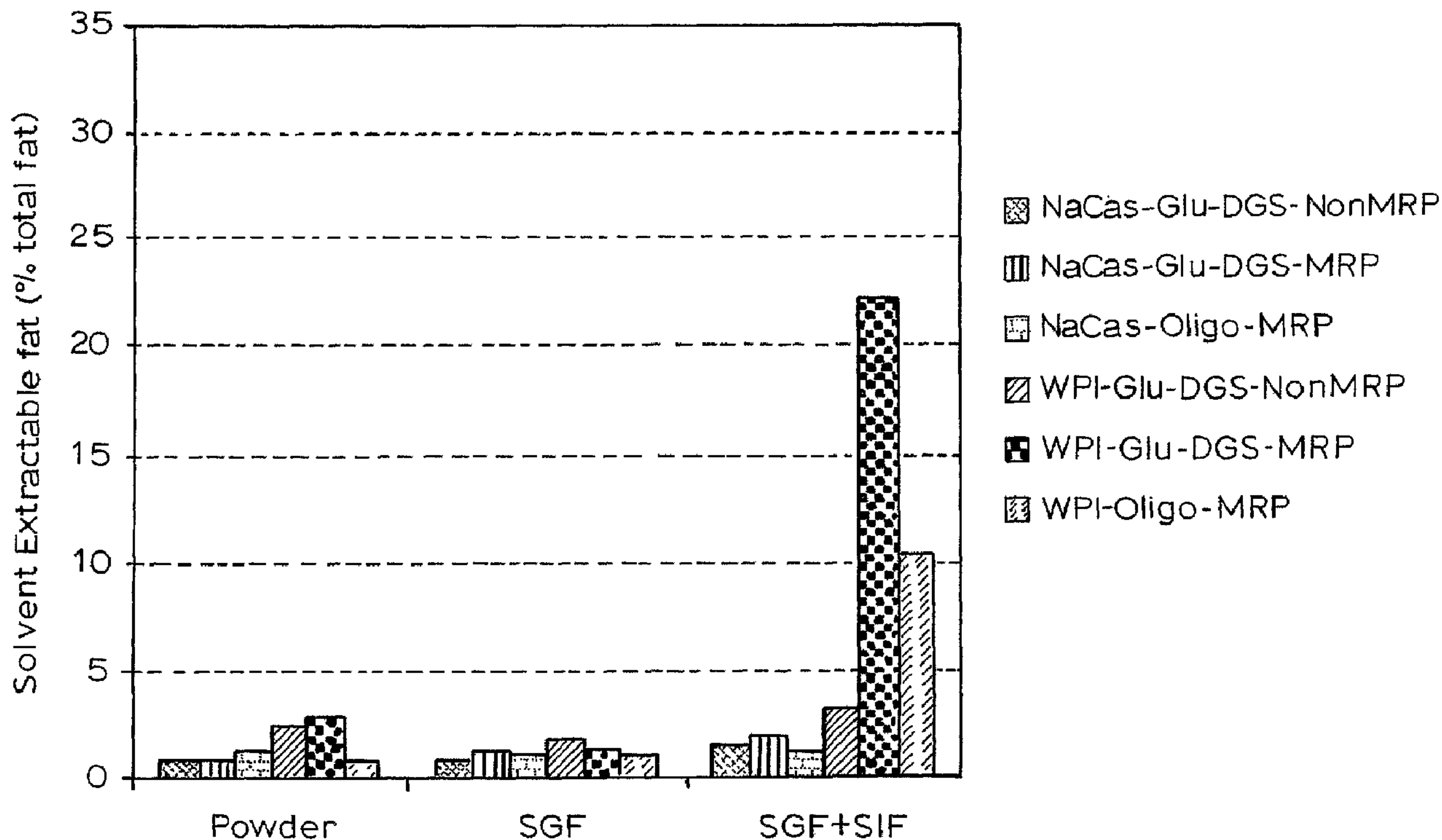




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 (72) Inventeurs/Inventors:  
 AUGUSTIN, MARY ANN, AU;  
 SANGUANSRI, LUZ, AU;  
 HEAD, RICHARD, AU  
 (73) Propriétaire/Owner:  
 COMMONWEALTH SCIENTIFIC & INDUSTRIAL  
 RESEARCH ORGANISATION, AU  
 (74) Agent: KIRBY EADES GALE BAKER

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 (54) Title: PROTEIN-POLYSACCHARIDE MICROCAPSULES FOR DELIVERING ACTIVE AGENTS TO THE GI TRACT



(57) Abrégé/Abstract:

A micro encapsulation material for use with storage unstable, therapeutic and nutritional agents which release the therapeutic and nutritional agents in predetermined locations in the gastro intestinal tract in which the encapsulation material is formed by combining a food grade treated carbohydrate with a water soluble food grade protein. The therapeutic and nutritional agents form an oil phase that is emulsified with the water dispersed or dissolved encapsulant to encapsulate the therapeutic and nutritional agents. These agents may be oils or oil soluble or oil dispersible. The agents that may be encapsulated include lipids (oils including

(57) **Abrégé(suite)/Abstract(continued):**

oxygen sensitive oils, fatty acids, triglycerides) and oil soluble and oil dispersible ingredients (including pharmaceuticals, probiotics, protein therapeutics and bioactives). The protein used may include any film forming water soluble protein or hydrolysed protein and includes milk proteins such as casein and its derivatives or whey proteins. The carbohydrate component may be those containing reducing sugar groups, oligosaccharides and starches (raw, modified, resistant, acetylated, propionylated and butylated starches).

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VIC 3030 (AU). **HEAD, Richard** [AU/AU]; 10 Pulteney  
Street, Adelaide, S.A. 5000 (AU).

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(74) Agent: **MISCHLEWSKI, Darryl**; I P Strategies, P.O.  
Box 1254, Camberwell, VIC 3124 (AU).

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MONWEALTH SCIENTIFIC & INDUSTRIAL  
RESEARCH ORGANISATION** [AU/AU]; Limestone  
Avenue, Campbell, ACT 2601 (AU).

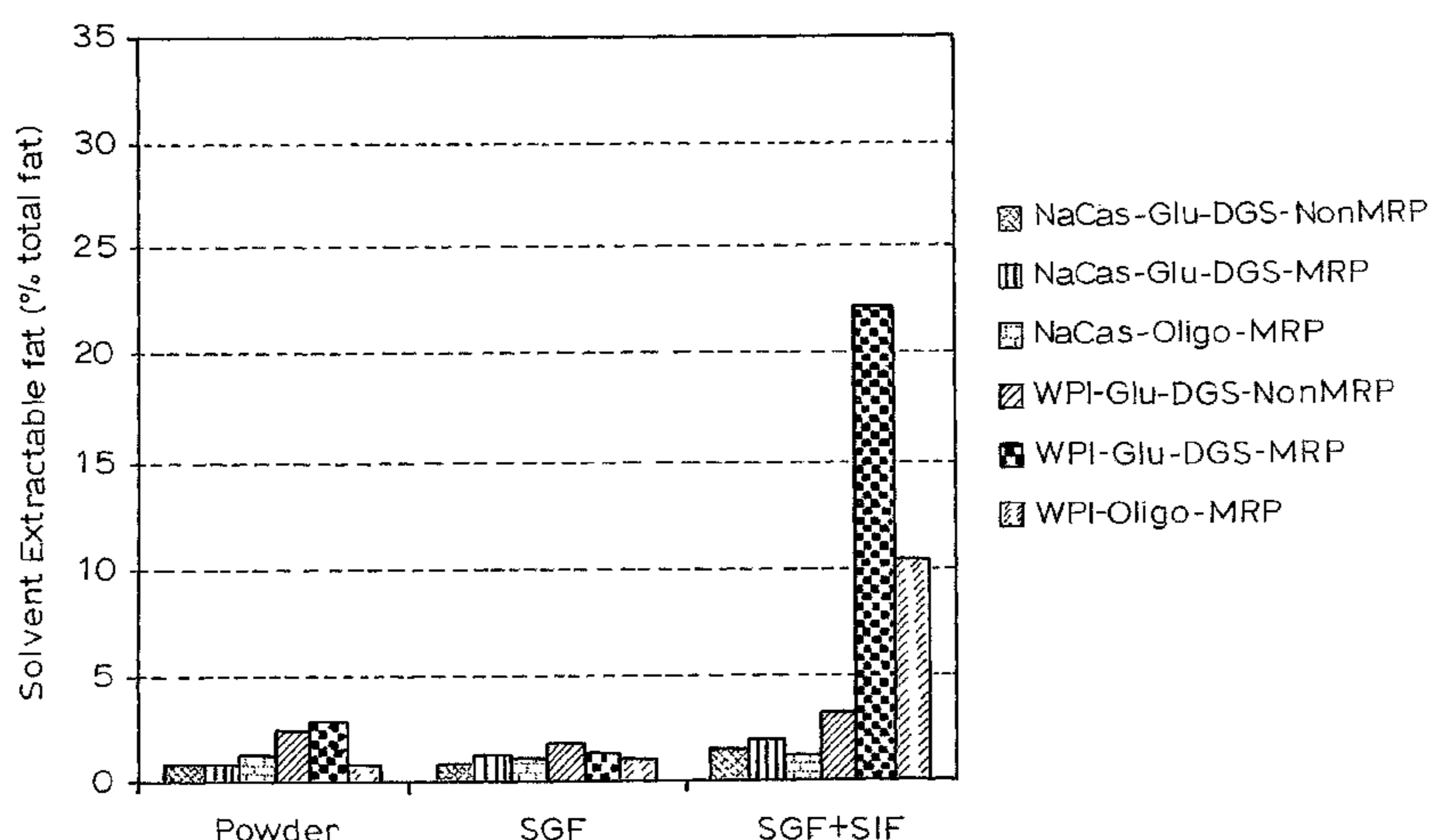
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(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **AUGUSTIN, Mary,  
Ann** [MY/AU]; Sneydes Road, Werribee, VIC 3030 (AU).  
**SANGUANSRI, Luz** [AU/AU]; Sneydes Road, Werribee,

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(54) Title: G I TRACT DELIVERY SYSTEMS



(57) **Abstract:** A micro encapsulation material for use with storage unstable, therapeutic and nutritional agents which release the therapeutic and nutritional agents in predetermined locations in the gastro intestinal tract in which the encapsulation material is formed by combining a food grade treated carbohydrate with a water soluble food grade protein. The therapeutic and nutritional agents form an oil phase that is emulsified with the water dispersed or dissolved encapsulant to encapsulate the therapeutic and nutritional agents. These agents may be oils or oil soluble or oil dispersible. The agents that may be encapsulated include lipids (oils including oxygen sensitive oils, fatty acids, triglycerides) and oil soluble and oil dispersible ingredients (including pharmaceuticals, probiotics, protein therapeutics and bioactives). The protein used may include any film forming water soluble protein or hydrolysed protein and includes milk proteins such as casein and its derivatives or whey proteins. The carbohydrate component may be those containing reducing sugar groups, oligosaccharides and starches (raw, modified, resistant, acetylated, propionylated and butylated starches).

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## PROTEIN-POLYSACCHARIDE MICROCAPSULES FOR DELIVERING ACTIVE AGENTS TO THE GI TRACT

This invention relates to microencapsulated formulations for delivery of nutritional and pharmaceutical agents to the gastro intestinal tract and in particular the colon.  
5 The compositions may be used for protection and delivery of nutrients or nutraceuticals in processed foods.

### Background to the invention

Microencapsulation involves the packaging of small particles of solid, liquid or gas  
10 within a secondary material to form a microcapsule. It has been used for targeted delivery of drugs in the body in the pharmaceutical industry. It is increasingly being seen as a technology that offers novel food processing solutions. With the use of microencapsulation, possible undesirable interactions between the added nutraceutical and other components in the food or its environment can be avoided  
15 and the site of release of the added component can be manipulated. The appropriate application of microencapsulation technology enables the fortification of food without affecting the taste, aroma or texture of food. It can afford protection to sensitive food ingredients and enhance the shelf-life and stability of fortified foods (Brazel, C.S. (1999) Microencapsulation: offering solutions for the food  
20 industry. *Cereal Foods World* 44(6): 388-393; Augustin, M.A., Sanguansri, L., Margetts, C. and Young. B. (2001) Microencapsulation of food ingredients. *Food Australia* 53 220-223).

Microencapsulation can serve both the purposes of the food and health industries, as it is a key technology with potential for the delivery of dietary bioactives and  
25 development of successful marketable functional foods. Addressing this challenge, requires tailoring the performance of food grade microcapsules in a food processing environment so that essential sensitive components are protected during food manufacture and the microcapsules can also meet the need for site specific delivery within the gastrointestinal tract.

30 Directing nutraceuticals and therapeutics of the colon is of interest for treatment of colon diseases (Rubinstein, A., Tirosh, B., Baluom, M., Nassar, T., David, A., Radai, R., Gliko-Kabir, I. And Friedman, M. (1997). The rationale for peptide drug delivery to the colon and the potential for polymeric carriers as effective tools. *J.*

*Controlled Release* **46**, 59-73). Targeting to colon has been carried out by formation of pro-drugs which are enzymatically cleaved in the colon, and multi-coats with pH sensitive and pressure dependent release. Often enteric acrylic polymers are used to protect cores in colon-delivery formulations. Biopolymers, particularly polysaccharides, may be used for targeting cores to the colon where the release of cores is triggered by the microflora in the colon. A range of polysaccharides such as chitosan, pectin, arabinoxylan, arabinogalactan, xylan, cellulose dextrans, guar gum, amylose, inulin and mixtures of these have been examined and shown to have potential as colon-delivery systems (Rubinstein, A. (2000) Natural Polysaccharides as targeting tools of drugs to the human colon. *Drug Development Research* **50**, 435-439; Sinha, V.R. and Kumaria, R. (2001) Polysaccharides in colon-specific drug delivery *Int. J. Pharmaceutics* **224**, 19-38; Vandaamme, Th.F., Lenourry, A., Charrueau, C. and Chaumeil, J.-C. (2002) The use of polysaccharides to target drugs to the colon. *Carbohydrate Polymers* **48**, 219-231; Sinha, V.R. and Kumaria, R. (2003) Microbially triggered drug delivery to the colon. *Eur. J. Pharmaceutical Sciences* **18**, 3-18).

There have been a number of attempts to use biopolymers for colon delivery and for treating colonic diseases

US Patent 5,952,314 discloses an enteral product comprising an oil blend with fatty acids {EPA (C20:5) and DHA(C22:6)} and a source of indigestible carbohydrate which is metabolised to short chain fatty acids in the colon. It has use for improving nutritional status and treating ulcerative colitis

US5108758 discloses a glassy amylose matrix for delivery of medication to the colon

US 5840860 is concerned with delivery of short chain fatty acids (SCFA) to the colon by way of a modified starch.

Japanese patent 10324642 discloses a colon delivery system for delivery of bioactives (eg peptides) comprising inner layer of chitosan and outer-layer of gastric resistant material such as wheat gliadin or zein.

US 5866619 discloses a colonic delivery system for drugs such as proteins and peptides comprising a saccharide containing polymer

US 6368629 discloses a drug coated with an organic acid-soluble polymer and a saccharide for colon delivery.

US 544054 discloses a method of treating colitis with a composition containing oil blend (with DHA/EPA) and a source of indigestible carbohydrate (CHO) which is metabolised to short chain fatty acids.

US 5952314 is concerned with an enteral nutritional product for treatment of colitis  
5 which comprises oil containing EPA/DHA and a source of indigestible carbohydrate which is metabolised to short chain fatty acids.

US 6531152 describes a drug delivery system containing a water soluble core (Ca pectinate or other water-insoluble polymers) and outer coat which bursts (eg hydrophobic polymer - Eudragit) for delivery of enterally-administered drugs to  
10 specific locations along the gastrointestinal tract

There are proposals using combinations of proteins and polysaccharides for the formation of coating systems.

US 6234464 discloses a system in which oils / polyunsaturated fatty acids (PUFA)/ fatty acids are provided with capsules comprised of two layers in which the inner  
15 layer consists of gelatin, casein or alginate and the outer layer consists of gelatin, gum arabic, chitosan to provide a product stable in boiling water

US 6403130 discloses a coating composition comprising a polymer containing casein and high methoxy pectin (amide formed by reaction of ester group  $R'COOCH_3$  of pectin with free amino group of protein  $R''NH_2$ )

WO 01/74175 discloses the encapsulation of oxygen sensitive materials such as polyunsaturated oils in a protein carbohydrate film treated to form a Maillard reaction product.

It is an object of this invention to provide a gastrointestinal delivery system that can be used with storage unstable ingredients as well as providing protection during  
25 delivery through the gut.

### **Brief description of the invention**

To this end the present invention provides a micro encapsulation material for use with storage unstable, therapeutic and nutritional agents which release the  
30 therapeutic and nutritional agents in predetermined locations in the gastrointestinal tract in which the microencapsulation material is formed by combining a food grade treated carbohydrate with a water soluble food grade protein.

