Enzymes of Bright and Burley Tobaccos

ROBERT E. BARRETT
Battelle Memorial Institute,
505 King Ave., Columbus 1, Ohio

Using manometric, colorimetric, titrimetric, and viscometric techniques, marked differences in enzyme activity were shown between unaged flue-cured tobacco (bright) and unaged air-cured tobacco (burley). The only appreciable enzyme activity found in unaged bright tobacco resulted from decarboxylases. Enzyme activity in unaged burley tobacco was shown for an oxidase (similar to laccase), catalase, protease, invertase, cellulase, tyrosinase, and probably decarboxylase and peroxidase. Except for the decarboxylases, the enzymes found in burley tobacco either were undetected or exhibited only slight activity in bright tobacco. Amylase, urease, cytochrome c oxidase, glucose oxidase, and certain deaminases and other enzymes were not detected in either unaged bright or burley tobaccos. Aging of bright tobacco involves primarily chemical effects and aging of burley tobacco possibly involves both chemical effects and enzymes.

UNAGED CIGARETTE TOBACCOS have been characterized as having an irritating effect on the membrane tissues and lacking taste and aroma. As bright (flue-cured) and burley (air-cured) tobaccos are the major ingredients in cigarette tobacco, considerable effort has been directed toward determining their properties before and after aging. It is customary to age these tobaccos for at least 18 months.

The mechanisms which are important in aging of these tobaccos probably involve naturally occurring enzymes, bacterial-released enzymes, and chemical effects.

It is generally recognized that enzymes are present in air-cured tobaccos such as burley tobacco. However, the presence and importance of enzymes in flue-cured tobaccos are still a matter of considerable dispute (*1*, *4*). Chemical analyses of these tobaccos before and after aging have shown only slight differences in chemical composition. Dixon and coworkers (*2*) concluded that enzymes are not important in the aging of flue-cured tobacco.

The present investigation was undertaken to help resolve the conflicting views of mechanisms of aging, especially of flue-cured (bright) tobacco.

Experimental Methods

Selection of Tobacco and Enzyme Preparations Unaged bright and burley tobaccos were obtained from stocks of 1952 and 1953 crops of the American Tobacco Co. The tobacco was cured, not redried, and was unaged when received. It was stored at 35° F. until used. For many experiments the tobacco was used intact except for grinding to pass a 20-mesh screen. Other experiments required a more elaborate extraction procedure in which enzymes were released from cellular structure with the aid of sucrose, iron-free sea sand, and the Waring Blendor or colloid mill.

After the tobacco had been cut into pieces less than 1 inch square, 30 grams of tobacco and 500 ml. of a sodium carbonate–sodium bicarbonate buffer at pH 9.5 and 0.5 ionic strength were placed in the colloid mill. Extractions made at pH's between 6 and 7 produced extracts with lower enzyme activity than higher pH's. Although some activity of certain enzymes may be destroyed at pH 9.5, extracts were kept cool to minimize this effect. The mill was run first at large openings and then at smaller openings until the solution flow stopped. Ice water was circulated around the mill to prevent overheating. The solution from the mill was centrifuged for 20 minutes at 22,000 g. The supernatant liquid was called J1 and the sediment J2. Recentrifuging of J1 at 27,000 g. for 30 minutes gave the supernatant liquid J3 and the sediment J4. Precipitation of J3 to a final adjusted concentration of 10% glacial acetic acid and its resuspension in buffer at pH 9.5 gave J5. Precipitation to a final adjusted concentration of 50% saturated ammonium sulfate and the resuspension in buffer at pH 9.5 gave J6.

In addition to the release of enzymes from cellular structure by the colloid-mill process, Roberts (*11*) has pointed out that polyphenols which are present will also be released. Large amounts of oxygen will be consumed and will give secondary oxidations which result in carbon dioxide being evolved from carbohydrates. These reduced polyphenols will, in turn, be reoxidized and oxidative enzyme activity would be difficult to determine. Because of this, additional extractions of unaged tobaccos were made in an effort to minimize this effect. Polyphenols and like compounds were removed by pre-extraction with acetone below 0° C., with the aid of a dry ice and dry ice–acetone bath. After extraction of the tobacco until no positive test was given with ferric chloride, the tobacco was dried under vacuum below 0° C.

