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Homogenized Leaf Curing

I. Theoretical Basis and Some Preliminary Results*

by

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INTRODUCTION

Tobacco curing is a vital process, which falls into the category of starvation phenomena of excised plant parts (1). The purpose of curing is to transform the freshly harvested leaves into a physical and chemical condition suitable for manufacture of tobacco products.

In the United States there are three basic methods of curing tobacco. They are air-curing, flue-curing, and fire-curing. The major types are either air-cured under natural conditions, using little or no heat, or flue-cured by heated air at gradually rising temperatures. These conventional curing methods are time-consuming, but they bring out the quality latent in the mature leaf. They offer little opportunity to manipulate chemical or physical leaf characteristics.

Labor of tobacco harvest and post-harvest handling may amount to 50–55 % of the total required to produce the crop. Many attempts have been made to modify conventional harvesting and curing systems for reasons of economics and labor saving. Some of these modifications include curing midrib-free lamina, shredding of tobacco before curing, bulk curing of leaf, and others (2, 3). These modified approaches generally retain the original cellular structure, and bulk curing of the bright type is widely accepted.

One of the most significant developments in the tobacco industry in recent years is the use of reconstituted sheets in manufactured products. Before reconstitution, conventionally cured leaf and leaf scrap is ground into "fines" (4). It is, therefore, not essential that tobacco leaf be kept in the "whole" form in order to be useful to the tobacco industry.

This paper reports a new procedure for curing leaf tobacco, through homogenization, incubation, and dehydration. The objectives of this new homogenized leaf curing (HLC) process are threefold: To reduce production cost by elimination of a large part of the hand labor, to reduce or eliminate undesirable factors that may be associated with the smoking-health problem, and to improve tobacco usability by enhancing certain physical and chemical factors. The present report deals with the theoretical basis for the HLC process and presents some preliminary results. Other papers in this series of reports will discuss specific HLC processes for bright and burley tobaccos, respectively.

THEORETICAL BASIS AND EXPERIMENTAL APPROACHES

The process of transforming ripe tobacco leaf into aromatic leaf tobacco by curing is a complicated series of chemical, physical, and biological phenomena. Some components, such as carbohydrates and ether-soluble organic acids, change drastically, and others, such as certain nitrogenous components, undergo limited changes. Components of the leaf framework and cell walls are relatively inert to changes. Under normal conditions, biochemical changes in the early stage of leaf curing are actually the continuation of the vital process of leaf senescence. The principal difference is the rate of dehydration, which significantly influences the chemical changes, especially those involving enzyme activities. In senescence, there is little dehydration. In air-curing, the rate of dehydration is much slower than that in flue-curing, because elevated temperatures are

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not used in the former process. However, the initial chemical changes are generally the same (1).

The chemical conversions during curing are dominated by hydrolytic enzymes. Oxidative reactions also take place, but on a relatively smaller scale. The success of a curing process depends on the degree of leaf maturity, temperature, and humidity, which control the biochemical processes and rate of dehydration.

Under normal conditions of conventional curing of mature tobacco, all changes follow a predesignated pattern, and there is little room for manipulation. Physical structure, such as leaf thickness and porosity, is fixed by its genetic and cultural background. Chemical properties of the cured leaf are limited by the basic constituents of the leaf at the time of harvest. Therefore, within a definite frame of varietal and cultural conditions, there is a built-in maximum usability factor under conventional curing. The usability factor includes desired physical properties and chemical components.

To achieve the desired changes of conventional curing and at the same time to improve the usability factor, an alternative is the homogenized curing procedure. The basic concept is to bring about the required chemical changes in a homogenized slurry instead of in the intact whole leaf, and to reconstitute the "cured" mass into a sheet form for commercial use. The advantages of this novel process are: [1] To save labor from handling of individual leaves, [2] To provide suitable conditions for removal of undesirable components (which may have been essential products during plant growth), [3] To provide better opportunities for controlling the biochemical changes, [4] To provide a feasible environment for enhancing desirable chemical components, and [5] To control the physical properties by reconstitution. There are, of course, many technical difficulties that must be overcome. One major problem is the control of browning which results from immediate contact between enzyme and substrate when cell walls are broken during homogenization. Another problem is providing adequate conditions for hydrolytic enzyme functions. Polysaccharides must be hydrolyzed to sugars, but most sugars should be retained in order to maintain the desired leaf quality of bright-type tobacco. These processes require specific conditions, which include proper leaf maturity, homogenization, chemical additives (as a reducing agent or enzyme inhibitors), suitable conditions for incubation, and an adequate dehydration system.

