

1

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PROCESS FOR THE TREATMENT OF TOBACCO STEMS

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7 Claims

ABSTRACT OF THE DISCLOSURE

This disclosure relates to a process for the treatment of tobacco stems. More particularly, the disclosure relates to a process for the treatment of tobacco stems which comprises soaking tobacco stems in an aqueous solution of polysaccharide-hydrolyzing enzymes exhibiting catalytic pectinase, cellulase and hemicellulase activity, permitting the enzymes to act on the stems for a period of from about 15 minutes to about 48 hours, preferably between one and 12 hours, pressing or rolling the treated and softened stems to flatten them, and drying and cutting for use in tobacco products. The aqueous solution contains an amount of enzyme equivalent to an enzyme to stem weight ratio of between about 1 to 10,000 and 1 to 10, preferably between 1 to 5,000 and 1 to 250. The pH of the aqueous solution will generally be from about 3.5 to about 5.5 and the concentration of water should be from about 50 to about 1,000%, preferably from about 100 to about 400% of the weight of the dry stems.

BACKGROUND OF THE INVENTION

Tobacco leaf midribs or stems have generally been found to be objectionable in smoking tobacco blends, particularly in blends for cigars and cigarettes, because of their undesirable burning qualities and their stiffness which may lead to deformed or punctured wrappers. It is, therefore, customary to separate the stems from the leaf and to process the stems separately. In many of the currently used processes the separated stems are ground, mixed with leaf dust and converted into a synthetic leaf. Such processes result in improved burning qualities and eliminate the stiffness of the original stems. Such processes, however, demand a large investment, huge facilities and an expensive daily operation. For these reasons a much simpler process is still currently operating at various tobacco processing facilities. This process consists in softening the stems by steam and then flattening them under pressure to compress and spread the stem to a form which may be shredded in the manner of tobacco leaf. The flattened stems are an improvement over untreated stems, but they are still somewhat stiff, and the burning qualities are not greatly improved. Furthermore, the pressed stems are rather brittle and fragile, and this results in a high amount of unusable dust and undesirable fine particles when these flattened stems are shredded for incorporation into a leaf blend for cigarettes. In addition, the stems tend to resume their unflattened condition as they cool and dry. Efforts to plasticize the rolled stems by application of softeners such as glycerine have not had enough effect to overcome these problems. The stiffness of the rolled product makes it capable at times of puncturing the wrapper in the manner of the untreated stems.

Certain components of tobacco smoke are sometimes regarded as undesirable, and decreased delivery of such components as total particulate matter and nicotine is advantageous.

2

SUMMARY

The present invention provides a method for converting tobacco stems into a product which may be employed in admixture with tobacco leaf to make cigarettes or which may be otherwise incorporated into tobacco products, which process comprises treating such stems by soaking the same in an aqueous solution of polysaccharide-hydrolyzing enzymes exhibiting catalytic pectinase and hemicellulase activity which aqueous solution penetrates the stem, swells and softens the same and breaks down some of the high-molecular weight polysaccharides into low-molecular weight ones, and flattening, drying and shedding the enzyme treated stems. The resulting material may then be air-dried or freeze-dried. Such a process provides a product useful as a cigarette filler with increased filling power and one which retains its flattened shape and pliability when crushed and dried to room conditions. The filling power of the treated stems may be further improved by freeze-drying, for example, by the process set forth in my copending application, Ser. No. 693,090 filed even date herewith. The air-dried material was found in some instances to have a higher, thus more desirable filling power than factory rolled stems of current production. Within the frame of this disclosure, it is thus possible but not necessary to prepare flattened stems of high filling power. Filling power also depends upon the amount of solubles such as humectants, tobacco solubles and other casings carried by the aqueous solution, deposited in the stems and remaining therein after pressing. Such extracts, humectants and other solubles aid and increase the softening of the stems.

The present invention provides a process for converting tobacco leaf stems into a cigarette filler product exhibiting excellent smoking properties, low tar delivery which is less than flattened stems produced without the addition of stem-softening enzymes and which process can be carried out with low capital investment utilizing readily available enzyme preparations and processing equipment.

