Abstract:
The present invention relates to a colorant comprising a water-soluble natural pigment, such as betanin (beetroot red), formulated in a water-in-oil-in-water (W/O/W) double emulsion using a gelling agent in the internal aqueous phase (W₂).
DOUBLE EMULSION-TYPE COLORANT WITH GELLING AGENT IN THE INTERNAL AQUEOUS PHASE

FIELD OF THE INVENTION

The present invention relates to a colorant comprising a water-soluble natural pigment, such as betanin (or beetroot red), formulated in a water-in-oil-in-water (Wi/0/W₂) emulsion using a gelling agent in the internal aqueous phase (Wi). Furthermore, the present invention relates to a process for preparing a Wi/0/W₂ double emulsion type colorant comprising a water-soluble natural pigment, such as betanin, in the internal aqueous phase and to methods for coloring of products, such as food- and feedstuffs, pharmaceuticals and cosmetics, using a Wi/0/W₂ double emulsion type colorant.

TECHNICAL BACKGROUND AND PRIOR ART

Water-in-oil-in-water (Wi/0/W₂) double emulsions, or multiple emulsions, are emulsion systems where small water droplets are entrapped within larger oil droplets that in turn are dispersed in a continuous water phase. Double emulsions find many applications in industries such as food, pharmaceuticals and cosmetics by enclosing useful substances inside the small water droplets.

Double emulsions, either Wi/0/W₂ or Oi/W₀/W₂ emulsions, are generally prepared using a 2-step procedure. For Wi/0/W₂ emulsions, first, a water-in-oil (Wi/O) emulsion is formed by blending a first (internal) aqueous phase (Wi) and an oil phase (O) together in the presence of a suitable oil-soluble (e.g. low hydrophilic-lipophilic balance (HLB) number) emulsifier. This emulsifier adsorbs to the surface of the water droplets and forms a protective coating that prevents their subsequent aggregation. Furthermore, the emulsifier reduces the interfacial tension between the oil and the water phase, favoring the formation of droplets, and increasing the stability of emulsions. Second, a Wi/0/W₂ emulsion is formed by blending the Wi/O emulsion with a second (external) aqueous phase (W₂) containing a suitable water-soluble (e.g. high HLB number) emulsifier. This emulsifier adsorbs to the surface of the oil droplets and forms a protective coating that prevents their subsequent aggregation. The emulsifier also reduces the interfacial tension between the water and the oil phase, favoring the formation of droplets, and increasing the stability of the emulsions. The first step is usually carried out in a high-shear device to produce very fine droplets. The second emulsification step is carried out in a low-shear device to avoid rupturing the multiple droplets.
WO 2012/059590 (Mason and Bonnet) relates to a multiple emulsion of the W/O/W type for stabilization of natural coloring agents against changes in pH values, oxidation and light as well as ingredient interactions.

McClements et al. (WO 2008/021531) describe multi-phase emulsion compositions comprising in a first aqueous phase a biopolymeric gelling component.

US 5,332,595 (Gaonkar) is directed to stable W/O/W and 0/W/O multiple emulsion which include a substantially continuous gelatinous membrane at an aqueous/oil interfacial region of the emulsions and a concentrated flavor encapsulated in the internal phase.

Due to consumer preferences synthetic food colorants are steadily being replaced by natural ones. However, in comparison with the natural coloring agents, the synthetic coloring agents show greater resistance and stability when exposed to oxidation, changes in temperature, pH and other factors.

Anthocyanins (polyphenolic pigments) are natural, water-soluble, nontoxic pigments displaying a variety of colors from orange to blue. Anthocyanin-rich extracts from fruits and vegetables can be used as food colorants.

Van den Horst and Langelaan (1999) analyzed the separation of the aroma and color compounds of red cabbage concentrate in multiple emulsions.

However, the main drawback in the use of anthocyanins as food colorants is their low stability. In fact, the color stability of anthocyanins depends on a combination of factors: structure and concentration of anthocyanins, pH, temperature, and presence of complexing agents (phenols, metal ions) (Markakis, P. Stability of anthocyanins in foods. In Anthocyanins as Food Colors; Markakis, P., Ed.; Academic Press: New York (1982); pp 163-180.). Anthocyanins are stable red-colored compounds when the pH of the bulk medium is around 4. At pH 6-7, which is typical of many foods including milk products, anthocyanins are no longer red but instead violet-blue. Since the color of anthocyanins changes with pH, it can be challenging to achieve a red color in many pH-neutral foods using anthocyanins.

Carminic acid is the free coloring principle extracted from the female cochineal insects Dactylopius coccus costa (Coccus cacti L). It is a water-soluble natural color that is orange at low pH (where it is most stable), purple at neutral pH, and blue at alkaline
Carmine is the aluminum or calcium-aluminum lake of carminic acid on an aluminum hydroxide substrate. It is dispersible but not soluble in water, although it can be dissolved in acidic or alkaline solutions.

Betanin is a glucosid. Its aglycone, obtained by hydrolyzing away the glucose molecule is betanidin.

Betanin (or beetroot red) is a water-soluble pigment usually purified from red beet juice concentrate. It has been approved by the European Union as a food additive with the E-number E162.


The color shades of betanin enables broadening of the palette of natural colorants and make it a useful alternative to using other red/pink pigments, such as e.g. carminic acid which is extracted from female cochineal insects and the carotenoids such as lycopene, in cosmetics and edible products, especially food products. However, betanin breaks down in the presence of oxygen, heat, and light, with increased kinetics at pH<3.0 or pH>5.0 and has found use as a coloring agent mainly in frozen food products, such as ice cream, meat and sausages, as well as food products with a short shelf-life.

In conventional Wi/O/W₂ double emulsions betanin may, depending on the oil component used, come in contact with unsaturated fatty acids, which can initiate the oxidation of the pigment. So far, betanin has mainly been used in the form of red beet juice concentrate for coloring of edible products.

Thus, the sensitivity of betanin especially towards oxidation, oxidizing fatty acids and free radicals is a commonly known problem and pose a limitation to the applications in which the natural pigment can be used.

There is, thus, a continuous industrial need for natural pigment, and especially betanin, colorants with improved stability and methods for coloring food products, cosmetics and pharmaceutical products these colorants and for methods to render these coloring agents more stable to external factors during use.
SUMMARY OF THE INVENTION

It is an object of the present invention to provide means for stabilizing and protecting water-soluble natural pigments, such as betanin, towards factors such as oxidation, ingredient interactions and temperature fluctuations.

Additionally, it is an object of the present invention to provide a method for coloring edible products, such as foodstuffs and beverages, with a stable \( W_{1}/0/W_{2} \) emulsion type colorant comprising an internal aqueous phase comprising water-soluble natural pigments, such as betanin, and a gelling agent.

Other objects, features, benefits and advantages of the present invention will be apparent from the summary and the following description and will be readily apparent to those skilled in the art.

The inventors of the present invention have found that by encapsulating betanin in the internal aqueous phase (\( W_{1} \)) of a \( W_{1}/0/W_{2} \) emulsion the coloring agent could be stabilized against exposure to heat and storage. It was found that the use of a gelling agent, optionally combined with a salt (to facilitate gelling and to prevent releasing behavior), in the internal aqueous phase lead to a surprising improvement in the stability of betanin towards heat treatment, and oxidation during process or storage when the internal aqueous phase was allowed to gelate during preparation of the double emulsion.

In addition, the \( W_{1}/0/W_{2} \) emulsion proved to stabilize the betanin against other changes in the environment such as ingredient interactions.

