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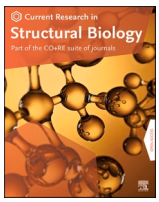

Nature's soothing solution: Harnessing the potential of food-derived polysaccharides to control inflammation

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ABSTRACT

Reducing inflammation by diet is a major goal for prevention or lowering symptoms of a variety of diseases, such as auto-immune reactions and cancers. Natural polysaccharides are increasingly gaining attention due to their potential immunomodulating capacity. Structures of those molecules are highly important for their effects on the innate immune system, cytokine production and secretion, and enzymes in immune cells. Such polysaccharides include β-glucans, pectins, fucoidans, and fructans. To better understand the potential of these immunomodulatory molecules, it is crucial to enhance dedicated research in the area. A bibliometric analysis was performed to set a starting observation point. Major pillars of inflammation, such as pattern recognition receptors (PRRs), enzymatic production of inflammatory molecules, and involvement in specific pathways such as Nuclear-factor kappa-B (NF-kB), involved in cell transcription, survival, and cytokine production, and mitogen-activated protein kinase (MAPK), a regulator of genetic expression, mitosis, and cell differentiation. Therefore, the outcomes from polysaccharide applications in those scenarios are discussed.

1. Introduction

Food-derived polysaccharides, which are complex carbohydrates composed of long chains of variable sugar units, have been found to possess anti-inflammatory properties. These compounds are abundant in various natural sources such as plants, fungi, and seaweeds. They are often important for tissue organization and structural management of metabolic processes in organisms. Several *in vitro* and *in vivo* studies have highlighted the influence of different polysaccharides or polysaccharide-enriched extracts, in regulation of inflammatory processes (Beukema et al., 2020; Prado et al., 2020).

One mechanism by which polysaccharides have exhibited anti-inflammatory action is by inhibiting the production of pro-inflammatory molecules, such as cytokines and chemokines, and by promoting the secretion of other anti-inflammatory molecules. Polysaccharides can interfere with the signaling pathways at gene and protein expression levels involved in the production of these inflammatory mediators, thereby reducing inflammation (Hou et al., 2020; Yang et al., 2022; J. Zhang et al., 2020).

Moreover, food-derived polysaccharides can also inhibit the activity of enzymes involved in inflammation, immune cell activity modulation, and possess antioxidant properties. For example, they can inhibit the activity of cyclooxygenase (COX) enzymes, which are responsible for the production of prostaglandins, potent mediators of inflammation (Hou et al., 2020).

It is worth noting that the specific mechanisms and the magnitude of regulation of anti-inflammatory actions may vary depending on the type of polysaccharide and its food source. Different polysaccharides have been studied extensively, including β-glucans, pectins, fucoidans, and fructans (Apostolova et al., 2020; Blanco-Pérez et al., 2021; Murphy et al., 2022; B. Xiong et al., 2021; Zhu et al., 2020).

In summary, polysaccharides derived from food sources exhibit anti-inflammatory action through multiple mechanisms, including inhibition of pro-inflammatory molecules, modulation of enzyme activity in immune cells, regulation of immune cell function, and antioxidant effects. These properties make those polysaccharides an interesting area of research for the development of natural anti-inflammatory agents with potential therapeutic applications.

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Due to the high variations in basic structures of polysaccharides depending on their source, it is helpful to understand some fundamental molecular aspects before exploring their anti-inflammatory properties.

2. Pectins

Pectins are heterogenous polysaccharides found in vegetable cell wall material, and are highly present in fruits and vegetables. They have very variable molecular sizes and are predominantly composed of α-1,4-D-Galactupyranuronic acid (Gal*p*A) units, which make up around 60–70% of their composition (homogalacturonans regions). As further discussed below, these units can be methylated at the carboxylic functional group (*O*-6), which can influence their biological effects. Other common structural monosaccharides are linked to α-L-Rhamnopyranose (Rha*p*) residues, which are sometimes intercalated with Gal*p*A units, forming the regions known as type 1 rhamnogalacturonan (RG-I; →α-1,2-Rha*p*-α-(1–4)-Gal*p*A→). The potential side chains of pectins often consist of α-L-Arabinofuranose, β-D-Galactopyranose, or a mixture of both bound to the Rha*p* residues (α-1,5-L-Ara*f* and β-1,4-D-Gal*p*, respectively) (L. D. F. Pedrosa et al., 2022). Minor quantities of other saccharides might be encountered in type 2 rhamnogalacturonan (RG-II), and chains consisting of xylogalacturonans are also often reported (e.g. β-Xyl*p*-(1–3)-α-Gal*p*A; Patova et al., 2021).

3. β-glucans

β-glucans are components of cell wall structures found in yeasts, mushrooms, cereals, and to a lesser extent, in seaweeds. They are composed of repeated D-glucose units, in different degrees of polymerization (DP, length of the chain). The main type of bonding within their molecular chains depends on the source of β-glucan. Cereal glucans have a combination of β-1,3 and β-1,4 linkages. Fungi and yeasts primarily have β-1,3 linkages, but fungi often have more frequent β-1,6 branching, while yeasts tend to form overall larger structures. Some seaweed β-glucans have mannitol as a terminal unit, but overall, they have smaller molecular sizes compared to other sources (Cerletti et al., 2021; Nakashima et al., 2018).

4. Fucoidans

Fucoidans are mainly found in brown algae sources. They are composed of repeated fucopyranosil (Fuc*p*) residues, with intercalating α-1,2 or α-1,3 linkages in the linear chain. Fucoidans also have α-1,4 linkages that generate side chains, which are usually sulfated at positions *O*-2, *O*-3, or to a lesser extent, *O*-4. The sulfur groups can also be substituted by acetyl groups or other fucose residues (Apostolova et al., 2020; Oliveira et al., 2020). It is constitutive of the intercellular tissue of their extracted organisms. Therefore, alterations such as the degree of sulfated units and the presence of other monosaccharides besides Fuc*p*, are highly variable, which illustrates the constant need for structural characterization of different extracted polysaccharides.

5. Fructans

This class is mainly composed of Fructofuranose (Fru*f*) units bound by β-2,1 in linear structures (determined as inulin fructans). Inulin-type fructans (ITF) are constituted of linear β-2,1-Fru*f*, with their terminal end linked to one α-1,2-Glucopyranoside (Glu*p*) residue. Linear chains of β-2,6 fructofuranose with external additional glucopyranoside residues and β-2,1 branching are named graminan-type fructans (GTF). Another type, linear but with β-2,6 linkages, is denoted as levan-type fructans (LTF). Fructans are widely used in the food industry in cookies, ice creams, apple slices, yogurts, and others, either as a stabilizer, fat substitute or to improve texture. They can be extracted from chicory and Jerusalem artichokes (García-Villalba et al., 2022; Mueller et al., 2016; L. D. F. Pedrosa and Fabi, 2023), while the GTF type is mostly extracted from agave and onions, being widely used in Latin America (Fernández-Lainez et al., 2022, 2023).

