

EMULSIFIED FOOD PRODUCT CONTAINING EGG PROTEIN AND IRON

TECHNICAL FIELD

5 This invention relates to an emulsified food product and a process for preparing an emulsified food product. In particular, the invention relates to an EDTA-free mayonnaise, and the use of egg proteins, for example livetin or phosvitin, to stabilise mayonnaise against the degradative effects of iron ions present in the mayonnaise.

BACKGROUND

A perception of naturalness is increasingly becoming an important attribute for food products. More and more, consumers demand that foods do not contain any additives, especially additives that are perceived to be non-natural or artificial. Cold sauces such as mayonnaise are no exception to this consumer demand. The additive EDTA
15 (ethylenediaminetetraacetic acid) is particularly relevant for mayonnaise products. The presence of EDTA in a food product requires an E number (E385) on the product packaging, and this is recognised by consumers as an additive that is non-natural and so can influence purchasing decisions.

The reason EDTA is added to many mayonnaise products is to counter the fishy and
20 metallic flavour off-notes that develop after some time. Mayonnaise products contain unsaturated fatty acids, especially omega-3 fatty acids, many of which are polyunsaturated acids. These compounds decompose on storage through oxidation catalysed by trace amounts of iron ions to give the undesirable off-notes. Iron is naturally present in egg yolk and so the presence of iron is unavoidable in any food product containing egg yolk. EDTA is
25 a well-known chelating agent and is added to bind the iron ions making them unavailable for catalysing the oxidation of unsaturated fatty acids. The fatty acids are therefore stabilised and the off-notes are minimised or prevented altogether.

EDTA is the only solution known for inhibiting the formation of fishy and rancid off-
notes in mayonnaises having a content high in omega-3 fatty acids. Its use can extend the
30 shelf-life of such mayonnaise products from, for example, 1 month to 9 months.

Some food products are known that rely on additives other than EDTA to stabilise unsaturated fatty acids. These may be traditional phenolic synthetic antioxidants, e.g. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), TBHQ (tert-butylhydroquinone), and propyl gallate, or natural antioxidants, e.g. green tea extract (which
35 has a high content of catechins and polyphenolic), rosemary extracts (which are rich in phenolic diterpenes) and tocopherol extracts (which are derived from soya oil). Citric acid and certain sulphites are also used in some mayonnaise products.

Different classes of molecules are potential natural metal-chelators including flavonoids, polyphenols, proteins, peptides and polysaccharides.

Proteins have been widely reported to be efficient in delaying metal-catalysed lipid oxidation in emulsions. The mechanism of protection depends on the location of the protein in the emulsion system. When adsorbed at the oil/water interface, proteins can repel iron electrostatically and thus reduce interactions between iron and lipid hydroperoxides (Kellerby *et al.*, *Journal of Agricultural and Food Chemistry*, 2006, 54, 7879–7884). When present in the aqueous phase, proteins can bind to the iron, with caseinate for example, having better chelating activity than whey proteins (Faraji *et al.*, *Journal of Agricultural and Food Chemistry*, 2004, 52, 4558–4564).

Milk proteins and peptides are known to have good antioxidant properties (Elias *et al.*, *Critical Reviews in Food Science and Nutrition*, 2008, 48, 430–441). This is mainly attributed to the presence of phosphoserine amino acid residues, which can bind to metal ions. For example, casein phosphopeptides can inhibit the oxidation of corn oil emulsions at acidic and neutral pH (Diaz *et al.*, *Journal of Agricultural and Food Chemistry*, 2003, 51, 2365–2370). Casein hydrolysates are also excellent inhibitors of lipid oxidation in emulsions because they have both metal-chelating and radical-scavenging properties (Diaz *et al.*, *Journal of Agricultural and Food Chemistry*, 2003, 51, 2365–2370). Because they have fewer clusters of phosphoserine residues, whey proteins have a lower iron-binding activity than caseins (Sugiarto *et al.*, *Dairy Sci. Technol.*, 2009, in press, DOI: 10.1051/dst/2009053).

Egg yolk is a good source of natural antioxidants. Egg yolk granules have been reported to significantly delay the oxidation of linoleate emulsions (Yamamoto *et al.*, *Agricultural and Biological Chemistry*, 1990, 54, 3099–3104), the native lipoprotein and the phosvitin being responsible for the observed strong antioxidant properties. The egg yolk protein phosvitin can chelate iron and copper ions, and it can inhibit iron-catalysed oxidation of phospholipid emulsions if a 1:30 phosvitin:iron ratio is used (Lu and Baker, *Poultry Science*, 1986, 65, 2065–2070, and *Journal of Food Science*, 1987, 52, 613–616). Egg yolk protein hydrolysates (M.W. < 1000 Da; amino acid number = 2.6) and amino acids can also delay oxidation of linoleic acid emulsions, at small concentrations, typically 0.025% (Sakanaka, *Food Chemistry*, 2004, 86, 99–103). Iron chelation can also be achieved using partially hydrolyzed egg white proteins (WO 2000/051447). These complexes are sufficiently stable to be used in food applications.

US patent application 12/004,777 describes the use of egg yolk proteins to stabilise oil-in-water emulsions, such as mayonnaises and salad dressings. Examples are described using egg yolk granule fractions, which may be phosvitin, and egg yolk plasma proteins, which may be livetin. The proteins are said to stabilise oil-in-water emulsions having a reduced fat or oil content and still provide a spoonable product having a smooth thick mouth

feel similar to full fat products. However, this approach does not provide a solution to spoilage due oxidation of lipids, and in all examples described EDTA is added.

US patent application 12/642,223 describes one way to avoid the addition of EDTA to food products which would otherwise develop an unpleasant taste or aroma through lipid oxidation. The method involves preparing an iron-reduced egg yolk. Iron and phosvitin are extracted from the egg yolk. The iron is removed from the phosvitin and the phosvitin is then added back to the egg yolk. A mayonnaise containing no iron can then be prepared from this egg yolk. This method is, however, time consuming and complicated, which is unlikely to be economically viable for mayonnaises and similar food products.

The object of the present invention is to improve the state of the art and to provide improved ways of producing emulsified food products having a source of iron and varying levels and types of unsaturated fatty acids, and especially of omega-3 fatty acids; which are resistant to oxidation, decomposition and spoilage; which have a prolonged shelf-life making them useful to be used as an industrial food product; which do not develop an off-taste and/or off-flavour upon storage or extended shelf-life; and which yet do not contain EDTA or other non-natural chelating agents for binding with iron.

An object of the present invention is therefore to provide an emulsified food product that at least goes part way to overcoming one or more of the above disadvantages of existing products, or at least to provide a useful alternative.

The object of the present invention is achieved by the subject matter of the independent claims. The dependent claims further develop the idea of the present invention.

SUMMARY OF THE INVENTION

Accordingly, in a first aspect of the invention there is provided a shelf-stable emulsified food product comprising:

- a) oil and/or fat, which contains at least 0.5 wt% omega-3 fatty acids per total of the emulsified food product;
- b) 0.05 to 5.0 wt% egg protein per total emulsified food product; and
- c) at least 10 ppm iron ions per total emulsified food product;

wherein the food product contains no EDTA (ethylenediaminetetraacetic acid).

The food product of the invention comprises at least 0.8 wt% omega-3 fatty acids, preferably at least 1.2 wt%, more preferably at least 2.0 wt%, or even more preferably at least 4.0 wt% omega-3 fatty acids calculated as to total weight of the emulsified food product.

The food product preferably comprises at least 1 %, more preferably at least 5 %, and even more preferably at least 10 %, by weight oil and/or fat. The oil and/or fat preferably contains at least 0.1 %, more preferably at least 1 %, and even more preferably at least 5 %, by weight of omega-3 fatty acids. In some preferred embodiments of the invention, the oil and/fat contains 8 to 12 % by weight omega-3 fatty acids. In other preferred embodiments, the oil and/fat contains 20 to 40 % by weight omega-3 fatty acids.

In some embodiments, the food product comprises 15 to 35 % by weight oil and/or fat. In other embodiments, the food product comprises 70 to 85 % by weight oil and/or fat.

Preferably, the food product comprises 0.1 to 3 % by weight of egg protein. It is also preferred that the food product comprises at least 20 ppm iron ions, preferably at least 50 ppm iron ions.

The egg protein is preferably a phosphorylated protein, for example phosvitin. The egg protein may also be livetin or yolk soluble protein or yolk albumin.

The food product may comprise egg yolk, which may be fresh egg yolk or powdered egg yolk. The egg yolk is preferably present in the amount of at least 1 % by weight.

In preferred embodiments of the invention, the food product is a mayonnaise, salad dressing, beverage, creamer, infant formula, baby food, dairy product, cereal products, functional drink, or pet food.

In a second aspect of the invention, there is provided a process for preparing an emulsified food product that contains no EDTA (ethylenediaminetetraacetic acid) comprising the step of mixing together:

- a) oil and/or fat;
- b) 0.05 to 5.0 wt% egg protein per total emulsified food product;
- c) a source of iron which contains at least 10 ppm iron ions per total emulsified food product; and
- d) water;

wherein the oil and/or fat contains at least 0.5 wt% omega-3 fatty acids calculated per total of the emulsified food product.

Preferably the egg protein is a phosvitin or a livetin.

The process may further comprise mixing an acidifier with components a) to d). In some embodiments, the acidifier is mixed with components b) to c), and then a) is added and emulsified. In other embodiments, components b) and c) are mixed together, then the acidifier is added and mixed, and then a) is added and emulsified. In other embodiments, components b) and c) are mixed together, then a) is added and emulsified, and then the acidifier is added and mixed.

In a still further aspect, the invention relates to a use of an egg protein in an emulsion comprising an oil and/or fat containing at least 0.5 wt% omega-3 fatty acids per total of the emulsion, at least 10 ppm iron ions and water, for preventing or retarding oxidation of the omega-3 fatty acids upon storage of the emulsion. Preferably, storage is for at least 1
5 months at refrigerated or room-temperature, more preferably for at least 4 months or at least 9 months under refrigerated or room-temperature conditions.

BRIEF DESCRIPTION OF THE FIGURES

10 Figure 1 shows the iron binding activity of phosvitin and livetin in water compared with EDTA.

Figure 2 shows the effect of process on iron binding activity of phosvitin at acidic pH.

15 Figure 3 shows the iron binding activity of livetins at acidic pH.

Figure 4 shows the iron binding activity of defatted egg yolk.

20 DETAILED DESCRIPTION

The term “shelf-stable” as it relates to the emulsified food product of this invention means that the food product can be safely stored in a sealed container at room or ambient temperature for a useful and reasonably long period of time without an organoleptic degradation. Particularly, this period of time or shelf-life is long enough to make an industrial
25 production feasible, to allow commercial distribution and reasonable storage time of the product at a distributor and also at a consumer’s home. Typically, such a shelf-life period is at least 1 month as from the production date and can extend for 9 months or even longer.

The term “emulsified” as it relates to the food product of this invention means oil droplets dispersed in a water matrix, i.e. an oil-in-water emulsion.

30 The term “omega-3 fatty acid” means an unsaturated fatty acid having a double bond starting after the third carbon atom from the end (known as the omega end) of the carbon chain.

The term “iron ions” refers to Fe^{2+} or Fe^{3+} or any combination thereof.

35 The term “protein” means a combination of chemically bound amino acid residues, and includes peptides, hydrolysed proteins, and any protein or peptide fragment.

The term “acidifier” means any ingredient that is used to decrease pH, and also may be referred to as acid, acidity regulator, acidic buffering agent, pH adjusting agent etc. The acidifier used in this invention may be, but is not limited to, vinegar, mustard, citric acid,

lemon juice, acetic acid, hydrochloric acid, phosphoric acid, malic acid, lactic acid, ascorbic acid, tartaric acid, fumaric acid, alginic acid, or succinic acid.

The term "livetin" refers to the serum proteins or the water-soluble protein fraction of egg yolk or yolk albumin, and may comprise any one or more of α -livetin (serum albumin), β -livetin (α -glycoprotein) and γ -livetin (serum γ -globulin or immunoglobulin Y).

The term "phosvitin" refers to the protein fraction present as lipoprotein in native egg yolk granules, and comprises one or both of α -phosvitin and β -phosvitin.

As used in this specification, the words "comprises", "comprising", and similar words, are not to be interpreted in an exclusive or exhaustive sense. In other words, they are intended to mean "including, but not limited to".

Further, any reference in this specification to prior art documents is not intended to be an admission that they are widely known or form part of the common general knowledge in the field.

The invention provides an emulsified food product comprising oil and/or fat (at least 0.5 wt% as of the total product is omega-3 fatty acids), egg protein in the amount of 0.05 to 5 % by weight, and iron ions in the amount of at least 10 ppm, wherein there is no EDTA in the food product. As described above, the presence of EDTA in many food products is undesirable, but excluding EDTA then requires an alternative way to avoid the undesirable fishy off-notes that result from the interaction between iron ions and unsaturated fatty acids, particularly omega-3 fatty acids.

Incorporating egg protein into emulsified food products that contain iron has now been found to preserve omega-3 fatty acids such that the presence of EDTA is not required to negate the action of iron by chelation. Thus, emulsified food products are obtained that contain no EDTA and have a higher level of omega-3 fatty acids which are well-known to be beneficial to human health.

Many food products inherently contain some iron ions. For example, any food product made from egg, especially egg yolk, will contain iron. Iron is a known catalyst for a range of chemical processes, one of which is the oxidation of unsaturated fatty acids. When omega-3 fatty acids in particular are oxidised, fishy and rancid flavours and aromas are commonly recognised by consumers. Thus, there is a need to remove the iron to render the iron ions inactive in some way. Removal of the iron is technically difficult and adds expense to food products. It has therefore been necessary to inactivate the iron present. The common method for doing this is to add a food grade chelating agent, such as EDTA, that will bind to the iron ions thereby inactivating the omega-3 fatty acid oxidation process.

The inventors have now found that egg protein can be included as an ingredient in emulsified food products to bind the iron ions present such that the addition of EDTA is not needed.

The egg protein used may be any such protein capable of binding iron ions. However, phosphorylated egg proteins or egg proteins containing glutamine, glutamic acid, asparagine, aspartic acid, cysteine or methionine amino acid residues are preferred. The phosphate groups, glutamic acid, aspartic acid, cysteine or methionine residues are thought to be necessary for binding with the iron ions. Livetins and phosvitins are the preferred egg proteins of the invention.

Binding of iron to egg proteins at neutral pH can be achieved with a one-step process by directly mixing the iron ions with the egg protein. However, at low pH and/or high ionic strength, the iron-binding activity of egg proteins is significantly reduced and complexes do not form when mixing the iron ions and the egg protein directly. It is known that, at least in the case of phosvitin, iron-binding activity is strongly dependent on pH and ionic strength. The maximum iron-binding activity is obtained at neutral pH and low ionic strength. The inventors have found that a two-step process, including pre-mixing of the egg protein with iron ions at neutral pH, followed by acidification to acidic pH, was necessary to allow complexation of iron ions by the egg protein and a much higher iron-binding activity than the standard one-step process.

The food product of the invention contains oil or fat. It is not important whether the product contains oil or contains fat, or a combination of the two. The food product preferably comprises at least 1 %, more preferably at least 5 %, and even more preferably at least 10 %, by weight oil and/or fat. The oil and/or fat component of the food product contains at least some omega-3 fatty acids, preferably at least 0.1 %, more preferably at least 1 %, and even more preferably at least 5 %. In preferred embodiments of the invention, the oil and/fat contains 8 to 12 % by weight omega-3 fatty acids, which is typical of products containing rapeseed oil. In other preferred embodiments, the oil and/fat contains 20 to 40 % by weight omega-3 fatty acids, which is typical of products containing fish oil. These fatty acids may be, but are not limited to, any of the nutritionally important fatty acids α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), all of which are polyunsaturated.

The amount of oil and/or fat in the food product may vary considerably. For example, a salad dressing or a low-fat mayonnaise may contain 15 to 35 % by weight oil and/or fat, and a full-fat mayonnaise may contain 70 to 85 % by weight oil and/or fat.

The egg protein may be any protein obtained from egg yolk or egg white, provided that it does give the desired benefit, i.e. interacts with iron or unsaturated fatty acids in some way to prevent or minimise the production of fishy off-notes. While not limiting to any particular theory, it is considered that the egg protein binds in some manner (e.g. by chelation or complexation) to iron ions present and therefore renders them inactive to catalysing oxidation of the unsaturated fatty acids. The egg protein is preferably a

phosphorylated protein or preferably contains glutamine, glutamic acid, asparagine, aspartic acid, cysteine or methionine amino acid residues. It is thought that the phosphate groups and the above amino acid residues aid the binding of iron. The most preferred proteins of the invention are phosvitins and livetins.

5 The egg protein may be incorporated into the food product using egg yolk or egg white itself, or may be incorporated in the form of an extract from egg yolk or egg white. The egg yolk or egg white may be fresh (i.e. viscous liquid) or may be in powdered form.

 The food product may be any emulsified food product that contains omega-3 fatty acids and iron ions and therefore where there is a need to minimise or prevent the formation
10 of fishy off-notes. Particularly suited products are mayonnaise, salad dressing, various beverages, creamer, infant formula, baby food, dairy products such as yoghurt and ice cream, cereal products, functional drinks, and pet food. The products may be full-fat or reduced-fat products, e.g. low fat mayonnaise.

 The inventors have also found that the method of preparation of the food product of
15 the invention can have an impact on the effectiveness of the egg protein for countering the effect of the iron ions on the fatty acids. Thus, in a one-step process the egg protein may be mixed with an ingredient inherently containing iron ions before oil addition and emulsification. This process is particularly suited for neutral pH food products. For acidic pH products, an acidifier may be mixed with the egg protein and the iron source before the addition of oil and
20 emulsification. In a two-step process (see Example 2 below), the egg protein may first be mixed with an ingredient already containing iron ions, and then an acidifier may be added. In this two-step process, the oil may be added and emulsified either before or after addition of the acidifier. The two-step process is particularly suited to acid pH products (e.g. mayonnaise products or salad dressings containing vinegar or citric acid as acidifier) as it
25 enhances the effectiveness of the egg protein in preventing fishy off-note formation in the final emulsified product. This two-step process is also especially suited to egg yolk or egg white peptides, which may have very low effectiveness in the food product if prepared in a one-step process but a higher effectiveness if prepared using the two-step process.

30 Those skilled in the art will understand that they can freely combine all features of the present invention disclosed herein. In particular, features described for the product of the present invention may be combined with the process of the present invention, and vice versa. Further, features described for different embodiments of the present invention may be combined.

35 The invention is further described with reference to the following examples. It will be appreciated that the invention as claimed is not intended to be limited in any way by these examples.

EXAMPLES

Example 1: Iron binding activity of phosvitin and livetin compared to EDTA

5 Livetins (or water soluble proteins) were extracted from egg yolk as follows:
Pasteurized egg yolk (400g) and acidified water (3600g, pH 2.85) were mixed for 5 min. The
pH was adjusted to 5.2 with 1 M hydrochloric acid, and the solution was stirred for 5 min. The
egg solution was left for 6 hours at 4 °C and stirred again for 5 min. The solution was then
centrifuged for 25 min at 10000 g and 4 °C. The supernatant was collected (3585 g) and
10 freeze-dried.

Phosvitin from egg yolk was obtained from Sigma (product number: P1253).

Different EDTA solutions (5–50 µM) were prepared by dissolving 10 mg EDTA in 100
ml Millipore water, and further dilution of this 0.34 mM solution to obtain lower EDTA
concentrations. The different egg protein solutions (1–50 µM) were prepared by dissolving
15 some protein sample in 100 ml Millipore water and mixing for 1 hour. A 0.5 mM iron chloride
solution was prepared by dissolving 13 mg in 200 ml Millipore water. Iron calibrating
solutions were prepared by several dilution of the above solution. A 1 mM ferrozine solution
was prepared by dissolving 25 mg of ferrozine in 50 ml in Millipore water.

The iron calibration was performed by mixing 135 µl Millipore water and 30 µl of the
20 above iron chloride solutions. The iron–chelator complexes were prepared by mixing 135 µl
of EDTA or egg protein solutions at different concentrations and 30 µl of 0.5 mM iron chloride
solution for 20 min at 26 °C. The free iron was measured by addition of 150 µl of 1mM
ferrozine to the solutions, stirring during 5 min at 26 °C, and measurement of the absorbance
of the solutions at 562 nm using a UV/Vis-Spectrometer with microplate reader (Varioskan
25 Flash).

All samples were analysed in quadruplicate. Blank samples (EDTA and egg protein
without iron) were prepared replacing 30 µl of 0.5 mM iron chloride solution with 30 µl of
Millipore water in the procedure described above. The absorbance value of the blank sample
(without iron) was deducted from the reading of the sample (with iron).

30 Figure 1 shows that when the concentration of phosvitin or livetin increases, the
concentration of bound iron ions increases in water, up to a maximum value (50 µM)
corresponding to binding of all iron ions present in solution. Phosvitin and livetins bind more
iron ions than EDTA at the same concentration and are thus more efficient iron-chelators.

Example 2: Effect of process on iron binding activity of phosvitin at acidic pH

Samples for the one-step process were prepared as follows:

A 0.1 M acetate buffer solution was prepared by mixing 463 ml of a 0.2 M acetic acid solution (5.7 ml glacial acetic acid in 494.3 ml Millipore water) with 37 ml of a 0.2 M acetate solution (1.64 g anhydrous sodium acetate with 98.36 g of Millipore water). The pH of the 0.1 M acetate buffer solution was 3.6 ± 0.1 . The different egg protein solutions (1–50 μM) were prepared by dissolving some protein sample in 100 ml of 0.1 M acetate buffer solution and mixing for 1 hour. A 0.5 mM iron chloride solution was prepared by dissolving 13 mg in 200 ml of 0.1 M acetate buffer solution. Iron calibrating solutions were prepared by several dilution of the above solution. A 1 mM ferrozine solution was prepared by dissolving 25 mg of ferrozine in 50 ml 0.1 M acetate buffer solution.

The iron calibration was performed by mixing 135 μl of 0.1 M acetate buffer solution and 30 μl of the above iron chloride solutions. The iron–chelator complexes were prepared by mixing 135 μl of egg protein solutions at different concentrations and 30 μl of 0.5 mM iron chloride solution for 20 min at 26 °C. The free iron was measured by addition of 150 μl of 1mM ferrozine to the solutions, stirring during 5 min at 26 °C, and measurement of the absorbance of the solutions at 562 nm using a UV/Vis-Spectrometer with microplate reader (Varioskan Flash). All samples were analysed in quadruplicate. Blank samples (egg protein without iron) were prepared replacing 30 μl of 0.5 mM iron chloride solution with 30 μl of 0.1 M acetate buffer solution in the procedure described above. The absorbance value of the blank sample (without iron) was deducted from the reading of the sample (with iron).

Samples for the two-step process were prepared as follows:

A 0.2 M acetate buffer solution was prepared by mixing 463 ml of a 0.4 M acetic acid solution (11.4 ml glacial acetic acid in 488.6 ml Millipore water) with 37 ml of a 0.4 M acetate solution (3.28 g anhydrous sodium acetate with 96.72 g of Millipore water) and 500 ml of Millipore water. The pH of the 0.2 M acetate buffer solution was 3.6 ± 0.1 . The different egg protein solutions (10–200 μM) were prepared by dissolving some protein powder in Millipore water different concentrations and mixing for 1 hour. A 18 mM iron chloride solution was prepared by dissolving 231 mg in 100 ml of 0.1 M acetate buffer solution. A 1 mM ferrozine solution was prepared by dissolving 25 mg of ferrozine in 50 ml 0.1 M acetate buffer solution. Iron calibrating solutions (10–100 μM) were prepared in 0.1 M acetate buffer (following the method described for the one-step process).

The iron–chelator complexes were prepared by mixing 25 ml of the above egg protein solutions with 0.25 ml of 18 mM iron chloride solution for 20 min. Acidification was performed by addition of 25 ml of pH 3.6 0.2 M acetate buffer. The free iron was measured by addition of 150 μl of 1mM ferrozine to 165 μl of iron–chelator solutions, stirring during 5 min at 26 °C, and measurement of the absorbance of the solutions at 562 nm using a UV/Vis-Spectrometer with microplate reader (Varioskan Flash). All samples were analysed in

quadruplicate. Blank samples (egg protein without iron) were prepared replacing 0.25 ml of 18 mM iron chloride solution with 0.25 ml of Millipore water in the procedure described above. The absorbance value of the blank sample (without iron) was deducted from the reading of the sample (with iron).

5 For both the one-step and the two-step process, the final iron–protein solutions all contained acetate buffer (pH 3.6, 0.1 M).

Figure 2 shows that the iron-binding activity of phosvitin depends on the method of preparation of the complexes. The iron-binding activity is higher when the iron ions and the phosvitin are pre-mixed at neutral pH before acidification with pH 3.6 acetate buffer,
10 compared to when they are directly mixed in pH 3.6 acetate buffer.

Example 3: Iron binding activity of livetins at acidic pH

Iron–livetin complexes were prepared at neutral pH according to the method described in Example 1, and at acidic pH according to the one-step process described in
15 Example 2.

Figure 3 shows that livetins surprisingly do not lose any iron-binding activity at pH 3.6 in acetate buffer, in contrast to phosvitin. The iron-chelating activity of livetins at acidic pH is very similar to that at neutral pH, i.e. all iron ions are bound with 33 μ M livetins. Livetins are an efficient iron-chelator at acidic pH, more efficient than the highly phosphorylated
20 phosvitin.

Example 4: Defatted egg yolk (yolk protein powder).

Yolk protein powder was prepared as follows: Pasteurized egg yolk was mixed with 2 volumes of (3:1) hexane:ethanol. The mixture was stirred for 3 hours and
25 filtered under vacuum on a Büchner funnel (Paper filter S&S N° 595, diameter 11 cm, retention 4-7 μ m). The sediment was then collected and dried at room temperature overnight.

Iron–egg protein complexes were prepared at neutral pH according to the method described in Example 1, and at acidic pH according to the one-step and two-step processes described in Example 2.

30 Figure 4 shows that the protein-rich defatted egg yolk powder has a some iron-binding activity at neutral pH. The iron-binding activity of the defatted yolk extract is significantly reduced at acidic pH in acetate buffer, but can be improved by using a two-step acidification process, i.e. by pre-mixing the iron and egg extract in water at neutral pH and further acidifying with acetate buffer. The efficiency of defatted yolk powder to bind iron at
35 acidic pH using the two-step method was surprisingly even higher than that obtained at neutral pH where maximum efficiency was expected.

