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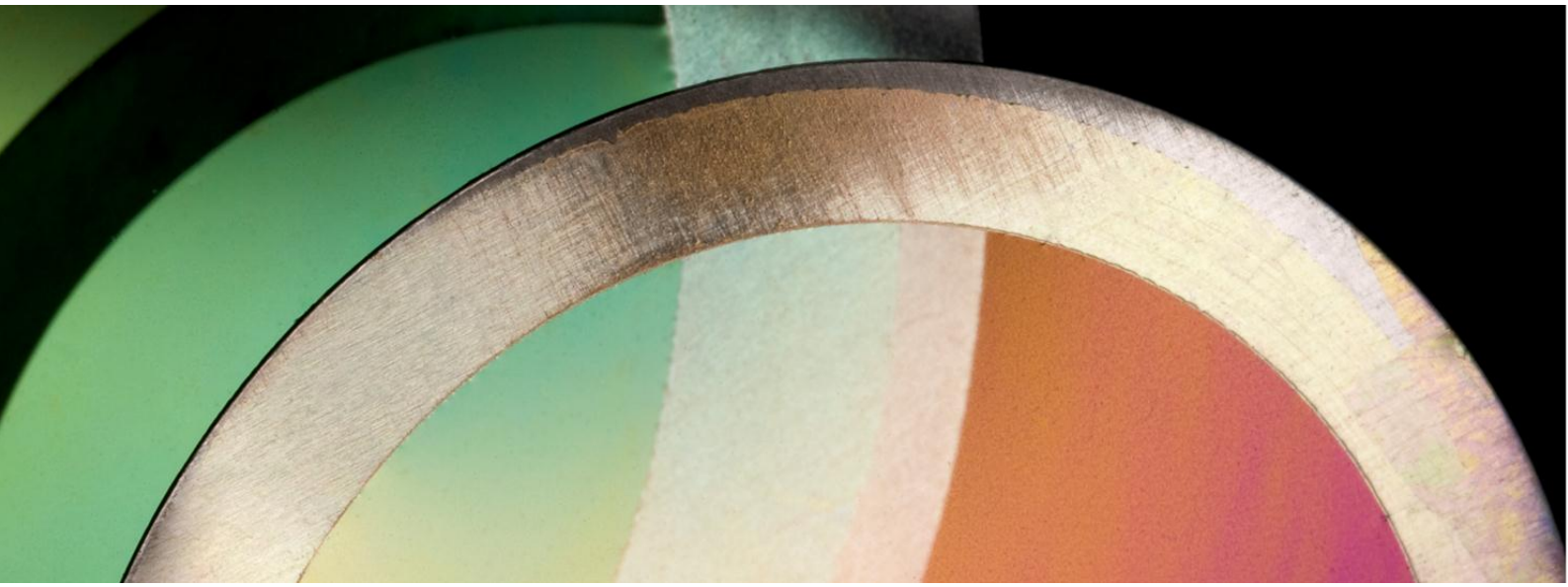

Provision of scientific and technical support with respect to the classification of extracts/concentrates with colouring properties either as food colours (food additives falling under Regulation (EC) No 1333/2008) or colouring foods

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Abstract

The Standing Committee on the Food Chain and Animal Health endorsed in 2013 the Guidance notes for the classification of food extracts with colouring properties. For the purpose of classification a term "enrichment" factor is introduced by the Guidance notes, calculated based on reference values, which should be listed in an Anne III to the Notes. The source material reference values might have a significant impact on the enrichment factor calculations. The support of the Joint Research Centre (JRC), one of the Directorates General of the European Commission, was requested by DG SANTE to search for and compile the necessary reference values, documenting the biological variation as far as possible. JRC was asked to produce nutrients reference values and reference values for the aromatic and colouring constituents in the source materials, requested by stakeholders. Analytical methods suitable for the determination of the aromatic and colouring compounds had to be identified as well, when possible. In this report a reference values/ranges were derived based on critical analysis of the information, compiled from food compositing databases, scientific books and peered reviewed articles. Bibliographic data were included as references for the analytical methods, suitable for determination of the colouring and aromatic constituents when feasible.

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Executive Summary

The European Commission's Standing Committee on the Food Chain and Animal Health endorsed the Guidance notes for the classification of food extracts with colouring properties in 2013.

As a supplement to the existing EU regulations on the classification and declaration of products used to provide colour, the objective of the guidance notes is to provide simple and practical criteria for the differentiation between foods with colouring properties (so-called Colouring Foods) and food colours, which are considered to be additives within the EU. Based on an easily applicable decision tree, they serve as an important working and decision tool for the food industry and enforcement authorities of the EU Member States

Whether an extraction process for obtaining colouring food is selective or not depends, according to Regulation (EC) No 1333/2008, on the ratio of the pigments relative to the nutritive or aromatic constituents in the obtained extract on the one hand and in the source material on the other. If the enrichment of the colouring principles is below a certain threshold (enrichment factor) the extract is regarded as colouring food. For the estimation of the enrichment factor reference values for the colouring principles and the nutritive and aromatic constituents of the source material are needed. The guidance notes recommend using reference values based on the scientific literature, relevant to the edible part from which the colouring food is extracted. The source material reference values might have significant impact on the enrichment factor calculations. The Guidance notes also require that "the reference values for the source materials are to be determined in cooperation with the Joint Research Centre. Cooperation and an input from the stakeholders are expected."

This report gives an overview of currently available data on nutritional information, and pigment and aroma constituents of twenty two source materials used for production of colouring food, requested to be included in Annex III of the guidance notes by the stakeholders.

The nutrient reference values were compiled after consulting publicly available databanks provided by EuroFIR (European Food Information Resource, which is an international non-profit association) and the US Department of Agriculture including food composition data from more than 30 European countries, USA, Canada and Australia. Two German databases were used as well for complementing the survey with additional data.

Compilations for colouring and aromatic constituents in the source materials were performed using more specialised databases such as eBasis, PhenolExplorer, but mainly from peer reviewed articles in scientific journals, attempting to cover as much as possible the existing variability in those parameters reflecting the geographical and genetic factors, growing conditions, stage of maturity and post-harvest handling.

Reference values for the nutritive constituents were derived based on robust statistic, when applicable. Reference ranges/values for the main colouring constituents were proposed considering the fact that the source materials with the highest available pigment content would be used for the purpose of producing colouring food.

A critical overview was performed on the analytical methods available for the determination of the pigments in the source materials. Fit for purpose methods were included as references in the report.

Defining characteristic aroma constituents and their contents in the source materials was a challenge. After having consulted a considerable number of books, peer reviewed articles and available databanks on volatile substances it was concluded that for many of the source material a clear definition of the key aroma constituents was not feasible. Character aroma impact compounds and their ranges were mentioned when available in the literature for those source materials with characteristic compounds contributing to its flavour/aroma.

The Annex contains detailed information regarding the composition (nutritive, colouring and aromatic constituents) of relevant source materials for producing colouring food and are an integral part of this report. The suggested reference values/ranges can be used for calculating the enrichment factors to decide whether a preparation is a colouring food or a food colour.

Background as provided by DG Health and Food Safety (SANTE)

When a product is obtained from a food for the primary function of colouring, the key to determining whether or not the product is a colour (i.e. food additive) is whether it has been obtained by way of 'selective extraction'. This leaves some room for preparations obtained from foods using a process of physical and/or chemical extraction which may be interpreted as not being selectively extracted. If it concerns a food normally consumed as such or normally used as a characteristic ingredient of food, these preparations can be called 'colouring food'.

Extraction can range from simple extraction, to degrees of selective extraction up to isolation of the pure pigments. To decide upon the classification of the product it is essential to identify when the product is no longer "a food normally consumed as such or normally used as a characteristic ingredient of food", but a colour which needs approval.

Whether an extraction is selective or not depends, according to Regulation (EC) No 1333/2008, on the ratio of the pigments relative to the nutritive or aromatic constituents. Once the pigments are selectively extracted relative to the nutritive or aromatic constituents the extract is a colour within the meaning of Regulation (EC) No 1333/2008. No other guidance (numerically expressed) is provided by the legislation.

The relationship between the ratio of the pigment(s) content to the nutritive or aromatic constituents in the colouring product (primary extract) compared to the corresponding ratio of the pigment(s) content to the nutritive or aromatic constituents of the source material can be expressed as an enrichment factor, which would allow verifying whether the primary extract's composition is not significantly different from the source material and therefore could be still considered to be a food or a characteristic ingredient of food.

For reasons of practicability and feasibility it is recommended to base the classification primarily on the nutritive enrichment factor in cases in which it is not feasible to check also the aromatic enrichment factor due to the specific character of the source material. However, the content of the aromatic constituents should be checked if it is needed to verify whether the aromatic substances have not been removed from the extract given that such extract would also be regarded as a food additive.

It is recommended to use total solids (i.e. everything but water) as the reference basis for the nutritive enrichment factor calculation. The data on the composition of the source material should be related to the parts of the source material from which the extract is obtained and expressed on a dry weight basis. Aromatic constituents could represent a broad range of very different substances and therefore, no general reference basis is recommended in the Guidance notes (Guidance notes on the classification of food extracts with colouring properties) developed by DG SANCO[1]. Instead, the relevant aromatic constituents should be determined on a case by case basis reflecting the source material under consideration.

The legislation does not directly address a common situation when the source material contains more pigments. Based on the legislation, the ratio between pigment(s) relative to the nutritive or aromatic constituents seems to be the decisive factor for the classification of the extraction no matter whether all pigments present in the source material are present also in the extract. Indeed, only the pigment(s) present in the extract should be compared with the same pigment(s) in the source material in order to properly reflect the ratio between the pigment(s) and the nutritive or the aromatic constituents when calculating the enrichment factor.

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[1] http://ec.europa.eu/food/food/fAEF/additives/docs/guidance_en.pdf

Technical specifications as provided by DG SANTE

Objectives and Scope

The source material reference values might have a significant impact on the enrichment factor calculations. For practical reasons (source material might not be available to enforcement officers, especially for the imported products) it is recommended to use reference values based on the literature.

The compilation by JRC of such reference values for a number of source materials (resources and timeframe were estimated based on 30-40 different source materials to be studied) used for the production of colouring food is the main purpose for commissioning this work. The reference values shall be compiled in Annex III of the Guidance notes on the classification of food extracts with colouring properties.

Cooperation and input from the stakeholders will be a key element for the successful completion of the assignment. Only for those source materials that are identified by stakeholders within three months after signature of the Administrative Arrangement reference values and the associated analytical method(s) will be entered to Annex III by JRC.

Tasks

The JRC will, once stakeholders have identified the source materials that are commonly used for the manufacture of colouring foods, search for and compile the necessary reference values, documenting the biological variation as far as possible. The information provided by stakeholders should include also what part(s) of the source material will be used for extraction as this might have an influence on the naturally occurring levels of nutrients as well as aromatic and colouring constituents.

1. Nutrient (carbohydrates, fat, proteins, minerals, and total solids) reference values of those source materials, will be produced by consulting publicly available databanks such as the Bundeslebensmittelschlüssel, EuroFIR (European Food Information Resource Network, the world-leading European Network of Excellence on Food Composition Databank systems), and the USDA National Nutrient Database for Standard Reference.

2. The same databanks will be interrogated to retrieve data related to aromatic and colouring constituents. As it is expected that the standard databanks will contain only limited information regarding the content of colouring pigments in the source materials, more specialised databanks will be consulted (eBASIS bioactive database, PhenolExplorer database, ePlantLIBRA database). In addition, literature searches will be done using search engines such as Scopus and scientific journals. As far as possible not only the main pigments occurring in the source material but also minor colouring principles will be documented.

3. Analytical methods suitable for the determination of the aromatic and colouring compounds will be identified, as far as possible, and the relevant bibliographic data will also be included in Annex III for information.

Introduction

The Standing Committee on the Food Chain and Animal Health endorsed the Guidance notes for the classification of food extracts with colouring properties, providing a tool for deciding whether a substance is a colour (i.e. a food additive) or not.

The guidance notes are a supplement to the existing EU regulations on the classification and declaration of colour ingredients used in food and beverages. These guidance notes provide an explanation of the differentiation between foods with colouring properties and food colours, which are food additives.

Provided that these foods or food ingredients retain their essential characteristics, foods with colouring properties should not be regarded as food colours whether used in the raw state or in a processed form, e.g. by concentration, drying, cooking or milling. For example spinach used in the manufacture of noodles as such or dried or in the form of concentrated juice, without a selective extraction of pigments, would be considered as a food ingredient and not as a food colour. On the other hand if pigments are 'selectively extracted' from the spinach and added to noodles in order to add colour, then these are regarded to be food additives (i.e. food colours - chlorophylls and chlorophyllins (E140))

Foods with colouring properties are called "COLOURING FOODSTUFFS", made from edible fruits, vegetables and/or other edible plants, such as fruit juices (for example, cherry juice added to yoghurt), tomato concentrates, or coffee. They are manufactured by physical processes resulting in concentrates in which the pigments have not been selectively extracted. Such foods would be regarded as ingredients and would have to be labelled as such, even when added principally for colouring purposes. On the other hand, products which are extracted from those foods by other processes than drying or concentration to be used in food for their colouring properties should not automatically be regarded as colouring food (unless they are normally consumed as such). Extraction can range from simple extraction, to varying degrees of selective extraction up to isolation of the pure pigments. To decide upon the classification of the product, it is essential to identify when the product is no longer "a food normally consumed as such or normally used as a characteristic ingredient of food", but a colour which needs approval.

From the 1st of January 2014 it is recommended that colouring products which are introduced to the market should be in line with the guidance notes, meaning that colour ingredients which do not meet the criteria for Colouring Foods will be considered as additives and need to be labelled with their specific name or E-number. Colour ingredients that are in line with the guidance notes are food ingredients with colouring properties and do not need authorisation.

For the purpose of classification a term "enrichment" factor was introduced by the guidance notes. The relationship between the ratio of the pigment(s) content to the nutritive or aromatic constituents in the colouring product (primary extract) compared to the corresponding ratio of the pigment(s) content to the nutritive or aromatic constituents of the source material is expressed as an enrichment factor.

The guidance notes on the classification of food extracts with colouring properties envisage the establishment of Annex III containing reference values for the source materials used for the production of colouring foods. The source material reference values could have a significant impact on the enrichment factor calculations. Therefore the guidance notes recommend using reference values for the content of the nutrients, pigments and where feasible aromatic constituents of the source material, based on the scientific literature. These reference values should be compiled in Annex III of the document, which is the main objective of this work. Additionally, the analytical methods for the determination of the colouring and aromatic constituents should be identified and relevant references included in the Annex III.

Approach/Methodology

A kick-off meeting took place at JRC-IRMM (Geel) on 19/11/2014. Details of the project were discussed and agreed upon between JRC-IRMMIRMM and DG SANTE.

The execution of the project comprised of several steps:

- establishing a list of the source materials, to be included in Annex III;
- establishing a list of colouring principles for which data would be searched for;
- identification of suitable databases and obtaining access to them;
- compiling information for the content of the nutrients in the source materials from the established list;
- compiling information for the content of the pigment constituents in the source materials from the established list;
- compiling information available for the content of the aroma constituents in the source materials from the established list;
- preparation of "Information on the source material" files for any of the source materials from the established list summarising the compiled data and proposing high, medium and low reference ranges for the content of the colouring and aroma constituents
- compilation on the methods for the determination of the content of the colouring and aromatic constituents in the raw source materials;

The first two steps were supposed to be executed in close cooperation with industry. Details of the methodology applied for obtaining the information necessary for the project were in chronological order.

List of the source materials requested for inclusion in Annex III;

According to the technical specifications, the source materials that are commonly used for the manufacture of colouring foods should be identified by the stakeholders. Following that, a letter (Annex 1) and a "source material template" (Annex 2) were prepared jointly by DG SANTE and JRC-IRMM and sent to the stakeholders by DG SANTE in the middle of September 2014. The deadline for collecting the filled in templates, one per each source materials requested by the industry to be included in Annex III, was 30th October. Natural Food Colours Association (NATCOL) requested an extension of the deadline until the end of the year. The extension of the deadline was granted partially to the stakeholders with the possibility to add by the end of the year new data to the templates, already submitted in October. The templates requested description of the source material itself and the part of it used for production of colouring food, some information on the process applied as well as any data available to the company on the content of nutrients, colouring and aroma compounds.

At that stage the stakeholders requested the following 25 source materials (see Annex 3) to be included in Annex III of the Guidance notes.

Alfalfa	Apple	Aronia (Chockberry)	Blackberry
Blackcurrant	Blueberry	Carrot Black	Carrot orange
Cherry	Elderberry	Grapes	Hibiscus (Roselle)
Lemon	Pumpkin	Raspberry	Red beet
Red Bell Pepper	Red cabbage	Red radish	Safflower
Spinach	Spirulina	Sweet purple potato	Tomato
Turmeric			

Later on, it has been clarified that apple and the lemon juices are not added to food primarily for colouring purposes, but as ingredients. Therefore, by default, they were not regarded as colouring food and were not consider in this report.

List of colouring constituents

Colours in fruits and vegetables are mainly due to three families of pigments: chlorophylls, carotenoids and anthocyanins, responsible for green, red-yellow and red to blue-purple colours, respectively. In some cases it is possible to find members of the three pigment families in a ripe fruit or vegetable, but in most cases only one or two kinds of pigments are found.

Therefore for the purpose of the project the stakeholders has been asked to provide information on the main pigment from any source material used in their colouring food preparations, for which a reference value should be established. Based on the data submitted (see Annex 3), the main colouring constituents in the source materials used in the production of colouring food were identified as chlorophylls, carotenoids, anthocyanins, and more specific ones as betanin/betalanin, phycocyanins, curcumoids, shafflomin A and B.

Chlorophylls

The green colour in all higher plants, including fruits, is due to chlorophylls, which participate in photosynthesis, one of the most important life processes, converting energy from light into chemical energy. Chlorophyll is a term used for several closely related green pigments found in the chloroplasts of algae and plants.

Two chlorophylls are important today as food colorants, chlorophyll a and chlorophyll b. These pigments are obtained from land plants and differ only by the replacement of a methyl group by a carbonyl group on carbon 7. Small differences in chlorophyll structures are enough to produce specific wavelength absorptions and consequently a variety of green hues. Colours range from yellow-green to blue-green, and derivatives of these chlorophylls would be likely to produce orange or, under drastic chemical conditions, even red colours [1].

However, the chlorophyll structure has an inherent instability, which is the major drawback for its application as colouring foodstuff.

Carotenoids

After chlorophylls, carotenoids are the most widely distributed group of pigments, occur naturally in large quantities, and are known for their structural diversity and various functions.

Carotenoids are lipid-soluble pigments responsible for many of the brilliant red, orange, and yellow colours in edible fruits, vegetables, fungi, flowers, and also in birds, insects, crustaceans, and trout.

Today more than 650 different carotenoids have been isolated from natural sources and identified, and more than 100 have been found in fruits and vegetables [1]. They are split into two classes - the xanthophylls yellow pigments (which contain oxygen) and the orange to red pigments of carotenes (which are purely hydrocarbons, and contain no oxygen). The most important carotenes are α-carotene, β-carotene, lycopene, and the most important xanthophylls are β-cryptoxanthin, lutein and zeaxanthin.

The carotenoid compositions can be very complex; in *Capsicum annuum* more than 35 carotenoids have been detected by HPLC and many of these identified [2, 3]. In contrast, in tomato (*Lycopersicon esculentum*) the only major carotenoid is lycopene [4].

Source materials contain the different carotenoid pigments in different ratios depending on the type of the fruit/vegetable, but very often also depending on the variety. For most of the source material where carotenoids are mentioned as major colouring principle for which reference value (ranges) should be derived, the value will reflect the total carotenoid content.

For the purpose of the project, the total carotenoid content was searched for in the respective source materials, unless otherwise requested by the stakeholders (only lycopene in tomatoes).

Anthocyanins

Anthocyanins are water soluble glycosides and acylglucosides of anthocyanidins. The aglycones (anthocyanidins) are found in fresh plant material only in trace quantities, because they are quite unstable [5].

More than 90% of all anthocyanins isolated in nature are based on the following six anthocyanidins: pelargonidin (plg), cyanidin (cyd), peonidin (pnd), delphinidin (dpd), petunidin (ptd), and malvidin (mvd), which are differentiated by the substitution pattern on the B ring. As of 2006, more than 550 different anthocyanins had been reported [1]. The difference in chemical structure that occurs in response to changes in pH is the reason why anthocyanins are often used as pH indicators, as they change from red in acids to blue in bases.

The occurrence of anthocyanins in fruits and vegetables depends on the kind of fruit or vegetable, cultivar, structure of tissues, geographical location, cultivation conditions and mostly on the maturity of the material.

The anthocyanin compositions of the source materials can be very complex. For the purpose of the project, reference values (ranges) were derived for the total anthocyanin content as requested by the stakeholders.

Betanin/betalanin

Betalains are water-soluble nitrogen-containing pigments, which are synthesised from the amino acid tyrosine into two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins [6]. More than 50 betalains are known.

Anthocyanins and betalains have never been reported in the same plant, seeming to be mutually exclusive in the plant kingdom. The major commercially exploited betalain crop is red beetroot (*Beta vulgaris*), which contains two major soluble pigments, betanin (red) from betacyanins and vulgaxanthine I (yellow) from betaxanthins.

As requested by stakeholders, the main pigment used for production of colouring food for which reference values were proposed is betanin.

Phycocyanins

Phycocyanin is a pigment-protein complex from the light-harvesting phycobiliprotein family, along with allophycocyanin and phycoerythrin. It is an accessory pigment to chlorophyll.

Curcumoids

Curcumin is a main colouring substance in *Curcuma longa* and two related compounds, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) are altogether known as curcuminoids.

Shafflomin A and B

Carthamus Yellow, a flavonoid, is obtained by extracting the corolla (petals) of *Carthamus tinctorius* L. with water or slightly acidified water and drying the extract. The principal colouring matters are safflomin A (hydroxysafflor yellow A) and safflomine B (safflor yellow B).

Identification of suitable databases

For establishing the nutrient (carbohydrates, fat, proteins, minerals, and total solids) reference values of the source materials from the established list, querying a number of international food composition databases (FCDBs) was required, reflecting the variability in the composition of foods between countries.

EuroFIR (European Food Information Resource Network, the world-leading European Network of Excellence on Food Composition Databank systems) and the U.S. Department of Agriculture (USDA) National Nutrient Database for Standard Reference were the major sources used to compile the required information.

EuroFIR draws together the best available food information globally from 26 compiler organisations in Europe, Australia, USA and Canada http://www.eurofir.org/. FoodEXplorer is an innovative new interface which allows users to search information from different databanks simultaneously. The EuroFIR partners are listed in Annex 4.

USDA National Nutrient Database for Standard Reference (SR) is the major source of food composition data in the United States http://ndb.nal.usda.gov/. It provides the foundation for most food composition databases in the public and private sectors. As information is updated, new versions of the database are released. This version, Release 26 (SR26), contains data on 8,463 food items and up to 150 food components. It replaces SR25 issued in September 2012.

