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(54) Title: Nutrition for improving muscle strength in elderly

(57) Abstract: The invention pertains to a composition for stimulating muscle strength in elderly, comprising whey protein, free BCAA and probiotic bacteria, wherein the composition comprises one or more free BCAAs selected from the group consisting of leucine, isoleucine and valine, and at least 50 g whey protein per 100 g dry weight of the total composition. Also, the invention pertains to a composition additionally comprising one or more dietary fibers selected from the group of pectins, pectates, alginates, fructans, fructooligosaccharides, indigestible dextrins, galactooligosaccharides, xylooligosaccharides, arabinooligosaccharides, glucooligosaccharides, mannoooligosaccharides and fucooligosaccharides, and to the use of such composition in dietary management of malnutrition of elderly.



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Nutrition for improving muscle strength in elderly

The invention is in the field of providing nutrition to elderly, particularly for improving muscle strength in elderly.

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BACKGROUND OF THE INVENTION

Old age consists of ages nearing or surpassing the average life span of human beings, and thus the end of the human life cycle. Euphemisms and terms for old people include seniors — chiefly an American usage — or elderly. As occurs with almost any
10 definable group of humanity, some people will hold a prejudice against others — in this case, against old people. This is one form of ageism.

Worldwide, the number of people 65 or older is increasing faster than ever before. Most of this increase is occurring in developed countries. In the United States the
15 percentage of people 65 or older increased from 4 percent in 1900 to about 13 percent in 1998. In 1900, only about 3 million of the nation's citizens had reached 65. By 1998, the number of senior US citizens had increased to about 34 million. Population experts estimate that more than 50 million Americans — about 17 percent of the population — will be 65 or older in 2020. The number of old people is growing around the world
20 chiefly because more children reach adulthood.

Old people have limited regenerative abilities and are more prone to disease, syndromes, and sickness than younger adults. Old people's energy levels decrease, resulting in a decreased level of muscle strength. This decrease in muscular strength
25 then leads to immobility, and decreased cellular insulin sensitivity resulting from a lack of exercise and decreased appetite. This decreased appetite often results in mal- or undernourishment leading to a further decreased muscle strength and so a downward spiral is created. In addition to that, it is postulated that undernourishment leads to damage to the gut epithelium, causing decreased gut mediated immunity, reduced
30 absorption of essential dietary components, also leading to loss of appetite, and thus

adding to the downward spiral ultimately leading to loss of muscle strength and immobility.

Therefore, a need exist for a treatment to break this downward spiral.

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It is known that as people get older they have reduced levels of friendly bacteria and increased levels of disease-causing bacteria in the gut. At about the age of 60 there is a big drop in bacteria levels, and older people have 1,000-fold less lactic acid (probiotic) bacteria than other younger adults. They are also more susceptible to gastrointestinal infections and bowel conditions, like Irritable Bowel Syndrome.

10

Malnutrition is remarkably common. It has been estimated that approximately 30% of the world's population suffer from malnutrition in some degree, and it is by no means confined to developing countries. Two studies in the past 10 years showed that nearly 50% of patients admitted to hospitals in Europe and the USA were either malnourished or considered at risk thereof. This condition is well recognized among the elderly. It is commonly found that 7% of those living at home and that are apparently well, fall within the category of being malnourished or considered at risk thereof, and the incidence doubles in subjects aged over 80 years old. Being housebound or ill from additional risk factors.

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US 2002/044988 discloses a composition that stimulates body protein synthesis and can improve muscle mass maintenance and recovery. The composition contains a protein source which provides at least about 8 % total calories of the composition and which includes at least about 50 % by weight of whey protein.

25

WO-2004/026294 discloses the use of nutritional compositions enriched in essential amino acids for tumor induced weight loss and promotion of muscle protein synthesis.

Outside the field, RU 2 105 491 discloses a method for preparing a beverage in which granulated sugar is added to dairy whey before pasteurization, and wherein fermentation is carried out with ferment prepared from a dry bacterial preparation.

