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(54) Title: METHODS OF SELECTIVELY FORMING SUBSTITUTED PYRAZINES

(57) Abstract: Methods of selectively forming substituted pyrazines are provided. Methods of the present invention can include receiving a reaction solution including at least one carbon source and at least one nitrogen source, and heating the reaction solution to a reaction temperature and holding the reaction solution at the reaction temperature for a time sufficient to produce a reaction product comprising at least one substituted pyrazine. The carbon source can be selected from the group consisting of hydroxy ketone(s), sugar(s) treated with at least one buffer, and combinations thereof. Tobacco products incorporating substituted pyrazines are also provided.



METHODS OF SELECTIVELY FORMING SUBSTITUTED PYRAZINES

FIELD OF THE INVENTION

The present invention relates to methods of forming substituted pyrazines. Of particular interest are methods of selectively forming targeted substituted pyrazines.

BACKGROUND OF THE INVENTION

5 Pyrazines are produced by reactions (e.g., Maillard) of a carbon source with nitrogenous compounds, such as amino acids and bases (e.g., diammonium phosphate (DAP), NaOH). In many conventional reaction pathways intended to produce pyrazines, a sugar (e.g., fructose, glucose, fructose/glucose mixtures, rhamnose) is used as the carbon source. To date, the vast majority of model reactions and fortified natural products which result in pyrazine-rich formulations upon heating have used
10 sugars such as fructose, glucose, fructose/glucose mixtures, and rhamnose as the carbon source components of the formulations. These sugars have been shown to serve as carbon sources for the formation of the pyrazine aromatic ring structure. *See, e.g.*, U.S. Pat. App. Pub. Nos. 2004/0173228 to Coleman, 2010/0037903 to Coleman et al., 2012/0152265 to Dube et al., 2012/0260929 to Coleman et al., 2013/0125907 to Dube et al., 2013/0337418 to Anuradha, 2015/0040922 to Dube et al., and 2015/0122271 to Chen et al.; U.S. Pat. Nos. 5,258,194 to Anderson et al., 6,298,858 to Coleman; 6,325,860 to Coleman, 6,440,223 to Dube et al., 6,499,489 to Coleman; 6,591,841 to White et al., 6,695,924 to Dube et al., 8,434,496 to Chen et al., 8,944,072 to Brinkley et al., 8,955,523 to Coleman et al., 8,991,403 to Chen et al., 9,010,339 to Dube et al., 9,254,001 to Byrd et al., 9,265,284 to Junker et al., and 9,402,415 to Coleman et al.; and Coleman III, On the synthesis and characteristics of aqueous formulations rich in pyrazines, in
15 Flavor Fragrance and Odor Analysis, Second Edition, Ray Marsili, ed., Chapter 7, pp 135-182, CRC Press, Boca Raton, 2012; each of which is herein incorporated by reference in its entirety.

Most often, these reactions with sugar(s) and a nitrogen source have employed ammonium hydroxide and/or free amino acids as nitrogen sources that furnish the nitrogen bond within the pyrazine structure. *See, e.g.*, Effect of time, temperature, and reactant ratio on pyrazine formation in model system, T. Shibamoto, R.A. Bernhard, J. Agric. Food Chem., 24, (1976) p. 847. The reaction produces a complex
25 mixture of many substituted pyrazines. When a sugar serves as the sole carbon source in the reaction to produce pyrazines, the molecules pyrazine and methylpyrazine are the dominant pyrazines produced, often much greater than 60% of the total pyrazine yield. Even when free amino acids are employed as co-reagents in an attempt to reduce the amount of pyrazine and methylpyrazine molecules produced, this trend is
30 evident.

From a sensory perspective, the molecules pyrazine and methylpyrazine possess neither desirable sensory notes nor acceptable volatility characteristics for use in tobacco products. Thus, their presence in a mixture of pyrazines is a less than desirable characteristic for certain applications.

As such, it would be desirable to provide methods for selectively producing certain desirable substituted pyrazines in greater amounts.

SUMMARY OF THE INVENTION

5 The present invention provides methods of selectively forming certain substituted pyrazines. A method of selectively producing pyrazines can comprise receiving a reaction solution comprising at least one tobacco-derived carbon source (e.g., a hydroxy ketone and/or at least one sugar treated with a buffer) and at least one tobacco-derived nitrogen source (e.g., a protein and/or an amino acid), and heating the reaction solution to a reaction temperature and holding the reaction solution at the reaction temperature for a
10 time sufficient to produce a reaction product comprising at least one substituted pyrazine. The nitrogen source can be selected from the group consisting of amino acids, ammonium ions, and combinations thereof.

In various embodiments, the at least one substituted pyrazine can be selected from the group consisting of 2,6-dimethylpyrazine; 2,5-dimethylpyrazine; 2-ethyl-5-methylpyrazine; 2-ethyl-6-methylpyrazine; 2,3,5-trimethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; 2-ethyl-2,5-dimethylpyrazine;
15 2,3,5,6-trimethylpyrazine; 2,3,5-trimethyl-6-ethylpyrazine; 2,6-dimethyl-3-propylpyrazine; 2,5-diethyl-3,6-dimethylpyrazine; 2,6-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(3-methylbutyl)pyrazine; 2,5-dimethyl-3-propylpyrazine; 2,5-dimethyl-3-cis-propenylpyrazine; 2-isopropenyl-3,6-dimethylpyrazine; 2-(2-methylpropyl)-3,5-dimethylpyrazine; 2,6-dimethyl-3-isobutylpyrazine; 2-(2-methylpropyl)-3,5,6-trimethylpyrazine, 2,3-dimethylpyrazine; trimethylpyrazine;
20 furaneol, 2,5-dimethyl-3-ethylpyrazine; tetramethylpyrazine; 2,3-diethyl-5-methylpyrazine; 2,5-dimethyl-3-propenylpyrazine; 2,3,5-trimethyl-6-isopropylpyrazine; 2-acetyl-4,5-dimethylpyrazine; 3,5-dimethyl-2-methylpropylpyrazine; 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-Methyl-2,n-propyl-6(1-butenyl)pyrazine; 2,5,7-trimethyl-6,7-dihydro-5H-cyclopentapyrazine; and 2,5-
25 dimethyl-3-ethylpyrazine; and combinations thereof.

In some embodiments, the at least one substituted pyrazine is disubstituted, trisubstituted, or tetrasubstituted. In certain embodiments, the at least one substituted pyrazine comprises at least one substituent group having 2 or more carbon atoms. In various embodiments, the at least one substituted pyrazine comprises at least one substituent group having 3 or more carbon atoms.

30 In various embodiments, the method of selectively forming certain substituted pyrazines can further comprise isolating the at least one substituted pyrazine from the reaction product. The step of isolating the at least one tobacco-derived pyrazine from the reaction product can comprise at least one of liquid-liquid extraction of the reaction product, liquid-solid extraction of the reaction product, and simple distillation of the reaction product, for example.

35 The methods of the present invention can further comprise incorporating at least one substituted pyrazine into a tobacco product. In certain embodiments, the tobacco product can be a smoking article. In some embodiments, the tobacco product can be a smokeless tobacco product.

In various embodiments of the present invention, a method of forming pyrazines is provided, the method comprising receiving a reactant solution comprising at least one alpha-hydroxy ketone and at least one nitrogen source, and heating the reactant solution to a reactant temperature and holding the reactant solution at the reactant temperature for a time sufficient to produce a reactant product comprising at least one substituted pyrazine. Various embodiments of the method can further comprise isolating the at least one substituted pyrazine from the reactant product. In certain embodiments, the at least one hydroxy ketone can comprise acetol and the at least one substituted pyrazine can be selected from the group consisting of: 2,3-dimethylpyrazine; 2,6-dimethylpyrazine; 2-ethyl-6-methylpyrazine; 2-ethyl-5-methylpyrazine; trimethylpyrazine; furaneol; 2,5-dimethyl-3-ethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; tetramethylpyrazine; 2,5-dimethyl-3-propenylpyrazine; 2,3,5-trimethyl-6-isopropylpyrazine; 2-acetyl-4,5-dimethylpyrazine; 3,5-dimethyl-2-methylpropylpyrazine, and combinations thereof. In some embodiments, the at least one hydroxy ketone can comprise acetoin and the at least one substituted pyrazine can be tetramethylpyrazine. In various embodiments, the at least one hydroxy ketone can comprise 1-hydroxy-2-butanone and the at least one substituted pyrazine can be selected from the group consisting of: 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-Methyl-2,n-propyl-6(1-butenyl)pyrazine; 2,5-dimethyl-3-ethylpyrazine; and combinations thereof.

In various embodiments of the present invention, the method can further include adding free amino acids to the reaction solution comprising at least one alpha-hydroxy ketone and at least one nitrogen source. In some embodiments, the method can further include adding at least one aldehyde to the reaction solution comprising at least one alpha-hydroxy ketone and at least one nitrogen source.

In various embodiments of the present invention, a method of forming pyrazines is provided, the method comprising receiving a carbon source solution comprising at least one sugar and at least one buffer such that an optimized amount of at least one hydroxy ketone is provided from the at least one sugar, mixing the carbon source solution with at least nitrogen source to form a reaction solution, and heating the reaction solution to a reaction temperature and holding the reaction solution at the reaction temperature for a time sufficient to produce a reaction product comprising at least one substituted pyrazine. Various embodiments of the methods herein can further comprise isolating the at least one substituted pyrazine from the reaction product. The at least one sugar can be selected from the group consisting of glucose, fructose, rhamnose, and combinations thereof. In certain embodiments, the buffer can be selected from the group consisting of: sodium hydroxide, a phosphate buffer, and combinations thereof. In certain embodiments, the buffer can buffer in a pH range of about 6.5 to about 7.5. Methods of the present invention can further comprise adding ammonium ions to the reaction solution comprising a carbon source comprising at least one sugar and at least one buffer.

The invention includes, without limitation, the following embodiments:

Embodiment 1: A method of forming pyrazines comprising: receiving a reaction solution comprising at least one alpha-hydroxy ketone and at least one nitrogen source; and heating the reaction solution to a reaction temperature and holding the reaction solution at the reaction temperature for a time sufficient to produce a reaction product comprising at least one substituted pyrazine.

5 Embodiment 2: A method of any preceding embodiment, wherein the at least one hydroxy ketone comprises acetol.

Embodiment 3: A method of any preceding embodiment, wherein the at least one substituted pyrazine is selected from the group consisting of: 2,3-dimethylpyrazine; 2,6-dimethylpyrazine; 2-ethyl-6-methylpyrazine; 2-ethyl-5-methylpyrazine; trimethylpyrazine; furaneol; 2,5-dimethyl-3-ethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; tetramethylpyrazine; 2,5-dimethyl-3-propenylpyrazine; 2,3,5-trimethyl-6-isopropylpyrazine; 2-acetyl-4,5-dimethylpyrazine; 3,5-dimethyl-2-methylpropylpyrazine, and combinations thereof.

Embodiment 4: A method of any preceding embodiment, wherein the at least one hydroxy ketone comprises acetoin.

15 Embodiment 5: A method of any preceding embodiment, wherein the at least one substituted pyrazine is tetramethylpyrazine.

Embodiment 6: A method of any preceding embodiment, wherein the at least one hydroxy ketone comprises 1-hydroxy-2-butanone.

Embodiment 7: A method of any preceding embodiment, wherein the at least one substituted pyrazine is selected from the group consisting of: 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-methyl-2,n-propyl-6(1-butenyl)pyrazine; 2,5-dimethyl-3-ethylpyrazine; and combinations thereof.

Embodiment 8: A method of any preceding embodiment, wherein the nitrogen source is selected from the group consisting of amino acids, ammonium ions, and combinations thereof.

Embodiment 9: A method of any preceding embodiment, further comprising adding free amino acids to the reaction solution.

Embodiment 10: A method of any preceding embodiment, further comprising adding at least one aldehyde to the reaction solution.

30 Embodiment 11: A method of any preceding embodiment, further comprising isolating the at least one substituted pyrazine from the reaction product.

Embodiment 12: A method of any preceding embodiment, wherein the step of isolating the at least one substituted pyrazine from the reaction product comprises at least one of liquid-liquid extraction of the reaction product, liquid-solid extraction of the reaction product, and simple distillation of the reaction product.

Embodiment 13: A method of any preceding embodiment, wherein the reaction temperature is about 90°C to about 150°C.

Embodiment 14: A method of any preceding embodiment, wherein the at least one substituted pyrazine is selected from the group consisting of: 2,6-dimethylpyrazine; 2,5-dimethylpyrazine; 2-ethyl-5-methylpyrazine; 2-ethyl-6-methylpyrazine; 2,3,5-trimethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; 2-ethyl-2,5-dimethylpyrazine; 2,3,5,6-trimethylpyrazine; 2,3,5-trimethyl-6-ethylpyrazine; 2,6-dimethyl-3-propylpyrazine; 2,5-diethyl-3,6-dimethylpyrazine; 2,6-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(3-methylbutyl)pyrazine; 2,5-dimethyl-3-propylpyrazine; 2,5-dimethyl-3-cis-propenylpyrazine; 2-isopropenyl-3,6-dimethylpyrazine; 2-(2-methylpropyl)-3,5-dimethylpyrazine; 2,6-dimethyl-3-isobutylpyrazine; 2-(2-methylpropyl)-3,5,6-trimethylpyrazine, 2,3-dimethylpyrazine; trimethylpyrazine; furaneol, 2,5-dimethyl-3-ethylpyrazine; tetramethylpyrazine; 2,3-diethyl-5-methylpyrazine; 2,5-dimethyl-3-propenylpyrazine; 2,3,5-trimethyl-6-isopropylpyrazine; 2-acetyl-4,5-dimethylpyrazine; 3,5-dimethyl-2-methylpropylpyrazine; 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-methyl-2,n-propyl-6(1-butenyl)pyrazine; 2,5,7-trimethyl-6,7-dihydro-5H-cyclopentapyrazine; and 2,5-dimethyl-3-ethylpyrazine; and combinations thereof.

Embodiment 15: A method of any preceding embodiment, wherein the at least one substituted pyrazine is disubstituted.

Embodiment 16: A method of any preceding embodiment, wherein the at least one substituted pyrazine is trisubstituted.

Embodiment 17: A method of any preceding embodiment, wherein at least one substituted pyrazine is tetrasubstituted.

Embodiment 18: A method of any preceding embodiment, wherein the at least one substituted pyrazine comprises at least one substituent group having 2 or more carbon atoms.

Embodiment 19: A method of any preceding embodiment, wherein the at least one substituted pyrazine comprises at least one substituent group having 3 or more carbon atoms.

Embodiment 20: A method of any preceding embodiment, wherein the reaction product is substantially free of the molecule pyrazine and the molecule methylpyrazine.

Embodiment 21: A method of any preceding embodiment, further comprising incorporating the at least one substituted pyrazine into a tobacco product.

Embodiment 22: A method of any preceding embodiment, further comprising incorporating the at least one substituted pyrazine into a tobacco product, wherein the tobacco product is a smoking article or a smokeless tobacco product.

Embodiment 23: A method of forming pyrazines comprising: receiving a carbon source solution comprising at least one sugar and at least one buffer; mixing the carbon source solution with at least nitrogen source to form a reaction solution; and heating the reaction solution to a reaction temperature and holding the reaction solution at the reaction temperature for a time sufficient to produce a reaction product comprising at least one substituted pyrazine.

Embodiment 24: A method of any preceding embodiment, wherein the at least one sugar is selected from the group consisting of glucose, fructose, rhamnose, and combinations thereof.

Embodiment 25: A method of any preceding embodiment, wherein the nitrogen source is selected from the group consisting of amino acids, ammonium ions, and combinations thereof.

5 Embodiment 26: A method of any preceding embodiment, wherein the buffer is selected from the group consisting of: sodium hydroxide, a phosphate buffer, and combinations thereof.

Embodiment 27: A method of any preceding embodiment, wherein the buffer buffers in a pH range of about 6.5 to about 7.5.

10 Embodiment 28: A method of any preceding embodiment, further comprising adding ammonium ions to the reaction solution.

Embodiment 29: A method of any preceding embodiment, further comprising isolating the at least one substituted pyrazine from the reaction product.

15 Embodiment 30: A method of any preceding embodiment, wherein the step of isolating the at least one substituted pyrazine from the reaction product comprises at least one of liquid-liquid extraction of the reaction product, liquid-solid extraction of the reaction product, and simple distillation of the reaction product.

Embodiment 31: A method of any preceding embodiment, wherein the reaction temperature is about 90°C to about 150°C.

20 Embodiment 32: A method of any preceding embodiment, wherein the at least one substituted pyrazine is selected from the group consisting of: 2,6-dimethylpyrazine; 2,5-dimethylpyrazine; 2-ethyl-5-methylpyrazine; 2-ethyl-6-methylpyrazine; 2,3,5-trimethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; 2-ethyl-2,5-dimethylpyrazine; 2,3,5,6-trimethylpyrazine; 2,3,5-trimethyl-6-ethylpyrazine; 2,6-dimethyl-3-propylpyrazine; 2,5-diethyl-3,6-dimethylpyrazine; 2,6-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(3-methylbutyl)pyrazine; 2,5-dimethyl-3-propylpyrazine; 2,5-
25 dimethyl-3-cis-propenylpyrazine; 2-isopropenyl-3,6-dimethylpyrazine; 2-(2-methylpropyl)-3,5-dimethylpyrazine; 2,6-dimethyl-3-isobutylpyrazine; 2-(2-methylpropyl)-3,5,6-trimethylpyrazine; 2,3-dimethylpyrazine; trimethylpyrazine; furaneol; 2,5-dimethyl-3-ethylpyrazine; tetramethylpyrazine; 2,3-diethyl-5-methylpyrazine; 2,5-dimethyl-3-propenylpyrazine; 2,3,5-trimethyl-6-isopropylpyrazine; 2-acetyl-4,5-dimethylpyrazine; 3,5-dimethyl-2-methylpropylpyrazine; 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-
30 ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-methyl-2,n-propyl-6(1-butenyl)pyrazine; 2,5,7-trimethyl-6,7-dihydro-5H-cyclopentapyrazine; and 2,5-dimethyl-3-ethylpyrazine; and combinations thereof.

Embodiment 33: A method of any preceding embodiment, wherein the at least one substituted pyrazine is disubstituted.

35 Embodiment 34: A method of any preceding embodiment, wherein the at least one substituted pyrazine is trisubstituted.

Embodiment 35: A method of any preceding embodiment, wherein at least one substituted pyrazine is tetrasubstituted.

Embodiment 36: A method of any preceding embodiment, wherein the at least one substituted pyrazine comprises at least one substituent group having 2 or more carbon atoms.

5 Embodiment 37: A method of any preceding embodiment, wherein the at least one substituted pyrazine comprises at least one substituent group having 3 or more carbon atoms.

Embodiment 38: A method of any preceding embodiment, wherein the reaction product is substantially free of the molecules pyrazine and methylpyrazine.

