

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
23 December 2010 (23.12.2010)

PCT

(10) International Publication Number
WO 2010/147456 A1

(51) International Patent Classification:

A61P 31/12 (2006.01) A61K 31/716 (2006.01)
A23L 1/308 (2006.01) A61K 31/732 (2006.01)
A61K 31/7004 (2006.01)

(21) International Application Number:

PCT/NL2009/050363

(22) International Filing Date:

19 June 2009 (19.06.2009)

(25) Filing Language:

English

(26) Publication Language:

English

(71) Applicant (for all designated States except US): **N.V. NUTRICIA** [NL/NL]; Eerste Stationsstraat 186, NL-2712 HM Zoetermeer (NL).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **VAN NORREN, Klaske** [NL/NL]; Fluitersdreef 4, NL-6871 LP Renkum (NL).

(74) Agent: **KETELAARS, Maarten**; J.W. Frisolaan 13, NL-2517 JS The Hague (NL).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: INHIBITION OF NFK-B MEDIATED VIRUS REPLICATION WITH SPECIFIC OLIGOSACCHARIDES

(57) Abstract: The inventors surprisingly found that specific oligosaccharides are capable of inhibiting viral replication through inhibiting NF-κB activation. The invention thus pertains to a composition comprising pectin (in the form of digalacturonic acid, trigalacturonic acid, polygalacturonic acid), Arabinoxylan from rice bran, β-glucan from bakers yeast, D-Ribose or mixtures thereof for inhibiting viral replication in a mammal with a viral disease.



WO 2010/147456 A1

Inhibition of NFK-B mediated virus replication with specific oligosaccharides

Background

Gut-derived bacterial products such as lipopolysaccharides (LPS) and peptidoglycans (PG) contribute to hyper-immune activation resulting in increased disease progression of HIV-1 infection. This hyper-immune activation might be due to chronic LPS or PG induced Toll-like receptor (TLR)-mediated NF- κ B activation, leading to increased HIV-1 replication. Dietary Acidic Oligosaccharides from pectin hydrolysate (pAOS) have been suggested to inhibit adhesion of bacterial pathogens in the gastrointestinal tract.

Summary of the invention

The inventors surprisingly found that specific oligosaccharides are capable of inhibiting viral replication through inhibiting NF- κ B activation. The experimental data show that in an *in vitro* model the Toll like receptor induced and the TNF- α induced NF- κ B stimulation can be inhibited. Further experiments show that the production of human immunodeficiency virus was inhibited with specific oligosaccharides.

Therefore a preferred embodiment according to the invention is the use of pectin in the form of or comprising digalacturonic acid, trigalacturonic acid, polygalacturonic acid, arabinoxylan (preferably from rice bran), β -glucan, preferably β -1,3/1,6-glucans such as derived from yeasts, D-ribose, and/or mixtures thereof, for inhibiting viral replication in a mammal with a viral disease, wherein the viral disease is selected from the group consisting of AIDS (HIV)– AIDS Related Complex, Chickenpox (Varicella), common cold, cytomegalovirus Infection, colorado tick fever, dengue fever, ebola hemorrhagic fever, hand, foot and mouth disease, hepatitis, herpes simplex, herpes zoster, herpes Papiloma Virus. influenza (Flu), lassa fever, measles, marburg hemorrhagic fever, infectious mononucleosis, mumps, norovirus, poliomyelitis, progressive multifocal leukencephalopathy, rabies, rubella, SARS, smallpox (Variola), viral encephalitis, viral gastroenteritis, viral meningitis, viral pneumonia, west nile disease, yellow fever.

In one preferred embodiment, the invention pertains to a composition comprising one or more polyuronic acids, preferably pectin AOS, more preferably digalacturonic acid, trigalacturonic acid and/or polygalacturonic acid, Arabinoxylan (preferably from rice bran), β -glucan from bakers yeast, D-Ribose, or mixtures thereof, for inhibiting viral replication in a mammal with a viral disease as disclosed above.

Also, the invention pertains to the use of one or more of:

- polyuronic acids, preferably digalacturonic acid, trigalacturonic acid and/or polygalacturonic acid (preferably pectin comprising digalacturonic acid, trigalacturonic acid and/or polygalacturonic acid), pectin comprising or in the form of digalacturonic acid, trigalacturonic acid and/or polygalacturonic acid,
- Arabinoxylan, preferably from rice bran,
- β -glucan from bakers yeast, preferably β -1,3/1,6-glucans such as derived from yeasts,
- D-Ribose,

and/or mixtures for the manufacture of a composition for inhibiting viral replication in a mammal with a viral disease as disclosed above. Also, the invention pertains to a method of treating a person suffering from viral disease, or at risk thereof, said method comprising administering to said person the aforementioned composition.

In a preferred embodiment, the composition comprises polyuronic acids, preferably digalacturonic acid, trigalacturonic acid and/or polygalacturonic acid, and cysteine equivalents, preferably N-acetyl cysteine (NAC), preferably having a weight ratio of polyuronic acid and cysteine equivalents between 10:1 and 1:10, more preferably 5:1 – 1:5. Alternatively or additionally, the composition comprises 100 mg – 10 g of polyuronic acids, preferably pectin AOS per daily dosage. The same weight amounts and ratios are preferred in case of other saccharides defined in the description.

All saccharides preferably have chain length having 2 – 250 units. These can be derived from natural sources, if necessary by acid or enzymatic hydrolysis as known in the art.

