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(54) **TOBACCO PRODUCT LABELING SYSTEM**

(76) Inventors: **Susan Reich**, Mamaroneck, NY (US); **Mark Conkling**, Chapel Hill, NC (US); **Steve Zelson**, Mamaroneck, NY (US)

Correspondence Address:
KNOBBE MARTENS OLSON & BEAR LLP
2040 MAIN STREET
FOURTEENTH FLOOR
IRVINE, CA 92614 (US)

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(57) **ABSTRACT**

The disclosed invention relates to visual content indicators of a tobacco product. More specifically, the invention includes labeling systems that provide at least one visual indicator of a tobacco product, methods for using the systems, and tobacco products that display one or more visual indicators of content. The present invention generally relates to tobacco products, which have a reduced amount of nicotine and tobacco specific nitrosamines (TSNAs), and contain one or more nicotine substitutes. The use of these tobacco products to reduce the exposure of an individual to nicotine, as well as, facilitating tobacco-use cessation is disclosed.

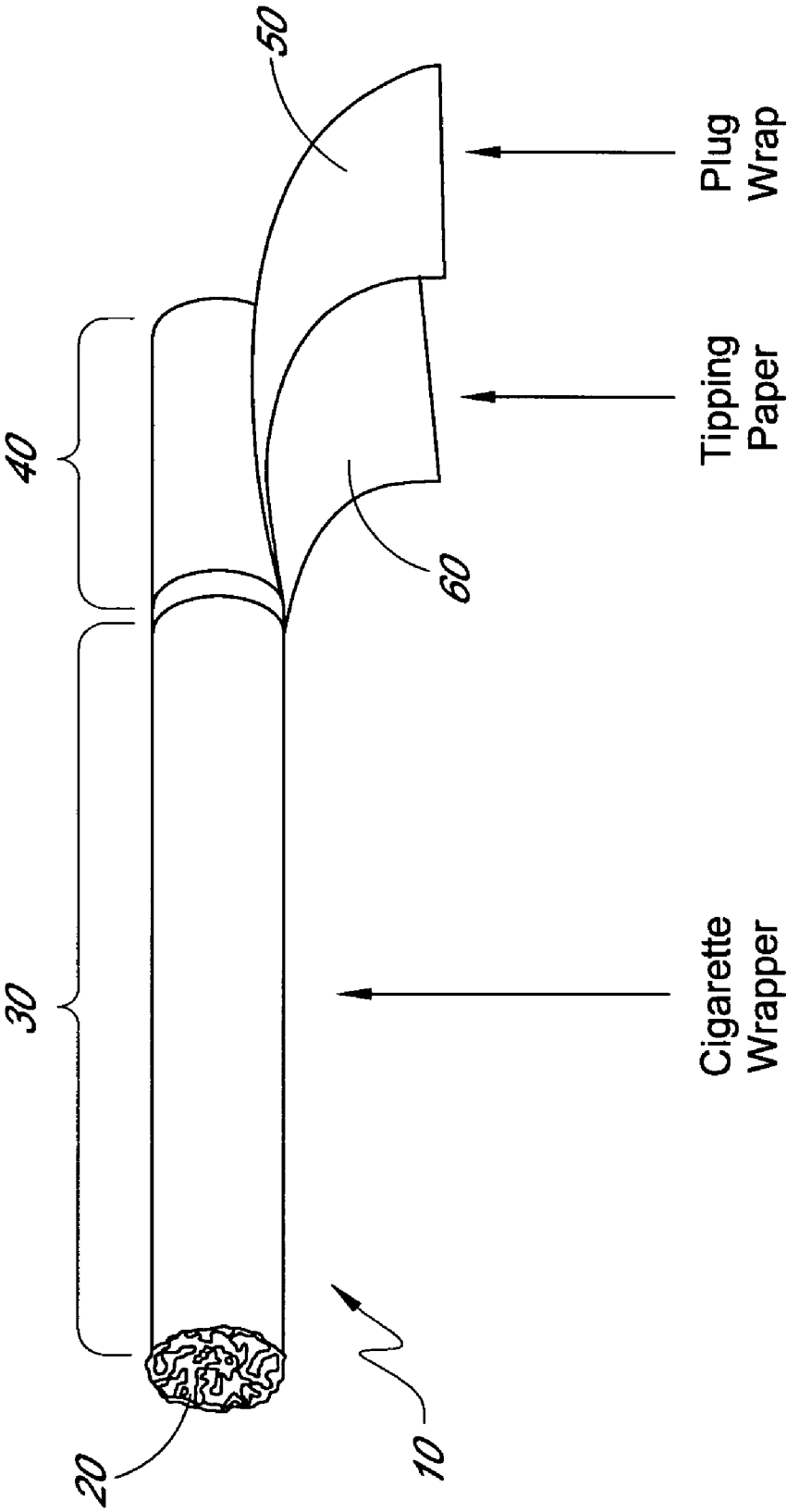

FIG. 1

TOBACCO PRODUCT LABELING SYSTEM

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This Application is a continuation of and claims the benefit of priority to PCT/US2004/029953, which designates that United States and was published in English, filed Sep. 13, 2004, which claims the benefit of priority to U.S. Provisional Application No. 60/509,042, filed Oct. 2, 2003; all of the above-referenced applications are hereby expressly incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention relates to visual indicators that denote the presence or content level of one or more substances found in a tobacco product. More specifically, tobacco product labeling systems that provide at least one visual content indicator of a tobacco product, methods for using these systems, and tobacco products that display one or more visual indicators of content are disclosed. The present invention generally relates to tobacco products, which have a reduced amount of nicotine and tobacco specific nitrosamines (TSNAs), and contain one or more nicotine substitutes. The use of these tobacco products to reduce the exposure of an individual to nicotine, as well as, facilitating tobacco-use cessation is disclosed.

BACKGROUND OF THE INVENTION

[0003] Tobacco product packaging comes in a variety of forms. Typically the packaging is directed to branding, where the packaging provides visual clues to consumers allowing them to recognize their preferred product. In many cases, these visual clues are trademarks or trade dress associated with a particular manufacturer.

[0004] Labeling of cigarettes is discussed, for example, in U.S. Pat. No. 4,700,725. Methods of marking cigarette papers are also known. For example, see U.S. Pat. No. 4,319,587, which provides a graduated or labeled cigarette paper that allows a smoker to determine how much a cigarette to consume. Other means of labeling cigarettes include a bar code that can be scanned with a laser (U.S. Pat. No. 5,377,697), a tactile indicator (U.S. Pat. No. 4,699,158), and coding of cigarettes by means of perforations in the filter (U.S. Pat. No. 4,766,911). One drawback of these labeling systems is that they can be very complicated and thus make it difficult for a tobacco user to decipher. For example, the labeling system provided in U.S. Pat. No. 5,377,697 requires the use of a laser scanner to read. In addition, the system in U.S. Pat. No. 4,766,911 uses small perforations to code cigarettes, thereby allowing the manufacturer to trace and identify these cigarettes. Accordingly, there is a need in the art for a labeling system that denotes the contents of a tobacco product in a way that is readily and quickly identifiable to a tobacco user.

[0005] The addictive properties of tobacco products are largely attributable to the presence of nicotine and the habitual use of the delivery system (e.g., the oral fixation associated with the act of smoking or chewing tobacco, smoke intake, and taste). Many tobacco-use cessation programs involve the use of nicotine replacement therapy (NRT), wherein various amounts of nicotine are given to the individual as a replacement for tobacco use. The factors involved with the habitual use of the delivery system are hereinafter referred to as "secondary factors of addiction." In addition to the fact that conventional NRT does little to quell the secondary factors of addiction, NRT has had only limited success in enabling people to quit tobacco use. Accordingly, there remains a need for products that address both primary and secondary factors of tobacco addiction and tobacco-use cessation programs that employ these products.

SUMMARY OF THE INVENTION

[0006] Some embodiments of the invention relate to cigarettes labeled with a visual content indicator, wherein the visual content indicator denotes a content level of one or more substances contained within the cigarette. In one aspect of this embodiment, the visual content indicator denotes a pH content level, a nicotine content level, one or more additive levels, one or more carcinogen levels, a carbon monoxide level, a nitric oxide level, a tar content level, or one or more tobacco-specific nitrosamine (TSNA) content levels of the cigarette. In another aspect of this embodiment, the content level is selected from the group consisting of a base content level, a reduced content level, and an essentially free content level.

[0007] Other embodiments of the invention provide for cigarettes labeled with a base nicotine content indicator. In a specific aspect of the invention, the cigarette labeled with the base nicotine content indicator contains approximately 0.6 mg nicotine, although any quantity of nicotine may be present and indicated in the cigarette or other tobacco product. An example of a particular base nicotine content indicator is a ring or bar on a cigarette paper.

[0008] Some embodiments of the disclosed invention relate to a cigarette labeled with a reduced nicotine content indicator. In one aspect of this embodiment, the reduced nicotine content indicator denotes the presence of approximately 0.3 mg nicotine in the labeled cigarette. The exact quantity of nicotine present and indicated in the cigarette or other tobacco product is not essential to the invention, so long as the content of nicotine in the reduced nicotine product is less than that present in the base nicotine product. An example of a particular reduced nicotine content indicator is two rings or two bars on a cigarette paper.

[0009] Another embodiment of the invention relates to a cigarette labeled with an essentially nicotine-free content indicator. In one aspect of this embodiment, the essentially nicotine-free content indicator denotes the presence of approximately 0.05 mg nicotine in the labeled cigarette. The exact quantity of nicotine present and indicated in the cigarette or other tobacco product is not essential to the invention, so long as the content of nicotine in the essentially nicotine-free nicotine product approaches less than one third of that found in a product labeled with a reduced content indicator. For example, an essentially nicotine-free content indicator can denote the presence of approximately 0.05 mg of nicotine in the labeled product. An example of a particular essentially nicotine-free content indicator is three rings or three bars on a cigarette paper.

[0010] Another embodiment of the invention relates to a cigarette having a visual content indicator denoting the content of one or more carcinogens selected from the group consisting of 4-aminobiphenyl, benzene, cadmium, chromium, 2-naphthylamine, nickel, polonium-210 (radon),

vinyl chloride, acrylonitrile, benzo[a]pyrene, 1,3-butadiene, dibenz(a,h)anthracene, formaldehyde, N-nitrosodiethylamine, N-nitrosodimethylamine, acetaldehyde, benzo[b]fluoranthene, benzoo]fluoranthene, benzo[k]fluoranthene, dibenz[a,h]acridine, dibenz[a,j]acridine, 7H-dibenz[c,g]carbazole, dibenzo(a,i)pyrene, dibenzo(a,l)pyrene, 1,1-dimethylhydrazine, hydrazine, indeno[1,2,3-cd]pyrene, lead, 5-methylchrysene, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 2-nitropropane, N-nitrosodiethanolamine, N-nitrosomethylethylamine, N-nitrosomorpholine, N'-nitrosonornicotine (NNN), N-nitrosopyrrolidine, quinoline, ortho-toluidine, urethanechrysene, crotonaldehyde, N'-nitrosoanabasine (NAB), and N'-nitrosoanatabine (NAT).

[0011] Another embodiment of the invention relates to a tobacco product labeling system. Typically the labeling system comprises a content indicator, wherein the content indicator denotes relative content levels of a tobacco product labeled with the content indicator, wherein the content indicator is applied to the tobacco product. In further embodiments, the content indicator is a non-alpha/numerical indicator. In particular embodiments, the tobacco product is labeled with a plurality of content indicators, wherein each indicator denotes the content of a different tobacco product component. In one aspect of this embodiment, the content indicator denotes a pH content level, a nicotine content level, one or more additive levels, one or more carcinogen levels, a carbon monoxide level, a nitric oxide level, a tar content level, or one or more TSNA content levels of a tobacco product. In another aspect, the content indicator is a nicotine content indicator selected from the group consisting of a base nicotine content indicator, a reduced nicotine content indicator, and an essentially nicotine-free content indicator. Typically, the reduced nicotine content indicator will appear on a tobacco product containing approximately half the nicotine of a tobacco product labeled with the base nicotine content indicator. Frequently, the essentially nicotine-free content indicator will appear on a tobacco product containing approximately one sixth the nicotine of a tobacco product labeled with the reduced nicotine content indicator. In various embodiments, the tobacco product labeling system is utilized to label a cigarette, a cigarette package, a carton of cigarette packages, tobacco gum and their wrappers and containers, tobacco lozenges and their wrappers and containers, snuff containers, and chewing tobacco containers.

[0012] In another embodiment, the tobacco product labeling system is used to label a cigarette with a base nicotine content indicator. In one aspect of this embodiment, the cigarette labeled with the base nicotine content indicator contains approximately 0.6 mg nicotine. The exact quantity of nicotine present and indicated in the cigarette or other tobacco product is not essential to the invention. An example of a base nicotine content indicator is a ring, stripe, or bar.

[0013] Other embodiments of the invention provide for the tobacco product labeling system being used to label a cigarette with a reduced nicotine content indicator. In one aspect of this embodiment, the cigarette labeled with the reduced nicotine content indicator contains approximately 0.3 mg nicotine. The exact quantity of nicotine present and indicated in the cigarette or other tobacco product is not essential to the invention, so long as the content of nicotine in the reduced nicotine product is less than that present in the base nicotine product. An example of a reduced nicotine content indicator is two rings, two stripes, or two bars.

[0014] Another embodiment of the invention provides for the tobacco product labeling system being used to label a cigarette with an essentially nicotine-free content indicator. In one aspect of this embodiment, the cigarette labeled with the essentially nicotine-free content indicator contains approximately 0.05 mg nicotine. The exact quantity of nicotine present and indicated in the cigarette or other tobacco product is not essential to the invention, so long as the content of nicotine in the essentially nicotine-free nicotine product approaches less than one third of that found in a product labeled with a reduced content indicator. An example of an essentially nicotine-free content indicator is three rings, three stripes, or three bars.

[0015] Another embodiment of the invention relates to a method for labeling tobacco products. This method includes the steps of providing a system of labeling comprising a content indicator, wherein the content indicator denotes relative content of a tobacco product component in a tobacco product labeled with the content indicator and labeling the tobacco product with the content indicator that reflects the content of the tobacco product component in the tobacco product system of labeling. In certain embodiments, the content indicator is a non-alpha/numerical indicator. In other embodiments, an alpha/numerical indicator is used.

[0016] In other embodiments, the tobacco product is labeled with a plurality of content indicators, wherein each content indicator denotes the content of a different tobacco product component. In certain embodiments the content indicator reflects the content of the tobacco product component in the tobacco product the system of labeling. In one aspect of this embodiment, the content indicator denotes a pH content level, a nicotine content level, one or more additive levels, one or more carcinogen levels, a carbon monoxide level, a nitric oxide level, a tar content level, or one or more TSNA content levels of the tobacco product. In another aspect of this embodiment, the content indicator is a nicotine content indicator selected from the group consisting of a base nicotine content indicator, a reduced nicotine content indicator, and an essentially nicotine-free content indicator.

[0017] In one embodiment of the invention, the reduced nicotine content indicator appears on a tobacco product containing approximately half the nicotine of a tobacco product labeled with the base nicotine content indicator. An aspect of this embodiment provides that the essentially nicotine-free content indicator appears on a tobacco product containing approximately one sixth the nicotine of a tobacco product labeled with the reduced nicotine-level indicator. In this embodiment, the tobacco product is a cigarette, cigarette package, or cigarette carton.

[0018] In another embodiment, a cigarette labeled with a base nicotine content indicator that denotes the presence of approximately 0.6 mg nicotine in the labeled cigarette. The exact quantity of nicotine present and indicated in the cigarette or other tobacco product is not essential to the invention. An example of a base nicotine content indicator is a ring, stripe, or bar.

[0019] In a further embodiment, a cigarette is labeled with the reduced nicotine content indicator that denotes the

presence of approximately 0.3 mg nicotine. The exact quantity of nicotine present and indicated in the cigarette or other tobacco product is not essential to the invention, so long as the content of nicotine in the reduced nicotine product is less than that present in the base nicotine product. An example of a reduced nicotine content indicator is two rings, two stripes, or two bars.

[0020] Another embodiment of the disclosed invention provides for a cigarette labeled with the essentially nicotine-free content indicator. In an aspect of this embodiment, the cigarette labeled with the essentially nicotine-free content indicator contains approximately 0.05 mg nicotine. The exact quantity of nicotine present and indicated in the cigarette or other tobacco product is not essential to the invention, so long as the content of nicotine in the essentially nicotine-free nicotine product approaches less than one third of that found in a product labeled with a reduced content indicator. An example of an essentially nicotine-free content indicator is three rings, three stripes, or three bars.

[0021] Aspects of the invention include a tobacco product comprising a modified tobacco that has less nicotine than a tobacco of the same variety and a nicotine substitute comprising Cytisine. In some embodiments, the tobacco is a genetically modified tobacco, which may be, for example, a Virginia Flue tobacco, an Oriental tobacco, or a Burley tobacco. In some embodiments the modified tobacco comprises 0.6 mg of nicotine or less, in others, the modified tobacco comprises 0.3 mg of nicotine or less, and in still others, the modified tobacco comprises 0.05 mg of nicotine or less.

[0022] The nicotine substitute can be added to the tobacco of said tobacco product and, in preferred embodiments, the tobacco product is a cigarette. In some embodiments, the nicotine substitute is added to the paper or filter of the cigarette. Preferably, the tobacco product comprises an amount of Cytisine that is sufficient to deliver less than 2.0 mg, 1.5 mg, 1.0 mg, or 0.05 mg of Cytisine to a consumer.

[0023] Additional embodiments concern methods of reducing the exposure of a tobacco consumer to nicotine comprising providing to said tobacco consumer a tobacco product comprising a modified tobacco that has less nicotine than tobacco of the same variety, and a nicotine substitute comprising Cytisine. In some embodiments, the modified tobacco can be a Burley tobacco, a Virginia Flue tobacco, or an Oriental tobacco. Preferably, the modified tobacco is a genetically modified tobacco. The modified tobacco in these methods can comprise 0.6 mg of nicotine or less, 0.3 mg of nicotine or less, or 0.05 mg of nicotine or less. The nicotine substitute is preferably added to the tobacco of said tobacco product in these methods and the tobacco product can be a cigarette. In some applications, the nicotine substitute is added to the paper or filter of said cigarette.

[0024] Preferably, in the methods above, the tobacco product comprises an amount of Cytisine that is sufficient to deliver less than 2.0 mg, 1.5 mg, 1.0 mg, or 0.05 mg of Cytisine to a consumer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **FIG. 1** shows a schematic representation of a cigarette.

DETAILED DESCRIPTION OF THE DISCLOSED INVENTION

[0026] The disclosed invention relates to visual indicators that indicate a content level of one or more substances present in a tobacco product. More specifically, the invention includes a tobacco product labeling system that provides a visual indication of the contents of a tobacco product, a method for using the system, and tobacco products that display one or more visual indicators of content. The visual indicators of content can be used to denote the presence or content level of a variety of ingredients or components in a tobacco product.

The Visual Indicators of Content

[0027] A wide variety of visual indicators can be used to provide an outward indication of one or more content levels of a tobacco product. In general, content indicators can denote whether a particular compound is actually present within a tobacco product. In more specific embodiments, content indicators can denote the amount or level of a compound present in a compound. In certain aspects, the amount or level of a compound can be indicated as a mass, a percentage of total mass of the tobacco product, a volume, or as a percentage of total volume of the tobacco compound. Further, the amount or level of a particular compound can be identified as a specific value, an approximate value, or as a range of values.

[0028] In other embodiments content indicators can denote a tobacco product's composition of smokable material (e.g., tobacco blend) or composition of materials from which the tobacco product is manufactured (e.g., filter material or wrapping material). In other embodiments, the use of visual content indicators to denote a tobacco product's composition of materials is expressly excluded. In certain embodiments, the content indicators denote the presence or level of one or more particular chemical compounds. In more specific embodiments, denoted chemical compounds can include compounds whose presence or content levels have not been traditionally indicated on a tobacco product. Examples of such compounds include nicotine and TSNAs.

[0029] In one aspect of the invention, the content indicator can be alpha/numerical. Alpha/numeric indicators of content include letters, numbers, or other symbols, such as punctuation marks and mathematical symbols. In some embodiments, numbers, such as 1, 2, and 3, can be used as visual alpha/numeric indicators of content. In certain embodiments the alpha/numeric indicators can include, for example, only numbers, only letters, only punctuation marks, or only mathematical symbols. In other embodiments the alpha/numeric indicator can include some combination of the four or other alpha/numeric characters. For example, the alpha/numeric indicator can include a combination of letters, numbers and mathematical symbols. As another example, a combination of letters, numbers, mathematical symbols, and punctuation marks can be used as an alpha/numeric indicator.

