# Comparison of the behavior of fungal and plant cell wall during gastrointestinal digestion and resulting health effects: A review

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## 1. Introduction

An increasing body of evidence is showing that health benefits of food depend on the food structure (or matrix) rather than its individual components. So often, only the macro or micronutrient content of a food has been considered for their nutritional properties and biological effects. However, the food structure is gaining more attention for its role in digestion and the modulation of subsequent physiological responses (Mackie, 2017). Several food processing stages, such as thermal processing, extrusion, fermentation, homogenization, drying, and milling, can modify the food structure. These methods applied to foods can also enhance the bioaccessibility and digestibility of nutrients within those foods (Grundy, Wilde, Butterworth, Gray, & Ellis, 2015; Mulet-Cabero, Mackie, Wilde, Fenelon, & Brodkorb, 2019) or, conversely, reduce the nutrition quality of micronutrients (Oghbaei & Prakash, 2016). The cell wall is an example of a food structure in both plants and fungi on a microscopic level. The cell wall itself is largely a polymeric structure, mainly composed of fiber such as cellulose in plants and β-glucans in fungi (Kang et al., 2018; Keegstra, 2010).

From a nutritional viewpoint, the cell wall is considered the primary source of dietary fiber (DF) and can also present a physical barrier that controls the bioaccessibility of nutrients contained within the plant/fungal cells. DF is classified as a carbohydrate which is indigestible to human mammalian enzymes (Dhingra, Michael, Rajput, & Patil, 2012). The monosaccharides of DF often have β-links which human enzymes are not able to hydrolyze. However, DF also comprises non-digestible α-linkages such as Rhamnogalacturonans I pectins (Lemaire et al., 2020).

The fungal cell wall (FCW) is mainly present in the diet as edible mushrooms, which are the spore-bearing fruiting bodies of filamentous fungi, primarily belonging to the *Basidiomycota* division. All mushrooms are considered fungi, but not all fungi can produce mushrooms. Filamentous fungi can be either multicellular organisms organized as hyphae that

all together compose the mycelium or unicellular organisms such as yeasts. The latter is mainly used for fermentation processes such as bread baking and beverages brewing (e.g., beer, wine, kombucha). In the same way, filamentous fungi can be used for fermentation purposes (e.g., koji with *Aspergillus oryzae*, tempeh with *Rhizopus oligosporus*, mold-ripened cheeses with *Penicillium camemberti* or *roqueforti*) and can also be used in association with yeasts (e.g., cured meats with *Debaryomyces hansenii* and *Penicillium* species, sake or rice wine with *Aspergillus oryzae* and *Streptomyces cerevisiae*, shoyu (soy sauce) with *Aspergillus oryzae or sojae, Hansenula spp.* and *Zygosaccharomyce rouxii*) (Venturini Copetti, 2019). Another way to consume filamentous fungi is represented by mycoprotein, which is not a mushroom, but the fermented mycelial biomass of the filamentous fungus *Fusarium venenatum* (ATCC PTA-2684). This fungus is a member of the *Ascomycota* division, which contains many plants and animal pathogens, but very few species produce edible fruiting bodies. The biomass is processed to have a similar texture to meat and is used as the main ingredient in all Quorn™ products (Denny, Aisbitt, & Lunn, 2008).

In contrast, the plant cell wall (PCW) is consumed in the human diet under different forms such as vegetables, legumes, fruits, nuts, seeds, and cereals. Plants are the principal component of diets known for their health benefits (e.g., Mediterranean diet) (Martínez-González, Gea, & Ruiz-Canela, 2019). Hence, the broader consumption of PCW than FCW has led to an extensive and accurate investigation of the health effects and mechanisms mediated by plant-based foods. Conversely, the effects and mechanisms by which fungal products and FCW can improve human health require more investigation. Despite some similarities, such as fibrous cell walls, it is not known whether compositional and structural differences between the two kingdoms may have different effects in modulating physiological responses. Hence, we aim to review the chemical and structural properties of FCW and PCW as sources DF, with a particular interest in the matrix effect and its impact on digestion and

subsequent physiological responses. The mechanisms that trigger the health effects promoted by PCW and FCW are discussed, and the gaps in our knowledge of the literature are highlighted.

## 2. Plant and fungal biology

The plants and fungi kingdoms of life have similarities as well as significant biological differences. **Table 1** is an overview that compares plant and fungi characteristics, which is admittedly a generalization as exceptions exist. Briefly, the two organisms are eukaryotic as their cells have nuclei. Cell walls are the outer layer that defines the cellular structure and retains membrane-bound organelles. Plants are capable of photosynthesis, which transforms light energy into chemical energy (autotrophic). On the other hand, fungi secrete enzymes into the extracellular environment to digest and absorb nutrients (heterotrophic). The glucose storage polymer of plants is starch, while glycogen is present in many fungi. Furthermore, the gametes for higher plants are eggs and pollen, whereas fungi use spores. Ergosterol is the principal sterol of the fungal cell membrane, while plants have different phytosterols such as sitosterol. The structural organization and the composition of PCW and FCW are similar as both cell walls are primarily composed of polysaccharides with β-links that are indigestible in the human upper gastrointestinal tract (GIT). PCW and FCW share the presence of β-glucans in their cell walls. However, the glucosyl residues composing β-glucans are linked by β-1-3, β-1-4 bonds in PCW, and β-1-3, β-1-6 in FCW. Another key difference between FCW and PCW is that fungi have chitin, a linear polymer of N-acetylglucosamine units, whereas plants have cellulose, a polymer of D-glucose units, and pectin composed of galacturonic acid units.

The dimensions of the cell walls differ substantially between the two kingdoms of life. The spatial distribution and organization of plant and fungal cells are also different as plant cells tend to form tissue structures comprising close-packed, interconnecting cells while fungi

form separated hyphae/mycelium that can potentially be considered more diffuse (e.g., high contact surface for enzymes) compared to plant tissues. These differences can influence the bioaccessibility of nutrients retained within the cell walls and may influence digestion physiology differently. This hypothesis is discussed in section 4.1.

