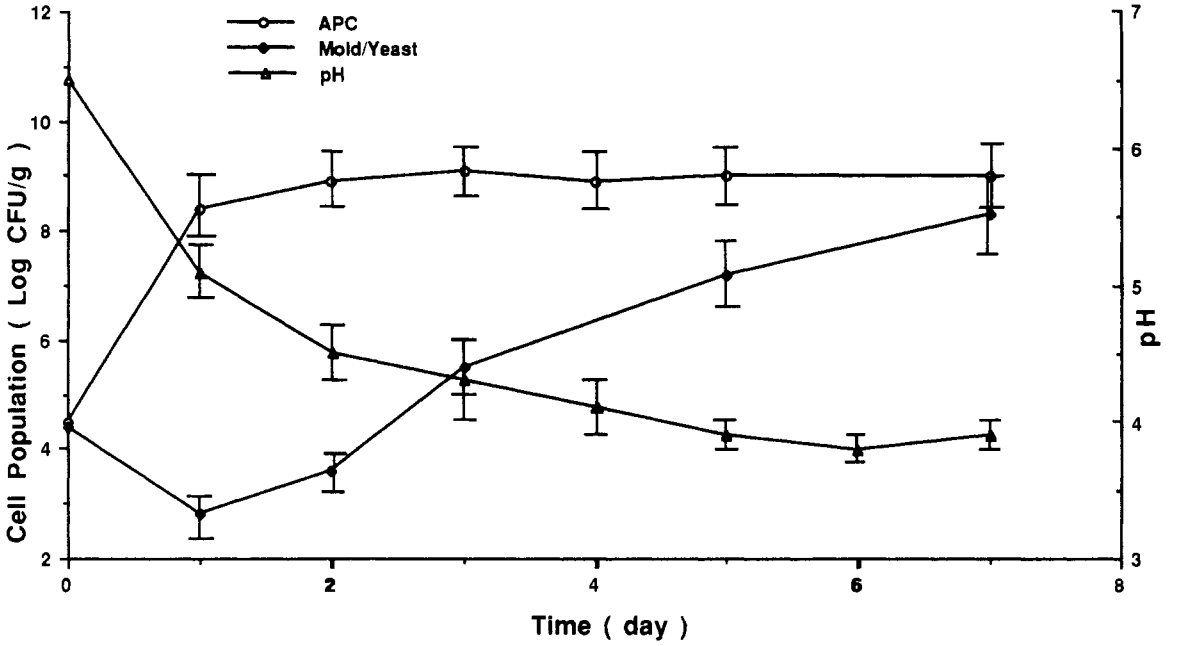

Figure 1. Values of pH and logarithm numbers of APC and mold/yeast counts in poi stored at 20 °C. 95% confidence intervals are indicated.

was collected. The eluate was filtered through a 0.2-μm microfilter prior to HPLC analyses.

The HPLC system was composed of a HPXL solvent delivery system (Rainin Instrument, Woburn, MA) fitted with a Rheodyne 7125 20-μL loop injector, and a RID-6A refractive index detector (Shimadzu Scientific, Columbia, MD). The column was a Spheri-5 Amino cartridge (100 × 4.6 mm) protected by a NewGuard Amino cartridge (15 × 3.2 mm, 7 μm) (both from Applied Biosystems, Foster City, CA) and developed isocratically with a mobile phase of acetonitrile/water (82/18 v/v) (AOAC, 1990). The flow rate was 2.0 mL/min.

Individual sugar standards (fructose, glucose, sucrose, and maltose, from Sigma Chemical, St. Louis, MO) were dried for 12 h at 60 °C under vacuum prior to use. Standard solutions of different sugars were prepared in ethanol/water (1 + 1) in the range 0.05–5.0 mg/mL. Retention times of the standards were compared with the sample to identify the varieties of sugar in poi. The presence of each of these sugars was further confirmed by spiking a poi extract prior to HPLC injection with each of the sugars individually. An internal standard method of adding a lactose standard in the poi extract was also used. Lactose was chosen because it was well separated from the other monosaccharides of interest and was not present in poi. Average recovery of spiked standard sugars was 95% with a coefficient of variability of 3–5%. Sugar content in extracts was quantitatively measured according to standards with comparable (within 10%) concentrations (AOAC, 1990).