As the two German Food Compositional databases - **Bundeslebensmittelschlüsse**l (BLS-MRI) http://bls.nvs2.de and **Souci-Fachman-Kraut** (SFK) http://www.sfk-online.net were not covered by EuroFIR, access to them was gained additionally.

It was expected that the standard databanks would contain only limited information regarding the content of the colouring substances in the source materials; therefore more specialised databanks were looked for.

eBASIS (Bioactive Substances in Food Information Systems) bioactive database is a unique database that contains critically evaluated data on the content and biological effects of bioactive constituents in plant based foods in 15 EU languages. Over 300 major European plant foods are listed and information on 17 bioactive compound classes (e.g. phytosterols, polyphenols, glucosinolates and lignans) is provided with data sourced from peer-reviewed literature http://ebasis.eurofir.org/Default.asp

ePlantLIBRA database - containing validated scientific information on Plant Food Supplement bioactive compounds

PhenolExplorer database - Phenol-Explorer is the first comprehensive database on polyphenol content in foods. The database contains more than 35,000 content values for 500 different polyphenols in over 400 foods. These data are derived from the systematic collection of more than 60,000 original content values found in more than 1,300 scientific publications http://phenol-explorer.eu/

USDA Database for the Flavonoid Content of Selected Foods - The database contains values for 506 food items for five subclasses of flavonoids: FLAVONOLS: FLAVONES: FLAVANONES: FLAVAN-3-OLS: ANTHOCYANIDINS http://ndb.nal.usda.gov/

Volatile Compound in Food (VCF-online) database - The VCF database contains information on published volatile compounds which have been found in natural (processed) food products http://www.vcf-online.nl/VcfHome.cfm . One drawback is that the references in the database are quite old, dating back to the 1970's-80's.

In addition, especially for the colouring and aromatic constituents in the source materials, literature searches were carried out, using search engines such as Scopus and scientific journals. More than 400 articles from peer review scientific journals were downloaded and more than 20 scientific books on food composition, colours and flavours were purchased for the purpose of the project.

Nutrient content of source materials

Definition of the parameters

Proper understanding of the figures behind any parameter reported in a food composition database is a prerequisite for the reliability and the comparability of the data from different databases. Consequently, the first step was to examine carefully each of the databases used in the study for the way the published nutritional data were derived and to harmonise them.

Total lipid (or fat), sometimes referred to as "crude fat", includes the mass of all lipid components, including triacylglycerols, phospholipids, sterols and related compounds determined by gravimetric methods after extraction. Sometimes the term is used for the triglyceride equivalent of fatty acids and differs from the gravimetrically determined value as it does not include other lipid components soluble in the solvent system.

The values for **protein** were calculated from the amount of total nitrogen (N) in the food, using the specific conversion factors for different food items. Raw protein also includes low-molecular N compounds such as free amino acids and peptides (fruits, vegetables and fish).

The **ash (minerals)** content of foods is determined by gravimetric methods or by summing up of minerals

The **moisture (or water)** content of foods is determined by (vacuum) oven drying and gravimetry

Total dietary fibre, – are plant polysaccharides and lignin not digested by enzymes in the human digestive tract

Total carbohydrates consist of total digestible and indigestible carbohydrates, including **total dietary fibres**. The parameter could be calculated by difference or by summation. By difference it is determined as the difference between 100 and the sum of the percentages of water, protein, total lipid (fat) and minerals (ash):

Total carbohydrates = 100 - (water + protein + fat + minerals).

By summation the total carbohydrates consisting of total dietary fibre, total sugars, and starch:

Total carbohydrates = total dietary fibre + total sugars + and starch

Because the analyses of total dietary fibre, total sugars, and starch are performed separately and reflect the analytical variability inherent to the measurement process, the sum of these carbohydrate fractions, may not equal the total carbohydrate-by-difference value

Available carbohydrates are the total carbohydrates without total dietary fibre. They could be calculated as well by difference or by summation. The value could be determined as the sum of the individual data for mono-, oligo- and poly-saccharides (e. g. glucose, fructose, sucrose, lactose, maltose, dextrin and starch) and sugar alcohols (sorbitol, xylitol, glycerol) which can be utilized by the human digestive system. In cases where the relevant data was incomplete or entirely missing the amount of available carbohydrates could be calculated by a differential method.

There were cases in the databases, when the values for protein, lipid (fat), mineral (ash) and water are taken from different references, leading to difficulties in data interpretation. For example in some situations total carbohydrates showed higher value then the total solids. Consequently, the obtained value for total hydrocarbon content based on dry weight for some source materials was higher than 100%.

Structure of the MS Excel file for the nutrients

Data on the nutritive constituents of the source materials were compiled in an MS Excel file named *"raw materials list nutrients.xls"*. The first sheet named "by stakeholders' request" combines the demands made by different stakeholders for inclusion of the material in Annex III of the guidance note.

For each source material there is a separate MS Excel sheet, named after the corresponding source material. Each sheet contains information provided by stakeholders, as well as those retrieved by different database searches (Table 1).

Information provided by stakeholders on nutrient content in the source materials were placed in the first worksheet rows. It was followed by the data extracted from the different food composition databases.

For ensuring traceability of the information, the first worksheet columns contain information on the data source (name of the database, code, country) and the material itself (original name in the database). The following two columns contain data on the water content (moisture) in % and the resulting total solids (g/100g) of the source material. The content of the total carbohydrates (incl. total dietary fibres), total dietary fibres, proteins, total lipids (fat) and minerals (ash) are presented as reported in the databases based on fresh weight. Next to that the same data were recalculated based on the solids content of the respective source material taking into account the reported moisture (water) content.

DB	ID Eurofir	Country ID	Country	Plant name in DB	water content %	total solids g/100g	carbohydrate, total, g	fibre, g	proteins, g	total lipids, g	minerals, mg	carbohydrate, total	fibre	protein	total lipids	minerals
							/100g source material					g/100g dry matter				

Table 1: Structure of the nutrient content data in the MS Excel file "raw materials list nutrients.xls".

For some source materials such as aronia (chokeberry), hibiscus (roselle), and turmeric, data available in the databases were scarce, while for safflower petals and alfalfa they were missing at all. Safflower is used for human consumption only as cooking oil from safflower seeds. The petals are used as a source of medicinal preparations, natural food colour and dyes for colouring fabrics, but details for their direct consumption as a food are scarce and related to some tea infusions. Therefore, reference values for nutritive constituents of safflower petals were those provided by stakeholders. For alfalfa data were related only to sprouts, while stakeholders requested data for leaves and stalks. Due to the lack of any information on that part of the source material and lack of information from stakeholders as well, we were not in a position to propose reference values at that stage.

After collection, the data were checked for severe outliers. During that process it was noted that some of the data sources were not very reliable. For example, in certain cases the reported values for total carbohydrates in the dry matter, were higher than the values of total solids, in others cases data were lacking for dietary fibres making impossible the calculation of the total carbohydrates. Such data were excluded from the data pool for deriving the reference values.

Statistics and establishing the recommended reference values

Minimum, maximum and average values were calculated for the nutrients of each source material. After thorough critical review and due to the wide range of some data, robust statistic has been applied in order to eliminate the influence of outliers. The median could be regarded as a good estimate of the robust mean, but only in cases when the number of available data was comparatively high. Therefore, the robust mean values and robust standard deviations were computed by application of an MS Excel Add-in for Robust Statistics elaborated by Analytical Method Committee of the Royal Society of Chemistry, Cambridge.

Approximations to the robust mean values were suggested as reference values for the nutrient constituents of the source materials (Annex 6). In some cases these values were associated with a rather high variance due to the widespread of the available data. It should be mentioned that the SUM of the suggested reference values for nutrients for the source materials were not equal to 100 and were not adjusted to 100%.

For some of the source materials, the data for the content of nutrients in the databases and in the literature were very scarce. For such materials as aronia, elderberry, hibiscus, safflower and turmeric, mainly information provided by the industry were taken as a basis for deriving the

reference values. The lack of any relevant information on the composition of alfaalfa's leaves and stalks from the literature as well as from the industry makes it impossible to propose reference values for alfalfa at that stage.

Content of the colouring constituents

Structure of the MS Excel file for the colouring constituents

A second MS Excel file was created, named "raw materials lists colours.xls" with a sheet corresponding to the each of the source materials, named after it (Table 2).

The first columns of any sheet gain information about the reference from where the data were retrieved; full description of the plant, and the compound class/constituent, to which the following information was related. The next four columns represent the content/range of the respective compound in the source material based on fresh weight or dry weight, as it was reported in the literature. The next column gives short information, whenever possible, on the analytical method or any other important details, and the last column containes a link to the information reference.

DB	ID Eurofir	Part of the plant	Plant name in DB	Scientific name	Compound class	coloring principle	mg/100g FW	range mg/100g FW	mg/100g DW	range mg/100g DW	Details	References

Table 2: Structure of the colouring pigment content data in the MS Excel file "raw materials lists colours.xls".

Data compilation

From stakeholders

As a first step following the objective to establish reference values for the content of the colouring constituents in the source materials, data submitted by the stakeholders via filled "source material datasheet" have been reviewed critically. They were placed at the beginning of each worksheet in the first rows.

From specialised databases

Specialised databases have been queried for obtaining the required data for the content of the colour constituents in the respective source materials.

EuroFIR - contains information either for individual carotenoids (α-carotene, β-carotene, lycopene, β-cryptoxanthin, lutein, zeaxanthin) or for total carotenoids in the source material of our interest. However, availability of data for only some of the individual carotenoids (e.g. only β-carotene) did not mean that the others were not present in the source material,, making the available information on the individual carotenoids irrelevant for the purpose of the project, which required reporting of the total carotenoids.

e-Basis - Data extraction from EuroFIR eBASIS uses a sophisticated data retrieval software system, searchable by compound classes, compounds, food or biological effect, allowing users full control over the data selected for output. A thorough search was performed for the two major classes of bioactive compounds with biological effects and colouring properties - carotenoids and anthocyanins. However, while for anthocyanins total anthocyanins were one of the parameters included in the database, total carotenoids were not available as parameter. Instead only individual values were reported for the following carotenoids,

Antheraxanthin	Lycopene	Xanthophyll
Cryptoxanthin	Neoxanthin	Zeaxanthin
Lutein	Violaxanthin	

α– and β-carotene were not included in the database. Consequently, the data were not suitable for the purpose of the project, as the total carotenoids content was the parameter of interest. Nevertheless, the content of individual carotenoids was filled in the datasheets of the Excel files for information purposes.

USDA Database for the Flavonoid Content of Selected Foods, Release 3.1 (December 2013) –The content of anthocyanins as one of the flavonoid subclasses was included. However, the general approach during the USDA database compilation was that only those data generated by analytical procedures, which led to good separation of flavonoid compounds (e.g., high-performance liquid chromatography [HPLC]) were included. Studies that contained data generated by thin layer or paper chromatography, pH differential methods or only spectrophotometric quantitation were not retained due to the lack of specificity of these methods. Consequently, the total anthocyanin content should be determined as SUM of the individual anthocyanins, assuming that all the individual components were quantified. Unfortunately data for the source materials of our interest were very scarce.

PhenolExplorer database - contains information for individual anthocyanins, analysed by chromatography and for total anthocyanins by pH differential methods. Unfortunately, only 3 fruit berries from the source materials of interest in this project are included in the database.

From the scientific publications

The most extensive and time-consuming step in data compilation for this project was the search performed in the peered reviewed scientific literature via scientific search engines such as "Scopus" and "Science Direct". More than 400 articles and 20 books were purchased and reviewed for the project. Attention was focused on those source materials where only limited data for the colouring constituents were obtained in the previous steps.

Critical review, statistics and deriving of ranges with low, medium and high levels of content of the main colouring principle in the source materials

The occurrence of pigments in the source materials such as fruits and vegetables can vary significantly because of natural differences related to

- the cultivar,
- the geographical location,
- the cultivation conditions, harvest year
- the maturity stage, harvest time
- the storage time and conditions (temperature, length of storage time).

As expected the variations in the pigment content data compiled for each of the source materials were very large. When possible (enough number of data sets available) histograms were derived from the data, showing the frequency distribution of the pigments content

While collecting data from the databases, books and review articles, in a number of cases the data found in e.g. a review paper did not agree to the data from the respective original reference. For examples, data reported for one species in fact concerned another, or data reported based on dry matter referred in fact to fresh weight. Often discrepancy between the measurement units was noticed.

Consequently, careful crosschecking was performed on all the data from review papers.

The need for data validation complicated the process of data compilation.

Another issue was the recalculation of the content of a colouring substance reported on a fresh weight base to the content on a dry matter base, when the respective moisture content was unknown/not reported. Recalculation of the concentrations based on the average water content would introduce an uncertainty, which could reach a factor of 2. For example Uyan [7] reported for carrots a content of anthocyanins of 93.3 mg/100 g on wet weight base; in the same article a content of 494.96 mg/100g dry matter is mentioned . This corresponds to a dry matter content of about 18 %, while the proposed reference value (approximated by the robust mean) for dry matter in carrots is only 10 %. Therefore, in the final tables, reference values were

proposed on fresh weight or dry weight bases, depending on the way the majority of the raw data were reported in the literature.

Variability in the compiled data for some of the source materials was introduced by the different analytical methods and measurement techniques applied in the studies. Comparability of the data obtained by applying different analytical methods were addressed and summarised in the chapter on analytical methods.

After a careful review of the data, all the compiled results were subjected to statistical evaluation. Histograms were constructed when enough data sets were available, representing the distribution of the compiled data over the whole concentration range. Robust statistics was applied in order to establish the robust mean and the robust standard deviation of the data by using Huber's H15 method. In some cases, when the dataset contained limited data the robust standard deviation is very similar to the robust mean itself, showing the huge variety in the compiled results.

As a final step reference value for the content of the main colouring constituent in any of the source materials were proposed. Leading principle was the understanding that for the purpose of colouring food production highly pigmented cultivars would be preferred over ordinary ones as source materials. Consequently, the robust mean value plus the robust standard deviation could serve as a good estimate of the reference value for the purpose of the Annex III of the Guidance Note. The proposed reference values were higher than those mentioned by industry for some source materials as spinach, spirulina and turmeric; the reason for the discrepancy is that for those source materials the scientific literature reports mostly maximum values, instead of averages or ranges for those source materials.

Since it was impossible to assess all the literature data available on the topic and sometimes data were scarce, the approach suggested shall only be regarded as an approximation. In all instances the threshold value for the enrichment factor (which is six) as laid down in the Guidance notes, was chosen to cover all seasonal and geographical differences, being at the same time not too high and assuring that primary concentrates/extracts do not overlap with food colour specifications.

Occurrences of other minor pigments were also mentioned when available.

Content of the aroma constituents

Flavour is determined by taste sensations and odour-active compounds. Taste is perceived on the tongue and odour by the olfactory system. The olfactory system is extremely sensitive; it can detect odours in amounts of parts per trillion, whereas receptors on the tongue can detect flavour compounds in amounts of parts per hundred. Sugars, acids, salts and compounds that contribute to bitterness and to adstringency such as phenolic acids, flavonoids, alkaloids, tannins, are important for the taste of food.

Odour in general is a clearly recognizable smell that can be both pleasant and unpleasant, while aroma refers only to pleasant smells. Some source materials for colouring food could have an unpleasant and undesirable off-flavour. Consequently odour specific compounds were searched for in the source material of interest; however they will be named aroma compounds according to the technical specification of the project.

A chemical compound has a smell or odour when it is sufficiently volatile to be transported to the olfactory system in the upper part of the nose. Many factors affect the volatile composition of the raw source material, e.g. genetics, maturity, growing conditions and postharvest handling.

Odour is a complex mixtures of volatile compounds. However, a vast majority of volatile chemicals that have been isolated from natural flavour extracts do not cause significant aroma contributions to the flavour. For instance, ethyl butyrate provides a nondescript “fruity” aroma to blackberries, raspberries, and pears, but it does not distinctly describe the flavour quality of any of these individual fruits. It has long been the goal of flavour chemists to elucidate

the identity of pure aroma chemicals that possess the unique flavour character of the natural fruit, vegetable or spice that they were derived from. Frequently, these unique flavour substances are referred to as "aroma active compounds", "key odour compounds", "key aroma compound" or "aroma impact compounds" [8].

The presence of such unique aroma impact compounds was looked for in the scientific literature for the purpose of the project.

Compilation of the identified volatile compounds, published in peer review articles, could be found in the TNO's VCF database [9] for most of the source materials. However, the database contains lists of 100-200 and even more compounds per source material, which were downloaded in another Excel file "raw material list_volatiles.xlsx". For part of them even occurrence data are available. However, in the absence of clearly identified aroma impact components, no reference values for aroma constituents could be proposed.

The following paragraph summarises the available information on the odour/aroma impact compounds by source material

Alfaalfa

No data available in the literature.

Aronia (Chokeberry)

The profiles of volatile constituents of the fruit of different *Aronia melanocarpa* genotypes were evaluated by different techniques [10]. In total, 74 volatile compounds were identified in chokeberry juice, 3-penten-2-one, 3,9-epoxy-*p*-menth-1-ene, and benzaldehyde being the most abundant constituents; however, their concentrations were remarkably different. Twenty two aroma-active compounds were detected and characterized by trained panellists. Olfactometry revealed that ethyl-2-methyl butanoate, ethyl-3-methyl butanoate, ethyl decanoate ("fruity" aroma notes), nonanal ("green" notes), an unidentified compound possessing "moldy" odor, and some other volatiles may be very important constituents for the formation of chokeberry aroma.

Hirvi and Honkanen [11] identified 48 compounds in the volatile fraction of a pressed juice from *Aronia*. The main compounds were benzaldehyde cyanohydrin, hydrocyanic acid and benzaldehyde. Furthermore, a series of benzene derivatives have also been reported as aroma substances like benzyl alcohol, 2-phenylethanol, phenylacetaldehyde, salicylaldehyde, acetophenone, 2-hydroxyacetophenone, 4-methoxyacetophenone, phenol, 2-methoxyphenol and methyl benzoate

Aronia melanocarpa berries possess some typical, however not very pleasant smell notes, which are rather disliked by consumers. The bitter-almond like odour of the berries was attributed to some majour flavour-active benzaldehyde derivatives including benzaldehyde itsef and benzaldehyde cyanohydrin and cyanogenic precursors, such as amygdalin [12]. **Amygdalin**, a cyanogenic glycoside isolated from the berries, is the mainly responsible for the bitter-almond smell of the fresh fruits as it degrades with liberation of hydrocyanic acid and benzaldehyde.

Blackberry

Approximately 150 volatiles have been isolated from blackberries and reported in the VCF database [9]. The aroma profile is complex, as no single volatile is described as an impact compound. Several compounds have been suggested as being of importance for the aroma of blackberries, e.g. ethyl hexanoate, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, 2-heptanone, 2-undecanone, 2-heptanol, 2-methylbutanal, 3-methylbutanal, hexanal, (E)-2-hexenal, furaneol, thiophene, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, 2-methylthiophene, methional, α-pinene, limonene, linalool, sabinene, α-ionone and β-ionone; however, these volatiles may vary between growing regions [13].

Wang et al. [14] demonstrated that the same cultivar grown in different regions in the USA had similar aroma compositions; however, in one region ethyl butanoate (fruity, apple-like), linalool

(floral, perfume), methional (cooked potato), (E,Z)-2,6-nonadienal (green cucumber), (Z)-1,5-octadien-3-one (green grass) and furaneol (sweet, strawberry-like) were prominent, while ethyl butanoate, linalool, methional, methyl 2-methylbutanoate (fruity), β-damascenone (rose-like, berry) and geraniol (sweet, rose-like) were prominent volatiles in another region.

Recently Du et al. [15] studied the variability in aroma compounds among cultivars. Although seasonal variations were present, the overall volatile profiles in two cultivars ("Marion" and "Black Diamond") were very similar, but the concentrations of some aroma compounds varied greatly. Odour-activity values (OAV) indicated that furaneol, linalool, β-ionone, and hexanal could be most important in "Marion", while in "Black Diamond", the most important compounds were linalool, β-ionone, furaneol, and 2-heptanol [16].

Blackcurrent

The aroma profile of black currant shares similarities with that of other berry fruits, although terpenes are more abundantly present in black currant. Over 150 volatile compounds have been reported from either black currant berries and/or juice, of which the major groups are monoterpenes, sesquiterpenes, esters and alcohols [17, 18]. Processing of berries to juice has been shown to lead to major changes in the aroma composition. The most important volatile compounds for black currant berry and juice include esters such as 2-methylbutyl acetate, methyl butanoate, ethyl butanoate and ethyl hexanoate with fruity and sweet notes, nonanal, *β*-damascenone and several monoterpenes (*α*-pinene, 1,8-cineole, linalool, terpinen- 4-ol and *α*-terpineol) as well as aliphatic ketones (e.g. 1-octen-3-one) and sulfur compounds such as 4-methoxy-2-methyl-butanethiol [Berger R. 1991]. **4-Methoxy-2-methylbutanethiol** has a characteristic "catty note" and is very important to black currant flavour [13].

Blueberry

Blueberry consists of cultivated highbush blueberries (*Vaccinium corymbosum*) and wild lowbush blueberries (*Vaccinium angustifolium*). The aroma of cultivated and wild blueberries is dominated by long-chain alcohols, esters and terpenoids. Forney [19] reported that γ-butyrolactone, α-terpineol, 6-ethyl 2,6-decadiene-4,5-diol, linalool, benzaldehyde and 2-ethyl-2-hexenal contribute to the aroma of fresh, whole highbush blueberries using gas chromatography-olfactometry (GC-O) analysis. In another study, Parliament and Scarpellino [20] reported that a combination of linalool and (Z)-3-hexen-1-ol produced a blueberry-like flavour, while Horvat and Senter [21] reported that a mixture of (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, (E)-2-hexenal, linalool and geraniol gave an aroma similar to the aroma isolated from blueberries.

The odour-active volatiles of intact lowbush blueberries include methyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, methyl butanoate and linalool. The major contributors to the aroma profile of blueberry juice are hexanal, (E)-2-hexenal and (E)-2-hexen-1-ol, limonene, linalool, α-terpineol, geraniol and nerol [13].

Carrots

More than 90 volatile compounds have been identified from carrots [9] while non-volatile polyacetylenes and isocoumarins contribute significantly to the bitterness of carrots. Carrot volatiles consist mainly of terpenoids in terms of numbers and amounts and include monoterpenes, sesquiterpenes and irregular terpenes. Monoterpenes and sesquiterpenes account for about 98% of the total volatiles in carrots. α-Pinene, sabinene, myrcene, limonene, β-ocimene, γ-terpinene, p-cymene, terpinolene, β-caryophyllene, α-humulene, (E)-γ-bisabolene and β-ionone were found to be the aroma impact compounds of raw carrots [22].

Monoterpenes like sabinene, myrcene and p-cymene seem to be important contributors to "green", "earthy" or "carrot top" flavour with relatively high odour activity values. Sesquiterpenes like β-caryophyllene and α-humulene contribute to "spicy" and "woody" notes, whereas a "sweet" note is mainly due to β-ionone [13]. The main non-terpenoid volatiles in carrot are 3-hydroxy-2-butanone, ethanol, hexanal, acetic acid, and erythro- and threo-2,3-butanediol [23] which also contribute to the carrot aroma. The characteristic impact compound

for raw carrot is **2-sec-butyl-3-methoxypyrazine** (625 ng/l juice), which has an extremely low (2 ng/l) threshold value [24]. By using GC–O it has been described as "raw carroty". Unsaturated aldehydes contribute to the flavour of cooked carrot, the most significant being (E)-2-nonenal (fatty-waxy) [25].

Sour cherry

Benzaldehyde has been determined to be the most important aroma compound in sour cherries, but benzyl alcohol, eugenol and vanillin are also important flavour compounds [26]. Growing and storage conditions affect the concentration of benzaldehyde, benzyl alcohol, eugenol and vanillin, and cold and rainy weather produces sour cherries with a less delicate sour cherry aroma [13].

Elderberry

The characteristic elderberry odour has been shown to be correlated to β-damascenone, dihydroedulan and ethyl 9-decenoate with elderberry-like notes, and to some extent also to 2-phenylethanol, phenylacetaldehyde and nonanal with elderflower-like notes [13]; however, only nonanal, dihydroedulan and β-damascenone have repeatedly been identified in various investigations as character-impact compounds for elderberry odour. The fruity-sweet flavours in elderberry juice and products have primarily been associated with aliphatic esters such as ethyl 2-methylbutanoate, ethyl 3- methylbutanoate, methyl heptanoate, methyl octanoate, methyl nonanoate, alcohols (2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol) and the aldehydes pentanal, heptanal and octanal [27].

Grape

365 volatile compounds are listed in the VCF database [9] for different cultivars of *Vitis vinifera*. The large diversity of volatiles contributing to the complex flavour and aroma of the grape, depending on the cultivar makes it not feasible to identify and quantify key aroma compounds.

Hibiscus

There have been few reports regarding the aroma of roselle (*Hibiscus sabdariffa*) because its unique flavour is very delicate, and trace amounts were too difficult to detect.

A direct analysis of fresh roselle volatiles was done by Chen et al. [28]. More than 37 compounds were characterized. They were classified into four groups: fatty acid derivatives, sugar derivatives, phenolic derivatives, and terpenes [29]. Large amounts of the aliphatic C6 lipid derivative (aldehydes and alcohols), which contributes to the green-note aromas (hexanal), were in the fresh roselle, while only trace amounts were found in the frozen and air-dried samples. In the air-dried roselles, significant amounts of furfural and 5-methyl-2-furfural were formed, while only minimal amounts were detected in the fresh samples. There were no obvious changes in phenolic derivatives (eugenol) among the fresh and dry samples. Terpenoids and and their oxides could also be isolated after simultaneous distillation-extraction (SDE). The drying process reduced them dramatically, especially the amount of R-terpineol, linalool oxide, and limonene. Roselle tea aromas depend upon a quantitative balance of various components [28].

Gonzalez-Palomares [30] and Camello-Mendez [31] confirmed that a combination of the terpene derivatives with fragrance notes (**geraniol** 7.1-9.73 mg/kg and linalool), and the sugar derivatives as furfural and 5-methyl-2-furfural with a caramel-like odour are responsible for the roselle aroma.

Pumpkin

About 30 compounds have been identified in the volatile extracts of raw pumpkin, belonging to aliphatic alcohols and carbonyl compounds, furan derivatives and sulfur-containing compounds [[9]]. Hexanal, (E)-2-hexenal, (Z)-3-hexen-1-ol and 2,3-butanedione have been identified as being important for the flavour of freshly cooked pumpkins [24]. Studies using GC-O techniques are needed to get a better understanding of the character-impact compounds of pumpkins [13].

Purple Sweet Potato

The tuber of potato (*Solanum tuberosum*) is eaten boiled, baked or fried. Raw potato possesses little aroma. Approximately 50 compounds have been reported to contribute to raw potato aroma [9]. It is reported that two compounds represent the typical potato aroma in raw potato: methional and (E,Z)-2,6-nonadienal. Other important volatiles in raw potatoes produced via degradation of fatty acids are 1-penten-3-one, heptanal, 2-pentyl furan, 1-pentanol and (E,E)-2,4-heptadienal. Pyrazines such as 3-isopropyl-2-methoxypyrazine could be responsible for the earthy aroma of potato [13].

Raspberry

Approximately 230 volatile compounds have been identified in raspberry [32]. The aroma of raspberries is composed of a mixture of ketones and aldehydes (27%) and terpenoids (30%), alcohols (23%), esters (13%) and furanones. The **raspberry ketone 1-(4-hydroxylphenyl)-3-butanone** along with α-ionone and β-ionone have been found to be the primary character-impact compounds in raspberries. Other compounds such as benzyl alcohol, (Z)-3-hexen-1-ol, acetic acid, linalool, geraniol, α- pinene, β-pinene, α-phellandrene, β-phellandrene and β-caryophyllene contribute to the overall aroma of mature red raspberries [13].

Red beet

Studies of volatile components of raw beets have been principally concerned with the identification of those compounds responsible for its unique earthy aroma; the strongly odorous 2-isopropyl-, 2-sec-butyl- and 2-(methylpropyl)-3-methoxypirazidines and geosmin were identified [24]. Of these compounds it is believed that 2-sec-butyl-3-methoxypyrazidine and **geosmin**, which have aroma threshold concentration of 2 and 2 ng/l, respectively, are the majour contributors to the earthy, musty aroma of this vegetable.

The VCF [9] database reported a concentration range for geosmin in beetroot from 0.6-3.7 µg/kg, however the data were based on the literature from the 1970's. An improved analytical method for the determination of geosmin in red beets was developed using headspace solid-phase microextraction (HSPME) by Lu at al. [33] The concentrations of geosmin in four beet cultivars analysed by this method ranged from 9.7 to 26.7 µg/kg, depending on cultivar. Freiding in his recent study [34] confirmed a similar concentration range for geosmin in beet roots from 1.4 to 39.2 µg/kg, depending of the cultivars (11) and environmental conditions (field or greenhouse).

Red bell pepper

In total 97 volatile compounds were identified as in the aroma of bell pepper [9]. The most important odour compounds in raw sweet pepper are 2-(2-methylpropyl)-3-methoxypyrazine (0.09-0.12ppm), (E,Z)-2,6-nonadienal, and (E,E)-2,4-decadienal, having very low aroma threshold values, while the major aroma compounds in cooked sweet pepper are 2-(2-methylpropyl)-3-methoxypyrazine, 1-nonen- 4-one, 2-nonen-4-one, (E,Z)-2,6- nonadienal, (E,E)-2,4-decadienal, and linalool, as reviewed by Whitfiel and Last [24].

In 2008 Naef at al. [35] added two more sulphur containing heptane derivatives to the list of the character impact compounds in bell paper being (E)-3-heptene-2-thiol and (E)-4-heptene-2-thiol [25].

Red Cabbage

Raw cabbage contains nearly 100 volatile compounds according to the VCF database and includes aliphatic alcohols, aldehydes, and esters as well as isothiocyanates and other sulphur-containing compounds [19]

Bitterness, pungency, and sweetness together with fruity and vegetal flavour are dominant aroma descriptors of fresh cabbage flavour. Important contributors to fruity and grassy/green flavours likely include 2-trans-hexenal), 3-cis-hexen-1-ol (is), 2,4-trans , trans-heptadienal, 2,4-trans , trans-decadienal, benzaldehyde, and phenylacetaldehyde [13].

Bitterness is negatively associated with consumer preference, although it generally is of less importance in raw cabbage than other flavour characteristics. The two primary bitter components in cabbage are the intact glucosinolate sinigrin and 5-vinyl-oxazolidine-2-thione (goitrin), a hydrolysis product of 2-hydroxy-3-butenyl glucosinolate (progoitrin) [36].

Pungency, reminiscent of mild horseradish or black mustard, is the most important flavour characteristic of fresh cabbage. Pungency has been previously assumed to contribute positively to the overall consumer acceptance of cabbage flavour. However, despite the positive contribution of pungency to the overall perception of flavour, even low levels of pungency may be unacceptable in some markets. Descriptors generally grouped together as off - flavours in cabbage include "sulfurous" "earthy" and "musty" [37]. **Allyl isothiocyanate** (AITC), a hydrolysis product of allyl glucosinolate (sinigrin), is the primary chemical influencing pungency in cabbage [38]. According to the VCF database the content of the allyl isothianate in raw freshly disrupted cabbage tissues is in the range 0.04-2.9 ppm

Other dominant sulfides contributing to the sulfurous aromas that may be considered undesirable in cabbage include dimethyl sulfide, butylmethyl sulfide, dipropyl sulfide, dimethyl disulfide, methylbutyl disulfide, methylpropyl disulfide, dimethyl trisulfide, and methylethyl trisulfide [36].

Red radish

The sharp, pungent flavour of radish is a major obstacle preventing widespread use of radish anthocyanin products as food colourants because it is imparts an undesirable flavour for preparations aiming to be used as food colorant. The off-flavour often results from the degradation of glucosinolates (sulphur-containing compounds in cruciferous vegetables) during mastication or processing [39]. The reported content of the total glucosinolates ranged from 60-164 mg/100 g fresh weight in Chinese radishes.

Different radish varieties likely contain different **glucosinolates**, which in turn can produce different off-flavours upon degradation. Four radish glucosinolates (glucoraphenin, dehydroerucin, glucobrassicin, and glucoerucin) were identified by LC–MS from root extracts and dehydroerucin was found to be the major glucosinolate in red radish roots [40].

The major volatile compound derived from the break-down of glucosinolate (GS) in black, white, and red radish roots is the sulfur-containing aroma component, 4-methylthio-3-butenyl-isothiocyanate [41]. Other relatively minor sulfur-containing aroma components identified by this study were 5-methylthio-pentyl isothiocyanate, dimethyl trisulfide 2-phenylethyl isothiocyanate, and 5-methylthio-(E,Z)-4-pentenenitrile [42].

Intact roots of 109 radish (*Raplzanus sativus* L.) cultivars were analyzed for GSand found to contain primarily 4-methylthio-3-butenyl-GS with small amounts of 4-methylsulfinylbutyl-, 4-methylsulfinyl-3-butenyl-, and 3-indolylmethyl-GS. Cultivars included food radishes (ssp. radicola) available in European, European-American, Japanese, and Korean markets. Regarding total GS, 80% or more of the red European-American radishes had 45-90 mg/100 g, the Korean 45-135 mg/100g, and the Japanese 45-135 mg/100g [43].

Safflower

Carthamus yellow colorants, which are flavonoid colorants, are widely used, mainly in the colouring of beverages and other edible products. However, a Carthamus yellow colorant has a distinctive odour that comes from its raw material, safflower (*Carthamus tinctorius* L.), so when it is used in foods and other products, this odour can sometimes undesirably taint the flavour and taste of these foods.

Safflower similar to saffron is used as a spice. Safflower has its own very pleasant taste and ability to colour food attractively (being used both as a textile dye, as is saffron, and as a food-colorant for margarine)

Choi et al. [44] identified as main volatile components in safflower terpene compounds, including p-cymene, limonene, α-phellandrene, γ-terpinene, camphor, 4-terpineol, selinene, β-caryophyllene, torreyol, β-eudesmol, and 10 acids including 3-methylbutanoic acid, 2-

methylbutanoic acid, and acids of C2, C5-C11 [44], but no character-impact compounds are reported in the literature for the safflower petals.

Spirulina

The aroma is not relevant to spirulina as this source material is not characterised with any specific flavour/aroma.

Spinach

Gas chromatography-olfactometry of headspace samples revealed that (Z)-3-hexenal, methanethiol, (Z)-1,5-octadien-3-one, dimethyl trisulphide, octanal, 2-isopropyl and 2-sec-butyl-3-methoxypyrazine are potent odorants of raw spinach. A change was observed after drying and storage of raw spinach: methylpropanal, 2- and 3-methylbutanal and propanal were identified as the odorants with higher odour activity values. It was also found that (Z)-1,5-octadien-3-one and methional, in a concentration ratio of about 1 : 100, are responsible for the fishy off-flavour of dry spinach stored at lower temperatures under nitrogen. The hay-like flavour was caused by oxidative degradation of furan fatty acids, yielding 3-methyl-2,4-nonanedione [45].

Tomato

The fruit of the tomato plant (*Lycopersicon esculentum*) is eaten raw, boiled, baked or fried. Tomato is also canned either whole or pureed. More than 400 volatile compounds have been identified in fresh tomato in more than 70 publications, of which 16 or so have odour-threshold values that indicate that they contribute to tomato flavour. The nature and relative amount of volatiles in tomato seem to depend on variety, maturity and preparation of the product more than in any other vegetable. The most important volatile compounds in tomatoes are 3-methylbutanal, hexanal, (Z)-3-hexenal, (E)-2-hexenal, 3-methyl-1-butanol, 1-hexanol, (Z)-3-hexen-1-ol, 1-penten-3-one, 6-methyl-5-hepten-2-one, β-ionone, β-damascenone, 2-phenylethanol, methyl salicylate, furaneol and 2-isobutylthiazole, and of these, (Z)-3-hexenal and β-ionone have the highest odour values [13].

The character impact compounds of fresh tomato are 2-iso-butylthiazole (0.00043-0.7 mg/kg) and (Z)-3-hexenal, with modifying effects from β-ionone and β-damascenone [25, 46], whereas dimethyl sulphide and furfural are major contributors to the flavour of thermally processed tomato paste [47] after the almost complete loss of cis-3-hexenal during processing.

Turmeric

About 40 volatile compounds are reported in the VCF database for the turmeric. The major compounds identified in turmeric essential oil are ar-turmerone, turmerone, ar-curcumene, zingiberene, α-phellandrene, curlone, 1,8-cineol and some other sesquiterpenes. Turmerones are sesquiterpenoid cyclic ketones accounting for 40-50% of the essential oil [48]. **Turmerone and ar-turmerone** are known to be the character impact compounds of turmeric contributing to the dry turmeric aroma [49]. These compounds together with 1:8 cineol, which imparts a camphory note, has been reported to be responsible for the top aroma note of the spice [50].

Source material profiles

A "JRC Information on the source material.docx" file for all source materials from the established list was prepared, summarising the compiled data (Annex 6).

Under the column "Content – literature data " the ranges were presented in which the respective parameters vary. The column "Suggested reference range/value" contains the proposal for reference ranges and reference values. As mentioned before, the proposals for nutritive reference values were derived by robust statistics of the data collected from a variety of databases and reflect the variety of cultivars, geographical regions, growing conditions, stage of maturity etc. Sometimes these reference values were associated with a high standard deviation, which were shown in the tables.

For colouring constituents, the ranges of low, medium and high pigment content in the source material were shown either for the fresh source material (FW - fresh weight) or based on dry weight (DW), depending on the availability of data in the literature. The proposed reference ranges and values were included in the last column.

Minor pigments were listed with their concentration levels when available under the main pigment.

Some information on the key aroma (character-impact) compounds was included in case they were available in the literature.

These files could serve as a background for Annex III of the Guidance note. All the information on the proposed values was summarised in the file "Basic compositional table" (Annex 5).

Analytical methods for the determination of the content of pigments.

Modern instrumental methods such as electrospray ionization mass spectrometry techniques (ESI-MS) are very suitable for the characterisation of colouring substances in complex food matrices. Liquid chromatography with mass spectrometry (MS) and/or nuclear magnetic resonance (NMR) spectroscopy are possibly the most powerful methods for the structural elucidation of substances with colouring principle. However the aim of the project was to determine the total content of these colouring substances in their source material, e.g. raw fruit or vegetable, which simplify considerably the tasks of the analytical chemist. Therefore our attention has been directed to more classical, simple and straightforward methods providing the required information less costly and time consuming.

Carotenoids

Analytical methods for carotenoid determination in source materials could be divided in two main groups: classical spectrophotometric and more contemporaneous HPLC methods.

Today, carotenoids are often measured by reversed-phase high-performance liquid chromatography (RP-HPLC), allowing for individual carotenoid quantification with the disadvantage of being more time and cost intensive. However spectrophotometric measurements are still widely used.

The traditional method to measure total carotenoids is via basic spectrophotometric methods after extraction from the matrix following AOAC official method 970.64-1974 [51]. However, without prior separation, carotenoids will be determined together with chlorophylls if present, absorbing at similar wavelengths [52].

Carotenoid analysis traditionally consists of extraction of the pigments from tissues, sometimes followed by purification by saponification, and quantification by spectrometry for total carotenoids, or via separation by chromatography and quantitation of the individual carotenoids by optical detectors.

Extraction has the aim of removing the hydrophobic carotenoids from a hydrophilic matrix. Many different organic solvents have been used in the analysis of carotenoids and selection of the appropriate one is not always easy. The different polarities of the existing carotenoids and the structure of the analytical matrix and its components also play important roles when selecting a solvent for extraction. Usually, non-polar solvents, such as hexane, are a good choice for non-polar (carotenes) or esterified carotenoids, while polar solvents, such as ethanol and acetone, are more appropriate for polar carotenoids (xanthophylls).

If the obtained extract is clear, free of turbidity and interference from non-carotenoid pigments, the carotenoid content may be calculated from its absorbance of the extract at 400-500 nm or after a chromatography.

Saponification is a common step in carotenoid analysis to remove neutral fats, fatty acids, and many esters. Another advantage of saponification is chlorophyll destruction in the saponified samples. Most carotenoids are stable under alkaline treatments although it was reported that

carotene is sensitive to alkaline saponification, thus mild saponification conditions should be applied [53].

For reducing the effect of co-extracted chlorophyll on the estimation of the carotenoid content Lichtenthaler [54] applied a mathematical correction of the chlorophyll content using wavelengths where carotenoids do not absorb. The method was created for the parallel quantification of chlorophylls and carotenoids in green leaves or separated chloroplasts but it is a method leading to higher mean concentrations compared to all other methods.

Biehler [52] compared the spectrophotometric methods of Hornero-Mendez and Minguez-Mosquera [55] and Lichtenthaler [51] with their in-house method based on a mean molar absorption coefficient at a wavelength of 450 nm for estimating the total carotenoid concentration using different chlorophyll and non-chlorophyll containing matrices. For validation, results were additionally analysed by an HPLC protocol adapted from Gorocica-Buenfil and others [56].No significantly different results for various carotenoid containing matrices was obtained with good correlation to the HPLC method.

Other more reliable methods include chromatographic separation prior to quantification. Some methods employ TLC [1] and others HPLC [57-59], [60]. Such methods yield information about the individual pigments present and the total carotenoid content of the sample.

For quantification of individual carotenoids separated by HPLC a major problem is the availability of commercial standards. There are no commercially available standards for all the carotenoids that have to being measured, and, in many cases, only one isomeric form is available [61]. Also, the susceptibility of carotenoids to oxidation has to be considered when developing a method for carotenoid extraction. These molecules are relatively stable in the matrix, but carotenoids in solution may be very sensitive to light, heat, acid or oxygen exposure.

In 1996 a European interlaboratory comparison study was organised for the analysis of carotenoids in a mixed vegetable material [62]. The study investigated possible problem area including chromatographic systems, standardisation of standard stock solutions via purity determination, extraction procedures and data handling. The results suggested that the effect of the chromatographic system is probably not a major variable. For the more experienced laboratories, variation in the standardisation of the carotenoid solution was not a significant problem as well. However, the preparation of the carotenoid extract may account for significant variance.

It appears that the typical overestimation by spectrophotometric methods due to minor compounds and degradation products is somewhat balanced by carotenoid losses due to saponification, resulting in a close estimation of the carotenoid content compared to HPLC [52].

Anthocyanins

While there are six common anthocyanidins, more than 540 anthocyanin pigments have been identified in nature.

The extraction of anthocyanins is the first step in the determination of both total and individual anthocyanins in any type of plant tissue. The following quantitation can be achieved by a variety of classical (spectrophotometric) or contemporary methods – HPLC coupled with a various types of UV detectors or mass spectrometers.

The methods of quantitation of anthocyanins can be divided into three major groups: methods for total anthocyanins in samples with few or no interfering compounds, methods for total anthocyanins in samples with interfering compounds that absorb in the 480 to 550 nm range, and methods for quantitation of individual anthocyanin components.

Spectrometric methods

The total anthocyanin content can often be determined in crude extracts containing other phenolic materials by measuring absorptivity of the solution at a single wavelength. This is possible because anthocyanins have typical absorption bands in the 490 to 550 nm region of the

visible spectra — far from the absorption bands of other phenolics with spectral maxima in the UV range [63].

For samples where the presence of carotenoids influence the anthocyanin determination, as in purple carrots or purple sweet potatoes, Lazcano [64] suggested a methods based on the preliminary removal of the carotenoids.

In some cases, a simple total anthocyanin determination via direct spectrophotometric measurement is inappropriate because of interference from polymeric anthocyanins, anthocyanin degradation products, or melanoidins from browning reactions.

The pH-differential method is a rapid and easy procedure for the quantitation of monomeric anthocyanins [65]. It relies on the structural transformation of the anthocyanin chromophore as a function of pH, which can be measured using optical spectroscopy. Degraded anthocyanins in the polymeric form are resistant to color change with change in pH. Therefore, polymerized anthocyanin pigments are not measured by this method because they absorb both at pH 4.5 and 1.0. In addition, other auxiliary spectrophotometric techniques are used to measure the extent of anthocyanin polymerization and browning.

The level of anthocyanins degradation of an aqueous extract, juice, or wine can be estimated from a few absorbance readings of a sample that has been treated with sodium bisulfite. Polymerized coloured anthocyanin-tannin complexes are resistant to bleaching by bisulfite, whereas the bleaching reaction of monomeric anthocyanins will rapidly go to completion. The absorbance at 420 nm of the bisulfite-treated sample serves as an index for browning. The ratio between monomeric and total anthocyanin can be used to determine a degradation index.

In 2005 the method has been approved as an AOAC Official Method 2005.02-2005 after a collaborative trial had been conducted [66, 67]

HPLC methods

Alternative methods for determining total anthocyanins are the separation of the anthocyanins by HPLC, measurements of the amounts of individual pigments by use of an external standard, and then summation of the individual anthocyanins.

Reversed phase HPLC coupled with photodiode array detection has been the most widely used tool for the identification, and quantification of anthocyanins. Individual anthocyanins can be separated by their polarity, which cause them to elute at different times. The anthocyanins can be quantitated with an external standard (cyanidin-3-glucoside or any purified anthocyanin standard). However, HPLC can result in an underestimation of the amount of anthocyanins present in samples that contain different anthocyanidin glycosides when using only one standard for quantification. Typically, cyanidin-3-glucoside is selected as the external standard. The sum of the peak area at a certain wavelength is used when quantifying anthocyanins by HPLC. This approach to quantitation is subject to the same concerns regarding purity and cost of external standards.

In their study, Jungmin Lee et al [68] demonstrated the high correlation between the AOAC pH differential method and HPLC when determining the amount of anthocyanins. For laboratories that do not have the capability for HPLC analysis, the pH differential is a simple and economical method to determine total anthocyanins.

The study also demonstrated the importance of reporting the reference standard used to express the values. In general, the amount of total anthocyanins was greater when related to malvidin-glucoside than as cyanidin-glucoside, despite the same extraction/separation method was used. This stresses the importance of access to anthocyanin (certified) reference materials.

Quantification by spectrophotometry at 530nm, the differential pH method, and HPLC, expressed as mg cyaniding equivalent, give similar results for source material that did not contain carotenoids in significant quantities. When the carotenoid level is significant, a first step for removing them is required when using simple spectrometry at 530 nm. Both other methods are robust in so far as the carotenoids do not interfere.

As an overview of the compiled from the literature data, the pH differential method played major role for determination of total anthocyanin content in source materials.

Chlorophylls

The concentrations of chlorophylls a and b from spinach can be determined easily based on the spectrophotometric method of Vernon from early 1960 [69] and later on of Robertson and Swinburne [70]. The AOAC method 942.04-1942 "Chlorophyll in plants. Spectrophotometric method for total chlorophyll and the a and b components" [71] is still in use.

Several HPLC methods have been developed for the analysis of chlorophyll and carotenoid pigments in green vegetables based on the determination of the individual substances. Total chlorophylls were calculated as a sum of chlorophyll a and b, chlorophyll a-1 and b-1, chlorophyll a' and b', and pheophytin a and b [72-74].

Betanin/Betalains

Similarly to carotenoids and anthocyanidins, the determinations of betalaines may be divided into two main groups of methods. Depending on the analytical method applied for determination of the concentration of the pigments, spectrophometry or HPLC, two different parameters are measured: either the total pigment content (betacyanins or betaxanthins), or individual constituents, – which are the betacyanins (betanin, isobetanin, probetanin, neobetanin) and betaxanthns (vulgaxanthin, indicaxanthin).

Nilsson [75] proposed a method for the simultaneous spectrophotometric quantification of betaxanthins and betacyanins in red beets. Although spectrophotometry was reported to yield higher pigment contents than the true values, Nilsson's method is still recommended for red beet samples today [76].

In comparison with the generally applied spectrophotometric method, the light-reflectance technique was found to be very accurate and reliable for determining the main beet pigments and could be used for rapid determination [77]. It has been shown that betalain quantitation by capillary zone electrophoresis is in close agreement with values produced by HPLC [78]

Today, the method of choice for quantitation of betalains is HPLC [79, 80].

Phycocyanins

Phycocyanin is a water soluble blue pigment. It is extracted from algal cells and determined according to the method reported by Bossiba and Richmond [81], Bennet [82], Sigelman and Kycia [83].

A single-laboratory validation study was conducted in 2008 [84] for a 2-wavelength spectrophotometric method for the determination of c-phycocyanin (cPC) and allophycocyanin (aPC) in *Spirulina* supplements and raw materials. The absorption maxima of cPC and aPC are at 620 and 650 nm, respectively. The concentrations of the analytes were calculated from measurement of absorbance at two wavelengths. The validation study showed that the method is precise with good specificity, linearity, and recoveries. The two-wavelength method has proven to be effective in the simultaneous determination of cPC and aPC.

Curcumoids

Curcumin is an oil soluble pigment, practically insoluble in water at acidic and neutral pH, but soluble in alkali. A variety of methods for the quantification of the curcuminoids have been reported. Most of them are spectrophotometric methods, expressing the total "colour" content of the sample [85].

Quantitative estimation of total curcuminoids can be carried out spectrophotometrically based on its absorbance at 420 nm [86, 87]. Standard solutions were prepared by dissolving standard curcumin in methanol in different concentrations. However, it is not possible to quantify the individual curcuminoids with spectrophotometric methods.

Several methods have been reported for the estimation of individual cucuminoids as HPLC, HPTLC, GC, LC/MS [88-90]. Jayaprakasha [89] determined the curcuminoid levels in rhizomes of *Curcuma longa* by a simple analytical procedure requiring minimal sample preparation. The method described is suitable for the routine analysis of a large number of commercial samples of C. longa.

Shafflomin A and B

Dried safflower powder was extract to isolate the soluble yellow pigment and the water insoluble carthamin. The pigment in the extracts were identified by using thin-layer chromatography and spectrophotometery [91]. Spectrophotometeric absorption spectra showed the absorption maxima for carthamin red at 520 nm in acetone. Similarly the water soluble safflower yellow pigment showed an absorption maximum at 405 nm [92]. The content of pigments were determined using the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specification for the food additive Carthamus Yellow [93].

CONCLUSIONS

- The source materials to be included in Annex III of the Guidance notes and the colouring principles for which reference values should be proposed were identified jointly with the stakeholders;
- Information for the content of the nutrients in the source materials from the established list has been compiled using food composition databases of more than 30 countries in Europe, USA, Canada and Australia, reflecting as much as possible the geographical and genetic variability of the cultivars and growing seasons;
- Information for the content of the colouring constituents in the source materials from the established list has been compiled from specialised databases, but mostly from the peered reviewed papers in scientific journals and books;
- Key-aroma constituents have been identified for those source materials from the established list for which the information is available in the scientific literature. However quantitative data were very scarce for proposals of reference values;
- Overview of the compiled data was summarised in "Information on the source material" files, prepared for each of the source materials from the established list;
- Proposals were included for

  - reference ranges and, based on robust statistics, reference values for the nutritive constituents of the source materials;

  - high, medium and low reference ranges and, based on robust statistics, reference values for the content of the colouring constituents;

  - identified key-aroma compound (upon availability of information)

- An overview of the existing methods for the determination of the content of the colouring constituents in the source materials has been prepared and the list with the bibliographic references has been included;

References

1. Artés, F, Minguez, M. I., and Hornero, D., *Analysing changes in fruit pigments*, in *Colour in food*, D.B. MacDougall, Editor. 2002, Woodhead Publishing Limited: North America by CRC Press LLC. p. 248-283.
2. Deli, J. and G. Tóth, *Carotenoid composition of the fruits of Capsicum annuum Cv. Bovet 4 during ripening.* Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 1997. **205**(5): p. 388-391.
3. Deli, J., Z. Matus, and G. Tóth, *Carotenoid Composition in the Fruits of Capsicum annuum Cv. Szentesi Kosszarvú during Ripening.* Journal of Agricultural and Food Chemistry, 1996. **44**(3): p. 711-716.
4. Shi, J. and M. Le Maguer, *Lycopene in tomatoes: chemical and physical properties affected by food processing.* Crit Rev Biotechnol, 2000. **20**(4): p. 293-334.
5. Clifford, M.N., *Anthocyanins – nature, occurrence and dietary burden.* Journal of the Science of Food and Agriculture, 2000. **80**(7): p. 1063-1072.
6. Carle, S.F.C.a.R., *Analysis of Betalains*, in *Food Colorants. Chemical and Functional Properties*, C. Socaciu, Editor. 2008, Taylor & Francis Group, LLC: 6000 Broken Sound Parkway NW, Suite 300.
7. Uyan, S.E., et al., *Effects of drying process on antioxidant activity of purple carrots.* Food / Nahrung, 2004. **48**(1): p. 57-60.
8. Robert, J.M., *Character-impact flavor compounds*, in *Sensory-Directed Flavor Analysis*. 2006, CRC Press. p. 223-267.
9. Nijssen, L.M.I.-V., C.A. van; Donders, J.J.H. [eds], *VCF Volatile Compounds in Food* in *version 15.2*. 1963-2014, TNO Triskelion, Zeist, The Netherlands.
10. Kraujalytė, V., E. Leitner, and P.R. Venskutonis, *Characterization of Aronia melanocarpa Volatiles by Headspace-Solid-Phase Microextraction (HS-SPME), Simultaneous Distillation/Extraction (SDE), and Gas Chromatography-Olfactometry (GC-O) Methods.* Journal of Agricultural and Food Chemistry, 2013. **61**(20): p. 4728-4736.
11. Hirvi, T. and E. Honkanen, *Analysis of the volatile constituents of black chokeberry (Aronia melanocarpa Ell.).* Journal of the Science of Food and Agriculture, 1985. **36**(9): p. 808-810.
12. Kulling, S.E. and H.M. Rawel, *Chokeberry (Aronia melanocarpa)– A Review on the Characteristic Components and Potential Health Effects.* Planta Med, 2008. **74**(13): p. 1625-1634.
13. Christensen, L.P., M. Edelenbos, and S. Kreutzmann, *Fruits and Vegetables of Moderate Climate*, in *Flavours and Fragrances*, R. Berger, Editor. 2007, Springer Berlin Heidelberg. p. 135-187.
14. Wang, Y., C. Finn, and M.C. Qian, *Impact of Growing Environment on Chickasaw Blackberry (Rubus L.) Aroma Evaluated by Gas Chromatography Olfactometry Dilution Analysis.* Journal of Agricultural and Food Chemistry, 2005. **53**(9): p. 3563-3571.
15. Du, X., C.E. Finn, and M.C. Qian, *Volatile composition and odour-activity value of thornless 'Black Diamond' and 'Marion' blackberries.* Food Chemistry, 2010. **119**(3): p. 1127-1134.
16. El Hadi, M., et al., *Advances in Fruit Aroma Volatile Research.* Molecules, 2013. **18**(7): p. 8200.
17. Boccorh, R.K., A. Paterson, and J.R. Piggott, *Sources of variations in aroma-active volatiles, or flavour components, of blackcurrant concentrates.* Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 1999. **208**(5-6): p. 362-368.

18. Boccorh, R.K., A. Paterson, and J.R. Piggott, *Extraction of aroma components to quantify overall sensory character in a processed blackcurrant (Ribes nigrum L.) concentrate.* Flavour and Fragrance Journal, 2002. **17**(5): p. 385-391.

19. Forney, C.F., *Horticultural and other Factors Affecting Aroma Volatile Composition of Small Fruit.* HortTechnology, 2001. **11**(4): p. 529-538.

20. Parliment, T.H. and R. Scarpellino, *Organoleptic techniques in chromatographic food flavor analysis.* Journal of Agricultural and Food Chemistry, 1977. **25**(1): p. 97-99.

21. Horvat, R.J. and S.D. Senter, *Comparison of the Volatile Constituents from Rabbiteye Blueberries (Vaccinium ashei) During Ripening.* Journal of Food Science, 1985. **50**(2): p. 429-431.

22. Kreutzmann, S., et al., *The role of volatile compounds on aroma and flavour perception in coloured raw carrot genotypes.* International Journal of Food Science & Technology, 2008. **43**(9): p. 1619-1627.

23. Soria, A.C., J. Sanz, and M. Villamiel, *Analysis of volatiles in dehydrated carrot samples by solid-phase microextraction followed by GC-MS.* Journal of Separation Science, 2008. **31**(20): p. 3548-3555.

24. Whitfield, F., Last, JH, *Vegetables in Volatile Compounds in Foods and Beverages*, in *Volatile Compounds in Foods and Beverages*, M. H., Editor. 1991, Marcel Dekker: New York. p. 203-282.

25. Robert, J.M., *Character-impact flavor and off-flavor compounds in foods*, in *Flavor, Fragrance, and Odor Analysis, Second Edition*. 2011, CRC Press. p. 207-262.

26. Petersen, M.B. and L. Poll, *The influence of storage on aroma, soluble solids, acid and colour of sour cherries (Prunus cerasus L.) cv. Stevnsbær.* European Food Research and Technology, 1999. **209**(3-4): p. 251-256.

27. Kaack, K., et al., *The relationship between sensory quality and volatile compounds in raw juice processed from elderberries (Sambucus nigra L.).* European Food Research and Technology, 2005. **221**(3-4): p. 244-254.

28. Chen, S.-H., et al., *Extraction, Analysis, and Study on the Volatiles in Roselle Tea.* Journal of Agricultural and Food Chemistry, 1998. **46**(3): p. 1101-1105.

29. Jung, E., Y. Kim, and N. Joo, *Physicochemical properties and antimicrobial activity of Roselle (Hibiscus sabdariffa L.).* Journal of the Science of Food and Agriculture, 2013. **93**(15): p. 3769-3776.

30. Gonzalez-Palomares, S., et al., *Effect of the Temperature on the Spray Drying of Roselle Extracts (Hibiscus sabdariffa L.).* Plant Foods for Human Nutrition, 2009. **64**(1): p. 62-67.

31. Camelo-Méndez, G.A., et al., *Comparative Study of Anthocyanin and Volatile Compounds Content of Four Varieties of Mexican Roselle (Hibiscus sabdariffa L.) by Multivariable Analysis.* Plant Foods for Human Nutrition, 2013. **68**(3): p. 229-234.

32. R., B., *Fruits I in Volatile Compounds in Foods and Beverages*, in *Volatile Compounds in Foods and Beverages*, M. H., Editor. 1991, Marcel Dekker: New York. p. 283-387.

33. Lu, G., et al., *Quantitative Determination of Geosmin in Red Beets (Beta vulgaris L.) Using Headspace Solid-Phase Microextraction.* Journal of Agricultural and Food Chemistry, 2003. **51**(4): p. 1021-1025.

34. Freidig, A.K. and I.L. Goldman, *Geosmin (2β,6α-Dimethylbicyclo[4.4.0]decan-1β-ol) Production Associated with Beta vulgaris ssp. vulgaris Is Cultivar Specific.* Journal of Agricultural and Food Chemistry, 2014. **62**(9): p. 2031-2036.

35. Naef, R., A. Velluz, and A. Jaquier, *New Volatile Sulfur-Containing Constituents in a Simultaneous Distillation−Extraction Extract of Red Bell Peppers (Capsicum annuum).* Journal of Agricultural and Food Chemistry, 2008. **56**(2): p. 517-527.

36. Radovich, T.J.K., *Cabbage Flavor*, in *Handbook of Fruit and Vegetable Flavors*. 2010, John Wiley & Sons, Inc. p. 741-750.

37. Radovich TJK , K.M., Sanchez - Vela A , Scheerens JC , Schult B *Fresh cabbage sensory quality: Components and the impact of production factors.* Acta Hortic 2003. **628**: p. 787-795.

38. Chin, H.W., Q. Zeng, and R.C. Lindsay, *Occurrence and Flavor Properties of Sinigrin Hydrolysis Products in Fresh Cabbage.* Journal of Food Science, 1996. **61**(1): p. 101-104.

39. Jing, P., et al., *Anthocyanin and glucosinolate occurrences in the roots of Chinese red radish (Raphanus sativus L.), and their stability to heat and pH.* Food Chemistry, 2012. **133**(4): p. 1569-1576.

40. Gao, R., et al., *Removal of off-flavours from radish (Raphanus sativus L.) anthocyanin-rich pigments using chitosan and its mechanism(s).* Food Chemistry, 2014. **146**(0): p. 423-428.

41. Blažević, I. and J. Mastelić, *Glucosinolate degradation products and other bound and free volatiles in the leaves and roots of radish (Raphanus sativus L.).* Food Chemistry, 2009. **113**(1): p. 96-102.

42. Ong, P.K.C. and S.Q. Liu, *Flavor and Sensory Characteristics of Vegetables*, in *Handbook of Vegetables and Vegetable Processing*. 2010, Wiley-Blackwell. p. 59-82.

43. Carlson D, M.E.D., and C.H. VanEtten, C.B. Hill and P.H. Williams, *Glucosinolates in Radish Cultivars.* Journal of the American Society for Horticultural Science, 1985. **110**(5): p. 634-638.

44. Choi, S.-H.I., Sung-Im ; Jang, Eun-Young ; Cho, Young-Su ;, *Volatile Components of Flower and Seed of Safflower.* Korean Journal of Food Science and Technology, 2004. **36**(2): p. 196-201.

45. Masanetz, C., H. Guth, and W. Grosch, *Fishy and hay-like off-flavours of dry spinach.* Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 1998. **206**(2): p. 108-113.

46. Buttery, R.G., *Quantitative and sensory aspects of flavour of tomato and other vegetables and fruits.*, in *Flavor Science: Sensible Principles and Techniques,* , a.R.T. ed. T. E. Acree, Editor. 1993, ACS Books, American Chemical Society: Washington, DC.

47. Marković, K., et al., *Aroma volatiles of tomatoes and tomato products evaluated by solid-phase microextraction.* Flavour and Fragrance Journal, 2007. **22**(5): p. 395-400.

48. Govindarajan, V.S. and W.H. Stahl, *Turmeric — chemistry, technology, and quality.* C R C Critical Reviews in Food Science and Nutrition, 1980. **12**(3): p. 199-301.

49. Richard, D.H., H. Chi-Tang, and T.R. Robert, *The Characterization of Volatile and Semivolatile Components in Powdered Turmeric by Direct Thermal Extraction Gas Chromatography?Mass Spectrometry*, in *Spices*. 1997, American Chemical Society. p. 80-97.

50. Surwase, V.S.L., K. S.; Kale, R. V.; Hashmi, S. I.; Lokhande, S. M. , *EXTRACTION AND ISOLATION OF TURMERONE FROM TURMERIC.* Electronic Journal of Environmental, Agricultural and Food Chemistry, 2011. **10**(5): p. 2173-2179.

51. Chemists, A.-A.o.O.A., *Method 970.64-1974 Carotenes and Xanthophylls in Dried Plant Materials and Mixed Feeds - Spectrophotometric Method*. 1974, Association of Official Analytical Chemists.

52. Biehler, E., et al., *Comparison of 3 Spectrophotometric Methods for Carotenoid Determination in Frequently Consumed Fruits and Vegetables.* Journal of Food Science, 2010. **75**(1): p. C55-C61.

53. Delgado-Vargas, F., A.R. Jiménez, and O. Paredes-López, *Natural Pigments: Carotenoids, Anthocyanins, and Betalains — Characteristics, Biosynthesis, Processing, and Stability.* Critical Reviews in Food Science and Nutrition, 2000. **40**(3): p. 173-289.

54. Lichtenthaler, H.K. and C. Buschmann, *Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy*, in *Current Protocols in Food Analytical Chemistry*. 2001, John Wiley & Sons, Inc.

55. Hornero-Méndez, D. and M.I. Mínguez-Mosquera, *Rapid Spectrophotometric Determination of Red and Yellow Isochromic Carotenoid Fractions in Paprika and Red Pepper Oleoresins.* Journal of Agricultural and Food Chemistry, 2001. **49**(8): p. 3584-3588.

56. Gorocica-Buenfil, M.A., et al., *Effect of low vitamin A diets with high-moisture or dry corn on marbling and adipose tissue fatty acid composition of beef steers.* J Anim Sci, 2007. **85**(12): p. 3355-66.

57. Minguez-mosquera, M.I., et al., *Determination of chlorophylls and carotenoids by HPLC during olive lactic fermentation*. 1991.

58. Minguez-Mosquera, M.I., B. Gandul-Rojas, and M.L. Gallardo-Guerrero, *Rapid method of quantification of chlorophylls and carotenoids in virgin olive oil by high-performance liquid chromatography.* Journal of Agricultural and Food Chemistry, 1992. **40**(1): p. 60-63.

59. Minguez-Mosquera, M.I. and D. Hornero-Mendez, *Separation and quantification of the carotenoid pigments in red peppers (Capsicum annuum L.), paprika, and oleoresin by reversed-phase HPLC.* Journal of Agricultural and Food Chemistry, 1993. **41**(10): p. 1616-1620.

60. Müller, H., *Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection.* Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 1997. **204**(2): p. 88-94.

61. Amorim-Carrilho, K.T., et al., *Review of methods for analysis of carotenoids.* TrAC Trends in Analytical Chemistry, 2014. **56**(0): p. 49-73.

62. Scott, K.J., et al., *Interlaboratory studies of HPLC procedures for the analysis of carotenoids in foods.* Food Chemistry, 1996. **57**(1): p. 85-90.

63. Jing, G.M.M.P., *Analysis of Anthocyanins*, in *Food colorants : chemical and functional properties*, C. Socaciu, Editor. 2008, CRC Press

64. Lazcano, C.A., K.S. Yoo, and L.M. Pike, *A method for measuring anthocyanins after removing carotenes in purple colored carrots.* Scientia Horticulturae, 2001. **90**(3): p. 321-324.

65. Giusti, M.M. and R.E. Wrolstad, *Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy*, in *Current Protocols in Food Analytical Chemistry*. 2001, John Wiley & Sons, Inc.

66. Chemists, A.-A.o.O.A., *Method 2005.02-2005 Total Monometric Anthocyanin Pigment Content* 2005, Association of Official Analytical Chemists.

67. Lee, J., R.W. Durst, and R.E. Wrolstad, *Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study.* Journal of AOAC International, 2005. **88**(5): p. 1269-1278.

68. Lee, J., C. Rennaker, and R.E. Wrolstad, *Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods.* Food Chemistry, 2008. **110**(3): p. 782-786.

69. Vernon, L.P., *Spectrophotometric Determination of Chlorophylls and Pheophytins in Plant Extracts.* Analytical Chemistry, 1960. **32**(9): p. 1144-1150.

70. Robertson, G.L. and D. Swinburne, *Changes in Chlorophyll and Pectin After Storage and Canning of Kiwifruit.* Journal of Food Science, 1981. **46**(5): p. 1557-1559.

71. Chemists, A.-A.o.O.A., *Method 942.04-1942 Chlorophyll in plants. Spectrophotometric method for total chlorophyll and the a and b components*. 1942, AOAC International.

72. Edelenbos, M., L.P. Christensen, and K. Grevsen, *HPLC Determination of Chlorophyll and Carotenoid Pigments in Processed Green Pea Cultivars (Pisum sativum L.).* Journal of Agricultural and Food Chemistry, 2001. **49**(10): p. 4768-4774.

73. Khachik, F., G.R. Beecher, and N.F. Whittaker, *Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography.* Journal of Agricultural and Food Chemistry, 1986. **34**(4): p. 603-616.

74. Kidmose, U., et al., *Chromatographic Determination of Changes in Pigments in Spinach (Spinacia oleracea L.) During Processing.* Journal of Chromatographic Science, 2005. **43**(9): p. 466-472.

75. Nilsson, T., *Studies into the pigments in beetroot (Beta vulgaris L. vulgaris var. rubra L.),.* Lantbrukshög. Ann.,, 1970. **36**: p. 179.

76. Schwartz, S.J., B.E. Hildenbrand, and J.H. Von Elbe, *Comparison of Spectrophotometric and HPLC Methods to Quantify Betacyanins.* Journal of Food Science, 1981. **46**(1): p. 296-297.

77. Cohen, E. and I. Saguy, *A rapid method of determination of betanin and vulgaxanthin I in beet powder using a general purpose tristimulus colorimeter.* Zeitschrift für Lebensmittel-Untersuchung und Forschung, 1982. **175**(1): p. 31-33.

78. Paredes-López, D.-V.F.a.O., *Anthocyanins and Betalains*, in *Natural Colorants for Food and Nutraceutical Uses*. 2002, CRC Press.

79. Kujala* T., J.L.a.K.P., *Betalains and Phenolics in Red Beetroot (Beta vulgaris) Peel Extracts: Extraction and Characterisation.* Z. Naturforsch., 2001. **56c**: p. 343-348.

80. Kujala T., M.S.V.K.D.K., Jyrki M. Loponen · Kalevi Pihlaja, *Betalain and phenolic compositions of four beetroot (Beta vulgaris) cultivars.* Betalain and phenolic compositions of four beetroot (Beta vulgaris) cultivars, 2002. **214**: p. 505-510.

81. Boussiba, S. and A. Richmond, *Isolation and characterization of phycocyanins from the blue-green alga Spirulina platensis.* Archives of Microbiology, 1979. **120**(2): p. 155-159.

82. Bennett, A. and L. Bogorad, *Properties of subunits and aggregates of blue-green algal biliproteins.* Biochemistry, 1971. **10**(19): p. 3625-3634.

83. Siegelman H, J.H.K., *Handbook of Phycological Methods, Physiological and Biological Methods*, in *Algal biliproteins*, J.S.C. Johan A. Hellebust, Editor. 2011, Cambridge University Press. p. 71-78.

84. Yoshikawa, N. and A. Belay, *Single-laboratory validation of a method for the determination of c-phycocyanin and allophycocyanin in Spirulina (Arthrospira) supplements and raw materials by spectrophotometry.* J AOAC Int, 2008. **91**(3): p. 524-9.

85. Paramasivam, M., et al., *High-performance thin layer chromatographic method for quantitative determination of curcuminoids in Curcuma longa germplasm.* Food Chemistry, 2009. **113**(2): p. 640-644.

86. Paisooksantivatana, T.R.a.Y., *Variation of Total Curcuminoids Content, Antioxidant Activity and Genetic Diversity in Turmeric (Curcuma longa L.) Collections.* Kasetsart J. (Nat. Sci.), 2009. **43**: p. 507-518.

87. Kulkarni S.J., K.N.M., M.P.Budre & R.P.Mahajan, *Extraction and purification of curcuminoids from Turmeric (curcuma longa L.).* International Journal of Pharmacology and Pharmaceutical Technology (IJPPT),, 2012. **1**(2): p. 81-84.

88. Himesh Soni*, P.S.S., Mishra K, Nayak Govind, Singhai AK, *QUALITATIVE AND QUANTITATIVE PROFILE OF CURCUMIN FROM ETHANOLIC EXTRACT OF CURCUMA LONGA.* International Research Journal of Pharmacy, 2011. **2**(4): p. 180-184.

89. Jayaprakasha, G.K., L. Jagan Mohan Rao, and K.K. Sakariah, *Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin.* J Agric Food Chem, 2002. **50**(13): p. 3668-72.

90. THOMAS Elizabeth, T.J.Z., S. SYAMKUMAR AND B. SASIKUMAR, *Curcuminoid profiling of Indian turmeric.* Journal of Medicinal and Aromatic Plant Sciences, 2011. **33**(1).

91. Fatahi N, J.C.a.R.H., *Spectrophotometric Measurement of Valuable Pigments from Petals of Saffiower (Carthamus tinctorius L.) and their Identification by TLC Method.* Research Journal of Biological Sciences, 2008. **3**(7): p. 761-763.

92. Carra, P.O., *Algal biliproteins.* Biochemical Journal, 1970. **119**(3): p. 2P-3P.

93. (JECFA), J.F.W.E.C.o.F.A., *CARTHAMUS YELLOW*, in *Online Edition: "Combined Compendium of Food Additive Specifications" of FAO of the US*. 1998, FAO/WHO.

Annexes

Annex 1. Letter sent to the stakeholders with the request for information

Annex 2. Template sent to the stakeholders for collecting the information on the source materials to be included in Annex III

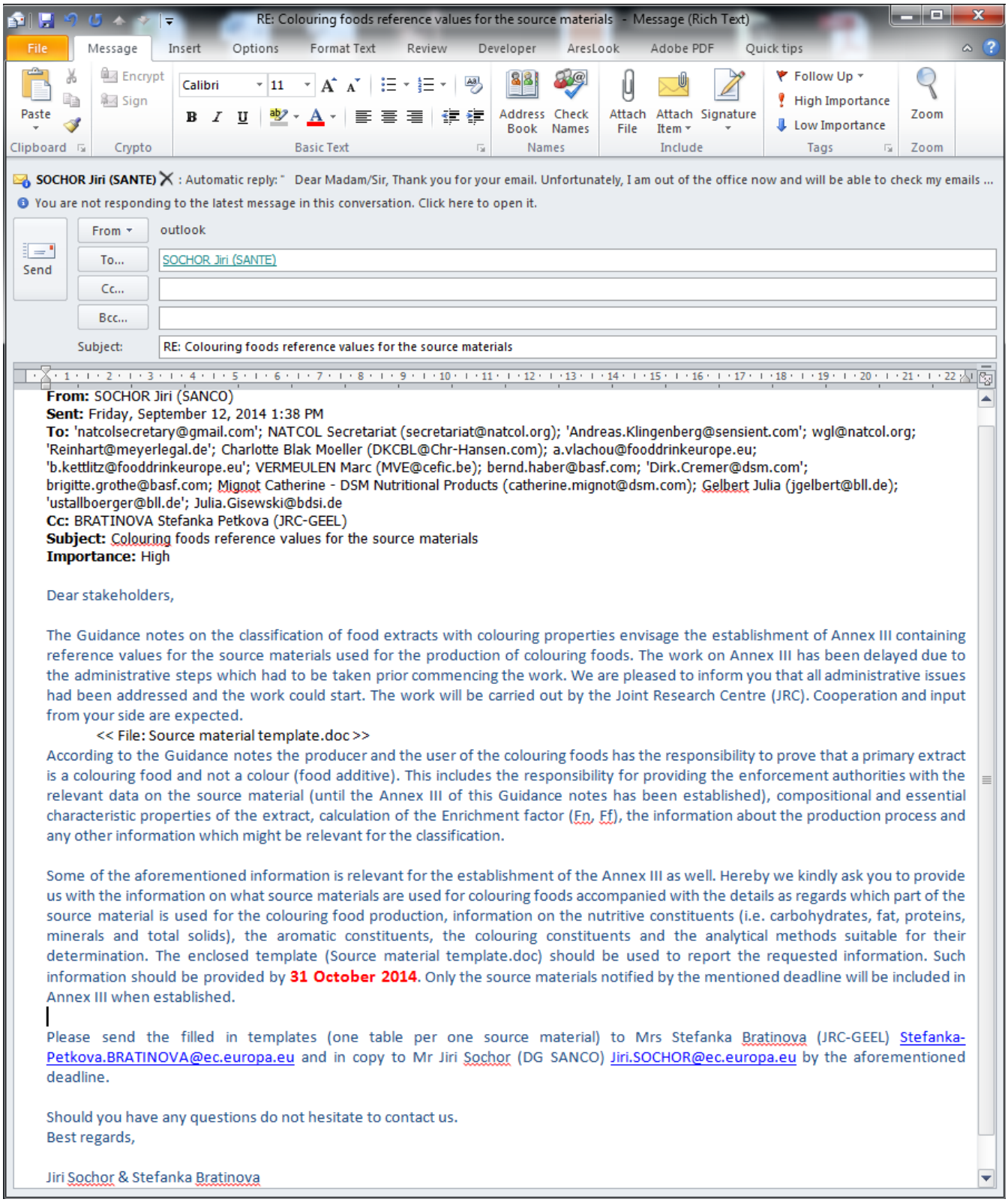
Annex 3. List of the source materials to be included in Annex IIIas requested by stakeholders

Annex 4. Food composition databases included in EuroFIR FoodExplorer.

Annex 5. Basic compositional table with proposed reference values.

Annex 6. JRC Information on the source materials.

Annex 1. Letter sent to the stakeholders with the request for information



RE: Colouring foods reference values for the source materials - Message (Rich Text)

File | Message | Insert | Options | Format Text | Review | Developer | AresLook | Adobe PDF | Quick tips

Paste | Encrypt | Sign | Calibri | 11 | Address Book | Check Names | Attach File | Attach Item | Signature | Follow Up | High Importance | Low Importance | Zoom

Clipboard | Crypto | Basic Text | Names | Include | Tags | Zoom

SOCHOR Jiri (SANTE) : Automatic reply: " Dear Madam/Sir, Thank you for your email. Unfortunately, I am out of the office now and will be able to check my emails ...

You are not responding to the latest message in this conversation. Click here to open it.

Send | From outlook | To... SOCHOR Jiri (SANTE) | Cc... | Bcc... | Subject: RE: Colouring foods reference values for the source materials

From: SOCHOR Jiri (SANCO)
Sent: Friday, September 12, 2014 1:38 PM
To: 'natcolsecretary@gmail.com'; NATCOL Secretariat (secretariat@natcol.org); 'Andreas.Klingenberg@sensient.com'; wgl@natcol.org; 'Reinhart@meyerlegal.de'; Charlotte Blak Moeller (DKCBL@Chr-Hansen.com); a.vlachou@fooddrinkeurope.eu; 'b.kettlitz@fooddrinkeurope.eu'; VERMEULEN Marc (MVE@cefic.be); bernd.haber@basf.com; 'Dirk.Cremer@dsm.com'; brigitte.grothe@basf.com; Mignot Catherine - DSM Nutritional Products (catherine.mignot@dsm.com); Gelbert Julia (jgelbert@bll.de); 'ustallboerger@bll.de'; Julia.Gisewski@bdsi.de
Cc: BRATINOVA Stefanka Petkova (JRC-GEEL)
Subject: Colouring foods reference values for the source materials
Importance: High

Dear stakeholders,

The Guidance notes on the classification of food extracts with colouring properties envisage the establishment of Annex III containing reference values for the source materials used for the production of colouring foods. The work on Annex III has been delayed due to the administrative steps which had to be taken prior commencing the work. We are pleased to inform you that all administrative issues had been addressed and the work could start. The work will be carried out by the Joint Research Centre (JRC). Cooperation and input from your side are expected.

<< File: Source material template.doc >>

According to the Guidance notes the producer and the user of the colouring foods has the responsibility to prove that a primary extract is a colouring food and not a colour (food additive). This includes the responsibility for providing the enforcement authorities with the relevant data on the source material (until the Annex III of this Guidance notes has been established), compositional and essential characteristic properties of the extract, calculation of the Enrichment factor (Fn, Ff), the information about the production process and any other information which might be relevant for the classification.

Some of the aforementioned information is relevant for the establishment of the Annex III as well. Hereby we kindly ask you to provide us with the information on what source materials are used for colouring foods accompanied with the details as regards which part of the source material is used for the colouring food production, information on the nutritive constituents (i.e. carbohydrates, fat, proteins, minerals and total solids), the aromatic constituents, the colouring constituents and the analytical methods suitable for their determination. The enclosed template (Source material template.doc) should be used to report the requested information. Such information should be provided by **31 October 2014**. Only the source materials notified by the mentioned deadline will be included in Annex III when established.

Please send the filled in templates (one table per one source material) to Mrs Stefanka Bratinova (JRC-GEEL) Stefanka-Petkova.BRATINOVA@ec.europa.eu and in copy to Mr Jiri Sochor (DG SANCO) Jiri.SOCHOR@ec.europa.eu by the aforementioned deadline.

Should you have any questions do not hesitate to contact us.
Best regards,

Jiri Sochor & Stefanka Bratinova

Annex 2

Template sent to the stakeholders for collecting the information on the source materials to be included in Annex III

Information on the source material used for the production of extracts with colouring properties

Organisation:	*Insert the name of the organisation*	**Date of submission:**	*Insert the date*
Contac person (name + email):	*Insert the name and email of the contact person*		
Source material:	*Insert the name of the source material + information from which parts of the source material is the extract obtained*		
Colouring principle of the extract/concentrate and/or extraction/concentration method:	*Insert the colouring constituents present in the extract/concentrates and information about the extraction/concentration method(s)*		

Data available on the source material as regards the content of:

	List	**Data from literature available about the content**	**Analytical data available/ method for analysis**
Pigment(s)[1]	*pigment(s) present in the source material + proposal and justification as regards pigment(s) that should be used as a reference*		
Nutritive constituents	*Information on carbohydrates, fat, proteins, minerals and total solids*		
Aromatic constituents[2]	*Aromatic constituents present in the source material + proposal and justification as regards aromatic constituent(s) that should be used as a reference*		
Files enclosed containing the requested information	*Put the name of the file(s) or refer to the information provided for the road-test exercise*	*Put the name of the file(s) or refer to the information provided for the road-test exercise*	*Put the name of the file(s) or refer to the information provided for the road-test exercise*

Annex 3. List of the source material requested to be included in Annex III of the Guidance notes by stakeholders

Stakeholder	Raw materials	source material	coloring principle
NATCOL	Black Carrot	roots	Anthocyanins
NATCOL	Elderberry	Whole fruit	Anthocyanins
NATCOL	Hibiscus	Dried flowers (calyces)	Anthocyanins
NATCOL	Orange Carrot	roots	Carotenoids
NATCOL	Pumpkin	fruit/pulp	Total carotenoids expressed as lutein
NATCOL	Red Beet	roots	betanin/betalanin
NATCOL	Red Cabbage	leaves	Anthocyanins
NATCOL	Red Bell Pepper	Red bell pepper fruit	Carotenoids
NATCOL	Red Radish	tuberous roots	Anthocyanins
NATCOL	Safflower	petals	Safflomin A and B/ carthamin
NATCOL	Spirulina	algae	Phycocyanins (C-phycocyanin and Allophycocyanin); others - chlorophyll+carotenoids
NATCOL	Tomato	whole fruit	Lycopene
NATCOL	Alfalfa	(stalks & leaves)	Chlorophylls
NATCOL	Grapes	berries	Anthocyanins
NATCOL	Purple Sweet Potato	from tubers	Anthocyanins
NATCOL	Spinach	leaves	Chlorophylls
NATCOL	Turmeric	Turmeric rhizomes	*Curcuminoids*
C Hansen	Aronia	berries	Anthocyanins
C Hansen	Black current	roots	Anthocyanins
C Hansen	Black Carrot	roots	Anthocyanins
C Hansen	Carthamus (Safflower)	flowers	Safflomin A and B
C Hansen	Grapes	berries	Anthocyanins
C Hansen	Elderberry	berries	Anthocyanins
C Hansen	Hibiscus	flowers (calyces)	Anthocyanins
C Hansen	Orange Carrot	roots	Carotenoids
C Hansen	Pumpkin	fruit	Carotenoids
C Hansen	Red Beet	roots	betanin
C Hansen	Red Radish	roots	Anthocyanins
C Hansen	Spirulina	algae	Phycocyanins (C-phycocyanin and Allophycocyanin)
C Hansen	Sweet Potato	tubers	Anthocyanins
SVZ (KleurCraft)	Apple	NA	NA
SVZ (KleurCraft)	Aronia	NA	NA
SVZ (KleurCraft)	Blackberry	NA	NA
SVZ (KleurCraft)	Blackcurrant	NA	NA
SVZ (KleurCraft)	Blueberry	NA	NA
SVZ (KleurCraft)	Carrot	NA	NA
SVZ (KleurCraft)	Cherry	NA	NA
SVZ (KleurCraft)	Elderberry	NA	NA
SVZ (KleurCraft)	Lemon	NA	NA
SVZ (KleurCraft)	Pumpkin	NA	NA
SVZ (KleurCraft)	Raspberry	NA	NA
SVZ (KleurCraft)	Red beet	NA	NA
SVZ (KleurCraft)	Red bell pepper	NA	NA
SVZ (KleurCraft)	Red radish	NA	NA
SVZ (KleurCraft)	Safflower	NA	NA
SECNA (Spain)	black carrot	roots	Anthocyanins
SECNA (Spain)	red cabidge	leaves	Anthocyanins
SECNA (Spain)	red beet	roots	Anthocyanins
SECNA (Spain)	grape	fruit	Anthocyanins
GNT	Carrot orange	NA	NA
GNT	Carrot Black	NA	NA
GNT	Elderberry	NA	NA
GNT	Hibiscus	NA	NA
GNT	Pumpkin	NA	NA
GNT	Safflower	NA	NA
GNT	Spirulina	NA	NA
GNT	Red cabbage	NA	NA
GNT	Aronia	NA	NA
GNT	Blackcurrant	NA	NA
GNT	Blueberry	NA	NA
GNT	Chery	NA	NA
GNT	Grape	NA	NA
GNT	Radish	NA	NA
GNT	Red cabbage	NA	NA
GNT	Sweet purple potato	NA	NA
GNT	Tomato	NA	NA

Annex 4. Food composition databases included in EuroFIR FoodExplorer.

* EuroFIR partner/national food composition database compilers

Country	Institution	Database name	Status
Austria	* UVI	OENWT Österreichische Nährwerttabelle	online
Belgium	* NUBEL	NIMS	online
Bulgaria	* NCH	FCTBL_BG (food composition tables – Bulgaria)	
Canada	* Government of Canada	Canadian Nutrient Files	online
Czech Republic	* Institute of Agricultural Economics and Information & Food Research Institute	Czech Food Composition Database	online
Denmark	* DTU	Danish Food Composition Databank	online
Finland	* THL	Fineli	online
France	* ANSES	CIQUAL French food composition table	online
Germany	* MRI	German Food Code and Nutrient Data Base	online
Germany	MedPharm	Souci-Fachman-Kraut; Food Composition and Nutrition Tables – Online Database	online
Greece	* HHF	Composition tables of foods and Greek dishes	online
Greece	Medical School of Crete	Food Composition Tables of Greek Foods	online
Iceland	* MATÍS	ÍSGEM	online
Ireland	* UCC	Irish Food Composition Database	online
Israel	BGU	BGU	online
Italy	* INRAN	Banca Dati di Composizione degli Alimenti	online
Italy	* IEO	Food Composition Database for Epidemiological Studies in Italy	online
Lithuania	* NNC	Respublikinis Mitybos Centras – EuroFIR Food Classification	online
Netherlands	* RIVM	NEVO	online
New Zealand	The New Zealand Institute for Plant and Food Research Limited & Ministry of Health	New Zealand Food Composition Database	online
Norway	* UiO	Norwegian Food Composition Tables	online
Poland	* NFNI	Food Composition Tables	online
Portugal	* INSA	Tabela de Composição dos Alimentos – INSA	online
Serbia	* IMR	Serbian Food and Nutrition Database	online
Slovakia	* FRI	Slovak Food Composition Data Bank	online
Spain	* UGR	Base de Datos Española de Composición de Alimentos – RedBEDCA	online
Sweden	* NFA	NFA Food Composition Database	online
Switzerland	* FSVO	Swiss Food Composition Database	online
Turkey	* TUBITAK	Turkey is currently developing a new Turkish food composition database system	
UK	* IFR	McCance and Widdowson's The Composition of Foods integrated dataset	online

Annex 5. Basic compositional table with proposed reference values.

		water	total solids	Carbohydrates*	Fibres	Proteins	Total lipids	Minerals			
		%	%	% on a dry weight					%, DW	colouring principle	Key-Aroma Compounds
Alfalfa	stalk and leaves								Robust		
Aronia/ Black chokeberry	berries	73	27	47	39	4.6	1.9	1.8	2.8	anthocyanins total	amygdalin
Blackberry	berries	85.5	14.5	44	31	8.7	5.6	2.6	1.35	anthocyanins total	
Blackcurrant	berries	80.7	19.3	48	28	7	2	2.5	1.66	anthocyanins total	4-Methoxy-2-methylbutanethiol
Blueberry	berries	85.5	14.5	60	24	4.5	4	1	1.75	anthocyanins total	
Carrot Black	root	90	10	54	27	7.5	2.2	4.2	1.80	anthocyanins total	2-sec-butyl-3-methoxypyrazine
Carrot orange	root	90	10	54	27	7.5	2.2	4.2	0.15	carotenoids total	2-sec-butyl-3-methoxypyrazine
Cherry	fruit	84	16	75	9.1	6.1	2	1.8	0.76	anthocyanins total	
Elderberry	berries	82.5	17.5	41	29	9.8	6.9	4	6.8	anthocyanins total	
Grape	fruit	81	19	82	7.1	3.3	1.3	1.5	2.7	anthocyanins total	
Hibiscus / Roselle	petals	86	14	30	42	5.5	2	7	2.5	anthocyanins total	geraniol
Pumpkin	fruit/pulp	93	7	52	21	13	2.4	6	0.2	carotenoids total	
Raspberry, Black	berries	85.5	14.5	41	38	8.8	4.2	2.4	4.2	anthocyanins total	raspberry ketone 1-(4-hydroxylphenyl)-3-butanone
Red beet	root	87.7	12.3	60	19	12	1	4.9	0.6	betanin by HPLC	geosmin
									1.6	by spectroscopy	
Red bell pepper	pericap	91.5	8.5	50	23	13	3.6	3.5	1.05	carotenoids total	
Red Cabbage	leaves	91	9	41	26	17	2.2	4.5	2.6	anthocyanins total	allyl isothiocyanate
Red radish	root	94.6	5.4	47	30	16	2.1	7.5	3.2	anthocyanins total	glucosinolates
Safflower	petals	22	78	24	23.8	13	5.5	12.8	3-12	Safflomin A+B	
Spinach	leaves	92	8	14	26	33	5	11	1.2	chlorophylls	
Spirulina	algae	90.5	9.5	23	4	60	4.2	4.4	19.4	phycocyanin	
Sweet Potato, Purple	root	75	25	78	10	5.6	1	2.2	0.63	anthocyanins total	
Tomato	fruit	94	6	48	20	15	3.8	5.8	0.18	lycopene	
Turmeric	root	11.3	88.7	59	16	9.7	7.2	4.5	7.9	curcuminoids	turmerone and ar-turmerone

* Carbohydrates are without fibres

Annex 6. JRC Information on the source material

Information on the source material used for the production of extracts with colouring properties

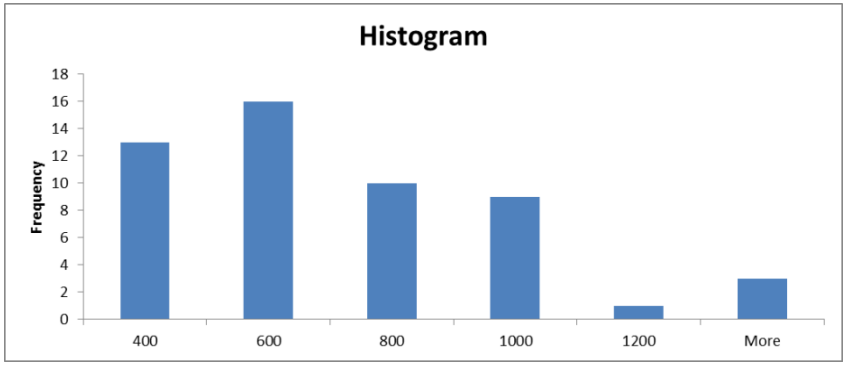
Source material:	**Aronia /Chokeberry *(Aronia Melanocarpa)***
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Typical process of the concentrate/extract : Berries --> juice extraction by traditional processes -> concentration through different processes like filtration, etc…

Data available on the source material as regards the content of:

	List	**Data from literature available about the content**	**Suggested reference value**
Nutritive constituents	Water Dry Matter- Total Solids Carbohydrates Fibres Fat Proteins Minerals (Ash)	Water (moisture): 60.7 – 84.4% fresh base Dry matter(Total solids): 15.6 – 39.3% fresh base	Moisture: 73.0% Total solids (TS): 27.0%

	%, FW base	% DW base
Carbohydrates total (without fibres)	7.5– 18.0	34.7 – 81.4
Fibres	2.1 – 20.0	9.5 – 73.7
Proteins	0.4 – 3.0	2.6 – 7.7
Fat	0.10 – 1.69	0.9 – 4.3
Minerals (Ash)	0.21 – 0.90	1.08 – 5.8

	%, DW	% SD
Carbohydrates total (without fibres)	56	
Fibres	42	
Proteins	5.2	
Fat	2.3	
Minerals (Ash)	3.2	

	List	Data from literature available about the content	Suggested reference range/value
Pigment(s)	The main colouring principles of aronia (black chokeberry) berries are anthocyanins: Determination of **total anthocyanins** Total anthocyanin amount, calculated as cyanidin-3-glucoside, was determined by means of the pH differential method or HPLC. **HPLC and spectrophotometric methods give similar results,** expressed as cyanidin 3-glucoside Two major anthocyanins are identified by HPLC: cyaniding 3-galactoside (65%) and cyaniding 3-arabinoside (29%) and several minor The highest reported values 1790 was not proved to be reliable  Carotenoids - 4.9 mg/100g FW	Total anthocyanin (based on 18 peered review articles) 135-1790 mg/100 g FW; Robust mean 585 mg/100g DW Robust SD 310 mg/100g DW 500-6629 mg/100 g DW (recalculated based on 27 % TS) Robust mean 2500 mg/100g DW Robust SD 1340 mg/100g DW	2800 mg/100 g DW (2.8 %)
Aromatic constituents	**Amygdalin,** a cyanogenic glycoside isolated from the berries, is responsible for the often off-flavoured bitter-almond smell of the fresh fruits. Amygdalin (laetrile) - 20.1 mg/100 g FW Olfactometry revealed that **ethyl-2-methyl butanoate, ethyl-3-methyl butanoate, ethyl decanoate** ("fruity" aroma notes), **nonanal** ("green" notes), **unidentified compound** possessing "moldy" odor, and **some other volatiles** may be very important constituents in formation of chokeberry aroma in different cultivars	Amygdalin 20.1 mg/100 g FW	Not feasible to suggest reference value due to non-identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Blackberry *(Rubus fruticosus L. s.l.)***
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Typical process of the concentrate/extract : Berries --> juice extraction by traditional processes -> concentration through different processes like filtration, etc…

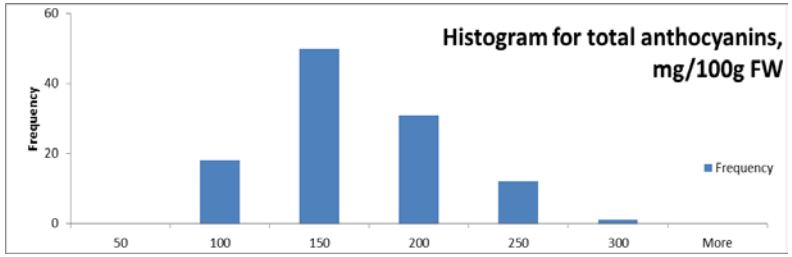
Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 82-88 % fresh base
Dry matter(Total solids): 12- 18 % fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>2.8 – 13.0</td><td>19 – 83.2</td></tr>
<tr><td>Fibres</td><td>2.7 – 7.3</td><td>15.2 – 47.9</td></tr>
<tr><td>Proteins</td><td>0.8 – 1.4</td><td>5.9 – 11.9</td></tr>
<tr><td>Fat</td><td>0.4 – 1.1</td><td>2.2 – 9.0</td></tr>
<tr><td>Minerals(Ash)</td><td>0.1-0.5</td><td>0.8 – 3.7</td></tr>
</table>
</td><td>Moisture: 85.5 %
Total solids: 14.5 %

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>44</td><td>10.55</td></tr>
<tr><td>Fibres</td><td>31</td><td>11.7</td></tr>
<tr><td>Proteins</td><td>8.7</td><td>2.2</td></tr>
<tr><td>Fat</td><td>5.6</td><td>2.6</td></tr>
<tr><td>Minerals(Ash)</td><td>2.6</td><td>1.1</td></tr>
</table>
</td></tr>
</table>

	List	Data from literature available about the content	Suggested reference range/value
Pigment(s)	The main colouring principles of blackberry berries are anthocyanins: Cyanidin 3-glucoside is the major pigment in all blackberry samples analyzed with a mean level of 82.9% of total peak area  Total anthocyanin amount, calculated as cyanidin-3-glucoside, was determined by means of the pH differential method or after identification by HPLC. Both methods spectrophotometric and HPLC give similar results; expressed as cyanidin 3-glucoside in most of the cases *Carotenoids - beta-carrotene*	Total anthocyanin (based on 10 peer reviewed articles, data from 3 databases and data provided by the industry) 67-326 mg/100 g FW Robust mean 147 mg/100 g FW Robust SD 50 mg/100 g FW 465-2250 mg/100 g DW (recalculated based on 14.5% TS) Robust mean 1011 mg/100 g DW Robust SD 340 mg/100 g DW 0.12-0.48 mg/ 100 g FW	Robust mean + robust SD 1350 mg/100 g DW
Aromatic constituents	The aroma profiles of blackberries are complex, as no single volatile was unanimously described as characteristically “blackberry”. Flavours of importance include ethyl butyrate, Furaneol, and other furanones, linalool, and β-damascenone.		Not feasible to suggest reference value due to non-identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Blackcurrent *(Ribes nigrum L. - fruit)***
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Typical process of the concentrate/extract : Berries --> juice extraction by traditional processes -> concentration through different processes like filtration, etc…

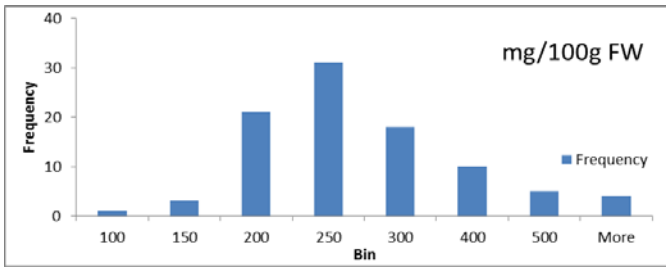
Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 76.0% – 85.5% fresh base
Dry matter(Total solids): 14.5% – 24.0% fresh base

<table><tr><th>Range</th><th>%, FW base</th><th>% DW base</th></tr><tr><td>Carbohydrates, (without fibres)</td><td>6.1 – 11.7</td><td>29.2 – 83.3</td></tr><tr><td>Fibres</td><td>3.5 – 8.7</td><td>15.9 – 45.7</td></tr><tr><td>Proteins</td><td>0.9 – 2.0</td><td>4.0 – 12.0</td></tr><tr><td>Fat</td><td>0.1 – 1.7</td><td>03 – 10.5</td></tr><tr><td>Minerals (Ash)</td><td>0.25 – 0.9</td><td>1.3 – 7.9</td></tr></table></td><td>Moisture: 80.7%
Total solids: 19.3%

<table><tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr><tr><td>Carbohydrates, (without fibres)</td><td>48</td><td>10.5</td></tr><tr><td>Fibres</td><td>28</td><td>9.2</td></tr><tr><td>Proteins</td><td>7</td><td>1.9</td></tr><tr><td>Fat</td><td>2</td><td>1.3</td></tr><tr><td>Minerals (Ash)</td><td>2.5</td><td>0.9</td></tr></table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference range/value
Pigment(s)	The main colouring principles of blackberry berries are anthocyanins. A total of 14 anthocyanins were detected across all black currant cultivars, with 4 of them most abundant - cyanidin 3-glucoside, delphinidin 3-glucoside, delphinidin 3-rutinoside and cyanidin 3-rutinoside. Almost all data are produced based on pH differential method with the exception of one article with higher values where HPLC-ESI-MS/MS is applied.  Carotenoids – beta carotene	**Total anthocyanin** (based on 12 peer reviewed articles, inputs in 2 databases and data provided by industry) 96-840 mg/100 g FW Robust mean 244 mg/100 g FW Robust SD 76 mg/100 g FW 500-4350 mg/100 g DW (recalculated based on 19.3% TS) Robust mean 1266 mg/100 g DW Robust SD 394 mg/100 g DW Carotenoids 0.03 - 0.55 mg/100 g FW 0.05 - 1.2 mg/100 g DW;	Robust mean + robust SD 1660 mg/100 g DW (1.66 %)
Aromatic constituents	The key aroma component in blackcurrant is **2-methoxy-4-methyl-4-butanethiol** giving rise to the characteristic catty note of blackcurrants, have in low amounts, varying between cultivars from **trace to 0.04 %,** identified in aromatic varieties The typical black currant aroma resulted from the presence of methyl and ethyl butyrates, eucalyptol, diacetyl, and a trace amount of a compound with an odour of cat urine	2-methoxy-4-methyl-4-butanethiol - from trace to 0.04 %,	Not feasible to suggest reference value due to scarce data on the key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	Blueberry (bilberry, huckleberry, hurtleberry, whortleberry) *Vaccinium sp*
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Made of sound and mature fruit which have been selected, washed, crushed, extracted and concentrated by evaporation

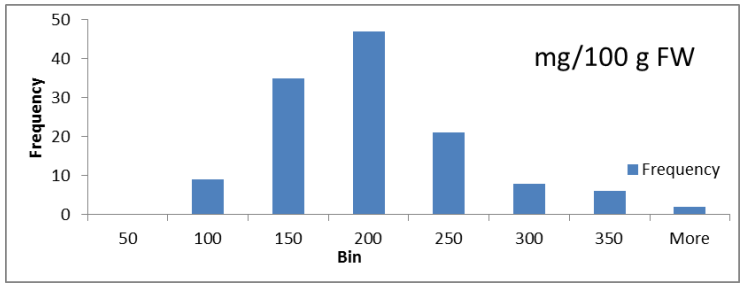
Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 83.0% – 88.7% fresh base
Dry matter(Total solids): 11.3% – 17.0% fresh base

<table>
<tr><th>Range</th><th>%, FW base</th><th>% DW base</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>1.2 – 14.2</td><td>39.3 – 79.9</td></tr>
<tr><td>Fibres</td><td>1.5 – 5.1</td><td>11.2 – 35.4</td></tr>
<tr><td>Proteins</td><td>0.4 – 1.2</td><td>3.0 – 8.4</td></tr>
<tr><td>Fat</td><td>0.2 – 0.8</td><td>1.1 – 6.4</td></tr>
<tr><td>Minerals (Ash)</td><td>0.1 – 0.3</td><td>0.14 – 2.2</td></tr>
</table></td><td>Moisture: 85.5%
Total solids: 14.5%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>60</td><td>14.13</td></tr>
<tr><td>Fibres</td><td>24</td><td>9.6</td></tr>
<tr><td>Proteins</td><td>4.5</td><td>1.3</td></tr>
<tr><td>Fat</td><td>4</td><td>1.6</td></tr>
<tr><td>Minerals (Ash)</td><td>1</td><td>0.8</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference range/value
Pigment(s)	The main colouring principles of blueberries are anthocyanins: Blueberries in comparison with other kinds of small fruits contain a wide range of anthocyanins. In the fruit dominate malvinidin and delphinidin, in lower concentrations are represented peonidin, cyanidin and petunidin  Total anthocyanin amount, calculated as cyanidin-3-glucoside, was determined mostly by means of the pH differential method although HPLC-MS/MS data were also aincluded. Both HPLC and spectrophotometric methods give similar results; expressed as cyanidin 3-glucoside in most of the cases. Carotenoids - low levels with lutein most abundant one	**Total anthocyanin** (based on 17 peer reviewed articles, 2 databases and data provided by the industry) 63-430 mg/100 g FW Robust mean 244 mg/100 g FW Robust SD 76 mg/100 g FW 430-3000 mg/100 g DW (recalculated based on 14.5% TS) Robust mean 1245 mg/100 g DW Robust SD 506 mg/100 g DW Carotenoids 0.1-2.4 mg/100 g DW	Robust mean + robust SD 1750 mg/100 g DW (1.75 %)
Aromatic constituents	Some authors determined that a combination of linalool and (Z)-3-hexen-1-ol produced a blueberry-like flavour, while others reported that a mixture of (Z)-3-hexen-1-ol, (E)-2- hexen-1-ol, (E)-2-hexenal, linalool and geraniol gave an aroma similar to the aroma isolated from blueberries.		Not feasible to suggest reference value due to non-identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Carrots Black *(Daucus Carota)*, roots**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Black carrot roots-> juice extraction by traditional processes-> Concentration through different steps like membrane-filtration, etc.

Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)

Nutritive data are for Carrots, without distinguishing between Black and Orange varieties.</td><td>Water: 80.4 – 92.0 fresh base
Dry matter(Total solids): 8.0 – 19.6 fresh base

<table>
<tr><th>Range</th><th>%, FW base</th><th>% DW base</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>2.8 – 8.8</td><td>24.5 – 90.5</td></tr>
<tr><td>Fibres</td><td>2.0 – 3.6</td><td>14.3 – 41.9</td></tr>
<tr><td>Proteins</td><td>0.4 – 1.3</td><td>3.9 – 16.2</td></tr>
<tr><td>Fat</td><td>0.1 – 0.5</td><td>0.3 – 4.6</td></tr>
<tr><td>Minerals(Ash)</td><td>0.2 – 0.9</td><td>1.7 – 7.3</td></tr>
</table></td><td>Moisture: 90%
Total solids: 10%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>54</td><td>14.41</td></tr>
<tr><td>Fibres</td><td>27</td><td>6.1</td></tr>
<tr><td>Proteins</td><td>7.5</td><td>1.6</td></tr>
<tr><td>Fat</td><td>2.2</td><td>1.1</td></tr>
<tr><td>Minerals(Ash)</td><td>4.2</td><td>1.4</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference range/value
Pigment(s)	The main colouring principles of purple carrots are anthocyanins: Polyphenols that are commonly quantified in purple carrots include 5 anthocyanins. The basic anthocyanin backbone is called cyanidin. The R groups are various glycosides. Contents of individual compounds indicated great differences in the potential of anthocyanin accumulation both between different cultivars and carrots of the same cultivar. Total anthocyanin amounts ranged from 45.4 mg/kg dry matter to 17.4 g/kg dry matter. Industry reported analytical data up to 5760 mg/100 DW, mentioning that "No literature inputs are reflecting the actual range of Anthocyanin contents found in the source material (black carrot" The carotene interference could cause an over-estimation of the total anthocyanin content when analysed spectrophotometrically without prior removal. Carotenoids in some purple carrots cultivar could be more than in orange varieties	**Total anthocyanin** (based on 12 peer reviewed articles and data provided by industry) 5-5760 mg/100 g DW Robust mean 1050 mg/100 g DW Robust SD 730 mg/100 g DW *(Average 1118 mg/100 g DW)* Carotenoids 5-250 mg/100 g DW	Robust mean + robust SD 1780 mg/100 g DW (1.8 %)
Aromatic constituents	More than 90 volatile compounds have been identified from carrots [VCF]. The carrot volatiles consist mainly of terpenoids in terms of numbers and amounts and include monoterpenes, sesquiterpenes and irregular terpenes. Monoterpenes and sesquiterpenes account for about 98% of the total volatile mass in carrots. The characteristic flavour of carrots depends on the composition of different volatiles. α-Pinene, sabinene, myrcene, limonene, β-ocimene, γ-terpinene, p-cymene, terpinolene, β-caryophyllene, α-humulene, (E)-γ-bisabolene and β-ionone are found to be the key faroma compounds of raw carrot. **2-sec-butyl-3-methoxy-pyrazine** - 624 ng/l juice - compound has extremely low threshold (2ng/l) and could be expected to have major role in the flavour of raw carrot as its aroma at a GC sniffing port has been described as "raw carroty".		Not feasible to suggest reference value due to scarce data on the key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Carrots Orange *(Daucus Carota)*, roots**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Black carrot roots-> juice extraction by traditional processes-> Concentration through different steps like membrane-filtration, etc.

Data available on the source material as regards the content of:

	List	**Data from literature available about the content**	**Suggested reference value**
Nutritive constituents	Water Dry Matter- Total Solids Carbohydrates Fibres Fat Proteins Minerals (Ash) Nutritive data are for Carrots, without distinguishing between Black and Orange varieties.	Water: 80.4 – 92.0 fresh base Dry matter(Total solids): 8.0 – 19.6 fresh base **Range** / **%, FW base** / **% DW base** Carbohydrates, (without fibres): 2.8 – 8.8 / 24.5 – 90.5 Fibres: 2.0 – 3.6 / 14.3 – 41.9 Proteins: 0.4 – 1.3 / 3.9 – 16.2 Fat: 0.1 – 0.5 / 0.3 – 4.6 Minerals(Ash): 0.2 – 0.9 / 1.7 – 7.3	Moisture: 90% Total solids: 10% **Ref. value** / **DW, %** / **SD, %** Carbohydrates, (without fibres): 54 / 14.41 Fibres: 27 / 6.1 Proteins: 7.5 / 1.6 Fat: 2.2 / 1.1 Minerals(Ash): 4.2 / 1.4

	List	Data from literature available about the content	Suggested reference range/ value
Pigment(s)	The main colouring principles of orange carrots are carotenoids: Carrot roots are rich in carotenoids. Six carotenes (α-, β-, γ -, and ζ -carotenes, β-zeacarotene, and lycopene) can be routinely separated and quantified in typical and dark orange carrots. The predominant carotenoids are the provitamin A carotenes, that is, α- and β-carotene, accounting for 13% to 40% and 45% to 80% of the carotenoids in orange carrots, respectively There are dark orange carrots with higher carotenoid content than normal orange carrots. Most probably they are used as source material for colouring food production. Industry reported analytical data up to 300 mg/100 DW for total carotenoids, mentioning that "No literature inputs are reflecting the actual range of carotenoid contents found in the source material orange carrot"	**Total carotenoids** (based on 8 peer reviewed articles, 4 databases and data provided by industry) 5-300 mg/100 g DW Robust mean 103 mg/100 g DW Robust SD 50 mg/100 g DW	Robust mean + robust SD 150 mg/100 g DW (0.15 %)
Aromatic constituents	More than 90 volatile compounds have been identified from carrots [VCF]. The carrot volatiles consist mainly of terpenoids in terms of numbers and amounts and include monoterpenes, sesquiterpenes and irregular terpenes. Monoterpenes and sesquiterpenes account for about 98% of the total volatile mass in carrots. The characteristic flavour of carrots depends on the composition of different volatiles. α-Pinene, sabinene, myrcene, limonene, β-ocimene, γ-terpinene, p-cymene, terpinolene, β-caryophyllene, α-humulene, (E)-γ-bisabolene and β-ionone are found to be the key faroma compounds of raw carrot. **2-sec-butyl-3-methoxy-pyrazine** - 624 ng/l juice - compound has extremely low threshold (2ng/l) and could be expected to have major role in the flavour of raw carrot as its aroma at a GC sniffing port has been described as "raw carroty".		Not feasible to suggest reference value due to scarce data on the key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Cherry Sour *(Prunus cerasus L.)*, berries (with or without pits)**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Made of sound and mature fruit which have been selected, washed, crushed, extracted and concentrated by evaporation

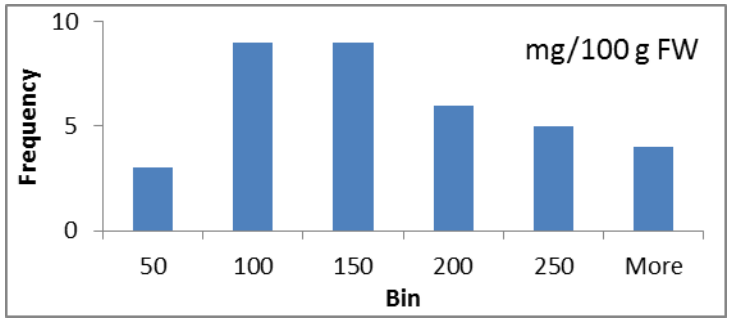
Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 75 - 90 % fresh base
Dry matter (Total solids): 10 - 25 % fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>8.1 – 17.4</td><td>52.9 – 89.0</td></tr>
<tr><td>Fibres</td><td>0.2 – 2.6</td><td>1.2 – 16.5</td></tr>
<tr><td>Proteins</td><td>0.7 – 1.5</td><td>2.2 – 13.3</td></tr>
<tr><td>Fat</td><td>0.1 – 0.7</td><td>0.3 – 4.0</td></tr>
<tr><td>Minerals(Ash)</td><td>0.1 – 0.6</td><td>0.66 – 4.21</td></tr>
</table></td><td>Moisture: 84 %
Total solids: 16 %

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>75</td><td>9.3</td></tr>
<tr><td>Fibres</td><td>9.1</td><td>2.5</td></tr>
<tr><td>Proteins</td><td>6.1</td><td>1.1</td></tr>
<tr><td>Fat</td><td>2</td><td>1.2</td></tr>
<tr><td>Minerals(Ash)</td><td>1.8</td><td>0.7</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference range/ value
Pigment(s)	The main colouring principles of cherries are anthocyanins: Usually sour cherries have more anthocyanins then sweet cherries. Most of the world production of sour cherries goes for juice preparations. Sour cherries had cyanidin 3-glucosylrutinoside as the major anthocyanin, whereas sweet cherries had cyanidin 3-rutinoside as the major anthocyanin 34 cultivar studied; 10 with AC >200 mg/100g FW; 10 with 21- 100  Carotenoids - mainly beta-catotene	Total anthocyanin (based on 17 peer reviewed articles, 1 database) 0.5-450 mg/100 g FW Robust mean 65 mg/100 g FW Robust SD 58 mg/100 g FW 3-2800 mg/100 g DW (recalculated based on 16% TS) Robust mean 400 mg/100 g DW Robust SD 360 mg/100 g DW Carotenoids 0.2-5.6 mg/100 g DW	Robust mean + robust SD 760 mg/100 g DW (0.76 %)
Aromatic constituents	Benzaldehyde has been determined to be the most important aroma compound in sour cherries, but benzyl alcohol, eugenol and vanillin are also important flavour compounds		Not feasible to suggest reference value due to the lack of reliable identification of key-aroma compounds and their content in the sour cherries.

Information on the source material used for the production of extracts with colouring properties

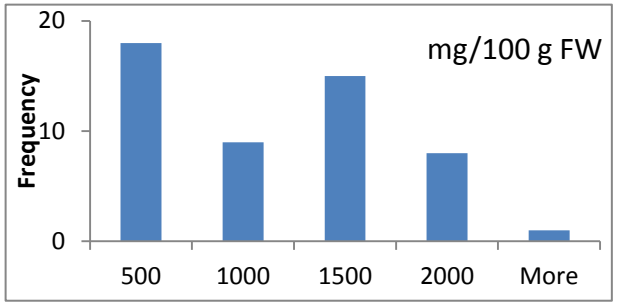
Source material:	**Elderberry *(Sambucus nigra)* , berries**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Elderberry concentrate obtained from aqueous extraction & pressing and subsequent concentration

Data available on the source material as regards the content of:

	List	**Data from literature available about the content**	**Suggested reference value**
Nutritive constituents	Water Dry Matter- Total Solids Carbohydrates Fibres Fat Proteins Minerals (Ash)	Water: 79.8 – 87.8% fresh base Dry matter(Total solids): 12.2 – 20.2% fresh base	Moisture: 82.5% Total solids: 17.5%

	%, FW base	% DW base
Carbohydrates total (without fibres)	2.5 – 11.4	15.4 – 56.4
Fibres	3.2 – 7.0	24.5 – 34.7
Proteins	0.7 – 2.6	3.3 – 15.6
Fat	0.2 – 1.8	1.5 – 10.4
Minerals(Ash)	0.4 – 2.0	1.8 – 7.0

	% DW	SD, %
Carbohydrates total (without fibres)	41	
Fibres	29	
Proteins	9.8	
Fat	6.9	
Minerals(Ash)	4	

	List	Data from literature available about the content	Suggested reference range/ value
Pigment(s)	The main colouring principles of elderberries are anthocyanins: Two main anthocyanidins cyanidin-3-sambubioside and cyanidin-3-glucoside and at least 6 minors Quantification by HPLC, expressed as mg cyaniding equivalent or by pH differential method. Huge variations in TA content reported depending on the maturity and cultivar/selection  Carotenoids	**Total anthocyanin** (based on 17 peer reviewed articles, 1 database) 14-2020 mg/100 g FW Robust mean 702 mg/100 g FW Robust SD 593 mg/100 g FW 80-11500 mg/100 g DW (recalculated based on 17.5% TS) Robust mean 3830 mg/100 g DW Robust SD 3000 mg/100 g DW Carotenoids 1.5-3.0 mg/100 g DW	Robust mean + robust SD 6800 mg/100 g DW (6.8 %)
Aromatic constituents	nonanal, dihydroedulan and β-damascenone have repeatedly been identified in various investigations as character-impact compounds for elderberry odour		Not feasible to suggest reference value due to non-identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Grapes *(Vitis Vinifera (L.),* fruits/ berries**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Grapes, Grape concentrate obtained from aqueous extraction & pressing and subsequent concentration

Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 78-84 % fresh base
Dry matter(Total solids): 16-22 % fresh base

<table>
<tr><th>Range</th><th>%, FW base</th><th>% DW base</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>13-19</td><td>63-93</td></tr>
<tr><td>Fibres</td><td>0.5-2.1</td><td>3-11</td></tr>
<tr><td>Proteins</td><td>0.3-0.8</td><td>1.4-4.4</td></tr>
<tr><td>Fat</td><td>0.1-0.6</td><td>0.5-3.1</td></tr>
<tr><td>Minerals(Ash)</td><td>0.2-0.5</td><td>1-2.5</td></tr>
</table></td><td>Moisture: 81%
Total solids: 19%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>82</td><td>3.6</td></tr>
<tr><td>Fibres</td><td>7.1</td><td>1.8</td></tr>
<tr><td>Proteins</td><td>3.3</td><td>0.6</td></tr>
<tr><td>Fat</td><td>1.3</td><td>0.8</td></tr>
<tr><td>Minerals(Ash)</td><td>1.5</td><td>0.7</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference range/value
Pigment(s)	The main colouring principles grapes are anthocyanins: The colour of red grapes is due to the anthocyanins. The composition of anthocyanins is primarily determined by genetic factors. Thus, a first distinction could be made between Vitis vinifera and others species of Vitis (i.e., muscadine grapes) according to the presence or lack of anthocyanidin diglucosides. In Vitis vinifera grapes there are only monoglucosides of five anthocyanidins: delphinidin, cyanidin, petunidin, peonidin and malvidin. Up to 21-22 different anthocyanins identified in some varieties. In all highly pigmented grape cultivar and selections most abundant are non-acylated monoglucosides, accounting for 61-95% of the total anthocyanin content (TA), followed by acylated monoglucosides and non-aqcylated diglucosides. Most data for anthocyanin content in grapes referred to grape skin or pomace as by-product of wine production, used for production of colouring food.	**Total anthocyanin** (based on 8 peer reviewed articles, 1 database and data reported by industry) n = 84 4-800 mg/100 g FW Robust mean 294 mg/100 g FW Robust SD 237 mg/100 g FW 20-4200 mg/100 g DW Robust mean 1500 mg/100 g DW Robust SD 1250 mg/100 g DW	Robust mean + robust SD 2700 mg/100 g DW (2.7 %)
Aromatic constituents	365 volatile compounds are identified in the VCF database for different cultivars of Vitis Vinifera species. The large diversity of volatiles contributing to the complex flavour and aroma of the grape, depending on cultivar variety makes it not feasible to identify and quantify key aroma compounds	No key aroma compounds identified and quantified	Not feasible to suggest reference value due to non-identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Hibiscus, *(Hibiscus sabdariffa)* , dried flower (calyces)**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Hibiscus flowers, aqueous extraction, subsequent concentration

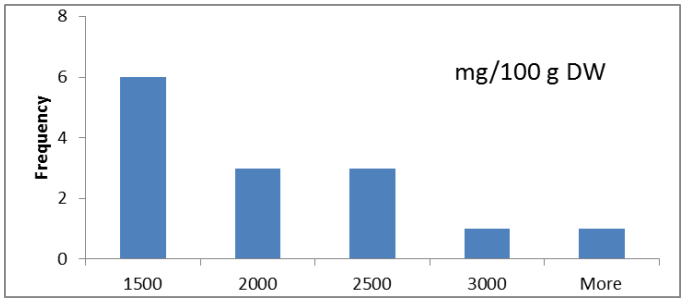
Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 6 – 89.5 % fresh base
Dry matter(Total solids): 10.5 – 94.0% fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Total carbohydrates, (without fibres)</td><td></td><td>20-42</td></tr>
<tr><td>Fibres</td><td></td><td>40-46</td></tr>
<tr><td>Proteins</td><td></td><td>4-7</td></tr>
<tr><td>Fat</td><td></td><td>0.3-4</td></tr>
<tr><td>Minerals(Ash)</td><td></td><td>4-11</td></tr>
</table>
</td><td>Moisture: 86%
Total solids: 14%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Total carbohydrates, (without fibres)</td><td>30</td><td></td></tr>
<tr><td>Fibres</td><td>42</td><td></td></tr>
<tr><td>Proteins</td><td>5.5</td><td></td></tr>
<tr><td>Fat</td><td>2</td><td></td></tr>
<tr><td>Minerals(Ash)</td><td>7</td><td></td></tr>
</table>
</td></tr>
</table>

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	The main colouring principles of hibiscus are anthocyanins: From the pigments of hibiscus calyces, three different anthocyanins were isolated: delphinidin-3-sambubioside (hibiscin), delphinidin-3-glucoside and cyanidin-3-glucoside (chrysanthenin). The results are very similar obtained by both methods: HPLC and pH differential spectroscopy.  Carotenoids - beta-carotene, lycopene	**Total anthocyanin** (based on 14 peer reviewed articles, and data reported by industry); n = 28 150-3220 mg/100 g DW Robust mean 1450 mg/100 g DW Robust SD 985 mg/100 g DW Carrotenoids 0.2-1.9 mg/100 g DW	Robust mean + robust SD 2500 mg/100 g DW (2.5%)
Aromatic constituents	a combination of the terpene derivatives with fragrance notes (geraniol 7.1-9.73 mg/kg and linalool), and the sugar derivatives as furfural and 5-methyl-2-furfural with a caramel-like odour are responsible for the Roselle aroma		Not feasible to suggest reference value due to scarce data on the key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Pumpkin (*Cucurbita maxima duchesne L.*) , fruit/pulp**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Carotenoids** Pumpkin -> juice extraction by traditional processes-> Concentration through different steps like membrane-filtration, etc.

Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 89.9 – 96.6% fresh base
Dry matter(Total solids): 3.4 – 10.1% fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>1.2 – 6.5</td><td>44.0 – 77.8</td></tr>
<tr><td>Fibres</td><td>0.5 – 2.8</td><td>6.0 – 40.0</td></tr>
<tr><td>Proteins</td><td>0.3 – 2.2</td><td>8.8 – 31.2</td></tr>
<tr><td>Fat</td><td>0.1 – 0.3</td><td>1.2 – 5.9</td></tr>
<tr><td>Minerals(Ash)</td><td>0.2 – 1.2</td><td>3.5 – 14.5</td></tr>
</table>
.</td><td>Moisture: 93%
Total solids: 7%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>52</td><td>7.7</td></tr>
<tr><td>Fibres</td><td>22</td><td>6.5</td></tr>
<tr><td>Proteins</td><td>13</td><td>2.2</td></tr>
<tr><td>Fat</td><td>2.4</td><td>1.4</td></tr>
<tr><td>Minerals(Ash)</td><td>6</td><td>2.5</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	The main colouring principles - Carotenoids Distinct differences could be observed in the different Cucurbita varieties as well as pulp and peel of the same variety. As peels are not edible the data focused on pulp. B-carotene is present in all varieties, while the rest of the carotenoids are different : lutein, Zeaxanthin, Antheraxanthin or a-carrotenoid As pumpkins mature, chlorophylls are replaced with carotenoids, and that signature orange colour develops, indicating ripeness. Mature pumpkin contains some trace of chlorophylls. NA data on the content	**Total carotenoids** (based on 8 peer reviewed articles, 4 databases and data provided by industry) 1-400 mg/100 g DW Robust mean 97 mg/100 g DW Robust SD 91 mg/100 g DW	Robust mean + robust SD 200 mg/100 g DW (0.2 %)
Aromatic constituents	About 30 compounds have been identified in the volatile extracts of raw pumpkin, with the major classes of compounds being aliphatic alcohols and carbonyl compounds, furan derivatives and sulphur-containing compounds [VCF].	No character-impact compounds of pumpkins identified	Not feasible to suggest reference value due to non-identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Raspberry *(Rubus), berries*** *red raspberry* ***RUBUS IDAEUS L*** *black raspberry:* ***Rubus leucodermis,*** *native to western North America* ***Rubus occidentalis****, native to eastern North America* ***Rubus coreanus****, also known as Korean black raspberry, native to Korea, Japan, and China*
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Typical process of the concentrate/extract : Berries --> juice extraction by traditional processes -> concentration through different processes like filtration, etc…

Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 80.3 – 90.0% fresh base
Dry matter (Total solids): 10.0 – 19.7% fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>3.2 – 15.7</td><td>24.6 – 80.1</td></tr>
<tr><td>Fibres</td><td>2.5 – 7.4</td><td>20.8 – 51.4</td></tr>
<tr><td>Proteins</td><td>0.9 – 1.8</td><td>5.2 – 14.9</td></tr>
<tr><td>Fat</td><td>0.3 – 1.9</td><td>1.9 – 9.9</td></tr>
<tr><td>Minerals (Ash)</td><td>0.2 – 0.7</td><td>1.2 – 6.2</td></tr>
</table></td><td>Moisture: 85.5%
Total solids: 14.5 %

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>41</td><td>10.9</td></tr>
<tr><td>Fibres</td><td>38</td><td>9.1</td></tr>
<tr><td>Proteins</td><td>8.8</td><td>1.6</td></tr>
<tr><td>Fat</td><td>4.2</td><td>2.2</td></tr>
<tr><td>Minerals (Ash)</td><td>2.4</td><td>0.9</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference range/value
Pigment(s)	The main colouring principles of raspberry berries are anthocyanins: Anthocyanins from Rubus fruit are unique in that they are predominately cyanidin based in the non-acylated form; Acylated pigments are occasionally found in Rubus fruit at low concentrations Black raspberries are with much higher levels of anthocyanins then the rest (red or yellow) Determination of **total anthocyanins** Total anthocyanin amount, calculated as cyanidin-3-glucoside, was determined mostly by means of spectroscopy and mainly following the pH differential method *Carotenoids – total* *Major carotenoid being lutein*	**Total anthocyanins** (based on 6 peer reviewed articles, and data reported by industry) n = 16 110-812 mg/100 g FW Robust mean 360 mg/100 g FW Robust SD 260 mg/100 g FW 760-5600 mg/100 g DW Robust mean 2500 mg/100 g DW Robust SD 1700 mg/100 g DW *Carotenoids total - 0.100-1.2 mg/ 100 g DW*	Robust mean + robust SD 4200 mg/100 g DW (4.2%)
Aromatic constituents	In addition to the character-impact compound of raspberry, 4-(4-hydroxyphenyl)- butan-2-one (raspberry ketone), alpha- and beta-ionone, geraniol, linalool, Furaneol, maple furanone, and other furaneols were concluded to be of importance to raspberry aroma. The odour threshold of raspberry ketone was measured at 1 to 10 µg/kg.	4-(4-hydroxyphenyl)- butan-2-one (raspberry ketone) - 0.009-4.3 ppm	Not feasible to suggest reference value due to the very scarce data on the content of the key-aroma compound

Information on the source material used for the production of extracts with colouring properties

Source material:	**Red beet (*Beta vulgaris* L.), roots**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: betanin Typical process of the concentrate/extract : Beet roots -> juice extraction by traditional processes -> Concentration through different steps like membrane filtration, etc

Data available on the source material as regards the content of:

	List	**Data from literature available about the content**	**Suggested reference value**
Nutritive constituents	Water Dry Matter- Total Solids Carbohydrates Fibres Fat Proteins Minerals (Ash)	Water: 86-92 % fresh base Dry matter(Total solids): 8- 14 % fresh base	Moisture: 87.7 % Total solids: 12.3 %

Range	FW base,%	DW base,%
Carbohydrates, (without fibres)	6.1 – 11.4	64.5-84.8
Fibres	1.7 - 2.9	15.2-34.2
Proteins	1.0 - 2	8.3-16.1
Fat	0.03 - 0.30	0.2-2.1
Minerals (Ash)	0.4 - 1	2.9-9.9

Ref. value	DW, %	SD, %
Carbohydrates, (without fibres)	60	4.8
Fibres	19	4.4
Proteins	12	1.6
Fat	1	0.4
Minerals (Ash)	4.9	1.6

	List	Data from literature available about the content	Suggested reference range/value
Pigment(s)	The main colouring principles of beetroot are betalains. These compounds can be sub-divided into the red-violet betacyanins and the yellow-orange betaxanthins. Betanin is the most abundant colorant accounting for 75-95% of the total betacyanin content. Depending on the analytical method applied for determination of the concentration of pigments - spectrophomethry or HPLC, two different parameters are measured - total pigment content (betacyanins or betaxanthins) and individual constituents in the pigments - betacyanins (betanin, isobetanin, probetanin, neobetanin) and betaxanthns (vulgaxanthin, indicaxanthin) The concentration of betanin depend whether with/without peel or only peals and even depend on the circle in the flesh from where the subsample is taken. Isobethanin has also significant contribution (25-30%) more in the peel then the flesh Pradhan 2010 reported extremely high betanin levels *	**Total betacyanins by spectrometry** (based on 4 peer reviewed articles, and data reported by industry) n = 60 350-2500 mg/100 g DW Robust mean 1100 mg/100 g DW Robust SD 500 mg/100 g DW **Individual components by HPLC - betanin** (based on 4 peer reviewed articles) n = 18 290-760 (3200*) mg/100 g DW Robust mean 457 mg/100 g DW Robust SD 134 mg/100 g DW	**For betacyanins by spectrometry** Robust mean + robust SD 1600 mg/100 g DW (1.6%) **For betanin by HPLC** (as required by stakeholders) Robust mean + robust SD 600 mg/100 g DW (0.6 %)
Aromatic constituents	2-sec-butyl-3-methoxypyrazidine and geosmin, which have aroma threshold concentration of 2 and 2 ng/l, respectively, are the major contributors to the earthy musty aroma of this vegetable. Intentionally removed as off flavour from the concentrates produced to be a colour.	Geosmin 0.15 - 3.9 μg/100g FW	Not feasible to suggest reference value due to very scarce data on geosmin content

Information on the source material used for the production of extracts with colouring properties

Source material:	**Red bell pepper (*Capsicum annuum L.*) , fruit**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Carotenoids** Red pepper -> mechanical separation by traditional processes -> concentration through different processes like membrane, filtration, etc…

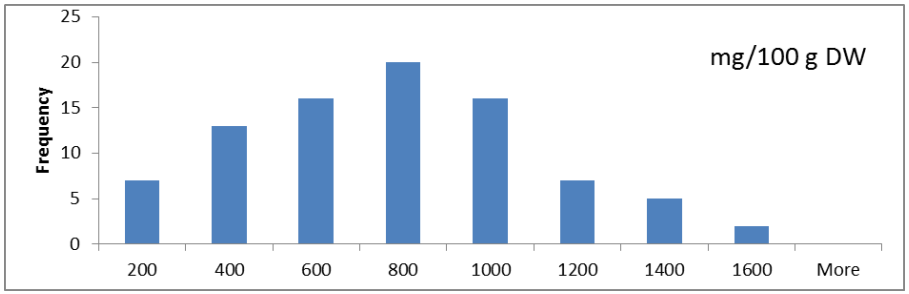
Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 90.2 – 94.0% fresh base
Dry matter(Total solids): 6.0 – 9.8% fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>4.4 – 7.4</td><td>55.1 – 84.1</td></tr>
<tr><td>Fibres</td><td>1.4 – 2.3</td><td>17.0 – 32.3</td></tr>
<tr><td>Proteins</td><td>0.8 – 1.3</td><td>9.2 – 17.9</td></tr>
<tr><td>Fat</td><td>0.1 – 0.5</td><td>1.1 – 6.2</td></tr>
<tr><td>Minerals (Ash)</td><td>0.1 – 0.6</td><td>0.5 – 7.1</td></tr>
</table></td><td>Moisture: 91.5%
Total solids: 8.5%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>50</td><td>5.2</td></tr>
<tr><td>Fibres</td><td>23</td><td>4.6</td></tr>
<tr><td>Proteins</td><td>13</td><td>2.7</td></tr>
<tr><td>Fat</td><td>3.6</td><td>1.4</td></tr>
<tr><td>Minerals (Ash)</td><td>3.5</td><td>1.1</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	The main colouring principles - Carotenoids. Lutein was the predominant carotenoid for immature green pepper while â-carotene was the predominant pigment for green peppers. The concentrations of xanthophylls such as capsorubin, cis-capsanthin, and ciszeaxanthin appeared only when the peppers reached the red stage. The total carotenoid pigments increased four times for red ripe fruits; capsanthin was the major pigment among them (50%) The red stage also has a high content of provitamin A due to the high concentrations of â-carotene and â-crytoxanthin. Different pigments, like capsanthin including ester derivatives, zeaxanthin, β-carotene, lutein, cryptoxanthin, capsorubin, are present within red pepper and can fluctuate depending on the variety 38 carotenoids are identified. In the ripened fruits, capsanthin and zeaxanthin accounted for about 29 and 15% of the total, respectively, â-carotene and â-cryptoxanthin for about 9 and 5%, respectively, and cucurbitaxanthin A for about 6%. 	**Total carotenoids** (based on 9 peer reviewed articles, and data reported by industry) n = 97 102-1500 mg/100 g DW Robust mean 700 mg/100 g DW Robust SD 350 mg/100 g DW .	Robust mean + robust SD 1050 mg/100 g DW (1.05%)
Aromatic constituents	In total 97 compounds were identified as volatile constituents of the bell pepper [VCF]. The most important odour compounds in raw sweet pepper are **2-(2-methylpropyl)-3- methoxypyrazine (0.09-0.12ppm)**, (E,Z)-2,6-nonadienal, and (E,E)-2,4-decadienal, having very low aroma threshold values	**2-(2-methylpropyl)-3-methoxypyrazine (0.09-0.12ppm)**	Not feasible to suggest reference value due to scarce data on the key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Red cabbage *(Brassica oleracea),* leaves**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Red Cabbage leaves -> juice extraction by traditional processes-> Concentration through different steps like membrane-filtration, etc.

Data available on the source material as regards the content of:

	List	**Data from literature available about the content**	**Suggested reference value**
Nutritive constituents	Water Dry Matter- Total Solids Carbohydrates Fibres Fat Proteins Minerals (Ash)	Water: 89.9 – 93.5% fresh base Dry matter (Total solids): 6.5 – 10.1% fresh base Range / FW base,% / DW base,% Carbohydrates, (without fibres): 0.7 – 5.3 / 8.4-70.4 Fibres: 1.0 – 3.6 / 13.0 – 40.0 Proteins: 0.8 – 2.0 / 10.6 – 24.7 Fat: 0.1 – 0.3 / 0.7 – 3.8 Minerals (Ash): 0.3 – 0.7 / 3.6 – 7.9	Moisture: 91% fresh base Total solids (TS): 9% fresh base Ref. value / DW, % / SD, % Carbohydrates, (without fibres): 41 / 8.9 Fibres: 26 / 5.9 Proteins: 17 / 3.5 Fat: 2.2 / 0.5 Minerals (Ash): 4.5 / 1.0

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	The main colouring principles of red cabbage are anthocyanins: Red cabbage products contained twenty different nonacylated and acylated anthocyanins with main structure of cyanidin-3-diglucoside-5-glucoside Quantification by absorption spectrophotometry at 530nm; pH differential method; HPLC, expressed as mg cyaniding equivalent give similar results Stakeholders stated that no literature inputs were found to reflect the actual range of Anthocyanin contents found in the source material (red cabbage). According to their analytical data obtained spectrophotometrically, the range of anthocyanins content in red cabbage leaves is 200-300 mg/100 g FW and 2000-3000 m g /100g DW. Similar even higher levels are found in USDA database - max. 475 mg/kg FW with the average of 209 mg/kg FW, although others reported in the literature levels are much lower. Carotenoids - beta carotene, lutein	**Total anthocyanins** (based on 14 peer reviewed articles, and data reported by industry) n = 21 80-4750 mg/100 g DW Robust mean 1300 mg/100 g DW Robust SD 1300 mg/100 g DW Carotenoids 0.100-0.400 mg/100 g DW	Robust mean + robust SD 2600 mg/100 g DW (2.6%)
Aromatic constituents	Pungency, reminiscent of mild horseradish or black mustard, is the most important flavour characteristic of fresh cabbage. Allyl isothiocyanate (AITC), a hydrolysis product of allyl glucosinolate (sinigrin), is the primary chemical influencing pungency in cabbage. According to VCF database the content of the allyl isothianate in raw freshly disrupted cabbage tissues is in the range 0.04-2.9 ppm	allyl isothianate 0.04-2.9 ppm	Not feasible to suggest reference value due to scarce data on the key-aroma compound

Information on the source material used for the production of extracts with colouring properties

Source material:	**Red radish *(Raphanus sativus))*, tuberous roots**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Red Radish roots -> juice extraction by traditional processes-> Concentration through different steps like membrane-filtration, etc

Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 92.6 – 95.6% fresh base
Dry matter (Total solids): 4.4 – 7.4% fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>2.5 – 6.5</td><td>56.8-108.3</td></tr>
<tr><td>Fibres</td><td>0.9 – 2.4</td><td>18.0 – 62.7</td></tr>
<tr><td>Proteins</td><td>0.6 – 1.1</td><td>11.1 – 20.0</td></tr>
<tr><td>Fat</td><td>0.10 – 0.15</td><td>1.7 – 2.5</td></tr>
<tr><td>Minerals(Ash)</td><td>0.3 – 0.9</td><td>4.4 – 15.9</td></tr>
</table></td><td>Moisture: 94.6%
Total solids: 5.4%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>47</td><td>18.2</td></tr>
<tr><td>Fibres</td><td>30</td><td>3.5</td></tr>
<tr><td>Proteins</td><td>16</td><td>3.6</td></tr>
<tr><td>Fat</td><td>2.1</td><td>0.3</td></tr>
<tr><td>Minerals(Ash)</td><td>7.5</td><td>2.8</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	The main colouring principles of red radish are anthocyanins. The major anthocyanidin present in red radish is pelaragonidin and its derivatives Spring cultivars (n=22) had pigmentation in the root from 11 to 32 mg/100 g FW, while in the skin, ranging from 39.3 to 185 mg ACN/100g skin. Red-fleshed winter cultivars (n=5) had pigment content ranging from 12.2 to 53 mg ACN/100g root. ACN profiles were similar for different cultivars, the major pigments being pelargonidin-3-sophoroside-5-glucoside, mono- or di-acylated with cinnamic and malonic acids; individual proportions varied among cultivars. Quantification by absorption spectrophotometry at 530nm; pH differential method; HPLC, expressed as mg cyaniding equivalent give similar results Carotenoids - beta carotene, lutein	**Total anthocyanins** (based on 8 peer reviewed articles, and data reported by industry) n = 24 5-350 mg/100 g FW Robust mean 93 mg/100 g FW Robust SD 84 mg/100 g FW 93-6485 mg/100 g DW Robust mean 1700 mg/100 g DW Robust SD 1550 mg/100 g DW Carrotenoids 0.1-0.4 mg/100 g DW	Robust mean + robust SD 3200 mg/100 g DW (3.2%)
Aromatic constituents	The specific sharp, pungent flavour of radish result from the degradation of glucosinolates (sulphur-containing compounds in cruciferous vegetables) during mastication or processing. Four radish glucosinolates (glucoraphenin, dehydroerucin, glucobrassicin, and glucoerucin) were identified by LC–MSn from root extracts and dehydroerucin was found to be the major glucosinolate in red radish roots. The reported content of the total glucosinolates ranged from 45-164 mg/100 g fresh weight in Chinese radishes	Total glucosinate - 45-164 mg/100 g FW	50 mg/100 g FW

Information on the source material used for the production of extracts with colouring properties

Source material:	**Safflower *(Carthamus tinctorius L.)* , petals**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Safflomin A and B** Typical process of the concentrate/extract: Dried petals -> Water extraction -> filtration -> concentration/evaporation

Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 4.7 - 45.0% fresh base
Dry matter(Total solids): 55.0 – 95.3% fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>10.3 – 35.0</td><td>11.6 – 36.7</td></tr>
<tr><td>Fibres</td><td>11.6 - 40.0</td><td>12.2 – 43.5</td></tr>
<tr><td>Proteins</td><td>1.8 – 16.0</td><td>1.9 – 21.8</td></tr>
<tr><td>Fat</td><td>0.5 – 8.5</td><td>0.9 – 9.2</td></tr>
<tr><td>Minerals(Ash)</td><td>6.5 – 14.0</td><td>7.6 – 18.2</td></tr>
</table></td><td>Moisture: 22.0%
Total solids: 78.0%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>24</td><td></td></tr>
<tr><td>Fibres</td><td>23.8</td><td></td></tr>
<tr><td>Proteins</td><td>13</td><td></td></tr>
<tr><td>Fat</td><td>5.5</td><td></td></tr>
<tr><td>Minerals(Ash)</td><td>12.8</td><td></td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	Safflower flowers contain two pigments viz. red (carthamin) which is insoluble in water and yellow (carthamidin) which is soluble in water. Cartamidin is called also Carthamus Yellow, a flavonoid, is obtained by extracting the corolla (petals) of Carthamus tinctorius L. with water or slightly acidified water and drying the extract. The principal colouring matters are safflomin A (hydroxysafflor yellow A) and safflomine B (safflor yellow B) Almost no data from any literature found on the pigment content in safflower petals. Therefore the ranges were derived from the information provided by stakeholders. Safflower petals contain about 30% yellow pigment and 0.83% red pigment (Nagaraj, et al., 2001; Kulkarni et al.,2001). The content of the yellow pigment is not confirmed. Only abstract from the proceedings, not possible to find the article. Carthamin – minor pigment The carthamin content in extract depends on the color of the flower. Thus, the extract from orange-red flowers contained the maximum amount of carthamin, whereas carthamin was not detected in the extract from yellow flowers	Safflomin A+B 0.2-12 g/100 g DW reported by the industry.	3-12 g/100 g DW
Aromatic constituents	Main volatile components in flower were terpene compounds, including p-cymene, limonene, camphor, 4-terpineol, selinene, torreyol and 10 acids including 3-methylbutanoic acid, 2-methylbutanoic acid…		Not feasible to suggest reference value due to non-identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Spinach (*Spinacia oleracea L.*) leaves**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: Chlorophylls Typical process of the concentrate/extract : Extraction from dried spinach leaves

Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 90.1 – 94.0 % fresh base
Dry matter(Total solids): 6.0 – 9.9 % fresh base

<table><tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr><tr><td>Carbohydrates, (without fibres)</td><td>0.3 – 4.1</td><td>3.3 - 41.7</td></tr><tr><td>Fibres</td><td>1.2 – 2.9</td><td>10.0 – 35.9</td></tr><tr><td>Proteins</td><td>1.5 – 3.7</td><td>22.8 – 40.2</td></tr><tr><td>Fat</td><td>0.2-0.8</td><td>2.0 – 8.6</td></tr><tr><td>Minerals(Ash)</td><td>0.4 – 1.8</td><td>3.8 – 25.5</td></tr></table></td><td>Moisture: 92 %
Total solids: 8%

<table><tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr><tr><td>Carbohydrates, (without fibres)</td><td>14</td><td>8.0</td></tr><tr><td>Fibres</td><td>26</td><td>6.3</td></tr><tr><td>Proteins</td><td>33</td><td>4.4</td></tr><tr><td>Fat</td><td>5</td><td>2.0</td></tr><tr><td>Minerals(Ash)</td><td>11</td><td>5.2</td></tr></table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	The colour of spinach, is attributable the presence of various pigments, which primarily are the green chlorophylls and the yellow, orange, and red carotenoids however only the green chlorophylls are seen because they mask the bright colours of the carotenoids. Eight individual chlorophylls (chlorophyll b, chlorophyll a, chlorophyll b', chlorophyll a', chlorophyll b-1, chlorophyll a-1, pheophytin b, and pheophytin a), 8 individual xantophylls [(all-E)-neoxanthin, (9'Z)-neoxanthin, (all-E)-violaxanthin, neochrome, (all-E)-lutein epoxide, (all-E)-lutein, neolutein B, and neolutein A), and b-carotene were separated and identified in the spinach samples	**Chlorophylls** (based on 9 peer reviewed articles and data reported by industry) n = 14 220-1800 mg/100 g DW Robust mean 940 mg/100 g DW Robust SD 307 mg/100 g DW **Lutein** 40-130 mg/ 100 g DW **Beta carotene** 20-80 mg/100 d DW **Total carotenoids** 110-180 mg/100 d DW	Chlorophylls Robust mean + robust SD 1200 mg/100 g DW (1.2 %)
Aromatic constituents	(Z)-3-hexenal, methanethiol, (Z)-1,5- octadien-3-one, dimethyl trisulphide, octanal, 2-isopropyland 2-sec-butyl-3-methoxypyrazine are odorants of raw spinach.	No quantitative data	Not feasible to suggest reference value due to non-identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Spirulina (*Arthrospira platensis*) algae**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: Phycocyanins (C-phycocyanin and Allophycocyanin) Typical process of the concentrate/extract : Algae -> Water extraction -> filtration -> concentration/evaporation

Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 90.5 – 91.6% fresh base
Dry matter(Total solids): 8.4 – 9.5% fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>2.0 – 2.5</td><td>21.3 – 28.6</td></tr>
<tr><td>Fibres</td><td>0.3 – 0.5</td><td>3.6 – 6.0</td></tr>
<tr><td>Proteins</td><td>5.90 – 5.92</td><td>61.8 – 63.7</td></tr>
<tr><td>Fat</td><td>0.1 – 0.4</td><td>4.18 – 4.2</td></tr>
<tr><td>Minerals(Ash)</td><td>0.3 – 0.6</td><td>2.9 – 6.3</td></tr>
</table></td><td>Moisture: 90.5%
Total solids: 9.5%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>23</td><td>2.5</td></tr>
<tr><td>Fibres</td><td>4</td><td>0.2</td></tr>
<tr><td>Proteins</td><td>60</td><td>28.4</td></tr>
<tr><td>Fat</td><td>4.2</td><td>2.4</td></tr>
<tr><td>Minerals(Ash)</td><td>4.4</td><td>1.5</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference range/ value
Pigment(s)	Spirulina containes three biliproteins: c-phycocyanin, allophycocyanin and phycoerythrin. c-Phycocyanin is water soluble blue pigment, the major phycobiliprotein in Spirulina and may constitute up to 20% of the dry weight of Spirulina. Spirulina is good source of carotenoids and chlorophyll as well.	**Phycocyanins** (based on 12 peer reviewed articles and data reported by industry) n = 24 1-22.3 g/100 g DW Robust mean 12.9 g/100 g DW Robust SD 6.5 g/100 g DW **Carotenoids** (beta carotene and lutein) 0.3-3.0 mg/100 g DW **Chlorophyll a** 0.8-1.7 mg/100 g DW	**Phycocyanins** Robust mean + robust SD 19.4 g/100 g DW (19.4 %)
Aromatic constituents	*No data*	No data	Not feasible to suggest reference value due to non-identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Purple-fleshed sweet potato, Ipomoea batatas (L.), tubers (storage root)**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Anthocyanins** Extraction from tubers with water and citric acid, filtration and concentration.

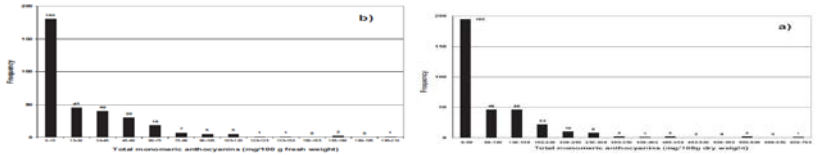
Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 67.2 – 80.3% fresh base
Dry matter(Total solids): 19.7 – 32.8% fresh base

<table>
<tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>11.6 – 28.3</td><td>58.9 – 88.4</td></tr>
<tr><td>Fibres</td><td>1.7 – 3.14</td><td>5.1 – 13.7</td></tr>
<tr><td>Proteins</td><td>1.0 – 1.7</td><td>3.0 – 7.0</td></tr>
<tr><td>Fat</td><td>0.1 – 0.3</td><td>0.7 – 1.5</td></tr>
<tr><td>Minerals (Ash)</td><td>0.4 – 1.1</td><td>1.3 – 2.8</td></tr>
</table></td><td>Moisture: 75%
Total solids: 25%

<table>
<tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr>
<tr><td>Carbohydrates, (without fibres)</td><td>78</td><td>5.2</td></tr>
<tr><td>Fibres</td><td>10</td><td>3.4</td></tr>
<tr><td>Proteins</td><td>5.6</td><td>1.6</td></tr>
<tr><td>Fat</td><td>1</td><td>0.5</td></tr>
<tr><td>Minerals (Ash)</td><td>2.2</td><td>0.6</td></tr>
</table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	Purple colour in sweet potatoes is due to anthocyanins that are mono- or diacylated forms of peonidin and cyanindin. The anthocyanin in sweet potatoes  content varied widely from 0 to 663 mg /100 g dry powder. Most of the genotypes (85%) contained <150 mg TA/100 g dry powder; 12% of the genotypes had 150-300 mg TA/100 g powder and about 3% of the genotypes has >300 mg TA/100g powder. On the fresh weight basis, the TMA contents were in a range of 0–210 mg/100 g with about 80% of the genotypes had <50 mg TA/100 g, 16% contained 50–100 mg TA/100 g, and about 4% of the genotypes contained >100 mg TA/100 g. The sweet potato genotypes with an anthocyanin content of >150 mg/100 g fresh weight (>400 mg/100 g dry weight) had a very intense purple-flesh colour. There is potential for further increasing the anthocyanin levels in PFSP through breeding efforts. In the past few years, sweet potato cultivars with deep purple flesh (PFSP) were developed in Japan, Korea, New Zealand, and other countries - Yamagawa-murasaki and Ayamurasaki cultivars.. Higher pigment content was reported for purple cultivar Urenika, containing an average of 184 mg petunidin and malvidin-based anthocyanins/100 g tuber flesh. Andian purple sweet potato confirmed an average of 182 mg/100g FW or 618 md/100 g DW Carotenoids	**Total anthocyanins** (based on 6 peer reviewed articles, and data reported by industry) n = 36 5-1440 mg/100 g DW Robust mean 340 mg/100 g DW Robust SD 290 mg/100 g DW Carotenoids 2-28 mg/100 g DW	Robust mean + robust SD 630 mg/100 g DW (0.63%)
Aromatic constituents	Approximately 50 compounds have been reported to contribute to raw potato aroma [VCF]. It is reported that two compounds represent typical potato aroma in raw potato: methional and (E,Z)-2,6-nonadienal. Other important volatiles in raw potatoes produced via degradation of fatty acids are 1-penten-3-one, heptanal, 2-pentyl furan, 1-pentanol and (E,E)-2,4-heptadienal. Pyrazines such as 3-isopropyl-2-methoxypyrazine could be responsible for the earthy aroma of potato.		Not feasible to suggest reference value due to scarce data and no n identified key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Tomato *(Lycopersicon esculentum L.)*, whole fruit**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Lycopene** Fresh tomatoes Crushing-filtration -pasteurisation-centrifugation and/or fluidised bed drying

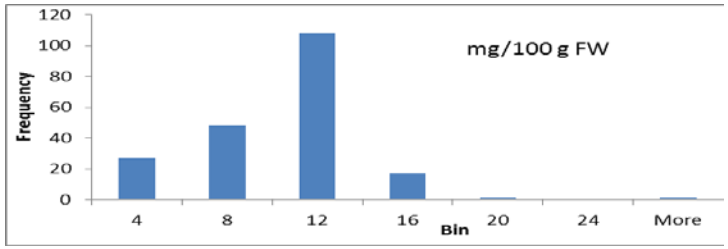
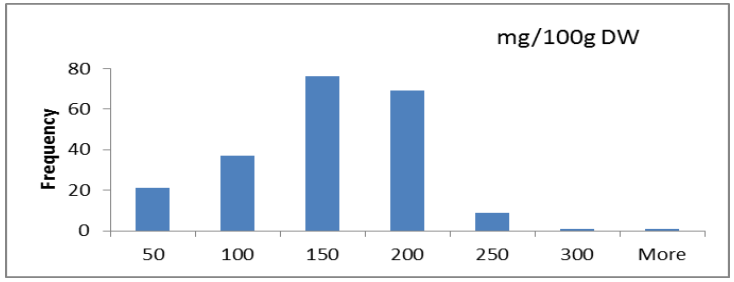
Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 90.6 – 95.4% fresh base
Dry matter(Total solids): 4.6 – 9.4% fresh base

<table><tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr><tr><td>Carbohydrates, (without fibres)</td><td>0.6 – 5.7</td><td>11.1 – 81.9</td></tr><tr><td>Fibres</td><td>0.5 – 4.0</td><td>8.7 – 44.0</td></tr><tr><td>Proteins</td><td>0.6 – 1.3</td><td>8.6 – 22.0</td></tr><tr><td>Fat</td><td>0.1 – 0.8</td><td>1.44 – 8.7</td></tr><tr><td>Minerals(Ash)</td><td>0.3 – 0.7</td><td>3.9 – 10.5</td></tr></table></td><td>Moisture: 94 %
Total solids: 6%

<table><tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr><tr><td>Carbohydrates, (without fibres)</td><td>48</td><td>9.9</td></tr><tr><td>Fibres</td><td>20</td><td>5.2</td></tr><tr><td>Proteins</td><td>15</td><td>3.4</td></tr><tr><td>Fat</td><td>3.8</td><td>1.1</td></tr><tr><td>Minerals(Ash)</td><td>5.8</td><td>1.7</td></tr></table></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	The carotenoid composition of tomatoes is relatively simple with lycopene accounting for 90–95% of total carotenoids (other study 80-90%)  	**Total lycopene** (based on 17 peer reviewed articles, 1 database and data reported by industry) n = 248 0.1-25 mg/100 g FW Robust mean 8.1 mg/100 g FW Robust SD 3.8 mg/100 g FW 8-474 mg/100 g DW Robust mean 130 mg/100 g DW Robust SD 54 mg/100 g DW Beta-carotene 10-17 mg/100 g DW upper range (6.3% total solids); < 5 mg/100 g DW lower range (6.3% total solids); Lutein 1.5-3.2 mg/100 g DW upper range (6.3% total solids); < 0.5 mg/100 g DW lower range (6.3% total solids);	Robust mean + robust SD 180 mg/100 g DW (0.18 %)
Aromatic constituents	The character impact of fresh tomato is delineated by 2-iso-butylthiazole (0.00043-0.7 mg/kg) and (Z)-3-hexenal, with modifying effects from β-ionone and β-damascenone. Alternatively, dimethyl sulphide and furfural are major contributors to the flavour of thermally processed tomato paste after the almost complete loss of cis-3-hexenal during processing.	2-iso-butylthiazole (0.00043-0.7 mg/kg)	Not feasible to suggest reference value due to scarce data on the key-aroma compounds

Information on the source material used for the production of extracts with colouring properties

Source material:	**Turmeric *(Curcuma Longa L.)*, rhizomes**
Colouring principle of the extract/concentrate and/or extraction/concentration method:	Colouring principle: **Curcuminoids** *Typical process of the concentrate / extract:* Turmeric root -> mechanical separation -> concentration and filtration

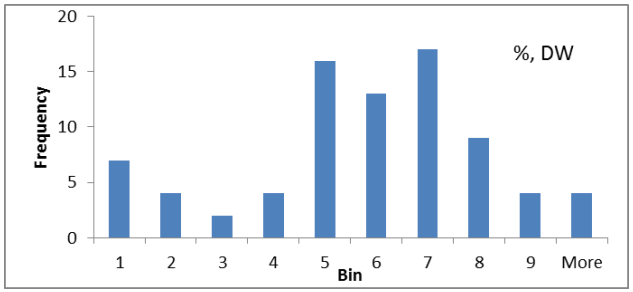
Data available on the source material as regards the content of:

<table>
<tr><th></th><th>List</th><th>Data from literature available about the content</th><th>Suggested reference value</th></tr>
<tr><td>Nutritive constituents</td><td>Water
Dry Matter- Total Solids

Carbohydrates
Fibres
Fat
Proteins
Minerals (Ash)</td><td>Water: 6.0 – 16.4% fresh base
Dry matter(Total solids): 83.6 – 94.0% fresh base

<table><tr><th>Range</th><th>FW base,%</th><th>DW base,%</th></tr><tr><td>Carbohydrates, (without fibres)</td><td></td><td>49.4 – 73.0</td></tr><tr><td>Fibres</td><td></td><td>5.9 – 26.</td></tr><tr><td>Proteins</td><td></td><td>7.4 – 12.9</td></tr><tr><td>Fat</td><td></td><td>3.0 – 11.2</td></tr><tr><td>Minerals(Ash)</td><td></td><td>3.3 – 11.9</td></tr></table>.</td><td>Moisture: 11.4 %
Total solids: 88.6 %

<table><tr><th>Ref. value</th><th>DW, %</th><th>SD, %</th></tr><tr><td>Carbohydrates, (without fibres)</td><td>59</td><td></td></tr><tr><td>Fibres</td><td>16</td><td></td></tr><tr><td>Proteins</td><td>9.7</td><td></td></tr><tr><td>Fat</td><td>7.2</td><td></td></tr><tr><td>Minerals(Ash)</td><td>4.5</td><td></td></tr></table></td></tr>
<tr><td>11.4</td><td></td><td></td><td></td></tr>
</table>

	List	Data from literature available about the content	Suggested reference value
Pigment(s)	The characteristic yellow-orange curcuminoids found in rhizomes are used for colouring food and textiles. The main yellow bioactive substances in the rhizomes are due to curcumin, demethoxycurcumin and bisdemethoxycurcumin. The Thai Herbal Pharmacopoeia recommended that dried turmeric should contain not less than 6.0% v/w of turmeric oil and 5.0% w/w of total curcuminoids (THP, 1995). Quantitation of total curcuminoids were performed by spectroscopy or HPLC after identification. 	**Total curcuminoids** (based on 10 peer reviewed articles and data reported by industry) n = 90 0.1-12 g/100 g DW Robust mean 5.3 g/100 g DW Robust SD 2.6 g/100 g DW	Robust mean + robust SD 7.9 g/100 g DW (7.9 %)
Aromatic constituents	Turmerone and ar-turmerone are known to be the character impact compounds of turmeric contributing to the dry turmeric aroma. These compounds together with 1:8 cineol that imparts a camphory note has been reported to be responsible for the top note of the spice	7-25 g/100 g DW ar-turmerone; 7-30 g/100 g DW turmerone	

European Commission
EUR 27425 EN – **Joint Research Centre – Institute for** Reference Material and Measurement

Title: **Provision of scientific and technical support with respect to the classification of extracts/concentrates with colouring properties either as food colours (food additives falling under Regulation (EC) No 1333/2008) or colouring foods**

Author(s): Stefanka Bratinova

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