Substance Sameness Statement of the Vinasses Consortium

1 Preamble

This sameness statement concerns the REACH registration of: Vinasses, a by-product obtained after the fermentation of molasses/sugar/other feedstock, using different microbial strains, in the production of alcohol, yeast and other organic substances. It is explained that five different types, or sub-groups, of Vinasses substances have been identified and therefore different registration dossiers for Vinasses will be submitted for each type. This document will be part of the joint registration dossiers.

2 Identity in relation to EINECS numbers

"The REACH Regulation does not define "sameness" and it does not foresee any formal role for ECHA in confirming the establishment of sameness or in the formation of a SIEF. The assessment of the exact nature of an EINECS entry and the different substances it may cover can only be carried out by the manufacturers or importers who are best placed to assess the composition of their substances. It is, therefore, up to them to take the responsibility for defining precisely the substance(s) for which a SIEF will be formed."[1]

Manufacturers and importers have pre-registered their Vinasses products under various EINECS- / EC- numbers given in Table 1, because a clear EINECS identity for this type of product is missing.

Table 1: EINECS- / EC- numbers under which VINASSES-products were pre-registered

EC- number	Name
231-915-5	Potassium Sulphate
231-915-5 / 293-805-3	Reaction mass of Potassium Sulphate and Vinasse and Distillery slops
293-805-3	Vinasse and distillery slops
297-918-9	Vinasse and distillery slops, tartrate free, dried
302-644-0	Molasses, yeast fermentation, distillation residues
@919-670-9	Concentrated by-product obtained after the fermentation of molasses/sugar/other feedstock, using different strains, in the production of alcohol, yeast and other organic substances
@920-615-6	Molasses, amino acid residues
@926-059-0	UVCB Filtration residue of fermentation of *Penicillium Chrysogenum*

[1] "Guidance on data sharing" September 2007, 4.5, page 34

The pre-registrations of Vinasses were reviewed and it was concluded that in some cases the definitions given in the EINECS inventory for the various EC numbers are very similar. For many products it is not clear which of the EC numbers is most appropriate, neither do they fit to the criteria for the identification of UVCB substances.
The best solution to cope with the various types of Vinasses is to create new EC numbers based on different processes and microorganisms (see chapter 5).

3 Definition of Vinasses, being an UVCB substance

Vinasses result from a strictly controlled fermentation processes involving complex biological raw materials and different micro-organisms. Thus, it is a typical substance of unknown or variable composition of biological origin (UVCB).
The Guidance for substance identification[2] states that such a product shall be defined by:

- The source: culture medium and micro-organism applied
- The process: fermentation, isolation of products, purification steps
- Other identifiers: type of products, known composition

In the Guidance for substance identification the following comparable cases are mentioned.

Regarding plant extracts the Guidance indicates[3]:
"At the time of the setting of the EINECS inventory, extracts from different processes, different solvents and even physical or chemical derivates were often covered under one single entry. These substances may be registered as a single substance under REACH, provided that the hazardous properties do not differ and warrant the same classification."

With respect to enzymes, it is stated in the Guidance[4] that:
"The enzyme substance typically contains 10 – 80% (w/w) of the enzyme protein. The other constituents vary in percentage and depend on the production organism used, the fermentation medium, and operational parameters of the fermentation process as well as the downstream purification applied, but the composition will typically be within the ranges indicated in the following table."

Active enzyme protein	10 - 80%
Other proteins + peptides and amino acids	5 - 55%
Carbohydrates	3 - 40%
Lipids	0 - 5%
Inorganic salts	1 - 45%
Total	100%

Conclusion: although enzyme substances typically contain 20-90% non-enzyme substances, resulting from the production organism, fermentation medium etc., the EINECS number – and

[2] "Guidance for identification and naming of substances under REACH" June 2007, Table 4.2, page 20
[3] "Guidance for identification and naming of substances under REACH" June 2007, 4.3.1.2 3, page 34
[4] "Guidance for identification and naming of substances under REACH" June 2007, 4.3.2 3, page 41

thus the substance sameness definition – does not necessarily include the source organism or the substrate. Constituents >10% should be identified (page 47).

As stated above, Vinasses are fermentation by-products and there is an inherent variability of the production processes and thus the composition of Vinasses.

