# Acetylated polysaccharides: Synthesis, physicochemical properties, bioactivities, and food applications

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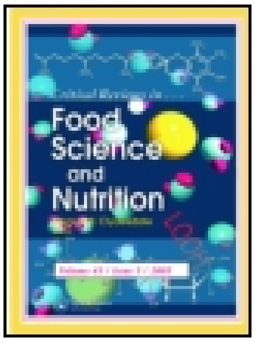

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REVIEW

# Acetylated polysaccharides: Synthesis, physicochemical properties, bioactivities, and food applications

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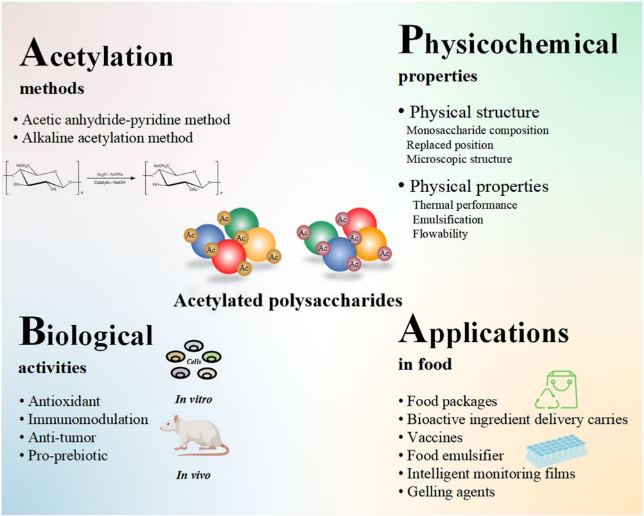
## ABSTRACT

Polysaccharides are biomacromolecular widely applied in the food industry, as gelling agents, thickeners and health supplements. As hydrophobic groups, acetyls provide amphiphilicity to polysaccharides with numerous hydroxyl groups, which greatly expand the presence of polysaccharides in organic organisms and various chemical environments. Acetylation could result in diverseness and promotion of the structure of polysaccharides, which improve the physicochemical properties and biological activities. High efficient and environmentally friendly access to acetylated derivatives of different polysaccharides is being explored. This review discusses and summarizes acetylated polysaccharides in terms of synthetic methods, physicochemical properties and biological activities and emphasizes the structure-effect relationships introduced by acetyl groups to reveal the potential mechanism of acetylated polysaccharides. Acetyls with different contents and substitution sites could change the molecular weight, monosaccharide composition and spatial architecture of polysaccharides, resulting in differences among properties such as water solubility, emulsification and crystallinity. Coupled with acetyls, polysaccharides have increased antioxidant, immunomodulatory, antitumor, and pro-prebiotic capacities. In addition, their possible applications have also been discussed in green food materials, bioactive ingredient carriers and functional food products, indicating that acetylated polysaccharides hold a clear vision in food health and industrial development.

## KEYWORDS

Acetylated polysaccharide; modification; property; bioactivity; application

## GRAPHICAL ABSTRACT



## HIGHLIGHTS

- Different catalysts and reagents could be chosen for acetylated polysaccharides preparation.
- Changes in structure properties of acetyl groups can affect physical and biological activities.
- Acetylated polysaccharides possess a lot of improved biological activities.
- Acetylated polysaccharides have great potential in food, pharmaceutical and biomaterial areas.

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## 1. Introduction

Polysaccharide, an essential nutrient for living organisms, is widely found in nature (Xie, Jin, et al. 2016). In recent years, natural polysaccharides have caught more and more attention due to their multiple biological activities, such as anti-tumor (Wu et al. 2020), anti-oxidant (Xie, Shen, Wen, et al. 2020), anti-bacterial (Lin, Zhu, et al. 2018), anti-inflammatory (Wang et al. 2020) and immunomodulation (Wang et al. 2019), which are widely used on food supplements or medical treatments (Huang et al. 2019; Li and Hu 2022). The advantages of stability and safety are also significant factors for their popularity (Xie, Shen, Hong, et al. 2020). However, it is a fact that not all natural polysaccharides extracted from sundry sources are quite satisfied bioactivities or even not (Liu et al. 2017). Molecular modifications could change physical and biological properties via the introduction of groups in the chain of polysaccharides (Shao et al. 2014).

In nature, polysaccharides consisting of acetyl glucosamine and acetylmuramic acid are widely found in bacterial cell walls and capsular, which protect cells from damage and exert well immunogenic properties (Xu et al. 2022). Chitosan is derived from chitin's deacetylation, part of the structural skeleton for crustacean shells and insect exoskeletons. It contains acetyls with various biological activities like antibacterial, antitumor, and antioxidant, and is widely used in antibacterial films, food packaging and nanocarriers (Benchamas et al. 2021; Lin, Liao, et al. 2018). Acetyls play an essential role in polysaccharide behaviors for various biological activities. Sera from mice vaccinated with deacetylation capsular polysaccharides did not recognize native capsular polysaccharides, suggesting that acetyl is essential to exert immune actions (Szu et al. 2014). Acetyl could also impact the antimicrobial activity of chitosan by influencing the protonation of $NH_2$ groups attached at the C-2 position (Rodrigues et al. 2021).

Introducing acetyls into polysaccharides could significantly modify their physicochemical properties and change their biological activities. Acetylation could be referred to as the process by which acetyls are connected to hydroxyl oxygen or amino nitrogen in a nucleophilic substitution mode to form polysaccharide esters (Huang et al. 2020). After acetyl groups are introduced, solubility is one of the most noticeable changes, closely related to the strength of physiological activities (Chen and Huang 2019). Along with acetyls and increased water-solubility, bitter gourd polysaccharides, tea polysaccharides and *Ganoderma atrum* polysaccharides exhibited stronger antioxidant, anticoagulant, and immune activity, respectively (Chen and Huang 2019; Chen et al. 2014; Liang et al. 2008). It is precisely because the original spatial structure was changed, exposing more hydroxyl groups and reactive groups that the water-solubility of polysaccharides was increased. Therefore, the relationship between structure and bioactivities of acetylated polysaccharides cannot be ignored. Many studies have shown that physicochemical properties are strongly connected to complicated molecular structures, including molecular weight (*Mw*), degree of substitution (DS), monosaccharide compositions, spatial conformation, and functional groups (Xie, Tang, et al. 2016). After acetylation, arabinose (Ara) and galactose (Gal) contents were changed, and the antioxidant activity of *Cyclocarya paliurus* polysaccharide ($CPP_{0.1}$) was subsequently increased (Zhao et al. 2021).

As hydrophobic groups, acetyls provide amphiphilicity to polysaccharides with numerous hydroxyl groups, which greatly expand the presence of polysaccharides in organic organisms and various chemical environments, which offers a good platform for better behavior of more and more acetylated polysaccharides. Acetylation has been considered an excellent way to gain enhanced biological activity of polysaccharides. In recent years, acetylated polysaccharides have continued to receive attention from scholars (Figure 1) and several review articles on acetylated polysaccharides have appeared. Simsek et al. (2021) reviewed the physical, chemical and biological properties of acetylated polysaccharides in the context of molecular modifications. Xue et al. (2022) partially summarized the effect of acetylation modification on the biological activities of polysaccharides in the context of pharmacognosic polysaccharides. Xie, Shen, Hong, et al. (2020) then outlined the preparation methods of acetylated polysaccharides, their antitumor activity and action mechanisms. Golachowski et al. (2015) conducted a review on the preparation and properties of acetylated starches. However, the above-articles are only partial descriptions of acetylated polysaccharides under one broad direction or are only oriented to overview of acetylated starches. Therefore, we have summarized and analyzed the methods of acetylation modification, physical structure and properties, biological activities and applications in the food industry, mostly from the point of non-starch polysaccharides and slightly from the point of starch, in order to better developing and utilizing acetylated polysaccharides.