It is contemplated that other water-soluble pigments, such as carminic acid and anthocyanins, may benefit from encapsulation in an internal aqueous phase (\( W_{1} \)) of a \( W_{1}/0/W_{2} \) emulsion wherein the internal aqueous phase comprises a gelling agent and the internal aqueous phase is allowed to gelate during preparation of the double emulsion.

In a first aspect the present invention provides a \( W_{1}/O/W_{2} \) emulsion colorant comprising:

a) an internal aqueous phase (\( W_{1} \)) comprising a water-soluble natural pigment, such as betanin, and at least one gelling agent,

b) an oil phase (\( O \)) comprising at least one oil-soluble emulsifier, and
c) an external aqueous phase \((W_2)\) comprising at least one water-soluble emulsifier.

A second aspect relates to a method for preparing the Wi/0/W \(_2\) emulsion colorant according to the first aspect of the invention; the method comprising the steps:

a) mixing a water-soluble natural pigment, such as betanin, and at least one gelling agent with water to form a first aqueous solution (Wi) under conditions wherein no significant gelling occurs,

b) mixing an oil component with at least one oil-soluble emulsifier to form an oil solution,

c) blending or homogenizing the first aqueous solution and the oil solution together under conditions wherein no significant gelling occurs to create a water-in-oil (Wi/O) emulsion,

d) inducing at least partial gelation of the betanin and the gelling agent within the first/internal aqueous phase \((Wi)\),

e) mixing a second aqueous solution with at least one water-soluble emulsifier to form a second aqueous solution \((W_2)\), and

f) blending or homogenizing the Wi/O emulsion with the second aqueous solution to create the Wi/0/W \(_2\) emulsion colorant.

A third aspect of the present invention relates to a method for coloring edible products, such as food- and feedstuffs and pharmaceutical products, the method comprising; providing an edible product, and incorporating into the edible product the Wi/0/W \(_2\) emulsion colorant according to the first aspect of the invention.

In a fourth aspect the present invention is directed to an edible product comprising the Wi/0/W \(_2\) emulsion colorant according to the first aspect of the invention.

In a fifth aspect the present invention is directed to the use of a Wi/0/W \(_2\) emulsion colorant comprising a water-soluble pigment, such as betanin, and a gelling agent in the internal aqueous phase for coloring of an edible product.
A sixth aspect of the present invention is directed to the use of a Wi/0/W \textsubscript{2} emulsion colorant according to the first aspect of the invention for coloring of an edible product.

A seventh aspect of the present invention relates to the use of a Wi/0/W \textsubscript{2} emulsion colorant comprising a water-soluble pigment, such as betanin, and a gelling agent in the internal aqueous phase for protecting the water-soluble pigment against oxidation.

**BRIEF DESCRIPTION OF THE FIGURES**

FIGURE 1 shows the global spectrum (Figure 1A), the chromatogram at 280 nm (Figure 1B) and the chromatogram at 480 nm (Figure 1C) of red beet juice concentrate.

FIGURE 2 shows the global spectrum (Figure 2A), the chromatogram at 280 nm (Figure 2B) and the chromatogram at 480 nm (Figure 2C) of betanin, vacuum-dried.

FIGURE 3 depicts double emulsions according to the invention with 0.3% betanin.

FIGURE 4 shows an example of the particle size distribution of a betanin double emulsion according to the invention.

FIGURE 5 shows an example of a microscopic image (x100) of a betanin double emulsion according to the invention.

FIGURE 6 shows smears on a piece of cardboard of acidic slurry comprising W/O/W emulsion of betanin in low/average concentration and high concentration (Figure 6A) and increasing concentrations of betanin in yoghurts prepared with slurries comprising increasing concentrations of W/O/W emulsion of betanin (Figure 6B).

FIGURE 7 depicts yoghurt samples prepared with a slurry colored with a betanin Wi/0/W \textsubscript{2} emulsion containing a gelling agent in the internal aqueous phase (Wi) (Figure 7A) and yoghurt samples prepared with a slurry colored with betanin W\textsubscript{3}/O/W \textsubscript{2} emulsion without gelling agent (Figure 7B). Np: Before pasteurization of the colored slurry; pasto: Directly after pasteurization of the colored slurry; pasto J+7; After pasteurization of the colored slurry followed by storage of the colored yoghurt for 7 days.
at 4°C in the dark; pasto J+14; After pasteurization followed by storage of the colored yoghurt for 14 days at 4°C in the dark; pasto J+21; After pasteurization followed by storage of the colored yoghurt for 21 days at 4°C in the dark; pasto slurry M+1; After pasteurization of the colored slurry followed by storage of this slurry for 30 days at 4°C in the dark.

FIGURE 8 depicts yoghurt samples colored with standard betanin colorant at betanin concentrations of 5 ppm, 10 ppm, 15 ppm and 20 ppm (Figure 8A) or with betanin double emulsion colorant according to the invention at betanin concentrations of 5 ppm, 10 ppm, 15 ppm and 20 ppm (Figure 8B). The colorants were employed in an acidic slurry (pH 3.8) before mixing with the yoghurt, and samples were taken out before heat treatment of the colored slurry (top row), after pasteurization at 90°C for 5 min of the colored slurry (middle row) and after 28 days storage of the colored yoghurts at 4°C in the dark (bottom row).

FIGURE 9 depicts samples of meat sausage colored with standard betanin color at a concentration of 35 ppm betanin (Figure 9A) and with the betanin double emulsion colorant according to the invention at a concentration of 22 ppm betanin (Figure 9B) and then submitted to heat treatment.

DETAILED DISCLOSURE OF THE INVENTION

The inventors of the present invention have by extensive research found that the stability of betanin can be increased by solubilizing betanin and a gelling agent in the internal aqueous phase of a Wi/O/W_2 double emulsion and allowing gelation of the international aqueous phase to take place. Especially, the stability of betanin towards storage and heating (pasteurization) is increased considerably by encapsulation in the double emulsion according to the invention.

Accordingly, a first aspect of the present invention relates to a water-in-oil-in-water (Wi/O/W_2) double emulsion colorant comprising:

a) an internal aqueous phase comprising a water-soluble natural pigment and at least one gelling agent,

b) an oil phase comprising an oil-soluble emulsifier, and

c) an external aqueous phase comprising a water-soluble emulsifier.
In a preferred embodiment the water-soluble natural pigment is selected from the groups comprising betanin, carminic acid and other anthraquinones, norbixin anthocyanins, chlorophyll, carthamus, caramel, malt and carbo vegetabilis.

In a more preferred embodiment the water-soluble natural pigment is betanin.

Also, other pigments for shifting the shade of betanins may further be included in the double emulsion, like water soluble pigments (for instance carminic acid or anthocyanins) in the internal or in the external aqueous phases, or oil-soluble pigments (for instance carotenes, xanthophylls, paprika, turmeric, annatto) in the oil phase of the double emulsion.

The term "gelling agent" refers herein to a substance which modifies the properties of the colloidal mixture of an aqueous gellable composition by forming a gel.

The term "gel" refers herein to a more or less firm cohesive internal structure behaving like a solid (no flow when in the steady-state).

It is part of the present invention that the at least one gelling agent is gelled within the internal aqueous phase.

The presence of a gelling agent in the internal aqueous phase modifies the texture of the internal aqueous phase and makes it behave like a solid, thus, limiting exchanges.

In some cases it may be desirable to limit gelation to a gelatinous layer at the surface of the internal aqueous droplets. In any case the gelation should provide a substantially continuous barrier or shell constituting a physical protection of the water-soluble natural pigment. The extent and thickness of the gel may be varied by adjusting the concentrations of gelling agent and salt as will be clear to the skilled person.