10 Embodiment 39: A method of any preceding embodiment, further comprising incorporating the at least one substituted pyrazine into a tobacco product.

Embodiment 40: A method of any preceding embodiment, further comprising incorporating the at least one substituted pyrazine into a tobacco product, wherein the tobacco product is a smoking article or a smokeless tobacco product.

15 These and other features, aspects, and advantages of the disclosure will be apparent from a reading of the following detailed description together with the accompanying drawings, which are briefly described below. The invention includes any combination of two, three, four, or more of the above-noted embodiments as well as combinations of any two, three, four, or more features or elements set forth in this disclosure, regardless of whether such features or elements are expressly combined in a specific embodiment description herein. This disclosure is intended to be read holistically such that any separable features or
20 elements of the disclosed invention, in any of its various aspects and embodiments, should be viewed as intended to be combinable unless the context clearly dictates otherwise.

BRIEF DESCRIPTION OF THE DRAWINGS

25 In order to provide an understanding of embodiments of the invention, reference is made to the appended drawings, which are not necessarily drawn to scale, and in which reference numerals refer to components of exemplary embodiments of the invention. The drawings are exemplary only, and should not be construed as limiting the invention.

FIG. 1 is a flow chart describing methods of selectively forming substituted pyrazines;

FIG. 2 is a flow chart describing methods of selectively forming substituted pyrazines;

30 FIG. 3 shows the GC/MS analysis of glucose reacted with a phosphate buffer at 140°C for 60 min and extracted with dichloromethane (DCM);

FIG. 4 shows the GC/MS analysis of pyrazines extracted using DCM from a reaction mixture of (1 gram of glucose reacted with 25mL of 40% phosphate buffer at 140°C for 60 min) with 1 mL of NH₄OH at 140°C for 17 hours;

35 FIG. 5 shows the GC/MS analysis of pyrazines extracted using DCM from 25 mL of a reaction mixture of (0.1 N NaOH reacted with 0.5 gram of glucose at 140°C for 60 min) reacted with 1 mL of NH₄OH at 140°C;

FIG. 6 is an exploded perspective view of a smoking article having the form of a cigarette, showing the smokable material, the wrapping material components, and the filter element of the cigarette;

FIG. 7 is a top view of a smokeless tobacco product embodiment, taken across the width of the product, showing an outer pouch filled with a tobacco material; and

5 FIG. 8 is a sectional view through an electronic smoking article comprising a cartridge and a control body and including a reservoir housing according to an example embodiment of the present disclosure.

DETAILED DESCRIPTION

The present invention now will be described more fully hereinafter. This invention may, however, 10 be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art. As used in this specification and the claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Reference to "dry weight percent" or "dry weight basis" refers to weight on the basis of dry 15 ingredients (i.e., all ingredients except water).

The present invention provides methods of forming selected pyrazines. Pyrazines display many different flavor profiles, including, but not limited to, roasted, toasted and nutty notes. For example, pyrazines with cyclopentyl derivatives are known for their positive sensory attributes at very low levels, ppb. Pyrazines are formed by heating mixtures of carbon sources and nitrogen sources. Methods of the present 20 invention not only minimize the formation of the molecules pyrazine and methylpyrazine, but also tailor the reaction so that other desired substituted pyrazines can be produced in a controlled fashion.

Selective Formation of Pyrazines Using a Carbon Source Other than Sugar(s)

Conventionally, sugar(s) have been used as the carbon source in reactions to form pyrazines. 25 Several reaction pathways are known in the art to produce pyrazine-rich formulations using sugar(s), including: 1) hydrolysis of protein into free amino acids followed by reaction of those free amino acids with sugar(s) such as glucose and/or high fructose syrup; and 2) biotechnical synthesis of free amino acids employing glucose and nitrogen (e.g., ammonium ions) followed by reaction of these free amino acids with sugar(s) such as glucose and/or high fructose syrup. When sugars are employed as an intact molecule and 30 reacted with a nitrogen source (e.g., ammonium hydroxide, amino acids), an array of pyrazines are produced, including the molecules pyrazine and methylpyrazine as the dominate pyrazines, with much lesser amounts of dimethylpyrazines, and significantly less amounts of higher molecular weight pyrazines.

As used herein, the term "the molecule pyrazine" refers to a heterocyclic organic compound with the chemical formula $C_4H_4N_2$. This is different from the general term "pyrazine(s)" used herein, which refers to 35 the group of compounds produced from the reaction of a carbon source with a nitrogen source.

As used herein, the term "nitrogen source" refers to a nitrogen containing compound that is reactive with a carbon source to form at least one pyrazine. In various embodiments, a nitrogen source can comprise

ammonium ions (NH_4^+), amino acids, proteins, or a combination thereof. In some embodiments, ammonium ions (NH_4^+) can be provided by compounds such as ammonium hydroxide, diammonium phosphate (DAP), etc. Amino acids can be derived from hydrolysis of protein, for example. In some embodiments, Amino acids can be derived from hydrolysis of tobacco-derived protein, as discussed in U.S. Pat. Appl. No.

5 15/009,199 to Dube et al. filed January 28, 2016, which is herein incorporated by reference in its entirety. Other compounds containing nitrogen that are known in the art and are reactive with a carbon source to form at least one pyrazine can also be used as nitrogen sources in embodiments of the invention disclosed herein.

In various embodiments of the present invention, the carbon source can comprise a hydroxy ketone. A hydroxy ketone is a functional group of a ketone flanked by a hydroxyl group, In two main classes of
10 hydroxy ketones, the hydroxyl group can be placed in the alpha position (i.e., an alpha-hydroxy ketone having the formula $\text{RCR}'(\text{OH})(\text{CO})\text{R}$), or in the beta position (i.e., a beta-hydroxy ketone having the formula $\text{RCR}'(\text{OH})\text{CR}_2(\text{CO})\text{R}$). The structures of alpha and beta hydroxy ketones are illustrated below.

15 In various embodiments of the present invention, the carbon source can comprise at least one alpha-hydroxy ketone. In various embodiments of the present invention, R and R' functional groups of the at least one hydroxy ketone can be H or a substituent independently selected from the group consisting of halo (e.g., Cl, F, or Br), OH, optionally substituted C1-10 alkyl, optionally substituted C1-10 alkoxy, optionally substituted
20 C2-4 alkenyl, optionally substituted C2-4 alkynyl, NR_6R_7 , NR_6COR_7 , $\text{NR}_6\text{CO}_2\text{R}_7$, $\text{CR}_6\text{R}_7\text{OR}_8$, CONR_6R_7 , CO_2R_6 , CN, CF_3 , NO_2 , N_3 , C1-3 alkylthio, R_9SO , R_9SO_2 , CF_3S , and CF_3SO_2 . In certain embodiments of the present invention, R and/or R' functional groups are a C1-6 alkyl.

The term "alkyl" as used herein means saturated straight, branched, or cyclic hydrocarbon groups (i.e., cycloalkyl groups), as well as unsaturated versions of the saturated examples (e.g., propenyl). In
25 particular embodiments, alkyl refers to groups comprising 1 to 10 carbon atoms ("C1-10 alkyl"). In further embodiments, alkyl refers to groups comprising 1 to 8 carbon atoms ("C1-8 alkyl"), 1 to 6 carbon atoms ("C1-6 alkyl"), or 1 to 4 carbon atoms ("C1-4 alkyl"). In other embodiments, alkyl refers to groups comprising 3-10 carbon atoms ("C3-10 alkyl"), 3-8 carbon atoms ("C3-8 alkyl"), or 3-6 carbon atoms ("C3-6 alkyl"). In specific embodiments, alkyl refers to methyl, trifluoromethyl, ethyl, propyl, isopropyl,
30 cyclopropyl, butyl, isobutyl, t-butyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, cyclohexylmethyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl.

“Optionally substituted” in reference to a substituent group refers to substituent groups optionally substituted with one or more moieties selected from the group consisting of, for example, halo (*e.g.*, Cl, F, Br, and I); alkyl (*e.g.*, C1-10 alkyl), halogenated alkyl (*e.g.*, CF₃, 2-Br-ethyl, CH₂F, CH₂Cl, CH₂CF₃, or CF₂CF₃); C2-4 alkenyl, C2-4 alkynyl; hydroxyl; amino; amido; carboxylate; carboxamido; carbamate; carbonate; urea; acetate; alkylamino; arylamino; C1-10 alkoxy; aryl; aralkyl, aryloxy; nitro; azido; cyano; thio; alkylthio; sulfonate; sulfide; sulfinyl; sulfo; sulfate; sulfoxide; sulfamide; sulfonamide; phosphonic acid; phosphate; and/or phosphonate.

The term “alkenyl” as used herein means alkyl moieties wherein at least one saturated C–C bond is replaced by a double bond. In particular embodiments, alkenyl refers to groups comprising 2 to 10 carbon atoms (“C2-10 alkenyl”). In further embodiments, alkenyl refers to groups comprising 2 to 8 carbon atoms (“C2-8 alkenyl”), 2 to 6 carbon atoms (“C2-6 alkenyl”), or 2 to 4 carbon atoms (“C2-4 alkenyl”). In specific embodiments, alkenyl can be vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, or 5-hexenyl.

The term “alkynyl” as used herein means alkyl moieties wherein at least one saturated C–C bond is replaced by a triple bond. In particular embodiments, alkynyl refers to groups comprising 2 to 10 carbon atoms (“C2-10 alkynyl”). In further embodiments, alkynyl refers to groups comprising 2 to 8 carbon atoms (“C2-8 alkynyl”), 2 to 6 carbon atoms (“C2-6 alkynyl”), or 2 to 4 carbon atoms (“C2-4 alkynyl”). In specific embodiments, alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butyne, 2-butyne, 3-butyne, 1-pentyne, 2-pentyne, 3-pentyne, 4-pentyne, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl.

The term “alkoxy” as used herein means straight or branched chain alkyl groups linked by an oxygen atom (*i.e.*, –O-alkyl), wherein alkyl is as described above. In particular embodiments, alkoxy refers to oxygen-linked groups comprising 1 to 10 carbon atoms (“C1-10 alkoxy”). In further embodiments, alkoxy refers to oxygen-linked groups comprising 1 to 8 carbon atoms (“C1-8 alkoxy”), 1 to 6 carbon atoms (“C1-6 alkoxy”), 1 to 4 carbon atoms (“C1-4 alkoxy”) or 1 to 3 carbon atoms (“C1-3 alkoxy”).

The term “halo” or “halogen” as used herein means fluorine, chlorine, bromine, or iodine.

The term “amino” as used herein means a moiety represented by the structure NR₂, and includes primary amines, and secondary and tertiary amines substituted by alkyl or aryl (*i.e.*, alkylamino or arylamino, respectively). Thus, R₂ may represent two hydrogen atoms, two alkyl moieties, two aryl moieties, one aryl moiety and one alkyl moiety, one hydrogen atom and one alkyl moiety, or one hydrogen atom and one aryl moiety.

Alkyl (amino) is a moiety represented by the structure -RNR₂ and includes an alkyl group as defined above attached to an amino group as defined above, wherein the moiety is attached to another portion of a molecule via the alkyl group.

The term “cycloalkyl” means a non-aromatic, monocyclic or polycyclic ring comprising carbon and hydrogen atoms.

The term “derivative” as used herein means a compound that is formed from a similar, beginning compound by attaching another molecule or atom to the beginning compound. Further, derivatives, according to the invention, encompass one or more compounds formed from a precursor compound through addition of one or more atoms or molecules or through combining two or more precursor compounds.

5 In one embodiment of the present invention, acetoin has been successfully used as the precursor to make tetramethylpyrazine (TMP). Specifically, acetoin has successfully been reacted with ammonium hydroxide and phosphoric acid (or diammonium phosphate) to yield tetramethylpyrazine (TMP) in essentially quantitative yields (>80% yield) with an accompanying significant degree of purity. The reaction between acetoin and ammonium hydroxide produces almost exclusively TMP with little to no detectable
10 amounts of the molecules pyrazine and methylpyrazine. As used herein, the terms “little to no detectable amounts”, “substantially no”, and “substantially zero” are used to indicate that the identified compound is present in an amount of less than 1.0% by weight, less than 0.5% by weight, or less than 0.1% by weight, based on the total weight of a reaction product.

Without being limited by theory, the different resulting pyrazines synthesized from the reactions
15 with sugar(s) versus acetoin as the carbon source, surprisingly indicate that the carbon source dictates, to a significant degree, the distribution of pyrazines in reactions between carbon sources and nitrogen sources such as ammonium ions and/or amino acids. In particular, it was surprisingly discovered that using different hydroxy ketones as the carbon source in the reaction to form pyrazines can produce an array of specific substituted pyrazines in a controlled fashion.

20 In certain embodiments, at least one substituted pyrazine produced according to methods described herein is disubstituted. In some embodiments, at least one substituted pyrazine produced according to methods described herein is trisubstituted. In various embodiments, at least one substituted pyrazine produced according to methods described herein is tetrasubstituted. In various embodiments, at least one substituted pyrazine produced according to methods of the present invention comprises at least one
25 substituent group having 2 or more carbon atoms. In certain embodiments, at least one substituted pyrazine produced according to methods of the present invention comprises at least one substituent group having 3 or more carbon atoms.

In some embodiments, at least one substituted pyrazine produced according to methods described herein is a branched pyrazine. As used herein, the term “branched pyrazine” refers to the inclusion of alkyl
30 groups on the pyrazine ring that are not linear in nature. For example, isobutyl, sec-butyl, and *tert*-butyl groups instead of the n-butyl group (this similarly applies to propyl and pentyl groups). As described in more detail below, it was surprisingly discovered that by varying the alpha hydroxy ketone, the distribution of the pyrazines produced can be dictated. For example, use of acetoin in the reaction yields only tetramethylpyrazine (about 99.5% or greater pure). Use of acetol in the reaction yields mostly
35 dimethylsubstituted pyrazines (about 95% or greater of the yield of pyrazines).

As illustrated in FIG. 1, for example, heated formulations comprising nitrogen sources and at least one hydroxy ketone can produce pyrazines. Pyrazine rich solutions can be prepared in various ways. For

example, one method can involve microwave heat treatment of a solution comprising at least one amino acid and at least one hydroxy ketone. As illustrated at operation 100 of FIG. 1, for example, an aqueous reaction solution comprising at least one amino acid and at least one hydroxy ketone can be formed. As illustrated at operation 104 of FIG. 1, for example, the reaction solution can be heated to a reaction temperature and held at the reaction temperature for a reaction time which is sufficient to allow the reactions to undergo a reaction to form pyrazines. As illustrated at operation 106 of FIG. 1, for example, pyrazines can then be optionally isolated from the reaction product using simple distillation or other separation techniques known in the art.

In one embodiment, pyrazines are isolated from the reactant product by first using simple distillation to provide a distillate comprising mostly water and pyrazines. This distillate can then be subjected to liquid liquid extraction with cyclohexane. The amount of cyclohexane used in the liquid liquid extraction can be equal to about half the amount of the distillate used. For example, if 10L of distillate is used, 5L of cyclohexane can be used. The liquid liquid extraction of the distillate with cyclohexane can be repeated multiple times. In some embodiments, the liquid liquid extraction of the distillate with cyclohexane can be repeated at least 5 times. Following the liquid liquid extraction, the cyclohexane containing the extracted pyrazines can be dried with any drying agent known in the art. For example, sodium sulfate, magnesium hydroxide, and/or molecular sieves can be used to dry the cyclohexane. After drying, the pyrazines can be isolated (i.e., the cyclohexane can be removed) by simple distillation and/or rotary evaporation.

As discussed above, using different hydroxy ketones as the carbon source in the reaction to form pyrazines can produce an array of specific substituted pyrazines. In certain embodiments, the carbon source comprises acetoin. As discussed in more detail in Example 1 below, for example, when acetoin serves as the sole carbon source in reactions with a nitrogen source (e.g., ammonium hydroxide (NH₄OH) and phosphoric acid (H₃PO₄), diammonium phosphate, etc.) to make pyrazines, the only pyrazine produced is tetramethylpyrazine. Furthermore, branched pyrazines, such as isopropylpyrazine, are not synthesized via the addition of amino acids (such as, for example, leucine or free amino acids from hydrolyzed F1 protein) to a reaction which contains acetoin, NH₄OH and H₃PO₄. Heating the reaction at higher temperatures or for longer periods of time does not change the result (i.e., that tetramethylpyrazine (TMP) is the sole pyrazine synthesized from acetoin). Hence, carbon sources other than acetoin can be synthesized if pyrazines other than TMP are desired from this synthetic approach.

In some embodiments, the carbon source can comprise 1-hydroxyacetone (also referred to as 1-OH-acetone, acetol, or 1-hydroxy-2-propanone). Acetol, an alpha-hydroxy ketone, is the simplest hydroxy ketone. As discussed in more detail below, acetol can be produced by the degradation of various sugars. For example, it can be formed as an intermediate in a Maillard reaction (i.e., reaction of sugar(s) and amino acid(s) to form pyrazines), and then the acetol can react further to form other compounds. In some embodiments of the present invention, the carbon source can comprise 1-hydroxy-2-butanone. The structures of 1-OH-acetone and 1-OH-2-butanone appear below. It is noted that for 1-OH-acetone, a methyl group is bound to one side of the carbonyl and for 1-OH-2-butanone, an ethyl group is bound to one side of the carbonyl. Without being limited by theory,

these structural features of the two α,β -hydroxyketones can dictate the structure of the resulting pyrazines.

1-hydroxyacetone
(Acetol)

1-hydroxy-2-butanone

5

As illustrated in Example 2 below, for example, reaction of 1-hydroxyacetone as a carbon source and NH_4OH as a source of base and nitrogen can produce an array of pyrazines. When 1-hydroxyacetone (acetol) serves as the carbon source, an array of specific alkyl substituted pyrazines can be produced. For example, exemplary pyrazines provided from the reaction of 1-hydroxyacetone with NH_4OH can include:

10 2,6-dimethylpyrazine; 2,5-dimethylpyrazine; 2-ethyl-5-methylpyrazine; 2-ethyl-6-methylpyrazine; 2,3,5-trimethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; 2-ethyl-2,5-dimethylpyrazine; 2,3,5,6-trimethylpyrazine; 2,3,5-trimethyl-6-ethylpyrazine; 2,6-dimethyl-3-propylpyrazine; 2,5-diethyl-3,6-dimethylpyrazine; 2,6-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(3-methylbutyl)pyrazine; 2,5-dimethyl-3-propylpyrazine; 2,5-dimethyl-3-cis-propenylpyrazine; 2-isopropenyl-
15 3,6-dimethylpyrazine; 2-(2-methylpropyl)-3,5-dimethylpyrazine; 2,6-dimethyl-3-isobutylpyrazine; 2-(2-methylpropyl)-3,5,6-trimethylpyrazine; 2,3-dimethylpyrazine; trimethylpyrazine; furaneol, 2,5-dimethyl-3-ethylpyrazine; tetramethylpyrazine; 2,3-diethyl-5-methylpyrazine; 2,5-dimethyl-3-propenylpyrazine; 2,3,5-trimethyl-6-isopropylpyrazine; 2-acetyl-4,5-dimethylpyrazine; and 3,5-dimethyl-2-methylpropylpyrazine.