The therapeutic and nutritional agents form an oil phase which is emulsified with the water dispersed or dissolved encapsulant to encapsulate the therapeutic and nutritional agents. These agents may be oils or oil soluble or oil dispersible which in the latter case may include water soluble ingredients.

5 The agents that may be encapsulated include lipids (oils including oxygen sensitive oils, fatty acids, triglycerides) and oil soluble and oil dispersible ingredients (including pharmaceuticals, probiotics, and bioactives). Water dispersible components including those that partition between oil and water phases may also be encapsulated. When water dispersible therapeutic and  
10 nutritional agents are used they may not be encapsulated with the oil phase but may be dispersed in the encapsulant film. The emulsions may be used as food ingredients or therapeutic agents but preferably the emulsions are dried to form powders.

Prior art encapsulation systems did not consider the use of combinations of  
15 proteins with other biopolymers for formation of capsules for target delivery of sensitive cores to the colon.

The delivery systems of this invention enable pharmaceutical and food manufacturers to offer a range of nutritionally and physiologically functional food ingredients and bioactive compounds in convenient formats and using all natural  
20 ingredients which will also enable the delivery of these products to the colon.

Some of the encapsulants used for colon delivery in this invention have the added benefits of being effective matrices for encapsulating oxygen sensitive ingredients. The film-forming and anti-oxidant properties of some of the encapsulants used work synergistically to preserve sensitive ingredients such as polyunsaturated fatty  
25 acids from being oxidised during storage and also protects them during exposure to high temperature, pressure and moisture encountered during the processing of foods. In addition, this invention uses readily available proteins and carbohydrates. There are no solvents used in the preparation of the encapsulated formulations as the process is an all-aqueous based system. The processes can be easily  
30 incorporated or adapted to suit most food and pharmaceutical manufacturing plants with drying operations.

The protein used may include any film forming water soluble protein or hydrolysed protein and includes milk proteins such as casein and its derivatives or whey

proteins. The carbohydrate component may be those containing reducing sugar groups, oligosaccharides and starches (raw, modified, resistant, acetylated, proprionated and butylated starches).

5 The proteins and carbohydrates may be reacted in aqueous solutions to obtain conjugates. The reaction, which occurs, can be between free amine groups of amino acids in the protein and reducing sugar groups in the carbohydrate. This type of reaction is generally termed a Maillard reaction typically occurring in the non-enzymatic browning of foods. This reaction occurs during heat processing of foods and has previously been shown to be beneficial for engineering desirable  
10 encapsulating properties for protection of oxygen sensitive components. For example, microencapsulated formulations containing oxygen sensitive oils are protected against oxidation as the Maillard reaction products [MRP] in the encapsulating matrix are good film-formers and also exhibit anti-oxidation activity as disclosed in WO 01/74175.

15 The starches used in the formulations may also be pre-processed using conventional and emerging processing technologies to modify the starch properties to provide improved processing characteristics during the preparation of the delivery systems. The pretreatments are chosen to break down the long starch molecules so that they form more stable emulsions and also to provide a larger  
20 number of terminal sugar reducing groups for Maillard reaction with the protein component of the encapsulant.

Colon delivery systems may be used for range of bioactives (e.g. oils), pharmaceuticals and therapeutics, which are unstable in the upper gastrointestinal tract. The protection afforded to the encapsulated components by the  
25 encapsulating material enable target release in the colon where the release is achieved after the encapsulant is degraded (e.g. by the action of microbial enzymes in the colon). Delivery of bioactives, pharmaceuticals and therapeutic components to the colon is desirable for treatment and prevention of diseases of the colon such as colorectal cancer, ulcerative colitis and inflammatory bowel  
30 disorder.

In some cases the encapsulants used in the formulations such as selected polysaccharides, can also serve as gut wall adherents or as prebiotics that facilitate growth of beneficial bacteria, and can offer added advantages. For

example delivery systems containing resistant starch have potential benefits on colonic health.

#### **Detailed Description of the invention**

5 A number of formulations will be described, some according to the invention and some for comparative purposes to show that some formulations are suitable to delivery to the colon whilst others could be more suitable for release in the small intestine. These formulations demonstrate that the core is protected from digestion in the stomach and the environment in the small intestine.

10 Figures 1 to 19 of the drawings graphically illustrate the solvent extractable fat content and other properties of the formulations of the invention as illustrated in examples 1 to 19 below.

The process of microencapsulating the active component involves the following manufacturing steps:

- 15 (a) Selection of the biologically active core (e.g. oil, oil soluble or oil dispersible material, bioactives, therapeutics, pharmaceuticals)
- (b) Dispersion of the protein and carbohydrates (or starch that has been pre-processed by conventional means such as heating or extrusion or by the use of emerging processing technologies such as high pressure processing, microfluidisation or ultrasonics) in the aqueous phase and treatment of the mixture. If desired, the protein-carbohydrate blends may be further heat
- 20 processed to induce the formation of conjugates (e.g. Maillard reaction products)
- (c) Mixing the core with the encapsulant (i.e. protein-carbohydrate mixture) and homogenizing the mixture to obtain an emulsion, in which the core is
- 25 surrounded by the encapsulant.
- (d) Optionally, spray drying the emulsion to obtain a powdered formulation in which the core is surrounded by the encapsulating matrix

6a

In one particular embodiment, there is provided a method of preparing microcapsules for use in storing storage unstable, therapeutic and nutritional agents for targeted delivery to predetermined locations in the gastrointestinal tract which includes the steps of:

- 5 a) selecting a nutritional or therapeutic oil, oil soluble or oil dispersible nutritional or therapeutic agent to form an oil phase
- b) dispersing a water soluble film forming protein and a treated resistant starch in the aqueous phase
- 10 c) mixing component (a) with component (b) and homogenizing the mixture to obtain an oil-in-water emulsion
- d) optionally drying the emulsion to obtain a powdered formulation in which the nutritional or therapeutic oil or agent is surrounded by the component (b)

thereby encapsulating the storage unstable, therapeutic or nutritional agent.

15 In another embodiment there is provided an encapsulation material for use with storage unstable, therapeutic and nutritional agents which releases the therapeutic and nutritional agents in predetermined locations in the gastrointestinal tract and in which the encapsulation material is formed by combining a food grade treated resistant starch with a water soluble food grade protein.

20 The invention further provides an orally administrable nutritional or therapeutic product for delivery of a nutritional or therapeutic agent to the gastrointestinal tract in which the agent comprises an oil or an oil soluble or dispersible component which is encapsulated in a material as described herein.

#### 25 **Emulsion formulations**

Tuna fish oil was used as an oil of choice in most of these examples since it contains a high amount of long chain polyunsaturated fatty acids and this needs to be protected from oxidation prior to consumption. In addition there is interest in

delivering these to the colon because of their potential for prevention of colorectal cancer and promotion of bowel health (Karmeli, R A. (1996) Historical Perspective and Potential Use of n-3 Fatty Acids in Therapy of Cancer Cachia. *Nutrition*, Vol 12 (1) S2-S4; Dommels Y E M, Alink, G M, van Bladeren, P J, van Ommen, B (2002) Dietary n-6 and n-3 polyunsaturated fatty acids and colorectal carcinogenesis: results from cultured colon cells, animal models and human studies, *Environmental Toxicology and Pharmacology*, Vol 12 (4), 233-244.). Tributyrin and lutein were also included as examples. The encapsulation of probiotics (i.e. an example of a water dispersible component) using this technology has been previously disclosed in WO 01/74175.

A range of formulations was prepared using protein and/or carbohydrate (raw or pre-processed) and oil mixtures at different ratios. The formulations were made-up to contain 25 and 50% fat in the final powder.

The protein used in these examples were sodium caseinate, whey protein isolate and hydrolysed milk proteins. The carbohydrates used, alone or in combination, were glucose, oligosaccharides, dried glucose syrup, modified starches, resistant starches and native starches. Polysaccharides, including high-methoxy pectin, alginate, carrageenan, guar gum, were added to protein-carbohydrate mixtures in some formulations.

20

### **Manufacture of microcapsules**

#### Materials

The core materials used in the examples include: tuna oil, tributyrin and 15% (w/w) lutein (mostly as dipalmitate and dimyristate lutein esters) in soy bean oil.

25 Proteins used as encapsulant in the examples include: sodium caseinate (NaCas), whey protein isolate (WPI), hydrolysed casein protein (HCP) and hydrolysed whey protein (HWP).

30 Carbohydrates used in the examples include: dextrose monohydrate (Glu), waxy maize, maize starch, dried glucose syrup (DGS), wheat starch, oligofructose (oligo), tapioca dextrin (K4484), modified starch (Capsul™), modified starch (Hi-Cap™ 100), Hi-Maize™, Hylon™ VII, Novelose™ 260 and Novelose 330, potato starch, sodium alginate, kappa carrageenan, high methoxy pectin (HMP) and guar gum.

### Preparation of protein-carbohydrate encapsulants

In some cases, unreacted blends of protein and carbohydrates (referred to as NonMRP formulations since these were not heated to induce the formation of Maillard reaction products) were used as the encapsulating matrix. For the  
5 preparation of reacted protein-carbohydrate encapsulants (referred to as MRP formulations as these were heated to induce the formation of Maillard reaction products), protein was dissolved in 60°C water, using a high shear mixer, and then the sugars, starch or the selected carbohydrate were added. Where a polysaccharide was also added, the polysaccharide was first allowed to hydrate in  
10 water at 90°C temperature before addition into the protein-sugar mixture. The pH of the protein-sugar/starch /gum mixtures was adjusted to 7.5. The mixture were then filled into 3 litre cans, sealed and heated in the retort to 98°C and held for 30 minutes, then cooled down to room temperature. Microcapsule formulations are given in the examples below together with the methods used for the manufacture  
15 of microcapsules.

### Preparation of protein-starch encapsulants

Protein was dissolved in 60°C water to make 15% total solids (TS) solution, using a high shear mixer. Starch (raw or heated, heated and microfluidised, extruded, high pressure processed and ultrasonicated) was prepared and processed  
20 separately to make a 10% TS solutions or dispersions in 70°C water (See Preparation of Starches for Microencapsulation detailed below). The 15%TS protein solution were mixed together with the 10%TS starch to get a 12%TS mixture with a 1:1 protein:starch ratio. Where MRP was required, the mixture were then filled into 3 litre cans, sealed and heated in the retort to 98°C and held for 30  
25 minutes, then cooled down to 60°C.

### Preparation of Starches for Microencapsulation

#### *Raw or Unprocessed*

10% TS starch dispersion (no pre-treatment applied) was mixed with 15% TS of protein solution at 60°C.

#### *Heat processing*

30 20% TS of each starch dispersion (except for potato starch where a 10% TS dispersion was used due to high viscosity at 20% TS) were heated at 121°C for 60 minutes in a 73x82 mm cans. Once heat processed, 70°C deionised water was

added to dilute the sample to 10% TS in a high shear mixer. This heat processed starch was mixed with 15% TS of protein solution at 60°C. This mixture was then used for microencapsulation of bioactives.

*Heat processing and Microfluidisation treatment*

- 5 20% TS of each starch dispersion (except for potato starch where a 10% TS dispersion was used due to high viscosity at 20% TS) were heated at 121°C for 60 minutes in a 73x82 mm cans. Once heat processed, 70°C deionised water was added to dilute the sample to 10% TS in a high shear mixer, and processed at 60°C through a pilot scale M-210B EH microfluidiser (MFIC, Newton MA, USA).
- 10 The plant was operated at 800 bars and 3 passes using a combination of 425 µm Q50Z auxiliary processing module and 200 µm E230Z interaction chamber (for dispersion and cell disruption). The microfluidised (MF) starch was mixed with 15% TS of protein solution at 60°C for microencapsulation.

*Heat processing and Ultra-high pressure treatment*

- 15 20% TS of a starch dispersion was heated at 121°C for 60 minutes in a 73x82 mm cans. Once heat processed, 70°C deionised water was added to dilute the sample to 10% TS in a high shear mixer, and processed by ultra-high pressure treatment at 6,000 bars for 15 minutes using HPP-QFP 35L unit. The ultra-high pressure treated (HPP) starch was mixed with 15% TS of protein solution at 60°C for
- 20 microencapsulation.

*Heat processing and Ultrasonics treatment*

- 20% TS of a starch dispersion was heated at 121°C for 60 minutes in 73x82 mm cans. Once heat processed, 70°C deionised water was added to dilute the sample to 10% in a high shear mixer, and processed with ultrasound treatment at 50
- 25 ml/min @ 380 watts using 20KHz unit. The ultrasound treated (US) starch was mixed with 15% TS of protein solution at 60°C for microencapsulation.

*Extrusion*

- Resistant starches were processed using a twin-screw extruder (model MPF 40, APV Baker, Peterborough PE3-6TA, England) 40mm screw diameter and length to
- 30 diameter ratio of 25:1, and a low shear screw configuration. A 4 mm die was used throughout the trial. Raw materials were fed into feed port 1 at 15 kg h<sup>-1</sup> for resistant starch processing using a gravimetric feeder (Ktron Soder AG CH-5702, Niederlenz) and water was injected into port 2 with a volumetric pump (Brook

Crompton, Huddersfield, England). Barrel moisture was injected at 20-40 % and the die melt temperature was varied from 140 to 178°C with increasing screw speed from 150-250rpm. The extruded resistant starches were milled to 0.2mm particle size powder. 10% TS extruded starch dispersion was mixed with 15% TS of protein solution at 60°C for microencapsulation.

#### Preparation of oil in water emulsions

The protein-carbohydrate mixtures and the tuna oil were pre-heated to 60°C separately. The bioactive core was added into the protein-carbohydrate mixture using a Silverson high shear mixer. The mixture were then homogenised at 350 and 100 bar pressures in two stages using a Rannie homogeniser.

#### Spray drying of emulsions

The homogenised emulsions were spray dried at 50-60°C feed temperature, 180°C inlet temperature and 80°C outlet temperature using a Niro production minor spray dryer. The powder was collected from the main chamber and packed.

#### **Estimation of solvent extractable fat in tuna oil powders**

The estimation of solvent-extractable was based on the method by Pisecky (Handbook of Milk Powder Manufacture, 1997) except that petroleum ether was used in place of carbon tetrachloride. Fifty ml of petroleum ether (b.p. 40-60°C) was added to 10g powder. The mixture was agitated in a stoppered flask for 15 minutes. The mixture was filtered and the solvent evaporated at 60°C using a rotary evaporator. The remaining fat residue was then dried in an oven at 105°C for 1h.

#### **In-vitro testing of microcapsules**

The stability of the microcapsules in the stomach and the small-intestine was estimated by assessment of oil-release properties of microcapsules (a) incubated in simulated gastric fluid (SGF) (pH 1.2) for 2h at 37°C and 100 rpm in a shaker water-bath incubator and (b) incubated in SGF (2 h at 37°C and 100 rpm in a shaker water-bath incubator) followed by exposure to simulated intestinal fluid (SIF) (pH 6.8) (3 h at 37°C and 100 rpm). SGF and SIF were prepared according to the methods given in the US Pharmacopeia (US Pharmacopeia 2000 & National Formulary (USP 24 NF 19), Rockville, MD)

For estimation of released oil from microcapsules in-vitro:

The solvent extractable fat from the incubated samples were measured. The sample was transferred into a 250 ml stoppered separating funnel and extracted with petroleum ether (75 ml plus 2x25ml). The sample was filtered through a phase separation filter paper to obtain the solvent phase after each extraction. The solvent was removed to recover the oil released.

For estimation of released lutein in-vitro:

The microcapsule containing the lutein (1.0 g) was incubated sequentially with SGF (pH 1.2) and SIF (pH6.8) as outlined above. For estimation of released lutein, the solvent extractable lutein from the incubated samples was measured. The extraction was performed in a centrifuge tube. The sample was extracted with petroleum ether (15 ml plus 2x10ml). The sample was centrifuged (2000 rpm for 10 min) after each extraction and the top solvent layer removed. The combined solvent extracts were filtered through a phase separation filter paper prior to dilution with petroleum ether. The absorbance of the diluted extract was measured at 444 nm and the concentration of extracted lutein was determined.

For estimation of released Tributyrin in-vitro:

The microcapsule containing the tributyrin (1.0 g) was incubated sequentially with SGF (pH 1.2) and SIF (pH 6.8) as outlined above. For estimation of released tributyrin samples that were exposed to SGF only were used directly and that exposed sequentially to SGF and SIF was adjusted to pH 2. To this mixture was added 2.5 g NaCl and 15 ml dichloromethane and the mixture was centrifuged at 2500 rpm for 10 min at 5 C. The aqueous layer was removed and kept while the dichloromethane layer was decanted into a conical flask without disturbing the gelatinous precipitate floating on top of the dichloromethane layer. The aqueous layer with the gelatinous precipitate was extracted with another 15 ml dichloromethane. The dichloromethane extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, before filtering (0.45 µm PTFE syringe filter). The dichloromethane was removed under Nitrogen in a warm water bath. The extracted material was dissolved in 10 ml hexane/iso-propyl alcohol (99:1, v/v) and the solution stored in freezer. The amount of tributyrin and butyric acid in the extract was analysed by normal-phase HPLC. [Column: PVA-Sil guard and analytical (250mm x 4.6 mm I.D.) columns; UV detector (210 nm)].