Therefore it can be concluded that a similar approach for identification of Vinasses as in above examples can be used.

4 Factors to be considered for classification

Concerning Vinasses, there are several aspects to be considered:

- The micro-organisms used for fermentation are qualified as safe micro-organisms, either having a QPS[5] status (EFSA opinion on safety of micro-organisms: Qualified Presumption of Safety), or belonging to a safe strain lineage[6] (see section 4.1).
- The fermentation ingredients qualify for use in food or feed production processes. Typical Vinasses are by-products, being a mixture of residual nutrients and cell contents. These Vinasses-solutions are comparable irrespective of the type of micro-organism used, provided that the biomass is removed.
- Process variations will not affect the hazardous properties or the substance classification.
- Vinasses have been sold as feed[7] for several decades.
- Vinasses are also used as organic fertilisers in agriculture and are therefore subject to national standards in several countries, e.g. France, Germany or the Netherlands.

4.1 Micro-organisms:

In case of the Vinasses types A1 and A2 (see section 5), the micro-organism biomass is removed from the fermentation broth and, thus, is not a constituent of the Vinasses and does not determine its identity. Even if the micro-organism biomass may be present in the vinasses as residues, it should be noted that it either has the QPS[5] status or complies with the "Safe Strain Lineage Concept"[6]. This "Safe Strain Lineage Concept" implies that no toxic secondary metabolites are produced by the micro-organism that could end up in the Vinasses.

In the case the micro-organism biomass becomes a part of the Vinasses, since it has not been removed from the fermentation broth, it co-determines the substance identity (Vinasses types B1, B2 and B3).

4.2 Culture medium:

The raw materials used in the fermentation consist of:

- substrates, mostly of vegetable origin of quality appropriate for a use in food or feed production process, for example: beet or cane molasses, sugar syrup, cereals and products containing starch, grapes, alcohol, distillery residues, fruit juice, whey, lactic acid, sugar, hydrolyzed vegetable fibres, etc.
- fermentation nutrients such as for example ammonia, mineral salts, vitamins etc.

In addition, processing aids such as pH controlling agents and defoamers are used during the fermentation.

Most of the manufacturers of the Vinasses use various substrates in the fermentation processes. For that reason it is not possible to define the culture medium precisely.

[5] http://www.efsa.europa.eu/en/biohaztopics/topic/qps.htm
[6] "The Safe Strain Lineage Concept.pdf" included in chapter 13 of the registration dossier (together with this document)
[7] Feed catalogue: (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:077:0017:0032:EN:PDF)

The absence of hazardous components is further secured by:

- the usage of substrates in food or feed production processes
- the requirement for by-products of the fermentations to be used in feed and as fertilizer and therefore not permitted to be contaminated with hazardous substances.

4.3 Removal of the primary product:

Since Vinasses is the by-product of the fermentation process, it is not isolated or purified itself. However, the main products (yeast, ethanol, organic acids etc.) are removed from the fermentation broth by using techniques such as filtration, precipitation, chromatography or distillation.
The manufacturer is responsible for ensuring that there are no (potentially) hazardous residues present in the Vinasses product, which could contribute to a classification of the Vinasses.
In a case in which a residue would lead to classification of a company's Vinasses, this company would need to opt-out from the joint classification and registration.

4.4 Concentration process

After the fermentation and the removal of biomass (if applicable) and primary product (if applicable) the fermentation broth is usually concentrated by physical processing steps (evaporation, centrifugation and/or micro-filtration). During this processing step processing aids such as pH controlling agents and defoamers are used.
The concentration process is a removal of water (being the solvent) and does not change the composition of the dry matter. According to Article 3.1 of the REACH regulation, this does not affect the substance identity. The dry matter of the Vinasses is regarded as an UVCB substance. Consequently, the Vinasses products of various degrees of concentration consist of Vinasses dry matter and water in different proportions.

5 Vinasses subgroups

5.1 Identification of 5 different subgroup Vinasses substances

Based on identified process variations, the Consortium has defined five groups that represent five substances within the Vinasses spectrum.
For each of these groups the identifiers and Reference Substance are specified separately.