The variables or combinations of variables studied are so numerous that it is not possible to describe all of them in detail or to present all data. We shall, however, list most of the variables investigated, and present select data for illustration.

Experimental Variables

In the process of developing this new HLC procedure, major emphasis was given to bright and burley-type tobaccos, with limited attention to the Maryland type.

Table 1. Experimental variables.

Main areas	Variables
A. Plant materials	
1. Type and variety	Bright type: NC 2326, Pale Yellow Burley type: Ky 14, Burley 21 Maryland type: Catterton
2. Maturity	Overmature Mature Undermature
3. Color	Natural yellow Forced yellow (pile yellow) Induced yellow (ethephon)
4. Pre-treatment	Rinse leaf with pet. ether vs. control (before homogenization)
5. Post-treatment	Extract slurry with pet. ether vs. control (after homogenization)
B. Homogenization	
1. Method	Food chopper (5/64-inch particle size) Waring Blendor
2. Fluid	Water vs. EtOH vs. control
3. Reducing agents	Ascorbic acid vs. sodium metabisulfite vs. control
4. Enzymes	Amylase, pectinase, protease vs. control
C. Incubation	
1. Acidity	Control (pH 5.4) vs. pH 5.8, 6.6, and 7.4 (adjusted with K_3PO_4)
2. Temperature	25°, 30°, 50°, 60°, 70°, 80° C
3. Duration	0, 4, 6, 12, 24, and 36 hours
4. Aeration	Control vs. O_2 vs. N_2
5. Agitation	Control vs. constant stirring
6. Lighting	Room light vs. darkness
D. Dehydration	Air-dry Forced oven-dry Freeze-dry Vacuum-dry

These studies will be extended to cigar tobacco types in the near future.

The experimental variables include four main areas: plant materials, additives for homogenization, conditions for incubation, and methods of dehydration. Variables investigated are shown in Table 1.

Results of Selected Experimental Samples

1. Burley tobacco, cv. Ky. 14, harvested at full maturity, was subjected to conventional air-curing (CC) and homogenized leaf curing (HLC). Leaves used for HLC were ground in a commercial-size Waring Blendor*, with 40 ml of water added for every 100 g

* Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

Figure 1. Normally flue-cured leaf of bright-type tobacco.

tobacco, and incubated at 40° C for 24 h. The resulting mixture was spread 0.75 cm thick onto a glass plate, then placed into a forced-draft oven at 55° C overnight for dehydration.

Chemical analyses on both HLC and CC samples were made for nitrogenous fractions, sugars, total polyphenols, and inorganics, as shown in Table 2.

Table 2. Comparison of certain chemical components in homogenized leaf curing (HLC) and conventionally cured (CC) burley-type tobacco.

	Bottom leaf		Middle leaf		Whole plant	
	HLC*	CC**	HLC	CC	HLC	CC
Nicotine, %	2.10	2.73	2.88	3.39	3.00	3.33
Nitrate N, %	0.85	1.02	0.65	0.98	0.70	0.90
Ammonia N, %	0.02	0.11	0.01	0.16	0.02	0.23
Total N, %	3.65	4.08	4.15	4.52	4.35	4.40
Sugar, %	2.08	1.12	1.28	1.18	1.50	1.18
Phenols, %	0.60	0.57	0.58	0.73	0.66	0.76
Potassium, %	5.40	6.15	5.12	5.92	5.02	5.60
Calcium, %	4.38	5.00	3.45	4.52	3.38	4.28
Phosphorus, %	0.17	0.18	0.20	0.19	0.22	0.22

* HLC: Homogenized leaf curing. ** CC: Conventional curing.

2. Bright-type tobacco varieties Pale Yellow and N.C. 2326 were harvested from the field and divided into three groups. One group was homogenized when leaf was in the mature stage with slightly green color. The second group was piled on the floor and covered with plastic sheet for 12 h to accelerate yellowing and then homogenized. The third group was also piled, but was sprayed with ethephon and covered with plastic for 12 h and then homogenized. Each group was subdivided into two sets. One set was homogenized with the addition of 10 ml of water to each 100 g of tobacco, and the other set with the same amount of 10% aqueous ethanol. Homogenates were incubated at 5° and 25° C for 24 h, with or without agitation. They were then spread in sheet form and air-dried at room temperature. Treatment with 10% EtOH, cv. Pale Yellow, 24-hour incubation at 25° C with agitation, appeared better than other combinations. Ethephon sprayed after harvesting caused brownish spots on the leaves.