**Table 1.** Comparison of general biological characteristics of fungus and plant kingdoms (exceptions exist). Adapted from Deacon (2013).

| Character | Fungus | Plant |
|---|---|---|
| ***Growth*** | Hyphal tip or budding yeast | Multicellular tissues |
| ***Nutrition*** | Heterotrophic | Autotrophic |
| ***Cell wall*** | Chitin, α– and β–glucans | Cellulose, hemicellulose, pectin, lignin |
| ***Carbon storage*** | Glycogen, lipids, trehalose | Starch, lipids, non-starch polysaccharides |
| ***Membrane sterol*** | Ergosterol | Sitosterol, other plant sterols |

### *2.1 Plant cell wall composition*

The following is a general description of the composition of a primary PCW (**Fig. 1**) PCW can be generally described as an envelope composed of a skeletal core of cellulose (unbranched and linear β-1-4 D-glucose units) that is combined with a hydrated-gel matrix comprising several polysaccharides (e.g., pectin and hemicelluloses such as mixed-linkage β-glucans composed by linked glucosyl residues). The middle lamella is a pectin-rich layer that serves to link two plant cells together (Jarvis, Briggs, & Knox, 2003). The structure and composition of primary PCW varies according to the plant species, cell types of the same plant species, and growth stage (Yokoyama, 2020). However, two main primary PCW types have

been described; Legumes and other dicotyledonous plant seeds have Type 1 primary cell walls, which are rich in pectic polysaccharides and xyloglucans, while cereals and other monocotyledonous grains have Type II cell walls which tend to be lower in pectin but rich in arabinoxylans and/or mixed-linkage 1-3, 1-4 β-D-glucans. A recent comparative study of processing and digestion behaviors of chickpea (type I) and durum wheat (type II) tissues revealed the different and mechanisms by which these cell wall types control nutrient bioaccessibility (Edwards, Ryden, Mandalari, Butterworth, & Ellis, 2021).

Miscellaneous components such as glycoproteins, phenolic acids, minerals, and lignin contribute to completing the PCW (Holland, Ryden, Edwards, & Grundy, 2020). Furthermore, some plant cells tend to accumulate cellulose and lignin to form what is defined as the secondary cell wall. The latter is less hydrated than primary cell walls and, in some plants, the primary structural elements appear to be microfibrillar bundles (Cosgrove & Jarvis, 2012). Overall, the dietary fibre in the human diet is mainly consumed in the form or primary cell walls as the lignification can decrease the food palatability (Holland, Ryden, Edwards, & Grundy, 2020; Waldron, Parker, & Smith, 2003).

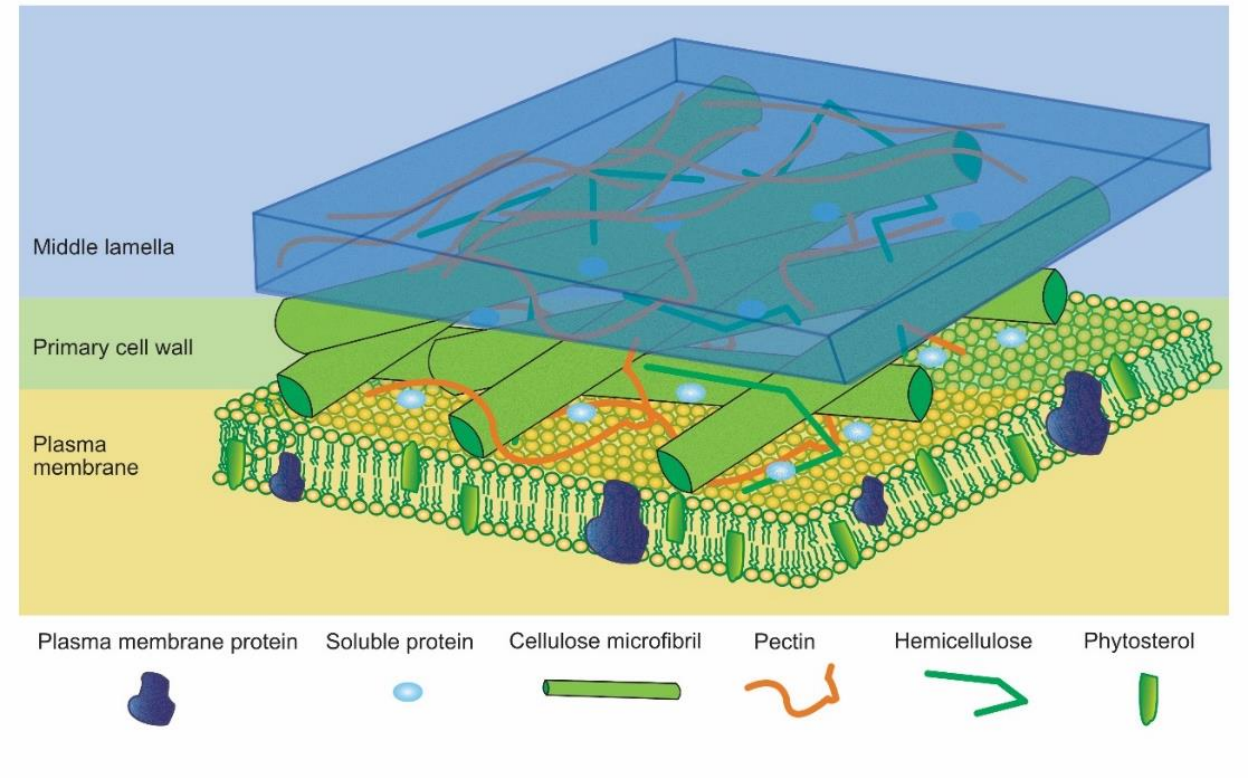


**Fig. 1**. Simplified representation of the primary plant cell wall. Adapted from Scheller and Ulvskov (2010).

### *2.2 Fungal cell wall composition*

The FCW organization can vary between species and according to the growth stage (Gow, Latge, & Munro, 2017). Another difference can be observed in the content of glucans that is higher in the cell walls of the fruiting bodies than the mycelium (McCleary & Draga, 2016). Likewise, Bak, Park, Park, and Ka (2014) showed that different sections of the fruiting bodies of *Lentinula edodes* differ in the β-glucans content. The stipe, which is the stem that supports the cap (pileus), showed the highest β-glucans content also compared with the mycelium. This section provides a general overview of the FCW (**Fig. 2**) that applies to filamentous fungi. The FCW is a biological envelope whose organization changes between different fungal phyla. However, the core skeletal components of the cell wall are β-1-3 (45-55% dw) and β-1-6 (5% dw) glucans and chitin (1-2% dw) (Ruiz-Herrera & Ortiz-Castellanos, 2019). The FCW is composed of three main layers. The inner layer, which is hydrophobic and

rigid and mainly composed of α-glucan and chitin, a medium inner layer that is hydrated and mobile, and an outer layer that is hydrated and highly mobile. The matrix also comprises various components such as glycoprotein (mannoprotein), melanin, lipids, and polyuronides (Gow, Latge, & Munro, 2017). Glucans and chitin form the rigid and hydrophobic inner portion of the FCW, while glycoprotein and α-1,3-glucan form an external and hydrated compartment characterized by high motility (Kang et al., 2018).

