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(54) Title: HOP PRODUCTS

(57) Abstract: The present invention relates to hop products, in particular to hop products for flavouring beer and enhancing the aroma of beers, ales and other brewed beverages. More specifically, the present invention relates to such products which are 100% hop-derived and which are readily dispersible in cold wort and beer. As a result of our research, we have determined that polar hop extracts, in particular aqueous extracts of hops, have emulsifying properties for hop oil or hop oil-based hop extract products. This has allowed us to develop hop flavouring and aroma products for beer consisting solely of natural products, without the need for artificial emulsifiers and thickening agents or solvents. Surprisingly and unexpectedly, we have found that our combinations of hop-derived constituents is readily dispersible in cold wort and in beer, even when formulated as a 100% hop-derived paste, and provides excellent hop flavour and aroma to the finished beer. Accordingly, the present invention provides a hop composition comprising a mixture of a polar extract of hops and a hop oil-containing component.

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HOP PRODUCTS

The present invention relates to hop products, in particular to hop products for flavouring beer and enhancing the aroma of beers, ales and other brewed beverages. More specifically, the present invention relates to such products which are 100% hop-derived and which are readily dispersible in cold wort and beer.

In order to enhance the flavour and aroma of beer, a range of hop-oil or hop oil-containing extracts can be added. Hop oil and hop oil-containing extracts are mixtures of non-polar compounds and, because of this, do not dissolve very readily in aqueous-based solutions such as beer or wort, especially when cold. In order to aid dissolution in beer, hop oil can be formulated in a number of ways including the use of a carrier solvent such as propylene glycol or ethanol; alternatively, the oil can be suspended as fine droplets in the form of aqueous emulsions. Addition to the hot wort can aid dispersion and solubility but the valuable volatile aroma compounds will be lost to some degree. Other formulations also used include hop oil being suspended on an inert solid support such as silica.

Products currently available on the market include hop oils, or hop oil fractions dissolved in propylene glycol (propane-1,2-diol, PG) or ethanol; or conventional hop-based emulsions.

Although the use of propylene glycol is permitted in food systems (in beer up to 0.1%) and it is a chemically inert substance, it is synthetic. ethanol, although a natural product, has several issues including its flammability, leading to high transport costs and safety concerns. The addition of ethanol into beer is not liked by brewers, who have a preference that all alcohol in the beer is derived from the brewing process; and, unlike propylene glycol, ethanol can interact with components in hop oil chemically, to change flavour. Consequently, of these two, currently propylene glycol is the favoured carrier solvent.

Conventional hop oil or hop oil-containing extract-based emulsions have been used in the brewing industry for many years. This type of product, however, contains natural but non-hop derived ingredients (e.g. Xanthan gum, starch) and synthetic food grade emulsifiers, such as polysorbate 80. Although use of these emulsions offers significant advantages over more traditional practices,

such as dry hopping (speedier processing and reduced beer losses), the flavours imparted tend to be less complex and balanced more towards aroma, rather than taste.

There is therefore a need for a hop oil or hop oil-containing extract product which provides an improved and more complex hop flavour, which is readily dispersible in beer and which is prepared from wholly natural products. We have sought to develop a product which is derived solely from hops.

As a result of our research, we have determined that polar hop extracts, in particular aqueous extracts of hops, have emulsifying properties for hop oil or hop oil-based hop extract products. This has allowed us to develop hop flavouring and aroma products for beer consisting solely of natural products, without the need for artificial emulsifiers and thickening agents or solvents. Surprisingly and unexpectedly, we have found that our combinations of hop-derived constituents are readily dispersible in cold wort and in beer, even when formulated as a 100% hop-derived paste, and provide excellent hop flavour and aroma to the finished beer.

The polar extracts can be obtained by extraction with cold, hot or boiling water of whole hops, hop pellets or spent hops (such as following a CO_2 extraction process to extract hop oil), or with other food-grade acceptable solvents, such as aqueous solvents of lower alcohols, such as ethanol.

The hop oil or hop oil-containing extract can be a CO_2 extract (typically from a liquid (subcritical) CO_2 extraction or supercritical CO_2 extraction), an oil-enriched CO_2 extract or distilled hop oils (obtained by molecular, steam or hydro distillation).

Accordingly, in its broadest sense, the present invention provides a hop composition comprising a mixture of a polar extract of hops and a hop oil-containing component.

Preferably, the polar extract of hops is an aqueous extract of hops.

More specifically, the present invention provides a hop composition comprising a mixture of an aqueous extract of hops having a water content of 55wt% or lower and a hop oil-containing component.

Preferably, the extract of hops is prepared by extracting hops or by extracting spent hops.

Preferably, the extract of hops is obtainable by extracting hops or spent hops with water, preferably with hot water, optionally with boiling water. Alternatively, the extract of hops is a de-solventised extract obtainable from an ethanolic hop extraction process.

Preferably, the hop oil-containing component is a mixture of hop oils or an oil-rich extract of hops.

Preferably, the polar or aqueous extract of hops and the hop oil-containing component are in a ratio of at least 1:1, preferably at least 3:2, more preferably about 7:3.

Preferably, the water content of the polar or aqueous extract of hops is 50% w/w or less, preferably 40% w/w or less; more preferably between 20% w/w and 40% w/w; even more preferably between 25% w/w and 35% w/w.

The present invention further provides an emulsion comprising a composition as defined above and further comprising water, preferably in an amount of up to about 99.8% w/w.

Suitably, the composition has a hop oil content of 5% by weight or less.

The present invention also provides the use of a polar extract of hops as an emulsifier for a non-polar extract of hops.

More generally, the present invention also provides the use of a polar extract of hops as an emulsifier for non-hop oils, preferably natural or synthetic essential oils.

Preferably, the polar extract of hops is an aqueous extract of hops.

Preferably, the water content of the polar or aqueous extract of hops is 55% w/w or less, preferably 50% w/w or less, more preferably 40% w/w or less; even more preferably between 20% w/w and 40% w/w; most preferably between 25% w/w and 35% w/w.

Preferably, the extract of hops is prepared by extracting hops or by extracting spent hops.

Preferably, the extract of hops is obtainable by extracting hops or spent hops with water, preferably with hot water, optionally with boiling water. Alternatively, the extract of hops is a de-solventised extract obtainable from an ethanolic hop extraction process.

Typically, the non-polar extract of hops is a mixture of hop oils or a hop oil-containing component, preferably an oil-rich extract of hops.

Preferably, the polar or aqueous extract of hops and the hop oil or hop oil-containing component are emulsified in a ratio of at least 1:1, preferably at least 3:2, more preferably about 7:3.

In a yet further aspect, the present invention also provides the use of a polar extract of hops as a foam enhancing agent in the brewing of beer.

In a modification of this aspect, the present invention also provides the use of a composition as defined above comprising a polar extract of hops and a non-polar extract of hops as a foam enhancing agent in the brewing of beer.

The present invention further provides a method of enhancing foam characteristics in a beer, the method comprising the addition of a composition as defined above prior to or at the stage of fermentation of the beer.

The present invention further provides a method of preparing a hop-flavouring composition, the method comprising the steps of preparing a polar extract of hops having a water content of 55wt% or lower and preparing a hop oil-containing component; and mixing the polar extract and the hop oil-containing component together.

Preferably, the polar extract of hops is an aqueous extract of hops.

Preferably, the extract of hops is prepared by extracting hops or by extracting spent hops.

Preferably, the extract of hops is obtainable by extracting hops or spent hops with water, preferably with hot water, optionally with boiling water. Alternatively, the extract of hops is a de-solventised extract obtainable from an ethanolic hop extraction process.

Preferably, the hop oil-containing component is a mixture of hop oils or an oil-rich extract of hops.

Preferably, the polar or aqueous extract of hops and the hop oil-containing component are in a ratio of at least 1:1, preferably at least 3:2, more preferably about 7:3.

Preferably, the water content of the polar or aqueous extract of hops is 50% w/w or less, preferably 40% w/w or less; more preferably between 20% w/w and 40% w/w; even more preferably between 25% w/w and 35% w/w.

Preferably, the step of mixing is carried out at a temperature of 20°C or more, preferably above about 35°C.

Optionally, the method further comprises a step of adding water to mixture of the polar extract and the hop oil-containing component to give a final water content of up to about 99.8% w/w.

The above and other aspects of the present invention will now be described in further detail, by way of example only, with reference to the following examples.

Broadly speaking, the present invention combines (i) a polar extract obtained by aqueous extraction of hops or spent hops with (ii) hop oil-containing component, as the hop flavouring and hop aroma component. The combination may, for example, be formulated as a binary paste or formed as an aqueous emulsion. In some variants, additional components may be added, such as thickening agents, for example xanthan gum.

Surprisingly and unexpectedly, we have found that such a combination of hop-derived constituents provides excellent flavouring and is readily dispersible in beer, even when formulated as a paste.

The hop oil-containing component can be any conventional flavour or aroma-providing hop extract as would be used by a brewer. For example, the component can be the product of any conventional

process, such as CO_2 or ethanol extraction, and may be followed by distillation or a combination of processes. The hop oil-containing component may essentially be a substantially pure mixture of hop oils or an extract containing hop oils, which may be a concentrated extract of hop oils.

In the following examples, we will describe the preparation of our hop compositions formulated, initially, as emulsions followed by formulation as pastes. We will then describe the results of various brewing trials with a range of hop varieties, from which it will be appreciated that the compositions of the present invention provide true-to-type hop flavouring and aroma with compositions that are readily dispersible both in actively fermenting beer, as the cold wort, or in beer itself.