6. Overall bioactivity

Different polysaccharidic samples, either isolated or in food products, have been reported to have positive effects in human health. Slower glucose absorption and insulin secretion, chelating power of heavy metals and other toxic compounds, cholesterol and (secundairy) bill acids carry over to excretion, dysbiosis reversion and healthy-microbiota proliferation, anticancer and antioxidant effects, are only a part of what has been studied regarding the biological activities of those macromolecules (Chandel et al., 2022; Cui et al., 2022; Eliaz et al., 2006; Ren et al., 2023). However, all of those can be somehow linked to a similar background: reducing or preventing inflammation.

Inflammation has been defined as an inherent response to an alteration of the homeostatic environment, which cannot be restored by homeostasis-related mechanisms (Meizlish et al., 2021). This response guarantees that, at least in some scenarios, the human body can successfully neutralize threats, such as pathogen infections. The opposite is also true, where some diseases have their pathogenesis linked to a constant "false alarm" to the immune system, which can, in response to this alarm, try to neutralize regulatory cells, and wrongly assume that the homeostasis has been perturbed. Some cardiological, neurological, and absorptive dysfunctions, such as coronary heart and inflammatory bowel diseases, and even depression, are deeply linked to local or systemic inflammation (Barberio et al., 2021; Peikert et al., 2020; Yang et al., 2022).

7. Bibliometric analysis

Polysaccharide applications in the study of inflammation, whether for inhibition or stimulation, have garnered increasing interest in the literature. A bibliometric analysis was conducted using the Scopus database to examine the trend over the past decade. The analysis revealed a noticeable growth in research output on the topic. Except for the years 2017 (reason unknown) and 2021 (likely due to the limitations imposed by the Sars-CoV-2 pandemic, hindering laboratory work), there has been a consistent increase in the number of articles published each year. Reviews, on the other hand, were less affected in 2021 and were also not less published in 2017. As of June 2023 (the latest check), the number of articles published has already exceeded that of the previous year, while the number of reviews is nearly reaching the same level. These findings suggest an increase in research efforts on the application of complex carbohydrates to regulate inflammation and a promising outlook for both areas in the current year.

The search criteria included only journal articles in their final stage of publication, excluding books, and were limited to the English language for relevance (see Fig. 1). Journal articles published from 2014 to 2023 were extracted from the Scopus database using the terms "natural", "polysaccharides", "effects", and "inflammation", with the Boolean operator "AND" used to refine the search. A total of 19,742 articles were imported into VOSViewer for a title text occurrence analysis to identify the most relevant keywords within the published data. Binary counting (presence or absence of a term in a title) was chosen for analysis. A filter was applied to include terms with at least 50 occurrences, resulting in 174 terms. The program suggested a standard retrieval of 60% of terms for further analysis, leading to the selection of 104 terms. Two terms ("use" and "review") were manually removed as they were deemed irrelevant. The final selection for clustering consisted of 102 terms, which are shown in Fig. 2.

Five clusters are identified, with three of them being prominently delineated.

Cluster 1 (red) is defined as the polysaccharide structural cluster, primarily encompassing terms related to the processes involved in obtaining and defining polysaccharide structures. Examples of

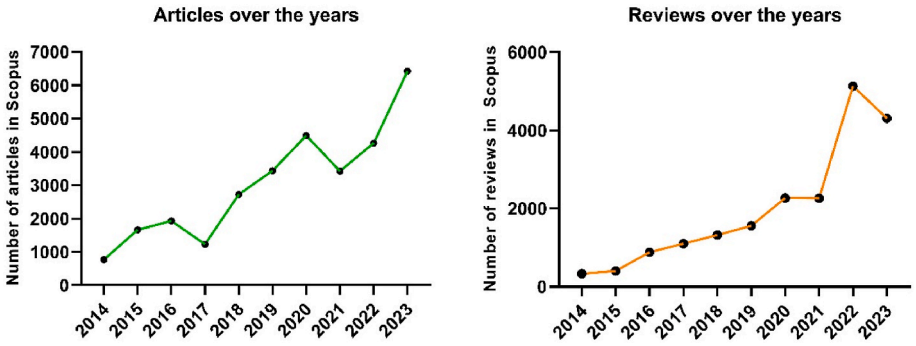

Fig. 1. Publications of both articles (left) and reviews (right) about natural polysaccharides and their effect on inflammation. Data retrieved from Scopus Database, from 2014 onward. Search was performed with only final publication journal materials, and in English.

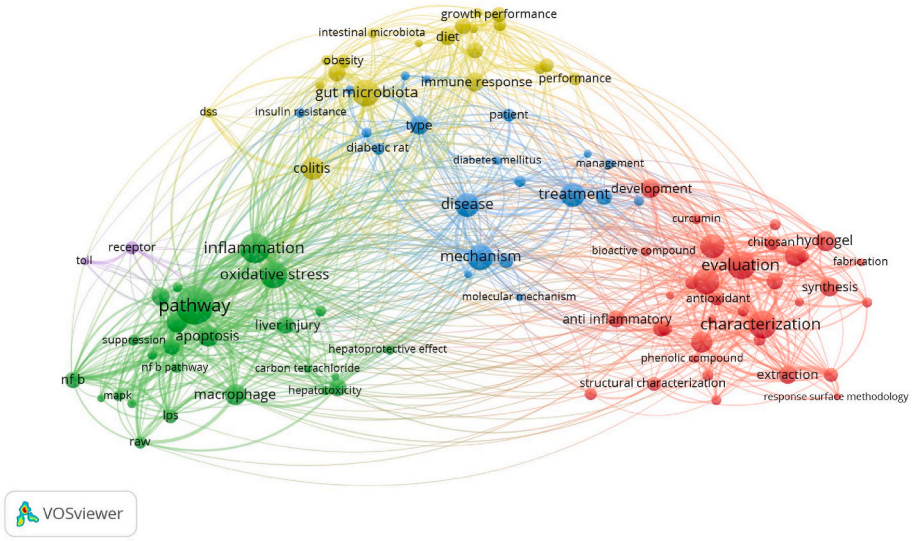

Fig. 2. Clustering of Title-text term occurrence. All the settings were standard of the program (approximation 2, repulsion 1).

significant terms include “evaluation”, “characterization”, “development”, “extraction”, and “synthesis”. Some minor terms such as “phenolic compound” and “curcumin” may be associated with comparing the effects of polysaccharides to those of other molecules in experiments related to oxidative stress.

Cluster 2 (green) is more focused on the observed effects of polysaccharides. Key examples of terms in this cluster include “inflammation”, “oxidative stress”, “apoptosis”, “MAPK”, “macrophage”, and “NF-κB pathway".