[0030] In other embodiments, non-alpha/numeric indicators can be used as a content indicator. Non-alpha/numeric indicators of content include non-alpha/numeric symbols, such as patterns, lines, waves, dotted bands, colors, and the like. Graphics, such as pictures or images are also included

under the rubric of non-alpha/numeric indicators of content. In some embodiments, rings, geometric symbols, colors, stripes, bars, shades of colors, or combinations thereof can be used as non-alpha/numeric visual indicators of content. For example, in some embodiments, a single ring can be used to indicate the presence of a base content level of a particular component of a tobacco product. Two rings can be used to indicate the presence of a reduced content level of the particular component of the tobacco product. Three rings can be used to indicate that the labeled product is essentially free of the particular component of interest. In certain embodiments, the rings are imprinted on the tobacco product.

[0031] In another embodiment, tobacco products with visual indicators denoting a base content level, a reduced content level and an essentially free content level can be used as part of a tobacco-use cessation program. In one example, these content indicators can denote the level of nicotine in a tobacco product, such as a cigarette. A subject who wishes to stop using tobacco can start with a tobacco product having a particular base nicotine content indicator. After a certain time period, the user can begin using a tobacco product with a reduced nicotine content indicator. The tobacco user can then use a tobacco product having an essentially nicotine-free content indicator. After a certain period, the tobacco user can stop using tobacco products altogether. In another embodiment, a tobacco user can start the tobacco-use cessation program by first using a tobacco product having a reduced nicotine content indicator, and then move to a tobacco product having an essentially nicotine-free content indicator, before quitting altogether. Tobacco-use cessation programs are provided in detail in U.S. Provisional App. No. 60/475,945, entitled "Method of Reducing the Harmful Effects of Orally Transdermally Delivered Nicotine," filed on Jun. 4, 2003, U.S. Provisional Application No. 60/371,635, entitled "Tobacco Having Reduced Nicotine and Nitrsoamines," filed on Apr. 9, 2002, and U.S. Provisional Application No. 60/486,875, entitled "Tobacco Products Containing Low Nicotine Tobacco and Nicotine Replacement Compounds," filed on Jul. 10, 2003 (attorney docket No. VTOB.273PR), all of which are hereby incorporated by reference in their entireties. Visual indicators can be used with these programs and other tobacco-use cessation programs.

[0032] In more specific embodiments, a tobacco product with a base nicotine content indicator can contain approximately 0.6 mg of nicotine. In other embodiments, the base nicotine tobacco product can contain approximately 11.0 mg, 10.0 mg, 9.0 mg, 8.0 mg, 7.0 mg, 6.0 mg, 5.0 mg, 4.0 mg, 3.0 mg, 2.0 mg, 1.0 mg, or 0.5 mg of nicotine, for example. In a particular embodiment, a tobacco product with a reduced nicotine content indicator can contain approximately 0.3 mg of nicotine. In other embodiments, the reduced nicotine tobacco product can contain approximately 10.9 mg, 10.0 mg, 9.0 mg, 8.0 mg, 7.0 mg, 6.0 mg, 5.0 mg, 4.0 mg, 3.0 mg, 2.0 mg, 1.0 mg, 0.5 mg, 0.4 mg, 0.2 mg, or 0.15 mg of nicotine, for example. In another embodiment, a tobacco product with an essentially nicotine-free content indicator contains no more than 0.05 mg of nicotine. In other embodiments, the essentially nicotine-free tobacco product can contain no more than approximately 1.0 mg, 0.9 mg, 0.8 mg, 0.7 mg, 0.6 mg, 0.5 mg, 0.4 mg, 0.3 mg, 0.2 mg, 0.15 mg, or 0.1 mg of nicotine, for example.

[0033] In other embodiments, the number of non-alpha/numeric symbols can be constant while different colors, shading, or patterns can be used to visually indicate the content of a particular additional component of a tobacco product. For example, a single ring can be placed on a tobacco product to indicate nicotine content while the ring can be presented in different colors to indicate content levels of another ingredient in the tobacco product such as, for example, presence or absence of a flavorant such as menthol.

[0034] Various combinations of visual content indicators can also be used to provide content information regarding a particular tobacco product. For example, in particular embodiments, a single ring can be placed on a tobacco product to indicate the nicotine content of the tobacco product while one or more additional visual indicators can also be present to indicate the content of another tobacco component, such as tar, carbon monoxide, nitric oxide, one or more carcinogens, one or more additives, and the like. Various exemplary types of tobacco-product contents that can be represented by visual indicators are discussed more fully below.

[0035] The visual indicators of content can be presented using colors, printed textures, or using other artistic media of expression that allow one to discern one indicator from another. In some embodiments, the content indicators are imprinted on the tobacco product. Standard printing techniques can be used to place the visual indicators on the tobacco products. In embodiments directed to directly labeling lozenges and gum, the visual content indicator can be impressed or stamped directly onto the lozenge or gum. Additionally, visual indicators can be presented as holograms. Methods of making holograms are found in U.S. Pat. No. 5,796,500, to Hart, entitled "Methods and Apparatus for Making Holograms," issued on Aug. 18, 1998, and methods of impressing holograms onto paper are provided in U.S. Pat. No. 5,862,750, to Dell'Olmo, entitled "Method for Impressing Directly on Paper Holograms, Kinetic Holograms, Diffraction Patterns or Microengravings Producing other Optical Effects," issued on Jan. 26, 1999; each of the foregoing is hereby expressly incorporated by reference in its entirety. Visual indicators, such as holograms, can also be affixed directly to a tobacco product.

[0036] In certain embodiments, the content indicators can include perforations in the tobacco product. In other embodiments, the use of perforations as content indicators is expressly excluded. It is important to note that for embodiments wherein the use of perforations as content indicators is expressly excluded, the tobacco product can still have perforations for other purposes, such as for intermixing air with smoke. In further embodiments, the visual indicators can include a bar code on the tobacco product that can be read by a laser scanner. In other embodiments, the use of bar codes as content indicators is expressly excluded. In further embodiments, content indicators that require a laser scanner to be discerned are also expressly excluded. Still in further embodiments, a content indicator can be identified tactilely. In other embodiments the use of bar codes and/or tactile content indicators is expressly excluded.

[0037] In still other embodiments, the tobacco product bares content indicators that can be readily or quickly identified by a tobacco user. The ease of identifying a content indicator typically depends on the total number and

types of content indicators present on a tobacco product. In general, the fewer total number and types of content indicators used, the more readily a tobacco user can identify the contents of the tobacco product.

[0038] In other embodiments, the tobacco products described herein can include a relatively large number and/or variety of content indicators. In certain aspects, the tobacco products can include at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more, content indicators, which can be the same or different in type, size, and shape.

[0039] The visual indicators can be located anywhere on the tobacco product. For example, one or more visual indicators can be located on a tobacco product package or wrapper. For example, in some embodiments, such indicators can be included on a cigarette carton, cigarette package, cigar, cigar wrapper or container, tobacco gum, tobacco gum wrapper or container, tobacco lozenge, tobacco lozenge wrapper or container, chewing tobacco container, or a snuff container. In another embodiment, the tobacco product is a cigarette and the one or more visual indicators of content can be located anywhere on the cigarette.

[0040] **FIG. 1** shows a schematic representation of a cigarette **10**. The cigarette comprises a tobacco rod **20**. The tobacco rod typically comprises a combined form of shredded and blended tobacco wrapped in cigarette paper **30**. Some cigarettes further comprise a filter or plug **40**, which is shown in **FIG. 1** as being wrapped with plug wrap **50** and tipping paper **60**. One or more visual indicators of content can be placed anywhere on the cigarette, for example, on the cigarette paper **30**, the tipping paper **60**, anywhere on the filter or plug **40**, or at the junction between the cigarette paper and the tipping paper. In further embodiments, the filter end of the cigarette is the primary location of any content indicator(s).

[0041] Visual indicators of content can be placed on a tobacco product in any orientation. For example, the visual indicators can be placed circumferentially on a tobacco product. In an alternate embodiment, one or more visual indicators of content are placed axially on a tobacco product.

[0042] The above-description of visual indicators is applicable to the tobacco products, tobacco product labeling systems, and methods of labeling tobacco products provided herein. Specifically, the tobacco products can be labeled with these visual indicators, and the tobacco product labeling systems and methods of labeling can be used to label tobacco products with these visual indicators.

[0043] The present invention concerns nicotine reduction and/or tobacco-use cessation programs, which involve the use of modified tobacco products that have reduced amounts of nicotine and TSNAs, but contain one or more nicotine substitutes. While most tobacco cessation programs rely exclusively on nicotine replacement therapy (NRT) or the administration of a pharmaceutical containing a nicotine substitute, many of the embodiments described herein provide and maintain the secondary factors of addiction, such as smoke intake, oral fixation, and taste, while gradually reducing the amount of nicotine ingested and, in some products and applications, substituting the nicotine in a reduced nicotine tobacco product with a nicotine substitute.

[0044] Some embodiments of the invention concern the use of reduced nicotine and TSNA tobacco products that have burning and taste characteristics that are virtually indistinguishable from conventional tobacco products. To these tobacco products, as well as conventional tobacco products, nicotine substitutes are added. While there are many ways to create reduced nicotine and/or TSNA tobacco, the preferred methods use techniques in plant genetic engineering to reduce or eliminate enzymes involved in nicotine biosynthesis. Preferably, techniques in plant genetic engineering are used to selectively reduce the amount of the enzyme quinolate phosphoribosyl transferase (QPTase), which is involved in the production of nicotine at the root cortex. A co-pending United States Patent Application entitled "Modifying Nicotine and Nitrosamine levels in Tobacco" (WO02100199), which was published in English designating the United States of America and claiming priority to U.S. Provisional Application No. 60/371635, is hereby incorporated by reference in its entirety. Additionally, U.S. patent application Ser. No. 09/941,042, entitled "Transgenic Plants Containing Molecular Decoys that Alter Protein Content Therein" and U.S. patent application Ser. No. 09/963,340, entitled "Regulation of Quinolate Phosphoribosyl Transferase Expression" are hereby expressly incorporated by reference in its entirety. There may be many ways to reduce levels of QPTase in tobacco plants, given the teachings described herein and the level of skill in the art, however, the preferred methods involve the use of antisense technology or molecular decoy technology.

[0045] By one approach, for example, a DNA construct encoding an antisense RNA that complements at least a portion of the QPTase gene is prepared such that transcription of the complementary strand of RNA reduces expression of the endogenous quinolate phosphoribosyl gene, which, in turn, reduces the amount of nicotine and, concomitantly, the amount of TSNA in the tobacco plant. By another approach, transcription factor molecular decoys for the QPTase gene, which are nucleic acid fragments that correspond to the **5'** upstream regulatory elements (e.g., Nic 1 and Nic 2 transcription factor binding sites) are inserted into the plant cell. The transcription factors bind to the decoy fragments rather than the endogenous transcription factor binding sites and a reduction in the level of transcription of QPTase is obtained.

[0046] Once the transgenic tobacco plants having reduced nicotine and/or TSNA are made, the tobacco is harvested and cured by conventional methods and is incorporated into a variety of tobacco products. Preferably, the transgenic tobacco is blended such that specific amounts of nicotine and/or TSNA are obtained in specific products. That is, the blending is conducted so that tobacco products of varying amounts of nicotine and/or TSNAs are made. In this manner, a step-wise tobacco-use cessation program can be established, wherein a program participant begins the program at step 1 with a tobacco product having only slightly less nicotine; at step 2 the program participant begins using a tobacco product with less nicotine than the products used in step 1; at step 3, the program participant begins using a tobacco product with less nicotine than the products in step 2; and so on, for as many steps as desired for a particular tobacco-use cessation program. Ultimately, the tobacco product used by the program participant can have an amount of nicotine that is less than that which is required to become addictive or maintain an addiction. In this manner, the exposure of a program participant to nicotine is reduced,

however, the secondary factors of addiction, including but not limited to, smoke intake, oral fixation, and taste are retained.

[0047] One or more nicotine substitutes (e.g., Cytisine) are then incorporated into the step-wise tobacco products above as complete substitutes for the amount of nicotine in the tobacco product or as a partial substitute such that the amount of nicotine substitute is gradually increased as the amount of nicotine in the product is reduced. For example, by one approach, a program participant begins the program at step 1 with a tobacco product having only slightly less nicotine than the product typically consumed by the individual but the loss of nicotine is compensated for by a nicotine substitute; at step 2 the program participant begins using a tobacco product with less nicotine than the products used in step 1 but more nicotine substitute; and at step 3, the program participant begins using a tobacco product with less nicotine than the products in step 2 but even more nicotine substitute; and so on, for as many steps as desired for a particular tobacco-use cessation program. By another approach, a program participant begins the program with a step 1 tobacco product that contains an amount of nicotine substitute sufficient to quell the urge for nicotine. At step 2, a tobacco product with less nicotine substitute than that used in step 1 is employed; and at step 3, a tobacco product with even less nicotine substitute than that in step 2 is used. The nicotine substitute can be added to the tobacco product by any way known in the art to associate compounds with tobacco or a tobacco product (e.g., reconstitution, fermentation, casing, processing, spraying, dipping, addition to the paper, filler, or the filter).

Tobacco Product Components

[0048] Tobacco products typically comprise tobacco and other components. Aspects of the invention can be used to provide visual indicators that represent the presence and quantity of one or more particular components of tobacco products. Various tobacco product components that can be represented using the visual indicators of content are discussed below. Examples include visual content indicators that denote a pH content level, a nicotine content level, one or more additive levels, one or more carcinogen levels, a carbon monoxide level, a nitric oxide level, a tar content level, or one or more TSNA content levels of a tobacco product.

Nicotine and TSNAs

[0049] The visual indicators of content disclosed herein can be used to indicate the quantity of nicotine present in a tobacco product. Health conscious consumers of tobacco products are becoming mindful of the additive characteristics of certain tobacco products and the role that nicotine plays in establishing such addictions. Nicotine is present in most tobacco products. For example, cigarettes typically comprise chopped tobacco leaf blended from two main varieties. The first type, yellowish 'bright', also known as Virginia flue where it was originally grown, typically contains around 2.5-3% nicotine. The second type, Burley tobacco, typically has a higher nicotine content of around 3.5-5%. Some blends, however, contain Oriental tobacco, which is also typically a low nicotine variety. As will be discussed in more detail below, methods are available to lower the naturally occurring nicotine content in tobacco.

[0050] Nicotine ($C_{10}H_{14}N_2$) is a naturally occurring alkaloid that has a potent stimulating effect. The body absorbs nicotine when one inhales the smoke from a tobacco product or liberates the compound when a tobacco product is masticated. Nicotine stimulates the central nervous system, increasing heart rate and blood pressure. Nicotine is toxic in large quantities and can cause death by paralyzing muscles involved in respiration.

[0051] Nicotine has been reported to be extremely addictive, on par with cocaine and heroin. Individuals that have become habituated to nicotine intake will typically experience negative physiological effects when nicotine is withdrawn. These physiological effects include strong cravings to ingest nicotine. These strong cravings are often accompanied by feelings of anxiety and irritability. Individuals suffering from nicotine withdrawal will frequently become restlessness, may gain weight, and may suffer from decreased concentration.

[0052] While it has not been scientifically demonstrated that nicotine itself is a carcinogen, TSNAs are highly carcinogenic. Formation of TSNAs occurs during curing and burning of tobacco. Nitrosamines are formed by the nitrosation of secondary and tertiary amines. These amines are present in tobacco products as nicotine, nornicotine, anabasine, and anatabine. Exemplary TSNAs include N-nitrosonornicotine (NNN), 4-(methylnitrosamineo)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), N'nitrosoantabine (NAT), N'-nitrosoanabasine (NAB), 4-(methylnitrosamino)-4-(3-pyridyl)butanal (NNA), 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (iso-NNAL), and 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid (iso-NNAC).

[0053] Tobacco-specific nitrosamines are found in smoking and smokeless tobacco products. Tobacco-specific nitrosamines are known to induce tumors of the lung, oral cavity, esophagus, pancreas, and liver. There is also evidence that TSNAs may be linked to cervical cancer and may also cause reproductive damage. TSNAs are known to bind to DNA and to hemoglobin. In fact, the TSNA adduct in blood is used to determine whether a subject has been exposed to tobacco smoke. Due to the harmful effects caused by nicotine and TSNAs, it would be advantageous to reduce the amounts of these compounds in tobacco products.

[0054] Accordingly, in some embodiments, one or more visual content indicators can be used to denote the content level of nicotine and/or one or more TSNAs in tobacco products containing reduced levels of nicotine and/or one or more TSNAs. As discussed in detail below, several methods for reducing endogenous levels of nicotine and TSNAs in a tobacco plant have been discovered. These approaches can be used to create the tobacco products described herein. In some embodiments, tobacco plants having a reduced amount of nicotine and/or TSNAs that retain good smoking characteristics and taste, when manufactured into tobacco products, can be used.

Approaches to Make Tobacco Products Having Reduced Nicotine and/or TSNA Levels

[0055] Nicotine is produced in tobacco plants by the condensation of nicotinic acid and 4-methylaminobutanal. Two regulatory loci (Nic1 and Nic2) act as co-dominant regulators of nicotine production. These two loci are unlinked and the gene action is semi-dominant and primarily additive (Legg et al. (1969) J. Hered., 60, 213-217).

[0056] Genetic and enzyme analyses have been used to investigate the Nic1 and Nic2 genes. Collins et al. ((1974) Crop Sci., 14, 77-80) prepared doubled haploid tobacco breeding lines of these four alkaloid genotypes. The genotype of standard cultivars is Nic1/Nic1 Nic2/Nic2 and that of low nicotine lines is nic1/nic1 nic2/nic2. Nic1/Nic1 nic2/nic2 is a high intermediate and nic1/nic1 Nic2/Nic2 is a low intermediate (Legg and Collins (1971) Can. J. Genet. Cytol. 13, 287-291). These lines are similar in days-to-flower, number of leaves, leaf size, and plant height. Enzyme analyses of roots of single and double Nic mutants show that the activities of two enzymes, quinolate phosphoribosyl transferase (QPTase) and putrescine methyl transferase (PMTase), are directly proportional to levels of nicotine biosynthesis (Saunders and Bush (1979) Plant Physiol 64:236). Both Nic1 and Nic2 affect PMTase and QPTase activities in roots, and thus, regulate nicotine synthesis (Leete (1983) In: Alkaloids: Chemical and Biological Perspectives, S. W. Pelletier, ed. John Wiley & Sons, pp. 85-152).

[0057] Enzyme analyses of roots of single and double Nic mutants show that the activities of QPTase and PMTase are directly proportional to levels of nicotine biosynthesis. An obligatory step in nicotine biosynthesis is the formation of nicotinic acid from quinolinic acid, which step is catalyzed by QPTase. QPTase appears to be a rate-limiting enzyme in the pathway supplying nicotinic acid for nicotine synthesis in tobacco (See, e.g., Feth et al., Planta, 168, pp. 402-07 (1986) and Wagner et al., Physiol. Plant., 68, pp. 667-72 (1986), herein expressly incorporated by reference in its entirety). A comparison of enzyme activity in tobacco tissues (root and callus) with different capacities for nicotine synthesis shows that QPTase activity is strictly correlated with nicotine content (Wagner and Wagner, Planta 165:532 (1985), herein expressly incorporated by reference in its entirety). In fact, Saunders and Bush (Plant Physiol 64:236 (1979), herein expressly incorporated by reference in its entirety, showed that the level of QPTase in the roots of low nicotine mutants is proportional to the levels of nicotine in the leaves.

[0058] Hibi et al. ((1994) Plant Cell, 6, 723-735) isolated the cDNA encoding PMTase, PMT, and showed that PMT transcript levels are regulated by Nic1 and Nic2. The QPTase cDNA and genomic clones (NtQPT1) have also been isolated and the transcript levels of NtQPT1are also regulated by Nic1 and Nic2. Thus, it appears that the Nic genes regulate nicotine content by regulating the transcript levels of genes encoding the two rate-limiting enzymes, PMTase and QPTase. Further, Nic1 and Nic2 have been shown to be positive regulators of NtQPT1transcription and that promoter sequences upstream of the transcription initiation site contain the cis-acting sequences necessary for Nic gene product activation of NtQPT1transcription. Because expression of QPTase and PMTase are coordinately-regulated by the Nic gene products, it likely that the Nic gene products also directly regulate transcription of the PMT gene.