Manometric Techniques Most of the enzymes studied were capable of taking up oxygen and/or giving off carbon dioxide when in the presence of a suitable substrate. If the substrate used was in the tobacco itself, the experiments or runs were called endogenous runs. The equipment used was a rotary refrigerated Warburg apparatus manufactured by the American Instrument Co. Both the 15-ml. single-side-arm and 16-ml. double-side-arm flasks were used. Most runs were performed at 30° C. for 6 hours with the flasks oscillating at 112 strokes per minute and at maximum amplitude. A monobasic sodium phosphate–dibasic sodium phosphate buffer at pH 6.5 and 0.5 ionic strength was used in the majority of runs. The center well of the flask was filled with 0.2 ml. of 20% potassium hydroxide or water. Generally, 200 mg. of dry tobacco or 2 ml. of its equivalent in

an enzyme was placed in the flask and 1 or 2 ml. of buffer added. The side arm was filled with 0.5 ml. of substrate (usually 0.02*M*) or water (for endogenous experiments).

Nonmanometric Methods

Invertase. Although there are several methods for measuring invertase activity, qualitative determination was made using glucose solutions and Benedict's solution. Because of the interfering substances in tobacco solutions which give rise to foreign colors, direct comparisons between photelometer readings of known concentration of glucose and unknown tobacco-glucose solutions could not be made. However, approximate percentages were arrived at by diluting the tobacco-glucose solutions to give visual appearances and photelometer readings similar to known glucose solutions.

Tobacco extracts were prepared as follows: A 2-ounce bottle containing 3 grams of ground tobacco and 30 ml. of buffer at pH 7 and 0.2 ionic strength was suspended in the Warburg and shaken for 4 hours. Control runs were shaken for only 5 minutes. The tobacco was then pressed through a cheesecloth and centrifuged at 5000 g. for 20 minutes, and the supernatant liquid was diluted with water for the measurements.

Protease. A modified Conway method was used for the determination of proteolytic enzyme activity in tobacco, which depends upon the release of ammonia from proteins and peptides. Protease as well as many other proteolytic enzymes give this action. The ammonia was absorbed in boric acid and the relative enzyme activity measured by titrating the ammonium borate formed with a mineral acid (hydrochloric acid). The procedure was modified to make use of the Warburg apparatus.

Double-side-arm flasks were used. In one side arm 0.5 ml. of 4% boric acid and 2 drops of bromocresol green indicator were placed. A 0.2-gram sample of tobacco plus 2 ml. of buffer at pH 7 was placed in the center of the flask. After adding 1 ml. of 1% casein (Coleman & Bell), the entire system was closed and placed in the Warburg. The flask was shaken for 45 minutes, and saturated potassium carbonate was added to the second side arm. After closing the system once more, the carbonate was dumped into the center well and any ammonia absorbed in the center solution was released rapidly and absorbed by the boric acid. After an additional 45-minute shaking period in the Warburg, the boric acid solution was removed to a small Erlenmeyer flask, which was titrated with 0.00246*N* hydrochloric acid.

Urease. The modified Conway method used in determining proteolytic activity was used for the determination of urease activity. The Conway method was used to measure the ammonia given off by 0.2 ml. of 0.25% urea. Manometric methods were also used to measure the carbon dioxide from the breakdown of urea by urease.

Amylase. A standard method was used for measuring amylase activity which involved the breakdown of starch by the enzyme, as determined by an iodine indicator. The amount of the enzyme solution and iodine indicator was varied to ascertain the optimum conditions for the test. After thermostating at 30° C., the time was noted for the disappearance of the blue color.

Cellulase. Cellulase activity was determined by the rate of viscosity decrease of an aqueous solution of sodium carboxymethylcellulose at pH 5 and 30° C. (*13*).

The procedure used consisted of mixing 10 ml. of 5% aqueous solution of carboxymethylcellulose in an acetate buffer (pH 5 and 0.1 ionic strength) with a colloid-mill enzyme extract. Enzyme solutions equivalent to 0.45 gram of tobacco were used for these measurements. Both carboxymethylcellulose and cellulose in these extracts were broken down by cellulase.

