[redacted]

ROBERT H. SINDT
ATTORNEY AT LAW

1850 M Street, N.W., Suite 400
Washington, D.C. 20036
Phone 202-466-4500 • Fax 202-466-5777 • E-mail rsindt@krooth.com

December 19, 2000

Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St., SW
Washington, DC 20204

Re: GRAS Notice for Specified Uses of Egg White Lysozyme
GRAS Notice for Specified Uses of Nisin
GRAS Notice for Specified Uses of Hops Beta Acids

Dear Sir or Madame:

On behalf of my client, Rhodia, Inc., please accept the attached documentation, in compliance with the GRAS notification procedure set out in the April 17, 1997 Federal Register (62 FR 18937), as submissions of notices of GRAS exemption claims for the above referenced substances, i.e. specified uses of egg white lysozyme, specified uses of nisin, and specified uses of hops beta acids. As specified in the aforementioned proposed rule, each GRAS notice is submitted in triplicate and contains: a signed exemption claim; detailed information on the substance, on any self-limiting levels of use, and on the basis for the determination; and an appendix containing further referenced and substantiating information on the substance.

Please promptly contact me should you have any question regarding any of the submitted notices. We look forward to receiving acknowledgment of receipt of the notices and to a response for each noticed substance. Thank you.

Sincerely,

Robert H. Sindt

Enc.
RHS/bs

[redacted]

ROBERT H. SINDT
ATTORNEY AT LAW

1850 M Street, N.W., Suite 400
Washington, D.C. 20036
Phone 202-466-4500 •Fax 202-466-5777 •E-mail rsindt@krooth.com

December 15, 2000

Dr. Linda Kahl
Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Re: GRAS Notice-Exemption Claim for Specified Uses of Hops

Dear Dr. Kahl:

On behalf of my client, Rhodia Inc., and in accordance with FDA's proposed rule of April 17, 1997 (62 FR 18938) relating to the filing of generally recognized as safe (GRAS) notices, please accept this claim and the attached information, submitted in triplicate, for that purpose as it relates to the use of hops, as hops beta acids, in certain foods. Specifically, Rhodia claims that use of hops, as hops beta acids, as an antimicrobial agent for frankfurters, and for cooked meat and poultry products sold ready-to-eat, is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on its determination that such use is GRAS. In conformity with the requirements outlined in the proposed rule, the following information is included with this exemption claim:

(i) Name and Address of the Notifier: Rhodia Inc.
CN 7500
259 Prospect Plains Road
Cranbury, NJ 08512-7500

(ii) Common or Usual Name of Notified Substance: Hops, as hops beta acids

(iii) Applicable Conditions of Use: Hops, as hops beta acids, are manufactured in compliance with current Good Manufacturing Practice as specified in 21 CFR Part 110 and the Food Chemicals Codex, Fourth Edition and any subsequent amendment thereto, and meeting the requirements for essential oils, oleoresins (solvent-free), and natural extractives (including distillates) for hops (from the plant source, humulus lupulus L.), listed at 21 CFR 184.1538. Hops, as hops beta acids, is proposed for use as an antimicrobial agent on casings for frankfurters at a

nisin

000024

[redacted]

[redacted]

concentration of 2.5 mg hops beta acids/lb of frankfurter, approximately 5.5 mg hops beta acids/kg of food, and for use as an antimicrobial agent on cooked meat and poultry products sold ready-to-eat at 2.0 mg hops beta acids/lb of cooked meat or poultry product, approximately 4.4 mg hops beta acids/kg of food. All persons greater than two years of age are expected to consume the substance.

(iv) Basis for the GRAS Determination: Scientific procedures and Common use in food

(v) Availability to FDA of Data and Information that are Basis of Determination: The data and information forming the basis for Rhodia's GRAS determination and the exemption claim asserted herein are available for FDA review and copying during reasonable business hours at the following address, or will be sent to FDA upon request: Robert H. Sindt, Attorney at Law
Suite 400
1850 M Street, NW
Washington, DC 20036
Phone: (202) 466-4500

Consequently, on the basis of the above specified information, and the additional requested information as specified in the proposed rule and submitted with this letter, please accept this as Rhodia's claim of exemption from the statutory premarket approval requirements for the use of hops, as hops beta acids, as an antimicrobial agent for frankfurters and for cooked meat and poultry products sold ready-to-eat. Should you have any questions regarding the submission of this notice, please contact me at the above number. Thank you for your prompt consideration of, and response to, this notice.

Sincerely,

Robert H. Sindt

RHS:bs

Attachments

P:\Rhodia\Hops GRAS Notice Claim.doc

[redacted]

HOPS BETA ACIDS
GRAS NOTICE INFORMATION

Rhodia

[redacted]

[redacted]

(2) DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE (§170.36(c)(2))

- Common and Usual Name of the Food Grade Substance: Hops beta acids

- Chemical Name for Hops Beta Acids: None

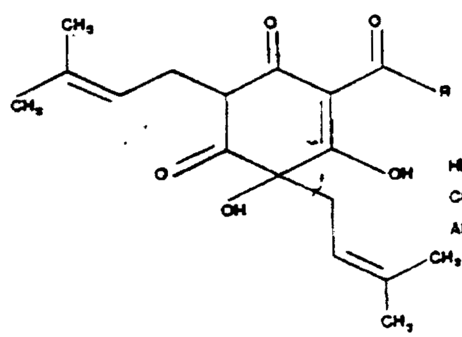
- Chemical Abstract Service (CAS) Registry Number for Hops Beta Acids: None

- Empirical Formula for Hops Beta Acids: Lupulone: $C_{27}H_{38}O_4$

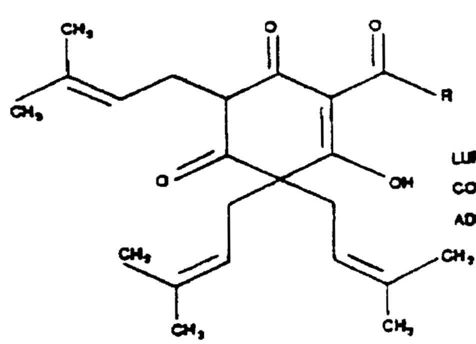
  Colupulone: $C_{26}H_{37}O_4$

  Adlupulone: $C_{27}H_{38}O_4$

- Structural Formula for Hops Beta Acids:



α-Acids
HUMULONE R = $CH_2CH(CH_3)_2$
COHUMULONE R = $CH(CH_3)_2$
ADHUMULONE R = $CH(CH_3)CH_2CH_3$



β-Acids
LUPULONE R = $CH_2CH(CH_3)_2$
COLUPULONE R = $CH(CH_3)_2$
ADLUPULONE R = $CH(CH_3)CH_2CH_3$

000027

[redacted]

- Quantitative Composition for Hops Beta Acids: Hops beta acids is a commercially available food ingredient, prepared from purified (80-90% pure) beta acids in liquid form that are dried on a carrier, such as maltodextrin. The purified hops beta acids are extracted from hops in comformity with current good manufacturing practice (GMP), and the GRAS listing for essential oils, oleoresins (solvent-free), and natural extractives (including distillates) at 21 CFR 182.20.

- Method of Manufacture for Hops Beta Acids: Hops beta acids are manufactured in compliance with current Good Manufacturing Practice (GMP) specified in 21 CFR, part 110, and in the Food Chemicals Codex, Fourth Edition and any subsequent amendment thereof.

  Hops processors extract component natural alpha and beta acids from resins contained in hops flower petals utilizing commonly accepted extraction methodology. The two acid fractions are then separated using liquid carbon dioxide under supercritical conditions or by chromatographic procedures. The resulting purified liquid extract normally contains 80-90% pure hops beta acid, and is typically dried on a carrier, such as maltodextrin, for commercial use.

- Characteristic Properties of Hops Beta Acids: Hops beta acids are contained along with alpha acids in resins which are found in small yellow vesicles called lupulin glands on the petals of hops flowers. Once extracted and isolated from the alpha acids, the primarily hops beta acid extract has antimicrobial activity against select

[redacted]

Gram positive spoilage bacteria, and has also been shown to inhibit the growth of *L. monocytogenes* in foods. Hops beta acids is the common and usual name for the family of chemically-related compounds. The empirical formulas for the primary hops beta acids are: lupulone-$C_{27}H_{38}O_4$; coluplone-$C_{26}H_{37}O_4$ and; adlupulone-$C_{27}H_{38}O_4$.

- Content of Potential Human Toxicants for Hops Beta Acids: None.

- Specifications for Food Grade Hops Beta Acids: Hops beta acids are extracted from hops flowers utilizing accepted extraction methodology in conformity with current GMP, and are dried for commercial food use on a carrier, such as maltodextrin. Specifications for a typical hops beta acids extract are as follows:

INGREDIENTS:	Maltodextrin & Hops Beta Acids
DESCRIPTION:	Powdered carbohydrate
AROMA:	Slightly aromatic
FLAVOR:	Slight astringency
COLOR:	Off-white to light yellow
MOISTURE:	6% max
ASH:	2% max
pH:	>7
TOTAL PLATE COUNT:	<2,500
LACTIC ACID COUNT:	<200
YEAST and MOLD:	<10
SALMONELLA:	None
COLIFORM:	None
LEAD (as Pb)	<2 ppm

[redacted]

HOPS, AS HOPS BETA ACIDS-GRAS NOTICE INFORMATION

(3) INFORMATION ON SELF-LIMITING LEVELS OF USE, IF ANY (§170.36(c)(3))

- No information on self limiting levels of hops beta acids use is noted.

[redacted]

(4) DETAILED SUMMARY OF THE BASIS FOR GRAS DETERMINATION (§170.36(c)(4))

(i) GRAS Determination Through Scientific Procedures

Rhodia's determination, that the notified uses of hops, as hops beta acids (as an anitmicrobial agent on casings for frankfurters and for cooked meat and poultry products sold ready-to-eat) are exempt from premarket approval requirements because such uses are GRAS, is based both on scientific procedures and on experience based on common use in food. The determination was confirmed by an independent panel of scientific experts convened by Rhodia to conduct such a critical review. Each member of the independent expert panel was qualified by their scientific training and experience to evaluate the safety of substances used in food. The independent expert panel's report and determination, dated November 2000, is included in its entirety in the Appendix attached hereto.

A. Safety of Hops Beta Acids:

Hops beta acids, for the uses proposed herein, meets the GRAS substance requirements for essential oils, oleoresins (solvent-free) and natural extractives (including distillates) for hops, from the plant source Humulus lupulus L., as listed by FDA at 21 CFR 182.20, when extracted utilizing commonly accepted extraction methodology in conformity with current GMP.

In connection with its determination, Rhodia requested Novigen Sciences, Inc. (Novigen) to conduct an estimated dietary intake assessment for the proposed uses of hops beta acids. The complete intake assessment from Novigen is included in the Appendix attached hereto. Of particular significance are the following passages from the Novigen assessment: "At the request of Rhodia, Inc. (Rhodia), Novigen Sciences, Inc. (Novigen) has estimated the dietary intake of hops beta acids (HBA) by the U.S. population and selected population subgroups in support of a self-assessment of GRAS status. Hops beta acids are proposed for use in casings for frankfurters at concentrations of 2.5 mg HBA/lb frankfurter (approximately 5.5 mg HBA/kg food) and in cooked meats sold ready-to-eat at 2.0 mg HBA/lb cooked meat (approximately 4.4 mg HBA/kg food).

"Intakes were estimated from food consumption data collected by the U.S. Department of Agriculture (USDA). For the overall U.S. population, 2-day average per-user intakes ranged from 0.21 mg/person/day at the mean to 0.52 mg/person/day at the 95th percentile of intake. On a per-capita basis, intakes for the U.S. population ranged from 0.09 mg/person/day at the mean to 0.38 mg/person/day at the 95th percentile.