Analysis of Organic Acids. The organic acids were extracted with 1 N HCl according to a modified procedure of Holloway et al. (1989). The extraction started with mixing 5-g (weighed to 0.01 g) poi samples with 15 mL of the acid in a 50-mL volumetric flask. The flask was then tightly capped and placed in a boiling water bath for 18 min. After the flasks were cooled to room temperature and filled up to volume with distilled water, the mixture was filtered through a fast, ashless Whatman No. 542 filter paper. Five replications were performed for each poi sample. The filtrate was again filtered through a 0.45-μm membrane filter prior to HPLC analyses. The extraction procedure was validated by obtaining an average of 97% recovery of known quantities of added acetic acid, lactic acid, oxalic acid, and succinic acid (Sigma).

The HPLC analyses were performed with a SP8800 programmable isocratic solvent delivery unit (Spectra-Physics, San Jose, CA) equipped with a variable-wavelength UV detector (Spectra 100, Spectra-Physics) and a conductivity detector (LDC Conductomonitor, Milton Roy, Riviera Beach, FL). The sample was injected through a Rheodyne 7125 loop injector that was fitted with either a 20- or a 100-μL sample loop. There were two types of column systems used. The Aminex column was used to measure lactic acid, acetic acid, and other weak organic acids (Picha, 1985; Holloway et al., 1989). The system included an Aminex HPX-87H cation-exchange column (300 × 7.8 mm, Bio-Rad Laboratories, Richmond, CA) which was developed isocratically with a 0.005 M H_2SO_4 mobile phase at a flow rate of 0.6 mL/min. The UV detector was set at 214 nm. The quantitative measurement of oxalic acid used a method developed by Huang and Tanudjaja (1992). The system included a Universal anion column (150 × 4.6 mm, Alltech Associates Inc., Deerfield, IL) and a mobile phase of 4 mM phthalic acid (adjusted to pH 4.5 with lithium hydroxide). The flow rate was 1 mL/min and the measurement of the LDC conductomonitor was recorded in micro-Siemans. Chromatograms were recorded and peak areas were analyzed using a digital integrator (Shimadzu CR601).

Data Analysis. Measurements of replicate samples were averaged to obtain a single datum point. Differences in averages were analyzed for statistical significance using either Student's t-test or Duncan's multiple-range test. Statistical analyses were conducted using the STATPAK software program (Northwest Analytical Inc., Portland, OR).

RESULTS AND DISCUSSION

Numbers and Types of Microorganisms. Commercially made fresh poi contained about 4.5 log CFU/g of aerobic plate count (APC), which increased rapidly to 8.0–9.0 log CFU/g in the first 24 h at 20 °C. Correspondingly, the pH of poi changed from 6.4 to 5.1 during the same period. The APC stabilized at 9.0 log CFU/g until the end of the seventh day, while the pH of poi continued falling until it reached 3.9 on about the fifth day (Figure 1). Approximately 85% of the microflora was identified as acid-producing bacteria of pinpoint size white colonies. They were Gram-positive cocci, catalase-negative, and could be grown in both aerobic and anaerobic conditions. They were identified to be *Lactococcus lactis* using the API Rapid STREP. The lactobacilli counts were less than 5% of the APC.

The mold/yeast counts were approximately equivalent to APC in fresh poi (Figure 1). In the first 24 h, the mold/yeast counts decreased while APC increased. Consequently, the mold/yeast counts constituted only a small fraction of the APC in those sour poi products. After the

Table I. Content of Sucrose, Fructose, and Glucose (Weight Percent, Dry Weight Basis) in Fresh Poi and Sour Poi Stored at 20 °C[a]

length of storage (days)	sucrose	fructose	glucose
0 (fresh)	4.4 ± 1.5	0.15 ± 0.02	0.16 ± 0.01
1	2.4 ± 0.4	0.54 ± 0.07	0.08 ± 0.01
2	1.2 ± 0.2	1.2 ± 0.1	0.13 ± 0.02
3	0.21 ± 0.03	1.3 ± 0.1	0.25 ± 0.03
4	0.05 ± 0.01	0.48 ± 0.05	0.37 ± 0.03
5	nd	0.11 ± 0.02	0.53 ± 0.08
6	nd	nd	0.8 ± 0.2
7	nd	nd	1.1 ± 0.3

