Nutrition & Food Science International Journal
ISSN 2474-767X

Research Article
Volume 12 Issue 5 - January 2024
DOI: 10.19080/NFSIJ.2024.12.555848

Nutri Food Sci Int J

Prebiotic Constituent of Mushroom

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Submission: November 22, 2023; **Published:** January 24, 2024

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Abstract

Edible mushrooms are fungi and belong to the class Basidiomycetes. Mushroom is a prebiotic as it contains nutrients that can stimulate the growth of beneficial microbiota and modulate several disease conditions such as diabetes, obesity, and cancer. Oligosaccharides and fibers are the major constituents of prebiotics found in mushrooms. Prebiotics are foods that contain non-digestible food ingredients that selectively stimulate the favourable growth and/or enhance the activities of indigenous probiotic bacteria. The important sources of prebiotics in mushrooms are non-digestible mushroom polysaccharides which can inhibit pathogen proliferation by enhancing the growth of probiotic bacteria in the gut. Prebiotics are produced from lactose, sucrose, starch, microalgae, pectin and recently mushroom has been considered as a source of prebiotic as it contains carbohydrates like chitin, hemicellulose, β and α -glucans, mannans, xylans and galactans. Prebiotic are used in the food industry to produce functional foods or nutraceuticals that have adverse health benefit to the body.

Keywords: Prebiotics; Mushroom; Functional food; Nutraceuticals

Background of the Study

Prebiotics are food ingredients that can stimulate the growth of beneficial microbiota. Oligosaccharides and fibers are the major constituents of prebiotics. Recent trend in food science and technology has shown the association of prebiotics to modulate the human gut microbiota and attenuate several disease conditions such as diabetes, obesity, and cancer. The important sources of prebiotics in mushrooms are non-digestible mushroom polysaccharides which can inhibit pathogen proliferation by enhancing the growth of probiotic bacteria in the gut [1].

Many studies have reported the use of prebiotic substances from mushroom extraction as non-digestible food ingredients to demonstrate probiotic growth stimulation and pathogenic microbial inhibition. For example, medicinal mushrooms were extracted and non-digestible carbohydrates were separated to be used for growth stimulation of beneficial bacteria in the gastrointestinal tract [2]. Extracts from some edible mushrooms such as Lentinus edodes and Pleurotus eryngii demonstrated high potential anti-microbial effects against Bacillus cereus, Staphylococcus aureus and Salmonella typhimurium. Edible mushrooms contain high bioactive polysaccharides which is interesting as a functional food as well as a safe food, especially as they are a good source of prebiotic substances which contain glucose, galactose, fructose and N-acetylglucosamine [3] which are non-digestible carbohydrates that stimulate the growth of beneficial microorganisms These microorganisms function as probiotics because of their potential to inhibit pathogenic microorganisms in the gastrointestinal tract. In addition, prebiotic compounds are gastrointestinal tolerant in the presence of salivary amylase, gastric juice or bile extract, maintaining properties to activate the beneficial microbes for host health.

This paper reviews the concept of prebiotics, mushroom classification and types, criteria for classification as prebiotic. The interaction of mushroom prebiotics to health and the microbiota was also reviewed with its uses in the new product formulations.

Concept of Prebiotics

The word ‘prebiotic’ was first introduced by Gibson and Roberfroid [4] and is defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health.” In common terms, bacterial species that are believed to be beneficial for health and well-being of humans use prebiotics as food. According to Dorna et al. [5], there are many types of prebiotics and most are a subset of carbohydrate groups and are mostly oligosaccharide carbohydrates (OSCs). These include:

Fructans

This category consists of inulin and fructo-oligosaccharide (FOS) or oligofructose. Their structure is a linear chain of fructose with β2-1 linkage. They usually have terminal glucose units with β2-1 linkage. Inulin has degree of polymerization (DP) of up to 60, while the degree of polymerization (DP) of FOS is less than 10. Some studies implicated that fructans can stimulate lactic acid bacteria selectively. The chain length of fructans is an important criterion to determine which bacteria can ferment them. Other bacterial species can also be promoted directly or indirectly by fructans [6].

Galacto-oligosaccharides

Galacto-oligosaccharides (GOS), the product of lactose extension, are classified into two subgroups:

i. The GOS with excess galactose at C3, C4 or C6.

ii. The GOS manufactured from lactose through enzymatic trans-glycosylation.

