

SPECIFICATION

NUTRITIONAL COMPOSITION FOR INHIBITING GROWTH OF TUMOR

TECHNICAL FIELD

[0001] The present invention relates to nutritional compositions useful for nutritional management and therapy of tumor patients. Furthermore, the present invention relates to nutritional compositions useful for pathological improvement of patients with colon solid tumors.

BACKGROUND ART

[0002] In recent year, using nutritional compositions to curing the patients suffering from tumor are reported. In a Chinese patent application whose published number is CN20061054681, soybean milk, vegetable juice and egg nutritious rice flour and wheat flour foods are reported.

[0003] The soy-bean milk with isoflavone components that can effectively inhibit the occurrence of leukemia, colon cancer, lung cancer and stomach cancer, etc.; the flavone and abundant anti-oxidation components in carrot juice has strong anti-cancer effect to human body, and egg has rich proteins to supplement nutrition to, and improve immunizing ability and prevent the occurrence of diseases of human body.

[0004] In another Chinese patent application whose published number is CN20081006602, a special nutrition food formulation containing short peptide and probiotic is described. The food formulation uses

food-originated short peptide as a raw material which is added with the probiotic and functional oligosaccharide to form a compound formulation. With the formulation, dry powder foods can be prepared and are taken with cooled water.

[0005] The foods prepared according to the formulation can improve the nutritional status of organism proteins, optimize the micro-ecological environment in intestines, regulate the functions of intestinal tracts, prevent constipation, diarrhea and colon cancer, enhance the immunity, whiten skins, and strengthen the physique comprehensively.

[0006] In international patent application whose published number is WO2004002238, a nutritional compositions that combine the added effects of both a prebiotic source and a phytochemical capable of inducing enzymatic activity in mammals are reported. The nutritional compositions capable of reducing the risk of cancer are provided.

[0007] In international patent application whose published number is WO2007107295, a low glycemic food composition which comprises isomaltulose, leucrose, trehalulose and / or turanose as a low glycemic carbohydrate source are provided. The low glycemic food is used for the treatment and/or prevention of malignant neoplastic diseases of the human or animal body.

[0008] In Japanese patent application whose published number is JP10-203994, a composition comprising a peptide 500-5000 in molecular weight formed by making a protease act on at least one kind of protein selected from those derived from cow milk, soybean and yeast as active ingredient are provided. The composition is excellent in immunopotentiative, antistress,

antioxidant, hypotensive, anticancer, neutral fat depressive and hyper-HDL-cholesterol activities.

[0009] Those are some patents and patent applications related to nutritional compositions regarded with tumor treatments, but the fluid diets type nutritional compositions in this field is inexistent, which contains protein, lipid and carbo-hydrate at a predetermined energy percentage and which is added with a viscous soluble food fiber and inulin or hydrolyzate.

PRIOR ART DOCUMENTS

PATENT LITERATURE

[0010] PLT 1 : CN20061054681
PLT 2 : CN20081006602
PLT 3 : WO2004002238 A1
PLT 4 : WO2007107295 A1
PLT 5 : JP10-203994
PLT 6 : JP2001-181303
PLT 7 : WO01049308

SUMMARY OF THE INVENTION

PROBLEMS TO BE RESOLVED BY THE INVENTION

[0011] An object of the present invention is to provide nutritional compositions to inhibit tumor growth, peculiarly to inhibit colon tumor growth.

MEANS OF SOLVING THE PROBLEMS

[0012] The present inventors relate nutritional compositions which can inhibit the growth of tumor such as reducing tumor weight and tumor volume, and having smooth edges and cells grew densely in Histopathologic

examination.

ADVANTAGEOUS EFFECTS OF THE INVENTION

[0013] The present invention provides a nutritional composition which is useful for nutritional management and therapy of tumor patients. For example, the ingested patients can inhibit growth of the cancer through taking fluid diet, enteral alimentation, oral nutrition, tubal feeding. Further, The present invention are better suited for nutritional management safety and during extended care.

[0014] The nutritional composition of the present invention can suppress the growth of a cancer while supplementing nutrition. The nutritional composition can be used in, for example, a liquid food, and oral/enteral/tube feeding nutrients to suppress the growth of a cancer of a person who has ingested it. Further, the composition of the present invention can be ingested safely and is hence suitable for the nutrition management in long-term medical treatment.

BRIEF DESCRIPTION OF DRAWINGS

[0015] Fig. 1 is a graph showing the Survival curve of S-180 tumor-bearing mice.

[0016] Fig. 2 is a graph showing the food intake of C26 tumor-bearing mice.

[0017] Fig. 3 is a graph showing the body weight of C26 tumor-bearing mice.

[0018] Fig. 4 is a photo showing the Tumors dissected from C26 tumor-bearing mice.

[0019] Fig. 5 is a graph showing the tumor volume of C26

tumor-bearing mice.

[0020] Fig. 6 is a photo showing the Histopathology of C26 tumor tissues (Hematoxylin-Eosin Stain, $\times 100$).

EMBODIMENT OF THE INVENTION

[0021] The present invention will be described below in detail.

[0022] 1. Protein

1-1. Milk protein hydrolysate

Casein, a whey protein (a whey protein concentrate (WPC), a whey protein isolate (WPI), α -lactalbumin (α -La), and β -lactoglobulin (β -Lg)), a milk protein concentrate (MPC or total milk protein (TMP)), and such can be used as protein sources.

[0023] Enzymes normally used for hydrolysis of whey proteins are, for example, pepsin, trypsin, and chymotrypsin. However, there are also reports of studies using plant-derived papain, and proteases derived from bacteria and fungi (Food Technol., 48: 68-71, 1994; Trends Food Sci. Technol., 7: 120-125, 1996; Food Proteins and Their Applications: pp.443-472, 1997). Whey protein-hydrolyzing enzyme activity varies greatly. Pepsin degrades denatured α -La and α -La, but not native β -Lg (Neth. Milk dairy J., 47: 15-22, 1993). Trypsin slowly hydrolyzes α -La but hardly degrades β -Lg (Neth. Milk dairy J., 45: 225-240, 1991).

[0024] Chymotrypsin rapidly degrades α -La, however slowly degrades β -Lg.

[0025] Papain hydrolyzes bovine serum albumin (BSA) and β -Lg, but shows resistance to α -La (Int. Dairy Journal 6: 13-31, 1996a). However, under acidic pH, α -La not

bound to Ca is completely degraded by papain (J. Dairy Sci., 76: 311-320, 1993).

[0026] By controlling the enzymatic degradation of a milk protein and by modifying the protein, the functional characteristics of that protein can be altered over a wide range of pH and processing conditions (Enzyme and Chemical Modification of proteins in Food proteins and their Applications pp.393-423, 1997 Marcel Dekker, Inc., New York, 1997; Food Technol., 48: 68-71, 1994).

[0027] Hydrolysis of peptide bonds increases the number of charged groups and hydrophobicity, decreases molecular weight, and modifies molecular configuration (J. Dairy Sci., 76: 311-320, 1993). Changes in functional properties depend greatly on the degree of hydrolysis.