Throughout the description which follows percentages are given as by weight (w/w) unless otherwise stated or unless the context indicates otherwise. The term HWE is an abbreviation of Hot Water Extract, which indicates a polar extract obtainable by an extraction of hops or spent hops using hot water. Hot water extracts of hops are commercially available or can be prepared in accordance with the examples which follow. The term ORE is an abbreviation of Oil-Rich Extract and refers to non-polar extracts of hops which are high in available hop essential oils. Such extracts are available commercially.

The term beer is used in this application in its broadest sense of encompassing all styles of beer, namely a beverage brewed, most usually, from cereal grains such as barley, wheat, maize or corn, although other ingredients are used in other traditional beers around the world. The present invention is concerned with adding hop flavour and aroma to any product to which it is desired to add or enhance hop flavour or aroma.

I - HOP EMULSIONS

Reagents:

1. All hop pellets, spent hops, oil-rich CO_2 extracts and hop oils commercially available from Barth-Haas UK Limited
2. Xanthan-Gum
3. Emulsifier (AdmulT80K)

4. Deionised water

In the first group of examples, we compared a standard emulsion prepared using a hop oil rich CO_2 extract (ORE) emulsified in water using a conventional emulsifier, polyoxyethylene sorbitan monooleate, (AdmulT80K), against emulsions of the invention. Both types of emulsion were thickened using Xanthan gum a natural ingredient obtainable through bacterial fermentation.

Comparative Example 1

A standard emulsion was prepared, using a standard emulsification method, from the following ingredients:

2 g CO_2 extract
0.4 g Xanthan-Gum
0.5 g Emulsifier
100 ml Water

100 ml of water was poured into a beaker (250 ml) and heated up to 50°C on a magnetic stirrer hot plate. 0.4 g of Xanthan gum was added into the water and dispersed by stirring the mixture for 20 min at 50°C. In a separate beaker (50 ml) 0.5 g of emulsifier and 2 g of ORE were mixed together with gentle stirring at 40°C on a hot plate. When fully mixed the extract/emulsifier mixture was slowly added to the aqueous xanthan gum dispersion and homogenised at 50 °C for 2 min at 4000 rpm using a Ultra-Turrex IKA T25 stirrer. The prepared emulsion was slightly yellow with a milk-like appearance and texture.

Inventive Example 1

Water extract (cold) of spent hops (not concentrated)

Aqueous extract preparation:

10 g of Citra spent hops and 200 ml of water (20°C) were placed into a 300 ml Duran bottle and then shaken (Stuart Scientific Shaker) for 30 min at 600 rpm. Collected extract including residue was transferred into the centrifugation bottle and centrifuged at 3000 rpm for 30 min (Thermo Scientific, Megafuge16). The supernatant (155 ml) was decanted from the solid mass and collected.

Inventive emulsion preparation:

Following the same emulsification procedure as described in Comparative Example 1, 100 ml of the polar extract was poured into a beaker (250 ml) and heated up to 50°C on a magnetic stirrer hot plate. 0.4 g of Xanthan gum was added into the water and dispersed by stirring the mixture for 20 min at 50°C. ORE (2g) was added slowly to the aqueous xanthan gum dispersion and homogenised at 50 °C for 2 min at 4000 rpm using a Ultra-Turrex IKA T25 stirrer.

Inventive Example 2

50°C water extract of spent hops (not concentrated)

Aqueous extract preparation:

10 g of Citra spent hops and 200 ml of water (50°C) were placed into a 300 ml Duran bottle and then shaken (Stuart Scientific Shaker) for 30 min at 600 rpm. The collected extract including residue was transferred into the centrifugation bottle and centrifuged at 3000 rpm for 30 min (Thermo Scientific, Megafuge16). The supernatant (150 ml) was decanted from the solid mass and collected and allowed to cool.

Inventive emulsion preparation:

The inventive emulsion was prepared using the same emulsification method as Inventive Example 1.

Inventive Example 3

Boiling water extract of spent hops (not concentrated)

Aqueous extract preparation:

10 g of Citra spent hops and 200 ml of water (at ambient temperature) were placed into the round bottom flask and boiled for 30 min. The round bottom flask was covered with a lid which was attached to the condenser (circulation). After 30 min of boiling the whole system was cooled down. Next, collected extract including residue was transferred into the centrifugation bottle and centrifuged at 3000 rpm for 1 hour (Thermo Scientific, Megafuge16). The supernatant (148 ml) was decanted from the solid mass, collected and allowed to cool.

Inventive emulsion preparation:

The inventive emulsion was prepared using the same emulsification method as Inventive Example 1.

Comparative Example 2

70 % v/v acetone extract of spent hops (not concentrated/ aqueous solution)

Aqueous extract preparation:

100 g of Target spent hops and 1000 ml of 70 % acetone (v/v in water) were placed into the 2 L beaker then stirred using overhead stirrer for 1 hour. Collected extract was filtered using a filter paper (Whatman no.1). Next, acetone was removed using a rotary evaporator (BUCHI) under reduced pressure. The concentrated extract (without acetone but still including water) was placed into the fridge overnight, then again filtered using a filter paper (Whatman no.6).

Emulsion preparation:

The emulsion was prepared using the same emulsification method as Inventive Example 1.

Unlike with the other aqueous extracts described above, we were unable to form an emulsion with this aqueous acetone extract.

The resultant compositions were then assessed for stability as an emulsion. The results are shown in Table 1 below.

	Aqueous Extraction Solvent	***Emulsifier Source Material***	***Emulsion stability***
Comparative Example 1	Not applicable	Emulsifier (AdmulT80K)	Excellent
Inventive Example 1	Cold Water	spent hops	Excellent
Inventive Example 2	50°C Water	spent hops	Excellent
Inventive Example 3	Boiling Water	spent hops	Excellent
Comparative Example 2	70 % Acetone	spent hops	Poor

Table1

Influence of solvent and raw material on Emulsion stability (un-concentrated extracts)

Emulsion Stability Assessment Definitions:

Excellent – smooth, milk-like, emulsion, no separation

Poor – rapid separation, two phases.

It is noticeable that the aqueous acetone extract failed to provide satisfactory emulsifying properties. It is known that extraction of botanicals with aqueous acetone can cause denaturing of proteins. Accordingly, our result may tend to indicate that the active emulsifying component of the aqueous extracts of the present invention may be associated with proteins in the hops.

We then carried out further trials using *concentrated* aqueous extracts of spent hops/ hops to form emulsions.

Inventive Example 4

Boiling water extract of spent hops (concentrated)

Aqueous extract preparation:

150 g of Citra spent hops and 2000 ml of water were placed into a round bottom flask and boiled for 1 hour under reflux. After 30 min of boiling the mixture was allowed to cool to approx. 30°C. Collected extract including residue was transferred into a centrifugation bottle and centrifuged at 3000 rpm for 30 min (Thermo Scientific, Megafuge16). The supernatant (1700 ml) was decanted from the solid mass and then concentrated using a rotary evaporator at 70°C (BUCHI) under reduced pressure, until obtaining mustard paste-like consistency (35 g).

Inventive emulsion preparation:

The inventive emulsion was prepared using the same emulsification method as Inventive Example 1, except using 4 g of concentrate aqueous extract and making up to 100 ml with distilled water.

Inventive Example 5

Boiling water extract of hop pellets (concentrated)

Aqueous extract preparation:

150 g of Citra hop pellets and 2000 ml of water were placed into a round bottom flask and boiled for 1 hour under reflux. After boiling the mixture was allowed to cool to approx. 30°C. Next, collected extract including residue was transferred into a centrifugation bottle and centrifuged at 3000 rpm for 30 min (Thermo Scientific, Megafuge16). The supernatant (1650 ml) was decanted from the solid mass and then concentrated using a rotary evaporator at 70°C (BUCHI) under reduced pressure, until a mustard paste-like consistency was obtained (41 g).

Inventive emulsion preparation:

The inventive emulsion was prepared using the same emulsification method as Inventive Example 1, except using 4 g of concentrate aqueous extract and making up to 100 ml with distilled water.

Inventive Example 6

96 % v/v ethanol extract of spent hops (concentrated)

Aqueous extract preparation:

10 g of Citra spent hops and 200 ml of 96 % v/v ethanol (in water) were placed into a 300 ml Duran bottle and shaken (Stuart Scientific Shaker) for 30 min at 600 rpm. The collected extract including the residue was transferred into the centrifugation bottle and centrifuged at 3000 rpm for 30 min (Thermo Scientific, Megafuge16). The supernatant was decanted from the solid mass, collected and then concentrated using a rotary evaporator at 70°C (BUCHI) under reduced pressure, until dry (1.8 g).

Inventive emulsion preparation:

The inventive emulsion was prepared using the same emulsification method as Inventive Example 1, except using 0.9 g and 0.5 g of concentrate aqueous extract (Inventive Emulsions 6a and 6b respectively) and making each up to 100 ml with distilled water.

Inventive Example 7

4 % v/v ethanol extract of spent hops (partially concentrated)

10 g of Citra spent hops and 200 ml of 4 % v/v ethanol (the balance being water) were placed into a 300 ml Duran bottle and shaken (Stuart Scientific Shaker) for 30 min at 600 rpm. The extract including the residue was transferred into a centrifugation bottle and centrifuged at 3000 rpm for 30 min (Thermo Scientific, Megafuge16). Due to excessive foaming the evaporation was stopped before achieving dryness, hence experiments 7a-7d, using various ratios of the aqueous concentrate (of unknown activity level).