When 1-hydroxy-2-butanone is used as the sole carbon source, pyrazines having ethyl groups
20 attached can be produced. For example, as illustrated in Example 4 below, pyrazines synthesized from a reaction using 1-OH-2 Butanone and NH_4OH can include 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-methyl-2,n-propyl-6(1-butenyl)pyrazine; and 2,5-dimethyl-3-ethylpyrazine.

When 1-hydroxyacetone (acetol), 2-hydroxy-3-butanone (acetoin), and 1-hydroxy-2-butanone are
25 used as carbons sources in reactions with nitrogen sources, the pyrazine and methylpyrazine molecules do not appear in the array of structures and pyrazines produced. It is noted that other hydroxy ketones can be used to produce alternative arrays of pyrazine. Using different hydroxy ketones as the carbon source in a

reaction to produce pyrazines can not only minimize the formation of pyrazine and methylpyrazine, but also allows one to tailor the reaction so that other desired substituted pyrazines are produced in a controlled fashion.

As illustrated in FIG. 1 at operation 102 for example, amino acids and/or aldehydes can optionally be added to the reaction solution comprising at least one hydroxy ketone and a nitrogen source. As illustrated in Example 3 below, for example, addition of amino acids or selected aldehyde(s) can increase not only the number of synthesized pyrazines, but also can increase the yield of pyrazines. For example, in certain embodiments the reaction solution can comprise an amino acid selected from the group consisting of serine, alanine, leucine, isoleucine, valine, threonine, phenylalanine, and combinations thereof. Similarly, any alkyl aldehyde can be employed to increase the number of synthesized pyrazines as well as the yield of pyrazines. For example, in certain embodiments the reaction solution can comprise an alkyl aldehyde selected from the group consisting of acetaldehyde, propanal, isopropanol, butanal, isobutanal, sec butanal, and combinations thereof.

Increased reaction time and temperature can produce increased pyrazines yield up to the point where a black tar substance is produced. Reaction temperature can be about 30°C or greater, about 90°C or greater, about 100°C or greater, about 120°C or greater, or about 140°C or greater for example. In some embodiments, the reaction temperature can be about 90°C to about 150°C, or about 120°C to about 140°C. Reaction time can be about 4 hours or greater, 8 hours or greater, about 12 hours or greater, 16 hours or greater, or about 24 hours or greater, for example. In various embodiments, the reaction time can be about 4 to about 24, or about 12 to about 20 hours. In certain embodiments, the reaction time can be about 16 hours.

In various embodiments, the molar ratio of the hydroxy ketone to the nitrogen source can affect the yield of pyrazines. The molar ratio of hydroxy ketone: nitrogen source (e.g., NH₄OH) can be about 1:0.5, about 1:1, about 1:2, or about 1:2.5, for example. In certain embodiments, the ratio of hydroxy ketone to nitrogen source can be between about 1:0.5 to about 1:2.5, or between about 1:1 to about 1:2. In certain embodiments, the ratio of hydroxy ketone to nitrogen source can be about 1:2.

Increasing the pH of the reaction solution can also result in increased amounts of pyrazines. A favored pH range can be about 7.5 to about 10.5, or about 8.5 to about 9.5. In some embodiments, the pH of the reaction solution can be about 8.0 or greater, about 8.5 or greater, about 9.0 or greater or about 10.0 or greater. A small addition of NaOH or KOH, for example, can be used to increase the pH of the reaction solution.

Selective Formation of Pyrazines Using Sugar(s) as the Carbon Source

In various embodiments of the present invention, the selective formation of substituted pyrazines has been optimized using at least one sugar (e.g., glucose, high fructose tobacco syrup (HFTS)) as the carbon source and ammonium ions, protein and/or amino acids as the nitrogen source. As discussed above, various reaction pathways to pyrazine-rich formulations have been previously used to make pyrazines using a sugar as a carbon source. *See, e.g.*, U.S. Pat. App. No. 15/009,199 to Dube et al., filed January 28, 2016,

which is herein incorporated by reference in its entirety. However, when sugars are used as the sole source in the reaction with a nitrogen source (e.g., ammonium hydroxide) to produce pyrazines, the molecules pyrazine and methylpyrazine can be the dominant pyrazines produced. Even when free amino acids are employed as co-reagents, this trend is evident. It was surprisingly discovered that by adjusting the pH of the carbon source before introducing it to the nitrogen source, substituted pyrazines can be selectively produced.

As illustrated in FIG. 2, for example, heated formulations comprising amino acids and sugars can produce pyrazines. *See, e.g.*, U.S. Pat. Pub. No. 2010/0037903 to Coleman III et al.; and Coleman III, On the synthesis and characteristics of aqueous formulations rich in pyrazines, in Flavor Fragrance and Odor Analysis, Second Edition, Ray Marsili, ed., Chapter 7, pp 135-182, CRC Press, Boca Raton, 2012; which are herein incorporated by reference in their entireties. Pyrazine rich solutions can be prepared in various ways. For example, one method can involve microwave heat treatment of a solution comprising at least one amino acid and at least one sugar. As illustrated at operation 120 of FIG. 2, for example, an aqueous reaction solution comprising at least one amino acid and at least one sugar can be formed. As illustrated at operation 124 of FIG. 1, for example, the reaction solution can be heated to a reaction temperature and held at the reaction temperature for a reaction time which is sufficient to allow the reactions to undergo a reaction to form pyrazines.

As discussed above, acetol, a hydroxy ketone, can be produced by the degradation of various sugars. Furthermore, when used as a carbon source in reactions with a nitrogen source, an array of substituted pyrazines can be produced, which do not include the molecules pyrazine and methylpyrazine. Accordingly, acetol can be a key intermediate in the reactions between sugar(s) and free amino acids and/or ammonium ions to make pyrazines containing branched alkyl side chains.

Literature indicates that 1-hydroxy-2-propanone (acetol) can be produced from a C6 sugar such as glucose and a derivative of glucose, sorbitol. *See, e.g.*, M. H. Mohamad, et.al., "A review of acetol: application and production", Amer. J. Appl. Sci., 8, 1135-1139 (2011); M. A. Dasari, "Catalytic conversion of glycerol and sugar alcohols to value added products", Univ. Missouri-Columbia, ISBN-10, 0549727582, pp, 264; W. Yan, "Gas phase conversion of sugars to C3 chemicals, PhD Thesis, University of Missouri-Columbia, 2008; J. Hayami, Mechanism of acetol formation", Bull. Chem. Soc. Japan, 34, 927-932(1961); P. F. Shaw, et.al., "Base catalyzed fructose degradation", J. Agric. Food Chem., 16, 979-982(1968); H. Weenen and W. Apeldoorn, "Carbohydrate Cleavage in the Maillard Reaction", Flavor Science, Recent Developments, A. Taylor and D. Mottran, eds., Royal Society of Chemistry, Special Publication # 197, Cambridge, 1996; each of which are herein incorporated by reference in their entireties. These references report on the conversion of sugars to 1-hydroxy-2-propanone using phosphate buffers at elevated temperatures, a strong base such as NaOH at pH 11.5 while under reflux, Ni and Palladium catalysts under hydrogen pressure, and copper chromite catalysts in the gas phase of heterogeneous reactions. Copper-chromite has been considered to be the best catalyst. For most of the reactions, the conversion of substrates was greater than 91%. The yield of acetol when they used glycerol as carbon source was 32.2 % at 220°C. When sorbitol was used as a carbon source, the highest yield was 11.8% at 280°C and when glucose was used as a carbon source, the highest yield of acetol was 8.99% at 280°C.

Cellulose has been converted to acetol with a 30% yield using a Sn based catalyst system. *See, e.g.,* F. Chambon, et.al., Process for transformation of lignocellulose biomass or cellulose by catalysts based on Sn oxide and/or Sb oxide and a metal that is selected from Groups 8 to 11, US Patent Application, US2013/028174A1, 2013, which is herein incorporated by reference in its entirety.

5 Recently Novotny et al., (Czech J. Food Sci., 25, 119-130, 2007, which is herein incorporated by reference) synthesized α -hydroxycarbonyl and α -dicarbonyl compounds via the degradation of monosaccharides. They used three different models comprised of an aqueous solution of potassium peroxodisulfate, an alkaline solution of potassium peroxodisulfate, and a solution of sodium hydroxide, respectively. A total of six α -hydroxycarbonyl and six α -dicarbonyl compounds were identified via
10 GC/MS. The maximum yield of α -hydroxycarbonyl (glycolaldehyde, acetol, lactaldehyde, glyceraldehyde, 1,3-dihydroxyacetone and acetoin) when glucose or fructose reacted with sodium hydroxide was approximately 4%. The yield was much lower in the aqueous solutions of potassium peroxodisulfate (0.32%) and alkaline solution of potassium peroxodisulfate (1.1%). Acetol and 1,3-dihydroxyacetone had the highest yield (2.52 and 1.02 %, respectively) when sodium hydroxide was used.

15 As illustrated in Example 6 below, alpha hydroxy ketones (e.g., acetol) can be produced from sugar to ultimately be used as the carbon source in sugar ammonia reactions. The acetol can be isolated via both distillation and column chromatography and then used as a carbon source in the reactions described herein. However, both techniques are time consuming (distillation) and expensive (chromatography). As such, it can be preferable to avoiding having to isolate hydroxy ketones derived from a sugar source before using
20 these hydroxy ketones in reactions with a nitrogen source to produce pyrazines.

 As illustrated in Example 7 below, for example, it has been surprisingly discovered that substituted pyrazines can be selectively formed from a sugar carbon source without first isolating a hydroxy ketone (e.g., acetol, acetoin, etc.) formed from the degradation of the sugar(s). As illustrated at operation 110 of FIG. 2, for example, by treating the sugar(s) with a buffer before combining the sugar(s) with the nitrogen
25 source, a different array of pyrazines can be produced than the array of pyrazines produced from the reaction of sugar(s) that were not pre-treated with a buffer and a nitrogen source. The reactions can be optimized such that the maximum amount of acetol and acetol-like compounds are produced from the sugar carbon source. Without being limited by theory, the role of the buffer is to control the pH so that the maximum amount of hydroxy ketone(s) (e.g., acetol, acetoin, etc.) are produced from the degradation of the sugar.

30 In some embodiments, the buffer can be a sodium hydrogen phosphate/sodium hydroxide buffer with a pH of about 12. In various embodiments, the buffer can include a potassium phosphate buffer with a pH of about 6.5 to about 7.5. In certain embodiments, the buffer can include sodium carbonate, sodium sulfite, peroxodisulfate, sodium phosphate, or combinations thereof. The selection of the buffer type, capacity, pH, and reaction temperature can affect the synthesis of hydroxy ketones from the sugar carbon
35 source, and can therefore impact the array of pyrazines produced as well as the quantity of pyrazines produced from the subsequent reaction with a nitrogen source.

In various embodiments, the buffer can buffer at a pH of approximately neutral or in an alkaline range, such as at a pH greater than about 6, greater than about 8, or greater than about 10 (e.g., about 6 to about 12). For example, in certain embodiments, the pH of the sugar carbon source can be buffered to about 11 to about 12. In some embodiments, the pH of the sugar carbon source can be buffered to about 6.5 to about 7.5.

For example, glucose is a known carbon source for the production of pyrazines. When employed as an intact molecule and reacted with ammonium hydroxide, an array of pyrazines are produced, including the molecules pyrazine and methylpyrazine as the dominant pyrazines with much lesser amounts of dimethylpyrazines and significantly less amounts of higher molecular weight pyrazines. When the glucose is pre-reacted with NaOH at pH 12 followed by reaction with ammonium hydroxide, a very similar array of pyrazines are produced. However, it was surprisingly discovered that if the glucose was treated with a potassium phosphate buffer at pH 6.5 followed by reaction with ammonium hydroxide, only dimethylpyrazines and higher molecular weight pyrazines were produced, thereby excluding the production of the less desirable molecules pyrazine and methylpyrazine.

Increased reaction time between the buffered sugar(s) carbon source and the nitrogen source and temperature can produce increased pyrazines yield up to the point where a black tar substance is produced. Reaction temperature can be about 30°C or greater, about 90°C or greater, about 100°C or greater, about 120°C or greater, or about 140°C or greater for example. In some embodiments, the reaction temperature can be about 90°C to about 150°C, or about 120°C to about 140°C. Reaction time can be about 30 mins or greater, about 60 mins or greater, about 90 mins or greater, or about 120 mins or greater, for example. In various embodiments, the reaction time can be about 30 mins to about 150 mins, or about 60 mins to about 120 mins.

Increasing the pH of the reaction solution can also result in increased amounts of pyrazines. A favored pH range can be about 7.5 to about 10.5, or about 8.5 to about 9.5. In some embodiments, the pH of the reaction solution can be about 8.0 or greater, about 8.5 or greater, about 9.0 or greater or about 10.0 or greater. A small addition of NaOH or KOH, for example, can be used to increase the pH of the reaction solution.

In various embodiments of the present invention, as illustrated at operation 122 of FIG. 2 for example, the addition of NH₄OH to the amino acid/sugar reaction solution can increase the yield of pyrazines. The NH₄OH/sugar molar ratio can have a dramatic influence on the yield of pyrazines. For example, a molar ratio of sugar to NH₄OH of about 6:1 to about 1:1, or about 5:1 to about 2:1 (e.g., about 5:1, about 2.5:1, about 2:1, or about 1.5:1), followed by heat treatment can produce formulations rich in pyrazines. In some embodiments, aqueous NH₄OH can slowly be added into the amino acid/sugar solution over the course of the reaction.

Different sugars and amino acids affect the types of pyrazines formed. *See, e.g.,* Coleman and Steichen, 2006, Sugar and selected amino acid influences on the structure of pyrazines in microwave heat treated formulations, J. Sci. Food Agric., 86, 380-391, which is herein incorporated by reference in its

entirety. For example, leucine and valine produce more branched pyrazines with highly substituted subchains and lower odor thresholds. Highly substituted pyrazines are relatively more potent than pyrazines that are not as branched, and therefore can be desirable in some applications. The substitution in the pyrazine can be a result of the amino acid used in the reaction. Therefore, it can be advantageous to select amino acids with branching, highly substituted subchains. With regard to sugars, rhamnose can be an ideal sugar for pyrazine formation, followed by fructose and then glucose.

As illustrated at operation 126 of FIG 2, for example, following the reaction, optionally pyrazines can be isolated from the reaction product using simple distillation, rotary evaporation, or other separation techniques known in the art. In certain embodiments, rotary evaporation can be a preferred isolation technique in a scaled up process of deriving tobacco-derived pyrazines.

Uses of Substituted Pyrazines in Tobacco Products

As described above, pyrazines generated according to the present invention can be useful as components (e.g., flavorants) incorporated into tobacco products, for example. The tobacco product to which the materials of the invention are added can vary, and can include any product configured or adapted to deliver tobacco or some component thereof to the user of the product. Exemplary tobacco products include smoking articles (e.g., cigarettes), smokeless tobacco products, and aerosol-generating devices that contain a tobacco material or other plant material that is not combusted during use.

In various embodiments of the present invention, pyrazines can be incorporated into smoking articles in the form of a flavorant in a tobacco composition and/or in a filter element of a smoking article. For example, pyrazines can be incorporated into a top dressing or casing of a tobacco product. Referring to FIG. 6, there is shown a smoking article 10 in the form of a cigarette and possessing certain representative components of a smoking article that can contain products derived from the cellulosic sugar materials of the present invention. The cigarette 10 includes a generally cylindrical rod 12 of a charge or roll of smokable filler material (e.g., about 0.3 to about 1.0 g of smokable filler material such as tobacco material) contained in a circumscribing wrapping material 16. The rod 12 is conventionally referred to as a "tobacco rod." The ends of the tobacco rod 12 are open to expose the smokable filler material. The cigarette 10 is shown as having one optional band 22 (e.g., a printed coating including a film-forming agent, such as starch, ethylcellulose, or sodium alginate) applied to the wrapping material 16, and that band circumscribes the cigarette rod in a direction transverse to the longitudinal axis of the cigarette. The band 22 can be printed on the inner surface of the wrapping material (i.e., facing the smokable filler material), or less preferably, on the outer surface of the wrapping material.

At one end of the tobacco rod 12 is the lighting end 18, and at the mouth end 20 is positioned a filter element 26. The filter element 26 positioned adjacent one end of the tobacco rod 12 such that the filter element and tobacco rod are axially aligned in an end-to-end relationship, preferably abutting one another. Filter element 26 may have a generally cylindrical shape, and the diameter thereof may be essentially equal to the diameter of the tobacco rod. The ends of the filter element 26 permit the passage of air and smoke

therethrough. A plug wrap 28 enwraps the filter element and a tipping material (not shown) enwraps the plug wrap and a portion of the outer wrapping material 16 of the rod 12, thereby securing the rod to the filter element 26.

The filter element of the invention typically comprises multiple longitudinally extending segments. Each segment can have varying properties and may include various materials capable of filtration or adsorption of particulate matter and/or vapor phase compounds. Typically, the filter element of the invention includes 2 to 6 segments, frequently 2 to 4 segments. In one preferred embodiment, the filter element includes a mouth end segment, a tobacco end segment and a compartment therebetween. This filter arrangement is sometimes referred to as a “compartment filter” or a “plug/space/plug” filter. The compartment may be divided into two or more compartments as described in greater detail below.

In various embodiments, the filter element can comprise an adsorbent in the form of an activated carbon material, wherein the activated carbon is capable of removing at least one gas phase component of mainstream smoke is incorporated into the filter element. In certain embodiments, the filter element 26 can include ventilation holes 30 that extend through the tipping paper (not shown) and the plug wrap 28 and, thus, provide air dilution of mainstream smoke. The ventilation holes 30 may be configured as a single line of perforations extending circumferentially around the filter element 26 or may comprise several lines of perforations. As would be understood, the exact count and size of the ventilation holes 30 will vary depending on the desired level of air dilution.

In various embodiments of the present invention, pyrazines obtained through methods disclosed herein can be incorporated into smokeless tobacco products in the form of a flavorant in a smokeless tobacco formulation. The form of the smokeless tobacco product of the invention can vary. In one particular embodiment, the product is in the form of a snus-type product containing a particulate tobacco material and a flavorant comprising a pyrazine obtained through methods of the present invention. Manners and methods for formulating snus-type tobacco formulations will be apparent to those skilled in the art of snus tobacco product production. For example, as illustrated in FIG. 7, an exemplary pouched product 300 can comprise an outer water-permeable container 320 in the form of a pouch which contains a particulate mixture 315 adapted for oral use. The orientation, size, and type of outer water-permeable pouch and the type and nature of the composition adapted for oral use that are illustrated herein are not construed as limiting thereof.