## 2. Acetylation of polysaccharides

Acetylation of polysaccharides can be classified into aqueous and non-aqueous mediums according to their different reaction agents (Biswas et al. 2018; Golachowski et al. 2015). The acetic anhydride-pyridine system as non-aqueous media

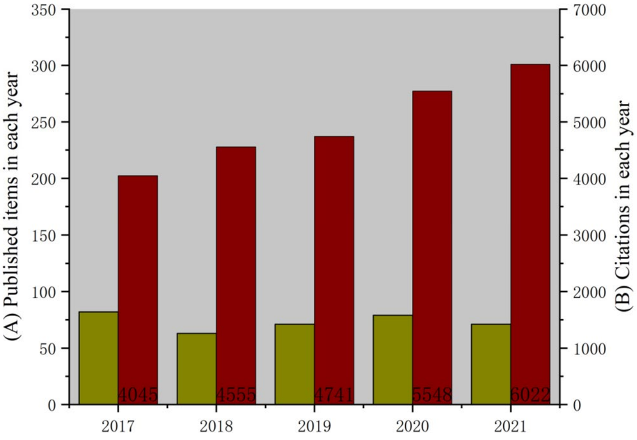

Figure 1. Research trends of acetylated polysaccharides in the past five years. (A) Citations in each year, and (B) Published Items in each year. Taken from the database of Web of Science (http://apps.webofknowledge.com). Topics entered: "acetylated polysaccharides." Retrieve date: August 2022.

is a more classical method, while alkaline acetylation as aqueous media is a more novel approach. The schematic diagram of acetylated polysaccharide is shown in Figure 2.

## 2.1. Acetic anhydride-pyridine method

As shown in Figure 3, the acetic anhydride-pyridine method is one of the popular synthetic methods for acetylated polysaccharides. The natural polysaccharides are dissolved in organic solvents, such as dimethyl sulfoxide (DMSO), pyridine (Py), formamide (FA), and dimethyl formamide (DMF), which are uniformly distributed by stirring and heating. Then, the primary acetylation reagents, acetic acid and acetic anhydride, are blended with catalysts before adding them to the reaction system which can permit hurrying up reactions (Zhang et al. 2014).

Usually, reaction time and temperature can be varied depending on natural polysaccharides and reagents as well as DS required. As reported, *Gracilariopsis lemaneiformis* polysaccharides (AGP) with 13.51% acetyls were successfully achieved after six hours at 80 °C (Wang et al. 2019). Acetylated maltogalactans could be prepared at 4 °C for four hours with a DS of 0.23 (Gu et al. 2018). For dissolving polysaccharides, FA is much better than other reagents. Under the same reaction time and temperature, DMSO and FA were added to obtain acetylated polysaccharides with DS of 0.13 and 0.31, respectively (Li et al. 2016).

Different catalysts are usually selected in acetylation for natural polysaccharides from various sources. Many studies have found that proper motivation plays a critical role in improving the efficiency of acetylation. For non-homogeneous polysaccharides, N-bromosuccinimide (NBS) is a good choice for low-cost and no toxicity with high-efficiency interaction compared with pyridine and 4-dimethylaminopyridine (4-DMAP). Adding 1% NBS of acetic anhydride, acetylated *Acanthopanax leucorrhizus* polysaccharides with 68.4% yield and DS of 0.42 were obtained (Hu et al. 2020). Using tetrabutylammonium bromide or 18-crown-6, acetylation-modified κ-carrageenan with 6-fold higher DS than conventional method was produced by phase transfer catalytic and ion exchange techniques (Tang et al. 2006). Tetrahydrofuran was applied as a catalyst for acetic anhydride reaction and prepared acetylated polysaccharides with the capacity to significantly inhibit tumor cell proliferation in vitro (Chan et al. 2011). Compared with metal chlorides and alkaline compounds for starch, ionic liquids allow green synthesis due to excellent solubility and little volatility. Highly acidified corn starch up to 2.63 was achieved using 1-ethyl-3-methylimidazole acetate as an ionic liquid catalyst (Ren et al. 2022). In addition, an acetylated cellulose membrane was prepared using sulfuric acid as a catalyst, effectively removing 99% of cations and anions from well water (Soto-Salcido et al. 2018).

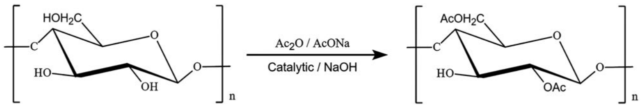

**Figure 2.** The esterification reaction of acetylated modification of polysaccharides.

The significant advantage of the acetic anhydride-pyridine method is its extensive compatibility with polysaccharides from different sources to obtain high DS values. However, it may not be suitable for large-scale industrial production. Applying organic reagents and complicated reaction processes in terms of optimization for catalysts, reaction reagents, duration time and temperature.

## 2.2. Alkaline acetylation method

For the alkaline acetylation method, the original polysaccharides are firstly dissolved in water and the pH of the solution should be adjusted to 8.0–9.0 by adding NaOH to keep alkaline conditions (Figure 3). The above reaction is carried out by adding acetic anhydride drop by drop while stirring (Kakar et al. 2021). With the pH of solution maintained at 8.0–8.5, reactions were carried out at room temperature for two hours by adding 1 mL, 4 mL and 6 mL of acetic anhydride to prepare acetylated polysaccharides with DS being 0.20, 0.29 and 0.40 (Yang et al. 2019).

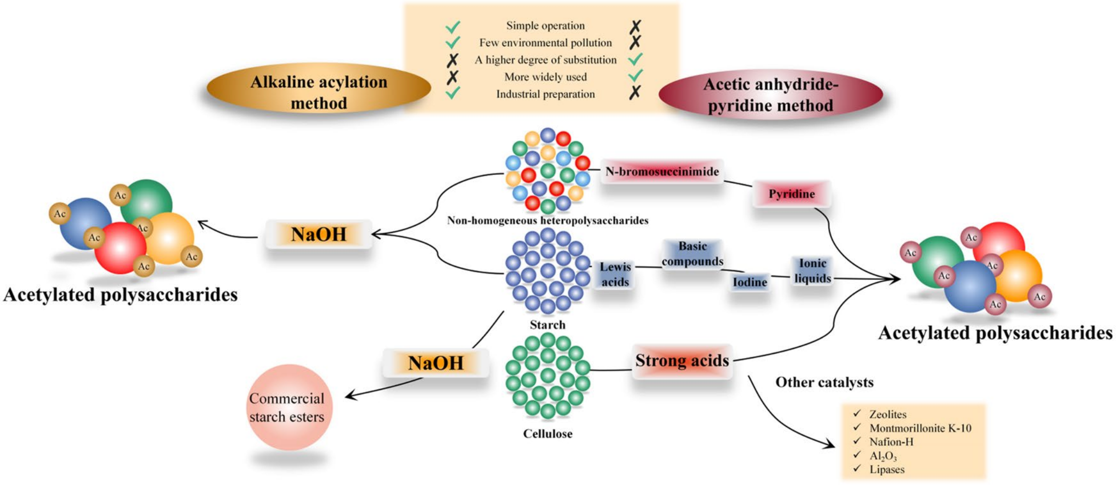

**Figure 3.** Preparation methods for acetylated polysaccharides.

Compared with the pyridine acetate method, acetylated polysaccharides obtained by alkaline are often synthesized with a lower DS value, usually less than 1 (Golachowski et al. 2015). However, due to the advantages of environmental protection, low cost and easy control, alkaline acetylation is gradually recognized and reported by more and more scholars. For example, the acetylation of polysaccharides isolated from plants and edible mushrooms, such as *Cyclocarya paliurus*, *Ganoderma atrum*, and *Pleurotus geesteranus*, was carried out by alkali acylation (Chen et al. 2014; Song et al. 2020; Xie et al. 2015). Acetylated Chinese yam polysaccharides with DS of 0.76 and 78.63% yield were prepared by reacting for 60 min (Zhou, Huang, and Chen 2021). Acetylated onion polysaccharide was produced by reaction at pH 9–10, followed by alcohol precipitation, dialysis, and acetyls were attached at C-6 position with DS of 0.78 (Zhou, Huang, and Huang 2022). The alkaline acetylation method was also used to prepare various acetylated starch esters, which can be applied in the food industry for cleanliness and safety. The acetylated corn starch could be added to beef patties as fat substitute to improve cooking properties and textural characteristics (Osman et al. 2022).