**In-vivo testing of microcapsules**

Male Sprague-Dawley rats, approximately 10 weeks of age were used for the in-vivo study. Rats were denied solid food for 24 hours prior to dosing, but were allowed free access to drinking water containing 2.5% glucose, 0.5% NaCl and

5 0.005% KCl (all w/v).

Preparation of radiolabelled tuna oil: 0.5 ml or 25  $\mu$ Ci radiolabelled tracer [ $^{14}$ C] 18:3 ([ $^{14}$ C] trilinolenin, 50-60 mCi/mmol; 50  $\mu$ Ci/mL) was added to 4.56 g tuna oil. Two lots of tuna oil samples with radiolabelled trilinolenin were prepared, one for encapsulated oil treatment (see example 19 for formulation and manufacture) and

10 one for free (unencapsulated) oil treatment.

Rat treatment: On the day of treatment rats were fed intra-gastrically using a stainless steel gavage needle either with 0.3 ml fish oil mixed with radiolabelled tracer [ $^{14}$ C] 18:3 (0.27 g tuna oil + 0.03 ml tracer [ $^{14}$ C] 18:3) for control treatment or 2 ml emulsion (0.09 g tuna oil + 0.01 ml tracer [ $^{14}$ C] 18:3) for the

15 microencapsulated treatment.

Tissue sampling: At time points of 4, 9 and 14 hours following treatment, rats were anaesthetised and a blood sample taken by cardiac puncture. The stomach, small intestine, caecum and colon were removed. The small intestine was divided into two sections, each GI tract segment was flushed with 0.9% NaCl and the washings

20 collected and frozen. The GI tract segments were then frozen for subsequent analysis. Faeces were also collected for analysis at time points. The tissues and faeces were weighed and samples taken for analysis and weighed.

Tissue sample analysis: Radioactivity of GI tract washings containing all unabsorbed oil (both released and encapsulated oil) was counted to estimate the

25 total amount of radioactivity. Tissue samples were dissolved overnight in BTS-450<sup>R</sup> tissue solubiliser. Faecal matters were dissolved in BTS-450<sup>R</sup>, with some prior treatment. The liquid scintillation cocktail Ready Organic<sup>R</sup> was added to each sample and the sample subjected to liquid scintillation counting in a Packard 1500 Tri-Carb Scintillation Counter.

**Example 1: Formulations and Manufacture of Powders with 25% oil loading with unheated or heated blends of protein-glucose/dried glucose syrup or protein-oligosaccharide as encapsulants**

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare NaCas solution (Alanate) at 60°C, add sugars [glucose and DGS (Maltostar)], (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Inlet temperature (Ti)/Outlet temperature (To).
Alanate 180	25.0%	7.7%	
Glucose.H <sub>2</sub> O	25.0%	7.7%	
Maltostar 30	25.0%	7.7%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

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Ingredient	powder composition	Emulsion composition	Processing steps
Water		69.2%	Prepare NaCas solution at 60°C, add oligosaccharide, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	7.7%	
Raftilose P95	50.0%	15.4%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare WPI solution (Alacen) at 60°C, add sugars, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	25.0%	7.7%	
Glucose.H <sub>2</sub> O	25.0%	7.7%	
Maltostar 30	25.0%	7.7%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare WPI solution at 60°C, add oligosaccharidesugars, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	25.0%	7.7%	
Raftilose P95	50.0%	15.4%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

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**Example 2: Formulations and Manufacture of Powders with 50% oil loading with unheated or heated blends of protein- glucose/dried glucose syrup or protein- oligosaccharide as encapsulants**

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Ingredient	powder composition	emulsion composition	Processing steps
Water		60.0%	Prepare NaCas solution at 60°C, add sugars, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	16.7%	6.7%	
Glucose.H <sub>2</sub> O	16.7%	6.7%	
Maltostar 30	16.7%	6.7%	
Tuna oil	50.0%	20.0%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		60.0%	Prepare NaCas solution at 60°C, add oligosaccharide (Raftilose), (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	16.7%	6.7%	
Raftilose P95	33.3%	13.3%	
Tuna oil	50.0%	20.0%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		60.0%	Prepare WPI solution at 60°C, add sugars, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	16.7%	6.7%	
Glucose.H <sub>2</sub> O	16.7%	6.7%	
Maltostar 30	16.7%	6.7%	
Tuna oil	50.0%	20.0%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		60.0%	Prepare WPI solution at 60°C, add oligosaccharidesugars, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	16.7.0%	6.7%	
Raftilose P95	33.3%	13.3%	
Tuna oil	50.0%	20.0%	
Total	100.0%	100.0%	

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**Example 3: Formulations and Manufacture of Powders with 25% oil loading with heated blends of protein- starch as encapsulants**

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare NaCas solution at 60°C, add starch (Capsul), (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	7.7%	
Capsul	50.0%	15.4%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare NaCas solution at 60°C, add starch (Hi-Cap), (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	7.7%	
Hi-Cap 100	50.0%	15.4%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare NaCas solution at 60°C, add dextrin, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	7.7%	
Tapioca dextrin K4484	50.0%	15.4%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare WPI solution at 60°C, add dextrin, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	25.0%	7.7%	
Tapioca dextrin K4484	50.0%	15.4%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

**Example 4: Formulations and Manufacture of Powders with 50% oil loading with heated blends of protein- starch as encapsulants**

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Ingredient	powder composition	emulsion composition	Processing steps
Water		60.0%	Prepare NaCas solution at 60°C, add starch, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	16.7%	6.7%	
Capsul	33.3%	13.3%	
Tuna oil	50.0%	20.0%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		60.0%	Prepare NaCas solution at 60°C, add starch, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	16.7%	6.7%	
Hi-Cap 100	33.3%	13.3%	
Tuna oil	50.0%	20.0%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		60.0%	Prepare NaCas solution at 60°C, add dextrin, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	16.7%	6.7%	
Tapioca dextrin K4484	33.3%	13.3%	
Tuna oil	50.0%	20.0%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		60.0%	Prepare WPI solution at 60°C, add dextrin, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	16.7%	6.7%	
Tapioca dextrin K4484	33.3%	13.3%	
Tuna oil	50.0%	20.0%	
Total	100.0%	100.0%	

5 **Example 5: Formulations and Manufacture of Powders with 25% oil loading with heated blends of protein-glucose/glucose syrup or protein-oligosaccharide in combination with gums as encapsulants**

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare NaCas solution at 60°C, add sugars and alginate (Protanal), (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	7.7%	
Glucose.H <sub>2</sub> O	25.0%	7.7%	
Maltostar 30	22.5%	6.9%	
Protanal	2.5%	0.8%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		77.7%	Prepare NaCas solution at 60°C, add oligosaccharide and guar gum solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	5.6%	
Raftilose P95	48.75%	10.9%	
Guar WW250F	1.25%	0.3%	
Tuna oil	25.0%	5.6%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		73.2%	Prepare NaCas solution at 60°C, add oligosaccharide and carrageenan solution (Gelcarin), (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	6.7%	
Raftilose P95	48.75%	13.0%	
Gelcarin GP 812	1.25%	0.3%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		73.1%	Prepare NaCas solution at 60°C, add oligosaccharide and high methoxy pectin (HMP) solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	6.7%	
Raftilose P95	47.5%	12.7%	
HMP RS400	2.5%	0.7%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		73.1%	Prepare NaCas solution at 60°C, add glucose-DGS and high methoxy pectin (HMP) solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	25.0%	6.7%	
1:1 Glu:DGS	47.5%	12.7%	
HMP RS400	2.5%	0.7%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		77.7%	Prepare WPI solution at 60°C, add oligosaccharide and guar gum solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	25.0%	5.6%	
Raftilose P95	48.75%	10.9%	
Guar WW250F	1.25%	0.3%	
Tuna oil	25.0%	5.6%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		73.2%	Prepare NaCas solution at 60°C, add oligosaccharide and 60°C carrageenan solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	25.0%	6.7%	
Raftilose P95	48.75%	13.0%	
Gelcarin GP 812	1.25%	0.3%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
water		73.1%	Prepare NaCas solution at 60°C, add oligosaccharide and 60°C HMP solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	25.0%	6.7%	
Raftilose P95	47.5%	12.7%	
HMP RS400	2.5%	0.7%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

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**Example 6: Formulations and Manufacture of Powders with 50% oil loading with heated blends of protein-glucose/glucose syrup or protein-oligosaccharide in combination with gums as encapsulants**

Ingredient	powder composition	emulsion composition	Processing steps
water		60.0%	Prepare NaCas solution at 60°C, add sugars and alginate, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	16.7%	6.7%	
Glucose	16.7%	6.7%	
Maltostar 30	15.0%	6.0%	
Protanal	1.7%	0.7%	
Tuna oil	50.0%	20.0%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.9%	Prepare HWP solution at 60°C, add oligosaccharide and guar gum solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
NaCas	16.7%	5.0%	
Raftilose P95	32.5%	9.8%	
Guar WW250F	0.8%	0.3%	
Tuna oil	50.0%	15.0%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		64.6%	Prepare NaCas solution at 60°C, add oligosaccharide and carrageenan solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	16.7%	5.9%	
Raftilose P95	32.5%	11.5%	
Gelcarin GP 812	0.8%	0.3%	
Tuna oil	50.0%	17.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		64.5%	Prepare NaCas solution at 60°C, add oligosaccharidesugars and HMP solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	16.7%	5.9%	
Raftilose P95	31.7%	11.2%	
HMP RS400	1.7%	0.6%	
Tuna oil	50.0%	17.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.9%	Prepare WPI solution at 60°C, add oligosaccharide and guar gum solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	16.7%	5.0%	
Raftilose P95	32.5%	9.8%	
Guar WW250F	0.8%	0.3%	
Tuna oil	50.0%	15.0%	
Total	100.0%	100.0%	

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Ingredient	powder composition	emulsion composition	Processing steps
Water		53.1%	Prepare WPI solution at 60°C, add oligosaccharide and carrageenan solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	16.7%	5.9%	
Raftilose P95	32.5%	11.5%	
Gelcarin GP 812	0.8%	0.3%	
Water for gum dispersion		11.5%	
Tuna oil	50.0%	17.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		53.3%	Prepare WPI solution at 60°C, add oligosaccharide and 60°C HMP solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alacen 895	16.7%	5.9%	
Raftilose P95	31.7%	11.2%	
HMP RS400	1.7%	0.6%	
Water for gum dispersion		11.2%	
Tuna oil	50.0%	17.8%	
Total	100.0%	100.0%	

**Example 7: Formulations and Manufacture of Powders with 25% oil loading with heated blends of protein hydrolysate - oligosaccharide in combination with gums as encapsulants**

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Ingredient	powder composition	emulsion composition	Processing steps
Water		73.2%	Prepare hydrolysed casein protein (HCP) solution at 60°C, add oligosaccharide, and 60°C carrageenan solution (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
HCP 102	25.0%	6.7%	
Raftilose P95	48.75%	13.0%	
Gelcarin GP 812	1.25%	0.3%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		73.1%	Prepare HCP solution at 60°C, add oligosaccharide and HMP solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
HCP 102	25.0%	6.7%	
Raftilose P95	47.5%	12.7%	
HMP RS400	2.5%	0.7%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		73.2%	Prepare hydrolysed whey protein (HWP) solution at 60°C, add oligosaccharide and carrageenan solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
HWP 205	25.0%	6.7%	
Raftilose P95	48.75%	13.0%	
Gelcarin GP 812	1.25%	0.3%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		73.1%	Prepare HWP solution at 60°C, add oligosaccharide and HMP solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
HWP 205	25.0%	6.7%	
Raftilose P95	47.5%	12.7%	
HMP RS400	2.5%	0.7%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		73.1%	Prepare HWP solution at 60°C, add glucose-DGS and HMP solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
HWP 205	25.0%	6.7%	
1:1 Glu:DGS	47.5%	12.7%	
HMP RS400	2.5%	0.7%	
Tuna oil	25.0%	6.7%	
Total	100.0%	100.0%	

**Example 8: Formulations and Manufacture of Powders with 50% oil loading with heated blends of hydrolysate - oligosaccharide in combination with gums as encapsulants**

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Ingredient	powder composition	emulsion composition	Processing steps
Water		64.6%	Prepare HCP solution at 60°C, add oligosaccharide and carrageenan solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
HCP 102	16.7%	5.9%	
Raftilose P95	32.5%	11.5%	
Gelcarin GP 812	0.8%	0.3%	
Tuna oil	50.0%	17.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		64.5%	Prepare HCP solution at 60°C, add oligosaccharide and HMP solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
HCP 102	16.7%	5.9%	
Raftilose P95	31.7%	11.2%	
HMP RS400	1.7%	0.6%	
Tuna oil	50.0%	17.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		64.6%	Prepare HWP solution at 60°C, add oligosaccharide and carrageenan solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
HWP 205	16.7%	5.9%	
Raftilose P95	32.5%	11.5%	
Gelcarin GP 812	0.8%	0.3%	
Tuna oil	50.0%	17.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		64.5%	Prepare HWP solution at 60°C, add oligosaccharide and HMP solution, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
HWP 205	16.7%	5.9%	
Raftilose P95	31.7%	11.2%	
HMP RS400	1.7%	0.6%	
Tuna oil	50.0%	17.8%	
Total	100.0%	100.0%	

**Example 9: Formulations and Manufacture of Powders with 25% oil loading with blends of sodium caseinate with raw or processed resistant starch (potato starch)**

Ingredient	powder composition	emulsion composition	Processing steps (using raw potato starch)
Water		84.6%	Prepare 10%TS starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Potato starch	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed potato starch)
Water		84.6%	Prepare 10%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Potato starch	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

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Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised potato starch)
Water		84.6%	Prepare 10%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Potato starch	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using extruded potato starch)
Water		84.6%	Prepare 10%TS extruded starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Potato starch	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

**Example 10: Formulations and Manufacture of Powders with 25% oil loading with blends of sodium caseinate with Hylon VII or pre-processed resistant starch (Hylon VII)**

Ingredient	powder composition	emulsion composition	Processing steps (using Hylon VII starch)
Water		84.6%	Prepare 10%TS starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

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Ingredient	powder composition	emulsion composition	Processing steps (using heat processed Hylon VII starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised Hylon VII starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using extruded Hylon VII starch)
Water		84.6%	Prepare 10%TS extruded starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

**Example 11: Formulations and Manufacture of Powders with 25% oil loading with blends of sodium caseinate with Hi-Maize 1043 or pre-processed resistant starch (Hi-Maize 1043)**

Ingredient	powder composition	emulsion composition	Processing steps (using Hi-Maize starch)
Water		84.6%	Prepare 10%TS starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hi-Maize 1043	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

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Ingredient	powder composition	emulsion composition	Processing steps (using heat processed Hi-Maize starch)
Water		84.6%	Prepare 20%TS starch dispersion at 60°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hi-Maize 1043	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised Hi-Maize starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hi-Maize 1043	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using extrude Hi-Maize starch)
Water		84.6%	Prepare 10%TS extruded starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hi-Maize 1043	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

**Example 12: Formulations and Manufacture of Powders with 25% oil loading with blends of sodium caseinate with Novelose 260 or pre-processed resistant starch (Novelose 260)**

Ingredient	powder composition	emulsion composition	Processing steps (using Novelose 260 starch)
Water		84.6%	Prepare 10%TS starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Novelose 260	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

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Ingredient	powder composition	emulsion composition	Processing steps (using heat processed Novelose 260 starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Novelose 260	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised Novelose 260 starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Novelose 260	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		84.6%	Prepare 10%TS extruded starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Novelose 260	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

**Example 13: Formulations and Manufacture of Powders with 25% oil loading with blends of sodium caseinate with Novelose 330 or pre-processed resistant starch (Novelose 330)**

Ingredient	powder composition	emulsion composition	Processing steps (using Novelose 330 starch)
Water		84.6%	Prepare 10%TS starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Novelose 330	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

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Ingredient	powder composition	emulsion composition	Processing steps (using heat processed Novelose 330 starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Novelose 330	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised Novelose 330 starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Novelose 330	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using extruded Novelose 330 starch)
Water		84.6%	Prepare 10%TS extruded starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Novelose 330	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

**Example 14: Formulations and Manufacture of Powders with 25% oil loading with blends of sodium caseinate with Hylon VII or high pressure processed or ultrasonicated resistant starch (Hylon VII)**

Ingredient	powder composition	emulsion composition	Processing steps (using Hylon VII starch)
Water		84.6%	Prepare 10%TS starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

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Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised x1 pass Hylon VII starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-1 pass. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bars, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised x3 pass Hylon VII starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bars, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using extruded Hylon VII starch)
Water		84.6%	Prepare 10%TS extruded starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bars, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

