



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) **Date de dépôt PCT/PCT Filing Date:** 2022/06/30
(87) **Date publication PCT/PCT Publication Date:** 2023/01/05
(85) **Entrée phase nationale/National Entry:** 2023/12/15
(86) **N° demande PCT/PCT Application No.:** EP 2022/068192
(87) **N° publication PCT/PCT Publication No.:** 2023/275323
(30) **Priorités/Priorities:** 2021/06/30 (EP21382576.3);
2022/02/11 (EP22382114.1)

(51) **Cl.Int./Int.Cl. A23L 33/14** (2016.01),
A23L 33/16 (2016.01)
(71) **Demandeur/Applicant:**
AB MAURI (UK) LTD, GB
(72) **Inventeurs/Inventors:**
DE LECEA, CARLOS, ES;
CUNE CASTELLANA, JORDI, ES;
TINTORE GAZULLA, MARIA, ES
(74) **Agent:** GOWLING WLG (CANADA) LLP

(54) **Titre : COMPOSITIONS DE LEVURE PHYSIOLOGIQUEMENT ACCEPTABLES DESTINEES A ETRE UTILISEES DANS LE
TRAITEMENT D'UN TROUBLE GASTRO-INTESTINAL**
(54) **Title: PHYSIOLOGICALLY ACCEPTABLE YEAST COMPOSITIONS FOR USE IN THE TREATMENT OF A GASTRO-INTESTINAL
DISORDER**

(57) **Abrégé/Abstract:**

A physiologically acceptable composition comprising at least one component selected from the group consisting of *S. boulardii* yeasts, *S. boulardii* lysates, *S. boulardii* cell wall components, and *S. boulardii* extracts, further comprising at least one component selected from the group consisting of *K. marxianus* yeasts, *K. marxianus* lysates, *K. marxianus* cell wall components and *K. marxianus* extracts. Optionally, the composition further comprises at least one component selected from the group of *S. cerevisiae* yeasts, *S. cerevisiae* lysates, *S. cerevisiae* cell wall components and *S. cerevisiae* extracts. The invention further relates to the composition for use as a medicament.



Date Submitted: 2023/12/15

CA App. No.: 3223134

Abstract:

A physiologically acceptable composition comprising at least one component selected from the group consisting of *S. boulardii* yeasts, *S. boulardii* lysates, *S. boulardii* cell wall components, and *S. boulardii* extracts, further comprising at least one component selected from the group consisting of *K. marxianus* yeasts, *K. marxianus* lysates, *K. marxianus* cell wall components and *K. marxianus* extracts. Optionally, the composition further comprises at least one component selected from the group of *S. cerevisiae* yeasts, *S. cerevisiae* lysates, *S. cerevisiae* cell wall components and *S. cerevisiae* extracts. The invention further relates to the composition for use as a medicament.

Title: Physiologically acceptable yeast compositions for use in the treatment of a gastro-intestinal disorder

The invention relates to a physiologically acceptable composition, comprising yeast for use in the treatment of a gastro-intestinal disorder, which treatment may be a preventive treatment or the treatment of an individual having said disorder. In particular, a composition according to
5 the invention is suitable for use in the treatment of diarrhoea. In particular, a composition according to the invention is suitable for use in the treatment of a gastro-intestinal disorder caused by a viral infection such as an infection by a rotavirus. The invention further relates to a physiologically acceptable composition comprising inactivated yeast cells. The invention
10 further relates to the composition for use as a medicament, as a food additive or functional ingredient for nutraceuticals, food for special medical purposes and functional foods.

Probiotics are live microorganisms, which confer a health benefit to the host, when administered in adequate amounts. Already for centuries, long
15 before becoming aware of their potential health benefits, humans have benefitted from microorganisms in food, for example in fermented milk and yoghurt. Modern probiotics-containing nutrients and pharmaceuticals are direct derivatives of the early fermented food. To date, the most common probiotics are from the bacterial genera *Lactobacillus* and *Bifidobacterium*,
20 however also yeasts are increasingly being considered as effective probiotic organisms.

Yeast-based probiotics are being recommended by several international guidelines to prevent or treat acute gastrointestinal disorders such as diarrhoea or chronic conditions such as inflammatory bowel disease (IBD)
25 and irritable bowel syndrome (IBS). The probiotic activities of these yeasts are considered multifactorial and includes improvement of gut barrier

function, pathogen competitive exclusion, production of antimicrobial peptides, immune modulation, modulation of the microbiota and trophic effects. Yeast-based probiotics have many advantages over bacterial probiotics, such as a better withstanding of the extreme environments of the stomach to reach the intestines and an insensitivity to antibiotics providing the possibility to have probiotic effects during antibiotic treatment. Several yeast species have been shown to have a probiotic effect. Several yeasts have been suggested to act as probiotics such as some strains belonging to the genera *Chrysonilia*, *Debaromyces*, *Hanseniaspora*, *Kluyveromyces*, *Lachanencea*, *Metschnikowia*, *Pichia*, *Saccharomyces*, *Torulaspora* and *Yarrowia* (Ogunremi *et al.*, 2015. J appl microbiol 117: 797-808; Sugiharto *et al.*, 2018. J adv vet 5(3):332-342; Agarbati *et al.*, 2020. Foods 9(3):287; Dufossé *et al.*, 2021. J Fungi 7(3): 177).

In order to address a need for novel strains of microorganisms which may exert a beneficial effect on health preventively and/or curatively on either specific pathologies or dysfunctions or on both physical and psychic general health conditions, US2010/303778 provides a specific *Saccharomyces cerevisiae* strain (deposited at the Collection Nationale de Cultures de Microorganismes under No. CNCM I-3856) and a specific a *Saccharomyces* var. *boulardii* yeast strain (deposited at the Collection Nationale de Cultures de Microorganismes under No. CNCM I-3799). Said yeasts may e.g. be used as in compositions for treating an intestinal disorder.

Acute diarrhoea is a severe health problem that affects mainly children. Among the causative agents of this disease, rotavirus accounts for a great proportion of severe cases. Before the introduction of oral live vaccines against rotavirus, the virus caused an estimated 111 million episodes of diarrhoea requiring only home-care, 25 million clinic visits, 2 million hospitalizations, and 352,000–592,000 deaths (median 440,000 deaths) in children <5 years of age each year (Parashar *et al.*, 2003. Emerg

Infect Dis 9: 565–572). Despite de availability of new oral live vaccines against the virus since 2006, 65 % of children worldwide remain unvaccinated. To overcome these issues, different nutritional interventions have been tested such as probiotics including *S. boulardii*, *Lactobacillus*
5 *rhamnosus GG (LGG)*, *Lactobacillus reuteri* and *Bifidobacterium*, prebiotics and bioactive components from milk.

There is a continuing need to provide alternative treatment possibilities of medical disorders, in particular gastro-intestinal disorders, such as diarrhoea, IBD or IBS. In particular there is a continuing need to
10 provide alternative treatment possibilities of gastro-intestinal disorders caused by a viral infection, such as an infection by a rotavirus. There is further a continuing need to provide alternative products suitable for improving gut health or improving gastro-intestinal functioning. It is in particular an object to provide a composition, which is suitable to provide an
15 improved gut barrier function, an improved anti-inflammatory effect or improved protection against a gastro-intestinal infection, in particular against a gastro-intestinal viral infection, compared to a known probiotic composition, comprising a yeast such as described in the above discussed prior art. One or more further objects that may be addressed will follow
20 from the description herein below.

We have now found that one or more of said objects are realized by providing a specific composition comprising at least two different yeasts.

Accordingly, the invention relates to a physiologically acceptable composition comprising, typically as active ingredients, (i) at least one
25 component selected from the group consisting of (inactivated) *S. boulardii* yeasts, *S. boulardii* lysates, *S. boulardii* cell wall components, and *S. boulardii* extracts and further comprising (ii) at least one component selected from the group consisting of (inactivated) *K. marxianus* yeasts, *K. marxianus* lysates, *K. marxianus* cell wall components and *K. marxianus*
30 extracts, for use in the treatment of a medical disorder, in particular a

gastro-intestinal disorder. A treatment in accordance with the invention can be prophylactic (preventive) or the treatment can be the treatment of an individual having the medical disorder to be treated. Such treatment can comprise curing the medical disorder, reducing suffering, alleviating or
5 relieving one or more symptoms associated with said medical disorder.

Preferred gastrointestinal disorders to be treated are disorders related to the impairment of the gut barrier function. In a particularly preferred embodiment, the composition according to the invention is for use in the treatment of diarrhoea. Preferred gastrointestinal disorders to be treated
10 are disorders caused by a viral infection, preferably an infection by a rotavirus, such as (acute) diarrhoea in an individual having the viral infection.

In particular in the treatment of an individual having a gastro-intestinal viral infection, good results have been achieved with a
15 composition comprising inactivated *S. boulardii* yeast and inactivated *K. marxianus* yeast, which composition is essentially free of living and of inactivated *S. cerevisiae*, more in particular with a composition wherein the yeast components of the composition essentially consist of inactivated *S. boulardii* yeast and inactivated *K. marxianus* yeast. More in particular such
20 treatment is useful for the treatment of acute diarrhoea.

In addition, the invention relates to a physiologically acceptable composition comprising inactivated *S. boulardii* yeast cells selected from the group consisting of *S. boulardii* DSM 33954, *S. boulardii* CNCM I-745, *S. boulardii* Hansen CBS 5926, *S. boulardii* BLD-3, *S. boulardii* CCTCC
25 M2012116, *S. boulardii* CNCM I-1079, *S. boulardii* ATCC MYA-796, *S. boulardii* Unique28, *S. boulardii* Kirkman, *S. boulardii* Unisankyo and *S. boulardii* CNCM I-3799 and inactivated *K. marxianus* yeast cells selected from the group consisting of *K. marxianus* AS41, *K. marxianus* B0399, *K. marxianus* CIDCA 8154, *K. marxianus* CBS1553, *K. marxianus* M3, *K.*
30 *marxianus* V21/012435 and *K. marxianus* Z17.

Furthermore, the invention relates to said physiologically acceptable composition, for use in maintaining or improving of gut health or gastrointestinal functioning, for use in the prophylactic treatment of an individual or the therapeutic treatment of an individual with a medical disorder, such as a gastrointestinal disorder or a disorder defined by one or more pro-inflammatory markers. Preferred gastrointestinal disorders to be treated are disorders related to the impairment of the gut barrier function. In a particularly preferred embodiment, the composition according to the invention is for use in the treatment of diarrhoea, IBD or IBS. Regarding the use in the treatment of, preferably one or more markers are selected from the group consisting of IL-8, IP-10, MCP-1, TNF α and TNF α /IL-10. Regarding, IL-10, it is observed that this is modulatory / anti-inflammatory compound, of which the concentration may be reduced and result in an increased TNF α /IL-10 ratio, also if the TNF α itself does not increase; this ratio is also a relevant maker for a disorder defined by one or more pro-inflammatory markers. Preferably, a disorder defined by one or more pro-inflammatory markers to be treated in accordance with the invention is selected from the group consisting of rheumatoid arthritis, osteoarthritis, topical dermatitis, psoriasis, allergy and obesity.

The physiologically acceptable composition (for use) according to the invention may be used as a food additive, a feed additive, a functional food in human nutrition, a functional food in animal nutrition, a food additive or functional ingredient for nutraceuticals.

Furthermore, the physiologically acceptable composition (for use) according to the invention may be used as a probiotic, a postbiotic, a paraprobiotic, a prebiotic, a symbiotic or a probiotic-substitute.