### 2.3. Degree of acetylation

Table 1 shows the degree of acetylation from recent literature. How much acetyl groups modify on glycan chains is expressed by the degree of substitution (DS)—the number of hydroxyl groups of each monomeric unit on polymeric chains that acetyls have replaced. DS could be mainly assayed by hydroxylamine colorimetry, saponification and NMR spectroscopy. DS is determined as follows (Liu et al. 2017):

$$\mathrm{DS} = \left(162 \times \mathrm{AG}\%\right) / \left[4300 - \left(43 - 1\right) \times \mathrm{AG}\%\right]$$

Here 162 is the molecular weight of dehydrated glucose, 43 is the molecular weight of acetyl, 1 is the atomic mass of hydrogen, and AG% is the acetyl content.

The DS is mainly determined by the saponification method, which is based on the hydrolysis of acetate by polysaccharide and strong alkali, and then titration of excess alkali with standard acid to obtain DS of acetyls (Chen and Huang 2019). With this method, the DS of acetylated $\mathrm{CPP}_{0.1}$ was determined to be 0.18 (Zhao et al. 2021) and the DS of acetylated corn starch was raised with acetic anhydride dosage (Luo and Shi 2018). The degree of substitution of acetylated polysaccharides is closely related to its biological activity. A higher DS means larger acetyls which adjust the electrical charge density and polarity of previous chemical compounds, and activate hydrogen atoms on heterotopic carbon, thus boosting antioxidant activity and immunomodulation (Zhang et al. 2014). Compared to *Konjac* and *Aloe*, *Dendrobium officinale* polysaccharides contained more acetyls exhibited superior colitis recovery and immunomodulatory abilities (Zhang, Huang, et al. 2019).

## 3. Physicochemical properties

### 3.1. Physical structure

Substitution positions of activity groups, molecular weight, monosaccharide composition and chain length, which produce differences in physical structure, are directly reflected in physicochemical properties and biological activities. Table 2 summarizes the central structural characterization of acetylated polysaccharides.

#### 3.1.1. Monosaccharide composition and Mw

Acetylation can change the monosaccharide composition and *Mw* of polysaccharide due to its depolymerization in organic

**Table 1.** Some acetylated polysaccharides preparation and DS determination methods.

| Source | Name | Methods | Reagents | DS | DS determination methods | References |
|---|---|---|---|---|---|---|
| *Cyclocarya paliurus* | Ac-$\mathrm{CPP}_{0.1}$ | Alkaline acetylation | $\mathrm{Ac_2O}$-NaOH | 0.18 | Saponification | (Zhao et al. 2021) |
| *Gracilariopsis lemaneiformis* | AGP | $\mathrm{Ac_2O}$-Py | $\mathrm{Ac_2O}$-FA-1%NBS | 0.59 | – | (Wang et al. 2019) |
| Bitter gourd | Ac-P | Alkaline acetylation | $\mathrm{Ac_2O}$-NaOH | 0.27 | Saponification | (Chen and Huang 2019) |
| Starch | – | $\mathrm{Ac_2O}$-Py | $\mathrm{Ac_2O}$-Py-Lewis acids, basic compounds, iodine and ionic liquids | – | NMR spectroscopy, Saponification | (Golachowski et al. 2015; Ren et al. 2022) |
|  | – | Alkaline acetylation | $\mathrm{Ac_2O}$-NaOH | – |  |  |
| *Ophiopogon japonicus* | ROH05A | $\mathrm{Ac_2O}$-Py | $\mathrm{Ac_2O}$-Py-DMSO | 0.23 | Hydroxylamine colorimetric | (Gu et al. 2018) |
| *Artemisia sphaerocephala* Krasch. | ASKP | $\mathrm{Ac_2O}$-Py | $\mathrm{Ac_2O}$-Py-DMSO/FA | 0.14 or 0.38 | NMR spectroscopy | (Li et al. 2016) |
| *Acanthopanax leucorrhizus* | A-ALP | $\mathrm{Ac_2O}$-Py | $\mathrm{Ac_2O}$-FA-1%NBS | 0.42 and 0.87 | Saponification | (Hu et al. 2020) |
| *Morchella angusticeps* peck | Ac-$\mathrm{PMEP}_{1–3}$ | Alkaline acetylation | $\mathrm{Ac_2O}$-NaOH | 0.20, 0.29 and 0.40 | Saponification | (Yang et al. 2019) |
| *Enteromorpha linza* | AEP1, AEP9 and AEP4 | $\mathrm{Ac_2O}$-Py | $\mathrm{Ac_2O}$-FA-1%NBS | 0.14,0.21 and 0.35 | Hydroxylamine colorimetric | (Zhang et al. 2014) |
| Sugar beet pulp | A-ASP2 | Alkaline acetylation | $\mathrm{Ac_2O}$-NaOH | 0.598–0.915 | NMR spectroscopy | (Ai et al. 2022) |
| *Pleurotus geesteranus* | AcPPS | Alkaline acetylation | $\mathrm{Ac_2O}$-NaOH | 0.32 | Hydroxylamine colorimetric | (Song et al. 2020) |
| *Sterculia striata* | ASSP | $\mathrm{Ac_2O}$-Py | $\mathrm{Ac_2O}$-Py-FA | 1.68, 1.35, 0.84 and 0.48 | Saponification | (Sombra et al. 2019) |

*Note*: Py, pyridine; FA, formamide; NBS, N-bromosuccinimide; DMSO, dimethyl sulfoxide; DS, degree of substitution.

