



(51) International Patent Classification:

C12C 3/12 (2006.01) *A23L 2/44* (2006.01)
A23L 3/3463 (2006.01) *C12P 7/06* (2006.01)
A23L 3/3508 (2006.01) *A23C 3/08* (2006.01)
C12H 1/00 (2006.01) *C12N 1/04* (2006.01)
C13B 10/00 (2011.01)

(21) International Application Number:

PCT/US2022/013341

(22) International Filing Date:

21 January 2022 (21.01.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/140,712 22 January 2021 (22.01.2021) US

(71) Applicant: JOHN I. HAAS, INC. [US/US]; 5185

MacArthur Boulevard NW, Suite 300, Washington, District of Columbia 20016 (US).

(72) Inventors: FAOUR, Sami; 5185 MacArthur Boulevard

NW, Suite 300, Washington, District of Columbia 20016 (US). KARAVOLOS, Michail; BetaTech Hop Products,

Malvern Hills Science Park Geraldine Road, Great Malvern Worcestershire WR14 3SZ (GB). VASQUEZ, Guillermo Guerrero; BetaTech Hop Products, Malvern Hills Science Park Geraldine Road, Great Malvern Worcestershire WR14 3SZ (GB).

(74) Agent: EIDT, Chandra, E. et al.; Seed Intellectual Property

Law Group LLP, Suite 5400, 701 Fifth Avenue, Seattle, Washington 98104-7064 (US).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

(54) Title: IMPROVED HOP ACIDS FORMULATIONS AND METHODS

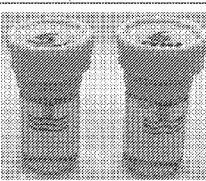
Sample name	Hop derived Alpha acids	Hop derived Alpha acids	Hop derived Alpha acids with 1%MA
Sample number	Sample 1 and 2	Sample 3 and 4	Sample 5 and 6
conditions	pH 8.31	pH 11.58	pH 11.67
Observations	 Resin precipitation after treating at 3°Cx24h	 suspended precipitate after treating at 0°Cx24h  Resin precipitation after treating at -2°Cx24h	 Stable product until -12°C

FIG. 3

(57) **Abstract:** The present disclosure provides compositions and methods for improved hop acids formulations comprising myristic acid which have improved stability characteristics. The formulations may be used as an anti-bacterial agent in fermentation processes.



UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

IMPROVED HOP ACIDS FORMULATIONS AND METHODS

TECHNICAL FIELD

The present disclosure relates generally to compositions and methods for improved hop acids formulations. More specifically, the disclosure relates to an improved formulation of hop derived alpha acids extracts comprising myristic acid and having improved stability characteristics.

BACKGROUND

Hops have been used in brewing for well over one thousand years. Hop cones contain lupulin glands that have two important bittering substances: alpha acids and beta acids. These acids are sometimes called humulones and lupulones, respectively. Hop acids were initially used as a preservative agent for beer prior to the existence of refrigeration. Today, they are primarily used to create the bitter taste and flavor of beer.

The term "hop acids," as used herein, means alpha acids, beta acids, mixtures of these acids, and/or other components found in hop extracts; for example, beta fraction, essential oils, waxes, and uncharacterized resins. The term "hop acids" also includes modified hop acids. Hop alpha acids consist of a mixture of analogues, including humulone, cohumulone, and adhumulone. Alpha acids make-up 10 to 15 percent w/w in dry hops and over 50 percent by weight of carbon dioxide hop extract. During the brewing of beer, hops are boiled and the alpha acids undergo thermal isomerization forming a new compound known as isoalpha acids. Isoalpha acids are the actual bittering and preserving compounds found in beer. Derivatives of isoalpha acids are made by performing simple chemical reductions. These reduced isoalpha acids include rhoisoalpha acids, tetrahydroisoalpha acids (THIAA), and hexahydroisoalpha acids (HHIAA). Thus the term "alpha acids" includes isoalpha acids, tetrahydroisoalpha acids, rhoisoalpha acids, and hexahydroisoalpha acids. Beta acids also consist of a mixture of analogues, including lupulone, colupulone, and adlupulone. The term "beta acids" also includes modified beta acids, such as hexa-hydro-beta acids.

Difficulty of maintaining a sterile condition free from bacteria is an important concern in conventional fermentation systems. The conditions used typically for fermentations in these systems are also conducive for bacterial growth. A single occurrence of contamination involves costly and time-consuming sterilization and loss of materials and production. The economics and efficiency of fermentation processes are frequently such that they cannot tolerate any such loss of production.

Current methods used to kill these unwanted microorganisms, among others, often involve introduction of foreign agents, such as antibiotics, heat, and strong chemical disinfectants, to the fermentation before or during production. Antibiotics and chemical disinfectants are expensive and can add greatly to the costs of large-scale production. Likewise, the use of heat requires substantial energy to heat the fermentation or yeast propagation vessels as well as, possibly requiring the use of special, pressure-rated vessels that can withstand the high temperatures and pressures generated in such heat sterilizing processes. Moreover, these chemicals are often hazardous materials requiring special handling and environmental and safety precautions, and are not “green”, i.e., are not organic. Thus, existing methods of controlling growth of microorganisms add to the time and costs of production processes.

Aqueous formulations of hop alpha acids can be used as an anti-bacterial agent in fermentation processes and obviate the need for antibiotic or hazardous chemical use. Moreover, as a traditional component of beer for many centuries, hop acids are a proven organic consumable. Hops are one of the basic ingredients of beer and, as such, hops and hop extracts are considered GRAS (Generally Recognized as Safe) by the U.S. Food and Drug Administration (FDA).

Currently available formulations, however, form a resin precipitate when exposed to temperatures below 4°C. This separation/precipitation is not easily reversed upon return to normal room temperatures, making these formulations difficult to work with.

There is a need in the art for stable hop acid compositions for use in controlling, preventing, or reducing microorganism growth in processes utilizing fermentation in order to increase product yield without significant cost and time.

BRIEF SUMMARY

5 As described below, the present disclosure features compositions comprising hop acids and methods for utilizing these compositions, for example but not limited to, for reducing and/or preventing microorganism growth in processes utilizing fermentation, brewing, and distilling.

10 The present disclosure provides compositions comprising a hop derivative, myristic acid, and water. In certain embodiments, the hop derivative may comprise an alpha acid, beta acid, or a combination of alpha and beta acids. In some embodiments, the hop alpha acids or hop derived alpha acids may comprise reduced isoalpha acids. In some embodiments, the hop alpha acids or hop derived alpha acids may comprise reduced isoalpha acids including tetrahydroisoalpha acids (THIAA), hexahydroisoalpha
15 acids (HHIAA), or a combination of tetrahydroisoalpha acids (THIAA) and hexahydroisoalpha acids (HHIAA). In another embodiment, the composition may further comprise hop beta acids or hop derived beta acids. In any of the previous embodiments, the composition is any one or more of a liquid, a powder, a colloid, an oil, and an emulsion.

20 In some embodiments, the hop alpha acids are present in an amount from about 1% to about 30% (w/w) and the myristic acid is present in an amount from about 0.05% to about 15% (w/v). In some embodiments, the hop alpha acids are present in an amount from about 1% to about 30% (w/w) and the myristic acid is present in an amount from about 0.2% to about 15% (w/v). In certain embodiments, the composition
25 comprises hop alpha acids in an amount from about 1% to about 30% (w/w) and the myristic acid is present in an amount from about 0.05 to about 0.2% MA(w/v). In these embodiments, the composition does not form a precipitate when cooled to -8°C and recovers its initial properties when warmed up to room temperature. In some embodiments, the composition comprises hop alpha acids in an amount from about 1%

to about 30% (w/w) and the myristic acid is present in an amount from about 0.3% to 1.5% MA (w/v). In these embodiments, the composition does not form a precipitate when the composition is cooled to -12°C and recovers its initial properties when warmed up to room temperature. In certain embodiments, the composition comprises
5 hop alpha acids in an amount from about 1% to about 30% (w/w) and the myristic acid is present in an amount from about 0.3% to 0.4% MA (w/v). In these embodiments, the composition does not form a precipitate when cooled to -16.7°C and recovers its initial properties when warmed up to room temperature.

In some embodiments, the hop alpha acids are present in an amount from about
10 1% to about 30% (w/w) and the myristic acid is present in an amount from about 0.25% to about 15% (w/v). In certain embodiments, the composition does not form a precipitate when cooled to 2°C. In some embodiments, a resin precipitates when the composition is cooled below -12°C. In further embodiments, a resin precipitates when the composition is cooled to -12°C and resolubilizes when the composition is warmed
15 to 20°C and gently agitated.

The present disclosure further provides methods of controlling bacterial growth in a fermentation process, comprising adding a composition comprising hop alpha acids, myristic acid, and water to a fermentation vessel, a yeast propagation tank, or both. In some embodiments, the fermentation process is used for ethanol production.
20 In certain embodiments, the fermentation process is used for yeast production. In a further embodiment, the method comprises adding an aqueous solution of a composition comprising hop alpha acids, myristic acid, and water to a process medium. The process medium can have a pH ranging from about 2 to about 6. Accordingly, a method is disclosed for controlling micro-organisms in an aqueous process medium
25 comprising adding an effective amount of the compositions disclosed herein.

In certain embodiments, the disclosure provides a kit for reducing or preventing the growth of microorganisms comprising the compositions disclosed herein in a form suitable for delivery to a target site. In some embodiments, the target site is a fermentation vessel, a yeast propagation tank, or both. In certain embodiments, the
30 target site is a heat exchanger, a liquefaction tank, or a seed tank.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color.

- 5 Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figures 1A and 1B depict the results of cooling and rewarming several different hop acids formulations. Figure 1A shows the results for a formulation of 9% (w/w) hop beta acids and 10% (w/v) myristic acid (“beta-derived solution”, second
10 column), a formulation of 10% (w/w) hop alpha acids having pH 9.94 (“alpha derived solution”, third column), and a formulation of 10% (w/w) hop alpha acids having pH 12.37 (“alpha derived solution”, fourth column). Figure 1B shows the results for two different formulations of 10% (w/w) hop alpha acids with varying concentrations of myristic acid. The second column shows the results for a 5% (w/v) myristic acid
15 formulation, and the third column shows the results for a 10% (w/v) myristic acid formulation.

Figure 2 depicts chromatograms of a 10% hop alpha acid formulation with 1% (w/v) myristic acid before (top panel) and after (bottom panel) freezing at -16.7°C. Both chromatograms were generated using an Agilent 1220 high-performance liquid
20 chromatograph with the following parameters. Mobile phase: MeOH-Water 79%, H₃PO₄ 1%, EDTA 0.001%, flow 1.5, injection 10 µl.

Figure 3 depicts the results of cooling and rewarming several different 10% (w/w) hop alpha acids formulations. A formulation of hop alpha acids having pH 8.31 is shown in column two, a formulation of hop alpha acids having pH 11.58 is shown in
25 column three, and a formulation of hop alpha acids and 1% myristic acid having pH 11.67 is shown in column four.

Figure 4 depicts samples of a 10% (w/w) hop alpha acids formulation without myristic acid at different temperatures. The left image shows the formulation at -2°C,

the center image shows the formulation at room temperature, and the right image shows the formulation after cooling to -12°C, rewarming to room temperature, and mixing.

Figure 5 depicts samples of a 10% (w/w) hop alpha acids formulation with 1% myristic acid at different temperatures. The top panel shows the formulation at -11.9°C (left image) and after cooling to -12°C and rewarming to room temperature (center and right images). The bottom panel shows the formulation at -16.7°C (left image), after cooling to -16.7°C and rewarming to room temperature (center image), and after cooling to -16.7°C, rewarming to room temperature, shaking, and standing at room temperature for three days (right panel).

