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(54) Process for enzymatic treatment of tobacco materials.

(57) This invention relates to processes for the enzymatic treatment of tobacco materials. More particularly, the present invention relates to processes for the partial enzymatic modification of tobacco materials by treatment with a cellulolytic or a pectolytic enzyme. Enzymatic treatment carried out according to the processes of the invention facilitates homogenization, fibrillation and processability of tobacco materials and improves the characteristics of the smoke of tobacco products made from the treated materials.

Croydon Printing Company Ltd

PROCESS FOR ENZYMATIC TREATMENT OF TOBACCO MATERIALS

TECHNICAL FIELD OF THE INVENTION

This invention relates to the enzymatic treatment of tobacco materials. More particularly, the present invention relates to processes for the partial enzymatic modification of tobacco materials, using cellulase or pectinase, to permit their use in tobacco manufacturing processes. The processes of this invention facilitate the processability of tobacco materials and improve the characteristics of the smoke of tobacco products made from these materials.

BACKGROUND ART

During processing of tobacco materials for the manufacture of tobacco products, substantial quantities of tobacco are rejected by, or fall out of, the processing equipment. As a result, significant amounts of tobacco by-products, including class tobacco, tobacco dust, fines, stems, veins and stalks are generated. Because these by-products cannot automatically be incorporated into the tobacco manufacturing process, they are either discarded or treated in an effort to make them useful in tobacco products.

High cellulose or pectin content is a characteristic which renders some of the tobacco by-

products, such as stems, veins and stalk, subjectively problematic for use in smoking products. Cellulose and pectin are high molecular weight, insoluble polysaccharides, which are particularly resistant to degradation. The enzymes cellulase and pectinase decrease the molecular weight and degree of polymerization of, respectively, cellulose and pectin, converting them to soluble monosaccharides, disaccharides, oligosaccharides and low or intermediate molecular weight polysaccharides.

A number of mechanical, chemical and enzymatic methods have been proposed for the processing of tobacco by-products. Among the enzymatic treatments, United States Patent 3,517,857 refers to a triple enzyme system, consisting of a cellulase, a hemi-cellulase and a pectinase, which is used to treat tobacco stems. United States Patent 3,240,214 employs the same triple enzyme system to treat ground tobacco stems and fines for use in the manufacture of reconstituted tobacco sheets. British Patent Specification 2,069,814 refers to a method for processing tobacco by treatment with one or more oxidoreductase, lyase or hydrolase enzymes.

United States Patent 4,298,013 refers to the treatment of cellulosic tobacco waste materials with cellulases, such as those derived from Trichoderma viride, to completely convert cellulose to simple sugars for use in tobacco manufacturing. The process proposes steps of comminution and base treatment of the tobacco materials to render them more susceptible to enzymatic degradation.

United States Patent 3,616,222 relates to a process for the saccharification of cellulosic and woody tissues by mixtures of a fungal culture which attacks the cellulose molecule from the end, yielding soluble sugar products and a culture which cleaves the cellulose molecule internally at random points

to yield shorter fibers but not soluble sugars; or mixtures of enzymes derived from such cultures.

United States Patent 3,132,651 refers to the treatment of tobacco with cellulase to effect rapid aging and to simultaneously eliminate or substantially reduce nicotine content of the tobacco.

United States Patent 4,307,733 refers to a process for treating tobacco materials with a buffered solution containing an enzyme exhibiting cellulase activity to enhance the expansion capability and filling capacity of the materials.

United States Patents 3,353,541; 3,386,449; 3,409,026; 3,411,515; 3,420,241 and 3,435,829 and British Patent Specification 1,153,120 refer to methods for producing reconstituted tobacco products. According to these methods, tobacco plant parts are treated, to release pectins, with solutions of, respectively, diammonium acid phosphate, diammonium acid phosphate followed by alkaline earth metal phosphate, alkali carbonate, sodium or potassium orthophosphate, ammonium orthophosphate, inorganic acid followed by alkaline material or ammonium sulfate optionally followed by a pectic enzyme.

United States Patent 3,636,097 refers to a process for obtaining malic acid from tobacco by extraction of water solubles including malic acid and water soluble pectins, followed by treatment with pectinase enzyme and alkaline earth metal oxide or hydroxide. United States Patent 3,747,608 refers to a method for the digestion of pectin-bound plant material by pectolytic enzyme-producing microorganisms under controlled pH, moisture, time and temperature conditions.

DISCLOSURE OF THE INVENTION

The present invention provides processes for the partial enzymatic modification of tobacco

materials using cellulase or pectinase. It advantageously permits formerly problematic or non-useful tobacco materials to be modified for use in tobacco manufacturing processes.

In one embodiment of the present invention, cellulosic tobacco materials are treated with a cellulolytic enzyme to a saccharification level of less than 100%. According to another embodiment of this invention, pectic woody tobacco materials are treated with a pectolytic enzyme to a saccharification level of less than 100% which permits fibrillation of the materials and improvement of their subjective characteristics. Advantageously, materials to be treated according to this invention need not be subjected to any mechanical, chemical or enzymatic pre-treatment in order to facilitate the desired degree of enzymatic modification.

By virtue of this invention, it is possible to control the degree of modification of the enzymatically treated tobacco materials and to permit their use in tobacco products.

DETAILED DESCRIPTION OF THE INVENTION

In order that the invention herein described may be more fully understood, the following detailed description is set forth. In the description, the following terms are employed:

American Cigarette Blend- A mixture of different types of tobacco such as, for example, Flue-cured, Burley, Maryland, Oriental, and reconstituted tobacco, in various proportions.

Oven Volatiles (OV)- The percent weight loss of tobacco in a circulating air oven in three hours at 100°C. "OV" is used herein to represent the moisture content of the tobacco.

Cellulolytic Microorganism- A microorganism that produces an enzyme capable of degrading native, crystalline cellulose.

Cellulolytic Enzyme- An enzyme that is capable of depolymerizing and hydrolyzing native cellulose to simple sugars or to lower molecular weight polysaccharides. An enzyme that is capable of cleaving a cellulose molecule internally at random points yielding shorter fibers but not simple sugars.

Class Tobacco- Any form of tobacco that has fallen out of the tobacco making machinery during the tobacco manufacturing process.