The end product of this reaction is mainly a mixture of tri- to pentasaccharides with galactose in β1-6, β1-3 and β1-4 linkages. Galacto-oligosaccharides (GOSs) can greatly stimulate Bifidobacteria and Lactobacilli. Enterobacteria, Bacteroidetes, and Firmicutes are also stimulated by GOS, but to a lesser extent than Bifidobacteria. There are some GOSs derived from lactulose which are also considered as prebiotics [7].

Starch and glucose-derived oligosaccharides

There is a kind of starch that is resistant to the upper gut digestion and which are known as resistant starch (RS). RS can promote health by producing a high level of butyrate [8].

Pectic oligosaccharides

Some oligosaccharides originate from a polysaccharide known as pectin. This type of oligosaccharide is called pectic oligosaccharide (POS). They are based on the extension of galacturonic acid (homogalacturonan) or rhamnose (rhamnogalacturonan I). The carboxyl groups may be substituted by methyl esterification, and the structure can be acetylated at C2 or C3. Various types of sugars such as arabinose, galactose, and xylose or ferulic acid are linked to the side chains with their structures varying significantly depending on the sources of POSs [9].

Non-carbohydrate prebiotics

Although carbohydrates are more likely to meet the criteria for prebiotics definition, there are some compounds that are not classified as carbohydrates but are recommended to be classified as prebiotics; example is cocoa-derived flavanols. Experiments demonstrate that flavanols can stimulate lactic acid bacteria [10].

Mushroom classification and types

The word Mushroom is derived from the French word for Fungi and Mold. They belong to the class Basidiomycetes and around 2,000 species exist in nature out of which around 25 are widely accepted as food. Mushrooms have high nutritional and functional value and they are also accepted as nutraceutical foods. They have their own medicinal properties and economic significance [11]. There are several mushrooms that are used for medicinal purposes and edible as food. Edible mushroom species have therapeutic properties [12]. The most cultivated edible mushrooms are: Agaricus bisporus, Lentinus edodes and *L. pleurotus*. Lentinus edodes is the most studied specie and have an antimicrobial action against gram positive and gram-negative bacteria. Mushrooms are a source of antimicrobial compound such as benzoic acid derivatives, terpenes and steroids. They are rich in protein, amino acid and fiber, which is why they are used in human diet to promote health because of the synergistic effects of the bioactive compounds [13].

Mushrooms are fruit bodies of macroscopic, filamentous and epigeal fungi. They are made up of hypha which form an interwoven web of tissue known as mycelium in the substrate upon which the fungus feeds. Most often their mycelia are buried in the tissue of a tree trunk, on a fallen log of wood or in other nourishing substrates. They are cosmopolitan, heterotrophic organisms that are specific in their nutritional and ecological requirements. Nigeria with her unique climatic conditions of tropical rain forest in the south and sub-saharan condition in the north is a home to diverse species of mushrooms. The common mushrooms in Nigeria include Termitomyces, Pleurotus, Lentinus, Lenzites, Trametes, Ganoderma, Pycnoporus, Coriolopsis and others [14] some of which are shown in Figure 1.

Criteria for classification of mushroom as prebiotic

The following criteria are used to classify a compound as a prebiotic:

i. It should be resistant to the acidic pH of stomach, cannot be hydrolyzed by mammalian enzymes, and also should not be absorbed in the gastrointestinal tract

ii. It should be fermented by intestinal microbiota. The growth and activity of the intestinal bacteria should be selectively stimulated by this compound and this process improves host's health (Gibson et al. [15]). Although not all the prebiotics are carbohydrates, the following two criteria can be exploited to distinguish fiber from carbohydrate-derived prebiotics:

(i) Fibers are carbohydrates with a degree of polymerization (DP) equal or higher than 3

(ii) Endogenous enzymes in the small intestine cannot hydrolyze them. It should be taken into account that the fiber solubility or fermentability is not crucial.

Wang [16] however grouped the criteria for classification of a food as prebiotic into five (5) as shown in Figure 2 namely:

Resistance to upper gut tract: It is the first criteria for prebiotics and actually to ensure that the prebiotics can withstand digestive processes before they reach the colon, thus stimulate the beneficial bacteria; bifidobacteria and lactobacilli

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effectively. Resistance to digestive processes includes resistance towards gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption (Gibson et al. [15]). Some of the non-digestible oligosaccharides presently available or in development as food ingredients include carbohydrates in which the monosaccharide unit is fructose, galactose and glucose. The non-digestible oligosaccharides are made of one, two or even three monosachharides and have no nutritional significant. It is water soluble and exhibits some sweetness. However, the degree of sweetness depends on the chain length. It was reported that inulin with degree of polymerization more than ten does not taste sweet anymore [16].