[0028] For example, the greatest changes commonly observed in whey protein functionality are increased solubility and decreased viscosity. Often when the degree of hydrolysis is high, hydrolysates will not precipitate, even upon heating, and are highly soluble at pH 3.5 to 4.0. Hydrolysates also have far lower viscosity than intact proteins. This difference is especially prominent when protein concentration is high. Other effects include altered gelation properties, enhanced thermostability, increased emulsifying and foaming abilities, and decreased emulsion and foam stabilities (Int. Dairy journal, 6: 13-31, 1996a; Dairy Chemistry 4, pp. 347-376, 1989; J. Dairy Sci., 79: 782-790, 1996).

[0029] Various bioactive oligopeptides derived from milk proteins are known. For example, A cancer metastasis inhibitor containing a treated material

obtained by incubating a protein digestive enzyme, e.g. pepsine (JP62059220(A)).

[0030] For example, it is known that an autolysate of a protein digestive enzyme such as pepsin suppresses cancer metastasis via the platelet agglomeration induction activity of cancer cells(JPA62-059220).

[0031] Milk protein-derived various physiologically active peptides are known. For example, it is known that an autolysate of a protein digestive enzyme such as pepsin suppresses cancer metastasis via the platelet agglomeration induction activity of cancer cells. Further, it is also known that a lactoferrin hydrolysate enhances the cytotoxic activity of an antibody preparation in the antibody therapy.

[0032] In addition to the references cited above , many patents (published patent applications and patents) exist with regards to milk protein hydrolysates. Examples include: a patent on the separate hydrolysis of casein and whey protein, followed by adsorption and removal of the hydrophobic portion, and then mixing of the casein and whey proteins in a designated ratio (Japanese Patent No. 2,986,764) ; a patent on the hydrolysis of whey protein with proteases derived from Bacillus and from actinomycetes , followed by the removal of the enzyme and insoluble hydrolysis products (Japanese Patent No. 3,222,638) ; a patent on a peptide mixture in which the mole ratio of branched-chain amino acids/aromatic amino acids, achieved by enzymatic degradation of β -lactoglobulin, is 10 weight percent or more, where aromatic amino acids are less than 2.0 weight percent, and where the average molecular weight is several hundred to several thousand (Japanese Patent No. 3,183,945); a patent on the selective enzymatic

degradation of β -lactoglobulin in whey protein (Japanese Patent No. 2,794,305) ; and a patent on using proteases derived from *B . licheniformis* and/or *B . subtilis* to hydrolyze whey proteins by the non-pH-stat technique to 15% to 30% (Dextrose Equivalent; DE) , and then obtaining the permeate from an ultrafiltration membrane with a cutoff value greater than 10,000 (Japanese Patent No. 3,167,723); and the present invention includes patents and unexamined published patents other than these patents and patent applications.

[0033] The protein hydrolysates of Whey Protein could be prepared by following five steps. For a whey protein hydrolysate, a preparation method comprising the following (1) to (5) steps can be described.

[0034] (1) An isolate of whey protein having a protein content of about 90% (w/w) as a dried product (WPI, Davisco Foods International, Inc.) was dissolved in distilled water so as to give a protein concentration of 8% (w/v), thereby obtaining an aqueous solution of the protein.

[0035] (2) The aqueous solution was heat treated at 85°C for 2 minutes to denature the protein. The heat treated aqueous solution had a pH of about 7.5.

[0036] (3) Then, 2.4 L of Alcalase (enzyme, Novozymes) was added at 2.0% (w/w) with respect to the protein (substrate) concentration and the aqueous solution was hydrolyzed while maintained at 55°C for 3 hours.

[0037] (4) Subsequently, PTN 6.0S (enzyme, Novozymes Japan Ltd.), pig-derived trypsin, was added at 3.0% (w/w) with respect to the protein (substrate)

concentration and the aqueous solution was hydrolyzed while maintained at 55 C for 3 hours. In other words, the total hydrolysis time was 6 hours. The pH of the aqueous solution at the completion of these hydrolysis reactions was about 7.0.

[0038] (5) The whey protein hydrolysate was centrifuged (20,000 x g, 10 minutes) and treated using an ultrafiltration (UF) membrane having a molecular weight cutoff of 10,000 (Millipore Corporation, Ultrafree MC).

[0039] In the method for preparing a protein hydrolysate, With regards to the preparation methods of protein hydrolysis, there are, for example, preliminary heating, enzyme substrate ratio (E/S), pH, hydrolyzing temperature, and hydrolyzing time are selected as five parameters for optimization. Preliminary heating: 65-90°C, E/S: 0.01-0.2 Hydrolyzing temperature: 30-65 °C, Hydrolyzing time: 3 hours to less than 20 hours.

[0040] Examples of the enzymes used for the preparation of protein hydrolysates For the preparation of a protein hydrolysate include the following enzymes from Nova Nordisk:

1) Endoproteases

B . licheniformis-derived : Alcalase
B . lentus-derived: Esperase
B . subtilis-derived: Neutrase
Bacteria-derived: Protamex
Porcine pancreas-derived: PTN (trypsin)

2) Exoproteases

Aspergillus oryzae-derived: Flavorzyme
Porcine or bovine viscera-derived:
carboxypeptidase.

[0041] Examples other than the above-mentioned enzymes include animal-derived pancreatin, pepsin, plant-derived papain, bromeline, endoprotease and exoprotease derived from microorganisms (for example, *Lactobacillus*, yeasts, molds, and mycobacteria), and their crudely purified material and bacterial homogenates. Furthermore, combinations of *B. licheniformis*-derived Alcalase and porcine pancreas-derived PTN (trypsin) are often used when combining enzymes.

[0042] The protein hydrolysates of the present invention include: enzyme hydrolysates; retained solutions or permeates after ultrafiltration; and commercial milk protein hydrolysates which show similar activity. For example, a protein hydrolysate of the present invention could use retained solutions of ultrafiltration membrane having 10,000 molecular weight cut off. For example, a retention liquid in the ultrafiltration membrane having a molecular weight cutoff of 10,000 can be used as the protein hydrolysate of the present invention.

[0043] The combination ratio of milk protein could be adjusted by the amount of adding a hydrolysate of milk protein can be suitably adjusted in accordance with an addition amount of other components (fermented milk protein, oleic-acid containing fats and oils, milk phospholipid, soybean lecithin, isomaltulose or the like), pathology, symptoms, age, body weight, of a cancer patient, purpose of use or the like. Specifically, examples of the addition amount of a hydrolysate of milk protein include 0.9 to 5.0 g, preferably 0.9 to 3.0 g, more preferably 1.0 to 2.5 g, and further preferably 1.2 to 2.0 g, per 100 mL of the

nutritional composition, but not limited to these ranges.

[0044] 1-2 Fermented Milk Protein

Fermented milk (any products obtained by fermenting using a starter such as *Lactobacillus* a liquid milk prepared by combining one or more livestock milks such as cow's milk, buffalo milk, goat milk, sheep milk, or mare's milk and/or a milk raw material such as partially skimmed milk, skim milk, reduced whole milk, reduced skim milk, reduced partially skimmed milk, butter, cream thereof or the like) can be used as an origin of the fermented milk protein of the present invention. For example, fresh cheese, natural cheese, yogurt and whey cheese are encompassed in the fermented milk of the present invention. Also, the cheese of the present invention refers to those obtained by fermenting milk, buttermilk or cream using *Lactobacillus* or curds obtained by adding an enzyme to milk, buttermilk or cream, from which whey is removed, and the presence of solidification or aging is irrelevant. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* can be mostly used as starters for producing fermented milk, but not limited thereto, and examples include *Lactobacilli* and *bifidobacteria* such as *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus diacetylactis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Lactobacillus casei*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosis*, *Lactobacillus plantarum*, *Lactobacillus murinus*, *Lactobacillus reuteri*, *Lactobacillus brevis*, *Lactobacillus gasseri*, *Bifidobacterium longum*,

Bifidobacterium bifidum, and *Bifidobacterium breve*. In addition, microorganisms such as *Propionibacterium* used when producing fermented milk can be used in combination. The nutritional composition of the present invention may be prepared using any fermented milk, but preferably prepared using fresh cheese or yogurt, more preferably quark or yogurt.