The internal aqueous phase (\(w_1\)) preferably constitutes from 10 to 25 wt.-% of the double emulsion, such as from 12 to 23 wt.-%, such as from 14 to 20 wt.-%, such as from 15 to 18 wt.-% of the double emulsion. The internal aqueous phase comprises purified betanin pigments, at least one gelling agent, optionally at least one salt (depending on the gelling agent and osmotic pressures) and optionally ascorbic acid.

Betanin pigments must represent from 1.5 to 5.0 wt.-% of the internal aqueous phase, more preferably from 2.0 to 4.0 wt.-%, such as from 2.5 to 3.5 wt.-%, and most preferably from 3.0 to 3.2 wt.-%.
The gel increases the stability of the double emulsion and controls the release of pigment molecules from the internal aqueous phase to the external aqueous phase, thus limiting the oxidation of the natural pigment. The gel induces stasis and limits exchanges, thus improving the isolation of the natural pigment from oxygen. This also protects the natural pigment from the oil phase and potentially oxidizing fatty acids or free radicals.

In a preferred embodiment the at least one gelling agent is a heat-induced gelling agent. The heat-induced gelling agent must have the property to be in a liquid form at high temperatures (at least over 40°C) and to gel after cooling down (known as being "thermo-reversible"). The choice of gelling agent will determine the heating temperature at which to prepare the W₁ and O phases, which must be blended to form the Wi/O emulsion over the gelling point of the chosen gelling agent (from 20 to 70°C depending on gelling products).

In an even more preferred embodiment the gelling agent is selected from the group consisting of fish gelatin, pork gelatin, bovine gelatin, poultry gelatin, collagen, albumins, pectins, chitosan, agar, gellan gum, locust bean gum and carrageenans.

In an even more preferred embodiment the at least one gelling agent is gelatin or iota and/or kappa carrageenans, more preferably the at least one gelling agent is iota carrageenans.

The at least one gelling agent is used in a concentration of from 0.5 to 10.0 wt.-% of the internal aqueous phase, preferably from 1.0 to 9.0 wt.-%, such as from 2.0 to 8.0 wt.-%, such as from 2.5 to 7.0 wt.-%, such as from 3.0 to 6.0 wt.-%.

In a preferred embodiment, salts, such as sodium chloride (NaCl), sodium citrates (Na₃C₆H₅O₇, Na₂C₆H₂O₇, or Na₃C₆H7O7), sodium hexametaphosphate (Na₆(P₂O₇)₆), sodium sulfate (Na₂SO₄), calcium chloride (CaCl₂), calcium lactate (CaC₆H₁₀O₇), calcium lactate gluconate (CaC₆H₁⁶O₁₀), calcium gluconate (CaCl₂H₂2O₁₄) is added to the internal aqueous phase to prevent releasing behavior. For instance, if gelatin is used as gelling agent, NaCl can with benefit be added at a weight ratio of 1/1. If iota carrageenans are used as gelling agent, NaCl at a weight ratio of 2/1 and CaCl₂ at a weight ratio of 0.1/1 can with benefit be added. Other specific mixtures of iota and kappa carrageenans might not require additional salts.

Thus, in a preferred embodiment the Wi/O/W₂ emulsion colorant further comprises at least one salt in the internal aqueous phase (Wi), in a concentration sufficient to enable a firm texture upon gelation.

A hydrophilic antioxidant may be added to the internal aqueous phase.
In a preferred embodiment the hydrophilic antioxidant is natural or synthetic ascorbic acid. The concentration of the hydrophilic antioxidant is preferably from 0 to 10 wt.-% of the water phase, more preferably from 0.5 to 9 wt.-%, such as from 1 to 8 wt.-%, such as from 1.5 to 7 wt.-%, such as from 2 to 6 wt.-%, such as from 2.5 to 5 wt.-%.

Preferably, the oil phase (O) constitutes from 15 to 30 wt.-%, such as from 17 to 29 wt.-%, such as from 19 to 28 wt.-%, such as from 21 to 27 wt.-%, such as from 23 to 26 wt.-% of the double emulsion. The oil phase comprises oil, an oil-soluble emulsifier and optionally a hydrophobic antioxidant.

For the oil phase, vegetal oils, oils of animal origin and mineral oils can be used for the formation of the Wi/O emulsion including but not limited to fatty acids (saturated or unsaturated), glycerols, glycerides and their respective derivatives, phospholipids and their respective derivatives, glycolipids, phytosterol and/or sterol esters (e.g. cholesterol esters, phytosterol esters and derivatives thereof), as may be required by a given food or beverage end use application.

Examples of saturated fatty acids that may be mentioned include palmitic acid, stearic acid and behenic acid.

Examples of unsaturated fatty acids that may be mentioned include myristoleic acid, palmitoleic acid, oleic acid, crucic acid, linoleic acid, linolenic acid, arachidonic acid and ricinoleic acid, and also mixtures thereof.

As examples of oils of animal origin, mention may be made of sperm whale oil, whale oil, sardine oil, herring oil, shark oil, and cod liver oil; pig or sheep fat (tallow).

As examples of vegetal oils, mentions may be made of rapeseed oil, canola oil, sunflower oil, oleic sunflower oil, groundnut oil, olive oil, walnut oil, maize oil, soybean oil, linseed oil, hemp oil, grape seed oil, coconut oil, palm oil, cottonseed oil, babassu oil, jojoba oil, sesame seed oil, castor oil, or oil from nuts or triglycerides, such as medium chain triglycerides (MCT).

As regards the mineral oils, mention may be made of naphthenic oils, paraffinic oils and polybutenes.

The products derived from the alcoholysis of the abovementioned oils may also be used.

The oil is preferably a vegetal oil. In a preferred embodiment, the oil contains mainly saturated and mono-unsaturated fatty acids and a limited amount of poly-unsaturated fatty acids, and is selected from the groups consisting of oleic sunflower oil, rapeseed oil and triglycerides.
Especially, rapeseed oil and coconut medium chain triglycerides are known to have a good resistance to heat-treatment, limited off-flavors and a low impact on the pigment final shade.

Thus, in an even more preferred embodiment the oil is rapeseed oil or coconut medium chain triglycerides.

The amount of the oil component to be added may be determined depending on e.g., the type of product to be manufactured, but its maximum amount will be determined by the maximum volume percentage of the Wi/O emulsion in the Wi/O/W or double emulsion and also by the minimum volume percentage of the water phase in that Wi/O emulsion.

In a preferred embodiment, the oil constitutes from 75 to 95 wt.-% of the oil phase, such as from 80 to 90 wt.-%, such as from 82 to 88 wt.-%, such as from 84 to 86 wt.-% of the oil phase.

The oil-soluble emulsifier to be used for the formation of the W/O emulsion from the oil components described above should have such an appropriate HLB that the oil component used can form the dispersed phase of the W/O emulsion. Particularly, polyglycerol esters of fatty acids, such as polyglycerol polyricinoleate (PGPR), can be used as emulsifier. Other Examples of food-grade, oil-soluble emulsifiers which can be used include but is not limited to emulsifier Mono-Di Mor 50 (Danisco Ingredients), enzyme-modified soy lecithin (Cargill), sunflower lecithin (Cargill), lecithin (Cargill), glycerol monooleate, phospholipid, sorbitan ester and sucrose ester. A combination of two or more oil-soluble emulsifiers may be used.

In a preferred embodiment the oil-soluble emulsifier is polyglycerol polyricinoleate (PGPR). The concentration of the oil-soluble emulsifier is from 4 to 8 wt.-% of the oil phase, more preferably from 5 to 7 wt.-%, and most preferably from 5.5 to 6.5 wt.-%.

A hydrophobic antioxidant may be added to the oil phase.

