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(54) **TOBACCO MATERIAL HAVING ITS STIMULATION/HOT FLAVOR AT SMOKING REDUCED, SMOKING FLAVOR IMPARTING AGENT, REGENERATED TOBACCO MATERIAL, PROCESS FOR PRODUCING TOBACCO MATERIAL, AND PROCESS FOR PRODUCING SMOKING FLAVOR IMPARTING AGENT**

(57) Tobacco material is reduced in stimulus and pungency in its smoking stage by decreasing a content of a colored acidic water-soluble polymer substance.

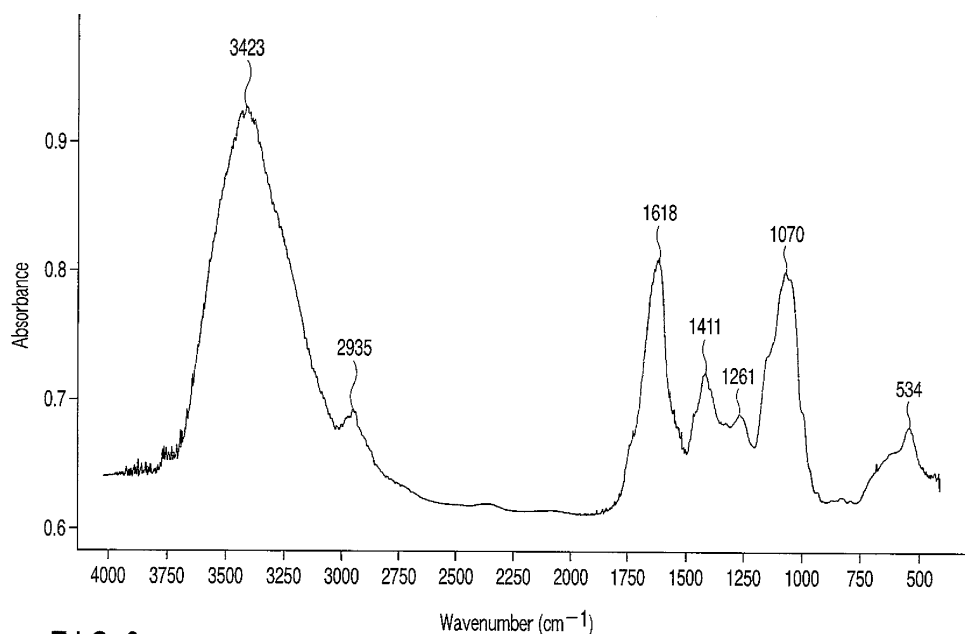


FIG. 3

Description

Technical Field

5 **[0001]** The present invention relates to the field of improving the flavor and taste of tobacco, and more particularly, relates to a tobacco material, a flavoring agent, and a regenerated tobacco, which are reduced in stimulus and pungency in the smoking stage, and also relates to a method of preparing the tobacco material and a method of preparing the tobacco flavoring agent.

10 Background Art

[0002] Natural tobacco material exhibits undesired tastes such as stimulus and pungency. Particularly, Burley tobacco is strong in the undesired tastes such as stimulus and pungency and, thus, various improvements have been applied to the tobacco by means of, for example, addition of a perfume or by a heat treatment. However, the causes of the undesired tastes have not yet been clarified. The perfume technology and the treating method employed in the past for improving the tobacco taste are based on experiences and are unsatisfactory in terms of the efficiency and the cost (Tobacco Reporter, July 2003, pp. 64-70).

15 **[0003]** If the component causing the stimulus and the pungency in natural tobacco material, particularly, Burley tobacco, can be chemically clarified, it is considered possible to efficiently improve the flavor and taste of natural tobacco material by applying an appropriate treatment for decreasing the components in question.

Disclosure of Invention

25 **[0004]** Accordingly, an object of the present invention is to provide, by chemically clarifying the component causing the stimulus and pungency in natural tobacco material, particularly Burley tobacco, measures to decrease the component causing the stimulus and pungency in view of the physical and chemical properties of the component, thereby improving the flavor and taste of the natural tobacco material.

[0005] The present inventors have fractionated an aqueous extract liquid of Burley tobacco by various chromatographic technologies, and made cigarettes by using each fraction. Then, the component causing the stimulus and pungency was purified, based on the expression of the stimulus and pungency by an organoleptic evaluation. As a result, it has been clarified that the component causing the stimulus and pungency (hereinafter referred to as "present causal component") is a colored acidic water-soluble polymer substance that is contained in the aqueous extract liquid. In the process of the fractionation purification, the physical and chemical properties leading to the development of a method for decreasing the present causal component have been clarified. Incidentally, since the present causal component is non-volatile, it is thus considered that the present causal component does not directly produce the stimulus and pungency, and is a precursor component which produces the components generating the stimulus and pungency in the smoking stage. The present invention is based on these findings.

35 **[0006]** More specifically, according to a first aspect of the present invention, there is provided a tobacco material characterized in that the stimulus and pungency in the smoking stage have been reduced by decreasing the content of a colored acidic water-soluble polymer substance.

[0007] According to a second aspect of the present invention, there is provided a tobacco flavoring agent, characterized by containing a tobacco extract liquid reduced in the component causing the stimulus and pungency by decreasing the content of a colored acidic water-soluble polymer substance.

45 **[0008]** According to a third aspect of the present invention, there is provided a regenerated tobacco material characterized in that it comprises a regenerated tobacco web comprising fibers, which are an extraction residue of natural tobacco material, added with a flavoring agent comprising an aqueous extract liquid from natural tobacco material, and the flavoring agent is a flavoring agent according to the present invention.

[0009] According to a fourth aspect of the present invention, there is provided a method of preparing a tobacco material, characterized by decreasing a colored acidic water-soluble polymer substance.

50 **[0010]** Further, according to a fifth aspect of the present invention, there is provided a method of preparing a flavoring agent, characterized by decreasing a colored acidic water-soluble polymer substance from an aqueous extract liquid of natural tobacco material.