Furthermore, the invention relates to a medical device comprising a physiologically acceptable composition according to the invention or physiologically acceptable composition (suitable) for use according to the invention.

As illustrated in the Examples, below, a composition (for use) according to the invention has been found effective in improving one or more relevant markers for gut health, such as a reduction of pro-inflammatory cytokine production by gut epithelial cells and immune cells (i.e. indicating an anti-inflammatory effect) and an increase of trans epithelial electric resistance (i.e. indicating an improved protection of the gut barrier function). Thus, the composition comprising a *S. boulardii* based fraction, *K. marxianus* based fraction and optionally a *S. cerevisiae* or lysates, cell wall components or extracts thereof can be used in a treatment for maintaining gut health. The effect on these markers are indicative of a positive effect for use in the treatment of gastro-intestinal disorders characterized by inflammation and/or a loss of epithelial integrity, like diarrhoea, IBD or IBS. Further, the results are supporting that the composition is also effective for the treatment of a gastrointestinal disorder, such as (acute) diarrhoea or a gastrointestinal infection, because simulating an acute gastrointestinal infection by using a pro-inflammatory stimulus or infecting epithelial cells with a typical bacteria strain causing diarrhoea, such as an *E. coli* strain, several markers for gut health improve. Additionally, the results are supporting that the composition is also effective in the treatment of a gastrointestinal disorder caused by a viral infection, because a protective effect of the composition against rotavirus infection was observed. Thus, it may be used as a prophylactic or to treat a subject experiencing the gastrointestinal disorder. In particular, a surprising effect is obtained by combining inactivated *S. boulardii*, and inactivated *K. marxianus*. Example 5 supports synergy in the treatment of (acute) virally induced diarrhoea for a combination of (inactivated) *S. boulardii*, and (inactivated) *K. marxianus*, without any *S. cerevisiae*-based fraction. Dependent on the intended use it may be preferred to additionally include a *S. cerevisiae*-based fraction, such as inactivated *S. cerevisiae*. In the examples 1-4, it is illustrated how several markers for gut health are improved by such combinations whilst individual

yeasts are ineffective, less effective, or even have an adverse effect. Synergy of a combination of said three yeast fractions, in particular a combination of (inactivated) *S. boulardii*, (inactivated) *K. marxianus*, and (inactivated) *S. cerevisiae* is, amongst others, illustrated for MCP-1 (Figure 2), IL-8 (Figure 3), for IP-10 and MCP-1 after a TNF-alpha/INF-gamma challenge (Figures 4 and 5) and for the ratio TNF-alpha/IL-10 (Figure 7). Furthermore, it is illustrated how said yeast combination improves gut epithelium integrity, measured by the increase in trans epithelial electric resistance (TEER) over a gut cell monolayer (Figure 8, 9 and 10).

Said yeasts or part thereof can each independently be selected from viable yeast cells and non-viable yeast cells. As illustrated by the Examples these yeasts do not need to be viable. Accordingly, the inventors further conclude that one, two or each of said *S. boulardii*, *S. cerevisiae* and *K. marxianus* in the composition can be replaced fully or in part by a yeast lysate, cell wall material or a yeast extract of respectively *S. boulardii*, *S. cerevisiae* and *K. marxianus*

Fig. 1 depicts the *in vitro* IP-10 chemokine production by Caco-2 cells after incubation with 3 yeasts and a combination thereof, in the absence of a pro-inflammatory stimulus.

Fig. 2 describes the *in vitro* production by Caco-2 cells of MCP-1 chemokine after incubation with 3 yeasts and a combination thereof, in the absence of a pro-inflammatory stimulus.

Fig. 3 describes the *in vitro* production by Caco-2 cells of IL-8 chemokine after incubation with 3 yeasts and a combination thereof, in the absence of a pro-inflammatory stimulus.

Fig. 4 depicts the *in vitro* IP-10 chemokine production by Caco-2 cells after incubation with 3 yeasts and a combination thereof, in the presence of a pro-inflammatory stimulus (TNF- α /INF- γ) simulating an inflamed gut epithelium.

Fig. 5 describes the *in vitro* production by Caco-2 cells of MCP-1 chemokine after incubation with 3 yeasts and a combination thereof, in the presence of a pro-inflammatory stimulus (TNF- α /INF- γ) simulating an inflamed gut epithelium.

5 Fig. 6 describes the *in vitro* production by Caco-2 cells of IL-8 chemokine after incubation with 3 yeasts and a combination thereof, in the presence of a pro-inflammatory stimulus (TNF- α /INF- γ) simulating an inflamed gut epithelium.

Fig. 7 shows the *in vitro* reduction in TNF α /IL-10 ratio observed in
10 human THP-1 cells (macrophages).

Fig 8. depicts the protective effect on the gut epithelium by the different yeasts and combinations thereof. A comparison of the yeasts versus the negative control is shown at 1 hour (bars at the left) or 2 hours (bars at the right) of incubation with an infective agent known to disrupt the
15 epithelium monolayer (*E. coli* ETEC H10407). Gut epithelium integrity is measured by the increase in trans epithelial electric resistance (TEER) of the monolayer.

Fig. 9 shows the differentiation of the gut epithelium measured by an increase in TEER when incubating the Caco-2 cells monolayer in the
20 presence of the yeasts (top) and their combination (bottom). Note that some error bars are too little to be visible.

Fig. 10 compares the effect observed in Figure 9 (differentiation of the gut epithelium measured by an increase in TEER when incubating the Caco-2 cells monolayer in the presence of the yeasts and their combination)
25 by means of % of increase vs. the control. Regression formulae for the trend lines are as follows, *S. boulardii*: $y = -0.0374x + 1.1848$; *S. cerevisiae*: $y = 0.011x + 0.9844$; *K. marxianus*: $y = -0.0031x + 1.0408$; *S. boulardii* + *S. cerevisiae*: $y = 0.0081x + 0.9871$; and ABB C22: $y = 0.0142x + 0.9384$.

Fig. 11 shows results for a combination of tyndallized *S. boulardii* and tyndallized *K. marxianus*, supporting the use of this combination as a product to protect against rotavirus infection

For the purpose of clarity and a concise description, features are
5 described herein as part of the same or separate embodiments, however, it will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well. The term "or" includes any and all
10 combinations of one or more of the associated listed items, unless the context clearly indicates otherwise (e.g. if an "either ...or" construction is used). It will be understood that the terms "comprises" and "comprising" specify the presence of stated features but do not preclude the presence or addition of one or more other features. It will be further understood that
15 when a particular step of a method is referred to as subsequent to another step, it can directly follow said other step or one or more intermediate steps may be carried out before carrying out the particular step, unless specified otherwise.

The term "(at least) substantial(ly)" is generally used herein to indicate that
20 it has the general character or function of that which is specified. When referring to a quantifiable feature, this term is generally used to indicate that it is more than 50 %, in particular at least 75 %, more in particular at least 90 %, even more in particular at least 95 % of the maximum of that feature.

The term 'essentially free' is generally used herein to indicate that a feature
25 is not present or present in such a low amount that it does not significantly affect the property of the product.

In the context of this application, the term "about" means generally a deviation of 15 % or less from the given value, in particular a deviation of 10% or less, more in particular a deviation of 5% or less.

As is used herein, the term “physiologically acceptable composition”, refers to a composition that is suitable for administration to an individual such as an animal or a human.

As is used herein, the term “probiotics”, refers to live microorganisms, which when administered in adequate amounts, confer a health benefit to an individual. Probiotics include all sorts of microorganisms including bacteria and yeasts.

As is used herein, the term “inactivated”, or “dead”, or “non-viable”, refers to an organism, such as a yeast, not being capable of reproduction or colonization. An inactivated organism can have intact or broken cell membranes. The skilled person will be able to obtain an inactivated organism yeast based on common general knowledge and the information disclosed herein. Possible means include irradiation, heat inactivation, sonication, lyophilization and chemical inactivation.

As used herein, the term “heat-killed”, refers to an organism inactivated by heat-treatment and such not capable of metabolic activity nor colonization. Means of heat-treatment to inactivate an organism are known to a person skilled in the art and include tyndallization, pasteurization, ultra-high temperature (UHT) heating, Ohmic heating (or Joule heating), blanching, drying, boiling and sterilization.

As is used herein, the term “tyndallization”, refers to a sterilization process often used to heat-kill probiotic microorganisms. Tyndallization involves repeating a heat-killing step for a certain consecutive days, for example as described on page 11 in the Handbook of Microbiological Media (third edition, 2004, CRC Press) by Ronald M. Atlas. Therefore, the term “tyndallized”, refers to an organism having been heat-killed by tyndallization and such not capable of metabolic activity or colonization.

As used herein, the term “cell lysis” refers to any type of cell disruption that results in the release of intercellular biological components naturally contained in the cells of an organism. Therefore, the term “lysate”, refers to

a product obtained after cell lysis. A “lysate”, as used herein, means in particular essentially the entire lysate obtained by lysis of an organism and therefore comprises macromolecules such as DNA, RNA, proteins, peptides and lipids from the lysed cell; as well as cellular debris, including cell wall material, and cell membrane components from the lysed cells. Methods for
5 obtaining a lysate are known to a person skilled in the art and include enzymatic, physical and chemical methods. Cell wall components can be separated from the fluid part of the lysate e.g. by centrifugation or filtering.

As used herein, the term “extract” of a yeast, refers to a fluid part or
10 fraction of the yeast cell or lysate, in particular liquid contents of yeast cells, in particular obtainable by filtration or by centrifugation, or a fraction thereof, obtainable by extraction from the cells or the lysate, using an extracting phase,

As used herein, the term “metabolite”, refers to any substance derived
15 from the growth or maintenance of the yeast, persisting in the culture medium and with no need to be preserved by special techniques. Examples of metabolites are organic and inorganic acids, proteins, (poly)peptides, amino acids, (co)enzymes, fatty acids, (esterified) lipids, carbohydrates (including monosaccharides, disaccharides and polysaccharides),
20 lipoproteins, glycolipids, glycoproteins, sugar phosphates, vitamins, salts, metals, or nucleic acids.

As is used herein, the term “alive”, or “viable”, refers to an organism being capable of reproduction or colonization.

As is used herein, the term “individual”, refers to any living organism
25 such as an animal or human that can benefit from the administration of a physiologically accepted composition of the invention. The term “animal” as used herein, refers in particular to vertebrate animals including fish, birds, mammals, reptiles and amphibians. The animal can be a farm animal, domestic animal or laboratory animal. An individual to be treated in
30 accordance with the invention may in particular be selected from humans,

nonhuman primates and monkey species, cows, sheep, pigs, goats, horses, dogs, cats, rodents such as mice, rats and guinea pigs, poultry, such as chickens, hens, turkeys, ducks and geese and aquatic animals such as fish and shrimp. The term individual does not denote a particular age or sex
5 (such as male/female). Thus, humans of any age group, including adults (18 years of age and older) and children (0-17 years of age), e.g. newborn individuals (0-12 months of) or toddlers (12-36 months of age), can be treated in accordance with the invention. In a preferred embodiment, the composition is for use of the treatment of a human, for which the examples
10 in particular illustrate a beneficial, even synergistic, effect on intestinal cells.

As is used herein, the term “nutritional product”, refers to a composition intended for ingestion by an individual providing at least one nutrient to the individual. Nutritional products generally comprise one or
15 more components selected from the group consisting of protein, fat, carbohydrate and micro-nutrients.