**Table 2.** Part of biological effects and structure characterizations for acetylated polysaccharides.

| Biological activities | Source | Name | Mw (kDa) | Compositions | DS | The position of substituents | References |
|---|---|---|---|---|---|---|---|
| Anti-tumor activity | *Ginkgo biloba sarcotesta* | GBEPP11 | 3.4 | Rha, Glc | 0.38 | – | (Wu et al. 2011) |
| Antioxidant activity | *Cyclocarya paliurus* | Ac-CPP | 38.4 | Gal, Ara | 0.18 | O-2, O-6 | (Zhao et al. 2021) |
| Anti-inflammatory activity | *Amorphophallus rivieri* | AceKGM | – | Man, Glc | – | – | (Wang et al. 2020) |
| Immunomodulatory activity | *Gracilariopsis lemaneiformis* | AGP | 123.06 | Glc, Gal | 0.59 | – | (Wang et al. 2019) |
| Immunomodulatory activity | *Cyclocarya paliurus* | Ac-CP | 1050 | Glc, Gal, Ara, GalA, Man, Xyl, GlcA, Rha | 0.13 | O-2, O-6 | (Liu et al. 2017) |
| Anticoagulant activty | Tea | Ac-TPS | – | – | 0.34 | – | (Liang et al. 2008) |
| Immunomodulatory activity | *Ganoderma atrum* | Ac-PSG-1 | 1870 | Glc, Gal, Man | 0.71 | O-6 | (Chen et al. 2014) |
| Anti-tumor activity | *Ophiopogon japonicus* | ROH05A | 16.7 | Gal | 0.23 | O-2, O-3, O-6 | (Gu et al. 2018) |
| Antioxidant activity | *Acanthopanax leucorrhizus* | *A-ALP*$_{1-2}$ | 93, 62 | Gal, Glc, Man, Ara | 0.42, 0.87 | O-2, O-6 | (Hu et al. 2020) |
| Immunomodulatory activity<br>Anti-inflammatory activity | *Morchella angusticeps* peck | Ac-PMEP$_{1-3}$ | – | Glc, Gal, Man, Ara | 0.20, 0.29, 0.40 | – | (Yang et al. 2019) |
| Anti-inflammatory activity | *Aloe arborescens* Leaves | AAAP6 | 2 | Man, Glc | – | O-2 | (Nazeam, El-Hefnawy, and Singab 2020) |
| Anti-inflammatory activity | *Pleurotus geesteranus* | AcPPS | 12 | Glc, Gal, Man, Rib, GlcA, Xyl, Fuc, GlcN | 0.32 | – | (Sun et al. 2020). |
| Immunomodulatory activity<br>Antioxidant activity | *Aloe barbadensis* Miller | AcP | 1020 | Man, Glc, Gal | 1.01 | O-2, O-3, O-6 | (Kumar and Kumar 2019) |
| Antioxidant activity | Chinese yam | A-P | 72.8 | Glc, Gal, Man, Xyl, Ara | 0.76 | O-2, O-3, O-6 | (Zhou, Huang, and Chen 2021) |
| Antioxidant activity | Chitosan | CTS | 8 | CTS | 0.74 | O-2, O-6 | (Sun et al. 2020) |
| Antioxidant activity | *Dendrobium officinale* | p-DOP | 312 | Man, Glc | 0.37 | O-2, O-3 | (Huang et al. 2015) |
| Antioxidant activity | Onion | AOP | 112 | Glc, Man, Gal, Rha, Xyl, Ara, Fuc | 0.78 | O-6 | (Zhou, Huang, and Huang 2022) |
| Immunomodulatory activity | *Cucurbita moschata* Duch. | CMDP-4b | 31.97 | GalA, Rha, Ara, Gal | 0.12 | O-3 | (Huang et al. 2021) |
| Immunomodulatory activity | *Cordyceps militaris* | AEPS-II | 61.52 | Glc, Gal, GlcA, Ara, GalA, Man, Rha | 0.058 | O-6 | (Yu et al. 2022) |
| Immunomodulatory activity | *Tilia tomentosa* | PSIII | 382 | GlcA, Rha, GalA | 0.11 | O-3 | (Georgiev et al. 2017) |
| Anti-inflammatory activity | *Smilax china L.* | SCLP3-2 | 16.8 | GalA, Ara, Gal, Rha | – | O-2, O-3 | (Zhang, Pan, et al. 2019) |
| Anti-inflammatory activity<br>Anti-tumor activity | *Amorphophallus rivieri* | Ac-GM | 100 | Man, Glc | 1.8 | – | (Feng et al. 2019) |
| Anti-tumor activity | *Aloe vera* | ABPA1 | 208 | Man, Glc, Gal, Ara | 0.44 | O-2, O-3, O-6 | (Tong et al. 2022) |
| Anti-tumor activity | *Bletilla ochracea* Schltr. | BOP | 490 | Man, Glc | 0.26 | O-2, O-3 | (Niu et al. 2020) |
| Improvement of gut microbiota and SCFAs | *Dendronan* | DOP | 312 | Man, Glc | – | O-2, O-3 | (Zhang et al. 2016) |
| Improvement of gut microbiota and SCFAs | *Dendrobium officinale* | DOP | 141.2 | Man, Glc | – | O-2 | (Zhang et al. 2020) |
| Improvement of gut microbiota and SCFAs | *Picea abies* | AcGGM | – | Gal, Man | 0.35 | O-2, O-3, O-6 | (Michalak et al. 2020) |
| Improvement of gut microbiota and SCFAs | Birch wood | AcGGM | – | Man, Glc, Gal | 0.54 | O-2, O-3, O-6 | (La Rosa et al. 2019) |
| Antihyperlipidemic activity | *Ulva pertusa* | AU | – | Glc, Rha | – | – | (Qi et al. 2012) |
| Anti-viral activity | *Undaria pinnatifida* | MF | 9 | Gal, Fuc, Ayl, Man | 0.42 | O-2, O-3, O-6 | (Synytsya et al. 2014) |

*Note*: Glc, glucose; Gal, galactose; Man, mannose; Ara, arabinose; Rha, rhamnose; Xyl, xylose; Fuc, fucose; Rib, ribose; GlcA, Glucuronic acid; GalA, galacturonic acid; CTS, chitosan; Mw, average molecular weight; DS, degree of substitution.

and strong bases, with a slight decrease in total sugar and uronic acid. After acetylation, the total sugar content of AGP was reduced from 97.59% to 69.21% and uronic acid content was lowered from 10.67% to 9.22% (Wang et al. 2019). A similar result was observed in the acetylation of *Enteromorpha linza* polysaccharides (Zhang et al. 2014). After acetylation, the ratio of fucose, arabinose, galactose, glucose, mannose, xylose, galacturonic acid and glucuronic acid which consisted of *Millettia speciosa* Champ polysaccharides (MSCP) was converted from 0.02:0.24:0.13:0.53:0.01:0.01:0.05:0.01 to 0.09:0.43:0.21:0.16:0.03:0.01:0.05:0.02 (Huang, Zong, and Lou 2022). Galactose has been shown to may take a crucial part in the regulation of immunity. Compared to CPP, the galactose content of Ac-CPP increased from 1.59 to 1.67, along with the addition of secreted immune factors (Liu et al. 2017). Owing to the cleavage of sugar chain, acetylated polysaccharides resulted in a trend of lower molecular weight. A purified polysaccharide-ROH05 reduced *Mw* from 15 kDa to 8 kDa (Gu et al. 2018). In *Artemisia sphaerocephala* Krasch polysaccharide (ASKP), *Mw* was smaller than that of natural polysaccharides when DS was lower. But *Mw* was an upward trend with DS increased, which finally exceeded its original. It may be attributed to the effect of acetylation which outweighed unagglomeration (Li et al. 2016).

### 3.1.2. Thin structure and replaced position

Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectroscopy are routinely used to resolve the architecture of polysaccharides. In FT-IR, the appearance or enhancement of the C = O stretching vibration peak of ester at 1750 ~ 1735 $cm^{-1}$ is generally considered a sign of successful acetylation (Liang et al. 2008). As reported in acetylated konjac glucomannan, this peak absorption is progressively stronger with increasing DS. However, the typical peak with -OH at 3500–3400 $cm^{-1}$ decreases. The opposite trend indicated that acetyls had displaced their own hydroxyl groups (Wang et al. 2020).

By signal at 2.00 ppm in $^1$HNMR belonging to hydrogen on acetyl methyl (-$COCH_3$) and signs between 173–179 ppm in $^{13}$CNMR owning to the acetylated carbonyl carbon, (Deng et al. 2018) confirmed *O*-acetyl group existed. All sites may be targeted for substituents, with preferred at C-6, followed by C-2 and C-3, possibly either mono- or multi-attached. C-2 and C-6 slots are presumed to be switched on Ac-$CPP_{0.1}$ by *O*-acetyl through signals migrating to a lower field at 3.75, 4.30, and 4.17/65.3 ppm in HSQC (Zhao et al. 2021). Similarly, C-2 and C-3 poses of Gal are presumed to be contacted on ROH05A by acetyls judging from signals at 74.47/5.04 ppm of HSQC and 89.29/5.04 ppm of HMBC (Gu et al. 2018). Among natural polysaccharides with acetyls, like *Dendrobium Officinale* polysaccharides, C-2, C-3 and C-6 are major sites, providing a valuable direction for in-depth study (Huang et al. 2020).

