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CHEMICAL CHANGES IN THE HARVESTED TOBACCO LEAF*

Part II. Chemical and Enzymic Conversions during Fermentation and Aging

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This report is dedicated to the memory of BERNHARDT G. MEYER, *late president of the General Cigar Company, whose foresight and interest initiated scientific work on cigar tobacco in this company in 1940.*

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* Part I of this report appeared in *Advances in Enzymology,* Volume VI, pp. 309–387, 1946.

I. Chemical Changes Caused by Shed Curing

In Part I (59), a survey was presented on the chemical composition of the harvested tobacco leaf, and on the chemical conversions that occur in the leaves when they are subjected to the drying or curing* process immediately after their removal from the field. The pattern of these conversions is determined predominantly by those enzymes which remain active in the leaf tissues throughout the curing phase. This first phase of tobacco processing includes a starvation period followed by the death of the leaves. A considerable fraction of the leaf proteins undergo hydrolysis with the simultaneous appearance of amino acids, amides, and ammonia compounds in the form of water-soluble split-products; the polysaccharides are converted, again by hydrolysis, to simple sugars; small amounts of the pectins are partially attacked and broken up into lower molecular compounds such as pectic acid, uronic acid, and methyl alcohol. All these and some additional minor hydrolytic conversions differ basically from the chemical metabolism of the living leaf by the absence of synthetic processes, including the assimilation of carbon dioxide. Because of the lack of counterbalancing reactions, the hydrolytic processes of disintegration dominate the chemical conversions in the curing leaf.

Parallel to this one-sided trend in favor of lower molecular split-products, an additional set of chemical changes develops in the leaves with the progress of curing. The dynamic equilibrium between oxidative and reductive processes, as it was established in the living leaf, is thrown off balance, due to a drastic weakening of the reductive factors within the leaf tissues. As a result, the *oxidation* of certain leaf components becomes a major effect of the curing process simultaneously with, and in the wake of, the hydrolytic reactions. The carbohydrates are among the first compounds to be subjected to oxidation obviously owing to the influence of the same enzyme complex that had sustained the respiration of the living leaf. The transformation of malic acid into citric acid, a characteristic effect of the curing process, is probably interrelated with the carbohydrate oxidation via a set of reactions closely related to the tricarboxylic acid cycle (169, 217). Gradually, the oxidative actions spread further, involving additional compounds, such as other organic acids, phenols, polyphenols, and some of the resinous components of the leaves. Concurrently, chlorophyll disappears by a process the chemical nature of which is unknown.

* The term "curing," sometimes used loosely to designate the entire processing of tobacco, is applied here exclusively to the phase of "shed curing" which covers the various methods employed for the first phase of tobacco treatment, such as air, flue, fire, and sun curing.

These are the principal transformations which take place in the "air curing" of tobacco leaves, as far as our present knowledge goes. In "flue curing," as applied to certain types of cigarette tobacco, the oxidative reactions are kept to a minimum by subjecting the tobacco to a drastic temperature increase. The latter is timed with the progress of curing at such a rate that the enzymes catalyzing the oxidative conversions are inactivated at the appropriate moment (3,5,24,41,139, 173,232,247,248).

II. Description of the Fermentation and Aging Process

All these chemical effects of shed curing constitute preparatory rather than decisive steps on the way from the green tobacco leaf to a satisfactory and industrially acceptable smoking tobacco. Without a further specific treatment, even well-cured cigar, cigarette, or pipe tobaccos are still unfit to be smoked, at least as far as the standards of conscientious manufacturers go. Such leaves develop on burning a pungent and irritating smoke which has a harsh and bitter taste and which obliterates the agreeable effects of any aroma compounds that may be present in the smoke among the products of combustion and of dry distillation.

The experience of the trade over many decades has taught that tobacco—and this holds for every known type—has to be subjected to *one or more additional specific operations* in order to yield a product which, on burning, develops a mild and aromatic smoke, free of the pungent ingredients characterizing the smoke of merely shed-cured tobaccos.

A. TYPES OF PROCESSING

These additional methods of processing vary considerably from one tobacco type to the next. Table I presents a survey on several typical operations as they are applied to various kinds of tobaccos; they range from the mild "aging," which has been developed for the fluo-

(f) Evolution of Other Gases and Vapors. The evolution of *ammonia* vapors by fermenting tobacco, particularly by sweating cigar leaf filler tobacco, snuff, or chewing tobaccos, is well known to everybody who has entered the fermentation chambers in which such tobacco types are being processed. We shall see later that the total nitrogen content of "sweating" tobacco decreases usually by amounts between 0.01 and 0.60% of its dry weight, or by 0.25 to 12% of its original nitrogen content, as a result of the fermentation. A considerable part of these losses of total nitrogen must be attributed to a volatilization of nitrogenous compounds which escape from the fermenting leaves into the surrounding air. (As to another cause for nitrogen losses in flue-cured tobacco, see page 407).