The supernatant was separated from the solid mass, collected and concentrated using a rotary evaporator at 40°C (BUCHI) to give a 60 g of extract (including water). The extract was then used to make four emulsions in the following proportions:

Inventive Example 7a - Emulsion with 4 % ethanol extract of spent hops (100ml)

2 g CO_2 extract (ORE; 13 % oil)

0.4 g Xanthan-Gum

10 g de-alcoholised extract with 4 % ethanol extract of spent hops

90 g Water

Inventive Example 7b - Emulsion with 4 % ethanol extract of spent hops (100ml)

2 g CO_2 extract (ORE; 13 % oil)

0.4 g Xanthan-Gum

20 g de-alcoholised extract with 4 % ethanol extract of spent hops

80 g Water

Inventive Example 7c - Emulsion with 4 % ethanol extract of spent hops (100ml)

2 g CO_2 extract (ORE; 13 % oil)

0.4 g Xanthan-Gum

30 g de-alcoholised extract with 4 % ethanol extract of spent hops

70 g Water

Inventive Example 7d - Emulsion with 4 % ethanol extract of spent hops (100 ml)

2 g CO_2 extract (ORE; 13 % oil)

0.4 g Xanthan-Gum

60 g de-alcoholised extract with 4 % ethanol extract of spent hops

40 g Water

Four emulsions were prepared for comparison because, as will be appreciated, the precise composition of the ethanol extract is unknown. The emulsions were tested for stability and the results are shown in Table 2 below.

	Polar Extract Solvent	***Polar Extract Source Material***	***Emulsion Stability***
Inventive Example 4	boiling water	spent hops	Excellent
Inventive Example 5	boiling water	hop pellets	Excellent
Inventive Example 6a Inventive Example 6b-	96 % ethanol	spent hops	Excellent Excellent
Inventive Example 7a Inventive Example 7b Inventive Example 7c Inventive Example 7d	4 % ethanol	spent hops	Excellent Excellent Excellent Good

Table2

Influence of solvent and raw material on Emulsion stability (concentrated extracts)

Emulsion Stability Assessment Definitions:

Excellent – smooth, milk-like, emulsion, no separation

Good – milk like with some waxy particle evident, no separation

Poor – separation, two phases

Flavour evaluation

A flavour evaluation of inventive emulsions in comparison with standard emulsion and traditional dry hopping with hop pellets was carried out. Samples of a commercial beer were treated with 6 different hop products in order to evaluate differences and/or similarities:

1. A Standard Emulsion (Comparative Example 1)
2. Hop Pellets T90 (a conventionally dry-hopped beer)
3. Emulsion formulated with Cold Water Extract (Inventive Example 1)
4. Emulsion formulated with 96 % ethanol Water Extract (Inventive Example 6)
5. Emulsion formulated with 4 % ethanol Water Extract (Inventive Example 7b)
6. Emulsion formulated with Hot Water Extract) (Inventive Example 2)

Six panellists were asked to describe the flavour of the samples and additionally, to rank the four inventive emulsions in terms of complexity of hop flavour on an arbitrary scale. The results are shown in Chart 1. It will be seen that the standard emulsion falls at one end of the scale and

conventional dry-hopped beer (T90 pellets) at the other with the selected inventive emulsions forming a cluster between the two.

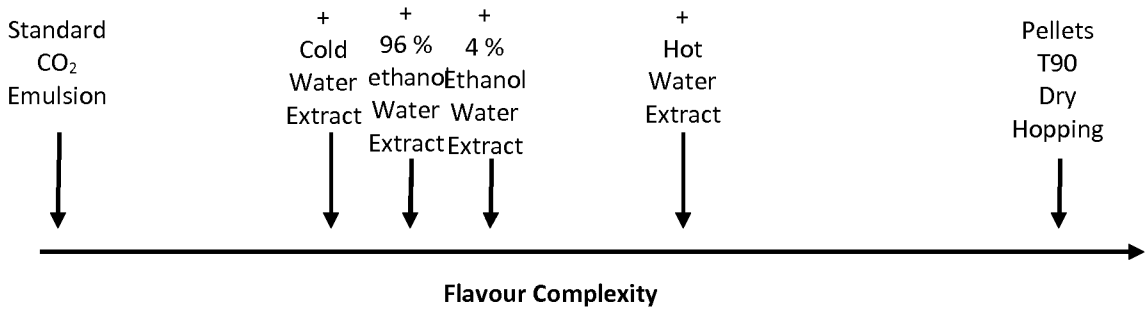

Chart 1.

It appeared that emulsion enhanced with a *HOT* water extract was the closest to dry hopped beer in terms of flavour and aroma. The two samples were not identical but were described as having a lot in common (mouthfeel, vegetal and hoppy notes, and complex bitterness). All of the inventive emulsions were considered to be improvements on the standard emulsion and closer to a traditional dry hopped flavour.

II - HOP PASTES

Further work showed, surprisingly, that our extracts can be formulated as simple, concentrated pastes which have excellent dispersibility in cold wort and beer and provide excellent hop flavours, without the need for an emulsifier such as AdmulT80K or thickening agent such as Xanthan gum. Pastes were prepared using a polar extract of spent hops/hops with a non-polar, hop-derived aroma fraction, for example, a CO_2 extract or hop oil obtained by distillation or hop oil fractions.

Inventive Example 8

Boiling Water Extract of spent hops (concentrated)

The concentrated boiling water extract of spent Citra hops (aqueous extract preparation) obtained in Inventive Example 4 was mixed with the Citra oil rich CO_2 extract (ORE; 13 % oil content) in a ratio of 60:40 w/w to form a paste (containing 5.2 % oil). The paste was found to have a very good stability and was surprisingly easy to disperse in beer.

Inventive Example 9

Boiling Water Extract of Hop Pellets (concentrated)

The concentrated boiling water extract of Citra hop pellets (aqueous extract preparation) obtained in Inventive Example 5 was mixed with the Citra oil rich CO_2 extract (ORE: 13 % oil content) in a ratio of 60:40 w/w to form a paste (5.2 % oil content). The paste was found to have a stability and dispersibility properties similar to Inventive Example 8.

Inventive Example 10

Different compositions of boiling water extract concentrate and ORE

Pastes were prepared consisting of varying proportions (see table below) of an oil-rich CO_2 extract (ORE) from Target hops, containing 5.5 % v/w oil as the hop oil-containing component with 1.5 g of the aqueous extract prepared in accordance with Inventive Example 4.

The mixtures were combined manually at ambient temperature. We found that it was not necessary to use a high shear mixer or elevated temperatures to ensure adequate mixing.

The ORE pastes were assessed for appearance, dispersibility in a commercial (finished) beer and flavour (taste and aroma) properties given to the beer. Dispersibility in beer was tested by placing a small amount of paste on a spatula and stirring into the beer for a few seconds and visually assessing what was left on the spatula and how evenly was the paste dispersed in the beer. Results are shown in Table 3 below.

Example	Formulation [g]		Oil in paste [%]	Appearance when stirred	Dispersibility in beer	Flavour properties	Paste stability
	Boiling water extract	ORE					
Inventive Example 10a	1.50	0.50	1.38	Smooth, mustard paste-like, mobile	Good	Positive, mild, delicate	Poor

Inventive Example 10b	1.50	1.00	2.20	Smooth, mustard paste-like	Excellent	Positive, balanced	Good
Inventive Example 10c	1.50	1.50	2.75	Smooth, mustard paste-like	Excellent	Sharp, resinous	Good
Inventive Example 10d	1.50	1.75	3.00	Smooth, more sticky, less mobile	Good	Sharp, resinous	Excellent
Inventive Example 10e	1.50	2.00	3.15	Smooth, sticky, ORE-like	Good	Sharp, resinous, burns	Good

Table 3

Influence of the Paste formulation, prepared from a Target ORE, on its appearance, ease of dispersion and flavour properties.

Definitions for Dispersibility in Beer:

Excellent – disperses totally leaving a clean spatula

Good- majority of paste disperses living a small amount of residue on a spatula

Poor- did not disperse at all

Definitions for Paste Stability:

Excellent – smooth, mustard-paste like, no phase separation

Good – mustard paste-like with some waxy particles, slight phase separation evident

Poor- separation, two phases

Inventive Example 11

Different compositions of hot water extract concentrate (HWE) and ORE

Pastes were prepared consisting of varying proportions (see table 4 below) of an oil-rich CO_2 extract (ORE) from Goldings hops, containing 10% v/w oil as the non-polar extract with 1.5 g of a commercially available hot water extract obtained from NATECO2 GmbH & Co KG.

The mixtures were combined manually at ambient temperature. Again, it was not necessary to use a high shear mixer or elevated temperatures to ensure adequate mixing.

The ORE pastes were assessed for appearance, dispersibility in a commercial (finished) beer and flavour (taste and aroma) properties given to the beer. Dispersibility in beer was tested by placing

a small amount of paste on a spatula and stirring into the beer for a few seconds and visually assessing what was left on the spatula and how evenly was the paste dispersed in the beer. Results are shown in Table 4 below.

