(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 February 2007 (08.02.2007)

PCT

(10) International Publication Number
WO 2007/016619 A2

(51) International Patent Classification:
A61Q 11/00 (2006.01) *G01N 33/497* (2006.01)

(21) International Application Number:
PCT/US2006/030069

(22) International Filing Date: 2 August 2006 (02.08.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/704,597 2 August 2005 (02.08.2005) US

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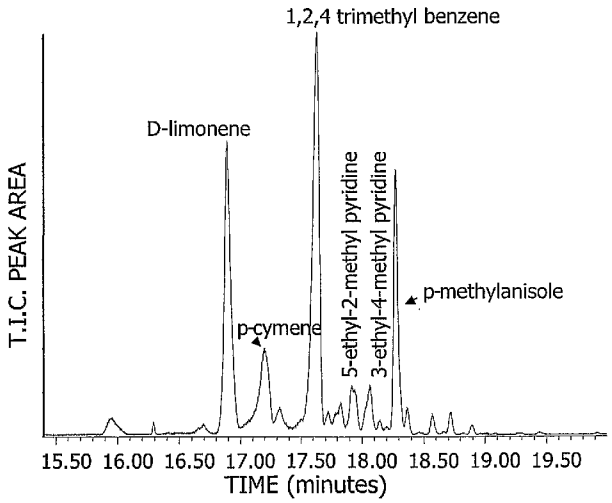
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** SCREENING METHOD FOR THE IDENTIFICATION OF COMPOSITIONS SUITABLE FOR THE TREATMENT OF ORAL CAVITY MALODOR ASSOCIATED WITH SMOKING A TOBACCO PRODUCT



(57) **Abstract:** This invention generally relates to a screening method for identifying compositions suitable for use in an oral composition (e.g., a confection or chewing gum product) effective for the treatment of oral cavity malodor associated with smoking a tobacco product. In particular, this invention relates to a screening method for determining the ability of a composition to reduce the concentration of a pyridine or pyrazine compound present in a model sample or solution which is representative of the oral cavity of a subject after smoking a tobacco product, as an indicator of the effectiveness of that composition in the treatment of oral malodor associated with smoking a tobacco product.

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SCREENING METHOD FOR THE IDENTIFICATION OF COMPOSITIONS SUITABLE FOR THE TREATMENT OF ORAL CAVITY MALODOR ASSOCIATED WITH SMOKING A TOBACCO PRODUCT

FIELD OF THE INVENTION

[0001] This invention generally relates to a screening method for identifying compositions suitable for use in an oral composition (e.g., a confection or chewing gum product) effective for the treatment of oral cavity malodor associated with smoking a tobacco product. In particular, this invention relates to a screening method for determining the ability of a composition to reduce the concentration of a pyridine or pyrazine compound present in a model sample or solution which is representative of the oral cavity of a subject after smoking a tobacco product, as an indicator of the effectiveness of that composition in the treatment of oral malodor associated with smoking a tobacco product.

BACKGROUND OF THE INVENTION

[0002] It has been estimated that more than 1.2 billion people worldwide smoke tobacco products. It is known that pyrolyzed tobacco often results in a lingering odor in the oral cavity of persons who smoke tobacco products. In addition, it is readily apparent to smokers and their associates that pyrolyzed tobacco volatiles are released from the smoker's oral cavity, air passageways and lungs and are present in the smoker's breath in sufficient quantity to be perceived by others. These odors are also generally perceived as aftertaste by the smoker, but in a manner consistent with the concept of odor adaptation; that is, constant exposure to the odor decreases perception over time such that the smoker may over time become immune to at least one of the indicators of the unpleasant odor which may be perceived by others.

[0003] The widespread use of tobacco products and oral malodor associated therewith has created a consistent demand for breath freshening products. However, it has been

estimated that approximately 4,800 compounds may be generated upon pyrolysis of tobacco. Furthermore, smoking the tobacco product also results in pyrolysis of additives found in cigarettes, and up to about 600 different additives may be utilized in cigarettes.

[0004] Successful strategies for the amelioration of oral malodor associated with tobacco smoke have to-date been difficult to develop, at least in part due to the myriad possible sources of oral malodor. In addition, in order for a substance to possess aroma, it typically is volatile and passes through a person's nasal epithelium retronasally (i.e., through the mouth) or orthonasally (i.e., by sniffing).

SUMMARY OF THE INVENTION

[0005] Briefly, therefore, the present invention is directed to methods for identifying a composition suitable for use in an oral composition (e.g., a confection or chewing gum product) effective for reducing oral malodor associated with tobacco smoke and/or preparing an oral composition effective for reducing oral malodor associated with tobacco smoke. In one embodiment, the method comprises contacting in a vessel a test composition and a model solution comprising a pyridine or pyrazine compound present in tobacco smoke, determining the ability of the test composition to reduce the concentration of the pyridine or pyrazine compound in a headspace of the vessel, and preparing an oral composition comprising the test composition. In this and/or other embodiments of the present invention detailed elsewhere herein, the test composition comprises cranberry extract, crabapple extract, hawthorn berry extract, plum extract, prune extract, grape seed extract, grape skin extract, cardamom oil, alfalfa extract, honeysuckle extract, rosemary extract, basil extract, thyme extract, aloe extract, chrysanthemum extract, green tea extract, coffee berry extract, licorice, parsley seed oil, pine extract, coffee extract, ginseng extract, dandelion root extract,

chlorogenic acid, ascorbic acid, caffeic acid, zinc lactate, silica gel, citric acid, maleic acid, tartaric acid, eugenol, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, quinic acid, activated carbon, or combinations thereof.

[0006] In another embodiment, the method comprises contacting in a vessel a test composition and a model solution comprising tobacco smoke odorants, the odorants comprising pyridine, 2-ethyl pyridine, 3-ethyl pyrazine and ethyl pyrazine, and determining the ability of the test composition to reduce the concentration of one or more of the odorants in the model solution in the headspace of the vessel.

[0007] In a further embodiment, the method comprises preparing a plurality of test compositions, individually contacting in a vessel each of the test compositions and a model solution comprising a pyridine or pyrazine compound present in tobacco smoke, determining the ability of each of the test compositions to reduce the concentration of the pyridine or pyrazine compound in the headspace of each of the vessels and identifying one or more test compositions as effective to reduce the concentration of the pyridine or pyrazine compound by at least about 50%.

[0008] The present invention is also directed to methods for treatment of oral malodor associated with tobacco smoke. In one such embodiment, the method comprises administering to a subject an oral composition (e.g., a confection or chewing gum) comprising an ingredient recognized to reduce the concentration of a pyridine or pyrazine compound present in the subject's oral cavity as a result of smoking a tobacco product.

[0009] In another such embodiment, the method comprises administering to a subject an oral composition comprising an ingredient effective to reduce the concentration of tobacco smoke odorants present in the subject's oral cavity as a result of smoking a tobacco product by at least about 50%.

The odorants comprise 2,4,6-trimethyl pyridine, 2,6-dimethyl pyridine, 3-ethyl pyridine and 2-ethyl-3-methyl pyrazine.

[0010] In still another such embodiment, the method comprises distributing an oral composition containing an ingredient recognized to reduce a concentration of a pyridine or pyrazine compound present in a subject's oral cavity as a result of smoking a tobacco product and encouraging a subject to consume or chew the product to ameliorate oral malodor resulting from smoking a tobacco product.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Fig. 1 is a flame ionization detector (FID) chromatogram prepared as described in Example 1.

[0012] Fig. 2 is a total ion current (TIC) chromatogram prepared as described in Example 1.

[0013] Fig. 3 is an overlay of an aromagram and TIC chromatogram described in Example 1.

[0014] Fig. 4 shows the headspace concentration change of odorous components following treatment of a model solution with Applephenon® as described in Example 2.

[0015] Fig. 5 shows the results of sensory analysis of model solutions treated with Applephenon® as described in Example 2; error bars indicate standard error.

[0016] Fig. 6 shows the percent reduction in headspace concentration of various components following treatment of a model solution with Applephenon® as described in Example 3.

[0017] Fig. 7 shows sensory analysis results comparing aftertaste intensity for subjects after smoking cigarettes and after chewing a control gum and a gum containing added active (Applephenon®) as described in Example 4.

[0018] Fig. 8 shows sensory analysis results comparing aftertaste intensity for subjects after smoking cigars and after chewing a control gum and a gum containing added active (Applephenon®) as described in Example 5.

[0019] Fig. 9 shows cranberry extract efficacy for reducing headspace concentration of odorants from a model solution headspace as described in Example 6; error bars indicate standard error.

[0020] Fig. 10 shows cardamom oil efficacy for reducing headspace concentration of odorants from a model solution headspace as described in Example 6; error bars indicate standard error.

[0021] Fig. 11 shows the effect of cranberry extract in reducing odor from pyridine and pyrazine components of a tobacco smoke model solution in terms of percent decrease vs. control as described in Example 6; error bars indicate standard error and sensory analysis was conducted utilizing a 0-10 point line scale.

[0022] Fig. 12 shows the effect of cardamom oil in reducing odor from pyridine and pyrazine components of a tobacco smoke model solution in terms of percent decrease vs. control as described in Example 6; error bars indicate standard error and sensory analysis was conducted utilizing a 0-10 point line scale.

[0023] Fig. 13 shows cardamom oil release (percent cardamom over time) as described in Example 7.

[0024] Fig. 14 shows cardamom oil release (amount of cardamom over time) as described in Example 8.

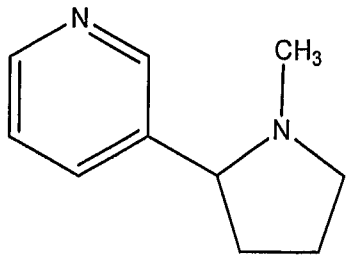
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0025] In accordance with the present invention, and as further detailed herein below, it has been discovered that contributors to oral cavity malodor associated with smoking a tobacco product, such as pyridine and/or pyrazine compounds, may be used to prepare model samples or solutions for the purpose of evaluating or screening potential active or test compositions (e.g., Applephenon®, cardamom oil or cranberry extract) for their ability or effectiveness in reducing the concentration of these indicator or target compounds in the

gaseous atmosphere, or headspace, of a vessel in which they are contained. It has been still further discovered that the ability or effectiveness of an active or test composition to reduce the concentration of these indicator compounds in the headspace of the vessel is also indicative of the ability or effectiveness of that composition to reduce the concentration of these volatile, odor-causing compounds that are present in a subject's oral cavity after smoking a tobacco product. Accordingly, such an active or test composition may be well-suited for incorporation into an oral composition including, but not limited to, a confection, chewing gum, lozenge, pressed tablet, edible film, mouthspray, mouthwash, or toothpaste product suitable for treatment of oral cavity malodor associated with smoking a tobacco product.

Odor-causing Compounds

[0026] Various aromatic nitrogenous compounds, including pyridine and/or pyrazine compounds, have been discovered to contribute to the oral malodor associated with smoking a tobacco product. In particular, it has been discovered that di- and tri-substituted pyridine and/or pyrazine compounds, including for example lower alkyl (e.g., C_1, C_2, C_3, C_4) substituted pyridine and/or pyrazine compounds (such as methyl, ethyl, propyl, butyl and/or cyclobutyl, di- or tri-substituted pyridine and/or pyrazine compounds), contribute to this oral malodor. Without being held to a particular theory, it is presently believed that the various pyridine and pyrazine compounds may be by-products of nicotine pyrolysis; in particular, in the case of pyridine compounds, pyrolysis is believed to result in cleavage of the bond between carbon number 2 and number 3 in the pyrrolidine moiety of nicotine (i.e., 3-(1-methyl-2-pyrrolidinyl) pyridine, illustrated below).



[0027] In addition to the various pyridine and pyrazine compounds present, a pyrrole (e.g., ethyl pyrrole) has also been identified and linked to tobacco smoke malodor. Non-nitrogenous compounds, such as acetophenone (i.e., 1-phenylethanone) and diacetyl (i.e., 2,3-butanedione), have been identified and linked to tobacco smoke malodor, as well.

[0028] Exemplary odorous components responsible for oral malodor caused by smoking a tobacco product identified in accordance with the present invention are listed in Table 1, provided below in Example 1. Certain of these odor-generating substances present in the smoke of tobacco products may be present at extremely low levels (e.g., parts per million or parts per billion levels), yet still contribute to noticeable odor. For example, pyridine has been reported to have an odor threshold value in water of 840 parts per billion (Flavor Base software, Leffingwell and Associates, Canton, GA, 2004).

Techniques for Identifying Odor-causing Compounds

[0029] In accordance with the present invention, techniques known in the art were utilized to identify the above-noted compounds as a primary source of odor in a subject's oral cavity, after smoking a tobacco product. Generally speaking, odorous tobacco smoke compounds may be extracted from the oral cavity of a subject who has smoked a tobacco product using various techniques known in the art, including for example swabbing the subject's tongue to provide a sample to be analyzed to determine the particular compounds contributing to the malodor. Verification of the presence of tobacco smoke odorants on the swab is typically carried out by

trained odor judges, who smell the swabs. Odorous tobacco smoke compounds may additionally, or alternatively, be collected by taking breath samples from the oral cavity of a subject who has smoked a tobacco product, also using various techniques known in the art. For example, the subject may exhale into a vessel containing simulated saliva (e.g., a solution containing sodium chloride, sodium bicarbonate and potassium bicarbonate in deionized water, prepared as described below in Example 1) in order to collect a sample.

[0030] Once collected, the odor-causing compounds present in these samples which contribute to the oral malodor associated with smoking a tobacco product may be identified using a variety of techniques known in the art. For example, the headspace of an airtight or hermetically sealed vessel in which the substances are contained (e.g., a vessel containing a swab taken from a smoker's oral cavity or a portion of a liquid sample containing tobacco smoke odorants (e.g., simulated saliva)) may be isolated by solid phase microextraction (SPME) and subjected to further analysis (e.g., gas chromatography, used in combination with a device suitable for compound detection or identification, such as a mass spectrometer) to determine the individual compounds contributing to the malodor.

[0031] Solid phase microextraction is a well-known method suitable for extracting a sample containing odorous components from a subject's oral cavity for subsequent analysis. (See, for example, *Released Oral Malodors Measured by Solid Phase Microextraction/Gas Chromatography Mass Spectrometry (HS-SPME-GC-MS)*, Payne, R., Labows, J., Liu, X, Proceeding of ACS - Flavor Release No. 0841236925, 2000.) For example, hydrogen sulfide, methyl mercaptan and dimethyl sulfide have been detected by extraction of mouth air with a gas tight syringe followed by separation with a packed column and detection with a flame photometric detector. (See, for example, *Direct Gas Chromatograph Analysis of Sulphur Compounds in Mouth Air in*

Man, Tonzetich, J., Archs, Oral Biol. 1971, 16, 587-597.) In particular, SPME has proven to be suitable for extracting volatile components from the headspace of vessels containing unconventional odorous substances for subsequent analysis for odor by gas chromatography-olfactometry (GCO) analysis and gas chromatography-mass spectrometry (GC/MS) analysis. (See, for example, *Headspace Solid Phase Microextraction*, Zhang, Z., Pawliszyn, J., Anal. Chem. 1993, 65, 1843-1852.) Benefits of SPME include few requirements with respect to sample preparation, little need for solvent, and relatively fast extraction times. Suitable apparatus for the GC/MS analysis include, for example, an Agilent 6890 gas chromatograph (GC) / mass spectrometer (MS) available from Agilent Technologies (Palo Alto, CA).