Homogenization of Tobacco- A part of the reconstitution process which consists of putting tobacco materials in water through a refiner which squeezes and mixes the tobacco materials contained therein with water to an essentially uniformly consistent slurry.

Enzymatic Modification of Tobacco- The enzymatic saccharification of any part of a tobacco mix to simple sugars or to lower molecular weight polysaccharides and/or the enzymatic cleavage of a cellulose or pectin molecule internally at random points yielding shorter fibers but not simple sugars.

Fibrillation of Tobacco- The division of tobacco fiber bundles into smaller bundles, the separation of small bundles of tobacco fibers into individual fibers, and the cutting of fibers into fibers of shorter length.

Pectolytic Microorganism- A microorganism that produces an enzyme capable of degrading native pectin.

Pectolytic Enzyme- An enzyme that is capable of depolymerizing and hydrolyzing native pectin to simple sugars or to lower molecular weight polysaccharides. An enzyme that is capable of cleaving a pectin molecule internally at random points yielding shorter fibers but not simple sugars.

$$\% \text{ Saccharification} = \frac{\text{Weight of Glucose} \times 0.9}{\text{Weight of Substrate}} \times 100$$

The present invention relates to processes for the enzymatic modification of cellulosic tobacco

materials, including by-products such as class tobacco, tobacco dust, fines, shreds, stems, veins, and stalks. The processes of the invention also permit the enzymatic modification of pectic woody tobacco materials such as stems, veins and stalks.

These processes facilitate homogenization of cellulosic tobacco materials and the application of such homogenized tobacco materials to other tobacco products. In addition, these processes improve the characteristics and sheet casting ability of such tobacco materials.

The processes of this invention permit the fibrillation of woody tobacco materials, such as stems, veins, and stalks, and reduction of the particle size and viscosity of fine tobacco materials, such as dust, fines, and shreds. Enzymatic treatment of both of these types of tobacco materials according to the processes of the present invention also provides a product having improved self-adhesive, film-forming properties.

Broadly stated, according to one embodiment of this invention, cellulosic tobacco materials are contacted with at least one enzyme capable of degrading tobacco cellulose under conditions suitable for hydrolysis of the cellulose, so that less than 100% of the cellulose contained in the tobacco materials is saccharified or converted to simple sugars or to lower molecular weight polysaccharides. The tobacco products produced by such partial enzymatic modification can then be recycled and incorporated in the tobacco manufacturing process or processed further for the preparation of reconstituted tobacco sheets and/or for other such uses known to those skilled in the art.

According to another embodiment of this invention, pectic tobacco materials, such as woody stems, stalks and veins, are treated with at least

one enzyme capable of degrading tobacco pectins, under conditions suitable for hydrolysis of the pectins, to a saccharification level of less than 100%, such that the materials are fibrillated and softened by the treatment.

Enzymatic treatment of different tobacco materials according to the processes of this invention yields different results. For example, enzymatic treatment of woody tobacco materials, such as stems, veins, and stalks, using cellulase or pectinase enzymes results in desirable fibrillation and softening of the tissue structure of these tobacco materials and enhances the subjective characteristics of processed tobacco products made from these materials. The treated woody tobacco materials are softened and therefore require less mechanical energy to be flattened and processed than untreated materials.

Enzymatic treatment of fine tobacco materials, such as dust, fines, and shreds, with cellulase reduces the particle size and viscosity of these materials, thereby enhancing the sheet-forming ability of these materials for use in the production of reconstituted tobacco products.

Cellulase treatment of both woody and fine types of tobacco materials according to the processes of this invention yields a tobacco product having improved self-adhesive, film-forming properties characteristic of tobacco products containing a high degree of a binder such as gum. These self-adhesive properties are attributable to the lower molecular weight cellulose fragments formed by the enzymatic treatment and which function as cement as well as plasticizers.

It should be understood that the processes of this invention may be employed to modify all types of cellulosic tobacco materials, including, but not limited to, tobacco stems, midribs, veins, stalks,

lamina, fines, shreds, strips, dust, class tobacco, and combinations thereof. In addition, pectic woody tobacco materials, such as stems, stalks and veins, may be treated by the processes of this invention. As used herein, unless specified to the contrary, references to tobacco by-products and tobacco materials are to be understood to include all such forms of tobacco, including green, cured, partially cured, or stored tobacco.

It should also be understood that treatment of cellulosic or pectic tobacco materials according to the processes of this invention need not produce any measurable quantity of simple sugars or lower molecular weight polysaccharides. Significant improvements in the processability of woody tobacco materials are achieved by enzymatic treatment that merely fibrillates and softens the cellulosic or pectic tobacco materials.

The cellulase or pectinase enzyme used may be derived from bacterial cultures, fungal sources or plant extracts. Any commercially available cellulase or pectinase may also be used. In the preferred embodiments of the invention, the cellulase or pectinase employed is derived from a microbial source.

Cellulase or pectinase produced, respectively, by a cellulolytic or pectolytic microorganism, may be contacted with the tobacco materials by adding a solid culture of the microorganism, a filtrate or extract of such a culture, or an aqueous culture mass to the materials to be treated.

Alternatively, microbially-produced cellulase or pectinase may be added in the form a broth which has been concentrated by conventional methods such as freeze drying or protein precipitation techniques.

Microorganisms useful in the processes of this invention, or as sources of enzymes useful in

these processes, include microorganisms of the genera: Chaetomium, Xylaria, Polyporous, Rhizopus, Aspergillus, such as Aspergillus niger, Aspergillus terreus and Aspergillus oryzae, Trametes, such as Trametes sanguinea, Cellulomonas, Myrothecium, such as Myrothecium verrucaria, Penicillium, such as Penicillium expansum, Pencilillium funiculosum and Penicillium variable, Trichoderma, such as Trichoderma viride and Trichoderma koningii, Fusarium, such as Fusarium solani and Fusarium javonicum, Bacillus, such as Bacillus subtilis, Basidiomycetes, Erwinia and Streptomyces. Preferably, the microbial source of cellulase is of the genus Trichoderma, more preferably, Trichoderma viride.