### 3.1.3. Particle size and zeta potential

Acetylation can result in particle size changes of polysaccharides. The particle size of Sterculia striata acetylated polysaccharides (ASSP) was increased from 241.8 nm to 273.9 nm with increasing DS from 1.35 to 1.68 (Sombra et al. 2019). It is most likely attributed to the higher acetyls with a more considerable spatial resistance than hydroxyls. When DS was increased to 0.56, the same addition in average droplet size of Ac-MSCP was shown, but it showed a more significant negative potential than the original one due to more acetyls (Huang, Zong, and Lou 2022). The introduction of acetyls activates hydrogen atoms on heterocapital carbon, promoting hydrogen supply capacity and more negative charges, with similar results found in ASKP (Li et al. 2016). In contrast, the absolute value of zeta potential for beet pulp polysaccharide (ASP2) was slightly decreased when the amount of acetic anhydride exceeded 3%, which may be due to acetylation masking carboxyl groups from the surface by invoking conformational changes, thus inhibiting carboxyls, and higher *Mw* A-ASP2 possessing higher viscosity to lower surface charge measured (Ai et al. 2022).

### 3.1.4. Microscopic structure

Before and after acetylation, the following transformations of basic structure, microstructure and solution conformation are also different. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) are reasonable means to characterize polysaccharides (Li et al. 2020). For acetylated polysaccharides from *Pleurotus geesteranus* (AcPPS), an irregularly layered structure with smaller pores and particles aggregated into clusters could be noticed by SEM (Song et al. 2020). Biofilms obtained from acetylated konjac glucomannan (Ac-KGM) for wound healing purposes displayed a porous texture (Wang et al. 2020). Outwardly, ASSP whose DS was 1.68 presented a spherical shape and a smooth outer surface by AFM, which possessed good antibacterial ability in vitro (Sombra et al. 2019). The morphology of *Atractylodes lancea* polysaccharide (A-ALP-1) was flake-like with a rough surface. Still, it looked pretty smooth under SEM after acetylation (Henriques et al. 2020), which was consistent with results obtained from observing the morphology of acetylated polysaccharide from *Cyclocarya paliurus* leaves (AC-CP), tending toward becoming smooth, which might be related to conditions of high temperature and strong alkali, with increased molecular cross-linking and changed structure and conformation (Xie et al. 2015).

## 3.2. Physical properties

### 3.2.1. Solubility

During acetylation, the extension of polysaccharides chain and modification of spatial structure lead to more exposure of hydroxyl radicals, which results in more excellent water solubility. Acetylated bitter gourd polysaccharide significantly improved water solubility and enhanced antioxidant activity (Chen and Huang 2019). For Ac-KGM, all DS of polysaccharides were soluble in polar organic reagents, and their hydrophilicity eventually increased by 109.98% with increasing DS (Wang et al. 2020). Solubility of acetylated ALP-1 with DS of 0.62 was also increased substantially, from 15.0 mg/mL to 137.5 mg/mL, much higher than that of phosphorylated derivative (Liu et al. 2022). Nevertheless, the hydrophobicity of acetyl could not be neglected and water solubility may be reduced or even insoluble for higher substituted derivatives (Masina et al. 2017). When corn starch substitution was raised to 2.63, it showed a more increased contact angle value at the interface with water, indicating that the sample's hydrophilicity was significantly reduced. As the same time, hydrophobicity was enhanced (Ren et al. 2022).

### 3.2.2. Thermal performance

When many acetyls are introduced, potential resistance could weaken remaining hydroxyls to form hydrogen bonds, which are reasons for polysaccharides to shape highly ordered crystal structures, thus decreasing crystallinity and increasing thermal stability. Thermogravimetric analysis (TGA) of acetylated acemannan revealed that thermal decomposition temperature increased with DS (Kumar and Kumar 2019). X-ray diffractometry (XRD) results showed that the double helical structure of amaranth starch was disrupted and crystallinity was dropped from 16.16% to 12.75%. As the same time, an increase in thermal stability might be attributed to fewer remaining hydroxyls after acetylation allowing a slower dehydration reaction between molecules. Thus a higher decomposition temperature was needed (Gu et al. 2019; Sindhu, Devi, and Khatkar 2021). Acetylated ALP-1 was also changed from an apparent crystalline structure to a semi-crystalline substance showing a bread-like peak only at $2\theta = 23.20°$ with reduced crystallinity, which may be related to increased molecular cross-linking and solubility, and was offered more robust thermal stability in TGA curve with higher half-life temperature $T_{50}$ of 298.70 °C (Liu et al. 2022). According to differential scanning calorimetry (DSC) analysis, endothermic transition peaks of acetylated okra leaves polysaccharide (OLP) at 126.6 °C and 317.7 °C corresponded to water loss and polysaccharide degradation, respectively (Olawuyi and Lee 2021). The mean enthalpy change ($\Delta H$) was 878.23 J/g, indicating a high heat requirement for polysaccharide cleavage and ester bond disruption (Olawuyi and Lee 2021).

### 3.2.3. Flowability

As a result of acetyls' water repellency feature, glycan strands have more opportunities to contact each other, which increases the viscosity of polysaccharides, and acetyl helps own lower interfacial free energy and decrease the trend of surface tension. There was an upward apparent viscosity pattern of acetylated ASKP, which was attributed to the increasing *Mw* and hydrophobic association between acetyls of polysaccharides. Moreover, good surface activity was imparted and surface tension was decreased from 9.2 mN/m to 7.5 mN/m (Kakar et al. 2021). The more highly *O*-acetylation, the more helical and defined conformation was gained by typhoid capsular polysaccharide and its solution became more viscous (Hitri et al. 2019). For alkaline soluble polysaccharides, interfacial tension was reduced with amounts of acetic anhydride, which might be related to less protein and higher *Mw* (Ai et al. 2022). The flow profile of acetylation banana starch indicated that it was a shear-thinning fluid, and lower shear stress and higher fluidity were obtained for structural differences by acetyl groups (Sanchez-Rivera et al. 2013).

### 3.2.4. Emulsification

Acetylation could confer polysaccharides amphiphilic properties, whereas emulsification activity (EA) and emulsion stability (ES) are significantly increased. It was found that enhancement in EA mainly depends on reducing interfacial tension influenced by DS and increasing viscosity affected by *Mw*, while ES is controlled by electrostatic and spatial interactions (Kakar et al. 2021; Shao et al. 2020). The highest degree and largest *Mw* of Ac-MSCP3 exhibited the best EA, while the highest negative potential increased the most substantial electrostatic repulsion, giving low-substituted Ac-MSCP1 a good ES (Huang, Zong, and Lou 2022). In the study of acetylated alkaline soluble polysaccharide, average droplet size was decreased significantly from 0.61 μm to 0.29 μm due to the effect of unagglomeration which outweighed acetylation, and viscosity was raised from 11.8 mPa s to 15.6 mPa s (Ai et al. 2022). Smaller droplet size and higher viscosity enabled polysaccharides to possess an increased ability to retard droplet aggregation, resulting in better EA and ES. Similarly in tapioca starches, acetylated samples were found to hold better EA and ES values than natural starches (Zhao et al. 2022).

## 4. Biological activities

Through chemical reactions, acetyls can be connected with original natural polysaccharide, alteration of spatial structure and physical activity will generate differences in biological activities. Virtually many biological properties could be observed both in vivo and in vitro (Figures 4 and 5).

### 4.1. Antioxidant

Free radicals and reactive oxygen species (ROS) which play significant roles in symbolic transduction and genetic expression will imbalance the oxidation-reduction system in organisms and predispose various diseases when excessive (Chen et al. 2021; Xie, Wang, et al. 2016). Acetylated polysaccharides can enhance antioxidant activity through direct or indirect scavenging of free radicals and ROS.

