Vivinal® GOS PT

A submission to the UK Food Standards Agency requesting consideration of Substantial Equivalence in accordance with Regulation (EC) No 258/97 concerning novel foods and novel food ingredients

Non-Confidential Version

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I Administrative data

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Food ingredient

The food ingredient for which an opinion on Substantial Equivalence is requested is Vivinal® GOS PT

Date of application

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II The Issue

In recent years, galacto-oligosaccharides have been the subject of several Novel Food Applications submitted under Regulation No 258/97 in the European Union (EC, 1997).

All of these applications filed by several companies, including Yakult and Nestlé, pertained to notifications of substantial equivalence in which the comparator product was FrieslandCampina's Vivinal® GOS. The latter product was already marketed in the EU prior to 15 May 1997 and is therefore outside the scope of the novel food Regulation.

The current submission pertains to a similar request for an opinion on substantial equivalence of a new galacto-oligosaccharides-product (Vivinal® GOS PT) produced by FrieslandCampina Domo, derived from lactose and applying a β-galactosidase enzyme isolated from *Papiliotrema terrestris*.

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The *Papiliotrema* production strain, the source organism of the β-galactosidase used in the production of Vivinal® GOS PT, was isolated from soil in Myanmar and has been identified as belonging to the species *Papiliotrema terrestris*, based on identical morphology and 100% 26S rDNA-D1/D2 sequence homology to the *P. terrestris* type strain CBS10810^{T}.

Further information on the type strain *P. terrestris* CBS 10810^{T}, including the partial sequence of the D1/D2 region of the 26S rRNA gene, can be retrieved from the Westerdijk Fungal Biodiversity Institute (formerly known as CBS-KNAW).
http://www.westerdijkinstitute.nl/Collections/Biolomics.aspx?Table=CBS%20strain%20database

1.1 Taxonomy

The taxonomic lineage of *P. terrestris* can be obtained from the Excel sheet "Fungal Selection" (http://www.westerdijkinstitute.nl/Collections/) entry no. 7381 and at http://www.uniprot.org/taxonomy/425109 and is shown below.

Superregnum: Eukaryota

Regnum: Fungi

Subregnum: Dikarya

Phylum: Basidiomycota

Subphylum: Agaricomycotina

Classis: Tremellomycetes

Ordo: Tremellales

Familia: Rhynchogastremataceae

Genus: Papiliotrema

Species: Papiliotrema terrestris

1.2 Phylogenic placement of *Papiliotrema terrestris*

Families and genera assigned to *Tremellomycetes* have been mainly circumscribed by morphology and for the yeasts also by biochemical and physiological characteristics. This phenotype-based classification is largely in conflict with molecular phylogenetic analyses. Here the phylogenetic placement framework of *P. terrestris* is proposed (Fig 1; Machado Pagani et al., 2016).