The cellulase used in the processes of this invention may be derived from the following Trichoderma viride cultures which are available from the American Type Culture Collection in Rockville, Maryland:

Trichoderma viride QM 9414 - ATCC 26921
Trichoderma viride QM 9123 - ATCC 24449
Trichoderma viride QM 6a - ATCC 13631

Procedures used for the preparation of cellulase from microbial sources are disclosed generally by Mandels and Sternberg in "Recent Advances in Cellulase Technology", Journal Of Fermentation Technology, 54(4), pp. 267-86 (1976).

The pectinase used in the processes of this invention may be derived from the following cultures: Aspergillus, Erwinia, Bacillus, Penicillium, Streptomyces or Cellulomonas.

In the practice of the present invention, tobacco materials for contact with the cellulase or pectinase enzyme are produced by employing conventional techniques. For example, the tobacco materials may be in the form of an aqueous slurry, tobacco fiber, stem, or stalk. Preferably, for

treatment with a cellulase enzyme, the tobacco is present in the form of a suspension in water to form a slurry having a concentration of between about 3% and about 30% tobacco solids by weight on a dry weight basis, and more preferably between about 3% and about 20% tobacco solids by weight. If the tobacco to be treated with cellulase or pectinase is present in the form of stems, the stems may be suspended in water to a level of between about 30% and about 60% tobacco solids by weight.

For the treatment of solid tobacco materials, the tobacco may be prepared using conventional spraying techniques to provide a water content sufficient to permit survival of cellulase or pectinase-producing microorganisms. In any case, the tobacco material must be wet enough to support growth of the organisms; such necessary water content being conventionally determined by exercise of ordinary skill in the art. In addition, any of a number of additives, such as a carbon source, conducive to the growth of the microorganisms may be added to the tobacco materials.

A solution of cellulase or pectinase enzyme may be applied to the tobacco materials by spraying. Alternatively, the tobacco materials may be passed through or dipped into a solution of a cellulase or pectinase enzyme.

In accordance with the processes of this invention, when tobacco materials are to be contacted directly with the cellulolytic or pectolytic microorganisms, this contact may be accomplished in any of the conventional ways. For example, aqueous slurries, may be contacted with the microorganisms in a continuous batch or a fed batch process. In the case of solid tobacco materials, conventional methods of fermentation, sweating, and curing may be used.

In accordance with one embodiment of the process of this invention, the cellulose contained in tobacco materials is hydrolyzed with cellulase to a saccharification level of less than 100%. Preferably, the tobacco cellulose is saccharified to a level of between about 0.1% and about 63%, more preferably between about 0.2% and about 30%.

In an alternate embodiment of this invention, the pectin contained in tobacco materials such as woody stems, stalks and veins is hydrolyzed with pectinase to a saccharification level of less than 100%. Preferably, the tobacco pectin is saccharified to a level of between about 0.1% and about 63%, more preferably between about 0.2% and about 30%.

Preferably, the amount of cellulase added to the tobacco is between about 1% and about 16% by weight, on a dry weight basis, more preferably between about 1% and about 8% by weight, on a dry weight basis.

In an alternate embodiment of this invention, the amount of pectinase added to the tobacco is between about 0.5% and about 10% by weight, on a dry weight basis, more preferably between about 2% and about 5% by weight, on a dry weight basis.

The pH and other characteristics of the tobacco materials may be adjusted before or during treatment. In accordance with this invention, when the cellulase enzyme used is obtained from Trichoderma viride, the pH of the tobacco-enzyme mixture during treatment is preferably maintained in a range of between about 4.3 and 6.5, the normal pH range of tobacco, more preferably at about 5.3.

When a pectinase enzyme is used to treat the tobacco materials, the pH of the tobacco-enzyme mixture during treatment is preferably maintained in a range of between about 4.3 and 6.5, more pref-

erably at about 5.3, where the relative activity of the enzyme is greater than 70%.

Advantageously, tobacco materials to be treated in the processes of this invention need not be chemically or mechanically pre-treated prior to contacting with the cellulase or pectinase enzyme in order to enhance the susceptibility of the cellulose or pectin to treatment. For example, external buffering agents need not be added to the material to be treated.

Following application of cellulase or pectinase to the tobacco materials, the tobacco typically has a moisture content of between about 30 and 97% by weight. Preferably, the tobacco is contacted with cellulase or pectinase at a moisture level between about 40% and about 95%, most preferably greater than about 40%. Although the activity of the enzyme increases with increasing moisture levels, optimum results in terms of fibrillation of the tobacco materials are realized at moisture levels less than about 70%.

Preferably, the tobacco materials are treated with cellulase at a temperature of between about 20°C and about 60°C, more preferably at about 50°C. Tobacco materials are preferably treated with pectinase at a temperature of between about 10°C and about 60°C, more preferably at about 37°C.

It should be understood that optimal treatment conditions, such as the period of time for contacting the tobacco with the cellulase or pectinase, or the pH of the tobacco-enzyme mixture, will depend on factors such as the size and type of tobacco materials present, the desired quality of the end product, and the source, concentration and activity of the enzyme used. The treatment time should not, however, be long enough to permit the occurrence of side reactions such as fermentation.

Woody tobacco materials, such as stalks, stems and veins, require a longer time of contact with the enzyme than more particulate matter, such as tobacco fines, dust and shreds. Preferably, woody tobacco materials are treated with cellulase or pectinase for between about 0.5 and about 50 hours, more preferably for about 24 hours. Tobacco fines, such as tobacco dust, are treated with cellulase for between about 0.5 and about 10 hours, preferably for between about 1 and about 3 hours. During the treatment, the tobacco-enzyme mixture may be agitated on a continuous or periodic basis.

The enzymatic treatment is stopped by deactivating the enzyme. Preferably, the cellulase or pectinase enzyme is deactivated by heat treatment. For example, when the cellulase is obtained from Trichoderma viride, deactivation is accomplished preferably by heating at a temperature preferably between about 70°C and about 110°C for between about a few seconds and several hours depending on the treatment temperature. Alternatively, the enzyme is deactivated by reducing the moisture content of the treated substrate, preferably at a temperature greater than about 70°C, to below 30% moisture, preferably to below 20% moisture, a level at which the dried substrate will maintain its integrity. If the tobacco is present in the form of a slurry, deactivation of the enzyme is preferably effected by boiling the slurry for about 5 minutes.