Figures 6A-6C depict samples of a 20% (w/w) hop alpha acids formulation with varied amounts of myristic acid at different temperatures. Figure 6A shows samples of a 20% (w/w) hop alpha acids formulation with no myristic acid at room temperature (top left), at 2°C (top right), at -12°C (bottom left), after cooling to -12°C, rewarming to room temperature, and mixing (bottom center), and after cooling to -12°C, rewarming to room temperature, mixing, and standing at room temperature for three days (bottom right). Figure 6B shows samples of a 20% (w/w) alpha acids formulation with 4% (w/v) myristic acid at room temperature (top left), at -10°C (top center), after cooling to -10°C and rewarming to room temperature (top right), at -12°C (bottom left), after cooling to -12°C, rewarming to room temperature, and mixing (bottom center), and after cooling to -12°C, rewarming to room temperature, mixing, and standing at room temperature for three days (bottom right). Figure 6C shows samples of a 20% (w/w) alpha acids formulation with 5% (w/v) myristic acid at room temperature (top left), at -11°C (top center), after cooling to -11°C and rewarming to room temperature (top right), at -12°C (bottom left), after cooling to -12°C, rewarming to room temperature, and mixing (bottom center), and after cooling to -12°C, rewarming to room temperature, mixing, and standing at room temperature for three days (bottom right).

Figures 7A-7F shows the results of cooling and rewarming of formulation of hop alpha acids without MA(control)(Figure 7A) and formulations of hop alpha acids comprising MA in an amount of 0.5% (Figure 7B), 0.75%(Figure 7C), 1% (Figure 7D),

1.25% (Figure 7D), and 1.5% (Figure 7F) w/v (g/100mL). All formulations were cooled down to -12°C and rewarmed to room temperature with gentle mixing. Bottom panel for each figure shows formulations rewarmed to room temperature from -12°C.

Figures 8A-8F shows the results of cooling and rewarming of formulation of hop alpha acids without MA (control) (Figure 8A), and formulations of hop alpha acids comprising MA in an amount of 0.05%(Figure 8B), 0.1%(Figure 8C), 0.2%(Figure 8D), 0.3%(Figure 8E) and 0.4%(Figure 8F) w/v (g/100mL), cooled down to -9°C and -12°C before being rewarmed to room temperature. Control Hop acids formulation (Figure 8A, top row) was first cooled to 5°C and then to -12°C (Figure 8A, bottom panel), before being rewarmed to room temperature. Hop acids formulations comprising 0.05% and 0.1% (w/v) of MA were first cooled to -9°C before being rewarmed to room temperature. (Figures 8B, top panel and Figure 8C, top panel, respectively). Bottom panel for each formulation shows formulations rewarmed to room temperature from -12°C. All samples were rewarmed up to room temperature with gentle mixing by hand.

Figures 9A-9F shows the results of cooling and rewarming of formulation of hop alpha acids without MA (Control) (Figure 9A), and formulations of hop alpha acids comprising MA in an amount of 0.05%(Figure 9B), 0.1%(Figure 9C), 0.2%(Figure 9D), 0.3%(Figure 9E), and 0.4%(Figure 9F) w/v (g/100mL) cooled down to -16.7°C before being rewarmed to room temperature. All samples were cooled to -16.7°C and rewarmed to room temperature slowly with gentle mixing. Top panel for each formulation shows formulations cooled down to -16.7°C, and bottom panels for each formulation shows formulations rewarmed to room temperature from -12°C.

DETAILED DESCRIPTION

As described below, the present disclosure provides compositions comprising hop acids and/or their derivatives, and fatty acids. Upon exposure to low temperatures, aqueous hop alpha acid formulations form a resin precipitate and they freeze at temperatures lower than -11°C. Surprisingly, the present inventors have discovered that the addition of fatty acids to hop alpha acid formulations has several significant benefits. For example, the present inventors have discovered that the addition of

myristic acid(MA), to an aqueous hop alpha acid formulation results in a stable solution that stays precipitate-free even when exposed to low temperatures, and has a lowered freezing temperature of -11°C or -13°C. Further, in certain embodiments, when these compositions are cooled to -12°C or -16°C and rewarmed to room temperature with
5 gentle mixing, the composition is clear and contains no resin or oily precipitate. These formulations are useful for inhibition, prevention, and/or control of bacterial growth in, for example but not limited to, fermentation processes for yeast production and ethanol production.

The present disclosure also provides compositions effective for reducing or
10 preventing the growth of microorganisms. In certain embodiments, the present disclosure is directed to a process for controlling micro-organisms in an aqueous process medium comprising adding an effective amount of aqueous alkaline composition comprising hop acids and fatty acids to the process medium. Myristic acid has been previously added to hop beta acid formulations as an emulsifier, but it has
15 been found that such formulations do not have lower freezing temperatures than hop beta acid formulations without myristic acid. Additionally, the addition of myristic acid to hop beta acid formulations does not prevent the appearance of precipitates when the compositions are cooled to -2°C and re-warmed up to room temperature.

As used herein, the term “about,” as applied to a numeric value or numerical
20 range, means a value within +/- 10% of the stated value.

As used herein, “room temperature” refers to the range of air temperatures that most people prefer for indoor settings when wearing typical indoor clothing. Room temperature is typically considered to be between about 20°C and about 26°C.

As used herein, “an effective amount”, means an amount effective in reducing,
25 preventing, slowing the growth or proliferation of microorganisms.

As used herein, “hop alpha acids”, or “hop derived alpha acids extract”, or “alpha acids”, refers to hop derived alpha acids and includes derivatives thereof, such as, isoalpha acids, reduced or modified isoalpha acids including tetrahydroisoalpha acids (THIAA), hexahydroisoalpha acids (HHIAA), rhoisoalpha acids, or combinations
30 and mixtures thereof.

The compositions and/or formulations provided herein comprise hop alpha acids, fatty acids, and water. In certain embodiments, the hop acid is a natural hop acid or a derivative thereof, such as, alpha acid, beta acid, tetrahydroalpha acid (THAA), or hexahydrobeta acid (HHBA), or mixtures thereof; an isomerized hop acid or a derivative thereof, such as, isoalpha acid (IAA), rhoisoalpha acid (RIAA), tetrahydroisoalpha acid (THIAA) or hexahydroisoalpha acid (HHIAA) or mixtures thereof. Alpha acids contained in the hop acid may be transformed into isoalpha acids during the preparation of the hop acid solution and maintain their anti-bacterial/anti-microbial effect. The hop alpha acids or hop derived alpha acids may comprise hexahydroisoalpha acids and tetrahydroisoalpha acids. In any of the above embodiments, the hop alpha acids comprise hexahydroisoalpha acids and tetrahydroisoalpha acids at a ratio of about 1:1.

Depending on the hop acid product, the concentration of hop acids in the composition will vary. Generally, the final concentration of hop alpha acids ranges from about 1% to about 30% (w/w), in another aspect from about 5% to about 25%(w/w), in another aspect from about 10% to about 20%(w/w). In certain embodiments, the hop alpha acids are present in the amount of about 10% (w/w). In some embodiments, the hop alpha acids are present in the amount of about 20% (w/w). Higher concentrations may be appropriate where longer transport times are required. Generally, hop acids in their acid form exhibit low solubility in water. However, hop acids can be mixed with an alkali metal hydroxide, for example potassium hydroxide, to make a water soluble alkali metal salt of the hop acids. Accordingly, it is advantageous to use alkali hydroxides, for example potassium hydroxide or sodium hydroxide or a mixture thereof as the alkaline medium to control micro-organisms. The concentrations of the alkaline medium ranges from about 20% to about 45 wt. %, or in another aspect from about 20 wt. %. In any of the previous embodiments, the hop alpha acids or hop derived alpha acids are in the form of potassium salts of isoalpha acids.

In any of the above embodiments, the fatty acids may be or may comprise myristic acid. Myristic acid is a saturated fatty acid with the molecular formula $\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$, and the chemical name 1-tetradecanoic acid. Myristic acid has

been previously added to hop beta acid formulations as an emulsifier, but it has been found that such formulations do not have lower freezing temperatures than hop beta acids formulations without myristic acid. Additionally, the addition of myristic acid to hop beta acids formulations does not prevent the appearance of precipitates when the compositions are cooled to -2°C and re-warmed.

In some embodiments, the myristic acid is present in an amount from about 0.05% to about 15% (w/v), 0.25% to about 15% (w/v), from about 0.5% to about 10% (w/v), from about 1% to about 8% (w/v), or from about 2% to about 5% (w/v). In certain embodiments, the myristic acid is present in the amount of about 0.05% to 0.2% (w/v), 0.3% to about 1.5% (w/v), about 0.3% to about 0.4% (w/v). In further embodiments, the myristic acid is present in the amount of about 0.25% (w/v), about 0.5% (w/v), about 1% (w/v), about 2.5% (w/v), about 4% (w/v), or about 5% (w/v).

In some embodiments, the compositions provided herein comprise about 15% (w/w) hop alpha acids and 0.05% (w/v) myristic acid, about 15% (w/w) hop alpha acids and 0.1% (w/v) myristic acid, about 15% (w/w) hop alpha acids and 0.2% (w/v) myristic acid, about 15% (w/w) hop alpha acids and 0.3% (w/v) myristic acid, and about 15% (w/w) hop alpha acids and 0.4% (w/v) myristic acid. In certain embodiments, the composition provided herein comprises about 15% (w/w) hop alpha acids and 0.5% (w/v) myristic acid, about 15% (w/w) hop alpha acids and 0.75% (w/v) myristic acid, about 15% (w/w) hop alpha acids and 1.0% (w/v) myristic acid, about 15% (w/w) hop alpha acids and 1.25% (w/v) myristic acid, about 15% (w/w) hop alpha acids and 1.5% (w/v) myristic acid.

In some embodiments, the composition provided herein comprises 10% (w/w) hop alpha acids and 0.05% (w/v) myristic acid, 10% (w/w) hop alpha acids and 0.25% (w/v) myristic acid, 10% (w/w) hop alpha acids and 0.3% (w/v) myristic acid, 10% (w/w) hop alpha acids and 0.5% (w/v) myristic acid, 10% (w/w) hop alpha acids and 0.75% (w/v) myristic acid, 10% (w/w) hop alpha acids and 1% (w/v) myristic acid, 10% (w/w) hop alpha acids and 1.25% (w/v) myristic acid, 10% (w/w) hop alpha acids and 1.5% (w/v) myristic acid, 10% (w/w) hop alpha acids and 2.5% (w/v) myristic acid, 10% (w/w) hop alpha acids and 4% (w/v) myristic acid, 10% (w/w) hop alpha acids and

5% (w/v) myristic acid, or 10% (w/w) hop alpha acids and 10% (w/v) myristic acid. In further embodiments, the compositions provided herein comprise 20% (w/w) hop alpha acids and 0.25% (w/v) myristic acid, 20% (w/w) hop alpha acids and 0.5% (w/v) myristic acid, 20% (w/w) hop alpha acids and 1% (w/v) myristic acid, 20% (w/w) hop alpha acids and 2.5% (w/v) myristic acid, 20% (w/w) hop alpha acids and 4% (w/v) myristic acid, 20% (w/w) hop alpha acids and 5% (w/v) myristic acid, or 20% (w/w) hop alpha acids and 10% (w/v) myristic acid.

In certain embodiments, the compositions provided herein further comprise hop beta acids.

10 In some embodiments, the compositions provided herein have a pH between about 8 and about 12 at 60°C. In some embodiments, the pH is between about 10 and about 12 at 60°C. In some embodiments, the compositions provided herein have a pH between about 8 and about 13 at room temperature. In certain embodiments, the pH is between about 8 and about 13 at a temperature of about 20°C to about 26°C. In some
15 embodiments, the pH is between about 10 and about 13 at a temperature of about 25°C. In some embodiments, the pH is about 10.5 at a temperature between about 20°C and about 26°C.

In some embodiments, the compositions provided herein do not form a precipitate when cooled to 2°C. In certain embodiments, the compositions provided
20 herein do not form precipitates when cooled to -12°C or -16°C and warmed back up to room temperature. In some embodiments, the compositions provided herein do not form a precipitate when cooled to -9°C and warmed back up to room temperature. These compositions regain their initial properties when warmed to room temperature. In further embodiments, a resin precipitates is formed when the composition is cooled
25 to -12°C or -16°C and resolubilizes when the composition is warmed to 20°C and gently mixed. In all previous embodiments, the formulations described herein are warmed up to room temperature (without heating) with gentle shaking with “hand” if needed and no sonication. Once warmed up to room temperature, physical properties are observed (separation of resin formation, crystal formation or still a homogenous
30 solution).