As is used herein, the term “nutraceutical”, refers to any nutritional product providing extra health benefits in addition to the basic nutritional value found in foods.

20 As is used herein, the term “oral rehydration salt (ORS)”, refers to a saccharide-based salt solution suitable for use in oral rehydration therapy. ORS are recommended in the prevention of dehydration from diarrhoea from any cause in individuals of any age. ORS’s are further recommended to treat a dehydrated individual of any age.

25 As used herein, the term “prebiotic” refers to any substance that is selectively utilized by microorganisms conferring a health benefit to an individual. Prebiotics are in particular nondigestible food ingredients that stimulate the growth and/or activity of said microorganisms.

As used herein, the term “postbiotic” refers to any preparation of
30 inactivated microorganisms and/or their components that confers a health

benefit to an individual. The components that confer a health benefit may be a mixture of metabolic products secreted by probiotics in cell-free supernatants, such as enzymes, secreted proteins, short chain fatty acids, vitamins, secreted biosurfactants, amino acids, peptides, organic acids, etc.

5 As used herein, the term “paraprobiotic” refers to inactivated microorganisms and/or cell fractions that confers a health benefit to an individual..

As used herein, the term “synbiotic” or “symbiotic”, refers to any preparation comprising a mixture of probiotics and prebiotics.

10 Hereinbelow, the term “yeast material” or “yeast based fraction” is used as a genus for living yeast cells, inactivated yeast cells, yeast lysates, yeast cell wall components and yeast extracts. Likewise, the term “microbiological material” is used as a genus for living microorganisms, inactivated microorganisms, lysates of microorganisms, cell wall
15 components of microorganisms and extracts of microorganisms.

Components of a physiologically acceptable composition

In this invention, the *S. boulardii* can be any strain classified or classifiable as *S. cerevisiae* var. *boulardii*, in particular any such strain that
20 is probiotic in a living or inactivated form. In an embodiment, the composition comprises at least one strain selected from the group consisting of *S. boulardii* DSM 33954, *S. boulardii* CNCM I-745, *S. boulardii* Hansen CBS 5926, *S. boulardii* BLD-3, *S. boulardii* CCTCC M2012116, *S. boulardii* CNCM I-1079, *S. boulardii* ATCC MYA-796, *S. boulardii* Unique28,
25 *S. boulardii* Kirkman, *S. boulardii* Unisankyo and *S. boulardii* CNCM I-3799. As illustrated in the Examples, in particular good results have been achieved with a composition comprising *S. boulardii* DSM 33954.

In this invention, the *S. cerevisiae* can be any strain belonging to the species *S. cerevisiae*, in particular any strain that is probiotic in a living
30 or inactivated form, with the exception of strains classified or classifiable as

S. cerevisiae var. *boulardii*. The species *S. cerevisiae* is also referred to in the art as baker's yeast, brewer's yeast or *Candida robusta*.

In a composition in accordance with the present invention, the presence of *S. cerevisiae* based fraction is optional. The *S. cerevisiae* based fraction, in particular inactivated *S. cerevisiae* yeast, used as an active ingredient in combination with inactivated *S. boulardii* and inactivated *K. marxianus* as active ingredients has been found advantageous – amongst others in providing an anti-inflammatory effect (Examples 1 and 2) and in supporting intestinal barrier integrity (Examples 3 and 4). On the other hand, as supported in Example 5, a composition that is essential free of *S. cerevisiae* based fraction, is particularly effective in providing protection to intestinal cells against a viral infection. Good results have been achieved in particular with a mineral-enriched *S. cerevisiae*, such as zinc-enriched *S. cerevisiae*.

If *S. cerevisiae* is present in the composition, it preferably comprises *S. cerevisiae* based fraction, in particular inactivated yeast cells, selected from the group of *S. cerevisiae* CNCM I-3856, *S. cerevisiae* S288C, *S. cerevisiae* Y1529 and *S. cerevisiae* UFMG 905, of which *S. cerevisiae* S288C (as deposited at ATCC) and *S. cerevisiae* Y1529 (as deposited at ATCC) are particularly preferred. In particular good results have been achieved with *S. cerevisiae* Y1529.

In this invention, the *K. marxianus* can be any strain belonging to the species *K. marxianus*, This species is also referred to in the art as *Saccharomyces marxianus*, *Candida Kefyr*, *Candida pseudotropicalis*, *Kluyveromyces fragilis*, *Kluyveromyces cicerisporus*. In an embodiment, the composition comprises one or more strains selected from the group consisting of *K. marxianus* AS41, *K. marxianus* B0399, *K. marxianus* CIDCA 8154, *K. marxianus* CBS1553, *K. marxianus* M3, *K. marxianus* V21/012435 and *K. marxianus* Z17. Good results have in particular been achieved with a composition according to the invention comprises *K.*

marxianus V21/012435 cells. Accordingly, *K. marxianus* V21/012435 cells, an extract thereof, a lysate thereof or cell wall material thereof is present in a preferred composition of the invention.

A person skilled in the art will be able to determine if a given yeast strain is classified or classifiable as *S. cerevisiae* var. *boulardii*, as species *S. cerevisiae* or as *K. marxianus* by using standard references such as e.g. “The yeasts, a taxonomic study” (CP Kurtzman, JW Fell and T Boekhout), 5th edition, 2011, Elsevier. Furthermore, person skilled in the art will be able to differentiate *S. cerevisiae* var. *boulardii* from other yeast strains belonging to the species *S. cerevisiae* based on standard references such as e.g. Edwards-Ingram L, Gitsham P, Burton P, *et al.*, “Genotypic and physiological characterization of *Saccharomyces boulardii*, the probiotic strain of *Saccharomyces cerevisiae*”, Appl Environ Microbiol 2007; 73: 2458–67.

The relative amounts of the (i) at least one component selected from the group consisting of (inactivated) *S. boulardii* yeasts, *S. boulardii* lysates, *S. boulardii* cell wall components and *S. boulardii* extracts (also referred to herein as *S. boulardii*-based fraction) and the (ii) at least one component selected from the group consisting of (inactivated) *K. marxianus* yeasts, *K. marxianus* lysates, *K. marxianus* cell wall components and *K. marxianus* extracts (also referred to herein as *K. marxianus*-based fraction) can vary within wide limits.

Additionally, if present the relative amounts of the at least one component selected from the group of *S. cerevisiae* yeasts, *S. cerevisiae* lysates, *S. cerevisiae* cell wall components and *S. cerevisiae* extracts (also referred to herein as *S. cerevisiae*-based fraction), can also vary within wide limits.

Generally, the *S. boulardii*-based fraction, in particular said fraction essentially consisting of inactivated *S. boulardii* cells, of the composition (for use) according to the invention is: 0.05 – 99.95 wt.%, preferably 5 – 95

wt. %, more preferably 20-80 wt. %, in particular 20-60 wt.%, more in particular 25-50 wt.%, based on total yeast components.

Generally, if present in the composition (for use) according to the invention and the composition is for a different purpose than the treatment
5 of a diarrhoea, in particular a virally induced diarrhoea, the *S. cerevisiae*-based fraction, in particular said fraction essentially consisting of inactivated *S. cerevisiae* cells of the composition (for use) according to the invention is in the range of 0.05 – 90 wt.%, preferably 10-90 wt.%, more preferably 20 - 80 wt. %, based on total yeast components. This has been
10 found advantageous for certain anti-inflammatory effects and gut barrier integrity. For a composition intended to be used in the treatment of diarrhoea, in particular a diarrhoea associated with a viral infection, more in particular a diarrhoea associated with a rotavirus infection or an infection with a virus selected from the group of noroviruses, enteric
15 adenoviruses, astroviruses, hepatitis viruses and cytomegalovirus, the *S. cerevisiae*-based fraction usually is 0-30 wt.% based on total yeast components. In particular preferred is a content of 0-0.044 wt.%. Alternatively a content in the range of 0.045-20 wt. %, in particular 0.050-10 wt.%, more in particular in particular 0.5-5 wt.% based on total yeast
20 components can be used in the treatment of diarrhoea, in particular diarrhoea associated with a viral infection, such as a rotavirus infection.

Generally, the *K. marxianus*-based fraction of the composition (for use), in particular said fraction essentially consisting of inactivated *K. marxianus* cells according to the invention is: 0.05 – 99.95 wt.%, preferably 5 – 95 wt.
25 %, more preferably 20-80 wt. %, in particular 20-60 wt.%, more in particular 25-50 wt.% based on total yeast components.

Usually, the *S. boulardii*-based fraction, the *S. cerevisiae*-based fraction and the *K. marxianus*-based fraction form together at least 10 wt.% of the total microbiological material (such as bacterial, algae or fungal cells,
30 lysates thereof, extracts thereof, cell wall parts thereof), preferably at least

25 wt. %, more preferably at least 50 wt. %, in particular at least 75 wt.%. If desired, other microbiological material may be present in the composition, in particular a probiotic micro-organism or cell material of a probiotic micro-organism. Thus, the total of the *S. boulardii*-based fraction, the *S.*

- 5 *cerevisiae*-based fraction and the *K. marxianus*-based fraction is 100 % of the total microbiological material or less, for instance 99 wt. % or less, based on total microbiological material.

In an advantageous embodiment, the physiological composition comprises 5-95 wt.% *S. boulardii*-based fraction, 10-80 wt. % *S. cerevisiae*-
10 based fraction and 5-95 wt.% *K. marxianus*-based fraction (all based on based on total microbiological material). In particular, with such composition good results are achieved, e.g. with respect to several pro-inflammatory markers or TEER with a composition having a *S. boulardii*-based fraction content in the range of 15-50 wt. %, a *S. cerevisiae*-based
15 fraction content in the range of 15-50 wt. % and a *K. marxianus*-based fraction content in the range of 15-50 wt. %, all based on of the total microbiological material of the composition, with the proviso that the total of said three fractions is 100 wt. % or less. Herein, advantageously the yeast based fraction at least substantially consists of inactivated yeast cells. Herein,
20 advantageously, the total microbiological material at least substantially consists of inactivated yeast cells. As the skilled person will understand, based on the information disclosed herein and common general knowledge, different contents may be applied with satisfactory results.

In another advantageous embodiment, the physiological composition
25 comprises 5-95 wt.% *S. boulardii*-based fraction (in particular inactivated *S. boulardii*) and 5-95 wt.% *K. marxianus*-based fraction (in particular inactivated *K. marxianus*), based on total yeast components in the composition, and is essentially free of *S. cerevisiae* based fraction (essentially free of *S. cerevisiae* yeasts, *S. cerevisiae* lysates, *S. cerevisiae* cell
30 wall components and *S. cerevisiae* extracts). In particular, good results are

achieved, e.g. with respect to protection against a viral infection, such as a rotavirus infection, of a composition having a *S. boulardii*-based fraction content (in particular inactivated *S. boulardii* content) in the range of 20-80 wt. % and a *K. marxianus*-based fraction content (in particular inactivated *K. marxianus* content) in the range of 20-80 wt. %, all based on of the total yeast material of the composition. Herein, the total of said two fractions is 100 wt. % or less of total yeast components, preferably 50-100 wt.%, more preferably 80-100 wt.% of total yeast components, in particular 90-99 wt.%. As the skilled person will understand, based on the information disclosed herein and common general knowledge, different contents may be applied with satisfactory results.