Figure 1: Species of Mushroom Source: Mendel [31].

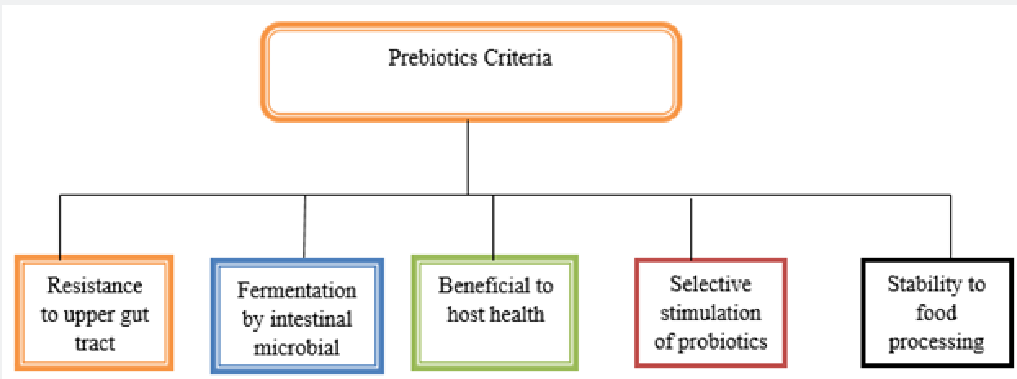

Figure 2: Criteria for Classification of food as Prebiotics Source: Wang [16].

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Fermentation by intestinal microbiota: The effects of this fermentation may lead to an increase in the expression or change in the composition of short-chain fatty acids, increased fecal weight, a mild reduction in luminal colon pH, a decrease in nitrogenous end products and reductive enzymes, an increased expression of the binding proteins or active carriers associated with mineral absorption and immune system modulation which could be beneficial to host health [16]

Beneficial to host health: The food must be able to stimulate beneficial bacteria in the colon with nutrient absorption that will be beneficial to the health of the host.

Selective stimulation of probiotic: Prebiotics must be particularly suited to the growth and activities of probiotics, bifidobacteria and lactobacilli [16] and suppress the growth of clostridia and bacteroides. Palframan et al. [17] came out with a comparative quantitative tool known as Prebiotic Index (PI) for measurement of prebiotic effects in vitro. Assumption from the equation is that the increases in the population of bifidobacteria and/or lactobacilli are considered a positive effect, while an increase in bacteroides and clostridia (histolyticum subgroup) are negative. PI can be calculated using the following equation:

Prebiotic index = Bif - Bac + Lac - Clo ……………………………………….1

Total Total Total Total

Where Bif is bifidobacterial numbers at sample time/numbers at inoculation, Bac is bacteroides numbers at sample time/numbers at inoculation, Lac is lactobacilli numbers at sample time/numbers at inoculation, Clos is Clostridia numbers at sample time/numbers at inoculation and total is total bacteria numbers at sample time/numbers at inoculation.

Stability to food processing: A prebiotic should withstand food processing conditions to remain intact not degraded or chemically altered. It must be available for bacterial metabolism in gut to improves gastrointestinal health of human. It has conclusively shown that only heating at low pH caused significant reduction in prebiotic activity of inulin, while fructooligosaccharides (FOS) contained product was observed to be the least stable.

Mushroom as a prebiotic

Prebiotics are typically oligosaccharides or more complex saccharides that are selectively used by commensal bacteria in the large intestine, including species considered to be beneficial for the human host to prevent viral or bacterial infection. The potential of mushrooms to enhance growth of bifidobacteria is used to measure the prebiotic index. Prebiotics must resist host digestion, absorption, and absorption before fermentation by one species of the resident microbiota.