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SUMMARY OF THE INVENTION

The inventors found that the elderly population would benefit from a nutritional treatment capable of increasing the energy levels and muscle strength, and improving the intestinal and immunological functions. The treatment involves the administration
10 of a nutritional product comprising a very high content of whey protein that is supplemented with at least free leucine, preferably with added other free branched chain amino acids (BCAA), probiotic bacteria and prebiotic dietary fibers selected from galactooligosaccharides (GOS), fructooligosaccharides (FOS) and acidic oligosaccharides (AOS), preferably a combination of the three fibers.

15

One aspect of the invention concerns a composition comprising whey protein, free added BCAA, and probiotic bacteria suitable for stimulating muscle strength in elderly, wherein the composition comprises free BCAA selected from the group consisting of leucine, isoleucine, valine, or a combination thereof, and wherein the composition
20 contains at least 50 g whey protein per 100 g dry weight of the total composition. In a preferred embodiment according to the invention, the composition is a liquid comprising at least 10 weight percent (wt%) dry matter, even more preferably between 12 and 20 wt%. The invention also pertains to the use of such composition for the aforementioned purposes, in elderly.

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In another aspect the invention pertains to a composition suitable for elderly, comprising whey protein, free added BCAA, probiotic bacteria and dietary fibers, wherein the composition comprises free BCAA selected from the group Leu, Ileu, Val, or a combination thereof, at least 50 g whey protein per 100 g dry weight of the total
30 composition, and dietary fibers selected from the group consisting of pectin, pectate, alginate, fructans, fructooligosaccharides (FOS) and polysaccharides (e.g. inulin),

indigestible dextrins, galactooligosaccharides (GOS), xylooligosaccharides, arabinooligosaccharides, glucooligosaccharides, mannoooligosaccharides, fucooligosaccharides and mixtures thereof. The invention also pertains to the use of such composition for the aforementioned purposes, in elderly.

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Also, the invention pertains to a method for stimulating muscle strength in elderly, comprising administering elderly with a composition having the aforementioned features. In yet another aspect the invention pertains to the use of the aforementioned composition in the manufacture of a composition/preparation for improving muscle

10 strength, in elderly.

In yet another aspect, the invention pertains to the manufacture of a composition as described above.

15 DETAILED DESCRIPTION OF THE INVENTION

Elderly

The term 'elderly' as used in the present invention means people aged 55 and older.

20 *Protein/ whey protein*

The inventors make use of the inherent differences in the ability of different protein sources to promote (muscle) protein synthesis: Animal protein sources (eg milk, egg, meat) appear more effective than vegetable proteins (eg soy, pea, wheat). Even within these groups, high quality sources like the milk proteins whey and casein show

25 differences in (muscle) protein synthesis. The main differences are:

- i. the essential amino acid content of a protein, in particular the leucine content.

Specifically, in terms of muscle protein synthesis, the addition of nonessential amino acids to an essential amino acid supplement does not provide an additional stimulatory effect the rate of protein-rich food digestion, splanchnic utilization and subsequent bioavailability of amino acids in the blood

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circulation. These influence muscle protein synthesis, since muscle protein

synthesis is positively related to the blood amino acid concentration. This also underlies the difference between fast- (whey) and slowly-absorbing (casein) proteins.

- 5 An important aspect in the elderly that addresses the importance of whey protein in relation to casein protein is that whey protein is more rapidly absorbed, resulting in a 25% higher blood concentration of essential amino acids, than casein that showed only a gradual but prolonged increase of amino acids in the blood. Enhanced protein synthesis following whey protein intake is found compared to the intake of casein
- 10 protein. The composition according to the invention therefore has high whey protein content.

- Preferably whey protein is present in an amount of at least 50 wt% based on dry weight of the composition, more preferably at least 55 weight percent (wt%) and even more
- 15 preferably between 55 and 75 wt% based on dry weight of the composition. Protein includes all nitrogen sources such as free amino acids, hydrolysates and intact protein.

- Compositions can be in liquid or powder form. Preferably the composition is in a liquid form such as - but not limited to - yoghurt drinks, sport drinks, medical nutrition drinks
- 20 including tube feeds and the like. The high amount of protein in the composition allows low volumes of liquid drink to be used, e.g. between 50 and 100 ml, that still contain an effective amount of protein. This small volume greatly improves the compliance of the product, particularly considering the target group. The amount of protein in the liquid composition is preferably between 6-20 gram per 100 ml even more preferably between
- 25 8 and 15 g per 100 ml.