"Intake estimates based on individual survey days (i.e., not two-day average) ranged from 0.09 mg/person/day (mean per-capita) to 0.50 mg/person/day at the 95th percentile per-capita intake. On a per-user

[redacted]

basis, intake estimates ranged from a mean of 0.34 mg/person/day to 0.81 mg/person/day at the 95th percentile of intake."

The independent expert panel convened by Rhodia engaged in a comprehensive safety review and stated in its report that "There are extensive data, including numerous published reports, to support the safety of hops when used in foods, especially beer, as an antimicrobial agent. Hops and their associated acids have long been recognized as effective bacteriological inhibitors, primarily against Gram positive bacteria. Recent studies also show that hops extracts of beta acids are extremely effective in controlling L. monocytogenes in foods, particularily certain cheeses and meat and poultry products. The antimicrobial efficacy demonstrated by the hops beta acids extracts is consistent in all respects with such functionality long exhibited by the use of hops in foods, especially beer. Microbial resistance varies among bacterial species and the literature supports that resistance develops only at high levels of usage (≥0.1%). The safety of hops and the component acids extracted therefrom is also supported by a long history of safe use in foods, especially beer, throughout recorded history.

"Estrogenic activity associated with hops harvesting and brewing has been reported in the scientific literature since the 1950s and anecdotally has been noted since the 15th century. Cold sludge baths containing 30 % hops were considered to have rejuvenating effects and were used for the treatment of gynecological disturbances. Menstrual

[redacted]

irregularities have been reported in female hops workers (cited in Fenselau and Talalay, 1973 and Milligan et al, 1999). Contradictory results have been reported in the scientific literature on the estrogenic activity of hops. Koch and Heim (1953, cited in Fenselau and Talalay, 1973 and Lilligan et al., 1999) and Zenisek and Bednar (1960) found extraordinary levels of estrogenic activity in beer, hops, or hops extracts. In contrast, Fenselau and Talalay (1973) reported no estrogenic activity of essential hops oil, alpha hops acid, beta hops acid, total hops resins, ethanolic extracts of hops, or commercial hops extracts. The variability of the results may be the result of the variability of the extracts and the specific assays used.

"Milligan et al (1999) reported on the estrogenic potential of hops extracts derived from 17 different hops varieties using two assays for estrogenic activity, Ishikawa cells and yeast cells. They found that the estrogenic activity was concentrated in the polar, polyphenol-containing fraction of all 17 varieties. The estrogenic potential of various hops extract components, including 8-prenylnarigenin and three other related hops prenylated polyphenols (xanthohumol, isoxanthohumol, and 6-prenylnarigenin) were compared to the estrogenic activity of 17 beta-estradiol, and the phytoestrogens coumestrol, genistein, and daidzein. Milligan and coworkers were able to clearly identify 8-prenylnarigenin as the substance responsible for the reported estrogenic activity of hops. Isoxanthohumol and 6-prenylnarigenin were only very weakly estrogenic. Xanthohumol was inactive in both estrogenic assays tested. No estrogenic

[redacted]

activity was detected in any of the other, less polar fractions of the hops extracts, including those of the alpha and beta hops acids which are responsible for the bitter taste of beer.

"The physiological properties of hops and their metabolism have been investigated by researchers in recent years. In addition to their sedative properties, phytoestrogenic activities have been proposed based on animal studies. However, these proposed physiological activities are observed only at very high dosages that well exceed the proposed use levels in this document. Duncan, et al (J.Am. Vet. Med. Assoc. 210:51, 1997) report anecdotally on 5 separate incidences of malignant hyperthermia in dogs that ingested large quantities of hops. Four of the five dogs were greyhounds, a breed that is very sensitive to malignant hyperthermia. This study is not relevant to the safety of hops beta acids since the doses were very high (in one dog that was subjected to gastric lavage, 250 ml of hops was recovered) and the condition is not common in humans. It can be triggered in susceptible pigs and dogs by stress or excitement but the most common triggers are anesthetic agents. Shipp, et al (Fd.Chem. Tox. 32:1007, 1994) reported on the effect of colupulone, a hops beta acid, on hepatic cytochrome P-450 enzymatic activity in the rat. Male Sprague-Dawley rats were fed a diet containing 0.36 % colupulone for 5 days. This relatively high dose of colupulone did not appear to have any functional effects on enzyme activity nor did it influence the mutagenicity of P-450-metabolized promutagens. The authors concluded

[redacted]

that "The effect of long-term colupulone administration of in vivo cytochrome P-450 enzymatic activity remains to be determined."

Finally, in making its determination Rhodia, Inc. conducted a comprehensive search of the scientific literature for safety, toxicity, efficacy, and tolerance on hops beta acids and made this information available to the Expert Panel, which in turn utilized it in concluding the safety of the proposed uses of hops beta acids. The literature search is included in the Appendix hereto.

(B) Information that may Appear Inconsistent with GRAS Determination:

In making its GRAS determination, Rhodia's independent expert panel stated that "No information on hops beta acids is noted that appears to be inconsistent with the determination of safety or general recognition of safety for the proposed uses. Indeed, it is noted that FDA lists the essential oils, oleoresins (solvent free), and natural extractives (including distillates) of hops as substances generally recognized as safe for their intended use in conformity with GMP at 21 CFR 182.20."

(C) Expert Concensus for GRAS Determination:

As stated in (A) above, Rhodia conducted a comprehensive search of the scientific literature for safety, toxicity, efficacy, and tolerance on hops beta acids, a copy of which is included in the Appendix hereto. In

[redacted]

addition thereto, a number of the studies are referenced in the excerpts from the independent expert panel report also cited above.

Based on the information contained in the exemption claim, the above additional and supplementary information, and the information contained in the Appendix attached hereto, an ample basis exists to support determination of general recognition of safety for the meat and poultry uses of hops beta acids proposed herein. Indeed, the independent expert panel indicated a consensus of common knowledge of safety of the proposed uses of hops beta acids among the qualified scientific community in concluding its review by determining that "The members of the Expert Panel, having independently and collectively critically evaluated the information summarized above and included in the appendices to this report, unanimously conclude that the natural extractive, hops beta acids, when produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is safe for use as an antimicrobial agent in certain foods.

"The members of the Expert Panel further concluded that the natural extractive, hops beta acids, produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is generally recognized as safe (GRAS), based on scientific procedures and common use in food throughout recorded history, for use as an antimicrobial for cooked meat and poultry products as specified herein. This conclusion is consistent with FDA's finding that natural extractives of hops are GRAS. 21 CFR 182.20."

(ii) GRAS Determination–Experience Based on Use in Food

Rhodia's determination, that the notified uses of hops, as hops beta acids (as an anitmicrobial agent on casings for frankfurters and for cooked meat and poultry products sold ready-to-eat) are exempt from premarket approval requirements because such uses are GRAS, is based both on scientific procedures and on experience based on common use in food. The determination was confirmed by an independent panel of scientific experts convened by Rhodia to conduct such a critical review. Each member of the independent expert panel was qualified by their scientific training and experience to evaluate the safety of substances used in food. The independent expert panel's report and determination, dated November 2000, is included in its entirety in the Appendix attached hereto.

(A) Safety of Hops Beta Acids:

The best indication of a showing of a substantial history of consumption by a significant number of consumers of hops beta acids is the discussion of the independent expert panel in its report of the history of use of hops. The independent expert panel stated "Hops are ubiquitous, naturally occurring plants. Throughout recorded history, hops flowers have been added as an essential ingredient in beer, and, as such, exhibit a substantial history of safe consumption. Hops flowers, and the acids or resins extracted therefrom, have long been recognized as bacteriological inhibitors, especially against Gram positive bacteria. Historically, hops has been used to inhibit lactobacilli and other bacterial contaminants in beer. The antimicrobial activity is due to the hops resins' humulone and lupulone and their chemical derivatives.

'Modern brewing hygienic practices have minimized the need for the use of hops and hops acids as antimicrobial agents. Instead, hops, commonly in the form of natural hops acid extracts, continue to be a required component of beer due to the necessity to maintain the bitter flavor it has historically imparted to beer. The natural bitter acids contained in hops are extracted and used primarily for this purpose. The most prevalent natural bitter acids in hops are alpha acids (humulones) and beta acids (lupulones), with the alpha acids preferred for bittering. Hops acids or resins are comprised of a family of compounds all with a basic allicyclic structure (2,4-cyclohexadiene-1-one). Existing techniques allow separation of natural hops acid extracts into primarily alpha acid and beta acid fractions. The separated natural acid extracts have been shown to maintain identity and to demonstrate functionality characteristics consistent with those recorded throughout history for hops, including as an antimicrobial agent. Natural hops beta acids, specifically, have been shown to be particularily efficacious against *Listeria monocytogenes* and against sporeforming bacteria in the genera *Bacillus* and *Clostridium.*

"The U.S. Food and Drug Administration (FDA) lists the essential oils,oleoresins (solvent-free) and natural extractives (including distillates) of hops to be substances generally recognized as safe for their intended use (21 CFR 182.20)."

Later in its report when discussing safety, the independent expert panel observed "There are extensive data, including numerous published reports, to support the safety of hops when used in foods, especially beer, as an antimicrobial agent. Hops and their associated acids have long been recognized as effective bacteriological inhibitors, primarily against Gram positive bacteria. Recent studies also show that hops extracts of beta acids are extremely effective in controlling *L. monocytogenes* in foods, particularily certain cheeses and meat and poultry products. The antimicrobial efficacy demonstrated by the hops beta acids extracts is consistent in all respects with such functionality long exhibited by the use of hops in foods, especially beer. Microbial resistance varies among bacterial species and the literature supports that resistance develops only at high levels of usage (≥0.1%). The safety of hops and the component acids extracted therefrom is also supported by a long history of safe use in foods, especially beer, throughout recorded history."

(B) Information That May Appear Inconsistent With GRAS Determination:

In making its GRAS determination, Rhodia's independent expert panel stated that "No information on hops beta acids is noted that appears to be inconsistent with the determination of safety or general recognition of safety for the proposed uses. Indeed, it is noted that FDA lists the essential oils, oleoresins (solvent free), and natural extractives (including distillates) of hops as substances generally recognized as safe for their intended use in conformity with GMP at 21 CFR 182.20."

(C) Expert Consensus for GRAS Determination:

As stated above, Rhodia conducted a comprehensive search of the scientific literature for safety, toxicity, efficacy, and tolerance on hops beta acids, a copy of which is included in the Appendix hereto. In addition thereto, a number of the studies are referenced in the excerpts from the independent expert panel report also cited above.

Based on the information contained in the exemption claim, the above additional and supplementary information, and the information contained in the Appendix attached hereto, an ample basis exists to support determination of general recognition of safety for the meat and poultry uses of hops beta acids proposed herein. Indeed, the independent expert panel indicated a consensus of common knowledge of safety of the proposed uses of hops beta acids among the qualified scientific community in concluding its review by determining that "The members of

[redacted]

the Expert Panel, having independently and collectively critically evaluated the information summarized above and included in the appendices to this report, unanimously conclude that the natural extractive, hops beta acids, when produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is safe for use as an antimicrobial agent in certain foods.

"The members of the Expert Panel further concluded that the natural extractive, hops beta acids, produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is generally recognized as safe (GRAS), based on scientific procedures and common use in food throughout recorded history, for use as an antimicrobial for cooked meat and poultry products as specified herein. This conclusion is consistent with FDA's finding that natural extractives of hops are GRAS. 21 CFR 182.20."