Cluster 3 (golden yellow) is associated with terms more related to *in vivo* effects. Some examples include “gut microbiota”, “colitis”, “obesity”, “diet”, and “immune response".

Cluster 4 (light blue) represents a more intersectional cluster. It contains generalistic terms such as “mechanism”, “treatment”, and “disease” that bridged the previous three clusters, establishing a broader relationship between them without specificity. This cluster also had strong connections with Cluster 3, with terms such as “insulin resistance” and “diabetic rat".

Finally, Cluster 5 (violet) consists of only two terms that were closely linked to Cluster 2, mainly because they pertained to mechanistic terms: “toll” and “receptor”, both of which could lead to Toll-like receptors.

8. Anti-inflammatory mechanisms

8.1. General cytokines effects and enzymatic pathways modulation

Core parts of the immunological response, and related to both innate and adaptative immune systems, are cytokines. These cytokines consist of small protein molecules that can either have pro- or anti-inflammatory modulation upon immune cells, such as on macrophages, B and T-cells, neutrophils, and others immune cells (Kany et al., 2019; L. Li, et al., 2020a,b). They are also responsible for signaling immune response transition. Some are deeply involved in specific immune cell differentiation (specialization). For example, IL-6 has been described as a B-cell stimulant and a cluster of differentiation (CD)4+ signaling molecule for T-cells. It is often used as an inflammatory biomarker due to its major inflammation-maintaining profile (Kany et al., 2019; Rose-John, 2023). However, IL-6 is also associated with more regulatory responses and therefore considered to be a major regulator of immunity. For example, IL-6 signaling in myeloid cells mitigates obesity-induced inflammation by stimulating macrophage alternative activation (see eg Mauer et al., 2014). Another cytokine, tumor necrosis factor-α (TNF-α), mainly acts as a cell survival regulator through activation of NF-κB protein but also has influences in other alternative systemic processes, such as coagulation (Kany et al., 2019).

Enzymes also play a key role in the inflammatory processes. The trending area of immunometabolism identifies metabolic enzymes, such as citrate synthase, that are involved with inflammation (e.g., citrate

leading to fatty acids, ROS, or prostaglandins production) (Pålsson-McDermott and O'Neill, 2020). Cyclooxygenases (COX) are enzymes that are involved in the conversion of arachidonic acid into prostaglandins (PGs). COX-1 is naturally expressed ubiquitously and is responsible for maintaining normal levels of PGs. On the other hand, COX-2 is more closely related to specific tissues, such as the brain and kidneys, and is expressed at very low levels under normal conditions (Andrade et al.). The production of high amounts of PGs is associated with COX-2 upregulation, particularly during inflammatory circumstances such as infections (where there is pathogenic recognition through LPS or other molecules) or cancers. In these situations, increased concentrations of iNOS and COX-2 lead to the production of macrophage-derived PGE2 (Hou et al., 2020).

Lipoxygenases (LOX), on the other hand, belong to another class of enzymes responsible for the peroxidation of polyunsaturated fatty acids. The isoform 5-LOX is associated with many autoimmune and inflammatory diseases.

Sulfated fucoidans from *Padina tetrastomatica* (an edible brown algae) were found to effectively reduce the activity of LOX-5 and COX-2 enzymes in rats, thereby reversing the inflammation induced by isoproterenol application. Inflammatory cytokines TNF-α and IL-6 showed downregulation in protein and mRNA expression in the groups treated with fucoidan, while upregulation of the anti-inflammatory cytokine IL-10 mRNA was observed (Lekshmi and Kurup, 2019). These polysaccharides may have a broad range of actions.

Sargassum horneri, another brown seaweed rich in sulfated fucoidans, has been studied for its bioactive effects. Pre-incubation of macrophage cells with purified fucoidans from this alga resulted in the down-regulation of iNOS and COX-2 protein expression after LPS induction. The highest dose (50 μg/mL) achieved a relative expression of less than 0.2-fold for iNOS and less than 0.4-fold for COX-2 (Sanjeewa et al., 2019). Another study also found similar results, this time with *Sargassum binderi*, another brown alga species. Interestingly, this group also observed a reduction in LPS-induced cell death and nitric oxide (NO) production in a zebrafish larva in an *in vivo* model (Je et al., 2021).

Ginkgo biloba L. is an ancient Chinese tree with various applications, mostly related to dietary supplementation and phytotherapy. Ye and colleagues (Ye et al., 2019) discovered that isolated polysaccharides from *G. biloba* L. sarcotesta significantly reduced the secretion of IL-1β, IL-6, and TNF-α cytokines, as well as the production of NO and PGE2, and the expression of COX-2 protein and mRNA in LPS-induced macrophages, without affecting cell viability. The molecular targets of this polysaccharide were found to be upstream checkpoints on the p38, JNK, and ERK/MAPK pathways. *Cyclocarya paliurus*, another Chinese herb, exhibits a dual effect. While it shows synergistic effects with LPS in promoting the release of NO and TNF-α, it acts antagonistically in the release of IL-1ß and PGE2 (L. Xiong et al., 2018). However, the authors did not delve further into investigating these effects, highlighting the need for additional studies to elucidate these observed effects and associated mechanisms.

A galacturonan polysaccharide derived from *Citrus grandis* fruit was found to upregulate both, iNOS and COX-2 mRNA expression, while also enhancing the secretion and expression of IL-6 and TNF-α in another study (Fan et al., 2020). This directly demonstrates the influence of polysaccharide structure on the outcomes observed in different biological models. *Astragalus membranaceous* (roots) is another well-known herbal medicine that contains polysaccharides as its main components. The most common polysaccharides found in *Astragalus* are characterized by a linear α-1,4-D-glucan backbone with α-1,6-D-glucan sidechains. This polysaccharide structure has been shown to influence the NF-κb and MAPK pathways by increasing IKβ-a levels and reducing p38 MAPK, p65 NF-κB, and ERK1/2 protein levels (Dong et al., 2019).

Studies conducted in BALB/c mice, *in vivo* models have demonstrated the significant protective effects of *Astragalus polysaccharides* against intestinal inflammation. The expression of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β was alleviated, and there was an increase in the expression of many tight junction proteins, including ZO-1, Occludin, and Claudin-1. These effects expedited the recovery of the morphology of the jejunal villi and ameliorated LPS-induced disruption (Dong et al., 2019, 2020).

Finally, Yu and colleagues (Yu et al., 2022) found that immune resistance was increased in the aquaculture of *Cyprinus carpio* (Jian carps) fishes when supplemented with polysaccharides derived from dandelion flower (*Taraxacum mongolicum,* TMP). The fishes were challenged with semi-lethal doses of *Aeromonas hydrophila,* and the groups receiving between 1 and 2 g/kg of TMP had higher survival rates. The authors argue based on gene expression that possibly the effects are derived from the oxidation-control mechanisms, such as the nuclear factor erythroid 2-related factor 2 (Nrf2), but also through antioxidant enzymes, which are further discussed below (Yu et al., 2022). However, such findings should not be projected in human effects, due to drastic differences between organisms. Nevertheless, this is a very substantial finding regarding this type of animal culture and can be suggested as an additional measure to guarantee fish culture welfare.