Results and Discussion

Parallel runs between unaged bright and burley tobaccos were generally made to give better comparison of results. Except where noted, values for oxygen uptake or carbon dioxide evolution in manometric experiments are reported in microliters per 5 hours. It was necessary to use this long period for comparison because of the slow penetration of substrates and buffer into the cellular structure of the ground tobacco leaf. Endogenous runs on unaged bright and burley tobacco were made at frequent intervals. The average oxygen uptake and carbon dioxide evolution at pH 6.5 and 30° C. for unaged bright tobacco are given along with the averages for unaged burley tobacco runs in Table I.

There appears to be reliable evidence that the endogenous activity exhibited both by bright and by burley tobaccos are nonenzymic in origin. This can be noted from Table I, which shows that autoclaving or boiling for 5 minutes has little or no effect on the activity. The addition of sodium cyanide by vacuum infiltration [see method of Li and Bonner (*8*)] had very little effect on the endogenous activity. These results suggest that, even if enzymes are present, these crude preparations do not allow them to come into direct contact with the naturally occurring substrates in the tobacco. The substrates or enzymes may interdiffuse so slowly that a long period, such as the aging period, is required for reaction. Retention of carbon dioxide in flue-cured tobacco leaf was determined by measuring the amount of gas evolved immediately after the addition of sufficient sulfuric acid. Considerable carbon dioxide was evolved, which may be the result of a fermentation process occurring during growing and/or curing of the tobacco. It appears that most of the carbon dioxide being measured in endogenous runs is that which is being released by the constant shaking during the run, rather than from enzyme activity.

Oxidases. Probably two of the most important classes of enzymes in plants, including tobacco, are the iron- and copper-containing enzymes. The activities of all of the enzymes in these classes involve oxidation processes and, in at least one case (peroxidase), may involve the evolution of carbon dioxide. Catalase, an iron-containing enzyme, has been studied the most extensively. Manometric results showed that catalase activity in unaged bright tobacco is nonexistent. The activity in burley tobacco, however, is proportional to the concentration of the hydrogen peroxide present. An attempt to inhibit the catalase activity with 0.01*M* sodium cyanide was unsuccessful, but 0.01*M* sodium azide reduced the activity to 5 μl. of oxygen evolved. Inhibition by autoclaving for 5 minutes further demonstrated catalase activity in unaged burley tobacco.

Activities displayed by the class of copper-containing enzymes were considered as a whole, with some distinction among monophenol oxidases, polyphenol oxidases, and ascorbic acid oxidases.

Monophenol oxidase and tyrosinase activities were assayed in unaged bright and burley tobaccos by using phenol, tyrosine, and *o*-cresol as substrates. Bright tobacco exhibited no oxidase activity toward phenol and tyrosine. As indicated in Table II, burley tobacco showed a little tyrosinase or monophenol oxidase activity on phenol and tyrosine, but not on *o*-cresol. An O value indicates little or no activity detected. The small amount of carbon dioxide given off by bright tobacco and greater amount by burley tobacco with tyrosine as a substrate suggested a possible decarboxylase system.

Another group of compounds, the polyphenols, were used to measure the activity of a wide group of oxidases falling in the copper-containing class. It was found that unaged bright tobacco had little or no activity toward the substrates catechol, resorcinol, pyrogallol, gallic acid, and phloroglucinol. However, considerable activity is shown by burley tobacco, which appears to be enzymic in most cases. It is difficult to name the exact enzyme or enzymes that give this activity. Nevertheless, an enzyme similar to laccase or the terminal oxidase that Roberts and Wood

(*12*) have found in tea leaves is indicated in unaged burley tobacco.

Catechol showed considerable activity toward burley tobacco. Boiled and autoclaved burley tobacco extracts had little or no activity towards catechol. Addition of 0.01*M* sodium cyanide reduces the activity to 6μl. of oxygen taken up in 300 minutes. Further evidence of polyphenol oxidase-type enzymes was shown by the inactivity of resorcinol and phloroglucinol with burley tobacco extracts.