Aida et al. [12] reviewed the prospect of mushrooms as a source of prebiotics that have the potential to promote human health; thus prebiotics was defined as selectively fermented food ingredients that induce changes in the composition and activity of the gastrointestinal tract microbiota that confer nutritional and health benefits to the host. Such compounds include mushroom polysaccharides, most of which are glucan polymers, with the main chain consisting of β1-3 linkages with some β1-6 branches as well as chitin, mannans, galactans, and xylans. Mushroom is a prebiotics as it contains carbohydrates like chitin, hemicellulose, β and α -glucans, mannans, xylans and galactans [12]. The prebiotic constituents of various mushroom are: beta glucan in Pleurotus ostreatus, Pleurotus eryngii, Pleurotus tuberregium, and Ganoderma lucidum. Chitin in Boletus spp and Agaricus spp, Lentinan in fruiting bodies of Lentinus edodes, 1- 3 alpha–d-glucan and beta 1-3 linked glucans in Ganoderma lucidum [18]. Beta-glucans, homo-glucans and heteroglycans with beta (1-3), beta(1-4) and beta(1-6) glucosidic linkages are responsible for the bioactivity of mushroom and its prebiotic properties. The prebiotic activity of mushroom is closely related to the presence of betaglucans and the fermentation of ability of the bacteria. Premsuda et al. [19] carried out a study to evaluate prebiotic property of five edible mushroom namely: Auricularia auricula-judae, Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus abalonus, and Volvariella volvacea. The mushroom samples were extracted separately to obtained soluble and insoluble polysaccharides. The mushroom were tested for their bifidogenic effect using 4 strains of Bifidobacterium sp. (B. bifidum TISTR 2129, B. breve TISTR 2130, B. longum TISTR 2194 and B. animalis TISTR 2195). The results showed that Pleurotus sajor-caju had the highest Prebiotic index value followed by Pleurotus abalonus and Pleurotus ostreatus. Auricularia auricula-judae and Volvariella volvacea had less stimulation of bacteria (bifidobacteria and lactobacilli) growth in the human gut to suppress harmful bacteria. Similarly studies by Bhakti and Harshita [18] on Pleurotus sajor-caju, Lentinus edodes and Pleurotus florida showed that extracts of Pleurotus sajor-caju produced good prebiotic index value when compared with Lentinus edodes and Pleurotus florida for *L. acidophilus*.

Digestive enzymes secreted by the pancreas cannot hydrolyze the glucosidic bond, which means the non-digestible mushroom carbohydrates can act as prebiotics in the digestive tract. These include ribose, xylose, fructose, mannose and glucose found in Pleurotus eryngii and Lentinus edodes [18]. Non-digestible oligosaccharides consist of two to three monosaccharides without nutritional value. For prebiotics from foods to have their beneficial effects they should not be structurally changed by food-processing conditions, such as heat and microwaves. Mushroom and mushroom-derived polysaccharides have been shown to have therapeutic properties against metabolic syndrome, which is characterized by obesity, hyperglycemia associated with diabetes, hypercholesterolemia, and hypertension.

Mushroom prebiotic and interaction with the microbiota

Gut microbiota is among the most complex microbial ecosystems. It crucial to human health through a variety of

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functions, such as the extraction of energy from foods, alterations in the appetite signaling pathway and involvement in host metabolic processes. It is also fundamental to host protection against pathogenic microorganisms, immune system development and function, imbalance in the dynamic interactions among microbial intestinal populations which led to inflammatory bowel disease, irritable bowel syndrome, obesity and colorectal cancer. Prebiotics play a key role in the restoration of the normal intestinal microbiota [19].

Prebiotics are post-ingestion fermentable food ingredients such as non-digestible polysaccharides like glucans and fibers that induce changes in the gastrointestinal microbiota that result in improved nutritional and health benefits to the host. The fermentation of non-digestible long-chain β-glucans from mushrooms to form short chain fatty acids (SCFAs) provides a potential source of new prebiotics [20]. Gut microbiota are also reported to affect obesity by helping to balance immunity and the nutritional status of the host [21]. Prebiotics are reported to lessen the symptoms of medical conditions associated with metabolic syndrome including diabetes. Prebiotics are also able to modify the environment of the gut because the fermentation products of prebiotics which are mostly acids decrease the gut pH [22].