The present invention provides an advantageous process for the refinement and/or homogenization of tobacco materials, such as by-products, for the preparation of homogeneous slurries, which may then be reused in the tobacco manufacturing process, for example, in the manufacture of reconstituted tobacco by conventional methods, such as slurry casting, papermaking, extrusion, dipping, coating, or

spraying. The treated slurries may be used in combination with reconstituted tobacco sheet or filler, tobacco strip, stems, or non-tobacco smoking materials. Treated tobacco products resulting from the processes of this invention are readily homogenizable by existing equipment and require a minimum of mechanical work. For example, cellulase treated tobacco material is softened to the point that it can be homogenized, if necessary, with conventional mixers, thus eliminating the need for colloidal mills and other special homogenizing techniques.

The homogeneous slurries produced by the processes of this invention have reduced viscosities and possess film-forming/adhesive properties which permit their use in sheet casting, extrusion, coating or spraying techniques. The slurries produced may be mixed to form a colloidal suspension which may then be applied to tobacco strips, filler, stems or other tobacco or non-tobacco materials using a spraying, dipping or coating technique. The coated tobacco may then be shredded or particulated into filler and dried to a moisture level of less than about 16% prior to being incorporated into smoking articles. Alternatively, the tobacco slurry may be coated onto a paper or other wrapper and used for wrapping cigarettes, cigars or cigarellos. In addition, tobacco slurries obtained according to this invention may be concentrated to between about 40% and 80% solids by vacuum evaporation and then extruded, calendered or molded, with or without the addition of binders, into smoking articles such as tubes, rods, flakes or ribbons, and dried.

In addition, treatment according to the processes of the present invention yields a tobacco product having improved subjectives and self-adhesive, film-forming properties. The self-adhesive quality of the treated tobacco advantageously eliminates the

need for addition of artificial adhesives or binders in order to incorporate fine tobacco particles into the tobacco. For example, the cellulase treated tobacco materials of the present invention adhere to a substrate without the addition of binding sugars or gums.

The processes of the present invention eliminate tobacco dust from the tobacco pulp and instead, incorporate it into a paste form for coating. Such processes improve the processing of reconstituted tobacco from tobacco slurries by the papermaking process by adding the treated tobacco dust in paste form onto a dried fibrous sheet, thereby providing for better drainage and tobacco sheet production at the wire.

It should be understood that the processes of this invention may be used to treat an entire portion of cellulosic or pectic tobacco materials generated as by-products of tobacco manufacturing process or only a fraction thereof. In the latter case, the treated tobacco fraction then acts to carry or support the untreated tobacco portion.

The tobacco products obtained in accordance with this invention may be combined with other tobacco materials and processed to a final smoking product or they may be recycled and reused in the tobacco manufacturing process, e.g., formation into sheets using conventional reconstitution methods.

Tobacco products, at least a portion of which contain tobacco material that has been treated in accordance with the processes of this invention, exhibit improved subjectives as compared with tobacco products prepared using wholly untreated cellulosic or pectic tobacco materials. Such products may include those consumed by smoking, such as, for example, cigars, cigarettes, cigarellos, pipe tobacco

and tobacco substitutes or by other means, e.g., chewing tobacco, and the like.

The following examples demonstrate the processes of the present invention. These examples are set forth for purposes of illustration only and are not be construed as limiting the scope of this invention in any manner.

EXAMPLES

Unless specified to the contrary in the following examples, viscosity measurements were taken using a Brookfield viscometer and the cellulase used was obtained from Trichoderma viride cultures (acquired from the Novo Company) by conventional methods known in the art. The pectinase used was obtained from Aspergillus niger cultures (acquired from the Novo Company) by conventional methods known in the art.

Example 1

2.7 kg of shredded bright tobacco stems were extracted with 18 liters of water at 60°C and dried to 12% OV in an air circulated oven at 60°C. The water extracted solubles were then concentrated with a thin film evaporator at 40°C under reduced pressure. Seven samples, one control and six experimental, of 25 g each, on a dry weight basis, of the extracted tobacco pulp were each mixed with 800 ml of water to form seven dilute tobacco slurries (suspensions). Two grams of cellulase were then added to each of the six experimental slurries and the slurries were incubated at 50°C for varying periods of time as indicated below in Table 1. After incubation, each slurry was boiled for 5 minutes to deactivate the enzyme and was then filtered. The filtrate from each slurry was concentrated with a thin film evaporator at 40°C under reduced pressure. The concentrated filtrate was recombined with both the cellu-

lase treated "pulp" from which it was derived and an appropriate quantity of the concentrated water solubles obtained from the initial water extraction step to yield a castable mixture. The resulting mixture was then mixed in a Waring blender for 1 minute at a medium rpm setting, cast onto stainless steel plates, and dried over a steam bath. Tobacco particle size was reduced by the treatment, a result indicated by the fact that the treated slurry became castable with a 15 mil opening casting knife. The results of these treatments are summarized below in Table 1.

This example demonstrates that treatment of tobacco stem slurries with cellulase according to the process of this invention softened the tissue structure and facilitated homogenization of the stem tobacco materials in comparison to untreated cellulosic stem tobacco materials. In addition, this example shows that reconstituted tobacco sheets produced from the cellulase treated bright tobacco stems had the desirable feel and appearance of a sheet containing a high degree of a binder, such as a gum, due to the adhesive and film-forming properties of the treated slurries.

TABLE 1

CELLULASE TREATMENT OF BRIGHT TOBACCO STEMS

Sample No.	Treatment Time (Hrs.)	Degree Of Saccharification (%)	Quality Of Cast Sheets
1 (control)	-	-	No sheet formation
2	0.5	0.9	Rough & weak (poor)
3	1.0	2.2	Good*
4	2.0	4.1	Very Good**
5	4.0	5.3	Very Good**
6	20.0	9.0	Very Good**
7	40.0	11.5	Very Good**

* The formed sheets were cohesively held together as if a gum was added.

** The degree of cohesiveness of these sheets was extensive and the sheets resembled those having a high level of added binder.