List of Figures

Figure 1 The effect of chain length of galacturonic acid oligosaccharides on NFkappaB inhibition. For inhibition by a polygalacturonic acid oligosaccharide a minimal chain length of 2 galacturonic acid moieties is needed. The best effect is obtained with oligosaccharides comprising at least 3 galacturonic acid moieties;

Figure 2. NFkappaB response in HEK293 cells after 22.5 hr. incubation with AOS and NAC (A) or L-cysteine (B) with TNF- α for 2.5 hr. Upon correction for the contribution of NAC or cysteine, the curves C and D are obtained, showing that the components are strictly additive;

Figure 3. Glutathione concentration at different NAC and AOS concentrations. The effect of the combination is more than the effect of the separate components over the complete curve.

Detailed description of the invention

CD4⁺ T cell count is a good indicator for AIDS progression, since HIV infects primarily CD4⁺ helper T cells, eventually leading to cell death. Chronic immune hyperactivation, a typical feature in progressive HIV disease, plays a crucial role in ongoing CD4⁺ T cell depletion. Brenchley *et al.* *Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med.* 2006;12:1365-1371. have proposed a major role for the gut in HIV-induced chronic immune activation. Increased gut permeability is common in HIV-infected patients and causes translocation of microbial products. Circulating lipopolysaccharide (LPS), a reliable marker of microbial translocation, has proved to be significantly raised in HIV-infected patients, correlating with immune hyperactivation. Moreover, LPS levels were decreased upon treatment with HAART. LPS is a major component of gram-negative bacterial cell walls and therefore a potent immunostimulatory product. These findings suggest that HIV infection causes impaired gut barrier function, followed by increased microbial translocation that eventually contributes to chronic immune activation. HIV replication seems to play a central role in maintaining this impaired gut barrier function.

The cause of gut permeability upon HIV infection is not completely understood, although it might be a result of CD4⁺ T cell infection. The GI tract is probably the largest lymphoid organ in the body, accounting for at least half of the total T cell load. Because CD4⁺ T cells are most abundant in the GI- tract, HIV infection creates a pro-inflammatory environment in the gut. It is hypothesized that this environment establishes a breakdown of the gastrointestinal mucosal barrier, and thereby augmenting microbial translocation. These bacterial products, like lipopolysaccharides and peptidoglycans (PG), induce an inflammatory response via binding to TLR receptors, which not only induces inflammation, but also triggers the HIV virus into replication. The released HIV particles infect other CD4⁺ T cells, which creates an even greater pro-inflammatory environment and increases gut permeability. This positive feedback-loop drives chronic immune activation.

Without being bound by theory, the inventors hypothesize that hyperimmune activation is mediated through LPS- or PG-induced toll-like receptor (TLR) activation. Each toll-like receptor subset is stimulated by a specific microbial product; LPS stimulates the TLR-4, while PG stimulates the TLR-2. The activation of TLRs induces a signaling pathway whereupon NF- κ B will be activated, leading to inflammation. NF- κ B is a dimer composed of two proteins of the Rel/ κ B family of transcription factors, which are both required for DNA binding. NF- κ B is constitutively expressed, but is present as an inactive complex with the I κ B inhibitor protein in the cytoplasm of unstimulated cells. When NF- κ B is released from its complex with I κ B, it is free to translocate to the nucleus where it functions as a transcription factor. Phosphorylation of NF- κ B increases nuclear localization, protein-protein interactions, and transcriptional activity.

When leukocytes are infected with HIV-1, NF- κ B translocation to the nucleus plays an additional role in virus replication. NF- κ B recognizes two 10bp stretches of DNA with the consensus sequence 5'-GGGPuNNPuPuCC-3' in the long terminal repeat (LTR) of HIV-1. In transient transfection studies, mutation of either κ B sites resulted in a dramatic loss of LTR promoter activity, suggesting an essential role for NF- κ B in HIV replication. Due to its this role, NF- κ B is a potential target for treatment of HIV-infected individuals. Indeed, it was found that inhibition of NF- κ B activation decreases HIV-1 replication *in vitro* (see examples), and may therefore postpone AIDS development *in vivo*.

It is hypothesized that the main site of action of the specific nutritional oligosaccharides as mentioned in example 1, is the intestine. Example 1 shows a large group of dietary fibers that were tested for the NF- κ B inhibiting capacity. It was found that only a small selection comprising Pectin (in the form of digalacturonic acid, trigalacturonic acid, polygalacturonic acid), Arabinoxylan from rice bran, β -glucan from bakers yeast, D-Ribose were capable of inhibiting NF- κ B in the present test system.

In the case of HIV infection, the gut is known to have high levels of viral replication in associated CD4⁺ T-lymphocytes during the acute and chronic phases of infection. It is further known that the gut is seen as a separate compartment that is difficult to reach with conventional anti retro viral therapy (e.g. blood viral levels can be reduced by antiretroviral therapy without influencing viral load in the intestine). The supposed ability of dietary oligosaccharides to modulate different pathways of NF- κ B activation leading to reduced viral production especially in the gut designates a potential beneficial role for dietary oligosaccharides in the management of HIV infection. It may result in a reduced viral load in the gut, which might systemically translate into either a reduction of viral load or an increase in CD4 count or a combination of both.

As a result of the reasoning above it could be that the viral load in the peripheral blood of the patients is not directly altered by a treatment with NF- κ B inhibitors such as AOS and/or NAC. This may be explained by the compartmentalization of the gut, where these nutritional ingredients are likely to have their strongest effect

Anti-viral medicaments

The supposed property of targeting a compartment difficult to address by anti retroviral therapy also implicates that the use of these specific oligosaccharides are likely to act synergistically with current anti viral therapies and in particular with an anti retroviral therapy (HAART). Due to the NF- κ B inhibiting activity of the oligosaccharides according to the invention, a preferred composition according to the invention comprises a combination of anti viral therapy (e.g. HAART) with the compositions according to the invention. For the treatment of HIV the compositions according to the claims may be used in combination with anti viral vaccines, with antiviral

pharmaceuticals like oseltamivir (Tamiflu), zanamivir (Relenza) and the like, or anti-retroviral therapeutics used for treatment of AIDS such as nucleoside and nucleotide reverse transcriptase inhibitors (nRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PIs), integrase inhibitors such as Raltegravir, entry inhibitors (or fusion inhibitors) such as Maraviroc and Enfuvirtide, or maturation inhibitors such as bevirimat and Vivecon.