Since safety issues with the use of live microorganisms have been arisen, for example in fragile or immunocompromised patient groups or neonates, interest in using non-viable inactivated probiotics has increased. Several inactivation methods are known to a person skilled in the art and include irradiation, heat inactivation, sonication, lyophilization and chemical inactivation. Tyndallization is a sterilization process often used to heat-kill probiotic microorganisms. Interestingly, in accordance with the invention tyndallized yeasts have shown to exert relevant biological responses such as restoring the normal intestinal homeostasis.

Accordingly, advantageously, the physiologically acceptable composition of the invention comprises at least one inactivated yeast selected from *S. boulardii*, *S. cerevisiae* and *K. marxianus*. Preferably inactivated *S. boulardii* and inactivated *K. marxianus* are present in the composition (for use) according to the invention. In an embodiment inactivated *S. cerevisiae* is further present in the composition according to the invention. If one or more of said yeasts is a mineral-enriched yeast, such as a zinc-enriched yeast, it is in particular preferred that the mineral-enriched yeast is inactivated. Preferably, the inactivated yeast is heat-killed. More preferably, the inactivated yeast is tyndallized. The

tyndallization may be based on tyndallization processes generally known in the art. In particular, good results have been achieved with a composition comprising a tyndallized *S. boulardii*, further a tyndallized *K. marxianus* and optionally further a tyndallized *S. cerevisiae*.

5 Although good results have been achieved with a compositions that is essentially free of viable yeast cells, , the physiologically acceptable composition of the invention may comprise at least one live yeast selected from *S. boulardii*, *S. cerevisiae* and *K. marxianus*.

 The *S. boulardii* as included in the physiologically acceptable
10 composition of the invention for administration into the gastrointestinal tract is usually present in a concentration ranging from 10^6 cells per gram (based on total weight of the yeast components) to 10^{11} cells per gram (based on total weight of the yeast components), preferably from 10^7 cells per gram (based on total weight of the yeast components) to 10^{11} cells per gram (based
15 on total weight of the yeast components), more preferably 10^9 cells per gram (based on total weight of the yeast components) to 2×10^{10} cells per gram (based on total weight of the yeast components).

 The *S. cerevisiae* - if included in the physiologically acceptable composition of the invention for administration into the gastrointestinal
20 tract is usually present in a concentration of up to 10^{11} cells per gram (based on total weight of the yeast components). When included in a composition intended for a use different from treatment of viral diarrhoea, in particular different from diarrhoea associated with a rotavirus infection, if present *S. cerevisiae* is preferably included in a concentration ranging from 10^6 to 10^{11}
25 cells per gram (based on total weight of the yeast components), preferably from 10^8 cells per gram (based on total weight of the yeast components) to 5×10^9 cells per gram (based on total weight of the yeast components). For a composition to be used in the treatment of diarrhoea in a person with a gastrointestinal viral infection, in particular diarrhoea associated with a
30 rotavirus infection, the *S. cerevisiae* concentration is advantageously less

than 10^6 cells per gram (based on total weight of the yeast components, in particular less than 10^5 cells per gram (based on total weight of the yeast components, more in particular $0-10^4$ cells per gram (based on total weight of the yeast components. In particular good results in the treatment of a viral diarrhoea have been achieved with a composition that is essentially free of a *S. cerevisiae* based fraction.

The *K. marxianus* as included in the physiologically acceptable composition of the invention for administration into the gastrointestinal tract is usually present in a concentration ranging from 10^6 cells per gram (based on total weight of the yeast components) to 10^{10} cells per gram (based on total weight of the yeast components), preferably from 10^7 cells per gram (based on total weight of the yeast components) to 5×10^9 cells per gram (based on total weight of the yeast components).

S. boulardii as included in the physiologically acceptable composition of the invention for another mode of administration (such as topical administration— in particular as part of a medical device) is usually present in a concentration ranging from 10^4 cells per gram (based on total weight of the yeast components) to 10^{11} cells per gram (based on total weight of the yeast components) , preferably 10^6 cells per gram (based on total weight of the yeast components) to 10^{10} cells per gram (based on total weight of the yeast components). *S. cerevisiae* if included in the physiologically acceptable composition of the invention for another mode of administration, such as topical administration, is usually present in a concentration ranging from 10^4 cells per gram (based on total weight of the yeast components) to 10^{11} cells per gram (based on total weight of the yeast components), preferably 10^6 cells per gram (based on total weight of the yeast components) to 10^{10} cells per gram (based on total weight of the yeast components). *K. marxianus* as included in the physiologically acceptable composition of the invention for another mode of administration, such as topical administration, is usually present in a concentration ranging from 10^4 cells

per gram (based on total weight of the yeast components) to 10^{11} cells per gram (based on total weight of the yeast components), preferably 10^6 cells per gram (based on total weight of the yeast components) to 10^{10} cells per gram (based on total weight of the yeast components).

5 Concentrations of live and inactivated yeasts are measured and expressed in cells per gram total yeast components. For live yeasts the concentration in 'cells per gram' is equivalent as 'colony forming units (CFU) per gram total yeast components. If a combination of live cells and inactivated cells (not colony forming) are present', the total of cells is will
10 usually be in the above mentioned usual range, preferably in an above mentioned preferred range or more preferred range. For a yeast lysate, for an extract or for cell wall components a suitable concentration usually corresponds to the amount of lysate, extract respectively cell wall components obtainable from 10^4 cells per gram (based on total weight of the
15 yeast components) to 10^{11} cells per gram (based on total weight of the yeast components).

The physiologically acceptable composition of the invention may comprise a yeast, that is mineral-enriched, e.g. a zinc-enriched *S. cerevisiae*
20 or (e.g. in the absence of *S. cerevisiae*) zinc-enriched *S. Boulardii* or zinc-enriched *K. Marxianus*. The physiologically acceptable composition of the invention may comprise a mineral salt, preferably zinc salt, most preferably a zinc sulphate.

Zinc-enriched yeast provide a natural source of highly bioavailable
25 zinc. Zinc is considered a key nutrient for immunity and diarrhoea management. Interestingly, it has been shown that zinc supplementation as organically-bound or blended zinc via yeast organisms results in a better bioavailability compared to inorganic zinc. Not only zinc, but several other minerals such as selenium, chromium, iron, copper, magnesium,
30 manganese, potassium, calcium and iodine have been shown to be beneficial

in restoring an individuals' mineral balance and can be enriched to yeasts to become a better bioavailability. A mineral-enriched yeast can be obtained by culturing a yeast in a medium in which one or more minerals are added, such as zinc, selenium, chromium, iron, copper magnesium, manganese, potassium, calcium or iodine. The mineral typically binds organically to yeast protein or is otherwise absorbed, resulting in yeasts having the one or more minerals absorbed in their cells. The minerals can be added to the medium before, during or after culturing. As used herein, a mineral-enriched yeast strain, is in particular a yeast strain fermented in the presence of a mineral salt or to which a mineral salt has been added after fermentation, containing a final concentration of such mineral up to 12 wt. % of the dry wt. of the whole product, in particular up to 5 wt. %, more in particular up to 2 wt.%, e.g. up to 1 wt. %. For zinc addition to a final concentration in the range of 1-12 wt. %, in particular of about 4 wt.% to about 10 wt.% is preferred.

Administration mode and formulations of a physiologically acceptable composition

The physiologically acceptable composition of the present invention is preferably administered into the gastrointestinal tract. Administration into the gastrointestinal tract is preferably orally. In a specific embodiment, the composition is administered by tube feeding or as a suppository.

Formulations of a composition according to the invention suitable for oral intake include but are not limited to: capsules, coated capsules, tablets, sachets, pills, pearls, softgels, vials, powders, granules, solutions, suspensions, emulsions, elixirs, syrups, sprays, lozenges, troches, gums, hard candies and gels. Formulations of a composition according to the invention suitable for topical administration include compositions suitable for application to a part of the gastro-intestinal tract at which the effect of

the yeast components is needed, such as a suppository or a gastro-intestinal medical device.

Topical administration in the treatment of a gastrointestinal disorder generally comprises administration at mucosa or epithelium of the gastro-
5 intestinal tract. A medical product comprising the composition (for use) according to the invention can be a product suitable to create a protective biofilm or the like at the surface of the gut epithelium. Such product can be based for instance on known products for treatment of IBS.

In a specific embodiment, the composition is to be administered as a
10 sustained release product. The composition (for use) according to the invention may be a food product. The food product may be a fermented food product or a non-fermented food product. Examples of a particularly suitable food products include dairy products such as a yogurt, a yogurt drink, cheese, milk, milk powder, infant formula, cream, ice-cream, cream
15 powder and butter; fruit-based products such as fruit juice, compote or fruit jelly; solid foodstuffs such as flours, cereals, a snack, a biscuit, and liquid formulations such as vegetable beverages, smoothies, isotonic drinks, salts solutions and enteral nutrition recipes. The physiologically acceptable composition (for use) according to the invention for intake by an animal may
20 be any appropriate food product for animals and include, in addition to the food products listed above, tablets, coated tablets, granules, cereal grains, (dried) meat, (dried) fish, oil meals, cakes, cookies, sugarcane, and roughages such as grasses, hays, silage, root crops, straw and stover.

The nutritional product (for use) according to the invention may be in
25 the form of a dietary supplement, a food additive, a feed additive, a functional food in human nutrition, a functional food in animal nutrition, a food additive or functional ingredient for nutraceuticals or food for special medical purposes.

The physiologically acceptable composition (for use) according to the
30 invention can be a pharmaceutical product or a nutraceutical product. Said

pharmaceutical product may further comprise a pharmaceutically acceptable adjuvant and/or excipient. Adjuvants and excipients are well known to a person skilled in the art.

An oral hydration salt (ORS) is a preferred example of a product according to the invention; an ORS which may be classified as a pharmaceutical (e.g. WHO) or as a food supplement, dependent on national regulations. Particularly suitable yeast cells for an ORS have been found to be *S. boulardii* DSM 33954 (available as ABB1 from ABBIOTEK - Spain, <https://www.abbiotek.com>) and *K. marxianus* V21/012435 (as deposited at the National Measurement Institute, Port Melbourne Vic 3207, Australia, available from ABBIOTEK as ABB7).

The physiologically accepted composition may further comprise a bulking agent, in particular a carbohydrate, for example maltodextrin.

An example of an ORS formulation in liquid format comprises *S. boulardii*, and *K. marxianus* and further water, glucose, sodium citrate, sodium chloride, maltodextrin, zinc sulphate, silicon dioxide), flavour, sweetener (acesulfame K) and acidifier (citric acid). The ORS formulation can be essentially free of *S. cerevisiae*. Optionally *S. cerevisiae* is also present.

An example of an ORS formulation in powder format comprises *S. boulardii* and *K. marxianus*; and further dextrose, lemon, flavour, citric acid, magnesium citrate, malic acid, sodium citrate, sodium chloride, potassium phosphate, calcium ascorbate, sucralose and riboflavin. The ORS formulation can be essentially free of *S. cerevisiae*. In an embodiment, *S. cerevisiae* is also present. In a specific embodiment, the combination of *S. boulardii*, *K. marxianus* and *S. cerevisiae* is ABB C22 available from ABBIOTEK (Spain, <https://www.abbiotek.com>).

The production of a physiologically acceptable composition

The physiologically acceptable composition can be made by combining the different components based on methodology known per se for making yeast preparation.

5 For instance, all yeasts may be produced from a non-GMO yeast strain. A fermentation process known per se for the yeast of interest can be used to produce a primary grown, yeast whose growth occurs under aseptic, aerobic conditions. The resulting product, or yeast cream may be held in refrigerated storage to maintain cell viability, if desired.