8.2. Antioxidant activity

Almost every step of energy-demanding cellular metabolism generates reactive oxygen species (ROS), which are highly reactive compounds that can cause cellular damage. Under normal conditions, ROS reactivity helps maintain homeostasis. However, immunological alterations can stimulate ROS production, leading to an uncontrolled environment. To counteract ROS abundance, endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH) play a crucial role (Kim et al., 2020; J. Zhang et al., 2020).

Polysaccharides also possess antioxidant properties, contributing to their anti-inflammatory effects. Inflammation often leads to the production of ROS, which can cause tissue damage and contribute to inflammation progression. Polysaccharides can scavenge these ROS, preventing oxidative stress, reducing inflammation, and protecting tissues from damage.

For example, *Panax ginseng* polysaccharides, both acidic and neutral, were tested in various antioxidant *in vitro* and *in vivo* (*C. elegans*) assays. The acidic fraction, with Gal, GalA, Glc, and Ara, demonstrated superior performance in all *in vitro* tests, possibly due to the presence of carboxylic acid groups and active hydroxyl groups. However, both polysaccharides exhibited similar protective effects against juglone-induced ROS production *in vivo* (Kim et al., 2020).

Unusual sources and structures, such as a glycosaminoglycan/xylopyranan from the buccinid gastropod mollusk *Babylonia spirata*, also exhibited significant antioxidant activity in DPPH and ABTS + scavenging tests, as well as Fe^{2+} chelating activity, comparable to standard α-tocopherol (Chakraborty and Salas, 2020).

In another study by Jayawardena et al. (2020), two types of polysaccharides were extracted from *Padina boyriana* brown algae. One was obtained through enzymatic cellulase action (PBE), and the other through alcohol precipitation (PBP). PBP, structurally similar to commercial fucoidans, outperformed PBE in all antioxidant tests, including DPPH, Alkyl, Hydroxyl, and hydrogen peroxide (H_2O_2) assays. In Vero kidney epithelial cells exposed to H_2O_2-induced oxidation, PBP lowered intracellular ROS levels in a dose-dependent way, partially restored cell viability, and prevented H_2O_2-induced apoptosis. Moreover, PBP increased the levels of SOD and CAT proteins. Similar protective effects were also observed in zebrafish models.

A polysaccharide from *Sargassum fulvellum* composed mainly of Gal, Xyl, and Fuc was found to prevent apoptotic body formation in Vero cells incubated with AAPH. It downregulated Bax and cleaved caspase-3 protein levels, while upregulating anti-apoptotic proteins Bcl-xL and PARP (L. Wang et al., 2019). These findings highlight the potential of different natural polysaccharides in modulating multiple biological pathways involved in inflammation and oxidation.

Despite all these positive findings, it is important to highlight that natural polysaccharide structures are not often considered as optimal antioxidant molecules when compared to synthetic polymers or polyphenols, as discussed in a comprehensive review by Zhang et al. (J. Zhang et al., 2020). The authors explore different mechanisms of ROS scavenging and discuss some synthetic modifications of natural polysaccharides, such as combining natural compounds, for example, the incorporation of polyphenols into polysaccharide backbones (J. Zhang et al., 2020).

8.3. Immune receptors interaction

Part of dysfunctional or dying cells, as well as many different types of pathogens, can be recognized by specific receptors due to similar structural patterns of cellular membrane components (damage or pathogen-associated molecular patterns – DAMPS or PAMPs, respectively) (H. Li et al., 2021). These types of receptors, therefore, are named pattern-recognition receptors (PRRs). Some main categories include nucleotide oligomerization domain receptors (NLRs), C-type lectin receptors (CLRs), and Toll-Like receptors (TLRs), and they are fundamental in supplying quick-inflammatory responses through innate-immune system when specialized B- and T-cells are not yet activated (H. Li et al., 2021).

Known as a β-glucan recognizing lectin, Dectin-1 is a dendritic CLR that plays a crucial role in detecting mostly fungal structures during exposition to those kinds of infections. However, its functions are not limited to infection responsiveness. It is primarily expressed in inflammatory cells such as dendritic cells (DCs), macrophages, monocytes, and T-cells. Dectin-1 recognizes β-glucans through its carbohydrate recognition domain (CRD), the region responsible for binding to these molecules. Metal ion-independent manner, involving conserved amino acid residues, degree of side chain branching, and polymer chain length are some of the mechanisms suggested to their binding affinity. Dectin-1 also mediates intracellular signaling through tyrosine-based motifs, leading to the recruitment of CARD9 and activation of the NF-κB pathway (Kalia et al., 2021; Tone et al., 2019).

A β-glucan extracted from bamboo mushroom (*Dictyophora indusiata*), named DIP, has been shown to activate macrophages in a Dectin-1-driven manner. Deng et al. (2018) observed that levels of cytokines IL-1β and TNF-α were higher in the group without anti-Dectin-1 antibody, indicating the involvement of Dectin-1 in the activation of macrophages by DIP. Additional consequences, such as the phosphorylation of ERK1/2, JNK, and p38, which are intracellular signaling pathways, were strongly reversed in the presence of the receptor antibody. However, it was also noted that Dectin-1 was not the only binding site that was involved, as observed through fluorescence detection of binding sites followed by anti-Dectin-1 labeling (Deng et al., 2018).

Apart from inflammatory cells, Dectin-1 has also been found in microglia and brain tissue. Li et al. (H. Li et al., 2021) injected *Ganoderma lucidum* polysaccharide (GLP) – mainly formed by β-glucans – into a specific mouse model and observed not only molecular anti-inflammatory effects but also behavioral changes suggested an impact on the gut-brain axis. The injection of GLP at a dose of 5 mg/kg was able to reverse depression-like symptoms in mice, as indicated by reduced immobility in forced swimming and tail suspension tests. Moreover, under constant provoked stress, mice exhibited a loss of sucrose preference, a symptom of anhedonia, which was also reversed by GLP treatment. At the molecular level, GLP enhanced the levels of Dectin-1, IL-10 (an anti-inflammatory cytokine), and BDNF (a neurotrophic factor), while decreasing the levels of inflammatory cytokines. The study also investigated astrocyte and microglia activation and found similar levels in the GLP-treated group compared to the non-stressed mice, indicating a potential role of GLP in modulating neuroinflammation. To further confirm the involvement of Dectin-1 in the observed effects, a Dectin-1 blocker called laminarin was used, and it nearly reversed the effects of GLP (H. Li et al., 2021).