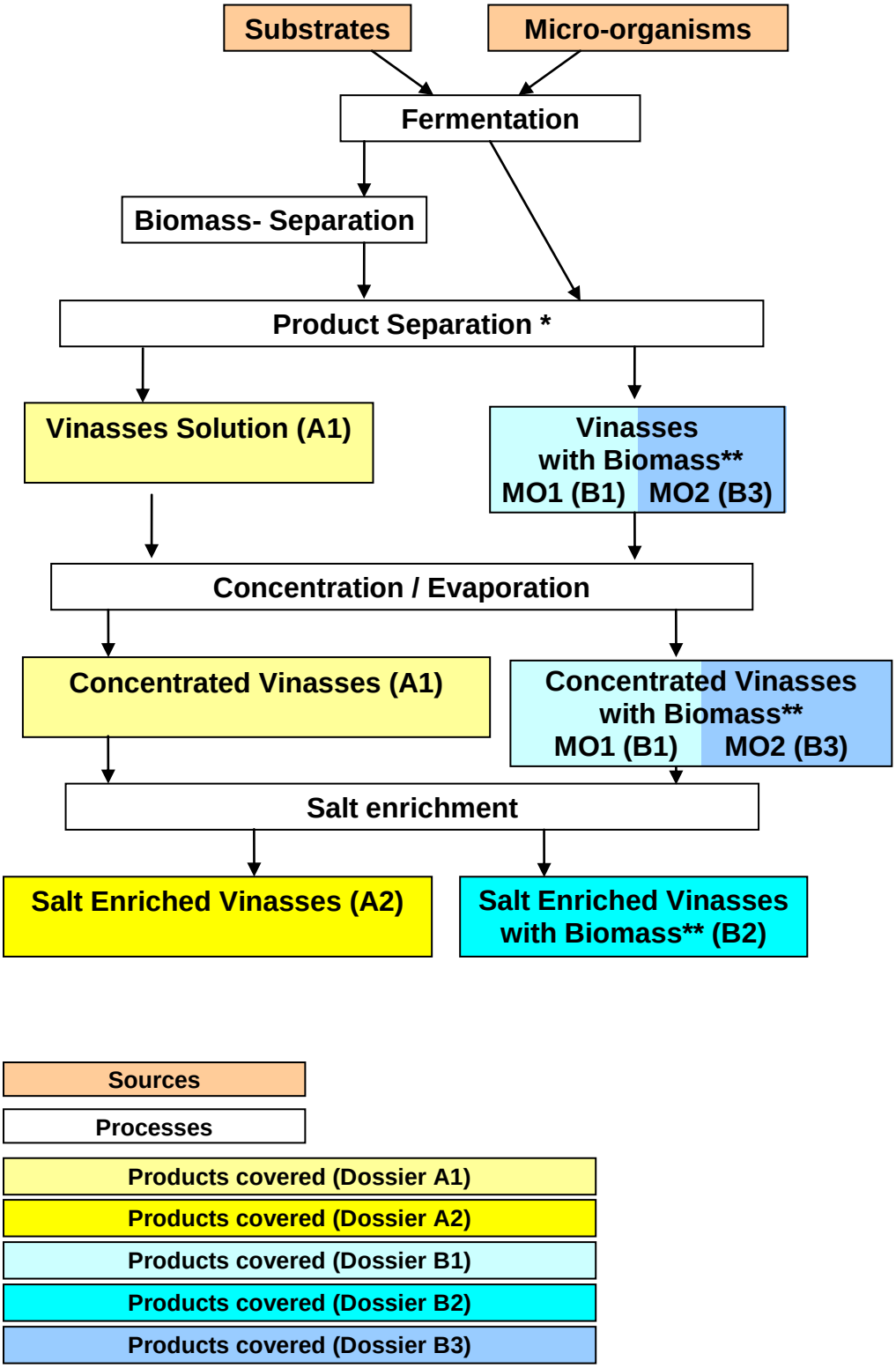
The first criterion of distinction is whether there is a biomass removal step in the production process (A or B). Where biomass is present in the Vinasses, the micro-organism plays a discriminating role.
A second criterion is whether there is a salt-enrichment[8] step in the production process.
Therefore the following groups are formed:

A Biomass removed:
   1. Vinasses, residue of fermentation
   2. Vinasses, residue of fermentation, salt-enriched

[8] The term "enrichment" is used in the sense of "increasing the concentration of a specific compound by a physical refinement process without leading to a purified substance".
It does not mean "addition of pure substance to increase the concentration" (fortification).