It is to be appreciated that although the invention has been described with reference to specific embodiments, variations and modifications may be made without departing from the scope of the invention as defined in the claims. Furthermore, where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in this specification.

Example 5: Sensory properties of rapeseed oil mayonnaise during storage at room temperature

The reference mayonnaise sample was prepared as follows: 1.36 kg egg yolk, 1.58 kg water, 500 g mustard, 328 g vinegar spirit 18% were mixed in a Stephan mixer. The dry mixture containing salt, sugar and flavours was added to the liquid ingredient mixture and this new mixture was stirred. Finally 16 kg rapeseed oil was emulsified into the previous mixture. The mayonnaise sample containing 75 ppm EDTA was prepared with the same process, EDTA was added to the dry mixture. Mayonnaise samples were conditioned into glass jars. Steam injection to reduce oxygen pressure was carried out on the samples before sealing with an aluminium lid. Mayonnaise jars were stored at 20 °C in the dark during several months.

The sensory attributes of the mayonnaise samples were assessed by a trained panel of 11 people. The samples were prepared in 20 ml transparent plastic cups (~15 g serving), and individually covered with food-grade plastic film. The samples were coded with random 3-digit numbers. The samples were distributed to the panellists in monadic order (one at the time) at 20 °C. The panellists described the rancid intensity in terms of aroma (odour in nose), flavour (taste in mouth) and persistency (after-taste after spitting out). The difference among samples was determined using analysis of variance ANOVA with a 5% limit of significance (alpha risk). The significant differences were further analyzed using the LSD multiple paired comparison test with a 5% limit of significance (alpha risk). The significant differences are shown with different letters in brackets in the following tables.

Sensory attribute	Reference mayonnaise	Mayonnaise with EDTA
Rancid aroma at 125 days	2.48 (A)	1.01 (B)
Rancid after-taste at 125 days	2.02 (A)	0.9 (B)

Example 5 clearly shows that rapeseed oil mayonnaise containing omega-3 fatty acids does not keep over storage because of significant rancid off note development (odour, taste and

after-taste up to 4 months of storage), as opposed to the rapeseed oil mayonnaise containing EDTA which does not get rancid over shelf life.

Example 6: Effect of egg livetins on rancidity development in rapeseed oil mayonnaise

Egg livetin fraction was prepared according to the method described in Example 1. Mayonnaise containing 2 wt% egg livetins was prepared as follows: 1.36 kg egg yolk, 400 g livetin fraction and 1.18 kg water were mixed in a Stephan mixer. 500 g mustard was added to the liquid ingredient mixture and this new mixture was stirred. 328 g vinegar spirit 18% containing salt, sugar and flavours was added and the mixture was stirred. Finally 16 kg rapeseed oil was emulsified into the previous mixture. The separate addition of mustard and vinegar allows step-wise acidification of the yolk/livetins mixture, thus formation of the iron-protein complexes. The mayonnaise sample containing 75 ppm EDTA was prepared according to the process described in example 5. Mayonnaise samples were conditioned and stored according to the method described in example 5.

Sensory assessment of the different mayonnaise samples was performed according to the procedure described in example 5. The significant differences are shown with different letters in brackets in the following table.

Sensory attribute	Reference mayonnaise	Mayonnaise with EDTA	Mayonnaise with livetin fraction
Rancid after-taste at 65 days	2.02 (A)	0.94 (B)	0.87 (B)

Example 6 shows that the use of livetins in rapeseed oil mayonnaise is as efficient at preventing rancidity development after 2 months of storage as EDTA, and livetins are an efficient natural solution to extend shelf life of omega-3 mayonnaise.

Example 7: Effect of hydrolyzed egg protein on rancidity development in rapeseed oil mayonnaise

Mayonnaise containing 1.2 wt% hydrolyzed egg white was prepared as follows: 1.36 kg egg yolk, 240g hydrolyzed egg white and 1.34 kg water were mixed in a Stephan mixer. 500 g mustard was added to the liquid ingredient mixture and this new mixture was stirred. 328 g vinegar spirit 18% containing salt, sugar and flavours was added and the mixture was stirred. Finally 16 kg rapeseed oil was emulsified into the previous mixture. The separate addition of mustard and vinegar allows step-wise acidification of the yolk/livetins mixture, thus the iron-protein complexes. The mayonnaise sample containing 75 ppm EDTA was prepared

according to the process described in example 5. Mayonnaise samples were conditioned and stored according to the method described in example 5.

- 5 Sensory assessment of the different mayonnaise samples was performed according to the procedure described in example 5. The significant differences are shown with different letters in brackets in the following table.