[0032] It is to be noted that each literature reference mentioned above, or elsewhere herein, is hereby incorporated by reference for all relevant purposes.

[0033] In one approach, a sample known to contain odorants may be analyzed using a gas chromatograph equipped with a mass spectrometer. Identification of the odor-causing components present therein may then generally be performed by matching sample spectra with a database (e.g., Wiley Registry of Mass Spectral Data, 7th Edition, John Wiley & Sons, Inc., 2000) and/or matching retention indices of components of the sample with known standards.

[0034] In an alternative approach, identification of the odorants may be conducted by gas chromatography-olfactometry (GCO) analysis, which comprises determining which portions of the GC column eluant exhibit tobacco smoke odor characteristics utilizing a sniff port and then subjecting those portions of the eluant to further analysis (e.g., mass spectrometry) to determine their composition. Various GCO techniques have been described in literature. One such method includes the Osme method, described in *Odor analysis of Pinot Noir Wines from Grapes of Different Maturities by a Gas*

Chromatography-Olfactometry Technique (Osme), Miranda-Lopez, R., Libbey, L.M., Watson, B.T., McDaniel, M.R., J. Food Sci., 1992, 57: 985-993, 1019. Another method includes CHARM analysis, described in *A procedure for the sensory analysis of gas chromatographic effluents*, Acree, T. E.; Barnard, J.; Cunningham, D. G, Food Chem. 1984, 41, 1698-1703. Still another method includes aroma extraction dilution analysis, described in *Characterization of saffron flavor by aroma extract dilution analysis*, Cadwallader, K. R.; Baek, H. H.; Cai, M, *Spices*; Shahidi, F.; Cadwallader, K. R., Eds.; American Chemical Society: Washington, DC, 1997, 66-79.

Model Solutions

[0035] In accordance with the present invention, once identified, the above-noted odor-causing compounds may be used to prepare solutions which model or mimic saliva present in the oral cavity of a subject who has smoked a tobacco product. These model solutions may be used to determine the efficacy of potential active compositions (e.g., test compositions) for ameliorating oral malodor attributed to smoking a tobacco product; that is, these model solutions may be used to determine the ability of a test composition to reduce the concentration of one or more of the odor causing compounds present in the gaseous atmosphere, or headspace, of a vessel in which the model solution is contained, which is in turn an indicator of the ability of the test composition to achieve a similar result in the oral cavity.

[0036] The similarity between the odor character and intensity of the model solution and the breath of a subject who has smoked a tobacco product may be determined by trained odor judges. In general, a model solution is prepared by adding one or more of the identified tobacco smoke odorant compounds to a liquid (e.g., aqueous) medium or solvent. The model solution may also be prepared by adding a liquid medium or solvent to a vessel containing one or more tobacco smoke

odorant compounds. The liquid medium typically comprises water, and may optionally comprise one or more additional components (e.g., an alcohol, such as ethanol or methanol). In various embodiments, the aqueous medium to which the tobacco smoke odorant compounds are added may comprise a combination of alcohol (e.g., ethanol) and water, at various concentrations or ratios. For example, a suitable aqueous medium typically contains from about 1% to about 20% (by weight) or from about 1% to about 10% (by weight) of an alcohol such as ethanol, and from about 80% to about 99% (by weight) water (e.g., a 5% ethanol/95% water solution (by weight) or a 1% ethanol/99% (by weight) water solution).

[0037] Generally, the model solution is prepared to assure, in the vessel in which it is contained, an initial and final odorant headspace concentration which exceeds the analysis method detection limit sufficiently such that errors in detection/measurement are minimized or avoided. Thus, typically, the model solution is prepared to provide a minimum headspace concentration of odorants in the vessel of at least about 10 parts per million (ppm), at least about 50 ppm, at least about 100 ppm, at least about 150 ppm, or at least about 250 ppm.

[0038] The model solution typically contains one or more tobacco smoke odorants such that the solution has a total odorant concentration of at least about 200 parts per million (ppm) (i.e., micrograms of odorant per milliliter solution), more typically at least about 600 ppm, still more typically at least about 1000 ppm and, still more typically, a total odorant concentration of at least about 1500 ppm. Preferably, the odorant or odorants are present in the model solution at a total concentration of from about 600 to about 2000 ppm, more preferably at a total concentration of from about 600 to about 1750 ppm and, more preferably, at a total concentration of from about 1000 to about 1750 ppm. The liquid (e.g., aqueous) medium or solvent (e.g., water, and optionally an alcohol or

some other component) typically makes up the remaining portion of the model solution.

[0039] Typically, the model solution comprises a pyridine or pyrazine compound and, in various embodiments, the model solution comprises both a pyridine compound and a pyrazine compound. In one preferred embodiment, the solution comprises a plurality of pyridine compounds and/or a plurality of pyrazine compounds. The pyridine compound(s) may be selected from, for example, 2,4,6-trimethyl pyridine, 2,6-dimethyl pyridine, 3,5-dimethyl pyridine, 2-ethyl pyridine, 3-ethyl pyridine and pyridine, or some combination thereof. The pyrazine compound(s) may be selected from ethyl pyrazine, 2,3-dimethyl pyrazine, 2,5-dimethyl pyrazine and 2-ethyl-3-methyl pyrazine, or some combination thereof. For example, in at least some embodiments, the model solution comprises 2,4,6-trimethyl pyridine, 2,6-dimethyl pyridine, 2-ethyl pyridine, 3-ethyl pyridine, pyridine, ethyl pyrazine, and/or 2-ethyl-3-methyl pyrazine. In still further embodiments, the model solution additionally or alternatively comprises pyridine, 2-ethyl pyridine, 3-ethyl pyrazine and/or ethyl pyrazine.

[0040] Additionally, it is to be noted that, optionally, one or more model solutions may also contain a pyrrole (e.g., ethyl pyrrole) and/or non-nitrogenous compounds such as acetophenone (i.e., 1-phenylethanone) and diacetyl (i.e., 2,3-butanedione).

[0041] In accordance with the present invention, various model solutions which are particularly representative of or similar to tobacco smoke odor have been identified. One such solution comprises pyridine, 2-ethyl pyridine, 3-ethyl pyrazine and ethyl pyrazine. Optionally, the model solution may additionally comprise these components as well as 2,4,6-trimethyl pyridine, 2,6-dimethyl pyridine, 3-ethyl pyridine, 2-ethyl-3-methyl pyrazine, or combinations thereof. These model solutions may optionally further comprise 3,5-dimethyl

pyridine, 2-ethyl pyridine, 3-ethyl pyridine, 2,3-dimethyl pyrazine, 2,5-dimethyl pyrazine, or combinations thereof.

[0042] In the case of one or more of the model solutions described above and/or elsewhere herein (e.g., a solution comprising pyridine, 2-ethyl pyridine, 3-ethyl pyrazine and ethyl pyrazine), the total odorant concentration is typically at least about 200 ppm, and preferably is at least about 600 ppm, and more preferably is at least about 1000 ppm, the concentration ranging for example from about 200 to about 1200 ppm, or from about 200 to about 800 ppm. By way of further example, in the case of a model solution which comprises pyridine, 2-ethyl pyridine, 3-ethyl pyrazine, ethyl pyrazine, 2,4,6-trimethyl pyridine, 2,6-dimethyl pyridine, 3-ethyl pyridine and 2-ethyl-3-methyl pyrazine, the total odorant concentration is typically at least about 400 ppm, and preferably is at least about 800 ppm, and more preferably is at least about 1000 ppm, the concentration ranging for example from about 400 to about 1400 ppm, or from about 800 to about 1400 ppm. More particularly, for one or more of the model solutions described herein above and/or elsewhere herein, each of the odorants may be present in the model solution at a concentration of 1 ppm, of at least about 50 ppm, preferably of at least about 75 ppm, and more preferably of at least about 100 ppm, the concentration of each odorant ranging for example from about 1 to about 200 ppm, from about 125 to about 175 ppm, or from about 75 to about 150 ppm.

Screening Method

[0043] In accordance with the present invention, the above-noted model solution may be utilized as part of a method for screening a composition to determine whether that composition is effective for reducing odorant concentration in the gaseous atmosphere or headspace of a vessel in which a model solution is contained. The vessel in which the model solution is contained is generally of a size appropriate to

provide the desired minimum odorant headspace concentration of the vessel in which the odorants are contained described elsewhere herein. In general, the screening method of the present invention comprises contacting the model solution and a test composition. Generally, the test composition may be in the form of an oil (e.g., cardamom oil), a solution of the test composition in an aqueous medium (e.g., water) or in the form of a solid which is dissolved upon contact with the model solution. The ability of the test composition to reduce the concentration of a pyridine or pyrazine compound in the headspace of the vessel in which the model solution containing the odorant(s) is contained may be determined using techniques known in the art. More specifically, this determination may be made by, for example, measuring the concentration of the pyridine or pyrazine compound in the headspace of the vessel containing the solution prior to contact with the test composition, and then measuring the concentration of the pyridine or pyrazine compound in the headspace of the vessel containing the solution after contact with the test composition. The difference between the initial and final concentration of the pyridine compound(s) and/or pyrazine compound(s) in the headspace of the vessel thus indicates the effectiveness of the test composition for reducing the odorant headspace concentration.

[0044] In this regard it is to be noted that, as further detailed elsewhere herein, there has been observed to be a correlation between a quantitative determination of effectiveness of a test composition for reducing odorant headspace concentration and the qualitative performance of an oral composition containing such a composition for treating oral cavity malodor associated with smoking a tobacco product.

[0045] Both the initial and final pyridine and/or pyrazine concentrations in the headspace of the vessel are generally determined using means known in the art including, as detailed elsewhere herein, by taking a sample of the

headspace and subjecting the sample portion of the vapors to analysis comprising separation, such as by chromatography, and detection, such as by mass spectrometry. Sampling of the headspace may be conducted by contacting the headspace with a fiber effective for absorbing a portion of the vapors comprising the pyridine or pyrazine compound in the headspace, or by contacting the headspace with a gas tight syringe effective for extracting a portion of the vapors comprising the pyridine or pyrazine compound in the headspace. Sampling or extracting a portion of the headspace is typically conducted at a temperature of from about 20°C to about 100°C or from about 20°C to about 40°C, preferably from about 20 to about 25°C and, still more preferably, at a temperature of about 22°C. In addition, the sampling or extracting of the headspace typically proceeds for at least about 5 minutes, at least about 10 minutes, at least about 20 minutes, at least about 30 minutes, or at least about 1 hour. Generally, sampling or extraction proceeds over a period of up to about 2 hours, up to about 3 hours, up to about 4 hours, up to about 6 hours, up to about 8 hours, or up to about 10 hours. In various other embodiments, sampling and extraction may proceed over the course of significantly longer periods of time (e.g., up to about 12 hours, up to about 24 hours, or up to about 48 hours). Multiple samples of the headspace may be taken consecutively and, in fact, multiple samples may be extracted during one or more of the sample times set forth above. Additionally or alternatively, samples may be taken intermittently in accordance with the sample times set forth above, with the interval between samplings not narrowly critical.

[0046] In this regard it is to be noted that sample analysis and the determination of the concentration of a given odor causing compound in the vessel headspace, either before or after contact with a test composition, may be performed

using other techniques or methodologies known in the art without departing from the scope of the present invention.

[0047] The model solution and the test composition are typically contacted in an airtight or hermetically sealed vessel. To ensure sufficient contact between the test composition and the model solution, the test composition and the model solution are typically contacted for at least about 5 minutes, at least about 10 minutes, at least about 20 minutes, at least about 30 minutes, or at least about 1 hour prior to determining the final pyridine and/or pyrazine concentration. Generally, the test composition and the model solution are contacted for a period of up to about 2 hours, up to about 3 hours, up to about 4 hours, up to about 6 hours, up to about 8 hours, or up to about 10 hours, prior to determining the final pyridine and/or pyrazine concentration. In various embodiments, the test composition and the model solution are contacted for significantly longer periods of time (e.g., up to about 12 hours, up to about 24 hours, or up to about 48 hours).

[0048] A suitable temperature for contacting the test composition and model solution is selected in order to preferably simulate conditions of the oral cavity of a subject consuming an oral composition (e.g., chewing a gum or consuming a confection), in which the test composition would be used. Thus, typically, the test composition and the model solution are contacted at a temperature of from about 20 to about 100°C, from about 20°C to about 40°C or from about 20°C to about 30°C, and preferably from about 20°C to about 25°C and, still more preferably, at a temperature of about 22°C.

[0049] The vessel containing the test composition and model solution may also be agitated, for example to simulate consumption (e.g., chewing) conditions. The degree and manner of agitation are not narrowly critical and may be conducted in accordance with methods known in the art.

[0050] Typically, the model solution is contacted with a quantity of the test composition that is representative of the amount of the test composition that would ultimately be used in an oral composition such as a chewing gum or confection product. Since the proportion of test composition incorporated into an oral composition may vary depending on, for example, the type of composition in which it is incorporated and the desired flavor of the composition, the quantity of test composition contacted with the model solution may likewise vary within relatively broad limits. For example, the model solution may in some embodiments of the present invention be contacted with at least about 0.01 milligram (mg) of the test composition per milliliter (ml) of solution, at least about 0.1 mg of the test composition per ml of solution, at least about 0.5 mg of the test composition per ml of solution, or at least about 1 mg of the test composition per ml of solution. In at least some embodiments, the model solution is contacted with from about 0.01 to about 1 mg of the test composition per ml of solution, from about 0.1 to about 0.8 mg of the test composition per ml of solution, or from about 0.2 to about 0.7 mg of the test composition per ml of solution. In various other embodiments, the model solution is typically contacted with from about 1 to about 75 mg of the test composition per ml of solution, more typically from about 5 to about 60 mg of the test composition per ml of solution, preferably from about 10 to about 50 mg of the test composition per ml of solution and, more preferably, from about 15 to about 30 mg of the test composition per ml of solution.

[0051] It is to be noted that the screening method of the present invention is amenable to testing a plurality of compositions using known combinatorial techniques. In such embodiments, a plurality of test compositions (e.g., a library or an array of, for example, at least about 5, at least about 10, at least about 15, at least about 20, at least about 25,

at least about 30, at least about 40, at least about 50 or more test compositions) may be prepared and contacted with the same, or a different, model solution in, for example, individual hermetically sealed vessels, or alternatively in individual hermetically sealed wells of a common substrate. Preferably, the plurality of test compositions are arranged in a spatially addressable format, such as in wells of a common substrate in a spatially addressable format (e.g., a microtiter plate), to enable the present method to be more easily carried out using commercially available automation (e.g., commercially available auto-sampling devices that may be used in combination with, for example, a commercially available GC/MS device). Advantageously, the ability of each of the plurality of test compositions to reduce the concentration of a pyridine or pyrazine compound in each of the vessels may be determined in parallel. In at least certain embodiments, an oral composition (e.g., confection or chewing gum) is prepared from at least 2 or more of the plurality of test compositions thus identified as effective for reducing pyridine and/or pyrazine concentrations.