Example 2

2.7 kg of shredded burley tobacco stems (rolled Kentucky stems) were extracted with 18 liters of water at 60°C and dried to 12% OV in an air circulated oven at 60°C, yielding water-extracted solubles and water-extracted pulp. The water-extracted solubles were then concentrated with a thin film evaporator at 40°C under reduced pressure to yield concentrated water-extracted solubles. The water-extracted tobacco pulp was slurried with water and incubated with 2 g of cellulase per 25 g of tobacco pulp. The incubation was carried out at 50°C for 24 hours to a saccharification level of 10%. The tobacco pulp-enzyme slurry was boiled for 5 minutes to deactivate the enzyme and was then filtered, yielding filtrate and pulp. The filtrate was concentrated with a thin film evaporator at 40°C under reduced pressure.

As in Example 1 (bright tobacco stems), cellulase treatment of burley tobacco stems softened the fibrous structure and facilitated homogenization of the stems. The resulting slurries, with and without sugars, all yielded cast tobacco sheets having the improved self-adhesive, film-forming properties discussed in Example 1.

The fractions obtained in various stages of the treatment of this Example were used to make reconstituted tobacco products by conventional methods known in the art for subjective testing as described below.

Sample 1: Amounts of cellulase-treated tobacco pulp, cellulase-treated filtrate, and non-cellulase treated water-extracted solubles were combined, mixed, cast as a tobacco sheet, and dried. This mixture yielded very good sheet.

Sample 2: Amounts of cellulase-treated tobacco pulp and non-cellulase treated water-extracted

solubles were combined, mixed, cast as a tobacco sheet and dried. This mixture also yielded very good sheet.

Sample 3: Amounts of non-cellulase treated water-extracted tobacco pulp and non-cellulase water-extracted solubles were mixed and dried to yield reconstituted shredded stems.

Each tobacco sheet was shredded and blended in an American cigarette blend to yield a final blend containing a 25% level of treated tobacco. Similarly, the reconstituted shredded stems (sample 3), which had not been treated with cellulase, were blended at the same blend level and used as the control in a subjective comparison by a trained panel of smokers. Test cigarettes made from the reconstituted sheets (samples 1 and 2) above were smoked against the control (reconstituted untreated, shredded stems, sample 3). The panel of expert smokers found that the test cigarette containing filler produced from sample 1 tasted fuller with heavier mouth coating than the control. The panel also found the test cigarette containing filler produced from sample 2 to be fuller and have a cleaner aftertaste than the control. In general, the control cigarette containing the shredded stems (from sample 3) was found to be more acrid and sour than cigarettes containing either of the fillers produced from samples 1 or 2 above.

This example demonstrates that in addition to the previously enumerated advantages, tobacco products made with tobacco stems treated according to the process of this invention also exhibit improved subjective characteristics.

Example 3

Four 25 g samples of water-extracted tobacco pulp were each mixed in a Waring blender with 800 ml of water to yield four tobacco slurries.

Three of the four slurries were subsequently inoculated, respectively, with 1 g, 2 g, and 4 g, on a dry weight basis, of cellulase and incubated for 24 hours at 50°C. The fourth slurry, which was not treated with cellulase, was used as a control. The saccharification level of each sample was determined at the end of 2 hour and 24 hour incubation times and is recorded below in Table 2.

The results summarized in Table 2 show that treatment of tobacco pulp with cellulase resulted in rapid and extensive saccharification of the tobacco, even when only relatively small quantities of cellulase were used. The control sample contained no detectable level of any sugars. This Example also demonstrates that saccharification of cellulosic tobacco materials was effectively and advantageously carried out without the use of added buffers.

TABLE 2

SACCHARIFICATION OF TOBACCO PULP WITH CELLULASE

Level of Cellulase In Tobacco Pulp(%)*	Treatment Time (Hrs.)	Saccharification** (%)
4	2	10.0
4	24	45.8
8	2	13.9
8	24	59.0
16	2	9.8
16	24	62.1

* On a dry weight basis.

** Calculated on the basis of the tobacco pulp weight.

Example 4

900 g of tobacco dust having a particle size less than 500 microns was combined with enough water to form a 6000 ml tobacco slurry. This slurry was divided into four equal samples: A, B, C and D. Sample A was used as a control. Samples B through D were treated, respectively, with 2.5 g, 5 g, and 10 g, or a dry weight basis, of cellulase. Each sample, including the control, was then homogenized by a 10-minute passage through a Gaulin homogenizer set at 6000 psi. Each sample was then incubated for 1, 2, and 3 hours at 50°C. The viscosity of the samples was measured at the end of each hourly time interval. The results are summarized below in Table 3.

As indicated by the data in Table 3, the viscosity of slurries containing small tobacco particles decreased significantly within a short period of time when the tobacco particles were treated with cellulase according to the process of this invention. The degree of decrease in viscosity was proportional to the amount of cellulase present. The greater the amount of cellulase present, the greater the degree of decrease in slurry viscosity.

TABLE 3

EFFECT OF CELLULASE TREATMENT ON TOBACCO SLURRY VISCOSITY

	Level Of Cellulase In Tobacco Slurry (%)*		Treatment Time (Hrs.)	Slurry Viscosity (cps)
	A	-	1	
	(control)	-	2	1680
		-	3	
	B	1	1	870
		1	2	676
		1	3	678
	C	2	1	459
		2	2	430
		2	3	450
	D	4	1	290
		4	2	220
		4	3	240

* On a dry weight basis.

Example 5

A tobacco slurry was prepared by mixing 1125 g of tobacco dust and 6375 ml water. The slurry was divided into four samples of equal weight: A, B, C and D. Sample A was used as a control. Samples B through D were treated, respectively, with 1%, 2% and 4% cellulase, on a dry weight basis. Each sample, including control A, was then mixed in a Waring blender for 5 minutes, incubated for 3 hours at 50°C, and homogenized for 5 minutes with a Gaulin homogenizer at a setting of 4000 psi. The viscosity of each slurry sample was measured before and after homogenization and is reported below in Table 4.

As shown in Table 4, cellulase treatment of tobacco dust slurries according to the process of this invention decreased the viscosity of the slurries even in the unhomogenized state.

TABLE 4

EFFECT OF CELLULASE TREATMENT ON TOBACCO SLURRY VISCOSITY

Sample	Cellulase On Tobacco Weight (%)*	Viscosity (cps) Before Homogenization	Viscosity (cps) After Homogenization
A (control)	-	1944	2448
B	1	660	1520
C	2	640	620
D	4	588	270

* On a dry weight basis.