10 The yeast cream can be subjected to an inactivation treatment, such as a high temperature sterilization or tyndallization to obtain a heat-treated version of the yeast. If desired, the yeast can be dried, for instance by spray drying, which preferably is done after inactivation (if non-viable yeast is to be used for the composition).

15 Maltodextrin or other bulking agents can be used as support agent to have standardized concentrations. The three yeasts are mixed, which mixing may be followed by a homogenization step.

20 Methods of treating an individual and improving an individual's gut health or gastrointestinal functioning

In accordance with the invention, the physiologically acceptable composition is advantageously used in the treatment of an individual with a gastrointestinal disorder, such as irritable bowel syndrome (IBS); an inflammatory bowel disorder (IBD), for instance Crohn's disease or
25 ulcerative colitis; functional constipation; diarrhoea, for instance antibiotic associated diarrhoea, traveler's diarrhoea, acute gastroenteritis, pediatric diarrhoea, dysbiotic diarrhoea or chronic diarrhoea, in particular in immunodeficient patients; functional abdominal pain; functional bloating, Postprandial Distress Syndrome; gastrointestinal allergy or intolerance;
30 necrotizing enterocolitis; gastrointestinal infections caused by bacteria, such

as *Escherichia*, *Salmonella*, *Shigella*, *Staphylococcus*, *Vibrio*,
Campylobacter, *Yersina*, *Clostridium* or *Helicobacter*; gastrointestinal
infections caused by a virus, such as norovirus, adenovirus,
cytomegalovirus, enterovirus or rotavirus; gastrointestinal infections caused
5 by a parasite, such as *Giardia*, *Entamoeba*, *Cryptosporidium*, *Cyclospora* or
Ascaris; and combinations thereof. Preferably, a gastrointestinal disorder is
treated, more preferably diarrhoea, is prevented or an individual having
the gastrointestinal disorder is treated. In a much preferred embodiment
the diarrhoea to be treated is caused by a viral infection such as a rotavirus
10 infection. Additionally preferred to be treated are individuals having IBD or
IBS.

Further, the physiologically acceptable composition, in particular such
composition comprising, as active ingredients, inactivated *S. boulardii*,
inactivated *K. marxianus* and inactivated *S. cerevisiae*, is advantageously
15 used in the treatment of an individual with a disorder defined by one or
more pro-inflammatory markers, said markers include IL-8, IP-10, MCP-1,
TNF α /IL-10 and TNF α . Disorders defined by pro-inflammatory markers
that are treatable by a composition of the invention include rheumatoid
arthritis, osteoarthritis, topical dermatitis, psoriasis, allergy and obesity.

20 Further, the physiologically acceptable composition of the invention
may be used for the treatment of inflammatory disorders defined by one or
more of said pro-inflammatory markers, in the absence of infection.

Further, the physiologically acceptable composition according to the
invention is particularly suitable to maintain or improve gut health or
25 gastrointestinal functioning, especially those conditions involving
impairment or weakness of the gut barrier function or alterations in the
inflammatory cytokines release. The term 'gut health' as described herein
means the health status of the gut. The gut health status of an individual
might be affected by, for example, infections causes or non-infectious causes,
30 such as a non-optimal diet. The term "gastrointestinal functioning" refers to

the operation of all of the organs and structures associated with the gastrointestinal system. Markers to determine gut health or gastrointestinal functioning are known to a person skilled in the art and include for example trans epithelial electrical resistance as an indication of epithelial barrier integrity and markers for inflammation and injury. Examples of markers are described in Celi *et al.* (2019) "Biomarkers of gastrointestinal functionality in animal nutrition and health" *Animal Feed Science and Technology* 250: 9-31.

The administration dosage, duration and frequency can be chosen within wide limits, dependent on the intended purpose and the subject to whom the composition is to be administered. The duration of treatment can be a relatively short period, e.g. of a week or less, or a day or less, e.g. for an acute manifestation of a disorder or symptom of a disorder, e.g. diarrhoea. The duration of a treatment can also be prolonged, e.g. for a week or more, a month or more or a year or more, e.g. in case of a chronic disorder such as IBD.

The physiologically acceptable composition for use according to the invention may be administered as a single dosage for a complete treatment, dependent on the application. If multiple dosages are intended, the number of dosages is generally 10 times per day or less. E.g. in case of the use of an ORS more than 3 dosages per day may be administered, but typically it is administered about 3 times per day or less, preferably about 2 times per day or less, in particular about once per day or less. In an embodiment, the composition is administered (on average) at least about once a week. Preferably, it is administered (on average) at least once per period of three days, more preferably (on average) at least once per period of two days.

The physiologically acceptable composition may comprise or can be co-administered with a(nother) probiotic, a prebiotic, a postbiotic, an antibiotic, an analgesic, an anti-inflammatory agent, an anti-diarrhoeal agent such as

an inhibitor of motility or secretion, an(other) oral rehydration salt, a laxative or a mixture thereof.

A person skilled in the art will be able to determine an appropriate dose of the physiologically acceptable composition to administer to an individual based on common general knowledge and the information disclosed herein without undue experimentation. As the skilled person will understand, an actual preferred dosage depends on a variety of factors, including the activity of the specific yeasts employed, the metabolic stability and length of action of that yeast, the age, body weight, general health, sex and species of the individual diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular disorder, and the individual. The dosages disclosed herein are indicative of an average case for a human individual (including adults and children). There can of course be individual instances where higher or lower dosage ranges are merited.

The usual effective daily dose for oral administration or other administration into the gastrointestinal tract, in particular for humans, is from about 100 mg (of the yeast components) to about 1000 mg (of the yeast components), preferably, from about 200 mg (of yeast components) to about 900 mg (of the yeast components), more preferably, from about 200 mg (of the yeast components) to about 650 mg (of yeast components). The usual effective daily dose for topical administration, in particular for humans, is from about 1 mg (of yeast components) to about 1000 mg (of the yeast components), preferably from about 5 mg (of the yeast components) to about 500 mg (of the yeast components).

The invention provides a physiologically acceptable composition for use in the treatment of a gastrointestinal medical disorder.

The invention provides a method of treating an individual in need of an improvement of gut health or in need of an improvement of gastrointestinal functioning, comprising the administration of an effective amount of the physiologically acceptable composition according to the invention, hereby

improving gut health or gastro-intestinal functioning. The invention has in particular found to be effective in improving the gut barrier function and in driving an anti-inflammatory status, as well as in the protection from viral, bacterial and yeast infection, while modulating the microbiota towards an eubiotic state.

The invention provides a method of treating an individual having a gastrointestinal disorder or preventing a gastrointestinal disorder in an individual, comprising the administration of an effective amount of the physiologically acceptable composition according to the invention. The effectivity of a prophylactic treatment can routinely be determined, e.g. by comparing cohorts or test animals treated with a composition for use according to the invention and a reference product (placebo), with a reduced incidence. The effect of a treatment of an individual having the disorder can be a complete cure, an alleviation of a symptom, reduced suffering etc.

The invention provides a method of treating an individual with a gastrointestinal disorder, such as diarrhoea, IBD, IBS or (other) gastrointestinal disorders related to the impairment of the gut barrier function, comprising the administration of an effective amount of the physiologically acceptable composition according to the invention to said individual.

The invention provides a method of treating an individual with a disorder defined by pro-inflammatory markers such as rheumatoid arthritis, osteoarthritis, topical dermatitis, psoriasis, allergy or obesity, comprising the administration of an effective amount of the physiologically acceptable composition according to the invention to said individual.

The invention provides the use of the physiologically acceptable composition according to the invention for use in the preparation of a product, which may be a medicament or a food product (such as a medical or clinical food), for use in the treatment of a gastrointestinal disorder, preferably a gastrointestinal disorder selected from the group consisting of diarrhoea,

IBD and IBS or a(nother) gastrointestinal disorder related to the impairment of the gut barrier function. Preferably, the diarrhoea is a diarrhoea of an individual infected with a virus, in particular a rotavirus. It is envisaged that not only adults but also children having an (acute) diarrhoea caused by a viral infection are effectively treatable.

The invention provides the use of the physiologically acceptable composition according to the invention for the preparation of a product to maintain or improve gut health or gastrointestinal functioning.

The invention will now be illustrated by the following examples, which are provided by way of illustration and it will be understood that many variations in the methods described and the amounts indicated can be made without departing from the spirit of the invention and the scope of the appended claims.

15

EXAMPLES

Example 1: Anti-inflammatory effect on gut epithelium cells.

20 Materials and methods

All yeasts were produced from a non-GMO yeast strain. The yeasts included in the experiment were *S. boulardii* DSM 33954 (as deposited at DSMZ, German Collection of Microorganisms and Cell Cultures, Germany), available from ABBIOTEK as ABB1; *S. cerevisiae* Y1529 (as deposited at ATTC), available from ABBIOTEK as ABB6 in zinc enriched form. and *K. marxianus* V21/012435 (as deposited at the National Measurement Institute, Port Melbourne Vic 3207, Australia), available from ABBIOTEK as ABB7. The combination of these yeasts, as used herein, is also available from ABBIOTEK as ABB22.

The fermentation process produced a primary grown, yeast whose growth occurs under aseptic, aerobic conditions. During fermentation, the temperature, pH, and growth rate were closely regulated. In the case of *S. cerevisiae*, zinc sulphate was added to the yeast cream at the end of the fermentation process to a concentration of about 10% based on dry weight. The resulting product, or yeast cream was held in refrigerated storage to maintain cell viability. Prior to spray drying, the chilled yeast cream was treated with a high temperature sterilization system to obtain a tyndallized version of the yeasts. Maltodextrin or other bulking agents can be used as support agent to have standardized concentrations.

The three heat-inactivated yeasts were premixed, followed by a homogenization step. For the in vitro studies described below, the mixture was made from stock solutions of each yeast.

The yeast concentration of each yeast stock was measured by flow cytometry. A sample standardized at 1×10^7 cells/mL in cell culture medium was prepared for each yeast. To obtain the combinations, samples of each yeast strain were mixed at a homogeneous ratio of each strain, i.e. each of the three strains in the mix were at a concentration of 0.333×10^7 cells/ml .

In vitro immune modulating activity of yeasts and combinations thereof was studied by chemokine production by Caco-2 cells in the presence and absence of a pro-inflammatory stimulus. Caco-2 cells were cultured to confluence in 96 well plates. At the start of the experiment, cells were washed once with antibiotic free culture medium. Monolayers were incubated with test components in triplicate for 1 hour at 37°C in antibiotic free medium. After that, the cells were incubated with medium containing the test components and 50 µg/ml gentamicin (Invitrogen), in duplicate. One of the duplicates was further stimulated with a mixture of recombinant TNFα (10 ng/ml) and recombinant IFNγ (5 ng/ml) (R&D systems), as pro-inflammatory stimulus (Figures 4-6). As blank control, medium only was used, without stimulation in case of Figures 1-3, a medium under a pro-

inflammatory stimulus (a mixture of recombinant TNF α (10 ng/ml) and recombinant IFN γ (5 ng/ml) for Figures 4-6.

Supernatants were collected 24h after stimulation and stored at 20°C. A Bio Plex assay (BioRad) was used to measure the IL-8, IP-10 and MCP-1
5 levels, according to the manufacturers' protocol.