In some embodiments, the composition comprises 10% (w/w) alpha acids and 1% or more (w/v) myristic acid. These compositions do not form a precipitate when cooled to -12°C. In some embodiments, the composition comprises 10% (w/w) alpha acids and between 0.1% and 1% (w/v) myristic acid. These compositions form some precipitate when cooled to -8°C, and the precipitate resolubilizes when the composition is warmed to room temperature.

In some embodiments, the composition comprises about 15% (w/w) alpha acids and between 0.05% and 0.2% (w/v) myristic acid. These compositions do not form a precipitate when cooled to -8°C. These compositions form precipitate when cooled to 12°C or below and warmed to room temperature. In some embodiments, the composition comprises about 15% (w/w) alpha acids and between 0.3% and 0.4% (w/v) myristic acid. These compositions do not form a precipitate when cooled to -12°C. In further embodiments, the composition comprises about 15% (w/w) alpha acids and between 0.3% and 0.4% (w/v) myristic acid. These compositions do not form a precipitate when cooled to -16.7°C and warmed to room temperature.

The present disclosure further provides methods of controlling, preventing, and/or reducing bacterial growth in for example, an ethanol production process. In some embodiments, the method comprises adding a composition comprising hop alpha acids, myristic acid, and water to a fermentation vessel, a yeast propagation tank, or both. In a further embodiment, the method comprises adding an effective amount of aqueous solution of the compositions disclosed herein to a process medium. The pH of the process medium may range from about 2 to about 6. In certain embodiments, the method may comprise adding the compositions disclosed herein to the process medium continuously or discontinuously, e.g., using shock dosage. For example, for shock dosage, the composition is periodically added to the process medium, e.g., the dosage is made at defined times within very short time intervals at which locally and for a short time interval high concentrations can be adapted. The high local concentrations achieved by this kind of dosing avoid the adaptation of the micro-organisms. The compositions disclosed herein may be manually dosed into the process medium. Alternatively, the solution may be added to the process medium through closed dosing

systems. That means that control of micro-organisms may be done under the use of the process installations (closed dosing systems) already available.

The method for controlling micro-organisms can be automated by the use of time controls for the dosing pumps and valves. The improved effect means that the overall concentration of active ingredients can be reduced, which produces a number of advantages. Either reduced costs are achieved through lower dosing or the same dosing produces a better effect.

The method for controlling micro-organisms using the compositions disclosed herein may be applied in an advantageous way in distilleries for the production of alcoholic drinks, specifically of spirits or in the production process of wine and wine containing drinks, further in the production of natural ethanol, fuel ethanol, sugar industry, and pharmaceutical drugs. The methods disclosed herein may also be used in the production of all kinds of dairy products, yeast, fruit juices, and tinned foods in aqueous solution. Furthermore, the methods may be used in the formulation of cosmetic and detergent compositions.

In certain embodiments, the disclosure provides a kit for reducing or preventing the growth of microorganisms comprising the compositions disclosed herein in a form suitable for delivery to a target site. In some embodiments, the target site is a fermentation vessel, a yeast propagation tank, or both. In certain embodiment, the target site is a heat exchanger, a liquefaction tank, or a seed tank.

EXAMPLES

EXAMPLE 1:

PRODUCTION OF A 10% (W/W) HOP ALPHA ACIDS FORMULATION WITH 1% (W/V) MYRISTIC ACID

A compound comprising 10% (w/w) alpha acids and 1% (w/v) myristic acid was formulated as follows. 100ml of alpha acids containing 50% hexa-hydro-iso-alpha acids (HHIAA) and 50% tetra-hydro-iso-alpha acids (THIAA) was heated to 60°C. To this solution was added 1.0109g of myristic acid at 60°C. The pH of this suspension

was adjusted to 10.71 using KOH. The solution was cooled to room temperature using an ice water bath, and the pH was measured to be 11.67. The concentration of hop alpha acids was determined by high performance liquid chromatography (HPLC), and measured to be 5% (w/w) hexa-hydro-iso-alpha acids and 4% (w/w) tetra-hydro-iso-alpha acids (see Figure 2, top panel).

EXAMPLE 2:

COMPARISON OF PROPERTIES OF 10% (W/W) HOP ALPHA ACIDS FORMULATIONS WITH OR WITHOUT MYRISTIC ACID

An alpha acid composition comprising about 10% (w/w) alpha acids and having pH of 8.31 at was cooled to 3°C for 24 hours. A resin precipitate was apparent following this treatment. See Figure 3, second column. An alpha acid composition comprising about 10% (w/w) alpha acids (having pH of 11.58 at room temperature) was cooled to 0°C for 24 hours. A suspended precipitate was apparent following this treatment. See Figure 3, third column, top image. A second sample of the same composition was cooled to -2°C for 24 hours. A resin precipitate was apparent following this treatment. See Figure 3, third column, bottom image. Another alpha derived composition comprising about 10% (w/w) alpha acids and (1% (w/v) myristic acid and having a pH of 11.67 was cooled to -12°C. No precipitate was observed to form following this treatment. See Figure 3, fourth column.

EXAMPLE 3:

COMPARISON OF PROPERTIES OF 9% (W/W) HOP BETA ACIDS FORMULATIONS AND 10% (W/W) HOP ALPHA ACIDS FORMULATIONS WITH VARYING AMOUNTS OF MYRISTIC ACID

A beta acid composition comprising about 9% (w/w) beta acids and about 10% (w/v) myristic acid and having a pH of 12.03 at room temperature was cooled to -2°C for 24 hours. At -2°C, the composition was observed to form a precipitate and crystals. See Figure 1A, second column.

Alpha acid compositions comprising about 10% (w/w) alpha acids were also tested. A composition having a pH of 9.94 was cooled to -2°C for 24 hours. At -2 C,

the composition formed a resin precipitate. After re-warming to room temperature, the resin precipitate persisted. See Figure 1A, third column. Similar results were obtained for an alpha acid composition having a pH of 12.37 at room temperature. See Figure 1A, fourth column.

- 5 Alpha acid compositions comprising about 10% (w/w) alpha acids and varying amounts of myristic acid were then tested. The alpha acid composition comprising 10% (w/v) myristic acid and having a pH of 12.73 was cooled to -2°C for 24 hours. At -2°C, resin or oil formation was not observed. See Figure 1B, third column. An alpha acid composition comprising 5% (w/v) myristic acid and having a pH of 12.06 was cooled to
10 -2°C for 24 hours and no precipitate or oil formation was observed. See Figure 1B, second column.

EXAMPLE 4:

COMPARISON OF PROPERTIES OF 10% (W/W) HOP ALPHA ACIDS FORMULATIONS WITH VARYING AMOUNTS OF MYRISTIC ACID

- 15 Alpha acid compositions with or without myristic acid were tested. An alpha hop acid composition comprising about 10% (w/w) alpha acids and no myristic acid was cooled to -2°C, which resulted in the formation of a resin precipitate. When the same formulation was cooled to -12°C and re-warmed to room temperature with mixing, the precipitate persisted. See Figure 4.
- 20 When myristic acid was added to an alpha acid hop composition, the composition could be cooled to a lower temperature without triggering the formation of a precipitate. An alpha hop acid composition comprising about 10% (w/w) alpha acids and about 1% (w/v) myristic acid having a pH of 11.9 could be cooled to -16.7°C without formation of a precipitate. An alpha hop acid composition comprising about
25 10% (w/w) alpha acids and about 0.5% (w/v) myristic acid having a pH of 11.35 could be cooled to -9°C without formation of a precipitate. An alpha hop composition comprising about 10% (w/w) alpha acids and about 0.25% (w/v) myristic acid having a pH of 11.3 could be cooled to -5°C without formation of a precipitate.

EXAMPLE 5:

COMPARISON OF PROPERTIES OF 10% (W/W) HOP ALPHA ACIDS FORMULATIONS WITH OR WITHOUT MYRISTIC ACID

An alpha hop acid composition with about 10% (w/w) alpha acids having a pH of 8.31 at room temperature was observed to form a precipitate upon cooling to 2°C.

An alpha hop acid composition with about 10% (w/w) alpha acids having a pH of 10.7 at room temperature was observed to form a precipitate upon cooling to -1°C.

However, an alpha hop acid composition with about 10% (w/w) alpha acids and about 1% (w/v) myristic acid having a pH of 10.7 at room temperature could be cooled to -12°C without formation of a precipitate. Upon re-warming of the composition to room temperature with gentle mixing, the composition regained its original properties. See Figure 5, top panel. When the same composition was cooled to -16.7°C and re-warmed to room temperature with gentle mixing, the composition regained its original properties and these properties, including a lack of precipitate, were maintained when the compositions was kept at room temperature for three days. See Figure 5, bottom panel.

EXAMPLE 6:

COMPARISON OF PROPERTIES OF 20% (W/W) HOP ALPHA ACIDS FORMULATIONS WITH VARYING AMOUNTS OF MYRISTIC ACID

An alpha hop acid composition with about 20% (w/w) alpha acids having a pH of 10.85 at 25°C was cooled to 2°C without formation of a precipitate. See Figure 6A, top panel. When the same formulation was cooled to -12°C and re-warmed to room temperature with gentle mixing, a large quantity of resin precipitate formed. The resin precipitate remained after three days at room temperature. See Figure 6A, bottom panel.

An alpha hop acid composition with about 20% (w/w) alpha acids and about 4% (w/v) myristic acid having a pH of 10.88 at 21.7°C was cooled to -10°C and re-warmed to room temperature. A small amount of resin precipitate was observed. See Figure 6B, top panel. When the same formulation was cooled to -12°C and re-warmed to room temperature with gentle mixing, a small amount of resin precipitate was observed. A

portion of the precipitate remained after three days at room temperature. See Figure 6B, bottom panel. An alpha hop acid composition with about 20% (w/w) alpha acids and about 5% (w/v) myristic acid was cooled to -11°C and re-warmed to room temperature. No resin precipitate was observed. See figure 6C, top panel. When the same formulation was cooled to -12°C and re-warmed to room temperature with gentle mixing, no resin precipitate was observed, and the lack of precipitate persisted after three days at room temperature. See Figure 6C, bottom panel.

EXAMPLE 7:

PREPARATION OF HOP ALPHA ACIDS FORMULATIONS WITH VARIOUS AMOUNTS OF MYRISTIC ACID (0.5% TO 1.5 %MA)

The general procedure for the preparation of hop derived alpha acids formulations comprising myristic acids (MA) was as follows: Hop alpha acid formulation comprising 17.36% Hop Acid liquid (pH 9.95 at 28°C and, 8.54 at 57.8°C) was heated up to 60°C and a determined mass of myristic acid (MA) ranging from 0.5, 0.75, 1, 1.25, and 1.5% w/v, was added. The amount of KOH added was stoichiometrically proportional to the content of MA. At this point, the pH of the mixture increases to range 10.0-10.2 at 60°C and then stabilized following stirring for 15 min. Then, the solution was cooled down to room temperature (r.t.) using an ice bath (pH range 10.4-10.7). The concentration of alpha acids was determined using HPLC.

The specific formulations with varying amounts of MA were prepared as follows:

To prepare a formulation of hop derived alpha acids comprising 0.5% MA: 0.2498g of MA was added to 15.74% total reduced hop acids at 60°C. Then, 1.43 eq, of KOH 40% was added dropwise to the emulsion. After adding the base the pH increased to 10.04 at 59°C and the mixture pH stabilised after stirring for 10 min. The solution was cooled down to room using an ice bath. The pH of the solution at r.t. was determined to be 10.53. The concentration of alpha acids was determined to be [%HHIAA] = 15.14; [%THIAA] = 0.6, total alpha acids = 15.74%.

To prepare a formulation of hop derived alpha acids comprising 0.75% MA, 0.3752g of MA was added to 16.69% total reduced hop alpha acids at 60°C (pH 8.54 at 58°C). Then, 1.3 eq, of KOH 40% was added dropwise to the emulsion. After adding the base the pH increased to 10.00 at 57.2°C and the mixture pH stabilised after stirring
5 for 10 minutes. The solution was cooled down to r.t. using an ice bath and the pH of the solution at r.t. was determined to be 10.4. The concentration of alpha acids was determined to be [%HHIAA] = 16.05: [%THIAA] = 0.64, total alpha acids = 16.69%.