King oyster mushroom (*Pleurotus eryngii*) is a widely used dietary fungus. Its extracted galactomannan polysaccharides were found to activate macrophages in mice. The described process resulted in increased phagocytic activity, NO production, and inflammatory cytokines (IL-6, IL-1β, and TNF-α) secretion in a dose-dependent manner. The authors investigated that the mechanism of action involved the inhibition of MAPK and NF-κB phosphorylation. To check which receptor was involved, inhibitors of TLR2, TLR4, and Dectin-1 were used (Yan et al., 2019). Among all of them, TLR2 inhibitor C29 was the only one able to reverse the observed macrophage activation, indicating that the galactomannan polysaccharide stimulated the pathways through TLR2 binding (Yan et al., 2019). However, in a study by Ellefsen et al. (2021), they used similar galactomannans from the same mushroom source, but no interaction with TLR2 or increased NO production was observed. Instead, TNF-α, and IL-6 were induced, and fractions with higher levels of β-glucans were found to interact with TLR2-TLR6 heterodimers and Dectin-1. However, the authors noted that these results were modest compared to the previous findings.

TLRs are a crucial component of the innate immune system. They are type-1 transmembrane proteins. The extracellular region is rich in leucine amino acids (Fitzgerald and Kagan, 2020). TLRs are recognition points of various molecules such as lipoproteins, polysaccharides, and peptidoglycans. Upon activation, TLRs trigger the transcription of inflammatory genes, leading to the production of chemokines, cytokines, and other signaling molecules (Kaur et al., 2021). TLR2 is a well-studied member of the TLR family, which can form heterodimers with TLR1 and TLR6 (TLR2-1, TLR2-6), expanding the range of possible ligands and making it a core player in PAMPs recognition. TLR4 is commonly activated by lipopolysaccharides (LPS), and each TLR4 homodimer can simultaneously interact with two LPS molecules. However, the MD2 protein forms a heterodimer with TLR4 to effectively bind to LPS (Fitzgerald and Kagan, 2020).

Pam3csk4 is a tri-acetylated lipoprotein that is a known TLR2/1 heterodimer agonist, promoting a significant TLR2-driven immune response (Y. Chen et al., 2019; Du et al., 2019; Jin et al., 2007). This molecule binds to an opening located between the C-terminal and central TLR2 domains, into an internal binding leucine-enriched pocket. Of the three hydrophobic chains, the two ester-bound are located in the TLR2 pocket, while the remaining amide-bound takes up a straight channel in TLR1. The vast majority of the TLR residues in this region are highly hydrophobic (Jin et al., 2007).

CU-CPT22 is a synthetic partial selective antagonist of TLR2/1 in mice. It is a competitive antagonist, as it also occupies the same binding pocket of Pam3csk4, described above (Grabowski et al., 2018, 2020). A preliminary molecular docking simulation utilizing the crystallized structure from a human TLR2/1 heterodimer activated by Pam3csk4 (PDBID: 2z7x, Jin et al., 2007) in the docking server DockThor (Guedes et al., 2021), demonstrated that different fragments of pectin could bind this receptor. This was attributed to one generally available polygalacturonic acid oligomer (non-methylated heptamer of GalA, Figs. 3, 4 and 5B), and a previous characterized rhamnogalacturonan oligomer (Figs. 3, 4 and 5C, Schols et al., 1994) in pectin, which had a spontaneous affinity to an hydrophobic binding pocket, with a binding affinity of −9.392 and −12.545, respectively, while the docking test with CU-CPT22 (Figs. 3, 4 and 5D) resulted in a binding affinity score of −9.589. The docking parameters were based on the Pam3csk4 size and center of mass (center of mass, x: 15.728; y: 13.387; z: 10.837; grid box size, x: 20; y: 35; z: 20).

This could potentially mean that some pectic fragments can occupy the TLR2/1 heterodimer binding pockets, being potential agonists or antagonists, such as will be discussed below.

Certain polysaccharides have been reported to interact with various TLR receptors, including TLR2/1, TLR3, TLR4, TLR5, TLR7, and TLR9, as well as NOD-like receptors. For example, pectins isolated from *Carica papaya* fruit were studied to evaluate how ripening affects their molecular characteristics and their modulation of TLRs. The higher

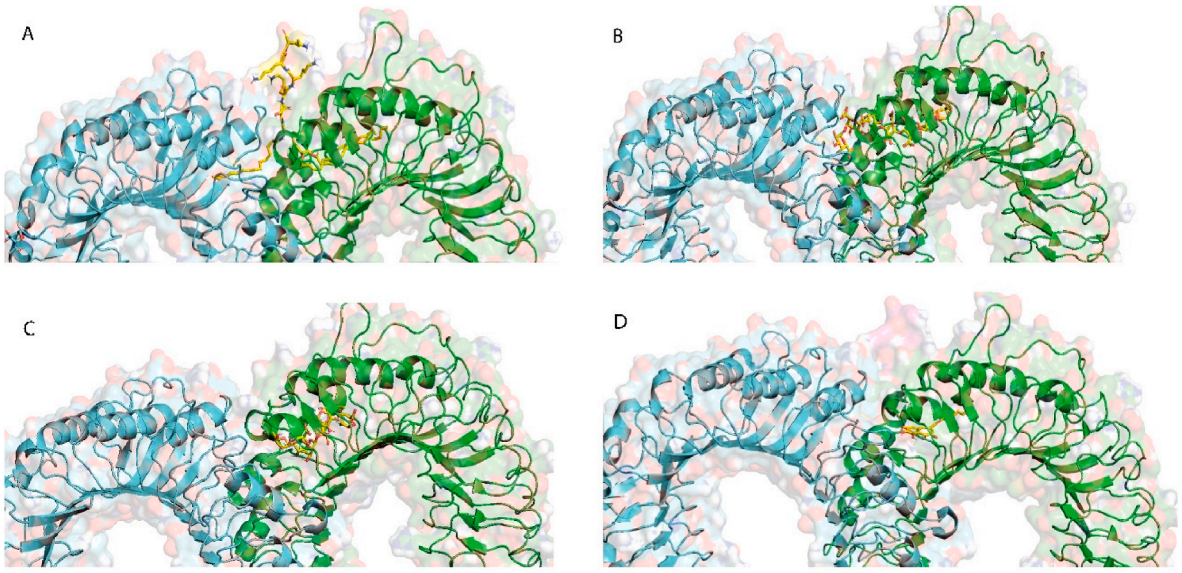

Fig. 3. Lateral visualization of molecular docking preliminary simulation with the TLR2/1 heterodimer activated by Pam3csk4 (PDBID: 2z7x, Jin et al., 2007). ***A: Pam3csk4; B: non-methylated homogalacturonan heptamer; C: A rhamnogalacturonan-I oligomer characterized by Schols and colleagues*** (Schols et al., 1994)***; D: CU-CPT22). Data were run by the authors on the DockThor docking system*** (Guedes et al., 2021).