[0052] Without being bound by a particular theory, reduction of the headspace concentration of a pyridine and/or a pyrazine compound attributed to contact with the test compositions as described herein is believed to proceed in accordance with one or more mechanisms.

[0053] One possible mechanism is the effect of addition of the test composition on the pH of the model solution. For example, model solutions containing pyridine and/or pyrazine compounds are typically neutral (i.e., have a pH of from about 6 to about 8). A drop in pH (i.e., acidifying the solution) was observed upon addition of various test compositions to such model solutions. For example, addition of Applephenon® to a model solution (as detailed in Example 2) produced an acidic solution having a pH of approximately 3.7. This drop in pH is presently believed to be attributed to a major

component of Applephenon®, the acidic polyphenol chlorogenic acid. In an acidified solution containing pyridine, pyridine (which has a pKa of 5.2) exists primarily as a cation. It is presently believed that hydrogen bonding of the charged pyridine species results in a protonated form having decreased volatility and, accordingly, reduced concentration in the headspace. In addition, a charged component of a model solution (positive or negative) is typically more water soluble than an uncharged species. An increase in solubility of odorous components is typically associated with greater salivary flow in the oral cavity, which can result in increased removal of the odorants from the oral cavity by swallowing prompted by the increased salivary flow. Removal of the soluble components from the oral cavity may also proceed by rinsing with water or other beverages.

[0054] In view of the foregoing, it is therefore to be noted that, in some embodiments, the screening method of the present invention may additionally, or alternatively, involve the screening of the same test composition and/or model solution, but at differing pH values, in order to evaluate the impact of pH on the performance of the test composition. For example, in such an embodiment, the contents of the test composition may be controlled so that, when contacted with the model solution, the resulting mixture or solution has a pH value of less than about 7, ranging for example from about 2 to about 6.

[0055] Another possible mechanism involves the effect of components of certain active compositions on salivary protein production. Polyphenols are components of certain of the active compositions (e.g., Applephenon® and cranberry extract) of the present invention. It is known that polyphenols, particularly tannins, precipitate salivary proteins. It is presently believed that volatile components adsorb to salivary proteins making them less likely to be released into the headspace of the solution. Also, a portion of the volatile

components are believed to be removed from the oral cavity by virtue of swallowing of the salivary proteins having volatile components adsorbed thereto.

[0056] Still another possible mechanism involves a reaction between a component of the active and an odor-causing compound. More particularly, a reaction between polyphenol (from a test composition such as cranberry extract) and nitrogenous compounds from tobacco smoke (e.g., pyridines), thereby reducing the concentration of these volatile components. Another active composition of the present invention, cardamom, contains terpenes, most notably limonene. These compounds possess antioxidant activity in a manner similar to polyphenols. Thus, it is presently believed that there may be a similar reaction between the terpenes associated with cardamom and pyridines and an attendant reduction in concentration of the volatile component.

[0057] Regardless of the mechanism by which the concentration reduction is achieved, it is to be noted that the present invention enables the screening of test compositions in a quantifiable, and/or analytical, way, in order to evaluate their potential use in an oral composition such as a chewing gum or confection product, without having to initially prepare such an oral composition. As such, a plurality of samples may be evaluated more rapidly and in a more efficient and cost effective manner. For example, in a preferred embodiment, the present invention may be utilized to screen a plurality of test compositions in order to more efficiently identify and select test compositions that are effective to reduce the concentration of one or more pyridine and/or pyrazine compounds in the headspace of a vessel containing a model solution (e.g., as determined by mass spectrometry using means known in the art) by at least about 20% or at least about 30%, preferably at least about 40%, more preferably at least about 50%, still more preferably at least about 60%, still more preferably at least about 70%, still

more preferably at least about 80%, still more preferably at least about 90%, and most preferably about 100%. Once identified, these compositions may then optionally be subjected to further testing, wherein they are formulated into an oral composition for further testing. In some instances, these resulting compositions (e.g., gums and/or confections) may achieve the same or similar reduction in the oral cavity of a test subject.

[0058] As previously noted, in order for a substance to possess aroma, it typically is volatile and passes through a person's nasal epithelium retronasally or orthonasally. Thus, reduction in headspace concentration of the volatile odorants of a model solution generally indicates effectiveness for reduction of volatile components present in a subject's oral cavity after smoking a tobacco product and, accordingly, treatment of oral cavity malodor caused by the presence of these volatile components. Accordingly, once a test composition has been successfully identified in accordance with the present invention to reduce the concentration of an odor causing compound, this test composition may then optionally be used to prepare an oral composition (e.g., chewing gum and/or confection product) using means known in the art, for further evaluation or use with human subjects.

[0059] Various compositions may be screened using the process described herein to determine their ability to reduce the odorant headspace concentration of model solutions, including compositions derived from various fruits (e.g., cranberries, apples, crabapple, hawthorn berries, plums, prunes and grapes), vegetables, and plants.

[0060] For example, compositions to be screened in accordance with the present invention may comprise, or alternatively consist essentially of, an extract, oil, compound, etc. selected from the group consisting of cranberry extract, Applephenon®, crabapple extract, hawthorn berry extract, plum extract, prune extract, grape seed extract,

grape skin extract, cardamom seed extract (e.g., cardamom oil), alfalfa extract, honeysuckle extract, rosemary extract, basil extract, thyme extract, aloe extract, chrysanthemum extract, green tea extract, coffee berry extract, licorice, parsley seed oil, pine extract, coffee extract, ginseng extract, dandelion root extract, chlorogenic acid, ascorbic acid, caffeic acid, zinc lactate, silica gel, citric acid, maleic acid, tartaric acid, eugenol, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, quinic acid, and combinations thereof.

[0061] By way of further example, compositions to be screened in accordance with the present invention may comprise, or alternatively consist essentially of, an extract, oil, compound, etc. selected from the group consisting of cranberry extract, crabapple extract, hawthorn berry extract, plum extract, prune extract, grape seed extract, grape skin extract, cardamom seed extract (e.g., cardamom oil), alfalfa extract, honeysuckle extract, rosemary extract, basil extract, thyme extract, aloe extract, chrysanthemum extract, green tea extract, coffee berry extract, licorice, parsley seed oil, pine extract, coffee extract, ginseng extract, dandelion root extract, chlorogenic acid, ascorbic acid, caffeic acid, zinc lactate, silica gel, citric acid, maleic acid, tartaric acid, eugenol, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, quinic acid, and combinations thereof.

[0062] In certain embodiments, the composition to be screened may be derived from a fruit including, for example, cranberry extract, crabapple extract, hawthorn berry extract, plum extract, prune extract, grape seed extract, grape skin extract, and combinations thereof. In still further embodiments, the composition to be screened may be derived from a plant including, for example, cardamom seed extract (e.g., cardamom oil), alfalfa extract, honeysuckle extract, rosemary extract, basil extract, thyme extract, aloe extract, chrysanthemum extract, green tea extract, coffee berry

extract, licorice, parsley seed oil, pine extract, coffee extract, ginseng extract, dandelion root extract, and combinations thereof. In still further embodiments, the composition to be screened comprises chlorogenic acid, ascorbic acid, caffeic acid, zinc lactate, silica gel, citric acid, maleic acid, tartaric acid, eugenol, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, quinic acid, and combinations thereof.

[0063] Carbon (e.g., activated carbon) obtained from, for example, wood or nutshells may also be screened in accordance with the present invention. In particular, Applephenon®, cardamom oil and cranberry extract have been determined to be effective for reducing odorant headspace concentration.

Use of Compositions in Oral Compositions

[0064] Compositions recognized as effective to reduce the concentration of a pyridine or pyrazine compound in the headspace of the vessel in which the substances are contained by at least about 50% (or greater) are particularly well-suited for incorporation into an oral composition including, but not limited to, a confection, chewing gum, lozenge, pressed tablet, edible film, mouthspray, mouthwash, or toothpaste product suitable for treatment of oral cavity malodor associated with smoking a tobacco product. In particular, a test, or an active, composition identified as effective for reducing the concentration of an odor causing compound in the headspace of a vessel in which the compound is contained, in accordance with the present screening method, is suitable for incorporation into a confection or chewing gum in accordance with methods known in the art as described, for example, in U.S. Patent No. 6,627,234, the entire contents of which is incorporated herein by reference.

[0065] The active composition may be incorporated into an oral composition without dilution, or it may be diluted prior to incorporation. In either case, the active composition may

be present in a confection or chewing gum, for example, at a concentration of at least about 0.1% by weight, more typically at least about 0.5% by weight and, still more typically, about 1% by weight. Preferably, the active composition is present in the confection or chewing gum at a concentration of from about 0.1% to about 5% by weight, more preferably from about 0.5% to about 2% by weight and, still more preferably, at a concentration of from about 0.5% to about 1% by weight.

Method for Treating Oral Malodor

[0066] Generally, treatment of oral malodor associated with tobacco smoke proceeds by administration to a subject an oral composition (e.g., one or more pieces of a confection or chewing gum product) containing an active composition identified in accordance with the present invention; that is, oral malodor may be treated in accordance with this invention by administering an oral composition recognized to reduce the concentration of tobacco smoke odorants in a subject's oral cavity. More particularly, oral malodor may be treated by administering to a subject a composition effective to reduce the concentration of a pyridine or pyrazine compound present in the subject's oral cavity as a result of smoking a tobacco product by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%. For example, in the case of an oral composition such as a chewing gum or confection, administration may comprise chewing multiple pieces of the oral composition. It is desirable for the duration of the product in the oral cavity, as well as the rate at which the active composition is released from the oral composition, to be controlled so as to optimize the effectiveness of the product in combating oral malodor caused by tobacco smoke. For example, in the case of a chewing gum, administration typically comprises chewing of the gum for at least about 5 minutes, more typically for about 5 to about 60

minutes, even more typically for about 10 to about 20 minutes and, still more typically, for about 20 minutes. In the case of a chewing gum, typically at least about 5%, at least about 10%, at least about 25%, at least about 50%, at least about 75%, or even about 100% of the active composition is released from the gum during the first few minutes (e.g., the first about 2 minutes, about 3 minutes, about 4 minutes, or even about 5 minutes) of chewing. More typically, at least about 25%, at least about 50%, at least about 75%, or even at least about 100% of the active composition is released from the gum during the first 20 minutes of chewing.

[0067] It is to be noted in this regard, however, that in various alternative embodiments, a more sustained delivery of active composition into the oral cavity may be desired. Thus, in such embodiments it may be desired for no more than about 25%, no more than about 50%, or no more than about 75% of the active composition to release into the oral cavity during the first few minutes (e.g., the first about 2 minutes, about 3 minutes, about 4 minutes, or even about 5 minutes) of administration. Likewise, it may be desired for no more than about 50% or no more than about 75% of the active composition to release into the oral cavity during the first 20 minutes of administration.

Method for Promoting the Use of a Composition

[0068] The present invention is also directed to a method for promoting an oral composition containing a composition effective to reduce the concentration of a pyridine or pyrazine compound present in a subject's oral cavity as a result of smoking a tobacco product. Generally, this process comprises distributing, to an end user or alternatively to someone who will in turn distribute to an end user, an oral composition including, but not limited to, a confection, chewing gum, lozenge, pressed tablet, edible film, mouthspray, mouthwash, or toothpaste product containing a composition

recognized to reduce a concentration of a pyridine or pyrazine compound present in a subject's oral cavity as a result of smoking a tobacco product to that subject, and encouraging a subject to consume or chew the product to ameliorate oral malodor resulting from smoking a tobacco product. For example, this encouragement may typically appear on a package containing a confection or chewing gum product and may be disseminated by conventional means (e.g., electronic or print media). Generally, the product is described as containing an ingredient recognized to reduce a concentration of a pyridine or pyrazine compound present in a subject's oral cavity as a result of smoking a tobacco product. In particular, the recognition of the ingredient's effectiveness is achieved by carrying out the screening method described herein. In the case of a chewing gum containing such an ingredient, generally the subject is encouraged to chew the gum for a certain period of time (e.g., at least about 5 minutes or about 20 minutes).

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[0069] The present invention is further illustrated by the following Examples. These Examples are not to be regarded as limiting the scope of the invention or the manner in which it may be practiced.

EXAMPLES

Example 1

[0070] This example details identification of the odorous components responsible for oral malodor caused by smoking a tobacco product.

[0071] The method for identification of the odorous components included swabbing the oral cavity of a subject prior to and after smoking a tobacco product and analysis utilizing solid phase microextraction (SPME), gas

chromatography-olfactometry (GCO) and gas chromatography/mass spectroscopy (GC/MS).

[0072] Testing for the odorous compounds was conducted at the Microanalytics lab (Microanalytics, A MOCON company Round Rock, TX) and utilized Macanudo cigars (Macanudo® robusto brand, Dominican Republic) using two panelists. The panelists did not eat or drink or use oral hygiene products for 2 hours prior to smoking. Panelists refrained from smoking or consuming any foodstuff except water one hour prior to smoking. Neither panelist regularly smoked, and panelists both possessed normal oral health. Each panelist smoked one-half of a cigar (approximately a 20 minute smoke).

[0073] Odorous tobacco smoke compounds were extracted from the panelists' oral cavities by swabbing the tongue's surface fore and aft, five strokes, with a nylon stemmed, nylon mesh coated swab (TX 714A, The Texwipe Co., Upper Saddle River, NJ). After verifying the swab possessed tobacco smoke odor by sensory screening (2 judges sniffed swabs and agreed on most odorous swabs), the polypropylene stem was cut off, and the swab head was sealed in a 40 ml glass vial with a plastic screw cap and Teflon septa for subsequent headspace sampling. Blank swabs taken from the panelists' oral cavities 10 minutes before smoking were analyzed as controls.

[0074] Headspace of the vial containing the swab head was extracted for 60 minutes utilizing solid phase microextraction (SPME) (Supelco, Bellefonte, PA) with a Carboxen - polydimethyl siloxane fiber (75 μm, 23 gauge).

[0075] The SPME fiber assembly was injected into an Agilent 6890 gas chromatograph (GC) / mass spectrometer (MS) modified for multidimensional analyses (Agilent Technologies, Palo Alto, CA) and equipped with a sniff port and Aroma Trax software (Microanalytics, Round Rock, TX) for the analyses. Fibers remained in the GC injection port for five minutes following injection. Initially, manual SPME extractions were conducted for analysis.

[0076] The GC/MS operating parameters included a He carrier gas flow rate of 6.5 ml/min, split mode (2:1) and the injector set at 250°C. Column 1 was 15 meter, 0.53 mm I.D, film thickness 1 μm with 5% phenyl methylpolysiloxane stationary phase (SGE BP5) and was operated with constant pressure mode at 16 psi at an average velocity 66 cm/sec. Column 2 was a 30 meter, 0.53 mm wax capillary with column film thickness 1 μm (SGE BP20) and was operated at a pressure of 5.7 psi at an average velocity 56 cm/sec. The oven was programmed to hold at 40°C for 3 minutes and then increase at 7°C/min to 220°C and hold for 20 minutes. The MS operated in Electron Impact Mode (E.I.) at 70 eV.

[0077] The GC sniff port was used to identify specific times of column eluant that exhibited odor characteristic of tobacco smoke; heartcuts (small segments) of chromatographic effluent that contained these odor peaks were selectively analyzed by the MS detector and sniff port for further evaluation and identification. The heart-cut valve was located between the first column and the second column. Second column eluant was split between the MS detector and sniff port (50:50) whereas eluant from the first column traveled exclusively to the flame ionization detector (FID) unless selectively sent to the second column by the heart-cutting valve or unless purged.

[0078] Additional testing and further refinement of aroma active compound identities were conducted at the Wrigley Chicago Research and Development facility using a Microanalytics GC/MS unit with identical features as that utilized previously, with the exception that the Chicago unit contained a Leap Technologies CombiPal autosampler capable of utilizing automated SPME (Leap Technologies, Carrboro, NC). The GC/MS unit also had a direct connection between the output of the first column and the sniff port to make heart cutting more accurate and precise. In the previous analysis utilizing manual sampling, the sniff port was connected to the output of

the second column only. The time utilized for heart-cuts was based on back-calculating when an odorous peak with a specific retention time at the MS and at the sniff port (at the output of the second column) had transited the first column (the heart cut valve was located on the output of the first column). In this instrument incorporating the autosampler the sniff port had plumbing that allowed for direct connection to the output of the first column, thus heart-cuts could be made based on odor detection time. The sniff port also had the option of connection to the output of the second column as before.

[0079] For components that exhibited odor activity but the identity of the component could not be firmly established, the heart-cut effluent was cryogenically focused onto the head of the second column (utilizing a feature of this instrument that contained a spray nozzle that utilized liquid CO_2) to provide additional peak separation. Headspace extraction times utilizing SPME were also extended up to 24 hours to fully load the fiber with headspace volatiles. Other than specified, columns, oven, and other analytical parameters remained the same as previously discussed.

[0080] Fig. 1 is an FID chromatogram showing extracted tobacco smoke components of a heart cut taken from 13.75 minutes to 14.25 minutes after injection of the sample in the chromatograph.

[0081] Fig. 2 is a total ion current (TIC) chromatogram TIC of heart cut effluent from the output of column 1 from 13.75 to 14.25 minutes after injection of the sample in the chromatograph.

[0082] Identification of the odorous components responsible for tobacco breath was also performed by entrapment of smoke volatiles in simulated saliva followed by SPME, GCO and/or GC/MS analysis. Simulated saliva was prepared in accordance with an in-house method by dissolving sodium chloride, sodium bicarbonate and potassium bicarbonate

in deionized water to produce a solution with electrolytes present in concentrations similar to saliva. This solution was utilized to measure residual tobacco smoke components following exposure in a manner similar to saliva exposure in the mouth. To prepare saliva-like solutions exposed to cigar smoke, a panelist drew smoke from a cigar (Onyx brand; Dominican Republic, mini Belicoso) and then gently blew four separate two second puffs with a standard drinking straw into the bottom of a 22.5 ml vial so that smoke bubbled through the solution. Liquid contents were then transferred to a separate vial to ensure that any smoke volatiles adsorbed on the glass surfaces were excluded.

[0083] Two panelists evaluated SPME extracts of the solution by gas chromatography olfactometry (GCO) analysis three times each and only peaks with the strongest odor intensities were further analyzed by heart cutting. The additional GCO analyses provided greater accuracy and precision than obtained previously. Confirmation of peak identities were made by comparing retention times of identified compounds with those of standard compounds and by comparing odors of identified components with standards.

[0084] Fig. 3 is an overlay of aromagram and TIC analysis of effluent from SPME headspace extraction of artificial saliva (23 hours extraction time at room temperature) followed by heart-cutting (30 second heart-cut) and cryogenic focusing (30 seconds before, during, and 30 seconds after the heart-cut) with odor intensity score and odor character displayed.

[0085] Compounds that possessed aroma activity in initial analyses conducted at Microanalytics were classified as Tier one, two or three. Those in Tier one exhibited the greatest aroma. In subsequent tests conducted at the Wrigley R&D facility, aroma active compounds were assigned numerical scores in accordance with aroma intensity (Table 1).

[0086] Identification of components was conducted by matching spectra utilizing a Wiley database (e.g., Wiley

Registry of Mass Spectral Data, 7th Edition, John Wiley & Sons, Inc., 2000), and by matching retention indices with authentic standards. Results are shown in Table 1.

Table 1

Preliminary identification of compounds responsible for the odor of tobacco smoke extracted from the oral cavity.

Odor intensity (0-100)	Retention Indice (Kovats)	Descriptor	Identity
67[1]	1046	Tobacco, musty	2,3,5-trimethylpyridine [3,4]
45[1]	945	Tobacco, earthy	4-ethyl-3-methyl pyridine [3,4]
50[1]	967	Nutty	2-ethyl-3,5-dimethyl pyridine [3,4]
58[1]	844	Savory	2,5-dimethyl pyrazine [3,4]
47[1]	972	Nutty	2,6-diethyl pyrazine [3]
45[1]	1034	Tobacco, musty	2,4,6-trimethyl pyridine (collidine)[3,4] and 2-ethyl-5-methyl pyridine [1]
45[1]	401	Floral	Acetophenone [3]
Tier 1[2]	979	Musty	ethyl pyrrole [3]
Tier 2[2]	856	Savory	2,3-dimethyl pyrazine [3,4]
Tier 3[2]	830	Nutty	methyl pyridine [3]
Tier 3[2]	945	Tobacco	cyclobutyl pyridine [3]
Tier 3[2]	525	Buttery	Diacetyl [3]

[1] Aroma active compounds measured utilizing simulated saliva blend.

[2] Aroma activity of compounds measured utilizing swabs and assigned position in 1st, 2nd, or 3rd Tier, according to aroma intensity, only.

[3] Compounds identified by correlation with Wiley mass spectrometry database.

[4] Compounds identified by matching retention indices of authentic standard.

Example 2

[0087] This example details *in vitro* GC/MS headspace and sensory measurement of Applephenon® (a natural apple extract powder high in short chained polyphenols and highly soluble in water; A.M. Todd Co. Kalamazoo, MI distributors of Applephenon® for Asahi Corp., Japan) vs. tobacco smoke odor in model solutions.

[0088] Measurement of the relationship between tobacco odorant headspace concentration and mass of active added (dose response) included preparation of a model solution composed of compounds with a similar combined odor character and intensity as that in the oral cavity following consumption of two Marlboro light cigarettes, or one-half robust cigar (such as Partagas 1845). These compounds were placed in a 1% ethanol/99% water solution, each at a concentration of 150 ppm (μg/ml solution) and included: collidine (2,4,6-trimethyl pyridine), lutidine (2,6-dimethyl pyridine), 2-ethyl pyridine, 3-ethyl pyridine, pyridine, ethyl pyrazine, 2-ethyl-3-methyl pyrazine.

[0089] In order for a substance to possess aroma, it typically passes through the nasal epithelium, via retronasal or orthonasal entrance. Saliva is 99% water and near pH 7 (actual pH depends on salivary flow rate). For this reason the tobacco smoke odor model solution containing 99% water was utilized for initial testing for active efficacy. By reducing headspace concentrations of the odors from aqueous solution, less aroma perception via retro or orthonasal means may result.

[0090] The similarity between odor character and intensity of the model solution and oral cavity tobacco smoke malodor was determined by ten R&D personnel experienced in sensory analysis. Odor judges smelled 22.5 ml vials with 5 ml of aqueous solution and assessed perceived aroma character and intensity.

[0091] A model solution (5 ml) was treated with the potential ameliorating active, Applephenon® (50 mg wt/wt). Headspace of the resulting aqueous solution was extracted in 22 ml vials with Teflon septa for 10 min. at 35°C with solid phase microextraction fiber (Stabilflex, Carboxen, PDMS, DVB) and analyzed using GC/MS and a CombiPal autosampler as described above in Example 1. Three replications were assessed for % relative standard deviation (RSD). Values in excess of 15% were repeated. Percentage of headspace reduction was assessed by comparison of headspace values of standard with no added actives. The results are shown in Fig. 4. As shown, all pyridine headspace concentrations were reduced by more than 75%.

[0092] The aqueous solution with pyrazines/pyridines had a pH 7. Following addition of polyphenol, the solution was pH 3.7 (the drop in pH is attributed to a major component of Applephenon®, the acidic polyphenol chlorogenic acid). Pyridine's pKa is 5.2, whereas pyrazine's is 1.1. In the solution with added polyphenol, pyridine existed primarily as a cation and pyrazine remained neutral. As a charged species, hydrogen bonding in the aqueous solution could account for the decreased volatility associated with pyridine in its protonated form. It was hypothesized the partition coefficient for pyrazine, under acidic aqueous conditions, favored the gaseous state thus the increased headspace concentration.

[0093] Sensory analysis of identical solutions was conducted with twenty panelists who evaluated the odor intensity of a model solution with and without added active. Odor intensity was assessed utilizing a 0-100 point scale ballot with 0 = no odor and 100 = very strong odor. Responses were averaged and analysis of variance (ANOVA) conducted to assess statistical significance. The results from sensory analysis of identical (but separate) solutions of odorants previously utilized for analytical testing are listed in Fig.

5. Panelists (N=10) found the solution with added polyphenol was significantly lower in tobacco odor intensity vs. the control with P value < 0.05. This corresponds with the decrease in headspace concentration of nitrogenous components previously discussed.

Example 3

[0094] Following initial *in vitro* tests that indicated potential active efficacy vs. tobacco odor, a preliminary test was designed to measure changes in residual tobacco smoke compounds present on the tongue and extracted with a swab as previously described.

[0095] Evaluation of active efficacy utilizing swabs by smoking one-half of an Onyx cigar and removing a sample of volatiles from the tongue with a Texwipe swab in the manner previously described, the effects of three treatments (Orbit® apple chewing gum commercially available from the Wm. Wrigley Jr. Company (Chicago, IL) plus 78 mg Applephenon®, Orbit® apple gum, and no gum) on residual oral cavity tobacco odor were evaluated by GC/MS. Gums were chewed for 20 minutes immediately following smoking. Swabs generated by two panelists were evaluated. Testing was held in late morning. Panelists consumed a light breakfast 2 hours before testing. Panelists were not cigarette smokers, and had no abnormal oral health problems.

[0096] Headspace analyses of panelist swabs before and after treatment with a cigar + no gum, cigar + control gum, and cigar + gum with active and no cigar base line were conducted. The results are shown in Fig. 6. The gum containing the active and the control gum dramatically reduced levels of pyridine (100% and 91% reduction, respectively), and 4-methyl pyridine (both gums reduced levels to below the detection limit) below the level found in the oral cavity following smoking a cigar and then not chewing any gum. Levels of 2-methyl pyridine were reduced 45% by gum with

active and 35% by gum without active. Ethyl pyrazine levels were reduced approximately the same by both gums (28% and 25%). Swabs obtained from oral cavities not exposed to cigar smoke did not possess any of the pyridines, but did possess low levels of ethyl pyrazine.

[0097] Because model solution headspace data analyzed in vitro (as previously explained) exhibited increased levels of pyrazines following treatment with the active, a reduction of ethyl pyrazine in the samples obtained following chewing gum + active below the level of that from the control gum was not expected and not observed. In general, differences between trace pyridine levels extracted from cigar + control gum and cigar + gum with active were negligible. This negligible difference between control and active gums may be attributed to the difficulty of extracting pyridines adsorbed to the surface of the tongue and then accurately measuring their concentration. Also, with two panelists statistical analyses were not meaningful. Nevertheless, the effect of chewing gum, either active or control, is readily evident in the decrease of odorant headspace concentrations.

Example 4

[0098] This example details a clinical assessment to determine if Orbit® Apple gum with added active (Applephenon®) reduced self-perceived malodor intensity caused by cigarettes as compared to a control gum without the active and using no gum at all.

[0099] A group of one hundred and two panelists were recruited by Tragon Corp. from the Chicago, IL area (Tragon, Buffalo Grove, IL) to smoke two Marlboro light cigarettes and then rate their tobacco aftertaste intensities at time 0 (immediately following smoking), and then at the following times in minutes following smoking: 1, 5, 10, 15, 20, 21, 25, 30, 35, 50, 60, 80 and 90. Three treatments (three sample complete block design) were utilized following smoking (each

panelist received a different treatment on three consecutive days and treatments were administered in random order). Test protocol included the following: consumers were asked not to eat, drink, smoke or use oral hygiene (includes brushing and mouthwash) 90 minutes prior to test. Each consumer evaluated all samples and treatments over 6 days; 1 sample per day. Sessions lasted two hours and each session accommodated 25-30 consumers. Four sessions per day were held. Consumers were selected based on smoking habits, all were gum chewers, all possessed no dental or other confounding health problems. Consumers did not consume food or beverage or use oral hygiene for 2 hours before each session. Consumers were given fifteen minutes or less to smoke two cigarettes. Consumers smoked in a designated area outside the building and immediately after smoking, consumers returned inside and rated intensity of tobacco aftertaste using a line scale for intensity.

[0100] Treatment one was chewing a serving of pellet gum (serving equal 2 pellets) with the active mixed into gum center (Orbit® Apple plus Applephenon®) for 20 minutes following smoking. Treatment two was chewing a control gum made in the identical manner as before, only with no active, and treatment three was no gum (panelists simply smoked the cigarettes and rated their aftertaste intensities at the appointed times). Panelists received no training on rating tobacco aftertaste and were simply asked to rate their aftertaste intensity based on the method they normally employ to discern if their breath is fresh. A repeated measures Analysis of Covariance was performed on the intensity data from all time points and a one tailed test was employed to determine level of significance.

[0101] The effect of added active (Applephenon®, 78mg) on perceived tobacco smoke aftertaste associated with smoking two Marlboro light cigarettes is shown in Fig. 7. Results indicated aftertaste intensities determined by a consensus of 102 untrained panelists after chewing Orbit® apple gum treated

with 78 mg active was lower than the control at all time points and significantly lower ($P < 0.05$) at times 20, 21, and 25. Additionally the gum treated with active had a significantly lower mean curve height than the control ($P < 0.05$). Both active and control gums were judged significantly better than chewing no gum at all vs. cigar aftertaste for all time points.

Example 5

[0102] This example describes a clinical assessment to measure efficacy of Wrigley's Orbit® Apple Gum with added active (Applephenon®) for reducing self-perceived breath malodor intensity caused by smoking a cigar.

[0103] Seven panelists were trained in five separate smoking sessions. In each session Onyx cigars were smoked in the manner previously described. After finishing one-half of the cigar panelists rated their self-perceived breath malodor intensity utilizing a 0-10 point line scale at identical times as described with cigarettes. Testing was conducted over four days (two days per week for two consecutive weeks). Four separate treatments (one treatment per day for four consecutive days) were evaluated over the 90 minute interval in random order and included no treatment, one serving of Eclipse Winterfresh™ gum commercially available from the Wm. Wrigley Jr. Company (Chicago, IL), one serving of Wrigley's Orbit® Apple gum, one serving of a Wrigley's Orbit® Apple gum with added active (Applephenon®). Following smoking, an initial self-perceived breath malodor assessment was made followed by 20 minutes of gum chewing. After expectoration, self perceived odor assessments were made at 30 minutes, 60 minutes and 90 minutes.

[0104] Preliminary testing by measuring self-perception of aftertaste following smoking cigars indicated potential active efficacy. The results are shown in Fig. 8. Results indicated aftertaste intensity was reduced the most, and

remained the lowest following chewing Orbit® apple flavored gum with 78 mg of active (Applephenon®). Aftertaste intensity scores were significantly lower than those obtained following chewing the control gum for each time period ($P < 0.05$). Additionally, aftertaste intensity scores obtained following chewing the gum with 78 mg active were significantly lower than scores measured after chewing Eclipse Winterfresh™ gum at times 60 min. and 90 min. Lastly, chewing gum, any gum, significantly reduced cigar aftertaste intensity vs. chewing no gum at all for each time period.

Example 6

[0105] A model solution containing 150 ppm (μg/ml) of each of pyridine, 2-ethyl pyridine, 3-ethyl pyridine and ethyl pyrazine, added to a 5% ethanol, 95% water solution was determined by consensus of odor judges to best represent tobacco smoke odor character and intensity.

[0106] A model solution (5 ml) was treated with each of two potential ameliorating actives in separate vials. The first potential active was cranberry extract, a highly concentrated natural cranberry extract powder soluble in water (Ocean Spray Corp., Winthrop, MA); analyses were conducted utilizing 50 mg, 100 mg, 150 mg, and 200 mg of the active. The second potential active was cardamom oil, a natural extract from crushed cardamom seed (Treatt Flavors, Lakeland, FL); analyses were conducted utilizing the potential active in the odorous solution at the following levels: 54 μg/ml, 156 μg/ml, 207 μg/ml and 642 μg/ml.

[0107] Headspace of the aqueous solution was extracted in 22 ml vials with Teflon septa for 10 min at 35°C using a solid phase microextraction fiber (Stabilflex, Carboxen, PDMS, DVB) and analyzed with GC/MS. A CombiPal autosampler was utilized as described above. Three replications were assessed for % relative standard deviation (RSD). Values in excess of 15% were repeated. Percentage of headspace reduction was assessed

by comparison of headspace values of a standard with no added actives.

[0100] In order for a substance to possess aroma, it typically is volatile and passes through the nasal epithelium, via retronasal or orthonasal entrance. Saliva is 99% water and near pH 7 (actual pH depends on salivary flow rate). For this reason the tobacco smoke odor model solution containing 99% water was utilized for initial testing for active efficacy. By reducing headspace concentrations of the odors from aqueous solution, less aroma perception via retro or orthonasal means may result.

[0101] Fig. 9 shows the results from the addition of 50 mg, 100 mg, 150 mg and 200 mg of cranberry extract. Tables 2-5 show the analytical dose response data for headspace concentration reduction associated with pyridine, 2-ethyl pyridine, ethyl pyrazine and 3-ethyl pyridine, respectively.

Table 2

Cranberry extract reduction of pyridine headspace concentration (expressed as mg cranberry per ml solution): analytical dose response data.

	GC/MS Peak Area				
	Control	40 mg/ml	30 mg/ml	20 mg/ml	10 mg/ml
Run 1	14444526	n.d.[1]	n.d.[1]	n.d.[1]	1558183
Run 2	14571161	n.d.[1]	n.d.[1]	n.d.[1]	1572148
Run 3	16085301	n.d.[1]	n.d.[1]	n.d.[1]	1579508
Mean	15033663	n.d.[1]	n.d.[1]	n.d.[1]	1569946
Standard Deviation	912943.9	n.d.[1]	n.d.[1]	n.d.[1]	10831.64
Standard Error	527088.4	n.d.[1]	n.d.[1]	n.d.[1]	6253.65
%RSD	6.07	n.d.[1]	n.d.[1]	n.d.[1]	0.69

[1] n.d. indicates that pyridine was not detected

Table 3

Cranberry extract reduction of 2-ethylpyridine headspace concentration (expressed as mg cranberry per ml solution): analytical dose response data.

	GC/MS Peak Area				
	Control	40 mg/ml	30 mg/ml	20 mg/ml	10 mg/ml
Run 1	160109870	1368141	1645724	1948092	6876637
Run 2	142768839	1496262	1565780	2264499	6660330
Run 3	173862114	1538198	1372219	1913381	6356517
Mean	158913607.7	1467533.6	1527907.6	2041990.6	6631161.3
Standard Deviation	15581117.4	88593.6	140630.6	193477.8	261283.9
Standard Error	8995762.3	51149.5	81193.1	111704.4	150852.3
% RSD	9.81	6.04	9.19	9.47	3.94

Table 4

Cranberry extract reduction of ethyl pyrazine headspace concentration (expressed as mg cranberry per ml solution): analytical dose response data.

	GC/MS Peak Area				
	Control	40 mg/mL	30 mg/mL	20 mg/mL	10 mg/mL
Run 1	35558030	76470454	71101842	73272062	82832901
Run 2	31179441	77280290	68358517	78616280	80690737
Run 3	36276405	76581435	66278482	73562475	74884246
Mean	34337959	76777393	68579614	75150272	79469295
Standard Deviation	2758839	439042.4	2419269	3005161	4112690
Standard Error	1592816	253481.3	1396766	1735030	2374463
% RSD	8.03	0.57	3.53	4.01	5.18

Table 5

Cranberry extract reduction of 3-ethylpyridine headspace concentration (expressed as mg cranberry per ml solution): analytical dose response data.

	GC/MS Peak Area				
	Control	40 mg/ml	30 mg/ml	20 mg/ml	10 mg/ml
Run 1	1.81×10^8	1096887	1365358	2109647	7721021
Run 2	1.61×10^8	1361864	1130701	2107292	7025718
Run 3	1.86×10^8	1201307	1193170	2357495	7174169
Mean	1.76×10^8	1220019	1229743	2191478	7306969
Standard Deviation	13293496	133475.9	121528.5	143779.8	366181
Standard Error	7675003	77062.35	70164.49	83011.28	211414.7
% RSD	7.55	10.94	9.88	6.56	5.01

[0102] Fig. 10 shows the results from addition of 54 μg/ml, 156 μg/ml, 207 μg/ml and 642 μg/ml levels of cardamom oil to the model solution containing pyridine, 2-ethyl pyridine, 3-ethyl pyridine and ethyl pyrazine. Tables 6-10 show the analytical dose response data for headspace concentration reduction associated with pyridine, 2-ethyl pyridine, ethyl pyrazine and 3-ethyl pyridine, respectively.

Table 6

Cardamom oil reduction of pyridine headspace concentration (expressed as μg cardamom oil per ml solution): analytical dose response data.

	GC/MS Peak Area				
	Control	642 μg/ml	207 μg/ml	156 μg/ml	54 μg/ml
Run 1	15992341	4784811	6911030	7410541	10584810
Run 2	15965030	4659265	7052210	7928636	11057573
Run 3	15177671	4650976	7387659	8581892	11536473
Mean	15711680.6	4698350.6	7116966.3	7973689.6	11059618.6
Standard Deviation	462667.5	74991.4	244824.0	586973.7	475834.7

Standard Error	267121.2	43296.3	141349.2	338889.4	274723.3
% RSD	2.94	1.59	3.44	7.36	4.31

Table 7

Cardamom oil reduction of 2-ethylpyridine headspace concentration (expressed as μg cardamom oil per ml solution): analytical dose response data.

	GC/MS Peak Area				
	Control	642 μg/ml	207 μg/ml	156 μg/ml	54 μg/ml
Run 1	184268848	112408826	94359048	97679714	122375677
Run 2	182003143	105790353	91684882	100144739	122903012
Run 3	173676457	97123074	90300102	101340819	124912319
Mean	179982816	105107417.7	92114677.3	99721757.3	123397002.7
Standard Deviation	5577721.5	7665725.9	2063323.4	1866844.2	1338528.3
Standard Error	3220299.0	4425808.9	1191260.3	1077823.0	772799.7
% RSD	3.09	7.29	2.24	1.87	1.08

Table 8

Cardamom oil reduction of ethylpyrazine headspace concentration (expressed as μg cardamom oil per ml solution): analytical dose response data.

	GC/MS Peak Area				
	Control	642 μg/ml	207 μg/ml	156 μg/ml	54 μg/ml
Run 1	40331343	9935627	12937889	13960096	20136295
Run 2	38850725	9687259	13204900	15240288	21393217
Run 3	36946768	9619715	13946772	16060245	22360332
Mean	38709612	9747533.6	13363187	15086876.3	21296614.6
Standard Deviation	1696694.32	166357.6	522735.4	1058445.9	1115161.0
Standard Error	979586.92	96046.6	301801.4	611094.0	643838.5
% RSD	4.38	1.71	3.91	7.02	5.24

Table 9

Cardamom oil reduction of 3-ethylpyridine headspace concentration (expressed as μg cardamom oil per ml solution): analytical dose response data.

	GC/MS Peak Area				
	Control	642 μg/ml	207 μg/ml	156 μg/ml	54 μg/ml
Run 1	185543821	52981160	70792235	75103881	103474622
Run 2	176086450	50707967	73101477	80706289	107019840
Run 3	168268511	50069965	75972042	85414846	111211778
Mean	176632927.3	51253030.6	73288584.6	80408338.6	107235413.3
Standard Deviation	8650610.4	1530223.8	2594967.6	5161935.7	3873080.1
Standard Error	4994432.3	883475.1	1498205.2	2980244.9	2236123.8
% RSD	4.89	2.98	3.54	6.42	3.61

[0103] With cranberry extract, all pyridine headspace concentrations were reduced by more than 85%. Rather than a decrease in headspace concentrations, pyrazine levels actually increased; this effect is believed to be attributed to the pH of the solution containing cranberry extract.

[0104] Sensory analyses of the model solutions containing cranberry extract and cardamom oil were conducted by ten panelists who evaluated the odor intensity of tobacco odorant model solutions with and without added active. Odor intensity was assessed utilizing a 0-10 point scale ballot with 0 = no odor and 10 = very strong odor. Responses were averaged and analysis of variance (ANOVA) conducted to assess statistical significance.

[0105] Fig. 11 and Table 10 show the panelist responses at the various amounts of cranberry extract active.

Table 10

Panelist responses (N=10) in rating aroma intensities of tobacco smoke odor solution (pyridines and pyrazines in water) treated with different concentrations of cranberry extract (expressed as mg cranberry/ml model solution)

	Panelist	40 mg/ml	30 mg/ml	20 mg/ml	10 mg/ml
1	DP	3	5	8	7
2	DC	2	2	5	5
3	CD	7	5	6	8
4	CM	3	2	5	10
5	DB	2	2	3	7
6	BP	1	3	2	3
7	RB	1	1	4	5
8	MT	1	1	1	2
9	LD	0	5	3	7
10	MD	1	2	1	2
	Mean	2.1	2.8	3.8	5.6
	Standard Deviation	1.868154	1.536229	2.135416	2.537716
	Standard Error	0.590762	0.485798	0.675278	0.802496

[0106] Fig. 12 and Table 11 show the panelist responses at the various amounts of cardamom oil active.

Table 11

Panelist responses (N=10) in rating aroma intensities of tobacco smoke odor solution (pyridines and pyrazines in water) treated with different concentrations of cardamom oil (expressed as μg cardamom oil/ml model solution)

	Panelists	25 μg/ ml	150 μg/ ml	630 μg/ ml
1	JK	8	7	3.5
2	SM	1	3	1
3	BM	5	4	1
4	DB	9	7	6
5	SM	8	10	2
6	LD	5	2	2
7	RB	0	0	0

8	MT	3	1	0
9	MH	1	3	0
10	MD	4	2	3
	Mean	4.4	3.9	1.85
	Standard Deviation	3.2	3.14	1.91
	Standard Error	1.0	0.99	0.60

[0107] As shown, the panelists (N=10) found the solutions with added actives were significantly lower in tobacco odor intensity vs. the control with P value < 0.05.

Example 7

[0108] This example details potential active component release from gum.

[0109] The method to measure active release and rate of release involved having 5 panelists chew one serving of gum each (2 pellets), for each of the following times: 0, 10, 20, 25 minutes. Following the chews gum cuds were collected (minimum of 6 cuds), dissolved in a chloroform solvent with undecane as internal standard. The solution was shaken for six hours to ensure solvation. Liquid was then removed and purified with solid phase extraction (SPE) utilizing Millipore (Billerica, MA) Millex-FH hydrophobic PTFE 0.4 μm.

[0110] For non-volatile active components, such as those in cranberry extract, the aqueous layer is removed for analysis conducted with high performance liquid chromatography (HPLC). Quinic acid is utilized as the indicator compound from cranberry (the percentage of quinic acid in cranberry is known, therefore by quantitative analysis of quinic acid in the gum, the amount of cranberry in the gum can be ascertained). Other HPLC operating parameters include the following: reversed phase; Restek Allure organic acids column with following dimensions: 5 micron 300 x 4.6 mm. The mobile Phase was 100% 0.1M phosphate buffer, pH of 2.5. Additional

conditions: flow = 0.3 ml/min, column temperature was 15°C, run time was 45 minutes, detector wavelength was 210 nm and injection volume was 10 ml.

[0111] For volatile components as in cardamom oil, undecane was utilized as an internal standard. The chloroform layer (bottom layer) was removed by Pasteur pipette and placed in a GC vial and capped with crimped cap and Teflon septa. Liquid is injected (in triplicate) into GC for subsequent active component quantification utilizing an Agilent 7683 Series Autosampler (Agilent Technologies, Palo Alto, CA) set to inject 5 μl.

[0112] Cranberry extract was placed in the gum center of a standard chewing gum formulation during production. The extract contained 2.3% quinic acid. Results indicated there was 1.5 mg (0.05%) quinic acid in a serving of gum (center plus coating). This equates to 65 mg cranberry in one serving of gum (2 pellets). No quinic acid was detected in gum chewed for 20 minutes, thus all cranberry was released from gum to the saliva during the 20 minute gum chew.

[0113] Table 12 summarizes the percent headspace reduction of pyridine and 3-ethyl pyridine and sensory odor intensity reduction. Table 13 summarizes the cranberry active release over the course of a 20 minute chew time.

Table 12

Cranberry summary In-vitro efficacy

Cranberry extract concentration (mg/ml model solution)	% pyridine headspace reduction	% 3-ethyl pyridine headspace reduction	Sensory % odor intensity reduction
10	90	96	44
20	100	98	62
30	100	99	72
40	100	99	79

Table 13

Cranberry release from formula

Chew time (min)	% in sample	mg found	% released	mg released
Test gum				
0	100	65	0	0
20	0	0	100	65

[0114] As shown, 100% of the cranberry extract, or 65 mg, is released from the gum during chewing for 20 minutes. Based on these results, it is believed that this amount of cranberry extract would reduce headspace levels of pyridines > 90% if added to 5 ml of model solution and would reduce odor intensity of 5 ml of model solution between 44% and 62%.