- The recommended dietary allowance (RDA) for protein, as promulgated by the Food and Nutrition board of the United States National Academy of Science, is 0.8 g protein/kg body weight/day for adults, regardless of age. This value represents the
- 30 minimum amount of protein required to avoid progressive loss of lean body mass in most individuals. In order to improve muscle mass, strength and function in elderly, it

is preferred to realise protein intake greater than the RDA. In addition, other factors, including immune status, wound healing, blood pressure and bone health may also benefit from the increasing protein intake above the RDA. Furthermore, the RDA does not address the recommended intake of protein in the context of a balanced diet. The

5 inventors believe that concerns about potential detrimental effects of increased protein intake on bone health, renal function, neurological function and cardiovascular function are unfounded in elderly and, on the contrary will be improved in elderly ingesting elevated quantities of protein. Therefore in a preferred embodiment of the invention the composition comprises a relatively high amount of protein, preferably between 35

10 and 100 energy percent (en%) of the composition is from protein, even more preferably between 50 and 90 en%, and even more preferably between 60 and 90 en% of the composition is from protein. En% protein means the percentage of calories delivered by protein in relation to the total caloric content of the composition. In a most preferred embodiment the protein is a fast absorbing protein such as bovine whey protein, or

15 protein hydrolysates.

Branched Chain Amino Acids

Branched Chain Amino Acids (BCAA) refer to those amino acids having aliphatic side-chains that are non-linear. These are leucine, isoleucine and valine. The

20 combination of these three essential amino acids make up for approximately 1/3rd of skeletal muscle in the human body, and play an important role in protein synthesis. BCAAs are currently used clinically to aid in the recovery of burn victims, as well as in supplements for providing strength to athletes.

25 According to the present invention it is preferred that at least free leucine is present in the composition in order to increase the total amount of leucine in the composition. The term 'free amino acid' as used in the present invention means that the amino acid is not part of a protein. Among the essential amino acids, leucine acts both as a substrate for protein synthesis and as a signaling molecule to trigger muscle protein synthesis. The

30 effects of leucine on muscle protein synthesis has been shown in the art (see e.g. Anthony, J.C., et al., *Leucine stimulates translation initiation in skeletal muscle of*

- postabsorptive rats via a rapamycin-sensitive pathway* J Nutr, 2000. 130(10): p. 2413-9; Katsanos, C.S., et al., *A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly* Am J Physiol Endocrinol Metab, 2006. 291(2): p. E381-7; Rieu, I., et al.,
- 5 *Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia.* J Physiol, 2006. 575(Pt 1): p. 305-15).

It is preferred to administer a daily dose of at least 1 gram leucine, for stimulating muscle strength. Therefore the composition according to the invention preferably

10 comprises at least 3 gram leucine per 100 g dry weight. The numbers on leucine content are the sum of leucine from protein and added free amino acid. More preferably, the amount of leucine in the composition is between 4.5 and 15 g per 100 g dry weight. In liquid nutrition, leucine preferably amounts to at least 1 g total leucine per 100 ml, more preferably between 1.2 and 5 g total leucine per 100 ml. In practice, if

15 bovine whey protein is incorporated in the composition, between 35 - 70% of leucine should be added as free amino acid. Bovine whey is a preferred protein source since it is rich in BCAA and in particular in leucine.

Probiotic bacteria

- 20 Probiotic bacteria and dietary fibers may be added to the composition to improve intestinal function, e.g. gut motility, barrier function, etc resulting in an improved appetite, therefore synergistically contributing to the effect of high-protein and high-leucine nutrition on muscle strength and mobility of elderly. Probiotics are bacterial components of the normal human intestinal flora, for example lactobacilli and
- 25 bifidobacteria, that produce as end products of metabolism lactate and short chain fatty acids such as acetate and butyrate. Certain specific probiotic strains have well defined and proven clinical effects for the treatment and/or prevention of diseases of intestinal and extra intestinal origin.
- 30 Probiotic bacteria, preferably gram-positive bacteria, are preferably lactic acid bacteria, more preferably bacteria of the genus *Lactobacillus* and/or *Bifidobacterium* and/or