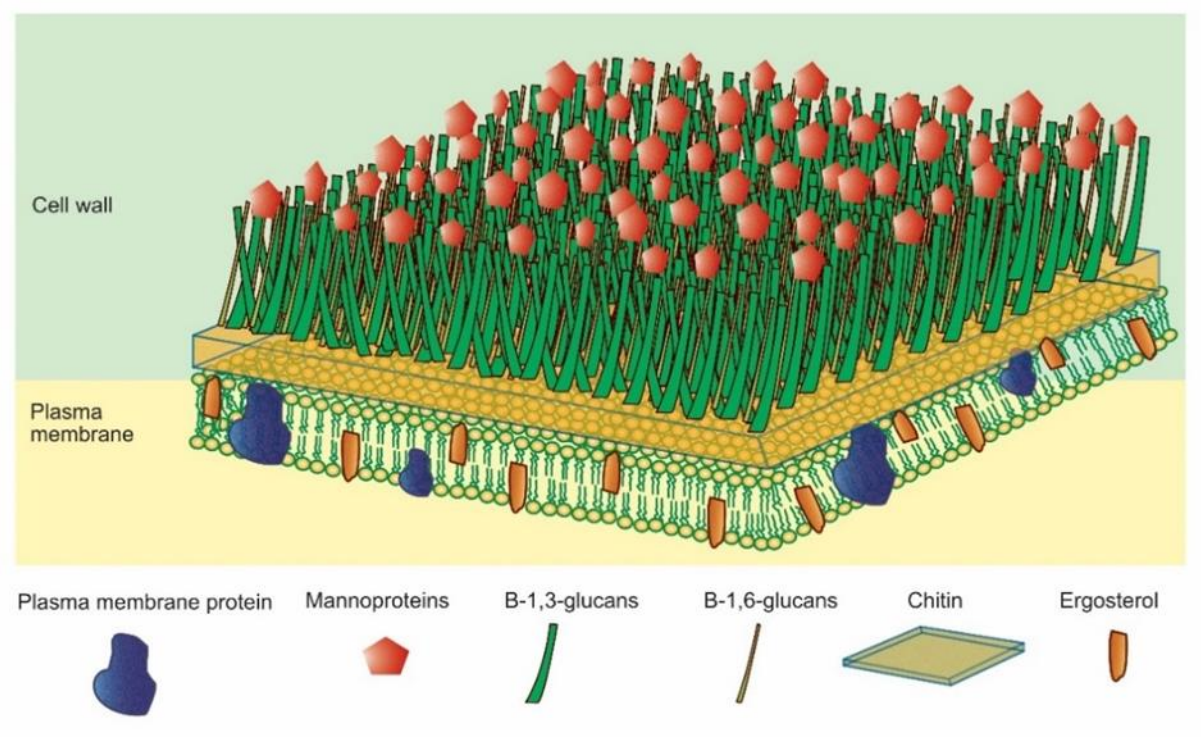


**Fig. 2**. Simplified representation of the fungal cell wall. Adapted from Vega and Kalkum (2012).

## 3. Physiological implications of cell wall fibers on human health

High consumption of plants and mushrooms is associated with reduced risk of food-related conditions such as type-2 diabetes (T2D) and cardiovascular diseases (CVD). The cell wall fibers are a critical component contributing to the prevention of these and other diseases (Scientific Advisory Committee on Nutrition, 2015 (Accessed 19/11/20)). The underlying mechanisms are not fully understood, and there is evidence that physicochemical factors of the

cell walls, such as solubility, viscosity, water-holding capacity, processing and intactness of its structure, are of mechanistic importance (Holland, Ryden, Edwards, & Grundy, 2020). Often considered an inert and undigestible component, the fibrous cell wall can influence digestion and the subsequent physiological responses in the GIT. The next sections will discuss the impact of PCW and FCW on glucose (3.1) and lipid (3.2) postprandial metabolism and satiety regulation. Furthermore, fungal components such as cell wall polysaccharides (β-glucans) may have an impact on human health by modulating anti-inflammatory responses (Muszyńska, Grzywacz-Kisielewska, Kała, & Gdula-Argasińska, 2018).

### *3.1 Glucose homeostasis regulation*

Briefly, the absorption of glucose released from dietary carbohydrates causes a surge in blood glucose. Glucose homeostasis is maintained primarily by insulin and glucagon, which regulate the uptake and release of glucose in the fed and fasted states, respectively. An imbalance in the glucose homeostasis, such as the development of insulin resistance, is one of the factors that contribute to T2D development. T2D complications also include CVD, retinopathy, nephropathy, neuropathy, and sexual dysfunction (Ceriello, 2005). There are several possibilities to prevent these pathologies, including a healthier lifestyle and dietary patterns. Several studies, which will be discussed in the next section, have shown that some foods rich in fiber can promote increased insulin sensitivity and lower insulin response. Thus, controlling glycemia and insulin responses is an essential tool for the prevention of T2D as well as CVD.

#### *3.1.1 Studies on plants*

The presence of intact fibrous structures that are not accessible to α-amylase have been linked to reduced starch digestion and, thus, can be a tool to help improve blood glucose

control in people with and at risk of T2D (Scazzina, Siebenhandl-Ehn, & Pellegrini, 2013). A meta-analysis reported an inverse correlation between T2D risk and DF intake, mostly when consumed from oatmeal and psyllium. These two DF sources have shown lower fasting blood glucose glycosylated hemoglobin (HbA1c) in individuals with diabetes and prediabetes compared to a placebo (Dreher, 2018).

These studies gave strong evidence on the correlation between DF intake and the prevention of T2D. Although, the fiber source seems to influence the physiological response as fruit or vegetables appear to have a negligible effect. This lack of effect may be due to the high sugar content of the fruit or structural differences in the cell walls that behave differently in the GIT. These inquiry lines require further investigation to understand how different fibrous structures can influence digestion, prevent T2D, and help develop healthier foods with a low glycemic index.

*3.1.2 Studies on fungi*

Studies have reported the effects of fungi consumption on attenuating postprandial glycemia and improving insulin response (Martel et al., 2017; Wu & Xu, 2015). For instance, incorporating fiber-rich mushroom powder (*Pleurotus sajor-caju*) into biscuits reduced starch hydrolysis (Ng, Robert, Ahmad, & Ishak, 2017). Despite the potential alteration of the FCW due to processing for incorporation to biscuits, the DF from FCW appeared to modulate starch digestion. This can improve glucose homeostasis and, therefore, reduce T2D risk. Likewise, a randomized, double-blind study on subjects (n = 120) with T2D showed that eating mushroom biscuits of *Pleurotus sajor-caju* or ajwain (annual herb) plus mushroom for three months reported a reduction of fasting blood glucose level and HbA1c (glycosylated hemoglobin) when compared to the ajwain control biscuit (Agrawal et al., 2010). Similarly, the oral administration of *Pleurotus ostreatus* has shown hypoglycemic effects in experimental rats

(Saritha & Usha, 2009). Comparable results have been observed from the consumption of mycoprotein based products. Turnbull and Ward (1995) found, in a cross-over study in healthy subjects (n = 19), a significantly lower postprandial serum glucose and insulin response to consumption of a mycoprotein-enriched milkshake compared to a nutritionally matched control milkshake (soy). However, Bottin et al. (2016) found no difference between the postprandial glycemic response of overweight and obese volunteers participants (n = 55) to a risotto made with chicken compared with mycoprotein in two randomized single-blinded controlled trials. Although the insulinemic response of mycoprotein compared with chicken was lower. Likewise, a randomized, single-blind, cross-over design study from Dunlop et al. (2017) conducted on young males subjects (n = 15) suggested that the insulin response is lower and more sustained on a mass-matched meal bolus, but comparable between mycoprotein and milk protein on a protein-matched meal bolus. This was supported by similar findings from the Monteyne et al. (2020) randomized, double-blind, parallel-group study in healthy resistance-trained male subjects (n = 20). The study showed that a leucine-matched bolus of mycoprotein had a lower insulin response in the first 15 min when compared to milk, but comparable in the subsequent time points.

These studies suggested that both mycelial biomass and fruiting bodies (mushrooms) can help promote and maintain healthy glucose homeostasis, and the presence of the FCW appeared to be a key component. Further investigation will be crucial to confirm these observations.

### *3.2 Hypocholesterolemic effects*

High levels of blood lipids are considered a risk factor for the development of CVD. A healthy lifestyle characterized by not smoking, moderate physical activity, and a balanced diet (e.g., Mediterranean diet) with foods rich in fiber is regarded as a preventive tool to reduce

234 the risk of developing CVD (Buttar, Li, & Ravi, 2005). Thus, DF sources such as PCW and
235 FCW are excellent candidates to prevent CVD development. In addition, as mentioned before,
236 DF can influence glucose homeostasis by reducing insulin resistance and controlling glycemia
237 that can also contribute to the prevention of CVD progression.
238
239 *3.2.1 Studies on plants*
240 Several types of fiber from plants may help prevent CVD (Lunn & Buttriss, 2007).
241 Queenan et al. (2007) reported that oat β-glucans could reduce total cholesterol and LDL
242 cholesterol in hypercholesterolemic men and women (n = 75) in a randomized, double-blind,
243 parallel-group design study. A meta-analysis with cohort study publications (n = 22) concluded
244 that the intake of total DF, insoluble fiber or fiber from cereals, vegetables, and fruits was
245 inversely associated with CVD risk (Threapleton et al., 2013). The DF source appeared to be
246 crucial in modulating a significant response, similar to what was reported in the studies on T2D
247 (section 3.1.1). Indeed, the fiber belonging to cereals showed a significant inverse correlation
248 with CVD mortality compared to other sources.
249 The vast number of findings and evidence reported by these meta-analyses suggests
250 that the DF intake from plants is an important critical factor for CVD and T2D prevention. It
251 is important to note that these meta-analyses do not generally consider the structural form of
252 the food (e.g., whole grain or tissue, milled flour, or extract), which is an important factor
253 controlling nutrient bioavailability. Therefore, it is crucial that future studies determine the
254 physicochemical properties of the food matrix and the cell walls and how they behave in the
255 GIT to understand better the correlation between the food matrix and the subsequent
256 physiological responses.
257
258 *3.2.2 Studies on fungi*

Studies have shown that consumption of edible mushrooms may have a preventative role for CVD (Jeong et al., 2010; Oyetayo, 2006). For instance, Kim et al. (2019) reported that the consumption of whole Portobello and Shitake mushrooms could reduce atherosclerosis in mice fed with a high-fat diet. Conversely, a review reported three studies focusing on CVD biomarkers in humans after edible mushroom consumption (Roupas, Keogh, Noakes, Margetts, & Taylor, 2012). Two studies reported significant hypolipidemic effects (Khatun, Mahtab, Khanam, Sayeed, & Khan, 2007; Mee-Hyang, Kwon, Kwon, Ma, & Park, 2002). However, one study reported no improvement in reducing CVD risk in a double-blinded placebo-controlled, cross-over intervention with eighteen participants (Wachtel-Galor, Tomlinson, & Benzie, 2004). Other pieces of evidence in the reduction of blood lipids were observed in studies on mycoprotein. Turnbull, Leeds, and Edwards (1992) investigated the impact of mycoprotein on blood lipids in participants (n = 21) with slightly raised blood cholesterol for eight weeks under free-living conditions. Mycoprotein was incorporated in a cookie matrix and compared with nutrient-balanced cookies without mycoprotein added. The results supported the previous outcome by showing a reduction in cholesterol and LDL levels, which was statistically lower than the control. Recently, a randomized, parallel-group study in twenty healthy adults showed that mycoprotein consumption could modify the plasma lipidome compared to meat or fish (Coelho et al., 2020). The study reported a decrease of lipoprotein fractions and cholesterol compared to the meat/fish control.

These studies provide evidence of the blood lipid-lowering effects promoted by the consumption of fungi, which could be excellent candidates for CVD prevention. However, studies have often focused on extracts and isolated components from edible mushrooms (Gil-Ramírez, Morales, & Soler-Rivas, 2018; Gil-Ramírez & Soler-Rivas, 2014) and did not consider the whole food matrix effect. Hence, the degradation of the food structure during

digestion needs to be further studied to understand its influence on physiological responses that mediate hypocholesterolemia.