[0115] Cardamom oil was added to the gum coating only of a standard chewing gum formulation. It was not added to the center; α-terpinyl acetate comprised 37% of the cardamom oil.

[0116] Although d-limonene also was present in cardamom oil in large amount, α-terpinyl acetate was chosen for this experiment because 1,8 cineole could not be separated from d-limonene as per TIC. Cardamom release results utilizing α-terpinyl acetate as the indicator compound are shown in Fig. 13. Cardamom release in terms of milligrams (mg) oil over time are shown in Fig. 14.

[0117] The majority of cardamom oil released from gum between 0-5 minutes of chewing (33% decrease in cardamom oil in gum between 0-5 minutes). Table 14 shows the cardamom release over the course of 25 minutes of chewing; as shown, 0.49 mg, or 33% of cardamom oil is released from the gum after chewing.

Table 14

Cardamom release from formula

Chew time (min)	% in sample	mg found	% released	mg released
Test gum				
0	0.05	1.46	0	0
5	0.03	0.97	33	0.49
25	0.03	0.97	33	0.49

[0118] Table 15 summarizes the percent headspace reduction of pyridine and 3-ethyl pyridine and sensory odor intensity reduction. This value of cardamom release would provide a reduction of approximately >60% of the tobacco smoke odor of 5 ml of model solution. Additionally, this quantity of cardamom oil release would reduce headspace concentrations of all volatiles, including pyrazines, in the model solution >50%.

Table 15

Cardamom summary In-vitro efficacy

Cardamom concentration (μg/ml model solution)	% pyridine headspace reduction	% 3-ethyl pyridine headspace reduction	Sensory % odor intensity reduction
25	-	-	56
54	29.6	39.2	-
150	-	-	61
156	49.3	54.4	-
207	54.7	58.5	-
630	-	-	82
642	70.1	70.9	-

- indicates not measured

- - - -

[0119] The present invention is not limited to the above embodiments and can be variously modified. The above

description of the preferred embodiments, including the Examples, is intended only to acquaint others skilled in the art with the invention, its principles, and its practical application so that others skilled in the art may adapt and apply the invention in its numerous forms, as may be best suited to the requirements of a particular use.

[0120] With reference to the use of the word(s) comprise or comprises or comprising in this entire specification (including the claims below), unless the context requires otherwise, those words are used on the basis and clear understanding that they are to be interpreted inclusively, rather than exclusively, and applicants intend each of those words to be so interpreted in construing this entire specification.

WHAT IS CLAIMED IS:

1. A method for preparing an oral composition effective for reducing oral malodor associated with tobacco smoke, the method comprising:

contacting in a vessel a test composition and a model solution comprising a pyridine or pyrazine compound present in tobacco smoke, wherein the test composition comprises cranberry extract, crabapple extract, hawthorn berry extract, plum extract, prune extract, grape seed extract, grape skin extract, cardamom oil, alfalfa extract, honeysuckle extract, rosemary extract, basil extract, thyme extract, aloe extract, chrysanthemum extract, green tea extract, coffee berry extract, licorice, parsley seed oil, pine extract, coffee extract, ginseng extract, dandelion root extract, chlorogenic acid, ascorbic acid, caffeic acid, zinc lactate, silica gel, citric acid, maleic acid, tartaric acid, eugenol, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, quinic acid, activated carbon, or a combination thereof;

determining the ability of the test composition to reduce the concentration of the pyridine or pyrazine compound in a headspace of said vessel; and

preparing an oral composition comprising said test composition.

2. A method as set forth in claim 1 further comprising diluting said test composition prior to said contacting.

3. A method as set forth in claim 1 or 2 wherein said oral composition comprises a confection, chewing gum, lozenge, pressed tablet, edible film, mouthspray, mouthwash, toothpaste product or combinations thereof.

4. A method as set forth in any one of the preceding claims wherein the ability of the test composition to reduce the concentration of a pyridine or pyrazine compound in the headspace of said vessel is determined by a method comprising:

measuring the concentration of the pyridine or pyrazine compound in the headspace of said vessel containing the model solution prior to contact with the test composition, to determine an initial pyridine or pyrazine compound concentration in said headspace;

measuring the concentration of the pyridine or pyrazine compound in the headspace of said vessel containing the model solution after contact with the test composition, to determine a final pyridine or pyrazine compound concentration in said headspace; and

determining the difference between the initial and final concentration of the pyridine or pyrazine compound in the headspace of the vessel.

5. A method as set forth in claim 4 wherein the test composition is contacted, and optionally agitated, with the model solution comprising the pyridine or pyrazine compound in the vessel for at least about 10 minutes at a temperature of about 20°C, prior to measuring the pyridine or pyrazine compound concentration in the headspace of the vessel containing the test composition and the model solution.

6. A method as set forth in claim 4 or 5 wherein measuring the concentration of the pyridine or pyrazine compound in the headspace of the vessel, before and/or after contact with the test composition, comprises:

sampling a portion of the vapors comprising the pyridine or pyrazine compound in the headspace; and

subjecting the sampled portion of the vapors to analysis comprising chromatography.

7. A method as set forth in claim 6 wherein said sampling comprises contacting the headspace with a gas tight syringe and extracting a portion of the vapors comprising the pyridine or pyrazine compound in the headspace from the vessel, wherein the vessel is hermetically sealed.

8. A method as set forth in claim 6 or 7 further comprising subjecting the sampled portion of the vapors to mass spectrometry.

9. A method as set forth in any one of the preceding claims wherein the test composition contacts a model solution comprising a pyridine compound.

10. A method as set forth in any one of the preceding claims wherein the concentration of a pyridine compound is reduced by at least about 50%.

11. A method as set forth in any one of the preceding claims wherein the test composition contacts a model solution comprising a pyrazine compound.

12. A method as set forth in any one of the preceding claims wherein the concentration of a pyrazine compound is reduced by at least about 50%.

13. A method as set forth in any one of the preceding claims wherein the test composition contacts a model solution comprising a pyridine compound and a pyrazine compound.

14. A method as set forth in claim 13 wherein the concentration of a pyridine compound and the concentration of a pyrazine compound is reduced by at least about 50%.

15. A method as set forth in any one of the preceding claims wherein the test composition comprises cardamom oil, cranberry extract or a combination thereof.

16. A method for identifying a composition suitable for use in an oral composition effective for reducing oral malodor associated with tobacco smoke, the method comprising:

contacting in a vessel a test composition and a model solution comprising tobacco smoke odorants, the odorants comprising pyridine, 2-ethyl pyridine, 3-ethyl pyrazine and

ethyl pyrazine, wherein the test composition comprises cranberry extract, crabapple extract, hawthorn berry extract, plum extract, prune extract, grape seed extract, grape skin extract, cardamom oil, alfalfa extract, honeysuckle extract, rosemary extract, basil extract, thyme extract, aloe extract, chrysanthemum extract, green tea extract, coffee berry extract, licorice, parsley seed oil, pine extract, coffee extract, ginseng extract, dandelion root extract, chlorogenic acid, ascorbic acid, caffeic acid, zinc lactate, silica gel, citric acid, maleic acid, tartaric acid, eugenol, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, quinic acid, activated carbon, or a combination thereof; and

determining the ability of the test composition to reduce the concentration of one or more of the odorants in said model solution in the headspace of said vessel.

17. A method as set forth in claim 16 wherein the model solution further comprises 2,4,6-trimethyl pyridine, 2,6-dimethyl pyridine, 3-ethyl pyridine, 2-ethyl-3-methyl pyrazine, or combinations thereof.

18. A method as set forth in claim 16 or 17 wherein the model solution further comprises 3,5-dimethyl pyridine, 2-ethyl pyridine, 3-ethyl pyridine, 2,3-dimethyl pyrazine, 2,5-dimethyl pyrazine, or combinations thereof.

19. A method as set forth in any one of claims 16 to 18 wherein said oral composition comprises a confection, chewing gum, lozenge, pressed tablet, edible film, mouthspray, mouthwash, toothpaste product or combinations thereof.

20. A method as set forth in any one of claims 16 to 19 wherein the ability of the test composition to reduce the concentration of one or more of the odorants in the headspace of said vessel is determined by a method comprising:

measuring the concentration of one or more of the odorants in the headspace of said vessel containing the model

solution prior to contact with the test composition, to determine an initial odorant concentration in said headspace;

measuring the concentration of one or more of the odorants in the headspace of said vessel containing the model solution after contact with the test composition, to determine a final odorant concentration in said headspace; and

determining the difference between the initial and final concentration of one or more of the odorants in the headspace of the vessel.

21. A method as set forth in any one of claims 16 to 20 wherein the concentration of an odorant is reduced by at least about 50%.

22. A method as set forth in any one of claims 16 to 21 wherein the test composition comprises cardamom oil, cranberry extract, or a combination thereof.

23. A method for treatment of oral malodor associated with tobacco smoke, the method comprising:

administering to a subject an oral composition comprising an ingredient effective to reduce the concentration of tobacco smoke odorants present in the subject's oral cavity as a result of smoking a tobacco product by at least about 50%, wherein said odorants comprise 2,4,6-trimethyl pyridine, 2,6-dimethyl pyridine, 3-ethyl pyridine and 2-ethyl-3-methyl pyrazine and the ingredient comprises cranberry extract, crabapple extract, hawthorn berry extract, plum extract, prune extract, grape seed extract, grape skin extract, cardamom oil, alfalfa extract, honeysuckle extract, rosemary extract, basil extract, thyme extract, aloe extract, chrysanthemum extract, green tea extract, coffee berry extract, licorice, parsley seed oil, pine extract, coffee extract, ginseng extract, dandelion root extract, chlorogenic acid, ascorbic acid, caffeic acid, zinc lactate, silica gel, citric acid, maleic acid, tartaric acid, eugenol, α-cyclodextrin, β-cyclodextrin,

γ-cyclodextrin, quinic acid, activated carbon, or a combination thereof.

24. A method as set forth in claim 23 wherein said oral composition comprises a confection, chewing gum, lozenge, pressed tablet, edible film, mouthspray, mouthwash, toothpaste product or combinations thereof.

25. A method as set forth in claim 23 or 24 wherein administration comprises chewing a gum comprising said composition for at least about 5 minutes.

26. A method as set forth in any one of claims 23 to 25 wherein administration comprises chewing the gum for from about 5 to about 60 minutes.

27. A method as set forth in any one of claims 23 to 26 wherein said odorants further comprise 3,5-dimethyl pyridine, 2-ethyl pyridine and pyridine.

28. A method as set forth in any one of claims 23 to 27 wherein said odorants further comprise ethyl pyrazine, 2,3-dimethyl pyrazine and 2,5-dimethyl pyrazine.

29. A method as set forth in any one of claims 23 to 28 wherein the ingredient comprises cardamom oil, cranberry extract, or a combination thereof.

30. A method as set forth in any one of the preceding claims wherein the test composition or ingredient is recognized as effective to reduce the concentration of a pyridine or pyrazine compound present in a subject's oral cavity as a result of smoking a tobacco product.

31. Use of a confection or chewing gum comprising a composition or ingredient recognized to reduce the concentration of a pyridine or pyrazine compound in accordance with any one of claims 1 to 30 to reduce the concentration of

a pyridine or pyrazine compound present in a subject's oral cavity as a result of smoking a tobacco product.

32. A composition effective to reduce the concentration of a pyridine or pyrazine compound present as a result of smoking a tobacco product by at least about 20%, for use in the treatment of oral cavity malodor in the oral cavity.

33. A composition effective to reduce the concentration of a pyridine or pyrazine compound present as a result of smoking a tobacco product by at least about 20%, for use in the manufacture of a medicament for the treatment of oral malodor in the oral cavity.

34. The use as set forth in claim 32 or 33 wherein the composition is effective to reduce the concentration of a pyridine or pyrazine compound present as a result of smoking a tobacco product by at least about 30%.

FIG. 1

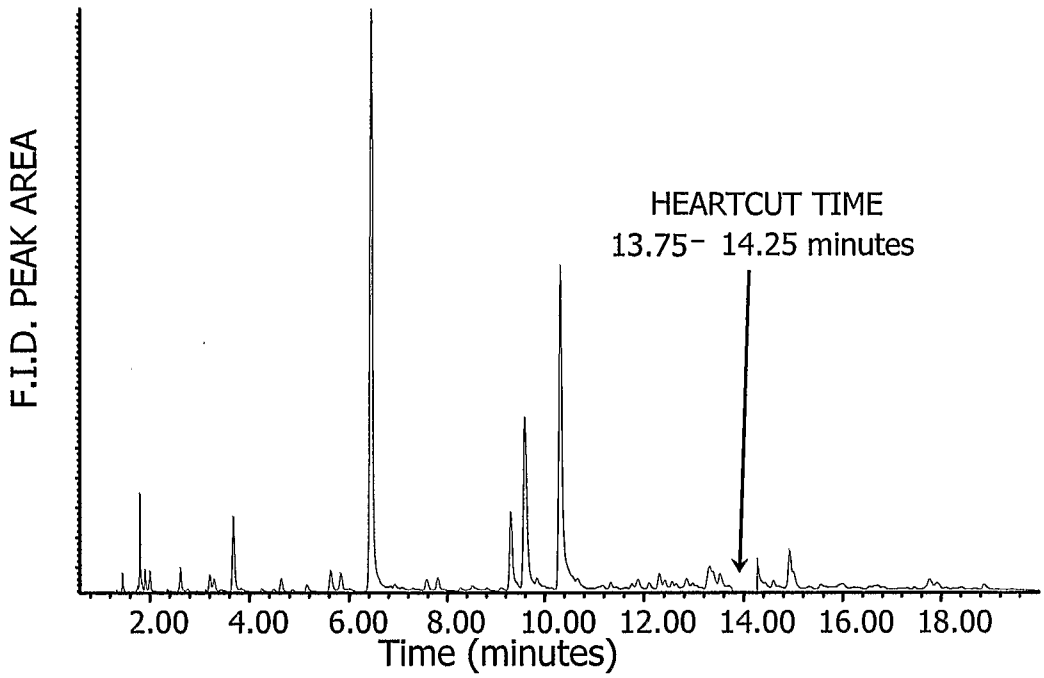

F.I.D. PEAK AREA
HEARTCUT TIME
13.75- 14.25 minutes
2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
Time (minutes)

FIG. 2

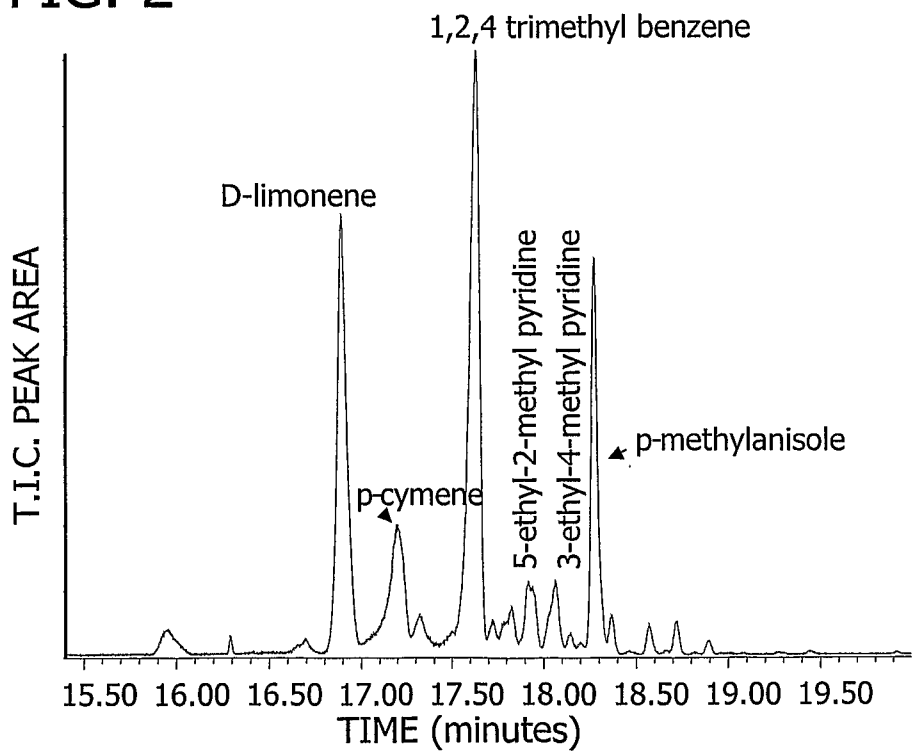

1,2,4 trimethyl benzene
D-limonene
p-cymene
5-ethyl-2-methyl pyridine
3-ethyl-4-methyl pyridine
p-methylanisole
T.I.C. PEAK AREA
15.50 16.00 16.50 17.00 17.50 18.00 18.50 19.00 19.50
TIME (minutes)

FIG. 3

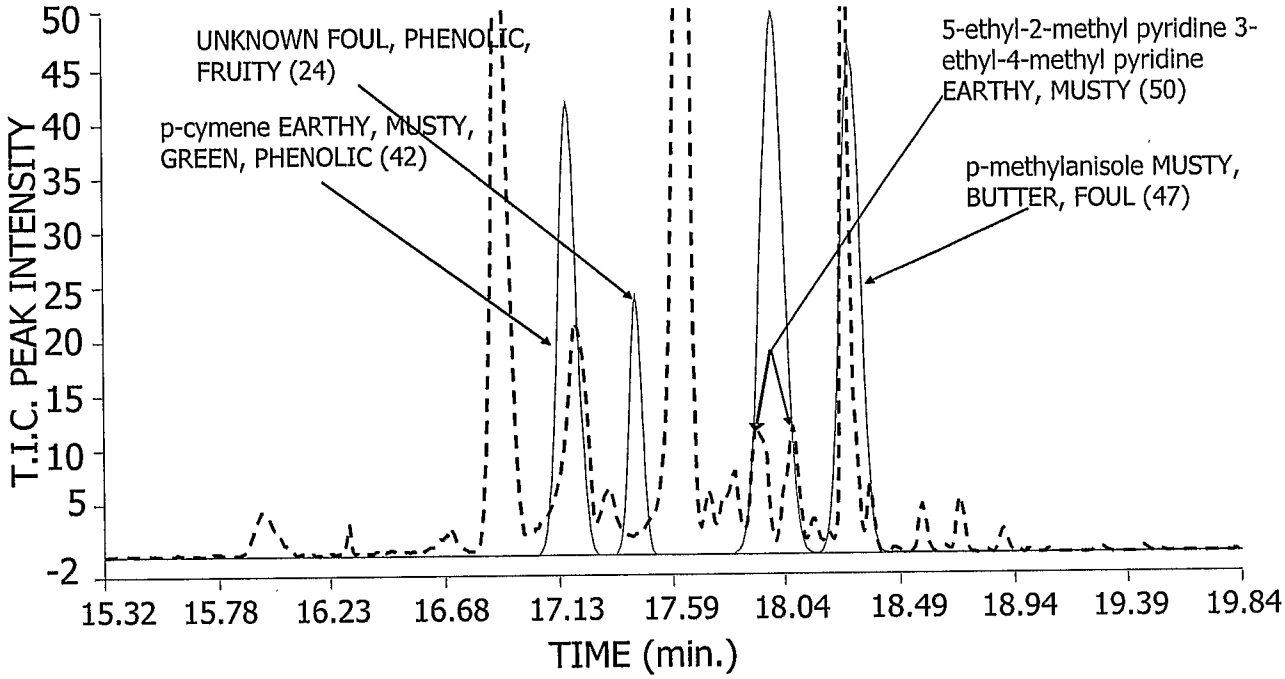

UNKNOWN FOUL, PHENOLIC, FRUITY (24)
p-cymene EARTHY, MUSTY, GREEN, PHENOLIC (42)
5-ethyl-2-methyl pyridine 3-ethyl-4-methyl pyridine EARTHY, MUSTY (50)
p-methylanisole MUSTY, BUTTER, FOUL (47)
T.I.C. PEAK INTENSITY
50
45
40
35
30
25
20
15
10
5
-2
15.32
15.78
16.23
16.68
17.13
17.59
18.04
18.49
18.94
19.39
19.84
TIME (min.)

FIG. 4

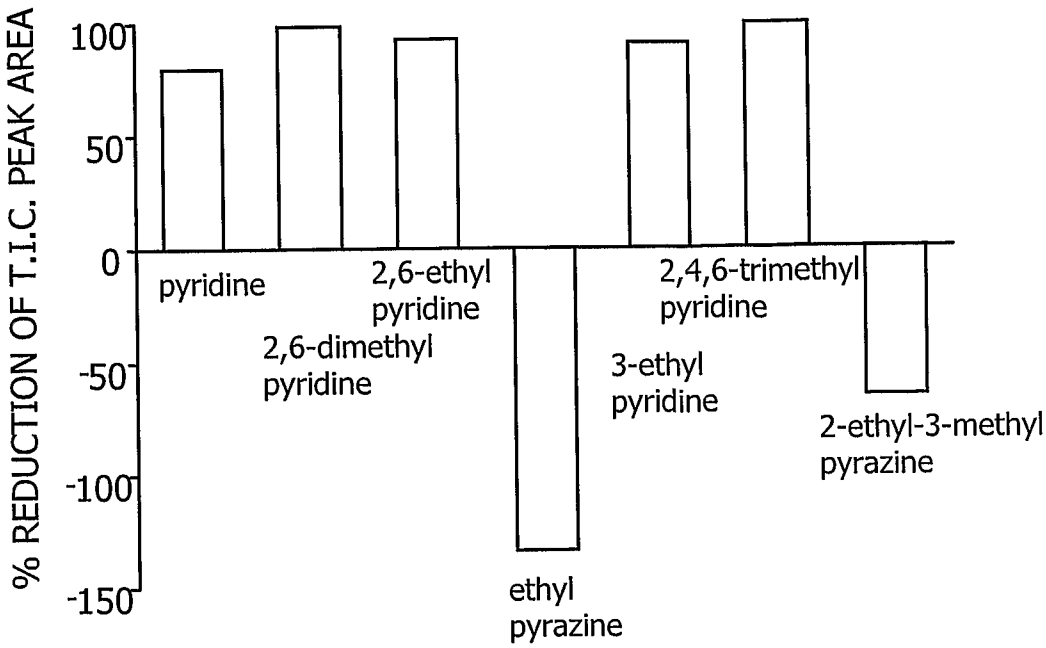

% REDUCTION OF T.I.C. PEAK AREA
100
50
0
-50
-100
-150
pyridine
2,6-dimethyl pyridine
2,6-ethyl pyridine
ethyl pyrazine
3-ethyl pyridine
2,4,6-trimethyl pyridine
2-ethyl-3-methyl pyrazine

FIG. 5

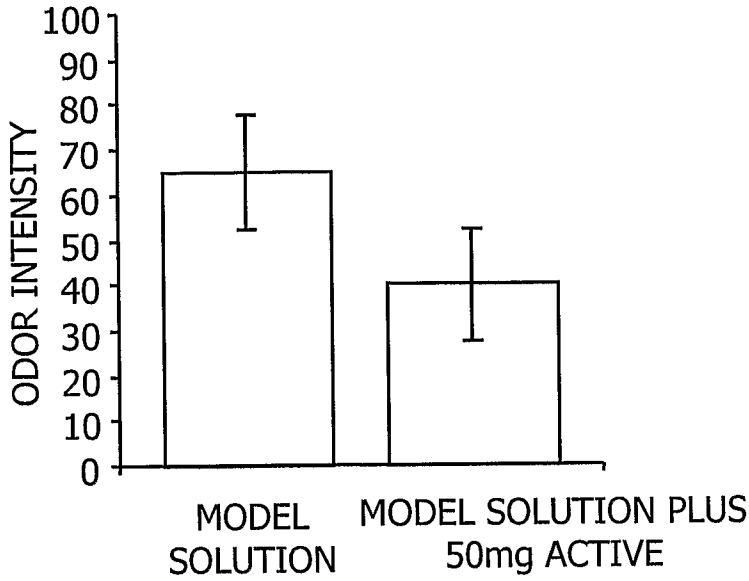

ODOR INTENSITY
100
90
80
70
60
50
40
30
20
10
0
MODEL SOLUTION
MODEL SOLUTION PLUS 50mg ACTIVE

FIG. 6

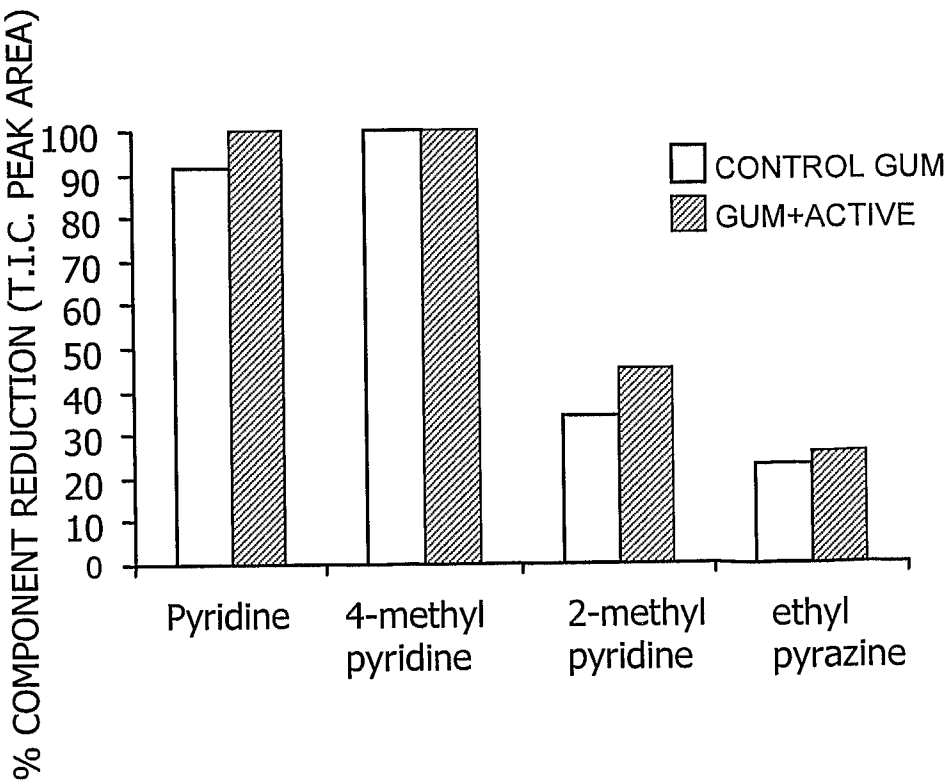

% COMPONENT REDUCTION (T.I.C. PEAK AREA)
100
90
80
70
60
50
40
30
20
10
0
CONTROL GUM
GUM+ACTIVE
Pyridine
4-methyl pyridine
2-methyl pyridine
ethyl pyrazine

FIG. 7

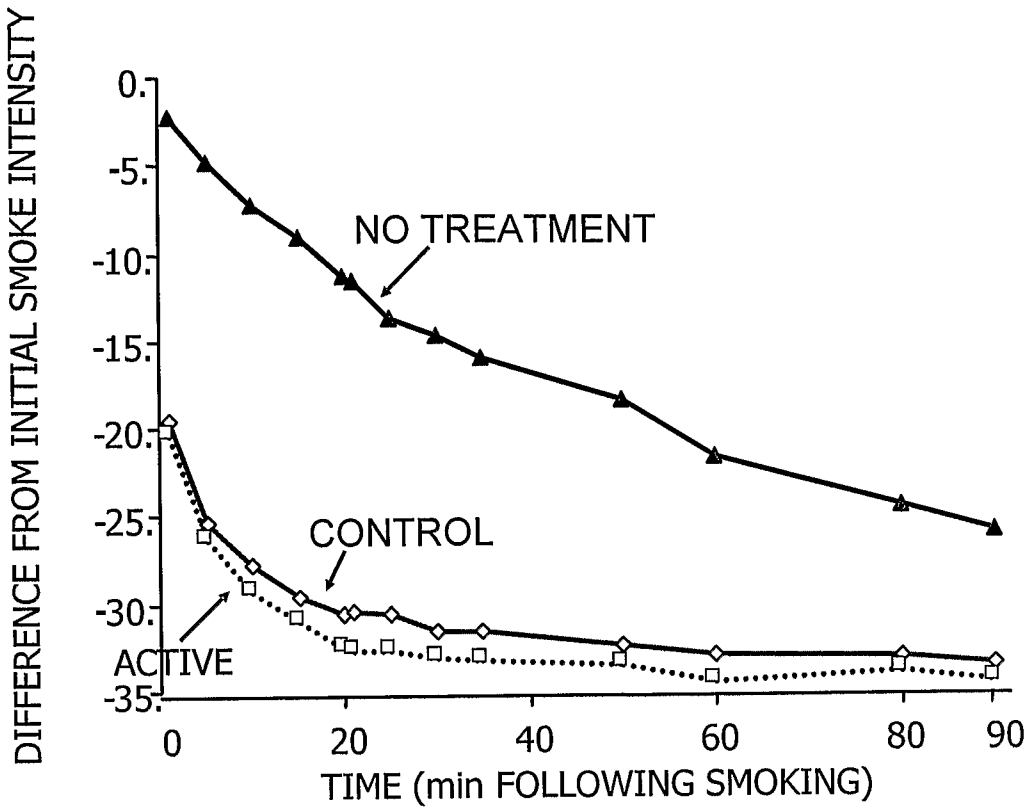

DIFFERENCE FROM INITIAL SMOKE INTENSITY
0
-5
-10
-15
-20
-25
-30
-35
NO TREATMENT
CONTROL
ACTIVE
0
20
40
60
80
90
TIME (min FOLLOWING SMOKING)