B Biomass not removed:

1. Vinasses, residue of fermentation containing biomass of bakers yeast (*Saccharomyces cerevisiae*)
2. Vinasses, residue of fermentation containing biomass of bakers yeast, salt-enriched
3. Vinasses, residue of fermentation containing biomass of *Corynebacterium glutamicum*
   (For other micro-organisms; separate dossier per MO would be required)



** In case that the product is not the biomass*
*** Different species lead to different substances (B1 and B3)*

Figure 1: Production Process of various Vinasses products

5.2 Read-across justification

The toxicological and ecotoxicological test data for all Vinasses dossiers are based on vinasses from group A1. This group consists of Vinasses that contain the components that remain in the liquid solution after the separation of the biomass and in some cases recovery of the fermentation products. These components are residues of the fermentation feedstock and minerals used in the fermentation and also residues of the cell content of production organism. All the identified Vinasses will contain the same types of constituents. These have been tested in various toxicity studies that are part of all joint registration dossiers. The read across principle is used. For the cases in which biomass is retained in the Vinasses this is then justified by the fact that only microorganisms are used that have the QPS[5] status (i.e. yeast) or comply with the Safe Strain Lineage concept[6]. Herewith it is assured that no hazardous properties are introduced in the Vinasses of group B1, B2 and B3.

6 Reference substance definitions

EC numbers under which Vinasses products are registered

Group	Description	EC number
A1	Vinasses, residue of fermentation	932-215-9
A2	Vinasses, residue of fermentation, salt-enriched	932-176-8
B1	Vinasses, residue of fermentation containing biomass of baker's yeast (Saccharomyces cerevisiae)	932-161-6
B2	Vinasses, residue of fermentation containing biomass of baker's yeast (Saccharomyces cerevisiae), salt-enriched	932-165-8
B3	Vinasses, residue of fermentation containing biomass of Corynebacterium glutamicum	932-179-4

A1. "Vinasses, residue of fermentation"

Vinasses, residue of fermentation, result from a fermentation process in which the micro-organism used has the QPS status (Qualified Presumption of Safety micro-organism recognized by the European Food Safety Authority) and/or complies with the "safe strain lineage concept". The fermentation process is performed with a culture medium consisting of substrates, mineral nutrients and vitamins, which quality is appropriate for a use in food or feed production processes. The micro-organism biomass and, if applicable, fermentation products (ethanol, amino acids or other products) are removed from the fermentation broth by appropriate methods. The resulting liquid is usually concentrated by physical steps (evaporation, centrifugation and/or micro-filtration steps).

The reference substance is defined as the dry matter content of the Vinasses with the following ranges of the constituents "g/100 g dry matter" :

Parameter	
Potassium	0-17% d.m.
Sodium	0-10% d.m.
Calcium	0-3% d.m.
Phosphorus	0-2% d.m.
Magnesium	0-1% d.m.
Sulfate	0-25% d.m.
Chloride	0-9% d.m.
Nitrate	0-11% d.m.
Ammonium	0-9% d.m.
Unknown mineral matter (expressed in oxides)	0-10% d.m.
Total amino acids	4-36% d.m.
Betain	0-30% d.m.
Lipids	0-3% d.m.
Sugars	0-25% d.m.
Organic acids	0-15% d.m.
Polyols	0-12% d.m.
Alcohols	0-1% d.m.
Unknown organic matter	0-40% d.m.
Mineral matter as crude ashes	3-40% d.m.
TOC	10-50% d.m.
Total N	1-15% d.m.

Additional information is given in the documents “Category justification document of the Vinasses Consortium” and “The Safe Strain Lineage Concept” documents, which are included in Chapter 13 of the dossier.