[0045] There are a wide variety of fresh cheese such as cottage, quark, string, Neuchâtel, cream cheese, mozzarella, ricotta, and mascarpone. Quark is a type of the unaged (fresh) cheese, has a low fat content and is characterized by fresh flavor and sour taste. A typical method for producing quark is described below. Skim milk is first pasteurized and starters *Lactobacilli* (mostly *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) are inoculated at 0.5 to 5% (w/w) for fermentation. When a pH of the solution reaches 4.6, a curd is formed, and the whey is centrifuged using a quark separator followed by cooling the obtained curd. An example of the quark composition of the present invention is 17 to 19% (w/w) of the total solid content, 11 to 13 % (w/w) of protein, below 1 % (w/w) of fat, 2 to 8% (w/w) of carbohydrate and below 2% (w/w) of lactose. Additionally, those coagulated using rennet are also encompassed in the quark of the present invention. Further, those cultured by adding to skim milk a mixed culture broth of *lactis* and *cremoris* belonging to genus *Lactococcus* and species belonging to genus *Leuconostoc* and the whey is removed therefrom are also encompassed in the quark of the present invention. Furthermore, those obtained by cutting a curd obtained in the same manner as in the above method using a cutter and separating the whey while heating the broth are also

encompassed in the fresh cheese of the present invention.

[0046] It is known that *Lactobacillus* and the culture supernatant thereof have an immunoregulatory function. For example, *Lactococcus lactis subsp. cremoris* and the polysaccharides derived from the culture supernatant thereof are known to be selectively toxic against transformed cells (tumors, cancer cells or the like) (Japanese Patent Laid-Open No. 2009-256312)

[0047] The amount of adding fermented milk protein can be suitably adjusted in accordance with an addition amount of other components (milk protein hydrolysate, oleic-acid containing fats and oils, milk phospholipid, soybean lecithin, isomaltulose or the like), pathology, symptoms, age, body weight, of an ingestion subject, purpose of use or the like. Specifically, examples of the addition amount of fermented milk protein include 0.5 to 6 g, preferably 2 to 6 g, and more preferably 2.5 to 4.5 g, per 100 mL of the nutritional composition in terms of the protein, but not limited to these ranges.

[0048] Japanese Ministerial Ordinance on Milk and Milk products concerning Compositional Standards (Ministry of Health and Welfare Ordinance No. 52, December 27, 1951) establishes a regulation of "banning milking from a cow, goat or ewe within 5 days from delivery" as the criterion for general compositional standard and preparation method for milk and the like. In other words, the Ministerial Ordinance restricts the use of colostrum in milk products.

[0049] In the present invention, it is preferable that the milk used for the preparation of a hydrolysate of milk protein and fermented milk protein be normal milk (or also referred to as mature milk). Milk may be

derived from any animal such as cow, buffalo, goat, sheep, horse, or human.

[0050] 2. Lipids

2-1. Phospholipids

As phospholipids, a combination of Milk phospholipid and soybean-derived lecithin or egg yolk lecithin is used. Milk phospholipid alone may also be used. In fields such as biochemistry, medicine and pharmacology, the term "lecithin" is used only for phosphatidylcholine . However in commercial or industrial fields, lecithin is used as a general term for phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol , phosphatidic acid, and a mixture of other phospholipids. In "Japan's Specifications and Standards for Food Additives" , 7th edition (1999) , lecithin is defined as "a substance obtained from oilseed or animal sources, whose main component is phospholipids".

[0051] Milk phospholipid comprises sphingo yelin (SM) , phosphatidylcholine (PC) , phosphatidylethanolamine (PE) , phosphatidylinositol (PI) , phosphatidylserine (PS) , and lysophosphatidylcholine (LPC) , and only exists in milk fat globule membranes (MFGM) . The composition of the MFGM phospholipid fraction is shown in Table 1 (Bulletin of Japan Dairy Technical Association, Vol. 50, pp. 58-91, 2000).

[0052] As indicated in Table 1, milk lecithin characteristically includes a large amount of SM, which is not contained in soybean lecithin.

[0053] [Table 1]

Phospholipid component	Weight %
Sphingomyelin	22
Shosphatidylcholine	36
Phosphatidylethanolamine	27
Phosphatidylinositol	11
Phosphatidylserine	4
Lysophosphatidylcholine	2

[0054] Soybean lecithin

While soybean lecithin is widely used as a natural food additive in the field of foods and food products, polyenephosphatidylcholine is also used as a drug (applications: for the improvement of liver function, fatty liver, and hyperlipidemia in chronic liver disease) .

[0055] "Natural" lecithin products are normally ranked according to their PC content. Various types of lecithins upgraded according to their use have been produced. As Table 2 indicates, soybean lecithin products are conveniently categorized according to differences in the main ingredient PC, based on purification and soybean lecithin fractionation (Fujikawa, T., Oil Chemistry, Vol. 40(10): 951-958, 1991) .

[0056] [Table2]

Table 2

Type	PC content (%)
Paste lecithin	15-20
Purified lecithin	20-25
Extracted lecithin	30-40
PC concentrated lecithin	45-60
PC highly purified lecithin	75-95
Phospholipids (PC, PE, PS, PG, etc.)	individually 98% or more

[0057] In the present invention, milk phospholipid and soybean lecithin may be used singly or in combination. The amount of adding milk phospholipid and/or soybean lecithin can be suitably adjusted in accordance with an addition amount of other components (milk protein hydrolysate, fermented milk protein, oleic-acid containing fats and oils, isomaltulose or the like), pathology, symptoms, age, body weight, of an ingestion subject, purpose of use or the like. Specifically, examples of the addition amount of milk phospholipid and/or soybean lecithin include the total amount of 0.01 to 0.5 g, 0.05 to 0.5 g, 0.1 to 0.5 g and 0.2 to 0.3 g, per 100 mL of the nutritional composition, but not limited to these ranges.

[0058] 2-2. Other lipids

The Ministry of Health, Labour and Welfare recommends that the preferred intake ratio of saturated fatty acids (SFA: palmitic acid, stearic acid, etc.) : monovalent unsaturated fatty acids (MUFA: oleic acid, etc.) : polyvalent unsaturated fatty acids (PUFA: linolic acid, linolenic acid, etc.) be changed from the former 1:1.5:1 to 3:4:3, and that the n-6 fatty acid: n-3 fatty acid ratio be made 4:1. One of the reasons for this recommendation is that in Japan it is difficult to practice a diet in which the intake ratio of MUFA is 1.5 times that of SFA and PUFA. Therefore, in the fatty acid composition of lipids, MUFA content is improved. oleic acid, which is a monovalent unsaturated fatty acid, is mixed into the fatty acid composition to compose more than 30%, or preferably 30-60% of the mixture. Lipid sources containing a large amount of oleic acid include high oleic acid-containing sunflower oil, rapeseed oil,

olive oil, high oleic acid safflower oil, soybean oil, corn oil, and palm oil. Furthermore, nutritionally adjusted oils and fats (NOF Corporation) are also a lipid source containing oleic acid. Sunflower oil, rapeseed oil, olive oil, and a mixture containing olive oil may be used.