The main feature of this invention is the use of stem-softening enzymes. Since, in this treatment, larger amounts of water have to be introduced than in the conventional steam-softening procedure, other water-soluble compounds can be introduced together with the enzyme, such as tobacco solubles, humectants, "casings," in order to improve the smoking aroma, and the smoke composition. If the process is employed in conjunction with a freeze-drying step, the stems must then be treated with sufficient water to obtain the benefits of the freeze-drying step. Thus, according to this invention, more liquid will generally be added to the stems than in the conventional processes of making flat stems by steam-softening and rolling.

In accordance with the present invention the softened stems require less pressure, thus less mechanical energy to be flattened and, when flattened, the resulting film or tissue is thinner than can be achieved without the use of the aforementioned enzymes. The low-molecular weight polysaccharides formed by the application of the enzymes possess the properties of a cement as well as of a plasticizer. This will result in less dust, less loss during shredding and in soft shreds.

DESCRIPTION OF THE INVENTION AND ITS PREFERRED EMBODIMENTS

The present invention comprises soaking tobacco stems in an aqueous solution of polysaccharide-hydrolyzing enzymes in catalytic amounts, allowing the enzymes to

3

act on the stems, draining and aging them for a time. The treated and softened stems are then pressed or rolled in the usual fashion on unheated or steam-heated rolls to flatten them. The stems may remain in the solution throughout the aging period, although this is not necessary for the action of the enzyme. When the stems remain in the solution, excess solution should be squeezed from the stems during the rolling process and then recovered. The product is dried and cut as required for use in smoking products.

The enzymes which are useful in the practice of the invention are pectinase, hemicellulase and cellulase. Pectinase, however, is the most essential of the enzymes as revealed by chemical analysis of the enzyme-treated stems. Cellulase is the least essential of the enzymes. However, certain commercial enzyme preparations designated as "cellulases" contain large amounts of pectinase and hemicellulase and are, therefore, useful enzyme preparations in the context of this invention. The presence of other enzymes in a commercial enzyme preparation such as proteases, lipases and diastases, does not interfere with the action of the pectinase, hemicellulase and cellulase. The presence of these other enzymes was not found to transmit undesirable properties to the enzyme-processed stems either. The polysaccharide-hydrolyzing enzymes useful in the context of this invention can be derived from plants, from animal organs, and from fungal and bacterial cultures. Industrially available enzyme materials considered effective in the present invention are usually derived from the growth of suitable microorganisms such as *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium chrysogenum notatum* and the like. Several commercial cellulase preparations and the organisms from which they are prepared are listed in a paper by C. S. Walseth (TAPPI 35:228, 1952).

Specific commercial enzyme preparations which have found to exert the softening action on stems include Cellulase 35 (a mixture of polysaccharases including cellulase, pectinase and hemicellulase), Pectinol R 10, Pectinol 100 D, Pectinol 41 P (Pectinol is a mixture of pectic enzymes and includes other enzyme such as cellulase; R 10 and 100 D include a diatomaceous earth filler), Lipase B (an enzyme preparation standardized with salt exhibiting protease activity at pH 5 and a hydrolysis of fats and fatty acid esters), all from Rohm and Haas Co., Philadelphia, Pa., Pectinase (Nutritional Biochemical Corp., Cleveland, Ohio), Cellulase 1000 (a fungal cellulase preparation derived from a selected strain of *Aspergillus niger*, and which contains trace amounts of protease, amylase, pectinase, beta galactosidase and cellobiase, from Wallerstein Co., Staten Island, N.Y.), Pectinase S.C. and Cellulase 4000 (Miles Laboratories, Elkhart, Ind.). A method for defining suitable enzyme activity is described in Example 2 of this application.

The pH of maximum activity for the enzyme is the natural pH for stems derived from flue-cured tobacco. This pH is between 3.5 and 5.0. However, some stems, such as those derived from burley tobacco, have a higher pH and in such cases it is advantageous to adjust the pH to the optimum range by adding acid.