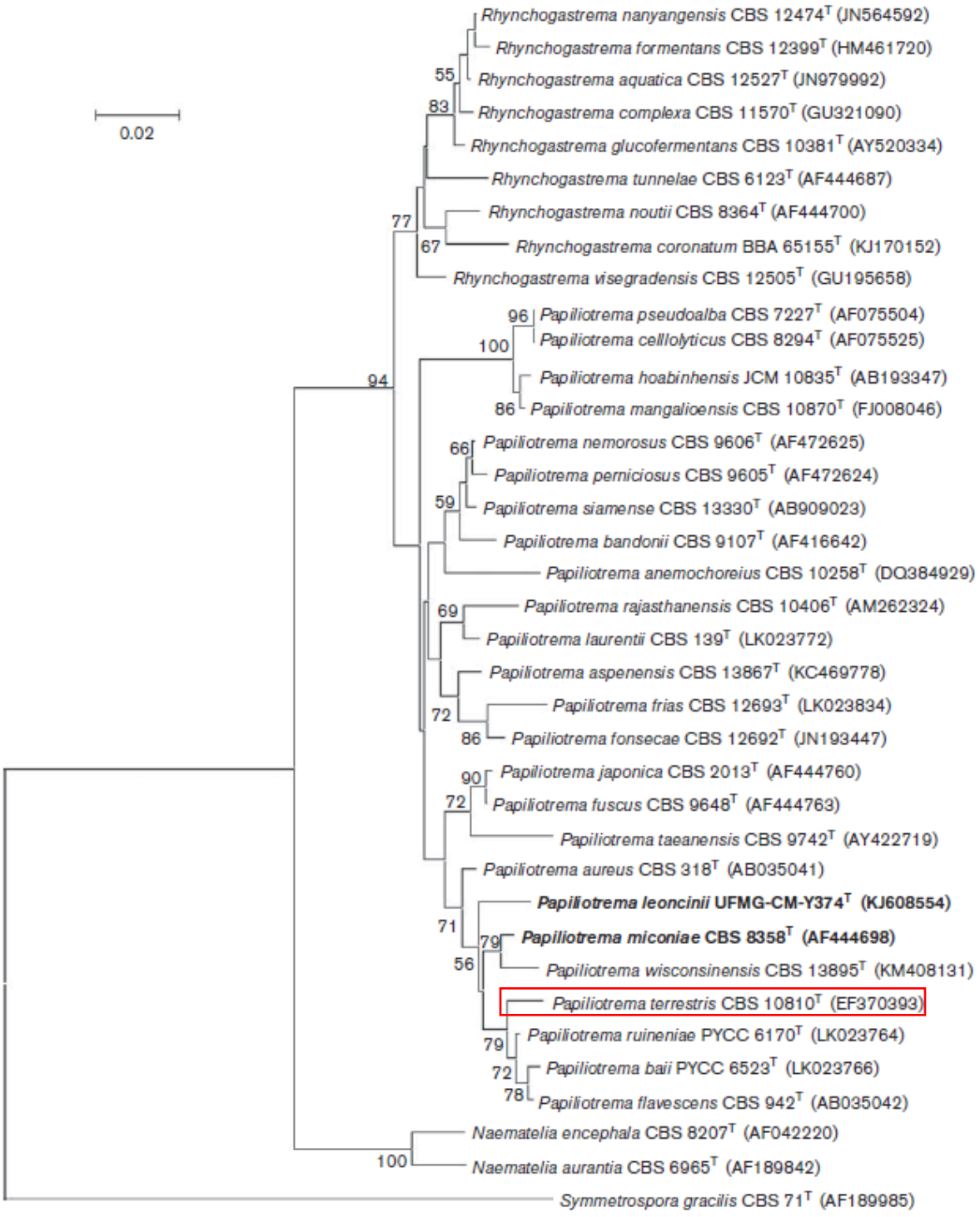


Figure 1. Phylogenetic placement of *P. leoncinii* sp. nov. and *P. miconiae* sp. nov. within the family *Rhynchogastremataceae* as obtained by neighbour-joining (Kimura's two-parameter distance method) analysis of the D1/D2 nt. Bootstrap values >50 % are shown (1000 replicates). Bar, 0.02 substitutions per nucleotide position. (*Source: Machado Pagani et al., 2016).*

Basidiomycetous yeasts are often difficult to classify due to their polyphyletic nature. These yeasts are classified in the *Ustilaginomycetes, Hymenomycetes, Urediniomycetes* and *Tremellomycetes*. Recently, Liu et al. (2015a, b) proposed an extensive reconstruction of the phylogenetic classification of the tremellomycetous yeasts based on multiple gene sequence analyses. Within this scheme of classification, Liu et al. (2015b) emended the family *Rhynchogastremataceae* with the genera *Rhynchogastrema* and *Papiliotrema.* On the basis of the multiple gene sequence analysis, *Papiliotrema terrestris* was placed in the genus *Papiliotrema.* (instead of *Cryptococcus*, as previously assigned).

1.3 Safety

With respect to Biosafety, the American Type Culture Collection (ATCC) has classified cultures and related products by biosafety level (BSL) for purposes of packaging for safe shipment. The classification is based on assessment of the potential risk using U.S. Public Health Service guidelines. Following an ATCC assessment, *Papilliotrema terrestris* strains were classified as BSL-1 (Biosafety level 1).