### *3.3 Satiety regulation*

The term satiety refers to the feeling of fullness and the suppression of hunger after eating. Satiety differs from satiation, which is the process that causes one to stop eating. Satiety is one of the main variables capable of influencing eating behavior that is metabolically regulated by gut peptide hormones such as glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK), amylin, and peptide tyrosine-tyrosine (PYY). DF is often associated with increased satiety (Slavin & Green, 2007). Correct nutrition characterized by appropriate satiety is thought to be important to reduce energy intake and improve weight management. This may minimize a high-caloric intake and, hence, reduce obesity and promote health, but the links with satiety and long term health benefits are not clear (Halford & Harrold, 2012).

#### *3.3.1 Studies on plants*

A randomized cross-over design was carried out to examine the effects on satiety of a processed-meat and cheese meal or a tofu vegan meal (matched in energy and macronutrients) in men with T2D (n = 20), obese men (n = 20), and healthy men (n = 20) (Klementova et al., 2019). The authors observed that the plant-based diet increased the secretion of gastrointestinal hormones that regulate appetite and promoted satiety more efficiently than the animal-based diet in all the groups. The meals were matched in terms of energy and macronutrients, except for fiber. Thus, the increased satiety appeared to be attributable to the DF component. However, the different protein profiles, the presence of minor compounds such as polyphenols, or protein-polyphenols bound particles may have contributed to increasing the concentration of gut hormones released and, therefore, satiety

(Foegeding, Plundrich, Schneider, Campbell, & Lila, 2017). A review and meta-analysis have reported that soluble fibers may increase satiety and reduce energy intake (German et al., 2009).

In these studies, satiety appears to be strongly influenced by viscosity promoted by soluble DF. Hence, it is crucial to estimate the degree of release of soluble DF from food matrices that may enhance viscosity in the physiological conditions of the GIT. The release of DF may vary from different sources and may depend on the cell wall organization and structure. Furthermore, the comparison between a plant-based meal and an animal-based meal is essential to understand the difference offered by the presence of fibrous cell walls in the release of nutrients that can impact satiety. Thus, studies that focus on the kinetics of nutrient released during digestion can be useful in comparing and investigating the correlation of specific nutrients (e.g., protein) in triggering the release of gut hormones that promote satiety (Wilde, 2009).

*3.3.2 Studies on fungi*

Previous studies have addressed the impact of mushroom consumption on satiety and food intake. Cheskin et al. (2008) reported no statistically significant difference in the satiety effect promoted by a white button mushroom meal compared to meat in a randomized, cross-over design study on normal-weight, overweight and obese individuals (n = 76). The study showed that matching the lunch meals by volume, a lower value of calories from mushrooms (339 kcal) was comparable in satiety to meat calories (783 kcal). Likewise, a one-year randomized clinical trial with seventy-three subjects (Poddar et al., 2013) showed that replacing red meat with mushrooms can increase weight loss, weight maintenance, and health parameters (e.g., lower systolic and diastolic pressure, lipid profile, and inflammatory markers). Furthermore, a decrease in body mass index (BMI) and waist circumferences was reported. Similarly, a randomized cross-over study showed that the consumption of mushrooms

(*Agaricus bisporus*) has an impact on satiety when compared to meat in a protein-matched meal in thirty-two healthy participants (Hess, Wang, Kraft, & Slavin, 2017).

Studies on mycoprotein consumption have also shown potential satiating effects in normal-weight participants (Turnbull, Walton, & Leeds, 1993), and over-weight individuals (Bottin et al., 2011). However, the SATIN project (European Commission project Satiety Innovation) recently reported that food that was known for reducing appetite effects, such as mycoprotein, did not reduce appetite nor promote weight loss in weight-reduced individuals (Andersen et al., 2020). This discrepancy of observations can be due to the testing performed in weight-reduced individuals whose body energy stores could have influenced both appetite and body weight assessments.

Overall, an increase in satiety appears associated with mushrooms consumption. A moderate energy intake that results in weight loss is crucial for preventing obesity and correlated complications (Martel et al., 2017). Low caloric density is mainly associated with the high content of fiber and, therefore, cell walls in fungal cells. The bulking action and low caloric content are mechanisms suggested behind the satiety enhancement. However, the effect of DF in the GIT can act on several fronts as discussed in the next section.

## 4. Mechanisms underlying the health effects

As discussed in section 3, foods rich in DF, and therefore cell walls, are often associated with promoting satiety and positive impacts on risk factors for diseases such as T2D and CVD. Several possible mechanisms have been suggested that underpin these impacts on health. The current knowledge suggests that DF can control the nutrient bioaccessibility, increase viscosity in the gut, promote the binding/sequestration of digestive components, and be fermented by the resident microbiota in the large intestine. These are the main mechanisms that are listed and discussed in this section. Nevertheless, other mechanisms mediated by DF (e.g., hormonal