[0059] One approach for reducing nicotine involves reducing the amount of a required enzyme (i.e. QPTase and PMTase) in the biosynthetic pathway leading to its production. Where the affected enzyme naturally occurs in a rate-limiting amount (relative to the other enzymes required in the pathway), any reduction in that enzyme's abundance will decrease the production of the end product. If the amount of the enzyme is not normally rate-limiting, its presence in a cell must be reduced to rate-limiting levels in order to diminish the pathway's output. Conversely, if the naturally-occurring amount of enzyme is rate limiting, then any increase in the enzyme's activity will result in an increase in the biosynthetic pathway's end product.

[0060] The modification of nicotine levels in tobacco plants by antisense regulation of PMTase expression is proposed in U.S. Pat. No. 5,369,023, entitled "Method of Purifying Putrescine N-Methyltransferase from Tobacco Plant Extract with an Anion Exchange Medium," issued on Nov. 29, 1994 and U.S. Pat. No. 5,260,205, entitled "Method of Purifying Putrescine N-methyltransferase from Tobacco Plant Extract with a Polyamine," issued on Nov. 9, 1993 to Nakatani and Malik, each of which is hereby expressly incorporated by reference in its entirety. PCT application WO 94/28142, entitled "Putrescine N-Methyltransferase, Recombinant DNA Molecules Encoding Putrescine N-Methyltransferase, and Transgenic Tobacco Plants with Decreased Alkaloid Content," filed Jun. 1, 1994, to Wahad and Malik describes DNA encoding PMT and the use of sense and antisense PMT constructs. Additionally, PCT Application WO 98/56923, entitled "Regultaion of Quinolate Phosphoribosyl Transferase Expression," filed Jun. 10, 1998, to Conkling et al. describes DNA encoding a plant QPTase enzyme, constructs comprising such DNA, and methods of altering QPTase expression to increase or decrease nicotine production in tobacco plants. Both of these PCT applications are expressly incorporated by reference in their entireties. Still further, U.S. patent application Ser. No. 09/941,042 to Conkling, entitled "Transgenic Plants Containing Molecular Decoys that Alter Protein Content Therein," filed on Aug. 28, 2001, which is hereby expressly incorporated by reference in its entirety, describes the use of DNA encoding regulatory sequences for the QPTase enzyme and methods of using these sequences as molecular decoys to sequester transcription factors at sites distant to the endogenous promoter for the QPTase gene, thereby decreasing nicotine production in tobacco plants.

[0061] More specific examples of generating reduced nicotine tobacco can be found in PCT application WO 02/100199, entitled "Modifying Nicotine and Nitrosamine Levels in Tobacco," and filed on Jun. 6, 2002, PCT application WO 02/018607, entitled "Transgenic Plants Containing Molecular Decoys that Alter Protein Content Therein," and filed on Aug. 28, 2001, PCT application WO 98/56923, entitled "Regulation of Quinolate Phosphoribosyl Transferase Expression," and filed on Jun. 10, 1998, U.S. Pat. No. 6,586,661, entitled "Regulation of Quinolate Phosphoribosyl Transferase Expression by Transformation with a Tobacco Quinolate Phosphoribosyl Transferase Nucleic Acid," and issued on Jul. 1, 2003, U.S. Pat. No. 6,423,520, entitled "Regulation of Quinolate Phosphoribosyl Transferase Expression," and issued on Jul. 23, 2002, U.S. application Ser. No. 09/963,340 entitled "Regulation of Quinolate Phosphoribosyl Transferase Expression," filed on Sep. 24, 2001, U.S. application Ser. No. 10/356,076, entitled "Regulation of Quinolate Phosphoribosyl Transferase Expression," and filed Jan. 31, 2003, and U.S. application Ser. No. 09/941,042, entitled "Transgenic Plants Containing Molecular Decoys that Alter Protein Content Therein," and filed on Aug. 28, 2001. Each of the foregoing is hereby expressly incorporated by reference in its entirety.

[0062] The following section describes in greater detail the antisense approach to making tobacco products having reduced nicotine and/or TSNA levels.

Antisense Technology can be Used to Create Tobacco Products Having a Reduced Level of Nicotine and/or TSNA

[0063] Antisense technology may be used to create tobacco plants with reduced nicotine levels. The preferred enzyme for antisense regulation of nicotine levels is the TobRD2 gene (see Conkling et al., Plant Phys. 93, 1203 (1990)) encoding a Nicotiana tabacum QPTase (see Example 1). In addition to the description of the technology provided herein, general aspects of the technology are described in PCT/US98/11893, which is hereby expressly incorporated by reference in its entirety.

[0064] Regulation of gene expression in plant cell genomes can be achieved by integration of heterologous DNA under the transcriptional control of a promoter which is functional in the host, and in which the transcribed strand of heterologous DNA is complementary to the strand of DNA that is transcribed from the endogenous gene to be regulated. The introduced DNA, referred to as antisense DNA, provides an RNA sequence which is complementary to naturally produced (endogenous) mRNAs and which inhibits expression of the endogenous mRNA. Although the mechanism of antisense is not completely understood, it is known that antisense constructs can be used to regulate gene expression. A preferred approach for reducing QPTase levels through molecular modification is provided in Example 2 and Example 3.

[0065] In some methods, the antisense product may be complementary to coding or non-coding (or both) portions of naturally occurring target RNA. The antisense construct can be introduced into the plant cells in any suitable manner, and can be integrated into the plant genome for inducible or constitutive transcription of the antisense sequence. Tobacco plants are then regenerated from successfully transformed cells using conventional techniques. It is most preferred that the antisense sequence utilized be complementary to the endogenous sequence, however, minor variations in the exogenous and endogenous sequences may be tolerated. It is also preferred that the antisense DNA sequence be of sufficient sequence similarity that it is capable of binding to the endogenous sequence in the cell to be regulated, under stringent conditions as described below.

[0066] Although the preferred enzyme for antisense regulation is QPTase, other enzymes that are suitable for antisense regulation include, for example, putrescine N-methyltransferase, N-methylputrescine oxidase, ornithine decarboxylase, S-adenosylmethionine synthetase, NADH dehydrogenase, phosphoribosylanthranilate isomerase, and any other enzyme linked to nicotine biosynthesis.

[0067] As an example of the use of antisense technology, tobacco having a reduced amount of nicotine and TSNA is generated from a tobacco plant that is created by exposing at least one tobacco cell of a selected tobacco variety (preferably Burley 21) to an exogenous DNA construct having, in the 5' to 3' direction, a promoter operable in a plant cell and DNA containing a portion of a DNA sequence that encodes an enzyme in the nicotine synthesis pathway or a complement thereof. The DNA is operably associated with said promoter and the tobacco cell is transformed with the DNA construct. The transformed cells are selected using either negative selection or positive selection techniques and at least one tobacco plant is regenerated from transformed cells. The regenerated tobacco plant or portion thereof is preferably analyzed to determine the amount of nicotine and/or TSNA present and these values can be compared to the amount of nicotine and/or TSNA present in a control tobacco plant or portion, preferably of the same variety.

[0068] The DNA constructs having a portion of a DNA sequence that encodes an enzyme in the nicotine synthesis pathway may have the entire coding sequence of the enzyme a complement of this sequence, or any portion thereof. A portion of a DNA sequence that encodes an enzyme in the nicotine synthesis pathway or the complement thereof may have at least 25, 27, 30, 35, 40, 45, 50, 60, 75, 100, 150, 250, 500, 750, 1000, 1500, 2000, 2500, or 5000 bases, or the entire coding sequence of the enzyme or complement thereof. Accordingly, these DNA constructs have the ability to perturb the production of endogenous enzyme in the nicotine biosynthesis pathway through either an antisense or cosuppression mechanism. It is contemplated that both antisense, RNAi, and cosuppression constructs are effective at reducing the levels of nicotine and/or nitrosamines in tobacco plants.

[0069] Nucleic acid sequences employed in the constructs described herein include those with sequence similarity to the gene encoding QPTase, and encoding a protein having quinolate phosphoribosyl transferase activity, including, for example, allelic variations in QPTase proteins. Thus, DNA sequences that hybridize to DNA of the QPTase-encoding gene and code for expression of QPTase, particularly plant QPTase enzymes, may also be employed in carrying out the present invention. Multiple forms of tobacco QPT enzyme may exist. Multiple forms of an enzyme may be due to post-translational modification of a single gene product, or to multiple forms of the NtQPT1gene.

[0070] As used herein, the term 'gene' can refer to a DNA sequence that incorporates (1) upstream (5') regulatory signals including the promoter, (2) a coding region specifying the product, protein or RNA of the gene, (3) downstream regions including transcription termination and polyadenylation signals and (4) associated sequences required for efficient and specific expression. In some contexts, a gene can include only (2), above, or some combination of items (1), (3), and (4) with (2). The DNA sequence may comprise or consist essentially of the sequence encoding the QPTase enzyme, or equivalent nucleotide sequences representing alleles or polymorphic variants of these genes, or coding regions thereof. Use of the phrase "substantial sequence similarity" in the present specification means that DNA, RNA or amino acid sequences which have slight and non-consequential sequence variations from the actual sequences disclosed herein are considered to be equivalent to the sequences of the present invention. In this regard, "slight and non-consequential sequence variations" mean that "similar" sequences (i.e., the sequences that have substantial sequence similarity with the DNA, RNA, or proteins disclosed and claimed herein) will be functionally equivalent to the sequences disclosed herein. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same compositions as the nucleic acid and amino acid compositions disclosed herein.

[0071] By one approach, a novel cDNA sequence encoding a plant QPTase may be used. As QPTase activity is strictly correlated with nicotine content, construction of transgenic tobacco plants in which QPTase levels are lowered in the plant roots (compared to levels in wild-type plants) result in plants having reduced levels of nicotine in the leaves. Embodiments of the invention provide methods and nucleic acid constructs for producing such transgenic plants, as well as the transgenic plants themselves. Such methods include the expression of antisense NtQPT1RNA, which lowers the amount of QPTase in tobacco roots.

[0072] Aspects of the present invention also concern sense and antisense recombinant DNA molecules encoding QPTase or QPTase antisense RNA molecules, and vectors comprising those recombinant DNA molecules, as well as transgenic plant cells and plants transformed with those DNA molecules and vectors. Transgenic tobacco cells and the plants described herein are characterized in that they have a reduced amount of nicotine and/or TSNA as compared to unmodified or control tobacco cells and plants.

[0073] Promoters to be linked to the antisense constructs of the present invention may be constitutively active promoters. Numerous constitutively active promoters which are operable in plants are available. A preferred example is the Cauliflower Mosaic Virus (CaMV) 35S promoter which is expressed constitutively in most plant tissues. In the alternative, the promoter may be a root-specific promoter or root cortex specific promoter, as explained in greater detail below.

[0074] Antisense sequences have been expressed in transgenic tobacco plants utilizing the Cauliflower Mosaic Virus (CaMV) 35S promoter. See, e.g., Cornelissen et al., "Both RNA Level and Translation Efficiency are Reduced by Anti-Sense RNA in Transgenic Tobacco", Nucleic Acids Res. 17, pp. 833-43 (1989); Rezaian et al., "Anti-Sense RNAs of Cucumber Mosaic Virus in Transgenic Plants Assessed for Control of the Virus", Plant Mol. Biol. 11, pp. 463-71 (1988); Rodermel et al., "Nuclear-Organelle Interactions: Nuclear Antisense Gene Inhibits Ribulose Bisphosphate Carboxylase Enzyme Levels in Transformed Tobacco Plants", Cell 55, pp. 673-81 (1988); Smith et al., "Antisense RNA Inhibition of Polygalacturonase Gene Expression in Transgenic Tomatoes", Nature 334, pp. 724-26 (1988); Van der Krol et al., "An Anti-Sense Chalcone Synthase Gene in Transgenic Plants Inhibits Flower Pigmentation", Nature 333, pp. 866-69 (1988).

[0075] Use of the CaMV 35S promoter for expression of antisense QPTase genes in the transformed tobacco cells and plants of this invention is preferred. Use of the CaMV promoter for expression of other recombinant genes in tobacco roots has been well described (Lam et al., "Site-Specific Mutations Alter In Vitro Factor Binding and Change Promoter Expression Pattern in Transgenic Plants", Proc. Nat. Acad Sci. USA 86, pp. 7890-94 (1989); Poulsen et al. "Dissection of 5' Upstream Sequences for Selective Expression of the *Nicotiana plumbaginifolia* rbcS-8B Gene", Mol. Gen. Genet. 214, pp. 16-23 (1988).

[0076] Other promoters which are active only in root tissues (root specific promoters) are also particularly suited to the methods of the present invention. See, e.g., U.S. Pat. No. 5,459,252 to Conkling et al.; Yamamoto et al., Plant Cell, 3:371 (1991). The TobRD2 root-cortex specific promoter may also be utilized. See, eg., U.S. patent application Ser. No. 08/508,786, now allowed, to Conkling et al; PCT WO 9705261. All patents cited herein are intended to be incorporated herein by reference in their entirety.

[0077] Some of the nucleic acids described herein may also be used in methods of sense co-suppression or RNAi-mediated suppression of nicotine production. Sense DNAs employed in these methods are preferably of a length sufficient to, when expressed in a plant cell, suppress the native expression of the plant QPTase protein as described herein in that plant cell. Such sense DNAs may be essentially an entire genomic or complementary DNA encoding the QPTase enzyme, or a fragment thereof, with such fragments typically being at least 15, 25, 27, 30, 35, 40, 45, 50, 60, 75, 100, 150, 250, 500, 750, nucleotides in length. Methods of ascertaining the length of sense DNA that results in suppression of the expression of a native gene in a cell are available to those skilled in the art.

[0078] In an alternate example, *Nicotiana* plant cells are transformed with a DNA construct containing a DNA segment encoding an enzymatic RNA molecule termed a "ribozyme", which enzymatic RNA molecule is directed against and cleaves the mRNA transcript of DNA encoding plant QPTase as described herein. Production of such an enzymatic RNA molecule in a plant cell and disruption of QPTase protein production reduces QPTase activity in plant cells in essentially the same manner as production of an antisense RNA molecule: that is, by disrupting translation of mRNA in the cell which produces the enzyme. The section below describes yet another method to decrease levels of specific enzymes involved in nicotine biosynthesis, using decoy nucleic acid fragments.

Molecular Decoy Technology to Lower Nicotine and/or TSNA Levels

[0079] The use of nucleic acid-based decoy fragments to reduce gene expression is referred to as "molecular decoys". In a preferred example, the "decoy fragment" corresponds to promoter sequences upstream of the QPTase, to reduce QPTase expression.

[0080] In some examples, an isolated nucleic acid, or a fragment thereof consisting of at least 20-450 consecutive nucleotides desirably, at least 30-400 consecutive nucleotides preferably, 50-350 consecutive nucleotides more preferably, and 100-300 or 200-400 consecutive nucleotides most preferably, that is or contains at least one cis-acting regulatory element, which exists upstream of the plant QPTase and/or putrescine methyl transferase PMTase coding sequences. Another example is the Nic gene product responsive element obtained from the sequence disclosed in U.S. Pat. No. 5,459,252, herein expressly incorporated by reference in its entirety. In some examples, the Nic gene product responsive element resides between −1000 and −600 or −700 bp of the NtQPT1promoter. Accordingly, some embodiments involve a 300-400 nucleotide long fragment of the NtQPT1 promoter that corresponds to the sequence of the NtQPT1 promoter between −1000 and −600 or −700, as disclosed in U.S. Pat. No. 5,459,252.

[0081] Thus, in several examples, the embodied nucleic acids have a structure that promotes an interaction with one or more transcription factors (e.g., Nic1 and Nic2), which are involved in initiating transcription of QPTase and/or

PMTase. Accordingly, said nucleic acids are said to be or contain at least one transcription factor (e.g., Nic1 and Nic2) binding sequences, which are also referred to as "cis-acting regulatory elements." By introducing multiple copies of these cis-acting regulatory elements (e.g., sequences that interact with Nic1 and/or Nic2) into a plant cell, the ability of the transcription factor to initiate transcription of the targeted gene (e.g., QPTase and/or PMTase genes) can be reduced or squelched.

[0082] By one approach, tobacco plants are transformed with an excess number of DNA sequences (cis-acting elements) from the promoters of genes encoding, but not limited to, QPTase and PMTase that are regulated in nicotine biosynthesis. These cis-acting elements are preferably integrated into the plant genome so as to allow for transfer to successive generations. Preferred approaches are provided in Example 4 and Example 5. Typically, the Nic1 and Nic2 DNA-binding proteins that interact with these cis-acting DNA sequences are expressed at relatively low levels in the cell, thus the excess of transgenic cis-acting elements will compete with the endogenous elements associated with the genes encoding, but not limited to, QPTase and PMTase for available Nic1 and Nic2 Accordingly, these cis-acting DNA sequences (and those of other cis-acting elements) are referred to herein as "decoys" or "molecular decoys". The competition decreases occupancy of trans-acting DNA-binding proteins on their cognate cis-acting elements, thereby down-regulating the synthesis of nicotine biosynthesis enzymes.

[0083] Embodiments of the present invention also provide are DNA molecules of cis-acting elements of QPTase or PMTase, and vectors comprising those DNA molecules, as well as transgenic plant cells and plants transformed with those DNA molecules and vectors. Transgenic tobacco cells and plants of this invention are characterized by lower nicotine content than untransformed control tobacco cells and plants.

[0084] Any of a variety of cis-acting elements can be used in carrying out the molecular decoy methods, depending upon the particular application. Examples of cis-acting elements (and corresponding transcription factors) that may be used, alone or in combination with one another, which may be used in embodiments of the present invention include, but are not limited to, AS-1 and ASF-1 (see U.S. Pat. Nos. 4,990,607 and 5,223,419), the AATT repeat element and PABF (see U.S. Pat. Nos. 5,834,236 and 6,191,258), a wounding-responsive cis-acting element from potato (Siebert et al., Plant Cell 1:961-8 (1989)), an embryo-specific cis-acting element from bean (Bustos et al, Plant Cell 1:839-853 (1989)), a root-specific cis-acting element from the tobacco RB7 promoter (U.S. Pat. No. 5,459,252 and Yamamoto et al., Plant Cell 3:371-382 (1991)), a positive poly(dA-dT) regulatory element and binding protein and negative CCCAA repeat element and binding protein (Wang et al., Mol. Cell Biol. 12:3399-3406 (1992)), a root-tip regulatory element from the tobacco phytochrome A1 promoter of tobacco (Adam et al., Plant Mol Biol 29:983-993 (1995)), an anaerobiosis-responsive element from the maize glyceraldehyde-3-phosphate dehydrogenase 4 gene (Geffers et al., Plant Mol Biol 43:11-21 (2000)), and a seed-specific regulatory region from an *Arabidopsis oleosin* gene (see U.S. Pat. No. 5,792,922), all of which are hereby expressly incorporated by reference in their entireties.

[0085] The status of the art is such that large databases list identified cis-acting regulatory regions (e.g., Plant Cis-acting Regulatory elements, "PLACE", with about 1,340 entries, and Plant Cis-acting Regulatory Elements "PlantCARE", which lists about 159 plant promoters. The listed cis-acting regulatory elements in these databases and the cis-acting regulatory elements that are provided in Raumbauts et al., Nucleic acids Research 27:295-296 (1999), and Higo et al., Nucleic acids Research 27:297-300 (1999) can be used with embodiments of the invention. Accordingly, the databases and references above are hereby expressly incorporated by reference in their entireties. The section below describes general methods for transformation of tobacco plants with modified sequences to create tobacco plants with low nicotine and/or TSNA levels.

Transgenic Plant Cells and Plants

[0086] DNA sequences provided herein can be transformed into a variety of host cells. A variety of suitable host cells, having desirable growth and handling properties, are readily available in the art. As used herein, a "native DNA sequence" or "natural DNA sequence" means a DNA sequence which can be isolated from non-transgenic cells or tissue. Native DNA sequences are those which have not been artificially altered, such as by site-directed mutagenesis. Once native DNA sequences are identified, DNA molecules having native DNA sequences may be chemically synthesized or produced using recombinant DNA procedures as are known in the art. A native plant DNA sequence typically can be isolated from non-transgenic plant cells or tissue.

[0087] DNA constructs, or "transcription cassettes," of the present invention may include, **5'** to **3'** in the direction of transcription, a promoter as discussed herein, a DNA sequence as discussed herein operatively associated with the promoter, and, optionally, a termination sequence including stop signal for RNA polymerase and a polyadenylation signal for polyadenylase. The term "operatively associated," as used herein, refers to DNA sequences on a single DNA molecule which are associated so that the function of one is affected by the other. Thus, a promoter is operatively associated with a DNA when it is capable of affecting the transcription of that DNA (i.e., the DNA is under the transcriptional control of the promoter). The promoter is said to be "upstream" from the DNA, which is in turn said to be "downstream" from the promoter.

[0088] In embodiments of the invention wherein a termination signal is used, any suitable termination signal may be employed in carrying out the present invention, examples thereof including, but not limited to, the nopaline synthase (nos) terminator, the octopine synthase (ocs) terminator, the CaMV terminator, or native termination signals derived from the same gene as the transcriptional initiation region or derived from a different gene. See, e.g., Rezian et al. (1988) supra, and Rodermel et al. (1988), supra. Alternatively, if nicotine levels are decreased by molecular decoy technology rather than by antisense or other methods, the molecular decoy fragments, with or without additional sequences, may be provided to the plant cell by any means. For example, the molecular decoy fragment may have an accompanying gene encoding a selectable marker, other suitable genes, or may be present as part of a plasmid vector. The molecular decoy fragment may consist of a single or double stranded DNA or RNA molecule. The molecular decoy may be integrated into the genome or may exist freely in the cell.

[0089] The transcription cassette may be provided in a DNA construct that also has at least one replication system. For convenience, it is common to have a replication system functional in *Escherichia coli,* such as ColEI, pSC101, pACYC184, or the like. In this manner, at each stage after each manipulation, the resulting construct may be cloned, sequenced, and the correctness of the manipulation determined. In addition, or in place of the *E. coli* replication system, a broad host range replication system may be employed, such as the replication systems of the P-1 incompatibility plasmids, e.g., pRK290. In addition to the replication system, there will frequently be at least one marker present, which may be useful in one or more hosts, or different markers for individual hosts. That is, one marker may be employed for selection in a prokaryotic host, while another marker may be employed for selection in a eukaryotic host, particularly the plant host. The markers may be protection against a biocide, such as antibiotics, toxins, heavy metals, or the like; may provide complementation, by imparting prototrophy to an auxotrophic host; or may provide a visible phenotype through the production of a novel compound in the plant.

[0090] The various fragments comprising the various constructs, transcription cassettes, markers, and the like may be introduced consecutively by restriction enzyme cleavage of an appropriate replication system, and insertion of the particular construct or fragment into the available site. After ligation and cloning the DNA construct may be isolated for further manipulation. All of these techniques are amply exemplified in the literature as exemplified by J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989)(Cold Spring Harbor Laboratory).

[0091] Vectors which may be used to transform plant tissue with nucleic acid constructs of the methods described herein include both *Agrobacterium* vectors and ballistic vectors, as well as vectors suitable for DNA.-mediated transformation. The term 'promoter' refers to a region of a DNA sequence that incorporates the necessary signals for the efficient expression of a coding sequence. This may include sequences to which an RNA polymerase binds but is not limited to such sequences and may include regions to which other regulatory proteins bind together with regions involved in the control of protein translation and may include coding sequences.

[0092] The QPTase recombinant DNA molecules and vectors used to produce the transformed tobacco cells and plants described herein may further comprise a dominant selectable marker gene. Suitable dominant selectable markers for use in tobacco include, inter alia, antibiotic resistance genes encoding neomycin phosphotransferase (NPTII), and hygromycin phosphotransferase (HPT). Other well-known selectable markers that are suitable for use in tobacco include a mutant dihydrofolate reductase gene that encodes methotrexate-resistant dihydrofolate reductase. DNA vectors containing suitable antibiotic resistance genes, and the corresponding antibiotics, are commercially available.

[0093] Transformed tobacco cells are selected out of the surrounding population of non-transformed cells by placing the mixed population of cells into a culture medium containing an appropriate concentration of the antibiotic (or other compound normally toxic to tobacco cells) against which the chosen dominant selectable marker gene product confers resistance. Thus, only those tobacco cells that have been transformed will survive and multiply. Additionally, the positive selection techniques described by Jefferson (e.g., WO 00055333; WO 09913085; U.S. Pat. Nos. 5,599,670; 5,432,081; and 5,268,463, hereby expressly incorporated by reference in their entireties) can be used.

[0094] Methods of making recombinant plants such as those described herein, in general, involve first providing a plant cell capable of regeneration (the plant cell typically residing in a tissue capable of regeneration). The plant cell is then transformed with a DNA construct comprising a transcription cassette of the present invention (as described herein) and a recombinant plant is regenerated from the transformed plant cell. As explained below, the transforming step is carried out by techniques as are known in the art, including but not limited to bombarding the plant cell with microparticles, carrying the transcription cassette, infecting the cell with an *Agrobacterium tumefaciens* containing a Ti plasmid carrying the transcription cassette or any other technique suitable for the production of a transgenic plant.

[0095] Numerous *Agrobacterium* vector systems useful in carrying out the methods described herein are known. For example, U.S. Pat. No. 4,459,355, herein expressly incorporated by reference, discloses a method for transforming susceptible plants, including dicots, with an *Agrobacterium* strain containing the Ti plasmid. The transformation of woody plants with an *Agrobacterium* vector is disclosed in U.S. Pat. No. 4,795,855, herein expressly incorporated by reference. Further, U.S. Pat. No. 4,940,838 to Schilperoort et al. discloses a binary *Agrobacterium* vector (i.e., one in which the *Agrobacterium* contains one plasmid having the vir region of a Ti plasmid but no T region, and a second plasmid having a T region but no vir region) useful in carrying out the methods disclosed herein.

[0096] Microparticles carrying a DNA construct of the present invention, which microparticle is suitable for the ballistic transformation of a plant cell, are also useful for making transformed plants described herein. The microparticle is propelled into a plant cell to produce a transformed plant cell, and a plant is regenerated from the transformed plant cell. Any suitable ballistic cell transformation methodology and apparatus can be used in practicing the present invention. Exemplary apparatus and procedures are disclosed in Sanford and Wolf, U.S. Pat. No. 4,945,050, and in Christou et al., U.S. Pat. No. 5,015,580, all of which are hereby expressly incorporated by reference. When using ballistic transformation procedures, the transcription cassette may be incorporated into a plasmid capable of replicating in or integrating into the cell to be transformed. Examples of microparticles suitable for use in such systems include 1 to 5 μm gold spheres. The DNA construct may be deposited on the microparticle by any suitable technique, such as by precipitation.

[0097] Plant species may be transformed with the DNA construct of the present invention by the DNA-mediated transformation of plant cell protoplasts; and subsequent regeneration of the plant from the transformed protoplasts in accordance with procedures well known in the art. Fusion of tobacco protoplasts with DNA-containing liposomes or via electroporation is known in the art. (Shillito et al., "Direct Gene Transfer to Protoplasts of Dicotyledonous and Mono-

cotyledonous Plants by a Number of Methods, Including Electroporation", Methods Enzymol. 153, pp. 313-36 (1987)).

[0098] As used herein, transformation refers to the introduction of exogenous DNA into cells, so as to produce transgenic cells stably transformed with the exogenous DNA. Transformed cells are induced to regenerate intact tobacco plants through application of tobacco cell and tissue culture techniques that are well known in the art. The method of plant regeneration is chosen so as to be compatible with the method of transformation. The stable presence and the orientation of the QPTase sequence in transgenic tobacco plants can be verified by Mendelian inheritance of the QPTase sequence, as revealed by standard methods of DNA analysis applied to progeny resulting from controlled crosses. After regeneration of transgenic tobacco plants from transformed cells, the introduced DNA sequence is readily transferred to other tobacco varieties through conventional plant breeding practices and without undue experimentation.

[0099] Any plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a vector of the present invention. The term "organogenesis," as used herein, means a process by which shoots and roots are developed sequentially from meristematic centers; the term "embryogenesis," as used herein, means a process by which shoots and roots develop together in a concerted fashion (not sequentially), whether from somatic cells or gametes. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, callus tissue, existing meristematic tissue (e.g., apical meristems, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem).

[0100] Plants of the present invention may take a variety of forms. The plants may be chimeras of transformed cells and non-transformed cells; the plants may be clonal transformants (e.g., all cells transformed to contain the transcription cassette); the plants may comprise grafts of transformed and untransformed tissues (e.g., a transformed root stock grafted to an untransformed scion in citrus species). The transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, first generation (or T1) transformed plants may be selfed to give homozygous second generation (or T2) transformed plants, and the T2 plants further propagated through classical breeding techniques. A dominant selectable marker (such as nptII) can be associated with the transcription cassette to assist in breeding.

[0101] As used herein, a crop comprises a plurality of plants of the same genus, planted together in an agricultural field. Thus, provided herein is a method of producing a crop of plants having lowered QPTase or PMTase activity and thus having decreased nicotine and/or TSNA levels, as compared to a similar crop of non-transformed plants of the same species and variety.

[0102] Levels of nicotine in the transgenic tobacco plants described herein can be detected by standard nicotine assays. Transformed plants in which the level of QPTase or PMTase is reduced compared to untransformed control plants will accordingly have a reduced nicotine level compared to the control.

[0103] The modified tobacco plants described herein are suitable for conventional growing and harvesting techniques (e.g. topping or no topping, bagging the flowers or not bagging the flowers, cultivation in manure rich soil or without manure). The harvested tobacco leaves and stems are suitable for conventional methods of processing such as curing and blending. The modified tobacco is suitable for use in any traditional tobacco product including, but not limited to, pipe, cigar and cigarette tobacco, and chewing tobacco in any form including leaf tobacco, shredded tobacco, or cut tobacco. The section below describes typical curing methods which may be used to prepare the tobacco once it is harvested.

Curing

[0104] The curing process, which typically lasts about 1 week, brings out the flavor and aroma of tobacco. Several methods for curing tobacco may be used, and indeed many methods have been previously disclosed. For example, U.S. Pat. No. 4,499,911 to Johnson; U.S. Pat. No. 5,685,710 to Martinez Sagrera; U.S. Pat. No. 3,905,123 to Fowler; U.S. Pat. No. 3,840,025 to Fowler; and U.S. Pat. No. 4,192,323 to Home, (each hereby expressly incorporated by reference in its entirety) describe aspects of the tobacco curing process which may be used for some embodiments of the present invention. Conventionally, "sticks" that are loaded with tobacco are placed into bulk containers and placed into closed buildings having a heat source known as a curing barn. A flue is often used to control the smoke (thus earning the term "flue-cured"). The method of curing will depend, in some cases, on the type of tobacco-use cessation product desired, (i.e., snuff, cigarettes, or pipe tobacco may preferably utilize different curing methods) and preferred methods may vary from region to region and in different countries. In some approaches, the stems and midveins of the leaf are removed from the leaves prior to curing to yield a high quality, low nitrosamine tobacco product.

[0105] "Flue curing" is a popular method for curing tobacco in Virginia, N.C., and the Coastal Plains regions of the United States. This method is used mainly in the manufacture of cigarettes. Flue curing requires a closed building equipped with a system of ventilation and a source of heat. The heating can be direct or indirect (e.g., radiant heat). When heat and humidity are controlled, leaf color changes, moisture is quickly removed, and the leaf and stems dry. Careful monitoring of the heating and humidity can reduce the accumulation of nitrosamines.

[0106] Another curing method is termed "air curing". In this method, an open framework is prepared in which sticks of leaves (or whole plants) are hung so as to be protected from both wind and sun. Leaf color changes from green to yellow, as leaves and stems dry slowly.

[0107] "Fire curing" employs an enclosed barn similar to that used for flue curing. The tobacco is hung over low temperature fire so that the leaves cure in a smoke-laden atmosphere. This process uses lower temperatures, so the process may take up to a month, in contrast to flue curing, which takes about 6 to 8 days.

[0108] A further curing method, termed "sun curing" is the drying of uncovered sticks or strings of tobacco leaves in the sun. The best known sun-cured tobaccos are the so-called oriental tobaccos of Turkey, Greece, Yugoslavia, and nearby countries.

[0109] The curing process, and most particularly the flue-curing process, is generally divided into the following four stages:

[0110] A) Firing Up: During this step, the tobacco leaves turn bright lemon-orange in color. This is achieved by a gradual increase in temperature.

[0111] B) Leaf Yellowing: In this step any moisture is removed. This creates the "yellowing" of the tobacco. It also prepares the tobacco for drying in the next step.

[0112] C) Leaf Drying: Leaf drying, an important step in the curing process, requires much time for the tobacco to dry properly. Additionally, air flow is increased in this step to facilitate the drying process.

[0113] D) Stem Drying: The drying process continues, as the stem of the tobacco leaf becomes dried.

[0114] The cured tobacco may then be blended with other tobaccos or other materials to create the product to be used for the tobacco-use cessation method. The section below describes typical methods of blending and preparing the tobacco product.

Tobacco Blending

[0115] It may be desirable to blend tobacco of varying nicotine levels to create the cessation product having the desired level of nicotine. This blending process is typically performed after the curing process, and may be performed by conventional methods. Preferred tobacco blending approaches are provided in Examples 6 and 7. In some embodiments, blending of the transgenic tobacco is conducted to prepare the tobacco so that it will contain specific amounts of nicotine and/or TSNA in specific products. Preferably, the blending is conducted so that tobacco products of varying amounts of nicotine and/or TSNAs are made in specific products.

[0116] A mixture which contains different types of tobacco is desirably substantially homogeneous throughout in order to avoid undesirable fluctuations in taste or nicotine levels. Typically, tobacco to be blended may have a moisture content between 30 and 75%. As an example, the tobacco is first cut or shredded to a suitable size, then mixed in a mixing device, such as a rotating drum or a blending box. One such known mixing device is a tumbling apparatus which typically comprises a rotating housing enclosing mixing paddles which are attached to and, therefore, rotate with the housing to stir the tobacco components together in a tumbling action as the drum turns.

[0117] After the desired tobaccos are thoroughly mixed, the resulting tobacco blend is removed from the mixing apparatus and bulked to provide a continuous, generally uniform quantity of the tobacco blend. The tobacco is then allowed to remain relatively undisturbed (termed the "bulking step") for the required period of time before subsequent operations are performed. The bulking step typically takes 30 minutes or less, and may be carried out on a conveyor belt. The conveyor belt allows the blended tobacco to remain in bulk form in an undisturbed condition while it is continuously moving the tobacco blend through the process from the mixing stage to the expansion stage.

[0118] The tobacco blend is typically expanded by the application of steam. The tobacco mixture is typically subjected to at least 0.25 pounds of saturated steam at atmospheric conditions per pound of blended tobacco for at least 10 seconds to provide an increase in moisture of at least 2 weight percent to the tobacco blend. After the tobacco blend has been expanded, it is dried. A typical drying apparatus uses heated air or superheated steam to dry the tobacco as the tobacco is conveyed by the heated air or steam stream through a drying chamber or series of drying chambers. Generally, the wet bulb temperature of the drying air may be from about 150 degrees F. to about 211 degrees F. The tobacco blend is typically dried to a moisture content of from about 60 percent to about 5 percent.

[0119] The visual indicators of content discussed above can be used to indicate the content of nicotine or TSNAs in a particular tobacco product. The particular tobacco product can have about 0.01 mg or less, 0.05 mg, 0.075 mg, 0.1 mg, 0.15 mg, 0.2 mg, 0.25 mg, 0.3 mg, **0.35**mg, 0.4 mg, **0.45**mg, 0.5 mg, **0.55**mg, 0.6 mg, **0.65**mg, 0.7 mg, **0.75**mg, 0.8 mg, 0.85 mg, 0.9 mg, 0.95 mg, 1.0 mg, 1.00 mg, 1.05 mg, 1.075 mg, 1.1 mg, 1.15 mg, 1.2 mg, 1.25mg, 1.3 mg, 1.35 mg, 1.4 mg, 1.45 mg, 1.5 mg, 1.55 mg, 1.6 mg, 1.65 mg, 1.7 mg, 1.75 mg, 1.8 mg, 1.85 mg, 1.9 mg, 1.95 mg, 2.0 mg, 2.01 mg, 2.05 mg, 2.075 mg, 2.1mg, 2.15 mg, 2.2 mg, 2.25 mg, 2.3 mg, 2.35 mg, 2.4 mg, 2.45 mg, 2.5 mg, 2.55 mg, 2.6 mg, 2.65 mg, 2.7 mg, 2.75 mg, 2.8 mg, 2.85 mg, 2.9 mg, 2.95 mg, 3.0 mg, 3.01 mg, 3.05 mg, 3.075 mg, 3.1 mg, 3.15 mg, 3.2 mg, 3.25 mg, 3.3 mg, 3.35 mg, 3.4 mg, 3.45 mg, 3.5 mg, 3.55 mg, 3.6 mg, 3.65 mg, 3.7 mg, 3.75 mg, 3.8 mg, 3.85 mg, 3.9 mg, 3.95 mg, 4.0 mg, 4.01 mg, 4.05 mg, 4.075 mg, 4.1 mg, 4.15 mg, 4.2 mg, 4.25 mg, 4.3 mg, 4.35 mg, 4.4 mg, 4.45 mg, 4.5 mg, 4.55 mg, 4.6 mg, 4.65 mg, 4.7 mg, 4.75 mg, 4.8 mg, 4.85 mg, 4.9 mg, 4.95 mg, 4.0 mg, 4.01 mg, 4.05 mg, 4.075 mg, 4.1 mg, 4.15 mg, 4.2 mg, 4.25 mg, 4.3 mg, 4.35 mg, 4.4 mg, 4.45 mg, 4.5 mg, 4.55 mg, 4.6 mg, 4.65 mg, 4.7 mg, 4.75 mg, 4.8 mg, 4.85 mg, 4.9 mg, 4.95 mg, 5.0 mg, 5.01 mg, 5.05 mg, 5.075 mg, 5.1 mg, 5.15 mg, 5.2 mg, 5.25 mg, 5.3 mg, 5.35 mg, 5.4 mg, 5.45 mg, 5.5 mg, 5.55 mg, 5.6 mg, 5.65 mg, 5.7 mg, 5.75 mg, 5.8 mg, 5.85 mg, 5.9 mg, 5.95 mg, 6.0 mg, 6.01 mg, 6.05 mg, 6.075 mg, 6.1 mg, 6.15 mg, 6.2 mg, 6.25 mg, 6.3 mg, 6.35 mg, 6.4 mg, 6.45 mg, 6.5 mg, 6.55 mg, 6.6 mg, 6.65 mg, 6.7 mg, 6.75 mg, 6.8 mg, 6.85 mg, 6.9 mg, 6.95 mg, 7.0 mg, 7.01 mg, 7.05 mg, 7.075 mg, 7.1 mg, 7.15 mg, 7.2 mg, 7.25 mg, 7.3 mg, 7.35 mg, 7.4 mg, 7.45 mg, 7.5 mg, 7.55 mg, 7.6 mg, 7.65 mg, 7.7 mg, 7.75 mg, 7.8 mg, 7.85 mg, 7.9 mg, 7.95 mg, 8.0 mg, 8.01 mg, 8.05 mg, 8.075 mg, 8.1 mg, 8.15 mg, 8.2 mg, 8.25 mg, 8.3 mg, 8.35 mg, 8.4 mg, 8.45 mg, 8.5 mg, 8.55 mg, 8.6 mg, 8.65 mg, 8.7 mg, 8.75 mg, 8.8 mg, 8.85 mg, 8.9 mg, 8.95 mg, 9.0 mg, 9.01 mg, 9.05 mg, 9.075 mg, 9.1 mg, 9.15 mg, 9.2 mg, 9.25 mg, 9.3 mg, 9.35 mg, 9.4 mg, 9.45 mg, 9.5 mg, 9.55 mg, 9.6 mg, 9.65 mg, 9.7 mg, 9.75 mg, 9.8 mg, 9.85 mg, 9.9 mg, 9.95 mg, 10.0 mg, 10.01 mg, 10.05 mg, 10.075 mg, 10.1 mg, 10.15 mg, 10.2 mg, 10.25 mg, 10.3 mg, 10.35 mg, 10.4 mg, 10.45 mg, 10.5 mg, 10.55 mg, 10.6 mg, 10.65 mg, 10.7 mg, 10.75 mg, 10.8 mg, 10.85 mg, 10.9 mg, 10.95 mg, 11.0 mg, 11.01 mg, 11.05 mg, 11.075 mg, 11.1 mg, 11.15 mg, 11.2 mg, 11.25 mg, 11.3 mg, 11.35 mg, 11.4 mg, 11.45 mg, 11.5 mg, 11.55 mg, 11.6 mg, 11.65 mg, 11.7 mg, 11.75 mg, 11.8 mg, 11.85 mg, 1.9 mg, 11.95 mg, or 12 mg of nicotine, for example. In other embodiments the tobacco product can have about less than 0.1 micrograms, 0.15 micrograms, 0.2 micrograms, 0.25 micrograms, 0.3 micrograms, 0.35 micrograms, 0.4 micrograms, 0.45 micrograms, 0.5 micrograms, 0.55 micrograms,

0.6 micrograms, 0.65 micrograms, 0.7 micrograms, 0.75 micrograms, 0.8 micrograms, 0.85 micrograms, 0.9 micrograms, 0.95 micrograms, 1.0 micrograms, 1.1 micrograms, 1.15 micrograms, 1.2 micrograms, 1.25 micrograms, 1.3 micrograms, 1.35 micrograms, 1.4 micrograms, 1.45 micrograms, 1.5 micrograms, 1.55 micrograms, 1.6 micrograms, 1.65 micrograms, 1.7 micrograms, 1.75 micrograms, 1.8 micrograms, 1.85 micrograms, 1.9 micrograms, 1.95 micrograms, 2.0 micrograms, 2.1 micrograms, 2.15 micrograms, or 2.2 micrograms of total TSNA, for example.

[0120] In some embodiments relating to tobacco products derived from Burley tobacco leaves, the tobacco products can have between about between about 0 and about 30,000 ppm nicotine and about 0 and about 8,000 ppb TSNA desirably, between about 0 and about 20,000 ppm nicotine and about 0 and about 6,000 ppb TSNA more desirably, between about 0 and about 10,000 ppm nicotine and about 0 and about 5,000 ppb TSNA preferably, between about 0 and about 5,000 ppm nicotine and about 0 and about 4,000 ppb TSNA more preferably, between about 0 and about 2,500 ppm nicotine and about 0 and about 2,000 ppb TSNA even more preferably, and most preferably between about 0 and about 1,000 ppm nicotine and about 0 and about 1,000 ppb TSNA. Embodiments of Burley leaf prepared by the methods described herein can also have between about 0 and about 1000 ppm nicotine and about 0 and about 500 ppb TSNA and some embodiments of Burley leaf prepared by the methods described herein have virtually no detectable amount of nicotine or TSNA.

[0121] Similarly, a Flue-cured tobacco leaf for use with the disclosed methods can have a reduced amount of nicotine which is between about 0 and about 20,000 ppm nicotine and about 0 and about 300 ppb TSNA desirably between about 0 and about 15,000 ppm nicotine and about 0 and about 250 ppb TSNA more desirably between about 0 and about 10,000 ppm nicotine and about 0 and about 200 ppb TSNA preferably between about 0 and about 5,000 ppm nicotine and about 0 and about 150 ppb TSNA more preferably between about 0 and about 2,500 ppm nicotine and about 0 and about 100 ppb TSNA and most preferably between about 0 and about 1,000 ppm nicotine and about 0 and about 50 ppb TSNA. Embodiments of flue-cured tobacco prepared by the methods described herein can also have between about 0 and about 500 ppm nicotine and about 0 and about 25 ppb TSNA and some embodiments of flue-cured tobacco prepared by the methods described herein have virtually no detectable amount of nicotine or TSNA.

[0122] Further, an Oriental tobacco for use with the embodied methods can have a reduced amount of nicotine having between about 0 and about 10,000 ppm nicotine and about 0 and about 100 ppb TSNA desirably between about 0 and about 7,000 ppm nicotine and about 0 and about 75 ppb TSNA more desirably between about 0 and about 5,000 ppm nicotine and about 0 and about 50 ppb TSNA preferably between about 0 and about 3,000 ppm nicotine and about 0 and about 25 ppb TSNA more preferably between about 0 and about 1,500 ppm nicotine and about 0 and about 10 ppb TSNA and most preferably between about 0 and about 500 ppm nicotine and essentially no TSNA. Embodiments of Oriental cured tobacco prepared by the methods described herein can also have between about 0 and about 250 ppm nicotine and essentially no TSNA and some embodiments of Oriental cured tobacco prepared by the methods described herein have virtually no detectable amount of nicotine or TSNA

[0123] It should be emphasized that in the context of tobacco products with reduced amounts of nicotine, TSNAs, or other components, the phrase "a reduced amount" is intended to refer to an amount of nicotine and or TSNA or indicated component in a treated or transgenic tobacco plant, tobacco, or a tobacco product that is less than what would be found in a tobacco plant, tobacco, or a tobacco product from the same variety of tobacco processed in the same manner, which has not been treated or was not made transgenic for reduced nicotine and/or TSNA. Thus, in some contexts, wild-type tobacco of the same variety that has been processed in the same manner is used as a control by which to measure whether a reduction in nicotine and/or TSNA has been obtained.

[0124] In some contexts, the phrase reduced amount of nicotine and/or TSNAs refers to the tobacco plants, tobacco and tobacco products of the invention that have less nicotine and/or TSNAs by weight than the same variety of tobacco grown, processed, and cured in the same way. For example, wild type tobacco can contain approximately 1-4% dry weight nicotine and approximately 0.2%-0.8% dry weight TSNAs depending on the variety, and the manner in which it was grown, harvested and cured. A typical cigarette has 11 mg of nicotine and 8 μg of TSNAs. Thus, the tobacco plants, tobacco and tobacco products of the invention can have, in dry weight for example, less than 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, 0.05%, 0.055%, 0.06%, 0.065%, 0.07%, 0.075%, 0.08%, 0.085%, 0.09%, 0.095%, 0.1%, 0.15%, 0.175%, 0.2%, 0.225%, 0.25%, 0.275%, 0.3%, 0.325%, 0.35%, 0.375%, 0.4%, 0.425%, 0.45%, 0.475%, 0.5%, 0.55%, 0.6%, 0.65%, 0.7%, 0.75%, 0.8%, 0.85%, 0.9%, 0.95%, and 1.0% nicotine and less than 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, 0.05%, 0.055%, 0.06%, 0.065%, 0.07%, 0.075%, and 0.08% TSNA.

[0125] In addition to nicotine and TSNA levels, the content levels of secondary or tertiary amine, or any other component of interest can be denoted by one or more visual content indicators. Examples of additional compounds are discussed in more detail below.

[0126] In certain embodiments, the visual content indicators described herein are used in accordance with Federal Trade Commission guidelines regarding such labeling. In the United States, the standard method of measuring and reporting tar, nicotine, and carbon monoxide yields from a cigarette is the methodology originally prescribed by the Federal Trade Commission in 1967 and modified in 1980. See, 32 Fed. Reg. 11,178 (1967) and 45 Fed. Reg. 46,483 (1980), herein expressly incorporated by reference in their entireties. This method prescribes reporting the tar content to the nearest milligram and the nicotine yield to the nearest one tenth of a milligram. 32 Fed. Reg. 11,178 (1967). The concentration of carbon monoxide is reported as milligrams per cigarette. 45 Fed. Reg. 46,483 (1980).

[0127] To accommodate these reporting parameters, yields of a particular component of a tobacco product will be rounded in accordance the traditional scientific rounding convention. The convention comprises rules for rounding when the last figure of the number to be rounded is 6 or

higher, 4 or less, and 5. According to this convention, when the last figure of a number to be rounded is 6 or above, the number is rounded up, and when last figure of the number to be rounded is 4 or less, the number is rounded down. Therefore, a tar yield of 1.6 milligrams (mg) or higher will be rounded up to 2 mg and a tar yield of 1.4 mg or less will be rounded down to 1 mg. Similarly, a nicotine yield of 0.26 mg or higher will be rounded up to 0.3 mg and a nicotine yield of 0.24 mg or less will be rounded down to 0.2 mg. According to the rounding convention, when the last figure in the number to be rounded is a 5 and the figure preceding the 5 is odd, the number is rounded up, but when the figure preceding the 5 is even, the number is rounded down. Following this convention, a tar yield of 1.5 mg is rounded up to 2 mg and a tar yield of 2.5 mg is rounded down to 2 mg. Similarly, a nicotine yield 0.35 mg is rounded up to 0.4 mg and a nicotine yield of 0.45 mg is rounded down to 0.4 mg. In embodiments of the present invention, when reference is made to a particular amount of a content or component of a tobacco product, such reference is generally intended to be inclusive of amounts within the range of amounts that would be included under the rounding convention. For example, a cigarette reported to have 0.6 mg of nicotine would be considered to be any cigarette having between 0.55 mg and 0.65 mg nicotine.

[0128] The Federal Trade Commission periodically publishes compilations of tar, nicotine, and carbon monoxide yields, also referred to as ratings, for domestic cigarettes. See, Federal Trade Commission, "Tar," Nicotine, and Carbon Monoxide of the Smoke of 1294 Varieties of Domestic Cigarettes for the Year 1998, 1 n.1 (2000), herein expressly incorporated by reference in its entirety. The tar, nicotine, and carbon monoxide ratings reported in these compilations are all determined by the prescribed Federal Trade Commission testing method. Id. at 4-8.

Tobacco Carcinogens

[0129] The visual indicators of content disclosed herein can be used to indicate the quantity of carcinogens present in a tobacco product. A carcinogen generally relates to a compound or agent that can cause cancer in an organism exposed thereto. Chemicals or compounds are typically acknowledged as being a carcinogen once research results indicates a statistical probability that exposure to the chemical leads to a cancerous growth. Tobacco smoke has long been recognized as a carcinogen.

[0130] Research into the components of tobacco smoke has identified a number of compounds within tobacco smoke that are or may be carcinogenic. Compounds in tobacco smoke that are recognized as being carcinogenic include: 4-aminobiphenyl, benzene, cadmium, chromium, 2-naphthylamine, nickel, polonium-210 (radon), and vinyl chloride. Compounds in tobacco smoke that are probably carcinogenic to humans include: acrylonitrile, benzo[a]pyrene, 1,3-butadiene, dibenz(a,h)anthracene, formaldehyde, N-nitrosodiethylamine, and N-nitrosodimethylamine. Compounds in tobacco smoke that are possibly carcinogenic to humans include: acetaldehyde, benzo[b]fluoranthene, benzodj]fluoranthene, benzo[k]fluoranthene, dibenz[a,h]acridine, dibenz[a,j]acridine, 7H-dibenz[c,g]carbazole, dibenzo(a,i)pyrene, dibenzo(a,l)pyrene, 1,1-dimethylhydrazine, hydrazine, indeno[1,2,3-cd]pyrene, lead, 5-methylchrysene, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 2-nitropropane, N-nitrosodiethanolamine, N-nitrosomethylethylamine, N-nitrosomorpholine, N'-nitrosonomicotine (NNN), N-nitrosopyrrolidine, quinoline, ortho-toluidine, and urethane. Compounds in tobacco smoke that may be carcinogenic to humans include: chrysene, crotonaldehyde, N'-nitrosoanabasine (NAB), and N'-nitrosoanatabine (NAT). One or more visual indicators of content can be used to label a tobacco product and thus indicate the presence of any of the compounds listed above, or other carcinogens/potential carcinogens. In other embodiments, the one or more visual indicators of content can be used to denote the presence and quantity of carcinogens in smokeless tobacco, such as chewing tobacco and snuff.

Tobacco Additives

[0131] The visual indicators of content disclosed herein can be used to indicate the quantity of one or more tobacco additives present in a tobacco product. In addition to the tobacco itself, a variety of additives are typically included in a tobacco product. There are more than **600** additives that can legally be added to tobacco products. These additives can be used, for example, to enhance the taste of the tobacco product or to facilitate nicotine uptake by a user of the tobacco product.

[0132] The following is a representative list of known tobacco additives: acetanisole, acetic acid, acetoin, acetophenone, **6**-acetoxydihydrotheaspirane, 2-acetyl-3-ethylpyrazine, 2-acetyl-5-methylfuran, acetylpyrazine, 2-acetylpyridine, 3-acetylpyridine, 2-acetylthiazole, aconitic acid, alanine, alfalfa extract, allspice extract, oleoresin, and oil, allyl hexanoate, allyl ionone, almond bitter oil, ambergris tincture, ammonia, ammonium bicarbonate, ammonium hydroxide, ammonium phosphate dibasic, ammonium sulfide, amyl alcohol, amyl butyrate, amyl formate, amyl octanoate, alpha-amylcinnamaldehyde, amyris oil, trans-anethole, angelica root extract, oil and seed oil, anise, anise star, extract and oils, anisyl acetate, anisyl alcohol, anisyl formate, anisyl phenylacetate, apple juice concentrate, extract, and skins, apricot extract and juice concentrate, 1-arginine, asafetida fluid extract and oil, ascorbic acid, 1-asparagine monohydrate, 1-aspartic acid, balsam peru and oil, basil oil, bay leaf, oil and sweet oil, beeswax white, beet juice concentrate, benzaldehyde, benzaldehyde glyceryl acetal, benzoic acid, benzoin, benzoin resin, benzophenone, benzyl alcohol, benzyl benzoate, benzyl butyrate, benzyl cinnamate, benzyl propionate, benzyl salicylate, bergamot oil, bisabolene, black currant buds absolute, borneol, bornyl acetate, buchu leaf oil, 1,3-butanediol, 2,3-butanedione, 1-butanol, 2-butanone, 4(2-butenylidene)-3,5,5-trimethyl-2-cyclohexen-1-one, butter, butter esters, and butter oil, butyl acetate, butyl butyrate, butyl butyryl lactate, butyl isovalerate, butyl phenylacetate, butyl undecylenate, 3-butylidenephthalide, butyric acid, cadinene, caffeine, calcium carbonate, camphene, cananga oil, capsicum oleoresin, caramel color, caraway oil, carbon dioxide, cardamom oleoresin, extract, seed oil, and powder, carob bean and extract, beta-carotene, carrot oil, carvacrol, 4-caryomenthenol, 1-carvone, beta-caryophyllene, beta-caryophyllene oxide, cascarilla oil and bark extract, cassia bark oil, cassie absolute and oil, castoreum extract, tincture and absolute, cedar leaf oil, cedarwood oil terpenes and virginiana, cedrol, celery seed extract, solid, oil, and oleoresin, cellulose fiber, chamomile flower oil and extract, chicory extract, chocolate, cinnamaldehyde, cinnamic acid, cinnamon leaf oil, bark oil,

and extract, cinnamyl acetate, cinnamyl alcohol, cinnamyl cinnamate, cinnamyl isovalerate, cinnamyl propionate, citral, citric acid, citronella oil, d1-citronellol, citronellyl butyrate, citronellyl isobutyrate, civet absolute, clary oil, clover tops, red solid extract, cocoa, cocoa shells, extract, distillate and powder, coconut oil, coffee, cognac white and green oil, copaiba oil, coriander extract and oil, corn oil, corn silk, costus root oil, cubeb oil, cuminaldehyde, para-cymene, 1-cysteine, dandelion root solid extract, davana oil, 2-trans, 4-trans-decadienal, delta-decalactone, gamma-decalactone, decanal, decanoic acid, 1-decanol, 2-decenal, dehydromenthofurolactone, diethyl malonate, diethyl sebacate, 2,3-diethylpyrazine, dihydro anethole, 5,7-dihydro-2-methylthieno(3,4-D) pyrimidine, dill seed oil and extract, meta-dimethoxybenzene, para-dimethoxybenzene, 2,6-dimethoxyphenol, dimethyl succinate, 3,4-dimethyl-1,2-cyclopentanedione, 3,5-dimethyl-1,2-cyclopentanedione, 3,7-dimethyl-1,3,6-octatriene, 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one, 6,10-dimethyl-5,9-undecadien-2-one, 3,7-dimethyl-6-octenoic acid, 2,4-dimethylacetophenone, alpha,para-dimethylbenzyl alcohol, alpha,alpha-dimethylphenethyl acetate, alpha,alpha dimethylphenethyl butyrate, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, dimethyltetrahydrobenzofuranone, delta-dodecalactone, gamma-dodecalactone, para-ethoxybenzaldehyde, ethyl 10-undecenoate, ethyl 2-methylbutyrate, ethyl acetate, ethyl acetoacetate, ethyl alcohol, ethyl benzoate, ethyl butyrate, ethyl cinnamate, ethyl decanoate, ethyl fenchol, ethyl furoate, ethyl heptanoate, ethyl hexanoate, ethyl isovalerate, ethyl lactate, ethyl laurate, ethyl levulinate, ethyl maltol, ethyl methyl phenylglycidate, ethyl myristate, ethyl nonanoate, ethyl octadecanoate, ethyl octanoate, ethyl oleate, ethyl palmitate, ethyl phenylacetate, ethyl propionate, ethyl salicylate, ethyl trans-2-butenoate, ethyl valerate, ethyl vanillin, 2-ethyl (or methyl)-(3,5 and 6)-methoxypyrazine, 2-ethyl-1-hexanol, 3-ethyl -2-hydroxy-2-cyclopenten-1-one, 2-ethyl-3, (5 or 6)-dimethylpyrazine, 5-ethyl-3-hydroxy-4-methyl-2(5h)-furanone, 2-ethyl-3-methylpyrazine, 4-ethylbenzaldehyde, 4-ethylguaiacol, para-ethylphenol, 3-ethylpyridine, eucalyptol, famesol, D-fenchone, fennel sweet oil, fenugreek, extract, resin, and absolute, fig juice concentrate, food starch modified, furfuryl mercaptan, 4-(2-furyl)-3-buten-2-one, galbanum oil, genet absolute, gentian root extract, geraniol, geranium rose oil, geranyl acetate, geranyl butyrate, geranyl formate, geranyl isovalerate, geranyl phenylacetate, ginger oil and oleoresin, 1-glutamic acid, 1-glutamine, glycerol, glycyrrhizin ammoniated, grape juice concentrate, guaiac wood oil, guaiacol, guar gum, 2,4-heptadienal, gamma-heptalactone, heptanoic acid, 2-heptanone, 3-hepten-2-one, 2-hepten-4-one, 4-heptenal, trans -2-heptenal, heptyl acetate, omega-6-hexadecenlactone, gamma-hexalactone, hexanal, hexanoic acid, 2-hexen-1-ol, 3-hexen-1-ol, cis-3-hexen-1-yl acetate, 2-hexenal, 3-hexenoic acid, trans-2-hexenoic acid, cis-3-hexenyl formate, hexyl 2-methylbutyrate, hexyl acetate, hexyl alcohol, hexyl phenylacetate, 1-histidine, honey, hops oil, hydrolyzed milk solids, hydrolyzed plant proteins, 5-hydroxy-2,4-decadienoic acid delta-lactone, 4-hydroxy-2,5-dimethyl-3(2h)-furanone, 2-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one, 4-hydroxy -3-pentenoic acid lactone, 2-hydroxy-4-methylbenzaldehyde, 4-hydroxybutanoic acid lactone, hydroxycitronellal, 6-hydroxydihydrotheaspirane, 4-(para-hydroxyphenyl)-2-butanone, hyssop oil, immortelle absolute and extract, alpha-ionone, beta-ionone, alpha-irone, isoamyl acetate, isoamyl benzoate, isoamyl butyrate, isoamyl cinnamate, isoamyl formate, isoamyl hexanoate, isoamyl isovalerate, isoamyl octanoate, isoamyl phenylacetate, isobornyl acetate, isobutyl acetate, isobutyl alcohol, isobutyl cinnamate, isobutyl phenylacetate, isobutyl salicylate, 2-isobutyl-3-methoxypyrazine, alpha-isobutylphenethyl alcohol, isobutyraldehyde, isobutyric acid, d,1-isoleucine, alpha-isomethylionone, 2-isopropylphenol, isovaleric acid, jasmine absolute, concrete and oil, kola nut extract, labdanum absolute and oleoresin, lactic acid, lauric acid, lauric aldehyde, lavandin oil, lavender oil, lemon oil and extract, lemongrass oil, 1-leucine, levulinic acid, licorice root, fluid, extract and powder, lime oil, linalool, linalool oxide, linalyl acetate, linden flowers, lovage oil and extract, 1-lysine, mace powder, extract and oil, magnesium carbonate, malic acid, malt and malt extract, maltodextrin, maltol, maltyl isobutyrate, mandarin oil, maple syrup and concentrate, mate leaf, absolute and oil, para-mentha-8-thiol-3-one, menthol, menthone, menthyl acetate, d1-methionine, methoprene, 2-methoxy-4-methylphenol, 2-methoxy-4-vinylphenol, para-methoxybenzaldehyde, 1-(para-methoxyphenyl)-1-penten-3-one, 4-(para-methoxyphenyl)-2-butanone, 1-(para-methoxyphenyl)-2-propanone, methoxypyrazine, methyl 2-furoate, methyl 2-octynoate, methyl 2-pyrrolyl ketone, methyl anisate, methyl anthranilate, methyl benzoate, methyl cinnamate, methyl dihydrojasmonate, methyl ester of rosin, partially hydrogenated, methyl isovalerate, methyl linoleate (48%), methyl linolenate (52%) mixture, methyl naphthyl ketone, methyl nicotinate, methyl phenylacetate, methyl salicylate, methyl sulfide, 3-methyl-1-cyclopentadecanone, 4-methyl-1-phenyl-2-pentanone, 5-methyl-2-phenyl-2-hexenal, 5-methyl-2-thiophenecarboxaldehyde, 6-methyl-3,5-heptadien-2-one, 2-methyl-3-(para-isopropylphenyl) propionaldehyde, 5-methyl-3-hexen-2-one, 1-methyl-methoxy-4-isopropylbenzene, 4-methyl-3-pentene-2-one, 2-methyl-4-phenylbutyraldehyde, 6-methyl-5-hepten-2-one, 4-methyl-5-thiazoleethanol, 4-methyl-5-vinylthiazole, methyl-alpha-ionone, methyl-trans-2-butenoic acid, 4-methylacetophenone, para-methylanisole, alpha-methylbenzyl acetate, alpha-methylbenzyl alcohol, 2-methylbutyraldehyde, 3-methylbutyraldehyde, 2-methylbutyric acid, alpha-methylcinnamaldehyde, methylcyclopentenolone, 2-methylheptanoic acid, 2-methylhexanoic acid, 3-methylpentanoic acid, 4-methylpentanoic acid, 2-methylpyrazine, 5-methylquinoxaline, 2-methyltetrahydrofuran-3-one, (methylthio)methylpyrazine (mixture of isomers), 3-methylthiopropionaldehyde, methyl 3-methylthiopropionate, 2-methylvaleric acid, mimosa absolute and extract, molasses extract and tincture, mountain maple solid extract, mullein flowers, myristaldehyde, myristic acid, myrrh oil, beta-napthyl ethyl ether, nerol, neroli bigarde oil, nerolidol, nona-2-trans, 6-cis-dienal, 2,6-nonadien-1-ol, gamma-nonalactone, nonanal, nonanoic acid, nonanone, trans-2-nonen-1-ol, 2-nonenal, nonyl acetate, nutmeg powder and oil, oak chips extract and oil, oak moss absolute, 9,12-octadecadienoic acid (48%) and 9,12,15-octadecatrienoic acid (52%), delta-octalactone, gamma-octalactone, octanal, octanoic acid, 1-octanol, 2-octanone, 3-octen-2-one, 1-octen-3-ol, 1-octen-3-yl acetate, 2-octenal, octyl isobutyrate, oleic acid, olibanum oil, opoponax oil and gum, orange blossoms water, absolute, and leaf absolute, orange oil and extract, origanum oil, orris concrete oil and root extract, palmarosa oil, palmitic acid, parsley seed oil, patchouli oil, omega-

