Behavior of Seedleaf Tobaccos in a Laboratory Fermentation Chamber*

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INTRODUCTION

After warehouse aging for 2 years, tobaccos destined for cigar or chewing tobacco manufacture are subjected to many weeks of either bulk sweating or forced fermentation in hogsheads. These fermentations are necessary to improve the taste characteristics of the chewing tobacco blend or to reduce the pungent cigar smoke which is unacceptable to the consumer.

Few systems have been developed in the laboratory to either monitor or predict fermentative events at the factory scale. One such system was developed in 1974 by Teague and DeVoe (1). Their apparatus consisted of a stainless steel cylinder of 31 cm outside diameter and a length of 51 cm, which was half loaded with one dark fired tobacco sample. Heat and humidified air were supplied as the enclosed system was rotated mechanically. This procedure shortened the fermentation time of snuff tobacco from months to days.

There are quantitative effects (2–4) which accompany tobacco fermentation, such as loss of dry weight, generation of heat, absorption of oxygen as well as evolution of carbon dioxide and ammonia, a change in pH, and a decrease in fire-holding capacity of the tobacco. Thus, a realistic tobacco fermentation system requires moisture, temperature control, a sizeable microbial population (5–6), and enough “breathing” to allow the exchange and release of gases. Perhaps the most critical factor in starting and controlling fermentation is the amount of water introduced into the leaf tissues (2). Also, periodic turning of the tobacco fosters a better fermentation process (7). A laboratory fermentation system of broader scope and versatility than previously known was needed.

The scope of this paper is to present the design and functionality of a laboratory fermentation system. The purpose of the work was to attain fermentation in the laboratory under accelerated, controlled conditions, not to isolate the causative factors leading to a successful fermentation. Details pertaining to physico-chemical characteristics other than routine leaf analysis data (e.g.: percentage of nicotine, pH, percentage of nitrogen) and organoleptic properties of fermented tobacco fall beyond the intent of this work, and will not be dwelled on in the subsequent discussion.

MATERIALS AND METHODS

Apparatus

Chamber: A fermentation chamber containing a tumbling fermentation device to achieve the above ends has been designed and made operational (Fig. 1). The environmental chamber (65 × 65 × 31 cm) is fabricated out of 9.5 mm Lexan® (polycarbonate, LNP Corp., Malvern, Pa., U.S.A.) sheet. There are two hatches, one at the top for sample introduction and one on a side for cleaning. A rectangular stainless steel water reservoir fits snugly at the bottom of the chamber.

Air heating is regulated by a proportional controller, connected to a precision thermistor in the inner center wall of the box. The air heater is a nichrome wire wound around a Vycor® (96% silica glass (Code 7913), Corning Glass Works) tube. A horizontal plate is located below the heat sensor. Thus no hot spot develops near the thermistor. A powerstat controls a coiled nichrome wire immersion heater in the water bath. Compressed air is bubbled into the water at an aeration rate of 600 ml per minute. Dry and wet bulb temperature probes can be inserted through the top of the box. Any humidity can be reached by varying the relative air and water temperatures in the box. The chamber can maintain a temperature within $\pm 0.2^\circ\text{C}$.

Windmill: An aluminum wheel or “windmill” (52 cm diameter × 4.8 mm thickness) is rotated by a 1/20 h.p., fixed speed Dayton Gearmotor (Dayton Electric Mfg. Co., Chicago, Ill., U.S.A.) mounted on an aluminum base outside the chamber. A glass plate, fixed at an angle above

Figure 1. Fermentation chamber.

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the wheel, serves as a canopy to prevent water droplets from wetting the fermentation vessels.

Vessels: The tobacco samples to be fermented are ground through a 2 mm screen on a Wiley mill, then sieved through an ASTM* No. 40 mesh sieve. Particles larger than 425 μ are retained, affording a reasonably homogeneous particle distribution. Leaf samples also underwent fermentation, as detected by leaf analysis. They were not chosen for systematic studies in order to minimize size and number of samples, and to enhance reproducibility.

The samples are tumbled at 2.4 r.p.m. in polypropylene screwcap bottles which are affixed to the wheel. The choice of this material was based on preliminary tests and on the nature of the plastic. It is biologically inert and high in chemical resistance. Each bottle has been modified for optimum gas exchange by introducing a 425 μ mesh stainless steel window in the side. Each of the eight vessels is held by five polypropylene arms from corresponding springs on the wheel. A vessel is half full when bearing 50 g of tobacco. The tobacco tumbling action is generated by the rotating motion of the wheel.