358 regulation) may also explain some of the health effects reported in the literature (Goff, Repin,
359 Fabek, El Khoury, & Gidley, 2018).
360
361 ***4.1 Control of nutrient bioaccessibility (barrier effect)***
362 Nutrients can be encapsulated within a food matrix, or they can be readily accessible
363 when no structure is present (e.g., juices). A growing body of studies has consistently shown
364 that fibrous cell walls control (limit or prevent) the release of nutrients from the food matrices.
365 Digestive enzymes are needed to gain access to and hydrolyze nutrients, and this property is
366 referred to as bioaccessibility. For instance, an intracellular nutrient from a plant or fungal cell
367 must be accessible to enzymes to be digested and then absorbed. This contact can happen in
368 two ways: first, the enzyme can diffuse through the cell wall to hydrolyze the nutrient, and later
369 the products of hydrolysis are released in the extracellular space. Second, the nutrient can be
370 released from a damaged or disrupted cell wall to become bioaccessible to the enzyme (**Fig.**
371 **3**).
372 As mentioned before, a controlled release of macronutrients may limit energy
373 availability from food and slow digestion, which can be beneficial in promoting satiety and
374 attenuating postprandial glycemia. These physiological responses may help maintain an
375 equilibrium in blood lipids and glucose homeostasis and prevent the onset of CVD and T2D.
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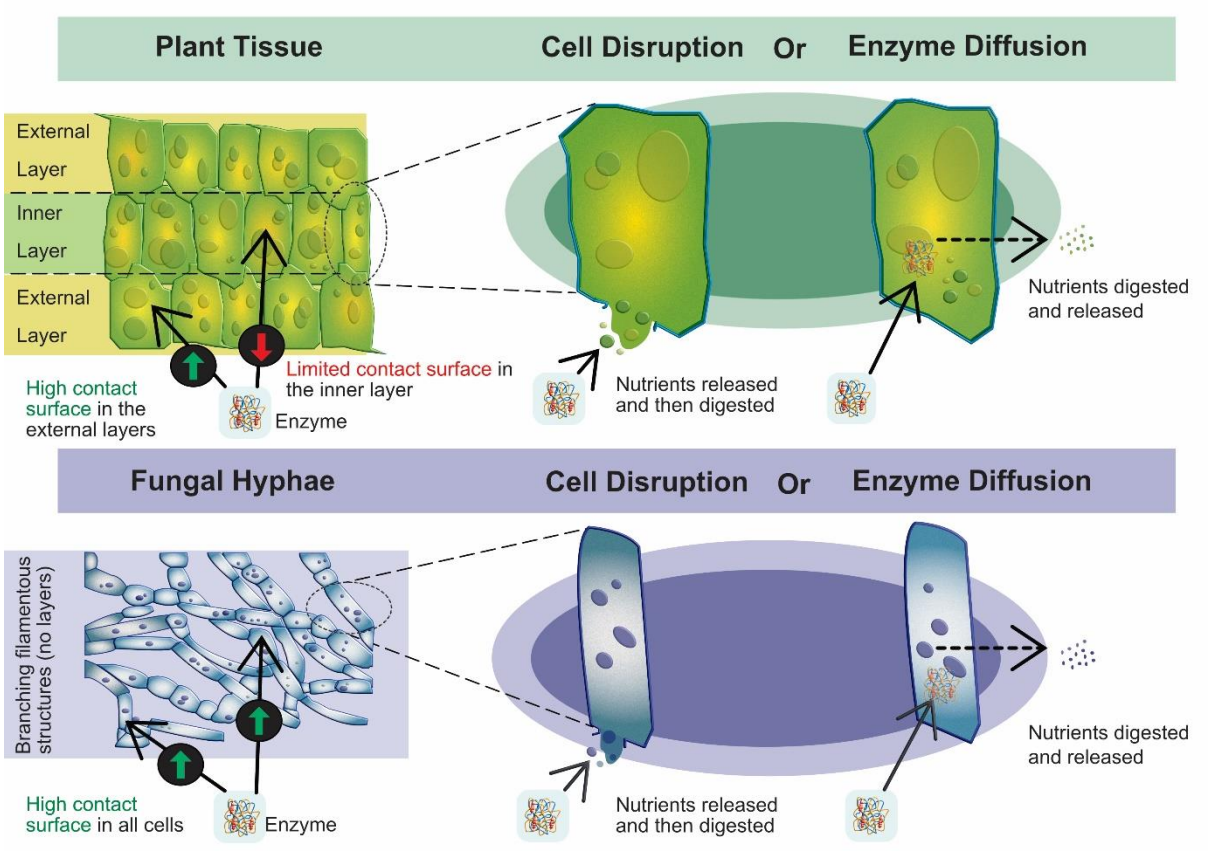


**Fig. 3**. Schematic representation of the structural/organizational difference between plant tissues and fungal hyphae; bioaccessibility of nutrients in plant and fungal cells.

The properties escribed in this section are specific to Type I or II cell walls. The cell wall of different plant sources has been shown to control the bioaccessibility of nutrients. For instance, the behavior of the PCW (mainly Type I walls) to act as an envelope with a crucial role in the control of enzyme accessibility and nutrient release has been described (Grundy, Wilde, Butterworth, Gray, & Ellis, 2015; Li, Zhang, & Dhital, 2019; Zahir, Fogliano, & Capuano, 2020). Processing such as particle size reduction (Edwards, Warren, Milligan, Butterworth, & Ellis, 2014) or hydrothermal conditions (Pallares et al., 2018) can alter the physicochemical properties of cell walls with marked effects on the availability of nutrients. For instance, in plant tissues where grinding, mastication, and cooking cause cell rupture or fracture (mainly type II cell walls), this could effectively increase the release and rate and extent of digestion of nutrients (Grundy, Wilde, Butterworth, Gray, & Ellis, 2015; Mandalari et al.,

2014). In other plant tissues, the hydrothermal processing enables plant cells to separate yet remain intact. These intact plant cells can persist through mastication and through upper gastrointestinal transit, such that the cell walls protect intracellular nutrients from digestion (Edwards, Ryden, Mandalari, Butterworth, & Ellis, 2021). The cell separation behavior of cooked pulses has been identified as a key mechanism that underpins the beneficial effects of pulse consumption on glycemic responses and cardiometabolic disease (Grundy, Wilde, Butterworth, Gray, & Ellis, 2015).

Cell wall intactness and encapsulation effects on nutrient bioavailability are now well-established in plants; On the contrary, little is known on FCW and its fate during gastrointestinal digestion. A recent investigation of the FCW from mycoprotein was carried out to investigate the role of the FCW in controlling the bioaccessibility of protein (Colosimo, Warren, Finnigan, & Wilde, 2020) and its effect in limiting starch hydrolysis (Colosimo, Warren, Edwards, Finnigan, & Wilde, 2020). Similarly to PCW, the FCW was shown to reduce the accessibility of nutrients leading to the slowest kinetic of digested nutrients (e.g., glycogen). However, although digestion was slow, the intracellular content was largely bioavailable as digestive enzyme were able to diffuse through the fungal cell wall and hydrolyse the nutrients. Furthermore, the disruption of the FCW did not lead to a significant increase in the yield of nutrients digested. In pulses and almonds (Type I PCW), an intact cell wall limits bioaccessibility of intracellular starch, lipid and protein, such that the highest rate and extent of digestion is achieved when cells are ruptured (Edwards, Ryden, Mandalari, Butterworth, & Ellis, 2021). Other plant tissues such as wheat endosperm (Type II PCW) are more permeable to digestive enzymes, so that cell wall encapsulation of nutrient mainly affects the rate of digestion rather than the final endpoint. In the ileostomy study by Edwards 2015, starch was progressively digested from seemingly intact endosperm cells from the particle periphery

towards the core, resulting in differences in rate of starch amylolysis and postprandial glycemic responses, but not the amount of resistant starch at the terminal ileum (Edwards et al., 2015).

Hence, differences in the cell wall structure between PCW and FCW may result in the differential release of digested nutrients, with different physiological and hormonal consequences. Future studies that compare PCW and FCW will be required to determine how structural differences can affect the digestion and release of nutrients and estimate the degree of damage/disruption of cell walls in the GIT. Enzymatic diffusion can also be altered by differences in the cell wall thickness and composition – such mechanisms are discussed in more detail below.