pentadecalactone, 2,3-pentanedione, 2-pentanone, 4-pentenoic acid, 2-pentylpyridine, pepper oil, black and white, peppermint oil, peruvian (bois de rose) oil, petitgrain absolute, mandarin oil and terpeneless oil, alpha-phellandrene, 2-phenenthyl acetate, phenenthyl alcohol, phenethyl butyrate, phenethyl cinnamate, phenethyl isobutyrate, phenethyl isovalerate, phenethyl phenylacetate, phenethyl salicylate, 1-phenyl-1-propanol, 3-phenyl-1-propanol, 2-phenyl-2-butenal, 4-phenyl-3-buten-2-ol, 4-phenyl-3-buten-2-one, phenylacetaldehyde, phenylacetic acid, 1-phenylalanine, 3-phenylpropionaldehyde, 3-phenylpropionic acid, 3-phenylpropyl acetate, 3-phenylpropyl cinnamate, 2-(3-phenylpropyl)tetrahydrofuran, phosphoric acid, pimenta leaf oil, pine needle oil, pine oil, scotch, pineapple juice concentrate, alpha-pinene, beta-pinene, d-piperitone, piperonal, pipsissewa leaf extract, plum juice, potassium sorbate, 1-proline, propenylguaethol, propionic acid, propyl acetate, propyl para-hydroxybenzoate, propylene glycol, 3-propylidenephthalide, prune juice and concentrate, pyridine, pyroligneous acid and extract, pyrrole, pyruvic acid, raisin juice concentrate, rhodinol, rose absolute and oil, rosemary oil, rum, rum ether, rye extract, sage, sage oil, and sage oleoresin, salicylaldehyde, sandalwood oil, yellow, sclareolide, skatole, smoke flavor, snakeroot oil, sodium acetate, sodium benzoate, sodium bicarbonate, 2,2,6-trimethylcyclohexanone, 2,3,5-trimethylpyrazine, 1-tyrosine, delta-undercalactone, gamma-undecalactone, undecanal, 2-undecanone, 10-undecenal, urea, valencene, valeraldehyde, valerian root extract, oil and powder, valeric acid, gamma-valerolactone, valine, vanilla extract and oleoresin, vanillin, veratraldehyde, vetiver oil, vinegar, violet leaf absolute, walnut hull extract, water, wheat extract and flour, wild cherry bark extract, wine and wine sherry, xanthan gum, 3,4-xylenol, and yeast. The visual indicators of content discussed herein can be used to indicate the content level of any tobacco additive present in a particular tobacco product, such as, for example, any of those listed above.

Tar

[0133] In other embodiments, the visual indicators of content disclosed herein can be used to indicate the quantity of tar present in or generated from the use of a tobacco product. Generally, most smoking tobacco products produce tar when used. Tar contains a number of known carcinogens. Tar is a mixture of many different chemicals that include cancer-causing agents such as formaldehyde, arsenic, cyanide, benzo[a]pyrene, benzene, toluene and acrolein. When smoke is inhaled, particles of tar can travel in the smoke to the lungs and respiratory system where they are absorbed. The visual indicators of content discussed herein can be used to indicate tar content or the content of various tar components in a particular tobacco product.

[0134] The addition of a nicotine substitute to the tobacco described herein can be at anytime during the processing, curing, blending or manufacture of the tobacco product, as described in greater detail below.

Nicotine Substitutes

[0135] In certain embodiments, it is advantageous to add a nicotine substitute to a tobacco product. More specifically, adding nicotine substitutes can be effective in tobacco-use cessation programs that are designed to alleviate the effects of nicotine withdrawal that tobacco users experience when trying to quit. Typically tobacco products containing a nicotine substitute have either a reduced level of nicotine or are essentially nicotine-free. Accordingly, in certain embodiments the content indicator can indicate the presence of a nicotine substitute within the tobacco product.

[0136] Several nicotine substitutes are known in the art and any one or more nicotine substitutes can be associated with, incorporated in or consumed in conjunction with the tobacco products described herein. See e.g., U.S. Pat. No. 5,780,051, to Eswara et al., entitled "Methods and Articles of Manufacture for Nicotine Cessation and Monitoring Nicotine Use," and issued on Jul. 14, 1998, U.S. Pat. No. 6,166,032, to Viner, entitled "Method for Controlling Tobacco Use and Alleviating Withdrawal Symptoms Due to Cessation of Tobacco Use," and issued on Dec. 26, 2000, U.S. Pat. No. 6,197,827, to Cary, entitled "Nicotine Addiction Treatment," issued on Mar. 6, 2001, U.S. Pat. No. 4,966,916, to Abood, entitled "Agonists and Antagonists to Nicotine as Smoking Deterrents," issued on Oct. 30, 1990, and U.S. Pat. No. 4,835,162, to Abood, entitled "Agonists and Antagonists to Nicotine as Smoking Deterents," issued on May 30, 1989. Each of the foregoing is hereby expressly incorporated by reference in its entirety. Preferably, Cytisine and Cytisine derivatives, including but not limited to purified forms, partially purified forms, isolated forms, synthesized forms, and natural sources of Cytisine, such as plants and plant components (e.g., leaves and stems) containing the compound, are used as the nicotine substitute. In some embodiments, the modified tobacco having a reduced amount of nicotine and/or TSNA described herein is associated with or provided in conjunction with 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more nicotine substitutes, as described above. The nicotine substitute can be applied to the tobacco itself at any stage of curing, processing, or manufacturing or to the tobacco product (e.g., paper, processed tobacco, filter).

[0137] The term "nicotine substitute" is used in a general sense to refer to either or both of two classes of compounds: (1) a compound that is a receptor binding nicotine substitute or (2) a compound that is a sensory altering nicotine substitute. A nicotine substitute as used herein does not include nicotine. The term "receptor binding nicotine substitute" generally refers a compound that binds with a specific affinity for one or more nicotinic receptors (e.g., Lobeline, Arecoline, Isoarecolone, Anabasine and Cytisine). The term "sensory altering nicotine substitute" generally refers to a compound which, when administered to a subject, alters the subjects sensory perception such that the subjects awareness of nicotine withdrawal is reduced (e.g., anti-anxiety agents, antidepressants, antiobsessional agents, and antipsychotic agents). The visual content indicators provided herein can denote the presence or content level of any of the above mentioned nicotine substitutes, or any other nicotine substitute. These substitutes and their uses are discussed further in U.S. Provisional Patent Application No. 60/486,875, entitled "Tobacco Products Containing Low Nicotine Tobacco and Nicotine Replacement Compounds," and filed on Jul. 10, 2003 (attorney docket No. VTOB.273PR), which is hereby incorporated by reference in its entirety.

[0138] In general, the nicotine substitute is a Cytisine or Cytisine-like compound that has an affinity for any nicotinic receptor but, preferably, the compound has an affinity to nicotinic receptor-enriched brain tissue, as determined by in vitro binding assays. The nicotinic receptor can also be

located, however, in neuronal tissue, muscular tissue, neuromuscular, and the like, for example.

[0139] The term "Cytisine," as used herein, also includes Cytisine free base and its various salts and Cytisine analogs. Functional groups may be added to or deleted from the general Cytisine chemical formula while retaining the physiological activity of Cytisine. Such alterations and deviations are encompassed by the term "Cytisine analogs". Cytisine, also known as Ulexin, Baptitoxine, and Sophora, is an alkaloid that can be obtained from a variety of leguminous plants (e.g., plants from the genus Cytisus, Genista, Sophara, Baptisia, and Anagyri). Cytisine is also commercially obtainable (e.g., Sopharma or Sigma C2899). In some embodiments, a Cytisine analog or Cytisine-like synthetic, such as Vareniclineg can be used. The Cytisine that is associated with or incorporated into the modified tobacco and tobacco products described herein can be in a purified form, as that which is commercially available, or can be present in a plant material (e.g., leaves and stems from plants of genus Cytisus, Genista, Sophara, Baptisia, and Anagyri) that is provided with (e.g., blended with) the modified tobacco described herein.

[0140] Nicotine substitutes can be added to tobacco at any suitable time during tobacco curing, processing or manufacturing. In some embodiments, the nicotine substitute is added to low nicotine and/or low TSNA tobacco before, during, or after the curing process. For example, in preferred embodiments, a nicotine substitute (e.g., leaves containing the Cytisine) is added during the blending, bulking, or expanding stages.

[0141] A nicotine substitute can be added to the tobacco by employing "additive technology," as is known in the art. In some embodiments, the nicotine substitute is present in a solution that is applied to the tobacco, filler, paper or filter. In preferred embodiments, the dissolved nicotine substitute is sprayed or atomized over bulk tobacco. In other embodiments, the solution containing the nicotine substitute is mixed with the tobacco in a slurry. When a nicotine substitute is dissolved in a solution, it is preferred that it is dissolved uniformly, through mixing or otherwise. The solution can also be added to cigarette paper or can be impregnated into the filter in a form that becomes volatilized when the cigarette is consumed.

[0142] In other embodiments, the nicotine substitute can be in the form of a dried powder. While directed specifically to flavoring tobacco, the methods provided in U.S. Pat. No. 4,617,945, to Vos et. al., can be used to add a powdered nicotine substitute onto tobacco, the paper, or filter, in accordance with the methods herein. This patent is hereby expressly incorporated by reference in its entirety.

[0143] Other detailed methods of incorporating nicotine substitute to low nicotine and/or TSNA tobacco are disclosed in U.S. Pat. No. 4,243,056, to de la Burde et al., which is hereby expressly incorporated by reference in its entirety. According to one method, a nicotine substitute is contacted to the tobacco through liquid carbon dioxide having the nicotine substitute dispersed therein. The liquid carbon dioxide is absorbed by the tobacco to conditions whereby it is converted to solid carbon dioxide. The solid carbon dioxide is then heated and allowed to evaporate. When the solid carbon dioxide vaporizes rapidly, the tobacco will be impregnated with the nicotine substitute and expand.

[0144] In still more embodiments, the nicotine substitute is incorporated into reconstituted tobacco. Reconstituted tobacco, in general, includes a combination of tobacco stems and leaf scraps that are ground into a pulp, occasionally extracted, and blended with additives and chemicals.

[0145] In other embodiments the nicotine substitute can be added to the filter of a cigarette or pipe containing low nicotine and/or TSNA tobacco. The nicotine substitute can be added to any suitable filter element such as tipping paper, shaped paper insert, mouthpiece plug, solid filter element, or free-flow filter element. In more specific embodiments the nicotine substitute can be incorporated into filter materials including cotton, paper, cellulose, and suitable synthetic fibers. In preferred embodiments, the nicotine substitute will be dissolved in a suitable solution and administered (e.g., spraying) to the filter element or material. In other embodiments the nicotine substitute can be administered to the cigarette paper or cigar wrap. Preferred methods of incorporating a nicotine substitute into a low nicotine and/or TSNA tobacco product are provided in Examples 8, and 9.

[0146] Effective and non-toxic amounts of the above-mentioned nicotine substitutes are readily known to those with skill in the art, or can be readily determined. Various amounts of nicotine substitutes can be incorporated into the tobacco product but preferably, the amount of nicotine substitute incorporated is an amount sufficient to quell the desire for nicotine. Because the amount of nicotine substitute that is roughly equivalent to an amount of nicotine is known in the field or is readily determinable, tobacco products containing various amounts of nicotine substitute are preferably provided. That is, the amount of nicotine substitute that is provided to the modified tobacco can be, in dry weight for example, less than 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, 0.05%, 0.055%, 0.06%, 0.065%, 0.07%, 0.075%, 0.08%, 0.085%, 0.09%, 0.095%, 0.1%, 0.15%, 0.175%, 0.2%, 0.225%, 0.25%, 0.275%, 0.3%, 0.325%, 0.35%, 0.375%, 0.4%, 0.425%, 0.45%, 0.475%, 0.5%, 0.55%, 0.6%, 0.65%, 0.7%, 0.75%, 0.8%, 0.85%, 0.9%, 0.95%, 1.0%. 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2.0%, 2.1%, 2.2%, 2.3%, 2.4%, 2.5%, 2.6%, 2.7%, 2.8%, 2.9%, 3.0%, 3.1%, 3.2%, 3.3%, 3.4%, 3.5%, 3.6%, 3.7%, 3.8%, 3.9%, **and 4.0**%. Generally, it is desired that tobacco that is used in earlier parts of the tobacco-use cessation program have relatively higher amounts of a nicotine substitute, and tobacco to be used in later parts of the cessation program have relatively lower amounts of a nicotine substitute.

[0147] While most embodiments provided herein have been directed to adding or incorporating a nicotine substitute into low nicotine and/or TSNA tobacco, it is also contemplated that the nicotine substitute can be administered to a tobacco user separately, but in close temporal proximity with low nicotine and/or TSNA tobacco. Close temporal proximity generally relates to a time period immediately preceding or following consumption of the tobacco product (e.g., within 1 minute, 5 minutes, 10 minutes, 20 minutes, thirty minutes, 45 minutes, 1 hour, 1.25 hours, 1.5 hours, 1.75 hours or 2 hours). In preferred embodiments the nicotine substitute is administered to a subject within less than about 110, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, 4, 3, 2, and 1 minutes of administration of the low nicotine and/or TSNA tobacco. The nicotine substitute can be separately adminis-

tered to a tobacco user before, after or at the same time the low nicotine and/or TSNA tobacco is administered.

Nicotine Reduction and/or Tobacco-use Cessation Programs Methods

[0148] It is also contemplated that the tobacco products labeled as described herein can be used in tobacco-use cessation programs. For example, low nicotine and/or TSNA tobacco containing one or more nicotine substitute, as described herein, can be processed and blended with conventional tobacco so as to create a wide-range of tobacco products with varying amounts of nicotine and/or nitrosamines. These blended tobacco products can be labeled or marked with the indicators described herein and used in nicotine reduction and/or tobacco-use cessation programs so as to move a consumer from a high nicotine and TSNA product to a low nicotine and TSNA product.

[0149] In some embodiments of the invention, a stepwise nicotine reduction and/or tobacco-use cessation program is established using the low nicotine, low TSNA products described above. As an example, the program participant initially determines his or her current level of nicotine intake. The program participant then begins the program at step 1, with a tobacco product having a reduced amount of nicotine, as compared to the tobacco product that was used prior to beginning the program. After a period of time, the program participant proceeds to step 2, using a tobacco product with less nicotine than the products used in step 1. The program participant, after another period of time, reaches step **3**, wherein the program participant begins using a tobacco product with less nicotine than the products in step 2, and so on. Ultimately, the program participant uses a tobacco product having an amount of nicotine that is less than that which is sufficient to become addictive or to maintain an addiction. The different tobacco products used at each step can be labeled with indicators as described herein, thereby facilitating participant compliance with the program. Using the methods and products described herein, the nicotine reduction and/or tobacco-use cessation program can limit the exposure of a program participant to nicotine and, concomitantly, the harmful effect of nicotine yet retains the secondary factors of addiction, including but not limited to, smoke intake, oral fixation, and taste. In some embodiments, the step 1, 2, and 3 products above have a nicotine substitute added. It will be appreciated that the content level of the substitute can also be labeled with indicators as described herein. The nicotine substitute can completely replace the nicotine or can be used as a supplement to the nicotine, in particular in the low nicotine tobacco products.

[0150] For example, a smoker can begin the program smoking blended cigarettes having 2 mg of nicotine, 1.5 mg nicotine substitute (e.g., Cytisine) and 1.5 kg of nitrosamine, gradually move to smoking cigarettes with 1 mg of nicotine, 1 mg nicotine substitute (e.g., Cytisine) and 1 μg of nitrosamine, followed by cigarettes having 0.5 mg nicotine, 0.5 mg nicotine substitute (e.g., Cytisine) and 0.5 μg nitrosamine, followed by cigarettes having 0.15 mg nicotine, 0.25 mg nicotine substitute (e.g., Cytisine) and 0.25 μg nitrosamine, followed by cigarettes having less than 0.1 mg nicotine and less than 0.1 μg TSNA until the consumer decides to smoke only the cigarettes having virtually no nicotine and nitrosamines or quitting smoking altogether. The cigarettes can be labeled appropriately with content indicators as described herein.

[0151] By another approach, a three-step program is followed whereby at step 1, cigarettes containing 0.6 mg nicotine and 1.5 mg nicotine substitute (e.g., Cytisine) and less than 2 μg/g TSNA are used; at step 2, cigarettes containing 0.3 mg nicotine and 1.0 mg nicotine substitute (e.g., Cytisine) and less than 1 μg/g TSNA are used; and at step 3, cigarettes containing less than 0.1 mg nicotine, and 0.5 mg nicotine substitute (e.g., Cytisine) and less than 0.7 μg/g TSNA are used. More preferably, a three-step program is followed whereby at step 1, cigarettes containing 0.6 mg nicotine, and 1.5 mg nicotine substitute (e.g., Cytisine) and less than 2 μg/g TSNA are used; at step 2, cigarettes containing 0.3 mg nicotine and 1.0 mg nicotine substitute (e.g., Cytisine) and less than 1 μg/g TSNA are used; and at step 3, cigarettes containing less than 0.05 mg nicotine and 0.5 mg nicotine substitute (e.g., Cytisine) and less than 0.7 μg/g TSNA are used. Accordingly, the blended cigarettes described herein provide the basis for an approach to reduce the carcinogenic potential in a human in a step-wise fashion. The blended cigarettes can be labeled or marked with appropriate indicators as described herein.