[0059] Oleic acid content could be adjusted with other constituents' content (such as fermented milk-derived protein)Milk lecithin and soybean lecithin may be used alone or in combination. The total content per 100 mL of product may be 0.1-0.5 g, or preferably 0.2-0.3 g. Oleic acid content may be 2-3 g, or preferably 2.1-2.5 g. Furthermore, polyvalent unsaturated fatty acids such as DHA, EPA, and arachidonic acid, and medium-chain fatty acids such as caprylic acid, capric acid, and lauric acid are added to adjust the SFA:MUFA: PUFA ratio to 3:4:3.

[0060] The amount of adding oleic acid can be suitably adjusted in accordance with an addition amount of other components (milk protein hydrolysate, fermented milk protein, milk phospholipid, soybean lecithin, isomaltulose or the like), pathology, symptoms, age, body weight, of an ingestion subject, purpose of use or the like. Specifically, examples of the addition amount of oleic acid include 25% or more, preferably 30% or more, and more preferably 30 to 50%, in the fatty acid composition of the nutritional composition of the present invention, but not limited to these ranges. Further, polyunsaturated fatty acids such as DHA, EPA, or arachidonic acid and medium chain fatty acids such as caprylic acid, capric acid, or lauric acid may be added to adjust so that the ratio of saturated fatty acid : monounsaturated fatty acid : polyunsaturated

fatty acid is close to 3 : 4 : 3.

[0061] 3. Carbohydrates and dietary fiber

The main carbohydrate as referred to in the present invention is isomaltulose. Examples of other carbohydrates include sugar alcohols (sorbitol, xylitol, maltitol, etc.) , honey, granulated sugar , glucose, fructose, and invert sugar.

[0062] Isomaltulose is a disaccharide composed of a molecule of glucose and a molecule of fructose bonded by α -1,6-linkage, a structural isomer of sucrose and is also termed as 6-O-(α -D-Glucopyranosyl)-D-fructose, isomaltulose or paratinose. Isomaltulose has a molecular weight of 342.297, Cas. No. 13718-94-0 and is used as a sweetener or the like. Isomaltulose is contained in an extremely small amount in honey, sugar cane and the like. Isomaltulose can also be produced by allowing *Protaminobacter rubrum*-derived α -Glucosyltransferase or the like to work on sucrose and causing the α -1,2 bond to transfer to α -1,6 bond. The sweetness of isomaltulose is close to that of sucrose but the degree of sweetness is about a half of that of sucrose. Isomaltulose orally ingested is decomposed by isomaltase in the digestive tract and digested into glucose and fructose as in sucrose before absorbed (Toshinao Goda et al. Nippon Eiyo Shokuryo Gakkaishi (in Japanese) (Journal of Japanese Society of Nutrition and Food Science), Vol. 36 (3): 169-173, 1983). Isomaltose, panose, isomaltotriose or the like also digested by isomaltase are considered to have suppressed digestion and absorption when isomaltulose is ingested to compete with the digestion of isomaltulose (Journal of Japanese

Society of Nutrition and Food Science), Vol. 36 (3), 169-173, 1983). Isomaltulose has the caloric value of 4 kcal/g. In the present invention, isomaltulose encompasses paratinose syrup, reduced paratinose, paratinose starch syrup and the like. Paratinose starch syrup is a starch syrup-like liquid comprising as the main component oligosaccharides such as tetrasaccharide, hexasaccharide, or octasaccharide produced by the dehydrocondensation of isomaltulose.

[0063] As for isomaltulose, it is known that a low-glycemic food composition containing isomaltulose or the like is used to treat or prevent malignant tumor diseases (National Publication of International Patent Application No. 2009-530326).

[0064] The amount of adding isomaltulose can be suitably adjusted in accordance with an addition amount of other components (milk protein hydrolysate, fermented milk protein, oleic-acid containing fats and oils, milk phospholipid, soybean lecithin or the like), pathology, symptoms, age, body weight, of an ingestion subject, purpose of use or the like. Specifically, examples of the addition amount of isomaltulose include 4 to 15 g, and preferably 5 to 7 g, per 100 mL of the nutritional composition, but not limited to these ranges (JPA2009-530326).

[0065] The nutritional composition of the present invention can adjust the caloric value thereof by suitably adding protein, lipid and sugar. Examples of the caloric value of nutritional composition of the present invention include 50 to 150 kcal, and preferably 80 to 120 kcal, per 100 ml of the nutritional composition, but not limited to these ranges.

[0066] In the nutritional composition of the present

invention, the energy ratio of protein, lipid and sugar with respect to the whole nutritional composition is in conformance with the 6th revised Recommended Dietary Allowance and Dietary Reference Intake for Japanese. Specifically, an example includes 15 to 25% of protein, 20 to 30% of lipid and 45 to 65% of sugar, but not limited to these ranges.

[0067] The nutritional composition of the present invention may also contain dietary fiber. Dietary fiber refers to substances in food which is not hydrolyzed by digestive enzymes of human and classified into water soluble dietary fiber and insoluble dietary fiber based on the affinity to water. For the water soluble dietary fiber, a non-digestible oligosaccharide such as lactulose, or lactitol, raffinose can be used. It is known that the physiological function of non-digestible oligosaccharides is to improve the enteral environment, i.e., regulate the functions of the intestines by reaching the large intestine while remaining undigested and contributing to the activation and proliferation of gut microorganisms belonging to genus Bifidobacterium. Other usable candidates of the water soluble dietary fiber include pectins (protopectin, pectinic acid and pectic acid), enzymatically decomposed guar gum, and tamarind seed gum.

[0068] Further, usable candidates of the water soluble dietary fiber include high molecular water soluble dietary fiber such as soybean polysaccharide thickeners, konnyaku glucomannan, alginic acid, low molecular alginic acid, psyllium, gum arabic, seaweed polysaccharides (cellulose, lignin-like substance, agar, carrageenan, alginic acid, fucoidan and laminarin), microbial gums (welan gum, curdlan, xanthan

gum, gellan gum, dextran, pullulan and rhamsan gum), and other gums (seed-derived locust bean gum, tamarind gum and tara gum, sap-derived karaya gum and gum tragacanth); low molecular water soluble dietary fiber such as polydextrose, non-digestive dextrin, pineapple fiber, and maltitol.

[0069] Insoluble dietary fiber increases the bulk of undigested material in the large intestine and shortens its passage time. This increases the frequency of defecation and the quantity of stool. Examples of candidates for insoluble dietary fiber include cellulose, hemicellulose, lignin, chitin, chitosan, soybean dietary fiber, wheat bran, pine fiber, corn fiber, beet fiber and oat Bran, wheat bran, adlay bran, rice bran, millet, foxtail millet, barnyard millet, common sorghum, Bran cereals, Legume bran, buck wheat, Sesame bran, bean curd refuse etc. Examples include millet brans such as oat bran, rye bran, tear grass bran, rice bran, common millet, foxtail millet, and barnyard millet, sorghum, leguminous (Fabaceae) brans, pseudocereal brans such as buckwheat, sesame bran, and okara.

[0070] In addition to the above proteins, lipids, sugars and dietary fibers, the nutritional composition of the present invention can use water, proteins, sugars, lipids, vitamins, minerals, organic acids, organic bases, fruit juice, flavors, emulsifiers, thickeners, stabilizers or the like. Examples of the protein include animal or plant proteins such as whole milk powder, skim milk powder, partial skim milk powder, casein, whey powder, whey protein, whey protein concentrate, whey protein isolate, whey protein hydrolysate, α -casein, β -casein, κ -casein,

β -lactoglobulin, α -lactalbumin, lactoferrin, soybean protein, egg protein, and meat protein, decomposed products thereof; various milk-derived components such as butter, whey mineral, cream, whey, non-protein nitrogen, sialic acid, phospholipid, and lactose. The nutritional composition may contain peptides and amino acids such as casein phosphopeptide, or lysine. Examples of the sugar include saccharides, processed starches (soluble starches, British starch, oxidized starches, starch ester, and starch ether, in addition to dextrin), and dietary fibers. Examples of the lipid include animal oils and fats such as lard, and fish oil, fractionated oils thereof, hydrogenated oil, transesterification oil; vegetable oils such as palm oil, safflower oil, corn oil, rapeseed oil, coconut oil, fractionated oils thereof, hydrogenated oil, and transesterification oil. Examples of the vitamins include vitamin A, carotenes, vitamin B complex, vitamin C, vitamin D complex, vitamin E, vitamin K complex, vitamin P, Vitamin Q, niacin, nicotinic acid, pantothenic acid, biotin, inositol, corrin, and folic acid; examples of the minerals include calcium, potassium, magnesium, sodium, copper, iron, manganese, zinc, and selenium. Examples of the organic acid include malic acid, citric acid, lactic acid, tartaric acid, and erythorbic acid. Additionally, the nutritional composition can contain a material having fecal odor reduction effects (for example, 5 mg to 500 mg (0.005% to 0.5%) of a champignon extract), a carotenoid preparation (for example, 10 μ g to 200 μ g (0.00001% to 0.0002%) of a preparation containing α -carotene, β -carotene, lycopene, or lutein) or an

antioxidant (catechin, polyphenol or the like). Two or more of these components can be used in combination, and synthetic products and/or food products containing these components in a large amount may be used. The form of food products may be any of solid, liquid, gel or the like.

[0071] The nutritional composition of the present invention can be produced by a method known in the art. A part or all of the raw materials described above are mixed and then homogenized as necessary. The homogenization refers to a procedure of homogenizing each component mixed by thoroughly blending while also mechanically microparticulating fat globules and coarse large particles of other components for the prevention of creaming and clustering of fat, thereby achieving a homogeneous emulsified state of the nutritional composition.

[0072] In the production of nutritional composition of the present invention, heat treatment or heat pasteurization is carried out. The heat pasteurization conditions can be typical pasteurization conditions for food products, and the heat pasteurization can be carried out using a common device. For example, the pasteurization at 62 to 65°C x 30 minutes, 72°C or higher x 15 seconds or longer or 72°C or higher x 15 minutes or longer, or 120 to 150°C x 1 to 5 seconds; sterilization at 121 to 124°C x 5 to 20 minutes or 105 to 140°C; retort (high-pressure high-temperature) pasteurization; autoclaved sterilization or the like can be employed, but not limited thereto. The heat pasteurization can be preferably carried out under applied pressure.

[0073] Further, examples include a method in which the liquid nutritional composition is heat-sterilized in advance and aseptically packaged in a container (for example, a combined method of the UHT sterilization method and an aseptic packaging method), a method in which the liquid nutritional composition is packaged in a container and heat-sterilized together with the container (for example, the autoclave method), and a method in which the composition is packaged in a can or various containers used for a liquid food or oral/tube feeding nutrient (so-called soft bags, nutrition bags or the like) for the retort pasteurization (for example, at 115 to 145°C for 5 to 10 seconds), subsequently heat-pasteurized at about 140 to 145°C for about 5 to 8 seconds, cooled and aseptically packaged, but not limited thereto.

[0074] The starter derived from raw material fermented milk (*Lactobacillus*, *Bifidobacterium*, *Propionibacterium* or the like) is killed by the heat treatment or heat pasteurization.

[0075] The composition of the present invention can be used to suppress the growth of a cancer. The nutritional composition of the present invention can be used for the nutrition management of patients on oral/enteral nutrients, old people, infants and the like, for example, in the form of liquid food, oral/tube feeding nutrients, drinks, and gel food products.

[0076] The osmotic pressure of the nutritional composition of the present invention is about 500 to 1000 mOsm/l, and an example of the osmotic pressure includes about 550 to 750 mOsm/l. When a viscosity is measured at room temperature, examples of the viscosity

of the nutritional composition include 20 to 100 cp (1 cp = 1 mPa·s), preferably 25 to 60 cp, and more preferably 30 to 50 cp, but not limited to these ranges.

Further, the pH of the nutritional composition of the present invention can be adjusted to 4.6 or lower, preferably 3.0 to 4.3, more preferably 3.8 to 4.2, but not limited to these ranges.

[0077] The nutritional composition of the present invention is effective for inhibiting the tumor growth of patients suffering from tumors. More specifically, it is useful for inhibiting the tumor growth of patients suffering from colon tumor, colon solid tumor, sarcoma cell tumor, lung cancer, thymoma, breast cancer, pharyngeal cancer, laryngeal cancer, gastric cancer, esophageal cancer, hepatocellular carcinoma, bile duct cancer, gallbladder cancer, pancreatic cancer, penile cancer, renal pelvis and ureter cancer, renal cell carcinoma, testicular tumor, prostate cancer, bladder cancer, vulvar cancer, cervical cancer, endometrial cancer, uterine sarcoma, vaginal cancer, ovarian cancer, ovarian germ cell tumor, or brain tumor, etc.

[0078] In an in vivo test using a mouse to which a tumor cell strain is transplanted, the nutritional composition of the present invention is found to have suppressed the proliferation of the tumor and the tumor infiltration into the peripheral tissues. When a tumor infiltrates the peripheral tissues, the tumor is likely to metastasize. When a tumor infiltrates an internal organ or a peripheral nerve, pain is caused. Accordingly, the composition of the present invention can suppress the growth of a tumor, infiltration/metastasis of a tumor or the occurrence of

pain caused by a tumor.

[0079] The dietary intake per day of the nutritional composition of the present invention in a pharmaceutical product or a drink or food product is not limited because it varies depending on pathology, age, symptoms, body weight, of an ingestion subject, purpose of use, whether the nutritional composition of the present invention is the only nutrient and the like. Specifically, examples of the dietary intake include, per adult per day, 400 to 1600 ml, preferably 600 to 1600 ml, and more preferably 800 to 1200 ml. For the case of an adult, for example, up to 3000 ml per day is acceptable. The dietary intake can also be determined by an attending doctor of an ingestion subject.

Further, the nutritional composition of the present invention may be used in combination with a conventionally known pharmaceutical product or food product having antitumor activities.