In vitro, after acetylation of Chinese yam polysaccharides, scavenging activities of hydroxyl radical and superoxide anion radical, reducing power against iron ions and anti-lipid peroxidation ability were increased close to control substance $V_C$ (Zhou, Huang, and Chen 2021). Because acetyls had activated hydrogen atoms on heterocapital carbon and affected charge density, hydrogen supply capacities and antioxidant

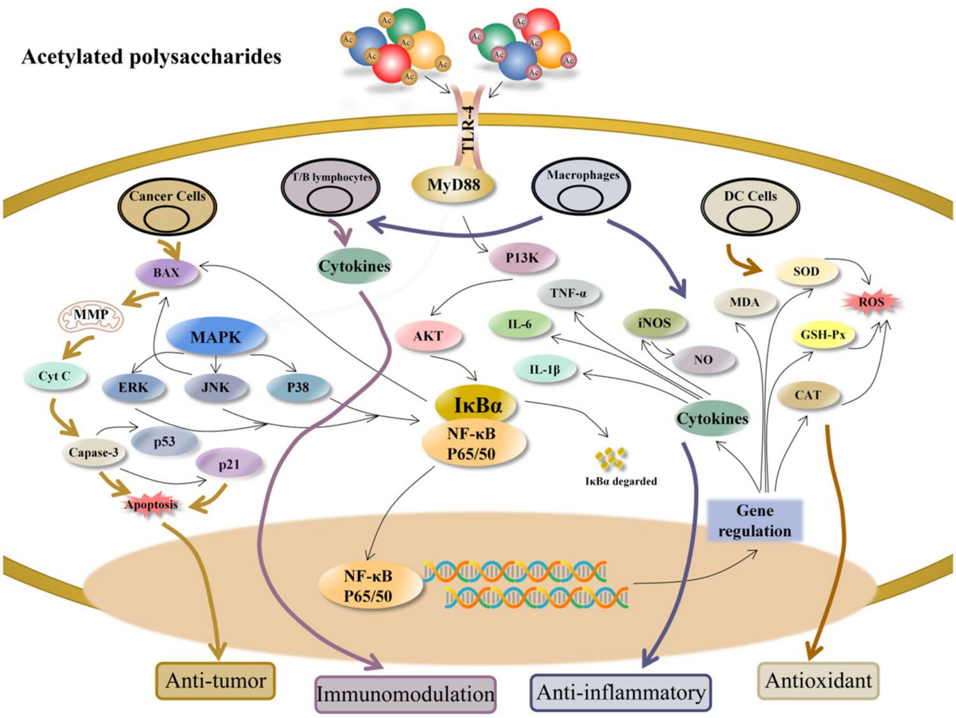

Figure 4. Possible actions of acetylated polysaccharides on biological effects in vitro (mainly in cells).

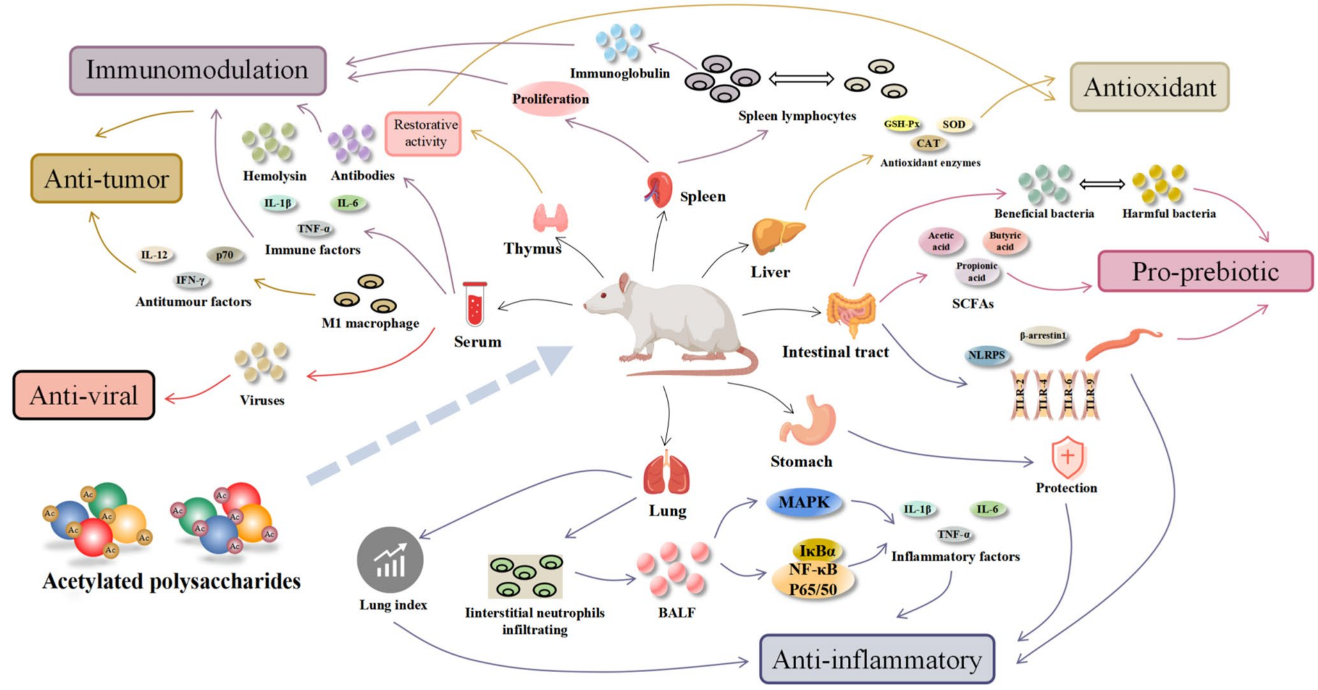

Figure 5. Possible actions of acetyltaed polysaccharides on biological effects in vivo.

properties of polysaccharides could be enlarged (Hu et al. 2020). Chitosan acetylated derivative was not toxic to cells at concentrations less than 500ug/mL, with good scavenging ability for free radicals (Sun et al. 2020). Upon acetylation, conformation of glycan chains was changed, exposing more reactive hydroxyl groups, more easily trapping free radicals, and blocking more chain reactions, as well as dose-dependently raising protein and mRNA expression of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), thus mitigating oxidative damage of DC cells by hydrogen peroxide (Zhao et al. 2021).

In vivo, oxidative stress was produced in the liver and thymus organs of cyclophosphamide (CTX) induced mice, *Dendrobium Officinale* O-acetyl-glucomannan displayed good restorative activity on the thymus while activating antioxidant enzymes of the liver to protect from injury (Huang et al.

2015). After hyperacetylation and deacetylation of acemannan, acetyls might play a significant role in reducing ROS levels within the spleen of mice, most likely because acetyl as an electron-donating group could neutralize excess free radicals well to maintain homeostasis of oxidation-reduction balance, and enhanced water solubility by acetyls which allowed polysaccharides to penetrate tissues and cells more quickly and more effectively to exert antioxidant effects (Kumar and Kumar 2019; Zhou, Huang, and Huang 2022).

### 4.2. Immunomodulation

The immunological system provides one of the critical barriers to keeping our bodies secure away from foreign pathogens and harmful agents (Barbosa and de Carvalho 2021; Fang et al. 2021). A purified acetylated polysaccharide structure could be recognized by toll-like receptor 4 (TLR4) receptors and trigger intracellular MAPK and NF-κB signaling pathways to increase pinocytic and phagocytic activities of RAW264.7 macrophages (Huang et al. 2021). Acetylated cyanocobalamin polysaccharides displayed better immunoreactivity at 200 μg/mL, with 94.2% increased secretion of interleukin-6 (IL-6) (Liu et al. 2017). Upon increases are likely tied to acetyls which have altered the original spatial structure and uncovered more mobile radicals. Vaccine agents containing *O*-acetylated capsular polysaccharides (CPS) had higher protection levels and produced higher titers of antibodies than deacetylated CPS vaccines (Hitri et al. 2019). Sera from mice vaccinated with deac-CPS did not recognize native CPS, suggesting that *O*-acetylation is essential for CPS to exert immune actions (Szu et al. 2014).

The presence of mannose and galactose may help polysaccharides to perform immune advantages. In vivo, *Cordyceps militaris* exopolysaccharides, primarily mannose and galactose, were shown to stimulate the proliferation of T/B lymphocytes in the spleen of mice, leading to raising of spleen organ finger and immunoglobulins including IgG, IgM and IgA (Yu et al. 2022). In addition, among three acetylated pectic polysaccharides, PSI could better activate macrophages and display immune activation, most likely due to smaller *Mw* allowing more accessible contact with receptors (Georgiev et al. 2017). In CTX induced immunosuppression mice model, the immunological function was probably recovered because of the balance of splenic lymphocyte subpopulation ratio and upregulation of serum hemolysin by acetyls (Wu et al. 2020).

### 4.3. Anti-inflammatory

Inflammation could occur in our tissues like the stomach, liver and intestines, a physiological phenomenon of a weakened body when injury, infection, and stress occur (Jin et al. 2021). Well-organized inflammation is necessary, but over-inflammatory reactions may turn into long-term or acute inflammation, inviting various diseases and even life-threatening (Wang et al. 2018).

AcPPS could regulate the expression of the p-IκBα protein in the NF-κB pathway and inhibit pro-inflammatory factors IL-6 and TNF-α secretion from attenuating weight loss, lung index reduction and interstitial neutrophil infiltration in mice (Song et al. 2020). Acetylated glucomannan could protect the stomach from gastric ulcer-induced gastric lesions caused by acidified ethanol (Parente et al. 2014). For Ac-KGM, acetyl was able to enhance the amphiphilic property of fibrous membrane and increase its stability in the physiological environment; on the other hand, acetyl had made a porous surface shape, added adhesion with macrophages and biocompatibility, thus better stimulating secretion of anti-inflammatory factors for skin remodeling which could significantly accelerate wound healing (Niu et al. 2017; Wang et al. 2020).

In vitro, *Morchella angusticeps* peck polysaccharide significantly down-regulated p65 and iNOS protein expression and phosphorylation of p38 at 50–200 μg/mL (Yang et al. 2019). Polysaccharide $\mathrm{SCLP}_{3\text{-}2}$, which underwent acetyl substitution at C-2 and C-3 positions, showed better anti-inflammatory activity by inhibiting LPS-induced NO, TNF-α, IL-6 production than $\mathrm{SCLP}_1$ without acetyl, which might be related to higher galacturonic acid and esterification (Zhang, Pan, et al. 2019). In addition, phenolic glucomannan with acetyls could stimulate the anti-inflammatory factor IL-12 by more than 10-fold compared to a positive control (Nazeam, El-Hefnawy, and Singab 2020). It is probably because acetyls can give polysaccharides a regular spherical shape by promoting internal polymerization, which allows them to spread widely in the organism (Feng et al. 2019).

### 4.4. Anti-tumor

When normal cells are transformed into cancer cells by genetic mutation under cancer-causing factors, they have abilities to proliferate indefinitely and destroy surrounding other normal cells and tissues, which is a severe threat to human health (Li et al. 2021). There has been much interest in the effects of acetylated polysaccharides on the regulation of tumor cell cycles, expression of apoptotic genes and related signaling pathways produced for suppressing malignant tumors.

Massive randomly distributed acetyls at C-2, C-3 and C-6 positions of mannan isolated from *Aloe vera* could initiate a mitochondria-mediated apoptotic pathway to induce apoptosis in colorectal cancer cells through up-regulation on pro-apoptotic protein Bax, cytochrome C and caspase-3 content and impact on mitochondrial metabolism (Tong et al. 2022). Acetylated polysaccharide Ac-GBEP modified from *Ginkgo biloba* sarcotesta could inhibit the proliferation of human leukemia cells (U937) in vitro. The inhibition rate increased with decreasing *Mw*, when *Mw* was reduced. $9.5 \times 10^5$ Da to $3.4 \times 10^4$ Da, the inhibition rate was also increased from 49.89% to 78.97%, which may be due to smaller *Mw* polysaccharides being more easily penetrated and recognized (Liu et al. 2020; Wu et al. 2011). ROH05A could inhibit the proliferation of pancreatic cancer cells via the outer death receptor pathway by upregulation of anti-tumor genes p53 and p21 and nuclear fragmentation and apoptosis (Gu et al. 2018).

In vivo, a dose-dependently rise in $\mathrm{CD4}^+$ T cells, triggering the onset of immune mechanisms to inhibit tumor growth rather than affecting tumor cells directly was found in treatment with *Bletilla ochracea* Schltr. Polysaccharide (BOP) containing high quantities of acetyls in tumor-bearing mice inoculated with CT26 cells (Niu et al. 2020). Acetyl brings immune enhancement to polysaccharides and enhances antitumor activity (Huang and Huang 2017). After acetylation, structurally altered glucomannan could specifically activate the TLR2 receptor, which then formed a dimeric complex with TLR6, activating the onset of adaptive immunity, stimulating the transformation of macrophages toward the anti-tumor M1 phenotype, promoting the uptake of anti-tumor cytokines (TNF-α, IL-12, p70 and IFN-γ), and attenuating tumor magnification in mice (Feng et al. 2019).

### 4.5. Pro-prebiotic

Gut microbiota can carry out metabolism and fermentation of food to produce short-chain fatty acids (SCFAs) that are beneficial to body (He et al. 2022; Wang et al. 2021). Acetylated polysaccharides could effectively inhibit harmful bacteria and promote healthy bacteria. Compared with the model group, acetylated polysaccharides significantly reduced weight loss, intestinal mucosal damage, colonic shortening and spleen hypertrophy in mice with dextran sulfate sodium (DSS)-induced colitis, significantly restored production of SCFAs (11.42 mmol/L vs. 15.26 mmol/L), as well as increased abundance and diversity of beneficial bacteria such as *Bacteroides*, *Lactobacillus* and *Ruminococcaceae*, indicating beneficial preventive effects on the progression of colitis (Zhang et al. 2016; Zhang et al. 2020). The introduction of acetyl is an esterification process, thereby adding an aliphatic group to the carbon chain and increasing polysaccharides' biocompatibility (Feng et al. 2019). In contrast, *O*-acetylated xylan significantly shortened defecation time in loperamide-induced constipation mice. It significantly up-regulated gastrointestinal transit rate (52.6% vs. 67.4%) and stool production (81 mg vs. 146 mg), along with reversed changes of gene expression on intestinal motility, water and ion transport, and inflammation in mice (Huang et al. 2022). Through targeting analysis, acetylated fibers acted on distal gut regions, matching the unique enzymatic capabilities of *Roseburia* and *Faecalibacterium* species to undergo secondary metabolic shifts (Michalak et al. 2020). It could be related to the increased solubility of polysaccharides. In vitro, acetylation also allowed a population of beneficial bacteria *Bifidobacterium* and butyrogenic fermentation, which indicates that acetylated polysaccharides have a good prebiotic ability to ensure intestinal health (La Rosa et al. 2019).

### 4.6. Others

Acetylated ulvans prepared by acetic anhydride significantly affected triglyceride and low-density lipoprotein cholesterol levels in serum with a negative dose-dependent manner, reducing by 25.3% and 20.6% at 125 mg/kg, respectively, suggesting that mice fed lower doses of acetylated polysaccharides might exhibit better anti-hyperlipidemic activity (Qi et al. 2012). This might be because hydrophobic acetyls can induce bile acid sequestering capacity, and altered polarity and viscosity may also be involved in the anti-hyperlipidemic activity. Also, acetylation could increase inhibitory on α-glucosidase and decrease inhibitory on hyaluronidase, which would help the application of polysaccharides from *Zizyphus jujuba* Fruit in health food and wellness industry (Jiao et al. 2009). Due to the increased water solubility and exposure to active hydroxyls after acetylation, tea polysaccharides were remarkably good at anticoagulant effect (Liang et al. 2008). Acetylated polysaccharides were shown a dose-dependently reduce viral titers in mice with the avian influenza virus. They inhibited virus replication, and immune systems were also triggered with higher antibody titers produced in serum, more likely due to the effect of acetyls on polysaccharide charge density and *Mw* (Synytsya et al. 2014). And induction of PC-12 cells neurogenesis by polysaccharide acetylation derivatives would also provide a possibility for preventing and treating neurodegenerative diseases (Jin et al. 2017).