In a preferred embodiment the hydrophobic antioxidant is natural or synthetic tocopherol. In an even more preferred embodiment the hydrophobic antioxidant is synthetic alpha tocopherol.

The concentration of the hydrophobic antioxidant is preferably from 0 to 15 wt.-% of the oil phase, such as from 1 to 14.5 wt.-%, such as from 2 to 14 wt.-%, such as from 3 to 13.5 wt.-%, such as from 4 to 13 wt.-%, such as from 5 to 12.5 wt.-%, such as
from 6 to 12 wt.-%, such as from 7 to 11.5 wt.-%, such as from 8 to 11 wt.-%, such as from 8.5 to 10 wt.-%.

Preferably, the ratio of internal aqueous phase to oil phase is between about 20:80 and about 65:35 by weight, such as between about 25:75 and about 40:60 by weight.

The blending of oil and water is homogenized using high pressure or shear or a membrane or ultrasound, by mixers known in the art. The blending speed and the duration of the shearing or mixing have an influence on the size of the water droplets inside the oil droplets, and thereby on the texture and consistency of the final double emulsion. The size of the water droplets influences the consistency of the final double emulsion and may be adjusted accordingly.

The external aqueous phase ($W_2$) in a preferred embodiment constitutes from 40 to 70 wt.-% of the double emulsion, such as from 44 to 68 wt.-%, such as from 48 to 66 wt.-%, such as from 52 to 64 wt.-%, such as from 56 to 62 wt.-%, such as from 58 to 61 wt.-% of the double emulsion. The external aqueous phase contains a water-soluble emulsifier, optionally at least one humectant, optionally a preservative and optionally a viscosifier.

The water-soluble emulsifier, or hydrophilic emulsifier, to be used in the present invention is an emulsifier having a HLB at which the aforementioned oil component can form the dispersed phase of the $0/W_2$ emulsion. As the water-soluble emulsifier, surface active agents which have various ranges of HLB may be used. Examples of food-grade water-soluble emulsifiers include but is not limited to Citrem LR10® (Danisco Ingredients), Citrem N12-Veg® (Danisco Ingredients), Panodan TR® (Danisco Ingredients), Purity Gum 2000® (National Starch), Modified starch - waxy maize (National Starch), pre-gelatinized starch sodium octenyl suc (Cargill), Polysorbate 80 (Croda), sucrose ester (Sisterna), sucrose monoester (Compass Food), calcium caseinate Lacprodan (Aria Foods Ingredients), sodium caseinate, fish gelatine, cow gelatine, beta pectin type beta, proteins, polysaccharides and polyoxyethylene sorbitan fatty acid esters, such as polyoxyethylene sorbitan mono-oleate, polyoxyethylene sorbitan mono-laurate, polyoxyethylene sorbitan mono-stearate).
In a preferred embodiment, the water-soluble emulsifier is selected from the group consisting of Polysorbate 80, calcium caseinate, Citrem N12-Veg®. In an even more preferred embodiment, the water-soluble emulsifier is Polysorbate 80.

The water-soluble emulsifier is dissolved in water to form an aqueous solution of the emulsifier.

The amount of the emulsifier to be added varies depending on the type of the emulsifier used and other conditions. Preferably, the water-soluble emulsifier is present in a concentration of from 2 to 15 wt.-% of the external aqueous phase (W2), such as from 3 to 13 wt.-%, such as from 4 to 11 wt.-%, such as from 5 to 9 wt.-%, such as from 5.5 to 7 wt.-% of the external aqueous phase (W2).

The term "humectant" as used herein refers to a hygroscopic substance (with an affinity to form hydrogen bonds with water molecules). It can be used to modify (preferably reduce) the water activity of the external water phase. Useful humectants include but are not limited to sugar polyols, such as glycerol, sorbitol, xylitol and maltitol, propylene glycol as well as hexylene glycol and butylene glycol.

The term "preservative" refers herein to a substance that is added to the double emulsion to prevent degradation by microbial growth or by undesirable chemical changes.

In a preferred embodiment the external aqueous phase comprises a preservative.

A suitable preservative is potassium sorbate in a concentration of from 0 to 0.6 wt.-% of the external aqueous phase, such as from 0.2 to 0.5 wt.-%, such as from 0.3 to 0.4 wt.-%.

Various additives may be added to the composition in order to further enhance or preserve its appearance and taste in foodstuffs. Accordingly, the external aqueous phase may further comprise a stabilizer, like starch or vegetal gum, in a concentration of from 5 to 20 wt.-% of the external aqueous phase, such as from 7 to 18 wt.-%, such as from 9 to 16 wt.-%, such as from 11 to 14 wt.-%, such as from 12 to 13 wt.-%, combined with osmotic agents, like dextrose or glucose, in a concentration of from 2 to 10 wt.-%, such as from 3 to 9 wt.-%, such as from 4 to 8 wt.-%, such as from 5 to 7 wt.-%.

The term "viscosifier" as used herein refers to an agent that increases the viscosity of a solution or of a liquid/solid mixture.
A viscosifier, such as starch, pectin, gum Arabic, locust bean gum, xanthan gum and carboxymethylcellulose, could be added in the external aqueous phase to favor the drop shearing.

Water-soluble molecules may further be included in the internal or in the external aqueous phase of the double emulsion.

Specifically, compounds for matching the osmotic balance, such as sugars, such as glucose, lactose and fructose, glycerol, proteins and electrolytes, such as salts, that can match the osmotic pressure from the internal to the external water phase can be added in the external aqueous phase (continuous phase). The aim is to maintain the compartmented structure of the double emulsions, reducing the water flows and the breakdown of the droplets.

The ratio of external aqueous phase (\(W_2\)) to water-in-oil (\(W_{i/O}\)) emulsion is preferably in the range of between 15:85 to 95:5. Preferably, it is in the range of between 20:80 to 80:20. More preferably, it is in the range of between 30:70 to 70:30.

In a preferred embodiment of the first aspect of the present invention the \(pH\) of the internal and external aqueous phase is at least 2.0 and at most 6.0, such as at least 3.0 and at most 5.0, and more preferably in the range of between 3.5 and 4.5.

In a preferred embodiment the particle size distribution of the oil droplets expressed as \(d[4,3]\) is between 1 \(\mu\text{m}\) and 100 \(\mu\text{m}\), such as between 2 \(\mu\text{m}\) and 60 \(\mu\text{m}\), such as between 3 \(\mu\text{m}\) and 50 \(\mu\text{m}\), such as between 4 \(\mu\text{m}\) and 40 \(\mu\text{m}\), such as between 5 \(\mu\text{m}\) and 35 \(\mu\text{m}\), such as between 10 \(\mu\text{m}\) and 30 \(\mu\text{m}\), such as between 15 \(\mu\text{m}\) and 25 \(\mu\text{m}\).

Example 5 herein describes a method for determining \(d[4,3]\). Other methods may be readily apparent to the skilled person.

The following describe the method for producing a \(W_{i/O}/W_2\) double emulsions type colorant with a water-soluble natural pigment, such as betanin, and at least one gelling agent in the internal aqueous phase by using the components described above.

First, pigment and at least one gelling agent are dissolved in water or an aqueous solution. A \(W_{i/O}\) emulsion is prepared with at least one oil component and the water or the aqueous solution containing pigment and at least one gelling agent. In this preparation
of the Wi/O emulsion, the oil-soluble emulsifier is added to and dissolved in the oil component to make oil phase, and a predetermined amount of the aqueous phase containing the pigment is added to the oil phase. The mixture is agitated, usually in a high-shear device, for a specific period of time so that the oil phase forms a continuous phase (i.e. dispersion medium), in which the aqueous phase is dispersed resulting in the formation of the Wi/O emulsion.