Cysteine or source of cysteine

The inventors found that N-acetyl cysteine (NAC) is also capable of blocking NF- κ B activation. The mechanism behind this suppressive capacity has not been cleared, but it has been hypothesized that it involves NAC's ability to decline oxidative stress indirectly via GSH level recovery. Oxidative stress has shown to promote NF- κ B activation, via a mechanism that is still debated and probably cell-type specific. However, most studies state ROS-induced NF- κ B activation to be dependent on the classical pathway by phosphorylating IKK indirectly via PKD (Protein Kinase D) activation.

Because the inventors found that NAC and AOS act together on NF- κ B activation, it was hypothesized that NAC and AOS also have additive effects on GSH levels. To determine this, HEK293 cells were incubated with AOS for 22.5 hr. and NAC in combination with TNF- α for 2.5 hr. Obtained GSH levels were corrected for cell numbers and results were compared levels with TNF- α -stimulated cells (control). The addition of NAC enhances GSH levels in a concentration of 10mM. AOS is able to significantly increase GSH levels after addition of 2 and 3 mg/l. The combination of NAC and AOS enhance GSH production up to 278% compared with control. Data sets were compared with their baseline controls (0 mg/ml AOS in combination with its specific concentration of NAC or L-cysteine) and presented in example 2. High concentrations of AOS and NAC result in a relative stronger increase in GSH levels. From this, it is concluded that AOS and NAC have synergistic effects on the production of GSH in HEK293 cells.

The compositions provided comprise in addition to one or more oligosaccharides as described above a suitable amount of cysteine and/or source of cysteine. The phrase “source of cysteine” refers herein to all compounds that contain a biologically available cysteine, in any form, and is calculated as the amount of cysteine amino acid that is present in a compound, or can be derived from a compound in the body after ingestion, on a molar basis.

Hereinbelow “cysteine equivalent” refers to an amount of cysteine as such or to an amount of cysteine that is present in a source of cysteine. For example 100 mg NAC (N-acetylcysteine; MW= 163.2) is equivalent to 74 mg cysteine (MW 121.15). Thus 100 mg NAC is 74 mg cysteine equivalent. Similarly this can be applied to proteins or peptides. When a peptide (MW = xDalton) contains 3 cysteine amino acids (3yDalton), than 100 mg of this peptide is equivalent to $100 \times 3Y/X$ mg cysteine. Thus 100mg of this peptide is $300y/x$ mg cysteine equivalent.

Suitable sources of cysteine according to the invention are, for example, proteins in denatured and/or undenatured form such as milk proteins e.g. whey or casein proteins. Egg proteins are rich in cysteine and are therefore also suitable. Plant proteins such as pea, potato, soy and rice can also be used to provide cysteine. Also hydrolysates of these protein sources can be used or fractions enriched for cysteine rich proteins or peptides (e.g. as described in EP1201137). Furthermore, synthetic cysteine equivalents, e.g. derivatives of cysteine, such as cysteine, cystine, cysteine salts, N-acetylcysteine and/or diacetylcysteine can be used.

25

The HIV infected subjects are suitably administered a daily dose of at least about 100 mg cysteine equivalent, preferably at least about 200, 400, or 600 mg cysteine equivalent per day, more preferably at least about 1000 mg cysteine equivalent per day. It is understood that a daily dosage can be subdivided into 2, 3 or more dosage units taken several times a day.

30

In yet another embodiment the compositions according to the invention comprise one or more compounds that stimulate glutathione levels. e.g. lipoic acid, pyruvate, oxaloacetate, oxaloaspartate, are capable in stimulating glutathione levels. Such glutathione level stimulating compounds may be used in addition to cysteine but also
5 instead of cysteine.

ω -3 polyunsaturated fatty acids

The compositions provided preferably comprise in addition ω -3 polyunsaturated fatty acids. The unsaturated fatty acid for a treatment in accordance with the invention is
10 preferably selected from the group of C18-C26 ω -3 polyunsaturated fatty acids.
Prefably ω -3 polyunsaturated fatty acids include eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), eicosatetraenoic acid (ETA) and docosapentaenoic acid (DPA). EPA showed NFkappaB inhibition in the same in vitro model as used for the testing of oligosaccharides. The daily dosage of C20-C26 ω -3 polyunsaturated fatty
15 acids can be between 200 mg – 2000 g.

Example 1.

Screening of oligosaccharides NFK-B inhibiting activity

(Oligo)saccharide	NF- κ B inhibition
1,1,1-kestopentaose	No
1,1-kestotetraose	No
1-kestose	No
Alginate	No
Amidated pectin	No
AOS from pectin	Yes
Apple pectin	Yes
Arabinogalactan	No
Arabinoxylan from rice bran	Yes
Digalacturonic acid	Yes
Fructose	No
Galactose	No
Galacturonic acid	No
Glucose	No
Iota-carrageenan II (irish moss)	No
Iota-carrageenan V (eucheuma spinosa)	No
Iota-carrageenan V (irish moss)	No
Maltodextrine	No
Palatinose	No
Polygalacturonic acid from citrus fruit	Yes
Polygalacturonic acid from oranges	Yes
Raffinose pentahydrate	No
Raftiline HP (long chain FOS)	No
Raftiline ST	No
Raftilose P95 (short chain FOS)	No
D-Ribose	Yes
β -D-glucan from barley	No

β -D-glucan from baker's yeast	Yes
Stachyose hydrate	No
Trigalacturonic acid	Yes
Vivinal-GOS	No
Λ -carrageenan (irish moss)	No

Example 2. Effect of oligosaccharides in combination with cystein on NF- κ B inhibition

Methods: HEK293 cells were stably transfected with a NF- κ B luciferase reporter gene construct. TNF- α was added to induce NF- κ B activation. citrus pectin oligosaccharide (pAOS) was added 18h prior to TNF- α addition (t=18h). As a cystein source, NAC was added at the same time as TNF- α . At t=20.5 the production of luciferase was measured by chemiluminescence.