Biosafety Level 1 is assigned to well-characterized agents suitable for work not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.
(*Source:* https://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_sect_iv.pdf)

2. β-galactosidase from *Papiliotrema terrestris*

2.1 β-Galactosidases

β-galactosidases (β-gal; EC 3.2.1.23) catalyze the hydrolysis and transgalactosylation of β-D-galactopyranosides (such as lactose). GOS are the products of transgalactosylation reactions catalyzed by β-galactosidases when using lactose or other structurally related galactosides as the substrate. β-galactosidases undergo a two-step mechanism of catalysis. First, this mechanism involves the formation of a covalently linked galactosyl-enzyme intermediate. Subsequently, the galactosyl moiety linked to the nucleophile in the active site is transferred to a nucleophilic acceptor. Water, as well as all sugar species present in the reaction mixture, can serve as a galactosyl acceptor. Hence, the resulting final mixture contains hydrolysis products of lactose, which are glucose and galactose, unconverted lactose as well as di-, tri and higher oligosaccharides *[reviewed by Intanon et al., 2014].*

β-Galactosidases are found in microorganisms (bacteria, fungi, and yeasts), plants especially in almonds, peaches, apricots, apples and animal organs. β-Galactosidases from microorganisms sources have been widely used for the hydrolysis of lactose because of the ease of fermentation and good stability. β-galactosidase activity is also abundantly present in the colon of human beings. It catalyzes the first step of lactose fermentation in colon and is often measured as an indication of the capacity of colonic microbiota to ferment lactose present in the intestine. *[reviewed by Intanon et al., 2014].*

2.2 Production of β-Galactosidase from *Papiliotrema terrestris*

In enzymology, a ß-galactosidase belongs to the family of glycosylase [EC 3.2.]. However it has not only a hydrolytic activity but also a transferase activity that catalyses the transfer of galactose residue of a lactose (donor) to another (acceptor). GOS and glucose can be the acceptor for the transfer reaction of β-galactosidase same as lactose. Therefore, β-galactosidase produces the various GOS having the different degrees of polymerization.

Safety analysis of a protein considers its origin and natural occurrence as well as the potential of the protein to elicit adverse effects. The potential of a food protein to elicit toxic or allergenic responses strongly depends on dose, absorption through the digestive system and protein conformation and integrity which is influenced by food processing.

2.5.5 Summary of safety aspects of different production steps

Taken together, regarding safety, the β-Galactosidase PT enzyme was shown to be non-mutagenic and non-clastogenic and to be non-toxic.

In addition, if present in a food, the β-Galactosidase PT enzyme, as applied in the production of Vivinal® GOS PT, is highly unlikely to elicit an allergic response.

Therefore, it is concluded that the β-Galactosidase PT enzyme, if present in a food, will not pose a risk to the consumer.