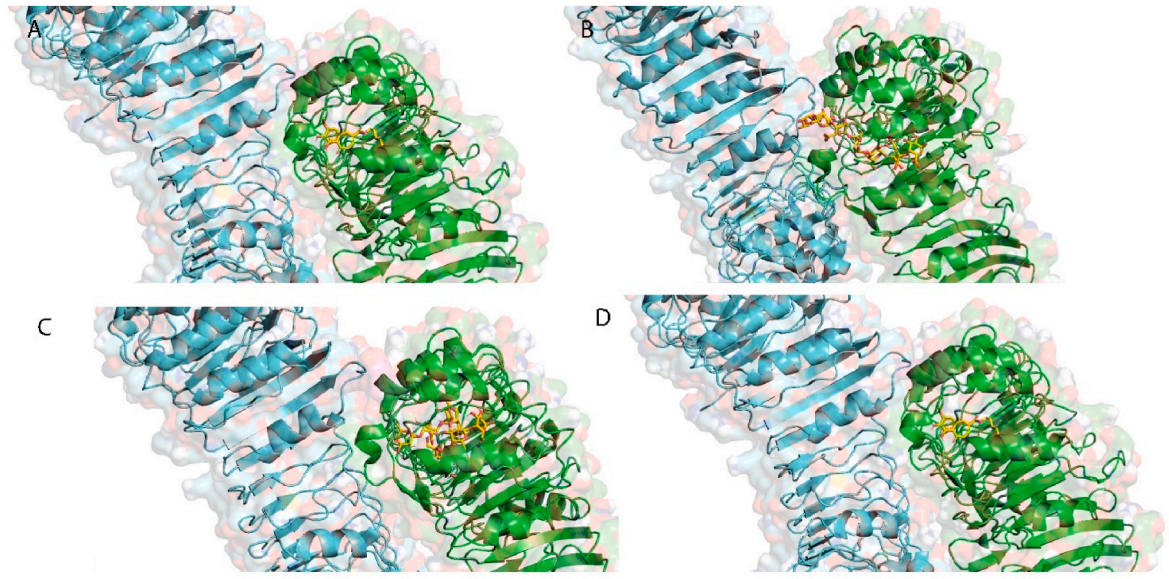

Fig. 4. Top visualization of molecular docking preliminary simulation with the TLR2/1 heterodimer activated by Pam3csk4 (PDBID: 2z7x, Jin et al., 2007). ***A: Pam3csk4; B: non-methylated homogalacturonan heptamer; C: A rhamnogalacturonan-I oligomer characterized by Schols and colleagues*** (Schols et al., 1994)***; D: CU-CPT22). Data were run by the authors on the DockThor docking system*** (Guedes et al., 2021).

molecular weight and less methylated pectins from unripe papayas did not activate TLR3, TLR5, and TLR9. They had an inhibitory effect on TLR3 and TLR9 activation. On the other hand, lower molecular weight pectins with a 40% degree of methylation from ripe fruits were able to activate all the tested receptors, similar to known agonists (Prado et al., 2020). Those facts demonstrate that even normal plant metabolism (ripening phenomenon), which influences polysaccharide structures, can generate novel interactions with human immune receptors. Artichoke pectin (AP) relieved disease activity index scores from colitis in mice, in a dose-dependent manner (). Also, the treatment decreased TNF-α, IL-1β, IL6, iNOS, ICAM-1, and TLR4 expressions. The authors even tested an enzymatic removal of the Ara and Gal residues and saw that this removal impaired the anti-inflammatory effects observed. At the highest dose (80 mg/kg), AP enhanced MUC1, MUC3, and Occludin expression, signaling an intestinal integrity restoration. The authors hypothesized that the effects started from a TLR4 interaction with AP (Sabater et al., 2019).

Beukema and colleagues conducted a study to test the inhibitory effects of four different citrus homogalacturonans with varying degrees of methylation (DM) on TLR2 in a murine *in vitro* model (Beukema et al., 2022). They found that the inhibition patterns remained consistent even after incubation with the TLR2 agonist Pam3csk4. The inhibitory effect was independent of the interval between methylation of galacturonic acid (known as the degree of blockiness, DB) for low DM pectins (18 and 19%), but it was significantly different for intermediate DM pectins (43 and 49%) (Beukema et al., 2022). This suggests that a certain level of demethylated galacturonic acid residues distributed throughout the homogalacturonan chain is necessary for effective TLR2 inhibition. In a

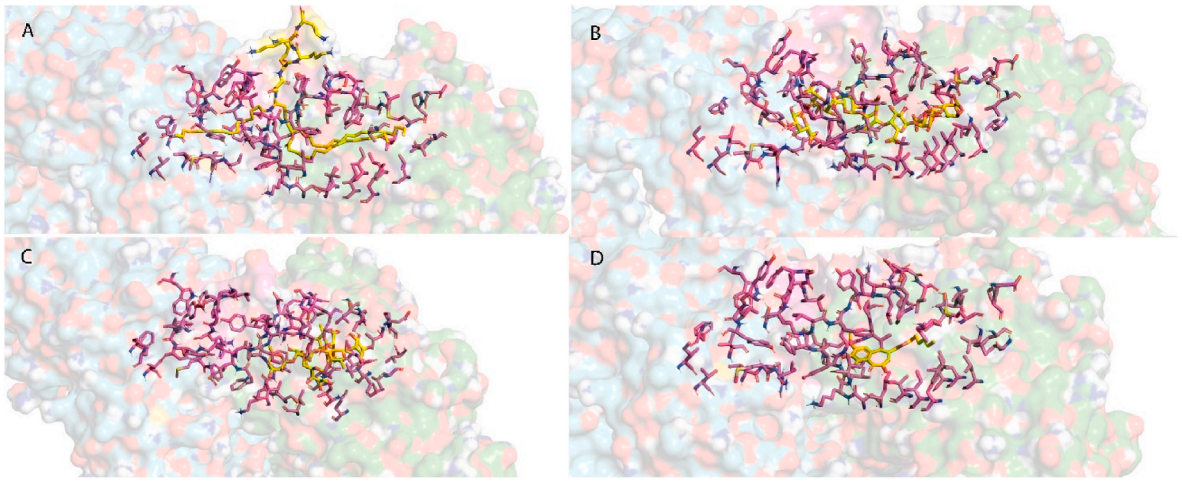

Fig. 5. Whole protein surface and specific binding residues of the Pam3csk4 binding pocket within TLR2/1 heterodimer sticks (around 5 Å) visualization of molecular docking preliminary simulation (PDBID: 2z7x, Jin et al., 2007). ***A: Pam3csk4; B: non-methylated homogalacturonan heptamer; C: A rhamnogalacturonan-I oligomer characterized by Schols and colleagues*** (Schols et al., 1994)***; D: CU-CPT22). Data were run by the authors on the DockThor docking system*** (Guedes et al., 2021).

mouse model of doxorubicin-induced mucositis, all homogalacturonan pectin samples were able to reduce neutrophil infiltration, as well as levels of IL6 and MCP-1 in the peritoneal cavity, and reduced the histopathological scores of the small intestine (Beukema et al., 2021). Jermendi et al. (2023) further investigated the differences in similar pectin structures with a wider range of DMs. Interestingly, they found that pectins with high DB but with varying DMs exhibited the strongest inhibitory potential on TLR2/1. Their analysis suggests that besides the size of the non-methylated galacturonic acid block, the distribution of remaining methylated residues also plays a role in TLR2/1 inhibition (Jermendi et al., 2023).

