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(71) Applicant *(for all designated States except US)*: **FRIESLAND BRANDS B.V.** [NL/NL]; Blankenstein 142, NL-7943 PE Meppel (NL).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **POORTINGA, Albert Thijs** [NL/NL]; Spadelaan 54, NL-7331 AL Apeldoorn (NL).

(74) Agent: **WINCKELS, J.h.f.**; C/o Vereenigde, Johan De Wittlaan 7, NL-2517 JR The Hague (NL).

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(54) Title: MICROCAPSULES

(57) Abstract: The invention relates to capsules, in particular to microcapsules which are suitable to be used in foods, and to methods for the preparation of such capsules. The invention further relates to foods, in particular dairy, such as cheese, in which such capsules have been processed. According to the invention, microcapsules are provided, comprising a capsule, which capsule comprises gelled casein.

Microcapsules

The invention relates to capsules, in particular to microcapsules which are suitable to be used in foods, and to methods for the preparation of such capsules. The invention further relates to foods, in particular dairy, such as cheese, in which such capsules have been incorporated.

Use of (micro)capsules for, for instance, pharmaceutical purposes is known per se. Also, microcapsules are used in the food industry, for instance for adding fragrances or flavorings.

In general, encapsulation takes place by emulsifying the material to be encapsulated (in liquid or solid form), or dispersing this material in an aqueous solution of the capsule material. By manipulating the solubility for the capsule material, for instance by adding particular ions, such as calcium (WO-A-03/018186), or by adding materials which form a coacervate with the already dissolved capsule material (EP-A-0 856 355), the capsule material precipitates on the core. Another method is to add a cross-linker to the core which cross-links dissolved capsule material on the core and thus makes it precipitate. Another possibility to enable a capsule to be formed is to make the solvent for the capsule material evaporate (US-A-5 601 760). In order to gain more control of the capsule size, an oil-in-water-in-oil emulsion (o/w/o emulsion) may also be made, with the primary emulsion containing the substance to be encapsulated and the water phase containing the capsule material. After cross-linking of the capsule material, the capsule can be separated from the oil (WO-A-04/022 220). These methods can only be used to encapsulate materials which are poorly soluble or insoluble in water or aqueous solutions.

With the aid of a variant of the above method, aqueous solutions can also be encapsulated. The aqueous solution is emulsified in a non-polar

substance (the 'oil' phase). In a number of cases, the oil phase contains the dissolved capsule material. A capsule can now be formed by evaporating the oil phase (CA-A-2 126 685), or, for instance, by slowly dissolving water in the oil phase, so that the oil-soluble capsule material precipitates (US-A-2002/0 160 109). Another possibility is having a water-soluble and an oil-soluble component react/complex in the water-oil interface, such as for instance in CA-A-1 148 800.

Further, protein-based capsules are known from, for instance, US-A-4 147 767, US-A-4 349 530 and GB-A-2 224 258. In all these publications, a protein solution (with the substance to be encapsulated added thereto) is emulsified in oil (with or without an emulsifier dissolved therein), the proteins are cross-linked and the particles thus created are separated from the oil, for instance by centrifuging, filtering, washing or a combination of these processes. In order to bring about the cross-linking of the proteins, heating takes place (US-A-4 147 767) or a cross-linker, such as glutaraldehyde (US-A-4 349 530) or a carbodiimide (GB-A-2 224 258) is added. These different manners of encapsulation have some drawbacks for use in foods. Thus, heating usually leads to protein denaturation, which usually causes undesired off-flavors. It is not allowed to use glutaraldehyde in the preparation of foods.

The present invention contemplates providing (micro)capsules which do not have the drawbacks of the capsules of the prior art. The present invention further contemplates providing a product which can be added to foods without any problems, for instance to specifically influence the structure, the viscosity and/or the texture of the food.

It has been found that this can be realized by making capsules from casein, which at least partly forms a network. Therefore, the present invention relates to at least one microcapsule, comprising a capsule, which capsule comprises gelled casein. "Gelled casein" is understood to mean that the casein is enabled to form physical bonds and/or chemical bonds and thus

form a network. Preferably, colloidal casein (calcium caseinate) is used, because this gels well by means of coagulation. In the literature, no mention has yet been made of capsules comprising gelled-out colloidal casein.

The formation of the casein network in the capsules according to the invention is preferably carried out enzymatically, in particular by means of coagulation (i.e. the gelling of casein by the enzyme chymosin). However, the network may also be formed by acidifying the casein solution.

A suitable method for forming the capsules according to the invention comprises the following steps:

- forming a solution of casein in a suitable solvent, in most cases water. A suitable concentration for the casein is between 0.5 and 15 wt.%. This solution is maintained at a low temperature, preferably lower than 15°C, more preferably 10°C or less. To the water, material to be encapsulated can be added;
- then, at a low temperature, the gelling agent, preferably coagulant, is added to this solution;
- the thus obtained solution is emulsified in a non-polar phase, in most cases in an oil, for instance sunflower oil or olive oil. Preferably, edible oils are used, but non-edible oils may optionally be used as well. However, in principle, any liquid in which water can be emulsified is usable. Preferably, for forming the emulsion, an emulsifier is used, such as Span™85 or Span™80. The HLB number ("Hydrophile-Lipophile Balance", as defined by W.C. Griffith in J. Soc. Cosmet. Chem., Vol. 1 (1949) 311) of the emulsifier used should preferably be lower than 7.
- The emulsion is subjected to conditions in which the network-forming reaction takes place, in most cases by heating, for instance to a temperature of more than 25°C, more preferably to a temperature of 26-35°C, for instance approx. 30°C.
- Then the casein capsules thus formed in the oil phase and filled with water (with or without a substance to be encapsulated therein) can be

transferred to an aqueous solution. This can be done by laying the oil with the casein capsules therein onto an aqueous solution. After some time, the capsules sink from the oil phase to the water phase under the influence of gravity. This process can be accelerated by centrifuging. The process can also be accelerated by choosing conditions in which the action of the emulsifier added to the oil phase is reduced or cancelled out. Examples thereof are choosing the right temperature (usually a lowest possible temperature) or adding an emulsifier with a high HLB number (higher than 7), such as for instance Tween™20, to the water phase on which the oil phase lies.

For some applications, it is not necessary to separate the capsules from the oil. The oil with the capsules therein can, for instance, be added directly to oil-continuous systems, such as for instance butter. Another option is again (mildly) emulsifying the oil with the casein capsules therein in an aqueous phase. This results in an emulsion of oil droplets in water, with the oil droplets being filled with the water trapped in the casein capsules.

Use of casein is very advantageous with use of the capsules in cheese because, in this case, use is made of a pure cheese-specific material.

In addition to the encapsulation use, the capsules can also be added to foods to provide them with structure/viscosity.

The capsules according to the invention contain approx. 95 wt.% or more water, preferably approx. 97 wt.% of water and approx. 3 wt.% of casein.

The microcapsules according to the invention preferably have an average diameter of between 0.1 and 1000 μm, more preferably between 1 and 500 μm, typically between 10 and 100 μm.

Use of the casein capsules according to the invention in, for instance, foods, in particular in dairy, such as cheese, can yield a number of advantages, such as for instance a considerable saving of materials.

Cheese, for instance, normally contains 40 wt.% of water and 30 wt.% of casein. Due to uses of the capsules according to the invention in cheese, the water/casein ratio becomes 33 instead of 1.3 on the plate where the capsule according to the invention ends up. In this manner, it is possible to reduce the protein content of the cheese, with an increase of the moisture content. Further, it is possible to replace a part of the amount of casein normally used in cheese by water, because, due to their structure, the capsules have a greater water-binding capacity than casein in a conventional form. This enables an increase in the moisture content of the cheese.

An important advantage of the capsules according to the invention as an additive to cheese over conventional additives, such as for instance whey protein-based or starch-based additives, is that casein is a "cheese-specific" material. So, with addition of the capsules to cheese, no ingredients are added to cheese which are not already normally present in cheese. Of course, this also holds for the addition to other foods, in particular to dairy. This means that adding the capsules need not lead to off-flavors.

A further advantage of the capsules according to the invention is that the casein can be cross-linked, that is, gelled by means of coagulation. Coagulation is a natural and food-grade manner of processing, unlike adding glutaraldehyde-based cross-linkers, or other cross-linkers, as is proposed in the prior art, and unlike making proteins cross-link by means of heating, which can lead to protein denaturation, which may result in undesired flavor changes.

Another advantage of the capsules according to the invention is that they can disintegrate at a low pH, in particular at a very low pH as it is prevalent in the stomach. This fact can be used, for instance by encapsulating components with a bad taste, while these components can subsequently still be digested well. Many of the capsules from the above-mentioned prior art do not have this property.

An interesting discovery was made when an attempt was made to make the capsules without using an emulsifier in the oil phase. It has been found that, in that case, aggregates of casein capsules are created in the oil. When the oil is subsequently emulsified in water, then these aggregates go over to the water phase, taking along the oil trapped among the aggregated capsules. Thus, an oil-in-water emulsion is created in which the fat droplets are filled with aggregated casein capsules. These emulsions can be used for low-fat applications. Also, this emulsion is excellently suitable for encapsulating water-soluble components in the casein capsules in the oil droplets. In fact, the emulsion described is a so-called double emulsion. Double emulsions of water-in-oil-in-water are known in the literature and are used more often to encapsulate water-soluble components in the innermost water phase. However, double emulsions known so far always use oil-soluble emulsifiers. It is known that these emulsifiers can increase the solubility in the oil of encapsulated material. As a result, encapsulated material generally escapes quickly from the innermost water phase. The double emulsion described herein will not have this drawback. In principle, a same double emulsion can be realized with the aid of capsules which are formed by cross-linked proteins other than casein. Therefore, the present invention also relates to an oil-in-water emulsion, in which the oil droplets are filled with capsules according to the invention, of which emulsion, the oil phase is preferably at least substantially emulsifier-free. More preferably, according to the invention, the oil phase is completely emulsifier-free.

The invention will now be explained on the basis of examples. Unless stated otherwise, all ratios are weight ratios.

Example 1

A solution of 1-5% of casein (casein obtained from milk by microfiltration and spray-drying of the retentate) was prepared in water

with a temperature of 10°C. Thereto, about 0.5 ml of coagulant/liter water was added. The obtained casein solution was then dispersed in a weight ratio of 1:5 in sunflower oil, to which 0.2% of Admul WOL™ emulsifier (HLB value of about 1) had been added, utilizing a stirrer (Kinematica Polytron PT3000, Switzerland). The oil was then heated to 30°C and maintained at this temperature for 20 minutes. A part of the thus obtained o/w emulsion was then poured onto a 1% aqueous solution of Tween™ 20 in a tube and was then centrifuged for 15 minutes at 5000 g.

The same treatment was carried out with pure water, i.e. water to which no Tween™ 20 had been added.

In both cases, the centrifuging step resulted in the transfer of an important part of the water droplets surrounded by casein to the aqueous phase. The thus obtained globules were dyed, utilizing Rhodamine B. Then, the samples were examined with the aid of a confocal scanning laser microscope (CSLM). The results of the Tween-free sample are shown in Fig. 1. The scale of Fig. 1 is the same as the scale of Fig. 2. At the bottom right in Fig. 2, a marking is shown which indicates a length of 80 μm.

It follows from Fig. 1 that the average diameter of the capsules was approx. 30 μm.

Example 2

Example 1 was repeated, but now Span™ 85 was used as an emulsifier. Span™ 85 is an oil-soluble emulsifier which is moderately hydrophilic (the hydrophilic/lipophilic balance, HLB is 1.8). Due to the higher HLB, the capsules could be transferred from the oil to the water phase even easier than in Example 1 by laying the capsule-containing oil on water. Under the influence of gravity, within an hour, the capsules largely moved from the oil to the water phase. A CSLM image of the thus obtained capsules is shown in Fig. 2.

Example 3

Example 1 was repeated, but now no emulsifier was added to the oil phase. After the heating step, the capsules were formed in the oil. Now, the capsules were found to have formed aggregates in the oil. Now, the (aggregates of) capsules were not separated from the oil, but the oil was emulsified (in a weight ratio of 1 to 10) with the aid of a stirrer in water with 0.5% of casein added thereto. A thus formed CSLM image of the oil droplets in water is shown in Fig. 3. In this Figure, the oil phase colors green, the protein phase red and the water phase black. Fig. 3 shows that oil droplets have been formed which are again filled with (aggregated) casein capsules.

CLAIMS

1. A microcapsule comprising a capsule, which capsule comprises gelled casein.

2. A microcapsule according to claim 1, wherein the casein has been gelled with the aid of enzymes, preferably with the aid of coagulant.

3. A microcapsule according to any one of the preceding claims, which is filled with a polar, preferably aqueous, liquid, or is filled with a gas, preferably air.

4. A microcapsule according to any one of the preceding claims, which is filled with a solution, a suspension or a dispersion of one or more components in a polar liquid.

5. A microcapsule according to any one of the preceding claims, with a diameter of between 0.1 and 1000 μm.

6. A microcapsule according to any one of the preceding claims, with a moisture content of 50 wt.% or more, preferably 80% or more, in particular 97% or more.

7. An oily phase with microcapsules according to any one of the preceding claims therein.

8. An aqueous phase with microcapsules according to any one of the preceding claims 1-6 therein.

9. An oil-in-water emulsion in which the oil droplets are filled with microcapsules according to any one of the preceding claims 1-6.

10. An emulsion according to claim 9, wherein the oil phase is at least substantially emulsifier-free.

11. An emulsion according to claim 10, wherein the capsules comprise cross-linked proteins other than casein.

12. A food, preferably dairy product, comprising microcapsules according to any one of claims 1-6.

13. A cheese according to claim 12.

14. A method for the preparation of microcapsules, comprising the steps of:

- emulsifying, at a temperature of <25°C, a casein solution with an enzymatic gelling agent, preferably coagulant, added thereto in an oil with an emulsifier added thereto;

- heating the thus obtained emulsion to a temperature at which the gelling agent becomes active;

- maintaining the emulsion at this temperature for a period which is sufficiently long to form a capsule; and

- optionally separating the microcapsules from the oil phase.

15. Microcapsules obtainable according to the method of claim 14.

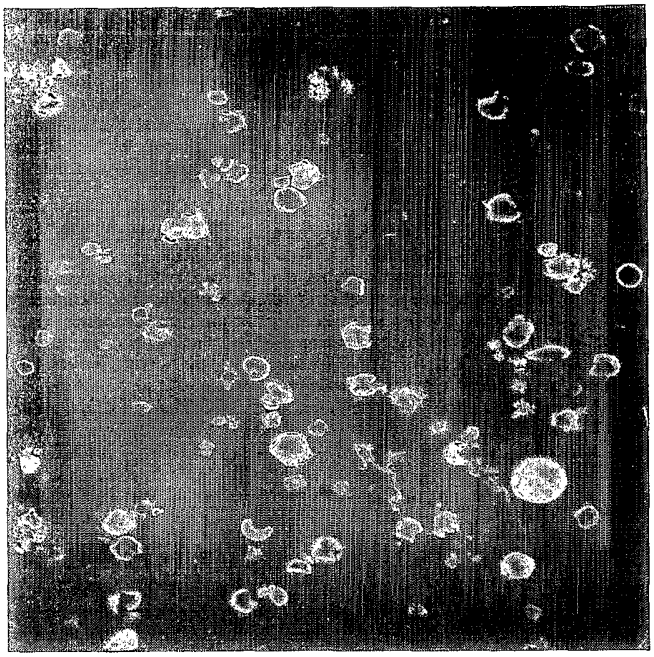

Fig. 1

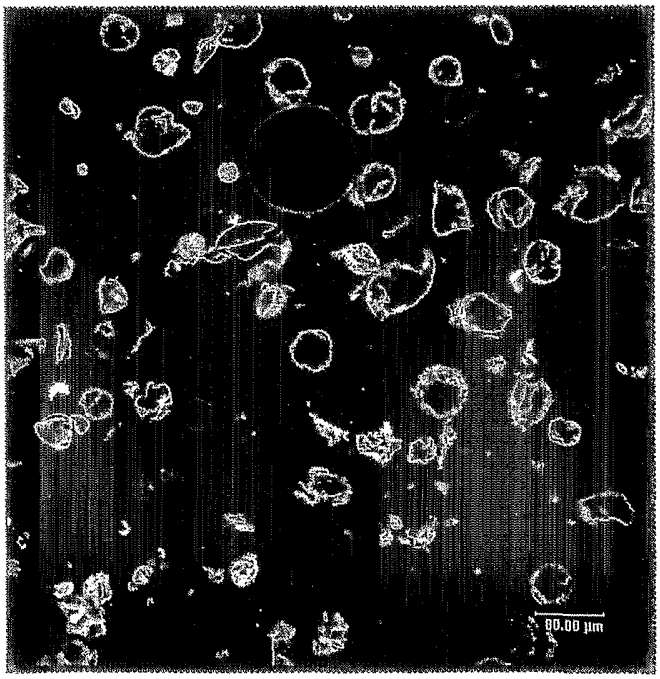

Fig. 2

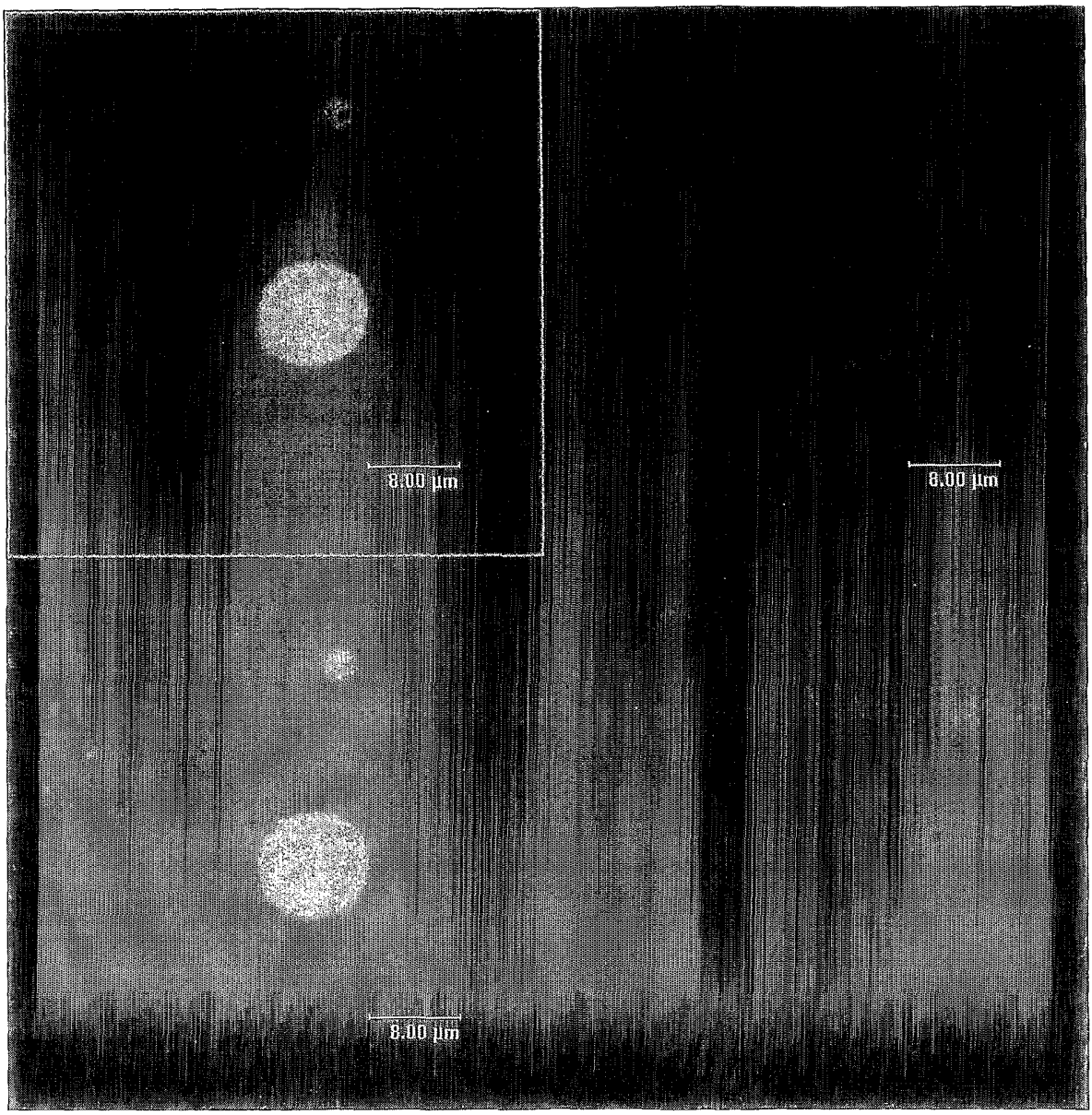

Fig. 3.

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2006/000092

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01J13/02 A23L3/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01J A23L

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, FSTA, BIOSIS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 006, no. 127 (C-113), 13 July 1982 (1982-07-13) & JP 57 053232 A (SNOW BRAND MILK PROD CO LTD), 30 March 1982 (1982-03-30) abstract	1-5,8, 12,14,15
X	US 4 729 792 A (SEITZ ET AL) 8 March 1988 (1988-03-08) claims 1-7	1,8

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 May 2006	19/05/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Willsher, C

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2006/000092

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2006/000092

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