Example 6

A tobacco slurry containing 300 g of tobacco powder and 2 liters of a tobacco casing, which contained humectants and sucrose, was prepared by homogenizing the tobacco mixture for 5 minutes with a Gaulin homogenizer at a setting of 4000 psi. The slurry was then divided into two equal samples, A and B. Sample B was treated with 6 g of cellulase (4% on a dry weight basis) and incubated for 3 hours at 50°C. Sample A was used as a control. The viscosity of each sample was then measured and compared. It was found that the cellulase treatment had no effect on slurry viscosity. The added sucrose in the slurry deactivated the enzyme.

To demonstrate the deactivating effect of added sucrose on cellulase, the above-described steps were carried out using the same type of tobacco dust and casing, with the exception that the casing did not contain sucrose. Three samples, C, D, and E, of slurry without added sucrose were prepared as described above. Sample C was used as a control. Samples D and E were each treated with 4% cellulase, on a dry weight basis. In sample E, the tobacco dust was extracted with water to reduce the sucrose level in the tobacco prior to cellulase treatment. The water-extracted solubles were then concentrated. The slurry was treated with cellulase, and then combined with the concentrated solubles. The results are summarized below in Table 5.

As demonstrated in Table 5 (samples A and B), sucrose added to tobacco slurries prior to cellulase treatment inactivated the cellulase and eliminated the beneficial viscosity-decreasing effect of the treatment. In addition, as evidenced by sample E, a reduction in tobacco sucrose level prior to treatment with cellulase, such as by extraction, further enhanced the cellulase activity, thereby yielding

slurries having lower viscosities as compared to slurries in which the tobacco sucrose level had not similarly been reduced.

When the cellulase treated tobacco slurries obtained in this Example, including the water-extracted slurry, were sprayed onto tobacco strip, rolled Kentucky stem samples, and water-extracted tobacco pulp samples, the tobacco slurry adhered to the substrate without the need for addition of a sugar or other adhesive solution.

TABLE 5

EFFECT OF CELLULASE TREATMENT
ON TOBACCO SLURRY VISCOSITY

Sample	Cellulase Level Of Tobacco Slurry (%)*	Slurry With And Without Sucrose **	Viscosity, After Incubation At 50°C For 3 Hours (cps)
A (control)	-	with sucrose	5800
B	4	with sucrose	5800
C (control)	-	without sucrose	5000
D	4	without sucrose	1680
E	4	without sucrose	1010

* On a dry weight basis.

** A significant amount of the sucrose inverted in the casing used. As a result, the tobacco casing contained a mixture of sugars which included sucrose, glucose and fructose.

Example 7

Tobacco water slurries containing 20% tobacco solids, on a dry weight basis, were prepared using homogenized class tobacco produced during the use of a Gaulin homogenizer or a Bead mill (a colloidal mill). The slurries were treated at 50°C with 4% cellulase, on a dry weight basis, for up to 2 hours.

The results summarized below in Table 6 demonstrate that cellulase treatment of homogenized class tobacco slurries significantly reduced the viscosity of these slurries.

TABLE 6

EFFECT OF CELLULASE ON
HOMOGENIZED TOBACCO SLURRY VISCOSITY

Sample	Slurry Viscosity Of Starting Material (cps)	Slurry Viscosity Of Cellulase Treated Material (cps)
Slurry from Bead Mill (1 pass)	2048	995
Slurry from Bead Mill (2 passes)	3984	1712
Slurry from Gaulin Homogenizer	1552	576

Example 8

A 40 pound sample of whole bright tobacco stems was divided into two equal parts. The first 20 pound sample was then divided into samples of 5 pounds each which were treated with either a 4% (DWB) or 8% (DWB) cellulase solution (Novo Celluclast 200L). Each sample was placed in an airtight container at either 45% or 55% OV and incubated for 24 hours in an air circulated oven at 50°C. Control samples from the second 20 pound sample were placed in airtight containers at either 45% or 55% OV and either incubated in an air circulated oven at 50°C or placed in a refrigerator at 5°C for a 24 hour period.

All of the samples were then processed at about one pound per minute through a Fitzmill Model M mill equipped with blunt blades rotating at 5,000 rpm and a screen having one-half inch diameter round holes. This processing caused the cellulase-treated softened stems to fibrillate to bundles of fibers. The more easily fibrillated material was air separated and recovered from the rest of the product which was then passed through the same mill equipped instead with a screen having one-quarter inch diameter round holes.

The fibrillated material from the second pass through the mill was air separated and then combined with the fibrillated product from the first pass. The resulting fibrillated product was then air dried at ambient temperature. Treatment conditions and results for the various samples are summarized in Table 7.

In this Example, it was observed that cellulase treatment of the stems yielded a shredded product that contained a higher amount of larger fibrous bundles than the controls. The control samples which were incubated at 50°C for 24 hours produced a better

fibrillated product than controls stored at 5°C or unaged samples but the product was not as satisfactory as the cellulase treated products.

The cellulase treated stems yielded a product containing longer fibers than those produced in the pectinase treatment of Examples 10 and 11.

Stems derived from the samples were used to prepare blended cigarettes at a 20% blend level (20% shredded stem, 80% American blend filler -- no reconstituted tobacco or other stem --) and were smoked by a panel of expert smokers. The panel found that the cellulase treated samples were smoother, more tobacco-strip like and preferable to cigarettes containing control sample stems at a corresponding level in a blend. Although both controls were judged to be low in tobacco flavor, the procedural control was found to be less stemmy than the untreated control. The 8% cellulase treatment exhibited a reduced stem character, smoother taste and more body with a slight sour, acrid taste which might have been a fermentation by-product. The 4% cellulase treatment yielded similar, but less pronounced results.