[a] Values are means ± SE of replicate analyses (n = 18). nd, not detectable.

second day, the mold/yeast counts increased from 3.6 to 8.3 log CFU/g on the seventh day. The total presumptive coliform counts were less than 1% of APC throughout the experimental period. Even though the poi products were commercially made, the tested samples exhibited remarkably uniform quality in terms of microbial counts. The low mold/yeast and coliform counts in sour poi support the observation by Allen and Allen (1933) that poi has a self-cleaning effect during souring due to the growth of the acid-producing bacteria.

Allen and Allen (1933) identified three *Lactobacillus* species and two *Streptococcus* (recently renamed to *Lactococcus*) species including *S. lactis* in poi. However, they did not identify the predominant species. The APC of fresh poi as reported by Allen and Allen was approximately 8.0 log CFU/g, but their APC of the second- and third-day poi was 7.0 log CFU/g. The discrepancy between their results and ours may reflect technological improvements in processing in the present-day poi factories. For instance, taro corms are now cooked in a pressure cooker or retort, in place of the open kettle used previously. Moreover, the current Good Manufacturing Practice (GMP), which did not exist 50 years ago, is now followed in poi factories. Consequently, it is reasonable to expect cleaner poi nowadays, as indicated by a lower APC but a more homogeneous microflora in the fresh poi. Possibly due to less competition from contaminating bacteria, a faster growth of *L. lactis* resulted in a rapid drop in pH and higher APC in the sour poi we studied. We could not compare sanitary conditions on the basis of coliform counts, because Allen and Allen (1933) did not report total coliform counts in their studies.

Profile of Simple Sugars. Most previous studies on taro and poi composition found a soluble sugar content of 1% or lower (Allen and Allen, 1933; Bilger and Young, 1935; Standal, 1970). These results were based on either measurement of the total reducing sugars or other less sensitive colorimetric tests. Recently, Hussain et al. (1984) employed a more specific enzymatic procedure that measured the sugar contents of sucrose, fructose, and glucose in taro to be 5.08, 0.43, and 0.33% on dry weight basis, respectively. In our study, the sugar content in fresh poi measured by HPLC was similar to that in the last paper (Hussian et al., 1984) at 4.4 ± 12, 0.15 ± 0.02, and 0.16 ± 0.01% for sucrose, froctose, and glucose, respectively (Table I). As the poi turned sour, its sucrose content decreased while fructose content increased. The fructose content peaked on the third day, decreasing thereafter. Sucrose was not detectable in poi after the fourth day, and fructose was not detectable after the fifth day. Glucose was only found in small quantities in poi 3 days old or less. Glucose content increased substantially after the third day. Maltose was not identified in either fresh poi or sour poi. The changes in sugar content during the poi-souring period were not previously reported.

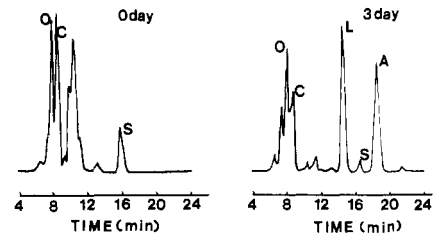

Figure 2. HPLC chromatograms of organic acids in fresh poi and 3-day-old poi. Chromatograms were developed by a mobile phase of 0.005 M H_2SO_4 at a flow rate of 0.6 mL/min on an Aminex HPX-87H column (300 × 7.8 mm). The organic acids were measured by an UV detector set at 214 nm: O, oxalic acid; C, citric acid; L, lactic acid; S, succinic acid; A, acetic acid.