[0080] The nutritional composition of the present invention can be used in either form of a pharmaceutical product or a drink or food product. For example, the nutritional composition of the present invention is expected to improve the gut flora when directly administered as a pharmaceutical product or when directly ingested as a food for specified dietary use such as food for specified health uses, a food product with nutrient function claims, a food product for nutritional supplement, a fluid food or a supplement. Alternatively, regardless of the form such as liquid, paste, or solid, powder, the composition may be added to various food products (cow's milk, soft drink, fermented milk, yogurt, cheese, bread, biscuit, cracker, pizza crust, formulated milk powder, liquid food, food

for specified dietary use, food product for the sick, nutritional food product, frozen food product, processed food product, other commercial food products and the like) and consumed. Further, when the form of the nutritional composition to be used is a powder, the composition can be produced, for example, by means of spray drying, or freeze drying.

[0081] The present invention could be administered by kinds forms as medicinal products or supplements, for example, when the nutritional composition of the present invention is used as a pharmaceutical product or a supplement, it can be administered in various forms. Examples of the form include oral administration such as tablets, capsules, granules, powders, and syrups. Each of these preparations can be prepared in accordance with a routine method using, as the main agent, a known auxiliary material for the preparation and typically usable in the field of pharmaceutical preparation technology such as an excipient, a binder, a disintegrator, a lubricant, a flavor, a solubilizing adjuvant, a suspension, or a coating agent. These preparations may further contain a proper amount of calcium. Furthermore, a proper amount of vitamins, minerals, organic acids, saccharides, amino acids, peptides or the like may be added.

EXAMPLES

[0082] Examples of the present invention will be shown to explain the present invention more specifically. They are only exemplification to explain the present invention, and the present invention is not limited by them.

Materials and Methods

[0083] Two commonly used cancer cell lines, mouse murine sarcoma tumor cell(S-180) and mouse colon tumor cell (C26), were used to build an S-180 mouse ascites tumor model and a C26 mouse solid tumor model. Mice were randomly divided into two groups of 18~20 each, half male and half female. From the inoculation day, mice were fed ad libitum with either the nutritional compositions of the present invention (which will hereinafter be called "test composition") diet or control diet AIN-93M for 14 days. For S-180 tumor-bearing mice, the survival time was recorded as the major indicator. For C26 tumor-bearing mice, food intake, body weight, tumor weight and tumor volume were recorded; serum TNF- α and IL-6 levels were determined; and the tumor histopathology was performed.

1. Test diets:

[0084] TEST COMPOSITION Yellow fine powder, produced by drying a liquid diet composition shown in Table 1 and Table3. Amount of oleic acid in the lipid is 39%, eicosapentaenoic acid is 1.2%, docosahexaenoic acid is 0.8% of total fatty acid composition.

AIN-93M White fine powder, provided by oriental Yeast Co., ltd, Japan. AIN-93M is a standard purified diet which is good for nutrition research in rats or mice, that had been published in 1993 by the United States National Institute of Nutrition. AIN-93M purified diet is standard for a period of growth was too mature animals(Table 4, Table5).

[0085] Table 3

Liquid diet composition for the preparation of TEST
COMPOSITION

Nutrition Composition		Liquid diet composition		solid content of liquid diet composition	
	Protein	5	g/100ml	21.83	g/100g
	Fat	2.8	g/100ml	12.23	g/100g
	Carbohydrate	14.5	g/100ml	63.32	g/100g
	Mineral	0.6	g/100ml	2.62	g/100g
	Water	84.4	g/100ml		
	Energy	100	kcal/100ml	436.7	kcal/100g
Protein					
	Whey Protein hydrolysate	2.0	g/100ml	8.7	g/100g
	Fermented milk protein	3.0	g/100ml	13.1	g/100g
Fat					
	Edible fat and oil	1.9	g/100ml	8.3	g/100g
	Milk Phospholipid	0.1	g/100ml	0.4	g/100g
	Medium Chain Triglycerides	0.6	g/100ml	2.6	g/100g
	Purified fish oil	0.2	g/100ml	0.9	g/100g
Carbohydrate					
	Dextrin	7.2	g/100ml	31.2	g/100g
	Isomaltulose	6.2	g/100ml	26.9	g/100g
	Dietary fiber	1.2	g/100ml	5.24	g/100g
Vitamin					
	VitaminA	150	mgRE/100ml	655	mgRE/100g
	Retinol	60	mgRE/100ml	262	mgRE/100g
	β -carotene	90	mgRE/100ml	393	mgRE/100g
	VitaminD	0.75	mg/100ml	3.275	mg/100g
	VitaminE	5	mg α -TE/100ml	21.83	mg α -TE/100g
	VitaminK	3.4	mg/100ml	14.85	mg/100g
	VitaminB1	0.25	mg/100ml	1.092	mg/100g
	VitaminB2	0.3	mg/100ml	1.31	mg/100g
	Niacin	4	mgNE/100ml	17.47	mgNE/100g
	VitaminB6	0.3	mg/100ml	1.31	mg/100g
	Folic acid	50	mg/100ml	218.3	mg/100g
	VitaminB12	0.6	mg/100ml	2.62	mg/100g
	Biotin	7.5	mg/100ml	32.75	mg/100g
	Pantothenic acid	1.2	mg/100ml	5.24	mg/100g
	VitaminC	50	mg/100ml	218.3	mg/100g
	Choline	9.2	mg/100ml	40.17	mg/100g
	Carnitine	15	mg/100ml	65.5	mg/100g
Mineral					
	Na	70	mg/100ml	305.7	mg/100g
	Ca	80	mg/100ml	349.3	mg/100g
	Fe	1	mg/100ml	4.367	mg/100g
	P	70	mg/100ml	305.7	mg/100g
	Mg	20	mg/100ml	87.34	mg/100g
	K	80	mg/100ml	349.3	mg/100g
	Cu	0.05	mg/100ml	0.218	mg/100g
	I	9.7	mg/100ml	42.36	mg/100g
	Mg	0.175	mg/100ml	0.764	mg/100g
	Se	5	mg/100ml	21.83	mg/100g
	Zn	1	mg/100ml	4.367	mg/100g
	Cr	2.96	mg/100ml	12.93	mg/100g
	Mo	2.5	mg/100ml	10.92	mg/100g
	Cl	80	mg/100ml	349.3	mg/100g
Energy valance					
	Protein	20	%		
	Fat	25	%		
	Carbohydrate	55	%		

[0086] Table 4

AIN-93M

AIN-93M	%
Casein	14.0
L-cystine	0.18
Cornstarch	46.5692
α — Cornstarch	15.5
Sucrose	10.0
Soybean oil	4.0
Cellulose powder	5.0
AIN-93M mineral MIX	3.5
AIN-93 vitamin MIX	1.0
Choline Bitartrate	0.25
tert-Butylhydroquinone	0.0008
total	100.0

AIN-93M mineral MIX	mg/100g
CaCO ₃	35700.0
KH ₂ PO ₄	25000.0
K ₃ C ₆ H ₅ O ₇ ·H ₂ O	2800.0
NaCl	7400.0
K ₂ SO ₄	4660.0
MgO	2400.0
FeC ₆ H ₅ O ₇ ·XH ₂ O	606.0
ZnCO ₃	165.0
MnCO ₃	63.0
CuCO ₃ ·Cu(OH) ₂ ·H ₂ O	32.4
KIO ₃	1.0
Na ₂ SeO ₄	1.025
(NH ₄)Mo ₇ O ₂₄ ·4H ₂ O	0.795
Na ₂ SiO ₃ ·9H ₂ O	145.0
CrK(SO ₄)·12H ₂ O	27.5
LiCl	1.74
H ₃ BO ₃	8.15
NaF	6.35
NiCO ₃ ·2Ni(OH) ₂ ·4H ₂ O	3.06
NH ₄ VO ₃	0.66
Sucrose	20978.32
total	100000.0