The enzyme treatment of the stems requires an establishment of enzyme concentration based upon the weight of stems, a required amount of moisture, a certain time of enzyme action and a specific temperature during the treatment. The concentration of a commercial enzyme preparation with regard to the stems may be as low as 0.01% and an excess up to 10% has no undesirable effect. For practical reasons an enzyme/stem ratio between 1 to 5,000 and 1 to 250 is preferred. Water is required to carry the enzyme inside the stems. The water at the same time swells the stems and increases their volume. The transport of the enzyme through the stems is facilitated if the stems have been predampened by steam, but this is not necessary. The amount of liquid to be applied may be from about 50 to about 1,000% but preferably from

4

about 100 to about 400% of the weight of the dry stems. This liquid, which may contain in addition to the enzyme tobacco extracts, humectants and casings, can be recirculated, i.e., part of the liquid can be squeezed out from the stems during the flattening procedure and used over for a new batch of stems.

The aforementioned humectants may comprise glycerine, propylene glycol or triethylene glycol or other plasticizers known in the tobacco processing industry. They aid and increase the softening action of the enzymes. Flavorants, tobacco solubles, and other casings may be included in the treating solution in order to improve the smoking aroma of the flattened stems.

The time of enzyme digestion may vary between 15 minutes and 48 hours, the lower limit being determined by the diffusion rate and by the action of the enzyme; the upper limit being determined by eventual spoilage of the stems. This spoilage can occur through fermentation if the digestion time is too long. The time of enzyme digestion is preferably between one and twelve hours. An increase in temperature accelerates the process but too high a temperature inactivates the enzymes. A temperature between 20° C. and 70° C. is, therefore, recommended.

The rolling may be replaced by a shearing process wherein the fibrous structure of the stems is torn apart. The enzyme treatment softens the intercellular binders, thus aiding in tearing apart the fibrous structure. If desired, a simple pressing between flat platens may be used.

The flattened product, while still wet, may be freeze-dried in accordance with the disclosure in copending application Ser. No. 693,090, by the same inventor, and filed of even date herewith.

The invention is illustrated by the following examples.

Example 1

Whole stems, from flue-cured tobacco, 20 g., were kept for 24 hours in 400 ml. of various enzyme solutions at room temperature. The enzyme preparations which exhibit the desired activity rendered the stem swollen and mushy. In the table below the minus (—) means that the stems were not softer than the control. For this control water alone was used and no enzyme was added. The sign plus (+) means activity resulting in a very soft, mushy stem material.

No added enzyme (control)	-----	—
Cellulase Meiji (Meiji Seika Kaisha, Ltd., Tokyo, Japan) 0.2%	-----	—
Endo-polygalacturonase (prepared in the laboratory from a <i>Saccharomyces fragilis</i> #351 culture)	-----	—
Pectin esterase (Nutr. Biochem. Corp.), 0.2%	-----	—
Hemicellulase (Nutr. Biochem. Corp.), 0.2%	-----	—
Hemicellulase (Miles Lab.), 0.2%	-----	—
Cellulase (Swiss Ferment Co. Ltd., Basle), 0.2%	-----	—
Rhozyme CL (Rohm and Haas Co.), 0.2%	-----	—
Rhozyme S (Rohm and Haas Co.), 0.2%	-----	—
Cellulase 35 (Rohm and Haas Co.), 0.2%	-----	+
Pectinol R 10 conc. (Rohm and Haas Co.), 0.2%	---	+
Pectinase S.C. (Miles Lab.), 0.2%	-----	+
Pectinol 100 D (Rohm and Haas Co.), 0.2%	-----	+

Example 2

The following method was employed to determine whether or not an enzyme preparation exhibits pectinase and hemicellulase activity. Five grams of pure citrus pectin (Pectin N.F. #3442 Sunkist Growers, Inc., Corona, Calif.), 5 g. of guar gum (Polymer Industries, Springdale, Conn., Guar type 501) and 5 g. of xylan (Plantstiehl Lab., Waukegan, Ill.) were separately admixed with 500 ml. portions of an acetic acid-acetate buffer solution having a pH of 4.5 and a molarity of 0.04. In the cases of pectin and guar gum, these materials are completely dissolved. However, in the case of the xylan, this material is present in suspension. Then one portion of

0.05 gram of the enzyme system is added to each mixture, and the mixtures maintained at 45° C. for a period for 24 hours. The resulting mixtures are then each placed in 1000 ml. of acetone, whereby precipitates form which comprise undegraded or little degraded pectin, guar gum, or xylan, respectively. These precipitates are separately filtered and dried at 45° C. in a forced air circulation oven to constant weight. Controls are also run for each of the materials, under identical conditions, except that no enzyme is present. Pectinase activity, as required in this application, is exhibited when the pectin shows a weight decrease after this treatment of 40% or more, as compared with a control which contained no added enzymes. Guar gum and xylan preferably also show a weight decrease, indicating suitable hemicellulase activity in the enzyme preparation. The table below shows the results obtained by this test with various commercial enzyme preparations. The first six enzyme preparations in the table are those which gave unsatisfactory results in the preceding Example 1. They all degrade less than 40% of the pectin.