Bio-active compounds in mushroom such as homo-polysaccharides and hetero-polysaccharides can influence the gut microbiota. This depends on factors starting with their frequency of consumption, the compounds concentration, the pattern of the bioactive compounds and the health of the organism. This directly and indirectly has effect on the modulation of GI microbiome. Mushrooms such as Ganoderma, Pleurotus, Boletus, Inonotus, Grifola, and Armillaria are used as prebiotics to improve the health of consumer. Edible mushroom serve as functional food to modulate the microbiota pattern and metabolomic function which result in antimicrobial and anti-inflammatory activities in the body. β-glucans made up of β-D-glucose units, which make up the cell-wall structure of mushrooms are responsible for modulating the immune response, regulating blood sugar and reducing dietary cholesterol absorption. β-glucans support the multiplication of favorable strains of genus Lactobacillus. It helps to mediate activation of T-cells and natural killer cells in the microbiota thereby boosting the immune system. The fermentative breakdown of the polysaccharides molecule stimulates the immune system, and protection against tumor cell proliferation. This modulates the microbiota and reduce the proportion of bacteria that synthesize products with carcinogenic potential [23]. Studies by Mitsou et al. [19] have shown that β-glucans, present in the species Pleurotus ostreatus, Pleurotus eryngii, Hericium erinaceus, as prebiotic had a targeted action on the elderly by increasing cell number of Lactobacillus spp strains' presence. This stimulates the synthesis of SCFAs, especially propionate and butyrate which are useful to modulate oxidative stresses in the body.

Functions and benefits of mushroom prebiotics to the body and health

As prebiotics in mushroom reach the colon (large bowel) without being digested because of their chemical nature. The whole length of human gut is occupied by microorganisms with population numbers and species distribution characteristic of specific regions of the gut. The colonic microflora derives their food from the undigested food such as non-digestible oligosaccharides, dietary fibre, and undigested protein. They also get their substrates from the mucin, the main glycoprotein constituent of mucus. Therefore, any undigested food that reaches the colon such as non-digested carbohydrates, some peptides and proteins, certain lipids are sources of prebiotics [24]. Interaction and effects of prebiotics in the body is shown in Figure 3 and the various benefits of mushroom to the body and health are:

I. Gastroenteritis

This is a common disease which usually occurs due to ingestion of food or water contaminated with pathogenic microorganisms and/or their toxins. The main pathogenic organisms responsible for gastroenteritis are Shigellae, Salmonellae, Yersina enterocolitica, Campylobacter jejuni, Escherichia coli, Vibro cholera and Clostridium perfringens. These pathogens grow and colonise the gastrointestinal tract and invade the host tissue or they secrete toxins in the foods before ingestion. These toxins disrupt the functions of the intestinal mucosa, causing nausea, vomiting and diarrhea. Prebiotics help to increase the population of useful organisms in large intestines that may possibly help in preventing gastroenteritis (Younis et al. [24]).

II. Inflammatory bowel disease

Inflammatory bowel disease has been related to the intestinal microbiota pathogenesis. One of the understandable ways for therapeutic intervention is probiotic treatment and mushroom as a prebiotic is rich in protein and hemicelluloses, the rich dietary fibre has prebiotic characteristics which reduce the incidence of bloody diarrhoea and mucosal injury (Younis et al. [24]).

III. Reduction of cancer risk

Upon the administration of prebiotics it has been found that the activity of genotoxic enzyme decreases. A study carried on feeding galacto-oligosaccharides to humans showed a decrease in nitroreductase (a mutagenic/carcinogenic substance) and decreased levels of indole and isovaleric acid produced due to proteolysis and deamination and markers of putrefaction. These interactions include apoptosis induction, a process which is inactivated in cancer cells which would generally lead to their elimination and an increase in the immunogenicity of cancer cells due to an increase in cell surface protein expression. However, the usual target bacteria for prebiotic use are bifidobacteria and lactobacilli but not butyrate producers and other gut flora components [24].