AIN-93 vitamin MIX	mg/100g
Nicotinic acid	300.0
Calcium pantothenate	160.0
Vitamin B ₆ (Pyridoxine hydrochloride)	70.0
Vitamin B ₁ (Thiamine hydrochloride)	60.0
Vitamin B ₂ (Riboflavin)	60.0
Folic acid	20.0
D-biotin	2.0
Vitamin B ₁₂ [0.1%]	250.0
Vitamin E[50%]	1500.0
Vitamin A[500,000U/g]	80.0
Vitamin D ₃ [400,000U/g]	25.0
Vitamin K ₁	7.5
Sucrose	97465.5
total	100000.0

[0087] Table 5

Comparison of TEST COMPOSITION and AIN-93M

	solid content of liquid diet composition (test composition)			AIN-93M		
Nutrition Composition						
	Protein	21.83	g/100g	Protein	14.18	g/100g
	Fat	12.23	g/100g	Fat	4.00	g/100g
	Carbohydrate	63.32	g/100g	Carbohydrate	77.07	g/100g
Protein						
	Whey Protein hydrolysate	8.7	g/100g	Casein	14.00	g/100g
	Fermented milk protein	13.1	g/100g	L-cystine	0.18	g/100g
Fat						
	Edible fat and oil	8.3	g/100g	Soybean oil	4.00	g/100g
	Milk Phospholipid	0.4	g/100g			
	Medium Chain Triglycerides	2.6	g/100g			
	Purified fish oil	0.9	g/100g			
Carbohydrate						
	Dextrin	31.2	g/100g	Cornstarch	46.57	g/100g
	Isomaltulose	26.9	g/100g	α - Cornstarch	15.50	g/100g
				Sucrose	10.00	g/100g
	Dietary fiber	5.24	g/100g	Cellulose powde	5.00	g/100g

2. Transplanted tumor cell lines:

[0088] S-180 cell line Provided by Shanghai Cancer Biotechnology Institute, China

C26 cell line Provided by Shanghai Cancer Biotechnology Institute, China

3. Animals

[0089] Species: BALB/c mice

Source: Shanghai SIPPR-BK Laboratory Animal Co.Ltd

Sex: Half males and half females

Age: 6~7 weeks

Body Weight: 18~20 g

Number: 76 (36 mice for S-180 experiment and 40 mice for C26 experiment)

Animal Production License No.: SCXK (Shanghai) 2008-0016

Animal Use Permit No.: SYXK (Shanghai) 2008-0027

Housing condition: SPF lab animal house, $24\pm 1^{\circ}\text{C}$, 40~70% relative humidity, 12/12 hour light/dark cycle, All mice were fed Chow ad libitum

4. Method

[0090] Recover the S-180 cell line and C26 cell line 1 week before inoculation. Use BALB/c mice to build the murine tumor models. Hold the mouse, disinfect skin, inoculate S-180 cells intraperitoneally to mice or inoculate C26 cells subcutaneously to mice near the right posterior axillary. On the inoculation day the animals were randomly divided into AIN-93M group or TEST COMPOSITION group, and were caged separately.

[0091] Thirty-six mice were used for the S-180 ascites tumor study; eighteen in each group. Six mice were kept in each cage in which two specially-made powder diet feeders each filled with about 40g of diet were placed. Mice were fed chow ad libitum. Food intake was weighed every other day, body weight was recorded every three days, and the number of dead mice was recorded.

[0092] Forty mice were used for C26 solid tumor study, twenty in each group. Five mice were kept in each cage in which two specially-made powder diet feeders each filled with about 40g of diet were placed. Mice were fed chow ad libitum. Tumor volume and food intake were weighed every other day, and body weight was recorded every three days. Fourteen days after inoculation, mice were sacrificed by cervical dislocation and eye blood was collected. Tumors were dissected out, tumor volume was measured, tumor weight was weighed, and tumor tissues were fixed in 10% neutral formalin for histopathologic examination.

[0093] Tumor Volume Calculation Formula:

$V(\text{cm}^3) = a \cdot b^2 / 2$ (a=longer diameter (cm); b=shorter diameter (cm))

Tumor Growth Inhibition Rate (%) = (Mean tumor volume of control group - Mean tumor volume of TEST COMPOSITION group) $\times 100\%$ / Mean tumor volume of control group,

Or

Tumor Growth Inhibition Rate (%) = (Mean tumor weight of control group - Mean tumor weight of TEST COMPOSITION group) $\times 100\%$ / Mean tumor weight of control group.

[0094] Efficacy Criteria: The difference of average tumor weight (or tumor volume) of two groups is statistically significant at $P < 0.05$.

5. Statistical Analysis

[0095] Data were analyzed using statistical software SPSS version 11.5. In the S-180 tumor study, the mean survival time and cumulative survival rates of two groups were compared with Survival Analysis Log-Rank test. In the C26 tumor study, the mean tumor weight and mean tumor volume of two groups were compared using student's t-test. $P < 0.05$ was considered statistically significant.

III Results

1. Murine S-180 ascites tumor study

[0096] The mean survival of mice in the TEST COMPOSITION group was 13.0 ± 0.3 day (average $\pm$ SE), and the mean survival of mice in AIN-93M group was 12.4 ± 0.4 day. There was no significant difference either in term of average survival or in terms of the cumulative survival rate

between the two groups ($P>0.05$, Log-Rank test of survival analysis) (Table 6, Table 7). The survival curves showed that the survival rate of mice in the TEST COMPOSITION group was higher than for the control group, and the two curves did not cross at any time point, indicating the survival time of mice in the TEST COMPOSITION group tended to be longer than that in the control group (Fig 1, Fig 2) although no significant difference was seen.

2. Murine C26 solid tumor study

2.1 Food intake and body weight

[0097] During the whole experiment, the average food intake of tumor-bearing mice in the TEST COMPOSITION group was 2.60 ± 0.49 g/mouse/day, and the average food intake of tumor-bearing mice in the control group was 3.37 ± 0.45 g/mouse/day (Table 8). There was no significant difference in food intake between the two groups ($P>0.05$) (Fig 2). Nor was there any significant difference in body weight change between the two groups ($P>0.05$) (Fig 3).

2.2 Tumor weight and tumor volume

[0098] As shown in Table 8, the mean tumor weight of mice in the TEST COMPOSITION group was 1.36 ± 0.54 g, and the mean tumor weight of control mice was 1.83 ± 0.89 g, showing significant difference ($P<0.05$). The tumor growth inhibition rate of TEST COMPOSITION group calculated according to tumor weight was 25.59%.

[0099] Similarly, the mean tumor volume of mice in the TEST COMPOSITION group was 1.868 ± 0.844 g, and the mean tumor volume of control mice was 2.582 ± 1.055 g. The mean tumor volumes measured at days 4, 7, and 11 were all

significantly larger in the TEST COMPOSITION group than in the control group ($P < 0.05$). The tumor growth inhibition rate of TEST COMPOSITION group calculated according to tumor volume was 27.65% (Table 9, Fig 4, Fig 5).