[0152] By still another approach, a three-step program is followed whereby at step 1, cigarettes containing 0.6 mg nicotine and less than 2 μg/g TSNA are used; at step 2, cigarettes containing 0.3 mg nicotine and less than 1 μg/g TSNA are used; and at step 3, cigarettes containing less than 0.1 mg nicotine, and less than 1.5 mg nicotine substitute (e.g., Cytisine) and less than 0.7 μg/g TSNA are used. More preferably, a three-step program is followed whereby at step 1, cigarettes containing 0.6 mg nicotine and less than 2 μg/g TSNA are used; at step 2, cigarettes containing 0.3 mg nicotine and less than 1 μg/g TSNA are used; and at step 3, cigarettes containing less than 0.05 mg nicotine and less than 1.5 mg nicotine substitute (e.g., Cytisine) and less than 0.7 μg/g TSNA are used. Accordingly, the blended cigarettes described herein provide the basis for an approach to reduce the carcinogenic potential in a human in a step-wise fashion while addressing both the primary and secondary factors of addiction. The cigarettes can be labeled or marked with appropriate indicators as described herein.

[0153] Embodiments also include stepwise blends of tobacco products, which are prepared with a variety of amounts of nicotine. These can be marked or labeled with appropriate indicators as described herein. These stepwise blends are made to have reduced levels of TSNAs and varying amounts of nicotine. As an example, cigarettes may contain, for example, 5 mg, 4, 3, 2, 1, 0.5, 0.1, or 0 mg of nicotine per cigarette. More preferably, blended cigarettes contain less than 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6% nicotine. Any of the cigarettes above can be supplemented with a nicotine substitute or the amount of nicotine in the cigarettes above can be replaced by the same amount of nicotine substitute or an amount that is approximately functionally equivalent (e.g., approximately 1.5 mg of Cytisine is functionally equivalent to the amount of nicotine in a typical cigarette, 2-5 mg).

[0154] In another aspect of the invention, the cigarettes of varying levels of nicotine and/or nicotine substitutes are packaged to clearly indicate the level of nicotine and/or nicotine substitute present, and marketed as a smoking cessation program. A preferred approach to produce a product for nicotine reduction and/or tobacco-use cessation pro-

gram is provided in Example **10**. Individuals may wish to step up the program by skipping gradation levels of nicotine per cigarette or staying at certain steps until ready to proceed to the next level. Significantly, aspects of the invention allow a consumer to individually select the amount of nicotine and/or nicotine substitute that is ingested by selection of a particular tobacco product described herein. Furthermore, because the secondary factors of addiction are maintained, dependence on nicotine can be reduced rapidly.

[0155] The nicotine reduction and/or tobacco-use cessation program described herein limits the exposure of a program participant to nicotine while retaining the secondary factors of addiction. These secondary factors include but are not limited to, smoke intake, oral fixation, and taste. Because the secondary factors are still present, the program participant may be more likely to be successful in the nicotine reduction and/or tobacco-use cessation program than in programs that rely on supplying the program participant with nicotine but remove the above-mentioned secondary factors. Ultimately, the program participant uses a tobacco product having an amount of nicotine that is less than that which is sufficient to become addictive, which may make it easier to quit smoking entirely.

[0156] In another aspect of the invention, individuals would choose to obtain only cigarettes with less than 0.05 mg nicotine per cigarette or cigarettes that contain 0.05 mg nicotine or less and a nicotine substitute. Some individuals, such as individuals needing to stop nicotine intake immediately (for example, individuals with medical conditions or individuals using drugs that interact with nicotine) may find these compositions useful.

[0157] In another aspect of the invention, packs of cigarettes containing the gradations of nicotine and/or nicotine substitute levels are provided as a "smoking cessation kit." The cigarettes in such "smoking cessation kits" can be labeled or marked with indicators as described herein to facilitate use of the kits. An individual who wishes to quit smoking can buy the entire kit of cigarettes at the beginning of the program. Thus any temptation that may occur while buying cigarettes at the cigarette counter is avoided. Thus, the success of this method may be more likely for some individuals. A preferred example of such a kit is provided in Example 11.

Systems

[0158] The disclosed invention also relates to tobacco product labeling systems. In certain embodiments these systems can be used to generate a plurality of visual indicators of content. In certain embodiments, the content indicators denote the relative content of one or more components of a tobacco product. The content indicator can be an alpha/numerical character or a non-alpha/numerical indicator. In one embodiment the content indicators are applied directly to a tobacco product, such as a cigarette paper, a cigarette filter, tipping paper, a lozenge, gum, or a cigar. In another embodiment, the content indicators are applied to a tobacco product package, such as a box, wrapper, carton, or container. Application may be in the form of ink or other printing media. Another alternative involves the use of symbols affixed to a tobacco product.

[0159] According to the disclosed invention, visual indicators of content are used in a predetermined manner to indicate content levels. In the case when tobacco products are available with varying levels of a particular component, a system of content indicators is applied to the various products that denotes the level of the component of interest. For example, a tobacco product that has a) a specified or base content level of nicotine, b) a reduced content level of nicotine relative to the first product, and c) a product that is essentially free of nicotine can each have a particular content indicator. Each of these indicators is cross-referenced against one another to allow one to visually determine the content of nicotine in a particular tobacco product. It is noted that nicotine content in a particular tobacco product is merely one example of a compound that can be denoted by a visual content indicator encompassed by the disclosed invention.

[0160] While each visual content indicator is selected and used to indicate the content of a particular tobacco component, it is noted that the selection and placement of particular visual indicators of content can be in many cases a design choice having ornamental and nonfunctional characteristics.

[0161] In some embodiments, the systems can include a printing apparatus for imprinting the visual content indicator onto the tobacco product. Examples of printing apparatuses that can be used to imprint content indicators on tobacco products are disclosed in U.S. Pat. No. 6,279,475 to Cardoso, entitled Apparatus for supplying flowable printing ink to a printer for cigarette paper webs, issued on Aug. 28, 2001, which is hereby expressly incorporated by reference in its entirety. In one aspect, the printing apparatus can be constructed and assembled to apply a flowable mixture of a printing ink and a compressed gas to a printer for the application of printing ink to a running elongated strip of imprintable material in the form of a pattern. In more specific embodiments, the imprintable material can be cigarette or tipping paper. In further aspects, the printing apparatus is capable of applying printing ink at a predetermined rate.

[0162] In other embodiments, the printing apparatus can include a nozzle having a mixture-receiving inlet and an outlet for directing the mixture against the printer, and means for supplying the mixture to the inlet in quantities which vary as a function of the predetermined rate.

[0163] The printing apparatus can further include means for moving the strip of imprintable material lengthwise and means for moving the nozzle relative to the imprintable material and the printer. In still further embodiments, the means for moving the nozzle can comprise means for reciprocating the nozzle, means for reciprocating an adjustable prime mover, and means for adjusting the prime mover as a function of the predetermined rate. The prime mover can include an adjustable electric motor, and the adjusting means can also include means for transmitting electric signals exhibiting characteristics dependent on the predetermined rate to the electric motor.

[0164] The electric motor or other selected adjusting means can be designed and mounted to reciprocate the nozzle, at least substantially transversely of the moving strip. For example, the means for reciprocating the nozzle at least substantially transversely of the moving imprintable material can comprise a variable-speed electric motor and means for transmitting to such motor electric signals to regulate the speed of the motor as a function of the predetermined rate.

[0165] The predetermined rate can be stored in a memory, in the form of information, which is addressed to operate the moving means. This information can be in the form of a so-called reference-print image, and the means for transmitting to the motor electric signals can comprise means for photoelectronically scanning the image and for generating signals for transmission to the moving means. The printing apparatus can also include a mobile printing ink applicator which is arranged to contact and to transfer printing ink onto the running imprintable material, and means for transferring ink from the outlet to the applicator.

[0166] Other examples of printing devices that can be used with the embodiments provided herein are found in U.S. Pat. No. 4,372,208, to Legardinier, entitled "Device for Supplying with Ink Printing Apparatus for Cigarette-Making Machines," and issued on Feb. 8, 1983, which is hereby expressly incorporated by reference in its entirety. Those with skill in the art will recognize that suitable printing apparatuses can be used in conjunction with various cigarette making machines. Examples of cigarette making machines that can be used herein are disclosed in U.S. Pat. No. 6,557,560, to Kastner, entitled "Cigarette Making Machine," and issued on May 6, 2003, U.S. Pat. No. 6,020,969, to Struckhoff et al., entitled "Cigarette Making Machine Including Band Inspection," and issued on Feb. 1, 2000, U.S. Pat. No. 5,819,751, to Barnes et al., entitled "Cigarette and Method of Making Same," and issued on Oct. 13, 1998, and U.S. Pat. No. 5,626,152 to Davis et al., entitled "Cigarette Making Machine," and issued on May 6, 1997. Each of the foregoing patents is hereby expressly incorporated by reference in its entirety.

[0167] In other embodiments, the visual content indicator can be imprinted on any suitable cigarette paper. Examples of cigarette paper that can be used with the embodiments herein can be found in U.S. Pat. No. 6,584,981, to Hampl Jr., entitled "Cigarette Paper Containing Carbon Fibers for Improved Ash Characteristics," and issued on Jul. 1, 2003, U.S. Pat. No. 5,540,242 to Chao et al., entitled "Cigarette Paper Having Reduced Sidestream Properties," and issued on Jul. 30, 1996, U.S. Pat. No. 5,109,876, to Hayden et al., entitled "Cigarette Paper and Cigarette Incorporating Same," and issued on May 5, 1992, and U.S. Pat. No. 5,062,434, to Aulbach et al., entitled "Cigarette Paper," and issued on Nov. 5, 1991. Each of the foregoing patents is hereby expressly incorporated by reference in its entirety. In some embodiments, the content indicator can be imprinted on any suitable tipping paper. Examples of tipping paper can be used with the embodiments herein are found in U.S. Pat. No. 4,094,324, to Bolsinger et al., entitled "Perforated Cigarette Tipping Paper," and issued on Jun. 13, 1978, and U.S. Pat. No. 5,830,318, to Snow et al., entitled "High Opacity Tipping Paper," and issued on Nov. 3, 1998. Each of the foregoing patents is hereby expressly incorporated by reference in its entirety.

[0168] In some embodiments, the systems provided herein can be used to manufacture the tobacco products described herein. In other aspects, the systems can be used with the methods of labeling described herein.

Methods

[0169] The disclosed invention further encompasses methods for labeling tobacco products with at least one visual indicator of content. In certain embodiments, the tobacco product labeling systems provided herein can be used with these methods. A preliminary step of such methods typically provides a system of labeling wherein particular content indicators are assigned to indicate the relative content of a particular component in a tobacco product. Tobacco products that will display the various content indicators are prepared. Once the system is determined and the products are prepared, the visual indicators of content are affixed to those tobacco products that contain the relevant quantity of the component of interest. As discussed above, the visual indicators can be affixed to the tobacco product itself, such as a label on an individual cigarette. In the alternative, the visual content indicator can be affixed to packaging that contains individual tobacco product units.

The Tobacco Products

[0170] A variety of tobacco products can be labeled with a visual content indicator. Examples of tobacco products include, individual cigarettes, packages of cigarettes, cartons of cigarettes, individual cigars, boxes of cigars, cigar wrappers or labels, cigar containers, pipe tobacco containers, tobacco lozenges and their wrappers and containers, tobacco gum and their wrappers and containers, chewing tobacco containers, and snuff containers.

[0171] The examples which follow are set forth to illustrate the present invention, and are not to be construed as limiting thereof.

EXAMPLE 1

Isolation and Sequencing

[0172] TobRD2 cDNA (Conkling et. al., *Plant Phys.* 93, 1203 (1990)) encodes QPTase, which is predicted to be a cytosolic protein. Comparisons of the NtQPT1amino acid sequence with the GenBank database revealed limited sequence similarity to certain bacterial and other proteins; quinolate phosphoribosyl transferase (QPTase) activity has been demonstrated for the *S. typhimurium, E. coli.* and *N. tabacum* genes. The NtQPT1encoded QPTase has similarity to the deduced peptide fragment encoded by an *Arabidopsis* EST (expression sequence tag) sequence (Genbank Accession number F20096), which may represent part of an *Arabidopsis* QPTase gene.

EXAMPLE 2

Transformation of Tobacco Plants

[0173] DNA of the QPTase gene, in antisense orientation, is operably linked to a plant promoter (CaMV 35S or TobRD2 root-cortex specific promoter) to produce two different DNA cassettes: CaMV35S promoter/antisense QPTase-encoding gene and TobRD2 promoter/antisense QPTase-encoding gene.

[0174] A wild-type tobacco line and a low-nicotine tobacco line are selected for transformation, e.g., wild-type Burley 21 tobacco (Nic1+/Nic2+) and homozygous Nic1-/Nic2-Burley 21. A plurality of tobacco plant cells from each line are transformed using each of the DNA cassettes. Transformation is conducted using an *Agrobacterium* vector, e.g., an *Agrobacterium*-binary vector carrying Ti-border sequences and the nptII gene (conferring resistance to kanamycin and under the control of the nos promoter (nptII)).

[0175] Transformed cells are selected and regenerated into transgenic tobacco plants called R_0. The R_0 plants are grown to maturity and tested for levels of nicotine; a subset of the transformed tobacco plants exhibit significantly lower levels of nicotine compared to non-transformed control plants.

[0176] R_0 plants are then selfed and the segregation of the transgene is analyzed in next generation, the R_1 progeny. R_1 progeny are grown to maturity and selfed; segregation of the transgene among R_2 progeny indicate which R_1 plants are homozygous for the transgene.

EXAMPLE 3

Tobacco Having Reduced Nicotine Levels

[0177] Tobacco of the variety Burley 21 LA was transformed with the binary *Agrobacterium* vector pYTY32 to produce a low nicotine tobacco variety, Vector 21-41. The binary vector pYTY32 carried the 2.0 kb NtQPT1 root-cortex-specific promoter driving antisense expression of the NtQPT1cDNA and the nopaline synthase (nos) 3' termination sequences from *Agrobacterium tumefaciens* T-DNA. The selectable marker for this construct was neomycin phosphotransferase (nptII) from *E. coli* Tn5 which confers resistance to kanamycin, and the expression nptII was directed by the nos promoter from *Agrobacterium tumefaciens* T-DNA. Transformed cells, tissues, and seedlings were selected by their ability to grow on Murashige-Skoog (MS) medium containing 300 μg/ml kanamycin. Burley 21 LA is a variety of Burley 21 with substantially reduced levels of nicotine as compared with Burley 21 (i.e., Burley 21 LA has 8% the nicotine levels of Burley 21, see Legg et al., *Can J Genet Cytol,* 13:287-91 (1971); Legg et al., *J Hered,* 60:213-17 (1969)).

[0178] One-hundred independent pYTY32 transformants of Burley 21 LA (T_0) were allowed to self. Progeny of the selfed plants (T_1) were germinated on medium containing kanamycin and the segregation of kanamycin resistance scored. T_1 progeny segregating 3:1 resulted from transformation at a single locus and were subjected to further analysis.

[0179] Nicotine levels of T_1 progeny segregating 3:1 were measured qualitatively using a micro-assay technique. Approximately ~200 mg fresh tobacco leaves were collected and ground in 1 ml extraction solution (Extraction solution: 1 ml Acetic acid in 100 ml H_2O). Homogenate was centrifuged for 5 min at 14,000×g and supernatant removed to a clean tube, to which the following reagents were added: 100 μL NH_4OAC (5 g/100 ml H_2O+50 μL Brij 35); 500 μL Cyanogen Bromide (Sigma C-6388, 0.5 g/100 ml H_2O+50 μL Brij 35); 400 μL Aniline (0.3 ml buffered Aniline in 100 ml NH_4OAC+50 μL Brij 35). A nicotine standard stock solution of 10 mg/ml in extraction solution was prepared and diluted to create a standard series for calibration. Absorbance at 460 nm was read and nicotine content of test samples were determined using the standard calibration curve.

[0180] T_1 progeny that had less than 10% of the nicotine levels of the Burley 21 LA parent were allowed to self to produce T_2 progeny. Homozygous T_2 progeny were identified by germinating seeds on medium containing kanamycin and selecting clones in which 100% of the progeny were resistant to kanamycin (i.e., segregated 4:0; heterozygous progeny would segregate 3:1). Nicotine levels in homozygous and heterozygous T_2 progeny were qualitatively determined using the micro-assay and again showed levels less than 10% of the Burley 21 LA parent. Leaf samples of homozygous T_2 progeny were sent to the Southern Research and Testing Laboratory in Wilson, N.C. for quantitative analysis of nicotine levels using Gas Chromatography/Flame Ionization Detection (GC/FID). Homozygous T_2 progeny of transformant #41 gave the lowest nicotine levels (~70 ppm), and this transformant was designated as "Vector 21-41."

[0181] Vector 21-41 plants were allowed to self-cross, producing T_3 progeny. T_3 progeny were grown and nicotine levels assayed qualitatively and quantitatively. T_3 progeny were allowed to self-cross, producing T_4 progeny. Samples of the bulked seeds of the T_4 progeny were grown and nicotine levels tested.

[0182] In general, Vector 21-41 is similar to Burley 21 LA in all assessed characteristics, with the exception of alkaloid content and total reducing sugars (e.g., nicotine and nor-nicotine). Vector 21-41 may be distinguished from the parent Burley 21 LA by its substantially reduced content of nicotine, nor-nicotine and total alkaloids. As shown below, total alkaloid concentrations in Vector 21-41 are significantly reduced to approximately relative to the levels in the parent Burley 21 LA, and nicotine and nor-nicotine concentrations show dramatic reductions in Vector 21-41 as compared with Burley 21 LA. Vector 21-41 also has significantly higher levels of reducing sugars as compared with Burley 21 LA.

[0183] Field trials of Vector 21-41 T_4 progeny were performed at the Central Crops Research Station (Clayton, N.C.) and compared to the Burley 21 LA parent. The design was three treatments (Vector 21-41, a Burley 21 LA transformed line carrying only the NtQPT1promoter [Promoter-Control], and untransformed Burley 21 LA [Wild-type]), 15 replicates, 10 plants per replicate. The following agronomic traits were measured and compared: days from transplant to flowering; height at flowering; leaf number at flowering; yield; percent nicotine; percent nor-nicotine; percent total nitrogen; and percent reducing sugars.

[0184] Vector 21-41 was also grown on approximately 5000 acres by greater than 600 farmers in five states (Pennsylvania, Mississippi, Louisiana, Iowa, and Illinois). The U.S. Department of Agriculture, Agriculture Marketing Service (USDA-AMS) quantified nicotine levels (expressed as percent nicotine per dry weight) using the FTC method of 2,701 samples taken from these farms. Nicotine levels ranged from 0.01% to 0.57%. The average percent nicotine level for all these samples was 0.09%, with the median of 0.07%. Burley tobacco cultivars typically have nicotine levels between 2% and 4% dry weight (Tso, T. C., 1972, Physiology and Biochemistry of Tobacco Plants. Dowden, Hutchinson, and Ross, Inc. Stroudsbury).

EXAMPLE 4

Regulation of NtOPT1 Gene Expression Using Molecular Decoys

[0185] Nucleotide sequence located between −1000 and −600 or −700 bp of the NtQPT1promoter is inserted in tandem arrays into a plant-*Agrobacterium* shuttle vector and subsequently transformed into tobacco via methods known

to one skilled in the art. Plants stably transformed with said vector are assessed for the level of expression of NtQPT1and for nicotine and/or TSNA content. These experiments demonstrate that tobacco transformed with molecular decoys that interact with Nic gene products exhibit a reduced level of expression of NtQPT1.

EXAMPLE 5

Tobacco Having Reduced Nicotine and/or TSNA Levels Generated Using Molecular Decoys

[0186] Multiple copies of an approximately 300 or 400 nucleotide long fragment of the NtQPT1promoter (e.g., including nucleotide sequence located between −1000 and −600 or −700 bp of the NtQPT1promoter), are affixed to microparticles (e.g., by precipitation) that are suitable for the ballistic transformation of a plant cell (e.g., 1 to 5 μm gold spheres). The microparticles are propelled into tobacco plant cells (e.g., Burley 21 LA) using any suitable ballistic cell transformation methodology, so as to produce transformed plant cells. Plants are then regenerated from the transformed plant cells. Burley 21 LA is a variety of Burley 21 with substantially reduced levels of nicotine as compared with Burley 21 (i.e., Burley 21 LA has 8% the nicotine levels of Burley 21, see Legg et al., *Can J Genet Cytol,* 13:287-91 (1971); Legg et al., *J Hered,* 60:213-17 (1969)).