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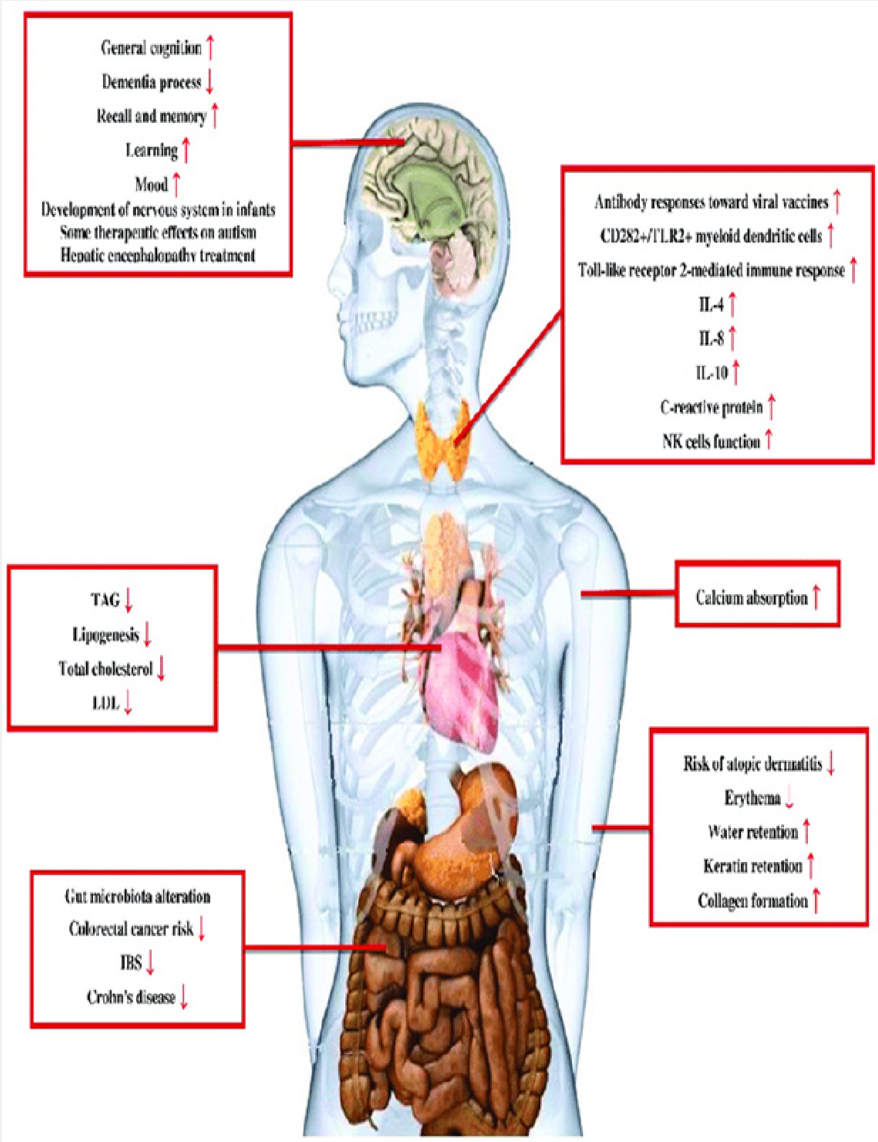

Figure 3: Prebiotics effects for health maintenance and protection against disorders Source: Dorna et al. [5].

III.13. Mushroom prebiotics and new product development

Prebiotics in mushrooms exhibit both important technological characteristics and interesting nutritional properties. Prebiotics can be used to offer a double benefit such as improved organoleptic quality and a better-balanced nutritional composition. Prebiotics are used in different foods as ingredients which help in the growth of health promoting colon microorganisms particularly probiotics and offer additional health benefits. Prebiotics in mushroom can be used in infant formula as stated by the European Commission's Scientific Committee on Food [25] that the addition of oligosaccharides to infant formulas had no major worry and including the studied infant formulas (formulas adapted especially for 6- to 12-month-old infants), up to a total concentration of 0.8 g/dL in ready-to-feed formula products.

Prebiotics can also be used in cheese, fermented milk and yoghurt. Ivana and Tri [26] used oyster mushroom powder as a prebiotic agent in yoghurt and recorded an increased lactic acid, reduced acidity, increased LAB viability. The addition of mushroom powder resulted to an increased yoghurt quality. Studies on supplementing bread with mushroom powder improved the dietary fiber (quality and quantity) which is a prebiotic that is important in the microbiota [27,28].

Milk mixed with residue from a hydroalcoholic extract of Agaricus blazei displayed a stronger antioxidant activity after digestion. Mixed cereal grains were prepared with Cordyceps militaris, a mushroom to develop an extruded product and upon utilization by exercise-induce fatigue animal model, the new extruded product was detected to have a significant enhancement

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of anti-fatigue property compared to the product of cereal grains only [29]. It can be concluded that mushroom has dietary fiber and important to the body as prebiotic and plays an important role in regulating the body mechanism [30,31].

Conclusion

Mushroom is composed of prebiotic constituents such as glucan polymers like β1-3 linkages and β1-6 branches with chitin, mannans, galactans, and xylans. There selectively ferment food ingredients that induce changes in the composition and activity of the gastrointestinal tract microbiota that confer nutritional and health benefits to the host.

Prebiotic constituents of edible mushroom serve as functional food to modulate the microbiota pattern and metabolomic function which result in antimicrobial and anti-inflammatory activities in the body. β-glucans made up of β-D-glucose units, which make up the cell-wall structure of mushrooms are responsible for modulating the immune response, regulating blood sugar, and reducing dietary cholesterol absorption.

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