Sensory attribute	Reference mayonnaise	Mayonnaise with EDTA	Mayonnaise with hydrolyzed egg white
Rancid aroma at 55 days	2.51 (A)	0.98 (B)	1.41 (B)

- 10 Example 7 shows that the use of hydrolyzed egg white in rapeseed oil mayonnaise prevents rancidity development after 2 months of storage in the same way as EDTA. Hydrolyzed egg white is thus an efficient solution to extend shelf life of omega-3 mayonnaise without using synthetic additives.

15 ***Example 8: Effect of two-step acidification process on rancidity development in rapeseed oil mayonnaise***

- Mayonnaise containing 1.2 wt% hydrolyzed egg white was prepared in a two-step process according to the method described in example 7. Mayonnaise containing 1.2 wt% hydrolyzed egg white in a one-step process was prepared according to the method described in example 5, where 240 g of hydrolyzed egg white powder was added to the dry mixture. Mayonnaise samples were conditioned and stored according to the method described in example 5. Sensory assessment of the different mayonnaise samples was performed according to the procedure described in example 5. The significant differences are shown with different letters in brackets in the following table.

Sensory attribute	Reference	One-step process	Two-step process
Rancid aroma at 55 days	2.51 (A)	2.15 (AB)	1.41 (B)

- 25 Example 8 shows that with the standard one-step process, the mayonnaise containing hydrolyzed egg white is not significantly different from the reference mayonnaise in term of rancid off note. However with the new two-step process, the mayonnaise containing hydrolyzed egg white is significantly less rancid than the reference mayonnaise. The use of a two-step process in rapeseed oil mayonnaise containing hydrolyzed egg white is thus more
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efficient at preventing rancidity development during storage than the one-step standard process.

CLAIMS

1. A shelf-stable emulsified food product comprising:
 - a) oil and/or fat, which contains at least 0.5 wt% omega-3 fatty acids per total of
5 the emulsified food product;
 - b) 0.05 to 5.0 wt% egg protein per total emulsified food product; and
 - c) at least 10 ppm iron ions per total emulsified food product;wherein the food product contains no EDTA (ethylenediaminetetraacetic acid).
- 10 2. The food product as of claim 1, which comprises at least 0.8 wt% omega-3 fatty acids, preferably at least 1.2 wt%, more preferably at least 2.0 wt%, or even more preferably at least 4.0 wt% omega-3 fatty acids per total of the emulsified food product.
- 15 3. The food product of claim 1 or 2, which comprises 15-35% or 70-85% oil and/or fat by weight of the total food product.
4. The food product of one of the above claims, which comprises 0.1 to 3 % by weight of
20 egg protein per total emulsified food product.
5. The food product of one of the above claims, which comprises at least 20 ppm iron ions, preferably at least 50 ppm iron ions.
6. The food product of one of the above claims, wherein the egg protein is a protein from
25 egg yolk.
7. The food product of claim 6, wherein the egg yolk protein is phosvitin, livetin or a combination thereof.
- 30 8. The food product of one of the above claims, which is a mayonnaise, salad dressing, beverage, creamer, infant formula, baby food, dairy product, cereal product, functional drink, ice cream, or pet food.
- 35 9. A process for preparing an emulsified food product that contains no EDTA (ethylenediaminetetraacetic acid) comprising the step of mixing together:
 - a) oil and/or fat;
 - b) 0.05 to 5.0 wt% egg protein per total emulsified food product;

c) a source of iron which contains at least 10 ppm iron ions per total emulsified food product; and

d) water;

wherein the oil and/or fat contains at least 0.5 wt% omega-3 fatty acids calculated per total of the emulsified food product.

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10. The process as of claim 9, wherein the egg protein is phosvitin, livetin or a combination thereof.

10 11. The process as of claim 9 or 10, further comprising mixing an acidifier with the components a) to d).

12. The process as of claim 11, wherein the components b) and d) are first mixed together; then the acidifier is added and mixed; and then a) is added and the entire mixture is emulsified.

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13. The process as of claim 11, wherein the components b) and d) are first mixed together; then a) is added and the mixture is emulsified; and then the acidifier is added and mixed with the emulsion.

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14. The process as of claim 11, wherein the components b) and d) and the acidifier are mixed together; and then a) is added and the entire mixture is emulsified.

15. Use of an egg protein in an emulsion comprising an oil and/or fat containing at least 0.5 wt% omega-3 fatty acids per total of the emulsion, at least 10 ppm iron ions and water, for preventing or retarding oxidation of the omega-3 fatty acids upon storage of the emulsion.

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