[redacted]

HOPS BETA ACIDS
SELF GRAS DETERMINATION

APPENDIX

Rhodia

[redacted]

HOPS BETA ACIDS GRAS NOTICE APPENDIX

INDEX TO INCLUDED ITEMS

[redacted]

INDEPENDENT GENERALLY RECOGNIZED AS SAFE DETERMINATION OF

HOPS BETA ACIDS

(IN CERTAIN COOKED MEAT AND POULTRY PRODUCTS)

NOVEMBER 2000

[redacted]

Independent GRAS Determination of Hops Beta Acids

1. Introduction

The undersigned, an independent panel of recognized experts (hereinafter, the Expert Panel), qualified by their scientific training and relevant national and international experience in evaluating the safety of food and food ingredients, were requested by Rhodia, Inc. to review and affirm the generally recognized as safe (GRAS) status of hops beta acids for use as an antimicrobial agent for certain cooked meat and poultry products as further specified herein. The members of the Expert Panel include Professor Joseph F. Borzelleca (Medical College of Virginia), Professor Eric A. Johnson (University of Wisconsin), and John Cerveny (formerly Director of Product Safety at Oscar Meyer). The qualifications of the members of the Expert Panel are evidenced in their curricula vitae with appear in Appendix 1.

2. Basis for GRAS Status

Rhodia, Inc. conducted a comprehensive search of the scientific literature for safety, toxicity, efficacy, and tolerance on hops beta acids and made this information available to the Expert Panel. In addition, Rhodia provided the Expert Panel with information and data on the chemical, physical, and antimicrobial properties, manufacture and processing, stability, conditions of anticipated use, estimated daily intakes resulting from these uses, and safety of hops beta acids. This information was consolidated by Rhodia in a document attached as Appendix 2 (the dossier). The Expert Panel independently and critically evaluated the information and data and other materials deemed appropriate or necessary, conferred by telephone, and then met in Chicago (26/27 July 2000) with technical representatives of Rhodia, Inc. and Viskase Corporation and other technical experts. The Expert Panel critically evaluated all the available information and unanimously concluded that hops beta acids, manufactured in accordance with current Good Manufacturing Practice (GMP) and meeting appropriate food grade specifications, are GRAS, by both scientific procedures and experience based on common use in food throughout history, for use as an antimicrobial agent for certain cooked meat and poultry products as specified herein at levels not to exceed current GMP.

3. History of Use

Hops are ubiquitous, naturally occurring plants. Throughout recorded history, hops flowers have been added as an essential ingredient in beer, and, as such, exhibit a substantial history of safe consumption. Hops flowers, and the acids or resins extracted therefrom, have long been recognized as bacteriological inhibitors, especially against Gram positive bacteria. Historically, hops has been used to inhibit lactobacilli and other bacterial contaminants in beer. The antimicrobial activity is due to the hops resins' humulone and lupulone and their chemical derivatives.

Modern brewing hygienic practices have minimized the need for the use of hops and hops acids as antimicrobial agents. Instead, hops, commonly in the form of natural hops acid extracts, continue to be a required component of beer due to the necessity to maintain the bitter flavor it has historically imparted to beer. The natural bitter acids contained in hops are extracted and used primarily for this purpose. The most prevalent natural bitter acids in hops are alpha acids (humulones) and beta acids (lupulones), with the alpha acids preferred for bittering. Hops acids or resins are comprised of a family of compounds all with a basic allicyclic structure (2,4-cyclohexadiene-1-one). Existing techniques allow separation of natural hops acid extracts into primarily alpha acid and beta acid fractions. The separated natural acid extracts have been shown to maintain identity and to demonstrate functionality characteristics consistent with those recorded throughout history for hops, including as an antimicrobial agent. Natural hops beta acids, specifically, have been shown to be particularily efficacious against *Listeria monocytogenes* and against sporeforming bacteria in the genera *Bacillus* and *Clostridium.*

The U.S. Food and Drug Administration (FDA) lists the essential oils,oleoresins (solvent-free) and natural extractives (including distillates) of hops to be substances generally recognized as safe for their intended use (21 CFR 182.20).

4. Characteristics of Hops Beta Acids

Hops beta acids are contained along with alpha acids in resins which are found in small yellow vesicles called lupulin glands on the petals of hops flowers. Once extracted and isolated from the alpha acids, the primarily hops beta acid extract has antimicrobial activity against select Gram positive spoilage bacteria, and has also been shown to inhibit the growth of *L. monocytogenes* in foods. Hops beta acids is the common and usual name for the family of chemically-related compounds. The empirical formulas for the primary hops beta acids are: lupulone-$C_{27}H_{38}O_4$; coluplone-$C_{26}H_{37}O_4$ and; adlupulone-$C_{27}H_{38}O_4$.

5. Manufacture

Hops beta acids are manufactured in compliance with current Good Manufacturing Practice (GMP) specified in 21 CFR, part 110, and in the Food Chemicals Codex, Fourth Edition and any subsequent amendment thereof.

Hops processors extract component natural alpha and beta acids from resins contained in hops flower petals utilizing commonly accepted extraction methodology. The two acid fractions are then separated using liquid carbon dioxide under supercritical conditions or by chromatographic procedures. The resulting purified liquid extract normally contains 80-90% pure hops beta acid, and is typically dried on a carrier, such as maltodextrin, for commercial use. Specifications for a typical hops beta acids extract are as follows:

INGREDIENTS:	Maltodextrin & Hops Beta Acids	pH:	>7
DESCRIPTION:	Powdered carbohydrate	TOTAL PLATE COUNT:	<2,500
AROMA:	Slightly aromatic	LACTIC ACID COUNT:	<200
FLAVOR:	Slight astringency	YEAST and MOLD:	<10
COLOR:	Off-white to light yellow	SALMONELLA:	None
MOISTURE:	6% max	COLIFORM:	None
ASH:	2% max	LEAD (as Pb)	<2 ppm

6. Uses

Hops beta acids are proposed for use on casings for frankfurters at concentrations of 2.5 mg hops beta acids/lb frankfurter or approximately 5.5 mg hops beta acids/kg food, and on cooked meat and poultry products sold ready-to-eat at 2.0 mg hops beta acids/lb of cooked meat or poultry product or approximately 4.4 mg hops beta acids/kg food (as specified in Exposure Assessment, Appendix 2).

No information on self-limiting levels of use was noted.

7. Exposure

Intakes were estimated from food consumption data collected by the U.S. Department of Agriculture (USDA). For the overall U.S. population, two-day average per-user intakes ranged from 0.21 mg/person/day at the mean to 0.52 mg/person/day at the 95th percentile of intake. On a per-capita basis, intakes for the U.S. population ranged from 0.09 mg/person/day at the mean to 0.38 mg/person/day at the 95th percentile.

Intake estimates based on individual survey days (i.e., not two-day average) ranged from 0.09 mg/person/day (mean per-capita) to 0.50 mg/person/day at the 95th percentile per-capita intake. On a per-user basis, intake estimates ranged from a mean of 0.34 mg/person/day to 0.81 mg/person/day at the 95th percentile of intake.

8. Safety

There are extensive data, including numerous published reports, to support the safety of hops when used in foods, especially beer, as an antimicrobial agent. Hops and their associated acids have long been recognized as effective bacteriological inhibitors, primarily against Gram positive bacteria. Recent studies also show that hops extracts of beta acids are extremely effective in controlling *L. monocytogenes* in foods, particularily certain cheeses and meat and poultry products. The antimicrobial efficacy demonstrated by the hops beta acids extracts is consistent in all respects with such functionality long exhibited by the use of hops in foods, especially beer. Microbial resistance varies among bacterial species and the literature supports that resistance develops only at high levels of usage ($\geq$0.1%). The safety of hops and the component acids extracted therefrom is also supported by a long history of safe use in foods, especially beer, throughout recorded history.

Estrogenic activity associated with hops harvesting and brewing has been reported in the scientific literature since the 1950s and anecdotally has been noted since the 15th century. Cold sludge baths containing 30 % hops were considered to have rejuvenating effects and were used for the treatment of gynecological disturbances. Menstrual irregularities have been reported in female hops workers (cited in Fenselau and Talalay, 1973 and Milligan et al, 1999). Contradictory results have been reported in the scientific literature on the estrogenic activity of hops. Koch and Heim (1953, cited in Fenselau and Talalay, 1973 and Lilligan et aL, 1999) and Zenisek and Bednar (1960) found extraordinary levels of estrogenic activity in beer, hops, or hops extracts. In contrast, Fenselau and Talalay (1973) reported no estrogenic activity of essential hops oil, alpha hops acid, beta hops acid, total hops resins, ethanolic extracts of hops, or commercial hops extracts. The variability of the results may be the result of the variability of the extracts and the specific assays used.

Milligan et al (1999) reported on the estrogenic potential of hops extracts derived from 17 different hops varieties using two assays for estrogenic activity, Ishikawa cells and yeast cells. They found that the estrogenic activity was concentrated in the polar, polyphenol-containing fraction of all 17 varieties. The estrogenic potential of various hops extract components, including 8-prenylnarigenin and three other related hops prenylated polyphenols (xanthohumol, isoxanthohumol, and 6-prenylnarigenin) were compared to the estrogenic activity of 17 beta-estradiol, and the phytoestrogens coumestrol, genistein, and daidzein. Milligan and coworkers were able to clearly identify 8-prenylnarigenin as the substance responsible for the reported estrogenic activity of hops. Isoxanthohumol and 6-prenylnarigenin were only very weakly estrogenic. Xanthohumol was inactive in both estrogenic assays tested. No estrogenic activity was detected in any of the other, less polar fractions of the hops extracts, including those of the alpha and beta hops acids which are responsible for the bitter taste of beer.

The physiological properties of hops and their metabolism have been investigated by researchers in recent years. In addition to their sedative properties, phytoestrogenic activities have been proposed based on animal studies. However, these proposed physiological activities are observed only at very high dosages that well exceed the proposed use levels in this document. Duncan, et al (J.Am. Vet. Med. Assoc. 210:51, 1997) report anecdotally on 5 separate incidences of malignant hyperthermia in dogs that ingested large quantities of hops. Four of the five dogs were greyhounds, a breed that is very sensitive to malignant hyperthermia. This study is not relevant to the safety of hops beta acids since the doses were very high (in one dog that was subjected to gastric lavage, 250 ml of hops was recovered) and the condition is not common in humans. It can be triggered in susceptible pigs and dogs by stress or excitement but the most common triggers are anesthetic agents. Shipp, et al (Fd.Chem. Tox. 32:1007, 1994) reported on the effect of colupulone, a hops beta acid, on hepatic cytochrome P-450 enzymatic activity in the rat. Male Sprague-Dawley rats were fed a diet containing 0.36 % colupulone for 5 days. This relatively high dose of colupulone did not appear to have any functional effects on enzyme activity nor did it influence the mutagenicity of P-450-metabolized promutagens. The authors concluded that "The effect of long-term colupulone administration of in vivo cytochrome P-450 enzymatic activity remains to be determined."

No information on hops beta acids is noted that appears to be inconsistent with the determination of safety or general recognition of safety for the proposed uses. Indeeed, it is noted that FDA lists the essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of hops as substances generally recognized as safe for their intended use in conformity with GMP at 21 CFR 182.20.

[redacted]

Summary and Conclusions

The members of the Expert Panel, having independently and collectively critically evaluated the information summarized above and included in the appendices to this report, unanimously conclude that the natural extractive, hops beta acids, when produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is safe for use as an antimicrobial agent in certain foods.

The members of the Expert Panel further concluded that the natural extractive, hops beta acids, produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is generally recognized as safe (GRAS), based on scientific procedures and common use in food throughout recorded history, for use as an antimicrobial for cooked meat and poultry products as specified herein. This conclusion is consistent with FDA's finding that natural extractives of hops are GRAS. 21 CFR 182.20.