Brief Description of Drawings

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[0011]

FIG. 1 is a gel filtration high performance liquid chromatogram of a colored acidic water-soluble polymer substance

(the component causing the stimulus and pungency);

FIG. 2 is an ultraviolet-visible absorption spectrum of a colored acidic water-soluble polymer substance (the component causing the stimulus and pungency); and

FIG. 3 is an infrared absorption spectrum of a colored acidic water-soluble polymer substance (the component causing the stimulus and pungency).

Best Mode for Carrying Out the Invention

[0012] The present invention will be described in more detail below.

[0013] In order to chemically clarify the causal component of the stimulus and pungency of natural tobacco material, particularly, Burley tobacco material, Burley tobacco material was extracted with an aqueous solvent to obtain an extract liquid. The extraction residue consists essentially of fibers. The extraction residue was formed into a slurry by an ordinary method, and the slurry was made into a regenerated web sheet, which was shredded to obtain regenerated tobacco shreds.

[0014] On the other hand, the extract liquid noted above was treated with an ultrafiltration membrane having a molecular weight cutoff of 10,000 daltons to obtain a fraction having a molecular weight lower than 10,000 daltons and another fraction having a molecular weight not lower than 10,000 daltons. The fraction having a molecular weight lower than 10,000 daltons was concentrated and, then, added to the regenerated tobacco shreds noted above, followed by preparing cigarettes by using the resultant tobacco shreds. The cigarettes were subjected to an organoleptic examination, and there was obtained an evaluation that the smoking taste was markedly improved in that the stimulus and pungency were scarcely felt, compared with the cigarettes prepared by using the regenerated tobacco shreds to which was added the extract liquid itself as it was. From this result, it was estimated that the present causal component would be contained in the water-soluble polymer fraction having a molecular weight not lower than 10,000 daltons.

[0015] However, since the most portion of the flavoring and tasting components such as sugars, amino acids and glycosides have been removed from the fraction having a molecular weight not lower than 10,000 daltons, it is impossible to evaluate the cigarette prepared from the regenerated tobacco shreds noted above added with the particular fraction. Such being the situation, the organoleptic test was conducted on evaluation cigarettes which were prepared separately and added with the fraction having a molecular weight not lower than 10,000 daltons. As a result, the stimulus and pungency similar to those of Burley tobacco were expressed, supporting the estimation given above.

[0016] Based on the above studies, each fraction was subjected to an ultrafiltration treatment with a molecular weight cutoff of 10,000 daltons in each of the following purification stages so as to carry out the evaluation in respect of the components having a molecular weight not lower than 10,000 daltons.

[0017] Also, evaluation cigarettes described below were prepared by using each fraction, and an organoleptic test was conducted on the evaluation cigarettes so as to permit the fractionation to proceed, with the expression of the stimulus and pungency used as an index.

<Organoleptic evaluation method for fractions>

[0018] The evaluation cigarettes added with each fraction were evaluated by the organoleptic evaluation performed by 10 expert panels as to whether the stimulus and pungency were expressed, compared with control cigarettes added with purified water alone. Cigarettes the same as commercially available cigarettes very low in nicotine and tar contents (1 mg of tar, and 0.1 mg of nicotine) except that no perfume was added were used as the evaluation cigarettes. These cigarettes exhibited a tendency that the tasting effect was small relative to the amount of the perfume added thereto. However, these cigarettes were substantially free from the effect of the cigarettes themselves on the taste and, thus, a satisfactory result was obtained in terms of the judgment of the tasting effect of each fraction. Each fraction was added by dissolving the fraction derived from 20g of Burley tobacco material in 1 mL of purified water and by injecting 10 μ L of the solution (corresponding to the fraction derived from 0.2g of the tobacco material) into the evaluation cigarette by using a syringe. The amount of the tobacco shreds per cigarette was about 0.6g and each fraction was added in an amount corresponding to about 1/3 of the amount of the tobacco shreds.

<Preliminary study of fractionation purification>

[0019] Various methods for fractionation purification of water-soluble polymers were studied in an attempt to perform the fractionation purification of the present causal component. However, it was difficult to carry out a systematic fractionation from the beginning because the polymers are insolubilized or are irreversibly adsorbed on the resin, etc., because of the change in the pH value or the ionic strength caused by the fractionation operation. Such being the situation, the information acquired in the various unit fractionation operations was straightened, and the combination of the unit fractionation methods was studied.

<Information acquired in unit fractionation operations>

[0020]

(1) The present causal component is dissolved more readily in an aqueous saline solution than in purified water, and also is dissolved more readily under basic conditions than under acidic conditions. However, the causal component could be subject to reaction and decomposition, if the aqueous solution has a high basicity. Therefore, an aqueous solution of a weakly acidic salt (sodium acetate) was used for the extraction from the natural tobacco material. The extracting temperature was set at room temperature (about 25°C), 60°C and 90°C, and the extract liquid obtained by the extracting treatment at room temperature was found to have exhibited the strongest stimulus and pungency.

(2) As described above, when a fractionating treatment was carried out by using an ultrafiltration membrane having a molecular weight cutoff of 10,000 daltons, the stimulus and pungency were recognized in the fraction having a molecular weight not lower than 10,000 daltons.

(3) Unlike the polysaccharides, the present causal component is adsorbed by reverse phase resins and is eluted by water/methanol, etc.

(4) The present causal component is adsorbed by an anion exchange resin such as diethylaminoethyl cellulose (DEAE cellulose) and exhibits properties of an acid.

(5) Unlike proteins and polysaccharides, the present causal component is not precipitated by a mixed solvent of methanol/water (volume ratio of 9/1), and the present causal component was present in the portion dissolved in the mixed solvent.

(6) Typical flavonoids present in tobacco do not exhibit the stimulus and pungency, but tend to associate with a polymer, and are colored. Therefore, the flavonoids were removed as interfering components (adsorption on a polyvinyl pyrrolidone resin).

[0021] Based on the preliminary studies described above, the fractionation purification scheme from which the best result was obtained will now be described in detail.

<Fractionation purification scheme of the component causing stimulus and pungency in Burley tobacco material>

[0022] Midribs (petiole, thick vein portions of the leaf) of Burley tobacco are thin in taste and prominent in the stimulus and pungency and, thus, used as the object of the study.

[0023] Midribs of Burley tobacco material were pulverized and the extraction was conducted by stirring at room temperature for 30 minutes in a 0.1M sodium acetate aqueous solution, which was 10 times as much in volume as the midribs. The extract liquid was filtered and, then, subjected to centrifugal separation to remove the fine powdery material. This extract liquid was put into a column of a reverse phase adsorbing material, and the column was washed with water. A dark brown solution eluted with a water/methanol or a water/acetonitrile, specifically, with a water/methanol (6/4), was freed from the organic solvent by the concentration under a reduced pressure, followed by adding a small amount of polyvinyl pyrrolidone and subsequently stirring and filtering to remove the polyphenols. Then, the processed liquid was subjected to an ion exchange chromatography using a DEAE-cellulose. The column was washed with water to remove the neutral and basic components, and the elution was conducted with an aqueous sodium hydroxide solution. The eluate was subjected to an ultrafiltration (a molecular weight cutoff of 10,000 daltons) to remove the low molecular weight components, washed with water and lyophilized. Further, the lyophilized material was subjected to fractional precipitation with methanol/water (9/1) and, then, subjected to centrifugal separation to remove the polysaccharides and proteins that were precipitated. The supernatant was concentrated under a reduced pressure and lyophilized, giving a final purified product. The final purified product of the present causal component was obtained from the raw material at a yield of about 0.2%. It was confirmed by all of the 10 expert panels that the stimulus and pungency peculiar to Burley tobacco were expressed from the evaluation cigarette having no perfume added thereto, but added with the final purified product at a concentration of about 400 ppm.

[0024] FIG. 1 shows a gel filtration high performance liquid chromatogram of the present causal component (see Example 1 below). As apparent from FIG. 1, the present causal component has a molecular weight of 10,000 to 60,000 daltons and its peak molecular weight was 16,000 daltons. As described above, the present causal component is an acidic substance, and an aqueous solution thereof exhibits a color of brown to dark brown depending on the concentration thereof (see the ultraviolet-visible absorption spectrum shown in FIG. 2). Also, unlike pectin, proteins, etc., i.e., the other water-soluble polymers contained in tobacco, the present causal component is soluble in a water-containing alcohol.

[0025] FIG. 3 shows the infrared absorption spectrum of the present causal component. As shown in FIG. 3, the present causal component exhibits absorption peaks at 3423, 2935, 1618, 1411, 1261, 1070, and 534 cm⁻¹.

[0026] The result of the elemental analysis of the present causal component was as follows:

C: 36.2%; H: 4.70%; N: 4.09%; ash: 12.6%.

[0027] The feature of the ultraviolet visible absorption spectrum of the present causal component resembles that of a brown polymer, which is called melanoidin, contained in general foods. Various researches have been conducted on melanoidin contained in foods. The Maillard (non-enzymatic browning) reaction of sugars and amino acids, and the oxidative polymerization reaction (enzymatic browning reaction) of polyphenols caused by the enzyme are said to be involved in the formation of melanoidin. However, since the relating components differ depending on the kind of food, melanoidin has different chemical composition and properties and, thus, the general chemical structure of melanoidin has not yet been confirmed (see Science of Food as Viewed from Color, compiled by Kazuhiko Takamiya, pp. 183-191 (2004), and Science of Starch, vol. 38, No. 1, pp. 73-79 (1991)).

[0028] On the other hand, a colored component contained in Burley tobacco material is called a brown pigment and resembles the present causal component in the chemical properties. Chemical studies have been conducted on the brown pigment. It is considered in Arch. Biochem. Biophys., 86, 94-101 (1960) and Arch. Biochem. Biophys., 93, 580-590 (1961) that the brown pigment is a polymer component formed of an oxidatively polymerized material (enzymatic browning reaction) of polyphenols (chlorogenic acid and rutin) conjugated with proteins, iron ions, etc. It has been clarified by the subsequent studies that the brown pigment contained in tobacco can be classified into several kinds differing from each other in the chemical composition (see J. Agric. Food Chem., 26 (1978), 380-385).

[0029] Further, an analytical method for a brown pigment contained in a leaf tobacco, as well as a discriminating method for discriminating normal leaf and the leaf of a deteriorated quality, which utilizes the analytical method, are proposed in Jpn. Pat. Appln. KOKAI Publication No. 11-83827. In this analytical method, a crude extract liquid of leaf tobacco is subjected to a reverse phase liquid chromatograph using a water/acetonitrile solvent system, with the proportion of acetonitrile successively increased, to separate the brown pigment components differing in polarity. The discrimination between the normal leaf and the leaf of the deteriorated quality is performed based on the ratio of the brown pigment components. The color constitutes a factor for determining the grade of the quality of the raw material leaf tobacco. In this sense, the brown pigment component constituting the main body of the color is important. However, no report has been made concerning the direct tasting effect of the brown pigment component.

[0030] Under the circumstances, it has been found for the first time that the colored acidic polymer component (present causal component) contained in natural tobacco material, particularly, in Burley tobacco material, expresses the stimulus and pungency inherent in Burley tobacco. It has also been clarified that the chemical properties of the present causal component resembles those of melanoidin and the component called brown pigment component of tobacco.

[0031] It follows that the stimulus and pungency can be decreased to effectively improve the flavor and taste of tobacco by reducing the present causal component from the natural tobacco material, particularly, Burley tobacco material. Thus, the present invention can provide a tobacco material characterized in that the stimulus and pungency in the smoking stage are decreased by decreasing the content of the colored acidic water-soluble polymer substance.