Gallic acid is extremely slow in activity toward enzymes which catalyze the oxidation of pyrogallol (*12*). Table II indicates that this was found for unaged burley tobacco. Again, both boiling and autoclaving reduce the activity considerably. A typical oxygen-uptake curve for pyrogallol and other substrates is shown in Figure 1. Bright tobacco shows little or no oxidase activity with pyrogallol and gallic acid. The evolution of carbon dioxide by unaged burley tobacco (see Table III) with pyrogallol may indicate a peroxidase activity.

Preliminary observations showed strong activity for both bright and burley tobaccos toward ascorbic acid. Unfortunately, ascorbic acid acts as an intermediate in both chemical and enzymic oxidation in the presence of many compounds, in particular, the quinone structures. There seems to be good evidence that most of the ascorbic acid activity is chemical, as neither boiling nor autoclaving reduces the activity appreciably. Purer enzyme preparations must be made from the tobacco to determine ascorbic acid–oxidase activity accurately. Using the colloid mill procedure outlined before, in which acetone-solubles had been extracted prior to milling, considerable activity to ascorbic acid by unaged burley tobacco was shown.

Although unaged burley tobacco showed activity for hydroquinone and *p*-phenylenediamine, bright tobacco, with only slight activity toward hydroquinone, showed stronger activity toward *p*-phenylenediamine than did burley tobacco. Considerable inactivation is obtained by boiling and autoclaving bright tobacco before adding *p*-phenylenediamine as a substrate. Further, lowering the pH to 5 reduces that activity to the level of the endogenous activity. Although laccase activity is again indicated for burley tobacco, there is considerable doubt as to the significance of the activity displayed by bright tobacco toward *p*-phenylenediamine.

Proteolytic Enzymes. The proteolytic activity of a known source of relatively pure protease, unaged bright, and unaged burley tobaccos was determined. An average of 2.00 ml. of 0.00246*N* hydrochloric acid was used to titrate the ammonia released from casein by pure protease. Unaged bright tobacco did not show any protease activity (see Table IV). Since both unaged bright and unaged burley tobacco contain some protein, they would be expected to show endogenous protease activity if this enzyme were present.

Unaged burley tobacco showed considerable protease activity with casein as a substrate (see Table IV). Without

Table I. Endogenous Activity of Unaged Bright and Burley Tobaccos

	Bright		Burley	
Conditions	O_2 uptake, μl.	CO_2 evolution, μl	O_2 uptake, μl.	CO_2 evolution, μl.
pH 6.5 and 30° C.	32	47	39	44
Average variation of above	±4	±5	±6	±6
pH 5.0 and 30° C.	33	46	31	37
Autoclaved 5 minutes	32	44	34	26
Boiled 5 minutes	23	49	25	43
0.01*M* NaCN added	42	26	19	25

Table II. Microliters of Oxygen Taken up by Action of Unaged Burley Tobacco Enzymes

Substrate	Unaged Tobacco	Aged Tobacco	Acetone Extract	Colloid Mill Extract	Autoclaved 5 Minutes
H_2O_2[a] (oxygen evolved)	122	126	127	89	25
Phenol	21	11	...	107	0
Tyrosine	20[b]	24[c]	30	...	0
o-Cresol	0	...	...	...	..
Guaiacol	0	...	...	...	..
Catechol	48–108	135	64	360	0
Resorcinol	0	...	0	...	..
Hydroquinone	133	105	184	329	9
Phloroglucinol	0	...	0	...	..
Pyrogallol	86	50	119	330	4
Gallic acid	13	28	22	...	0

[a] Values at 25–30 minutes.
[b] 28 μl. CO_2 evolved.
[c] 35 μl. CO_2 evolved.

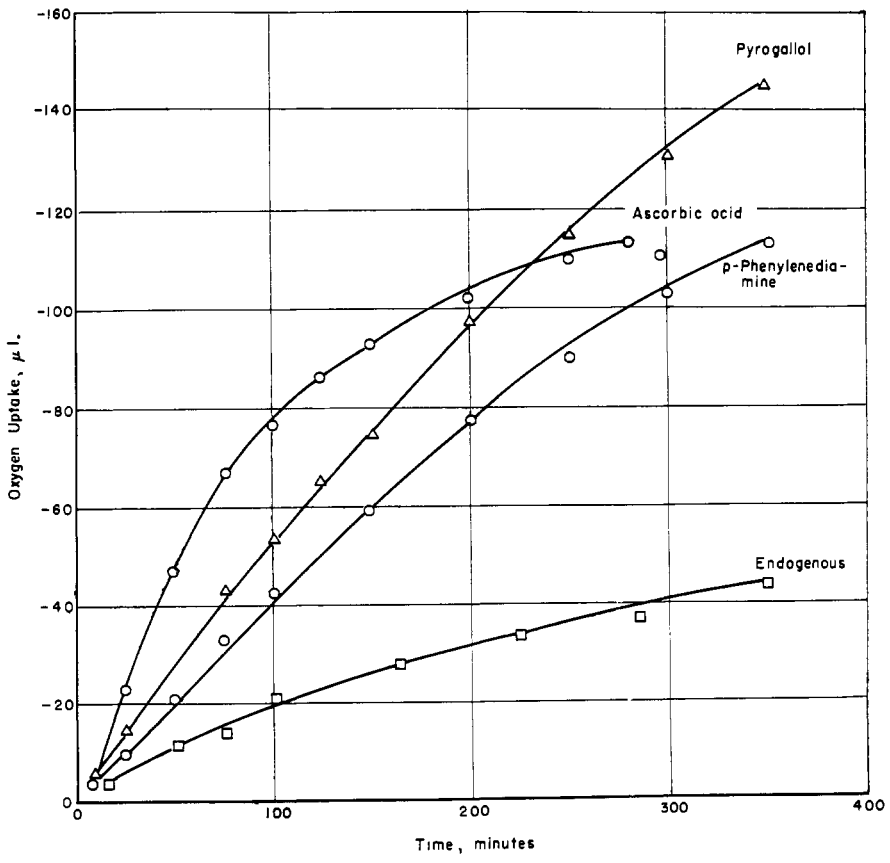

Figure 1. Typical oxygen uptake curves for burley tobacco

the casein, some endogenous activity (0.55 ml. of hydrochloric acid) was shown. Nearly as much hydrochloric acid was used for the burley tobacco as was used for the pure protease, indicating that burley tobacco has a rather large protease activity.

Urease. Although urease activity in unaged burley tobacco is shown for ground tobacco samples in Table III, no evidence was obtained for activity in any of the colloid-mill extracts. Boiling or autoclaving the tobacco failed to reduce consistently the carbon dioxide being evolved; this was also true of unaged bright tobacco which, upon occasion, gave varying amounts of evolved carbon dioxide. There appears to be a chemical mechanism involved here and an effort to determine urease activity by another method.

The Conway method for determining urease activity gave results which are summarized in Table V. Although the known solution of urease showed considerable activity, negative results were obtained for both unaged bright and burley tobaccos. Bright tobacco had no activity, with or without urea. Burley tobacco showed the same activity with the urea. However, this activity was due not to urease, but to the activity of proteolytic enzymes which give off ammonia with certain substrates—e.g., proteins—as does urease with urea. As burley tobacco contains both proteins and a proteolytic enzyme, the endogenous activity was significant, as shown by the 0.55 ml. of 0.00246*N* hydrochloric acid, recorded in Tables IV and V. If the endogenous activity resulting from protease is subtracted from the values in Table V for endogenous sample activity with urea, the differences are 0.00 and 0.05 ml. of hydrochloric acid, respectively, which represent the titratable ammonia produced by urease. This tends to rule out the presence of urease in either unaged bright or unaged burley tobaccos.

Decarboxylases. Decarboxylases (α-ketoglutaric acid and pyruvic acid decarboxylases) were found to be present in unaged bright tobacco. The decarboxylases have proved to be one of the most difficult classes of enzymes to confirm. Many naturally occurring substances in leaf tissue will decarboxylate spontaneously—especially after disintegrating cell walls by means of a colloid mill—and may thus mask the effect resulting from decarboxylases. Although various methods of extraction indicate different degrees of activity, it is evident from Table III that unaged bright tobacco does contain decarboxylases.