Example	Formulation [g]		Oil in paste [%]	Appearance when stirred	Dispersibility in beer	Flavour properties	Paste stability
	HWE	ORE					
Inventive Example 11a	1.50	0.10	0.63	Smooth, mustard paste like	Poor	N/A	Poor
Inventive Example 11b	1.50	0.50	2.50	Smooth, mustard paste like	Poor	N/A	Poor
Inventive Example 11c	1.50	0.75	3.33	Smooth, mustard paste like	Poor	N/A	Poor
Inventive Example 11d	1.50	1.00	4.00	Smooth, more sticky, less mobile	Good	Sharp, hoppy, resinous, vegetal,	Poor
Inventive Example 11e	1.50	1.25	4.55	Smooth, mustard paste like	Excellent	Sharp, hoppy, resinous, vegetal	Poor
Inventive Example 11f	1.50	1.5	5.00	Smooth, mustard paste like	Excellent	Sharp, hoppy, resinous, vegetal	Poor
Inventive Example 11g	1.50	1.75	5.38	Smooth, mustard paste like	Excellent	Sharp, hoppy, resinous, vegetal	Excellent
Inventive Example 11h	1.50	2.00	5.74	Smooth, mustard paste like	Excellent	Sharp, hoppy, resinous, vegetal, burns	Excellent
Inventive Example 11i	1.50	2.25	6.00	Smooth, mustard paste like	Excellent	Sharp, hoppy, resinous, vegetal, burns	Excellent
Inventive Example 11j	1.50	2.50	6.25	Smooth, sticky, ORE-like	Poor	N/A	Excellent
Inventive Example 11k	1.50	2.75	6.47	Smooth, sticky, ORE-like	Poor	N/A	Excellent

Inventive Example 11l	1.50	3.00	6.67	Smooth, sticky, ORE-like	Poor	N/A	Excellent

Table 4

Influence of the Paste formulation, prepared from a Goldings ORE (10%) and a concentrated hot water extract, on its appearance, ease of dispersion and flavour properties.

Definitions for Dispersibility in Beer:

Excellent – disperses totally leaving a clean spatula

Good- majority of paste disperses living a small amount of residue on a spatula

Poor- did not disperse at all

Definitions for Paste Stability:

Excellent – smooth, mustard-paste like, no phase separation

Good – mustard paste-like with some waxy particles, slight phase separation evident

Poor- separation, two phases

These results suggest that dispersibility of the paste in the beer is dependent upon the ratio of non-polar extract to polar extract, whereas stability of the paste varies with oil content.

Inventive Example 12

Hop oil pastes

A series of hop oil pastes were prepared consisting of 1.5 g of the aqueous extract prepared in Example 4 and 0.25 g of *Target* hop oil to investigate the stability (at 19°C over 10 days) and dispersibility in water of the hop oil paste. The results are shown in Table 5 below.

	Oil Content [g]	*Oil Content [% w/w]*	*Aqueous Extract Content [g]*	*Paste Stability*	*Paste Dispersibility in Water*
Inventive Example 12a	0.00	0.00	1.5	Excellent	Excellent
Inventive Example 12b	0.01	0.66	1.5	Excellent	Excellent
Inventive Example 12c	0.02	1.30	1.5	Excellent	Excellent

Inventive Example 12d	0.03	1.95	1.5	Excellent	Excellent
Inventive Example 12e	0.05	3.25	1.5	Good	Good
Inventive Example 12f	0.10	6.25	1.5	Good	Good
Inventive Example 12g	0.15	9.10	1.5	Good	Good
Inventive Example 12h	0.25	14.5	1.5	Good	Good
Inventive Example 12i	0.30	16.5	1.5	Poor	Poor
Inventive Example 12j	0.35	19.0	1.5	Poor	Poor
Inventive Example 12k	0.40	21.0	1.5	Poor	Poor
Inventive Example 12l	0.50	25.0	1.5	Poor	Poor
Inventive Example 12m	0.75	33.5	1.5	Poor	Poor
Inventive Example 12n	1.00	66.5	1.5	Poor	Poor
Inventive Example 12o	1.25	45.5	1.5	Poor	Poor
Inventive Example 12p	1.50	50.0	1.5	Poor	Poor

Table 5

Target Oil Paste stability

Definitions for Paste Stability: Excellent – smooth, mustard-paste like, no phase separation

Good – mustard paste-like with some waxy particles, slight phase separation evident

Poor- separation, two phases

Definitions for Dispersibility in Beer:

Excellent – disperses totally leaving a clean spatula

Good- majority of paste disperses living a small amount of residue on a spatula

Poor- did not disperse at all

As can been seen from these results, the aqueous hop extracts of the present invention are capable of providing stable pastes having good or excellent dispersibility in water at hop oil contents as least to as high an oil content as 14.5% w/w.

Inventive Example 13

β-acid content of the ORE and paste stability

Citra pastes containing a range of different concentrations of β-acids were prepared and monitored in terms of physical stability and dispersibility in water (Table 0). Oil content was constant for all the samples 7% w/w. The ratio between polar extract and ORE was 70:30. Two temperatures (20ºC and 40ºC) were tested for the mixing process.

Results:

It was observed (Table 6) that the concentration of β-acids had a significant impact on paste stability and paste dispersibility in water. The higher the concentration, the poorer the stability of the paste and the higher amount of crystals found in the sample. However, paste stability could be notably improved by heating up the ingredients up to above 35 °C before blending/ mixing together. Heating to improve mixing and subsequent stability did not have any noticeable impact on subsequent use of the pastes.

PASTE QUALITY VS BETA-ACID CONTENT				
SAMPLE	Beta-acid Content in Paste [%]	Paste Texture immediately after mixing	Stability [First 24 hours]	Dispersibility in Water
Temperature = 20◦C				
1	39.9	Smooth	Separation, Crystals	Average
2	34.4	Smooth	Separation, Crystals	Poor
3	31.6	Smooth	Separation, Crystals	Average
4	28.7	Smooth	Separation, Crystals	Poor
5	22.7	Smooth	Separation, Crystals	Average
6	16.1	Smooth	Separation, Crystals	Excellent
Temperature = 40◦C				
1	39.9	Smooth	Slight Separation, Crystals	Excellent (some mini waxy lumps on top)
2	34.4	Smooth	Slight Separation, Crystals	Excellent (some mini waxy lumps on top)

3	31.6	Smooth	Slight Separation, Crystals	Excellent
4	28.7	Smooth	Slight Separation, some Crystals	Excellent
5	22.7	Smooth	Stable, some Crystals	Excellent
6	16.1	Smooth	Stable	Excellent

Table 6

Paste Quality depending on β-acid content

Poor- did not disperse at all,

Average-some waxy lumps noticed,

Excellent – disperse completely

Consequently, for further studies, we heated both the aqueous extract (hot water extract) and the oil-containing components to 35°C, or more. The two components were mixed by stirring the aqueous extract and slowly adding the oil-containing component, followed by stirring over ten minutes.

As a result of these preparative steps, we determined that preferably the amount (by weight) of the aqueous extract preferably generally equals or exceeds that of the oil-containing component in order to produce a homogeneous paste with dispersibility in water.

The precise ratio of aqueous extract to oil-containing component varies from sample to sample, principally depending upon the oil and β-acid content of the oil-containing component as well as varying with the hop variety. Generally, the higher the oil content of the oil component, the lower the proportion of oil-containing component in the final paste. We also found that high β-acid content oil-containing components required heating to higher temperatures to ensure mixing to a homogeneous paste. There is also some variation with water content of the polar extract.

Accordingly, preferably the ratio of the polar extract to the oil-containing component is equal to or above 1:1; preferably 60:40 as above; more preferably 70:30 or above.

Inventive Example 14

Water content of aqueous extract

The impact of the water content of the aqueous extract on its emulsifying properties/miscibility with CO_2 extract was examined using an extract with a water content of 46.5% w/w. For small amounts of extract, the water content was adjusted to 50, 55, and 60% w/w and the extracts were mixed with Target CO_2 extract (8% w/w oil) at a ratio of 3:2 to produce a paste. The resulting pastes were examined under the microscope at 1000x and/ or 100x magnification. At 46.5% w/w and 50% w/w water content, the polar extract formed smooth pastes with the CO_2 extract with very small, homogenous droplets. At 55 and 60% water content, phase separation was observed and oily droplets formed that increased in size as the water content increased. Microscopic images for the four pastes are given in Figure 1.

Similar experiments were performed using a high-oil Mosaic extract (20.6% oil). Initially, the aqueous extract was mixed with the oil rich extract (ORE) at a ratio of 3:1. More ORE was then added slowly up to a ratio of 1:1. The resulting paste was smooth, paler than the aqueous extract itself and rather mustard-like. As the ORE content was increased, the paste became thicker. The water content was then adjusted to 50% in the final paste. The resulting paste was liquid, but still smooth, with a mustard-like colour and texture. It was stored for three weeks and showed very little separation. The few small, dark droplets formed could be easily eliminated by shaking.

Overall, the experiments showed that a maximum water content for the aqueous extract prior to combination with the hop-oil containing component of about 55wt% is important for paste production and stability. When using high-oil extracts, the rate of addition also has an impact on miscibility and the ORE needs to be added slowly to ensure full emulsification. Once a stable paste is made up, water can be added to improve the consistency (making dosing easier).

Inventive Example 15

<u>Comparison of time of paste addition during brewing process (laboratory scale)</u>

Investigation of the influence of the point of addition during brewing on the final flavour and aroma of the beer was carried out. Fermentation trials were set up to investigate three different times of addition (start of fermentation, start of maturation and end-of maturation) using the following hop paste products**:**

1) CO_2 ORE Citra Paste (5.2 % oil content)

2) CO_2 ORE El Dorado Paste (3.8 % oil content)

3) CO_2 Aurora Paste (4.0 % oil content)

4) CO_2 ORE Cascade Paste (3.2 % oil content)

5) Oil Equinox Paste (14 % oil content).

All the ORE pastes were made in accordance with Inventive Example 8 and hop oil paste in accordance with Inventive Example 12 (sample 8).