Fermentation chamber tests with the standardized window polypropylene bottles consistently exhibited moisture retention of 95%–100% at 90% relative humidity. This ambient relative humidity was empirically found to be necessary so as to retain tobacco moistures ranging from 30%–70%.

Fermentations

Fermentation of Pennsylvania (PA) and Wisconsin (WIS) Seedleaf tobaccos has been performed in this system at moistures ranging from 30% to 70% for 1 to 14 days. The temperature was kept at 45 °C. There was no evidence of mold formation even at the highest moisture level. Upon completion of a run, one could readily detect ammonia release.

Leaf Analyses

Standard leaf analyses provided a chemical profile of the fermenting tobacco. All analyses were performed on tobacco which had been dried at 41 °C for 18 hours. Thus, measurements were made on a dry basis. Since the tobacco samples had already been ground and sieved, the whole sample was analyzed before and after laboratory fermentation.

Total nitrogen was obtained by the *Kjeldahl* method, using a Labconco macrodigestion system. Total volatile bases (percentage of TVB) were determined with a Fisher potentiometric Titralyzer. Nicotine was determined by cyanogen bromide cleavage of its pyridine group and reaction with aniline in a buffered solution. The resulting yellow dye was measured colorimetrically at 460 nm on a Technicon AutoAnalyzer II system. Tobacco pH was determined with a Fisher automatic titrator.

RESULTS

Weight loss in the fermented tobacco samples was physicochemically quantitated in part by nitrogen, total volatile

bases, and nicotine determinations. It was not deemed necessary for our purpose to measure gross total weight loss nor ash content (i.e. gross level of inorganics present). Thus, the extent of fermentation was judged by decreases in the percentages of total nitrogen, TVB and nicotine, and an increase in pH of the tobacco. Some trends can be culled from the data tabulated for various crop types and years. All of these results were obtained at 45 °C and 90% relative humidity. *Reid et al.* (8), in studies with cured and fermented PA, WIS and Ohio cigar leaf tobacco, found an initial surge in aerobic bacteria, most likely spore formers and cocci, at the beginning of the fermentation. This was followed by a gradual decline in population as the bulk temperature increased and conditions became less aerobic.

The changes in leaf analysis data followed the expected direction, as is illustrated in Tables 1–3. The values are comparable to those obtained during large scale aging and forced fermentation. For example, PA 1973 tobacco had clearly fermented well at 70% moisture over a 4-day period, as evidenced in the pH increase from 6.04 to 8.03 (Table 1). A typical pH increase for this tobacco in a large scale fermentation is 1–1.5 pH units. By contrast, WIS S 1974, WIS N 1975 and WIS S 1975 all underwent huge changes, particularly in the percentage of TVB and pH, even at 60% moisture (Table 2). The leaf analysis data, especially the percentage of nicotine, were unusually low even before chamber fermentation because of extensive aging prior to sampling. Nicotine levels are generally lower in WIS tobacco than in PA, and, as a rule, faster fermentation occurs in WIS than in PA tobaccos. The great changes in WIS tobacco data are probably due to greater activity in WIS microflora. The nature and population of the micro-organisms may be different also. It was beyond the scope of this work to detect and identify microbial members of the mixed culture found on fermented tobaccos.

Table 1. Ground tobacco chamber fermentation of PA 1973 and PA 1976.

Time (days)	Moisture (%)	Nitrogen (%)	Total volatile bases (%)	Nicotine (%)	pH
PA 1973*					
0	7.2	3.68	1.05	2.50	6.04
4	60.6	3.51	0.77	2.36	6.39
4	70.8	3.50	0.40	2.24	8.03
PA 1976**					
0	10.0	4.02	0.95	2.61	5.80
4	51.0	3.89	0.94	2.40	—
4	60.0 ⁺	3.81	0.59	2.34	6.93
4	71.0	3.89	0.44	2.15	8.73

* Duplicate determinations were performed.

** Determinations were in duplicate except for line 3, which was carried out in quadruplicate. The mean variation was: percentage of nitrogen, ± 0.12 ; percentage of TVB, ± 0.02 ; percentage of nicotine, ± 0.28 ; pH, ± 0.08 .

⁺ Calculated initial moisture.

* American Society for Testing Materials.

Table 2. Ground tobacco chamber fermentation of WIS S 1974, WIS N 1975, and WIS S 1975.