Joseph F. Borzelleca, Ph.D.
Professor, Pharmacology & Toxicology
Medical College of Virginia

Eric A. Johnson, Sc.D.
Professor, Microbiology
University of Wisconsin

John G. Cerveny
Consultant, Microbiology and Food Safety

[redacted]

HOPS BETA ACIDS
SELF GRAS DETERMINATION

Rhodia

[redacted]

1% NFE (hop beta-acids)

Description and Nomenclature:

Hops are used by brewers primarily for their bittering characteristics. This bitterness comes from resins which contain ***humulone*** (a.k.a. ***alpha acids***) and and ***lupulone*** (a.k.a. ***beta acids***), which are found (along with the aromatic oils) in small yellow vesicles called ***lupulin glands***, found on the petals of the flower. Over the years, brewers have abandoned the use of native hops and have switched to the inclusion of hop extracts, provided by hop processors. In general, typical crude hop extracts contain a 2:1 ratio of alpha to beta acids. Since alpha acids are responsible for 90% of bitterness, and since beta acids dissolve poorly in normal wort, the percentage of beta acids is of little or no importance to the brewer. However, Bio-Technical Resources of Manitowoc, WI have discovered that the hop beta-acids, when separated from their alpha counterparts by liquid carbon dioxide under supercritical conditions, are effective bacteriocides against organisms such as *Listeria monocytogenes*.

Application or inclusion of hop acids can cause an objectionable flavor in many foods. But it has been found that when used at low levels, bitterness presents no problem. Food flavor is unaffected after treatment with an aqueous solution of beta-acids and the growth of Listeria can be significantly inhibited.

A. Common or Usual Name:

Hop beta acids

[redacted]

B. Chemical Name:

None

1. Chemical Abstract Service (CAS) registry number:

None

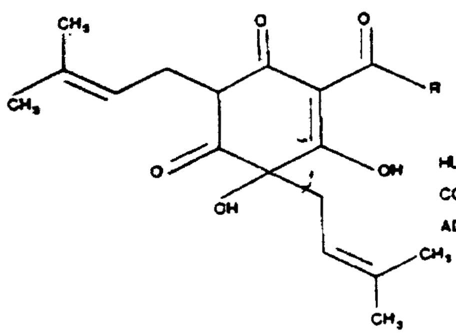
2. Empirical Formula:

Lupulone: $C_{27}H_{38}O_4$

Colupulone: $C_{26}H_{37}O_4$

Adlupulone: $C_{27}H_{38}O_4$

E. Structural Formulas:

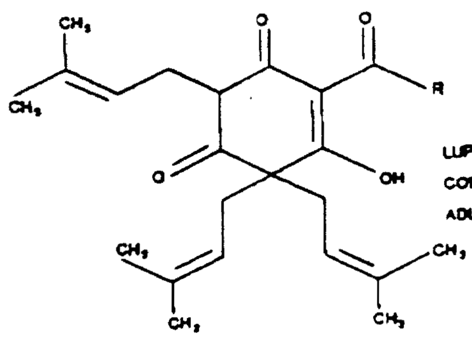


α-Acids

HUMULONE $R = CH_2CH(CH_3)_2$

COHUMULONE $R = CH_2(CH_3)_2$

ADHUMULONE $R = CH(CH_3)CH_2CH_3$



β-Acids

LUPULONE $R = CH_2CH(CH_3)_2$

COLUPULONE $R = CH_2(CH_3)_2$

ADLUPULONE $R = CH(CH_3)CH_2CH_3$

From Food Technology, January 1989, p. 139

[redacted]

F. Specifications for food grade material:

Included (as Appendix __) are the specifications of NFE (Rhodia's name for hop beta-acid blend). NFE is manufactured according to Good Manufacturing Practices as per 21 CFR, part 110. In addition the listed specifications reflect AOAC, 16th edition methods for moisture and pH and FDA/BAM methods for microbiological specifications. page 32

G. Quantitative compositions:

Purified (80-90% pure) beta-acids are received from the hop processor as a liquid and are dried directly on maltodextrin using GMP food grade facilities of All American Blending, Mankato, Minnesota.

1. **Formula and Product Label:**

Maltodextrin, hop beta-acids

H. Manufacturing Process:

The production of hop extracts in the USA began in the early 1960's. With hops generally containing from 2 to 12 weight % alpha-acids and 1 to 10 wt. % beta-acids, the beta-acids can be extracted to provide a product containing 50 to 80 wt. % beta-acids.

The solvents capable of being used are hexane and carbon dioxide. In Europe, ethyl alcohol is also used. All three solvents make quite acceptable products for brewing, but nevertheless, the brewing industry is very sensitive to any connotation of chemical processing and, therefore, attention has turned to ethyl alcohol and to carbon dioxide.

Once received from the hop extractor, the liquid stream of product (~80% beta-acids) can be dried on a maltodextrin carrier, or further fractionated using liquid CO_2 to yield a highly purified liquid (>90% pure beta-acids) which can be dried on a matrix of maltodextrin.

All purification and processing of hop beta-acids are done on/in high grade stainless steel in compliance with Good Manufacturing Practices specified in 21CFR, part 110, the Food Chemical Codex, Fourth Edition and any amendments thereof.

Quality Specifications and Packaging

NFE (Natural flavor extract)

INGREDIENTS	Maltodextrin and Hop Beta Acids
DESCRIPTION	Powdered carbohydrate
AROMA	Slightly aromatic
FLAVOR	Slight astringency
COLOR	Off-White to Light Yellow
MOISTURE	6% max.
ASH	2% max.
pH	> 7 (in a water slurry)
TOTAL PLATE COUNT	< 2,500
LACTIC ACID COUNT	< 200
YEAST and MOLD	< 10
SALMONELLA	None
COLIFORM	None

Packaged in 20 pound bag-in-a box

Rhodia Inc. — 2802 Walton Commons West, Madison, WI 53718-6785 Tel: 608.224.1850 Fax: 608.224.3130

[redacted]

[Code of Federal Regulations]
[Title 21, Volume 3, Parts 170 to 199]
[Revised as of April 1, 2000]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR182.20]

[Page 447-449]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (CONTINUED)

PART 182--SUBSTANCES GENERALLY RECOGNIZED AS SAFE--Table of Contents

Subpart A--General Provisions

Sec. 182.20 Essential oils, oleoresins (solvent-free), and natural extractives (including distillates).

Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are generally recognized as safe for their intended use, within the meaning of section 409 of the Act, are as follows:

Common name	Botanical name of plant source
Alfalfa	Medicago sativa L.
Allspice	Pimenta officinalis Lindl.
Almond, bitter (free from prussic acid)	Prunus amygdalus Batsch, Prunus armeniaca L., or Prunus persica (L.) Batsch.
Ambrette (seed)	Hibiscus moschatus Moench.
Angelica root	Angelica archangelica L.
Angelica seed	Do.
Angelica stem	Do.
Angostura (cusparia bark)	Galipea officinalis Hancock.
Anise	Pimpinella anisum L.
Asafetida	Ferula assa-foetida L. and related spp. of Ferula.

[redacted]

Balm (lemon balm)............... Melissa officinalis L.

Balsam of Peru.................. Myroxylon pereirae Klotzsch.

Basil.......................... Ocimum basilicum L.

Bay leaves...................... Laurus nobilis L.

Bay (myrcia oil)................ Pimenta racemosa (Mill.) J. W. Moore.

Bergamot (bergamot orange)...... Citrus aurantium L. subsp. bergamia Wright et Arn.

Bitter almond (free from prussic acid). Prunus amygdalus Batsch, Prunus armeniaca L., or Prunus persica (L.) Batsch.

Bois de rose.................... Aniba rosaeodora Ducke.

Cacao.......................... Theobroma cacao L.

Camomile (chamomile) flowers, Matricaria chamomilla L. Hungarian.

Camomile (chamomile) flowers, Anthemis nobilis L. Roman or English.

Cananga......................... Cananga odorata Hook. f. and Thoms.

Capsicum........................ Capsicum frutescens L. and Capsicum annuum L.

Caraway......................... Carum carvi L.

Cardamom seed (cardamon)........ Elettaria cardamomum Maton.

Carob bean...................... Ceratonia siliqua L.

Carrot.......................... Daucus carota L.

Cascarilla bark................. Croton eluteria Benn.

Cassia bark, Chinese........... Cinnamomum cassia Blume.

Cassia bark, Padang or Batavia.. Cinnamomum burmanni Blume.

Cassia bark, Saigon............ Cinnamomum loureirii Nees.

Celery seed..................... Apium graveolens L.

Cherry, wild, bark.............. Prunus serotina Ehrh.

Chervil......................... Anthriscus cerefolium (L.) Hoffm.

Chicory......................... Cichorium intybus L.

Cinnamon bark, Ceylon........... Cinnamomum zeylanicum Nees.

Cinnamon bark, Chinese.......... Cinnamomum cassia Blume.

Cinnamon bark, Saigon........... Cinnamomum loureirii Nees.

Cinnamon leaf, Ceylon........... Cinnamomum zeylanicum Nees.

Cinnamon leaf, Chinese.......... Cinnamomum cassia Blume.

Cinnamon leaf, Saigon........... Cinnamomum loureirii Nees.

Citronella...................... Cymbopogon nardus Rendle.

Citrus peels.................... Citrus spp.

Clary (clary sage).............. Salvia sclarea L.

Clover.......................... Trifolium spp.

[redacted]

Coca (decocainized)............ Erythroxylum coca Lam. and other spp. of Erythroxylum.

Coffee.......................... Coffea spp.

Cola nut........................ Cola acuminata Schott and Endl., and other spp. of Cola.

Coriander....................... Coriandrum sativum L.

Cumin (cummin).................. Cuminum cyminum L.

Curacao orange peel (orange, Citrus aurantium L. bitter peel).

Cusparia bark................... Galipea officinalis Hancock.

Dandelion....................... Taraxacum officinale Weber and T. laevigatum DC.

Dandelion root.................. Do.

Dog grass (quackgrass, triticum) Agropyron repens (L.) Beauv.

Elder flowers................... Sambucus canadensis L. and S. nigra I.

Estragole (esdragol, esdragon, Artemisia dracunculus L. tarragon).

Estragon (tarragon)............ Do.

Fennel, sweet................... Foeniculum vulgare Mill.

Fenugreek....................... Trigonella foenum-graecum L.

Galanga (galangal).............. Alpinia officinarum Hance.

Geranium........................ Pelargonium spp.

Geranium, East Indian........... Cymbopogon martini Stapf.

Geranium, rose.................. Pelargonium graveolens L'Her.

Ginger.......................... Zingiber officinale Rosc.

Grapefruit...................... Citrus paradisi Macf.

Guava........................... Psidium spp.

Hickory bark.................... Carya spp.

Horehound (hoarhound)........... Marrubium vulgare L.

Hops............................ Humulus lupulus L.

Horsemint....................... Monarda punctata L.

Hyssop.......................... Hyssopus officinalis L.

Immortelle...................... Helichrysum augustifolium DC.

Jasmine......................... Jasminum officinale L. and other spp. of Jasminum.

Juniper (berries)............... Juniperus communis L.

Kola nut........................ Cola acuminata Schott and Endl., and other spp. of Cola.

Laurel berries.................. Laurus nobilis L.

Laurel leaves................... Laurus spp.

Lavender........................ Lavandula officinalis Chaix.

Lavender, spike................. Lavandula latifolia Vill.

[redacted]

Lavandin....................... Hybrids between Lavandula officinalis Chaix and Lavandula latifol n Vill.

Lemon........................... Citrus limon (L.) Burm. f.

Lemon balm (see balm)...........

Lemon grass..................... Cymbopogon citratus DC. and Cymbopogon lexuosus Stapf.

Lemon peel...................... Citrus limon (L.) Burm. f.

Lime............................ Citrus aurantifolia Swingle.

Linden flowers.................. Tilia spp.

Locust bean..................... Ceratonia siliqua L,

Lupulin......................... Humulus lupulus L.