[0187] Transformed cells, tissues, and seedlings are grown on Murashige-Skoog (MS) medium (with or without the selection compound, e.g., antibiotic, depending on whether a selectable marker was used. One-hundred independent transformants of Burley 21 LA (T_0) are allowed to self. Progeny of the selfed plants (T_1) are germinated. Nicotine levels of T_1 progeny are measured qualitatively using a micro-assay technique. Approximately 200 mg fresh tobacco leaves are collected and ground in 1 ml extraction solution. (Extraction solution: 1 ml Acetic acid in 100 ml H_2O) Homogenate is centrifuged for 5 min at 14,000×g and supernatant removed to a clean tube, to which the following reagents are added: 100 μL NH_4OAC (5 g/100 ml H_2O+50 μL Brij 35); 500 μL Cyanogen Bromide (Sigma C-6388, 0.5 g/100 ml H_2O+50μuL Brij 35); 400 μL Aniline (0.3 ml buffered Aniline in 100 ml NH_4OAC+50 μL Brij 35). A nicotine standard stock solution of 10 mg/ml in extraction solution is prepared and diluted to create a standard series for calibration. Absorbance at 460 nm is read and nicotine content of test samples are determined using the standard calibration curve.

[0188] T_1 progeny that have less than 10% of the nicotine levels of the Burley 21 LA parent are allowed to self to produce T_2 progeny. Homozygous T_2 progeny are then identified. Nicotine levels in homozygous and heterozygous T_2 progeny are also qualitatively determined using the micro-assay. Leaf samples of homozygous T_2 progeny can also be sent to the Southern Research and Testing Laboratory in Wilson, N.C. for quantitative analysis of nicotine levels using. Gas Chromatography/Flame Ionization Detection (GC/FID). Homozygous T_2 progeny will have nicotine levels that are substantially reduced as compared to the untransformed tobacco (e.g., ~70 ppm). Because the nicotine levels in such plants are substantially reduced, the TSNA levels in these plants is concomitantly reduced.

[0189] These experiments demonstrate that tobacco transformed with molecular decoys that interact with Nic gene products exhibit a reduced amount of nicotine and/or TSNA. Plants with multiple tandem insertions of the molecular decoy that have reduced NtQPT1 expression and reduced nicotine/TSNA levels are used to generate commercially valuable tobacco products.

EXAMPLE 6

Low Nicotine and Nitrosamine Blended Tobacco

[0190] The following example describes several ways to create tobacco products having specific amounts of nicotine, and/or TSNAs through blending. Some blending approaches begin with tobacco prepared from varieties that have extremely low amounts of nicotine and/or TSNAs. By blending prepared tobacco from a low nicotine/TSNA variety (e.g., undetectable levels of nicotine and/or TSNAs) with a conventional tobacco (e.g., Burley, which has 30,000 parts per million (ppm) nicotine and 8,000 parts per billion (ppb) TSNA; Flue-Cured, which has 20,000 ppm nicotine and 300 ppb TSNA; and Oriental, which has 10,000 ppm nicotine and 100 ppb TSNA), tobacco products having virtually any desired amount of nicotine and/or TSNAs can be manufactured. Other approaches blend only low nicotine/TSNA tobaccos (e.g., genetically modified Burley, genetically modified Virginia flue, and genetically modified Oriental tobaccos that contain reduced amounts of nicotine and/or TSNAs). Tobacco products having various amounts of nicotine and/or TSNAs can be incorporated into tobacco-use cessation kits and programs to help tobacco users reduce or eliminate their dependence on nicotine and reduce the carcinogenic potential.

[0191] By one approach, a step 1 tobacco product is comprised of approximately 25% low nicotine/TSNA tobacco and 75% conventional tobacco; a step 2 tobacco product can be comprised of approximately 50% low nicotine/TSNA tobacco and 50% conventional tobacco; a step 3 tobacco product can be comprised of approximately 75% low nicotine/TSNA tobacco and 25% conventional tobacco; and a step 4 tobacco product can be comprised of approximately 100% low nicotine/TSNA tobacco and 0% conventional tobacco. A tobacco-use cessation kit can comprise an amount of tobacco product from each of the aforementioned blends to satisfy a consumer for a single month program. That is, if the consumer is a one pack per day smoker, for example, a single month kit would provide 7 packs from each step, a total of 28 packs of cigarettes. Each tobacco-use cessation kit would include a set of instructions that specifically guide the consumer through the step-by-step process. Of course, tobacco products having specific amounts of nicotine and/or TSNAs would be made available in conveniently sized amounts (e.g., boxes of cigars, packs of cigarettes, tins of snuff, and pouches or twists of chew) so that consumers could select the amount of nicotine and/or TSNA they individually desire. There are many ways to obtain various low nicotine/low TSNA tobacco blends using the teachings described herein and the following is intended merely to guide one of skill in the art to one possible approach.

[0192] To obtain a step 1 tobacco product, which is a 25% low nicotine/TSNA blend, prepared tobacco from an approximately 0 ppm nicotine/TSNA tobacco can be mixed with conventional Burley, Flue-cured, or Oriental in a 25%/75% ratio respectively to obtain a Burly tobacco product

having 22,500 ppm nicotine and 6,000 ppb TSNA, a Flue-cured product having 15,000 ppm nicotine and 225 ppb TSNA, and an Oriental product having 7,500 ppm nicotine and 75 ppb TSNA. Similarly, to obtain a step 2 product, which is 50% low nicotinerrSNA blend, prepared tobacco from an approximately 0 ppm nicotine/TSNA tobacco can be mixed with conventional Burley, Flue-cured, or Oriental in a 50%/50% ratio respectively to obtain a Burly tobacco product having 15,000 ppm nicotine and 4,000 ppb TSNA, a Flue-cured product having 10,000 ppm nicotine and 150 ppb TSNA, and an Oriental product having 5000 ppm nicotine and 50 ppb TSNA. Further, a step 3 product, which is a 75%/25% low nicotinerTSNA blend, prepared tobacco from an approximately 0 ppm nicotine/TSNA tobacco can be mixed with conventional Burley, Flue-cured, or Oriental in a 75%/25% ratio respectively to obtain a Burly tobacco product having 7,500 ppm nicotine and 2,000 ppb TSNA, a Flue-cured product having 5,000 ppm nicotine and 75 ppb TSNA, and an Oriental product having 2,500 ppm nicotine and 25 ppb TSNA.

[0193] It should be appreciated that tobacco products are often a blend of many different types of tobaccos, which were grown in many different parts of the world under various growing conditions. As a result, the amount of nicotine and TSNAs will differ from crop to crop. Nevertheless, by using conventional techniques one can easily determine an average amount of nicotine and TSNA per crop used to create a desired blend. By adjusting the amount of each type of tobacco that makes up the blend one of skill can balance the amount of nicotine and/or TSNA with other considerations such as appearance, flavor, and smokability. In this manner, a variety of types of tobacco products having varying level of nicotine and/or nitrosamine, as well as, appearance, flavor and smokability can be created.

EXAMPLE 7

Low Nicotine and Nitrosamine Blended Tobacco

[0194] By a preferred method, conventional Virginia flue tobacco was blended with genetically modified Burley (i.e., Burley containing a significantly reduced amount of nicotine and nitrosamine) to yield a blended tobacco that was incorporated into three levels of reduced nicotine cigarettes: a step 1 cigarette containing 0.6 mg nicotine, a step 2 cigarette containing 0.3 mg nicotine, and a step 3 cigarette containing less than 0.05 mg nicotine. The amount of total TSNA was found to range between approximately 0.1 μg/g-0.6 μg/g.

[0195] In some cigarettes, approximately, 28% of the blend was Virginia flue tobacco, approximately 29% of the blend was genetically modified (i.e., reduced nicotine Burley), approximately 14% of the blend was Oriental, approximately 17% of the blend was expanded flue-cured stem, and approximately 12% was standard commercial reconstituted tobacco. The amount of total TSNAs in cigarettes containing this blend was approximately 1.5 μg/g.

EXAMPLE 8

Incorporating a Receptor Binding Nicotine Substitute into Low Nicotine/TSNA Tobacco

[0196] Various tobacco products (e.g., cigarettes and chewing tobacco) are made to contain a reduced amount of nicotine and TSNA and are supplemented with a receptor binding nicotine substitute. Modified tobacco is obtained, preferably a blend of Burley and Virginia flue tobacco containing less than 0.1 mg/g nicotine, a quantity of nicotine that is generally considered to be non-addictive. A receptor binding nicotine substitute (e.g., Cytisine) is brought into solution at various concentrations. A prepared solution containing the receptor binding nicotine substitute is then sprayed onto the blended tobacco. The blended tobacco containing the nicotine substitute is then manufactured into a tobacco product (e.g., cigarette or chewing tobacco). Alternatively, the nicotine substitute is applied to the filter or paper of a cigarette. By adjusting the concentration of the solution of nicotine substitute used, one can make tobacco products having different concentrations of nicotine substitute.

[0197] A tobacco cessation program using the tobacco products described above can be followed. By one approach, a consumer will start the program with tobacco products containing relatively high amounts of the nicotine substitute. After a set period of time, the consumer will then switch to a tobacco product containing a moderate amount of the nicotine substitute and, gradually, the consumer will switch to a tobacco product having very small amounts of the nicotine substitute. Immediately, however, the consumer has removed themselves from the harmful effects of nicotine by switching to a product that contains a non-addictive nicotine substitute and, as the consumer progresses through the program, the consumer gradually reduces the exposure to the nicotine substitute.

EXAMPLE9

Low Nicotine and Nitrosamine Blended Tobacco Containing Cytisine

[0198] By a preferred method, conventional Virginia flue tobacco is blended with genetically modified Burley (i.e., Burley containing a significantly reduced amount of nicotine and nitrosamine) to yield a blended tobacco containing less than 0.05 mg nicotine. During processing of the blend, solutions of Cytisine, at various concentrations, are prepared and sprayed onto the tobacco or onto the paper or onto the filter such that the final concentration of Cytisine delivered to the consumer is any number less than 0.25 mg, 0.5 mg, 0.75 mg, **1.o** mg, 1.25 mg, 1.5 mg, 1.75 mg, 2.0 mg, 2.25 mg, 2.5 mg, 2.75 mg, or 3.0 mg. Tobacco products containing the differing concentrations of nicotine substitute are then prepared, packaged, and labeled so that a consumer can distinguish the amount of nicotine and/or nicotine substitute present in the product.

[0199] A tobacco cessation program using the tobacco products described above is then followed. By one approach, a consumer will start the program with tobacco products containing relatively high amounts of the Cytisine (e.g., 2.0-3.0 mg). After a set period of time (e.g., 2 weeks), the consumer will switch to a tobacco product containing a moderate amount of Cytisine (e.g., 1.0-1.5 mg) and, gradually, the consumer will switch to a tobacco product having very small amounts of the Cytisine (e.g., less than 1.0 mg). By following this program, the consumer is immediately removed from the harmful effects of nicotine and, gradually, the consumer will reduce the exposure to the nicotine substitute.

EXAMPLE 10

Nicotine Reduction and/or Smoking Cessation Programs

[0200] The following example provides a nicotine reduction and/or smoking cessation program utilizing the low nicotine, low TSNA tobacco products, which contain a nicotine substitute, as described herein. The modified tobacco containing very low levels of TSNAs and essentially no nicotine is mixed with tobacco having a known amount of nicotine to create specific, stepwise levels of nicotine per cigarette. As an example, Virginia flue tobacco was blended with genetically modified Burley (i.e., Burley containing a significantly reduced amount of nicotine and nitrosamine) to yield a blended tobacco that was incorporated into three levels of reduced nicotine cigarettes: a step 1 cigarette containing 0.6 mg nicotine, a step 2 cigarette containing 0.3 mg nicotine, and a step 3 cigarette containing less than 0.05 mg nicotine. These tobaccos can be supplemented with one or more nicotine substitutes (e.g., an amount of Cytisine sufficient to deliver 1.5 mg of the compound to the consumer). The Cytisine can be sprayed on the tobacco prior to or after blending or the Cytisine can be impregnated into the filter of a cigarette.

[0201] By another approach, genetically modified Burley tobacco having an amount of nicotine that is less than 0.05 mg/g is blended with genetically modified Virginia flue tobacco having less than 0.05 mg/g nicotine to yield a blended tobacco having less than 0.1 mg/g. The tobacco can be sprayed prior to or after blending with a solution containing a nicotine substitute (e.g., a solution of Cytisine that yields 1.5 mg Cytisine per gram of tobacco). Alternatively, the filters on the cigarette can be impregnated with a nicotine substitute such that the nicotine substitute is delivered to the consumer while smoking (e.g., an amount of Cytisine sufficient to deliver 1.5 mg to the consumer).

[0202] The stepwise packs of cigarettes are clearly marked as to their nicotine and/or nicotine substitute content, and the step in the stepwise nicotine reduction program is also clearly marked on the package. Each week, the user purchases packs containing cigarettes having the next lower level of nicotine, but limits himself to no more cigarettes per day than consumed previously. The user may define his/her own rate of nicotine reduction and/or smoking cessation according to individual needs by choosing a) the number of cigarettes smoked per day b) the starting nicotine and/or nicotine substitute levels c) the change in nicotine and/or nicotine substitute level per cigarette each week, and d) the final level of nicotine consumed per day. To keep better track of the program, the individual keeps a daily record of total nicotine intake, as well as the number of cigarettes consumed per day. Eventually, the individual will be consuming tobacco products with essentially no nicotine. Since the nicotine-free tobacco products of the final step are non-addictive, it should then be much easier to quit the use of the tobacco products altogether.

EXAMPLE 11

Nicotine Reduction and/or Smoking Cessation Kit Containing Packs of Cigarettes with Low TSNA Levels and Stenwise Reductions in Nicotine Levels

[0203] Various nicotine reduction and/or smoking cessation kits are prepared, geared to heavy, medium, or light smokers. The kits provide all of the materials needed to quit smoking in either a two-week period (fast), a one-month period (medium) or in a two-month period (slow), depending on the kit. Each kit contains a set number of packs of cigarettes modified according the methods described herein, containing either step 1 cigarettes containing 0.6 mg nicotine, step 2 cigarettes containing 0.3 mg nicotine, and step 3 cigarettes containing less than 0.05 mg nicotine. Alternatively, each kit contains a set number of packs of step 1 cigarettes containing 1.5 mg Cytisine, step 2 cigarettes containing 1.0 mg cytisine, and step 3 cigarettes containing less than 0.05 mg Cytisine. For example, 1 pack a day smokers would receive 7 packs of cigarettes, each pack containing the above amounts of nicotine per each cigarette. Several weeks worth of additional cigarettes containing less than 0.05 mg Cytisine/cigarette would also be provided in the kit, to familiarize the consumer with smoking no nicotine cigarettes. The content of nicotine and/or nicotine substitue(s) and/or other components of the cigarettes is demarked using the labels and indicators described herein. The kit would also contain a diary for keeping track of daily nicotine intake, motivational literature to keep the individual interested in continuing the cessation program, health information on the benefits of smoking cessation, and web site addresses to find additional anti-smoking information, such as chat groups, meetings, newsletters, recent publications, and other pertinent links.

[0204] Although the invention has been described with reference to the above embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All references cited herein are hereby expressly incorporated by reference.

What is claimed is:

1. A cigarette labeled with a visual content indicator, wherein the visual content indicator identifies the amount of a compound of the cigarette.

2. The cigarette of claim 1, wherein the compound is nicotine.

3. The cigarette of claim 1, wherein the visual indicator is a number.

4. The cigarette of claim 1, wherein the visual indicator is a color.

5. The cigarette of claim 1, wherein the compound is a carcinogen.

6. The cigarette of claim 1, wherein the visual indicator is a symbol.

7. The cigarette of claim 1, wherein the visual indicator is a letter.

8. The cigarette of claim 1, wherein the compound is a nicotine substitute.

9. A tobacco use cessation program comprising a cigarette that comprises a visual indicator of the amount of nicotine.

10. The tobacco use cessation program of claim 9, wherein the program comprises a first cigarette with a visual indicator that denotes reduced nicotine and a second cigarette with a visual indicator that denotes essentially nicotine-free.

11. The tobacco use cessation program of claim 9, wherein the visual indicator is a number.

12. The tobacco use cessation program of claim 10, wherein the visual indicators on the said first and said second cigarettes are numbers.

13. A cigarette comprising a tobacco and a nicotine substitute.

14. The cigarette of claim 13, wherein said tobacco is genetically modified.

15. The cigarette of claim 14, wherein the tobacco comprises a reduced amount of nicotine as compared to a conventional tobacco of the same variety.

16. The cigarette of claim 14, wherein the nicotine substitute is Cytisine.

17. This cigarette of claim 14, wherein the cigarette is labeled with a visual content indicator, wherein the visual content indicator identifies the amount of a compound of the cigarette.

18. The cigarette of claim 17, wherein the compound is nicotine.

19. The cigarette of claim 17, wherein the compound is a nicotine substitute

20. A tobacco use cessation program comprising a modified tobacco product, wherein said modified tobacco product comprises a modified tobacco that has less nicotine than a tobacco of the same variety and a nicotine substitute.

21. The tobacco use cessation program of claim 20, wherein said tobacco product further comprises a visual content indicator, wherein the visual content indicator identifies the amount of a compound of the tobacco product.

22. The tobacco use cession program of claim 20, wherein said tobacco is a genetically modified tobacco.

23. The tobacco use cessation program of claim 20, wherein said genetically modified tobacco is a Virginia Flue tobacco.

24. The tobacco use cessation program of claim 20, wherein said genetically modified tobacco is an Oriental tobacco.

25. The tobacco use cessation program of claim 20, wherein said genetically modified tobacco is an a Burley tobacco.

26. The tobacco use cessation program of claim 20, wherein said modified tobacco comprises 0.6 mg of nicotine or less.

27. The tobacco use cessation program of claim 20, wherein said modified tobacco comprises 0.3 mg of nicotine or less.

28. The tobacco use cessation program of claim 20, wherein said modified tobacco comprises 0.05 mg of nicotine or less.

29. The tobacco use cessation program of claim 20, wherein said nicotine substitute is Cytisine.

30. The tobacco use cessation program of claim 20, wherein said tobacco product is a cigarette.

31. The tobacco use cessation program of claim 30, wherein said nicotine substitute is added to the paper of said cigarette.

32. The tobacco use cessation program of claim 30, wherein said nicotine substitute is added to a filter of said tobacco product.

33. The tobacco use cessation program of claim 20, wherein said nicotine substitute Cytisine is a receptor binding nicotine substitute.

34. The tobacco use cessation program of claim 20, wherein said tobacco product comprises an amount of Cytisine that is sufficient to deliver less than 2.0 mg of Cytisine to a consumer.

35. The tobacco use cessation program of claim 20, wherein said tobacco product comprises an amount of Cytisine that is sufficient to deliver less than 1.5 mg of Cytisine to a consumer.

36. The tobacco use cessation program of claim 20, wherein said tobacco product comprises an amount of Cytisine that is sufficient to deliver less than 1.0 mg of Cytisine to a consumer.

37. The tobacco use cessation program of claim 20, wherein said tobacco product comprises an amount of Cytisine that is sufficient to deliver less than 0.5 mg of Cytisine to a consumer.

38. A method of reducing the exposure of a tobacco consumer to nicotine comprising:

providing to said tobacco consumer a tobacco product comprising a modified tobacco that has less nicotine than tobacco of the same variety, and a nicotine substitute.

39. The method of claim 38, wherein said tobacco product further comprises a visual content indicator, wherein the visual content indicator identifies the amount of a compound of the tobacco product.

40. The method of claim 38, wherein said modified tobacco is a Burley tobacco.

41. The method of claim 38, wherein said modified tobacco is a Virginia Flue tobacco.

42. The method of claim 38, wherein said modified tobacco is an Oriental tobacco.

43. The method of claim 38, wherein said modified tobacco is a genetically modified tobacco.

44. The method of claim 38, wherein said modified tobacco comprises 0.6 mg of nicotine or less.

45. The method of claim 38, wherein said modified tobacco comprises 0.3 mg of nicotine or less.

46. The method of claim 38, wherein said modified tobacco comprises 0.05 mg of nicotine or less.

47. The method of claim 38, wherein said nicotine substitute is Cytisine.

48. The method of claim 38, wherein said tobacco product is a cigarette.

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