3. Substantial equivalence of the manufacturing process of Vivinal® GOS PT

Figure 3 Flow diagram production of Vivinal® GOS PT Syrup

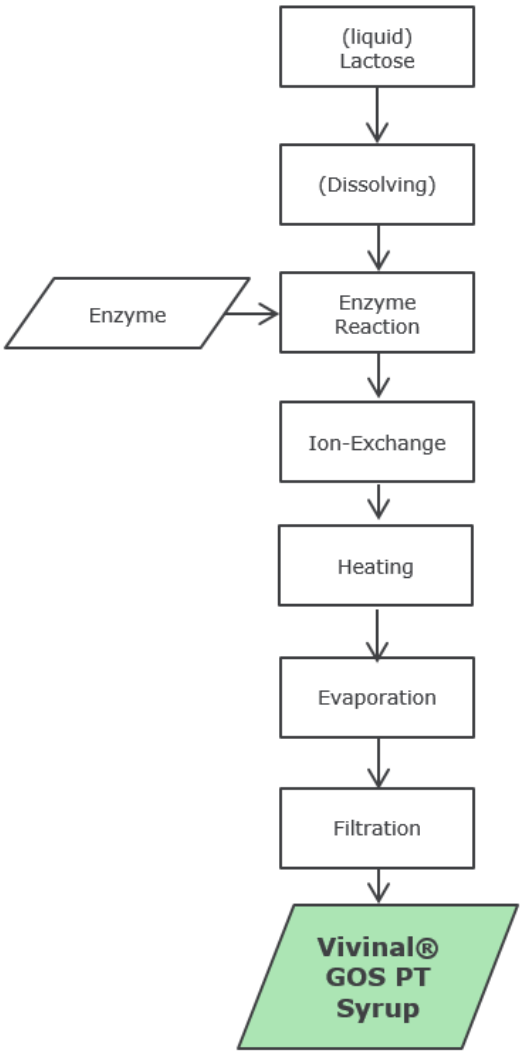
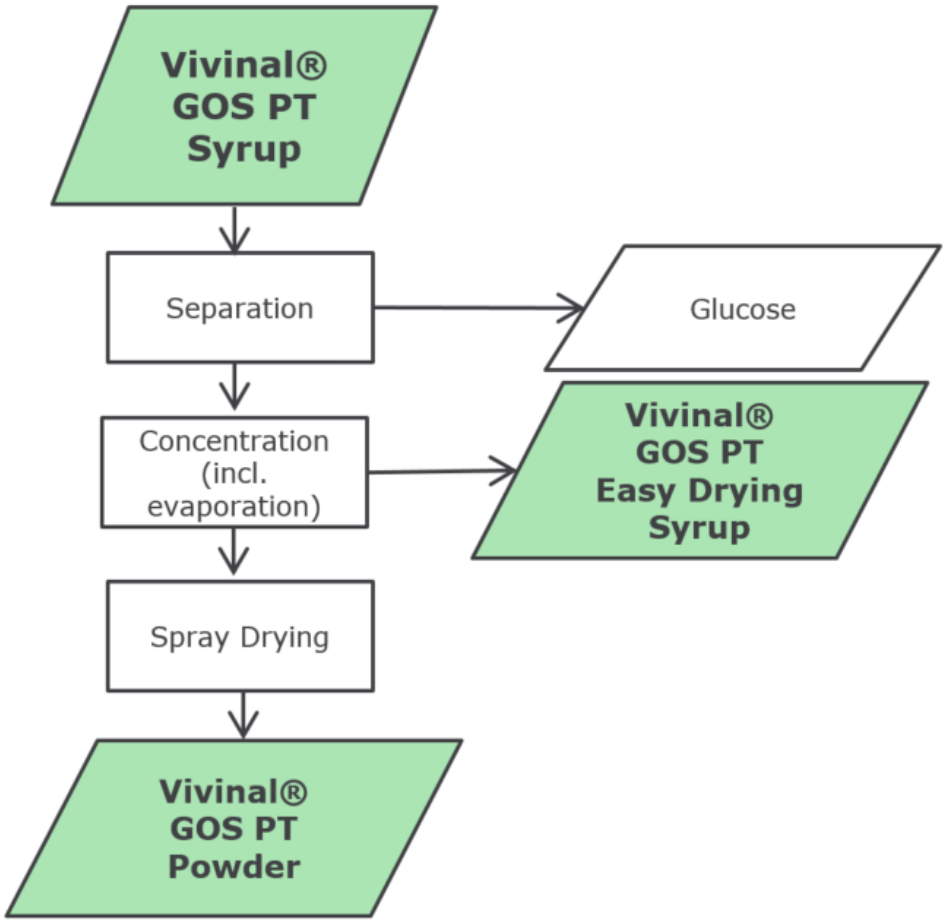


Figure 4 Flow diagram production of Vivinal® GOS PT Easy Drying Syrup & GOS PT Powder



4. Substantial Equivalence of Composition of Vivinal® GOS PT with Vivinal® GOS

4.1 Substantial Equivalence of Proximate Analysis of Vivinal® GOS PT Syrup

The data required to establish substantial equivalence and which are presented below, pertain to Vivinal® GOS PT Syrup and its comparator product Vivinal® GOS Syrup.

As shown in Figure 4, Vivinal® GOS PT Syrup is the starting material for further processing into Vivinal® GOS PT Easy Drying Syrup and Vivinal® GOS PT Powder. Therefore, below we only elaborate on the proximate analysis, degree of polymerization, nutritional value and levels contaminants of Vivinal® GOS PT Syrup and compare these parameters with their Vivinal® GOS counterpart. From the substantial equivalence thus shown for Vivinal® GOS PT Syrup, it is inferred that likewise Vivinal® GOS PT Easy Drying Syrup and Vivinal® GOS PT Powder will be substantially equivalent to their Vivinal® GOS comparator products (Vivinal® GOS Easy Drying Syrup and Vivinal® GOS Powder, respectively).

Applying the above described manufacturing process, the proximate analyses of the thus obtained Vivinal® GOS PT Syrup-batches are indicated in Table 4.1. In this table it is shown that the specifications of the four Vivinal® GOS PT Syrup batches produced by applying four different batches of *P. terrestris* β-Galactosidase enzyme, are highly consistent (low inter-batch variation) and are shown to be substantially equivalent with the specifications of a representative batch Vivinal® GOS Syrup, the latter representing the already established Vivinal® GOS Syrup specifications.