The gelation of the gelling agent within Wi in the Wi/O emulsion is induced. This can be done by slowly cooling down the Wi/O emulsion to a temperature suitable for solubilizing the gelling agent.

Then this Wi/O emulsion is slowly added to an aqueous solution prepared by dissolving water-soluble emulsifier in water and processed, usually in a low-shear device, to form a W/O/W2 double emulsion.

The optimal period of time required for shearing in preparing the W/O/W2 emulsion varies depending on the emulsification equipment used.

Accordingly, a second aspect of the present invention relates to a method for preparing the W/O/W2 emulsion colorant according to the first aspect of the invention, the method comprising the step:

a) mixing a water-soluble natural pigment, such as betanin, and the at least one gelling agent with water to form a first aqueous solution (Wi) under conditions wherein no significant gelling occurs,

b) mixing an oil component with at least one oil-soluble emulsifier to form an oil solution,

c) blending or homogenizing the first aqueous solution and the oil solution together to create a water-in-oil (Wi/O) emulsion under conditions wherein no significant gelling occurs,

d) inducing at least partial gelation of the gelling agent within the first/internal aqueous phase (Wi),

e) mixing a second aqueous solution with at least one water-soluble emulsifier to form a second aqueous solution (W2), and

f) blending or homogenizing the Wi/O emulsion with the second aqueous solution to create the W/O/W2 emulsion colorant.
In a preferred embodiment, the gelling agent is a heat-induced gelling agent and step d) comprises mixing the water-soluble natural pigment and the at least one gelling agent with water at a temperature suitable for solubilizing the gelling agent and maintaining it in a liquid form (this temperature will depend on the gelling agent chosen), mixing the oil component with the at least one oil-soluble emulsifier at a temperature suitable for solubilizing the gelling agent and maintaining it in a liquid and slowly cooling down the water-in-oil (Wi/O) emulsion to a temperature under the gel point of the gelling agent.

In an even more preferred embodiment the method comprises the steps:

a) mixing a water-soluble natural pigment, such as betanin, and at least one gelling agent with water to form a first aqueous solution (Wi) at a temperature of at least 40°C, such as at least 50°C, such as at least 60°C, such as at least 70°C, such as at least 80°C,

b) mixing an oil component with at least one oil-soluble emulsifier to form an oil solution,

c) blending or homogenizing the first aqueous solution and the oil solution together at a temperature of at least 40°C, such as at least 50°C, such as at least 60°C, such as at least 70°C, such as at least 80°C,

d) to create a water-in-oil (Wi/O) emulsion,

e) inducing at least partial gelation of the betanin and the gelling agent within the first/ internal aqueous phase (Wi) by cooling down the water-in-oil (Wi/O) emulsion to at least 30°C, such as to at least 25°C, such as to at least 20°C,

f) mixing a second aqueous solution with at least one water-soluble emulsifier to form a second aqueous solution (W₂), and

g) blending or homogenizing the Wi/O emulsion with the second aqueous solution to create the Wi/0/W₂ emulsion colorant.

The Wi/0/W₂ emulsion colorant may be used for coloring products, such as cosmetics and edible products including food- and feedstuff as well as pharmaceuticals. Particularly, the Wi/O/W₂ emulsion colorant according to the present invention can be used for coloring edible products, where there is a growing demand for non-synthetic and vegetal coloring agents.
A third aspect of the present invention relates to a method for coloring edible products such as food- and feedstuffs and pharmaceutical products.

The method for coloring edible products comprises providing the edible product and incorporating into the edible product the $W_1/O/W_2$ emulsion colorant prepared according to the invention.

The colorant may be incorporated into the edible product by any conventional method known in the art such as stirring or mixing the colorant into the edible product or an ingredient of the edible product. The edible product may also be colored by e.g. spraying the $W_1/O/W_2$ double emulsion type colorant onto the edible product or an ingredient of the edible product.

A fourth aspect of the present invention relates to an edible product comprising the $W_1/O/W_2$ double emulsion type colorant according to the first aspect of the invention.

In a preferred embodiment, the food product is selected from the group consisting of fermented dairy products, confectionary, fruit preparations, ice cream and prepared food, such as meat products, baked products, fillings and shortenings.

A fifth aspect of the present invention relates to the use of a $W_1/O/W_2$ emulsion colorant comprising a water-soluble natural pigment, such as betanin, and at least one gelling agent in the internal aqueous phase for coloring an edible product.

A sixth aspect of the present invention is directed to the use of a $W_1/O/W_2$ emulsion colorant according to the first aspect of the invention for coloring of an edible product.

In much preferred embodiments of the sixth and seventh aspect of the present invention the edible product is selected from the group consisting of fermented dairy products, confectionary, fruit preparations, ice cream and prepared food, such as meat products, baked products, fillings and shortenings.

A seventh aspect of the present invention relates to the use of a $W_1/O/W_2$ emulsion colorant comprising a water-soluble pigment and a gelling agent in the internal aqueous phase for protecting the water-soluble pigment against oxidation and/or heat treatment.
In a much preferred embodiment the water-soluble pigment is betanin (beetroot red).

The present invention is further illustrated by the following non-limiting examples.

5 EXAMPLES

Example 1

Preparation of betanin and comparison of the shades obtained after purification and drying of betanin pigments.

Betanin pigments can be purified in various ways. As an illustrative example, the procedure for the purification of betanin pigment for a colorant of the invention is hereby given.

Red beet juice concentrate is diluted and deposited on 20-60 mesh Sepabeads® SP-207 resin, composed of a brominated styrene-divinylbenzene matrix with ~1.20 mL/g pore volume, 105 Å mean pores size and 650 m²/g surface area. The betanin pigments are retained by the resin and sugars from the concentrate is eluted with water.

The purified pigment extract is eluted with water/ethanol 20/80 v/v, concentrated under low pressure and dried with 30 wt.-% maltodextrin. The extract is vacuum-dried at low temperature (< 50°C, preferably < 30°C) and low pressure (< 100 bars, preferably < 10 bars) to preserve the purple and pink shades of betanin pigments.

To compare the raw material (red beet juice concentrate) to the betanin preparation the shade index = Abs(480 nm) / Abs(535 nm) was measured by spectrophotometry in deionized water (Figures 1-2), at a concentration of about 0.0005-0.001 wt.-% betanin:

- red beet juice concentrate = 0.70-0.75
- betanin = ~0.60 - 0.70

Example 2

Preparation of a 0.5 wt.-% betanin Wi/Q/W double emulsion colorant

An example of preparation of a betanin double emulsion according to the invention:

- 36 g of betanin extract liquid (prepared by dissolving purified betanin concentrate or powder in deionized water, in appropriate proportions to obtain an extract containing 3.5% betanins) is mixed with 2.4 g NaCl, 1.2 g carrageenan and 0.12 g CaCl and
heated to 80°C (this temperature must be maintained the shortest time possible to preserve betanin pigments);

- 51 g of rapeseed oil (Lesieurs) is mixed with 5.4 g alpha tocopherol and 3.6 g polyglycerol polyricinoleate (PGPR) and heated to 80°C;

- the Wi/O emulsion is formed by incorporating the aqueous phase into the oil phase and blending the two at 80-85°C using a Silverson Mixer with a 4-cm rotor-stator at 8,000 rpm for 3 min.;

- the Wi/O emulsion is slowly cooled down to 10-15°C, to allow formation of the gel in the inner water phase, and left at room temperature (20-25°C);

- 140 g of a water-glycerol mixture (at a weight ratio of 54:46) is mixed with 10 g Polysorbate 80 at room temperature (20-25°C);

- The Wi/O/W₂ emulsion is formed by incorporating the Wi/O emulsion into the outer aqueous phase and blending the two at 20-25°C using a Silverson Mixer with a 4-cm rotor-stator at 1,000 rpm for 2 min. and 2,500 rpm for 4 min. until the emulsion drops have reached a small (around 25 µm) and homogenous size.

**Example 3**

Visual aspect of a double emulsion with 0.3 wt.-% betanin.

A double emulsion was made up with 0.3 wt.-% betanin prepared as described in Example 2 but using betanin extract liquid containing 2.1 wt.-% purified betanin.

Figure 3 shows the shade of the double emulsion with 0.3 wt.-% betanin.

**Example 4**

Examples of physicochemical characteristics of a double emulsion according to the invention.

A W₁/O/W₂ double emulsion according to the invention was prepared as described in Example 2 and characterized by measuring pigment strength, water activity, turbidity, pH and Brix.

- Pigment strength = 0.45-0.55 wt.-%

- Shade index with betanin = 0.60 - 0.70

- Water activity: 0.7-0.8

- Turbidity at 0.05 wt.% in deionized water = 100-200 NTU
pH = 3.0 - 4.0
- Brix = ~50°Bx

The pigment strength and shade index are measured by spectrophotometry according to internal method AM-1014-6-GB for betanin.

Water activity is measured at 24.8°C in Navasina aw measuring instrument aw-Lab IC-500.

Turbidity at 0.05 wt.-% was measured in Nephelometric Turbidity Units (NTU) on a VWR turbidimeter.

Brix was measured using an electronic Atago pocket refractometer.

**Example 5**

Examples of granulomere data of the double emulsion.

The particle size distribution of the W₁/O/W₂ double emulsion prepared in Example 4 was determined by measuring particle sizes (Figure 4) using a Mastersizer 2000 from Malvern Instruments.

The Mastersizer 2000 uses the method of laser diffraction and measures droplet size from analyzing the scattering pattern created by the droplets of a sample dispersed in a liquid.

Emulsion samples were dispersed in demineralized water before measuring, and the size distributions were determined using the Fraunhofer theory.

The d[4,3] moment-volume mean is calculated from the measured particle size distribution data using the formula below.

\[
d[4,3] = \frac{\sum d^4 \cdot \varphi}{\sum d^3 \cdot \varphi},
\]

where \( \varphi \) is the droplet volume fraction of diameter \( d \).

The d[4,3] represents the experimental data (unequally-sized particles) by a system of same-sized particles having two characteristics of the original distribution (moment and volume in this case).

\( d(0.1) \) is defined as the particle diameter where 10% of the population lies below this value.
d(0.5), the median, is the particle diameter where 50% of the population lies below this value.

d(0.9) refers to the particle diameter where 90% of the population lies below this value.

\[
\begin{align*}
5 \quad d[4,3] &= 15-25 \, \mu m \\
&d(0.1) = 5-15 \, \mu m \\
&d(0.5) = 12-22 \, \mu m \\
&d(0.9) = 28-35 \, \mu m
\end{align*}
\]

10 Figure 5 shows a microscopic photo of the double emulsion according to the invention.

**Example 6**

Application of a double emulsion with betanin pigments in acidic slurry for fruit preparation or dairy and in yoghurt.

15 A betanin double emulsion colorant according to the invention and as prepared in Example 2 was employed in an acidic slurry (at pH 3.8, composed of an aqueous base with sugar, thickeners and pH regulator) at dosages permitting to have respectively 5 to 20 mg/kg betanin in final yoghurt. The slurry mixture (Figure 6A) was pasteurized at 90°C for 5 min., cooled down and added to plain yoghurts (Figure 7B).

20 The samples were then subjected to colorimetric analysis in small petri boxes to measure the lightness \( (L^*) \), chroma \( (C^*) \) and hue \( (h^*) \). They all have \( h^* \) around 345.

Consequently, such colorant prepared according to the invention with purified betanin pigments is a good replacer of carmine and carmine derivate pigments (like the red acid stable citroammoniacal salt of carminic acid 4-amminocarminic acid) in dairy products.

**Example 7**

Comparison of the stability of betanin formulated in double emulsion with and without gelling agent in the internal aqueous phase

To evaluate the effect of gelling agent and salt in the internal aqueous phase two double emulsions at a final concentration of 0.25 wt.-% betanin were prepared, one with 6 wt.-%
gelatin and 6 wt.-% NaCl in the internal aqueous phase, and one without gelling agent and without salt.

Compositions and procedures:

- Stable betanin W/O/W emulsion containing a gelling agent: betanin extract liquid, gelatin, NaCl, vegetable oil, tocopherol, PGPR, water, PS-80:
  a) 36 g of betanin extract liquid (prepared by dissolving purified betanin concentrate or powder in deionized water, in appropriate proportions to obtain an extract containing 1.6% betanins) is mixed with 2.4 g NaCl and 2.4 g gelatin and heated to 60°C
  b) 51 g of rapeseed oil is mixed with 5.4 g alpha tocopherol and 3.6 g polyglycerol polyricinoleate (PGPR) and heated to 60°C;
  c) the Wi/O emulsion is formed by incorporating the aqueous phase into the oil phase and blending the two at 60°C using a Silverson Mixer with a 4-cm rotor-stator at 8,000 rpm for 3 min.;
  d) the Wi/O emulsion is slowly cooled down to 4°C, to allow formation of the gel in the inner water phase, and then left at room temperature (20-25°C);
  e) 140 g of a water-glycerol mixture (at a weight ratio of 54:46) is mixed with 10 g Polysorbate 80 at room temperature (20-25°C);
  f) The Wi/O/W₂ emulsion is formed by incorporating the Wi/O emulsion into the outer aqueous phase and blending the two at 20-25°C using a Silverson Mixer with a 4-cm rotor-stator at 1,000 rpm for 2 min. and 2,500 rpm for 4 min. until the emulsion drops have reached a small (around 25 μm) and homogenous size.

- Stable betanin W/O/W emulsion without gelling agent: betanin extract liquid, vegetable oil, tocopherol, PGPR, water, starch, glucose:
  a) 36 g of betanin extract liquid (prepared by dissolving purified betanin concentrate or powder in deionized water, in appropriate proportions to obtain an extract containing 1.6% betanins) is mixed with 4 g water.
  b) 51 g of rapeseed oil is mixed with 5.4 g alpha tocopherol and 3.6 g polyglycerol polyricinoleate (PGPR);
  c) the Wi/O emulsion is formed by incorporating the aqueous phase into the oil phase and blending the two at room temperature using a Silverson Mixer with a 4-cm rotor-stator at 8,000 rpm for 3 min.;
d) 22 g starch and 14 g glucose are mixed with 114 g water at 65°C, then left at room temperature (20-25°C);

e) The W₁/O/W₂ emulsion is formed by incorporating the Wi/O emulsion into the outer aqueous phase and blending the two at 20-25°C using a Silverson Mixer with a 4-cm rotor-stator at 1,000 rpm for 2 min. and 2,500 rpm for 4 min. until the emulsion drops have reached a small (around 25 µl) and homogenous size.

The samples were subjected to colorimetric analysis to measure the lightness (L*), chroma (C*) and hue (h*). DE*2000 gives the global difference of shade between two samples (here before and after heat-treatment): the value is acceptable when < 2.0.

First, the betanin concentration and the shade index (purple/brown index) were significantly more stable in the colorant formulated with the gelling agent during 3 months of storage of the bulk (data not shown).

Second, as can be seen from Table 1 and Figure 7, the results in dairy applications also highlighted that the presence of the gelling agent significantly increased betanin stability, especially towards storage after pasteurization.
Table 1:

<table>
<thead>
<tr>
<th>Yoghurt colored with a betanin W₁/O/W₂ emulsion containing gelling agent and salt</th>
<th>L*</th>
<th>C*</th>
<th>h*</th>
<th>DE*2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before pasteurization</td>
<td>68.69</td>
<td>28.26</td>
<td>347.26</td>
<td>ref.</td>
</tr>
<tr>
<td>After pasteurization</td>
<td>69.15</td>
<td>28.39</td>
<td>347.21</td>
<td>0.36</td>
</tr>
<tr>
<td>Pasteurization +7d</td>
<td>69.14</td>
<td>28.07</td>
<td>347.44</td>
<td>0.37</td>
</tr>
<tr>
<td>Pasteurization +14d</td>
<td>69.44</td>
<td>27.55</td>
<td>348.22</td>
<td>0.73</td>
</tr>
<tr>
<td>Pasteurization +21d</td>
<td>69.64</td>
<td>26.63</td>
<td>349.27</td>
<td>1.21</td>
</tr>
<tr>
<td>Pasteurized slurry +30d</td>
<td>70.11</td>
<td>27.36</td>
<td>345.64</td>
<td>1.28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Yoghurt colored with a betanin W₁/O/W₂ emulsion without gelling agent</th>
<th>L*</th>
<th>C*</th>
<th>h*</th>
<th>DE*2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before pasteurization</td>
<td>68.84</td>
<td>27.03</td>
<td>351.12</td>
<td>ref.</td>
</tr>
<tr>
<td>After pasteurization</td>
<td>70.15</td>
<td>25.41</td>
<td>353.34</td>
<td>1.42</td>
</tr>
<tr>
<td>Pasteurization +7d</td>
<td>70.65</td>
<td>24.20</td>
<td>354.99</td>
<td>2.23</td>
</tr>
<tr>
<td>Pasteurization +14d</td>
<td>71.48</td>
<td>22.97</td>
<td>357.35</td>
<td>3.32</td>
</tr>
<tr>
<td>Pasteurization +21d</td>
<td>72.04</td>
<td>21.85</td>
<td>359.89</td>
<td>4.28</td>
</tr>
<tr>
<td>Pasteurized slurry +1d</td>
<td>72.91</td>
<td>18.24</td>
<td>8.92</td>
<td>7.23</td>
</tr>
</tbody>
</table>

d: Days of storage at 4°C in the dark.

Example 8

Comparison of the stability of betanins in double emulsions prepared with rapeseed oil, oleic sunflower oil or medium chain triglycerides.

Different W₁/O/W₂ double emulsions with the same amount of betanin were prepared as described in Example 2 but using betanin extract liquid containing 0.5 wt.-% purified betanin, and using in the oil phase either rapeseed oil, rapeseed oil and oleic sunflower oil, oleic sunflower oil or medium chain triglycerides (MCT) enriched or not with delta and gamma tocopherol (500 ppm in the oils). Slurries were colored with those diverse double emulsions and submitted to pasteurization at 90°C for 5 min.

The slurries were then subjected to colorimetric analysis according to the CIELAB L*C*h* color system to measure the shade intensity or lightness (L*), chroma (C*) and hue (h*) of
solutions immediately after pasteurization and again after storage for 15 days at 4°C in the dark.

Colorimetric measurements were performed in small petri boxes using Spectraflash 650 (Datacolor) in transmission mode under D65 LOdeg illuminant.

As can be clearly seen from the colorimetric measurements in Table 2, betanin pigments are more stable (more intense) in formulation with MCT oil towards heat-treatment and storage; but the shade is the least bluish. Moreover, adding tocopherol to the oil does not significantly improve the stability.

Table 2:

<table>
<thead>
<tr>
<th>In freshly pasteurized slurries</th>
<th>Oils used for formulation</th>
<th>L*</th>
<th>C*</th>
<th>h*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapeseed</td>
<td>29.98</td>
<td>34.54</td>
<td>8.50</td>
<td></td>
</tr>
<tr>
<td>Rapeseed + tocopherol</td>
<td>30.37</td>
<td>34.25</td>
<td>9.85</td>
<td></td>
</tr>
<tr>
<td>Rapeseed + oleic</td>
<td>31.32</td>
<td>34.38</td>
<td>11.85</td>
<td></td>
</tr>
<tr>
<td>Rapeseed + oleic + tocopherol</td>
<td>30.54</td>
<td>34.09</td>
<td>11.28</td>
<td></td>
</tr>
<tr>
<td>Oleic</td>
<td>31.08</td>
<td>33.43</td>
<td>12.85</td>
<td></td>
</tr>
<tr>
<td>Oleic + tocopherol</td>
<td>30.49</td>
<td>34.32</td>
<td>11.50</td>
<td></td>
</tr>
<tr>
<td>MCT</td>
<td>29.58</td>
<td>36.33</td>
<td>14.72</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In pasteurized slurries stored for 15 days at 4°C in the dark</th>
<th>Oils used for formulation</th>
<th>L*</th>
<th>C*</th>
<th>h*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapeseed</td>
<td>32.00</td>
<td>33.61</td>
<td>358.82</td>
<td></td>
</tr>
<tr>
<td>Rapeseed + tocopherol</td>
<td>31.53</td>
<td>34.44</td>
<td>358.81</td>
<td></td>
</tr>
<tr>
<td>Rapeseed + oleic</td>
<td>31.76</td>
<td>33.88</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Rapeseed + oleic + tocopherol</td>
<td>31.40</td>
<td>33.98</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Oleic</td>
<td>31.08</td>
<td>33.38</td>
<td>2.37</td>
<td></td>
</tr>
<tr>
<td>Oleic + tocopherol</td>
<td>31.22</td>
<td>33.59</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>MCT</td>
<td>28.72</td>
<td>35.15</td>
<td>3.86</td>
<td></td>
</tr>
</tbody>
</table>

Rapeseed: Rapeseed oil; oleic: oleic sunflower oil; MCT: medium chain triglycerides.
Example 9

Improvement of the stability towards heat-treatments, of betanin pigments in double emulsions versus standard formulation in acidic slurry/yoghurts.

Standard betanin colorant (red beet juice concentrate) or betanin double emulsion colorant according to the invention and as prepared in Example 2 were employed in an acidic slurry (pH 3.8, as described in Example 6) at a dosage permitting to have 5 to 20 mg/kg betanin in final yoghurt. The slurry mixture was pasteurized at 90°C for 5 min., cooled down and added to plain yoghurts.

The samples were subjected to colorimetric analysis to measure the lightness (L*), chroma (C*) and hue (h*). DE*2000 gives the global difference of shade between two samples (here before and after heat-treatment): the value is acceptable when < 2.0.