Time (days)	Moisture (%)	Nitrogen (%)	Total volatile bases (%)	Nicotine (%)	pH
<i>WIS S 1974**</i>					
0	14.0	3.25	0.79	0.93	6.77
4	60.9	3.36	0.29	0.84	9.28
4	70.7	3.16	0.26	0.87	9.41
<i>WIS N 1975*+</i>					
0	26.0	3.30	0.38	0.53	7.21
4	61.8	3.36	0.25	0.39	9.20
<i>WIS S 1975*</i>					
0	19.3	3.51	0.71	1.53	6.12
4	59.7	3.40	0.31	1.18	9.55

* Duplicate determinations.

** Triplicate determinations.

+ This sample had already undergone extensive fermentation prior to chamber fermentation.

With PA 1976 tobacco, distinct changes occurred in the percentages of TVB and nicotine, and pH during 4-day fermentations (Table 1). These quantifiable events progressed nicely from 50% to 60% to 70% moisture levels, underscoring the important role which water plays in the fermentation process.

Tumbling of the tobacco samples is a virtue of the system described here, and is akin to tobacco sweating in bulk fermentation. It becomes only partial, however, at a tobacco moisture level above 70%. The advantage of total sample tumbling is steady exposure of tobacco surface area to ambient conditions, thus maximizing reproducibility. Plotting the decrease in the percentage of total volatile bases versus the percentage of moisture for PA 1977 ground tobacco fermentation shows that, between 50% and 60% moisture, the TVB level drops sharply (Fig. 2). Thus, 4-day runs at 60% moisture are sufficient and convenient. A broad view of PA 1977 fermentation behavior can be seen in the table showing time and moisture related changes. For example, even after 2 weeks at 30% moisture, no fermentation occurs; likewise, at 40% for 4 days. By contrast, both 60% and 70% moisture induce great changes (Table 3).

DISCUSSION

Relationships connecting fermentation events with leaf analysis measurements are interpreted below.

Percentage of Nitrogen: With some PA and WIS tobacco samples, the percentage of nitrogen first rises slightly and then diminishes after prolonged fermentation. This is not true with PA 1977. Generally, there is a weight loss for the whole sample throughout the fermentation period, which reflects loss of nitrogen-containing species as well as carboxylic acids, etc., the two events almost balancing each other analytically. It is thus reasonable that the change in

Table 3. Ground tobacco chamber fermentation of PA 1977.*

Time (days)	Moisture (%)	Nitrogen (%)	Total volatile bases (%)	Nicotine (%)	pH
<i>PA 1977</i>					
0	13.5	4.14	1.19	3.48	6.40
4	31.0	4.03	1.15	3.42	6.12
7	31.0	3.96	1.09	3.38	—
14	31.0	4.01	1.14	3.20	—
4	41.2	3.99	1.13	3.36	—
4	60.2	3.83	0.83	3.31	7.10
4	69.3	3.76	0.63	3.15	8.10
8	71.2	3.73	0.45	2.98	8.51

* Determinations were done in duplicate, except those reported on lines 6 and 7, which were done 17 and 5 times, respectively. The mean variation was: percentage of moisture, ± 1.25 ; percentage of nitrogen, ± 0.04 ; percentage of TVB, ± 0.12 ; percentage of nicotine, ± 0.31 ; pH, ± 0.10 .

concentration of nitrogenous material may be slight relative to any other measurement performed on the fermenting tobacco. Pounds (9) found that fermenting PA and flue-cured tobaccos left the total nitrogen basically unchanged even though nitrogen-bearing compounds are actively undergoing change.

Percentage of Total Volatile Bases (TVB): The decrease in the percentage of TVB for any of the tobacco types and years studied is the most dramatic of any of the measurements reported here; e.g. with PA 1973 tobacco, the TVB level drops by 62% over a 4-day period at 70% moisture. The slope depicting TVB drop varies among tobacco types and years. Southern Wisconsin tobacco shows a much greater TVB loss than any of the other tobaccos. Our most thoroughly studied system, PA 1977, lies in an intermediate position in TVB slope. Observe the decrease in the percentage of TVB for PA 1977 over a 14-day period (Fig. 3). A steady drop is noticed until the 7th day, then a plateau, followed by a second, less steep decrease beginning with the 11th day. The change in slope is

Figure 2. Change in the percentage of total volatile bases (TVB) versus moisture for PA 1977 tobacco during a four-day chamber fermentation.

Figure 3. Decrease in the percentage of total volatile bases (TVB) in 60% moisture PA 1977 tobacco during chamber fermentation.

marked. Multiple determinations give a weighted significance to the 14th day point.