2.3 Tumor pathology

[0100] During the anatomical dissection of tumors, it was observed that the integrity of the tumors in the TEST COMPOSITION group was better, having a clear margin and being easier to peel off. In contrast, the tumors in the AIN-93M group seemed to have invaded into the surrounding tissues and were difficult to peel off.

[0101] Histopathologic examinations showed that: the edges of tumors in TEST COMPOSITION group were smooth, with cancer cells and hemorrhagic lesions distributed in the margin areas; in the central area deep-stained cells grew densely (Figure 6A), and these cancer cells were of different sizes and shapes with deep stained nuclei (Figure 6B). The edges of tumors in the AIN-93M control group were obscure and large areas of hemorrhage could be seen (Figure 6C); cancer cells were dispersed, and necrosis was obvious and surrounded by hemorrhage (Figure 6D).

[0102] Pathological examination indicated that the malignant tumors in the TEST COMPOSITION group were less invasive than those of the control group, suggesting that TEST COMPOSITION has inhibitory effects on tumors to some extent.

[0103] A. TEST COMPOSITION group: edge of tumor in TEST COMPOSITION group is neat and smooth, cancer cells spread, and eosin-like homogeneous substance (i.e. hemorrhage) can be seen. Deep-stained cancer cells can

be seen in the central area.

[0104] B. TEST COMPOSITION group: cancer cells grow in density in the central area, cells are of different sizes and shapes, small areas of homogeneous eosin-like substances (i.e. hemorrhage) are seen. (Upper right, high magnification: cancer cells of various shapes and sizes, nuclei were deep stained. HE × 400)

[0105] C. Control group: tumor margin is not uniform, and there is a large area of homogeneous eosin-like substance (i.e. hemorrhage).

[0106] D. Control group: Cancer cells are dispersed, necrotic lesions are visible and surrounded by homogeneous eosin-like substances (i.e. hemorrhage).

[0107] [Table 6]

Table 6 Mean survival days of S180 tumor-bearing mice in two groups

Group	Survival Days		95% Conf. Interval
	Mean	Std. Err.	
TEST COMPOSITION	13	0.3	12.5~13.6
AIN-93M	12.4	0.4	11.8~13.1

P>0.05, Survival Analysis Log-Rank test

[0108] [Table 7]

Table 7 Cumulative survival rates of S180 tumor-bearing mice in two groups

Group	Day	Cumulative Survival Rate	Standard Error	Cumulative Event	Number Remainin
TEST COMPOSITION	10	1	0	0	18
	11	0.9444	0.054	1	17
	12	0.6111	0.1149	7	11
	13	0.2778	0.1056	13	5
	14	0.1667	0.0878	15	3
AIN-93M	10	0.9444	0.054	1	17
	11	0.7778	0.098	4	14
	12	0.3333	0.1111	12	6
	13	0.2222	0.098	14	4
	14	0.1667	0.0878	15	3

P>0.05, Survival Analysis Log-Rank Test

[0109] [Table 8]

Table 8 C26 tumor weight and tumor inhibitory rate

Group	Animal number	Mean±SE food intake (g/d)	Mean±SE body weight (g)		Mean±SE Tumor weight (g)	Tumor Inhibitory Rate (%)
			Start	End		
TEST COMPOSITION	20	2.60±0.49	21.80±0.56	24.44±0.85	1.36±0.54*	
AIN-93M	20	3.37±0.45	23.00±0.84	24.93±1.19	1.83±0.89	25.59

*P<0.05 compared with control group

[0110] [Table 9]

Table 9 C26 tumor volume and tumor inhibitory rate at different times

Group	Mean±SE Tumor volume (cm ³)				
	Day 1	Day 4	Day 7	Day 11	Day 14
TEST COMPOSITION	0.05±0.06	0.29±0.45*	0.59±0.43*	1.14±0.59*	1.87±0.84
AIN-93M	0.07±0.39	0.38±0.27	1.04±0.72	1.86±0.99	2.58±1.06
Tumor Inhibitory rate (%)	27.77	23.9	42.88	38.83	27.65

*P<0.05 compared with control group

Summary

[0111] Two commonly used murine cancer models, the S-180 ascites tumor model and the C26 mouse solid tumor model, were applied to preliminarily evaluate whether TEST COMPOSITION can inhibit tumor growth in mice. The results showed that the average survival time of S-180 tumor-bearing mice in the TEST COMPOSITION group was not significantly longer than that of control group mice ($P>0.05$); TEST COMPOSITION could inhibit the tumor growth in the C26 murine tumor model.

INDUSTRIAL APPLICABILITY

[0112] The nutritional compositions in the present invention are useful for the patients suffering from colon solid tumors, such as inhibiting the colon solid tumor growth and reducing the invasions of the malignant tumors.

CLAIMS

1. A nutritional composition suitable for tumor patients comprising : a milk protein hydrolysate and a fermented milk protein as proteins; a oleic acid-containing oil and milk phospholipid and/or soybean lecithin as lipids; and isomaltulose as a carbohydrate.
2. The nutritional composition according to claim 1, wherein said milk proteins selected from the group consisting of casein, a milk protein concentrate (MPC) ' a Whey protein concentrate (WPC), a wheyproteinisolate (WPI) , α - lactoalbumin , β - lactoglobulin , and lactoferrin.
3. The nutritional composition according to claim 1, wherein the amount of said milk protein hydrolysate in 100ml of the composition is 0.9g to 5.0g.
4. The nutritional composition according to claim 1, wherein said milk protein hydrolysate maybe obtained by hydrolyzing a whey proteinisolate (WPI) with alkalase from Bacilluslicheniformis, and trypsin from a porcine pancreas.
5. The nutritional composition according to claim 4, which is a permeate obtained by further treatment with an ultrafiltration membrane having a fractionation molecular weight of 10,000.

6. The nutritional composition according to claim 1, wherein said fermented milk protein derived from cheese.
7. The nutritional composition according to claim 6, wherein said cheese is quark.
8. The nutritional composition according to claim 1, wherein the amount of said fermented milk protein in 100ml of the composition is 0.5g to 6g.
9. The nutritional composition according to claim 1, wherein the amount of said isomaltulose in 100ml of the composition is 4g to 15g.
10. The nutritional composition according to claim 1, wherein the amount of oleic acid in the lipid is 30% or more of total fatty acid composition.
11. A growth of tumor inhibiting method, which comprises administering to a patient in need thereof, an effective amount of a nutritional composition comprising : a milk protein hydrolysate and a fermented milk protein as proteins; a oleic acid-containing oil and milk phospholipid and/or soybean lecithin as lipids; and isomaltulose as a carbohydrate.
12. Method according to claim 11, wherein the patient is suffering from colon solid tumor.
13. Method according to claim 11, wherein the

composition is an oral or tube feeding (enteral) nutrient.

14. An tumor treating or reducing method, which comprises administering to a patient in need thereof, an effective amount of a nutritional composition comprising : a milk protein hydrolysate and a fermented milk protein as proteins; a oleic acid-containing oil and milk phospholipid and/or soybean lecithin as lipids; and isomaltulose as a carbohydrate.
15. Method according to claim 14, wherein the tumor is colon solid tumor.
16. Method according to claim 14, wherein the composition is an oral or tube feeding (enteral) nutrient.