Mace............................ Myristica fragrans Houtt.

Mandarin........................ Citrus reticulata Blanco.

Marjoram, sweet................. Majorana hortensis Moench.

Mate............................ Ilex paraguariensis St. Hil.

Melissa (see balm)..............

Menthol......................... Mentha spp.

Menthyl acetate................. Do.

Molasses (extract).............. Saccarum officinarum L.

Mustard......................... Brassica spp.

Naringin........................ Citrus paradisi Macf.

Neroli, bigarade................ Citrus aurantium L.

Nutmeg.......................... Myristica fragrans Houtt.

Onion........................... Allium cepa L.

Orange, bitter, flowers......... Citrus aurantium L.

Orange, bitter, peel............ Do.

Orange leaf..................... Citrus sinensis (L.) Osbeck.

Orange, sweet................... Do.

Orange, sweet, flowers.......... Do.

Orange, sweet, peel............. Do.

Origanum........................ Origanum spp.

Palmarosa....................... Cymbopogon martini Stapf.

Paprika......................... Capsicum annuum L.

Parsley......................... Petroselinum crispum (Mill.) Mansf.

Pepper, black................... Piper nigrum L.

Pepper, white................... Do.

Peppermint...................... Mentha piperita L.

Peruvian balsam................. Myroxylon pereirae Klotzsch.

Petitgrain........................ Citrus aurantium L.

Petitgrain lemon................ Citrus limon (L.) Burm. f.

Petitgrain mandarin or tangerine Citrus reticulata Blanco.

Pimenta.......................... Pimenta officinalis Lindl.

Pimenta leaf..................... Pimenta officinalis Lindl.

Pipsissewa leaves............... Chimaphila umbellata Nutt.

Pomegranate...................... Punica granatum L.

Prickly ash bark................ Xanthoxylum (or Zanthoxylum) Americanum Mill. or Xanthoxylum clava-
herculis L.

Rose absolute.................... Rosa alba L., Rosa centifolia L., Rosa damascena Mill., Rosa gallica L.,
and vars. of these spp.

Rose (otto of roses, attar of roses). Do.

Rose buds....................... Do.

Rose flowers.................... Do.

Rose fruit (hips)............... Do.

Rose geranium................... Pelargonium graveolens L'Her.

Rose leaves..................... Rosa spp.

Rosemary........................ Rosmarinus officinalis L.

Saffron.......................... Crocus sativus L.

Sage............................ Salvia officinalis L.

Sage, Greek..................... Salvia triloba L.

Sage, Spanish................... Salvia lavandulaefolia Vahl.

St. John's bread................ Ceratonia siliqua L.

Savory, summer.................. Satureia hortensis L.

Savory, winter.................. Satureia montana L.

Schinus molle................... Schinus molle L.

Sloe berries (blackthorn Prunus spinosa L. berries).

Spearmint....................... Mentha spicata L.

Spike lavender.................. Lavandula latifolia Vill.

Tamarind........................ Tamarindus indica L.

Tangerine....................... Citrus reticulata Blanco.

Tarragon........................ Artemisia dracunculus L.

Tea............................. Thea sinensis L.

Thyme........................... Thymus vulgaris L. and Thymus zygis var. gracilis Boiss.

Thyme, white.................... Do.

[redacted]

Thyme, wild or creeping......... Thymus serpyllum L.

Triticum (see dog grass)........

Tuberose........................ Polianthes tuberosa L.

Turmeric....................... Curcuma longa L.

Vanilla......................... Vanilla planifolia Andr. or Vanilla tahitensis J. W. Moore.

Violet flowers.................. Viola odorata L.

Violet leaves................... Do.

Violet leaves absolute.......... Do.

Wild cherry bark................ Prunus serotina Ehrh.

Ylang-ylang..................... Cananga odorata Hook. f. and Thoms.

Zedoary bark.................... Curcuma zedoaria Rosc.

[42 FR 14640, Mar. 15, 1977, as amended at 44 FR 3963, Jan. 19, 1979; 47 FR 29953, July 9, 1982; 48 FR 51613, Nov. 10, 1983; 50 FR 21043 and 21044, May 22, 1985]

[[Page 450]]

US005286506A

United States Patent [19]

[11] **Patent Number: 5,286,506**

Millis et al.

[45] **Date of Patent: Feb. 15, 1994**

[54] **INHIBITION OF FOOD PATHOGENS BY HOP ACIDS**

[75] Inventors: **James R. Millis**, Kohler; **Mark J. Schendel**, Manitowoc, both of Wis.

[73] Assignee: **Bio-Technical Resources**, Manitowoc, Wis.

[21] Appl. No.: **969,698**

[22] Filed: **Oct. 29, 1992**

[51] Int. Cl.⁵ A23L 3/3508
[52] U.S. Cl. 426/335; 426/532
[58] Field of Search 426/335, 532

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,550,255 4/1951 Jensen .... 426/335 X

OTHER PUBLICATIONS

Banwart, Basic Food Microbiology, 1981, AVI Westport, Connecticut, p. 100.

S. Velani & R. J. Gilbert, "Listeria Monocytogenes in Prepacked Ready-to-Eat Sliced Meats," PHLS Microbiology Digest vol. 7 (1990).

Teuber & Schmalreck, Arch. Mikrobiol. 94 (1973), pp. 159-171.

Primary Examiner—Joseph Golian
Attorney, Agent, or Firm—Frank C. Hilberg, Jr

[57] **ABSTRACT**

The protection of food products from contamination by food pathogens, particularly Listeria monocytogenes, by incorporation of 6 to 50 ppm and preferably 6 to 15 ppm of beta-acids, as extracted from hops, into such food products.

7 Claims, No Drawings

000063

[redacted]

INHIBITION OF FOOD PATHOGENS BY HOP ACIDS

BACKGROUND OF THE INVENTION

The present invention relates to the use of beta-acids as extracted from hops for controlling Listeria and other food pathogens in food products intended for human consumption.

Recent studies have revealed that listeriosis in humans as caused by Listeria species and particularly Listeria monocytogenes is associated with the consumption of various types of foods, particularly soft cheese and pate as well as hams and other prepacked meat and poultry products. "Listeria Monocytogenes in Prepacked Ready-To-Eat Sliced Meats", a survey by the 16 public health laboratories in the PHLS food chain, by S. Velani and R. J. Gilbert, PHLS Microbiology Digest Vol. 7 (1990).

Hops and their associated acids have long been recognized as bacteriological inhibitors. More specifically, hop acids and resins have been shown to be primarily active against gram positive bacteria, e.g., Bacilli, Corynebacteria, Diplococci, Mycobacteria, Streptococci, Lactobacilli and Streptomyces. Most of the publications have focused on Lactobacilli, since this organism is a major contaminant in beer fermentation. Activity against gram negative bacteria is far less pronounced. Teuber and Schmalrek (Arch. Mikrobiol. 94, pp. 159-171, 1973) and Simpson and Hammond (European Brewery Convention, 1991) have suggested that the effect was due to induced permeability of the cell membrane in gram positive bacteria, but was inactivated by serumphosphatides in gram negative bacteria.

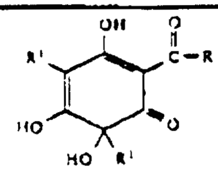
SUMMARY OF THE INVENTION

The present invention relates to the discovery that of the bitter acids contained in hops the beta-acids are useful as a bacteriocide against a dangerous food pathogen (Listeria) at levels below that at which a noticeable flavor from the beta-acids is detectable.

DETAILED DESCRIPTION

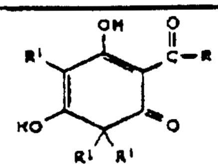
One of the historical roles of hops in beer making, namely the bacteriostatic function, has been made obsolete in the modern manufacture of beer by aseptic fermentation and packaging. The bitter acids component of the hops and particularly the beta-acids have now been found to be unexpectedly useful as bacteriocides in food products. The most prevalent groups of bitter acids found as components of hops are the alpha-acids and the beta-acids, also referred to as humulones and lupulones, respectively. Both contribute bitterness to beer, but the alpha-acids are much more intense in this regard than the beta-acids. Producers of hop extracts have thus recently developed a technique to separate the two acid fractions using liquid carbon dioxide under supercritical conditions. A by-product of the operation is a product which contains approximately 61 weight percent beta-acids, the remainder consisting essentially of hop resins.

The alpha-acids contained in hops have the structure:



	R	R¹
Humulone	$-CH_2CH(CH_3)_2$	
Cohumulone	$-CH(CH_3)_2$	$-CH_2-CH=C(CH_3)_2$
Adhumulone	$-CH(CH_3)CH_2CH_3$	

The alpha-acids form a precipitate with lead acetate. The beta-acids contained in hops have the structure:



	R	R¹
Lupulone	$-CH_2CH(CH_3)_2$	
Colupulone	$-CH(CH_3)_2$	$-CH_2-CH=C(CH_3)_2$
Adlupulone	$-CH(CH_3)CH_2CH_3$	

The beta-acids do not form a precipitate with lead acetate.

The beta-acids used herein are virtually insoluble in aqueous solutions at pH 5.2 and below, but are readily soluble above pH 7. Thus, the beta-acids are normally applied to the food being treated as an aqueous solution at pH 7 to 9.5. Generally the aqueous solution contains from 6 to 100 ppm beta acids with from 15 to 50 ppm being the preferred range. The aqueous solution of beta acids is applied to the food product being treated as a spray or the food product can be dipped in the solution of beta acids, prior to storage.

Hops are added to the wort during the brewing process at a rate of 1 to 8 grams per liter, depending on the fermentation process being used and the hop variety. Hops generally contain from 2 to 12 wt. % alpha-acids and 1 to 10 wt. % beta-acids. Thus the beta-acid content of the wort is from 0.001 to 0.08 wt. % (10 to 800 ppm) beta-acids. For use herein generally the beta-acids are extracted to provide a product containing 10 to 80 wt. % beta-acids, which is diluted or concentrated to provide the desired aqueous solution.

The present invention relates to the discovery that the carbon dioxide extract fraction which is rich in beta-acids is useful as a food preservative. The present invention targets the food pathogen Listeria monocytogenes. This pathogen is responsible for approximately 70 deaths per year, more than twice the number from any other food pathogen. It is generally believed that the predominant source of Listeria contamination is the food manufacturer. These facts have led to an intense search for new techniques to detect and inhibit Listeria.

It has now been found that the growth of Listeria monocytogenes is completely inhibited in liquid culture at a concentration of 6 parts per million (ppm) beta-acids. Our experiments indicate the effect to be bacte-

000064

nostatic. We have also noted growth inhibition by beta-acids with respect to *Salmonella enteritidis, Staphylococcus aureus* and *Clostridium perfringer*. No growth inhibitions by beta-acids have been found with respect to *Campylobectu sputorum bubulus, Saccharomyces cerevisieae, Aspergillus niger, Candida tropicalis*, Geotrichum sp., *Penicillium griseofulvum*, and *Fusarium tricintum*.

Taste evaluations indicate that purified beta-acids impart a noticeable flavor above 15 ppm, and an objectionable flavor above 50 ppm. Thus the beta-acids are generally applied to the food to be protected from contamination with Listeria monocytogenes at the level of 6 ppm to 50 ppm with from 6 ppm to 15 ppm being the preferred range, (as based on the weight of the food).

Example 2

A plurality of test tubes are charged with 5 ml of brain-heart broth (B-H broth). Tubes are inoculated with 100 microliters of a culture of Listeria that had been grown overnight in brain-heart broth. A solution of beta-acids, as prepared in Example 1, is added to each test tube to provide the beta-acid concentration reported in Table I below. Another set of test tubes containing brain-heart broth and beta-acids in concentrations is prepared corresponding to those prepared above. These later tubes were not inoculated with Listeria. All of the tubes are incubated together at 3°° C. for 24 hours.