10

Ingredient	powder composition	emulsion composition	Processing steps (using high pressure treated Hylon VII starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, HPP at 600 MPa, for 15 minutes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using ultrasound treated Hylon VII starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, US using 20 KHz unit @ 50 ml per minute, 380 Watts. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

**Example 15: Formulations and Manufacture of Powders with 25% oil loading with unheated and heated blends of sodium caseinate with raw starches or pre-processed starch**

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Ingredient	powder composition	emulsion composition	Processing steps
Water		84.6%	Prepare 10%TS starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Waxy maize	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, Microfluidise at 800 bar-1 pass. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Waxy maize	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		84.6%	Prepare 10%TS starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Maize starch	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, Microfluidise at 800 bar-1 pass. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C) Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Maize starch	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		84.6%	Prepare 10%TS starch dispersion at 70°C. Prepare 15%TS NaCas solution at 60°C and mix with starch dispersion above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C) Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Wheat starch	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, Microfluidise at 800 bar-1 pass. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C) Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Wheat starch	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

**Example 16: Formulations and Manufacture of Powders with 25 % (lutein-in-oil) in heated and unheated blends of protein-sugar-starch as encapsulants**

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare NaCas solution at 60°C, add DGS and starch, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add lutein heated to 90°C, homogenise at 350/100 bars, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	7.7%	
Maltostar 30	25.0%	7.7%	
Tapioca dextrin K4484	25.0%	7.7%	
Lutein in oil	25.0%	7.7%	
Total	100.0%	100.0%	

5 **Example 17: Formulations and Manufacture of Powders with 25% tributyrin in heated blends of protein-sugar or protein-sugar-RS starch as encapsulants**

Ingredient	powder composition	emulsion composition	Processing steps
Water		69.2%	Prepare NaCas solution at 60°C, add sugars, (preferably, adjust pH of solution to 7.5, heat to 98°C and hold for 30 minutes, cool down to 60°C), add tributyrin, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	7.7%	
Glucose	25.0%	7.7%	
Maltostar 30	25.0%	7.7%	
Tributyrin	25.0%	7.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised Hylon VII starch)
Water		69.2%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C, add sugar and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add tributyrin, homogenise at 350/100 bars, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	7.7%	
Glucose	25.0%	7.7%	
Hylon VII	25.0%	7.7%	
Tributyrin	25.0%	7.7%	
Total	100.0%	100.0%	

**Example 18: Formulations and Manufacture of Powders with 25% tuna oil in heated blends of NaCas-sugar-HylonMF or NaCas-HylonMF or NaCas-StarPlus MF as encapsulants**

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised Hylon VII starch)
Water		69.2%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C, add sugar and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add tuna oil, homogenise at 350/100 bars, spray dry at 180/80°C Ti/To.
Alanate 180	25.0%	7.7%	
Glucose	25.0%	7.7%	
Hylon VII	25.0%	7.7%	
Tuna oil	25.0%	7.7%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised Hylon VII starch)
Water		84.6%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Hylon VII	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

5

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised Star Plus A)
Water		84.6%	Prepare 20%TS Star Plus A dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Star Plus A	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

Ingredient	powder composition	emulsion composition	Processing steps (using heat processed and microfluidised Star Plus P)
Water		84.6%	Prepare 20%TS Star Plus P dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C and mix with processed starch above. (Preferably heat protein-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add oil heated to 60°C, homogenise at 350/100 bar, spray dry at 180/80°C Ti/To.
Star Plus P	37.5%	5.8%	
Alanate 180	37.5%	5.8%	
Tuna oil	25.0%	3.8%	
Total	100.0%	100.0%	

**Example 19: Formulations and Manufacture of Powders with 25% tuna oil (+ Radiolabelled tracer) in heated blends of protein-sugar-RS starch as encapsulants for in-vivo testing.**

Ingredient	Ingredient Wt. (g)	emulsion composition (%)	Processing steps (using heat processed and microfluidised Hylon VII starch)
Water	82.47	82.04%	Prepare 20%TS starch dispersion at 70°C, process in 73 x 82 mm cans at 121°C-60 minutes, cool down, add remaining water to make-up to 10% TS, microfluidise at 800 bar-3 passes. Prepare 15%TS NaCas solution at 60°C, add sugar and mix with processed starch above. (Preferably heat protein-glucose-starch mixture in cans at 98°C-30 minutes, cool down to 60°C). Add radiolabelled tuna oil, homogenise at 350/100 bars, spray dry at 180/80°C Ti/To.
Alanate 180	4.33	4.31%	
Glucose	4.33	4.31%	
Hylon VII	4.33	4.31%	
Tuna oil	4.56	4.54%	
Radiolabelled tracer [ <sup>14</sup> C] 18:3	0.50 ml (25 µCi)	0.50%	
Total solids	18.05	18.0%	
<b>Total</b>	<b>100.52</b>	<b>100.0%</b>	

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**Characteristics of microcapsules in-vitro**

The properties of the example 1 formulations are shown in figure 1 of the drawings. Solvent-extractable fat in all powders (25% fat in powder) were less than 3% (of total fat) indicating that the encapsulating efficiency was good. Released oil in SGF was less than 2% of total fat for all formulations. Released oil in SGF+SIF were less than 4% of total fat for casein based microcapsules and up to 22% of total fat for WPI based microcapsules. In these examples NaCas based formulations offer better protection than WPI based formulations. Also heat treatment applied to WPI-sugar encapsulant can increase the release in SGF+SIF. Depending on the type of protein and whether heat treatment is applied to the encapsulant the core may be released targeted to a specific site in the GI tract.

The properties of the example 2 formulations are shown in figure 2 of the drawings. Solvent-extractable fat in all powders (50% fat in powder) were less than 3% (of total fat) indicating that the good encapsulating efficiency was maintained when that ratio of the fat to encapsulating material was increased from 1:3 in 25% fat powders to 1:1 in 50% fat powders. Released oil in SGF was less than 2% of total fat for all formulations. Released oil in SGF+SIF were less than 4% of total fat for casein based microcapsules and up to 30% of total fat for WPI based microcapsules. The trend in the release properties of the microcapsules in Figure 2

25

with 50% fat powders mirror those observed in Figure 1 for 25% fat powders. In these examples NaCas based formulations offer better protection than WPI based formulations. Also heat treatment applied to WPI-sugar encapsulant can increase the release in SGF+SIF. Depending on the type of protein and whether heat  
5 treatment is applied to the encapsulant the core may be released targeted to a specific site in the GI tract.

The properties of the example 3 formulations are shown in figure 3 of the drawings. Formulations (25% fat powders) made with heated protein-starch as  
10 encapsulants had low solvent extractable fat (<1% of total fat). Released oil in SGF was less than 2% of total fat for all formulations. Released oil in SGF+SIF were less than 4% of total fat for casein based microcapsules and up to 12.5% of total fat for WPI based microcapsules. In these examples NaCas based formulations offers better protection than the WPI based formulation. Depending  
15 on the type of protein used the core may be released targeted to a specific site in the GI tract.

The properties of the example 4 formulations are shown in figure 4 of the drawings. Formulations (50% fat powders) made with heated protein-starch as  
20 encapsulants had higher solvent extractable fat (1 to 20 % of total fat) than corresponding formulation for 25% fat powders. Released oil in SGF was less than 2% of total fat for all formulations. Released oil in SGF+SIF were less than 5% of total fat for casein based microcapsules and up to 15% of total fat for WPI based microcapsules. In these examples NaCas based formulations offer better  
25 protection than the WPI based formulation. Depending on the type of protein used the core may be released targeted to a specific site in the GI tract. Solvent-extractable fat in powder was not related to solvent extractable fat in SGF and SIF fluids.

30 The properties of the example 5 formulations are shown in figure 5 of the drawings. For 25% fat powders the use of gums in combination with protein – glucose/dried glucose syrup or protein – oligosaccharide as encapsulant resulted in powders with low extractable fat in powder (<3% of total fat) and in SGF (<2% of

total fat). Released oil in SGF+SIF were less than 7% of total fat for casein based microcapsules and up to 22.8% of total fat for WPI based microcapsules.

Caseinate-based formulations with gums released more fat (figure 5) than similar formulations without gum (Figure 1) after sequential exposure to SGF and SIF. In these examples NaCas based formulations offer better protection than WPI based formulations. Depending on the type of protein used the core may be released targeted to a specific site in the GI tract.

The properties of the example 6 formulations are shown in figure 6 of the drawings. The trends observed for 50% fat powders containing gums in combination with protein-glucose/dried glucose syrup or oligosaccharide (Figure 6) are similar to those observed for compositions with 25% fat powders (Figure 5). All formulations had low extractable fat in powder (<4% of total fat) and SGF (<2% of total fat). Released oil in SGF+SIF were less than 5% of total fat for casein based microcapsules and up to 23% of total fat for WPI based microcapsules. The amount of oil released in 50% fat powders (Figure 6) is significantly more than that in 25% fat powders (figure 5) after sequential exposure to SGF and SIF for WPI based formulations. In these examples NaCas based formulations offers better protection than WPI based formulations. Depending on the type of protein used the core may be released targeted to a specific site in the GI tract.

The properties of the example 7 formulations are shown in figure 7 of the drawings. Hydrolysed milk proteins can be used in place of whole proteins for encapsulation of oil. For 25% fat powders use of hydrolysed protein in combination with oligosaccharide and polysaccharide as encapsulant resulted in powders with low extractable fat in powder (<3% of total fat). Released oil in SGF was less than 9% of total fat for all formulations. Released oil in SGF+SIF was less than 12% in all formulations. While combinations of hydrolysed casein with oligosaccharide and polysaccharides were less effective for protecting oils from release in SGF+SIF compared to corresponding formulations with the parent protein (Na caseinate), the reverse trend was found with the use of hydrolysed whey protein with oligosaccharide and carrageenan (Compare Figures 5 and 7).

The properties of the example 8 formulations are shown in figure 8 of the drawings. For 50% fat powders use of hydrolysed protein in combination with oligosaccharide and polysaccharide as encapsulant resulted in powders with low extractable fat in powder (<3% of total fat). While solvent-extractable fat in  
5 powders (50% fat) was low, the hydrolysed casein-based formulation containing carrageenan released a significant amount of the oil in SGF (77% of total fat) and in SGF+SIF (51% of total fat). This formulation will be a suitable delivery system if the site for target delivery is the stomach or small intestine. Those containing hydrolysed casein or hydrolysed whey protein with high methoxy pectin were  
10 comparatively better at protecting their load than those with carrageenan with release in SGF+SIF less than 3% of total fat. In these examples HWP based formulation offers better protection than HCP based formulation. Depending on the type of protein-polysaccharide combination used the core may be released targeted to a specific site in the GI tract.

15

The properties of the example 9 formulations are shown in figure 9 of the drawings. The results show that 25% fat powders made with unheated and heated combinations of caseinate and raw or pre-processed potato starch had solvent-extractable fat of between 3-8% of total fat, which was generally higher than those  
20 made with combinations of proteins with sugar/dried glucose syrup or oligosaccharides. All formulations with potato starch have very low oil release in-vitro. Exposure to SGF resulted in release of <0.6% of total fat and sequential exposure to SGF and SIF resulted in between 4-8% of total fat being released.

25 The properties of the example 10 formulations are shown in figure 10 of the drawings. The results show that 25% fat powders made with unheated and heated combinations of caseinate and unprocessed or pre-processed Hylon VII had solvent-extractable fat of between 13-26% of total fat, which was generally higher than those made with combinations of proteins with sugar/dried glucose syrup or  
30 oligosaccharides or potato starch indicating that encapsulation efficiencies of formulations with Hylon VII were significantly lower. Use of Hylon VII that had been subjected to microfluidisation or extrusion prior to combination with protein improved encapsulation efficiency. All formulations with Hylon VII have very low oil

release in-vitro. Exposure to SGF which results in hydration of the capsule resulted in minimal release of <0.8% of total fat and sequential exposure to SGF and SIF resulted in between 3–7% of total fat being released.

5 The properties of the example 11 formulations are shown in Figure 11 of the drawings. The results show that 25% fat powders made with unheated and heated combinations of caseinate and unprocessed or pre-processed Hi-Maize had solvent-extractable fat of between 13-26% of total fat. Use of Hi-Maize that had been subjected to microfluidisation or extrusion prior to combination with protein  
10 improved encapsulation efficiency. All formulations with Hi-Maize have very low oil release in-vitro. Exposure to SGF which results in hydration of the capsule resulted in minimal release of <1% of total fat and sequential exposure to SGF and SIF resulted in between 4–6% of total fat being released.

15 The properties of the example 12 formulations are shown in figure 12 of the drawings. The results show that 25% fat powders made with unheated and heated combinations of caseinate and unprocessed or pre-processed Novelose 260 had solvent-extractable fat of between 14-25% of total fat. Use of Novelose 260 that had been subjected to microfluidisation prior to combination with protein improved  
20 encapsulation efficiency. All formulations with Novelose 260 have very low oil release in-vitro. Exposure to SGF which results in hydration of the capsule resulted in minimal release of <1% of total fat and sequential exposure to SGF and SIF resulted in between 2–6% of total fat being released. The characteristics of formulations with Novelose 260 were similar to those observed for formulations  
25 with Hylon VII (Figure 10) or Hi-Maize (Figure 11), which like Novelose 260 (Figure 12) are RS2 type starches.

The properties of the example 13 formulations are shown in figure 13 of the drawings. The results show that 25% fat powders made with unheated and heated  
30 combinations of caseinate and unprocessed or pre-processed Novelose 330 (an RS3 type starch) had solvent-extractable fat of between 13-33% of total fat. Use of Novelose 330 that had been subjected to extrusion prior to combination with protein improved encapsulation efficiency. All formulations with Novelose 330 have

very low oil release in-vitro. Exposure to SGF which results in hydration of the capsule resulted in minimal release of <1% of total fat and sequential exposure to SGF and SIF resulted in between 3.1 – 8.0 % of total fat being released.

5 The properties of the example 14 formulations are shown in figure 14 of the drawings. The results demonstrate that pre-processing of starches using emerging food processing technologies (i.e. microfluidisation, high pressure processing or ultrasonication) and extrusion could improve the properties of starches used in combination with casein as delivery systems to the GI tract. Released oil in SGF  
10 was less than 1.2% of total fat for pre-processed starches. Released oil in SGF+SIF was less than 10% in pre-processed starches. All pre-processed starches have lower oil released in-vitro compared to the formulation containing unprocessed starch.

15 The properties of the example 15 formulations are shown in figure 15 of the drawings. The results demonstrate that use of native non-RS starch and their pre-processed counterparts in combination with protein produced powders with solvent extractable fat of between 5.5-13.6% of total fat. Released oil in SGF was less than 2% of total fat. Released oil in SGF+SIF was between 12-14% (Figure 15),  
20 which was slightly higher than that observed when resistant starches were used in combination with protein for microencapsulation (See Figures 9-14).

The properties of the example 16 formulations containing lutein-in-oil are shown in figure 16 of the drawings. The results demonstrate that lutein was protected in the  
25 powder microcapsule (0.4-2.5% unencapsulated lutein). Released lutein in SGF was also very low (2.5-4% of total lutein). Released lutein in SGF+SIF was between 34-51% (Figure 16).

The properties of the example 17 formulations containing tributyrin are shown in  
30 figure 17 of the drawings. All the tributyrin was released after sequential exposure to SGF and SIF in NaCas-sugar formulation, and up to 83% in NaCas-sugar-RS starch formulation. These results suggest that formulation with RS starch has improved the protection of tributyrin in the GI tract.

The properties of the example 18 formulations containing 25% tuna oil in heated blends of NaCas-sugar-HylonMF or NaCas-HylonMF or NaCas-StarPlus MF as encapsulants are shown in figure 18 of the drawings. The results demonstrate that addition of Glucose into an NaCas-Hylon formulation can improve the encapsulation efficiency of the powder microcapsule without affecting the release in SGF and SGF+SIF. Use of acetylated starch (StarPlus A) or proprionylated starch (Starplus P) in place of Hylon in formulations containing resistant starch in combination with NaCas increased the release in SGF+SIF from 5% for Hylon to 12% and 25% for Star Plus A and StarPlus P respectively (Figure 18), but there was no difference in the amount of release in SGF.

#### **Release characteristics of tuna oil microcapsules in-vivo**

The result of the in-vivo experiment (example 19 formulation) is shown in figures 19a and 19b of the drawings. Lumen contents were expressed as a percentage of dose of radioactivity given to indicate relative abundance between the treatment groups. The figures show the percentage of administered dose of radioactivity recovered after dosing with C14 trillinolein as free oil after 4, 9, and 14 hours. This includes lumen contents, tissue and faeces. Data is expressed as percentage of total lumen radioactivity to show relative distribution across the system. All rats n=5 in each case except for fig 19b at 14 hours where n=4.