Results: Both pAOS and NAC inhibited TNF- α -mediated NF- κ B activation dose dependently, although the timeframe of activity differed per component (figure 2A and 2B). AOS-mediated NF- κ B inhibition was optimal after an overnight pre-incubation period, while NAC-mediated inhibition was most effective after 2.5 hours. Upon correction for the contribution of NAC, the complete pAOS signal remained (figure 2c and 2D). This finding suggests that the components do not interact with each other, but inhibit NF- κ B activation in a different, additive way.

Conclusions: The addition of NAC to pAOS results in a strongly improved inhibition of NF- κ B. The lack of competition between the two components, together with different incubation times required, suggest different working mechanisms for both components.

Example 3. Effect of oligosaccharides on HIV production

Methods: HEK293 cells were calcium phosphate co-transfected with both a plasmid DNA to produce HIV-1 virus and a LAI construct containing either the CMV promoter or the phRL-TK promoter. HIV-1 virus concentrations were determined by measuring CA-p24 by an enzyme-linked immunosorbent assay. To correct inhibition of HIV-1 production for non specific inhibition of gene expression basal and TNF-alpha induced HIV-1 capsid production was calculated relative to that of a co-transfected LAI construct, which was arbitrarily set at a value of 1. To control for NF-kappaB specificity of the inhibition, this co-transfected LAI construct contained either a promoter containing NF-kappaB binding sites (CMV promoter) or not (phRL-TK promoter). AOS was added immediately at transfection. Two days after transfection, the culture medium was removed and HIV viral production was measured by measuring CA-p24 by an enzyme-linked immunosorbent assay as described above. The cells were washed once with phosphate-buffered saline and lysed by the addition of 200

5 μ l of reporter lysis buffer (Promega), and the sample was mixed for 45 min at room temperature. The lysate was collected in a tube, and the cell debris was removed by centrifugation for 15 min at 15,000 rpm in an Eppendorf centrifuge. The luciferase activity (in relative light units) was determined by a Berthold luminometer, model LB9501.

Results: All transiently co-transfected cells transfected with the complete HIV-1 genome combined with a control viral vector produced HIV-1 viral particles. This HIV-1 viral production was inhibited by AOS in all these cells. When the HIV-1 production was corrected for the activation of a promoter without NF- κ B binding sites, however, production remained significant. Again, this production was inhibited by AOS. Moreover, production was stimulated by TNF-alpha. This TNF-alpha induced production was also inhibited by AOS. When the viral HIV production was corrected for a viral promoter containing NF-kappaB binding sites (CMV) (a positive control), hardly any activity remained.

15 Conclusions: This study shows for the first time that specific dietary oligosaccharides (pAOS) are able to inhibit HIV-1 production in vitro. The HIV-1 production rate directly influences the HIV-1 replication rate. Moreover, the results from corrections with positive and negative controls (promoters containing NF- κ B binding sites or not), demonstrate that it is a general mechanism that applies to other viruses with promoters containing NF- κ B binding sites like CMV. Even more general, it is hypothesized that the components inhibit viruses that make use of NF- κ B activation via different pathways for their replication.

HIV production [Ca-p24] / Renilla activity phRL-TK promoter								
AOS concentration (mg/ml)								
	0		1		2		3	
unstimulated	2.4 $\pm$	0.2	1.4 $\pm$	0.1	0.7 $\pm$	0.1	0.9 $\pm$	0.1
TNF stimulated	8.0 $\pm$	0.6	3.5 $\pm$	0.5	1.4 $\pm$	0.3	2.4 $\pm$	0.2

25 HIV production when corrected for viral promoter activity of a viral promoter without NFkappaB binding sites.

HIV production [Ca-p24] / Renilla activity CMV								
	AOS concentration (mg/ml)							
	0		1		2		3	
unstimulated	3.0 ±	0.5	4.1 ±	0.8	4.8 ±	0.6	5.2 ±	0.5
TNF stimulated	3.7 ±	0.0	3.6 ±	0.0	3.4 ±	0.0	2.6 ±	0.0

HIV production when corrected for viral promoter activity of a viral promoter containing NFkappaB binding sites (CMV).

Example 4. Effect of oligosaccharides in combination with a cystein source on glutathione

Methods: HEK293 cells were incubated with citrus pectin oligosaccharide (pAOS) for 18 h, then as a cystein source, NAC was added and incubated for 2.5 h. After the incubation period, the cells were centrifugated (2000 rpm for 5 min). Medium was discarded and taken up into ice-cold perchloric acid (0.4 M) and resuspended. Samples were then centrifuged for 10 min. at 13.000 rpm. Supernatant was collected and used for glutathione measurements. Total glutathione measurements were performed in 10 or 20 times diluted samples.