In a more innovative application, Hu et al. (2021) developed pectin and alginate microcapsules for immunoprotection of pancreatic islets and evaluated their effects on immune and NF-κB activation in THP-1 macrophages *in vitro*. They observed lower damage-associated molecular pattern (DAMP)-related immune responses and NF-κB activation specifically in macrophages exposed to microcapsules having low DM pectin in their capsule wall. These low DM pectin/alginate capsules also reduced inflammatory cytokine expression in xenografted models. In mice with diabetes, grafts of islets in low-DM inflammation attenuating pectin based capsules successful reversed hyperglycemia for nearly 200 days, compared to less than 30 days in the control group that received only alginate capsules (Hu et al., 2021). High DM pectin/alginate capsules also exhibited a longer period of glycaemic control, although shorter than the low DM pectin/alginate capsules (around 100 days). These findings suggest that variable polysaccharides used as capsule components hold promise for more advanced xenografting techniques in the future.

Two ITFs composed of β-2,1-Fruf residues with between 2 and 3.5 kDa, extracted with water and alkali from *Atractylodis Macrocephalae*, which is an East Asian herb, were tested *in vitro* for immunoregulatory functions. The water-extracted ITF had activated mRNA expression of TLR3 and TLR12 while inhibiting TLR1 expression, with a significant increase of TNF-α mRNA expression as well. The other one activated TLR5, TLR6, TLR8, TLR11, and TLR12, but no TNF-α expression was identified (X. J. Li et al., 2023). The β-2,1-Fruf ITFs were also able to reduce T84 PMA-induced intestinal barrier disruption, and the data suggests that smaller sizes (DP ranging from 1 to 10) were the best intestinal model protectors. However, long DP polysaccharides (from 10 to 60) resulted in the highest TLR2 activation (Vogt et al., 2014).

Fructans have also shown potential in modulating TLR responses. Graminan-type fructans (GTP) exhibited the strongest NF-κB stimulation in human macrophages compared to inulin-type fructans (ITP). The high degree of polymerization (DP) of GTP inhibited TLR2, TLR4, and TLR9, while also synergistically activating TLR3 with a specific agonist. In contrast, high DP inulin did not inhibit any TLRs. However, lower DP inulin suppressed TLR5 and TLR9 (Fernández-Lainez et al., 2022). Once again, the size and structure of the fructans played a decisive role in their modulation of TLRs.

Further ahead *in vivo*: a slight glimpse into transcriptomics and metabolomics approaches regarding polysaccharide-treated animal models.

Although more and more models are designed and optimized to predict anti-inflammatory effects of complex polysaccharides, such as co-culture cellular spheroids or organoids, *in vivo* studies in experimental animals still play a major role to allow exploration to human health effects. However, combined with omics approaches which are instrumental in understanding and translation to humans, experimental animal models teach us what complex polysaccharides can do in disease prevention or lowering of symptoms. For example, transcriptomics, the study of genetic expression profiles, and metabolomics, the study of cell and tissue metabolites, are some of the powerful instruments that can be derived from animal models material harvest, still with higher degrees of complexity than *in vitro* models. Dexamethasone-induced osteoporotic rats had genetic reprogramming of the colonic epithelial cells. Some of the dysfunctions observed by the disease involved primary immunodeficiency and impairment of intestinal IgA production. The authors found that 53 genes that were modified in the model group, were recovered with the application of *Astragalus membranaceus* polysaccharides (APS) through gavage (Liu et al., 2021). Main reversions were in extracellular matrix proteins (ECM) secretions, such as collagen and fibronectin, but also maturation and differentiation of T or B lymphocytes. The authors also evaluated DNA methylome and found that the APS treatment was able to upregulate a series of genes related to immunity and inflammation parameters, such as the TLRs and PPAR signaling pathways. Dexamethasone was involved in downregulation of 55 genes, from a total of 57 changed expressions, with functions such as cytokine-cytokine or ECM-receptor interactions (Liu et al., 2021) being regulated. LPS-induced acute lung injury rats were treated with *Ganoderma atrum* polysaccharides. This intervention was able to reduce the excessive neutrophils and macrophage tissue infiltration from LPS induction, while its pretreatment at the highest doses (80 mg/kg) prevented the LPS-driven rise of TNF-α, IL-1β, and IL-6 cytokines, alongside NO production. Metabolomics analysis found that, besides higher SCFA production in the polysaccharide-treated animals, there was also reversion of the upregulation in L-histidine metabolism, which can be

converted to histamine, and in excess can trigger NF-kB expression, a major regulator of cell cytokine production and survival/apoptosis. L-tryptophan metabolism, which can also result in pro-inflammatory aberrant behavior, through indoleamine 2,3-dioxygenase activity and kynurenine metabolite production, contributing to oxidative stress, was downregulated in the treated group (L. Li, et al., 2020a). *Prunellus vulgaris*, known as the carpenter's herb, exerted anti-inflammatory effects with its extracted polysaccharides (PVP) in rats with hyperlipidemia. The PVP reduced TNF-α and malondialdehyde levels in the serum and enhanced GSH activity. There was also a reversion in immune cell infiltration and liver tissue damage observed. In the metabolites analysis through GC-MS, the PVP group had lower values of Alanine, Threonine, Succinic acid, Proline, Inositol, and Arachidonic acid, compared to the model group (Z. Zhang et al., 2020). The ulcerative colitis (UC) model has also been studied for metabolite profiling in rats, utilizing dextran-sulfate sodium (DSS) as induction of colitis, and *Holothuria leucospilota*, or black sea cucumber, polysaccharides (HLP) as colitis attenuator. While DSS has reflected in higher pyridoxal and 5-hydroxy valeric acid metabolites in the gut, and lower tryptophyl-tryptophan, spermidine, and L-tyrosine, the groups that received HLP in the highest dose (200 mg/kg, through gavage) have restored the values closer to the normal control (X. Zhang et al., 2023). HLP also contributed to modulating *Staphylococcus nepalensis* and *Corynebacterium glutamicum* amino acid metabolism regulation, such as 5-hydroxytryptamine precursion by 5-hydroxytryptophan. Overall, HLP was correlated to intestinal amino acid and antimicrobial peptide metabolisms, as well as energy metabolism in Sprague Dawley rats with the UC induction, mostly related to microbial community structuration (X. Zhang et al., 2023). Although both DSS and HLP had their sulfate groups, essential sugar composition is different, as HLP is rich in Fucose and Rhamnose residues, and this could be one of the reasons it is highly fermentable by beneficial microbiota, thus reverting DSS adverse effects. However, this still needs to be shrouded, and the precise mechanisms cannot be defined with the actual literature information. Finally, in another DSS-induced protocol, but now with azoxymethane used in combination to reverse symptoms in a colitis-associated colorectal cancer model, *Zizyphus jujuba* (also known as red/Chinese date) polysaccharides were compared to non-treated and commercial apple pectin-treated C57BL/6 mice. The very high dose group (1000 mg/kg of polysaccharides) had lower mortality and tumor burden, and both commercial apple pectin and red date polysaccharides were able to alleviate the colon length reduction in the AOM/DSS model. Pro-inflammatory cytokines were measured, and the polysaccharides successfully restored normal values similar to non-treated control mice for IL-6, IL-1β, and TNF-α in serum, but also in colonic tissue mRNA levels for the last two. Metabolite-wise, the expected high SCFAs were present in treated animals, and the authors found higher *Actinobacteria* and *Tenericutes* phylum populations. Once again, the metabolites regulations were around the tricarboxylic acid cycle, tryptophan, and tyrosine metabolisms (Ji et al., 2019). Those constantly found tendencies regarding especially amino acid metabolisms are trending areas for, for example, cecal microbiota fermentation patterns, and consequences for human health benefits, and should keep being targeted in future studies.