To prepare a formulation of hop derived alpha acids comprising 1% MA: 0.5003g of MA was added to 15.62% total reduced hop alpha acids at 60°C (pH 8.54 at
10 58°C). Then, 1.24 eq, of KOH 40% was added dropwise to the emulsion. After adding the base the pH increased to 10.09 at 58.2°C and the mixture pH stabilised after stirring for 10 min. The solution was cooled down to r.t. using an ice bath and the pH of the solution was determined to be 10.53. The concentration of alpha acids was determined to be [%HHIAA] = 15.0: [%THIAA] = 0.61, total alpha acids = 15.62%.

15 To prepare a formulation of hop derived alpha acids comprising 1.25% MA: 0.625g of MA was added to 16.89% total reduced hop alpha acids at 60°C (pH 8.54 at 58°C). Then, 1.16 eq, of KOH 40% was added dropwise to the emulsion. After adding the base the pH increased to 10.03 at 57.3°C and the mixture pH stabilised after stirring for 10 min. The solution was cooled down to r.t. using an ice bath and the pH of the
20 solution was determined to be 10.41. The concentration of alpha acids was determined to be [%HHIAA] = 16.23: [%THIAA] = 0.66, total alpha acids = 16.89%.

To prepare a formulation of hop derived alpha acids comprising 1.5% MA: 0.7503g of MA was added to 17.47% total reduced hop alpha acids at 60°C (pH 8.54 at 58°C). Then, 1.15 eq, of KOH 40% was added dropwise to the emulsion. After adding
25 the base the pH increased to 10.00 at 57.7°C and the mixture pH stabilised after stirring for 10 min. The solution was cooled down to r.t. using an ice bath and the pH of the solution was determined to be 10.54. The concentration of alpha acids was determined to be [%HHIAA]= 16.75 : [%THIAA]= 0.72, total alpha acids= 17.47%.

EXAMPLE 8:

STABILITY STUDY FOR A TEMPERATURE RANGE FROM 5 TO -12°C FOR FORMULATIONS OF HOP ALPHA ACIDS WITH VARIOUS AMOUNTS OF MYRISTIC ACID (0.5 TO 1.5 %(w/v)) MA

The formulated products were tested at appropriate low temperatures ranging from 5°C to -12°C to determine their chemical and physical properties. The temperature was dropped from 5°C down to -12°C in one-degree increments for 24 hrs each degree. Following which, the samples were warmed up to room temperature (without heating) with gentle shaking with “hand” if needed, and no sonication. Once warmed up to room temperature (r.t.), physical properties of the samples were observed (separation of resin formation, crystal formation or a homogenous solution). The determination of Hop α and β acids in Hop acid products in the samples exposed to low temperatures was performed using high-performance liquid chromatography (HPLC).

An improvement in the tolerance, of hop derived alpha acids formulations comprising myristic acid (MA) in percentages weight (g) per volume (100mL) as an emulsifier, to low-temperature changes, was observed (Figures 7B-7F). The formulations of alpha acids comprising MA (0.5% to 1.5% w/v) could be frozen below the limit of -9°C without display of any resin precipitation. These formulations recovered their initial properties after reaching a room temperature (r.t., see Table 1 and Figures 7B-7F) with the cooling recirculation system turned off. The improvement is significant because the hop alpha acid formulations comprising MA do not display any resin precipitation after thawing and warming up to r.t. These studies have determined that MA above 0.5% increased the tolerance of hop alpha acids to low temperatures with complete stability until -12°C, avoiding the resin precipitation in the range of pH 10.4-10.7 at r.t.

In comparison, hop derived alpha acid formulations without MA (control formulation) precipitate after reaching the temperature of 5°C (Figure 7A). Further, it was observed that the control formulation is unable to recover its initial characteristics after reaching room temperature: [%HHIAA] = 5.59; [%THIAA] = 17.91 Total alpha acids = 6.12%.

Table 1.

Concentration after thawing study from -12°C to r.t (*The Temperature of the bath and analyte** were 15.7°C after turn off the system by 15h).

Sample name	Control Hop derived Alpha Acid Extract	Hop derived Alpha Acid Extract 0.5%MA	Hop derived Alpha Acid Extract 0.75%MA	Hop Derived Alpha Acid Extract 1%MA	Hop Derived Alpha Acid Extract 1.25%	Hop Derived Alpha Acid Extract 1.5%MA
t=initial HPLC analysis	[%HHIAA]= 16.64 : [%THIAA]= 0.67, Total α acids= 17.31%.	[%HHIAA]= 15.14 : [%THIAA]= 0.6, Total α acids= 15.74%.	[%HHIAA]= 16.05 : [%THIAA]= 0.64, Total α acids= 16.69%.	[%HHIAA]= 15.0 : [%THIAA]= 0.61, Total α acids= 15.62%	[%HHIAA]= 16.23 : [%THIAA]= 0.66, Total α acids= 16.89%.	[%HHIAA]= 16.75 : [%THIAA]= 0.72, Total α acids= 17.47%.
t=final HPLC analysis	[%HHIAA]= 15.71 : [%THIAA]= 0.55, Total α acids= 16.25%. pH 9.46 at rt	[%HHIAA]= 16.12 : [%THIAA]= 0.56, Total α acids= 16.68%. pH 10.52 at rt	[%HHIAA]= 16.12 : [%THIAA]= 0.55, Total α acids= 16.62%. pH 10.34 at rt	[%HHIAA]= 15.84 : [%THIAA]= 0.54, Total α acids= 16.37%. pH 10.4 at rt	[%HHIAA]= 15.91 : [%THIAA]= 0.54, Total α acids= 16.45%. pH 10.31 at rt	[%HHIAA]= 15.67 : [%THIAA]= 0.54, Total α acids= 16.21%. pH 10.49 at rt
Final observation	Resin remaining precipitated.	Stable formulation.	Stable formulation.	Stable formulation.	Stable formulation.	Stable formulation.

5 ***The samples were only shaken (not heated up for homogenization) and sampling at 15.7°C.*

EXAMPLE 9:

PRODUCTION OF HOP DERIVED ALPHA ACIDS FORMULATIONS WITH VARIOUS AMOUNTS OF
MYRISTIC ACID (0.05% TO 0.4 % MA W/V)

The general procedure for the preparation of the hop derived alpha acid
10 formulations comprising 0.05% to 0.4 % MA w/v, subjected to stability testing was as
follows: Hop alpha acids 15.8% HHIAA:THIAA liquid (pH 9.95 at 28°C and, 8.54 at
57.8°C) was heated up to 60°C and to it was added a determined mass of myristic acid
ranging from 0.05, 0.1, 0.2, 0.3, and 0.4% w/v (the percentage amount of MA was
calculated as weight (g) per volume (100mL). At this point, the pH of the mixture
15 increases to range 10.0-10.2. Then, the solution was cooled down to r.t. using an ice
bath. The concentration of HHIAA and THIAA was determined using HPLC.

The specific formulations with varying amounts of MA were prepared as follows:

To prepare a formulation of hop derived alpha acids comprising 0.05% MA:
0.0375g of MA was added to 15.8% total reduced hop alpha acids at 60°C (pH 8.74 at 58.7°C). Then, 4.34 eq, of KOH 40% was added dropwise to the emulsion. Following
5 addition of the base, the pH of the mixture increased to 10.08 at 59.4°C and then stabilized after stirring for 10 min. The solution was cooled down to r.t. using an ice bath. The pH of the solution was determined to be 10.77. The concentration of alpha acids was determined to be [%HHIAA] = 15.37: [%THIAA] = 0.5, total alpha acids= 15.87%.

10 To prepare a formulation of hop derived alpha acids comprising 0.1% MA:
0.00033 mmol of MA was added to 15.92% total reduced hop alpha acids at 60°C (pH 8.74 at 58.7°C). Then, 2.61 eq of KOH 40% was added dropwise to the emulsion. Following addition of the base, the pH of the mixture increased to 10.11 at 57.9°C. The solution was cooled down to r.t. using an ice bath. The pH of the solution was
15 determined to be 10.51. The concentration of alpha acids was determined to be [%HHIAA] = 15.4: [%THIAA] = 0.52, total alpha acids= 15.92%.

To prepare a formulation of hop derived alpha acids comprising 0.2% MA:
0.15g of MA was added to 15.99% total reduced hop alpha acids at 60°C (pH 8.74 at 58.7°C). Then, 1.73 eq, of KOH 40% was added dropwise to the emulsion. Following
20 addition of the base, the pH of the mixture increased to 10.11 at 58.7°C. The solution was cooled down to r.t. using an ice bath. The pH of the solution was determined to be 10.48. The concentration of alpha acids was determined to be [%HHIAA] = 15.47: [%THIAA] = 0.52, total alpha acids= 15.99.

To prepare a formulation of hop derived alpha acids comprising 0.3% MA:
25 0.225g of MA was added to 16.02% total reduced hop alpha acids at 60°C (pH 8.74 at 58.7°C). . Then, 1.8 eq, of KOH 40% was added dropwise to the emulsion. Following addition of the base, the pH of the mixture increased to 10.03 at 59.5°C. The solution was cooled down to r.t. using an ice bath. The pH of the solution was determined to be 10.71. The concentration of alpha acids was determined to be [%HHIAA]= 15.5 :
30 [%THIAA]= 0.52, total alpha acids= 16.02%.

To prepare a formulation of hop derived alpha acids comprising 0.4% MA: 0.3009g, of MA (pH 7.82 at 53°C) was added to 15.89% total reduced hop acids at 60°C (pH 8.74 at 58.7°C). . Then, 1.63 eq, of KOH 40% was added dropwise to the emulsion. Following addition of the base, the pH of the mixture increased to 10.08 at 5 58.3°C. The solution was cooled down to r.t. using an ice bath. The pH of the solution was determined to be pH 10.46 at r.t. The concentration of alpha acids was determined to be [%HHIAA] = 15.39 : [%THIAA] = 0.5, total alpha acids = 15.89%.

EXAMPLE 10

10 FREEZING STABILITY STUDY 5 TO -12°C FOR FORMULATIONS OF HOP DERIVED ALPHA ACIDS WITH VARIOUS AMOUNTS OF MYRISTIC ACID (0.05 TO 0.4 %) MA

The formulated products were tested at appropriate low temperatures ranging from 5 °C to -12°C to determine their chemical and physical properties. The temperature was dropped from 5°C down to -12°C in one-degree increments for 24 hrs 15 each degree. Following which, the samples were warmed up to room temperature (without heating) with gentle shaking with “hand” if needed and no sonication. Once warmed up to room temperature (r.t.), physical properties of the samples were observed (separation of resin formation, crystal formation or a homogenous solution). The determination of Hop alpha (Hop α) and beta (Hop β) acids in Hop acid products in the 20 samples exposed to low temperatures was performed using high-performance liquid chromatography (HPLC).

The present assay describes the stability of hop derived alpha acids formulations below 0.5% MA as percent w/v (g/100mL). Figures 8A-8F and Table 2 summarize the most representative changes between a temperature range of 5°C to -12°C for 24 hrs 25 each degree. We have observed two sub-formulation ranges 0.05 to 0.2% MA that confers low temperature tolerance to alpha hop acid solution down to -8°C. The same formulations displayed some cloudiness or minor resin precipitation after reaching -9°C. Resin precipitant in this range of formulations at -12°C to r.t. (Figures 8A-8F) was observed. The next formulation range between 0.3 and 0.4% MA provides an

excellent tolerance to low temperatures since the samples can be frozen in the temperature range of -10°C to -12°C but does not display any resin precipitation and the sample recovered its initial properties (see Figures 8E-8F, table 2).

Table 2.

- 5 Concentration after thawing study from -12°C to r.t (*The Temperature of the bath and analyte were 12.8°C after turn off the system by 15h).