Results: Both pAOS and NAC inhibited TNF- α -mediated NF- κ B activation dose dependently (Figure 3), although the timeframe of activity differed per component. AOS-mediated NF- κ B inhibition was optimal after an overnight pre-incubation period, while NAC-mediated inhibition was most effective after 2.5 hours. Upon correction for the contribution of NAC, the complete pAOS signal remained. This finding suggests that the components do not interact with each other, but inhibit NF- κ B activation in a different, additive way.

Conclusions: The addition of NAC to pAOS resulted in a synergistic effect on glutathione. These results imply that disease states that show both an increase in oxidative stress and are related to infections with viruses that can be activated via NF- κ B, might especially benefit from proposed combination. These disease are: COPD , IBD, Crohn, hepatitis and HIV (AIDS). E.g. Rhinovirus, the organism most often responsible for causing the common cold, is also the most common infectious cause of chronic obstructive pulmonary disease exacerbations. Coronavirus, influenza, respiratory syncytial virus, parainfluenza, adenovirus, and metapneumovirus are other important viral causes of chronic obstructive pulmonary disease exacerbations. Adenovirus increases acute exacerbations via NF- κ B activated ICAM expression.

Claims

1. Composition comprising pectin (in the form of digalacturonic acid, trigalacturonic acid, polygalacturonic acid), Arabinoxylan from rice bran, β -glucan from bakers yeast, D-Ribose, or mixtures thereof for inhibiting viral replication in a mammal
5 with a viral disease.
2. Composition according to claim 1, wherein the pectin has an average degree of polymerization between 2 and 250 saccharide units.
3. Composition according to claim 1 or 2, wherein the viral disease is selected from the group consisting of AIDS, HIV infection and cytomegalovirus infection,
10 Adenovirus, Common cold (Rhino virus), Dengue fever, Enterovirus, Hepatitis (A, B, C), Herpes simplex and Influenza (Flu).
4. Composition according to claim 1 or 2, wherein the viral disease is selected from the group consisting of AIDS, HIV infection and cytomegalovirus infection.
5. Composition according to any of the previous claims, further comprising a cysteine
15 source selected from the group consisting of cystine, cysteine, cysteine salts, N-acetylcysteine and diacetylcysteine.
6. Composition according to any of the previous claims further comprising ω -3 polyunsaturated fatty acids.
7. Nutritional composition comprising at least 20 en% protein, at least 5 en% fat, and
20 at least 10 wt% based on dry weight of the composition of at least one carbohydrate selected from the group consisting of pectin (in the form of digalacturonic acid, trigalacturonic acid, polygalacturonic acid), Arabinoxylan from rice bran, β -glucan from bakers yeast and D-Ribose.
8. Nutritional composition according to claim 6 further comprising at least one
25 cysteine equivalent selected from the group consisting of cystine, cysteine, cysteine salts, N-acetylcysteine and diacetylcysteine.
9. Composition according to any of the previous claims further comprising anti-viral medicaments.