TABLE 7

RESULTS OF TREATMENT OF BRIGHT TOBACCO STEMS WITH CELLULASE

Sample	Cellulase On Tobacco Wt. (%)	Sample OV (%)	Incubation Temp. (°C)	Eq. OV (%)	Eq. CV (cc/10g)	Total Reducing Sugars (%)	Hot Water Solubles (%)
A (untreated control)	--	45	5	14.4	39	10.7	49
B (procedural control)	--	45	50	14.9	35	10.4	52
C	4	45	50	14.9	34	10.4	54
D	8	45	50	15.4	31	10.5	54
E (untreated control)	--	55	5	14.7	46	10.9	49
F (procedural control)	--	55	50	21.9	40	10.8	52
G	4	55	50	13.9	35	11.0	52
H	8	55	50	15.6	38	11.0	53

Example 9

A 20 pound sample of washed whole burley tobacco stems was divided into two equal parts. The first 10 pound sample was then divided into samples of 5 pounds each which were treated with either a 4% (DWB) or 8% (DWB) cellulase solution (Novo Celluclast 200L). Each sample was placed in an airtight container at 70% OV and incubated for 24 hours in an air circulated oven at 50°C. Control samples from the second 10 pound sample were placed in airtight containers at 70% OV and either incubated in an air circulated oven at 50°C or placed in a refrigerator at 5°C for a 24 hour period.

All of the samples were then processed at about one pound per minute through a Fitzmill Model M mill equipped with blunt blades rotating at 5,000 rpm and a screen having one-half inch diameter round holes. This processing caused the cellulase treated softened stems to fibrillate to bundles of fibers. The more easily fibrillated material was air separated and recovered from the rest of the product which was then passed through the same mill equipped instead with a screen having one-quarter inch diameter round holes.

The fibrillated material from the second pass through the mill was air separated and then combined with the fibrillated product from the first pass. The resulting fibrillated product was then air dried at ambient temperature. The specific treatment conditions and results for the various samples are summarized in Table 8.

The cellulase treated stems produced a better fibrillated product than the controls or the pectinase treated products of Example 12. Sample stems were used to prepare blended cigarettes at a 20% blend level (20% shredded stem, 80% American

blend filler -- no reconstituted tobacco or other stem --) and were smoked by a panel of expert smokers. The panel found that the cellulase treated stem samples produced a much smoother, less harsh and more tobacco-like smoking material than the control samples or the pectinase treated products of Example 12. The procedural control sample which was incubated at 50°C for 24 hours at 70% OV was also found to have improved subjectives in comparison to controls incubated at 5°C. This was probably due to naturally occurring enzymatic activity.

TABLE 8

RESULTS OF CELLULASE TREATMENT OF BURLEY TOBACCO STEMS

Sample	Cellulase On Tobacco Wt. (%)	Sample OV (%)	Incubation Temp. (°C)	Eq. OV (%)	Eq. CV (cc/10g)	Total Reducing Sugars (%)
A (untreated control)	--	70	5	14.9	78	0.24
B (procedural control)	--	70	50	14.4	82	0.33
C	4	70	50	13.6	76	0.48
D	8	70	50	13.7	73	0.54

Example 10

A 10 pound sample of whole bright tobacco stems was divided into two equal parts. The first 5 pound sample was then cased with a pectinase water solution to a 2% (DWB) pectinase (Novo Pectinex 3N) level. The sample was placed in an airtight container at 45% OV and incubated for 24 hours in an air circulated oven at 37°C. A control sample, from the second 5 pound sample, was placed in an airtight container at 45% OV and incubated in an air circulated oven at 37°C for a 24 hour period.

The samples were then processed at about one pound per minute through a Fitzmill Model M mill equipped with blunt blades rotating at 5,000 rpm and a screen having one-half inch diameter round holes. This processing caused the pectinase treated softened stems to fibrillate to bundles of long, thin and flexible fibers. The more easily fibrillated material was air separated and recovered from the rest of the product which was then passed through the same mill equipped instead with a screen having one-quarter inch diameter round holes.

The fibrillated material from the second pass through the mill was air separated and then combined with the fibrillated product from the first pass. The resulting fibrillated product was then air dried at ambient temperature.

In this Example, pectinase treated stems yielded a shredded product that contained primarily very thin fibers and, in general, was more fibrous in nature than the control. The control sample contained a large portion of flat and chunky short pieces.

Example 11

A 10 pound sample of whole bright tobacco stems was divided into two equal parts. The first

5 pound sample was then divided into samples of 1.25 pounds each which were cased with either a 2% (DWB) or 5% (DWB) pectinase solution (Novo Pectinex 3N). Each sample was placed in an airtight container at either 45% or 55% OV and incubated for 24 hours in an air circulated oven at 37°C. Control samples from the second 5 pound sample were placed in airtight containers at either 45% or 55% OV and either incubated in an air circulated oven at 37°C or placed in a refrigerator at 5°C for a 24 hour period.

All of the samples were then processed at about one pound per minute through a Fitzmill Model M mill equipped with blunt blades rotating at 5,000 rpm and a screen having one-half inch diameter round holes. This processing caused the pectinase treated softened stems to fibrillate to bundles of long, thin and flexible fibers. The more easily fibrillated material was air separated and recovered from the rest of the product which was then passed through the same mill equipped instead with a screen having one-quarter inch diameter round holes.

The fibrillated material from the second pass through the mill was air separated and then combined with the fibrillated product from the first pass. The resulting fibrillated product was then air dried at ambient temperature. Treatment conditions and results for the various samples are summarized in Table 9.

In this Example, the pectinase treated stems yielded a shredded product that was more fibrous in nature and more acceptable than the controls. The samples treated with the 5% pectinase solution fibrillated more easily than samples treated with the 2% pectinase solution and yielded shorter but thinner fibers.

The pectinase treated samples produced a product having shorter and thinner fibers than the cellulase treated products of Example 8.

Stems derived from the samples treated with the 5% pectinase solution were used to prepare blended cigarettes at a 20% blend level (20% shredded stem, 80% American blend filler -- no reconstituted tobacco or other stem --) and were smoked by a panel of expert smokers. The panel found that the procedural control had a clean taste, some harshness and less stemmy character than the untreated control. The pectinase treated samples exhibited a reduced stemmy character, with the flavor altered away from the bright tobacco smoke to a more blended tobacco note.