*4.1.1 Cell wall encapsulation and tissue structure*

Despite the barrier effect, the diffusion of enzymes through the PCW has been reported in the literature (Grundy et al., 2016). The permeability/porosity suggests that enzymes can permeate some cell walls to access the intracellular nutrients despite the enveloping function. Similarly, the cell wall permeability/porosity in fungi is known (Walker et al., 2018) and could be a crucial factor for the bioaccessibility of nutrients. The structural and chemical differences in the layers can influence the diffusion rate of enzymes through the cell wall. Furthermore, plant cells can be tightly packed together in tissues, bound together by polymers, whereas fungi, despite some agglomeration of the hyphae, tend to be in a more open structure. Therefore, the cell surface area accessible to enzymes can be higher in fungi compared to cells contained in plant tissues (**Fig. 3**). The permeability/porosity can also be affected by digestive processes that can increase the diameter of the pores and/or increase the diffusion of digestive enzymes. Further investigation is required better to understand these mechanisms in both PCW and FCW.

### *4.2 Binding and sequestration of digestive components*

The binding of digestive components (e.g., enzymes, bile salts) to DF is a known mechanism that potentially modulates digestion and subsequent physiological responses. The binding is often associated with sequestration, these two terms are usually used as synonyms, but they should be considered two separate concepts. The binding is a mechanism that requires a chemical bond (e.g., non-covalent) between molecules (e.g., fiber/protein from the cell wall with digestive enzymes), whereas sequestration may refer to the consequence of the binding, or entrapment, that leads to a reduced concentration, and hence activity, of the bound compound from solution. For instance, physical entrapment of enzymes into the cell wall, or a viscous matrix made of soluble fiber, does not necessarily involve a chemical bond. Eventually, more clarity between binding and sequestration would be required in future studies to understand better the mechanisms by which fiber modulates digestion.

In the case of bile salts binding by fiber, the interaction leads to a decrease of serum LDL cholesterol as the steroid bile acids are bound and eliminated in the feces (Goel et al., 1998). Consequently, the liver activates the endogenous cholesterol catabolism to produce new bile acids. Nonetheless, the bile salt binding could be influenced by increased viscosity promoted by dietary fiber (Zacherl, Eisner, & Engel, 2011) or the different solubility of the fiber itself (Wang, Onnagawa, Yoshie, & Suzuki, 2001). ~~A recent *in vitro* study conducted with foods enriched with fiber from plant origins has suggested that bile acid adsorption to fiber might be directly correlated with the hydrophobicity of the bile acids (Naumann, Schweiggert-Weisz, Eglmeier, Haller, & Eisner, 2019). Likewise, the hydrophobicity of protein subunits has been often associated with the capacity to bind to bile salts Guerin, Kriznik, Ramalanjaona, Le Roux, & Girardet, 2016). This suggests that the binding, due to hydrophobic interactions, may play a crucial role in the interaction between bile acids and DF. Similarly, Pabois et al. (2020) investigated the impact of methylcellulose, which is chemically produced from natural plant~~

~~cellulose, on lipolysis and the interaction with bile salts. Despite the lack of a whole-cell wall structure, the hydrophobic methylcellulose reduced the bile salts concentration and, by decreasing their activity, lipolysis was also reduced. This reduction in free bile salt concentration suggests a chemical bond of the fiber with bile acids is the potential mechanism.~~ The binding and consequent sequestration of bile acids has been examined extensively with plant samples (Gunness & Gidley, 2010).

In contrast, little is known on how FCW or fungal components (e.g., protein or isolated fiber) interact with bile salts despite the hypocholesterolaemic effects that have been reported by *in vivo* studies (section 3.2.2). A recent *in vitro* study investigated bile salt binding and lipolysis reduction mediated by mycoprotein and showed an inverse correlation between mycoprotein concentration and lipolysis activity. Moreover, bile salt binding was observed only when the FCW was exposed to a previous gastric acid environment (pH 3.0) and then washing to neutrality in the small intestinal step (pH 7.0). This suggested a chemical interaction between the fiber of the cell wall and bile salts (Colosimo et al., 2020).

Similarly, the binding of digestive enzymes has been observed in PCW and FCW. Cellulose has been reported to bind the enzyme α-amylase in a purified form or as a component of wheat bran (with cell walls) (Dhital, Gidley, & Warren, 2015), suggesting a binding mechanism more than sequestration. On the contrary, α-amylase appeared to be sequestered within the FCW of mycoprotein as shown by Colosimo, Warren, Edwards, Finnigan, and Wilde (2020).

The results of the binding and sequestration of enzymes/bile salts are both positively correlated to human health as they lead to slow and sustained digestion (as discussed before, section 4.1). However, many studies focused on the fiber as a general nutrient without considering the food matrix effect mediated by cell walls. Therefore, further studies are

required to understand better the binding or sequestration mechanisms mediated by PCW and FCW to help develop new products that can improve human health.

### *4.3 Increased viscosity in the gut*

Viscosity is generally defined as a physical property of fluids that show resistance to flow or mathematically defined as the shearing stress ratio to the velocity gradient in a liquid. An increased viscosity in the GIT that can be promoted by some types of DF has been extensively studied, especially in plants sources, for its inverse correlation with blood glucose and lipid-lowering effects or for enhancing satiety (Scazzina, Siebenhandl-Ehn, & Pellegrini, 2013).

The impact of fungal components on increasing viscosity *in vitro* and potentially promoting health benefits has been described by Wu, Chiou, Weng, Yu, and Wang (2014). The authors reported the hypoglycemic effects (adsorption of glucose, retardation of glucose diffusion, and reduction of the α-amylase activity) of hot water extract of *Auricularia polytricha* (wood ear mushroom) whose viscosity was comparable to psyllium. Although the hot water extract showed a hypoglycemic effect *in vitro*, it is not clear how the release of fibre from the FCW would have similar effects under physiological conditions. The release of soluble fiber from the food structure is a critical step that increases the viscosity of the digesta in the GIT, or the food structure itself can also modulate viscosity. On the other hand, the *in vitro* digestion of mycoprotein did not show any significant increase in the viscosity compared to the undigested sample (Colosimo et al., 2020). This may suggest that there was no soluble fiber release from the FCW. Alternatively, if fiber was released, the amount of fiber or its molecular characteristics (Bai et al., 2017) were not significant to increase viscosity.