Sample name:	Control Hop derived Alpha Acid Extract	Hop derived Alpha Acid Extract 0.05%MA	Hop derived Alpha Acid Extract 0.1%MA	Hop derived Alpha Acid Extract 0.2%MA	Hop derived Alpha Acid Extract 0.3%MA	Hop derived Alpha Acid Extract 0.4%MA
t=initial HPLC analysis	[%HHIAA]= 15.29 : [%THIAA]= 0.51, Total α acids= 15.8%. pH 9.09 at 25°C	[%HHIAA]= 15.37 : [%THIAA]= 0.5, Total α acids= 15.87%. pH 10.77 at 25°C	[%HHIAA]= 15.4 : [%THIAA]= 0.52, Total α acids= 15.92%. pH 10.51 at 25°C	[%HHIAA]= 15.47 : [%THIAA]= 0.52, Total α acids= 15.99%. pH 10.48 at 25°C	[%HHIAA]= 15.5 : [%THIAA]= 0.52, Total α acids= 16.02%. pH 10.71 at 25°C	[%HHIAA]= 15.39 : [%THIAA]= 0.5, Total α acids= 15.89%. pH 10.46 at 25°C
t=final HPLC analysis (at 12.8°C)	[%HHIAA]= 14.57 : [%THIAA]= 0.46, Total α acids= 15.03%. pH 9.12 at rt	[%HHIAA]= 15.24 : [%THIAA]= 0.5, Total α acids= 15.74%. pH 10.10 at rt	[%HHIAA]= 15.13 : [%THIAA]= 0.5, Total α acids= 15.63%. pH 10.10 at rt	[%HHIAA]= 15.05 : [%THIAA]= 0.49, Total α acids= 15.91%. pH 10.07 at rt	[%HHIAA]= 15.87 : [%THIAA]= 0.52, Total α acids= 16.39 %. pH 10.2 at rt	[%HHIAA]= 15.26 : [%THIAA]= 0.5, Total α acids= 15.68%. pH 10.02 at rt
Final Observation (at 12.8°C)	The resin remains precipitated after thawing and mixing.	The resin remains precipitated after thawing and mixing.	The resin remains precipitated after thawing and mixing.	The resin remains precipitated after thawing and mixing.	Stable formulation: The frozen samples recovered the initial properties after thawing.	Stable formulation.

***The samples were only shaken (not heat up for homogenization) and sampling at 12.8°C.*

EXAMPLE 11

HOP ALPHA ACIDS WITH MYRISTIC ACID (MA; 0.05, 0.1, 0.2, 0.3, 0.4% W/V), FREEZING STUDY AT -16.7°C:

The formulated products were tested at appropriate low temperatures ranging from 5°C to -12°C to determine their chemical and physical properties. The temperature was dropped down to -16.7°C in one-degree increments for 24 hrs each degree. Following which, the samples were warmed up to room temperature (without heating) with gentle shaking with “hand” if needed and no sonication. Once warmed up to room temperature (r.t.), physical properties of the samples were observed (separation of resin formation, crystal formation or a homogenous solution). The determination of Hop α and β acids in Hop acid products in the samples exposed to low temperatures was performed using high-performance liquid chromatography (HPLC).

Samples between 0.05 to 0.2% display resin precipitation after thawing and mixing (Figures 9B-9D). The present data provide conclusive information to demonstrate that formulations of Hop alpha acid solution with 0.3 and 0.4% MA as emulsifier confer full protection to avoid any resin precipitation after the samples were frozen at -16.7°C and subsequent thawing to r.t. (Figures 9E-9F and Table 3).

Table 3.

Concentration after thawing study from -16.7°C to r.t (23.7°C, Note: this is the temperature after 24h).

	Control Hop derived Alpha Acid Extract	Hop derived Alpha Acid Extract 0.05%MA	Hop derived Alpha Acid Extract 0.1%MA	Hop derived Alpha Acid Extract 0.2%MA	Hop derived Alpha Acid Extract 0.3%MA	Hop derived Alpha Acid Extract 0.4%MA
t=initial HPLC analysis	[%HHIAA]= 15.29 : [%THIAA]= 0.51, Total α acids= 15.8%. pH 9.09 at rt	[%HHIAA]= 15.37 : [%THIAA]= 0.5, Total α acids= 15.87%. pH 10.77 at rt	[%HHIAA]= 15.4 : [%THIAA]= 0.52, Total α acids= 15.92%. pH 10.51 at rt	[%HHIAA]= 15.47 : [%THIAA]= 0.52, Total α acids= 15.99%. pH 10.48 at rt	[%HHIAA]= 15.5 : [%THIAA]= 0.52, Total α acids= 16.02%. pH 10.71 at rt	[%HHIAA]= 15.39 : [%THIAA]= 0.5, Total α acids= 15.89%. pH 10.46 at rt
t=final HPLC analysis	[%HHIAA]= 14.87 :	[%HHIAA]= 16.24 :	[%HHIAA]= 15.01 :	[%HHIAA]= 15.39 :	[%HHIAA]= 15.23 :	[%HHIAA]= 15.62 :

	[%THIAA]= 0.49, Total α acids= 15.36%. pH 9.34 at rt	[%THIAA]= 0.53, Total α acids= 16.77%. pH 10.44 at rt	[%THIAA]= 0.5, Total α acids= 15.51%. pH 10.29 at rt	[%THIAA]= 0.52, Total α acids= 15.91%. pH 10.69 at rt *	[%THIAA]= 0.53, Total α acids= 15.76%. pH 10.38 at rt	[%THIAA]= 0.52, Total α acids= 16.14%. pH 10.43 at rt
Final observation	Resin remaining precipitated	Resin remaining precipitated	Resin remaining precipitated	Resin remaining precipitated	Not changes. Stable formulation.	Not changes. Stable formulation.

*No reduction. pH meter calibration (17-06-2021) = 96.6%, offset-26mV.

It was determined that hop derived alpha acids formulations with levels above 0.3% w/v (g/100mL) MA improve the tolerance to low-temperature exposure. These samples recovered their initial properties completely, even when thawed to 12.8°C after 15h. The formulation with 0.4 % w/v MA does not display any physical alteration after exposure to the complete range of temperatures used in this study (5 to -12°C).

On the other hand, hop derived alpha acids formulations with 0.3 to 0.4% w/v MA recovered their initial properties at 23.7°C from -16.7°C after 24h and a soft mixing (no resin precipitation was observed).

Based on these data (1st: Hop derived alpha acid extract with myristic acid (MA; 0.5, 0.75, 1, 1.25, 1.5% w/v), freezing study 5 to -12°C; 2nd: Hop derived alpha acid extract with myristic acid (MA; 0.05, 0.1, 0.2, 0.3, 0.4% w/v), freezing study 5 to -12°C; and 3rd: Hop derived alpha acid extract with myristic acid (MA; 0.05, 0.1, 0.2, 0.3, 0.4% w/v), freezing study at -16.7°C), it was determined that 0.3 to 0.4% w/v (g/100mL) MA is the optimal range that confers full protection to hop derived alpha acids extract at low temperatures without over-charging the composition with the emulsifier.

Table 4:

Summary of the Freezing stability studies with different ranges of Myristic Acid:

Sample name:	Hop derived alpha acid Extract	Re-formulation per cent of emulsifier (%w/v, g/100mL of myristic acid)									
Temperature (°C)	Control	0.05	0.1	0.2	0.3	0.4	0.5	0.75	1	1.25	1.5
5	P	S	S	S	S	S	S	S	S	S	S
4	P	S	S	S	S	S	S	S	S	S	S

3	P	S	S	S	S	S	S	S	S	S	S
2	P	S	S	S	S	S	S	S	S	S	S
1	P	S	S	S	S	S	S	S	S	S	S
0	P	S	S	S	S	S	S	S	S	S	S
-1	P	S	S	S	S	S	S	S	S	S	S
-2	P	S	S	S	S	S	S	S	S	S	S
-3	P	S	S	S	S	S	S	S	S	S	S
-4	P	S	S	S	S	S	S	S	S	S	S
-5	P	S	S	S	S	S	S	S	S	S	S
-6	P	S	S	S	S	S	S	S	S	S	S
-7	P	S	S	S	S	S	S	S	S	S	S
-8	P, F, NRi	PSR, S	PSR, S	PSR, S	S	S	S	S	S	S	S
-9	P, F, NRi	PF, PSR, Ri, NRi	PF, PSR, Ri, NRi	PF, S	PF, Ri, S	S	S	S	S	PF, Ri, S	S
-10	P, F, NRi	PF, Ri, S	PF, Ri, S	PF, Ri, S	F, Ri, S	S	F, Ri, S	S	S	F, Ri, S	PF, Ri, S
-11	P, F, NRi	F, Ri, S	F, Ri, S	F, Ri, S	S	S	S	S	PF, Ri, S	PF, Ri, S	PF, Ri, S
-12	P, F, NRi	F, NRi, P	F, NRi, P	F, NRi, P	F, Ri, S	S	PF, Ri, S	PF, Ri, S	S	PF, Ri, S	PF, Ri, S
-16.7	P, F, NRi	P, F, NRi	P, F, NRi	P, F, NRi	F, Ri, S	F, Ri, S	N/A	N/A	N/A	N/A	N/A
Keywords:		S: Stable product, P: Resin precipitation; NP: No resin precipitation; F: Frozen sample; PF: Partially frozen sample;									
		Ri: Recovery of the initial properties; NRi: Not recovery of the initial properties; PSR: A possible sign of resin precipitation									

The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including but not limited to U.S. Provisional Patent Application No. 63/140,712, filed on January 22, 2021, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with

the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. A composition comprising hop alpha acids in an amount from about 1% to about 30% (w/w), myristic acid in an amount from about 0.05% to about 15% (w/v), and water.
2. The composition of claim 1, wherein the hop alpha acids comprise hexa-hydro-iso-alpha acids and tetra-hydro-iso-alpha acids.
3. The composition of claim 2, wherein the hexa-hydro-iso-alpha acids and tetra-hydro-iso-alpha acids are present in a ratio of about 1:1.
4. The composition of claim 2 or claim 3, wherein the hop alpha acids are present as potassium salts.
5. The composition of any one of claims 1-4, wherein the hop alpha acids are present in an amount from about 10% to about 20% (w/w).
6. The composition of claim 5, wherein the hop alpha acids are present in an amount of about 10% (w/w).
7. The composition of claim 5, wherein the hop alpha acids are present in an amount of about 20% (w/w).
8. The composition of any one of claims 1-7, wherein the myristic acid is present in an amount from about 0.05% to about 2% (w/v).
9. The composition of any one of claims 1-7, wherein the myristic acid is present in an amount from about 0.3% to about 1.5% (w/v).

10. The composition of any one of claims 1-7, wherein the myristic acid is present in an amount from about 0.05% to about 0.25% (w/v).

11. The composition of any one of claims 1-7, wherein the myristic acid is present in an amount from about 0.3% to about 0.4% (w/v).

12. The composition of any one of claims 1-7, wherein the myristic acid is present in an amount from about 0.25% to about 15% (w/v).

13. The composition of claim 12, wherein the myristic acid is present in an amount of about 0.25% (w/v).

14. The composition of claim 12, wherein the myristic acid is present in an amount of about 0.5% (w/v).

15. The composition of claim 12, wherein the myristic acid is present in an amount of about 1% (w/v).

16. The composition of claim 12, wherein the myristic acid is present in an amount of about 2.5% (w/v).

17. The composition of claim 12, wherein the myristic acid is present in an amount of about 5% (w/v).

18. The composition of claim 12, wherein the myristic acid is present in an amount of about 10% (w/v).

19. The composition of any one of claims 1-18, wherein the composition has a pH between about 8 and about 12 at 60°C.

20. The composition of any one of claims 1-18, wherein the composition has a pH between about 8 and about 13 at a temperature between about 20°C and about 26°C.

21. The composition of claim 20, wherein the composition has a pH of about 10.5 at a temperature between about 20°C and about 26°C.

22. The composition of any one of claims 1-21, further comprising hop beta acids.

23. The composition of any one of claims 1-22, wherein the composition does not form a precipitate when cooled to -2°C.

24. The composition of any one of claims 1-22, wherein the composition does not form a precipitate when cooled to -12°C.

25. The composition of any one of claims 1-22, wherein the composition does not form a precipitate when cooled to -16°C.

26. A method of controlling bacterial growth in an ethanol production process, the method comprising adding the composition of any one of claims 1-25 to a fermentation vessel, a yeast propagation tank, or both.