Streptococcus. It is preferred to employ Gram-positive bacteria comprising at least one *Bifidobacterium* selected from the group consisting of *B.breve*, *B.infantis*, *B.bifidum*, *B.catenulatum*, *B.adolescentis*, *B.thermophilum*, *B.gallicum*, *B.animalis* or *lactis*, *B.angulatum*, *B.pseudocatenulatum*, *B.thermacidophilum* and *B.longum* more preferably *B. breve*, *B. infantis*, *B. bifidum*, *B. catenulatum*, *B. longum*, more preferably *B. longum* and *B. breve*, even more preferably *B. breve*, most preferably *B. breve* I-2219 deposited at the CNCM in Paris, France. Preferably the probiotic bacteria used according to the present invention comprise at least one, more preferably at least two, even more preferably at least three, most preferably at least four different *Bifidobacterium* species.

Preferably the probiotic bacteria used according to the present invention comprise at least one, more preferably at least two *Lactobacillus* species selected from the group consisting of *L. casei*, *L. reuteri*, *L. paracasei*, *L. rhamnosus*, *L. acidophilus*, *L. johnsonii*, *L. lactis*, *L. salivarius*, *L. crispatus*, *L. gasseri*, *L. zeae*, *L. fermentum* and *L. plantarum*, more preferably *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. johnsonii*, *L. acidophilus*, *L. fermentum* and even more preferably *L. paracasei*. Even more preferably the probiotic bacteria used according to the present invention comprise *Bifidobacterium breve* and/or *Lactobacillus paracasei*. In a preferred composition according to the invention the lactic acid bacteria used comprise at least *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Streptococcus thermophilus* *Prebiotic dietary fibers*

In the context of the invention, prebiotic dietary fibers follow the definition given by the American Association of Cereal Chemists: “the edible parts of plants or similar carbohydrates resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine.” Surprisingly, certain combinations of fibres show better stimulation of NK cell activity. Combinations of galactooligosaccharides (GOS), including trans galactooligosaccharides (TOS), fructooligosaccharides (FOS), including long chain FOS (lcFOS) and short chain FOS

(scFOS), and several combinations with acidic oligosaccharides such as pectin hydrolysate all give better effects than the individual fibres.

It was surprisingly found that a mixture of GOS, FOS and pectin hydrolysate had a better NK cell activity stimulating effect than the individual saccharides. As shown in example 4 where it can be seen that a mixture of oligo and polysaccharides stimulate NK T-cell activity in an ageing mouse model. Natural killer T-cell activity is known to be significantly decreased in elderly (Aging and innate immune cells, Journal of Leukocyte Biology Volume 76, 2004). Example 4 shows that in fast ageing mice a specific blend of dietary fibers is capable of stimulating NK cell activity, showing that dietary fibers have a potential strong effect in elderly humans. Therefore the composition according to the invention preferably comprises a mixture of two fibres, e.g. GOS and FOS, GOS and pectin hydrolysate, and FOS and pectin hydrolysate and more preferably comprises a mixture of three fibres, e.g. GOS and FOS and pectin hydrolysate.

Preferably the fibres are selected from the group consisting of GOS, FOS and pectin degradation products (e.g. pectin hydrolysates). Even more preferably the ratio of the GOS, FOS and pectin is in the range of approximately 5-9:1:2-10.

Pectin is divided into two main categories: high methoxylated pectin, which is characterized by a degree of methoxylation above 50% and low methoxylated pectin having a degree of methoxylation below 50%. As used herein, "degree of methoxylation" (also referred to as DE or "degree of esterification") is intended to mean the extent to which free carboxylic acid groups contained in the polygalacturonic acid chain have been esterified (e.g. by methylation).

The present acid oligosaccharide is preferably prepared from high methoxylated pectin. Preferably the acid oligosaccharides have a degree of methylation above 20%, preferably above 50 % even more preferably above 70%.