Thus, viscosity might have a role in improving T2D as well as CVD. Several studies of plant sources have been reviewed. However, more studies on fungal cells are required to

515 understand if fibers can be released from the FCW and promote a viscosity increase in the GIT.
516 Besides, further work is required to understand the physical basis underlying the role of
517 viscosity during digestion, specifically whether it is the viscosity of the whole digesta, or local
518 areas of high viscosity are capable of retarding digestion.
519
520 ***4.4 Colonic fermentation***
521 The area of research involving the GIT microbiota has gained a high interest in recent
522 years. A shift and/or increase in the bacterial population or specific bacterial activity have been
523 correlated with modulation of metabolic disorders and T2D (Cani, 2018). DF is a crucial
524 nutrient for gut microbiota. It has been shown that the lack of DF is detrimental for murine
525 colon health as the resident microbiota starts to degrade the colonic mucus barrier and increases
526 pathogen susceptibility (Desai et al., 2016). The fermentation of DF by the microbiota leads to
527 the production of short-chain-fatty-acids (SCFAs) such as acetate, propionate, and butyrate
528 (Harris, Morrison, & Edwards, 2020). SCFAs have been correlated to beneficial effects for
529 human health. For instance, SCFA can regulate blood pressure, appetite, glucose homeostasis,
530 and maintain gut integrity (Chambers, Preston, Frost, & Morrison, 2018). Propionate and
531 butyrate possess important protective activity against inflammation and colon cancer.
532 Propionate is also adsorbed and transported to the liver where it has been suggested to have
533 some beneficial effects on cholesterol reduction and glycemic control (Ramakrishna, 2013).
534 Once the SCFAs reach the blood circulation, they can modulate physiological processes such
535 as glucose storage in different tissues (e.g., muscle, fat) and organs (e.g., liver) that may help
536 in the control and/or prevention of T2D (Kim, 2018). For instance, an open-label, parallel-group
537 study reported that a diet high in DF, which is composed of whole grains, traditional Chinese
538 medicinal foods, and prebiotics, promoted changes in the gut microbiota and improved glucose
539 homeostasis in participants (n = 27) with T2D (Zhao et al., 2018).

Furthermore, the release of soluble DF from the PCW can be crucial for SCFAs production. For instance, an *in vitro* study using inocula from pigs found that the fermentation rate and SCFAs production from plant DF were higher in soluble substrates (arabinoxylan and mixed linkage β-(1-3)-(1-4)-β-glucans) compared to insoluble (insoluble arabinoxylan, maize and wheatstarch granules, and bacterial cellulose) (Williams, Mikkelsen, Le Paih, & Gidley, 2011).

Overall, the colonic fermentation of PCW and DF belonging to plants has been reviewed in the literature (Williams, Grant, Gidley, & Mikkelsen, 2017). On the other hand, the fungal DF literature still lacks enough studies to draw consistent conclusions. However, studies have shown that SCFAs are produced following fungal DF fermentation. Kawakami et al. (2016) showed that mushroom powders from white or brown *Agaricus bisporus* were fermented in rats. The SCFAs production was significantly higher in the white mushroom compared to the brown and the control. A recent *in vitro* study from Harris, Edwards, and Morrison (2019) has shown how the fermentation of whole mycoprotein or its isolated fiber can produce SCFAs. Marzorati, Maquet, and Possemiers (2017) reported that repeated and prolonged administration of isolated chitin/glucan, which are the two main components of FCW, can promote gradual changes in the bacterial population *in vitro*. A different SCFA production was reported between the low or high tested doses. The low dose was correlated with propionate production, whereas the high dose with both propionate and butyrate. The overall growth of both *Bacteroidetes* and *Firmicutes* was observed with the higher administration of chitin and glucans. However, a decrease in the ratio of *Bacteroidetes*/*Firmicutes* was observed during time, with *Bacteroidetes* taking more advantage of the presence of chitin and glucans. Similarly, a randomized, open-label cross-over study with participants (n = 32) eating meat or mushroom (*Agaricus bisporus*) diet reported a shift in *Bacteroidetes*/*Firmicutes* ratio that was in favor of *Bacteroidetes* after

564 the mushroom consumption. (Hess, Wang, Gould, & Slavin, 2018). Furthermore, no differences
565 in SCFAs concentrations were observed within the two diets.

566 More studies are required to determine the importance of the food matrix structure of
567 the FCW for colonic fermentation and health. Potentially, the rigid inner layer of the FCW may
568 have lower accessibility for fermentation by the large intestine microbiota, and this needs to be
569 tested in future studies if fungal-based foods are to be developed to optimize their impact on
570 health.

571

572 **5. Conclusions**

573 This review aimed to highlight similarities and differences between plants and fungi as
574 sources of DF and their impact on digestion and health, which arise from the characteristics of
575 their cell walls. The PCW has been studied during the last century, and its health effects are
576 well established. Nevertheless, more knowledge is coming out from recent studies that focus
577 on the importance of the whole intact food matrix on health effects. The cell wall, which is
578 strictly correlated with the structure of plants and fungal cells, can improve health by reducing
579 T2D and CVD risk or increasing satiety. Some of the plausible mechanisms in which the cell
580 walls can promote these effects have been reported and discussed. They include controlling the
581 bioaccessibility of nutrients in protein-rich fungi such as mycoprotein, whereas this effect can
582 be negligible in other fungal sources due to the modest content of macronutrients. Furthermore,
583 other mechanisms such as the binding and sequestration of digestive components (e.g.,
584 enzymes, bile salts), increasing viscosity, and colonic fermentation were discussed. The fungal
585 kingdom is gaining more attention in recent years as a third class of food for human
586 consumption. However, more research is required to fill the considerable gaps in our
587 knowledge when compared to plant counterparts. Besides sharing similarities, structural and
588 chemical differences between the two cell walls have shown divergent results (e.g., differential

release of digested nutrients) which are offering new insights. This understanding should be used to develop new lines of inquiry to fully understand how to control and optimize the impacts of fungal-based foods on health.

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**Author contributions**

**Raffaele Colosimo:** Writing – Original draft/review & editing, Software, Conceptualization, Visualization; **Frederick J. Warren:** Writing – review & editing, Supervision; **Cathrina H. Edwards:** Writing – review & editing, Supervision; **Peter Ryden:** Writing – review & editing; **Paul S. Dyer:** Writing – review & editing; **Tim J. A. Finnigan:** Writing – review & editing, Supervision; **Peter J. Wilde:** Writing – review & editing, Supervision, Project administration, Conceptualization, Visualization.

**Abbreviations**

DF: Dietary Fiber; PCW: Plant Cell Wall; FCW: Fungal Cell Wall.

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