FIG. 8

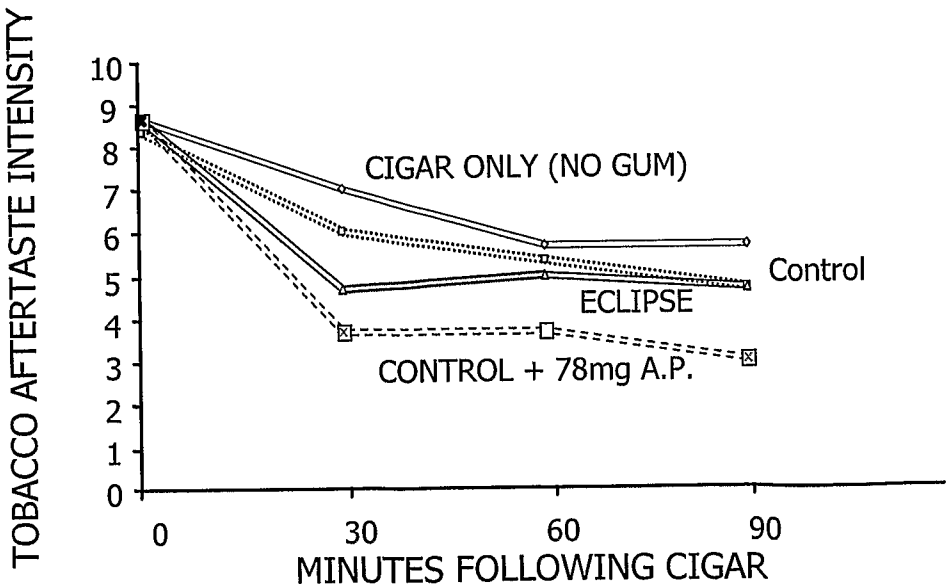

TOBACCO AFTERTASTE INTENSITY
10
9
8
7
6
5
4
3
2
1
0
CIGAR ONLY (NO GUM)
Control
ECLIPSE
CONTROL + 78mg A.P.
0
30
60
90
MINUTES FOLLOWING CIGAR

FIG. 9

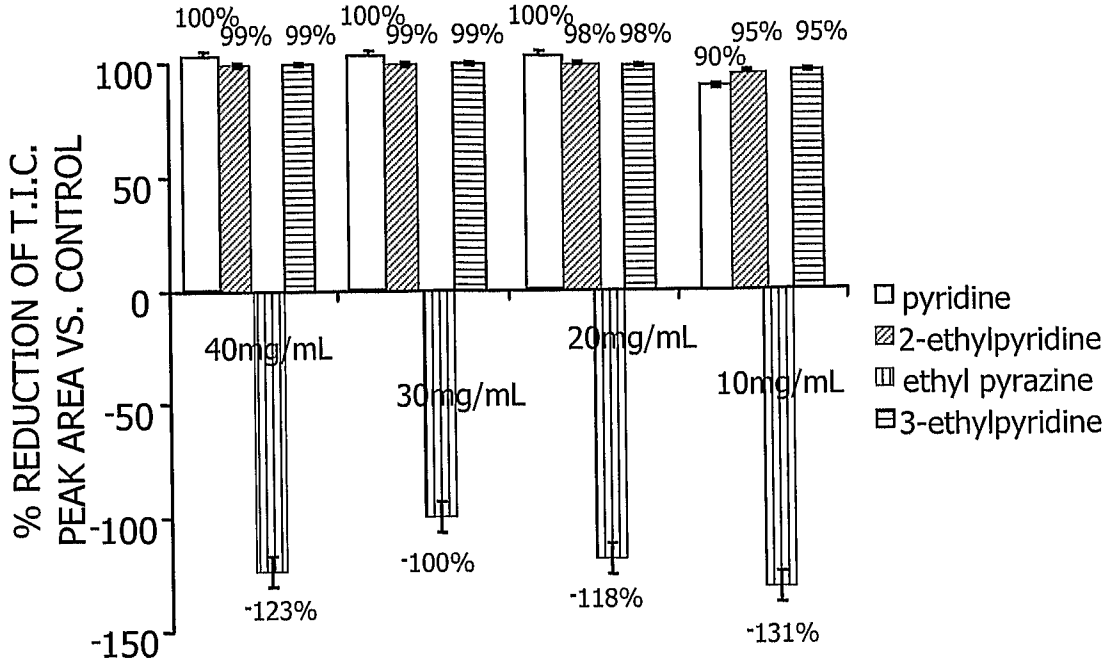

% REDUCTION OF T.I.C. PEAK AREA VS. CONTROL
100
50
0
-50
-100
-150
100% 99% 99%
100% 99% 99%
100% 98% 98%
90% 95% 95%
40mg/mL
30mg/mL
20mg/mL
10mg/mL
-123%
-100%
-118%
-131%
pyridine
2-ethylpyridine
ethyl pyrazine
3-ethylpyridine

FIG. 10

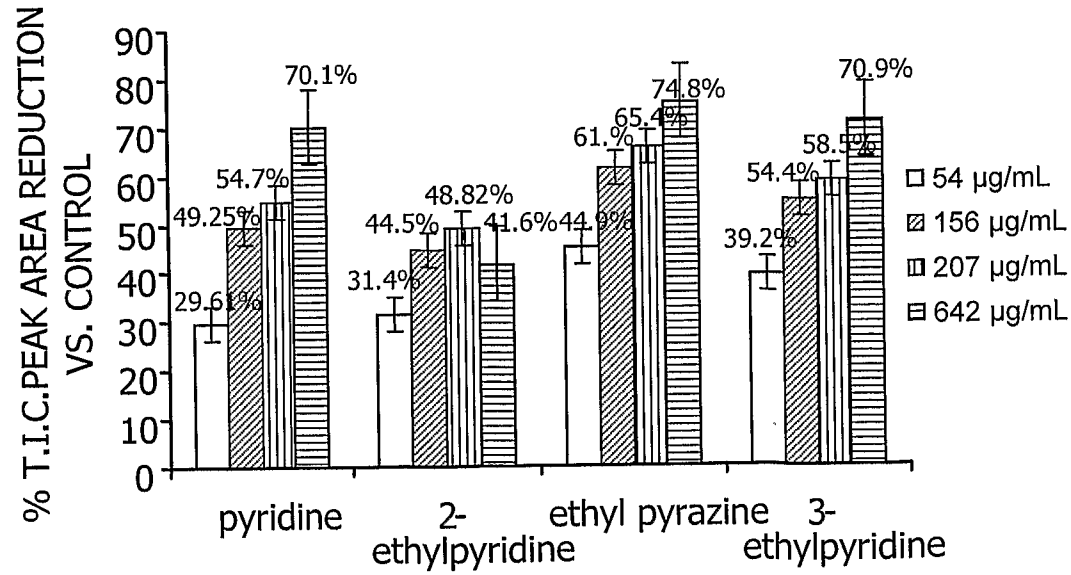
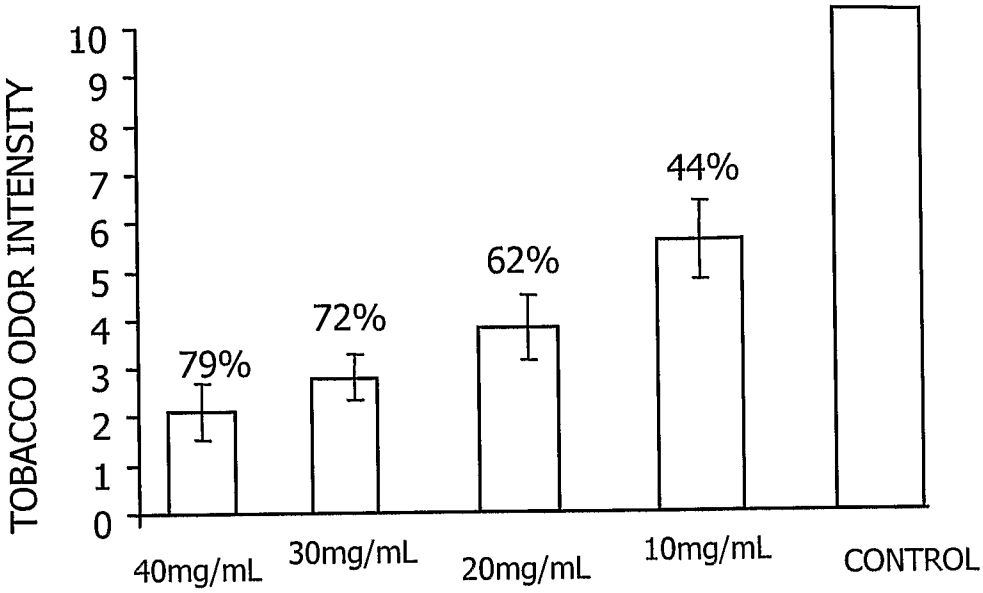
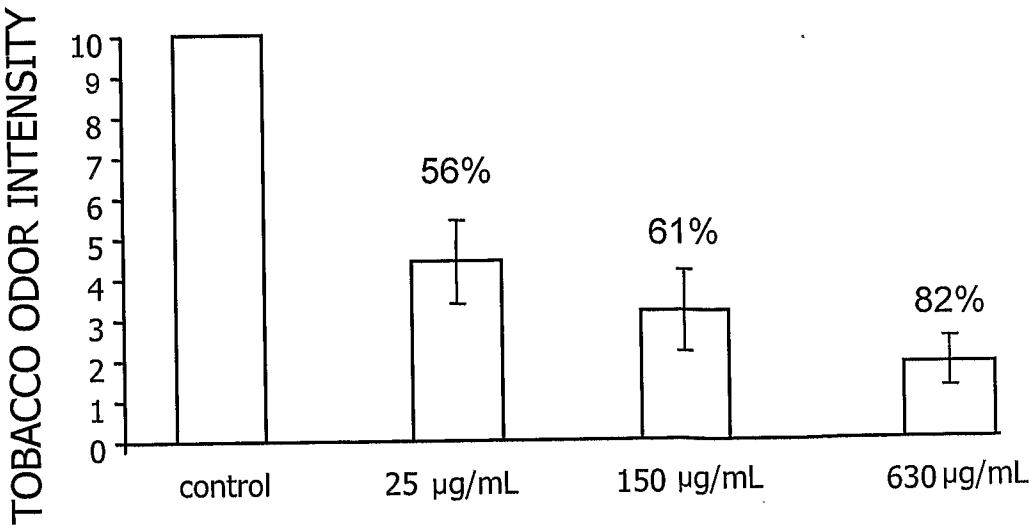

% T.I.C.PEAK AREA REDUCTION VS. CONTROL
90
80
70
60
50
40
30
20
10
0
29.6%
49.25%
54.7%
70.1%
31.4%
44.5%
48.82%
41.6%
44.9%
61.%
65.4%
74.8%
39.2%
54.4%
58.5%
70.9%
pyridine
2-ethylpyridine
ethyl pyrazine
3-ethylpyridine
54 µg/mL
156 µg/mL
207 µg/mL
642 µg/mL

FIG. 11



CRANBERRY EXTRACT ADDED TO 5mL TOBACCO SMOKE ODOR MODEL SOLUTION

FIG. 12



CARDAMOM OIL ADDED TO 5mL TOBACCO SMOKE ODOR MODEL SOLUTION

FIG. 13

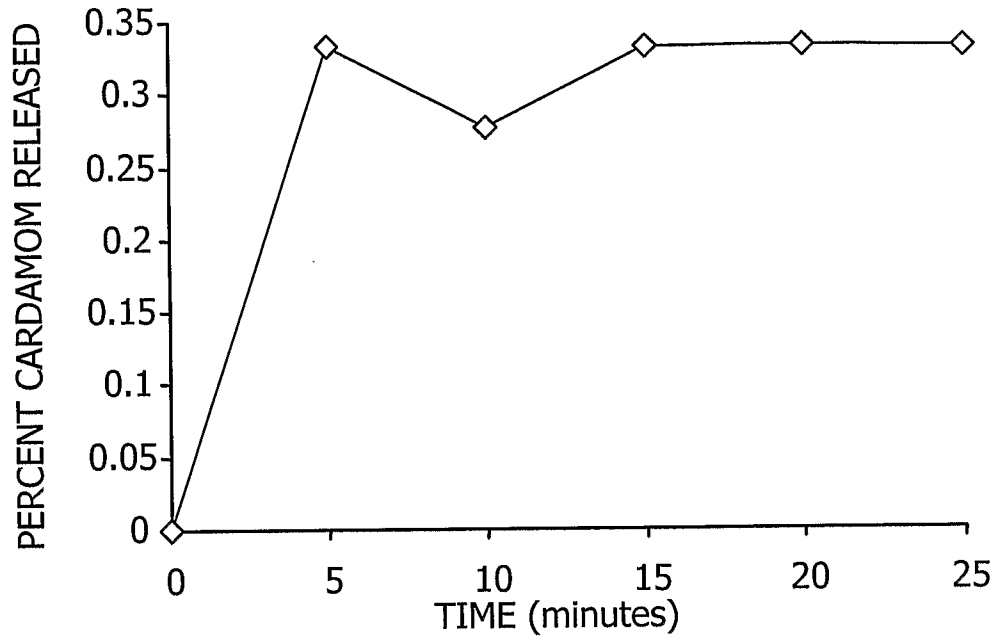
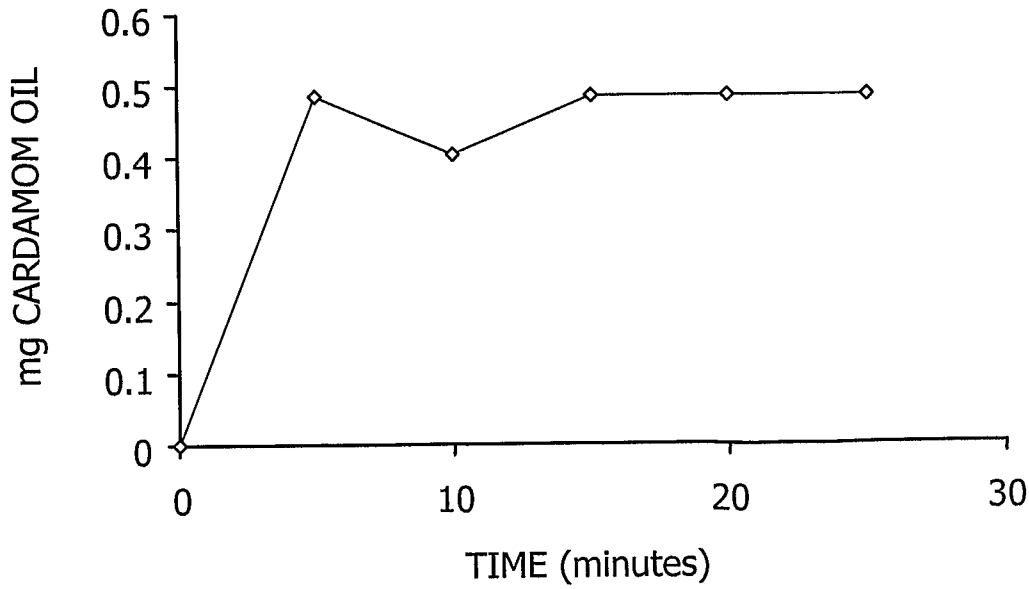

PERCENT CARDAMOM RELEASED
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
TIME (minutes)

FIG. 14


mg CARDAMOM OIL
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
TIME (minutes)