The colloid-mill procedure showed that unaged burley tobacco contains decarboxylase. In the present procedure, the J3 extract showed considerable decarboxylase activity and, upon purification with ammonium sulfate precipitation to give the J6 extract, strong decarboxylase activity was once more shown. Since J3 and J6 were extracted with the aid of the colloid mill, it appears that decarboxylases are probably unavailable to substrates added in the ground tobacco procedure.

Results with unaged burley tobacco indicate a different result. As burley tobacco contains little sucrose, a great endogenous activity would not be expected, even if invertase were present. This was found to be the case where the transmission percentages and general appearance of the endogenous solution were practically the same as those of the control sample. With the addition of sucrose, marked differences were noted in both transmission and appearance of the various dilutions. Although a 1 to 1 dilution showed no precipitate for the control or endogenous solution, a 1 to 18 dilution of the sample containing sucrose still showed a slight red precipitate. Invertase activity in unaged burley tobacco was inhibited by boiling the tobacco solution before adding sucrose.

Cellulase. A slight decrease in viscosity of solutions of carboxymethylcellulose and a colloid-mill extract of unaged burley was found. Other extracts of unaged burley tobacco indicated no cellulase activity. A very slight cellulase activity was noted for unaged bright.

Amylase. Initial tests for amylase activity on the J3 extract from the colloid-mill procedure in unaged bright and burley tobaccos showed no amylase activity. These extracts and crude enzyme solutions took up the iodine immediately and gave a premature disappearance of the blue color. Additional iodine indicator was added and the blue color reappeared. The procedure had to be repeated several times until several milliliters of iodine solution were added. Although these results are somewhat inconclusive, no amylase activity was indicated.

Other Enzymes. Miscellaneous investigations of enzymes in unaged bright and burley tobaccos were also made. Cytochrome c oxidase was examined in both unaged bright and burley tobaccos, as well as the J3 extract from burley tobacco. A manometric procedure was used in which a small amount of cytochrome c was added. The addition of cytochrome c did not enhance the activity when the substrate, hydroquinone, was used. This occurred for both bright and burley tobacco and implies the absence of any appreciable cytochrome c oxidase activity.

Initial attempts following Ettori's modified method (*3*) to establish definitely the presence or absence of peroxi-

Table III. Microliters of Carbon Dioxide Evolved by Action of Unaged Bright and Burley Tobacco Enzymes

Substrate	Unaged Tobacco	Aged Tobacco	Acetone Extract	Colloid Mill Extract	Autoclaved 5 Minutes
Pyrogallol					
Bright	14	0	15	Variable	14
Burley	55	20	64	173	0
Gallic acid					
Burley	4–22	16	20	...	0
Urea					
Bright	0	9	..	0	Variable
Burley	13–43	30	..	0	Variable
Pyruvic acid					
Bright	32	31	32	...	0
Burley	0	0	..	...	0
α-Ketoglutaric acid					
Bright	34	40	41	124–196	0
Burley	0	0	47	34–240	...

Table IV. Proteolytic Enzyme Activity of Pure Protease, Unaged Bright Tobacco, and Unaged Burley Tobacco

Enzyme Source	Substrate	0.00246N HCl Used,[a] Ml.
Pure protease	Casein	2.00
Bright tobacco	Endogenous	0.00
	Casein	0.10
Burley tobacco	Endogenous	0.55
	Casein	1.85
Burley tobacco (boiled)	Casein	0.30

[a] Average after blank has been subtracted.

Table V. Urease Activity of Unaged Bright and Burley Tobaccos

Enzyme Source	Substrate	0.00246N HCl Used,[a] Ml.
Urease	Urea	3.05
Bright tobacco	Endogenous	0.00
	Urea	0.00
Burley tobacco	Endogenous	0.55
	Urea	0.60

[a] Average after blank had been subtracted.