3. Bright-type tobacco, cv. NC 95, grown in the greenhouse, and yellowed with ethephon, was treated by adding enzymes. One-hundred g of leaf tissue was combined with 200 ml of water containing 0.01 M

Figure 2. Homogenized leaf curing (HLC) material of bright-type tobacco.

metabisulfite (final concentration) and homogenized. The slurry was divided into four samples: [a] control, [b] with 0.5 % amylase (diatase of malt), [c] with 0.5 % pectinase, and [d] with 0.5 % protease (bromelain). Each sample was stirred again 6 h later, and once more after 18 h. After 24 h of incubation, the slurry was spread out to dry in the dark, with a moving air current supplied by hood fan. Control, amylase-treated, and protease-treated dried samples were similar in color, aroma, and texture. The pectinase-treated sheet was lighter in color, more compact in texture, and less pronounced in odor than the others. Chemical data on these treatments are shown in Table 3.

4. Bright-type tobacco, cv. Pale Yellow, harvested from the field when fully mature, was homogenized in a food chopper. Metabisulfite was added (5 % EtOH, 200 ml 0.01 M per kg of tobacco). The slurry was incubated 48 h at room temperature without agitation and then freeze-dried. The comparative leaf analysis of HLC and the flue-cured control is shown in Table 4.
5. In a more recent test with bright-type tobacco, cv. C-319 leaf tobacco was harvested when fully matured in the field. Half of the samples were flue-cured as control and half were cured employing the homogenization process as described under 4. above, but dehydrated under a vacuum. The cured materials from

Table 3. Comparative chemical composition of HLC samples (bright tobacco) processed with various enzymes.

	% Total alkaloid	Sugar (mg/g sucrose)	Phenol (mg/g tyrosine)	Amino acid (mg/g glycine)	Protein (mg/g BSA)	Moisture retention (% at 25° C)
Control	1.02	56.25	43.42	73.0	15.0	5.0
+ Amylase	0.77	53.75	32.90	19.2	10.6	5.6
+ Pectinase	0.88	86.25	32.94	37.2	7.6	5.0
+ Protease	0.88	43.75	34.62	43.2	8.6	6.6

Figure 3. Reconstituted tobacco sheet of normally flue-cured leaf.

both the conventionally cured and HLC process were reconstituted into tobacco sheets using the slurry process. The color photographs illustrate: 1. Normally flue-cured leaf (Fig. 1), 2. HLC-processed material (Fig. 2), 3. Tobacco sheet made of normally flue-cured leaf material, as 1 above (Fig. 3), and 4. Tobacco sheet made of HLC-processed material, as 2 above (Fig. 4).

Removal of certain groups of compounds by solvent extraction, such as petroleum ether extraction, was also conducted. This extraction process can be carried out either before or after leaf homogenization. Extracts may be further fractionated. After separation of the undesirable fraction, the remainder of the extract can be returned to the homogenate during incubation or dehydration.

Table 4. Analysis of leaf and sheet material from flue-cured and HLC samples.

Samples	N_2 %	TVB** %	Nicotine %	Ash %	Reducing substance %
Flue-cured control	2.17	0.43	2.60	10.2	16.6
Reconstituted sheet control	1.71	0.30	1.62	15.0*	11.7
HLC	2.15	0.36	1.95	13.3*	11.8

* The increase may be resulted from addition of inorganic component in the sheet-making process. Calculated on corrected ash basis (10.2 %), the data are consistent with the control.

** TVB: Total volatile bases.

Evaluation of Smoke and Smoke Condensate

Experimental materials (HLC and CC control) were made into cigarettes of 70 mm length. These experimental cigarettes were used for limited smoking tests and also tested for total particulate matter delivery. A preliminary study of dimethylnitrosamine (5) and a short-term bioassay using sebaceous glands (6) were also conducted.

Table 5 shows the relative smoke-preference rating and dry total particulate matter (TPM) content of these experimental cigarettes. The control for comparison in this test was reconstituted sheet from conventionally flue-cured leaf. For bright tobacco, the different dehydration procedures appear to affect the TPM content. Freeze-drying appeared to deliver a lower TPM content than other dehydration processes in this preliminary test. HLC burley also delivers a lower TPM than control.

Figure 4. Reconstituted tobacco sheet of HLC material.