TABLE I

Beta-Acids (ppm)	0	1	3	5	7	10	12	15	25	50	75
50 ppm Beta Acids (ml)	—	0 04	0 12	0 2	0 28	0.4	0 48	0.06	1 0	2.0	.0
H_2O (ml)	5	4 96	4 88	—	4.72	4 6	4 52	4 4	4 0	3 0	.0
B-H Broth (ml)	5	5	5	5	5	5	5	5	5	5	:
O.D. 620 nm (1)	0.47	0 21	0 125	0	0.005	0	0 007	0	0 001	0 005	0 .001
O.D. 620 nm (2)	0.44	0 22	0 125	0 03	0.005	0.005	0	0	0	0	0 005
O.D. 620 nm (3)	0.48	0.22	0.085	0.005	0 005	0.01	0.005	0 005	0	0	0 01
NG/Growth	—	Lawn	Lawn	210 col	TNTC	4 col	20 col	23 col	1 col	11 col	3 col
620 nm	0 46	0 22	0 11	0 01	0 005	0 005	0.004	0.003	0 0	0 005	.005

Foods most likely to be contaminated with Listeria are solid foods which include cheese, particularly soft cheese, seafood, processed meats including hot dogs, sausages, hams, turkey and chicken.

EXAMPLES

Example 1

A 1.0 g sample of beta-acid fraction is weighed and placed in a large test tube. The beta-acid fraction was a commercial product obtained from John I. Haas, Inc. believed to be obtained by liquid carbon dioxide extraction of hops under super critical conditions. The beta-acid fraction contains 61% beta-acids and the remainder is essentially hop resins. Five ml of hexane is added to the test tube and the contents of the test tube vortexed until the beta-acids are dissolved.

Fifteen ml of 1M aqueous NaOH is added to the test tube, and the contents of the test tube vortexed well. The alkaline aqueous phase is removed by decantation and saved. Another fifteen ml aliquot of 1M aqueous NaOH is added to the test tube, and the contents of the test tube vortexed well. The alkaline aqueous phase is removed by decantation and combined with the alkaline aqueous phase saved above. The combined alkaline aqueous phase material is acidified to about pH 1 with concentrated hydrochloric acid. The acidified aqueous is then extracted three times with 125 ml aliquots of petroleum ether using a separatory funnel. The solution of beta-acids is dried over sodium sulfate at 23°-24° C., then evaporated in a laboratory Roto-Vec® at 45° C. to remove excess ether until only an oily residue remains. The oil is placed in a freezer at −20° C. Crystals

As can be seen from the above the beta-acids posses bacteriostatic activity which is effective against Listeria at levels of about 5 ppm and above.

In Table I the "NG/Growth" row reports values for plate counts, or the number of live colonies per ml of medium. Notations for "lawn" or "TNTC" indicate more than 300 colonies per ml.

EXAMPLE 3

A solution containing 100 ppm beta-acids is prepared as follows:

Ten mg of the beta-acid solution from Example 1 is charged into a 45 ml sterile test tube. Ten ml of 95% ethyl alcohol is added to test tube to dissolve the beta-acids. The resulting solution is added to a 100 ml volumetric flask. The contents of the flask are brought up to total 100 ml with 8.5 pH Mueller Hinton Broth. The resulting solution is filtered under sterile conditions using a filter having a 0.45 U in pore size. Thirty eight and four tenths ml of the filtered beta-acid solution and 1.6 ml of Mueller Hinton Broth is added to a sterile 125 ml shake flask fitted a stir bar. Twenty ml of Mueller Hinton Broth are added to each of 18 50 ml test tubes, along with enough of the above prepared beta-acid solution to bring the beta-acid level to that reported in Table II. When all the test tubes are filled, 2 5 ml is removed from each tube to record pH. Nine of the test tubes are inoculated with 25 Ul Listerium Mueller Hinton Broth (6N). All 18 test tubes are incubated at 35° C for 24 hours. The O.D. (600 nm) and the pH are recorded after the 24 hour incubation period. The results are reported in Table II.

TABLE II

Beta-Acids (ppm)	96	48	24	12	6	3	1 5	0 75	0
pH (start)	8 12	7 64	7 53	7 36	7 26	7 22	7 20	7.18	7 0
pH (after 24 hrs)	8 25	7 62	7.35	7 35	7 25	7 05	6 93	6 93	6 62
O D (24 hrs)	0 003	0 006	0 002	0 006	0 008	0 114	0 19	0 383	0 420

form which are isolated by filtration, then recrystallized from ether at −20° C. The beta-acids are stored in the freezer until used.

Example 4

A solution containing 100 ppm beta-acids is prepared as follows:

[redacted]

Ten mg of the beta-acid solution from Example 1 is charged into a 45 ml sterile test tube. Ten ml of 95% ethyl alcohol is added to the test tube to dissolve the beta-acids. The resulting solution is added to a 100 ml volumetric flask. The contents of the flask are brought up to total 100 ml with pH 8.5 Mueller Hinton Broth. The resulting solution is filtered under sterile conditions using a filter having a 0.45 Um pore size. Thirty eight and four tenths ml of the filtered beta-acid solution and 1.6 ml of brain-heart broth is added to a sterile 125 ml shake flask fitted with a stir bar. Twenty ml of brain-heart broth are added to each of 18 50 ml test tubes, along with enough of the above-prepared beta-acid solution to bring the beta-acid level to that reported in Table III. When all the test tubes are filled, 2.5 ml is removed from each tube to record pH. Nine of the test tubes are inoculated with 25 Ul Listeria in brain-heart broth (6N). All 18 test tubes are inoculated at 35° C. for 24 hours. The O.D. (600 nm) and the pH are recorded after the 24 hour incubation period. The results are reported in Table III.

TABLE III

Beta-Acids (ppm)	96	48	24	12	6	3	1.5	0.75	0
pH (start)	8.35	7.76	7.54	7.43	7.41	7.39	7.38	7.38	7.37
pH (after 24 hrs)	8.26	7.75	7.54	7.45	7.41	7.17	6.08	5.92	5.79
O.D. (24 hrs)	0.0	0.00	0.007	0.004	0.0	0.133	0.442	0.808	1.092

In the above examples the O.D. is taken at 600 nm using 2.5 ml of well mixed broth in disposable cavettes.

The results indicate that the beta-acids prevent the growth of Listeria down to 6 ppm for both the Mueller Hinton and Brain Heart broths.

The pH is affected by the concentration of beta-acids, but remains in the growth range for Listeria. So this should not be responsible for the growth inhibition of Listeria observed herein

We claim.

1. A process comprising applying a solution containing 6 to 100 ppm beta-acids as extracted from hops to a solid food product to incorporate from 6 to 50 ppm beta-acids in said food product to prevent growth of Listeria in said food product.

2. The process of claim 1 wherein the solution of beta-acids is applied to the food product by dipping the food product in the solution of beta acids.

3. The process of claim 2 wherein from 6 to 15 ppm beta-acids are incorporated into the food product.

4. The process of claim 1 wherein the solution of beta-acids is applied to the food product by spraying the solution of beta-acids onto the food product.

5. The process of claim 4 wherein from 6 to 15 ppm beta-acids are incorporated into the food product.

6. A packaged solid food product containing from 6 to 50 ppm beta-acids as extracted from hops to prevent growth of Listeria therein.

7. The food product of claim 6 wherein the food product contains from 6 to 15 ppm beta-acids.

* * * * *

[redacted]

NOVIGEN
SCIENCES, INC.
International

UNITED STATES
1730 Rhode Island Avenue NW
Ste. 1100, Washington, DC 20036
T (202) 293-5374 F (202) 293-5377
info@novigensci.com

UNITED KINGDOM
75 Graham Road
Malvern, Worcs, WR14 2HR
T (+44) 1684-588444 F (+44) 1684-588445
info@novigensci.co.uk

ESTIMATED DIETARY INTAKE OF HOPS BETA ACIDS AS PROPOSED FOR USE IN FOODS IN THE US

Prepared for:
Rhodia, Inc.
CN7500 Prospect Plains Road
Cranbury, NJ 08512-7500

Prepared by:
Novigen Sciences, Inc.
1730 Rhode Island Avenue, NW
Washington, DC 20036

June 2, 2000

[redacted]

ESTIMATED DIETARY INTAKE OF HOPS BETA ACIDS AS PROPOSED FOR USE IN FOODS IN THE US

I. INTRODUCTION

At the request of Rhodia, Inc. (Rhodia), Novigen Sciences, Inc. (Novigen) has estimated the dietary intake of hops beta acids (HBA) by the U.S. population and selected population subgroups in support of a self-assessment of GRAS status. Hops beta acids are proposed for use in casings for frankfurters at concentrations of 2.5 mg HBA/lb frankfurter (approximately 5.5 mg HBA/kg food) and in cooked meats sold ready-to-eat at 2.0 mg HBA/lb cooked meat (approximately 4.4 mg/kg food).

Intakes were estimated from food consumption data collected by the U.S. Department of Agriculture (USDA). For the overall U.S. population, 2-day average per-user intakes ranged from 0.21 mg/person/day at the mean to 0.52 mg/person/day at the 95th percentile of intake. On a per-capita basis, intakes for the U.S. population ranged from 0.09 mg/person/day at the mean to 0.38 mg/person/day at the 95th percentile.

Intake estimates based on individual survey days (i.e., not two-day average) ranged from 0.09 mg/person/day (mean per-capita) to 0.50 mg/person/day at the 95th percentile per-capita intake. On a per-user basis, intake estimates ranged from a mean of 0.34 mg/person/day to 0.81 mg/person/day at the 95th percentile of intake.

II. INTAKE ESTIMATES

A. Food Consumption Data

Detailed information on food and beverages consumed by the U.S. population is collected by the USDA in their Continuing Surveys of Food Intakes by Individuals (CSFII). The most recent survey, conducted between 1994 and 1996 (94-96 CSFII), has been used to estimate intake of HBA from selected foods (USDA 1998).

The 94-96 CSFII was conducted as three separate 1-year surveys. Each survey used a stratified area probability sample of individuals residing in all 50 states. The USDA developed statistical weights to adjust for over- and under-representation of certain population subgroups in the unweighted sample due to the sample design, nonresponse, and unequal interviewing across seasons and days of the week. Statistical weights were also developed to allow results of the three years of surveys to be combined for analysis.

Information on the amounts and kinds of foods and beverages consumed at home as well as away from home was collected by an in-person interviewer using a multiple-pass 24-hour recall. Quantities of foods and beverages consumed were recorded in household measures; USDA converted the quantities to grams. Each food reported in the survey was assigned a code by USDA and entered into the survey database; about 6,000 food codes were reported in the survey database.

Approximately 16,000 individuals participated in the surveys over the 3-year period. Individuals who took part in the survey were asked to provide two nonconsecutive days of dietary data. Although most participants reported consumption for both days of the survey, some individuals reported consumption for only one day. Separate statistical weights were developed for consumption data collected on Day 1 of the survey and for data reported by individuals participating in both days of the survey. Intake estimates presented in this report are based on data from only those respondents who provided consumption information for both days of the survey.

B. Proposed Uses and Use Levels

A list of foods included in the data analysis is presented in the Appendix. Estimated intake of HBA was based on the proposed use levels of 2.5 mg/lb frankfurter and 2.0 mg/lb cooked meat. Cooked "deli-type" meats such as turkey loaf, roast beef, ham, pastrami and other meats sold ready-to-eat were included in the category cooked meat. Note that, as a conservative estimate, all types of frankfurters (including turkey and chicken) reported consumed in the CSFII were assumed to contain HBA. Although the use of HBA for frankfurters is limited to the hot dog casing, it was assumed that all HBA would be transferred to the hot dog and that no HBA would be lost during cooking.