Table VI. Enzyme Activity in Unaged Bright and Burley Tobaccos

Enzyme	Tobacco	Barrett	Dixon and Coworkers (2)	Nakai and Inaba (9)	Hukusuma (7)	Oosthuizen and Shedd (10)
Tyrosinase	Burley	(S)	..	..	...	...
Protease	Bright	O	O	.	...	...
	Burley	X	..	..	...	(S)
Lipase	Burley	...	..	..	...	X
Emulsin	Burley	...	..	..	...	X
Amylase or diastase	Bright	O	O	X	...	...
	Burley	O	X	..	...	X
Invertase	Bright	(S)	O	X	...	...
	Burley	X	X	..	...	X
Oxidase or laccase	Bright	O	O	..	O	...
	Burley	X	..	..	...	X
Peroxidase	Bright	O	O	X	(S)	...
	Burley	(?)	..	..	...	...
Catalase	Bright	O	O	X	X	...
	Burley	X	X	..	...	...
Reductase	Bright	...	..	..	O	...
Decarboxylase	Bright	X	..	..	...	...
	Burley	(?)	..	..	...	...
Urease	Bright	O	..	..	...	...
	Burley	(?)	..	..	...	...
Cellulase	Bright	O	..	..	...	...
	Burley	(S)	..	..	...	...

O. Little or no activity.
X. Definite activity.
(S). Slight activity.
(?). Presence of activity questionable.

dase in tobacco have not been entirely successful. This method involves the standard reaction, catalyzed by peroxidase, between pyrogallol and hydrogen peroxide. The reaction products are purpurogallin, water, and carbon dioxide. The carbon dioxide is measured manometrically.

The determination of peroxidase activity in the presence of catalase activity is very difficult for the enzyme preparations used here. Some results, however, indicate that unaged burley tobacco contains peroxidase, and considerable carbon dioxide evolution is obtained.

Other manometric studies showed little or no enzyme activity by unaged bright and burley toward these substrates: histidine, alanine, lactic acid, glucose, fructose, and sucrose. There appears to be a slight activity toward acetic acid by both tobaccos and by unaged bright tobacco toward glycolic acid. A decarboxylating mechanism giving rise to carbon dioxide is indicated here.

In Table VI the presence or absence of enzyme activity in unaged bright and burley tobaccos, as determined in the present study, is compared with the findings of other investigators. The results of the present investigation generally support the findings of Dixon and coworkers (*2*) for unaged bright tobacco or flue-cured tobacco. The only enzymes found to have more than slight activity in unaged bright tobacco were the decarboxylases. Garner (*5*) summarizes for other investigators, who reported activity for many enzymes not detected in unaged bright tobacco in the present investigation.

Unaged burley tobacco contains several enzymes which have been found by others. No amylase was found in the present investigation, in disagreement with other reported findings. Pectin methylesterase has been found in both unaged bright and burley tobaccos by specific spectrophotometric micromethods (*6*).

Importance of Enzymes in Aging Tobacco. This subject has been discussed by Dixon and coworkers, Frankenburg, and others (*2, 4*). This investigation indicates that the aging process in bright tobacco is primarily a chemical one. Aging of burley tobacco appears to be chemical but enzymes may have an important and necessary part of the process. Speculation on the action of some enzymes found in unaged bright and burley tobaccos could show the following possible mechanisms involved in the aging process.

The decarboxylases give carbon dioxide and aldehydes from keto acids. Although this action decreases the acidity during aging (*2*), reactions involving the aldehydes formed can be very important with respect to the quality of the tobacco. Aldehydes can react with proteins and change the properties of the tobacco, including solubility.

Probably one of the most important classes of enzyme found in unaged burley tobacco was the oxidases. These were shown to be enzymes closely related to laccase and tyrosinase. Their action frequently involves the oxidation of hydroxyl structures in polyphenols, such as tannins, catechins, and simple polyphenols, to give quinonelike structures. In this way, oxidases may control the reaction which is believed to occur between polyphenols and α-amino nitrogen compounds during aging. Apparently, the insoluble compounds formed (similar to melanoids) are tied up closely with the smoking quality of cigarette tobacco.

Protease, which has been found in unaged burley tobacco, may initiate a reaction leading toward the formation of melanoids. Losses in α-amino nitrogen and sugars and an increase in water, shown chemically, could thus be accounted for in unaged burley tobacco.

Many other enzyme systems might have a bearing on the aging of flue-cured and air-cured tobaccos such as bright and burley. A more thorough investigation of the minor, more subtle, changes in chemical composition (chemical or enzymic in origin) of tobacco during the aging process should give added knowledge of the aging process.

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