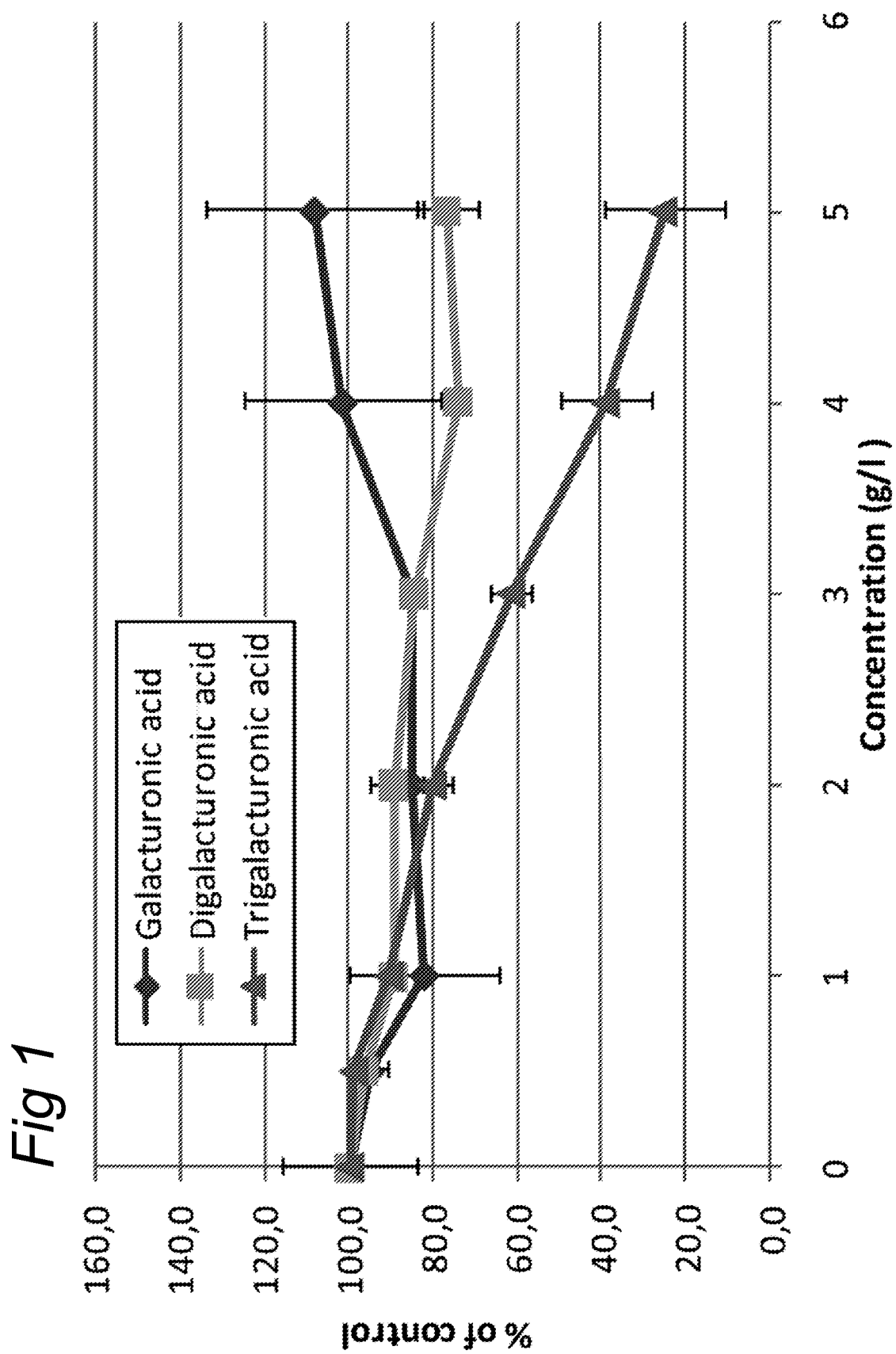
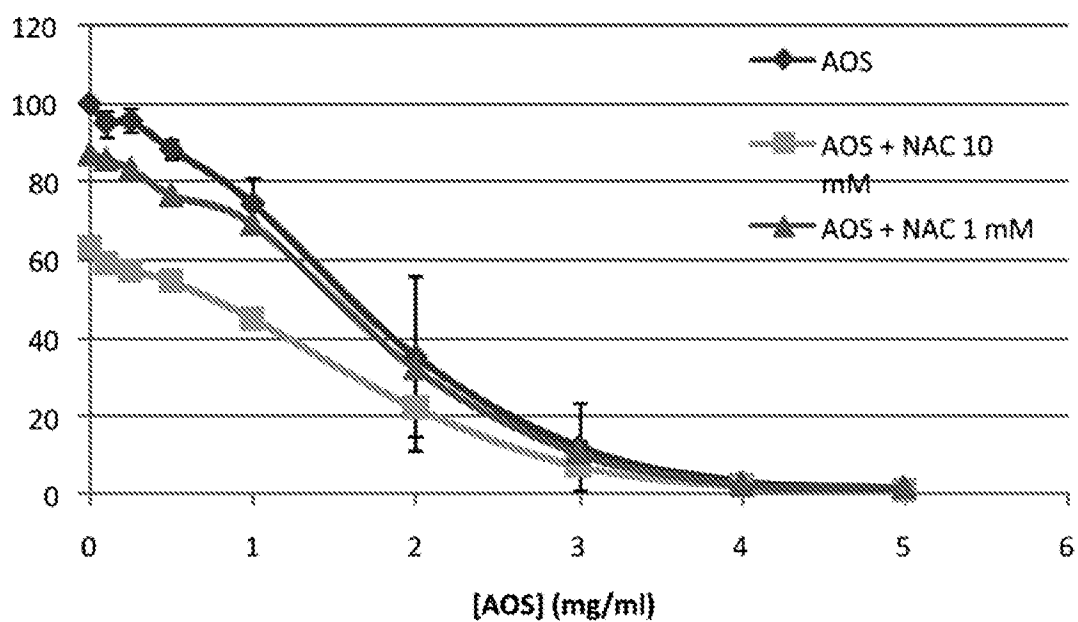
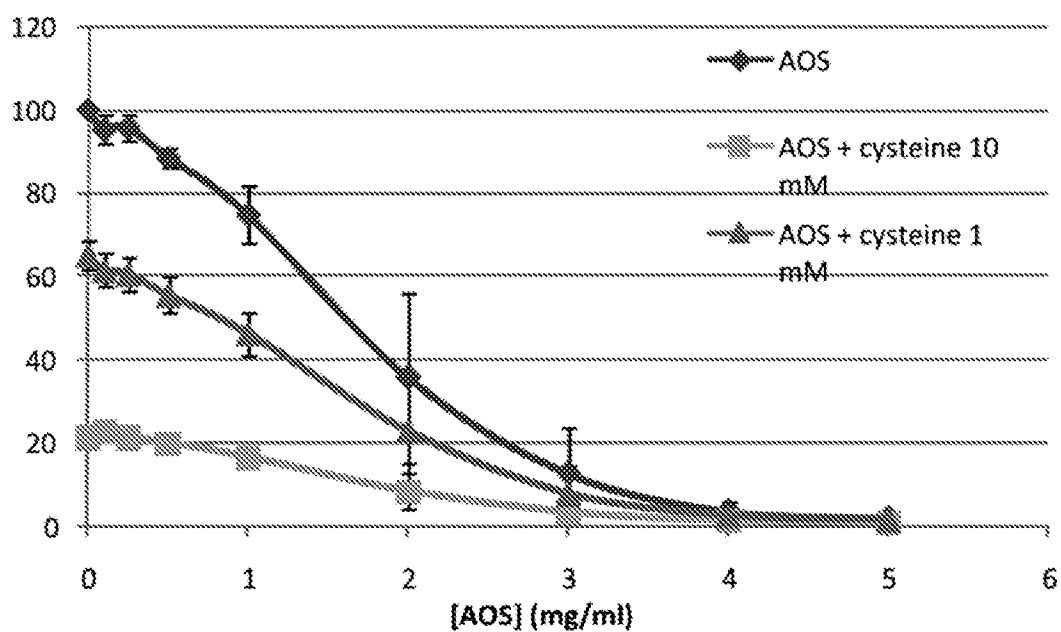