From Figure 8 and data in Table 3 it can be seen that with standard betanin colorant (Figure 8A) the shade of the slurry/yoghurt turned lighter and more red while it remained purple-pink with betanin double emulsion colorant (Figure 8B).
<table>
<thead>
<tr>
<th>5 ppm</th>
<th>In yoghurt with slurry</th>
<th>L*</th>
<th>C*</th>
<th>h*</th>
<th>DE*2000</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard color</strong></td>
<td>Before pasteurization</td>
<td>79.35</td>
<td>16.46</td>
<td>351.05</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After pasteurization</td>
<td>81.93</td>
<td>11.98</td>
<td>13.88</td>
<td>5.55</td>
</tr>
<tr>
<td></td>
<td>Pasteurization +28d</td>
<td>82.60</td>
<td>11.40</td>
<td>26.01</td>
<td>na</td>
</tr>
<tr>
<td><strong>Double emulsion</strong></td>
<td>Before pasteurization</td>
<td>80.02</td>
<td>16.99</td>
<td>349.72</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After pasteurization</td>
<td>81.02</td>
<td>15.32</td>
<td>353.38</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>Pasteurization +28d</td>
<td>81.69</td>
<td>14.67</td>
<td>359.27</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>10 ppm</strong></td>
<td>Before pasteurization</td>
<td>74.09</td>
<td>23.36</td>
<td>344.97</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After pasteurization</td>
<td>75.66</td>
<td>18.75</td>
<td>355.54</td>
<td>3.71</td>
</tr>
<tr>
<td></td>
<td>Pasteurization +28d</td>
<td>76.78</td>
<td>17.33</td>
<td>2.36</td>
<td>na</td>
</tr>
<tr>
<td><strong>Double emulsion</strong></td>
<td>Before pasteurization</td>
<td>75.01</td>
<td>23.87</td>
<td>344.59</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After pasteurization</td>
<td>75.65</td>
<td>23.37</td>
<td>345.38</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Pasteurization +28d</td>
<td>76.32</td>
<td>22.22</td>
<td>347.26</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>15 ppm</strong></td>
<td>Before pasteurization</td>
<td>70.44</td>
<td>27.80</td>
<td>343.42</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After pasteurization</td>
<td>71.56</td>
<td>23.99</td>
<td>349.98</td>
<td>2.70</td>
</tr>
<tr>
<td></td>
<td>Pasteurization +28d</td>
<td>72.57</td>
<td>22.03</td>
<td>355.11</td>
<td>na</td>
</tr>
<tr>
<td><strong>Double emulsion</strong></td>
<td>Before pasteurization</td>
<td>71.60</td>
<td>28.33</td>
<td>343.65</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After pasteurization</td>
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d: Days of storage at 4°C in the dark.
Example 10

Improvement of the stability towards heat-treatments, of betanin pigments in double emulsions versus standard formulation in meat products.

5 Standard betanin colorant (red beet juice concentrate) or betanin double emulsion colorant according to the invention and as prepared in Example 2 were employed in sausage meat at a dosage permitting to have the desired shade in final product. The meat was then pasteurized at 90°C for 5 min and cooled down.

From Figure 9 it can be seen that with standard betanin colorant (Figure 9A) the shade of the sausage turned orange-brown while it remained pink with betanin double emulsion colorant (Figure 9B).

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Claims

1. A water-in-oil-in-water (Wi / O/W₂) emulsion colorant comprising:
   a) an internal aqueous phase (Wi) comprising a water-soluble natural pigment and at least one gelling agent,
   b) an oil phase (O) comprising at least one oil-soluble emulsifier, and
   c) an external aqueous phase (W₂) comprising at least one water-soluble emulsifier.

2. The Wi / O/W₂ emulsion colorant according to claim 1, wherein the water-soluble natural pigment is betanin (beetroot red).

3. The Wi / O/W₂ emulsion colorant according to claim 2, wherein the betanin is present in a concentration of 1.5 to 5 wt-% of the internal aqueous phase.

4. The Wi / O/W₂ emulsion colorant according to any of claims 1 to 3, wherein the at least one gelling agent is present in a concentration of 0.5 to 10 wt-% of the internal aqueous phase.

5. The Wi / O/W₂ emulsion colorant according to claim 4, wherein the at least one gelling agent is selected from the group consisting of fish gelatin, pork gelatin, bovine gelatin, poultry gelatin, collagen, albumins, pectins, chitosan, agar, gellan gum, locust bean gum and carrageenans.

6. The Wi / O/W₂ emulsion colorant according to any of claims 1 to 5 further comprising at least one salt in the internal aqueous phase (Wi).

7. The Wi / O/W₂ emulsion colorant according to any of claims 1 to 6, wherein the average diameter in volume, d[4,3] of the oil droplets (Wi / O/W₂ emulsion) is between 1 μm and 100 μm.

8. A method for preparing the Wi / O/W₂ emulsion colorant according to any of claims 1 to 7, the method comprising the steps:
a) mixing a water-soluble natural pigment and at least one gelling agent with water to form a first aqueous solution (Wi) under conditions wherein no significant gelling occurs,

b) mixing an oil component with at least one oil-soluble emulsifier to form an oil solution,

c) blending or homogenizing the first aqueous solution and the oil solution together under conditions wherein no significant gelling occurs to create a water-in-oil (Wi/O) emulsion,

d) inducing at least partial gelation of the betanin and the gelling agent within the first/internal aqueous phase (Wi),

e) mixing a second aqueous solution with at least one water-soluble emulsifier to form a second aqueous solution (W₂), and

f) blending or homogenizing the Wi/O emulsion with the second aqueous solution to create the W₁/O/W₂ emulsion colorant.

9. The method according to claim 8, the method comprising the steps:

a) mixing the water-soluble natural pigment and at least one gelling agent with water to form a first aqueous solution (Wi) at a temperature of at least 40°C,

b) mixing an oil component with at least one oil-soluble emulsifier to form an oil solution,

c) blending or homogenizing the first aqueous solution and the oil solution together at a temperature of at least 40°C to create a water-in-oil (Wi/O) emulsion,

d) inducing at least partial gelation of the betanin and the gelling agent within the first/internal aqueous phase (Wi) by cooling down the water-in-oil (Wi/O) emulsion,

e) mixing a second aqueous solution with at least one water-soluble emulsifier to form a second aqueous solution (W₂), and

f) blending or homogenizing the Wi/O emulsion with the second aqueous solution to create the W₁/O/W₂ emulsion colorant.
10. The method according to claim 8, wherein the water-soluble natural pigment is betanin.

11. A method for coloring edible products, such as food- and feedstuffs and pharmaceutical products, the method comprising; providing an edible product and incorporating into the edible product the \( W_1/W_2 \) emulsion colorant according to any of claims 1 to 7.

12. An edible product comprising the \( W_1/W_2 \) emulsion colorant according to any of claims 1 to 7.

13. Use of a \( W_1/W_2 \) emulsion colorant comprising a water-soluble natural pigment and at least one gelling agent in the internal aqueous phase (\( W_1 \)) for coloring of an edible product.

14. Use according to claims 13 or 14, wherein the water-soluble natural pigment is betanin.

15. Use according to any of claims 13 to 16, wherein the edible product is selected from the group consisting of fermented dairy products, confectionary, fruit preparations, ice cream, prepared food, such as meat products, baked products, fillings and shortenings.

16. Use of a \( W_1/W_2 \) emulsion colorant comprising a water-soluble natural pigment and at least one gelling agent in the internal aqueous phase (\( W_1 \)) for protecting the water-soluble natural pigment against oxidation.

17. Use according to claim 16, wherein the water-soluble natural pigment is betanin.
Figure 2
Figure 5
A  

low/average concentration  

high concentration

$h^* = \sim 345$

(in acidic slurry)

B

Increasing concentrations

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<th>5 ppm bet.</th>
<th>10 ppm bet.</th>
<th>15 ppm bet.</th>
<th>20 ppm bet.</th>
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$L^* = 80-70$

$C^* = 15-30$

$h^* = \sim 345$

(in yoghurt)

Figure 6
Figure 7
Figure 8
A
35 ppm pigment,

B
22 ppm pigment

Figure 9
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C09B61/00
ADD.

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C09B A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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**X** Further documents are listed in the continuation of Box C. **X** See patent family annex.

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**“Z”** document member of the same patent family

**Date of the actual completion of the international search**

5 November 2014

**Date of mailing of the international search report**

21/11/2014

Name and mailing address of the ISA/

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Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

**Authorized officer**

Ketterer, Michael

Form PCT/ISA210 (second sheet) (April 2000)
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