Percentage of Nicotine: Probably the most significant, although small, change detected by leaf analysis of a fermented sample is the drop in the percentage of nicotine. The maximum decrease observed was about 25% over a 2-week period for PA 1977 at 60% moisture. A drop of 3%–5%, however, such as after 4 days at this moisture, is meaningful. The plot of the percentage of nicotine versus time (Fig. 4) shows a similar pattern to that for TVB. This may be due to the like nature of the basic structures detected. Again, a plateau ensues at days 7–8. A drop of greater slope starts at day 11 as in the case of the TVB-time plot. This pattern may indicate that over-fermentation does begin at about the 11th day under the conditions described.

Figure 4. Drop in the percentage of nicotine in 60% moisture PA 1977 tobacco during chamber fermentation.

pH: This determination correlates well with the TVB/nicotine data. In the stages of laboratory overfermentation (i.e. past day 11) there may be no net decrease in acid species versus bases so that the smoothly increasing pH pattern reaches a plateau.

As displayed in Table 2, Wisconsin tobaccos show much higher increases than Pennsylvania (e.g. from pH 6.12 to

Figure 5. pH Increase in 60% moisture PA 1977 tobacco during chamber fermentation.

9.55 at 60% moisture for WIS S 1975). This behavior is explainable by the greater activity of microflora associated with WIS tobaccos.

The plot of pH versus fermentation time for PA 1977 shows a rising convex curve, with a gradual tapering off by days 7–8, and an asymptote by the 14th day (Fig. 5). This pattern confirms the observation that, after 7 days at 60% moisture and 45 °C, a tumbled, ground Seedleaf tobacco sample is already well fermented. Therefore, it is felt that 4-day, 60% moisture runs may be sufficient for the gleaning of trends which could be extrapolated to large scale fermentation.

Taste testing of PA 1977 leaf samples, which had been chamber fermented at 50% and 60% moisture for 8 days, showed distinct improvement in the flavor of the fermented tobaccos, as judged by an expert taste panel with a limited number of samples. The 60% moisture fermented samples were milder than those fermented at 50% moisture. No attempt was made at this time to expand this small taste sampling to a larger study.

SUMMARY

A laboratory system which simulates factory fermentation of tobaccos has been designed and made operational. This system is of broader scope and versatility than the known laboratory tobacco fermenters. The device consists of a Lexan® (polycarbonate) humidified chamber containing a rotating aluminum wheel bearing 8 polypropylene vessels. Heating, humidification and aeration are controlled.

Routine fermentation conditions are 45 °C and 90% relative humidity for 4 days. Seedleaf tobaccos from 1973–1977 have been fermented in this chamber. Extent of fermentation was determined by decreases in the percentages of total nitrogen, total volatile bases and nicotine, and by an increase in pH of the dried tobacco.

ZUSAMMENFASSUNG

Eine Versuchsanlage, die die Simulation der industriellen Tabakfermentation gestattet, wurde bis zur Funktionsfähigkeit entwickelt. Im Vergleich zu den üblicherweise in

den Laboratorien benutzten Fermentationsgeräten verfügt diese Apparatur über einen umfangreicheren und vielseitigeren Anwendungsbereich. Sie besteht aus einem feucht gehaltenen Raum aus Lexan® (Polycarbonat), in dem ein Aluminiumrad mit acht Behältern aus Polypropylen rotiert. Beheizung, Befeuchtung und Belüftung der Anlage sind steuerbar.

In der Regel wird in dem Gerät bei 45 °C und 90%iger relativer Luftfeuchtigkeit vier Tage lang fermentiert. In der vorliegenden Arbeit wird über Fermentationsversuche mit amerikanischen „Seedleaf“-Tabaken aus Ernten der Jahre 1973 bis 1977 berichtet. Der Fermentationsgrad wurde ermittelt, indem die Abnahme des prozentualen Gehaltes an Gesamtstickstoff, gesamten flüchtigen Basen und Nikotin im getrockneten Tabak sowie das Ansteigen des pH-Wertes gemessen wurden.

RÉSUMÉ

On a conçu et mis au point un système permettant de simuler en laboratoire la fermentation industrielle des tabacs. Ce système offre plus de capacité et de versatilité que les installations en laboratoire usuelles. Il comprend une chambre humide en Lexan® (polycarbonate) contenant une roue tournante en aluminium portant huit récipients en polypropylène. Le chauffage, l'humidification et l'aération sont réglables.

En général, la fermentation est effectuée à 45 °C et à 90 % d'humidité relative pendant une période de 4 jours. Les tabacs «Seedleaf» des récoltes 1973 à 1977 ont été fermentés dans l'installation en question. Le degré de fermentation a été déterminé en mesurant la diminution des pourcentages, dans le tabac séché, de l'azote total, des bases volatiles totales et de la nicotine ainsi que l'augmentation du pH.

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