TABLE 9

RESULTS OF TREATMENT OF BRIGHT TOBACCO STEMS WITH PECTINASE

Sample	Pectinase On Tobacco Wt. (%)	Sample OV (%)	Incubation Temp. (°C)	Eq. OV (%)	Eq. CV (cc/10g)	Total Reducing Sugars (%)	Hot Water Solubles (%)
A (procedural control)	--	45	37	15.0	37	11.4	51
B	2	45	37	14.9	34	11.4	54
C	5	45	37	15.4	31	12.5	58
D (untreated control)	--	55	5	14.4	38	10.3	49
E (procedural control)	--	55	37	14.5	37	11.1	50
F	2	55	37	14.7	35	11.5	55
G	5	55	37	15.0	32	12.7	58

Example 12

A 10 pound sample of whole burley tobacco stems was divided into two equal parts. The first 5 pound sample was then divided into samples of 2.5 pounds each which were cased with either a 2% (DWB) or 5% (DWB) pectinase solution (Novo Pectinase 3N). Each sample was placed in an airtight container at 70% OV and incubated for 24 hours in an air circulated oven at 37°C. Control samples from the second 5 pound sample were placed in airtight containers at 70% OV and either incubated in an air circulated oven at 37°C or placed in a refrigerator at 5°C for a 24 hour period.

All of the samples were then processed at about one pound per minute through a Fitzmill Model M mill equipped with blunt blades rotating at 5,000 rpm and a screen having one-half inch diameter round holes. This processing caused the pectinase treated softened stems to fibrillate to bundles of long fibers. The more easily fibrillated material was air separated and recovered from the rest of the product which was then passed through the same mill equipped instead with a screen having one-quarter inch diameter round holes.

The fibrillated material from the second pass through the mill was air separated and then combined with the fibrillated product from the first pass. The resulting fibrillated product was then air dried at ambient temperature. The specific treatment conditions and results for the various samples are summarized in Table 10.

The pectinase treated stems produced a more easily fibrillated product than the controls. The pectinase treated samples yielded long fibrils

while the control samples produced a large quantity of short, flat pieces and much dust. Sample stems were used to prepare blended cigarettes which were smoked by a panel of expert smokers. The panel found that the pectinase treated stem samples produced a much smoother, less harsh and more tobacco-like smoking material than the control samples.

TABLE 10

RESULTS OF PECTINASE TREATMENT OF BURLEY TOBACCO STEMS

Sample	Pectinase On Tobacco Wt. (%)	Sample OV (%)	Incubation Temp. (°C)	Eq. OV (%)	Eq. CV (cc/10g)	Total Reducing Sugars (%)
A (untreated control)	--	70	5	14.9	78	0.24
B (procedural control)	--	70	37	14.2	86	0.55
C	2	70	37	14.8	71	0.59
D	5	70	37	14.7	67	1.04

CLAIMS

1. A process for the enzymatic modification of a tobacco material by contacting the material with a cellulase or pectinase enzyme characterised in that the material is contacted with the enzyme under conditions that permit less than 100% saccharification of the cellulose or pectin contained in the material.

2. The process according to claim 1, wherein the tobacco material is contacted with the enzyme at a pH of between 4.3 and 6.5, and preferably about 5.3.

3. The process according to claim 1 or 2, wherein the tobacco material is contacted with the enzyme for between 0.5 and 50 hours, and preferably for about 24 hours.

4. The process according to claim 1, 2 or 3, wherein the tobacco material is contacted with the enzyme at a moisture level of greater than 40%.

5. The process according to claim 1, wherein the enzyme is produced by a cellulolytic or pectolytic microorganism.

6. The process according to any of claims 1 to 5, wherein the tobacco material is tobacco stems and is first suspended in water to a level of between 30% and 60% solids by weight and the said suspension is then contacted with the enzyme.

7. The process according to any of claims 1 to 6, wherein the enzyme is subsequently deactivated by heat treatment.

8. The process according to any of claims 1 to 7,

wherein the tobacco material is cellulosic and is contacted with a cellulase enzyme.

9. The process according to claim 8, wherein the tobacco material comprises class tobacco, tobacco dust, stems, veins, shreds, fines, stalks or a combination thereof.

10. The process according to claim 8 or 9, wherein the level of saccharification of the cellulosic tobacco material is between 0.01% and 63%, and preferably between 0.2% and 30%.

11. The process according to claim 8, 9 or 10, wherein the tobacco material is contacted with cellulase at a temperature of between 20°C and 60°C and preferably at about 50°C.

12. The process according to any of claims 8 to 11, wherein the cellulase is produced by a cellulolytic microorganism selected from Chaetomium, Xylaria, Polyporous, Aspergillus, Rhizopus, Trametes, Cellutomonas, Myrothecium, Pencillium, Trichoderma, Fusarium, Bacillus, and Basidiomycetes.

13. The process according to claim 12, wherein the cellulolytic microorganism is Trichoderma viride.

14. The process according to any of claims 8 to 13, wherein the amount of cellulase contacted with the tobacco material is between 1% and 8% by weight of the material on a dry weight basis.

15. The process according to any of claims 8 to 14, wherein the tobacco material is first contacted with water to form a tobacco slurry having a concentration of between 5% and 30% solids by weight, and preferably

between 5% and 20% solids by weight, and the said slurry is then contacted with the cellulase.

16. The process according to any of claims 1 to 7, wherein the tobacco material is pectic and is contacted with a pectinase enzyme under conditions that permit fibrillation of said material.

17. The process according to claim 16, wherein the tobacco material comprises tobacco stems, veins, stalks, or a combination thereof.

18. The process according to claim 16 or 17, wherein the tobacco material is contacted with pectinase at a temperature of between 10°C and 60°C, and preferably at about 37°C.

19. The process according to claim 16, 17 or 18, wherein the pectinase is produced by a pectolytic microorganism selected from Aspergillus, Penicillium, Bacillus, Erwinia, Cellulomonas and Streptomyces.

20. The process according to claim 19, wherein the pectolytic microorganism is Aspergillus niger.

21. The process according to any of claims 16 to 20, wherein the amount of pectinase contacted with the tobacco material is between 0.5% and 10% by weight of the materials on a dry weight basis, and preferably between 2% and 5%.

22. A smoking product which comprises, at least in part, a tobacco material produced by a process according to any of claims 8 to 15 in the form of cigarettes, cigars, cigarillos, pipe tobacco, tobacco substitutes or chewing tobacco.

23. A tobacco product which comprises, at least in part, a tobacco material produced by a process according to any of claims 16 to 21 in the form of cigarettes, cigars, cigarillos, and chewing tobacco.