Table 4.1 Proximate analysis of Vivinal® GOS PT Syrup

Test Parameter	**Vivinal® GOS Specification**	**Vivinal® GOS PT 731**	**Vivinal® GOS PT 741**	**Vivinal® GOS PT 631**	**Vivinal® GOS PT 431**	**Vivinal® GOS 704212**
Dry matter	74 - 76 %	**76,08**	**75,09**	**76,39**	**76,79**	**74.26**
Galacto-oligosaccharides	Min. 57% on DM	62,2	63,14	63,8	64,82	58.14
Nitrogen	Max. 0.032% on DM	0,0016	<0,0016	<0,0016	<0,0016	0,0016
Protein		0,01	<0,01	<0,01	<0,01	0.01
Sulphated Ash	Max.0,3% on DM	< 0,01	0,02	0,02	0,02	0.01
Lactose	Max.23% on DM	18,3	17,6	16,62	17,07	20,04
Glucose	Max.22% on DM	18,4	18,16	18,39	17,24	20,36
Galactose	Min.0.8% on DM	1,03	1,1	1,19	0,88	1.46
Nitrite	Max. 2 ppm on DM	0,08	0,07	0,07	0,07	0.02
pH	2,8 – 3,8	3,34	3,24	3,08	3,2	2,9

4.2 Substantial Equivalence of composition of Vivinal® GOS PT Syrup

We have further analyzed the degree of polymerization (DP) of the four Vivinal® GOS PT Syrup batches and compared those with a typical DP pattern of a Vivinal® GOS Syrup batch (Table 4.2). It was shown that the DP among the four Vivinal® GOS PT Syrup batches was highly consistent. More importantly, the DPs of the Vivinal® GOS PT Syrup batches were substantially equivalent when compared to the DP of a typical Vivinal® GOS Syrup batch. However, it is noted that levels of DP2 through DP4 were slightly higher in Vivinal® GOS PT Syrup, whereas levels of DP5 and DP6 were lower. These slight differences notwithstanding, we consider Vivinal® GOS PT Syrup to be substantially equivalent with Vivinal® GOS Syrup.

Therefore, with respect to composition we consider Vivinal® GOS PT Syrup substantially equivalent to Vivinal® GOS Syrup.

Table 4.2 Comparison of degree of polymerization of Vivinal® GOS PT Syrup

	galactose	glucose	DP2	DP3	DP4	DP5	DP6	DP7	Total GOS
Vivinal® GOS PT 731	0,91	18,93	41,15	26,02	11,30	1,56	0,14	-	100
Vivinal® GOS PT 741	1,19	18,68	41,21	25,99	11,26	1,53	0,14	-	100
Vivinal® GOS PT 631	1,13	19,11	40,59	25,63	11,67	1,71	0,16	-	100
Vivinal® GOS PT 431	0,82	18,08	41,25	26,43	11,67	1,60	0,15	-	100
Vivinal® GOS	1,31	21,12	37,42	22,02	10,76	4,88	1,90	0,60	100

Additionally, we have determined the mineral content of the six most abundant minerals (Table 4.3) present in infant and follow-on formula (as per Annex I of Directive 2006/141/EC) in Vivinal® GOS PT Syrup and assured that addition of Vivinal® GOS PT Syrup to infant and follow-on formula (for Intended uses of Vivinal® GOS PT Syrup, paragraph 4.5) will not disturb the delicate mineral balance of these formula.

Table 4.3 Element analysis Vivinal® GOS PT Syrup

Test Parameter	Vivinal® GOS PT 731	Vivinal® GOS PT741	Vivinal® GOS PT 631	Vivinal® GOS PT 431	Vivinal® GOS 704212
Calcium (mg/kg)[+]	ND	ND	ND	ND	ND
Magnesium (mg/kg)*	ND	ND	ND	ND	ND
Phosphorus (mg/kg)[&]	ND	ND	ND	ND	ND
Potassium(mg/kg)[%]	ND	ND	ND	ND	ND
Sodium(mg/kg)[$]	ND	ND	ND	ND	ND
Chloride (%)[#]	ND	ND	ND	ND	ND

ND: not detected
Limits of Detection (LOD): [+]Calcium: 10 mg/kg, *Magnesium: 1 mg/kg, [&]Phosphorus: 10 mg/kg, [%]Potassium: 25 mg/kg, [$]Sodium: 20 mg/kg, [#]Chloride: 0.01%

4.3 Substantial equivalence in levels of undesirable substances

Heavy metal levels were determined and are presented in Table 4.4. The results pertain to the four batches of Vivinal® GOS PT which are compared with Vivinal® GOS.