The acid oligosaccharide, preferably pectin hydrolysate, is preferably administered in an amount of between 10 mg and 100 g per day, preferably between 100 mg and 50 g per day, even more between 0.5 g and 20 g per day.

- 5 Dietary fibres often have a large molecular weight. The fibres used preferably have an average degree of polymerization of at least 3 and no more than 250. This is because small oligosaccharides do not bind to Toll like receptors and are therefore expected not to be effective in our model systems. This requirement does not imply that no oligosaccharides with DP 1 or 2 may be present. Most commercially available
- 10 oligosaccharides consist in part of saccharides with a DP 1 and 2, while most have a DP 3 and higher. When calculating weight percentages of the oligosaccharide fraction the weight of all oligosaccharides, including the saccharides with DP 1 and 2 are used in the calculation. The total amount of fibres used in the composition therefore also depends on the source of oligosaccharides and the amount of oligosaccharides with DP
- 15 3 or higher present in the source. The maximum DP of about 250 is related to the effect on viscosity. Large molecular weight dietary fibres with a DP higher than about 250 will significantly increase the viscosity of a liquid product resulting in a decreased palatability of the product and possible difficulties during production of a liquid product.

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Calcium

- Calcium has a pivotal role in the control of muscle cell action. A decreased availability of calcium in the muscle tissues will inevitably lead to decreased muscle strength. The use of dietary fiber improves the calcium absorption in elderly, and therewith
- 25 stimulates muscle strength. Calcium intake is often low in elderly due to decreased intake of dairy and other calcium containing products. In a preferred embodiment according to the invention the composition comprises dietary fiber and calcium.

- Preferably the amount of dietary fibers in the present composition is between 2 – 10
- 30 wt% based on dry weight of the composition.

In a liquid composition preferably 0.2 to 10 g dietary fibers per 100 ml are present. For reasons outlined above, calcium may be present in an amount between 30 and 1000 mg per 100 ml or between 0.05 and 1 wt% based on dry weight of the composition.

5 *Vitamin E, zinc; other ingredients*

Nutrition plays a prominent role in immune response, and the elderly often suffer from malnutrition. Reduced caloric intake is known to slow the aging process and help maintain higher numbers of naive T cells and levels of IL-2. Vitamin E and zinc in particular are important nutrients for the proper functioning of the immune system.

- 10 Long-term zinc deficiency in the elderly causes a decrease in cytokine production and impaired regulation of helper T cell activity.

Therefore, according to a preferred embodiment the composition according to the invention additionally comprises vitamin E and/or zinc, preferably both.

15

In a preferred embodiment the composition according to the invention for the stimulation of the immune function and for the stimulation of muscle strength in elderly comprises in addition to high protein at least one selected from; vitamin E, zinc and dietary fibers. More preferably all of these ingredients are present.

20

Effect of nutritional composition on the immune system of elderly

Aging affects many components of the immune system, and since the immune system interacts with every organ in the body, a clearer understanding of the immunological changes due to aging is critical for designing effective health care for the elderly. The present inventors now realized that an improved immune function helps to prevent infections and thereby stimulate the mobility of the elderly patients resulting in improved muscle strength.

25

According to one aspect of the invention the composition according to the invention can be used for

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- a. increasing muscle mass, strength and function;
- b. improving physical capacity and agility; and/or
- c. improving vitality, i.e. the ability to perform activities of daily living.

- 5 The efficacy of the products can be established in a trial setting by either of the following measures:
- Muscle protein synthesis: incorporation of amino acids is a good indication for muscle strength
 - Muscle mass and strength: hand grip and leg strength can be measured
 - 10 • Physical capacity: can be measured by using an array of different exercise tests
 - Activities of Daily Living: can be measured using a score list
 - Quality of Life: is normally measured using a questionnaire, score list