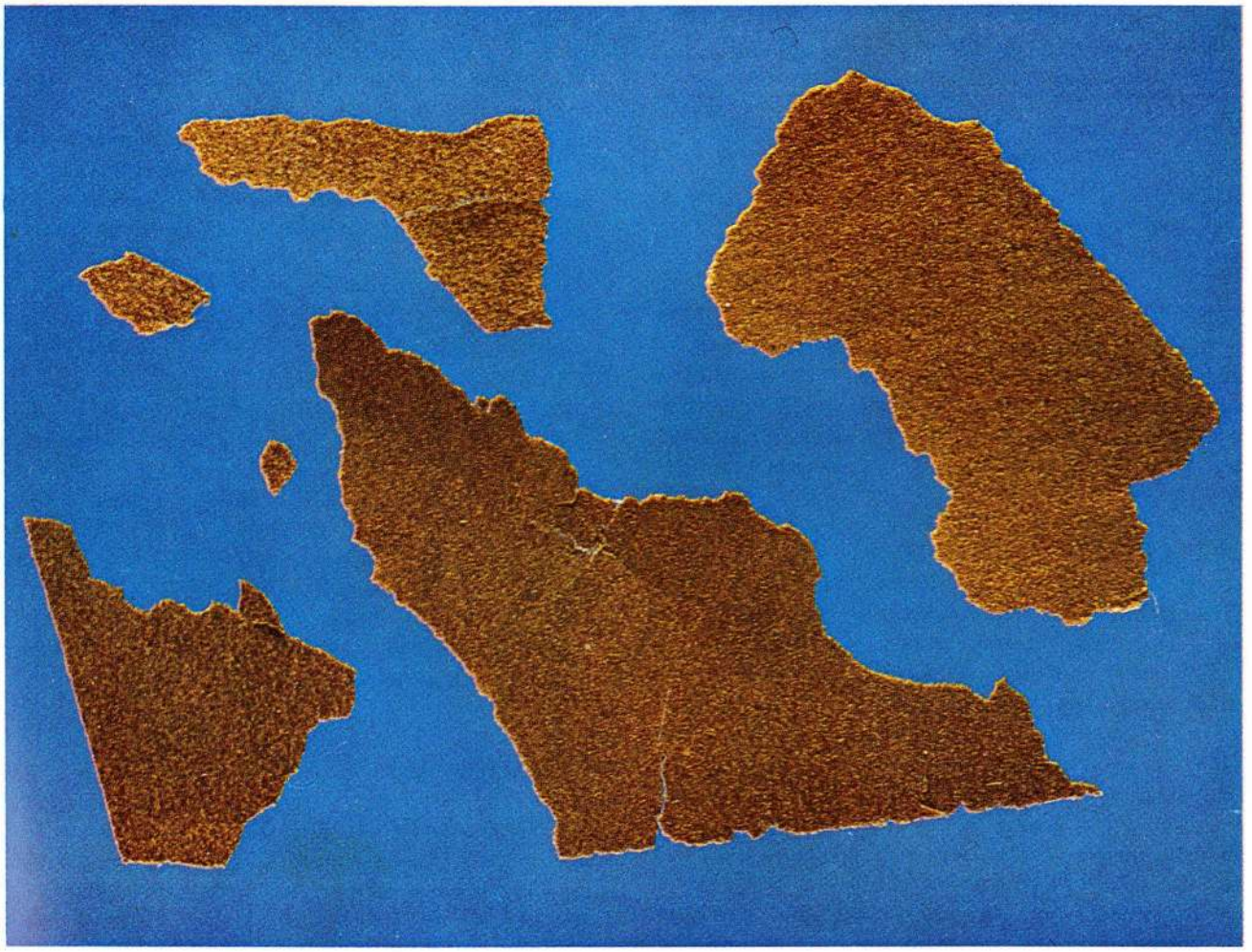

Table 5. Relative smoke-preference rating and TPM delivery.

Samples	Relative smoking preference rating*	Dry TPM (mg/g)
Bright-type tobacco		
Reconstituted sheet (RST) made from flue-cured control	10	22
RST of HLC-1 (freeze-dried for dehydration	9	17–19
RST of HLC-2 (forced hot-air-dried in rotary drum, natural yellow)	8	22
RST of HLC-3 (forced hot-air-dried in rotary drum, forced yellowed leaf)	8	23
Burley-type tobacco		
Reconstituted sheet made from air-cured control	N.A.	18
RST of HLC material	N.A.	16

* Scores from 10 to 0, with 10 as the highest score assigned to bright-type control.

A small sample was made available for dimethylnitrosamine (DMN) measurement in cigarette smoke (5). Burley tobacco is higher in DMN content than the bright type. In either type, the conventional controls were the highest, reconstituted sheets were medium, and the HLC cigarettes were the lowest in DMN content. Results are shown in Table 6.

Table 7 shows preliminary short-term bioassay results by sebaceous-gland tests of smoke condensate of experimental cigarettes (6). These results are relative activities of smoke condensates. In both burley and

Table 6. Nitrosamines in cigarette smoke.