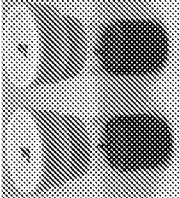
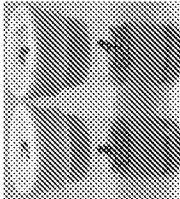
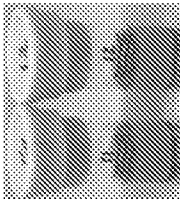
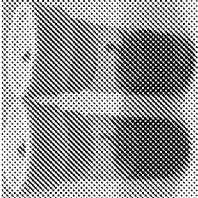
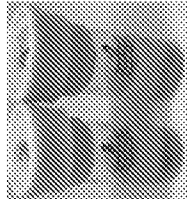
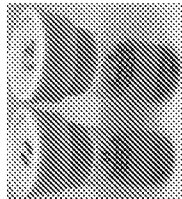
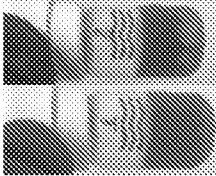
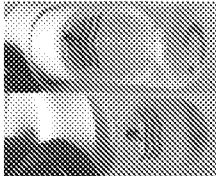
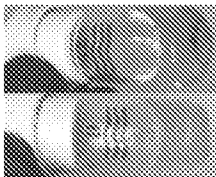
Sample	Hop Beta derived Extract	Hop Alpha derived Extract	Hop Alpha derived Extract
conditions	pH 12.03	pH 9.94	pH 12.37
t=0, T=r.t.			
t=24h, T=-2°C			
Observation	Brown solution with precipitates and crystals	Yellow-pale solution with resin precipitate	Yellow solution with resin precipitate
<i>Additional information</i>			

FIG. 1A

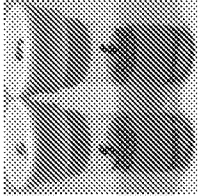
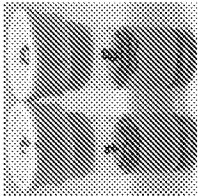
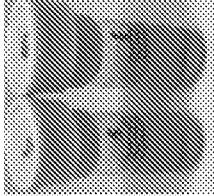
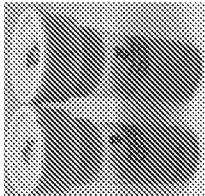
Sample	Alpha acid extract with Myristic acid (%)	
	5%	10%
conditions	pH 12.06	pH 12.73
t=0, T=r.t.		
t=24h, T=-2°C		
Observations	Stable yellow solution	Stable yellow solution

FIG. 1B

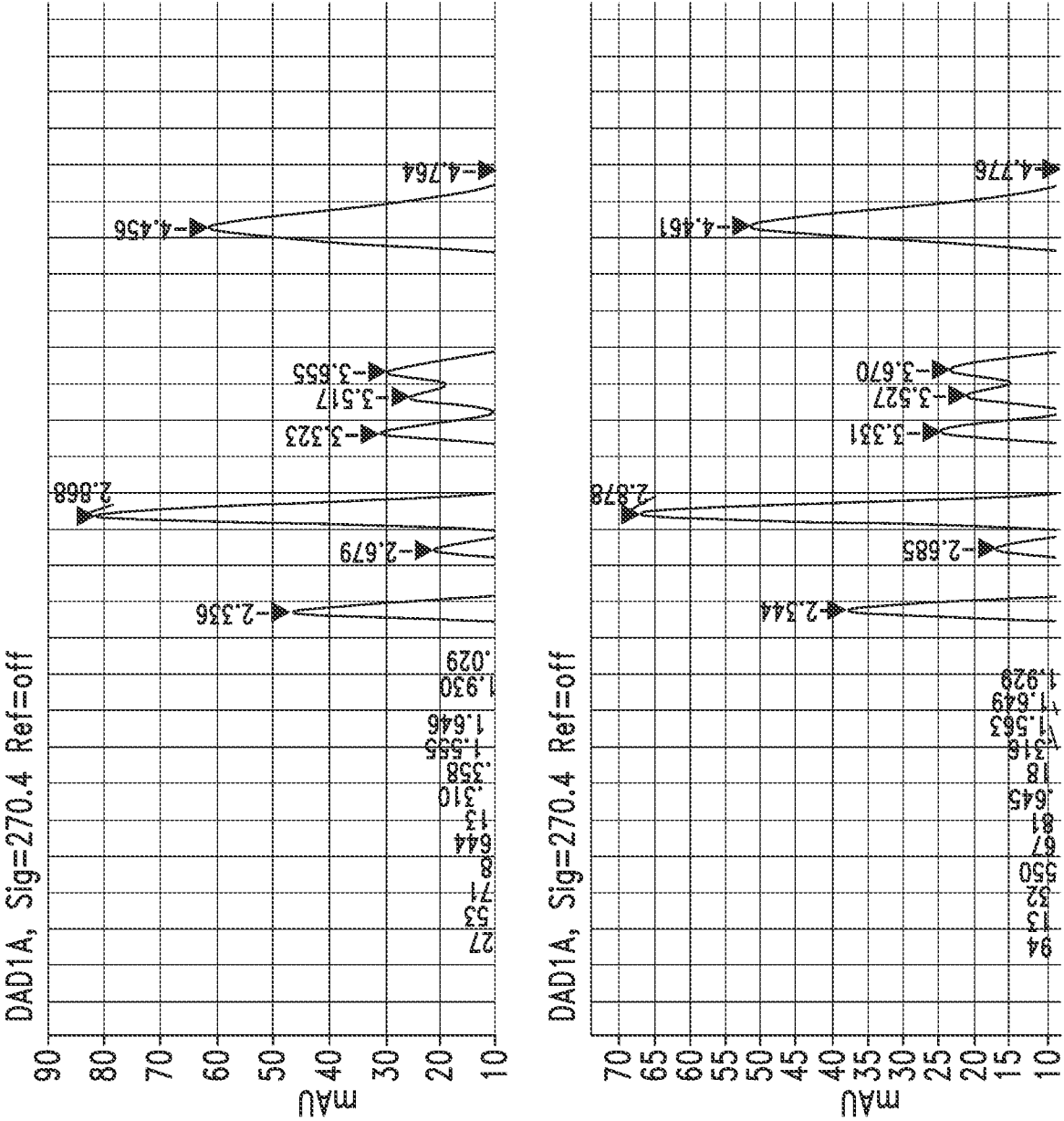


FIG. 2

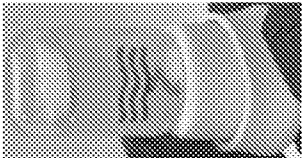
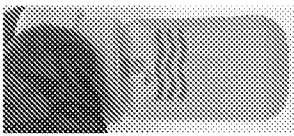
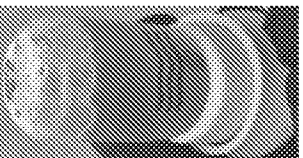
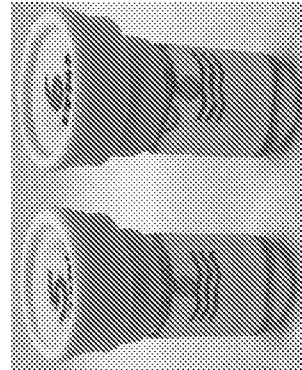
Sample name	Hop derived Alpha acids	Hop derived Alpha acids	Hop derived Alpha acids with 1%MA
Sample number	Sample 1 and 2	Sample 3 and 4	Sample 5 and 6
conditions	pH 8.31	pH 11.58	pH 11.67
Observations	 Resin precipitation after treating at 3°Cx24h	 suspended precipitate after treating at 0°Cx24h  Resin precipitation after treating at -2°Cx24h	 Stable product until -12°C