Muscle strength

- 15 Muscle strength adds to the quality of life and improved ability to perform activities of daily living. Loss of muscle mass and function in apparently healthy elderly is reflected in the increased difficulty that elderly experience in performing physical activities and activities of daily living, with walking as an obvious example. From 60 years of age loss of muscle mass and strength/function is accelerated and, as a consequence around
- 20 30% of muscle strength is lost at the age of 70. When people reach the age of 75 or older it has been demonstrated that the loss of mobility can be significantly impaired:
- 30% can no longer walk a 1 km distance (Lauretani, F., et al., *Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia*. J Appl Physiol, 2003. 95(5): p. 1851-60)
 - 25 • Walking (gait) speed declines by 20%
 - Activities of Daily Living decline: 50% can no longer lift a weight over 4.5kg (Baumgartner, R.N., et al., *Predictors of skeletal muscle mass in elderly men and women*. Mech Ageing Dev, 1999. 107(2): p. 123-36.)
- 30 To stay independent, people need to maintain or regain physical strength to continue the performance of activities of daily living. Therefore, the term 'muscle strength' as

used in the present invention can be measured by 'walking distance', 'walking speed', activity of daily living', 'hand grip strength'.

In one aspect, the invention thus pertains to the use of the composition having the
5 aforementioned characteristics in the manufacture of a preparation for stimulating muscle strength in elderly and/or for treating/preventing malnutrition (thus controlling dietary management) of elderly.

In one aspect, the invention thus pertains to a method for stimulating muscle strength
10 and or preventing/treating malnutrition in a person in need thereof, particularly elderly, by administering the composition having the aforementioned characteristics. In one aspect, the method is non-medical, i.e. non-therapeutic.

The invention also pertains to the non-medical or non-therapeutic use of the
15 composition having the aforementioned characteristics for stimulating muscle strength in elderly and/or for treating/preventing malnutrition (thus controlling dietary management) of elderly

Method for preparing high protein containing product

20 The method for preparing the compositions according to the invention involves a careful selection of steps with optimal ranges of the amount of ingredients and strict ranges for pH and temperatures. The most critical for making a fermented, stable product with such high protein content above 8 gram protein per 100 ml, is the pH of the product before emulsifying. This pH preferably is in the range of 3.5 and 4.5 and
25 even more preferably between 3.9 and 4.2. The upper limit of the final product is preferably set at pH 4.2 because at a higher pH the shelf-life of the product becomes problematic. Therefore in a preferred method according to the invention the pH is between 4.0 and 4.2. The effect of pH is demonstrated in Table 1 below.

30 Another critical step in preparing the compositions according to the invention is the late addition of the free amino acids, i.e. after the fermentation step. The inventors found

that during fermentation the probiotic bacteria preferably metabolize these free amino acids leading to a decreased amount of these added amino acids in the end product. Therefore, in the preferred method for preparing the compositions according to the invention, the amino acids are added after the fermentation step.

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Therefore, in one aspect the invention pertains to a method for preparing a composition according to the present invention, wherein the method comprises:

- a. Mixing whey protein and lactose with demineralized water;
- b. adding the bacterial cultures and ferment;
- 10 c. adding free amino acids selected from the group consisting of leucine, valine and isoleucine, and optionally add dietary fibers
- d. adjusting pH to 3.5 – 4.5, preferably between 4.0 and 4.2;
- e. pasteurization;
- f. homogenization;
- 15 g. if considered appropriate, adjusting pH to between 3.5 and 4.5, preferably between 4.0 and 4.3;
- h. optionally adding water to 100%; and
- i. sterilizing and packaging product.

20 **Table 1. Determination of the optimal pH of the product**

Product characteristics	pH					
	3.84	3.93	4.05	4.09	4.22	4.33
Particle size distribution D[4,3]	7.602	5.223	3.020	2..622	2.284	1.806
Particle size distribution D[0,5]	6.896	5.088	2.596	2.025	1.010	0.766
Viscosity (100 1/s)	32.80	32.40	22.50	20.60	19.70	17.70
Viscosity (1000 1/s)	20.00	21.90	16.90	15.70	15.10	13.80
sediment	36.88	33.43	8.61	6.97	6.90	5.99

As seen in Table 1, the shelf-life of the product increases when lowering the pH of the product. However, the upper-limit of the pH range is preferably set at pH 4.3 since above this pH microbial growth would make it necessary to sterilize the product for obtaining sufficient shelf-life, which than leads to deterioration of the product characteristics. Although less sediment and lower viscosities can be obtained when increasing the pH, the most optimal pH for this product is selected to be between 4.05 and 4.3. In this pH range the particle size distribution, viscosity and sediment formation are the most acceptable. Similar experiments with casein or other milk protein sources resulted in different optimal pH ranges.