Type	Samples	Dimethylnitrosamine (ng/g tobacco burned)
Burley	Control (conventionally cured)	97.0
	Reconstituted-sheet control	65.0
	HLC	20.0
Bright	Control (conventionally cured)	2.6
	Reconstituted-sheet control	1.7
	HLC	< 1.7

Table 7. Sebaceous-gland tests.

Type	Samples	Relative activities of smoke condensate (unit/g)
Burley type	Control	9.5
	Reconstituted-sheet control	< 8.0
	HLC	5.1
Bight type	Control	7.9
	Reconstituted-sheet control	6.3
	HLC	< 3.0

bright samples, smoke condensates derived from HLC materials were significantly and substantially less active than those derived from controls, including conventional and reconstituted materials.

DISCUSSION

Preliminary results, as presented in this report, indicate the potential of this new curing method. Labor saving and chemical and physical improvements are obvious advantages. Larger-scale pilot studies are under way for more comprehensive evaluation of leaf characteristics, smoke composition, and biological response.

Leaf maturity is an important factor in the HLC process. Induced yellowing by an ethylene-releasing agent on field tobacco is very desirable, so far as the color and the chemical composition of the finished product are concerned. There are also varietal effects because of differences in uniformity of leaf maturity as well as leaf coloring.

Ascorbic acid was not as beneficial as metabisulfite in this curing process. The latter served not only as a reducing agent but also as an inhibitor of polyphenoloxidase activity (7). The metabisulfite decreased or prevented browning after homogenization. The use of metabisulfite did not cause any pronounced changes of sulfur components in cigarette smoke. Only minor differences were observed, as shown in Table 8 (8).

Use of enzymes such as amylase, pectinase, or protease appear to cause considerable effects on certain organic components. However, the exact conditions for beneficial use of these and other enzymes will need further study.

Conditions and duration of incubation, as well as method of dehydration, affects significantly the quality of the final products. These variables reflect the nature and extent of biochemical changes and the exact cut-off point at which the most ideal chemical balance may occur. This varies according to tobacco types and the desired composition.. More detailed information will be pretented in the reports that will follow in this series.

Table 8. Sulfur components in smoke of metabisulfite-treated HLC and control cigarettes.

	COS	H_2S	SO_2
	(µg/cig.)	**(µg/cig.)**	**(µg/cig.)**
Flue-cured control	41	66	< 1
HLC	26	74	< 1
	(µg/puff)	**(µg/puff)**	**(µg/puff)**
Flue-cured control	3.1	5.1	< 0.1
HLC	3.2	9.2	< 0.1

SUMMARY

A new procedure (HLC) of curing tobacco leaf through homogenization, incubation, and dehydration is described. At the homogenate stage, chemical composition can be improved by controlled enzyme action, by extraction, or with chemical additives. Physical properties can be improved by reconstitution. Preliminary results show HLC may provide smoke with quality comparable to that from conventionally cured material, but with relatively lower biological response. There is a great potential in using the HLC procedure for labor saving as well as for improving leaf usability.

ZUSAMMENFASSUNG

Für die Trocknung von Blattabak wird ein neues Verfahren (HLC: homogenized leaf curing) beschrieben, das auf Homogenisierung, Inkubation und Wasserentzug beruht. Im Stadium der Homogenisierung kann die chemische Beschaffenheit des Tabaks durch gesteuerte Enzymaktivität, durch Extraktion oder durch chemische Zusätze verbessert werden. Die physikalischen Eigenschaften können durch Rekonstitution verbessert werden. Vorläufige Ergebnisse zeigen, daß mit Hilfe dieses Verfahrens Rauch erhalten werden kann, der in seinen Eigenschaften dem Rauch von herkömmlich behandeltem Tabak vergleichbar ist, dessen biologische Aktivität jedoch vergleichsweise geringer ist. Das Verfahren bietet günstige Möglichkeiten für die Einsparung von Arbeitskraft und für die Verbesserung der Blattnutzung.

RESUME

Un nouveau procédé de séchage du tabac en feuilles (HCL: homogenized leaf curing / procédé de séchage de feuilles homogénéisées) par homogénéisation, incubation et déshydratation est décrit. Durant la phase d'homogénéisation, la composition chimique peut être améliorée par l'action contrôlée d'enzymes, par extraction ou par des additifs chimiques. Les propriétés physiques peuvent être améliorées par reconstitution. Des résultats préliminaires tendent à démontrer que la méthode HCL peut produire une fumée de qualité comparable à celle des tabacs séchés de façon conven-

tionelle, mais avec une activité biologique plus faible. Le procédé HLC laisse prévoir de larges possibilités, tant en gain de main-d'oeuvre qu'en proportion de feuilles utilisables.

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