Fig 2a**AOS + NAC****Fig 2b****AOS + L-cysteine**

3/4

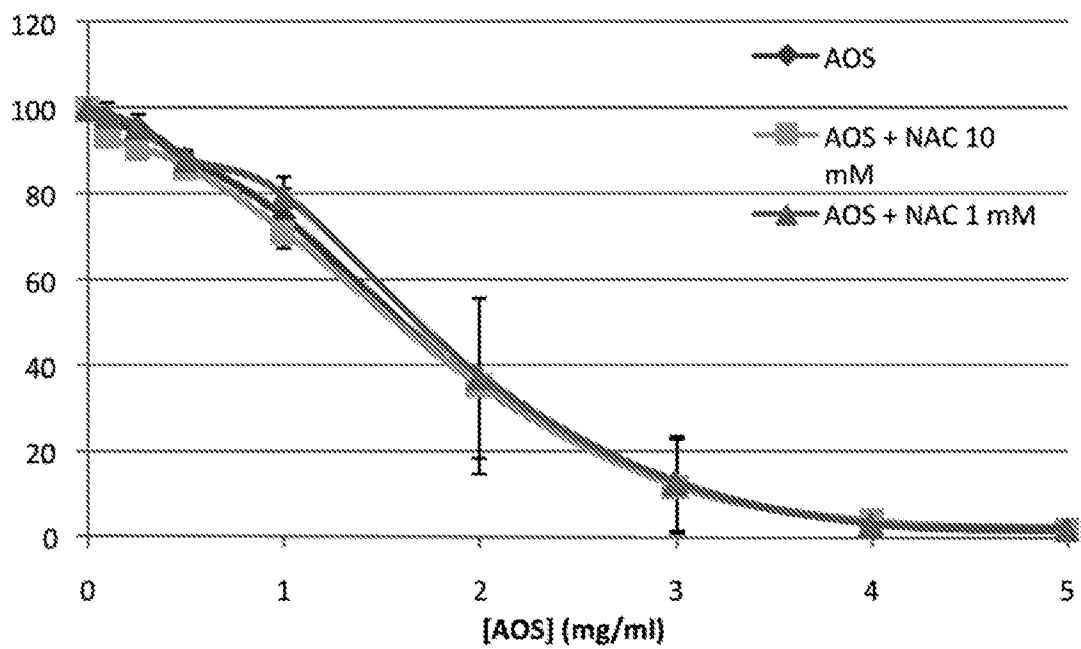
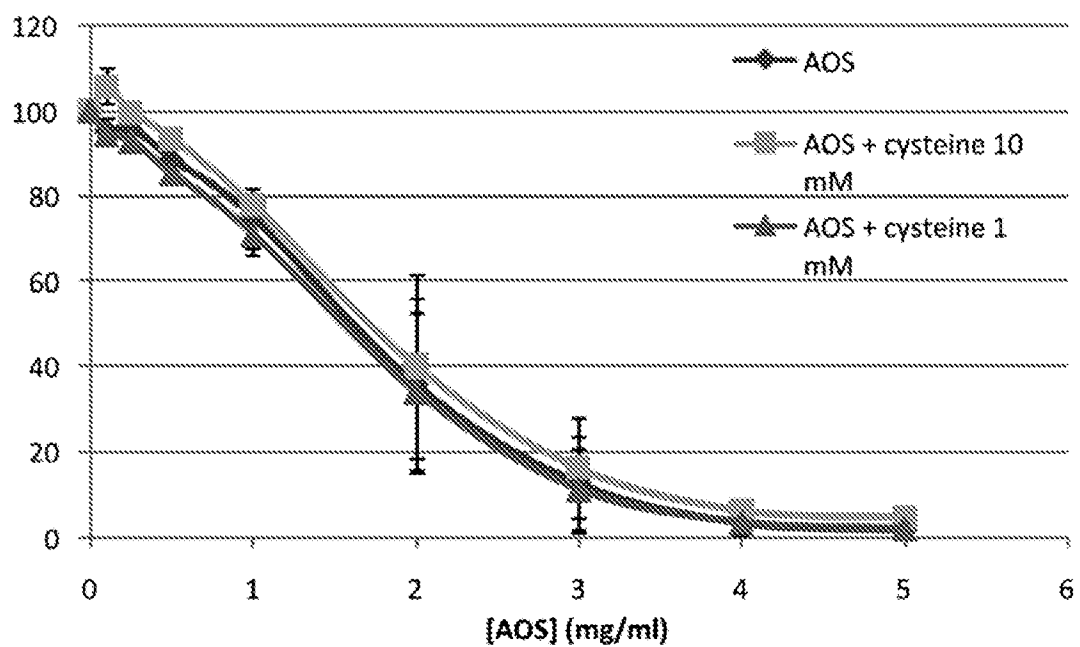
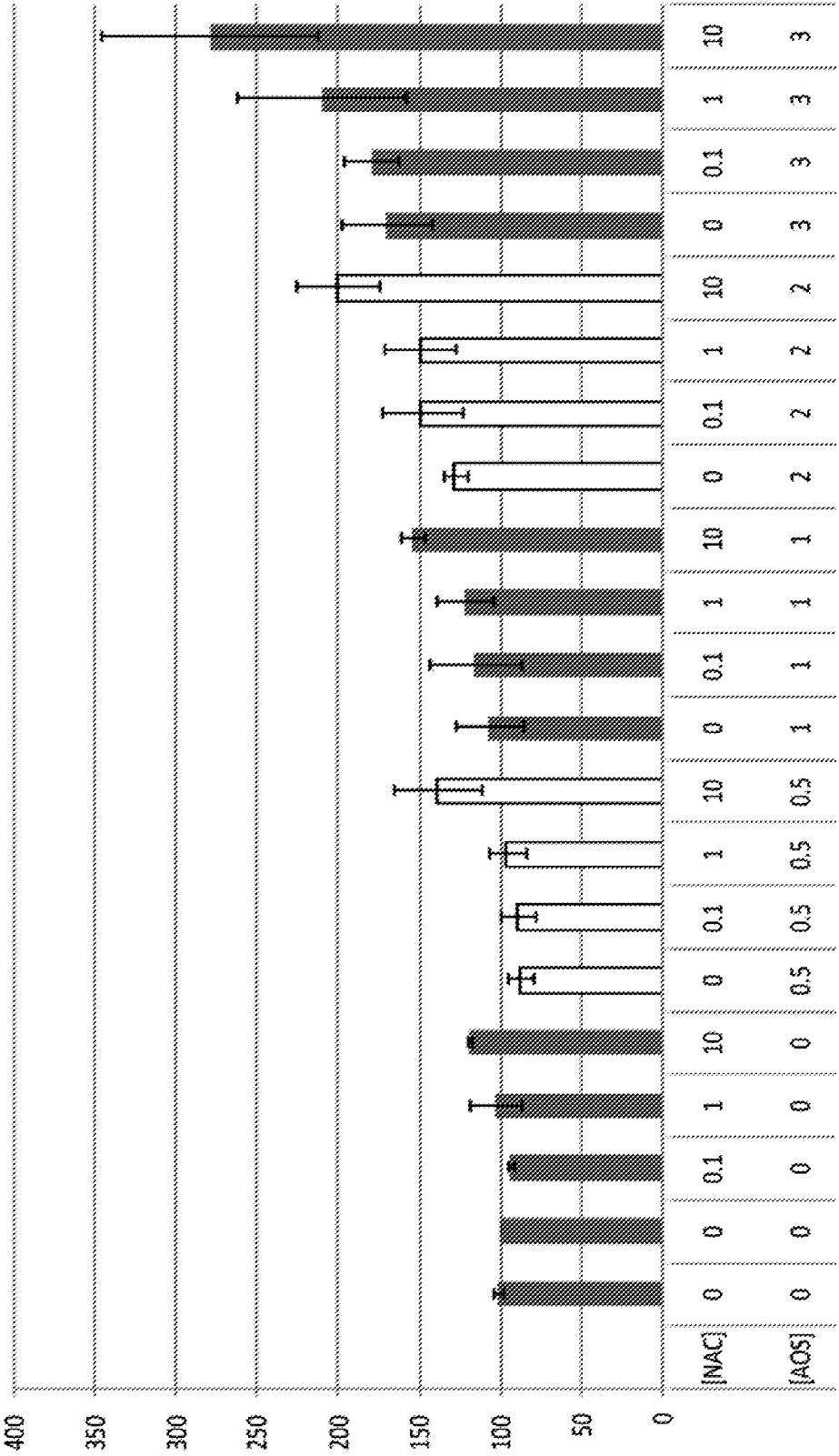
*Fig 2c***AOS + NAC***Fig 2d***AOS + L-cysteine**