8.4. Beyond the gut

For a long time, intact or polysaccharide fragments were not deeply investigated regarding oral absorption. Most of those polysaccharides are considered dietary fibers, and by definition, it means they are structures that are non-digestible by the human gastrointestinal tract. Although non-digestible, absorption mechanisms have been suggested, especially in recent years, with different techniques for identifying those molecules, such as fluorescent or radiolabelling, and immunoassays. Paracellular transport, Peyer patch and M cell absorption, vesicle-producing pathways, and other mechanisms are suggested throughout the literature as possible ways for polysaccharides to reach the intestinal lamina propria or even other tissues (Q. Chen et al., 2021; Sakai et al., 2019; Y. Wang et al., 2020). Zheng and colleagues have made a great review exploring this subject, where different sources and types of polysaccharides, ranging from heparin and chondroitin sulfate to pectin and chitosan, had varying degrees of absorption in both cells and animal models (Zheng et al., 2022). Although the polysaccharides mentioned in the present review, such as pectins, are usually considered to have poor absorption, they are a highly variable class of compounds with different monosaccharide proportions, molecular size, and molecular charge (influenced by the DM or degree of acetylation, DA) (Pedrosa et al., 2023). That is why different types of molecular modification are promising areas to explore since this could originate reproducible molecular patterns that are meaningful also for absorption. Therefore, Zheng et al. also gather in the review ways to modify distinct molecules to enhance this absorption pattern and deeply explore the possible mechanisms by which it could happen (Zheng et al., 2022). Overall, absorption models to evaluate possible systemic effects by polysaccharides are a trending area of science and can lead to promising new steps in human health modulation.

9. Conclusion

Polysaccharides are versatile and highly complex macromolecules, whose chemical structures are intimately linked to their biological activity potential. The differences in immunological modulation observed in the literature, induced by these polysaccharides, highlight the importance of studying the interplay between their structure and function. The chemical characterization of polysaccharides derived from fungi, algae, yeasts, cereals, fruits, and herbs is essential for determining methods to scale up their extraction, manipulation, and production. By employing a robust bioinformatics approach to mathematically correlate and establish trends, it is possible to enhance the targeting of immune receptors, immune-related enzymes, or specific interleukin signaling, as discussed in this review.

Numerous well-optimized inflammation models, both *in vitro* and *in vivo*, play a crucial role in defining the way forward. These models facilitate the dissection of action mechanisms, primary toxicological evaluations, assessments of nanocomposite stability, and more. It is also fundamental to state that there is still ignorance of detailed mechanisms for some contradicting effects, such as the briefly commented DSS colitis induction, and attenuation of this effect by another sulfated polysaccharide, even if they differ in sugar composition, for example, and mostly the results are regarding fermentability and microbiota modulation. This shows, therefore, that it remains necessary to replicate experimental conditions in varied models to ensure a more accurate mechanism of action, and then in healthy humans and later in patients. By adapting insights gained from previous experiments, usage and application strategies can be better delineated for the benefit of society (Fig. 6).

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CRediT authorship contribution statement

Lucas de Freitas Pedrosa: Conceptualization, Methodology, Investigation, Writing – original draft. **Paul de Vos:** Supervision, Writing – review & editing. **João Paulo Fabi:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

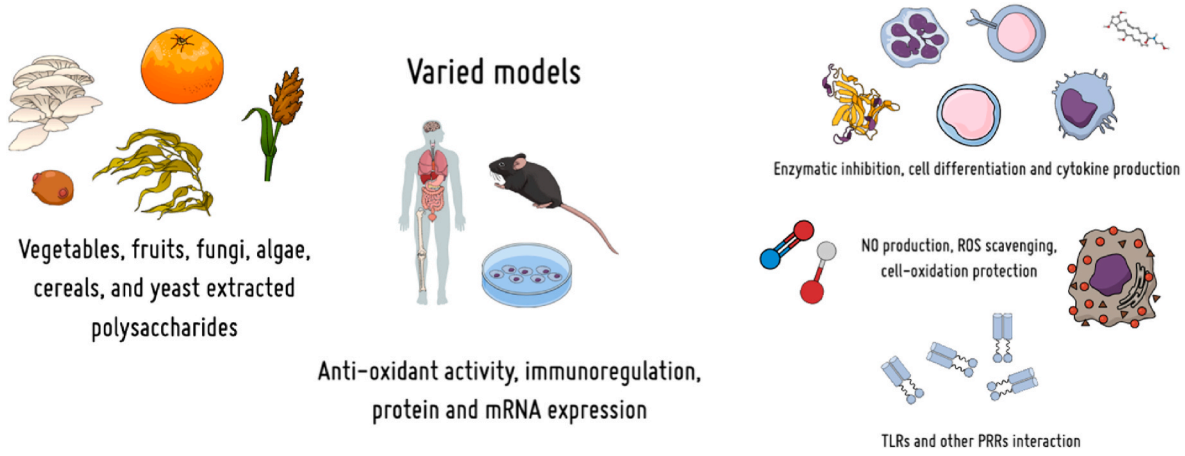

Fig. 6. The interaction between Natural Polysaccharides and many facets of the inflammation process.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Grammarly to check for English syntax mistakes. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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