[0032] An extract liquid obtained by reducing the present causal component from an extract obtained by extraction of natural tobacco material with an aqueous extracting solvent can be used as it is, or after concentrated or lyophilized, as a tobacco flavoring agent in which the stimulus and pungency are reduced. Further, a regenerated tobacco material can be obtained by adding the tobacco flavoring agent to a regenerated web sheet prepared by an ordinary method by using the fibrous material which is the extraction residue.

[0033] It is possible to use leaf, shreds, midribs, stalks and roots of tobacco as well as a mixture thereof as the natural tobacco material that is subjected to the extracting treatment. An aqueous solvent can be used as the extracting solvent. The aqueous solvent such as water may be alkaline or acidic. A mixture of water and an organic solvent miscible with water can be used as the aqueous extracting solvent. Examples of such an organic solvent include alcohols such as ethanol. It is possible to dissolve an inorganic salt such as sodium chloride in these extracting solvents. The extracting treatment can be performed usually at room temperature (about 25°C) to 100°C for about 5 minutes to 6 hours.

[0034] After completion of the extracting treatment, the extraction mixture is subjected to a separating operation by, for example, filtration under compression to separate the mixture into the extract liquid and the extraction residue (fibers). A tobacco flavoring agent of the present invention can be obtained by decreasing the content of the present causal component from the extract liquid. The tobacco flavoring agent can be used as it is or after concentrated or lyophilized.

[0035] On the other hand, a slurry is prepared by using the extraction residue, and is made into a regenerated tobacco web. The regenerated tobacco web may be entirely or partially formed of the extraction residue. Further, the extraction residue used may be the one obtained as a result of the extraction treatment directly intended to prepare the tobacco flavoring agent of the present invention, or may be the one obtained by the extraction of natural tobacco material intended for the other purposes.

[0036] Next, more specific methods of decreasing the present causal component from the extract liquid will be described. The decreasing methods directly utilize the physical and chemical properties of the present causal component. That is, the flavor and taste can be improved by effectively decreasing the present causal component utilizing the physical and chemical properties that the causal component is a polymer substance having a molecular weight of 10,000 to

60,000 daltons, that its polarity is low compared with that of tobacco components such as pectin, and that the present causal component is an acidic water-soluble polymer substance. The methods of decreasing the present causal component will now be described in detail. Incidentally, the methods for obtaining the extract liquid from the natural tobacco material are as described above.

<Method of decreasing the present causal component by ultrafiltration, etc.>

[0037] The extract liquid obtained from the natural tobacco material is subjected to ultrafiltration treatment using a semi-permeable membrane or a hollow fiber to remove the polymer components having a molecular weight not lower than 1,000 daltons, preferably not lower than 10,000 daltons. The removing operation can also be performed by a dialysis method or a gel filtration method. The fraction having a molecular weight lower than 1,000 daltons, preferably lower than 10,000 daltons, can be used as the tobacco flavoring agent.

<Method of decreasing the present causal component by ion exchange>

[0038] The extract liquid obtained from the natural tobacco raw material is subjected to an adsorbing treatment by using an anion exchange resin such as DEAE cellulose (DEAE Cellulofine (trade name)) and, then, the resin is removed by, for example, filtration. The present causal component is decreased in the resultant filtrate. This filtrate can be utilized as the tobacco flavoring agent. In this operation, an ion exchange membrane can also be used.

<Method of decreasing the present causal component by using adsorbing resin, etc.>

[0039] The extract liquid obtained from the natural tobacco raw material is subjected to an adsorbing treatment using an activated carbon, an adsorptive resin (such as a polystyrene/divinylbenzene copolymer, or a reverse phase adsorbent (representative example: octadecyl silica gel (ODS), etc.), followed by separating the adsorbent to obtain a processed liquid. The adsorbent is washed with water, a water/alcohol mixed system, or the like, and the washings are added to the processed liquid. This processed liquid can be utilized as the tobacco flavoring agent.

<Method of decreasing the present causal component by acid precipitation>

[0040] Since the present causal component is a polymer substance exhibiting acidic properties, it can be separated by lowering the pH value (hydrogen ion concentration) of the solution to make it insoluble (see the procedures described in J. Agric. Food Chem., 26 (1978), 380-385 referred to previously). Specifically, an inorganic acid (such as sulfuric acid or a phosphoric acid) or an organic acid (such as oxalic acid) is added to the extract liquid obtained from the natural tobacco material to lower the pH value to preferably 2 or less. After the precipitates are removed by filtration or centrifugal separation, an alkaline material (such as calcium hydroxide) is added to bring the pH value back to the original value. In the case of using oxalic acid, a treatment with calcium hydroxide forms water insoluble calcium oxalate is formed, which can be removed by, for example, centrifugal separation. The thus processed liquid can be used as the tobacco flavoring agent.

<Method of decreasing the present causal component by precipitation with solvent>

[0041] Unlike the other polysaccharides and proteins, the present causal component is soluble in a water/methanol mixed solvent (volume ratio of 1/9), but insoluble in a solvent having a lower polarity. By utilizing the particular properties, the present causal component can be separated from the other components. As the solvents having a lower polarity, alcohols, specifically aliphatic alcohols such as methanol (100%), ethanol and isopropanol or aromatic alcohols such as benzyl alcohol, or other solvents soluble in water such as acetone, methyl ethyl ketone and acetonitrile can be used singly or as a mixed solvent. In this case, a solvent not miscible with water such as hexane can also be used because it can be dissolved in a solvent containing an alcohol or the like. Further, the kind of the solvent can be changed stepwise so as to carry out fractional precipitation, and the fractions can be taken up or discarded, based on the analytical value of the present causal component.

<Method of decreasing the present causal component by combination of the treating methods described above>

[0042] For decreasing the present causal component from the tobacco materials, the decreasing methods given above can be used singly or in combination. Also, the decreasing amount of the causal component can be controlled by selecting the conditions, and the separated components can be added in an appropriate amount to the regenerated tobacco web.

[0043] It is also possible to mix at least two kinds of the tobacco flavoring agents obtained by the decreasing methods

given above.

[0044] As described above, it is possible to reduce the stimulus and pungency of the final tobacco product in the smoking stage by removing the colored acidic water-soluble polymer substance from the natural tobacco material (or its extract liquid). In this case, it is desirable for the colored acidic water-soluble polymer substance to be decreased in an amount not smaller than 10% (to 100%), more desirably not smaller than 50%, and most desirably not smaller than 80%, of the initial amount (the amount present in the natural tobacco material, or the amount in the extract liquid as extracted with an aqueous extracting solvent), in view of decrease of stimulus and pungency.

[0045] The present invention will now be described more specifically by way of Examples and Comparative Examples.

Example 1: Analytical method for the present causal component

[0046] As an index of the decrease of the present causal component, a simple analytical method for the present causal component was established by using a high performance liquid chromatography. 1.0g of Burley tobacco material (in the case of the extract liquid, pulverized was the extracted liquid, lyophilized one in an amount corresponding to 1.0g of the tobacco material) was pulverized, to which 10 mL of 0.1M aqueous solution of sodium acetate was added. The mixture was stirred at room temperature for 30 minutes, filtered and subjected to centrifugal separation. The supernatant was passed into a reverse phase solid phase extraction cartridge (Oasis HLB (6 cc, 500 mg, Waters Inc.), washed with water and eluted with 10 mL of acetonitrile/water (6/4). The eluate was concentrated under a reduced pressure to remove the acetonitrile, passed into a mini column of a polyvinyl pyrrolidone resin (Polyclear VT (Wako Junyaku K.K.)) and then into an anion exchange solid phase extraction cartridge (BondElute DEA (500 mg, Varian Inc.)), and was eluted with 4 mL of 0.4N sodium hydroxide after washing with water. The eluate was subjected as it was to centrifugal ultrafiltration and to the exchange of a buffer solution (0.1M phosphate buffer (pH 6.8)), containing 0.1M of table salt by using an ultrafiltration device (Amicon Ultra-4 (molecular weight cutoff of 10,000 daltons, Millipore Inc.). Finally, the amount of the sample was increased to 5 mL by adding the same buffer solution to the sample, and the sample was subjected to a high performance liquid chromatography analysis.

<Analytical Conditions>

[0047] High performance liquid chromatograph apparatus used: Type 1100, Agilent Inc.

Column: Protein pack 125 (Waters Inc.)

Solvent: 0.1M phosphate buffer solution (pH 6.8) containing 0.1M of table salt.

Solvent flow rate: 1.0 mL/min.

Detector: A diode array, measuring wavelength of 410 nm.

[0048] FIG. 1 shows a typical chromatogram. The integrated value of the peak at the wavelength of 410 nm in the chromatogram is used as an index for decreasing the present causal component.

[0049] The ultraviolet-visible absorption spectrum of the present causal component was as shown in FIG. 2, and the infrared absorption spectrum was as shown in FIG. 3. The result of the elemental analysis was as described previously.

[0050] Reference Example: Preparation of regenerated tobacco sheet:

[0051] 75g of Burley tobacco material (midrib : lamina weight ratio = 1 : 1) was dipped in 0.75L of warm water of 60°C for 30 minutes, and filtered under compression to separate the mixture into a filtrate (extract liquid) and an extraction residue (fibers). By an ordinary method, the fibers were formed into a slurry, which was made into a regenerated tobacco web sheet. The weight of the regenerated tobacco web sheet after a conditioning treatment was 20.6g. The regenerated web sheet was cut to obtain shredded tobacco.

[0052] On the other hand, the filtrate (about 0.5L) was concentrated to 50 mL or less under a reduced pressure, and sprayed onto the shredded tobacco, which was then dried. The dried material was subjected to a conditioning treatment (stored under the constant temperature and the constant humidity until the moisture equilibrium was reached) to obtain regenerated tobacco shreds. Further, cigarettes (shred amount of about 0.6g per cigarette) were manually prepared.

Example 2

[0053] The filtrate (0.5L, corresponding to 75g of the raw material) obtained in the Reference Example was subjected to centrifugal separation (3,000 rpm, 10 minutes) so as to remove the insoluble portion and, then, subjected to an ultrafiltration treatment by using an ultrafiltration disk using a semipermeable membrane made of a regenerated cellulose having a fractionating molecular weight cutoff of 10,000 daltons (Ultracell PLGC ultrafiltration disk, Millipore Inc., nominal molecular weight cutoff of 10,000 daltons). The yields of the permeate and the concentrated liquid, after lyophilization, were 12.8g and 2.1g, respectively. The lyophilized material of the permeate was dissolved in 50 mL of water, and the total amount of the resultant solution was sprayed onto the shreds obtained in the Reference Example to obtain regenerated tobacco shreds. Cigarettes were made as in the Reference Example by using the regenerated tobacco shreds.

Example 3

[0054] The filtrate (0.5L, corresponding to 75g of the raw material) obtained in the Reference Example was subjected to centrifugal separation (3,000 rpm, 10 minutes) so as to remove the insoluble portion, and added with 50 mL (volume of wet resin) of an anion exchange resin (DEAE Cellulofine available from Seikagaku Kogyo K.K.) to carry out adsorption by stirring, followed by filtration. The processed liquid was concentrated under a reduced pressure, and the total amount of the concentrate was sprayed onto the shredded tobacco obtained in the Reference Example to obtain regenerated tobacco shreds. Cigarettes were made as in the Reference Example by using the regenerated tobacco shreds.

Example 4

[0055] The filtrate (0.5L, corresponding to 75g of the raw material) obtained in the Reference Example was subjected to centrifugal separation (3,000 rpm, 10 minutes) so as to remove the insoluble portion, and was passed into a column of a reverse phase adsorbing resin (Oasis HLB; resin amount of 6g x 2, Waters Inc.) to adsorb the colored component. The processed liquid was concentrated under a reduced pressure, and the total amount of the concentrate was sprayed onto the shredded material obtained in the Reference Example to obtain regenerated tobacco shreds. Cigarettes were made as in the Reference Example by using the regenerated tobacco shreds.

<Analytical Value and Taste Evaluation of Reference Example and Examples 2 to 4>

[0056] There was obtained the analytical value (the area of the peak at the wavelength of 410 nm in the liquid chromatogram as referred to in Example 1; relative value). Also, the evaluation of decrease of the stimulus and pungency was expressed by the number of expert panels who evaluated that the stimulus and pungency were clearly decreased relative to the cigarette of the Reference Example, out of 10 expert panels. Table 1 shows the result.

Table 1

	Analytical value of the present causal component in the extract liquid	Evaluation of decrease of stimulus and pungency	Comments on evaluation of tobacco taste
Reference Example (no treatment; control)	454.8		Stimulus and pungency were strong and remained in the mouth afterward. Pungency like Burley tobacco, offensiveness and peculiarity were strong.
Example 2 (ultrafiltration treatment)	27.5	10/10	Substantially free from stimulus and pungency, and the feel in the mouth afterward was decreased. Offensiveness and the peculiarity were decreased so as to facilitate smoking.
Example 3 Example 3 (ion exchange treatment)	81.0	6/10	Stimulus and pungency were decreased. The feel in the mouth somewhat remained afterward. The pungency was decreased, but the acidic odor was somewhat intensified.

(continued)

	Analytical value of the present causal component in the extract liquid	Evaluation of decrease of stimulus and pungency	Comments on evaluation of tobacco taste
Example 4 (reverse phase resin treatment)	23.0	10/10	Substantially free from stimulus and pungency, and the feel in the mouth afterward was decreased. Somewhat malodorous.

[0057] The present invention has been described with reference to various embodiments and Examples, but the present invention is not limited to these embodiments and Examples. The causal component of the stimulus and pungency, which was found by the present inventor for the first time, is contained in Burley tobacco, and may be contained in the other species of tobacco (e.g., flue-cured tobacco, Orient tobacco and domestic tobacco). Needless to say, the present invention can be applied widely to the tobacco of these species and to the natural tobacco materials including these species of tobacco in general.

[0058] As described above, the stimulus and pungency of the tobacco material can be decreased by decreasing the colored acidic water-soluble polymer substance contained in the water-soluble portion of the tobacco materials according to the present invention, without adding various perfumes and without employing the process step employed in the past for improving the taste of the tobacco material.

Claims

1. A tobacco material which is decreased in stimulus and pungency in a smoking stage by decreasing a content of a colored acidic water-soluble polymer substance.
2. The tobacco material according to claim 1, wherein the colored acidic water-soluble polymer substance has a molecular weight of 10,000 to 60,000 daltons.
3. The tobacco material according to claim 1, wherein the tobacco material comprises Burley tobacco.
4. A tobacco flavoring agent comprising a tobacco extract liquid which has been decreased in a causal component causing stimulus and pungency by decreasing a content of a colored acidic water-soluble polymer substance.
5. The tobacco flavoring agent according to claim 4, wherein the colored acidic water-soluble polymer substance has a molecular weight of 10,000 to 60,000 daltons.
6. The tobacco flavoring agent according to claim 4, wherein the tobacco extract liquid is from Burley tobacco.
7. A regenerated tobacco material **characterized by** comprising a regenerated tobacco web comprising fibers which are an extraction residue of a natural tobacco material, added with a flavoring agent comprising an aqueous extract liquid from the natural tobacco material, wherein the flavoring agent is the tobacco extract liquid recited in claim 4.
8. A method of preparing a tobacco material, comprising decreasing a colored acidic water-soluble polymer substance.
9. A method of preparing a flavoring agent, comprising decreasing a colored acidic water-soluble polymer substance from an aqueous extract liquid of a natural tobacco material.