Fig 3



INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2009/050363

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61P31/12 A23L1/308 A61K31/7004 A61K31/716 A61K31/732

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 A61P

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.
X	US 2006/178341 A1 (MOON WON KOOK [KR] ET AL) 10 August 2006 (2006-08-10) claims 1-7	1
X,P	VAN NORREN K ET AL: "P272 PECTIN DERIVED ACIDIC OLIGOSACCHARIDES INHIBIT NFKB-MEDIATED HIV-1 PRODUCTION IN VITRO" CLINICAL NUTRITION SUPPLEMENTS, ELSEVIER LNKD- DOI:10.1016/S1744-1161(09)70322-9, vol. 4, no. 2, 26 August 2009 (2009-08-26), pages 139-140, XP026747344 ISSN: 1744-1161 [retrieved on 2009-01-01] abstract	1-6,9
Y		1-6,9
Y	WO 02/47612 A2 (MANNATECH INC [US]) 20 June 2002 (2002-06-20) claims 1-3,20	1-6,9
	----- -/-	

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

☒ See patent family annex.

* Special categories of cited documents:

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

2 June 2010

Date of mailing of the international search report

09/06/2010

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Herrera, Suzanne

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2009/050363

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2009/096772 A1 (NUTRICIA NV [NL]; VRIESEMA ADRIANUS JOHANNES MARIA [NL]; VAN HELVOORT) 6 August 2009 (2009-08-06) abstract; claims 1-3 -----	1-6, 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2009/050363

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
2(completely); 1, 3-6, 9(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2(completely); 1, 3-6, 9(partially)

Composition comprising pectin (in the form of digalacturonic acid, trigalacturonic acid, polygalacturonic acid) for inhibiting viral replication in a mammal with a viral disease.

2. claims: 1, 3-6, 9(all partially)

Composition comprising Arabinoxylan from rice bran for inhibiting viral replication in a mammal with a viral disease.

3. claims: 1, 3-6, 9(all partially)

Composition comprising Beta-glucan from bakers yeast for inhibiting viral replication in a mammal with a viral disease.

4. claims: 1, 3-6, 9(all partially)

Composition comprising D-Ribose for inhibiting viral replication in a mammal with a viral disease.

5. claims: 7, 8(completely); 9(partially)

Nutritional composition comprising at least 20 en% protein, at least 5 en% fat, and at least 10 wt% based on dry weight of the composition of at least one carbohydrate selected from pectin (in the form of digalacturonic acid, trigalacturonic acid, polygalacturonic acid), Arabinoxylan from rice bran, Beta-glucan from bakers yeast and D-Ribose.

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2009/050363

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 2006178341	A1	10-08-2006	CN	1809366 A	26-07-2006
WO 0247612	A2	20-06-2002	AU	4326702 A	24-06-2002
WO 2009096772	A1	06-08-2009	WO	2009096789 A1	06-08-2009