Table 4.4 Comparison of heavy metal contamination Vivinal® GOS PT Syrup

Test Parameter	Vivinal® GOS Norm	Vivinal® GOS PT 731	Vivinal® GOS PT 741	Vivinal® GOS PT 631	Vivinal® GOS PT 431	Vivinal® GOS 704212
Arsenic*	Max. 100 µg/kg	ND	ND	ND	ND	ND
Cadmium[&]	Max. 10 µg/kg	ND	ND	ND	ND	ND
Lead[%]	Max. 50 µg/kg	ND	ND	ND	ND	ND
Mercury[$]	Max. 50 µg/kg	ND	ND	ND	ND	ND
Aluminum[#]	Max. 4.8 mg/kg	ND	ND	ND	ND	ND

ND: not detected
Limits of Detection (LOD) *Arsenicum: 10 µg/kg, [&]Cadmium: 5 µg/kg, [%]Lead: 20 µg/kg, [$]Mercury: 3 µg/kg, [#]Aluminium: 0.2 mg/kg.

In conclusion and with respect to heavy metal contamination, Vivinal® GOS PT Syrup is considered substantially equivalent with Vivinal® GOS Syrup.

Microbiological information was determined for the microorganisms indicated in Table 4.5. The results pertain to the four batches of Vivinal® GOS PT Syrup compared with Vivinal® GOS Syrup.

Table 4.5 Microbiological analysis of Vivinal® GOS PT Syrup

Vivinal® GOS PT	**Vivinal® GOS Specification**	**Vivinal® GOS PT 731**	**Vivinal® GOS PT 741**	**Vivinal® GOS PT 631**	**Vivinal® GOS PT 431**	**Vivinal® GOS 704212**
Aerobic mesophilic count 30°C	Max. 3000 cfu/g	17	80	2	<1	<1
B. cereus	Max. 50 cfu/g	40	<10	<10	<10	<10
E. coli	Absent in 5 g	NEG	NEG	NEG	NEG	NEG
Enterobacetriaceae	Absent in 1 g	NEG	NEG	NEG	NEG	NEG
Yeasts	Max. 50 cfu/g	9	<1	<1	<1	<1
Salmonella	Absent in 125 g	NEG	NEG	NEG	NEG	NEG
S. aureus	Absent in 1 g	NEG	NEG	NEG	NEG	NEG
Moulds	Max. 50 cfu/g	<1	8	<1	<1	<1
SRC	Max. 30 cfu/g	<1	<1	<1	<1	<1

The data presented in Table 4.5 show that microbiological contamination of Vivinal® GOS PT Syrup is within specifications, and therewith confirms the substantial equivalence with microbiological contamination as reported for the comparator Vivinal® GOS Syrup.

Taken together, with respect to microbiological and heavy metal contamination, we consider Vivinal® GOS PT Syrup substantially equivalent to Vivinal® GOS Syrup. Further, from the data pertaining to the levels of contaminants and showing substantial equivalence of Vivinal® GOS PT Syrup with Vivinal® GOS Syrup, it is inferred that also the Easy Drying Syrup and Powder product variants of Vivinal® GOS PT will be substantially equivalent to their Vivinal® GOS comparator products with respect to heavy metal and microbiological contaminants.

4.4 Substantial equivalence of Nutritional Value and Metabolism

The minor differences in the proportional content of GOS, lactose, glucose and galactose will not have a significant impact on the nutritional value and metabolism of Vivinal® GOS PT Syrup when compared to Vivinal® GOS Syrup.
Indeed, the total energy of Vivinal® GOS PT Syrup is also substantially equivalent to Vivinal® GOS Syrup (241.5 kcal/100 g vs. 240 kcal/100 g, respectively: 1014 kJ/100 g vs 1008 kJ/100 g, respectively).

Therefore, with respect to nutritional value and metabolism, we consider Vivinal® GOS PT substantially equivalent to Vivinal® GOS. In addition, since the Vivinal® GOS PT Syrup derived product variants (Easy Drying Syrup and Powder) are obtained through the application of similar production and processing techniques (in the same production facility at FrieslandCampina Borculo, the Netherlands), it is inferred that the nutritional value and metabolism of Vivinal® GOS PT Easy Drying Syrup and Vivinal® GOS PT Powder are substantially equivalent to their Vivinal® GOS comparator products, respectively.