A2. "Vinasses, residue of fermentation, salt-enriched"

Vinasses, residue of fermentation, salt-enriched, result from a fermentation process, in which a micro-organism is used. This micro-organism has a QPS status (Qualified Presumption of Safety micro-organism recognized by the European Food Safety Authority) or complies with the "safe strain lineage concept".
The fermentation process is performed with a culture medium consisting of various substrates and nutrients. Processing aids as pH controlling agent and anti-foam are also used during the fermentation.
The fermentation process at industrial scale is performed under aerobic conditions, with continuous control of temperature, pH and foam level.
After the fermentation process is completed, the micro-organism biomass is removed by centrifugation, thus resulting into two distinct flows: yeast cream and wort.
The fermentation liquid without biomass is then concentrated by evaporation.
During the concentration process salt crystals mainly consisting of potassium sulphate (K2SO4) are formed, which are next separated from the liquid vinasses by centrifugation to obtain slurry that is clearly "salt-enriched" compared to the original vinasses.
The reference substance is defined as the dry matter content of the salt-enriched Vinasses.

Its typical composition is given in the following table:

Parameter	
Potassium	18-40% d.m.
Sodium	0-8% d.m.
Calcium	0-3% d.m.
Phosphorus	0-1% d.m.
Magnesium	0-0.5% d.m.
Sulfate	30-61% d.m.
Chloride	0-2% d.m.
Nitrate	0-0.6% d.m.
Ammonium	0-1% d.m.
Total amino acids	1-5% d.m.
Betaine	0-10% d.m
Organic acids	1-8% d.m.
Total sugar	0-5% d.m.
Polyols	0-2% d.m.
Alcohols	0-1% d.m.
Lipids	0-1% d.m.
Unknown mineral	0-32% d.m.
Unknown organic	0-40% d.m.

More details of a manufacturing process are presented in Chapter 3.1 of the dossier. Additional information is given in the "Category justification document of the Vinasses Consortium.pdf" and in the "The Safe Strain Lineage Concept.pdf" which are included in Chapter 13 of this dossier.

B1 "Vinasses, residue of fermentation containing biomass of bakers yeast"

Vinasses, residue of fermentation containing biomass of baker's yeast (Saccharomyces cerevisiae), result from a fermentation process, in which the micro-organism used is Saccharomyces cerevisiae (Genus: Saccharomyces; Species: Cerevisiae; Family: Saccharomycetaceae). This micro-organism has a QPS status (Qualified Presumption of Safety micro-organism recognized by the European Food Safety Authority) or complies with the "safe strain lineage concept".

The fermentation process is performed with a culture medium consisting of various substrates and nutrients. In addition, processing aids as pH controlling agent (i.e. Sulphuric acid or hydrochloric acid) and anti-foam are also used during the fermentation.

The alcoholic fermentation process at industrial scale is performed under anaerobic conditions, with continuous control of temperature, pH and foam level. Prior to this alcoholic fermentation step, a pre fermentation step can be performed under aerobic.

After the fermentation process is completed, the fermentation product (ethanol) is extracted from the fermentation broth by distillation. The liquid residue named Vinasses is then concentrated by evaporation.

The reference substance is defined as the dry matter content of the Vinasses.

The typical macro-composition lies in the following ranges, based on dry-matter content:

Parameter	
Potassium	0.04-19% d.m.
Sodium	0.03-9% d.m.
Calcium	0-2% d.m.
Phosphorus	0-1% d.m.
Magnesium	0-2% d.m.
Sulfate	0.2-15% d.m.
Chloride	0.15-10% d.m.
Nitrate	0-5% d.m.
Ammonium	0-5% d.m.
Total amino acids	1-44% d.m.

Parameter	
Betaine	2-59% d.m
Organic acids	1-25% d.m.
Total sugar	1-15% d.m.
Polyols	0.1-45% d.m.
Alcohols	0-2% d.m.
Lipids	0.24-8% d.m.
Unknown mineral	0-5% d.m.
Unknown organic	0-80% d.m.

More details of a manufacturing process are presented in Chapter 3.1 of the dossier. Additional information is given in the "Category justification document of the Vinasses Consortium.pdf" and in the "The Safe Strain Lineage Concept.pdf" which are included in Chapter 13 of this dossier.

B.2 Vinasses, residue of fermentation containing biomass of bakers yeast, salt-enriched

Vinasses, residue of fermentation containing biomass of bakers yeast, salt enriched, result from a fermentation process, in which the micro-organism used is Saccharomyces cerevisiae (Genus: Saccharomyces; Species: Cerevisiae; Family: Saccharomycetaceae). This micro-organism has a QPS status (Qualified Presumption of Safety micro-organism recognized by the European Food Safety Authority) or complies with the "safe strain lineage concept".
The fermentation process is performed with a culture medium consisting of various substrates and nutrients. Processing aids as pH controlling agent and anti-foam are also used during the fermentation.
The alcoholic fermentation process at industrial scale is performed under aerobic conditions for cell propagation and anaerobic conditions for alcohol fermentation, with continuous control of temperature, pH and foam level.
After the fermentation process is completed, the fermentation product (ethanol) is extracted from the fermentation broth by distillation. The liquid residue named Vinasses is then concentrated by evaporation. During this step, processing aids may be used: the final concentration of these processing aids is reduced as low as technically feasible.
During the concentration process salt crystals mainly consisting of potassium sulphate (K2SO4) are formed, which are next separated from the liquid Vinasse by centrifugation to obtain sludge of salts: the resulting slurry is clearly "salt-enriched" compared to original vinasse.
The reference substance is defined as the dry matter content of the Vinasses, salt-enriched.
The typical macro-composition lies within the following ranges:

The typical macro-composition lies within the following ranges:

Parameter	

Parameter	
Potassium	12-40% d.m.
Sodium	0.1-3.2% d.m.
Calcium	1.1-5% d.m.
Phosphorus	0-0.25% d.m.
Magnesium	0-0.17% d.m.
Sulfate	28-64% d.m.
Chloride	0-0.4% d.m.
Nitrate	0-0.4% d.m.
Ammonium	0-0.1% d.m.
Total amino acids	0.5-5% d.m.
Betaine	0-10% d.m
Organic acids	0.5-8% d.m.
Total sugar	0-3% d.m.
Polyols	0.5-8% d.m.
Alcohols	0-1% d.m.
Lipids	0-1% d.m.
Unknown mineral	0-30% d.m.
Unknown organic	0-20% d.m.

More details of a manufacturing process are presented in Chapter 3.1 of the dossier. Additional information is given in the "Category justification document of the Vinasses Consortium.pdf" and in the "The Safe Strain Lineage Concept.pdf" which are included in Chapter 13 of this dossier.

B.3. Vinasses, residue of fermentation containing biomass of Corynebacterium Glutamicum

Vinasses, residue of fermentation, result from a fermentation process, in which the micro-organism used is Corynebacterium Glutamicum. This micro-organism has a QPS status (Qualified Presumption of Safety micro-organism recognized by the European Food Safety Authority) or complies with the "safe strain lineage concept".
The fermentation process is performed with a culture medium consisting of:

- The substrates: cane or beet molasses, sugar and cereals.
- Phosphoric acid, as nutrient and pH-regulator
- Ammonia (NH3), as nutrient and pH-regulator.
- Polyglycol, as an anti-foaming agent.

The fermentation process at industrial scale is performed under aerobic conditions with continuous control of temperature, pH and foam level. After the fermentation process is completed, the micro-organism biomass is not removed.
Amino / Glutamic Acid is removed by crystallisation. Water is removed by evaporation and results in the liquid vinasses. To produce the dry prilled product, the liquid vinasses is spray-dried with hot air.
The reference substance is defined as the dry matter content of the Vinasses.
The typical macro-composition lies in the following ranges, based on dry-matter content:

The typical macro-composition lies within the following ranges:

Parameter	
Potassium	3-8% d.m.
Sodium	0.5-15% d.m.
Calcium	0.5-6% d.m.
Phosphorus	0-1% d.m.
Magnesium	0.1-3% d.m.
Sulfate	0.4-12% d.m.
Chloride	4-22% d.m.
Nitrate	0-0.5% d.m.
Ammonium	3-12% d.m.
Total amino acids	8-30% d.m.
Betaine	0-12% d.m
Organic acids	1-5% d.m.
Total sugar	3-12% d.m.
Polyols	0.1-4% d.m.
Alcohols	0-0.01% d.m.
Lipids	0-2% d.m.

Parameter	
Unknown mineral	0-70% d.m.
Unknown organic	0-70% d.m.

More details of a manufacturing process are presented in Chapter 3.1 of the dossier. Additional information is given in the "Category justification document of the Vinasses Consortium.pdf" and in the "The Safe Strain Lineage Concept.pdf" which are included in Chapter 13 of this dossier.