III. RESULTS

Estimated intake of HBA by the overall U.S. population is presented in Table 1. Mean, 90^{th} percentile and 95^{th} percentile of intake are reported.

IV. REFERENCE

U.S. Department of Agriculture (USDA), Agriculture Research Service. 1998. CSFII/DHKS Data Set and Documentation: The 1994-96 Continuing Survey of Food Intakes by Individuals and the 1994-96 Diet and Health Knowledge Survey. CD-ROM: PB98-500457. Springfield, VA: National Technical Information Service.

TABLE 1

INTAKE ESTIMATES: HBA IN FRANKFURTER CASINGS AND IN COOKED MEATS MG/PERSON/DAY

	2-day average intake		Person-day Intake	
	Per capita	**Per user**	**Per-capita**	**Per user**
Mean	0.09	0.21	0.09	0.34
90th percentile	0.27	0.40	0.33	0.61
95th percentile	0.38	0.52	0.50	0.81

000071

[redacted]

APPENDIX

PROPOSED FOOD USES INCLUDED IN THE ESTIMATED INTAKE OF HBA

22431000 Pork roll, cured, fried
24198660 Chicken, chicken roll, roasted, NS as to light or dark meat
24198640 Chicken, chicken roll, roasted, light meat
24198650 Chicken, chicken roll, roasted, dark meat
24204000 Turkey, roll, roasted
25210110 Frankfurter, wiener, or hot dog, NFS
25210120 Frankfurter or hot dog, breaded, baked
25210150 Frankfurter or hot dog, cheese-filled
25210160 Frankfurter or hot dog, bacon and cheese-filled
25210170 Frankfurter or hot dog, chili-filled
25210210 Frankfurter or hot dog, beef
25210220 Frankfurter or hot dog, beef and pork
25210230 Frankfurter or hot dog, beef and pork, lowfat
25210250 Frankfurter or hot dog, meat and poultry, fat free
25210280 Frankfurter or hot dog, meat and poultry
25210310 Frankfurter or hot dog, chicken
25210410 Frankfurter or hot dog, turkey
25210510 Frankfurter or hot dog, low salt
25210610 Frankfurter or hot dog, beef, lowfat
25210700 Frankfurter or hot dog, meat & poultry, lowfat
25220010 Cold cuts, NFS
25220390 Bologna, beef, lowfat
25220400 Bologna, pork and beef
25220410 Bologna, NFS
25220420 Bologna, Lebanon
25220430 Bologna, beef
25220440 Bologna, turkey
25220450 Bologna ring, smoked
25220460 Bologna, pork
25220470 Bologna, beef, lower sodium
25220480 Bologna, chicken, beef, and pork
25220490 Bologna, with cheese
25220500 Bologna, beef and pork, lowfat
25220510 Capicola
25221500 Salami, NFS
25221510 Salami, soft, cooked
25221530 Salami, beef
25230110 Luncheon meat, NFS
25230210 Ham, sliced, prepackaged or deli, luncheon meat
25230220 Ham, sliced, low salt, prepackaged or deli, luncheon meat
25230230 Ham, sliced, extra lean, prepackaged or deli, luncheon meat
25230310 Chicken or turkey loaf, prepackaged or deli, luncheon meat
25230410 Ham loaf, luncheon meat
25230430 Ham and cheese loaf
25230450 Honey loaf
25230510 Ham, luncheon meat, chopped, minced, pressed, spiced, not canned
25230520 Ham, luncheon meat, chopped, minced, pressed, spiced, lowfat, not canned
25230550 Ham, pork, and chicken, luncheon meat, chopped, minced, pressed, spiced, canned, reduced sodium
25230560 Liverwurst
25230610 Luncheon loaf (olive, pickle, or pimiento)
25230710 Sandwich loaf, luncheon meat
25230790 Turkey ham, sliced, extra lean, prepackaged or deli, luncheon meat

5230800 Turkey ham
25230810 Veal loaf
25230820 Turkey pastrami
25230840 Turkey salami
25230900 Turkey or chicken breast, prepackaged or deli, luncheon meat
25231110 Beef, sliced, prepackaged or deli, luncheon meat
25231150 Corned beef, pressed
27113200 Creamed chipped or dried beef
27120210 Frankfurter or hot dog, with chili, no bun
27120250 Frankfurters or hot dogs with tomato-based sauce (mixture)
27220080 Ham croquette
27420040 Frankfurters or hot dogs and sauerkraut (mixture)
27460490 Julienne salad (meat, cheese, eggs, vegetables), no dressing
27460510 Antipasto with ham, fish, cheese, vegetables
27500100 Meat sandwich, NFS
27510000 Beef sandwich, NFS
27510910 Corned beef sandwich
27510950 Reuben sandwich (corned beef sandwich with sauerkraut and cheese), with spread
27511010 Pastrami sandwich
27513010 Roast beef sandwich
27513020 Roast beef sandwich, with gravy
27513040 Roast beef submarine sandwich, on roll, with lettuce, tomato and spread
27513050 Roast beef sandwich with cheese
27513060 Roast beef sandwich with bacon and cheese sauce
27513070 Roast beef submarine sandwich, on roll, au jus
27516010 Gyro sandwich (pita bread, beef, lamb, onion, condiments), with tomato and spread
27520250 Ham on biscuit
27520300 Ham sandwich, with spread
27520310 Ham sandwich with lettuce and spread
27520320 Ham and cheese sandwich, with lettuce and spread
27520330 Ham and egg sandwich
27520340 Ham salad sandwich
27520350 Ham and cheese sandwich, with spread, grilled
27520360 Ham and cheese sandwich, on bun, with lettuce and spread
27520370 Hot ham and cheese sandwich, on bun
27520380 Ham and cheese on English muffin
27520390 Ham and cheese submarine sandwich, on multigrain roll, with lettuce, tomato and spread
27520540 Ham and tomato club sandwich, with lettuce and spread
27540110 Chicken sandwich, with spread
27540310 Turkey sandwich, with spread
27540330 Turkey sandwich, with gravy
27540350 Turkey submarine sandwich, on roll, with cheese, lettuce, tomato and spread
27560000 Luncheon meat sandwich, NFS, with spread
27560110 Bologna sandwich, with spread
27560120 Bologna and cheese sandwich, with spread
27560300 Corn dog (frankfurter or hot dog with cornbread coating)
27560310 Corny dog, with chili, on bun
27560320 Frankfurter or hot dog, plain, on bun
27560330 Frankfurter or hot dog, with cheese, plain, on bun
27560340 Frankfurter or hot dog, with catsup and/or mustard, on bun
27560350 Pig in a blanket (frankfurter or hot dog wrapped in dough)
27560360 Frankfurter or hot dog, with chili, on bun
27560370 Frankfurter or hot dog with chili and cheese, on bun

APPENDIX (CONT'D)

27560380 Pochito (frankfurter or hot dog and beef chili wrapped in tortilla)
27560400 Chicken frankfurter or hot dog, plain, on bun
27560510 Salami sandwich, with spread
27560910 Submarine, cold cut sandwich, on bun, with lettuce
32105030 Egg omelet or scrambled egg, with ham or bacon
32105060 Egg omelet or scrambled egg, with peppers, onion, and ham
32105080 Egg omelet or scrambled egg, with cheese and ham or bacon
32105085 Egg omelet or scrambled egg, with cheese, ham or bacon, and tomatoes
32202010 Egg, cheese, and ham on English muffin
32202020 Egg, cheese, and ham on biscuit
32202110 Egg and ham on biscuit
41206030 Beans and franks
58127210 Croissant, filled with ham and cheese
58127310 Croissant with ham, egg, and cheese
58132710 Spaghetti in tomato sauce w/frankfurters
58132713 Pasta in tomato sauce w/frankfurters, canned
58145160 Macaroni or noodles with cheese and frankfurters or hot dogs

§ 182.20 Essential oils, oleoresins (solvent-free), and natural extractives (including distillates).

Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are generally recognized as safe for their intended use, within the meaning of section 409 of the Act, are as follows:

Common name	Botanical name of plant source
Alfalfa	Medicago sativa L.
Allspice	Pimenta officinalis Lindl.
Almond, bitter (free from prussic acid).	Prunus amygdalus Batsch, Prunus armeniaca L., or Prunus persica (L.) Batsch.
Ambrette (seed)	Hibiscus moschatus Moench.
Angelica root	Angelica archangelica L.
Angelica seed	Do.
Angelica stem	Do.
Angostura (cusparia bark)	Galipea officinalis Hancock.
Anise	Pimpinella anisum L.
Asafetida	Ferula assa-foetida L. and related spp. of Ferula.
Balm (lemon balm)	Melissa officinalis L.
Balsam of Peru	Myroxylon pereirae Klotzsch.
Basil	Ocimum basilicum L.
Bay leaves	Laurus nobilis L.
Bay (myrcia oil)	Pimenta racemosa (Mill.) J. W. Moore.
Bergamot (bergamot orange)	Citrus aurantium L. subsp. bergamia Wright et Arn.
Bitter almond (free from prussic acid).	Prunus amygdalus Batsch, Prunus armeniaca L., or Prunus persica (L.) Batsch.
Bois de rose	Aniba rosaeodora Ducke.
Cacao	Theobroma cacao L.
Camomile (chamomile) flowers, Hungarian.	Matricaria chamomilla L.
Camomile (chamomile) flowers, Roman or English.	Anthemis nobilis L.
Cananga	Cananga odorata Hook. f. and Thoms.
Capsicum	Capsicum frutescens L. and Capsicum annuum L.
Caraway	Carum carvi L.
Cardamom seed (cardamon)	Elettaria cardamomum Maton.
Carob bean	Ceratonia siliqua L.
Carrot	Daucus carota L.
Cascarilla bark	Croton eluteria Benn.
Cassia bark, Chinese	Cinnamomum cassia Blume.
Cassia bark, Padang or Batavia	Cinnamomum burmanni Blume.
Cassia bark, Saigon	Cinnamomum loureirii Nees.
Celery seed	Apium graveolens L.
Cherry, wild, bark	Prunus serotina Ehrh.
Chervil	Anthriscus cerefolium (L.) Hoffm.
Chicory	Cichorium intybus L.
Cinnamon bark, Ceylon	Cinnamomum zeylanicum Nees.
Cinnamon bark, Chinese	Cinnamomum cassia Blume.
Cinnamon bark, Saigon	Cinnamomum loureirii Nees.