EXAMPLES

Example 1. Preferred method for making high protein Leucine containing yogurt

1. Mix whey protein, lactose with demineralized water
2. Pasteurisation step of 15 seconds at 75 degrees Celsius
3. Add and dissolve the bacterial culture
4. Fermentation between 16 and 18 hours
5. Add and dissolve Leucine, valine, isoleucine, dietary fibers and sugar
6. Add and dissolve vitamins and minerals
7. Adjust pH tot 3.5 – 4.5, preferably between 4.0 and 4.2.
8. Add fat and perform inline emulsifying step
9. Pasteurization step of 30s, at 75 degrees Celsius
10. Homogenization at 60 degrees Celsius, 250 -50 bar
11. Adjust to pH 4.2 and add water to 100%
12. Sterilize 30 seconds, at 92 degrees Celsius
13. Package in bottle

Example 2. Preferred composition fermented product.

Composition	Per 100 ml
Protein total (g)	9.7
Whey protein (g)	9.0
BCAA total free (g)	0.7

	Leucine free (g)	0.6
	Leucine total (g)	1.6
	Isoleucine free (g)	0.023
	Isoleucine total (g)	0.6
5	Valine free (g)	0.1
	Valine total (g)	0.6
	Fat (g)	1.50
	Rapeseed oil with low erucic acid	
	Carbohydrate (g)	
10	saccharose	2.50
	Prebiotic fiber	
	(pectin hydrolysate)	0.20
	Vitamins and minerals	
	Calcium (mg)	200
15	Zinc	
	vitamin D	200IU
	Vitamin E	
	Ferment	
	Probiotic bacteria <i>Bifidobacterium lactis</i> , <i>Lactobacillus acidophilus</i> , <i>Streptococcus</i>	
20	<i>thermophilus</i>	

Example 3. Sterilized composition with 10 g intact protein per 100ml.

<u>Composition</u>	Gram per 100 ml
Intact protein	10.00
Protein total	10.39
Leucine free	0.39
Leucine total	1.40
Isoleucine free	0.000
Isoleucine total	0.62
Valine free	0.00

Valine total	0.57
fat	1.50
carbohydrate	2.50
Dietary fibers	
GOS/Inulin/AOS	1.5
Probiotic bacteria	10^9 - 10^{10}
<i>Bifidobacterium</i>	heat killed
<i>lactis</i> ,	CFU
<i>Lactobacillus</i>	
<i>acidophilus</i> ,	
<i>Streptococcus</i>	
<i>thermophilus</i>	

Example 4. Stimulation of NK-cell activity with a fiber mix comprising AOS in fast-ageing mice

- 5 In order to investigate the effect of fibres on the NK-cell activity in aged mice, the NK-cell activity in splenocytes was measured in 8 months old fast ageing SAMP8 mice that received various doses of scGOS, lcFOS and acid oligosaccharide (AOS) from pectin hydrolysate. The animals were aged on a standard rodent chow, and received the supplemented diets or control diet for the last 43 days ('treatment protocol').

10

- NK cell activity was measured by a modified "JAM" assay. The method as described by P. Matzinger in The JAM test. A simple assay for DNA fragmentation and cell death. J Immunol Methods. 1991 Dec 15;145(1-2):185-92 was used, with the modifications and specifications described here. Yac-1 cells were used as target cells,
- 15 were labeled for 4-6 hours with 2.5 microCurie/ml tritiated thymidine (Perkin Elmer, Groningen, the Netherlands) and seeded at 5000 cells/well in round-bottom 96-well plates in cell culture medium (RPMI-1640 with 10% fetal calf serum). Murine splenocytes were added to the 96-well plates as effector cells in three different effector:target (E:T) ratios: 100:1, 50:1 and 25:1. Effector and target cells were

incubated for 4 hours at 37 degrees Celsius in an atmosphere containing 5% carbon dioxide. After incubation, the effector and target cells were harvested on Unifilter GF/c plates (Perkin Elmer). These filterplates were dried and counted in a scintillation counter (Wallac Microbeta, Perkin Elmer) and the percentage target cell lysis was
5 calculated as described by P. Matzinger. Statistics have been performed on the separate E:T ratios.