The results indicated that the treatment with microencapsulated oil at 9 hours resulted in greater caecum and colon (18% and 35%) radioactivity (Figure 19a) than treatment with free oil, where only about 5% in caecum at 4 hours and about 10% in colon at 4 hours, with minimal amounts of radioactivity at 9 hours (Figure 19b). Radioactivity levels in the lumen for the treatment with free oil were low at all time points, which indicates that even by 4 hours there may be significant uptake and metabolism to CO<sub>2</sub>. Overall the in-vivo study indicates that the process of microencapsulation was reasonably successful in protecting the fish oil against early uptake and metabolism in the stomach and upper GI tract. For the treatment with microencapsulated oils the recovery was high at 4 and 9 hours, and at these time points the radioactivity was either in the stomach at 4 hours or caecum and colon at 9 hours. High amounts in the caecum and colon indicates that the

microencapsulated oil passed the small intestine without significant absorption. For the free oil, smaller amounts reached the caecum and colon, primarily because the recovery of the given dose was low at all time points indicating greater metabolism. Even at 4 hour time point the oil had already transited the small  
5 intestine. There was little radiolabel retained in the tissues at 14 hour in either group, which indicates that conversion to endogenous lipids was not significant.

From the above those skilled in the art will see that the present invention provides a simple to use yet effective delivery vehicle to the colon as well as preserving  
10 sensitive core ingredients during storage and processing. Those skilled in the art will also realise that this invention can be implemented in a number of different embodiments by varying the encapsulant proteins and carbohydrates without departing from the teachings of this invention.

**CLAIMS**

1. A method of preparing microcapsules for use in storing storage unstable, therapeutic and nutritional agents for targeted delivery to predetermined locations in the gastrointestinal tract which includes the steps of:
- 5
- a) selecting a nutritional or therapeutic oil, oil soluble or oil dispersible nutritional or therapeutic agent to form an oil phase
  - b) dispersing a water soluble film forming protein and a treated resistant starch in the aqueous phase
  - 10 c) mixing component (a) with component (b) and homogenizing the mixture to obtain an oil-in-water emulsion
  - d) optionally drying the emulsion to obtain a powdered formulation in which the nutritional or therapeutic oil or agent is surrounded by the component (b)
- 15 thereby encapsulating the storage unstable, therapeutic or nutritional agent.
2. A method as defined in claim 1 in which the treated resistant starch is a resistant starch which has been treated to increase the number of sugar reducing groups.
- 20 3. A method as defined in claim 1 in which the protein is selected from milk proteins.
4. A method as defined in claim 3 wherein the milk proteins are selected from the group consisting of casein and whey proteins.
5. An encapsulation material for use with storage unstable, therapeutic and nutritional agents which releases the therapeutic and nutritional agents in predetermined locations in the gastrointestinal tract and in which the
- 25

encapsulation material is formed by combining a food grade treated resistant starch with a water soluble food grade protein.

6. An encapsulation material as claimed in claim 5 in which the treated resistant starch is a resistant starch which has been treated to increase the number of sugar reducing groups.
7. An encapsulation material as claimed in claim 5 in which the protein is selected from milk proteins.
8. An encapsulation material as claimed in claim 7 wherein the milk proteins are selected from the group consisting of casein and whey proteins.
9. An orally administrable nutritional or therapeutic product for delivery of a nutritional or therapeutic agent to the gastrointestinal tract in which the agent comprises an oil or an oil soluble or dispersible component which is encapsulated in a material as claimed in any one of claims 5 to 8.

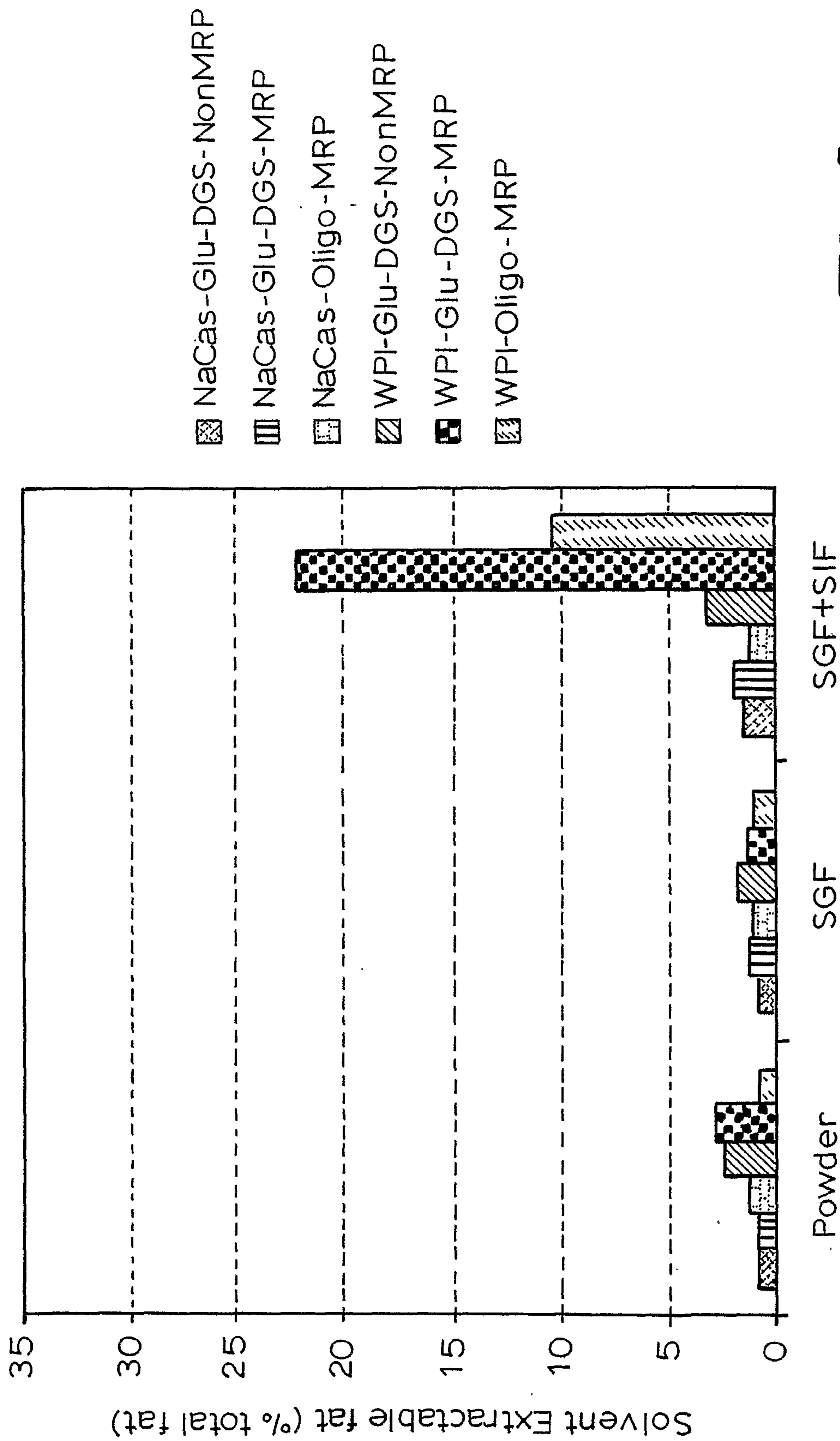


Fig. 1

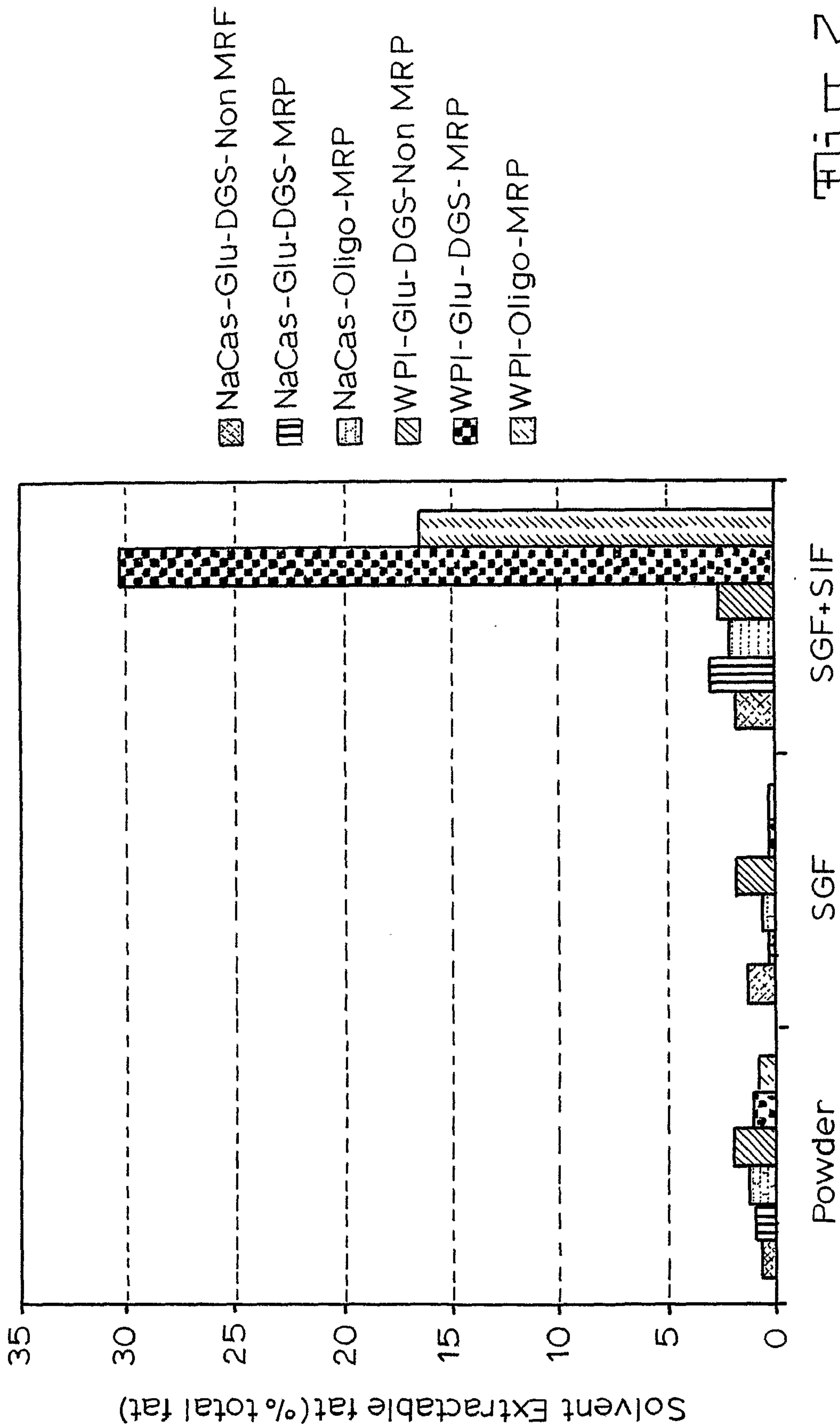


Fig. 2.

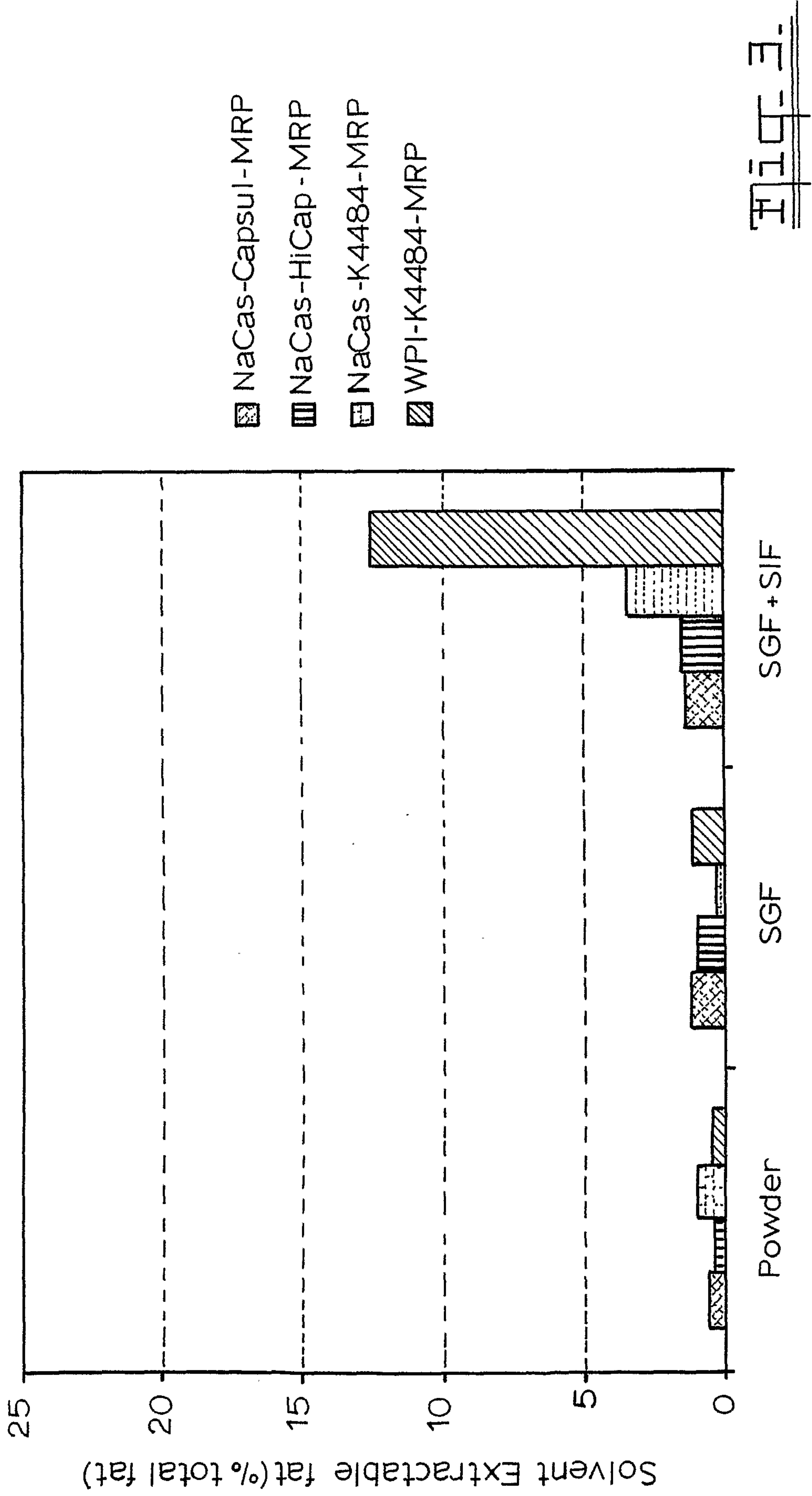


Figure 3.

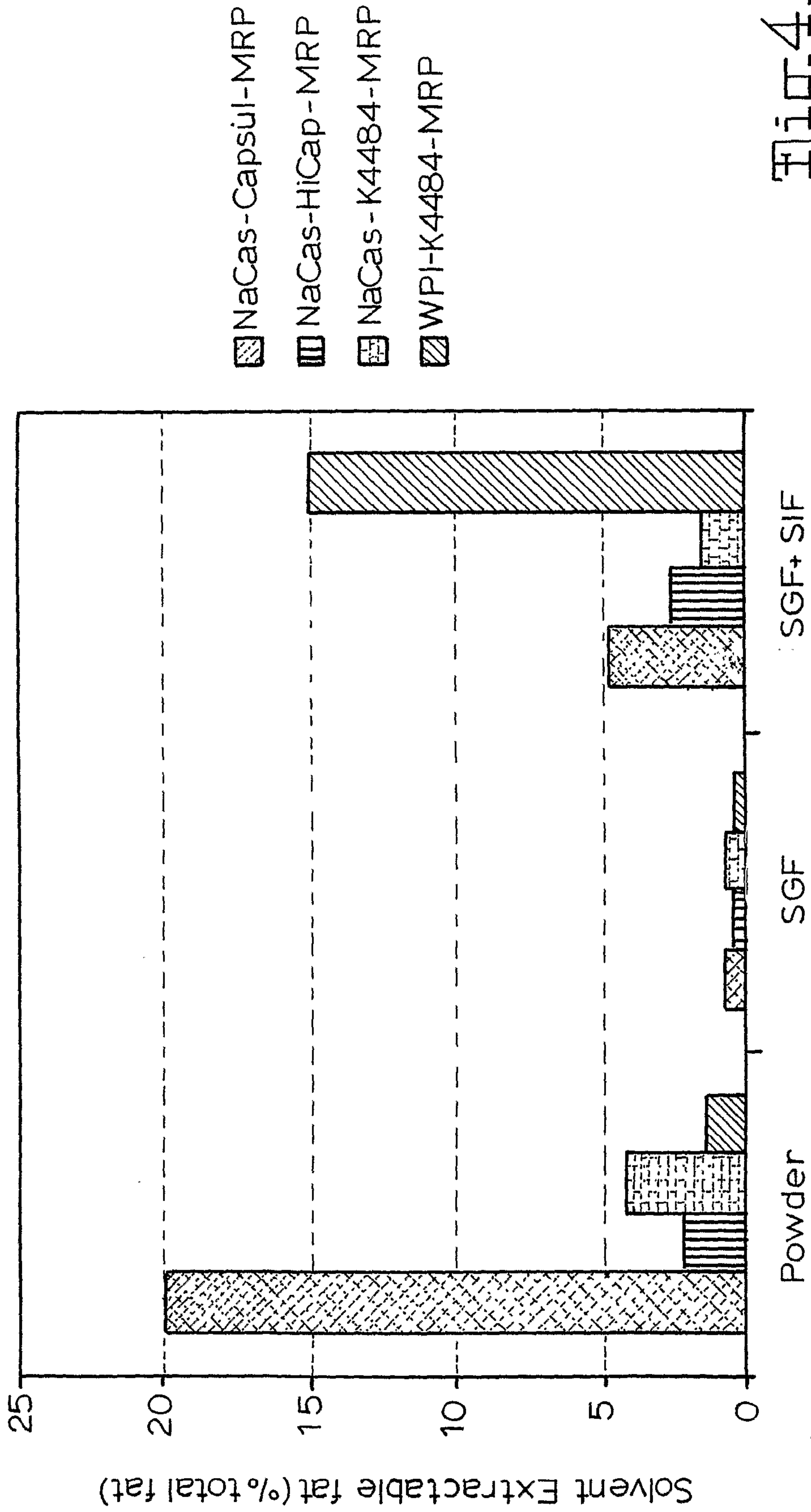
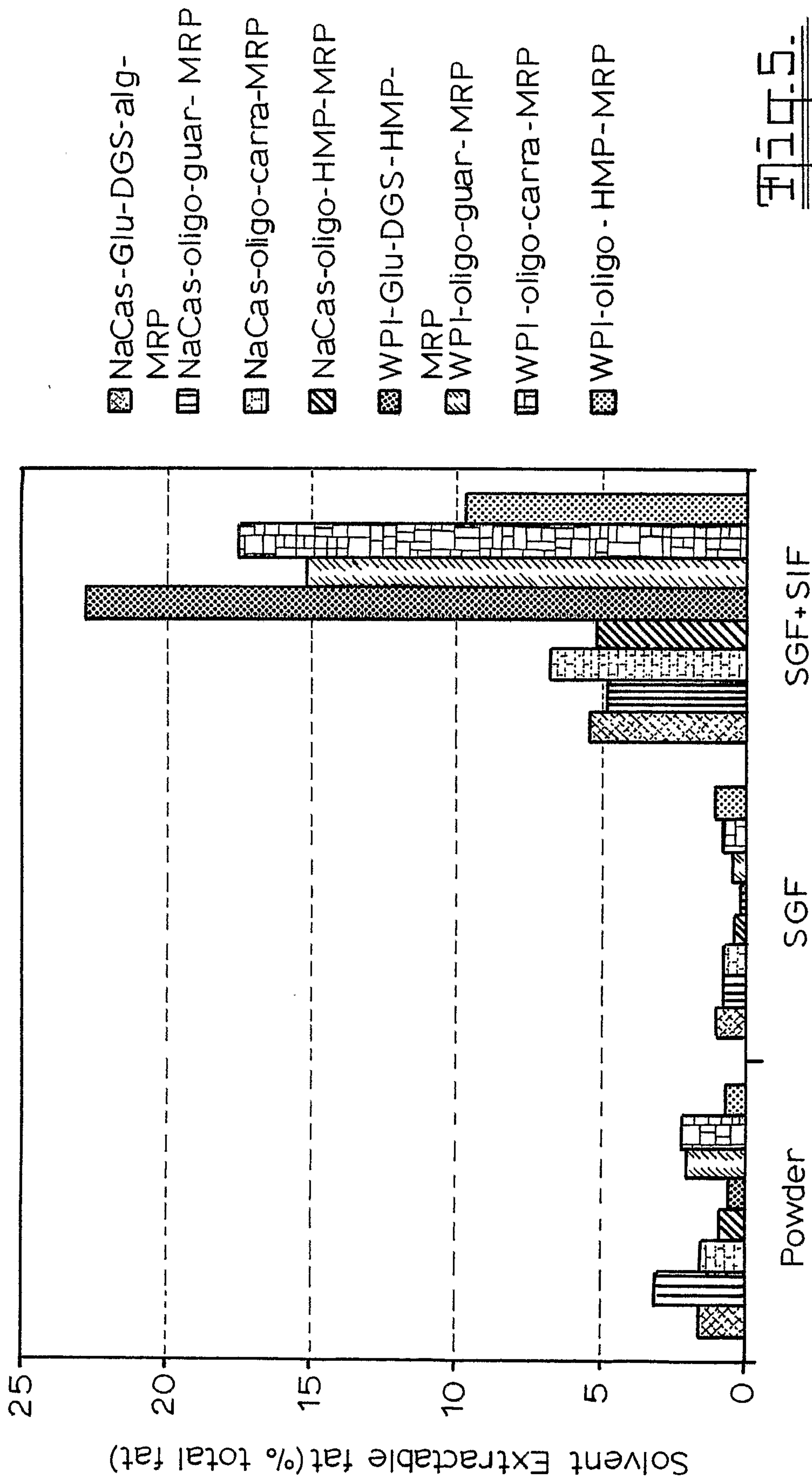


Fig. 4



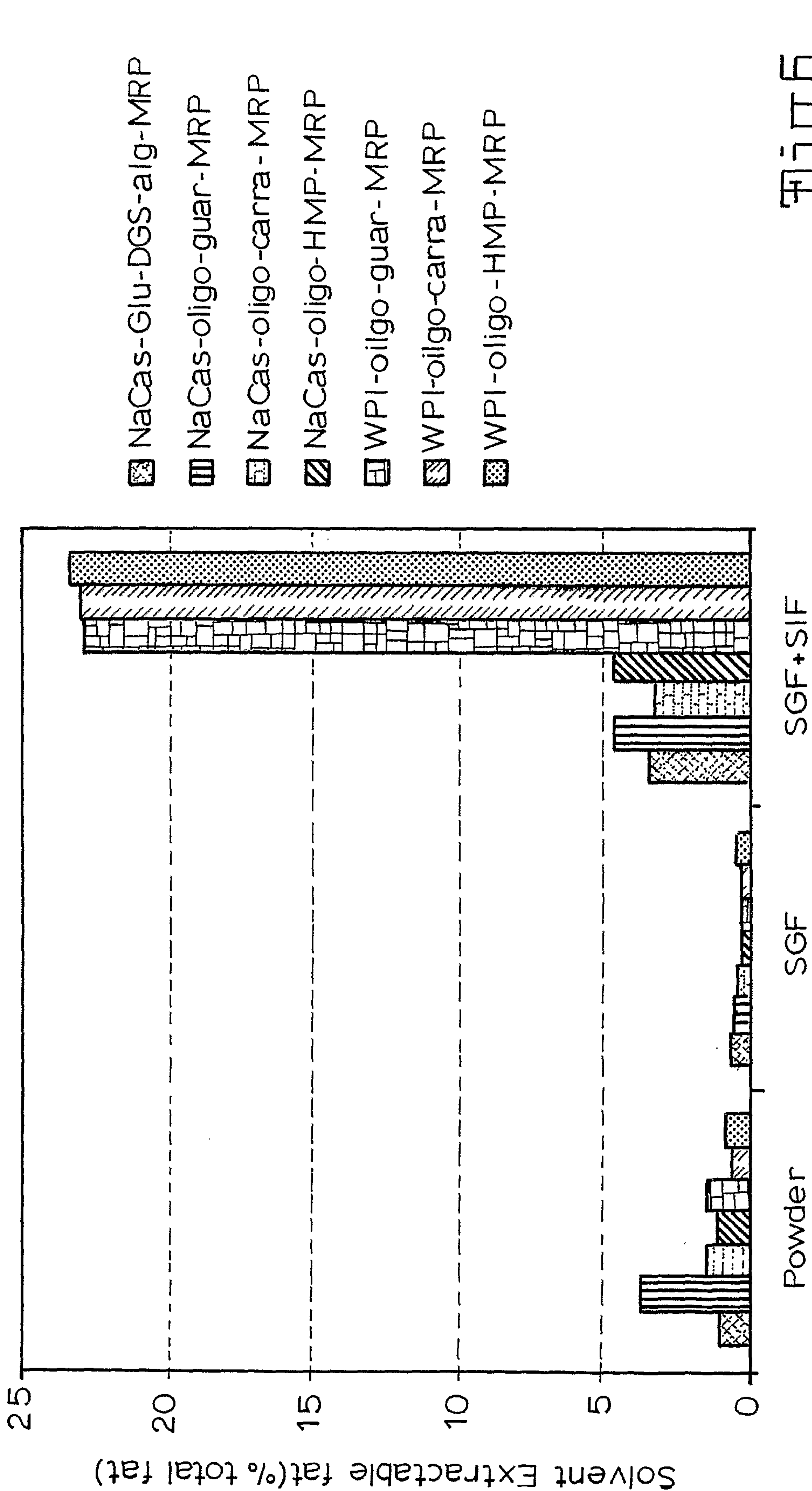


Fig. 6.

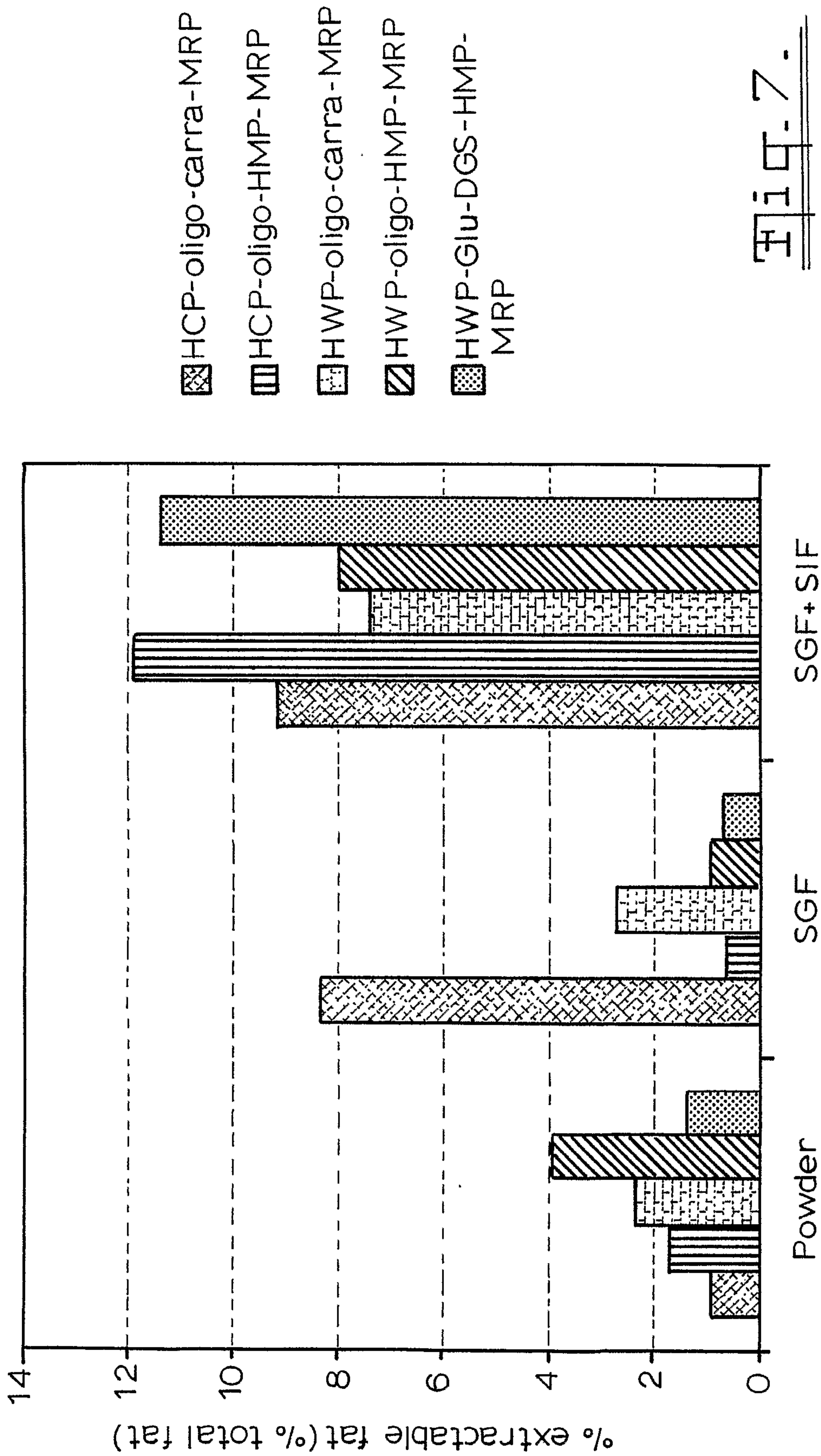


Fig. 7.

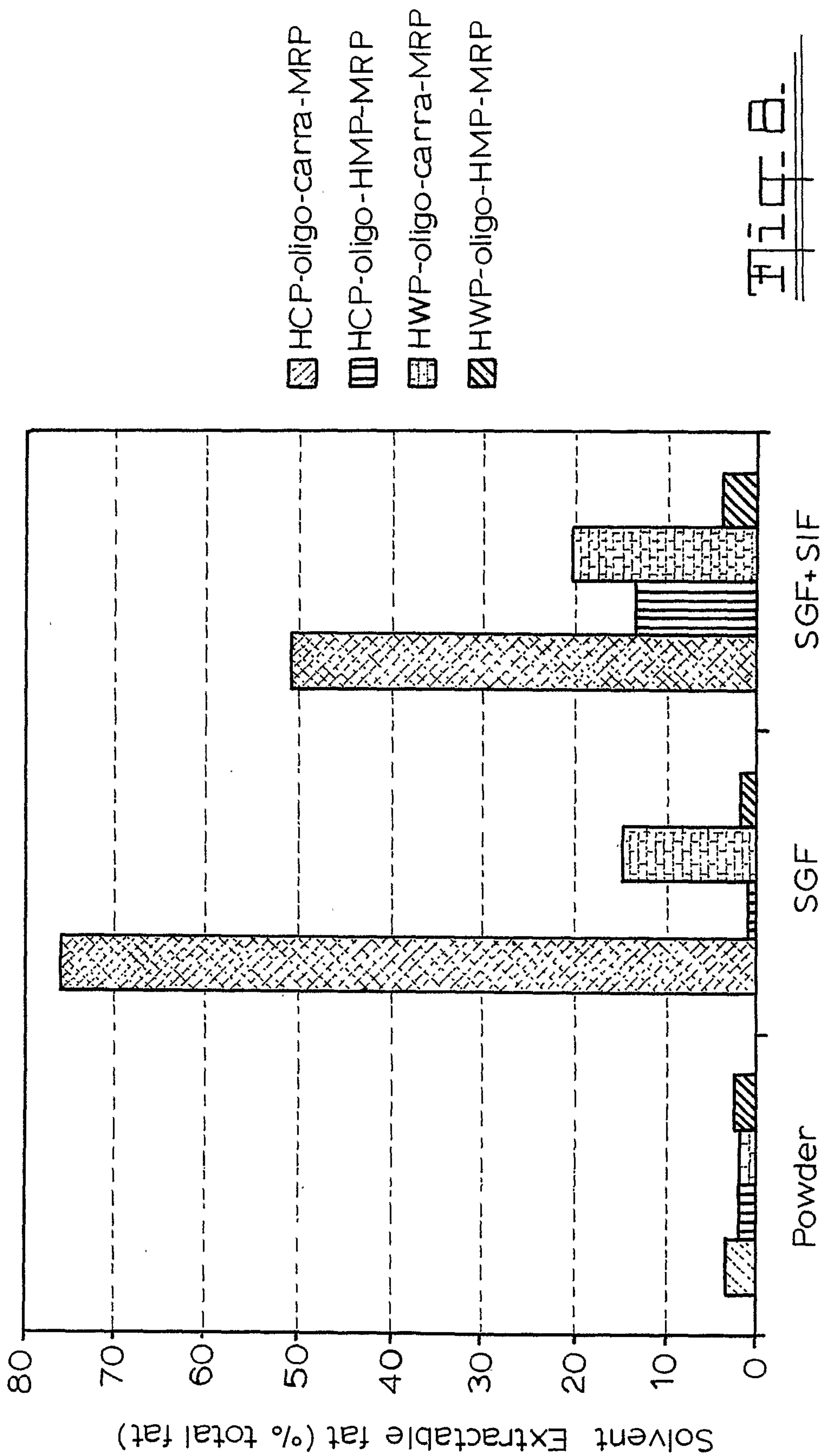


Fig. 8.

Figure 9.

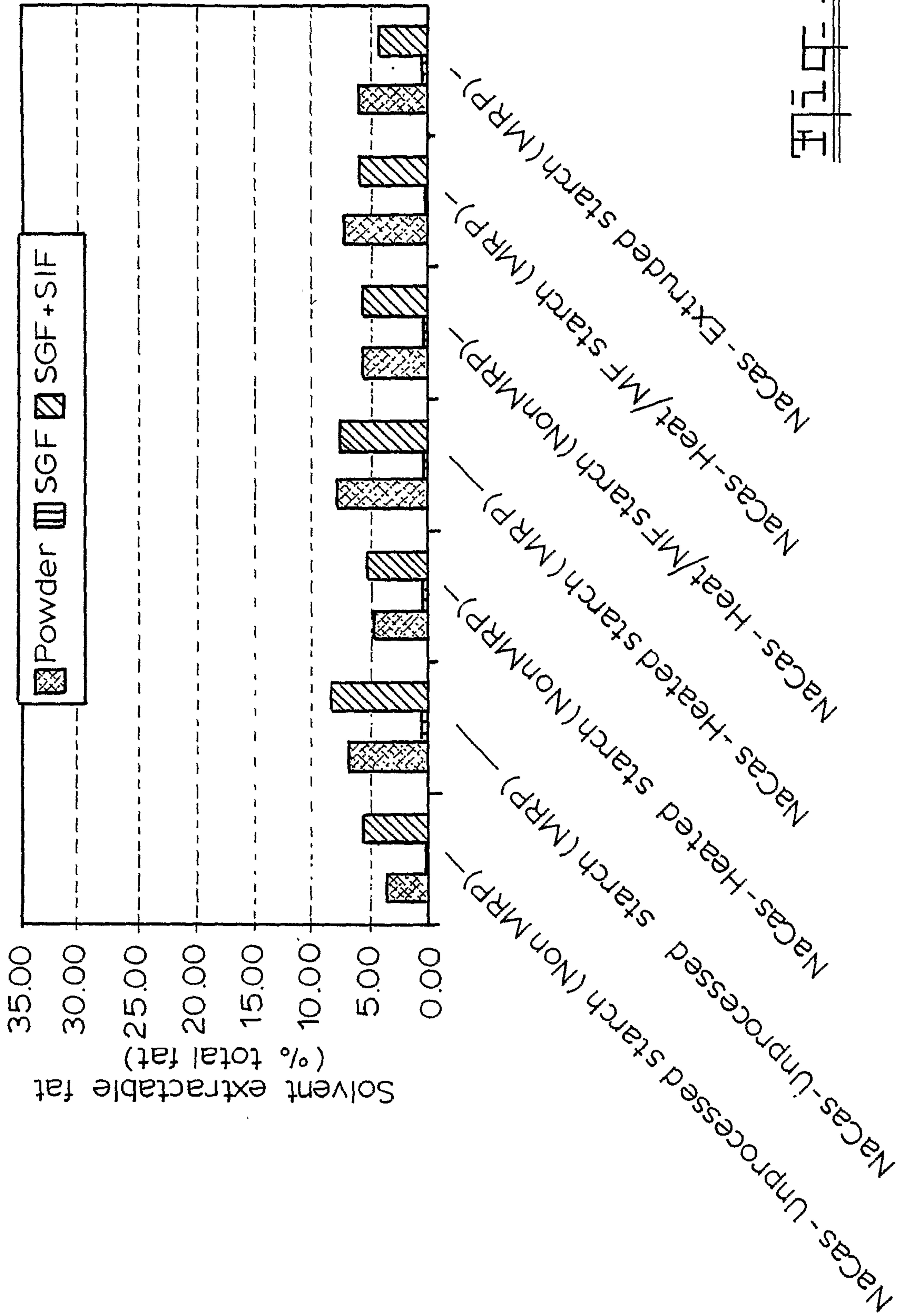
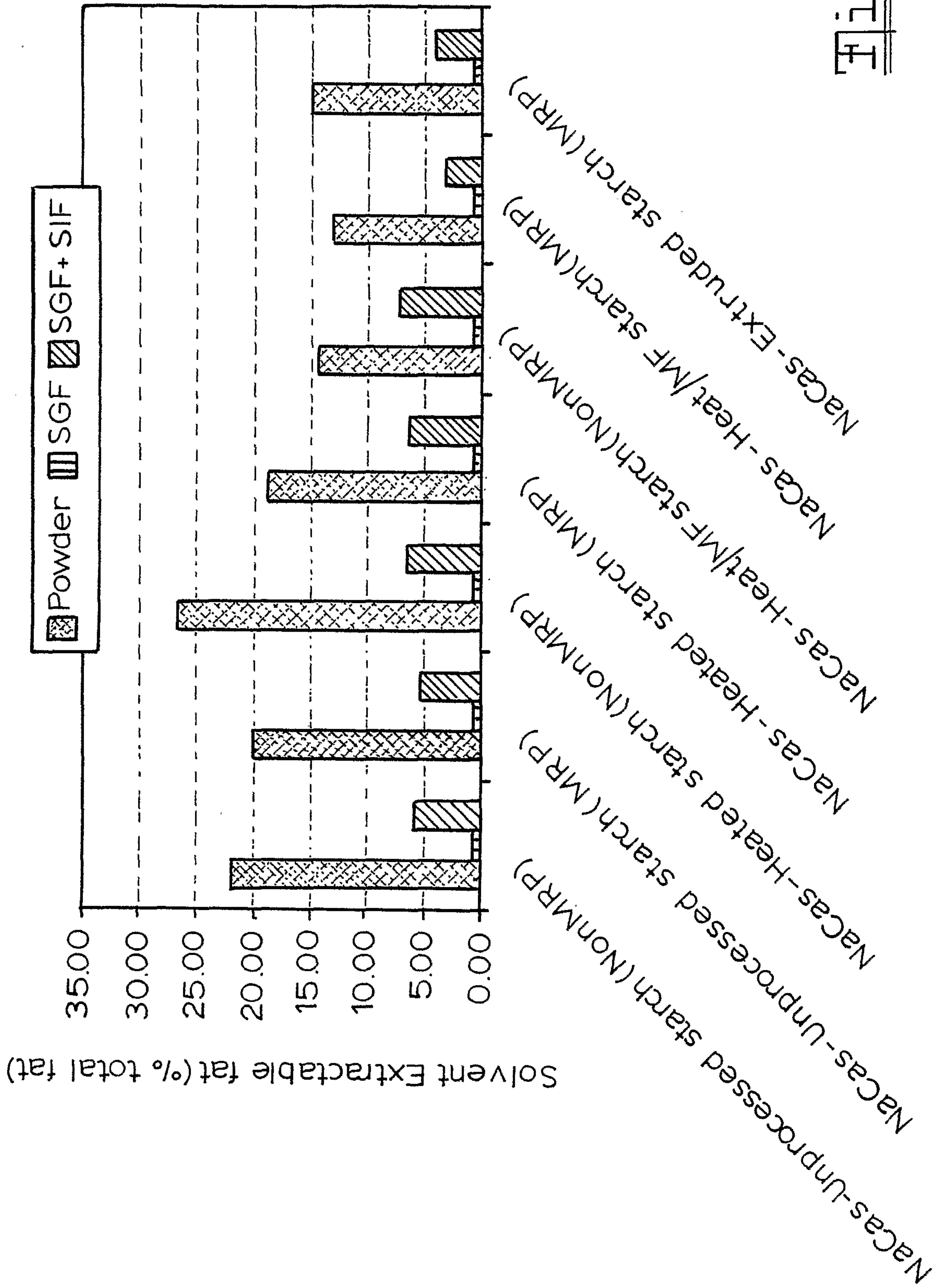


Figure 10





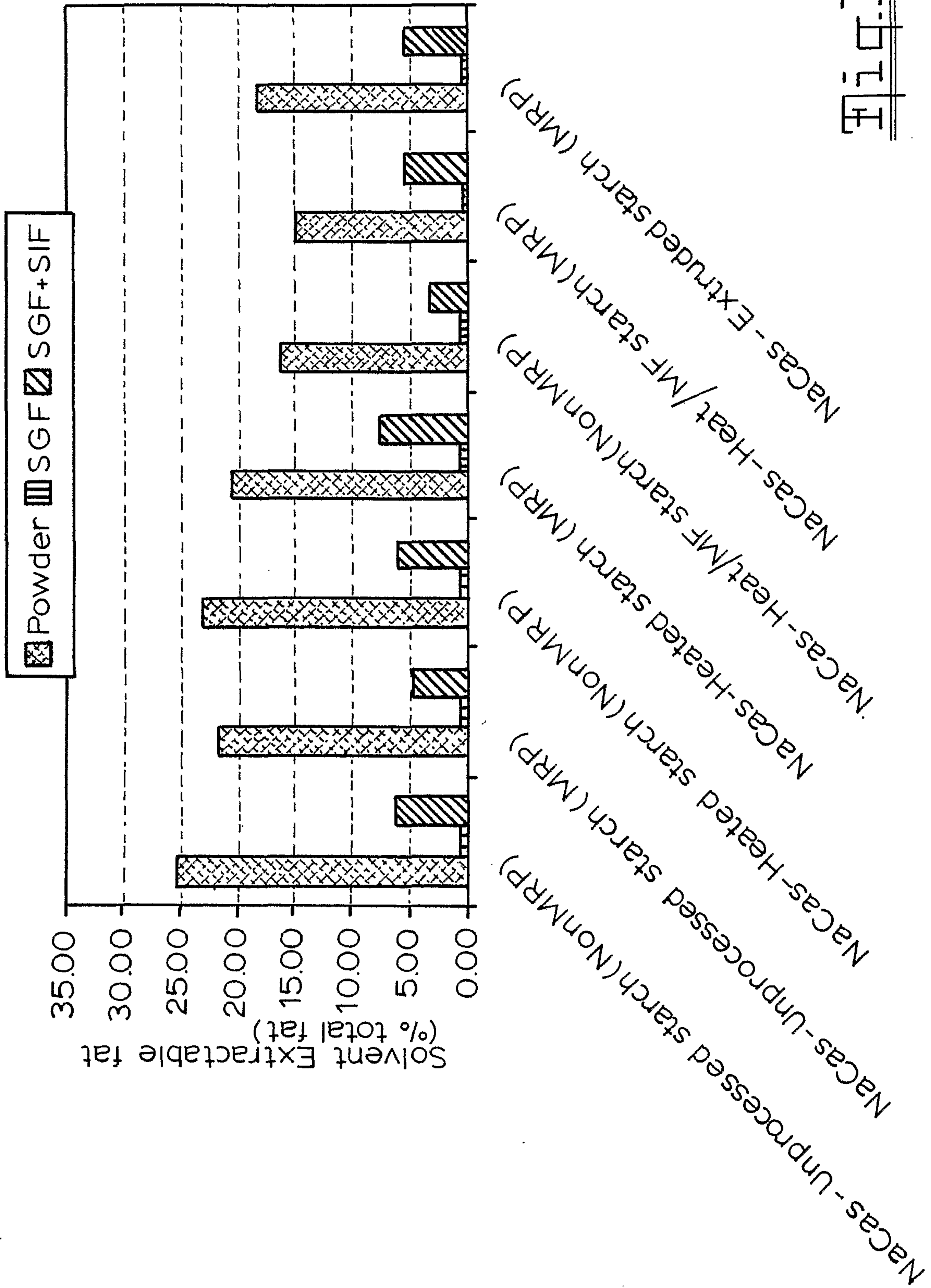
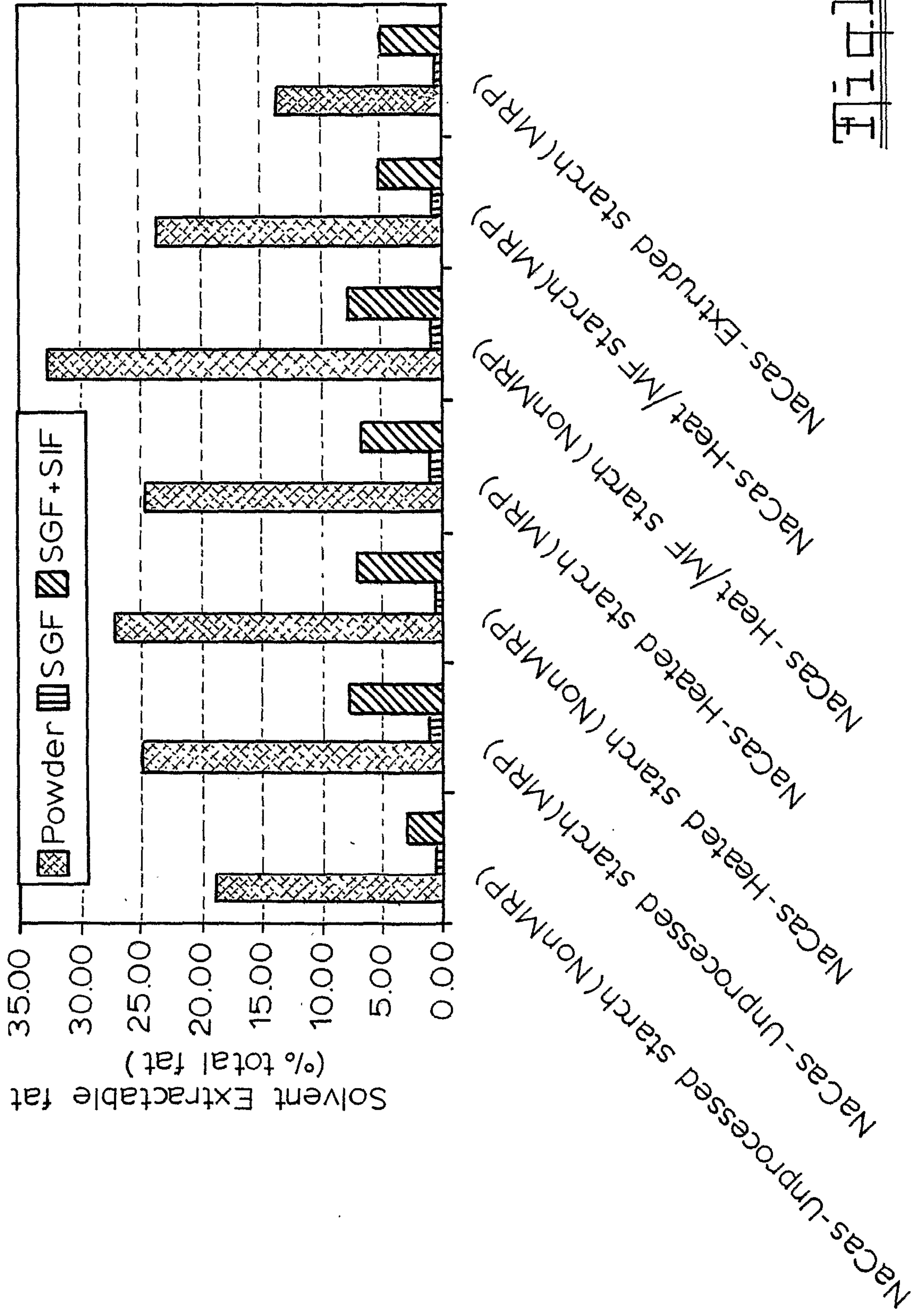


Figure 12

Figure



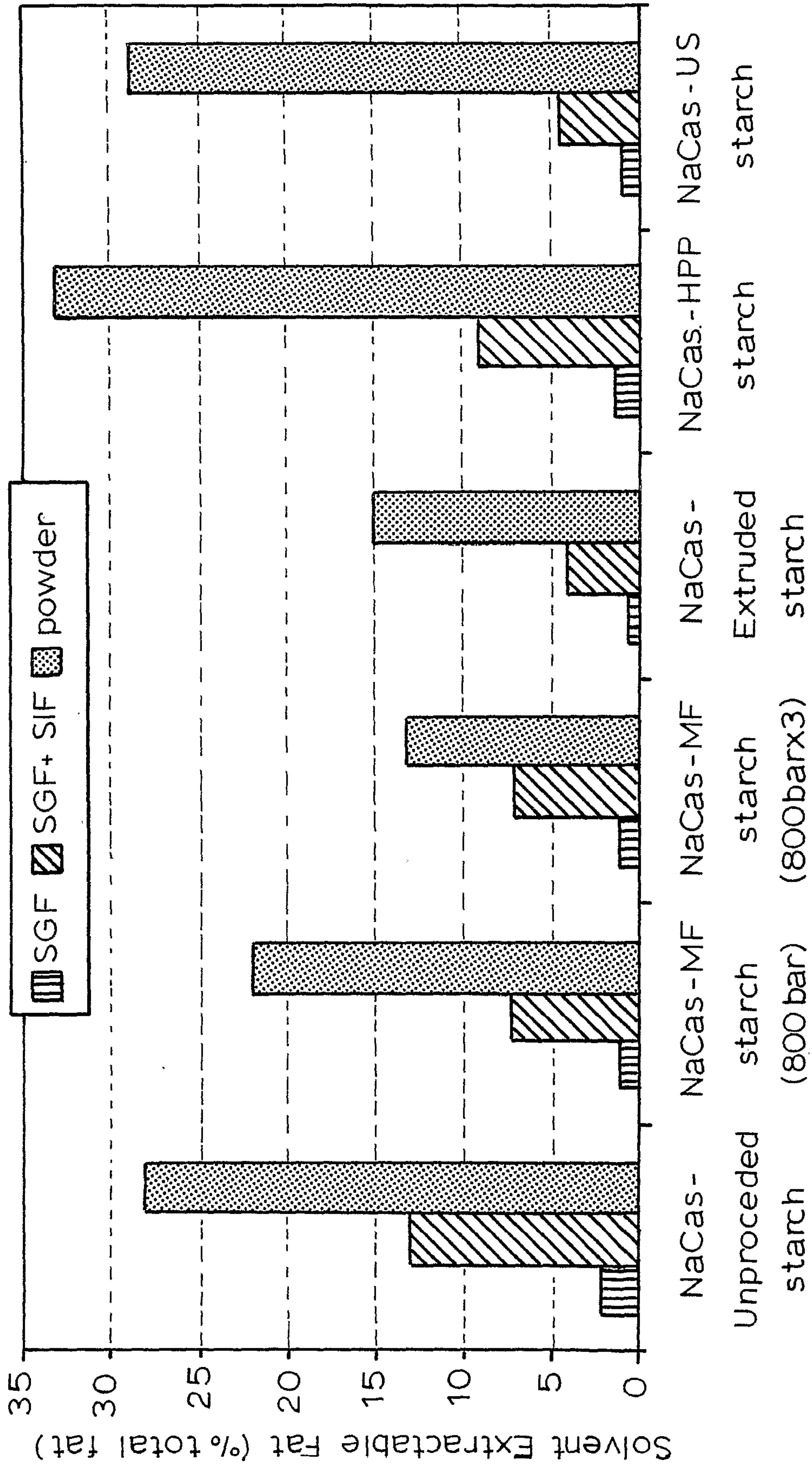
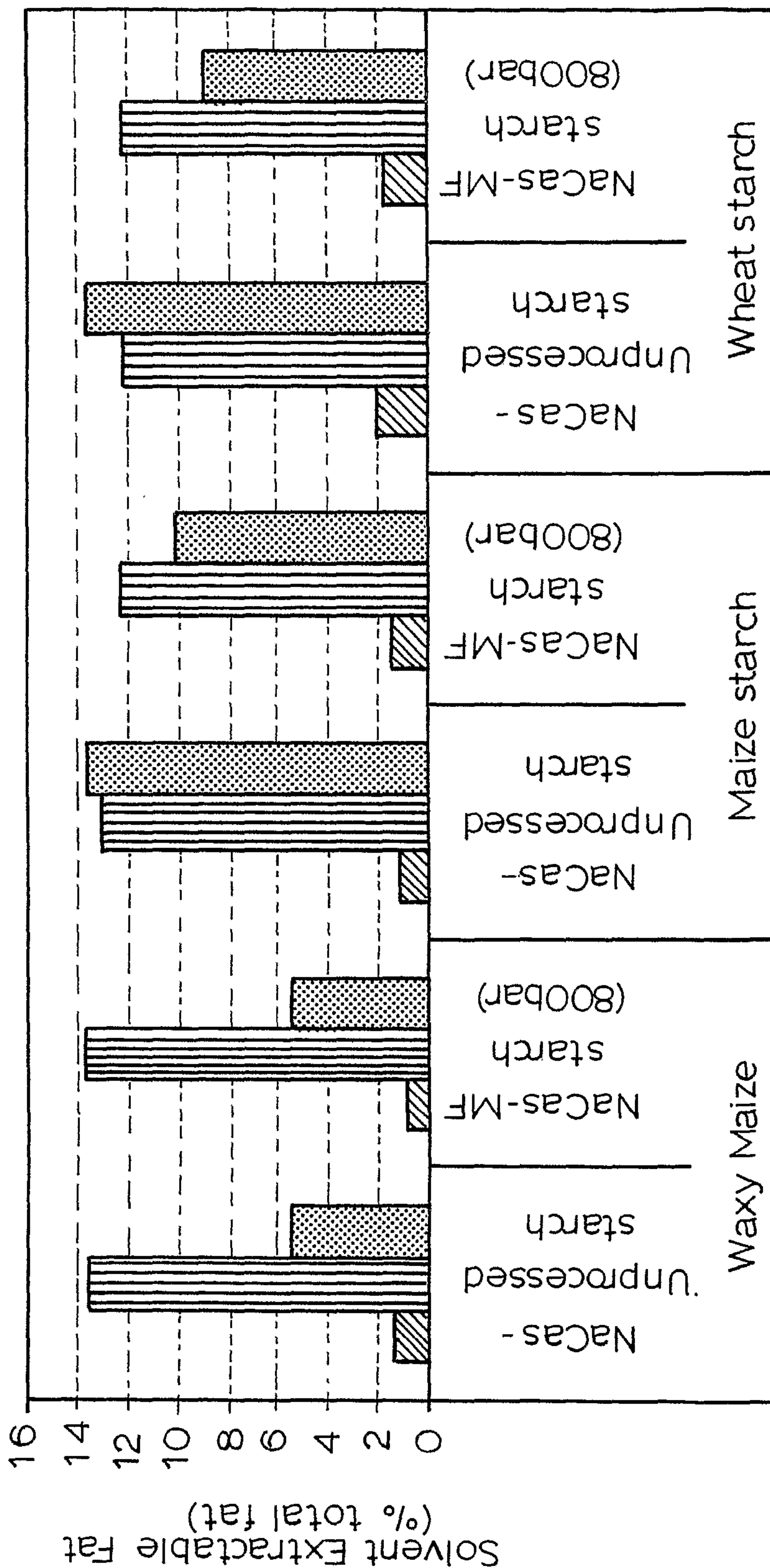


Fig. 14



SGF
 SGF+SIF
 Powder

Fig. 15.



Fig. 16.

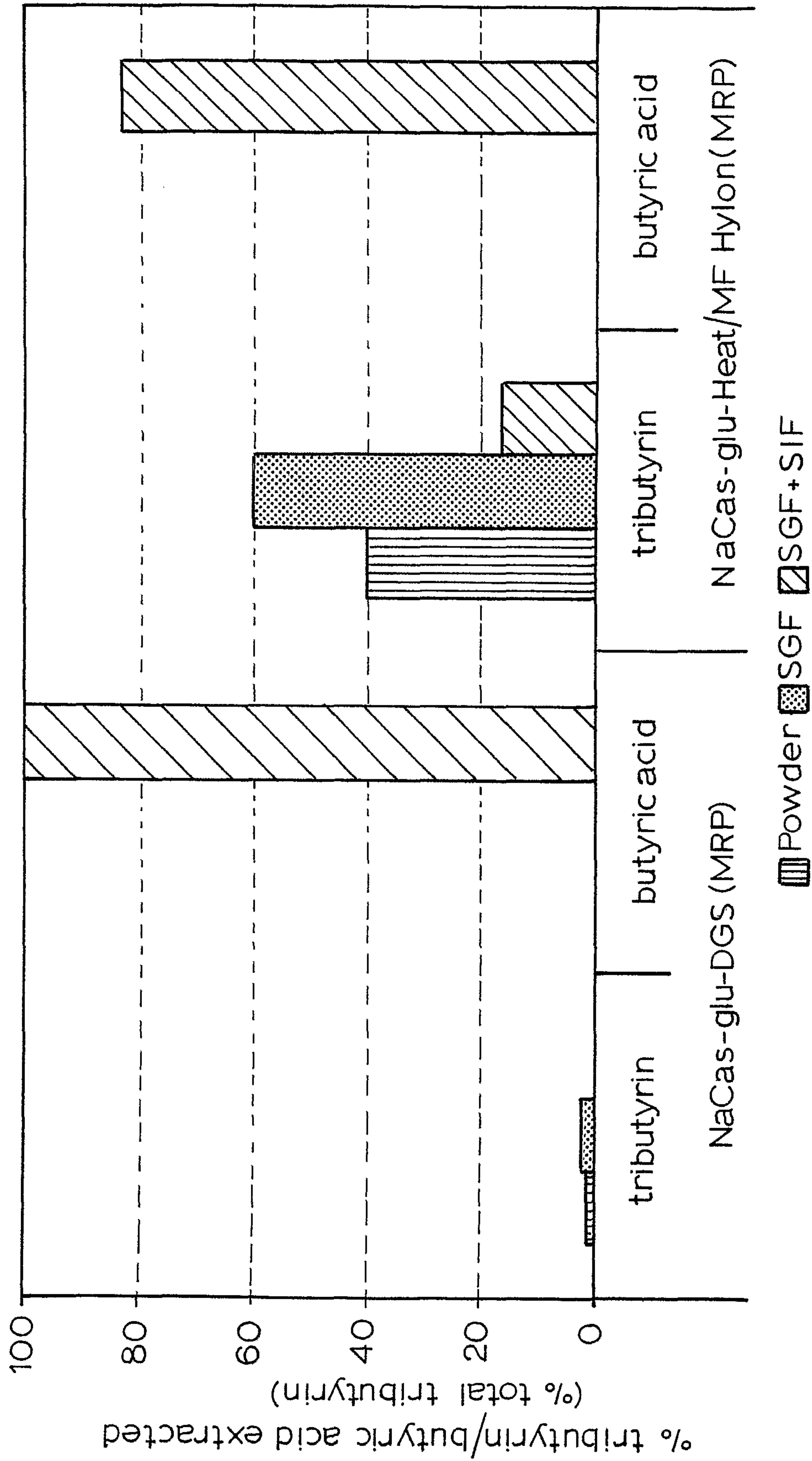


Fig. 17

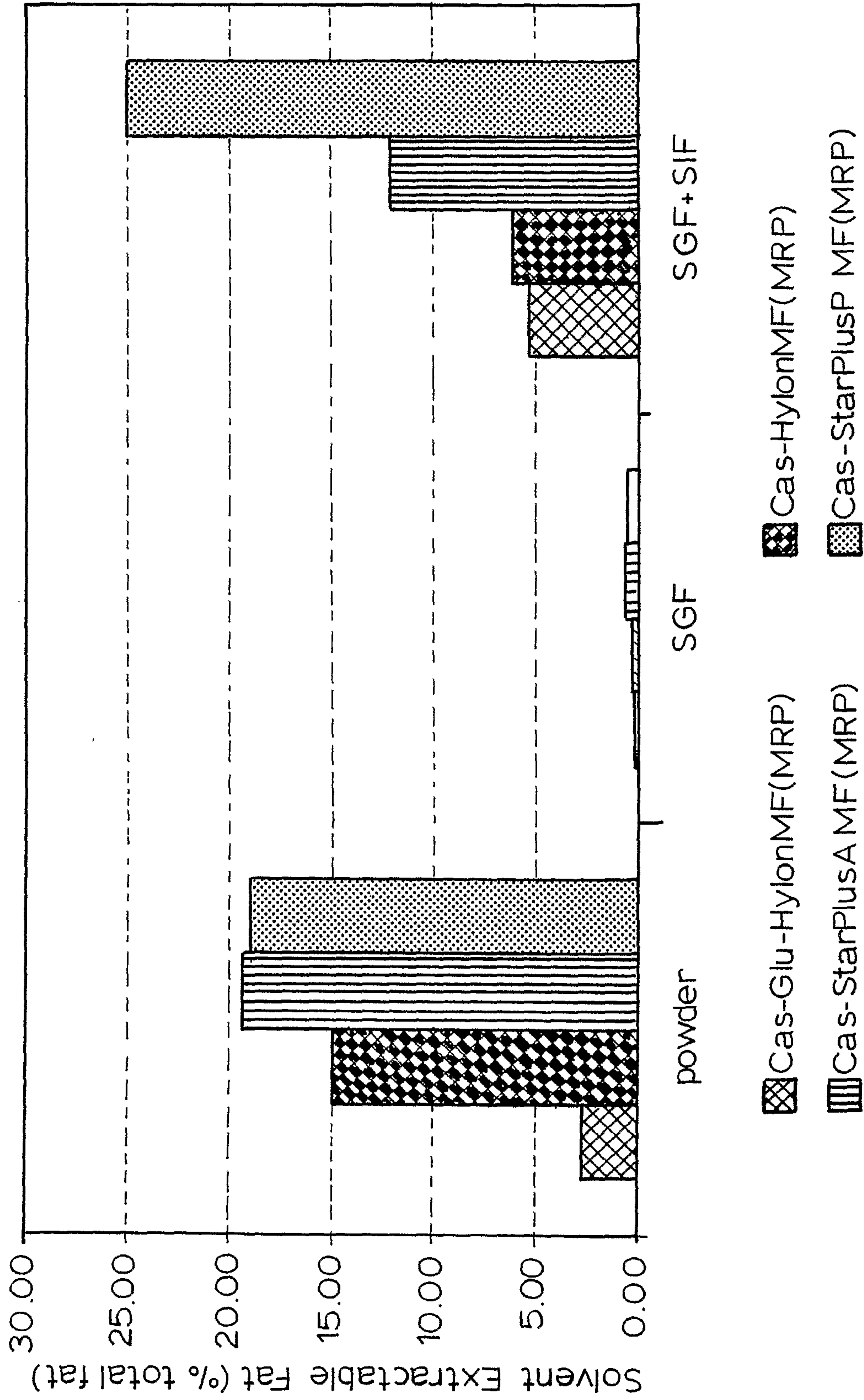


Fig. 18.

Fig. 19.

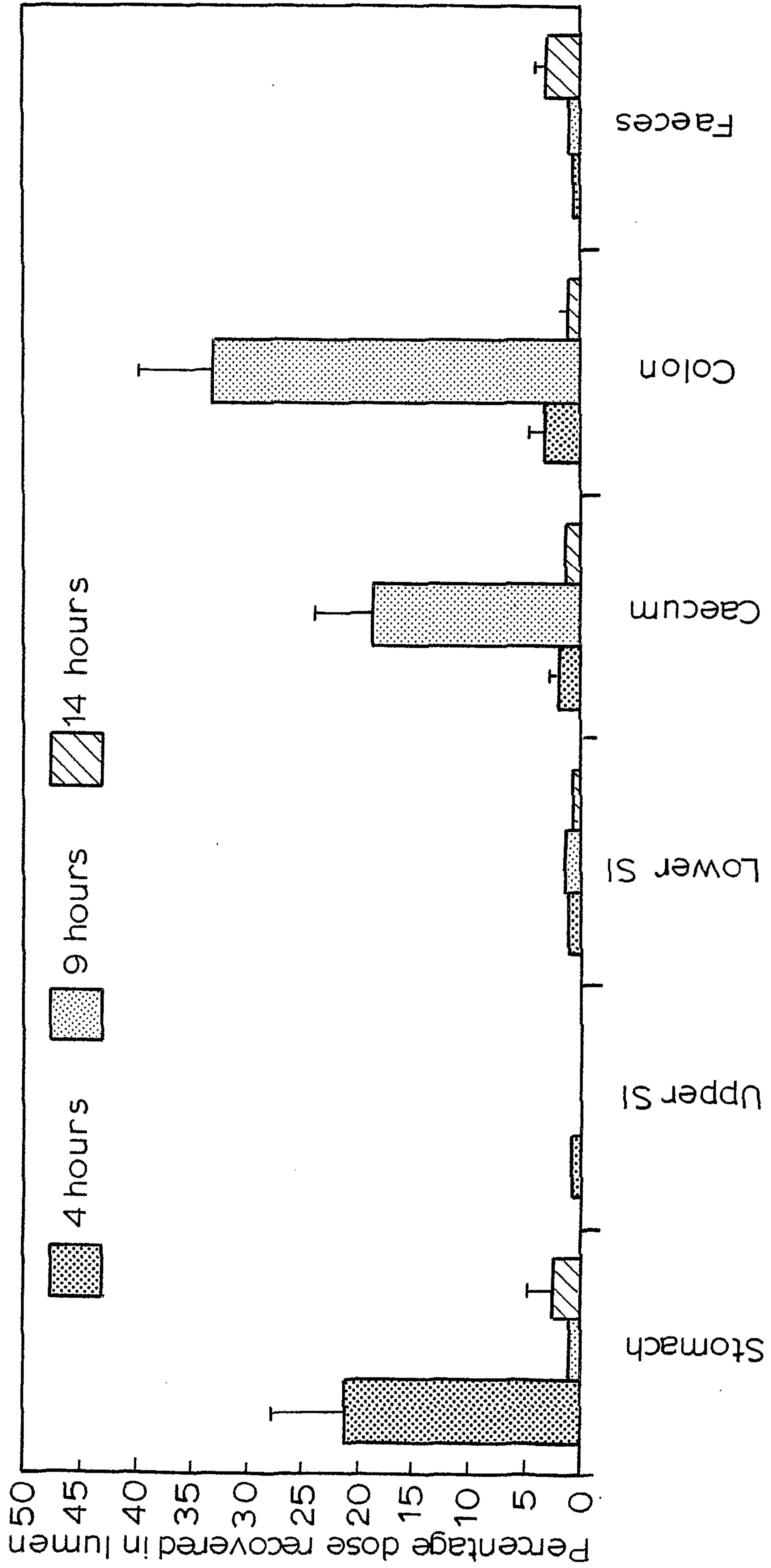


Fig. 19b.