An additional source of nitrogen losses is possibly caused by a translocation of nitrogenous compounds, during fermentation, from the leaf blades into the midribs of the leaves. As the midribs are usually removed before the analysis of leaf samples, such a migratory effect would cause a nitrogen loss from the leaf blades without a corresponding evaporation of nitrogenous substances from the bulk of fermenting tobacco. Zaporojanu (247) claims to have found indications for such a translocation of nitrogenous compounds from the blade into the midrib in the fermentation of a Rumanian cigar tobacco. Analyses of samples before and after fermentation which were made in the author's laboratory have shown that, in Pennsylvania cigar leaf tobacco, the loss of total nitrogen in per cent of its initial amount is practically the same in the leaf blades and in the midribs. This seems to prove that, with this tobacco type, translocation of nitrogen from the blades into the midribs does not occur during the fermentation.

A fraction of the nitrogen lost by evaporation corresponds to volatilized ammonia. An escape of ammonia from the leaves is not necessarily to be expected, even if ammonia and ammonia compounds increase in the tobacco leaves during the fermentation. Rather, it will depend on the pH of the tissues, on the temperature and moisture of the tobacco, and on the humidity and turbulence of the surrounding air, how much, if any, ammonia will be given off by the tobacco.

Experiments (58) which throw some light on the quantities of ammonia lost from fermented cigar leaf tobacco were carried out in the author's laboratory by withdrawing, in a vacuum, the vapors and gases given off by fermenting Pennsylvania Seedleaf tobacco at a certain stage of the resweat process. The condensate consisted mainly of water and amounted to roughly 1 liter per 110 kg. of the dry weight of tobacco. This one liter corresponds to about 1/14 of the total amount of water lost by this tobacco over the entire duration of its resweat. The aqueous condensate contained about 350 mg. NH_3 and 250 mg. nicotine per liter. If we assume that the relative amounts of water vapor, ammonia, and nicotine that

evaporate during fermentation would remain approximately constant, we derive a value of about 15.4 g. for the ammonia and 11.0 g. for the nicotine which would escape from 110 kg. tobacco during the entire fermentation process. Thus the losses would amount to roughly 0.014% ammonia and to about 0.010% nicotine (of the tobacco dry weight), or to about 0.011% ammonia nitrogen and to about 0.002% nicotine nitrogen. Both these values appear very small when compared with the total loss of nitrogen shown by the same tobacco sample at completion of fermentation—about 0.30%. An explanation of this drastic discrepancy could probably be provided by assuming that the basis of our calculation was wrong. It is very likely that the evolution of ammonia and of nicotine from the tobacco per unit of water evaporated will strongly increase toward the end of fermentation due to the increasing alkalinity of the tissues. More experimental work is needed to clarify this point.

Methyl alcohol has been found to escape in considerable amounts from fermenting tobacco. The quantities evolved during fermentation vary between 0.04 and 0.12% of the dry tobacco weight. It originates from the demethoxylation of pectins. Of other compounds, *essential oils* have been found, in amounts of the same order of magnitude as those of methyl alcohol, among the vapors in fermentation chambers (202). Furthermore, *volatile acids* (formic and acetic) seem to be exhaled, particularly from cigarette tobaccos, and *carbon monoxide* has been determined as a component of the gases in fermentation rooms in amounts equaling about 20% of those of the evaporating methyl alcohol.

That additional organic substances are volatilized from fermenting tobaccos may be suspected from the following consideration. An approximate figure for the loss of *total carbon* during the fermentation of Pennsylvania cigar leaf tobacco can be calculated from carbon determinations of this tobacco made with samples before and after their fermentation by Haley, Longenecker, and Olson (82). In assuming a weight loss for this tobacco of about 8% during fermentation, the total loss of carbon amounts to about 3.5% of the dry weight of the tobacco according to these determinations. If all this carbon were lost in the form of carbon dioxide, this would correspond to an evolution of about 129 g. CO_2/kg. tobacco. This amount is considerably larger than the values we estimated previously for CO_2 generation during the fermentation of an air-cured Oriental-type cigarette tobacco. Although it is possible that the more intensive sweat of cigar leaf tobacco results in the evolution of larger quantities of carbon dioxide than the fermentation of cigarette tobacco (compare the estimated value of 60 to 90 g. CO_2/kg. cigar leaf, page 343), the possibility cannot be excluded that an appreciable part of the loss of 3.5% of carbon is caused by the escape from the fermenting leaves of gases which, besides carbon dioxide, contain a number of volatile organic compounds. So far, only methyl alcohol, essential oils, and organic nitrogenous substances have been identified among these products.