The other six enzyme preparations listed in the table below give satisfactory results. They all degrade more than 40% of the pectin according to the described test. The very satisfactory Cellulase 35 also degrades substantial amounts of guar gum and xylan.

	Percent Degradation Due to Enzyme		
	Pectin	Guar	Xylan
Cellulase Meiji	15.5	25.3	87.9
Pectin esterase Nutr. Biochem	7.6	7.4	58.5
Hemicellulase Miles	6.2	25.5	1.0
Cellulase Ferment Co.	3.4	11.4	32.4
Rhozyme CL	36.0	14.0	0.0
Rhozyme S	25.7	34.7	39.0
Cellulase 35	90.0	49.3	41.8
Pectinol R 10 conc.	89.9	44.6	45.0
Pectinase S.C.	40.3	2.3	1.0
Pectinase Nutr. Biochem	85.5	11.1	23.3
Lipase B conc.	89.0	40.4	83.4
Cellase 1000	87.3	34.4	58.0

Example 3

Bright and burley tobacco stems, 20 g. dry weight, were immersed in 300 ml. enzyme solution buffered at pH 4.5. They were digested for 24 hours at 45° C., filtered, washed, and dried to constant weight at 45° C. The stems were then submitted to a sequential extraction procedure for the determination of benzene-ethanol and water solubles, for the determination of pectin (ammonium oxalate solubles), for the determination of lignin (sodium chloride solubles), for the determination of hemicelluloses (12% potassium hydroxide solubles), for the determination of cellulose (residue minus ash), and for the determination of residual ash after treatment with 12% KOH. The percentages from the analysis of the treated stems were converted into percent based on the weight of the original, dry stems. The results from the treatment of the stems with the enzyme preparations were compared to a control. In the control experiment the addition of enzyme was omitted, otherwise the stems were submitted to the same operations as in the case of added enzyme.

This sequential extraction procedure is a standard procedure in wood chemistry. In the table below only the values are listed which were obtained for pectin, hemicelluloses and cellulose. It can be seen from the table, that the active enzyme preparations, used in amounts of 5 and 10% based on the original stems, solubilized portions of the pectin, hemicelluloses and cellulose contained in the stems. It is implied, although not directly proven that the residual pectin, hemicelluloses and cellulose remaining in the stems have been drastically modified, although not to the point of being made soluble. It is also implied in this example that by using less than the 5 or 10% of enzyme preparation which were used, there will be a lesser amount of pectin, hemicelluloses

and cellulose rendered water soluble, but the polysaccharides remaining in the stems will be modified.

	Percent Material Present in Processed Stems		
	Pectin	Hemicelluloses	Cellulose
Flue-Cured Stems:			
Control, no enzyme added	9.0	12.2	15.4
Cellulase 35, 10%	3.8	7.9	12.2
Pectinol R 10, 10%	6.1	7.5	7.8
Burley Stems:			
Control, no enzyme added	10.2	16.4	21.5
Cellulase 35, 5%	9.8	13.6	16.4

Example 4

Bright and burley tobacco stems were soaked for 18 hours in various aqueous solutions as shown in the table below, drained, placed between cardboard sheets, and flattened with a hammer. The ease and degree of flattening were determined subjectively. The table below indicates that the enzyme-treated stems were softer, and necessitated less effort for flattening; they also stayed flatter than those treated with water. The solution in which burley stems were immersed required adjustment of pH to from 3.5 to 5.5 with acid for optimum enzyme activity; with bright stems this was unnecessary.

AQUEOUS ENZYME TREATMENT AND FLATTENING OF TOBACCO STEMS

	Soaking solution	Ratio Stems/soln.	Result	
			Stems	Result
30	0.2% Cellulase 35 ¹ (conc.)	1/20	Bright	Easily flattened.
	0.2% Cellulase 35 ¹ (conc.)	1/20	do	Easily flattened;
	5% glycerine.			stems stay flexible dried at 60% r.h.
	Water only	1/20	do	Very hard, not easily flattened.
35	0.08% Cellulase 35 ¹ (conc.), 10% glycerine, 2N acetic acid to pH 4.0 with stems present.	1/20	Burley	Soft, easily flattened.