Metabolic activity of the cells for confirming non-cytotoxicity of the test components, was analysed by WST-1 assay (Roche), according to the manufacturers protocol, after collecting the culture supernatants. The cells were found not to be metabolic active, indicating that any observed effect is
10 not due to metabolic alterations or by cytotoxicity.

As in the other examples, one-way ANOVA was performed after which statistical differences between the control condition and the test conditions were calculated by using Dunnett's post hoc test. Significance thresholds as used in the figures were as follows: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.
15

Results

Figure 1 depicts the *in vitro* IP-10 chemokine production by Caco-2 cells after incubation with 3 yeasts and a combination thereof, in the absence of a pro-inflammatory stimulus

20 A synergistic reduction of pro-inflammatory cytokines in gut epithelial cells was in particular observed for the yeast composition comprising *S. boulardii*, *S. cerevisiae* and *K. marxianus*, from now on called composition ABB C22, compared to the individual yeasts (Figures 2-3). A synergistic reduction of pro-inflammatory cytokines in gut epithelial cells after a pro-inflammatory challenge with TNF α /IFN γ was observed for the yeast
25 composition ABB C22 compared to the individual yeasts (Figure 4 - 5). Furthermore, it is observed that the composition ABB C22 outperforms a composition only comprising the yeasts *S. boulardii* and *S. cerevisiae* (Figures 2-6), indicating a crucial role of *K. marxianus* in obtaining a

positive effect for several marked, which is even synergistic in at least some aspects.

Example 2: Anti-inflammatory effect on immune cells.

5

Materials and methods

The yeasts and combinations thereof were prepared as in example 1.

In vitro immuno-modulating activity of yeasts and combinations thereof was studied by following the cytokine production by THP-1 cell line (macrophages).

The human THP-1 cell line was cultured 1×10^5 cells/ well in 96-well plates in the presence of 100nM phorbol12-myristate 13-acetate (PMA, Sigma) and incubated for 48 hours to induce differentiation of the THP-1 monocytes into macrophages. Cells were washed and incubated for another 72 hours in culture medium. After this, the cells were incubated for 1 hour with the test components, after which the cells were incubated for another 16 hours with and without LPS (100 ng/ml, Sigma) in the presence of the test components. All conditions were tested in triplicates.

Supernatants were collected after stimulation and stored at -20°C. An ELISA assay (IL-10 Human Uncoated ELISA Kit, TNF- α Human Uncoated ELISA Kit, Life Technologies) was used, to measure the TNF- α and IL-10 levels, according the manufacturers protocol. TNF- α /IL-10 ratio was calculated as a measure of anti-inflammatory effect of the tested components.

Metabolic activity of the cells, for confirming non-cytotoxicity of the test components, was analysed by WST-1 assay (Roche), according to the manufacturers protocol, after collecting the culture supernatants. The cells were found not to be metabolically active.

Results

30

Reduction of TNF α /IL-10 ratio, as a measure of anti-inflammatory effect, in immune cells was observed for the yeast composition ABB C22 compared to the individual yeasts and compared to a composition only comprising the yeasts *S. boulardii* and *S. cerevisiae* (Figure 7).

5

Example 3: Intestinal barrier integrity after challenge.

Materials and methods

The yeasts and combinations thereof were prepared as in example 1.

10 The effect of the yeasts and combinations thereof on gut barrier function upon a challenge was studied by following trans-epithelial electric resistance (TEER) over a gut cell layer.

Caco-2 cells were seeded (2×10^4 cells/cm²) and cultured on Transwell polycarbonate cell culture inserts with a mean pore size of 0.4 μ m and a
15 diameter of 0.33 cm² (Greiner Bio one) until full differentiation (1000 Ω). As indicative measure of barrier integrity, TEER was measured with an EVOM2 epithelial volt ohmmeter, (World Precision Instruments).

At the day of the experiment, the cells were washed and incubated for 1 hour at 37°C with antibiotic- and serum-free medium containing the test
20 components. Subsequently, the wells were exposed for 6 hours to ETEC H10407 (MOI 200:1) in the presence of the test components. TEER was measured before the start of the experiment ($t = 1$), 1 hour after exposure to the test components and before addition of the ETEC ($t = 0$), and 1 h, 2 h, 3h, 4h and 6h after exposure to ETEC (respectively $t = 1$, $t = 2$, $t = 4$ and
25 6h).

The TEER values of the individual conditions after exposure to the pathogens were related to their own TEER value at $t = 0$ and expressed as Δ TEER (Ω .cm²). A negative control (ETEC H10407 only) and a positive control which was not exposed to the pathogen nor to test components was
30 included. All conditions were tested in triplicate.

Transepithelial flux with FITC Dextran (Sigma) was measured at various timepoints after the TEER measurement.

Results

5 Protection of the gut epithelium integrity was measured after incubation with an infective agent known to disrupt the epithelium monolayer, here *E. coli* ETEC. The yeast combination ABB C22 has a higher increase in TEER relative to the negative control, compared to the individual yeasts or the combination of *S. cerevisiae* with *S. boulardii* after 1
10 and 2 hours of incubation (Figure 8).

Example 4: Intestinal barrier integrity formation.

Materials and methods

15 The yeasts and combinations thereof were prepared as in example 1.

 The effect of yeasts and combinations thereof on growth and differentiation of intestinal epithelial cells was followed during formation of the gut cell monolayer.

 Caco-2 cells were seeded (2×10^4 cells/cm²) on Transwell polycarbonate
20 cell culture inserts with a mean pore size of 0.4 µm and a diameter of 0.33 cm² (Greiner Bio one). After overnight attachment of the cells, the test components were added at the apical side of the cells.

 The test ingredients were prepared and stored at 20°C in aliquots. Every two days a new aliquot was taken to refresh the ingredients. To avoid
25 overgrowth of epithelial cells by the tested yeasts (when able to grow in aerobic conditions), 10% of conditioned medium of the yeasts and combinations thereof, together with the heat-killed yeasts were used.

 As indicative measure of cell growth and barrier integrity formation, TEER was measured every other 2 days, with an EVOM2 epithelial volt
30 ohmmeter (World Precision Instruments).

Results

Increase in TEER was used to measure the spontaneous formation of the gut epithelium over time. An increase in TEER is observed when compared to the negative control for the three yeasts after 16 days, and maintained until day 20 only for *S. cerevisiae* (Figure 9a, top). In contrast, the increase in TEER with respect to the control from day 16 observed for the combination ABB C22 is maintained and increased until day 22 (Figure 9b, bottom).

A higher slope for TEER increase, indicative for a faster TEER increase, is observed for the combination ABB C22 compared to individual yeast strains and the combination of *S. cerevisiae* and *S. boulardii* (slopes of trendlines in Figure 10).

Example 5: Protection conferred to intestinal epithelial cells against rotavirus infection.

Materials and methods

Viral expansion and harvesting

HT-29 cells were seeded on a T75 culture flask and were grown to 80% confluence. The culture medium was removed and half the contents of the vial containing the virus were added (500 µl), followed by incubation for 2h at 37°C. Afterwards, fresh medium was added until the monolayer was completely covered ('HT-29 cells growing medium', i.e. McCoy's 5A + 2mM Glutamine + 10% Foetal Bovine Serum FBS / FCS;

https://www.sigmaaldrich.com/ES/es/product/sigma/cb_91072201?gclid=CjwKCAiA866PBhAYEiwANkIneP61QoaUBsagNLA2fdSVt2ru3mbcwSxQH_VtHd4NcJel460L-9GpVxoCeEQQA_vD_BwE).

Cells were incubated for 24-96h without changing the culture medium, with frequent observations under the microscope until empty spaces became evident within the monolayer. Flasks were tapped on their side to detach

infected cells and the supernatant was harvested. The harvested supernatant was frozen and thawed at -80°C once to allow cell lysis and virus release. Eight serial dilutions were made of the harvested supernatant (namely 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256 and 1/512 dilutions).

5

Viral titration

HT-29 cells were seeded in a 96-well plate and were grown to 80% confluence. The culture medium was removed from the cells and replaced by 50 µl of the serial dilutions of the harvested supernatant, using one different dilution per row, followed by incubation for 1h at 37°C. Afterwards, 50 µl fresh culture medium was added. The plate was incubated for 24-96h at 37°C until differences between the dilutions were observed. The plate was visualized under the microscope and the 50% Tissue Culture Infectious Dose (TCID₅₀) was calculated using the Spearman-Kärber method as follows:

15 $\log(\text{TCID}_{50}) = -(\log \text{ of the highest virus concentration} - [(\text{sum of the percentage of affected wells at each dilution}/100 - 0.5) \times (\log \text{ of dilution factor})]$

Probiotic strains to be tested

20 The tested yeast strains are listed in table 1 and were prepared as in example 1.

Table 1: tested yeast strains

Code	Strain identification	Form
ABB1	<i>Saccharomyces boulardii</i> (deposit n° DSM 33954)	Live
ABB3	<i>Saccharomyces boulardii</i> (deposit n° DSM 33954)	Tyndallized
ABB7	<i>Kluyveromyces marxianus</i> (deposit n° V21/012435)	Live
ABB8	<i>Kluyveromyces marxianus</i> (deposit n° V21/012435)	Tyndallized

Measurement of the activity of probiotic strains and combinations of probiotic strains against rotavirus infection

Per tested yeast strain or combination, one row of a 96-well plate was seeded with HT-29 cells. Additionally, one 96-well plate was used as
5 negative control plate and one 96-well plate was used as rotavirus-infected control plate. The negative control plate contained the HT-29 culture medium, not PBS; the rotavirus-infected control plate contained HT-29 cells with culture medium and virus load. Plates were different from those with yeast to avoid cross contamination. All plates were grown up to 80%
10 confluence. The supernatant was removed and 50 µl of culture medium containing 2x final concentration for each well of yeast strain or combination was added, followed by incubation for 1h at 37°C. Afterwards, 50 µl of one of the viral supernatant was added to obtain a final concentration of the estimated TCID₅₀. The plates were incubated for 24-
15 96h at 37°C until empty spaces were observed. The cell culture supernatant was harvested to determine the presence of Rotavirus genes NSP3 and VP7.

Detection of rotavirus antigen by RT q-PCR

Detection of NSP3 and VP7 Rotavirus genes was performed by RT-
20 qPCR following the method described in C. Kottaridi et al. Journal of Virological Methods 180(2012) 49-53.

Results

Tyndallized *S. boulardii* (ABB3) and tyndallized *K. marxianus* (ABB8) did
25 not confer protection against rotavirus infection when each of these strains was tested alone (table 2). However, positive results were obtained by combining tyndallized *S. boulardii* and tyndallized *K. marxianus* (ABB3+ABB8, table 2 and Figure 11), supporting the use of this combination as a product to protect against rotavirus infection.

30

Table 2: Effect of different yeast strains and combinations of yeast strains on protection against rotavirus.