In various embodiments, a moisture-permeable packet or pouch can act as a container for use of the composition within. The composition/construction of such packets or pouches, such as the container pouch 320 in the embodiment illustrated in FIG. 7, may be varied as noted herein. For example, suitable packets, pouches or containers of the type used for the manufacture of smokeless tobacco products, which can be modified according to the present invention, are available under the tradenames CatchDry, Ettan, General, Granit, Goteborgs Rape, Grovsnus White, Metropol Kaktus, Mocca Anis, Mocca Mint, Mocca Wintergreen, Kicks, Probe, Prince, Skruf and TreAnkrare. A pouch type of product similar in shape and form to various embodiments of a pouched product described herein is commercially available as ZONNIC (distributed by Niconovum AB). Additionally, pouch type products generally similar in shape and form to various

embodiments of a pouched product are set forth as snuff bag compositions E-J in Example 1 of PCT WO 2007/104573 to Axelsson et al., which is incorporated herein by reference, which are produced using excipient ingredients and processing conditions that can be used to manufacture pouched products as described herein.

5 The amount of material contained within each pouch may vary. In smaller embodiments, the dry weight of the material within each pouch is at least about 50 mg to about 150 mg. For a larger embodiment, the dry weight of the material within each pouch preferably does not exceed about 300 mg to about 500 mg.

10 In some embodiments, each pouch/container can have disposed therein a flavor agent member, as described in greater detail in US Pat. No. 7,861,728 to Holton, Jr. et al., which is incorporated herein by reference. The flavor agent member can comprise a flavorant comprising a pyrazine derived via methods of the present invention, as discussed above. If desired, other components can be contained within each pouch. For example, at least one flavored strip, piece or sheet of flavored water dispersible or water soluble material (e.g., a breath-freshening edible film type of material) may be disposed within each pouch along with or without at least one capsule. Such strips or sheets may be folded or crumpled in order to be readily
15 incorporated within the pouch. See, for example, the types of materials and technologies set forth in US Pat. Nos. 6,887,307 to Scott et al. and 6,923,981 to Leung et al.; and The EFSA Journal (2004) 85, 1-32; which are incorporated herein by reference.

20 In various embodiments, the outer water-permeable pouch can comprise PLA or other pouch materials known in the art. Descriptions of various components of snus types of products and components thereof also are set forth in US Pat. App. Pub. No. 2004/0118422 to Lundin et al., which is incorporated herein by reference. See, also, for example, US Pat. Nos. 4,607,479 to Linden; 4,631,899 to Nielsen; 5,346,734 to Wydick et al.; and 6,162,516 to Derr, and US Pat. Pub. No. 2005/0061339 to Hansson et al.; each of which is incorporated herein by reference. See, also, the types of pouches set forth in US Pat. No. 5,167,244 to Kjerstad, which is incorporated herein by reference. Snus types of products can be
25 manufactured using equipment such as that available as SB 51-1/T, SBL 50 and SB 53-2/T from Merz Verpackungsmaschinen GmbH. Snus pouches can be provided as individual pouches, or a plurality of pouches (e.g., 2, 4, 5, 10, 12, 15, 20, 25 or 30 pouches) can be connected or linked together (e.g., in an end-to-end manner) such that a single pouch or individual portion can be readily removed for use from a one-piece strand or matrix of pouches.

30 The invention is not limited to snus-type smokeless tobacco products. For example, the mixture of tobacco material and flavorants comprising at least one pyrazine derived via the methods described herein can also be incorporated into various smokeless tobacco forms such as loose moist snuff, loose dry snuff, chewing tobacco, pelletized tobacco pieces, extruded tobacco strips or pieces, finely divided or milled agglomerates of powdered pieces and components, flake-like pieces (e.g., that can be formed by
35 agglomerating tobacco formulation components in a fluidized bed), molded tobacco pieces (e.g., formed in the general shape of a coin, cylinder, bean, cube, or the like), pieces of tobacco-containing gum, products incorporating mixtures of edible material combined with tobacco pieces and/or tobacco extract, products

incorporating tobacco (e.g., in the form of tobacco extract) carried by a solid inedible substrate, and the like. For example, the smokeless tobacco product can have the form of compressed tobacco pellets, multi-layered extruded pieces, extruded or formed rods or sticks, compositions having one type of tobacco formulation surrounded by a different type of tobacco formulation, rolls of tape-like films, readily water-dissolvable or water-dispersible films or strips (see, for example, US Pat. Appl. Pub. No. 2006/0198873 to Chan et al.), or capsule-like materials possessing an outer shell (e.g., a pliable or hard outer shell that can be clear, colorless, translucent or highly colored in nature) and an inner region possessing tobacco or tobacco flavor (e.g., a Newtonian fluid or a thixotropic fluid incorporating tobacco of some form).

In some embodiments, smokeless tobacco products of the invention can have the form of a lozenge, tablet, microtab, or other tablet-type product. See, for example, the types of lozenge formulations and techniques for formulating or manufacturing lozenges set forth in US Pat. Nos. 4,967,773 to Shaw; 5,110,605 to Acharya; 5,733,574 to Dam; 6,280,761 to Santus; 6,676,959 to Andersson et al.; 6,248,760 to Wilhelmsen; and 7,374,779; US Pat. Pub. Nos. 2001/0016593 to Wilhelmsen; 2004/0101543 to Liu et al.; 2006/0120974 to Mcneight; 2008/0020050 to Chau et al.; 2009/0081291 to Gin et al.; and 2010/0004294 to Axelsson et al.; which are incorporated herein by reference.

Depending on the type of smokeless tobacco product being processed, the tobacco product can include one or more additional components in addition to the tobacco material and the flavorants comprising at least one pyrazine derived from methods of the present invention. For example, the tobacco material and the tobacco-derived flavorants can be processed, blended, formulated, combined and/or mixed with other materials or ingredients, such as other tobacco materials or flavorants, fillers, binders, pH adjusters, buffering agents, salts, sweeteners, colorants, disintegration aids, humectants, and preservatives (any of which may be an encapsulated ingredient). See, for example, those representative components, combination of components, relative amounts of those components and ingredients relative to tobacco, and manners and methods for employing those components, set forth in US Pat. Pub. Nos. 2011/0315154 to Mua et al. and 2007/0062549 to Holton, Jr. et al. and US Pat. No. 7,861,728 to Holton, Jr. et al., each of which is incorporated herein by reference.

In various embodiments, at least one pyrazine derived from methods described herein can be incorporated into smokeless tobacco products in the form of a flavorant in an electronic smoking article. There have been proposed numerous smoking products, flavor generators, and medicinal inhalers that utilize electrical energy to vaporize or heat a volatile material, or attempt to provide the sensations of cigarette, cigar, or pipe smoking without burning tobacco to a significant degree. See, for example, the various alternative smoking articles, aerosol delivery devices and heat generating sources set forth in the background art described in U.S. Pat. No. 7,726,320 to Robinson et al., U.S. Pat. Pub. Nos. 2013/0255702 to Griffith Jr. et al., 2014/0000638 to Sebastian et al., 2014/0060554 to Collett et al., 2014/0096781 to Sears et al., 2014/0096782 to Ampolini et al., and 2015/0059780 to Davis et al., which are incorporated herein by reference in their entirety.

An exemplary embodiment of an electronic smoking article 200 is shown in FIG. 8. As illustrated therein, a control body 202 can be formed of a control body shell 201 that can include a control component 206, a flow sensor 208, a battery 210, and an LED 212. A cartridge 204 can be formed of a cartridge shell 203 enclosing a reservoir housing 244 that is in fluid communication with a liquid transport element 236 adapted to wick or otherwise transport an aerosol precursor composition stored in the reservoir housing to a heater 234. An opening 228 may be present in the cartridge shell 203 to allow for egress of formed aerosol from the cartridge 204. Such components are representative of the components that may be present in a cartridge and are not intended to limit the scope of cartridge components that are encompassed by the present disclosure. The cartridge 204 may be adapted to engage the control body 202 through a press-fit engagement between the control body projection 224 and the cartridge receptacle 240. Such engagement can facilitate a stable connection between the control body 202 and the cartridge 204 as well as establish an electrical connection between the battery 210 and control component 206 in the control body and the heater 234 in the cartridge. The cartridge 204 also may include one or more electronic components 250, which may include an IC, a memory component, a sensor, or the like. The electronic component 250 may be adapted to communicate with the control component 206. The various components of an electronic smoking device according to the present disclosure can be chosen from components described in the art and commercially available.

In various embodiments, the aerosol precursor composition can comprise a flavorant comprising at least one pyrazine derived according to methods of the present invention. Exemplary formulations for aerosol precursor materials that may be used according to the present disclosure are described in U.S. Pat. No. 7,217,320 to Robinson et al.; U.S. Pat. Pub. Nos. 2013/0008457 to Zheng et al.; 2013/0213417 to Chong et al.; 2014/0060554 to Collett et al.; and 2014/0000638 to Sebastian et al., the disclosures of which are incorporated herein by reference in their entirety. Other aerosol precursors that can incorporate the tobacco-derived pyrazines described herein include the aerosol precursors that have been incorporated in the VUSE® product by R. J. Reynolds Vapor Company, the BLU™ product by Imperial Tobacco, the MISTIC MENTHOL product by Mistec Ecigs, and the VYPE product by CN Creative Ltd. Also desirable are the so-called “smoke juices” for electronic cigarettes that have been available from Johnson Creek Enterprises LLC.

EXPERIMENTAL

Aspects of the present invention are more fully illustrated by the following examples, which are set forth to illustrate certain aspects of the present invention and are not to be construed as limiting thereof.

Example 1

Pyrazines are produced using acetoin (3-hydroxy-2-butanone) instead of sugar(s) as the carbon source in reactions with ammonia.

Acetoin, ammonium hydroxide (28-30%), leucine, dichloromethane, and phosphoric acid (H_3PO_4) are obtained from Sigma-Aldrich (St. Louis, MO). F1 protein is obtained from R.J. Reynolds Tobacco Co. (Winston-Salem, NC) and hydrolyzed. The weight percent of hydrolyzed amino acids in all of the hydrolyzed solutions is in the range of 50-55%. All pyrazine synthesis reactions are performed in a 40mL Parr vessel. In each reaction, 0.8 gram of acetoin is mixed with 1.8 mL of NH_4OH and 0.6 mL of H_3PO_4 , and then enough hydrolyzed F1 protein (20 mL) is added to make the mass of amino acids equal to 0.4 gram. For example, when 40 grams of F1 protein is hydrolyzed in 1 liter solution, the weight percent of amino acids in the solution is equal to 50%, which is equal to 20 grams of amino acids in 1 liter solution. In order to use 0.4 gram of amino acids in a reaction, only 20 mL of above solution is added to the reaction vessel. In some of the reactions, instead of hydrolyzed F1 protein, leucine is used as a source of amino acid and only 20 mL of water is added to adjust the volume. For all solutions, the pH is adjusted to 8.0 and then reaction is started. After completion of each reaction, the mixture is extracted with 30 mL of dichloromethane (DCM). Next, 200 μ L of the DCM extract is diluted to 1 mL using DCM and analyzed via GC/MS.

All GC/MS analyses are performed using a 6890 GC equipped with a 5973 Mass Selective detector (MSD) from Agilent (Wilmington, DE). Separations are obtained using a DB-WAXTER capillary column (30 m long x 250 μ m I.D. with a film thickness of 0.25 μ m) from J&W (Wilmington, DE). The following operating parameters are used for each analysis:

20	Injection Port Temp	260°C
	Purge Valve	3 mL/min
	Purge Time	1 min
	Total Flow	24 mL/min
	Constant Flow	1 mL/min
25	Injection Volume	2 μ L, split 1:20
	Column Oven Initial Temp	50°C
	Column Oven Initial Time	3 min
	Column Oven Ramp Rate	15°C/min
	Column Oven Final Temp	250°C
30	Column Oven Final Time	1 min
	MSD Transfer line Temp	260°C

MS Wiley library is used to identify each pyrazine.

In a first reaction, acetoin (0.8 gram) is reacted with NH_4OH (1.8 mL) and H_3PO_4 (0.6 mL) at pH=8 at 90°C for 12-15 hours. More than 90% of acetoin is converted to tetramethylpyrazine (TMP). Next, the reaction is repeated using identical conditions, but instead of heating the reaction at 90°C for 12-15 hours, it is heated only for 4 hours at 120°C. Results are similar to those obtained at 90°C and 12-15 hours.

Next, in order to determine if branched pyrazines can be synthesized, an amino acid (leucine) is added to the reaction reagent mixture. For this purpose, 0.8 gram acetoin + 1.8 mL of NH_4OH + 0.6 mL of H_3PO_4 and 0.25 gram of leucine are mixed with 20mL of H_2O and pH is adjusted to 8. Then the reaction is heated for 18

hours at 120°C and then the reaction mixture is extracted with 30 mL of DCM and analyzed via GC/MS. Only TMP ($t_R=8.2$ min) and some acetoin ($t_R=6.1$ min) are detected. No branched pyrazines are detected.

Next, a similar reaction is performed, but instead of leucine and H₂O, 20 mL of hydrolyzed F1 protein is used for the reaction. The reaction is performed in the Parr vessel at 120°C for 18 hours. After cooling the reactant, 30 mL DCM is used to extract the pyrazines. Again, no branched pyrazines were observed, only TMP.

In order to determine whether ammonia is consuming all the acetoin and thus preventing the amino acid(s) from reacting with acetoin, another source of base (NaOH) is used instead of NH₄OH to assure that basic, pH > 8, reaction conditions are maintained. For this purpose, two reactions are performed. In the first reaction, 0.8 acetoin is mixed with 0.25 gram of leucine and 20 mL of 0.1N NaOH (pH= 12), while in the second reaction the pH is adjusted to 8.2 using H₃PO₄. Both reactions are heated at 120°C for 8 hours using the Parr vessel. After cooling the reaction, the reactants are extracted with DCM and analyzed via GC/MS. No pyrazines, not even TMP, are detected.

The above reactions illustrate that the addition of amino acids to the reaction of acetoin and NH₄OH does not produce branched pyrazines. Furthermore, it is discovered that acetoin is not a key intermediate in the reactions between sugar(s) and free amino acids to make pyrazines containing branched alkyl side chains.

Example 2

Hydroxy ketone carbon sources other than acetoin are utilized to selectively produce pyrazines other than TMP.

1-OH-acetone, 1-OH-2-butanone, ammonium hydroxide (28-30%), phosphoric acid (H₃PO₄), isoleucine, threonine, and isovaleraldehyde are obtained from Sigma-Aldrich (St. Louis, MO). F1 proteins derived from a plant of the *Nicotiana* species are obtained from R.J. Reynolds Tobacco Co. (Winston-Salem, NC) and hydrolyzed to form amino acids. The weight percent of hydrolyzed amino acids in all of the hydrolyzed solutions are in the range of 50-55%.

All pyrazine synthesis reactions are performed in a 40 mL Parr vessel. In each reaction, 1 gram of 1-OH-acetone or 1-OH-2-butanone is mixed with 0.25, 0.5, 1 and 1.25 mL of NH₄OH and 10mL of H₂O. Each reaction is mixed and heated at different temperatures (100-140°C) for a period of 4-24 hours. The pH levels for most of the reactions are approximately 11 (no adjustments are made). However, in the reactions in which the pH level is adjusted to 8, concentrated H₃PO₄ is used to lower the pH.

After the completion of each reaction, the mixture is extracted with 20-25 mL of dichloromethane. In each extraction, 250 µg of deuterated 2-methylpyrazine is used as an internal standard for all quantifications. For all reactions, the mixtures are stirred using a magnetic stirrer during the reaction process.

All GC/MS analyses are performed using the same equipment and operating parameters as those used in Example 1 above. MS Wiley library is used to identify each pyrazine. For quantitative analysis, pyrazines are quantified using single ion monitoring mode. Each pyrazine is quantified against the mass of internal standard (250 µg) added to the extraction solvent.

Initially, 1-hydroxyacetone is reacted with NH_4OH at different ratios, temperatures, pH levels, and reaction times to maximize the percent yield of pyrazines. The pyrazines detected from the reaction of 1-OH-acetone with NH_4OH include: 2,6-dimethylpyrazine; 2,5-dimethylpyrazine; 2-ethyl-5-methylpyrazine; 2-ethyl-6-methylpyrazine; 2,3,5-trimethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; 2-ethyl-2,5-dimethylpyrazine; 2,3,5,6-trimethylpyrazine; 2,3,5-trimethyl-6-ethylpyrazine; 2,6-dimethyl-3-propylpyrazine; 2,5-diethyl-3,6-dimethylpyrazine; 2,6-dimethyl-3-(2-MethylButyl)pyrazine; 2,5-dimethyl-3-(2-MethylButyl)pyrazine; 2,5-dimethyl-3-(3-MethylButyl)pyrazine; 2,5-dimethyl-3-propylpyrazine; 2,5-dimethyl-3-cis-propenylpyrazine; 2-isopropenyl-3,6-dimethylpyrazine; 2-(2-methylpropyl)-3,5-dimethylpyrazine; 2,6-dimethyl-3-isobutylpyrazine; 2-(2-methylpropyl)-3,5,6-trimethylpyrazine.

10 In a first part of this study, reactions are performed at two different pH levels (8.0 and 11.0) to determine which pH levels provide the highest yield and greatest number of pyrazines. In a first reaction, 1mL of 1-OH-acetone is reacted with 0.5 mL of NH_4OH and 10 mL of H_2O . The pH level for this reaction is measured to be around 11.0. In a second reaction, the same volume of reactant is mixed, but the pH level is adjusted to 8.0 using concentrated H_3PO_4 . Both reactions are heated at 120°C for 12 hours. The percent yield of the pyrazines is
15 higher when the pH level is around 11.0. For this purpose, the pH level of later experiments is not adjusted and reactions are performed at pH 11 or higher.

The effects of temperature (100, 110, 120, 130 and 140°C) on the synthesis of pyrazines using 1-OH-acetone (1 g) and NH_4OH (1 g) with a 1:2 mole ratio of C:N in 10mL of H_2O (reaction time 12 hours) is tested next. Pyrazine yield increases as the temperature increases, still showing an increase in pyrazine yield as the
20 temperature reached 140°C .

The effect of varying reaction times (4, 8, 12, 16, and 24 hours) on the synthesis of pyrazines using 1-OH-acetone (1 g) and NH_4OH (1 g) with a 1:2 mole ratio of C:N in 10 mL of H_2O is tested. Maximum yields of pyrazines are obtained when the reaction time is 16 hours.

The effect of varying the 1-OH-acetone: NH_4OH molar ratio (1:0.5, 1:1, 1:2, and 1:2.5, 1-OH-acetone
25 and NH_4OH) reaction in 10 mL of H_2O on the yield of pyrazines at 120°C after 12 hours is tested. The optimum ratio is 1:2, which is equal to 1 gram of 1-OH-acetone and 1 mL of NH_4OH . When a higher volume of NH_4OH is used in the reaction, the yield of the reaction drops by more than 10%.