¹ Rohm & Haas, Inc.

Example 5

Bright tobacco stems were immersed overnight at room temperature in 0.1%, 1.0%, and 10% aqueous solutions of Rohm and Haas Cellulase 35 and then pressed. Stems were also immersed in water for the same period to provide a control. The stems were drained and pressed on a Carver laboratory hydraulic press (Fred S. Carver, Inc., Summit, New Jersey) at a load of 16,000 pounds. They were then dried. At conditions of equilibrium with an ordinary room (60% r.h.), the stems without enzyme treatment were brittle and had begun to lose their flatness; those treated with enzyme were pliable and flat.

Example 6

Burley tobacco stems (Experiments 1-5, as shown in the table below) were soaked for 24 hours at room temperature in 25 times their weight of water containing the additives shown in the table below. In experiment No. 6, the treatment of the stems was for 4 hours at 80° C. in 20 times their weight of water. The drained stems were flattened in a Carver laboratory press at 16,000 lbs. The conditions of treatment and the results are shown in the table below.

SOAKING AND PRESSING OF BURLEY STEMS

Experiment No.	Soaking solution	Results
1	10% sodium citrate	Stems very hard.
2	1% sodium citrate	Do.
3	10% sodium citrate, 2 N acetic acid to pH 4.5.	Stems hard.
4	0.02% Cellulase 35, 1% sodium citrate, 2 N acetic acid to pH 4.5.	Stems soft, well flattened.
5	10% diammonium phosphate, 0.2% Cellulase 35.	Stems very flat.
6	0.05% diammonium phosphate, 0.25% Cellulase 35, 2 N acetic acid to pH 4.5.	Stems soft, very flat.

Example 7

Whole bright tobacco stems, 150 g., were soaked in enzyme-containing solutions for 18 hours, drained, pressed in a Carver press at 15,000 lbs. and air-dried. A portion of the stems was analyzed (table below) by conventional procedures for tobacco to determine the total ash content, benzene-ethanol solubles, hot water solubles, glycerine, and total particulate matter (TPM). The stem extract used in the treatment was obtained from 500 g. of —50 mesh ground bright stems soaked in 2000 ml. of water for two hours at 45° C. and filtered to give 1480 ml. of extract. This extract was used to make up for solubles lost during pressing, simulating a commercial rolling operation where solubles would have to be recirculated. The analytical results show that, with allowance for glycerine, the treated stems were little changed from their original composition.

The pressed stems were shredded, hand-made cigarettes containing only the stems were prepared, and a commercial filter of cellulose acetate tow was attached. The cigarettes were smoked by machine by standard procedures and 55 mm. of the rod burned (=0.72 g. and 1.27 g. of filler for control and experimental, respectively). Total particulate matter (TPM) retained by a Cambridge glass microfiber filter was weighed. TPM, sometimes called “stars,” is often regarded as an undesirable component of smoke. The reduction, as shown in the table below, is significant.

ANALYSIS OF CIGARETTE FILLER AND SMOKE

	Bright stems	Bright stems	Bright stems	Commercial filler
Soaking solution (ratio to stems 7/1).	Stem extract, 1%; Cellulase 35, 10% glycerine.	Stem extract, 1%; Cellulase 35, 10% glycerine.	(1)	(1)
Filler:				
Total ash, percent.....	17.8.....	16.0.....	18.5.....	
Benzene-ethanol solubles, percent.....	40.4.....	38.9.....	39.4.....	
Hot water solubles, percent.....	18.1.....	18.2.....	20.2.....	
Glycerine, percent.....	10.3.....	11.3.....		
Smoke: TPM, mg./g. filler.....	6.5.....	7.3.....		21.5

¹ Untreated control.

Example 8

Bright stems were soaked in either water (control), enzyme in water or enzyme in stem extract to compensate for the loss of extract during pressing. (In a continuous operation the liquid squeezed out during pressing will be reused for the soaking of a next batch of stems.) The mixtures were digested for 15 hours at room temperature, pressed in the Carver press at 15,000 p.s.i. and air-dried. The flattened, dry stems were shredded and made into non-filter cigarettes. All cigarettes of same filler weight had an RTD (resistance to draw) of 1.0±0.2 inch of water.

The results show that the enzyme-treated stems delivered less tars to smoke than a control of pressed stems not enzyme-treated.

TPM AND PHENOLS DELIVERY OF CIGARETTES CONTAINING 100% PRESSED STEMS

Experiment.....	1 ¹	2	3
Amount enzyme used, percent based on weight of stems.....	0	2.3	0.2
Stem extract added.....	No	No	Yes
TPM (mg./cigarette).....	19	8	7

¹ Control

Example 9

Bright and burley tobacco stems, cured, were soaked overnight in water containing Pectinol 41 P conc., an enzyme preparation from the Rohm and Haas Co. The enzyme preparation is effective at a concentration of 0.02% based on the weight of the stems. After soaking, the softened stems were pressed in a laboratory hydraulic press manufactured by Fred S. Carver, Inc.

Half of the stems were freeze-dried and half were air-dried. Cross-sectional views at low magnification showed the comparatively, much more expanded structure of the freeze-dried stems. The air-dried stems had shrunk and also were noticeably darker. The table below gives comparative color coding, per Munsell Book of Color, Munsell Color Co., Inc., Baltimore, Md., 1958.

MUNSELL COLOR CODES FOR PRESSED STEMS

	Air-dried	Freeze-dried
Bright Stems.....	7.5 YR hue, 4/6.....	7.5 YR hue, 6/4.
Burley Stems.....	7.5 YR hue, 4/4.....	7.5 YR hue, 6/4.

Example 10

Portions weighing 100 g. each of whole bright tobacco stems and whole burley stems were immersed in 400 ml. of water containing 1 g. of Cellulase 35 conc. enzyme from Rohm and Haas Co. The stems were removed and drained after 15 minutes. The bright stems had absorbed 90 ml. of solution, the burley 125 ml. Both batches were left covered at room temperature for about 16 hours. The stems were flattened on a Carver laboratory press at 16,000 lbs. Both types flattened well, the bright somewhat more readily than the burley, but both very much better than stems containing approximately their own weight of water.

Example 11

Whole bright tobacco stems, 300 g., were soaked overnight in 2800 ml. of 0.04 M acetate buffer of pH 4.5 con-

taining 0.1% of Rohm and Haas Cellulase 35. The drained stems were pressed on a Carver laboratory press at 15,000 lbs. and air-dried, then shredded. Cigarettes made from various weights of the stems were compared in the following table for RTD (resistance to draw) with those from commercial rolled stems. Increased filling power is shown by greater RTD of a rod having an equal weight of filler.

COMPARATIVE FILLING POWER IN 68 mm. CIGARETTES

RTD, inches of water		
Filler weight g.	Enzyme-treated pressed stems	Factory rolled stems
1.0.....	2.0.....	
1.1.....	4.2.....	
1.2.....	4.5.....	1.7
1.3.....	6.6.....	2.2

The following measurements were obtained when enzyme-converted flue-cured pressed and shredded stems were blended with factory leaf filler and made into 68 mm. cigarettes with no filter attached.

FILLING POWER OF FILLER CONTAINING ENZYME-CONVERTED PRESSED STEMS

Total filler weight g.	Leaf filler, g.	Enzyme-converted pressed stems, g.	RTD, inches of water
1.1.....	1.1	0	2.2
1.1.....	1.0	0.1	2.2
1.1.....	0.9	0.2	2.3
1.1.....	0.8	0.3	2.0
1.15.....	0.85	0.3	3.5

Example 12

To test smoke flavor and aroma, enzyme-treated pressed and shredded stems from flue-cured leaf were blended with a commercial all-leaf filler and made into cigarettes with characteristics as follows: wt. stems 0.3 g., wt. leaf 0.8 g., RTD experimental cigarette 4.0 in., RTD control (factory pressed stems from flue-cured leaf, no enzyme used) cigarette 3.7 in. These cigarettes were smoked subjectively by a panel of expert smokers; of eight smokers, seven preferred the experimental blend.

Example 13

Six lots of unwashed, air-dry bright tobacco stems, each weighing 2500 g., were placed in polyethylene bags. A Waring blender was used to dissolve 4.6 g. portions of Cellulase 35 enzyme, activity $f=1.00$ (Rohm and Haas Co.), in various quantities of water as shown in the following table. The enzyme solutions were added to the tobacco and the bags were shaken to distribute the solutions evenly. After having stood for about 24 hours, the stems were rolled between factory rolls operating at a surface speed (both rolls) of 690 ft./min. at a clearance of 0.035 in. In some instances (see the following table) the rolls were heated by steam. The flattened stems were examined and oven volatiles (largely moisture) were measured, by weight loss in 4 hours at 95° C. under circulating air.

ENZYME TREATMENT AND ROLLING OF BRIGHT TOBACCO STEMS

	Volume of Steam water added, heated ml. rolls	Oven volatiles after rolling, percent
I.....	375 Yes.....	24
II.....	605 Yes.....	28.5
III.....	835 No.....	31
IV.....	1,295 No.....	39.7
V.....	1,885 No.....	47
VI.....	1,885 No.....	51

¹ No enzyme.

Example 14

Dry flue-cured stems, 19 g., were digested for 24 hours in 65 ml. of water containing 0.05% Rohm and Haas Co. Pectinol 41 P conc. All the liquid was absorbed by the stems. The stems were then pressed in the Carver press at 15,000 lbs. The pressed stems had a moisture content of 202% and twenty-seven ml. solution was squeezed out of the stems. This amount can be reused on a fresh batch of stems.

Example 15

Rolled flue-cured and burley stems of current production and stems according to the present invention as described in Examples 5 and 6 were moisture equilibrated and shredded. The enzyme-treated stems proved to be more plastic and to yield less dust and fines during shredding than the rolled stems of current production.

For the purposes of this invention the following methods may be used to determine enzyme activity.

One method which can be employed to determine whether or not an enzyme system exhibits cellulase activity is to place ten grams of cellophane in a solution containing one gram of the enzyme system and maintained at a pH of 4.5 and a temperature of 45° C. for a

period of 24 hours. After 24 hours, the remaining cellophane is removed from the solution by filtration, is washed with water and dried in a forced air circulation oven at a temperature of 45° C. until constant weight is attained. A control is run, using cellophane treated under exactly the same condition, but in the absence of any enzyme. Cellulase activity, as it is used in this application, is exhibited when the cellophane shows a weight decrease after the treatment of 0.5% or more, as compared with the control which contained no added enzymes. Methods employed to determine whether or not an enzyme system exhibits hemicellulase and/or pectinase activity are described in Example 2.

Obviously, other assay methods which will determine the existence of the three enzymes may also be employed.

I claim:

1. A process for treating tobacco leaf stems which comprises the steps of:

contacting tobacco stems with an aqueous solution of polysaccharide-hydrolyzing enzymes exhibiting pectinase, hemicellulase and cellulase activity, said aqueous solution comprising a concentration of enzymes equivalent to an enzyme to stem ratio of between about 1 to 10,000 and 1 to 10 by weight and a concentration of water equivalent to between about 100% and 1000% of the weight of the dry stems, said aqueous solution being maintained at a pH of between about 3.5 and 5.0, for a period of time of from about 15 minutes to about 48 hours at a temperature of from about 20° C. to about 70° C.; removing excess water from the resulting stems and flattening the enzyme-treated and softened stems;

drying the flattened enzyme-treated stems; and shredding the resulting product.

2. A process according to claim 1 wherein the enzyme digestion period is between about 1 and 12 hours.

3. A process according to claim 1 wherein the enzyme to stem ratio in the aqueous solution is between about 1 to 5000 and 1 to 250.

4. A process according to claim 1 wherein the concentration of water in the aqueous solution is between about 50 and 400% of the weight of the dry stems.

5. A process according to claim 1 wherein the flattened enzyme-treated stems are freeze-dried.

6. A process according to claim 1 wherein the polysaccharide-hydrolyzing enzymes are derived from the growth of microorganisms such as *Aspergillus niger*, *Aspergillus oryzae* and *Penicillium chrysogenum notatum*.

7. A process according to claim 1 wherein the aqueous solution also contains water soluble compounds such as humectants, tobacco solubles, flavorants and casings.

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