FIG. 3

5/12

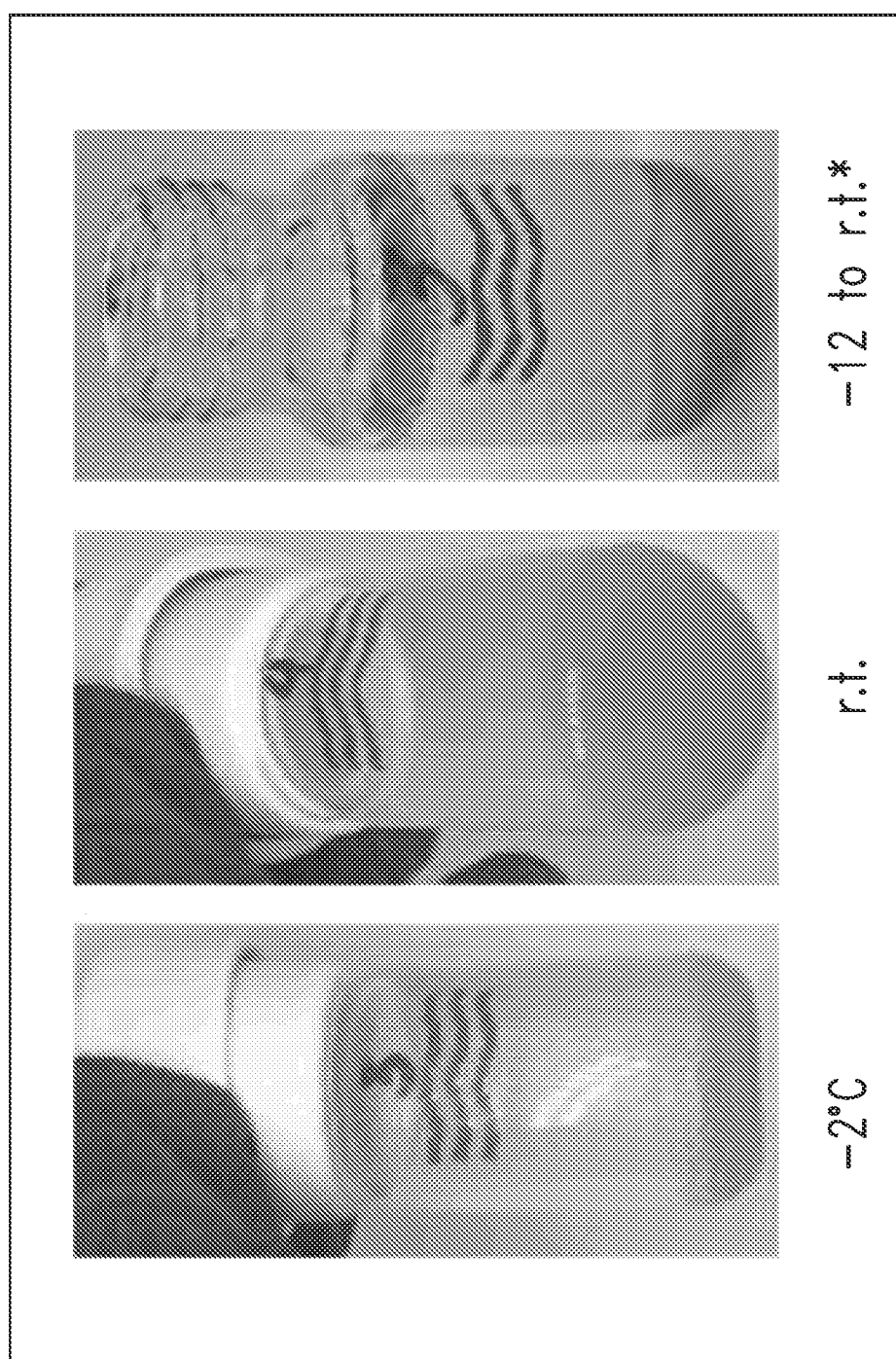


FIG. 4

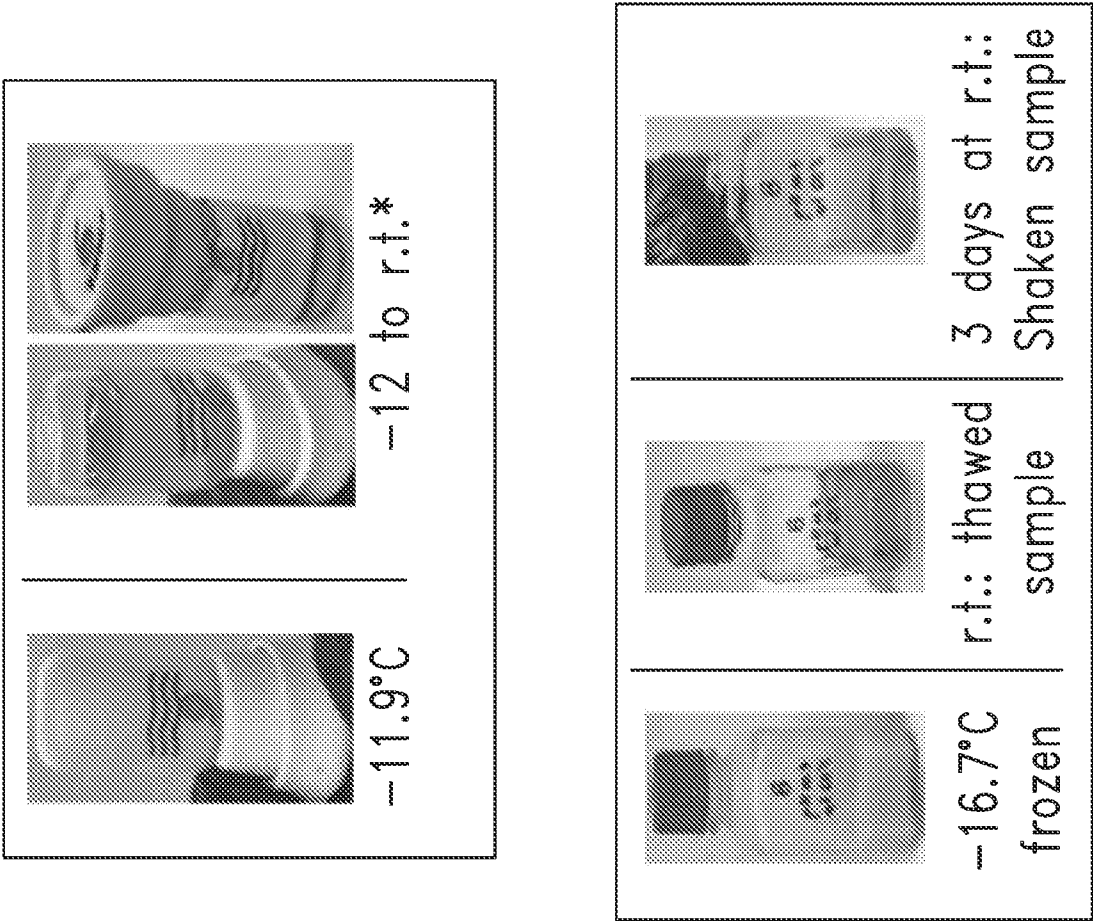


FIG. 5

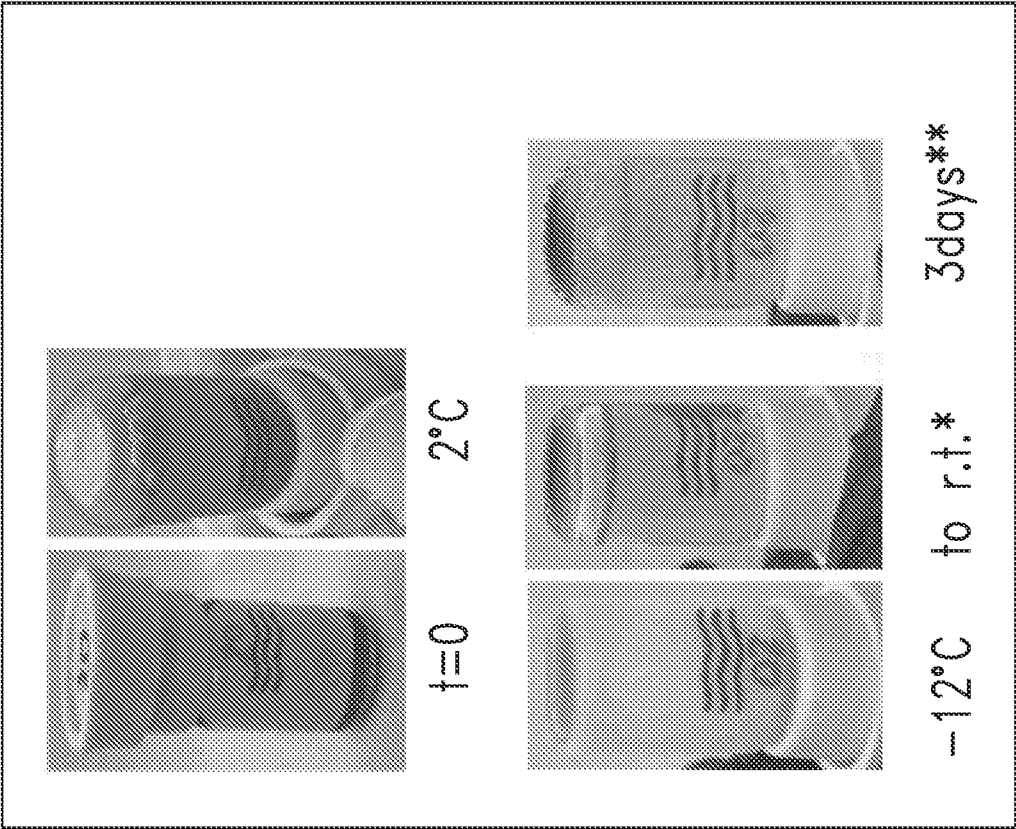
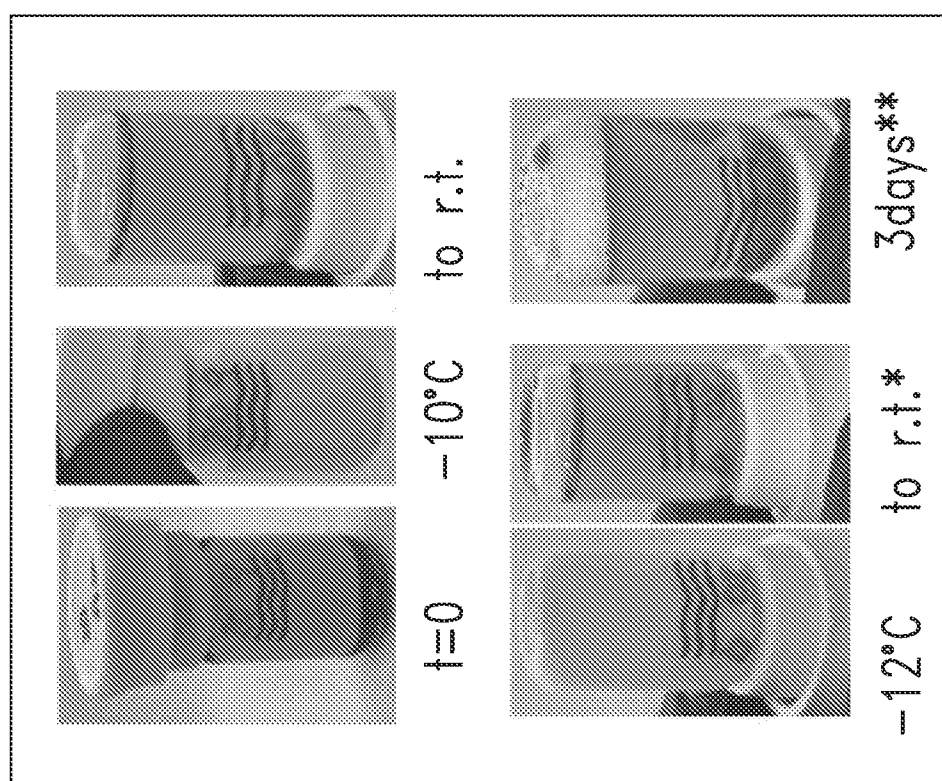


FIG. 6A

8/12

*FIG. 6B*

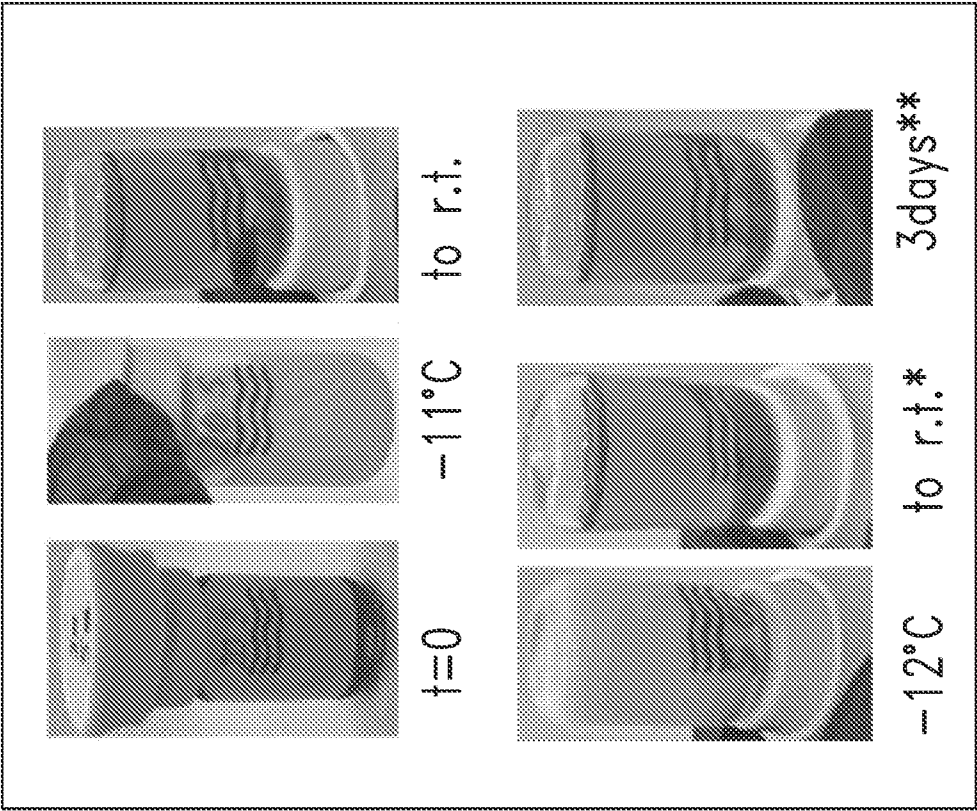
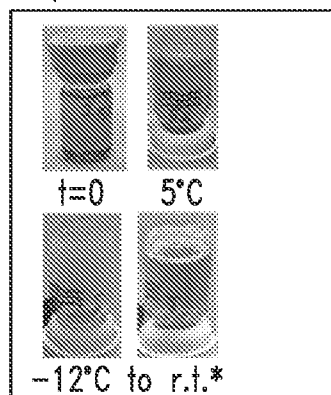


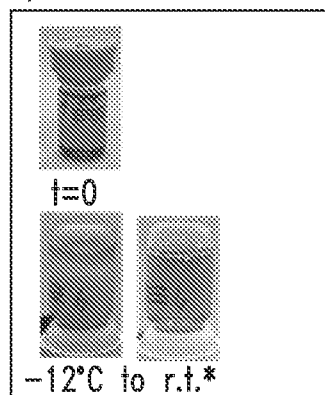
FIG. 6C

10/12

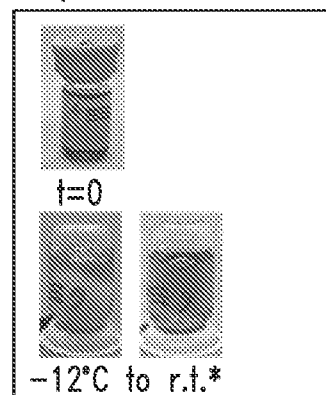
Control:
Hop derived alpha acids
pH 9.09 at 25°C

*FIG. 7A*

Hop derived alpha acids
with 0.5% w/v MA
pH 10.53 at 25°C

*FIG. 7B*

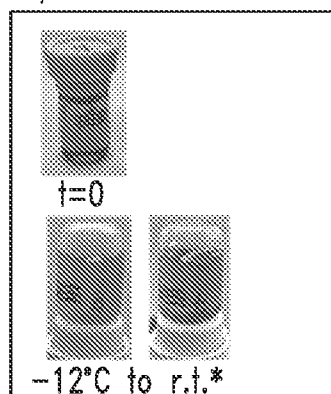
Hop derived alpha acids
with 0.75% w/v MA
pH 10.4 at 25°C



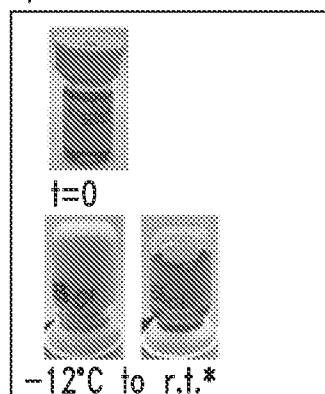
*warm up (r.t.) and mix.