The change in sugar profile of sour poi corresponded well with the growth of microorganisms. The rapid decrease in sucrose coincided with the rapid increase in APC in the first 2 days of poi fermentation. Glucose is the most preferred carbon source for *Lactococcus* bacteria (Skinner and Quesnel, 1978); therefore, the glucose from the breakdown of sucrose was consumed immediately. This explains the low glucose content in the first 3 days. Fructose, also produced from the breakdown of sucrose, was consumed at a slower rate in the beginning, which explains the accumulation of fructose in the first 3 days. After rapid depletion of sucrose by the bacteria population, the consumption of fructose increased. The initial amount of sucrose in taro, therefore, dictated the initial bacteria growth in poi. Taro starch may be used later by the yeasts and molds, which explains the surge in glucose content after the fourth day. However, most people would not eat sour poi that is moldy or older than 5 days. The initial sucrose content in taro corms, therefore, may be an important quality index for maintaining a consistent sour poi product. The low sugar content in sour poi (i.e., 2–3-day-old poi) is also a factor to be remembered in manufacturing sour poi by nonmicrobial means. To make a sour poi acceptable to people adapted to the plain sour taste, the sugar content may need to be kept low.

Organic Acid Profile. We positively identified and quantitatively measured the changes of four organic acids (acetic, lactic, oxalic, and succinic) in sour poi. The retention times and resolution factors between standards were in agreement with previously published data (Picha, 1985; Holloway et al., 1989; Huang and Tanudjaja, 1992). In Figure 2, the HPLC chromatogram of organic acids in a typical fresh poi was compared with that in a typical 3-day-old poi. The main differences were increases in acetic acid and lactic acid and decreases in oxalic acid and succinic acid. Citric acid was also identified in fresh poi, but we believed that the interference in poi extract to citric acid on this HPLC column was too great to accurately quantify its content. This interference was also noticed in fermenting cacao bean using the same HPLC method (Tomlins et al., 1990). We recently overcame the interferences to oxalic acid by using the Alltech universal anion column instead of the Bio-Rad column (Huang and Tanudjaja, 1992). A similar study is needed for citric acid quantification by HPLC. In Table II, the contents of the four organic acids in poi at different stages of fermentation are compared. Results show that both lactic acid and acetic acid contents increased rapidly in the first 24 h, coinciding with the drop in pH and the increase in the population of acid-producing bacteria. The increase continued into the second day but reached a plateau on the third day. The oxalic acid content decreased substantially in the first 2

Table II. Content[a] of Organic Acids Identified in Poi at Different Stages of Fermentation at 20 °C

fermentation stage (days)	lactic acid	succinic acid	acetic acid	oxalic acid
0 (fresh)	2.3 ± 0.2^{a}	142 ± 28^{a}	nd	86 ± 7^{a}
1	33 ± 4^{b}	126 ± 16^{a}	23 ± 2^{a}	74 ± 8^{a}
2	64 ± 8^{c}	77 ± 15^{b}	36 ± 4^{b}	69 ± 5^{ab}
3	82 ± 6^{cd}	29 ± 12^{c}	45 ± 4^{b}	54 ± 6^{b}
5	92 ± 5^{d}	14 ± 8^{c}	38 ± 3^{b}	55 ± 5^{b}
7	87 ± 6^{cd}	12 ± 5^{c}	45 ± 5^{b}	54 ± 5^{b}

[a] Mean ± SE (n = 18), expressed in milligrams/100 g of fresh weight; common superscript letter within a column indicates nonsignificance ($p < 0.05$).

days. The rate of decrease slowed after the third day. The succinic acid content, on the other hand, started decreasing after the second day and continued until it was almost depleted. These data indicate a change in the activities of the acid-producing bacteria, although the bacteria counts remained stable.

In addition to lactic acid and acetic acid, Bilger and Young (1935) also found some formation of formic acid in sour poi. Our HPLC method can clearly identify formic acid if it exists in the extract. However, we did not see any accumulation of formic acid in the acid extract of sour poi. This again may be a result of a more homogeneous bacteria population in presently manufactured poi. This study is part of a continuous effort to extend the shelf life and to modernize the manufacturing of poi and poi-based products.

ACKNOWLEDGMENT

This research was supported by the Governor's Agriculture Coordinating Committee of the State of Hawaii, Contract 90-07.

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Received for review June 7, 1993. Accepted October 4, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1993.