In summary, reaction conditions (temperature, time, C:N ratio, and pH) are optimized to maximize quantity of pyrazines. Results demonstrated that at optimized conditions (C:N = 1:2, temperature = 120°C ,
30 reaction time = 16 hours, and pH = 11-12) at least 19-20 different pyrazines are synthesized using hydroxyacetone as the sole carbon source. The absence of any detectable amounts of the molecules pyrazine and/or methylpyrazine from the synthesized pyrazines supports the discovery that the carbon source (i.e., the α,β -hydroxyketone) influences the structures of the pyrazines produced from reaction of the carbon source with a nitrogen source.

35 Example 3

The effects of amino acids and aldehyde additions into a separate reaction of 1-OH-acetone and NH_4OH , according to the parameters of Example 2 above, with C:N ratio of 1:2 mixed with 10 mL H_2O at 120°C for 12 hours are measured.

Two different amino acids are tested as an additional source of nitrogen. In each reaction, 0.2 grams of amino acid are added separately to each reaction so that it is possible to study how the additional amino acids affect the pyrazine synthesis and its yield. In a separate reaction, isovaleraldehyde is added to the optimized reaction to study the effect on pyrazine synthesis and yield. When the hydrolyzed F1 protein is used as a source of additional nitrogen, 10mL of hydrolyzed F1 proteins (which contain approximately 0.2 gram of different amino acids) was used. In this reaction, H_2O is not added since the hydrolyzed F1 proteins are contained within the 10mL of H_2O .

It is observed that when isoleucine is used as a possible additional source of nitrogen, the concentrations of 2,5-dimethyl-3-(2-methylbutyl) pyrazine and 2,5-dimethyl-3-(3-methylbutyl) pyrazine increase. In a separate reaction, when threonine is used as an additional source of nitrogen, the concentrations of 2,5-dimethyl-3-(2-methylbutyl) pyrazine and 2,5-dimethyl-3-(3-methylbutyl) pyrazine increase and the 2,6-dimethyl-3-(2-methylbutyl) pyrazine yield increases. It is interesting to note that the total yields of pyrazines are similar when threonine or isoleucine is added to the reaction. Both compounds increase the total yield of pyrazines by more than 7%.

The addition of isovaleraldehyde to a separate reaction of 1-OH-acetone and NH_4OH causes the percent yield of 2,5-dimethyl-3-(2-methylbutyl) pyrazine and 2,5-dimethyl-3-(3-methylbutyl) pyrazine to increase from 0 to 14 and 13 mg, respectively. The total yield of pyrazines increases by more than 20 percent.

Instead of a pure amino acid, a mixture of amino acids prepared from the hydrolysis of F1 proteins is used in the reaction. Since hydrolyzed F1 proteins are already in an aqueous solution, no water is added to the mixture. For this purpose, 10 mL of hydrolyzed F1 proteins which contained about 0.2 g of amino acid and 10 mL of H_2O is reacted with 1-OH-acetone and NH_4OH with a C:N ratio of 1:2 at 120°C for 16 hours. The yield of 2,5-dimethylpyrazine increases by more than 80% and the yield of 2,5-dimethyl-3-(2-methylbutyl) pyrazine and 2,5-dimethyl-3-(3-methylbutyl) pyrazine increases from 0 to more than 1 mg. Without being limited by theory, the alkyl portion of the amino acid is converted to a Strecker aldehyde which reacts with the ammonium hydroxide to form an imine which in turn is incorporated into a pyrazine structure.

The effects of different temperatures and C:N ratios (1:1 and 1:2) on the synthesis of pyrazines using 1-OH-acetone and NH_4OH in the presence of additional amino acids/aldehyde is tested. In these studies, increasing the temperature from 100 to 120°C and C:N ratio, increases the yield of pyrazines.

In summary, addition of amino acids, selected aldehydes, or hydrolyzed F1 protein increases not only percent yield of certain pyrazines, but also increases the number of synthesized pyrazines.

Example 4

Pyrazines are synthesized according to Examples 2 and 3 above, except 1-OH-2-butanone is used as a carbon source instead of 1-OH-acetone.

For this purpose, 1 gram of 1-OH-2-butanone is reacted with 1 mL of NH₄OH and 10 mL of H₂O at 120°C for 16 hours. No methyl pyrazines are formed. All pyrazines formed contain ethyl or higher branched alkanes. The yield of pyrazines is, however, not as high as when 1-OH-acetone is used. The pyrazines synthesized from a reaction using 1-OH-2-butanone and NH₄OH with C:N of 1:2 at 120°C for 16 hours includes 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-M-2,n-propyl-6(1-butenyl)pyrazine; 2,5-dimethyl-3-ethylpyrazine.

As mentioned earlier, with 1-OH-acetone as the carbon source, the molecules pyrazine and methylpyrazine are not produced. With 1-OH-2-butanone, the molecules pyrazine, methylpyrazine, and dimethylpyrazine are not produced, confirming that the carbon source is overwhelmingly dictating the structure of the pyrazines. Results further show that by changing the carbon source from 1-OH-acetone to 1-OH-2-butanone, one can control the type of pyrazines being synthesized.

Example 5

The reaction of 1-OH-acetone and NH₄OH according to Example 2 above is accomplished on a larger scale using a larger Parr reactor with greater reaction volume.

100 grams of 1-OH-acetone is reacted with 100 mL of NH₄OH and 1000 mL of H₂O at 120°C for 16 hours in a 1.5 liter Parr high pressure vessel. After the reaction is complete, the mixture is cooled and transferred into a glass bottle.

It is noted that in the bottom of the vessel is a considerable amount of a tar like material which is only soluble in MeOH. The addition of H₂O to the top of this material causes it to become hard. It is discovered that concentration can play a significant role in the presence or absence of the tar like material. For all optimization studies, the amount of tar at the bottom of reaction vessel is low. For this reason, a small volume of methanol (1 mL) is sufficient to dissolve everything and include it with the remaining reaction material. For large reactions, the mass of tar is higher and at least 100-200 mL of methanol is required to dissolve it.

After the completion of the reaction, the aqueous solution is distilled (3 x 375 mL) at 130-140°C. In each distillation (375 mL), approximately 175 mL of aqueous solution containing different pyrazines is collected (light yellow color – total volume about 500 mL). Next, the distilled materials (3 x 175 mL) are combined and passed through a C₁₈ column (15 x 2.5 cm packed with SPE material) in order to remove the pyrazines from the water. After removal of the water from the C₁₈ column, trapped pyrazines are eluted using ethanol. Next, ethanol is removed using a rotary evaporation and vacuum. Due to the presence of some water in the final product, pyrazines are extracted into MTBE and dried over sodium sulfate. Next, MTBE is removed using a rotary evaporator and vacuum. A vial with the **solution 1** label contains most of the pyrazines after MTBE is removed.

It is important to note here that three pyrazines are not distilled and remain in the reaction product due to their higher boiling points. This contributed to a lower percent yield. These pyrazines are identified as 2-(2-methylpropyl) 3,5-dimethylpyrazine (12.57 min), 2,6-dimethyl-3-isobutylpyrazine (12.74 min), and 2-(2-methylpropyl) 3,5,6-trimethylpyrazine (12.95 min).

DCM (200-250 mL) is used to extract the remaining three pyrazines from the reaction solution after distillation. Next, DCM is removed from the solution using a rotary evaporator and vacuum while the thick dark solution is later transferred to the second vial and labeled as **solution 2**. Figure 3 shows a chromatogram of this sample.

5 The yield of total pyrazines from distillation of 1200 mL of reaction solution (100 grams of 1-OH-acetone + 100 mL of NH₄OH) is about 60% compared to the 12 mL reaction (1 gram of 1-OH-acetone + 1 mL of NH₄OH). This yield does not include the three pyrazines that remained in the post distillation reaction mixture.

Solution 1 from above is used for the gas chromatography olfactometry (GCO) evaluations. The pyrazine sample is analyzed using an Agilent 7890A Series GC with 5975C MSD and ODP3 equipped with a Gerstel Multipurpose Sampler with SPME capability. The instrument method is developed for the sample to obtain better separation of the pyrazines and suitable analyzing duration for the olfactory analysts. The transfer line in ODP3 is heated to 260°C. The sample is prepared by pipetting two drops of the sample into a 20 mL SPME screw cap vial. An empty vial is analyzed before and after the sample. The gas chromatographic column separated the pyrazines and they were detected/evaluated by humans using a subjective smell test as they exited the column. The pyrazines identified include: methylpyrazine; 2,6-dimethylpyrazine; 2,5-dimethylpyrazine; 2-ethyl-5-methylpyrazine; 2-ethyl-6-methylpyrazine; trimethylpyrazine; 2,5-dimethyl-3-propylpyrazine; 3-ethyl-2,5-dimethylpyrazine; 2,5-dimethyl-3-isopropylpyrazine; 2-ethyl-3,5-dimethylpyrazine; tetramethylpyrazine; 2-methyl-5-propylpyrazine; 2,3,5-trimethyl-6-propylpyrazine isomer 1; 2,3-diethyl-5-methylpyrazine; 2,3,5-trimethyl-6-ethylpyrazine; 3,5-dimethyl-2-propylpyrazine; 2,3,5-trimethyl-6-propylpyrazine isomer 2; 2,3,5-trimethyl-6-propylpyrazine isomer 3; trimethyl-1-propenylpyrazine (Z)-isomer 1; 5H-cyclopentapyrazine, 6,7-dihydro-2,5-dimethyl pyrazine isomer 1; 5H-cyclopentapyrazine, 6,7-dihydro-2,5-dimethyl pyrazine isomer 2; trimethyl-1-propenylpyrazine (Z)-isomer 2; 2,3-dimethyl-3-(1-propenyl)pyrazine (Z) isomer 1; trimethyl-1-propenylpyrazine (Z)-isomer 3; 2,3-dimethyl-3-(1-propenyl)pyrazine (Z); 2-isopropenyl-3,6-dimethylpyrazine; trimethyl-1-propenylpyrazine (Z)-isomer 4; trimethyl-1-propenylpyrazine (Z)-isomer 5; trimethyl-1-propenylpyrazine (Z)-isomer 6; trimethyl-1-propenylpyrazine (E)-isomer 1; trimethyl-2-propenylpyrazine; trimethyl-1-propenylpyrazine (E)-isomer 2. It is noted that the methylpyrazine compound, although present, was present in a lower amount than typical for traditional sugar carbon source reactions.

Four flavor analysts evaluate the individual olfactory characters from the ODP portal in four separate sessions. The individual pyrazines, based on the four independent assessments, are very positive. Expected descriptors for the aroma of substituted pyrazines are found. Nutty, roasted, toasted, chocolate, peanut, musty, brown and complex are frequent descriptors. These are all positive flavor characteristics.

Example 6

Alpha hydroxy ketones (acetol) are produced from sugar to ultimately be used as the carbon source in sugar ammonia reactions. Sodium hydroxide is used as a base to optimize different parameters such as sugar type, temperature, reaction time, pH, and base concentration in order to maximize the yield of acetol.

Glucose, Fructose, 1-OH-acetone (acetol), 1-OH-2-butanone (acetoin), sodium hydroxide, sodium chloride, sodium sulfate anhydrous, hydrochloric acid, methanol, and dichloromethane are obtained from Sigma-Aldrich (St. Louis, MO). Synthesis reactions are performed in a 40mL Parr vessel or an open round bottle flask. For all reactions in which pH is controlled, reactions are performed in round bottom flasks under reflux at 100°C. In each reaction, 0.25, 0.5, or 1.0 gram of a different sugar (glucose, fructose or mixture of both) is mixed with 25 mL of 0.025, 0.05, 0.1 and 0.2 M NaOH. Each reaction is stirred and heated at different temperatures (90-140°C) for a period of 1-12 hours. The initial pH levels for most of the reactions are approximately 12 and no adjustments are made during the reaction. However, for the reactions in which the pH level is adjusted to 9, hydrochloric acid is used. For reactions in which the pH is kept constant at 12 during the process, 10-40µL of 10 M NaOH is used. After the completion of each reaction, the mixture is cooled and the pH is adjusted to 6.0-6.5 using 1 M HCl. Next, 1 mg of acetoin is added to the reaction mixture as an internal standard. The mixture is extracted 4 times with 8-10 mL of dichloromethane. All four extraction solvents are combined and dried with sodium sulfate.

All GC/MS analyses are performed using the same equipment and operating parameters as those used in Example 1 above. MS Wiley library is used to identify peaks. For quantitative analysis, the calibration curve is prepared using a concentration of acetol and a response factor ratio of acetol/acetoin. It is important to note here that acetoin is not detected in any reactions performed at 90, 100 and 120°C. However, acetoin residue is found in the reaction where the temperature is set to 140°C. For this purpose, acetoin concentrations are determined in the reaction and this value is accounted for in all calculations.

GC/FID is used to prepare the calibration curve for the quantification of acetol in all reactions. Next, the following parameters are varied to determine the optimum conditions for the synthesis of acetol.

First, two different type of sugars (glucose, fructose, and a mixture of both) are used to determine which sugar provides a higher yield of acetol. In each reaction, 0.5 grams of sugar is mixed with 25mL of 0.05M NaOH. Each reaction is heated for 60 minutes at 100°C. Table 1 below shows the reaction conditions and the corresponding mg of acetol that were obtained from each reaction. It can be observed that glucose generated a high mass of acetol when it was used in the reaction.

Table 1: Effect of sugar type on synthesis of acetol

Sugar	Wt. gr	pH	NaOH	Temp, °C	Time, min	mg acetol*	% Yield wt. base – mole base
Glucose (Glu)	0.5	12-7.5	0.05 M	100	60	3.77	0.75 – 1.8
Fructose (Fru)	0.5	12-7.5	0.05 M	100	60	2.97	0.59 – 1.4
Glu+Fru	0.25+0.25	12-7.5	0.05 M	100	60	3.18	0.64 – 1.5

*%RSD varied from 2-5

In this part of study, since it is required to adjust the pH during the reaction process, all reactions are performed under reflux in an open round flask instead of in a closed Parr reactor. Three different experiments are performed. In the first experiment, the pH is measured every 10 minutes and no adjustment

is made to the pH. In the second experiment, the pH is measured every 10 minutes and, if it is required, the pH is adjusted to the initial value (12.0) using 10M NaOH. In the third experiment, initial pH is adjusted to 9.0 and then the reaction is started, with pH measurements taking place every 10 minutes (without adjustments).

5 Results from the pH measurements show that the pH drops from 12.0 to around 9 within the first 10 minutes of the reaction at 100°C. At the end of 30 minutes, the pH of the reaction is approximately around 7-8 and remains constant during the remaining reaction time. For the experiments where the pH is maintained around 12.0, it becomes necessary to add approximately 15-20 μL of 10M NaOH to the reaction every 10 minutes. After 40 minutes, the pH remains constant around 11-12.

10 Table 2 below shows reaction conditions and the mg of acetol obtained from each reaction using different pH conditions. When the pH is kept constant around 12, the highest mass of acetol is obtained.

Table 2: Effect of pH on synthesis of acetol

Sugar	Wt. gr	pH	NaOH	Temp, °C	Time, min	mg acetol*	% Yield wt. base – mole base
Glu	0.5	12-7.5	0.05 M	100	60	2.79	0.56 – 1.4
Glu	0.5	12.0-12.0	0.05 M	100	60	4.94	0.99 – 2.4
Glu	0.5	9-7.5	0.05 M	100	60	0.00	0.00 – 0.0

*%RSD varied from 5-9

15 Three different sugar concentrations are tested in order to determine how sugar concentration effects acetol production. Therefore, reactions are performed using 0.25, 0.5 and 1 gram of glucose in 25mL of 0.05M NaOH solution. Each reaction is heated at 100°C for 60 minutes using a Parr reactor. Table 3 below shows the results of this study. It is observed that when 0.25 gram of glucose is used in a reaction, the highest amount of acetol is obtained.

20 Without being limited by theory, it is believed that when sugar concentration is high, acid formation during the reaction process minimizes the production of acetol. When the sugar concentration is less, acid formation will take longer while the pH is high enough to cause the formation of acetol.

Table 3: Effect of Sugar Concentration on synthesis of acetol

Sugar	Wt. gr	pH	NaOH	Temp, °C	Time, min	mg acetol*	% Yield wt. base – mole base
Glu	0.25	12-7.5	0.05 M	100	60	3.88	1.55 - 3.8
Glu	0.5	12-7.5	0.05 M	100	60	3.77	0.75 - 1.8
Glu	1	12-7.5	0.05 M	100	60	2.77	0.28 - 0.7

25 *%RSD varied from 5-7

30 Four different concentrations of NaOH (0.2, 0.1, 0.05 and 0.025M) are used for the preparation of acetol. Table 4 below shows results of this study. As can be observed, when the NaOH concentration is 0.025M, the least amount of acetol is obtained. However, as the concentration increases from 0.025 to 0.1M, the mass of synthesized acetol increases too. When the concentration of base increases to 0.2M, the

amount of acetol decreases. It is noted that the reaction solution smells of burnt sugar compared to the other reactions where the base concentration is lower.

Table 4: Effect of NaOH Concentration on synthesis of acetol

Sugar	Wt. gr	pH	NaOH	Temp, C	Time, min	mg acetol*	% Yield wt. base – mole base
Glu	0.5	12-11.5	0.2 M	100	60	3.35	0.67 – 1.6
Glu	0.5	12-8.5	0.1 M	100	60	5.38	1.08 – 2.6
Glu	0.5	12-7.5	0.05 M	100	60	3.77	0.75 – 1.8
Glu	0.5	12-6.5	0.025 M	100	60	2.01	0.40 – 1.0

5 *%RSD varied from 5-20 (%RSD was 20 for 0.2M NaOH reactions)

The effects of temperature on the synthesis of acetol using NaOH and glucose are investigated. For this purpose, different temperatures ranging from 90 to 140°C are studied. Results for reaction temperatures of 90, 100 and 120°C are similar while the reaction at 140°C yields approximately 25% more acetol than the other reactions with lower temperatures. Table 5 below shows results of this study.

Table 5: Effect of Reaction Temperature on synthesis of acetol

Sugar	Wt. gr	pH	NaOH	Temp, °C	Time, min	mg acetol*	% Yield wt. base – mole base
Glu	0.5	12- 7.0	0.05M	90	60	3.37	0.67 – 1.6
Glu	0.5	12-7.5	0.05 M	100	60	3.77	0.75 – 1.8
Glu	0.5	12-7.5	0.05 M	120	60	3.23	0.65 – 1.6
Glu	0.5	12-6.8	0.05 M	140	60	4.58	0.92 – 2.2

*%RSD varied from 2-8

15 The effects of reaction time on the synthesis of acetol is also studied. Reactions are performed using identical conditions while reaction times are varied from 60 to 720 minutes. Table 6 below shows the results of this study. The results show that with increasing reaction times, there is no significant change in the yield of acetol.

20 **Table 6:** Effect of Reaction Time on synthesis of acetol

Sugar	Wt. gr	pH	NaOH	Temp, °C	Time, min	mg acetol*	% Yield wt. base – mole base
Glu	0.5	12-7.5	0.05 M	100	60	3.77	0.75 – 1.8
Glu	0.5	12-6.8	0.05 M	100	120	3.24	0.65 – 1.6
Glu	0.5	12-6.8	0.05 M	100	240	3.72	0.74 – 1.8
Glu	0.5	12-6.9	0.05 M	100	720	3.38	0.68 – 1.6

*%RSD varied from 2-6

In the last part of this study, based on results obtained from previous reactions, different reactions are performed using optimized conditions. In reaction A, 1 gram of glucose is reacted with 0.05M NaOH

under reflux at 100°C, while the pH is kept constant around 11-12. This reaction continues for 120 minutes until the pH remains constant and does not change. The yield of acetol is 6.7 mg. In reaction B, a Parr vessel is used at 100°C to react 0.5 gram of HFTS with 25mL of 0.05M NaOH for 60 minutes. The acetol yield is 3.42 mg. When the same reaction is performed under optimized conditions, (0.1M NaOH and 140°C for 60 min) the acetol yield increases by 100% to 6.69 mg (Rxn D). Similar results are obtained when optimized conditions are applied to 0.5 grams of glucose (Rxn C). The acetol yield increases to 8.2 mg. Using identical reaction conditions, but using 50% less glucose (0.25 gram), the yield of acetol decreases to 5.32 mg.

10 **Table 7: Effect of Different Reaction Conditions on synthesis of acetol**

		Sugar	Wt. gr	pH	NaOH	Temp, °C	Time, min	mg acetol	% Yield wt. base – mole base
Rxn A	Reflux	Glu	1	12.0-11.0 ⁺	0.05 M	100	120	6.70	0.67 – 1.6
Rxn B	Parr	HFTS*	0.5	12.0-7.5	0.05 M	100	60	3.42	0.68 – 1.7
Rxn C	Parr	Glu	0.5	12.5-6.5	0.1 M	140	60	8.20	1.64 – 4.0
Rxn D	Parr	HFTS	0.5	12.5-6.7	0.1 M	140	60	6.69	1.34 – 3.3
Rxn E	Parr	Glu	0.25	12.0-8.5	0.1M	140	60	5.32	2.13 – 5.2

* “HFTS” stands for high fructose tobacco syrup

+ pH was kept constant around 12

In summary, different parameters such as sugar type, reaction temperature, reaction time, pH, base and sugar concentrations are optimized for the synthesis of acetol from reaction sugars and sodium hydroxide. It is demonstrated that the highest yield of acetol can be obtained when the reaction is performed at 140°C using 0.1M sodium hydroxide and 0.5 grams of glucose for 60 minutes. It is noticed that the pH of the reaction can be changed rapidly (within 10 minutes) from 12 to 6.5 if the base concentration is low or the sugar concentration is high. Also, it is noted that at high temperatures (140°C), the reaction product contains small amounts of acetoin (along with the acetol). It is important to note that hydroxy ketones (acetoin) have been prepared from glucose using biotechnical approaches with yield greater than 90%. However, published chemical conversions of glucose to hydroxy ketones are much less at ~9%.

Example 7

25 Pyrazines are produced using glucose as a carbon source.

As illustrated in Example 6 above, acetol can be synthesized via a reaction of 0.1 N NaOH and glucose using optimum conditions (0.5 gram glucose reacted with 25 mL of 0.1 N NaOH at 140°C for 60 min). The yield of acetol was approximately 2% based on the glucose weight.

A publication by Nodzu (R. Nodzu, On the action of phosphate upon hexoses, The formation of acetol from glucose in acidic solution of potassium phosphate. Bull Chem. Soc. Japan, 10, 122-130, 1935, which is herein incorporated by reference) demonstrated that acetol with 4% yield (based on the weight of the glucose) can be synthesized from a reaction of a 40% phosphate buffer solution with a pH of 6.5-6.8

with glucose at a temperature of 100-120°C. They demonstrated that a higher yield of acetol can be obtained at a pH of 7.0-7.1 and as pH decreased, the yield of acetol decreased.

This Example first illustrates the preparation of acetol via a reaction of 0.1 N NaOH and glucose under optimized conditions. A method to isolate the acetol from the reaction mixture is then illustrated.

5 Finally, it is demonstrated that the above mixture, without the isolation of acetol acetoin, can be reacted with NH₄OH to synthesize different branched pyrazines.

10 Glucose, 1-OH-acetone (acetol), 1-OH-2-butanone (acetoin), sodium hydroxide, di-sodium hydrogen phosphate / sodium hydroxide buffer solution pH=12, potassium phosphate dibasic, potassium phosphate monobasic, sodium chloride, sodium sulfate anhydrous, hydrochloric acid, methanol, and dichloromethane are obtained from Sigma-Aldrich (St. Louis, MO).

15 Synthesis of acetol is performed in a 40mL or 1.5L Parr vessel. To synthesize acetol, 0.5 grams of glucose is mixed for every 25 mL of 0.1 N NaOH or buffer solution. Each reaction is stirred and heated at 140°C for a period of 60 min. The initial pH levels for all reactions are approximately 12 and no adjustments are made during the reaction. After the completion of each reaction, the mixture is cooled and the quantity of both acetol and sugar are measured using GC and HPLC. For acetol quantification, 25 mL of solution is spiked with 1 mg of acetoin as an internal standard and extracted with 30-35 mL of DCM. Next, the extracted DCM solution is dried over sodium sulfate and analyzed via GC/FID for quantification.

20 The synthesis of pyrazines is performed in the same Parr vessel. Reactions are performed by reacting the acetol synthesized from the reaction of sugar and 0.1 N NaOH. In this reaction, every 25 mL of solution is reacted either with 0.25, 0.5 or 1 mL of NH₄OH. Reactions are obtained at 120 or 140°C for a period of 17 hours. Next, each reaction mixture is cooled and the pyrazines are extracted and quantified. In each extraction 0.25 mg of d₆-2 methyl pyrazine is added as an internal standard and the solution is extracted with 30-35 mL of DCM as a solvent. Next, the DCM solution is dried over sodium sulfate and analyzed via GC/MS for quantification. Extracted ions are used to quantify each pyrazine.

25 All GC/MS analyses are performed using the same equipment and operating parameters as those used in Example 1 above. MS Wiley library is used to identify each pyrazine. For quantitative analysis, the calibration curve is prepared using a concentration of acetol and a response factor ratio of acetol/acetoin. It is noted here that acetoin was not detected in any reactions performed at 90, 100 and 120°C. However, acetoin residue is found in the reaction where the temperature is set to 140°C. Therefore, acetoin concentrations are determined in the reaction and this value is accounted for in all calculations.

30 All HPLC/RI separations are performed using Sugar-Pak (300 x 6.5 mm) columns from Waters (Milford, MA). An Agilent 1100 series HPLC equipped with a quaternary pump, refractive index (RI) detectors, auto-sampler, and oven heater set to 80°C is employed. The isocratic mobile phase for analysis of sugar is 0.005% EDTA disodium dihydrate. The flow rate is set at 0.5 mL/min for these analyses.

35 First, acetol is synthesized using a buffer solution. Due to rapid changes in the pH of a solution in a reaction of glucose with 0.1 N NaOH solution for synthesis of acetol, a buffer solution with a pH of 12 (purchased from Sigma Aldrich) is used in the synthesis of acetol instead of a 0.1 N NaOH solution. In this

study, 25 mL of di-sodium hydrogen phosphate/sodium hydroxide buffer solution pH=12 is reacted with 0.25 or 0.5 gram of glucose at 140°C for 60 minutes. Results show that the concentration of acetol in the reaction product is much higher (2X) when a buffer solution is used to perform the synthesis. Table 8 below shows the results of this study.

5

Table 8 – Effect of glucose concentration on preparation of acetol using basic buffer solution from Sigma Aldrich

Solution	Glucose mg	Buffer Volume mL	pH	Rxn, Time Min	Temp., °C	Acetol, mass mg	% Yield based on Glucose
Buffer	0.25	25	12	60	140	6.53	5.2
Buffer	0.50	25	12	60	140	15.3	6.1

When a similar buffer is prepared (mixing 7.1 gram of Na₂HPO₄ and 1 gram of NaOH in 100 mL of H₂O with a pH of approximately 12 and capacity of 0.05 N) and reacted with glucose at 140°C for 60 minutes, the reaction mixture did not show a presence of acetol. This reaction is repeated three times and acetol is not found in any of the reaction products. However, when a similar buffer with the same pH but lower capacity (0.025 N) is used, acetol is found in the reaction product and concentration of it is much higher than when 0.1 N NaOH was used. As such, it is determined that the buffer type, capacity, pH, and reaction temperature have an effect on the synthesis of acetol using glucose.

Next, the acetol concentration in a reaction of glucose with 40% phosphate buffer with pH 6.5-7.0 is determined. 25 mL of a 40% potassium phosphate buffer pH=6.5-6.8 is reacted with 0.5 or 1 gram of glucose at 140°C for 60 minutes using a Parr reactor. Table 9 below shows the mass of acetol obtained from a reaction of a 40% phosphate buffer (25 mL) at a pH of 6.5-6.8 with different concentrations of glucose. It is noted here other types of hydroxy ketones such 3-OH-2-butanone and 1-OH-2-butanone are also formed in this reaction. The quantity of these hydroxy ketones is not measured. Figure 3 shows the GC/MS analysis of glucose reacted with a phosphate buffer at 140°C for 60 min and extracted with DCM.

Table 9 - Mass of Acetol obtained from reaction of 40% phosphate buffer with different concentration of glucose

Solution	Glucose (g)	Volume (mL)	Rxn, Time (min)	Temp. (°C)	Acetol, mass (mg)	% Yield based on Glucose
Buff. Phosph.	0.5	25	60	140	23.57	4.7
Buff. Phosph.	0.5	25	60	140	16.4	3.3
Buff. Phosph.	0.5	25	60	140	15.92	3.2
Buff. Phosph.	1	25	60	140	44.3	4.4
Buff. Phosph.	1	25	60	140	44.4	4.4

Next, the glucose and acetol concentrations in 300mL and 1L reactions are determined. 6 grams of glucose is reacted with 300 mL of 0.1 N NaOH for 60 min at 140°C. After the reaction is cooled, both

acetol and glucose concentrations in the solution are obtained. A small amount (0.964 mg/mL) of glucose is detected in the reactant. This corresponds to about less than 5% of the glucose that remained unreacted.

Table 10 below shows the concentration of acetol in this reaction. It can be determined that the

concentration of acetol is about 12 mg for every 25 mL. Two additional reactions are performed using 1

liter of 0.1 N NaOH and 20 grams of glucose using a high pressure reaction vessel. Both reactions are

performed at 130-140°C for 60 minutes. Both glucose and acetol concentrations are determined. In both 1

liter reactions, concentrations of glucose are less than 1 mg/mL (0.945 and 0.958 mg/mL). Table 10 below

also shows the concentration of acetol in each 1 liter reaction. Again, the acetol concentration is about 12

mg for every 25 mL.

Table 10 - Calculated mass of Acetol from reaction of 0.1N NaOH with different mass of glucose

Solution	Glucose (g)	Volume (mL)	Rxn, Time (min)	Temp. (°C)	Acetol, mass (mg)	% Yield based on Glucose
0.1N NaOH	6	300	60	140	140.26	2.3
0.1N NaOH	6	300	60	140	140.68	2.3
0.1N NaOH	20	1000	60	140	490.12	2.5
0.1N NaOH	20	1000	60	140	470.08	2.4

Next, acetol is isolated from the reaction of glucose with 0.1N NaOH. Different methods are used to

isolate acetol from the reaction of 0.1 N NaOH and glucose. Previous results for the isolation of pyrazines

via distillation demonstrated that pyrazines with a boiling point of 140°C were isolated from the reaction

mixture. Therefore, a distillation apparatus set at 120-140°C is used to isolate acetol from the reaction

mixture. For this purpose, 100mL of a reaction mixture of 0.1 N NaOH and glucose is distilled at 140°C.

After the collection of 40mL of distillation solution, both the distilled and the remaining materials are

extracted with DCM. GC/FID analysis shows that only 15-20% of acetol is distilled while more than 80-

85% of acetol remains in the reaction mixture.

Similar results are obtained when a reaction mixture of glucose with a 40% phosphate buffer at

140°C for 60 minutes is distilled. For this distillation, water is continuously added to the flask during the

distillation to keep the distilled solution volume constant. After the collection of about 10 mL solution via

distillation, approximately 10 mL H₂O is added to the distillation flask to compensate for the volume lost. A

total of 4x10 mL fractions are collected. Acetol is observed in each fraction. Analysis of the remaining

reaction mixture with DCM shows a presence of more acetol in the solution. The isolation of acetol can be

obtained if this distillation continues for several hours.

In a second method, column chromatography is used to isolate the acetol from the reaction mixture.

For this purpose a 30 x 2.0 cm glass (or metal) column (packed up to only 12-15 cm) with C₁₈ (particulate

size of 40-60 μ m and 90 Å pore size) is used to isolate the acetol. After washing the column with methanol followed by a 0.1% FA solution, 25 mL of the reaction mixture containing acetol is passed through the column. All of the solution is collected during this isolation. After pushing the solution through the column (fraction 1, 25 mL), 25 mL of deionized H₂O is used to wash the material from the column (Fraction 2).

5 Next, the column is washed with an additional 25 mL H₂O until clean H₂O is eluted from the column (Fraction 3). Then the column is dried with N₂, and the remaining analytes trapped in the column are eluted with 100% MeOH (Fraction 4). All fractions are extracted with DCM and analyzed via GC/FID. Results showed that most of the acetol is eluted with fractions 1 and 2 while fractions 3 and 4 did not contain any acetol. It is noted here that after passing the reaction mixture through the C₁₈ packing, it is very difficult to use the packing for another reaction mixture cleanup. It is very difficult to clean up the packing. In
10 summary, isolating the hydroxy ketones from the reaction product can be timely and expensive.

Next, pyrazines are synthesized from the reaction product of glucose and sodium hydroxide with NH₄OH, without first isolating the hydroxy ketones. After the preparation of acetol via reacting 0.5 grams glucose with 25 mL of 0.1 N NaOH at 140°C for 60 minutes, it is determined that the reaction mixture
15 contained about 10-12 mg of acetol/25 mL of solution. For this purpose, 25 mL of the solution is reacted with 0.5 or 1 mL of NH₄OH for a period of 17-18 hours at 120-140°C to determine the type and the percent yield of the pyrazines. Each reaction is repeated twice. Table 11 below shows list of pyrazines that are detected including their elution time from the GC column. Results are similar when the volume of NH₄OH is changed from 0.5 to 1 mL.

20

Table 11 - List of synthesized pyrazines

Retention time	Peak #	Analyte
5.82	1	Internal Standard
5.08	2	Pyrazine
5.82	3	2-Methylpyrazine
6.48	4	2,6-Dimethylpyrazine
6.56	5	2,5- Dimethylpyrazine
6.76	6	2,3- Dimethylpyrazine
7.178	7	2-Ethyl-5-Methylpyrazine
7.24	8	2-Ethyl-6-Methylpyrazine
7.4	9	2,3,5-Trimethylpyrazine
7.95	10	2,6- Dimethyl -3-Ethylpyrazine
8	11	2,Ethyl-2,5- Dimethylpyrazine
8.1	12	2,3,5,6-Trimethylpyrazine
8.44	13	3,5-Diethyl-2- Methylpyrazine
8.63	14	2,5-Dimethyl- 3Propylpyrazine
8.81	15	2,5-Diethyl-3,6- Dimethylpyrazine
9.4	16	6,7-DiHydrocyclo-5-

		Methylcyclopentapyrazine
9.669	17	2-Isopropenylpyrazine
9.69	18	2,3-Dimethyl-5-Isopentylpyrazine
9.89	19	2,5-Dimethyl-3-(3-methylbutyl)pyrazine
9.96	20	6,7-DiHydrocyclo-2,5-Dimethyl-5H-cyclopentapyrazine
10.238	21	2-Methyl-5H-6,7-diHydrocyclopentapyrazine

Similar reactions are performed in the 1 liter scale. Two batches of 1 liter solution are prepared. In each batch, 20 grams of glucose is reacted with 1000 mL of 0.1 N NaOH solution at 140°C for 60 min. Each solution is cooled and the acetol concentration is measured. Next, in one reaction mixture, 20 mL of NH₄OH is added, and in a second reaction mixture, 40 mL of NH₄OH is added. Each reaction is heated at 120-130°C for 17 hours with continuous mixing. After the reactions are cooled, 25 mL of each reaction is spiked with an internal standard (d₆-2-methylpyrazine) and extracted with DCM to determine the concentration and distribution of each pyrazine. Each remaining reaction solution is distilled and collected separately. Approximately 125 mL of distilled solution is collected for every 500 mL of reaction mixture. Next, the distilled solution (10 mL) is extracted with DCM and quantified via GC/MS. The total mass of pyrazines is higher than if the solution had not been extracted with DCM, which is due to a higher concentration of pyrazines in the distilled solution. The distribution of pyrazines is almost identical in both extracts. It is noted that both 2-methylpyrazine and pyrazine are detected in large quantities in all reactions when NH₄OH is reacted with the reaction mixture of glucose with 0.1 N NaOH. No pyrazines are detected in the extract of the remaining solution after distillation.

Next, pyrazines are synthesized from the reaction product of glucose and phosphate buffer with NH₄OH, without first isolating the hydroxy ketones. 25 mL of the reaction mixture of a phosphate buffer with pH = 6.5-6.8 and glucose (0.5 or 1 gram, solutions A and B, respectively) is reacted with 1 mL of NH₄OH at 140°C for 17 hours. After each reaction is cooled down, 0.25 mg of deuterated 2-methylpyrazine (internal standard) is added to each reaction mixture and the solutions are extracted with DCM. Each extract is analyzed using GC/MS and the mass of pyrazines and their percent distribution is calculated. It is noted that both pyrazine and 2-methylpyrazine are not synthesized in these reactions. Also, the total mass of pyrazines is higher by 20% in solution B when 1 gram of glucose is reacted with a phosphate buffer. Without being limited by theory, it is believed that this is due to a higher concentration of acetol in solution B compared to solution A. Figure 4 shows the GC/MS analysis of pyrazines extracted using DCM from a reaction mixture of solution B (1 gram of glucose reacted with 25mL of 40% phosphate buffer at 140°C for 60 min) with 1 mL of NH₄OH at 140°C for 17 hours. For comparison, Figure 5 shows the GC/MS analysis of pyrazines extracted using DCM from 25 mL of a reaction mixture of 0.1 N NaOH reacted with 0.5 gram of glucose at 140°C for 60 min and then reacted with 1 mL of NH₄OH at 140°C. In this reaction, both pyrazine and 2-methylpyrazine are detected.

In summary, acetol (and other hydroxy ketones) can be isolated from a reaction mixture of glucose and 0.1 N NaOH using both distillation and column chromatography. Distillation is very time and energy consuming while chromatography can become very expensive. However, it is possible to synthesize pyrazines without the isolation of acetol. When the acetol prepared from the reaction of 0.1 N NaOH and glucose reacted with NH_4OH at 140°C for 17 hours, an array of pyrazines were produced. The pyrazines were isolated via distillation. It is noted that pyrazine and 2-methylpyrazine were synthesized during this process. However, when the acetol prepared from the mixture of glucose and phosphate buffer reacted with NH_4OH , similar pyrazines were produced with a similar distribution, but no pyrazine or 2-methyl pyrazine was detected in the reaction mixture.

Many modifications and other embodiments of the invention will come to mind to one skilled in the art to which this invention pertains having the benefit of the teachings presented in the foregoing description. Therefore, it is to be understood that the invention is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

WHAT IS CLAIMED IS:

1. A method of forming pyrazines comprising:
receiving a reaction solution comprising at least one alpha-hydroxy ketone and at least one nitrogen source; and
heating the reaction solution to a reaction temperature and holding the reaction solution at the
5 reaction temperature for a time sufficient to produce a reaction product comprising at least one substituted pyrazine.
2. The method of claim 1, wherein the at least one hydroxy ketone comprises acetol.
- 10 3. The method of claim 2, wherein the at least one substituted pyrazine is selected from the group consisting of: 2,3-dimethylpyrazine; 2,6-dimethylpyrazine; 2-ethyl-6-methylpyrazine; 2-ethyl-5-methylpyrazine; trimethylpyrazine; furaneol; 2,5-dimethyl-3-ethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; tetramethylpyrazine; 2,5-dimethyl-3-propenylpyrazine; 2,3,5-trimethyl-6-isopropylpyrazine; 2-acetyl-4,5-dimethylpyrazine; 3,5-dimethyl-2-methylpropylpyrazine, and combinations thereof.
- 15 4. The method of claim 1, wherein the at least one hydroxy ketone comprises acetoin.
5. The method of claim 4, wherein the at least one substituted pyrazine is tetramethylpyrazine.
- 20 6. The method of claim 1, wherein the at least one hydroxy ketone comprises 1-hydroxy-2-butanone.
7. The method of claim 6, wherein the at least one substituted pyrazine is selected from the group consisting of: 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-methyl-2,n-propyl-6(1-butenyl)pyrazine; 2,5-dimethyl-3-ethylpyrazine; and
25 combinations thereof.
8. The method of claim 1, wherein the nitrogen source is selected from the group consisting of amino acids, ammonium ions, and combinations thereof.
- 30 9. The method of claim 1, further comprising adding free amino acids to the reaction solution.
10. The method of claim 1, further comprising adding at least one aldehyde to the reaction solution.

11. The method of claim 1, further comprising isolating the at least one substituted pyrazine from the reaction product.
12. The method of claim 11, wherein the step of isolating the at least one substituted pyrazine from the reaction product comprises at least one of liquid-liquid extraction of the reaction product, liquid-solid extraction of the reaction product, and simple distillation of the reaction product.
13. The method of claim 1, wherein the reaction temperature is about 90°C to about 150°C.
14. The method of claim 1, wherein the at least one substituted pyrazine is selected from the group consisting of: 2,6-dimethylpyrazine; 2,5-dimethylpyrazine; 2-ethyl-5-methylpyrazine; 2-ethyl-6-methylpyrazine; 2,3,5-trimethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; 2-ethyl-2,5-dimethylpyrazine; 2,3,5,6-trimethylpyrazine; 2,3,5-trimethyl-6-ethylpyrazine; 2,6-dimethyl-3-propylpyrazine; 2,5-diethyl-3,6-dimethylpyrazine; 2,6-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(3-methylbutyl)pyrazine; 2,5-dimethyl-3-propylpyrazine; 2,5-dimethyl-3-cis-propenylpyrazine; 2-isopropenyl-3,6-dimethylpyrazine; 2-(2-methylpropyl)-3,5-dimethylpyrazine; 2,6-dimethyl-3-isobutylpyrazine; 2-(2-methylpropyl)-3,5,6-trimethylpyrazine; 2,3-dimethylpyrazine; trimethylpyrazine; furaneol; 2,5-dimethyl-3-ethylpyrazine; tetramethylpyrazine; 2,3-diethyl-5-methylpyrazine; 2,5-dimethyl-3-propenylpyrazine; 2,3,5-trimethyl-6-isopropylpyrazine; 2-acetyl-4,5-dimethylpyrazine; 3,5-dimethyl-2-methylpropylpyrazine; 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-methyl-2,n-propyl-6(1-butenyl)pyrazine; 2,5,7-trimethyl-6,7-dihydro-5H-cyclopentapyrazine; and 2,5-dimethyl-3-ethylpyrazine; and combinations thereof.
15. The method of claim 1, wherein the at least one substituted pyrazine is disubstituted.
16. The method of claim 1, wherein the at least one substituted pyrazine is trisubstituted.
17. The method of claim 1, wherein at least one substituted pyrazine is tetrasubstituted.
18. The method of claim 1, wherein the at least one substituted pyrazine comprises at least one substituent group having 2 or more carbon atoms.
19. The method of claim 1, wherein the at least one substituted pyrazine comprises at least one substituent group having 3 or more carbon atoms.

20. The method of claim 1, wherein the reaction product is substantially free of the molecule pyrazine and the molecule methylpyrazine.
21. The method of any of claims 1 through 20, further comprising incorporating the at least one substituted pyrazine into a tobacco product.
22. The method of claim 21, wherein the tobacco product is a smoking article or a smokeless tobacco product.
23. A method of forming pyrazines comprising:
receiving a carbon source solution comprising at least one sugar and at least one buffer;
mixing the carbon source solution with at least nitrogen source to form a reaction solution; and
heating the reaction solution to a reaction temperature and holding the reaction solution at the reaction temperature for a time sufficient to produce a reaction product comprising at least one substituted pyrazine.
24. The method of claim 23, wherein the at least one sugar is selected from the group consisting of glucose, fructose, rhamnose, and combinations thereof.
25. The method of claim 23, wherein the nitrogen source is selected from the group consisting of amino acids, ammonium ions, and combinations thereof.
26. The method of claim 23, wherein the buffer is selected from the group consisting of: sodium hydroxide, a phosphate buffer, and combinations thereof.
27. The method of claim 23, wherein the buffer buffers in a pH range of about 6.5 to about 7.5.
28. The method of claim 23, further comprising adding ammonium ions to the reaction solution.
29. The method of claim 23, further comprising isolating the at least one substituted pyrazine from the reaction product.
30. The method of claim 29, wherein the step of isolating the at least one substituted pyrazine from the reaction product comprises at least one of liquid-liquid extraction of the reaction product, liquid-solid extraction of the reaction product, and simple distillation of the reaction product.
31. The method of claim 23, wherein the reaction temperature is about 90°C to about 150°C.

32. The method of claim 23, wherein the at least one substituted pyrazine is selected from the group consisting of: 2,6-dimethylpyrazine; 2,5-dimethylpyrazine; 2-ethyl-5-methylpyrazine; 2-ethyl-6-methylpyrazine; 2,3,5-trimethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; 2-ethyl-2,5-dimethylpyrazine; 5 2,3,5,6-trimethylpyrazine; 2,3,5-trimethyl-6-ethylpyrazine; 2,6-dimethyl-3-propylpyrazine; 2,5-diethyl-3,6-dimethylpyrazine; 2,6-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(2-methylbutyl)pyrazine; 2,5-dimethyl-3-(3-methylbutyl)pyrazine; 2,5-dimethyl-3-propylpyrazine; 2,5-dimethyl-3-cis-propenylpyrazine; 2-isopropenyl-3,6-dimethylpyrazine; 2-(2-methylpropyl)-3,5-dimethylpyrazine; 2,6-dimethyl-3-isobutylpyrazine; 2-(2-methylpropyl)-3,5,6-trimethylpyrazine, 2,3-dimethylpyrazine; trimethylpyrazine; 10 furaneol, 2,5-dimethyl-3-ethylpyrazine; tetramethylpyrazine; 2,3-diethyl-5-methylpyrazine; 2,5-dimethyl-3-propenylpyrazine; 2,3,5-trimethyl-6-isopropylpyrazine; 2-acetyl-4,5-dimethylpyrazine; 3,5-dimethyl-2-methylpropylpyrazine; 2,6-diethylpyrazine; 2,5-diethylpyrazine; 2-ethyl-3,5,6-trimethylpyrazine; 3,5(3,6-dimethyl)-2,N-propylpyrazine; 2,5-diethyl-3-methylpyrazine; 2,3-diethyl-5,6-dimethylpyrazine; trans-3-methyl-2,n-propyl-6(1-butenyl)pyrazine; 2,5,7-trimethyl-6,7-dihydro-5H-cyclopentapyrazine; and 2,5-15 dimethyl-3-ethylpyrazine; and combinations thereof.
33. The method of claim 23, wherein the at least one substituted pyrazine is disubstituted.
34. The method of claim 23, wherein the at least one substituted pyrazine is trisubstituted.
- 20 35. The method of claim 23, wherein at least one substituted pyrazine is tetrasubstituted.
36. The method of claim 23, wherein the at least one substituted pyrazine comprises at least one substituent group having 2 or more carbon atoms.
- 25 37. The method of claim 23, wherein the at least one substituted pyrazine comprises at least one substituent group having 3 or more carbon atoms.
38. The method of claim 23, wherein the reaction product is substantially free of the molecules pyrazine and methylpyrazine.
- 30 39. The method of any of claims 23 through 38, further comprising incorporating the at least one substituted pyrazine into a tobacco product.
- 35 40. The method of claim 39, wherein the tobacco product is a smoking article or a smokeless tobacco product.

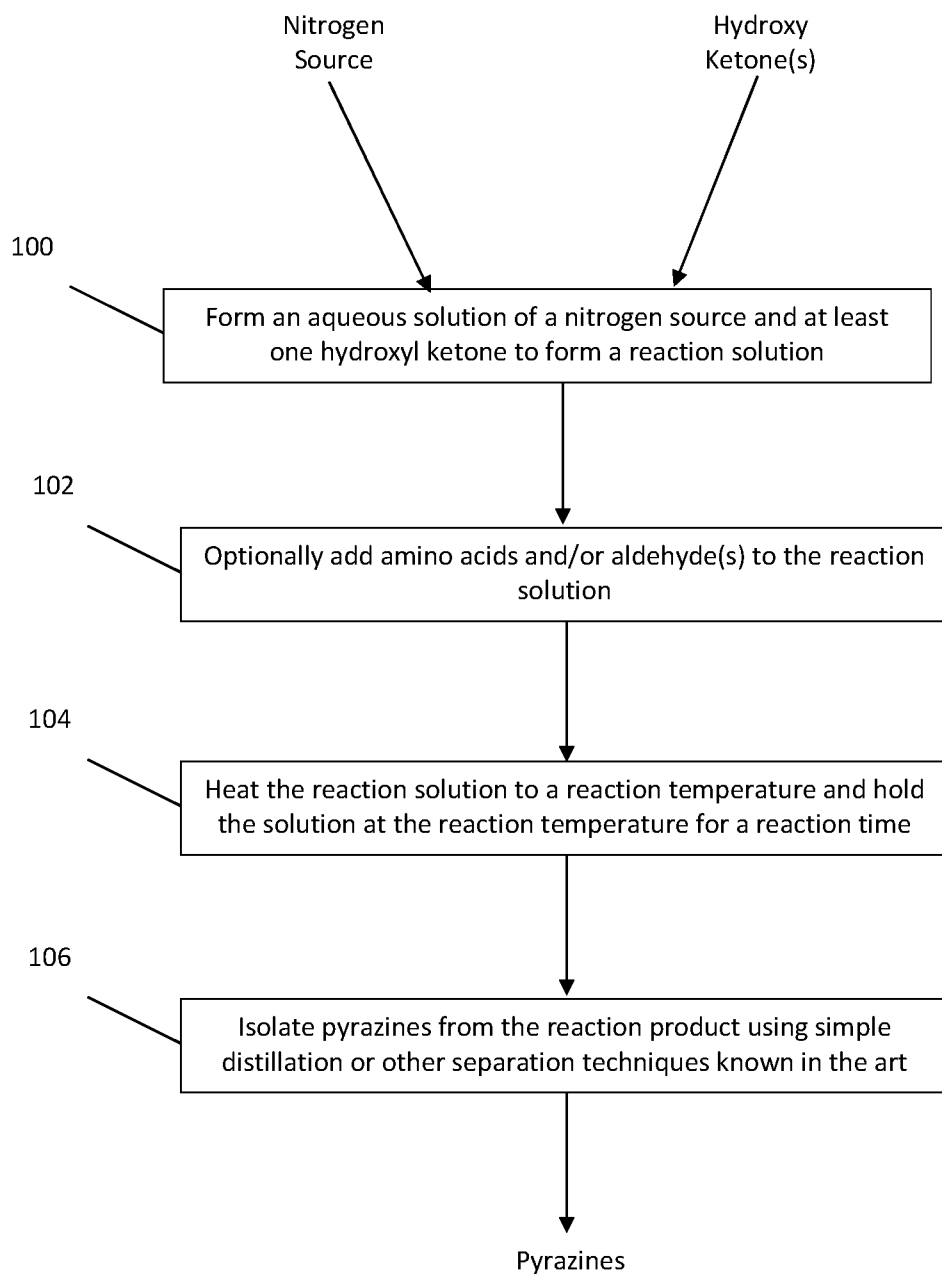


FIG. 1

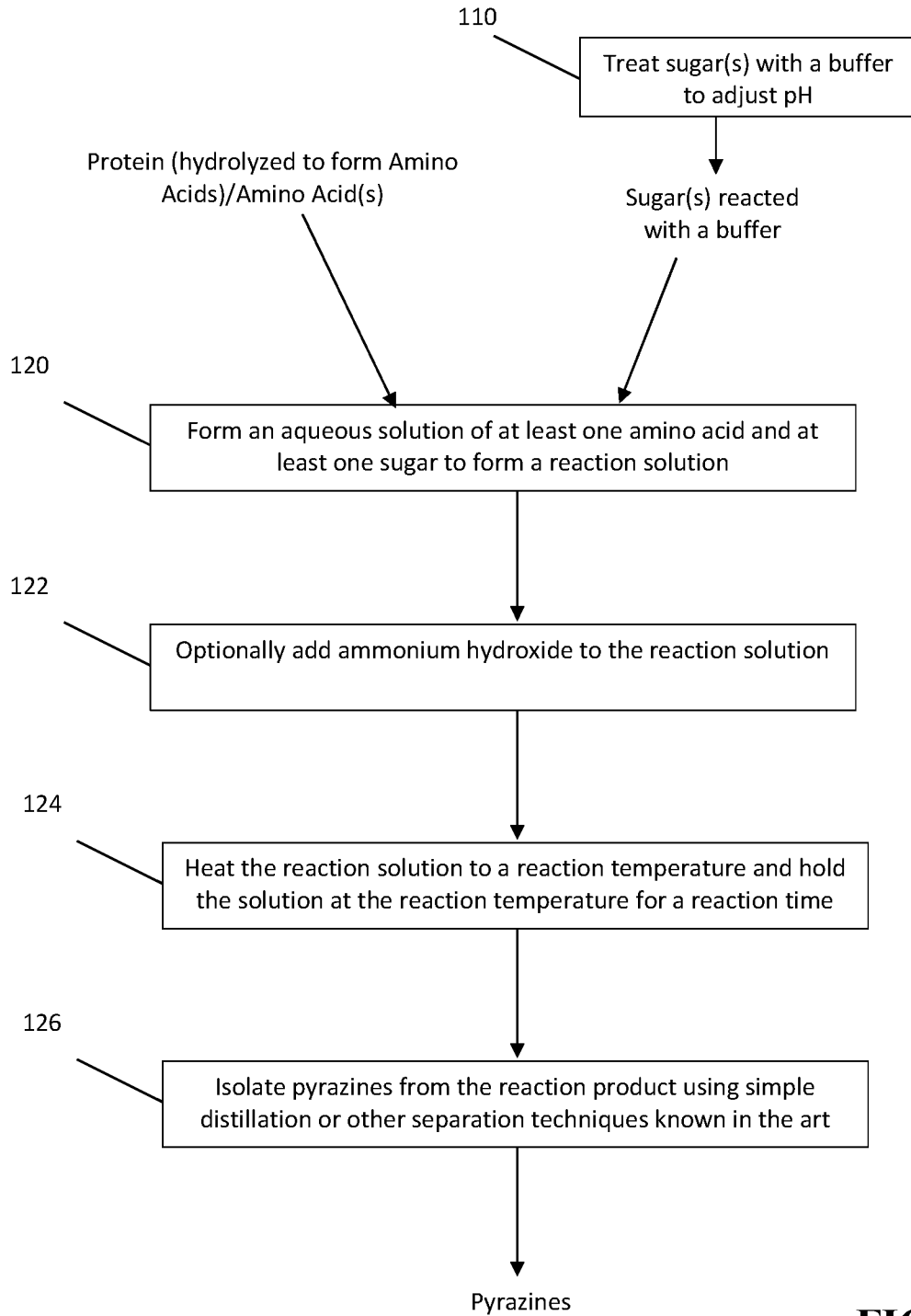


FIG. 2

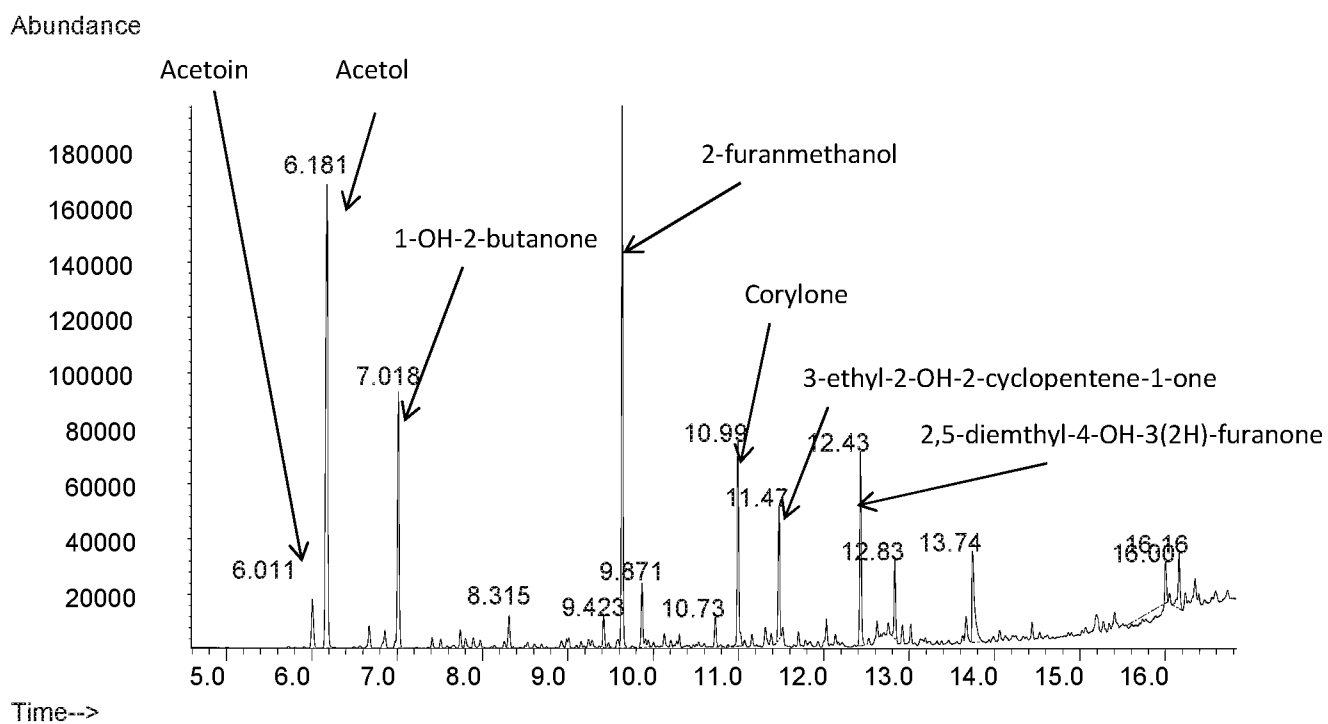


FIG. 3

Abundance

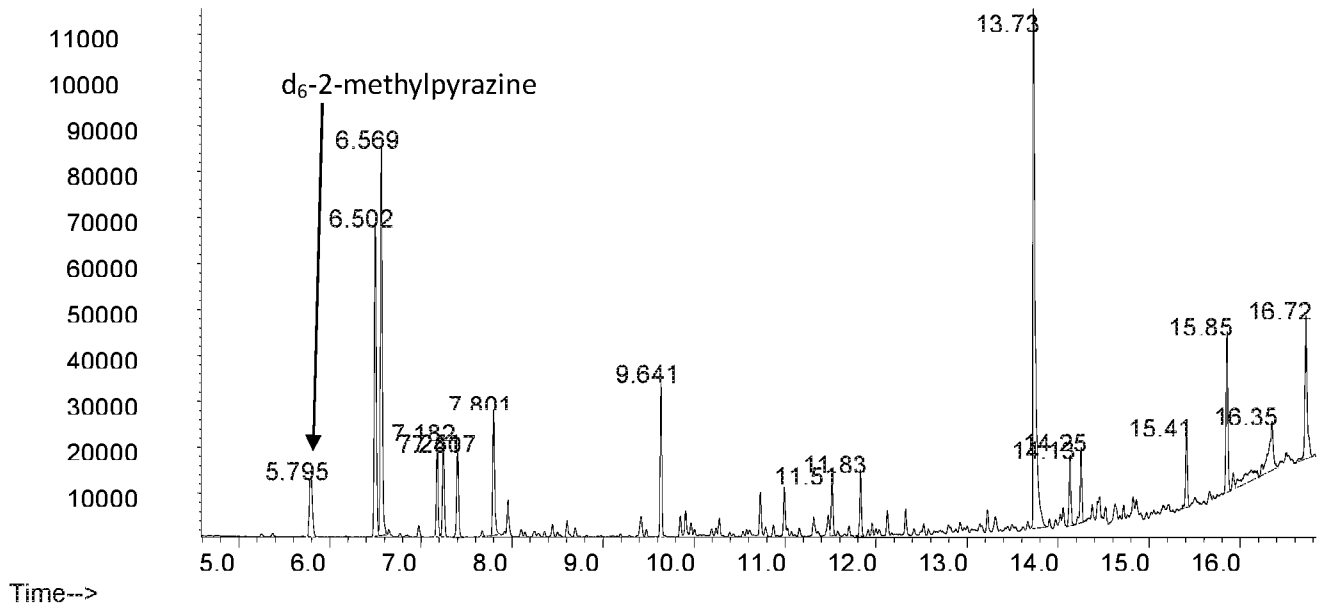


FIG. 4

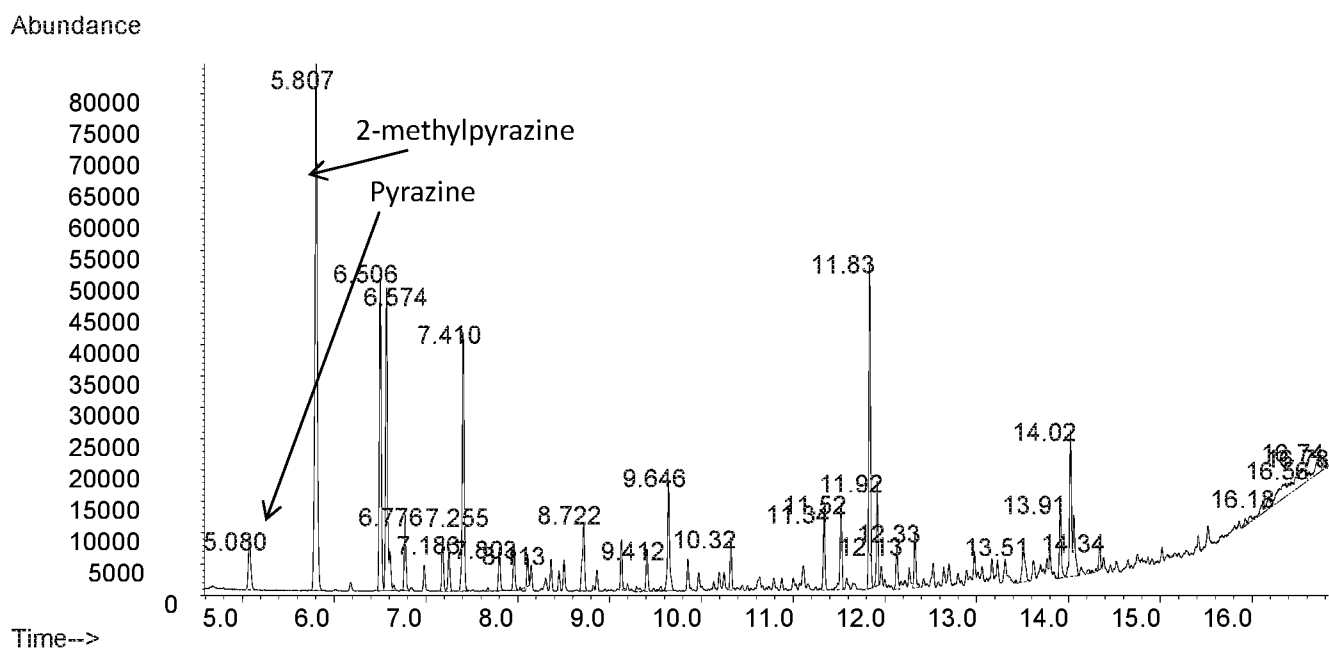


FIG. 5

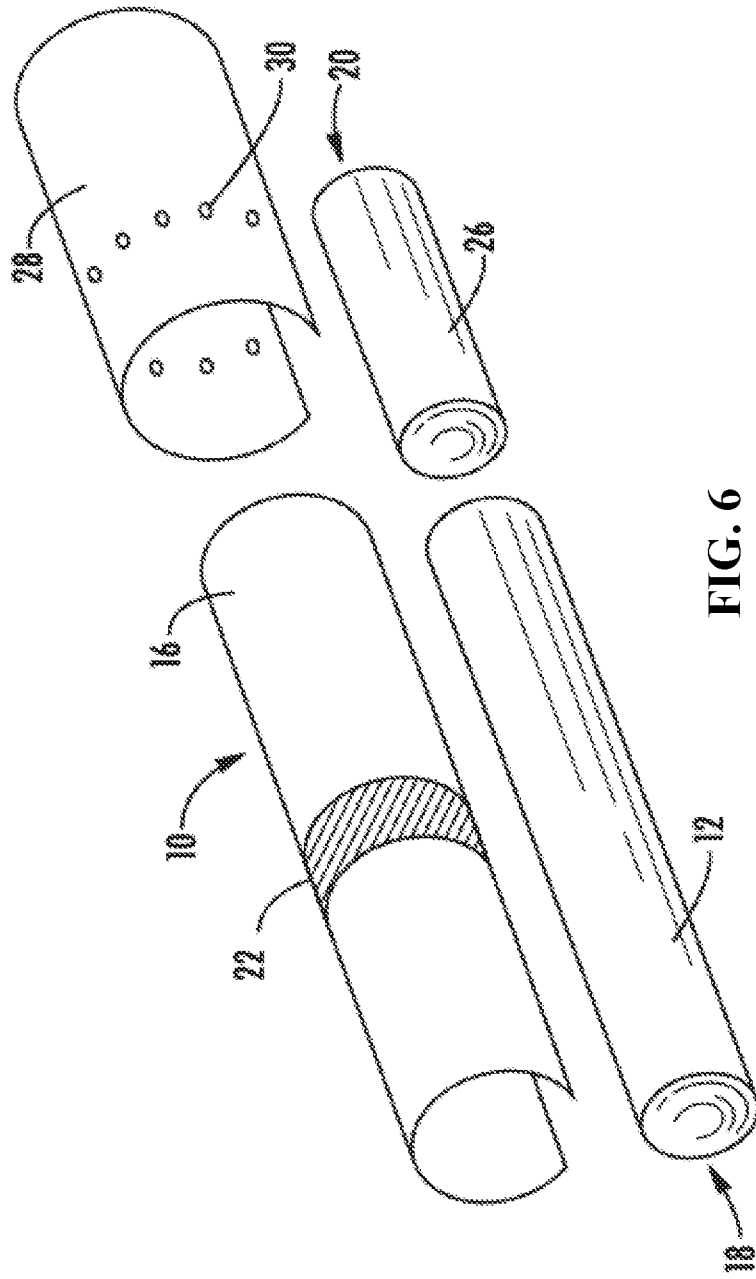
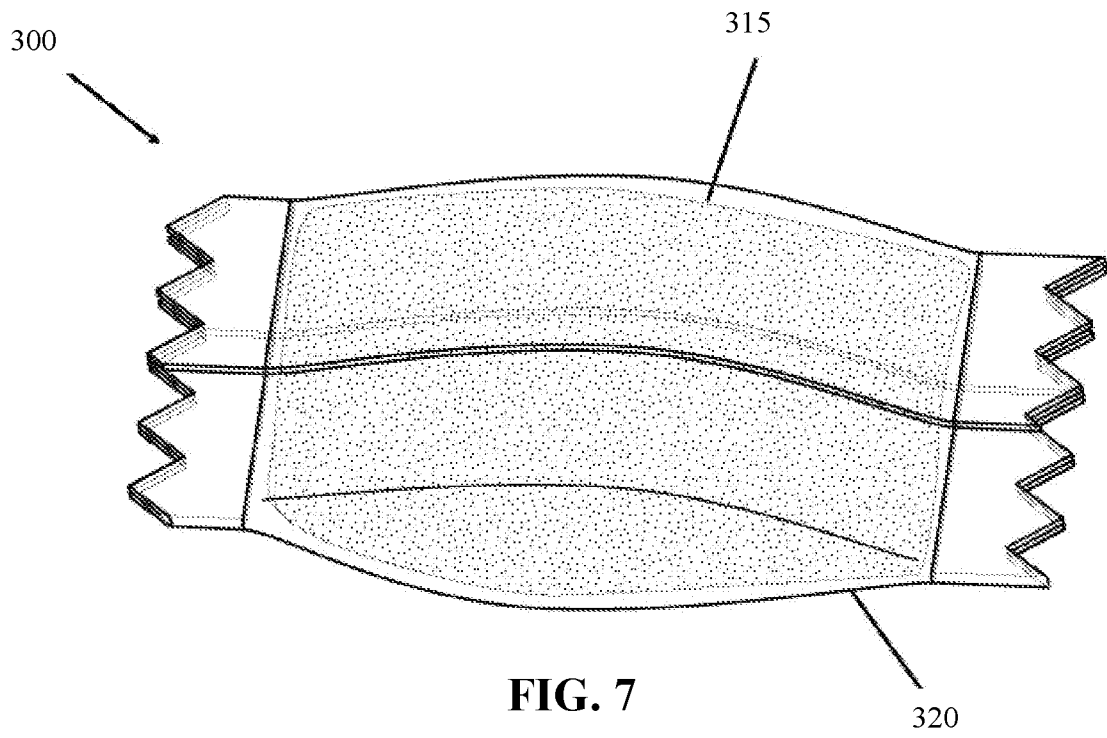


FIG. 6



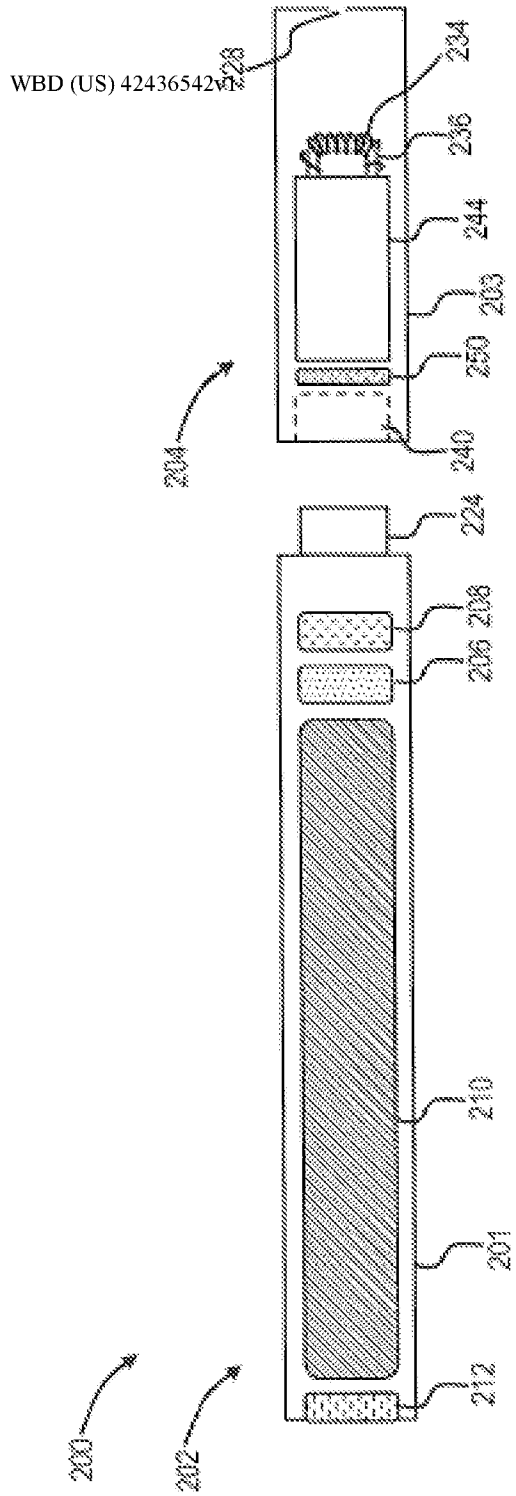


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2018/051994

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D241/12 A24B15/38 A24B15/42
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C07D A24B

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	LEI SONG ET AL: "One-pot synthesis of 2-hydroxymethyl-5-methylpyrazine from renewable 1,3-dihydroxyacetone", GREEN CHEMISTRY, vol. 19, no. 15, 5 May 2017 (2017-05-05), pages 3515-3519, XP055474494, GB ISSN: 1463-9262, DOI: 10.1039/C7GC00578D entries 7, 10 and 11 in Table 1 and Scheme 1 in p. 3516 ----- -/--	1-3,8, 13-15,20

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search 20 July 2018	Date of mailing of the international search report 08/08/2018
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Sahagún Krause, H
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INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2018/051994

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELMORE J S ET AL: "Effects of sulphur nutrition during potato cultivation on the formation of acrylamide and aroma compounds during cooking", FOOD CHEMISTRY, ELSEVIER LTD, NL, vol. 122, no. 3, 1 October 2010 (2010-10-01), pages 753-760, XP027039567, ISSN: 0308-8146 [retrieved on 2010-03-15]	1,6,8, 10,16, 18,20
Y	p. 755, 1st column l. 14-18 and 21-22 -----	9
X	WO 99/03842 A1 (RHONE POULENC RORER SA [FR]; BASHIARDES GEORGES [FR]; CARRY JEAN CHRIS) 28 January 1999 (1999-01-28) claim 6 and examples 1 and 3-8 -----	1,8,11, 13-15, 18-20
A	HILLEL R ALPERT ET AL: "A study of pyrazines in cigarettes and how additives might be used to enhance tobacco addiction", TOBACCO CONTROL, vol. 25, no. 4, 10 June 2015 (2015-06-10), pages 444-450, XP055361616, GB ISSN: 0964-4563, DOI: 10.1136/tobaccocontrol-2014-051943 box 1 -----	1-22
X	EP 0 505 891 A1 (NESTLE SA [CH]) 30 September 1992 (1992-09-30) -----	1-8, 11-15, 17,18,20
Y	examples and claim 1 -----	9
X	GUSTAVO LUIS LEONARDO SCALONE ET AL: "Influence of Free Amino Acids, Oligopeptides, and Polypeptides on the Formation of Pyrazines in Maillard Model Systems", JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 63, no. 22, 1 June 2015 (2015-06-01), pages 5364-5372, XP055493906, US ISSN: 0021-8561, DOI: 10.1021/acs.jafc.5b01129	23-26, 31-34, 36,38
Y	materials and methods in p. 5366-5368 ad Table 2 ----- -/--	27-30, 39,40

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2018/051994

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FIEN VAN LANCKER ET AL: "Formation of Pyrazines in Maillard Model Systems of Lysine-Containing Dipeptides", JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 58, no. 4, 24 February 2010 (2010-02-24), pages 2470-2478, XP055493914, US ISSN: 0021-8561, DOI: 10.1021/jf903898t	23-26, 31-38
Y	Model Reaction in Materials and Methods, p. 247 and Tables 1-3 -----	27-30, 39,40
A	BRANDES, S.: "Ueber pyrazine und piperazine", JOURNAL FUER PRAKTISCHE CHEMIE, vol. 2, no. 54, 1896, pages 481-495, XP002783209, p. 486-493 -----	23-40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2018/051994

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-22

A method of forming pyrazines comprising:
receiving a reaction solution comprising at least one
alpha-hydroxy ketone and at least one nitrogen source; and
heating the reaction solution to a reaction temperature and
holding the reaction solution at the reaction temperature
for a time sufficient to produce a reaction product
comprising at least one substituted pyrazine

2. claims: 23-40

A method of forming pyrazines comprising:
receiving a carbon source solution comprising at least one
sugar and at least one buffer;
mixing the carbon source solution with at least nitrogen
source to form a reaction solution; and
heating the reaction solution to a reaction temperature and
holding the reaction solution at the reaction temperature
for a time sufficient to produce a reaction product
comprising at least one substituted pyrazine.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2018/051994

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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			DE 69232923 D1 20-03-2003
			DE 69232923 T2 21-08-2003
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			JP H0597820 A 20-04-1993
			JP H0730050 B2 05-04-1995
			US 6225471 B1 01-05-2001