Composition	Ratio of strains	Protective
ABB1		Yes
ABB3		No
ABB7		No
ABB8		No
ABB3 + ABB8	1:1	Yes

Claims

1. A physiologically acceptable composition comprising (i) at least one component selected from the group consisting of inactivated *S. boulardii* yeasts, *S. boulardii* lysates, *S. boulardii* cell wall components, and *S. boulardii* extracts, and further comprising (ii) at least one component
5 selected from the group consisting of inactivated *K. marxianus* yeasts, *K. marxianus* lysates, *K. marxianus* cell wall components and *K. marxianus* extracts, for use in the treatment of a gastrointestinal disorder.
2. The physiologically acceptable composition for use according to claim 1,
10 wherein the gastrointestinal disorder is diarrhoea.
3. The physiologically acceptable composition for use according to claim 1 or claim 2, wherein the gastrointestinal disorder is caused by a viral infection, preferably an infection by a rotavirus, a norovirus, an enteric
15 adenovirus, an astrovirus, a hepatitis virus or cytomegalovirus, in particular by a rotavirus.
4. The physiologically acceptable composition for use according to any of claims 1-3, wherein the composition comprises inactivated *S. boulardii*
20 yeasts and inactivated *K. marxianus* yeasts, which yeasts are preferably heat-killed, more preferably tyndallized.
5. The physiologically acceptable composition for use according to any of claims 1-4, wherein the *S. boulardii* is present in a concentration of at least
25 10^6 cells per gram (based on total weight of the yeast components), preferably 10^9 cells per gram (based on total weight of the yeast components) to 4×10^{10} cells per gram (based on total weight of the yeast components), and wherein *K. marxianus* is present in a concentration of at least 10^6 cells per gram, preferably 10^6 cells per gram (based on total

weight of the yeast components) to 5×10^9 cells per gram (based on total weight of the yeast components).

6. The physiologically acceptable composition for use according to any of
5 claims 1-5, wherein the composition is a pharmaceutical product or a nutraceutical product.

7. The physiologically acceptable composition according to any of claims
1-6, wherein the composition is a nutritional product, preferably a product
10 selected from the group consisting of dairy products, infant formulae, fruit-based products, cereal products, snacks, vegetable beverages, smoothies and isotonic drinks.

8. The physiologically acceptable composition according to any of claims
15 1-7, wherein the composition is an oral rehydration salt.

9. The physiologically acceptable composition for use according to any of
claims 1-8, wherein the composition is to be administered into the
gastrointestinal tract, preferably orally.

20

10. The physiologically acceptable composition for use according to any of
the claims 1-9, wherein the composition is for use in the treatment of a
human.

25 11. The physiologically acceptable composition for use according to any of
the claims 1-10, wherein the composition comprises at least one component
selected from the group of *S. cerevisiae* yeasts, *S. cerevisiae* lysates, *S. cerevisiae*
cell wall components and *S. cerevisiae* extracts, preferably in a
total content in the range of 0.05 – 90 wt.%, 10 - 90 wt. %, or 10 - 80 wt. %,
30 based on total yeast components.

12. The physiologically acceptable composition for use according to claim 11, wherein the composition comprises an inactivated *S. cerevisiae* yeast, preferably a heat-killed *S. cerevisiae* yeast, more preferably a tyndallized *S. cerevisiae* yeast.

5

13. The physiologically acceptable composition for use according to claim 11 or claim 12, wherein *S. cerevisiae* is selected from the group consisting of *S. cerevisiae* CNCM I-3856, *S. cerevisiae* S288C, *S. cerevisiae* Y1529 and *S. cerevisiae* UFMG 905, preferably *S. cerevisiae* Y1529 or *S. cerevisiae* S288C, in particular *S. cerevisiae* Y1529.

10

14. The physiologically acceptable composition for use according to any of the claims 1-11, wherein the composition is essentially free of *S. cerevisiae* yeasts, in particular essentially free of *S. cerevisiae* yeasts and further *S. cerevisiae* yeast components (such as *S. cerevisiae* lysates, *S. cerevisiae* cell wall components and *S. cerevisiae* extracts).

15

15. The physiologically acceptable composition for use according to any of the claims 1-14, wherein the composition is essentially free of *S. cerevisiae* lysates, *S. cerevisiae* cell wall components and *S. cerevisiae* extracts.

20

16. The physiologically acceptable composition for use according to any of the claims 1-15, wherein *S. boulardii* is selected from the group consisting of *S. boulardii* DSM 33954, *S. boulardii* CNCM I-745, *S. boulardii* Hansen CBS 5926, *S. boulardii* BLD-3, *S. boulardii* CCTCC M2012116, *S. boulardii* CNCM I-1079, *S. boulardii* ATCC MYA-796, *S. boulardii* Unique28, *S. boulardii* Kirkman, *S. boulardii* Unisankyo and *S. boulardii* CNCM I-3799, preferably *S. boulardii* is *S. boulardii* DSM 33954.

25

17. The physiologically acceptable composition for use according to any of the claims 1-16, wherein *K. marxianus* is selected from the group consisting of *K. marxianus* AS41, *K. marxianus* B0399, *K. marxianus* CIDCA 8154, *K. marxianus* CBS1553, *K. marxianus* M3, *K. marxianus* V21/012435 and *K.*
5 *marxianus* Z17, preferably *K. marxianus* is *K. marxianus* V21/012435.

18. The physiologically acceptable composition for use according to any of the claims 1-17, wherein the inactivated *S. boulardii* content of the composition is 5 – 95 wt.%, preferably 20-80 wt.% , based on total yeast
10 components.

19. The physiologically acceptable composition for use according to any of the claims 1-18, wherein the inactivated *K. marxianus* content of the composition is 5 – 95 wt.%, preferably 20-80 wt.%, based on total yeast
15 components.

20. The physiologically acceptable composition for use according to claim 18 or 19, wherein the total content of inactivated *K. marxianus* plus *S. boulardii* is 50-100 wt.%, based on total yeast components, preferably 90-
20 100 wt.%, based on total yeast components.

21. The physiologically acceptable composition for use according to any of the preceding claims, wherein the composition is for use in the treatment of an individual having said gastrointestinal disorder.
25

22. The physiologically acceptable composition for use according to any of the claims 1-20, wherein the treatment is a prophylactic treatment.

23. A physiologically acceptable composition comprising inactivated *S. boulardii* yeast cells selected from the group consisting of *S. boulardii* DSM 33954, *S. boulardii* CNCM I-745, *S. boulardii* Hansen CBS 5926, *S.*
30

boulardii BLD-3, *S. boulardii* CCTCC M2012116, *S. boulardii* CNCM I-1079, *S. boulardii* ATCC MYA-796, *S. boulardii* Unique28, *S. boulardii* Kirkman, *S. boulardii* Unisankyo and *S. boulardii* CNCM I-3799 and inactivated *K. marxianus* yeast cells selected from the group consisting of *K.*
5 *marxianus* AS41, *K. marxianus* B0399, *K. marxianus* CIDCA 8154, *K. marxianus* CBS1553, *K. marxianus* M3, *K. marxianus* V21/012435 and *K. marxianus* Z17.

24. The physiologically acceptable composition according to claim 23,
10 wherein the composition comprises *S. boulardii* DSM 33954 and *K. marxianus* V21/012435.

25. The physiologically acceptable composition according to claim 23 or 24,
15 wherein the composition is essentially free of *S. cerevisiae* cells, preferably essentially free of a *S. cerevisiae*-based fraction.

26. The physiologically acceptable composition according to any of the claims 23-25, wherein the inactivated *S. boulardii* content of the composition is 5 – 95 wt.%, based on total yeast components and the
20 inactivated *K. marxianus* content fraction of the composition is 5 – 95 wt.%, based on total yeast components, said contents preferably both being in the range of 20-80 wt.%.

Figure 1:

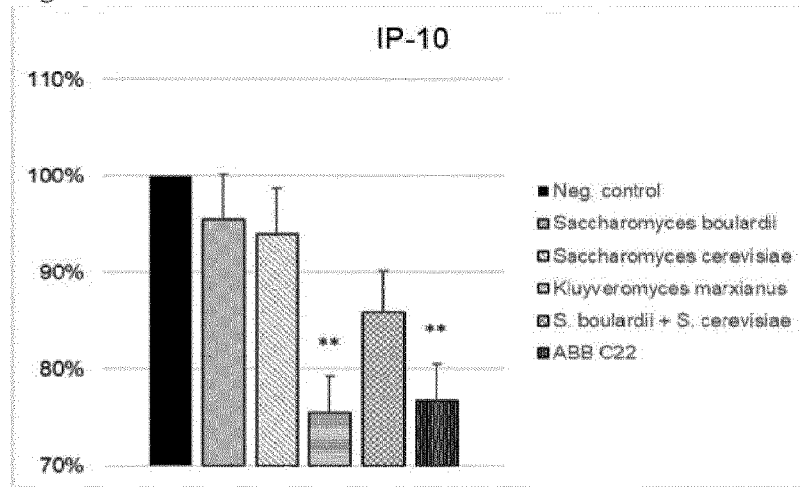


Figure 2:

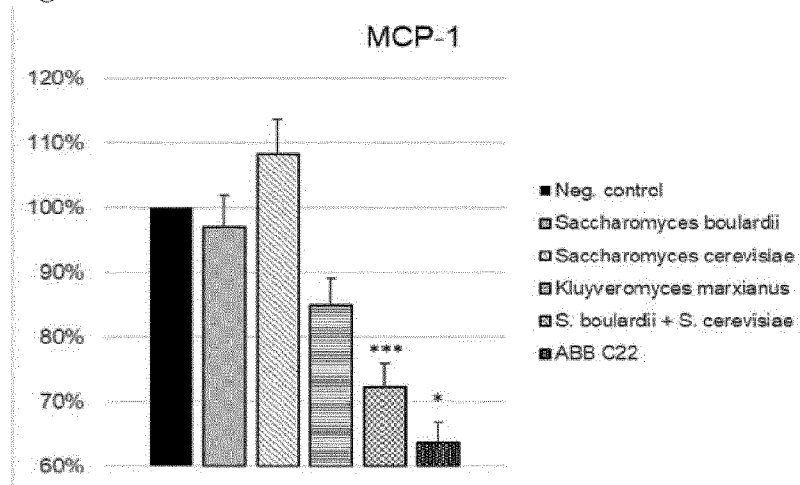


Figure 3:

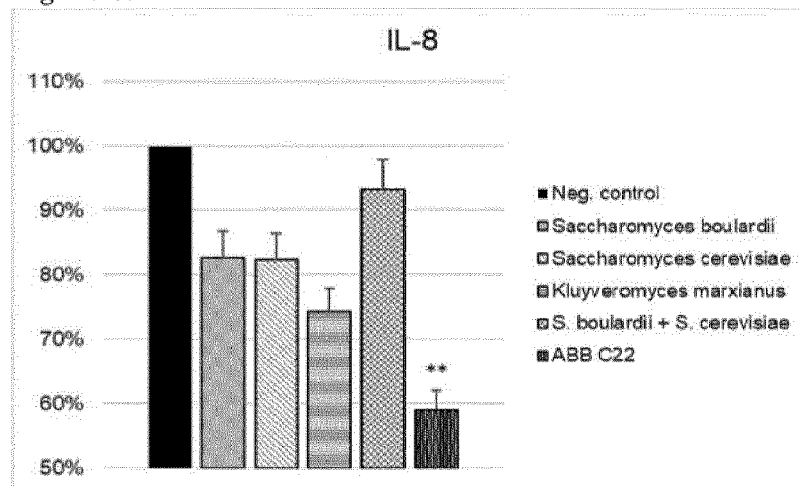


Figure 4:

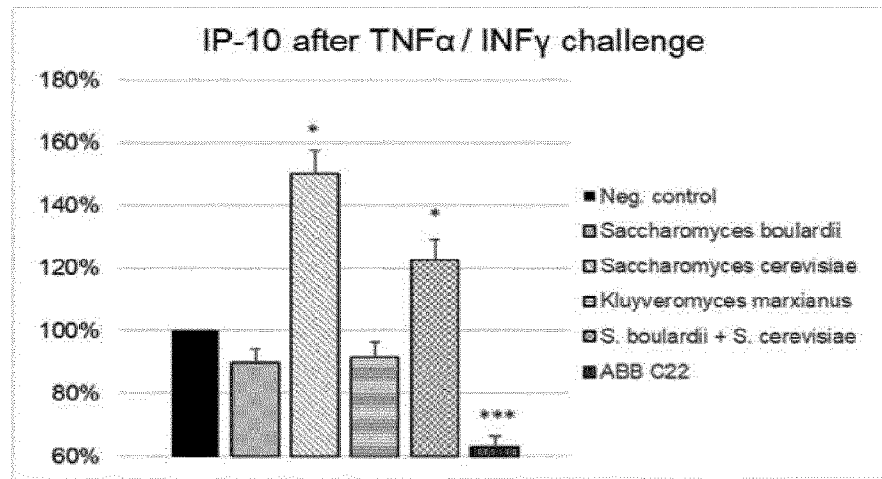


Figure 5:

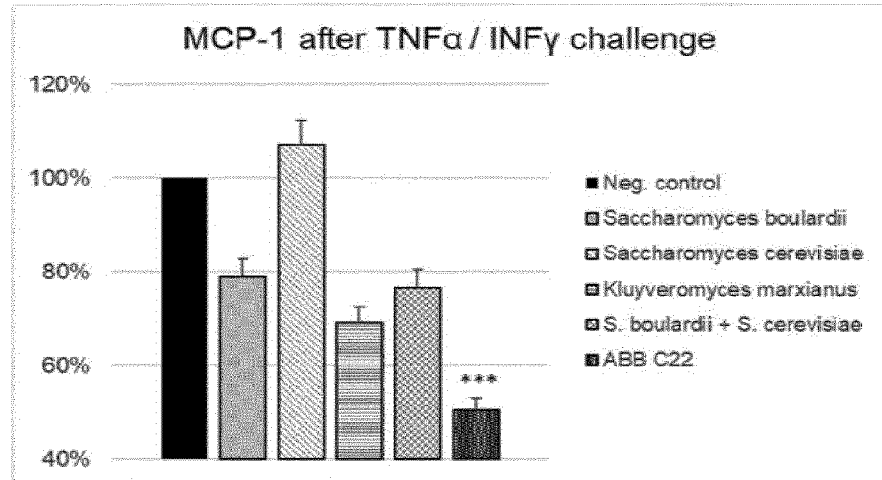


Figure 6:

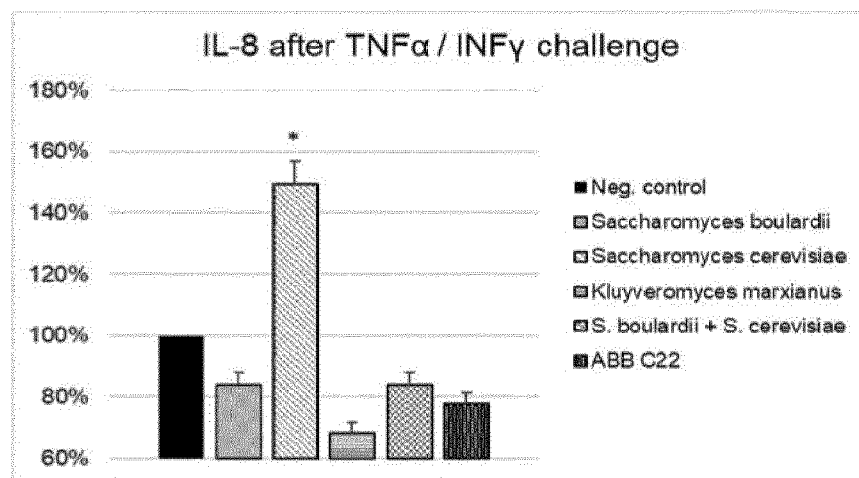


Figure 7:

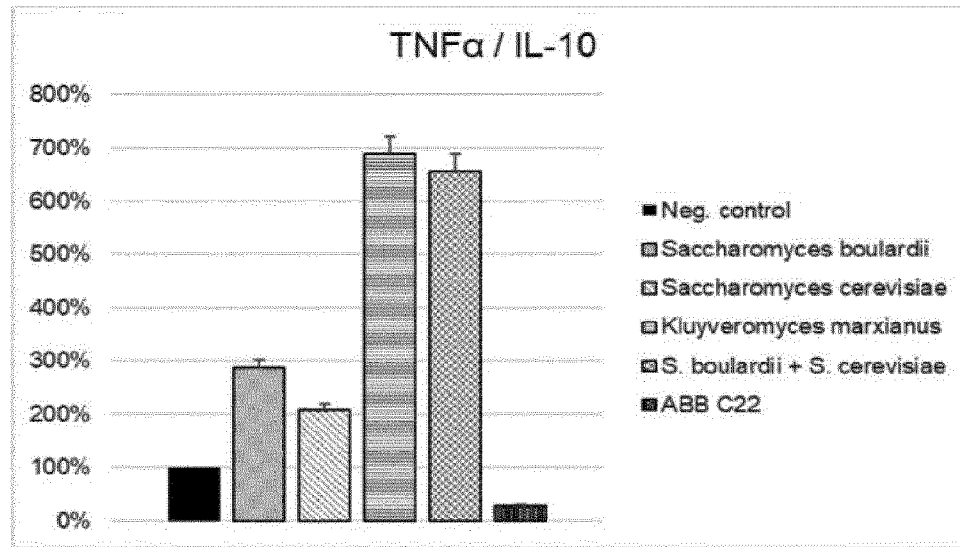


Figure 8:

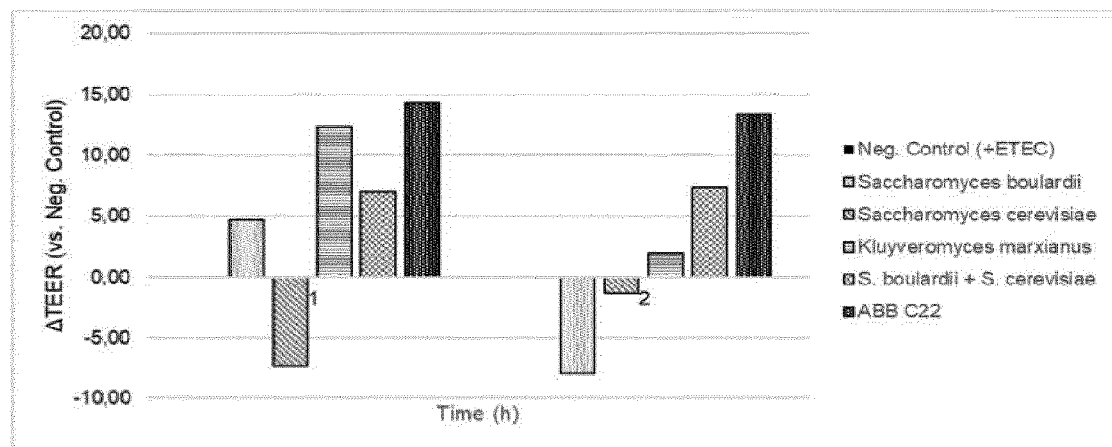


Figure 9a:

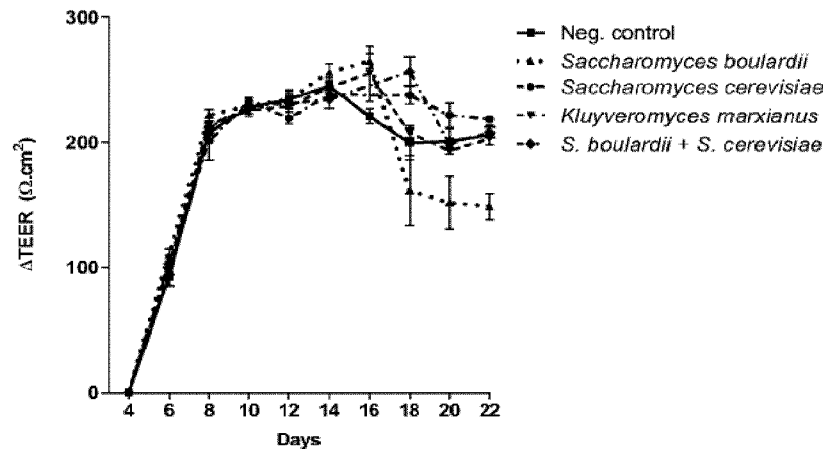


Figure 9b:

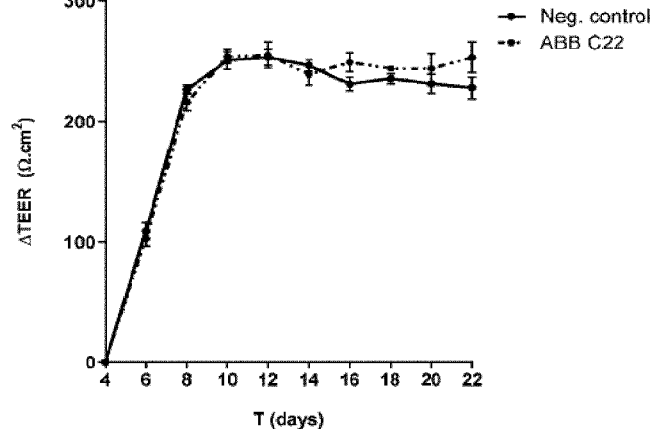


Figure 10:

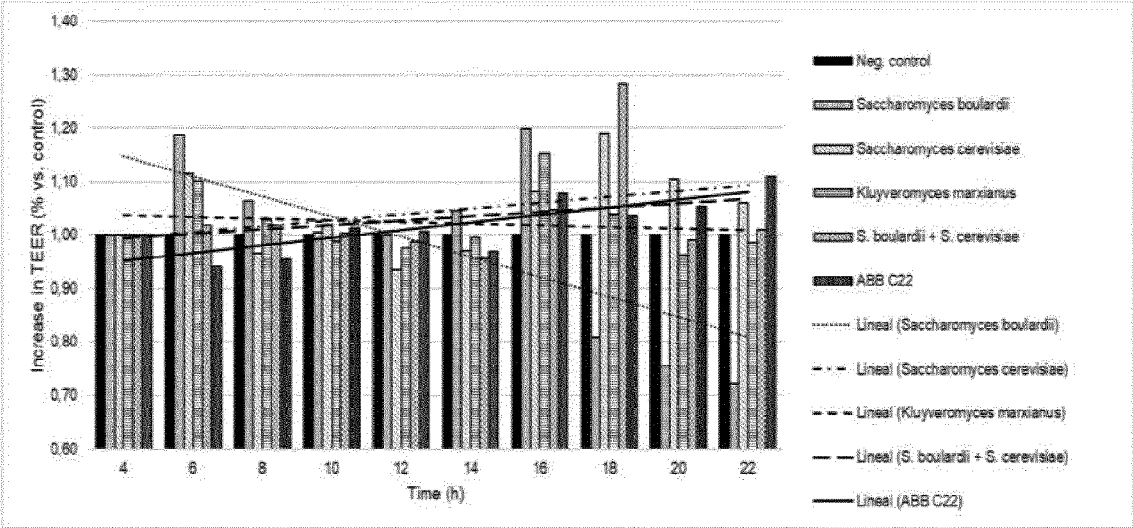


Figure 11:

