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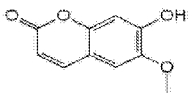
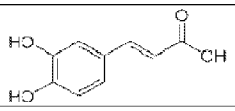
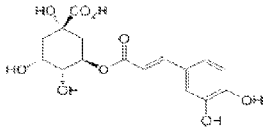
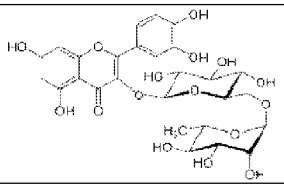
[Continued on next page]

(54) Title: TREATMENT OF TOBACCO MATERIAL

Figure 1

Polyphenol	Chemical Structure
Scopoletin	
Caffeic acid	
Chlorogenic acid	
Rutin	

(57) Abstract: A method is provided for treating a tobacco material, comprising treating the tobacco material by steam hydrolysis. Also provided is a tobacco material which has been treated by such a method, or a derivative thereof, and a smoking article which comprises a tobacco material treated by such a method.

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Treatment of Tobacco Material

Field of the Invention

The present invention relates to a method for the treatment of tobacco material.

Background

In some circumstances, it may be desirable to reduce the content of certain constituents from tobacco material before incorporating the tobacco material into a smoking article such as a cigarette.

Summary

According to a first aspect, there is provided a method for treating a tobacco material, wherein the method comprises treating the tobacco material by steam hydrolysis.

In some embodiments, the method results in a reduction in the polyphenol content of the tobacco material compared to the polyphenol content of the untreated tobacco material.

In some embodiments, the method of the invention does not substantially reduce the amount of nicotine in the tobacco material.

In some embodiments, the method results in a reduction in the protein content of the tobacco material compared to the protein content of the untreated tobacco material.

In some embodiments, the tobacco material is suspended in an aqueous medium which is heated to generate the steam for the steam hydrolysis. In some embodiments, the aqueous medium is water, an aqueous solution or an aqueous suspension.

In some embodiments, the ratio of aqueous medium to tobacco material is at least 1:1 by weight.

In some embodiments, the method comprises ohmic heating. In some further embodiments, the aqueous solution contains sufficient ions to provide electrical conductivity for ohmic heating.

In some embodiments, the method is carried out under elevated pressure. In some embodiments, the pressure is released after an optional period of time at the maximum pressure.

In some embodiments, the tobacco material treated by steam hydrolysis is subsequently separated from the aqueous medium in which it was suspended. This separation may involve, for example, filtration and/or centrifugation.

In addition to steam hydrolysis, the method of the invention may further comprise: treating the tobacco material with one or more enzymes; treating the tobacco material with one or more surfactants; treating the tobacco material with one or more adsorbents; and/or treating the tobacco material with one or more non-aqueous liquids.

According to a second aspect, there is provided a tobacco material which has been treated by a method according to the first aspect, or a derivative thereof.

According to a third aspect, there is provided a smoking article which comprises a tobacco material according to the second aspect or a derivative thereof.

According to a fourth aspect, there is provided a use of stream hydrolysis for removing one or more polyphenols from a tobacco material.

Brief Description of the Drawings

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 shows the chemical structure of the four reference polyphenol compounds detected and measured in experiments using High-Performance Liquid Chromatography (HPLC): scopoletin, caffeic acid, chlorogenic acid, and rutin.

Figure 2 shows an HPLC spectrum obtained for a sample containing each of the four reference polyphenol compounds at a concentration of 100 ppm.

Figure 3 shows an HPLC spectrum obtained for one of the steam hydrolysis experiments.

Figure 4 shows the calibration curve for converting light absorbance into units of GAE (Gallic Acid Equivalents) in the Folin-Ciocalteu (FC) assay.

Figure 5 shows a graph portraying the positive correlation between the amounts of removed polyphenol compound measured using HPLC and the FC assay.

Figure 6 shows the calibration curve for converting light absorbance into Bovine Albumin Serum concentration, which could be used as an approximation for the concentration of all types of protein in the Bradford assay.

Figure 7 is a schematic side view of a smoking article including treated tobacco material according to embodiments of the invention.

Detailed Description

There is provided a method for treating a tobacco material, wherein the method comprises treating the tobacco material by steam hydrolysis. In at least some embodiments, steam hydrolysis results in hydrolysis of cellulosic materials, thereby aiding the release of cell-bound components.

In the past, methods attempting to remove proteins or polyphenols have been proposed, but these have tended to be complex and expensive.

Treating the tobacco material by steam hydrolysis may be used for the purpose of modifying the tobacco material in any suitable way. In some embodiments, steam hydrolysis leads to the removal of one or more chemical substances. In particular, in some embodiments, steam hydrolysis leads to the removal of one or more chemical substances which are considered to be undesirable in the tobacco material in some circumstances. In some embodiments, steam hydrolysis leads to the removal of one or more polyphenols. In some embodiments, the steam hydrolysis may also lead to the removal of protein, for example by removing one or more proteins.

The treatment of tobacco material by steam hydrolysis comprises at least one step in which the tobacco material undergoes steam hydrolysis. In some embodiments where the method comprises more than one steam hydrolysis step, the same or different conditions may be used in each steam hydrolysis step.

The treatment of tobacco material by steam hydrolysis may result in the removal of at least one chemical substance from the tobacco material. In some embodiments, the treatment may lead to the removal of at least some of the polyphenol compounds present in the untreated tobacco material. Alternatively or in addition, the treatment

may lead to the removal of at least some of the protein present in the untreated tobacco material.

Treating tobacco material by steam hydrolysis can result in hydrolysis of the cellulosic material, thereby aiding the release of cell-bound components. Ohmic heating is a highly efficient and volumetric electrical heating method. In some embodiments, aqueous suspensions of tobacco are heated under pressure (i.e. under elevated pressure, that is pressure greater than atmospheric pressure) and the elevated pressure is then rapidly released. This release of pressure is believed to rupture the cell structure enabling intracellular components to be extracted into the aqueous phase.

Tobacco material comprises dead plant cells, and dead plant cells have many functional groups which are reactive towards water under favourable conditions. As a result, exposing tobacco material to water under favourable conditions is likely to result in the breakdown of different cellular structures, and the consequent release of different chemical substances. Most significantly, cellulose in the plant cell walls comprises glucose molecules linked by O-glycosidic bonds, which may be broken by hydrolysis under favourable conditions. This will cause the cell wall to rupture and, without the cell wall to balance the positive pressure potential of the water (Ψ_p), intracellular substances will be released.

The steam hydrolysis step in the treatment of tobacco material may be advantageous since it means that hydrolysis is likely to take place at a high temperature and/or pressure, this being beneficial because a high temperature and/or pressure is likely to enhance the rate of hydrolysis and, therefore, enhance the removal of certain components, such as polyphenols and/or proteins from tobacco material.

The treatment of tobacco material by steam hydrolysis may be applied to any suitable tobacco material. The tobacco material may be derived from any suitable part of any suitable tobacco plant of the plant genus *Nicotiana*. The tobacco material may then be treated in any suitable way, and may be cured using any suitable method of curing, before being treated by steam hydrolysis. In some embodiments, however, the tobacco material treated by steam hydrolysis has already been cured and may be cured cut rag and/or cured whole leaf tobacco. Examples of tobaccos which may be used in the treatment of tobacco material by steam hydrolysis include, but are not limited to: Virginia, Burley, Maryland, Oriental, and Rustica.

The treatment of tobacco material by steam hydrolysis may remove one or more chemical substances from the tobacco material. In some embodiments, one or more of the chemical substances removed from the tobacco material are polyphenols. Polyphenols which may be removed by steam hydrolysis include, but are not limited to: chlorogenic acid, caffeic acid, rutin, scopeletin, and quercetin.

The treatment of tobacco material by steam hydrolysis may comprise any suitable steps, and any suitable number of steps, in order to reduce the polyphenol and/or content of the tobacco material. The treatment may also further modify the tobacco material in any suitable way, for example by modifying the flavour it generates upon combustion, and/or removing other types of chemical substances.

In some embodiments, the treatment of the tobacco material by steam hydrolysis results in a reduction in the content of one or more polyphenols of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90% or at least 95%, based upon the polyphenol content of the untreated tobacco material.

In some embodiments, the treatment of the tobacco material by steam hydrolysis results in the extraction of one or more polyphenols in an amount of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90% or at least 95%, based upon the polyphenol content of the untreated tobacco material.

Alternatively or in addition, the treatment of the tobacco material by steam hydrolysis results in a reduction in the protein content of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, or at least 50%, based upon the protein content of the untreated tobacco material.

In some embodiments, the treatment of the tobacco material by steam hydrolysis results in the extraction of protein in an amount of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40% or at least 50%, based upon the protein content of the untreated tobacco material.

In some embodiments, the treatment of tobacco material by steam hydrolysis — in particular the steam hydrolysis step — reduces or minimises the removal of at least some of the chemical substances whose removal would be undesirable, which could be

the case for a variety of different reasons. One reason, for example, could be that the substance makes a positive contribution to the experience of smoking a smoking article which contains the treated tobacco material.

Nicotine may be an example of such a substance, and for this reason in some embodiments it is undesirable to remove this molecule. In some embodiments, the treatment of tobacco material by steam hydrolysis removes less than 50%, 40%, 30%, 20%, 10%, or 5% of the nicotine from the tobacco material; in further embodiments, the treatment removes less than 2%, 1%, 0.5%, or 0.1% of nicotine from the tobacco material; and, in further embodiments still, the treatment removes essentially no nicotine from the tobacco material.

In embodiments wherein treating the tobacco material by steam hydrolysis leads to the removal of one or more chemical substances from the tobacco material, one or more of these may be re-introduced into the material following treatment, and one or more of these may be substances whose removal would be undesirable, such as nicotine.

In some embodiments, the steam may be generated for steam hydrolysis by elevating the temperature of an aqueous medium. This aqueous medium may be water, but in some embodiments may be an aqueous solution or suspension. In some embodiments, the aqueous medium includes ions which will provide the aqueous medium with sufficient conductivity for ohmic heating. For example, the aqueous medium may be an aqueous saline solution (or brine). The temperature at which steam is generated may be modified using any suitable means, such as by regulating the pressure and/or regulating the solute content of the aqueous medium.

In some embodiments, the heating of the suspension of tobacco material in an aqueous medium is by an electrical or resistive heating method, such as by ohmic heating (also known as Joule heating). In some embodiments, other mechanisms of heating may be used alternatively or in addition to ohmic heating. As a non-limiting example, the alternative heating mechanism may include holding the suspension in a container having a heated jacket or including a heating element.

In some embodiments, the steam generated for steam hydrolysis may be superheated. This means that it is raised to a temperature greater than the boiling point of the water from which it is formed. In some embodiments, this may be beneficial because the

higher temperature provides the water molecules with greater kinetic energy and, therefore, greater reactivity. It should be noted that the treatment of tobacco material by steam hydrolysis disclosed herein does not use subcritical water.

In some embodiments, the method comprises one or more steam hydrolysis steps in which the tobacco material is suspended in an aqueous medium, such as an aqueous solution or suspension. The tobacco suspension may be held in a container. In some embodiments, the steam for the steam hydrolysis is generated from the aqueous medium. The aqueous medium may be pure water or may, for example, comprise any suitable solute or solutes, any suitable substance or substances in suspension, and/or any suitable immiscible liquid or liquids. As used herein, "pure water" relates to water treated to remove contaminants and/or impurities.

In embodiments in which the tobacco material is suspended in an aqueous medium, the aqueous medium may have any suitable weight and the tobacco material may have any suitable weight. In addition, the ratio of the aqueous medium to the tobacco material, by weight, may have any suitable value. In some embodiments, the ratio of tobacco material to aqueous medium results in the removal of a large quantity of polyphenols from the tobacco material. This ratio may be different for different aqueous media and tobacco materials, and for steam hydrolysis under different conditions.

In many embodiments, a greater quantity of polyphenols is likely to be removed when the weight of the aqueous medium is greater than or equal to the weight of the tobacco material. This is because, when the weight of the aqueous medium is greater than the weight of the tobacco material, there are more water molecules to carry out hydrolysis per unit mass of the tobacco material undergoing hydrolysis. In some embodiments, therefore, the weight of the aqueous medium is greater than the weight of the tobacco material and, in some embodiments, the ratio may be 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, or any suitable higher ratio.

The temperature of the aqueous medium may be increased to generate steam whilst the tobacco material is suspended within it. In some embodiments, this may be achieved by changing the pressure inside the container holding the suspension so that the pressure inside the container is lower than or equal to the vapour pressure of the water.

In addition or alternatively, the temperature inside the container may be adjusted to any suitable value. The temperature may be constant throughout the steam hydrolysis step or it may be varied. In some embodiments, it may be preferable for the temperature inside the container to be adjusted to at least 100°C.

The pressure and/or the temperature within the container holding the suspension of tobacco material and aqueous medium may be changed and controlled using any suitable mechanism. As a non-limiting example, the temperature within the container may be changed and controlled by providing the container with a heated jacket or a heating element. As a non-limiting example, the pressure within the container may be changed and controlled by using a container which is a pressure vessel, optionally including a valve or the like to adjust the pressure within the container.

In some embodiments, ohmic heating is utilised as the mechanism of heating the aqueous medium. In such embodiments, the aqueous medium may comprise salt in an amount to give sufficient electrical conductivity for the material to be heated ohmically. Any suitable salt or salts may be added to the aqueous medium in order to improve its capacity to carry charge. In some embodiments, the one or more salts added to the aqueous medium may have a high dissociation constant under the employed conditions and, in further embodiments, the one or more salts added to the aqueous medium may be strong electrolytes. An example of a suitable salt which may be added to the aqueous medium is NaCl.

The pressure inside the container may be adjusted to any suitable value, may be constant or variable, and may be changed using any suitable mechanism. In some embodiments, however, the pressure inside the container is initially increased to a maximum or peak pressure as the suspension of tobacco material and aqueous medium is heated. The pressure is then subsequently released so that it returns to atmospheric pressure. In some embodiments, the maximum or peak pressure is held or maintained for a period of time before release. This period (referred to as the hold time) may be, for example, at least about 1 minute, 90 seconds, 2 minutes, 150 seconds, 3 minutes, 4 minutes, 5 minutes, 10 minutes, 15 minutes, or at least about 20 minutes, or any suitable longer length of time. In other embodiments, the hold time is zero, the pressure being immediately released once the maximum or peak pressure has been reached. In some embodiments, the pressure is rapidly released. Without being bound by any particular theory, it is hypothesised that the release of the pressure, and

especially the rapid release of the pressure assists in the rupture of the cellular structure, enabling intracellular components to be extracted into the aqueous phase.

In some embodiments, the pressure inside the container is raised to at least 200 kPa, 300 kPa, 400 kPa, 500 kPa, 600 kPa, 700 kPa, 800 kPa, 900 kPa or at least 100 kPa (at least 2 bar, 3 bar, 4 bar, or 5 bar, 6 bar, 7 bar, 8 bar, 9 bar or 10 bar), or any suitable higher value.

After steam hydrolysis, the tobacco material may be separated from the aqueous medium (also referred to as the aqueous extract). This separation may involve any suitable filtration method, any suitable filtering medium pore size, and any suitable number of filtration steps. For example, the tobacco material may be filtered by nanofiltration, microfiltration, and/or ultrafiltration. Alternatively or in addition, the tobacco material may be separated from the aqueous medium by centrifugation using any suitable centrifuge system, any suitable angular velocity, and any suitable number of centrifugation steps.

Once separated from the aqueous medium, the tobacco material (also referred to as tobacco residue) may be washed any suitable number of times using any suitable liquid or liquids, such as water.

In addition to one or more steam hydrolysis steps, the treatment may comprise one or more further treatment steps and/or extraction processes. Further treatment steps or extraction processes may be particularly useful in the treatment of tobacco material by steam hydrolysis for the purpose of removing large quantities of protein. This is because steam hydrolysis is likely to rupture the plant cell walls in the tobacco material, thereby providing easier access to the intracellular components of the plant cells and the proteins found therein.

Suitable additional treatment steps include, but are not limited to: treating the tobacco material with one or more suitable non-ionic liquids, such as water; treating the tobacco material with one or more enzymes, which may be enzymes which catalyse the modification of polyphenols or proteins, such as phenol-oxidising and proteolytic enzymes; treating the tobacco material with one or more suitable surfactants, such as sodium dodecylsulfate (SDS), in any suitable solvent; treating the tobacco material with one or more suitable adsorbent materials, such as polyvinyl polypyrrolidone (PVPP),

hydroxylapatite, bentonite, activated carbon or attapulgite, in any suitable solvent if appropriate; and treating the tobacco material with one or more suitable non-aqueous liquids, such as ionic liquids.

Additionally or alternatively, the tobacco material subjected to steam hydrolysis may be subsequently subjected to further extraction processes.

Having undergone any of the previously-described treatment steps in accordance with the treatment of tobacco material by steam hydrolysis, the tobacco material may be dried and further modified in any suitable way before being incorporated into a smoking article. For example, certain chemical substances may be added to the tobacco material, such as flavourants where local regulations permit, and the tobacco material may be cut and/or shredded before being incorporated into a smoking article using any suitable method of incorporation.

As used herein, the term "smoking article" includes smokeable products such as cigarettes, cigars and cigarillos whether based on tobacco, tobacco derivatives, expanded tobacco, reconstituted tobacco or tobacco substitutes and also heat-not-burn products. The smoking article may be provided with a filter for the gaseous flow drawn by the smoker.

As used herein, the terms "flavour" and "flavourant" refer to materials which, where local regulations permit, may be used to create a desired taste or aroma in a product for adult consumers. They may include extracts (e.g., licorice, hydrangea, Japanese white bark magnolia leaf, chamomile, fenugreek, clove, menthol, Japanese mint, aniseed, cinnamon, herb, wintergreen, cherry, berry, peach, apple, Drambuie, bourbon, scotch, whiskey, spearmint, peppermint, lavender, cardamon, celery, cascarilla, nutmeg, sandalwood, bergamot, geranium, honey essence, rose oil, vanilla, lemon oil, orange oil, cassia, caraway, cognac, jasmine, ylang-ylang, sage, fennel, piment, ginger, anise, coriander, coffee, or a mint oil from any species of the genus Mentha), flavour enhancers, bitterness receptor site blockers, sensorial receptor site activators or stimulators, sugars and/or sugar substitutes (e.g., sucralose, acesulfame potassium, aspartame, saccharine, cyclamates, lactose, sucrose, glucose, fructose, sorbitol, or mannitol), and other additives such as charcoal, chlorophyll, minerals, botanicals, or breath freshening agents. They may be imitation, synthetic or natural ingredients or blends thereof. They may be in any suitable form, for example, oil, liquid, or powder.

In an exemplary embodiment of the invention a sample of cured whole leaf tobacco is added to water including a dissolved salt and the resultant suspension is held inside a container. The ratio of tobacco material to aqueous solution is 1:20 by weight. The temperature of the water is elevated to 100°C by passing an electric current through the aqueous solution, before the pressure inside the container is elevated to 300 kPa (3 bar), and then the pressure is quickly released after a hold time of 0 minutes. Steam hydrolysis is able to take place under these conditions to result in the extraction of chemical substances from the tobacco material, including polyphenol compounds. Following steam hydrolysis, the tobacco material is separated from the aqueous solution by filtration, dried, and modified in any suitable way before being incorporated into a smoking article.

Referring to Figure 7, for purpose of illustration and not limitation, a smoking article **1** according to an exemplary embodiment of the invention comprises a filter **2** and a cylindrical rod of smokeable material **3**, such as tobacco treated in accordance with the invention described herein, aligned with the filter **2** such that one end of the smokeable material rod **3** abuts the end of the filter **2**. The filter **2** is wrapped in a plug wrap (not shown) and the smokeable material rod **3** is joined to the filter **2** by tipping paper (not shown) in a conventional manner.

In some embodiments, the methods described herein may comprise one or more further steps to modify the tobacco material in any suitable way. For example, the tobacco material may be modified to provide it with one or more characteristics desirable for a tobacco material. For example, where the treated tobacco material is to be incorporated into a smoking article such as a cigarette, the tobacco material may be treated in order to modify the flavour it generates upon combustion, and/or may be treated in order to remove one or more of its chemical substances.

Experimental Work

A series of experiments were carried out in order to investigate how the treatment of a tobacco material by steam hydrolysis can affect the protein, polyphenol, and nicotine content of the tobacco material. The disclosed experimental work is not intended to limit the scope of the invention.

Experiments

A total of fourteen experiments were carried out, two of which were controls.

In the control experiments, the tobacco material was added to an acetone : water solvent (7:3 ratio), before the residual tobacco material was filtered from the solvent to provide two fractions for analysis — the filtrate and the residual tobacco material. The purpose of the two control experiments was to provide a measure of the quantity of readily-soluble polyphenols in the tobacco material. The acetone : water solvent is a very good solvent for polyphenols and, consequently, the quantity of polyphenol compounds measured in the filtrate following filtration could be taken as a measure of the quantity of readily-soluble polyphenols in the tobacco material. Many polyphenols would not have been readily soluble due to being bonded to, and/or trapped in, various cellular structures.

In the non-control experiments, a tobacco material was suspended in water (in the ratios specified in Table 1 below) to which a small amount of salt was added to give sufficient electrical conductivity for the material to be heated by ohmic heating. The ohmic heating was sufficient to result in steam hydrolysis. Following steam hydrolysis, the suspension was filtered to recover the residual tobacco material and the aqueous extract. Analysis was carried out on both.

Five variables were used in different combinations in the experiments: tobacco material to water ratio (the ratio of tobacco material to water by weight), maximum pressure (the maximum pressure reached during steam hydrolysis), hold time (the length of time over which the pressure is held at its maximum), and release speed (the relative speed at which the pressure is released after reaching its maximum). The conditions used in each of the experiments are detailed below in Table 1.

Table 1

Experiment	Tobacco Material	Tobacco Material:Water (by weight)	Maximum Pressure (kPa)	Hold Time (min)	Relative Release Speed
Control 1		1:40	n/a	n/a	n/a
Control 2		1:20	n/a	n/a	n/a
1	Whole leaf	1:2	600	0	Fast
2	Whole leaf	1:2	600	5	Fast
3	Whole leaf	1:10	300	0	Slow

4	Whole leaf	1:10	300	0	Fast
5	Whole leaf	1:10	300	5	Fast
6	Whole leaf	1:10	300	0	Fast
7	Whole leaf	1:3	100	5	Fast
8	Whole leaf	1:3	300	10	Fast
9	Cut rag	1:3	300	0	Fast
10	Cut rag	1:3	300	5	Fast
11	Cut rag	1:3	300	10	Fast
12	Whole leaf	1:20	300	0	Fast

Analysis: Polyphenol Content

Two analytical techniques were used to provide a measure of the quantity of polyphenol compounds removed from tobacco material in each of the experiments: High-Performance Liquid Chromatography (HPLC) and the Folin-Ciocalteu (FC) assay.

HPLC was used to measure the concentration of four reference polyphenol compounds, namely scopoletin, caffeic acid, chlorogenic acid, rutin in the aqueous filtrate following filtration. The chemical structures of these four reference polyphenol compounds are provided in Figure 1.

HPLC could only be used to measure the concentration of the four reference polyphenol compounds due to the way in which the analytical method was used to quantify their concentrations. Each polyphenol compound provides a peak at a particular position on an HPLC spectrum and, importantly, the position of this peak was only known for the four reference polyphenol compounds. Consequently, only the concentrations of the four reference polyphenols could be determined using HPLC: by comparing the peaks in the collected HPLC spectra with the peaks in an HPLC spectrum obtained for a sample containing known concentrations of the four reference polyphenol compounds.

An HPLC spectrum obtained for a sample containing each of the four reference polyphenols at a concentration of 100 ppm is provided in Figure 2. The figure illustrates the relative positions and integrals of the four peaks, which were used to qualitatively and quantitatively measure the four reference polyphenols in experimental samples.

The HPLC spectrum obtained for experiment 6 is provided as an example in Figure 3. In this spectrum there are two peaks, one of which is representative of the polyphenol chlorogenic acid, and one of which is representative of the polyphenol rutin.

After the concentrations of the four reference polyphenol compounds had been measured using HPLC, they were converted into masses, before these masses were then calculated as percentages of the mass of the original tobacco material on a Dry Weight Basis (% DWB).

The total mass of the four reference polyphenols measured in the filtrate could be taken as a measure of the total mass of the four reference polyphenols removed from the tobacco material. And, furthermore, the total mass of the four reference polyphenols removed from the tobacco material could be taken as an indication of the quantity of all types of polyphenol removed from the tobacco material — thereby acting as reference compounds.

The masses of the four polyphenol compounds (% DWB) measured in the filtrate in each of the experiments are detailed below in Table 2.

Table 2

Experiment	Mass of Four Polyphenols Removed (% DWB)
Control 1	0.80
Control 2	0.36
1	n/a
2	0.08
3	0.22
4	0.28
5	0.02
6	0.30
7	0.04
8	0.04
9	0.08
10	0.01
11	0.01
12	0.85

The results provided in Table 2 indicate that treating a tobacco material by steam hydrolysis can indeed lead to the removal of polyphenol compounds.

The Folin-Ciocalteu (FC) assay was used to provide a relative measure of the quantity of all types of polyphenol compound removed from tobacco material by steam hydrolysis in each of the experiments.

The FC assay is a colorimetric assay used to provide a measure of total polyphenol content in solution. In an FC assay, the magnitude of absorption at a particular radiation frequency — which polyphenols absorb — is measured for a sample. Following this, the measured magnitude of absorption is compared to the magnitude of absorption at the same radiation frequency for a solution of the polyphenol, Gallic Acid. The measured absorption of light may then be expressed in units of GAE (Gallic Acid Equivalents).

It is important to note that the FC assay only provides a qualitative measure of polyphenol content, and that the content of polyphenols in units of GAE is only correlated with the concentration of polyphenol compounds. This is for two reasons: firstly, different polyphenol compounds have different absorption coefficients, and secondly, there are various compounds besides polyphenols which absorb light at the detected radiation frequency — which is why qualitative results are more accurate when compared samples have similar compositions.

A calibration curve was prepared for converting the measured absorption of light into polyphenol content in units of GAE, and is pictured in Figure 4.

Having determined the concentration of all types of polyphenol compound in units of GAE, these were then converted into masses, before these masses were then calculated as percentages of the mass of the original tobacco material on a Dry Weight Basis (GAE as % DWB).

The masses of polyphenol compounds (GAE as % DWB) measured in each filtrate using the FC assay are detailed below in Table 3.

Table 3

Experiment	Mass of Polyphenols Removed (GAE as % DWB)
1	n/a
2	0.3
3	1.4
4	1.3
5	1.0
6	1.6
7	0.4
8	0.2
9	0.1
10	0.4
11	0.3
12	4.5

The results provided in Table 3 indicate that treating a tobacco material by steam hydrolysis does indeed lead to the removal of polyphenol compounds. As expected, the measured quantities of removed polyphenol compounds are higher than those measured using HPLC. Furthermore, it is also apparent that the results collected using the two analytical methods are well correlated, and a graphical illustration of this correlation is provided in Figure 5.

The results obtained using the two analytical methods may provide an indication of the way in which each of the tested variables affect the removal of polyphenol compounds from tobacco material. Conclusions which may be drawn from these results include, but are not limited to, the following. A higher ratio of water to tobacco material appears to reduce the polyphenol content of tobacco material more than a lower ratio; a relatively fast reduction in pressure appears to reduce the polyphenol content of tobacco material more than a relatively slow reduction in pressure; although the shorter hold time appears to reduce the polyphenol content more than a longer hold time, without being bound by any particular theory, this is most probably because holding the temperature and pressure so high for so long causes the removed polyphenols to react and therefore not be detected despite having been removed; and, finally, more experiments are required to determine the effect of the adopted maximum pressure.

Analysis: Protein Content

Two analytical techniques were used to provide a measure of the quantity of protein compounds removed from the tobacco material in each of the experiments: calculation based on Nitrogen content and the Bradford assay.

The total Nitrogen content of the collected samples was measured and, using a simple conversion protocol, used to provide a measure of the total quantity of protein removed from the tobacco material.

Proteins are molecules composed of amino acids, each of which contains one Nitrogen atom in its generic structure and possibly one or more Nitrogen atoms in its R group. By measuring the total Nitrogen content of a sample, therefore, the total protein content could be estimated by converting the total Nitrogen content into total protein content using a suitable conversion factor, known as the Jones factor.

A Jones factor of 6.25 was used in the experiments, which is the standard value used for a sample of protein without taking into account the particular type of protein being measured. If this conversion factor were used alone, however, it would significantly overestimate the content of protein in each sample, since there were many other nitrogenous compounds in each of the measured samples besides protein. One of these nitrogenous compounds was nicotine and so, in order to obtain a more accurate measure of protein content, the Nitrogen content attributable to nicotine was taken into account in the calculations.

The calculated masses of protein in the residual tobacco material (% DWB) and the filtrate (% DWB), and an estimate of the quantity of protein removed from the tobacco material (taking into account nicotine content) are detailed for each of the experiments below in Table 4.

Table 4

Experiment	Mass of Protein in Residual Tobacco Material (% DWB)	Mass of Protein in Filtrate (% DWB)	Mass of Removed Protein* (% DWB)
1	n/a	n/a	n/a
2	16.6	1.1	1.0
3	9.0	1.9	1.5

4	7.3	2.3	1.5
5	10.5	2.5	2.2
6	12.5	1.8	1.4
7	7.0	0.9	0.8
8	19.6	0.6	0.6
9	17.2	1.0	1.0
10	15.6	1.8	1.7
11	15.7	1.5	1.4
12	9.5	7.8	6.3

* including nicotine correction

The results provided in Table 5 suggest that treating tobacco material by steam hydrolysis does indeed result in the removal of some protein, although the results further suggest that the quantity of protein left in the material following hydrolysis is much greater than the quantity of protein removed from the material.

The quantity of protein removed from the tobacco material in experiment 12 was significantly higher than the quantity of protein removed in the other experiments, which seems to suggest that a high ratio of water to tobacco material results in the removal of a greater quantity of protein.

The Bradford Assay was also used to provide a measure of the quantity of protein removed from the tobacco material by steam hydrolysis. The assay may be used to detect the quantity of dissolved proteins in the filtrate following filtration by measuring the magnitude of absorption at the wavelength absorbed by proteins bonded to the Bradford reagent.

The results obtained using the Bradford assay may provide an accurate measure of the quantity of protein removed from the tobacco material, since the Bradford reagent complexes with very few compounds besides proteins.

A calibration curve was prepared for converting the measured magnitude of absorption into protein concentration, and is pictured in Figure 6. The curve was prepared using an aqueous solution of the protein Bovine Albumin Serum, and was linear in the concentration range tested.

In all of the experiments, the Bradford assay suggested that approximately 0.1% of the original tobacco material (% DWB) was removed as protein by steam hydrolysis. As expected, this percentage is lower than the results derived using Nitrogen content, since the Bradford assay essentially only detected protein molecules in the tested filtrates.

The results collected using the Bradford assay would suggest that the differences in the quantity of protein removed from tobacco material determined using Nitrogen content are largely due to the removal of nitrogenous compounds besides protein. Consequently, it is difficult to draw conclusions regarding how individual variables affect the removal of protein from tobacco material using steam hydrolysis.

Analysis: Nicotine Content

HPLC was used to provide a measure of the quantity of nicotine removed from whole leaf tobacco material.

As with the four reference polyphenol compounds, the molecule nicotine provides a peak at a known position on an HPLC spectrum following HPLC, and the quantity of nicotine in each of the analysed samples could therefore be measured by comparing the peak size in the collected HPLC spectra with the peak size in a spectrum obtained for a sample containing a known concentration of nicotine.

The total mass of nicotine measured in the extracts could be taken as a measure of the amount of nicotine removed from the tobacco material.

The masses of nicotine (% DWB) measured in the filtrate in each of the experiments are detailed below in Table 5.

Table 5

Experiment	Mass of Nicotine in Filtrate (% DWB)
1	n/a
2	0.1
3	0.4
4	0.7
5	0.4

6	0.3
7	0.1
8	0.0
9	0.1
10	0.1
11	0.1
12	1.4

The results provided in Table 5 indicate that treating a tobacco material by steam hydrolysis does not significantly reduce the nicotine content of the tobacco material, although the result also suggest that a high ratio of water : tobacco material may lead to the removal of a significant quantity of nicotine.

Analysis: Recovery of Tobacco Material

In each of the experiments, the residual whole leaf tobacco material which remained after treatment was weighed in order to determine the percentage of the material which remained after treatment (% DWB)

The masses of residual tobacco recovered in each of the two experiments are detailed below in Table 6.

Table 6

Experiment	**Mass of Residual Tobacco Material (% DWB)**
1	n/a
2	5.9
3	16.5
4	14.6
5	14.2
6	13.6
7	4.9
8	3.7
9	6.2
10	10.9
11	9.1
12	49.7

The results provided in Table 6 indicate that many chemical substances must be removed from tobacco material by steam hydrolysis aside from protein and polyphenol compounds. These chemical substances are likely to include various polysaccharides, such as cellulose and certain sugars, as well as nucleic acids, in the form of both DNA and RNA. Results further suggest that the higher the ratio of water to tobacco material, the greater the quantity of compounds removed from the tobacco material.

The series of experiments and their results discussed herein provide the skilled person with guidance as to how to select the starting material and/or the processing parameters to ensure that the treatment process provides a treated product with the desired properties, including the extent of reduction in polyphenol, protein or other compound content.

In order to address various issues and advance the art, the entirety of this disclosure shows by way of illustration various embodiments in which the claimed invention(s) may be practiced and provide for superior tobacco treatment, tobacco material, and products incorporating tobacco material. The advantages and features of the disclosure are of a representative sample of embodiments only, and are not exhaustive and/or exclusive. They are presented only to assist in understanding and teach the claimed features. It is to be understood that advantages, embodiments, examples, functions, features, structures, and/or other aspects of the disclosure are not to be considered limitations on the disclosure as defined by the claims or limitations on equivalents to the claims, and that other embodiments may be utilised and modifications may be made without departing from the scope and/or spirit of the disclosure. Various embodiments may suitably comprise, consist of, or consist essentially of, various combinations of the disclosed elements, components, features, parts, steps, means, etc. In addition, the disclosure includes other inventions not presently claimed, but which may be claimed in future.

Claims

1. A method for treating a tobacco material, wherein the method comprises treating the tobacco material by steam hydrolysis.

2. A method according to claim 1, wherein the method results in a reduction in the polyphenol content of the tobacco material compared to the polyphenol content of the untreated tobacco material.

3. A method according to any one of the preceding claims, wherein the method does not substantially reduce the amount of nicotine in the tobacco material.

4. A method according to any one of the preceding claims, wherein the method results in a reduction in the protein content of the tobacco material compared to the protein content of the untreated tobacco material.

5. A method according to any one of the preceding claims, wherein the tobacco material is suspended in an aqueous medium which is heated to generate the steam for the steam hydrolysis.

6. A method according to claim 5, wherein the ratio of aqueous medium to tobacco material is at least 1:1 by weight.

7. A method according to any one of the preceding claims, wherein the method comprises ohmic heating.

8. A method according to claim 7, wherein the aqueous solution contains sufficient ions to provide electrical conductivity for ohmic heating.

9. A method according to any one of the preceding claims, wherein the method is carried out under elevated pressure.

10. A method according to claim 9, wherein the pressure is released after an optional period of time at the maximum pressure.

11. A method according to any one of claims 5 to 10, wherein the tobacco material treated by steam hydrolysis is subsequently separated from the aqueous medium in which it was suspended.

12. A method according to any one of the preceding claims, wherein the method of the invention may further comprise: treating the tobacco material with one or more enzymes; treating the tobacco material with one or more surfactants; treating the tobacco material with one or more adsorbents; and/or treating the tobacco material with one or more non-aqueous liquids.

13. A tobacco material which has been treated by a method according to any one of the preceding claims, or a derivative thereof.

14. A smoking article which comprises a tobacco material according to claim 13 or a derivative of thereof.

15. Use of steam hydrolysis for removing one or more polyphenols from a tobacco material.

Figure 1

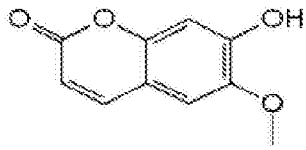
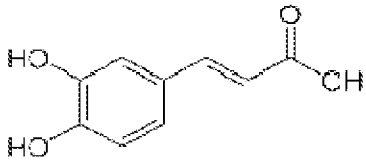
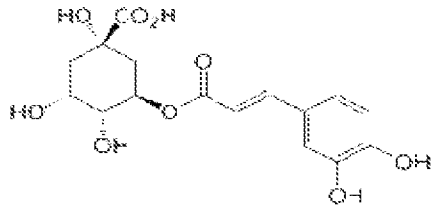
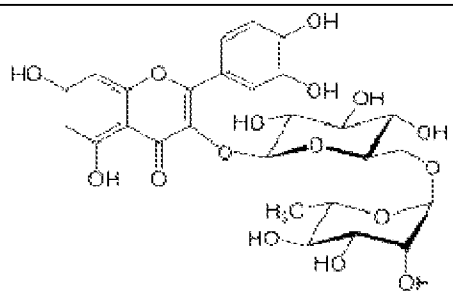
Polyphenol	Chemical Structure
Scopoletin	
Caffeic acid	
Chlorogenic acid	
Rutin	

Figure 2

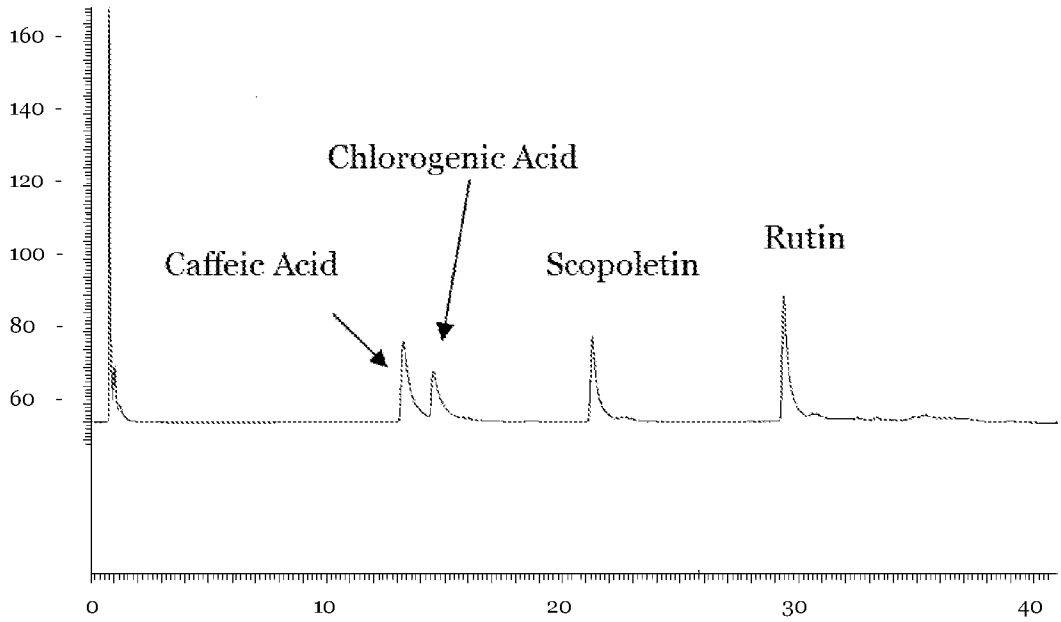


Figure 3

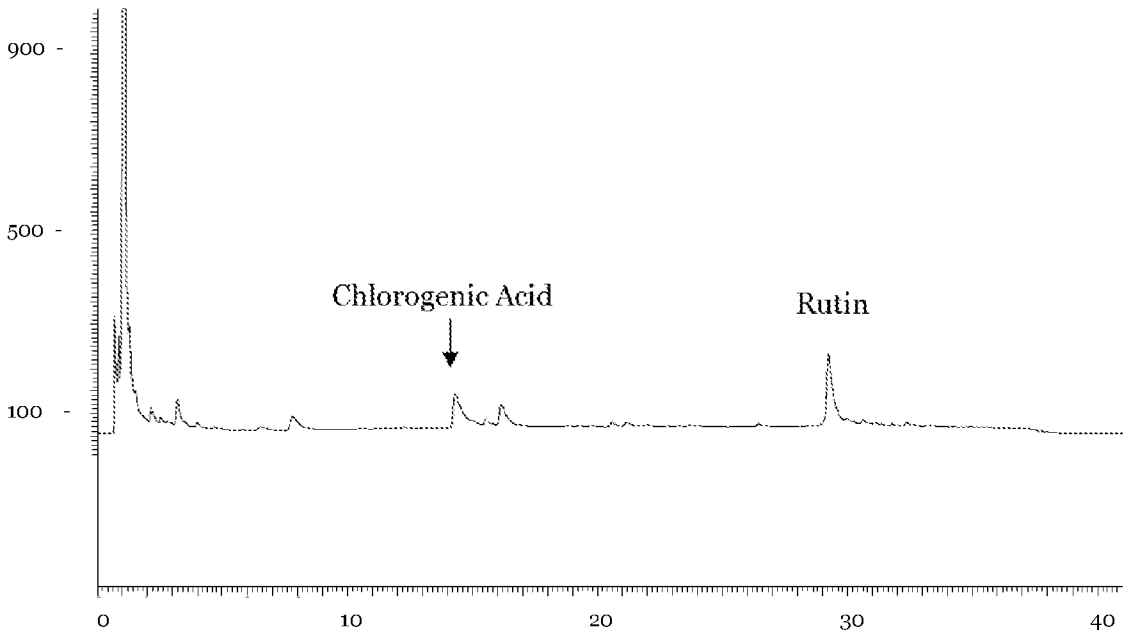


Figure 4

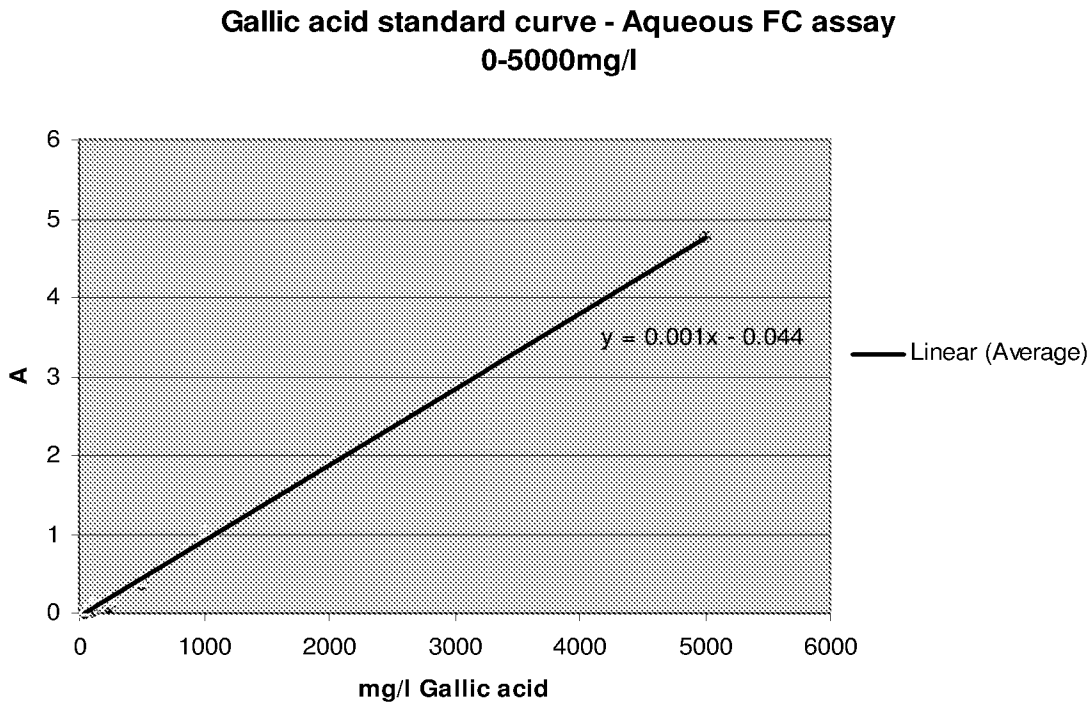


Figure 5

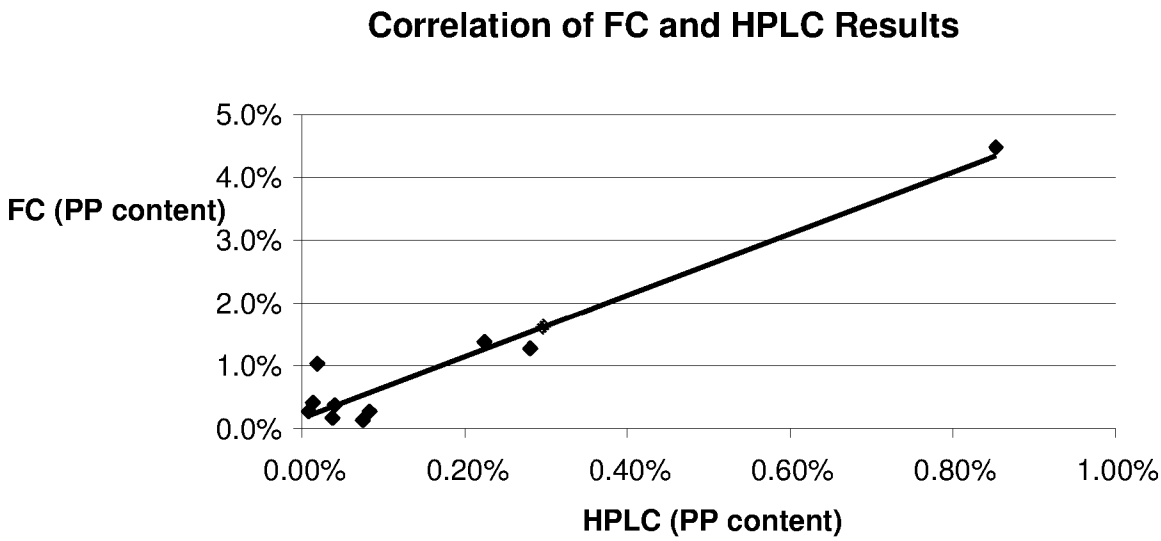


Figure 6

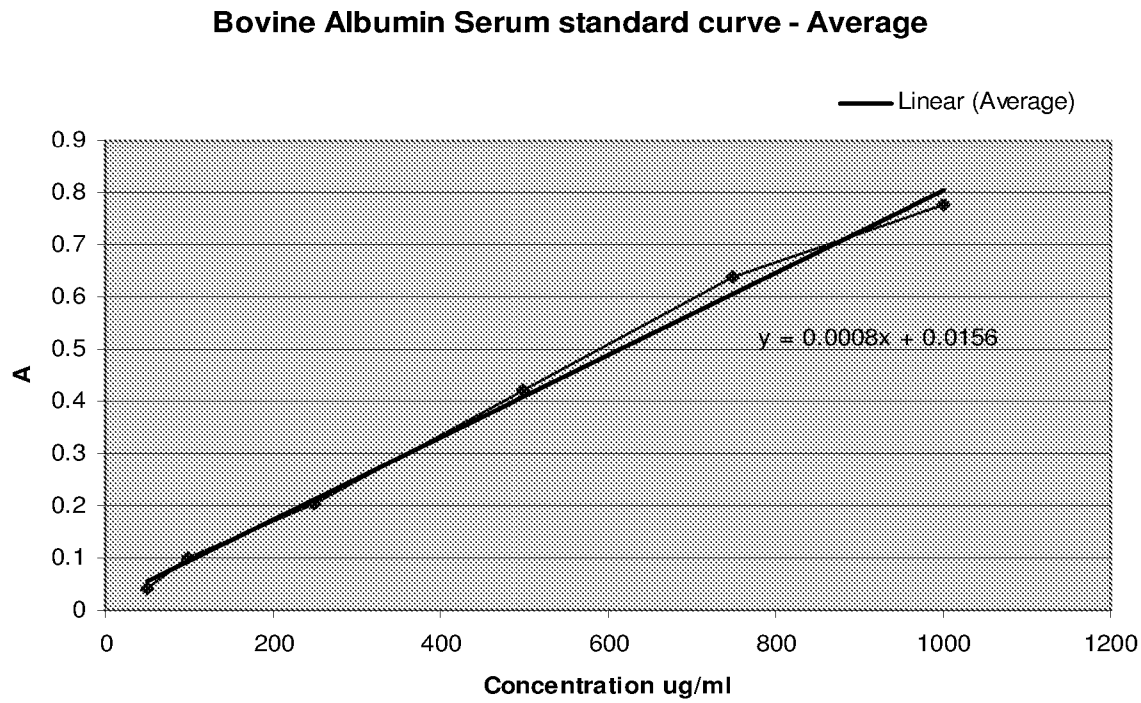
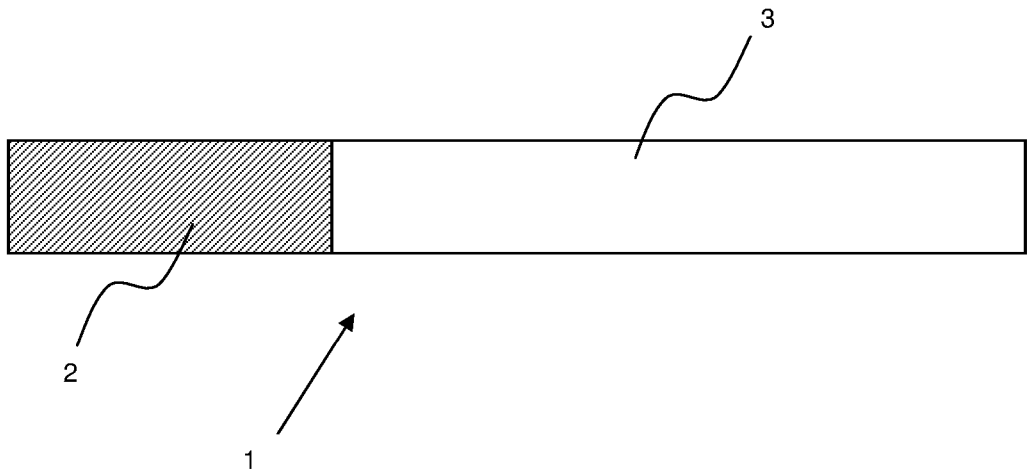


Figure 7



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/053104

A. CLASSIFICATION OF SUBJECT MATTER
INV. A24B15/18 A24B15/24
ADD.

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)
A24B

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 1 192 338 A (LI TIEYING [CN]) 9 September 1998 (1998-09-09)	1-14
A	the whole document	15

X	CN 102 391 390 A (CHONGQING HENGYUAN JINTONG TECHNOLOGY CO LTD) 28 March 2012 (2012-03-28)	1-14
A	the whole document	15

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2014	21/02/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Dimoula, Kerasina

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2013/053104

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 1192338 A	09-09-1998	NONE	
CN 102391390 A	28-03-2012	NONE	