000075

[redacted]

Cinnamon leaf, Ceylon...........	Cinnamomum zeylanicum Nees.
Cinnamon leaf, Chinese..........	Cinnamomum cassia Blume.
Cinnamon leaf, Saigon...........	Cinnamomum loureirii Nees.
Citronella......................	Cymbopogon nardus Rendle.
Citrus peels....................	Citrus spp.
Clary (clary sage)..............	Salvia sclarea L.
Clover..........................	Trifolium spp.
Coca (decocainized).............	Erythroxylum coca Lam. and other spp. of Erythroxylum.
Coffee..........................	Coffea spp.
Cola nut........................	Cola acuminata Schott and Endl., and other spp. of Cola.
Coriander.......................	Coriandrum sativum L.
Cumin (cummin)..................	Cuminum cyminum L.
Curacao orange peel (orange, bitter peel).	Citrus aurantium L.
Cusparia bark...................	Galipea officinalis Hancock.
Dandelion.......................	Taraxacum officinale Weber and T. laevigatum DC.
Dandelion root..................	Do.
Dog grass (quackgrass, triticum)	Agropyron repens (L.) Beauv.
Elder flowers...................	Sambucus canadensis L. and S. nigra I.
Estragole (esdragol, esdragon, tarragon).	Artemisia dracunculus L.
Estragon (tarragon).............	Do.
Fennel, sweet...................	Foeniculum vulgare Mill.
Fenugreek.......................	Trigonella foenum-graecum L.
Galanga (galangal)..............	Alpinia officinarum Hance.
Geranium........................	Pelargonium spp.
Geranium, East Indian...........	Cymbopogon martini Stapf.
Geranium, rose..................	Pelargonium graveolens L'Her.
Ginger..........................	Zingiber officinale Rosc.
Grapefruit......................	Citrus paradisi Macf.
Guava...........................	Psidium spp.
Hickory bark....................	Carya spp.
Horehound (hoarhound)...........	Marrubium vulgare L.
Hops............................	Humulus lupulus L

NCBI National Library of Medicine PubMed

PubMed | Nucleotide | Protein | Genome | Structure | PopSet | Taxonomy | OMIM

Search PubMed for colupulone

Limits | Preview/Index | History | Clipboard

About Entrez

Display Summary Save Text | Order Detach Add to Clipboard

Entrez PubMed
Overview
Help | FAQ
New/Noteworthy

PubMed Services
Journal Browser
MeSH Browser
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Cubby NEW

Related Resources
Order Documents
Grateful Med
Consumer Health
Clinical Alerts
ClinicalTrials.gov

Privacy Policy

Show: 20 Items 1-7 of 7 On

1: Stephan TE, Ngo EO, Nutter LM. Related
Hexahydrocolupulone and its antitumor cell proliferation activity in vitro.
Biochem Pharmacol. 1998 Feb 15;55(4):505-14.
PMID: 9514086; UI: 98173019

2: Mannering GJ, Shoeman JA. Related
Murine cytochrome P4503A is induced by 2-methyl-3-buten-2-ol, 3-methyl-1-pentyn-3-ol (meparfynol), and tert-amyl alcohol.
Xenobiotica. 1996 May;26(5):487-93.
PMID: 8736060; UI: 96338987

3: Shipp EB, Mehigh CS, Helferich WG. Related
The effect of colupulone (a HOPS beta-acid) on hepatic cytochrome P-450 enzymatic acti the rat.
Food Chem Toxicol. 1994 Nov;32(11):1007-14.
PMID: 7959454; UI: 95047955

4: Mannering GJ, Shoeman JA, Shoeman DW. Related
Effects of colupulone, a component of hops and brewers yeast, and chromium on glucose tolerance and hepatic cytochrome P450 in nondiabetic and spontaneously diabetic mice.
Biochem Biophys Res Commun. 1994 May 16;200(3):1455-62.
PMID: 8185600; UI: 94242008

5: Simpson WJ. Related
Application of in vivo bioluminescence to the study of ionophoretic action.
J Biolumin Chemilumin. 1993 May-Jun;8(3):147-52.
PMID: 8493884; UI: 93262998

6: Simpson WJ, Smith AR. Related
Factors affecting antibacterial activity of hop compounds and their derivatives.
J Appl Bacteriol. 1992 Apr;72(4):327-34.
PMID: 1517174; UI: 92388052

7: Mannering GJ, Shoeman JA, Deloria LB. Related
Identification of the antibiotic hops component, colupulone, as an inducer of hepatic cytochrome P-4503A in the mouse.
Drug Metab Dispos. 1992 Mar-Apr;20(2):142-7.
PMID: 1352202; UI: 92315830

Display Summary Save Text | Order Detach Add to Clipboard

000077

[redacted]

NCBI National Library of Medicine PubMed

☐ 1: Tagashira M, Watanabe M, Uemitsu N. Related

Antioxidative activity of hop bitter acids and their analogues.
Biosci Biotechnol Biochem. 1995 Apr;59(4):740-2.
PMID: 7772843; UI: 95290775

☐ 2: Wohlfart R, Wurm G, Hansel R, Schmidt H. Related

[Detection of sedative-hypnotic active ingredients in hops. 5. Degradation of bitter acids t methyl-3-buten-2-ol, a hop constituent with sedative-hypnotic activity].
Arch Pharm (Weinheim). 1983;316(2):132-7. German. No abstract available.
PMID: 6847343; UI: 83203341

☐ 3: Teuber M, Schmalreck AF. Related

Membrane leakage in Bacillus subtilis 168 induced by the hop constituents lupulone, hum isohumulone and humulinic acid.
Arch Mikrobiol. 1973 Dec 21;94(2):159-71. No abstract available.
PMID: 4205069; UI: 74102189

000078

NCBI National Library of Medicine PubMed

PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM

Search PubMed for hops

Limits Preview/Index History Clipboard

About Entrez

Summary

Show: 20 Items 1-14 of 14 One page.

Entrez PubMed
Overview
Help | FAQ
New/Noteworthy

PubMed Services
Journal Browser
MeSH Browser
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Cubby NEW

Related Resources
Order Documents
Grateful Med
Consumer Health
Clinical Alerts
ClinicalTrials.gov

Privacy Policy

1: Miranda CL, Yang YH, Henderson MC, Stevens JF, Santana-Rios G, Deinzer ML, Buhler DR. Related Articles

Prenylflavonoids from hops inhibit the metabolic activation of the carcinogenic heterocyclic amine 2-amino-3-methylimidazo[4, 5-f]quinoline, mediated by cDNA-expressed human CYP1A2.
Drug Metab Dispos. 2000 Nov;28(11):1297-302.
[MEDLINE record in process]
PMID: 11038156; UI: 20493202

2: Miranda CL, Stevens JF, Ivanov V, McCall M, Frei B, Deinzer ML, Buhler DR. Related Articles

Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro.
J Agric Food Chem. 2000 Sep;48(9):3876-84.
[MEDLINE record in process]
PMID: 10995285; UI: 20450950

3: Stevens JF, Taylor AW, Clawson JE, Deinzer ML. Related Articles

Fate of xanthohumol and related prenylflavonoids from hops to beer.
J Agric Food Chem. 1999 Jun;47(6):2421-8.
PMID: 10794646; UI: 20255326

4: Henderson MC, Miranda CL, Stevens JF, Deinzer ML, Buhler DR. Related Articles

In vitro inhibition of human P450 enzymes by prenylated flavonoids from hops, Humulus lupulus.
Xenobiotica. 2000 Mar;30(3):235-51.
PMID: 10752639; UI: 20214313

5: Dixon-Shanies D, Shaikh N. Related Articles

Growth inhibition of human breast cancer cells by herbs and phytoestrogens.
Oncol Rep. 1999 Nov-Dec;6(6):1383-7.
PMID: 10523716; UI: 99455162

6: Miranda CL, Stevens JF, Helmrich A, Henderson MC, Rodriguez RJ, Yang YH, Deinzer ML, Barnes DW, Buhler DR. Related Articles

Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (Humulus lupulus) in human cancer cell lines.
Food Chem Toxicol. 1999 Apr;37(4):271-85.
PMID: 10418944; UI: 99345312

7: Milligan SR, Kalita JC, Heyerick A, Rong H, De Cooman L, De Keukeleire D. Related Articles

Identification of a potent phytoestrogen in hops (Humulus lupulus L.) and beer.
J Clin Endocrinol Metab. 1999 Jun;84(6):2249-52.

000079

[redacted]

PMID: 10372741; UI: 99299956

8: Zava DT, Dollbaum CM, Blen M.

Related Articles

Estrogen and progestin bioactivity of foods, herbs, and spices.
Proc Soc Exp Biol Med. 1998 Mar;217(3):369-78.
PMID: 9492350; UI: 98151314

9: Duncan KL, Hare WR, Buck WB.

Related Articles

Malignant hyperthermia-like reaction secondary to ingestion of hops in five dogs.
J Am Vet Med Assoc. 1997 Jan 1;210(1):51-4.
PMID: 8977648; UI: 97132192

10: Shipp EB, Mehigh CS, Helferich WG.

Related Articles

The effect of colupulone (a HOPS beta-acid) on hepatic cytochrome P-450 enzymatic activity in the rat.
Food Chem Toxicol. 1994 Nov;32(11):1007-14.
PMID: 7959454; UI: 95047955

11: Mannering GJ, Shoeman JA, Shoeman DW.

Related Articles

Effects of colupulone, a component of hops and brewers yeast, and chromium on glucose tolerance and hepatic cytochrome P450 in nondiabetic and spontaneously diabetic mice.
Biochem Biophys Res Commun. 1994 May 16;200(3):1455-62.
PMID: 8185600; UI: 94242008

12: Mannering GJ, Shoeman JA, Deloria LB.

Related Articles

Identification of the antibiotic hops component, colupulone, as an inducer of hepatic cytochrome P-4503A in the mouse.
Drug Metab Dispos. 1992 Mar-Apr;20(2):142-7.
PMID: 1352202; UI: 92315830

13: Newmark FM.

Related Articles

Hops allergy and terpene sensitivity: an occupational disease.
Ann Allergy. 1978 Nov;41(5):311-2. No abstract available.
PMID: 717854; UI: 79060378

14: Fenselau C, Talalay P.

Related Articles

Is oestrogenic activity present in hops?
Food Cosmet Toxicol. 1973 Aug;11(4):597-603. No abstract available.
PMID: 4758964; UI: 74045709

Summary

Show: 20 . Items 1-14 of 14 One page.

000080

SUBMISSION END

000081

[redacted]

FD

ROBERT H. SINDT
ATTORNEY AT LAW

1850 M Street, N.W., Suite 400
Washington, D.C. 20036
Phone 202-466-4500 • Fax 202-466-5777 • E-mail rsindt@krooth.com

February 21, 2000

Dr. Lawrence Lin
Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St., SW
Washington, DC 20204

Re: Hops Beta Acids GRAS Notice (GRN) No. 000063

Dear Dr. Lin:

This will follow up our recent further conversation regarding the above referenced GRN No. 000063 for hops beta acids, filed on behalf of my client, Rhodia, Inc, relating to the quantitative composition of the ingredient listing on page 000006 of GRN No. 000063.

Upon further consideration, Rhodia has determined to provide FDA with the normal range of quantitative composition for the ingredients in question. Consequently, please utilize a composition range for maltodextrin of 95-99%, and a compositon range of 1-5% for hops beta acids for the ingredients listed on page 000006 of GRN No. 000063. Further, please disregard the confidentiality representation contained in my letter to you of February 9, 2001.

I hope you will find this information to be fully responsive to your earlier inquiry. Please let me know, if you have other questions, so that we may promptly respond to them. Thank you.

Sincerely

Robert H. Sindt

RHS:bs

[redacted]

AM

ROBERT H. SINDT
ATTORNEY AT LAW

1850 M Street, N.W., Suite 400
Washington, D.C. 20036
Phone 202-466-4500 • Fax 202-466-5777 • E-mail rsindt@krooth.com

February 9, 2001

Dr. Lawrence Lin
Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St., SW
Washington, DC 20204

Re: Hops Beta Acid GRAS Notice (GRN) No. 000063

Dear Dr. Lin:

This will follow up the recent conference call between Dr. Kahl, your other FDA colleagues, you, Jim Elfstrum of my client, Rhodia Inc., and me regarding the above referenced GRN No. 000063 filed on behalf of Rhodia, Inc. relating to specified uses of hops beta acids. Specifically, this letter is intended to address and clarify a matter you raised during the call regarding GRN No. 000063.

You noted that the specifications listed on page 000008 do not include quantitative compositions in the ingredient listing. As we discussed, the quantitative composition of the referenced ingredients is deemed to be proprietary information and, therefore, Rhodia Inc. maintains that specific ingredient percentages must remain confidential. For your information, however, it is noted that the ingredients are listed in order of predominance.

It is hoped the above clarifies the matter you raised. We appreciate your bringing this matter to our attention and for the opportunity to clarify it. Please promptly contact me should you have other questions in your review of GRN No. 000063. Thank you.

Sincerely

Robert H. Sindt

RHS/bs

[redacted]