FIG. 7C

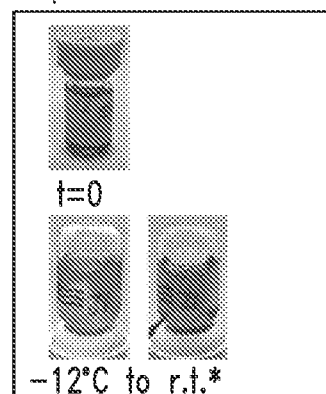
Hop derived alpha acids
with 1% w/v MA
pH 10.53 at 25°C

*FIG. 7D*

Hop derived alpha acids
with 1.25% w/v MA
pH 10.41 at 25°C

*FIG. 7E*

Hop derived alpha acids
with 1.5% w/v MA
pH 10.54 at 25°C



*warm up (r.t.) and mix.

FIG. 7F

11/12

Control:
Hop derived alpha acids
pH 9.09 at 25°C

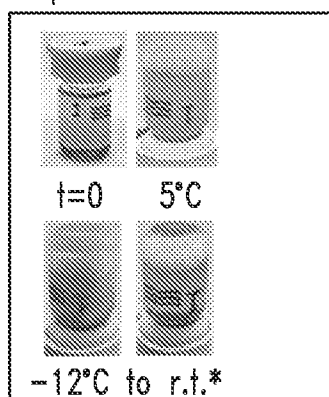


FIG. 8A

Hop derived alpha acids
with 0.05% w/v MA
pH 10.77 at 25°C

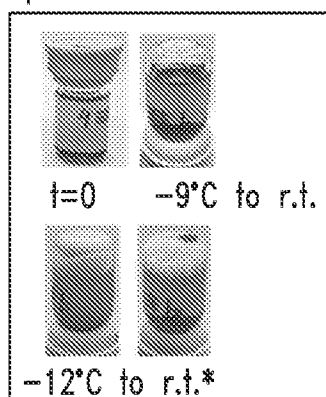
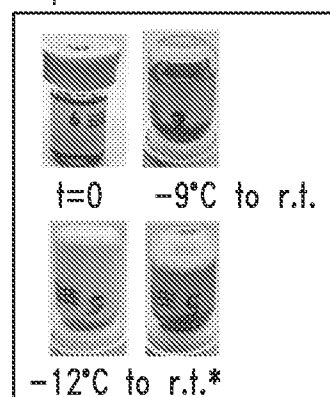


FIG. 8B

Hop derived alpha acids
with 0.1% w/v MA
pH 10.46 at 25°C



*warm up (r.t.) and mix.

FIG. 8C

Hop derived alpha acids
with 0.2% w/v MA
pH 10.48 at 25°C

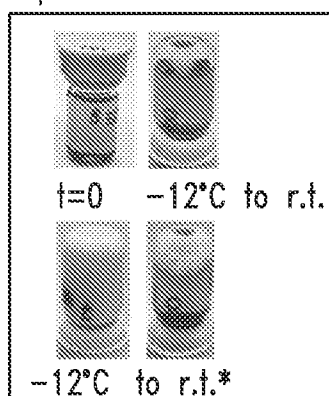


FIG. 8D

Hop derived alpha acids
with 0.3% w/v MA
pH 10.71 at 25°C

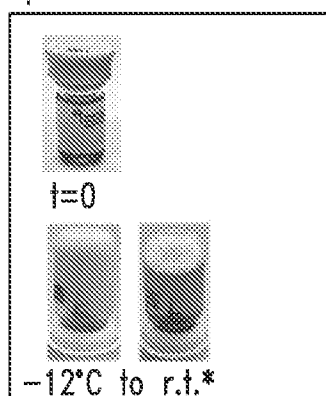
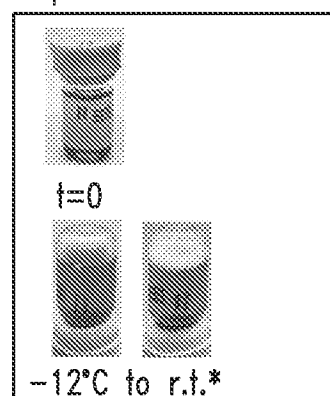


FIG. 8E

Hop derived alpha acids
with 0.4% w/v MA
pH 10.46 at 25°C

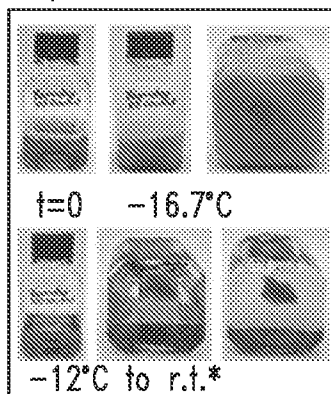


*warm up (r.t.) and mix.

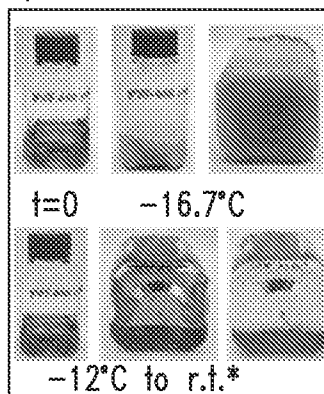
FIG. 8F

12/12

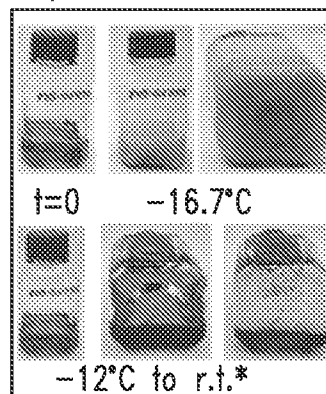
Control:
Hop derived alpha acids
pH 9.09 at 25°C

*FIG. 9A*

Hop derived alpha acids
with 0.05% w/v MA
pH 10.77 at 25°C

*FIG. 9B*

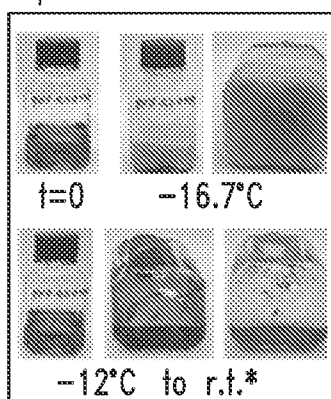
Hop derived alpha acids
with 0.1% w/v MA
pH 10.51 at 25°C



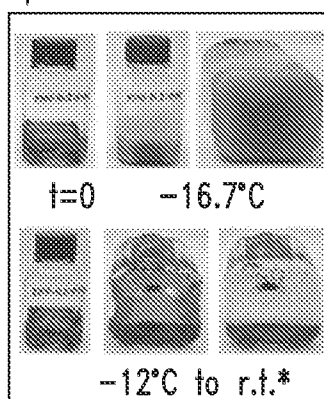
*warm up (r.t.) and mix.

FIG. 9C

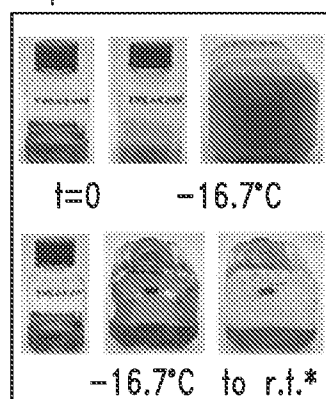
Hop derived alpha acids
with 0.2% w/v MA
pH 10.48 at 25°C

*FIG. 9D*

Hop derived alpha acids
with 0.3% w/v MA
pH 10.71 at 25°C

*FIG. 9E*

Hop derived alpha acids
with 0.4% w/v MA
pH 10.46 at 25°C



*warm up (r.t.) and mix.

FIG. 9F

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/013341

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	C12C3/12	A23L3/3463
	A23L2/44	C12P7/06
		A23C3/08
		C12H1/00
		C13B10/00
ADD.		C12N1/04
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
C12C A23L C12H C13B C12P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
EPO-Internal, BIOSIS, FSTA, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 8 778 646 B1 (CHAPMAN JOHN S [US] ET AL) 15 July 2014 (2014-07-15) claims 1-2, 4-7, 11, 13-16 column 4, lines 28-37 column 5, lines 55-67 column 6, lines 27-33 -----	1-26
A	DE 10 2006 022716 A1 (BETATEC HOPFENPRODUKTE GMBH [DE]) 15 November 2007 (2007-11-15) claims 2, 12-14, 17-20 paragraphs [0056] - [0058], [0078] -----	1-26
A	WO 2004/072291 A2 (HAAS INC JOHN I [US]; MAYE JOHN PAUL [US]) 26 August 2004 (2004-08-26) the whole document ----- -/-	1-26
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
4 April 2022		11/04/2022
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Heirbaut, Marc

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/013341

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 251 461 B1 (JOHNSON ERIC A [US] ET AL) 26 June 2001 (2001-06-26) the whole document -----	1-26
A	WO 00/52212 A1 (HAAS HOP PRODUCTS INC [US]; MAYE JOHN PAUL [US]; BEDDIE DAVID [GB]) 8 September 2000 (2000-09-08) the whole document -----	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/013341

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 8778646	B1	15-07-2014	BR 112015021883 A2	18-07-2017
			CA 2901154 A1	18-09-2014
			CN 105051199 A	11-11-2015
			EP 2971017 A1	20-01-2016
			ES 2683385 T3	26-09-2018
			HU E039660 T2	28-01-2019
			PL 2971017 T3	30-11-2018
			UA 118188 C2	10-12-2018
			US 8778646 B1	15-07-2014
			WO 2014143938 A1	18-09-2014

DE 102006022716 A1		15-11-2007	AU 2007251849 A1	22-11-2007
			BR PI0711412 A2	01-11-2011
			CA 2651662 A1	22-11-2007
			DE 102006022716 A1	15-11-2007
			EP 2024489 A1	18-02-2009
			EP 2088189 A2	12-08-2009
			ES 2542858 T3	12-08-2015
			PL 2024489 T3	30-11-2015
			WO 2007131669 A1	22-11-2007
			ZA 200809399 B	28-04-2010

WO 2004072291	A2	26-08-2004	AU 2004210945 A1	26-08-2004
			AU 2011202086 A1	26-05-2011
			BR PI0407365 A	10-01-2006
			CA 2515723 A1	26-08-2004
			EP 1611230 A2	04-01-2006
			EP 2650355 A1	16-10-2013
			KR 20050120629 A	22-12-2005
			MX PA05008493 A	10-03-2006
			US 2004044087 A1	04-03-2004
			US 2006263484 A1	23-11-2006
			US 2007036882 A1	15-02-2007
			US 2009042276 A1	12-02-2009
			US 2011054024 A1	03-03-2011
			WO 2004072291 A2	26-08-2004

US 6251461	B1	26-06-2001	DE 19846432 A1	22-04-1999
			GB 2330076 A	14-04-1999
			JP H11221064 A	17-08-1999
			US 6251461 B1	26-06-2001
			US 2001014365 A1	16-08-2001

WO 0052212	A1	08-09-2000	AU 3721300 A	21-09-2000
			WO 0052212 A1	08-09-2000