Fermentation:

All equipment (e.g. beakers, spatulas, flasks) used during fermentation were sterilised with boiling water and then with 70 % ethanol. 495 g of Amber Malt Extract (Thomas Coopers) was placed in a 5 L glass beaker and mixed with 3 L of boiling water. The mixture was stirred manually until all the extract was dissolved and allowed to cool slowly to 30°C before 0.45 g of Iso-hop (Barth Haas UK) and 0.216 g of yeast aid powder (Brupaks Yeast-Vit nutrient) were added. All ingredients were stirred together for 10 min using a magnetic stirrer. When the temperature of the solution reached 25°C a yeast slurry (3.18 g of dried yeast Safbrew T-58, Fermentis in 300 ml of water t = 30°C) was added. All ingredients were stirred using a magnetic stirrer for 3 min and then divided between 11 beakers (300 ml aliquots in each) for fermentation. Samples were kept at ambient temperature (17-19°C) for 3 days. After 3 days the yeast was separated from the 'green beer' and the beakers were then placed in a fridge at 5°C for a further 3 days (maturation). At the end of maturation, all beers were treated with Isinglass (approx. 0.3 %) and held at 5°C for a further 24 h. The beers were then tasted and the clarity measured (Hach 2100N Turbidimeter, 90°C). The same brewing conditions were applied for all the samples investigated. Results from taste assessments are presented in Table 7 below.

Sample	**Time of addition**	**Sensory evaluation**
Control	N/A	Beer-like, bitter
ORE Citra Paste	Start of Fermentation	Fruity, very soft, passion fruit, floral, balanced, peach, mouthfeel
ORE Citra Paste	Start of Maturation	Fruity, passion fruit, sharp, dry
ORE Citra Paste	Maturation	Fruity, sharp, hoppy, resinous mouthfeel

ORE El Dorado Paste	Start of Fermentation	Flora, rose-like, hoppy, balanced
ORE El Dorado Paste	Start of Maturation	Floral, hoppy, vegetal
ORE El Dorado Paste	Maturation	Hoppy, herbal
ORE Aurora Paste	Start of Fermentation	Lager-like, nutty, fruity, cherry-like,
ORE Aurora Paste	Start of Maturation	Fruity, fresh, citrusy, bitter, mouthfeel,
ORE Aurora Paste	Maturation	Very intense flavour, fruity, resinous, nectarine, passion fruit - like
ORE Cascade Paste	Start of Fermentation	Hoppy, herbal, smooth
ORE Cascade Paste	Start of Maturation	Hoppy, herbal, smooth, balanced
ORE Cascade Paste	End of Maturation	Intense hoppy, resinous, hoppy
Oil Equinox Paste	Start of Fermentation	Sweet, floral, tea-like (bergamot)
Oil Equinox Paste	Start of Maturation	Sweet, floral, fruity, bitter, citrusy, hoppy
Oil Equinox Paste	End of Maturation	Very intense, hoppy, bitter, citrusy, resinous

Table 7

Sensory evaluation of beer samples treated with paste and dosed at three different times during the fermentation process.

There is a correlation between addition time during the brewing process and the final flavour and aroma of the beer. When paste is added during fermentation it gives a complex, rich and balanced flavour, particularly fruity and floral, dependent on the variety. When added after fermentation mainly hoppy, herbal notes appear. When added at the end of maturation strong hoppy and resinous notes are detected.

We had observed that addition of the paste to the final beer, at the end of maturation or post-maturation, can cause the beer to become hazy. However, we found that when the paste is added

earlier in the process, before or after fermentation or during early maturation, the resulting beer is less hazy and can be visually bright.

We also examined the effect of filtration of the beers using 3, 1.2, and 0.45 micron filters and again evaluated the beers in terms of flavour and clarity. The results are shown in Table 8.

Sample	***Haze EBC at 20°C (before filtration)***	***Sensory evaluation***			
		No filtration (as Table 4)	***3 microns***	***1.2 microns***	***0.45 microns***
Control	3.10	Beer-like, bitter	Beer-like, bitter	Beer-like, bitter	Beer-like, bitter
ORE Citra Paste at start of fermentation	3.22	Fruity, very soft, passion fruit, floral, balanced, peach, mouthfeel	Hoppy, fruity, passion fruit like	Hoppy, fruity, passion fruit like	Control like, mouthfeel, fruity
ORE Citra Paste at start of maturation	2.89	Fruity, passion fruit, sharp, dry	Bitter, hoppy, mouthfeel, fruity	Bitter, hoppy, mouthfeel, similar to control	Control –like, bitter
ORE Citra Paste at end of maturation	4.33	Fruity, sharp, hoppy, resinous mouthfeel	Very bitter, dry, mouthfeel, fruity	Very bitter, dry, mouthfeel	Control-like, mouthfeel, fruity

Table 8

Effect of filtration

Initial haze is similar to the control for earlier additions of paste but starts to increase for later additions at the end of maturation. Haze of all beers were very similar after filtration regardless of dosing point.

In general, the earlier the addition of paste in the brewing process the fruitier and more floral the flavour achieved and with good beer clarity. Hence less loss of flavour during filtration. It also appears that flavour survives filtration better when the paste is added earlier in the brewing process.

Inventive Example 16

Foam enhancement

During the filtration experiment of Inventive Example 15 above, we noticed that the beers dosed with paste at the start of fermentation showed enhanced foam properties to the those dosed later and the control beer. The foam appeared to be more creamy and more stable.

This was verified in a small brewing trial in which beer was dosed as described above with Citra paste at the start of fermentation. Further beer samples were dosed with an equivalent amount of polar extract at the start of fermentation, at the start of maturation, and at the end of maturation. The foam properties of all beers were assessed using an Alka-Seltzer (RTM) test as described below. Tetrahop, a hop-derived foam enhancer, dosed at 4 ppm, was used as a positive control; and untreated beer was used as a negative control. 200 ml of each beer sample were poured onto an Alka Seltzer tablet (a pharmaceutical product that consistently and reproducibly releases a fixed volume of carbon dioxide when contacted with liquid (beer)) at the bottom of a 500 ml measuring cylinder and the time from pouring to collapsing of the foam (surface of the beer becomes visible) was measured. The results can be found in Table 9. Both addition of paste or pure polar extract early in the brewing process shows improved foam stability.

Beer sample	**Improvement (compared with control)**
Tetrahop (replication 1 added at start of fermentation)	37%
Tetrahop (replication 2 added at start of fermentation)	38%
Citra paste (replication 1 added at start of fermentation)	15%
Citra paste (replication 2 added at start of fermentation)	21%
Polar extract added at start of fermentation	53%
Polar extract added at maturation	24%
Polar extract added at end (to final beer)	2%

Table 9

Foam enhancement

Inventive Example 17

CO_2 Target paste versus Target pellets (standard dry hopping)

A comparative experiment was performed for Target pellets (0.8 % oil) and Target paste (10% oil). Both hop products (1g of pellets, 0.08g of paste both equivalent to 26 ppm of oil in beer) were added at the start of fermentation, start of maturation and at the end of maturation and then evaluated in terms of flavour and aroma. The results are shown in Table 10.

Sample	Sensory Evaluation
Target CO_2 Paste	
ORE Target Paste; Start of Fermentation	Floral, Fruity, complex, balanced,
ORE Target Paste; Start of Maturation	Balanced, bitter, hoppy
ORE Target Paste; End of Maturation	Balanced, hoppy, fruity, complex, resinous
Target Pellets	
Target Pellets; Start of Fermentation	Intense hoppy, vegetal, very bitter, toffee, oat-like, some paper notes
Target Pellets; Start of Maturation	Intense hoppy, vegetal, very bitter, toffee, oat-like, some paper notes
Target Pellets; End of Maturation	Intense hoppy, vegetal, very bitter, toffee, oat-like, some paper notes

Table 10

Sensory evaluation of beer samples treated with paste and pellets, and dosed at three different times during the fermentation process.

There are differences in flavour between beers treated with paste and those treated with pellets. Pellets delivered intense hoppy and vegetal flavour with toffee and oat-like notes, characteristic for a raw Target hops. Additionally, some paper-like notes were detected in all beers brewed with hop pellets. Samples treated with paste were preferred over pellets delivering complex, balanced flavours. Depending on the addition time different notes were detected with more floral, fruity notes being prevalent when the paste was added at the start of fermentation to hoppy, resinous flavours for later additions.

Inventive Example 18

Comparison of time of paste addition during brewing process (20 litre scale)

Brewing trials with addition of Citra Paste were carried out in the 20 L brewery in the offices of Joh. Barth & Sohn, in Nuremberg. Paste was added in the fermenter at two dose rates (equivalent to 14ppm, 28ppm oil), and at two different times: start of fermentation and at the start of maturation.

Brewing Details:

Weyermann Bavarian Pilsner malt extract was used.

4 kg were solved in 22 L water to achieve an original gravity of 12°Plato

A bitterness of 20 IBUs was achieved by dosing Isohop during wort boiling.

Three brews of 20 L each were made:

Trial 1: Control

Trial 2: Treatment at start of fermentation

Trial 3: Treatment at start of maturation

For sterilisation, the wort was boiled for 20 min. Precipitated proteins were separated by using whirlpool technique, with no hop addition in whirlpool, followed by a rest for 20 min. The wort was fermented at 24°C for six days with Fermentis Safale US 05 yeast strain.

Trial 1 - Fermentation was carried out over 6 days followed by maturation at <5°C for a further 6 days. The beer was then transferred into bottles and conditioned for two weeks at ambient temperature and then for a minimum of two weeks at <5°C.

Trial 2 – The wort (20 L) was divided into two portions (10 L each) and dry hopped by pre-mixing the paste (at two dose levels) in a small amount of wort and adding the mixed paste during wort aeration (rapid manual stirring) prior to pitching of yeast. Fermentation was carried out over 6 days followed by maturation at <5°C for a further 6 days. The beer was then transferred into bottles and conditioned for two weeks at ambient temperature and then for a minimum of two weeks at <5°C.

Low dosage: 2.5 g paste / litre (fermentation/maturation) ≡ 14 ppm of oil

High dosage: 5.0 g paste / litre (fermentation/maturation) ≡ 28 ppm of oil

Trial 3 - Fermentation was carried out over 6 days. The green beer (20 L) was divided into two lots (10 L each) and the paste was pre-mixed (at two dose levels) in a small amount of green beer before being added back into the remainder of the green beer. Another fermenter was used for maturation of the beer for six days at <5°C prior to bottling, conditioning for two weeks at ambient temperature and then for a minimum of two weeks at <5°C.

Low dosage: 2.5 g paste / litre (fermentation/maturation) ≡ 14 ppm of oil

High dosage: 5.0 g paste / litre (fermentation/maturation) ≡ 28 ppm of oil

Sensory evaluation showed that all treatments with the paste produced 'bright' beers that exhibited a significant and positive flavour impact. The results are shown in Table 11.

Sample	**Tasting results/ Flavour**
Dose rate: 14 ppm	
ORE Citra Paste; Start of Fermentation	Fruity, complex, balanced, passion fruit, peach-like; **Preferred**
ORE Citra Paste; Start of Maturation	Fruity, hoppy, herbal (less fruity than when added at the start of fermentation)
Dose rate: 28 ppm	
ORE Citra Paste; Start of Fermentation	Hoppy, herbal, little fruity
ORE Citra Paste; Start of Maturation	Very intense, balanced, fruity, hoppy, herbal, dry-hopping like; **Preferred**

Table 11

Sensory evaluation of beer samples treated with Citra paste and dosed at two dose rates and two different times during the fermentation / maturation process.

After maturation, all the beers were visually 'bright' with an intense hop aroma and taste. No distinctive preference was noted with some tasters expressing a personal preference for fermenter additions whilst others preferred the beers dose at the start of maturation. Other than expressing that personal preference, no tasters expressed a preference for the control.

We then conducted further experiments to seek to optimise conditions for the preparation of our inventive pastes and investigate further the effects of using the pastes compared with the use of pellets in the flavouring of beer.

Inventive Example 19

Brewing trial – timing of paste addition

A light lager beer was dosed with paste made from three different hop varieties at two stages of the brewing process – at the start of fermentation (FV) and at the start of maturation (MV). Half of the beer was filtered before bottling; the other half was bottled unfiltered. The pastes were formed by mixing an aqueous extract containing 40wt% water with an oil rich extract (ORE) in a ratio of 70:30 to giving a final equivalent of 25ppm of hop oil. Experiments were carried out at the research brewery in Train-St. Johann.

Results:

All beers were tested organoleptically and the results are summarised in Table 12 and Table ***13***13.

Sample	Aroma	Flavour
Citra 2 Fermentation Filtered	Passion fruit, citrus, nectarine, fruity, intense, beautifully balanced, floral – excellent [delivered typical Citra hops character]	Passion fruit, fresh, citrus, peach, fruity, intense, beautifully balanced, pleasant aftertaste
Citra 2 Maturation Filtered	Hoppy, little resinous, sweet, passion fruit, nectarine – very good	Fruity, sweet, little spicy, pleasant bitterness, some sweet resinous notes, balanced, more resinous than fermentation sample
Citra 2 Fermentation Unfiltered	More intense then FV and MV filtered, lost fruity character, less citrus [overdosed]	More intense than FV and MV filtered, lost fruity character [overdosed], bitter

Table 12

Tasting results for beers dosed with Citra paste.

Sample	Aroma	Flavour
Mosaic Fermentation Filtered	Fruity, nectarine, peach, little hoppy, vegetal, citrus, herbal, fresh - excellent	Peach, nectarine, fresh, dry, balanced, herbal, vegetal, hoppy, very pleasant mouthfeel
Mosaic Maturation Filtered	Fruity, sweet, passionfruit, citrus, grapefruit, herbal, vegetal, some sweet off notes	Dry, hoppy, herbal, nectarine, peach, pleasant
Mosaic Fermentation Unfiltered	Fruity, grapefruit, passionfruit, citrus, fresh, hoppy, herbal	Fruity, grapefruit, passion fruit, citrus, fresh, dry, mouthfeel, bitter – very pleasant
Ella Fermentation Filtered	Nectarine, peach, passion fruit, hoppy, citrus, grapefruit, less intense than Mosaic fermentation	Fruity, soft fruits, nectarine, peach, herbal, hoppy, very pleasant
Ella Maturation Filtered	Fruity, sweet, resinous, sweet, herbal vanilla, some cheesy notes, less intense than Mosaic maturation	Fruity, fresh, hoppy, herbal, balanced, herbal peach, nectarine
Ella Fermentation Unfiltered	Less fruity then filtered sample, some grassy notes still very fruity, herbal, vegetal, passion fruit like	Less fruity then filtered sample, some grassy notes, herbal, vegetal, still very fruity, passion fruit like, more mouthfeel, more bitter

Table 13

Tasting results for beers dosed with Ella and Mosaic pastes.

Conclusions

- When paste added into FV – more balanced, sweet, fruity character, compared with when paste added into MV, and when fewer less fruity, some resinous notes appear
- Citra - excellent results for both trials
- Mosaic versus Ella – both very good, Mosaic more intense, excellent flavour
- Filtration – filtered samples deliver more fruity character than unfiltered samples. However, unfiltered samples are more bitter, vegetal with a pleasant mouthfeel
- Unfiltered samples – lost fruity character in comparison with the filtered samples, still positive. 40% of the panellists preferred unfiltered samples.

All trial beers were analysed by SPME-GC-MS. The results are summarised in Figure 2. The SPME confirmed the intensity perception from the tasting - filtered beers were weaker than unfiltered beers, beers dosed with paste during fermentation were weaker than those dosed during maturation. Mosaic beers showed lower level of the compounds of interest than Citra beers and levels were lowest in beers dosed with Ella extract. The impact of filtration varied for different compounds and seemed to be particularly high for linalool and humulene.

Inventive Example 20

Dry hopping – paste versus pellets

In order to determine the advantage of dry hopping with paste over dry hopping with pellets a brewing trial on a pilot scale was carried out on site at Barth-Haas UK in Paddock Wood. The base beer was a pale ale. Citra pellets and Citra paste were added at two different stages of the brewing process (during fermentation and during maturation). The amount of paste and pellets added into the beer was recalculated on the oil content (25ppm, each sample). Fermentation took 1 week and maturation 2 weeks (3 weeks all together). 1L of each sample was collected and investigated in terms of flavour, HS-SPME profile, haze, ethanol content (GC) and utilization (yeast slurry). The rest of the beer was transferred into bottles, sparked with sugar (2g per bottle), sealed and then left to for one week to settle. Utilisation of key aroma compounds (by HS-SPME-MS), impact on bitter compound concentration, handling and final flavour of the beer were investigated. A second trial using lager as a base beer was performed and these samples were also analysed by HS-SPME GC-MS to investigate utilisation.

Results:

Sensory evaluation did not show significant differences between pellets and paste. All samples delivered fruity, passion fruit-like flavour and aroma.

The alcohol content was comparable for all the samples (approx.6.5%). It appeared that paste did not affect performance of the yeast.

The resultant yeast slurry was measured for all the samples by collecting the sediment from the kegs and letting them settle in the beakers (2 days). The assessment was made on a qualitative rather than accurate quantitative basis, but it showed that the collected sediment for pellets was significantly higher than for paste (250ml of sediment for the paste treated beer and 450ml for the pellet-treated beer, following maturation), demonstrating lower beer losses when using the inventive pastes.

Key aroma compounds were analysed in all beers using HS-SPME-GC-MS. Almost all compounds showed higher concentrations in the paste-dosed beer compared to the pellet-dosed beers and this effect was more pronounced for maturation addition than for fermentation addition (see Figure 3).

The samples from both trials were also analysed for iso-α-acids, as losses of iso acids through dry-hopping have been reported in the literature (https://www.hopsteiner.com/wp-content/uploads/2016/03/2015-06_TS_Humulinone-Utilization.pdf). Iso-β-acids are the principle source of bitterness in beers. This effect was confirmed in both trials and it was found to be substantially less pronounced for paste (loss of 4-5% on average) compared to pellets (loss of 10 to 17%) (see Table 14).

	Reduction in iso-α-acid concentration compared to control
Paste Fermentation	5%
Paste Maturation	4%

Pellets Fermentation	17%
Pellets Maturation	10%

Table 14

Reduction in iso-α-acid concentration in dry-hopped beers compared to control beers.

In assessing the effect of the pastes on the beer, the following observations were made.

When added directly into the final beer:

- As a general observation, beer treated with paste has a very complex, hoppy, herbal and vegetal flavour with a characteristic mouthfeel. Depending on the hop variety used, fruity, citrusy and floral notes might appear.
- Paste based on CO_2 oil rich extract gives a very rich, balanced and complex flavour, closer to dry-hopped beers than any other hop-derived extracts.
- Paste based on hop oil/ hop oil fraction delivers a very intense, hop oil-like aroma and a complex flavour with some mouthfeel and soft bitterness (floral, hoppy, and balanced).
- Some flavour will be lost depending on the filtration regime used to remove haze arising from addition of hop products.

When added during the brewing process

- There is a correlation between addition time during brewing process and the final flavour and aroma of the beer. When paste is added during fermentation it gives very complex, rich and balanced flavour (fruity, floral- depending on the variety). When added after fermentation mainly hoppy, herbal notes appear. When added at the end of maturation strong, hoppy, resinous notes are detected.
- Earlier addition produce a beer with good clarity.
- Losses of flavour due to filtration are less significant with earlier addition of the paste.

Our hop emulsions and pastes are surprisingly effective in terms of delivering complex and pleasant hop flavour and aroma which are characteristic of the hop variety used.

Accordingly, the hop emulsions of the present invention are superior to conventional products and disperse in beer as readily as conventional emulsions, providing excellent flavouring, in a naturally-based product. Additionally, empirical trials have shown that the presence of the polar (aqueous) extract, being a hop extract, delivers a more complex flavour and aftertaste to the beer, substantially true-to-type with traditional dry hopping, whilst still retaining the ease of use advantages of conventional hop emulsions.

Unexpectedly, aqueous extracts contain natural emulsifying agents which allow omission of synthetic, food grade emulsifiers, although an acceptable thickening agent may still be included.

Most surprisingly, we have been able to develop a purely hop-derived paste which, unexpectedly, is readily dispersible in cold wort and in beer and which can add excellent hop flavour without the need for solvent carriers, thickeners or emulsifiers.

Additionally, as the inventive emulsions and pastes are an extract of hops, beer losses which are typically incurred due to adsorption onto cellulose particles when dry hopping with hop cones or pellets (typically 15% in brewing of Craft beers) are minimal.

CLAIMS

1. A hop composition comprising a mixture of a polar extract of hops having a water content of 55wt% or lower and a hop oil-containing component.

2. A composition as claimed in claim 1, wherein the polar extract of hops is an extract of hops or spent hops.

3. A composition as claimed in claim 1 or claim 2 wherein the polar extract of hops is an aqueous extract of hops.

4. A composition as claimed in claim 3, wherein the aqueous extract of hops is obtainable by extracting hops or spent hops with water, preferably with hot water, optionally with boiling water.

5. A composition as claimed in claim 3, wherein the aqueous extract of hops is a de-solventised extract obtainable from a hop extraction process with a lower alcohol, preferably methanol, ethanol or iso-propanol, more preferably ethanol.

6. A composition as claimed in any preceding claim, wherein the hop oil-containing component is a mixture of hop oils or an oil-rich extract of hops.

7. A composition as claimed in any one of claims 1 to 6, wherein the aqueous extract of hops and the hop oil-containing component are in a ratio of at least 1:1, preferably at least 3:2, more preferably about 7:3.

8. A composition as claimed in any preceding claim, wherein the water content of the aqueous extract of hops is 50% w/w or less, preferably 40% w/w or less; more preferably between 20% w/w and 40% w/w; even more preferably between 25% w/w and 35% w/w.

9. An emulsion comprising a composition as claimed in any preceding claim, further comprising water, preferably in an amount of up to about 99.8% w/w.

10. An emulsion as claimed in claim 9, further comprising a thickening agent, preferably xanthan gum.

11. An emulsion as claimed in claim 9 or claim 10, having a hop oil content of 5% by weight or less.

12. Use of a polar extract of hops as an emulsifier for a non-polar extract of hops; or as an emulsifier for non-hop oils, optionally for natural or synthetic essential oils.

13. Use as claimed in claim 12, wherein the non-polar extract of hops is a hop oil-containing component.

14. Use as claimed in claim 13, wherein the hop-oil-containing component is a mixture of hop oils or an oil-rich extract of hops.

15. Use of a polar extract of hops as a foam enhancing agent in the brewing of beer.

16. Use of a composition as claimed in any one of claims 1 to 11 as a foam enhancing agent in the brewing of beer.

17. Use as claimed in any one of claims 12 to 16 wherein the polar extract of hops is an aqueous extract of hops.

18. Use as claimed in claim 17 wherein the aqueous extract of hops is an extract of hops or spent hops; preferably an aqueous extract of hops is obtainable by extracting hops with water, preferably hot water, optionally with boiling water; or is obtainable from an ethanolic hop extraction process.

19. A method of preparing a hop-flavouring composition, the method comprising the steps of preparing an aqueous extract of hops having a water content of 55wt% or lower and preparing a hop oil-containing component; and mixing the aqueous extract and the hop oil-containing component together.

20. A method as claimed in claim 19, wherein the step of mixing is carried out at a temperature of 20°C or more, preferably above about 35°C.

21. A method as claimed in claim 19 or claim 20, further comprising a step of adding water to give a final water content of up to about 99.8% w/w.

22. A method as claimed in any one of claims 19 to 21, wherein the composition is a composition as claimed in any one of claims 1 to 8 or an emulsion as claimed in any one of claims 9 to 11.

23. A method of enhancing foam characteristics in a beer, the method comprising the addition of a composition as claimed in any one of claims 1 to 8 or an emulsion as claimed in any one of claims 9 to 11 11 at the start of, during or after fermentation or maturation.

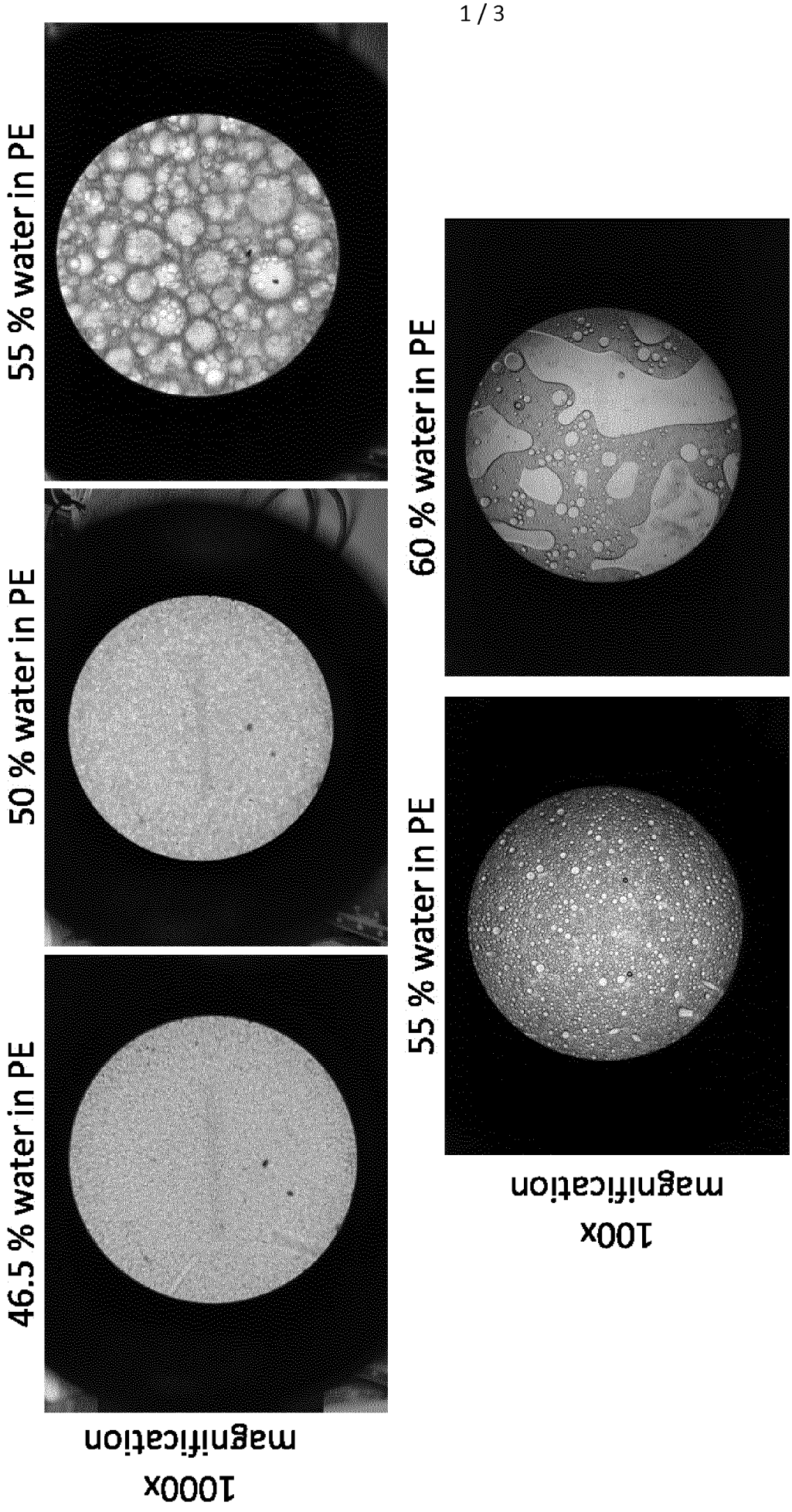

Figure 1: Microscopic images of pastes produced with polar extracts (PE) possessing different water contents; 1000x and 100x magnification.

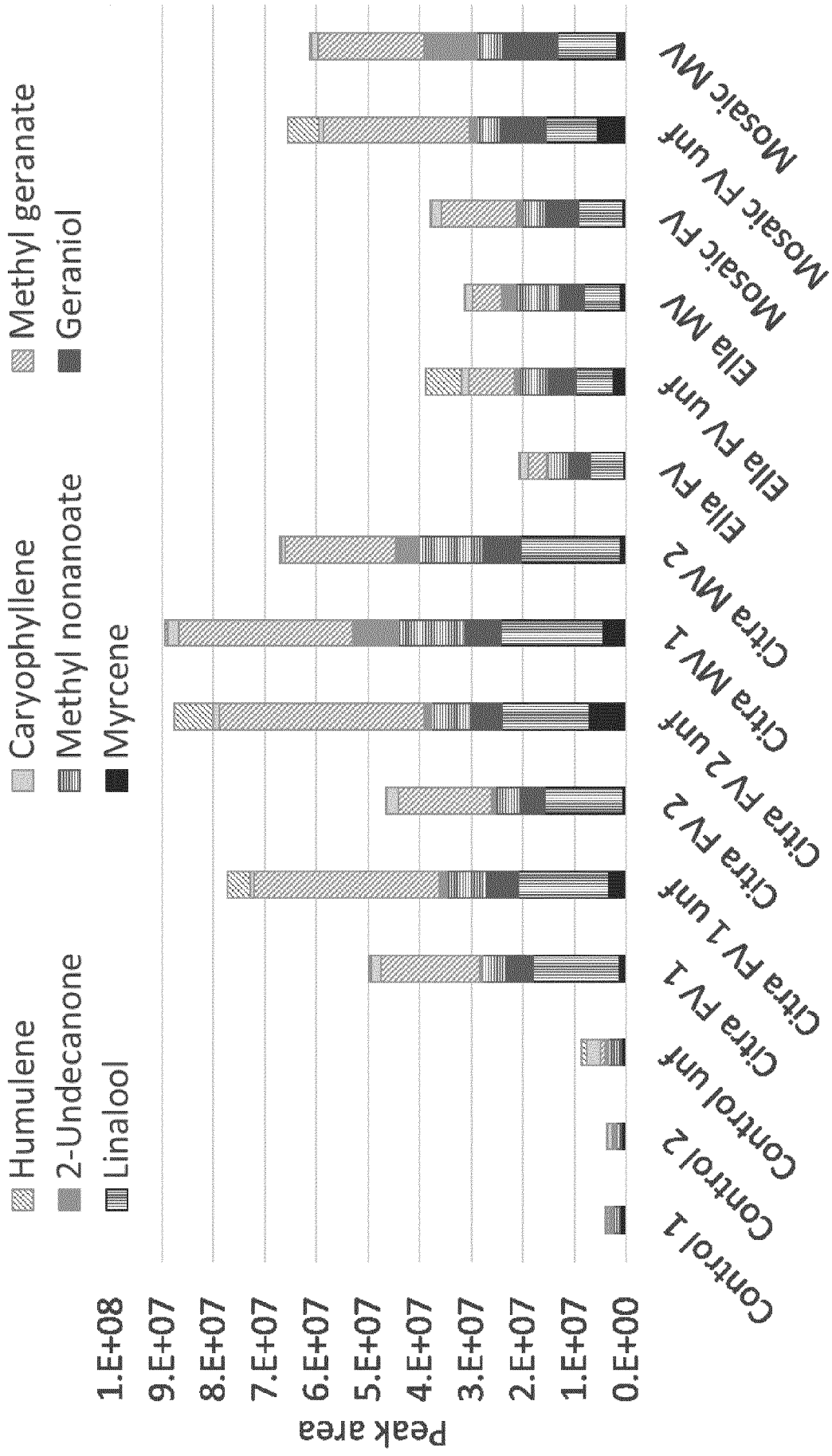

Figure 2: GC-MS areas for selected compounds of interest in beers dry-hopped with paste at different stages of the brewing process. FV – fermentation vessel; MV – maturation vessel; unf – unfiltered.

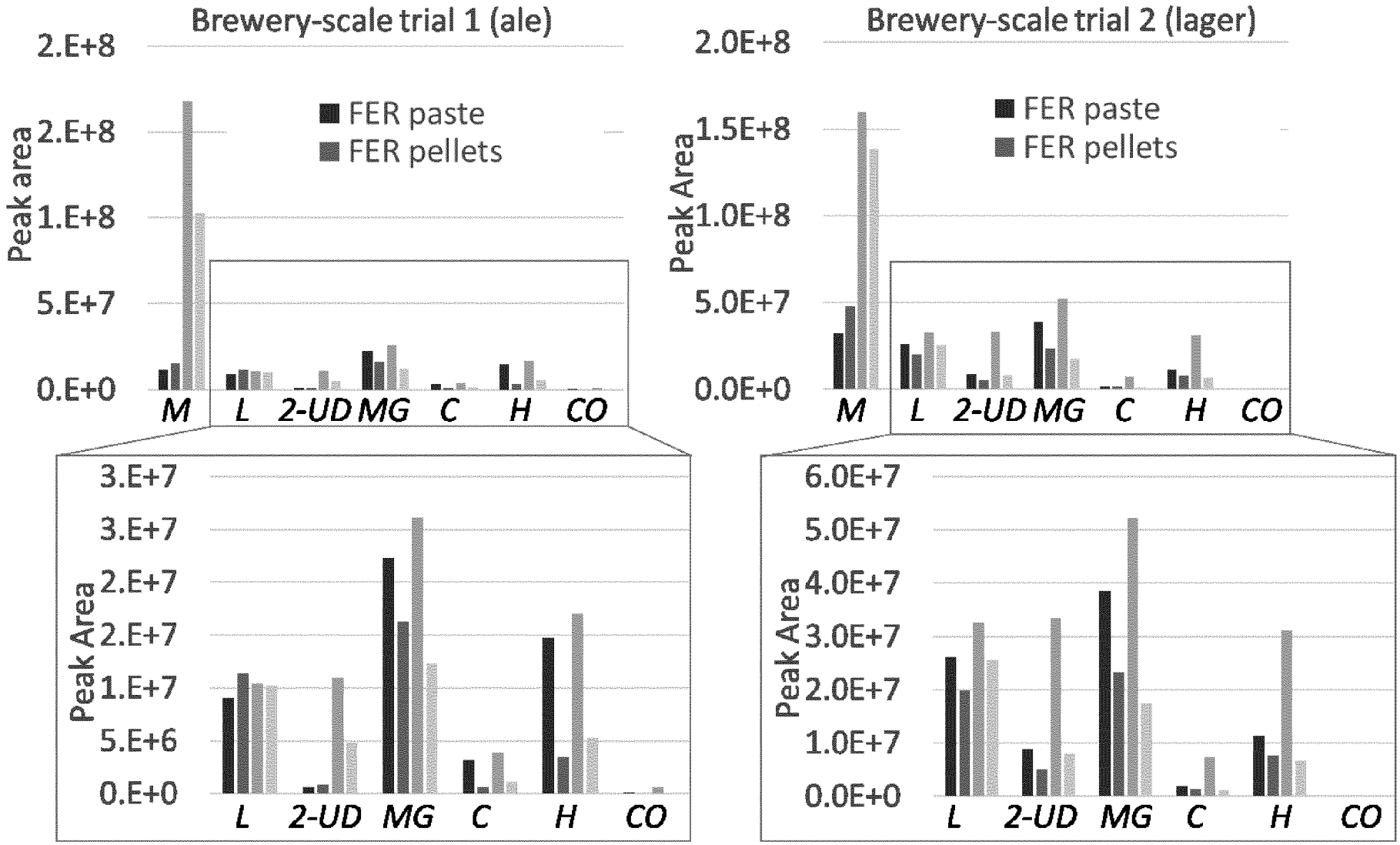

Figure 3: Comparison of analytical results for the two utilisation trials (dry hopping with paste vs. pellets); L – linalool, 2-UD – 2-undecanone, MG – methyl geranate, C – caryophyllene, H – humulene, CO – caryophyllene oxide; FER – fermentation, MAT – maturation.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/076788

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12C3/08 C12C3/10 C12C5/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12C

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.
X	GB 459 635 A (LOUIS ANTON FREIHERR VON HORST) 12 January 1937 (1937-01-12) page 5, lines 71-107 claims 1-5,7	1-11, 19-22
X	GB 1 048 912 A (MARIA BRIEM) 23 November 1966 (1966-11-23) the whole document	1-11, 19-22
X	US 2003/138546 A1 (GOLDSTEIN HENRY [US] ET AL) 24 July 2003 (2003-07-24) paragraphs [0017], [0019] - [0022], [0041] - [0044]; claims 1-6,10-11,26,28,30-31	1-11, 19-22
A	WO 93/15181 A1 (RHONE POULENC INC [US]) 5 August 1993 (1993-08-05) the whole document	1-11, 16-23

☐ 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
31 January 2018	05/04/2018

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2017/076788

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

1-11, 16, 19-23(completely); 17, 18(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-11, 16, 19-23(completely); 17, 18(partially)

   Hop composition (claims 1-8); emulsion comprising the hop composition (claims 9-11); use of the hop composition (claim 16; claims 17-18 (partially)); method of preparing the hop composition (claims 19-22); method of enhancing foam characteristics employing the hop composition (claim 23).

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2. claims: 12-14(completely); 17, 18(partially)

   Use of a polar extract of hops (claims 12-14); claims 17-18 (partially).

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3. claims: 15(completely); 17, 18(partially)

   Use of a polar extract of hops (claim 15); claims 17-18 (partially).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/076788

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 459635 A	12-01-1937	NONE	
GB 1048912 A	23-11-1966	NONE	
US 2003138546 A1	24-07-2003	AU 733954 B2	31-05-2001
		BR 9807919 A	22-02-2000
		CA 2285663 A1	08-10-1998
		EP 0975736 A1	02-02-2000
		NZ 338078 A	26-10-2001
		US 5972411 A	26-10-1999
		US 2003138546 A1	24-07-2003
		WO 9844087 A1	08-10-1998
WO 9315181 A1	05-08-1993	AU 3606393 A	01-09-1993
		CA 2127440 A1	05-08-1993
		CN 1082104 A	16-02-1994
		EP 0625188 A1	23-11-1994
		FI 943599 A	02-08-1994
		JP H08502641 A	26-03-1996
		NO 942868 A	30-09-1994
		NZ 249359 A	26-11-1996
		TW 199905 B	11-02-1993
		US 5387425 A	07-02-1995
		WO 9315181 A1	05-08-1993