Results:

At all three ratios, a significant dose-dependent increase in target cell lyses was found
10 using linear regression analysis (slope is significantly greater than zero). The lower three plots show these regressions, statistical testing was performed in SPSS.

The Figure shows an overview of all Effector:Target (E:T) ratios and all GFA doses. It can be seen that the NK-cell activity in the mice significantly increases with the dose of fibres indicating that AOS can actually stimulate the NK cell activity in the aged mice.

CLAIMS

1. A composition for stimulating muscle strength in elderly, comprising whey protein, free BCAA and probiotic bacteria, wherein the composition comprises
5 one or more free BCAAs selected from the group consisting of leucine, isoleucine and valine, and at least 50 g whey protein per 100 g dry weight of the total composition.
2. A composition comprising whey protein, free BCAA, probiotic bacteria, and a
10 dietary fiber, wherein the composition comprises one or more free BCAAs selected from the group consisting of leucine, isoleucine and valine, at least 50 g whey protein per 100 g dry weight of the total composition, and one or more dietary fibers selected from the group of pectins, pectates, alginates, fructans, fructooligosaccharides, indigestible dextrins, galactooligosaccharides,
15 xylooligosaccharides, arabinooligosaccharides, glucooligosaccharides, mannoooligosaccharides and fucooligosaccharides.
3. The composition according to claim 1 or 2 wherein the composition comprises
20 between 55 and 75 g whey protein per 100 g dry weight of the total composition.
4. The composition according to any one of claims 1 to 3, wherein the composition is a liquid comprising at least 10 wt% dry matter.
- 25 5. The composition according to claim 4, comprising between 12 and 20 wt% dry matter.
6. The composition according to claim 3, wherein the composition comprises
30 between 8 and 15 gram protein and a total of at least 1.4 gram leucine per 100 ml.

7. The composition according to any one of the preceding claims, wherein at least one of the probiotic bacteria is selected from the group of lactic acid bacteria of the genus *Lactobacillus*, *Bifidobacterium* and *Streptococcus*.
- 5 8. Non-medical use of the composition according to any one of claims 2 to 7, for the dietary management of malnutrition of elderly.
9. Use of the composition according to any one of claims 2 to 7 in the manufacture of a preparation for the dietary management of malnutrition of elderly.
- 10 10. A method for preparing a composition according to any of the claims 1 to 7 comprising:
- a. mix whey protein and lactose with demineralized water;
 - b. add probiotic bacterial cultures and ferment;
 - 15 c. add free amino acids selected from the group consisting of leucine, valine and isoleucine;
 - d. optionally add dietary fibers
 - e. adjust pH to 3.5 – 4.5, preferably between 4.0 and 4.2;
 - f. pasteurize;
 - 20 g. homogenize;
 - h. if appropriate, adjust pH to between 3.5 and 4.5, preferably between 4.0 and 4.3;
 - i. optionally add water to 100%;
 - j. sterilize and package product.
- 25 11. A composition for stimulating muscle strength in elderly, comprising whey protein, free BCAA and probiotic bacteria, wherein the composition comprises one or more free BCAAs selected from the group consisting of leucine, isoleucine and valine, and at least 50 g whey protein per 100 g dry weight of the
- 30 total composition.

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2010/050346

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/20 A61K35/66
ADD. A61P3/02

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 02/15720 A2 (NESTLE SA [CH]; AGRONOMIQUE INST NAT RECH [FR]) 28 February 2002 (2002-02-28) *cf. abstract, page 1, lines 10-16, page 2, lines 21-23 and line 25 bridging with line 21 on page 3, page 6, lines 6-9, page 7, last line extending to page 8, line 3, claims 1-15*	1-11
Y	US 2005/153019 A1 (FUCHS EILEEN C [US] ET AL) 14 July 2005 (2005-07-14) *cf. abstract, furthermore sections [0009] and [0011] on page 1*	1-11

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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