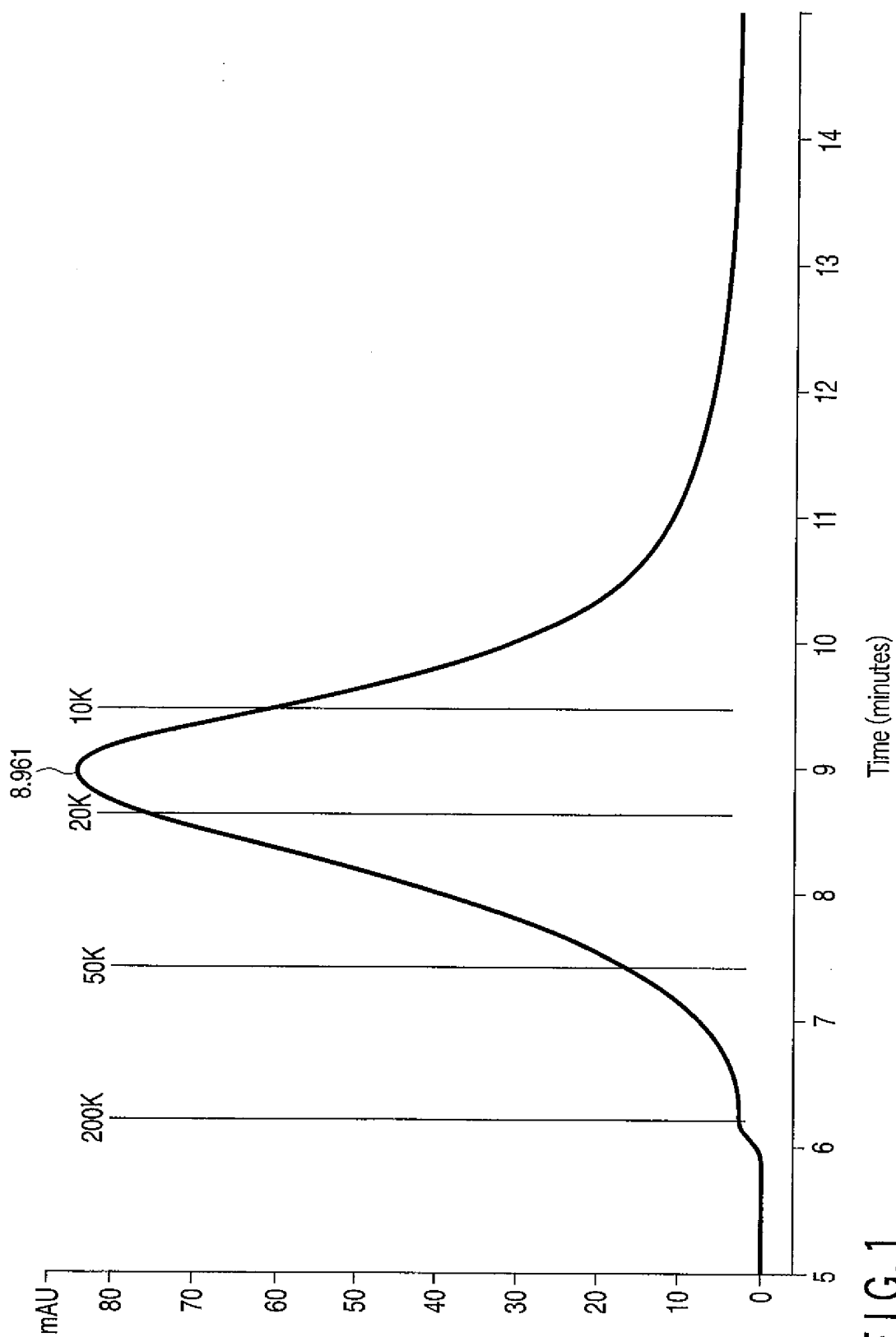


FIG.1

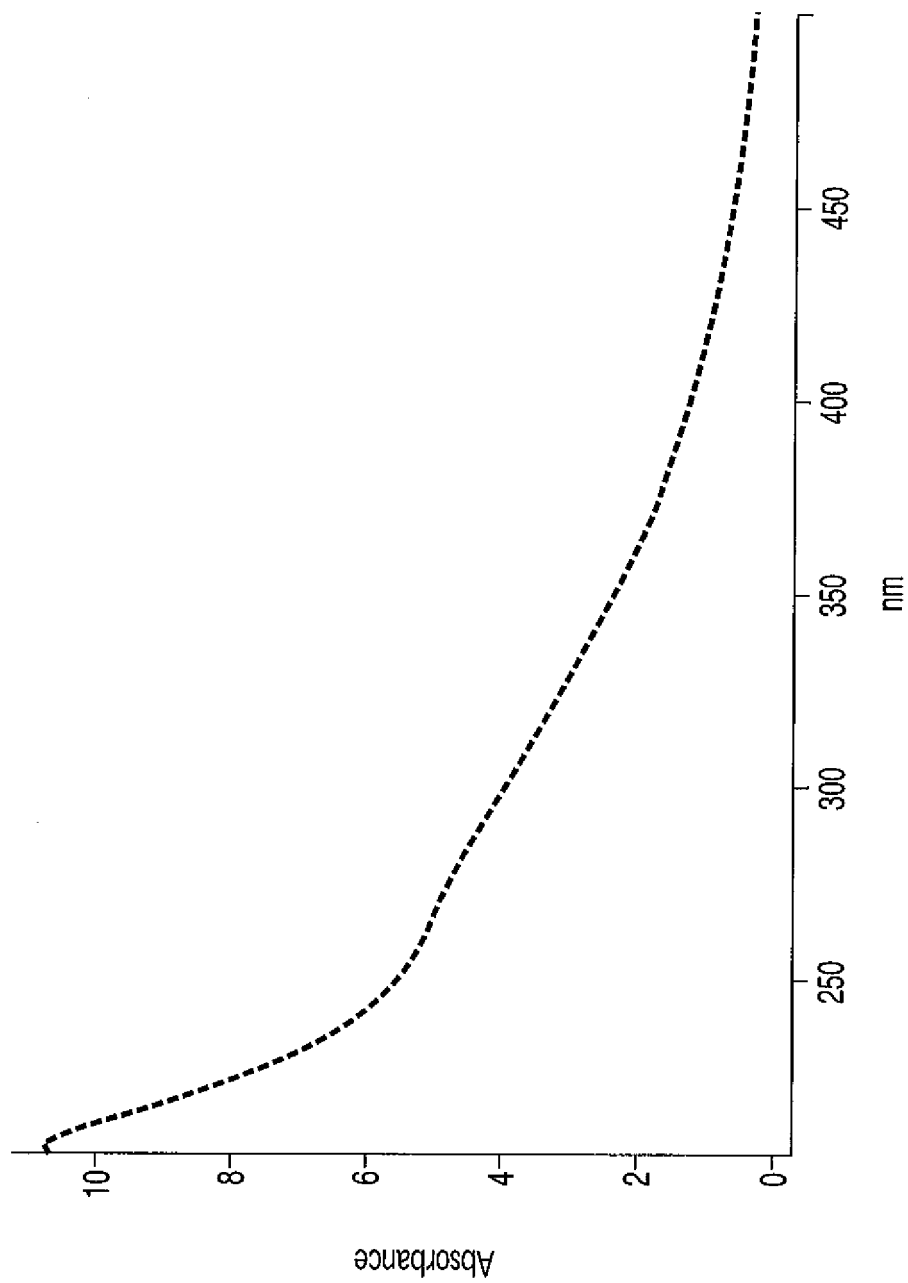


FIG. 2

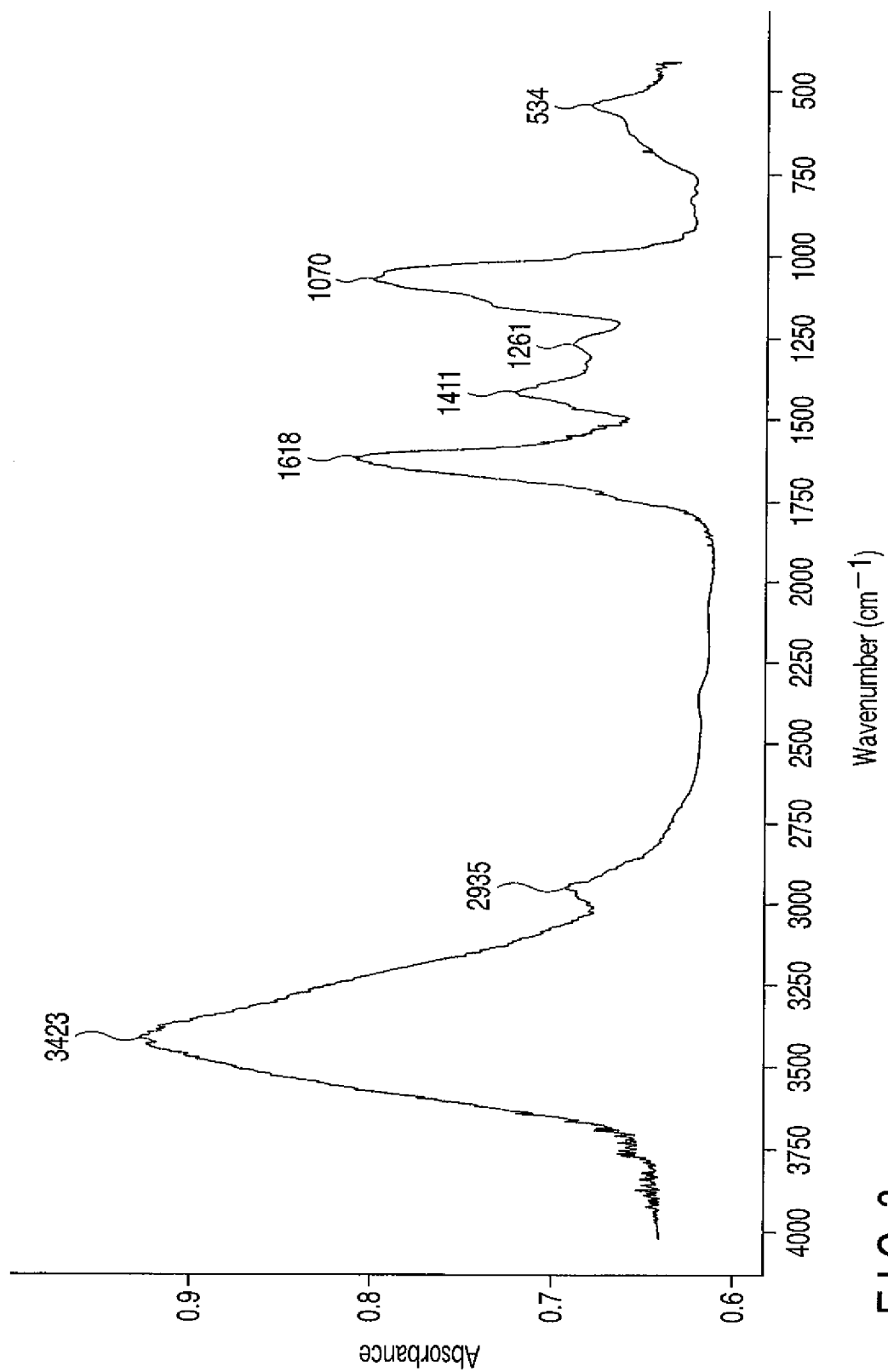


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2005/019510

A. CLASSIFICATION OF SUBJECT MATTER

A24B15/26(2006.01), **A24B3/12**(2006.01), **A24B15/12**(2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A24B15/26(2006.01), **A24B3/12**(2006.01), **A24B15/12**(2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2006

Kokai Jitsuyo Shinan Koho 1971-2006 Toroku Jitsuyo Shinan Koho 1994-2006

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2002-520005 A (Nobozaimusu A/S), 09 July, 2002 (09.07.02), Full text; all drawings & WO 00/2464 A & US 6298859 A & EP 1094724 A & DE 69903430 D & AU 4769899 A & BR 9911909 A & CA 2316249 A & AT 225612 A	1-9
Y	JP 60-23830 B (Nippon Senbai Kosha), 10 June, 1985 (10.06.85), Full text; all drawings & JP 56-69371 A	1-9

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search
24 January, 2006 (24.01.06)Date of mailing of the international search report
31 January, 2006 (31.01.06)Name and mailing address of the ISA/
Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2005/019510

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP 3-83570 A (R.J. Reynolds Tobacco Co.), 09 April, 1991 (09.04.91), Full text; all drawings & US 5025812 A & EP 412768 A & SU 1837813 A & CA 2021455 A & CN 1049275 A	1-9
Y	JP 5-236926 A (T. Hasegawa Co., Ltd.), 17 September, 1993 (17.09.93), Full text; all drawings (Family: none)	1-9
Y	JP 2001-525190 A (Brown & Williamson Tobacco Corp.), 11 December, 2001 (11.12.01), Full text; all drawings & US 5908034 A & EP 1039810 A & WO 99/29189 A & BR 9813444 A & CA 2313188 A & CN 1281342 A	1-9
A	JP 2-49572 A (R.J. Reynolds Tobacco Co.), 19 February, 1990 (19.02.90), Full text; all drawings & US 5435325 A & EP 338831 A	1-9

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REFERENCES CITED IN THE DESCRIPTION

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- *J. Agric. Food Chem.*, 1978, vol. 26, 380-385 [0028] [0040]