4.5 Substantial equivalence of Intended Uses

Vivinal® GOS PT is intended for use for the same purposes and at the same levels as Vivinal® GOS, including infant and follow-on formulae (Table 4.6).

Table 4.6. Intended uses of Vivinal® GOS PT

Final foods	Serving size (g)	Maximal GOS using amount (g)	Maximal ratio GOS/kg final food
Milk	244	5	0.020
Milk drinks	250	7.5	0.030
Meal replacement drinks	250	5	0.020
Milk substitutes	245	5	0.020
Yogurt	227	7.5	0.033
Dairy based deserts	70	3	0.043
Frozen dairy deserts	70	3	0.043
Fruit drinks and energy drinks	240	5	0.021
Fitness water and thirst quenchers	240	3	0.013
Juice	240	5	0.021
Fruit pie filling	85	5	0.059
Fruit prep	40	5	0.125
Food Supplements (Dietary supplements)	15	5	0.333
Food Supplements (Fiber supplements)	15	5	0.333
Bars	40	5	0.125
Cereals	40	5	0.125
Infant formula for term infants	1000	8	0.008
Infant meal replacement drinks	250	3	0.012
Baby juice	120	3	0.025
Baby yogurt drink	125	3	0.024
Baby desert	110	3	0.0.27
Baby snack	7	1	0.143
Baby cereals	110	3	0.027

4.6. In conclusion

With this application, we show Substantial Equivalence of our new Vivinal® GOS PT Syrup to Vivinal® GOS Syrup.

Since Vivinal® GOS PT Syrup is the starting material for further processing into Vivinal® GOS PT Easy Drying Syrup and Vivinal® GOS PT Powder, these latter two product variants of Vivinal® GOS PT are also considered to be substantially equivalent to the Vivinal® GOS Easy Drying Syrup and Powder counter products, respectively.

5. References

Aalberse RC. Structural biology of allergens. *J Allergy Clin Immunol.* 2000 Aug;106(2):228-38. Review.

AMFEP 1998 – Report WORKING GROUP ON CONSUMER ALLERGY RISK FROM ENZYME RESIDUES IN FOOD.

Bindslev-Jensen C, Skov PS, Roggen EL, Hvass P, Brinch DS. Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry. *Food Chem Toxicol.* 2006 Nov;44(11):1909-15.

COMMISSION DIRECTIVE 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ 30.12.2006, L 401/1 – 33.

EC 2012 - COMMISSION Implementing REGULATION (EU) 562_2012 - Amending Reg No 234_2011 - implementing rules risk assessment food enzymes.

EFSA 2010 - Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010; 8(7):1700.

FAO 2001 - Evaluation of Allergenicity of Genetically Modified Foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 22 – 25 January 2001. FIntanon M, Arreola SL, Pham NH, Kneifel W, Haltrich D, Nguyen TH. Nature and biosynthesis of galacto-oligosaccharides related to oligosaccharides in human breast milk. *FEMS Microbiol Lett.* 2014 Apr;353(2):89-97.

Koch C, Jensen SS, Oster A, Houen G. A comparison of the immunogenicity of the native and denatured forms of a protein. APMIS. 1996 Feb;104(2):115-25.

Liu, X.-Z., Wang, Q.-M., Theelen, B., Groenewald, M., Bai, F.-Y. & Boekhout, T. (2015a). Phylogeny of tremellomycetous yeasts and related dimorphic and filamentous basidiomycetes reconstructed from multiple gene sequence analyses. *Stud Mycol* 81, 1–26.

Liu, X.-Z., Wang, Q.-M., Göker, M., Groenewald, M., Kachalkin, A. V., Lumbsch, H. T., Millanes, A. M., Wedin, M., Yurkov, A. M. & other authors (2015b). Towards an integrated phylogenetic classification of the Tremellomycetes. *Stud Mycol* 81, 85–147.

Machado Pagani D, Brandão LR, Santos AR, Felix CR, Pais Ramos J, Broetto L, Scorzetti G, Fell JW, Augusto Rosa C, Valente P, Fontes Landell M. Papiliotrema leoncinii sp. nov. and Papiliotrema miconiae sp. nov., two tremellaceous yeast species from Brazil. *Int J Syst Evol Microbiol.* 2016 Apr;66(4):1799-806.

REGULATION (EC) No 258/97 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 January 1991 concerning novel foods and novel food ingredients.

REGULATION (EC) No 1331/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 establishing a common authorization procedure for food additives, food enzymes and food flavourings