## 5. Food applications

Acetyls bring the variation to original polysaccharides in terms of physiological and physical activities, while they have given more possibilities for food applications (Figure 6).

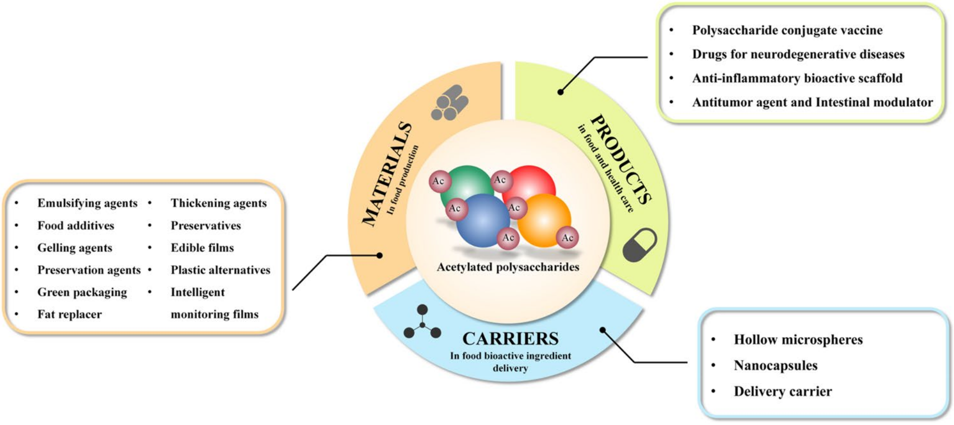

**Figure 6.** Applications for acetylated polysaccharides.

### 5.1. Products in food and health care

The application of acetylated polysaccharides has to be mentioned in vaccines. The multi-acylated Vi (a Virulence Antigen) polysaccharides extracted from *Salmonella enterica* serovar *Typhi* (S. *Typhi*) pods can potentially be used to create vaccines for typhoid fever which is a type of infectious intestinal disease (Hitri et al. 2019). Among them, acetyls displaced at the C-3 position were the primary epitope of Vi multisaccharide, which is a crucial part of antigenicity and immunogenicity. Also, the level of *O*-acetylation is considered a measure of vaccine efficacy (Berti, De Ricco, and Rappuoli 2018). A polysaccharide conjugate vaccine was formed by coupling *pneumococcal* serotype 31 polysaccharide, which contained abundant acetyls, with CRM197 elicited potently, and mice were given the vaccine to produce higher titers of antibodies and had better immune efficacy (Sun et al. 2022). In studies on the treatment of meningococcal meningitis, Neisseria meningitidis serogroup A capsular acetylated polysaccharide conjugate vaccine was also prepared using a feature that systemic antibodies preferentially recognize *O*-acetylated fraction and laid the foundation for the rational design of added therapies for meningitis (Henriques et al. 2020).

In addition, an effect of acetylated polysaccharides on prolonged neurosynapses of PC-12 cells would suggest possible drugs for neurodegenerative diseases (Jin et al. 2017). Ability to modulate intestinal micro-ecology showed that acetyl-modified polysaccharides could also be harnessed as agents for chronic-intestinal inflammation, colon cancer and cardiovascular disease (Michalak et al. 2020; Zhang et al. 2016; Zhang et al. 2020). Based on fighting inflammation properties, it can be made into cellulose film to accelerate wound healing which has the potential to be a bioactive scaffold for scar regeneration (Wang et al. 2020). Acetylated glucomannan can also act as an activator targeting TLR2 receptor because of antitumor activity (Feng et al. 2019).

### 5.2. Carriers in food bioactive ingredient delivery

A thin hydrophobic film can be formed on the surface of the sugar branch when acetyl is attached, which can be applied to controlled release and hollow microspheres. Due to their hydrophobicity and more considerable spatial resistance, after admission of acetyls into debranched starch, spherical micelle with the open and hydrophobic interior was organized by sugar self-assembled, which was bound through hydrophobic interactions and served as good nanocarrier for curcumin, with encapsulation and release rates up to 61.60% and 55%. Meanwhile, its low toxicity and high biocompatibility significantly promoted the application of curcumin in functional foods (Liu et al. 2021). In addition, starch could also be modified as encapsulating agent for gallic acid (GA). Increased thermal stability has enhanced microencapsulation encapsulation efficiency during spray drying. In contrast, increased swelling and solubility have allowed encapsulated particles with controlled release of GA in the hydrophilic system, suggesting that GA particles could be applied in dry mixtures or fast foods (Robert et al. 2012). All of the above indicates that changes in physicochemical properties and structure brought by acetyls expand the values of various matters. Acetylated *Sterculia striata* polysaccharides within high substitution can be served as nanocapsules to transport amphotericin B (AMB) drugs for curing systemic fungal infections, with an encapsulation rate as high as 99% and a release rate of 59.2% after 72 h plus 100% after 212 h, suggesting that structural changes give polysaccharides potential application as a delivery system (Sombra et al. 2019). Acetylated polysaccharide hydrophobic membranes fabricated with high DS could effectively delay protein release and apply to controlled slow delivery of protein drugs (Xiao et al. 2019).

### 5.3. Biomaterials in food production

The increased viscosity, emulsification and hydrophobicity of acetylated polysaccharides greatly extend their biocompatibility and have great potential for biomaterials in emulsifiers, gelling agents, food additives, and smart food packaging.

#### 5.3.1. Emulsifying agents

Acetylated glycans can survive as emulsifiers. Acetyl has imparted good emulsifying activity to MSCP and oil in water emulsifier with good storage and lipid oxidation stability was prepared (Huang, Zong, and Lou 2022). Modified beet pulp polysaccharide and spruce galactoglucomannans are also expected to be potential food-grade emulsifier (Ai et al. 2022; Bhattarai et al. 2019). Esterified starch, with acetyl groups shielding the surface hydroxyl groups, improves emulsification capacity and storage stability which is a promising stabilizer for surfactant-free Pickering emulsions (Yao et al. 2023). Study on artificially acetylated xylans and natural acetylated xylans have revealed that the degree of acetylation is an important parameter in regulating water solubility and emulsifying ability. Acetylated xylans can be used as an adsorption barrier and emulsifiers for loading of fat-soluble components in dairy products (Wang, Gao, et al. 2022). A novel acetylated exopolysaccharide from Bacillus haynesii CamB6 had more effective emulsifying activity and shown more stable emulsion formation compared to commercial chemical surfactant which could be a useful additive in the food industry (Banerjee et al. 2022).

#### 5.3.2. Gelling agents

Pectin extracted and modified from cocoa bean shells with higher acetyl content could produce acidic items due to gel-forming properties under low pH and low water activity (Vriesmann and Petkowicz 2017). Chitosan hydrogels with controlled acetylation can protect fruits and vegetables from microbial deterioration, and also improve solubility and stability of nutrients (fat soluble), preventing them from being oxidized, which can be used as food preservation agents (Manzoor et al. 2022). At 25 °C, acetyl can promote inter- and intra-molecular interactions of gellan gum, increase apparent viscosity, improve thickening ability in water, with a more compact and complex gel network structure and good viscoelasticity, which can be used as thickening agents, widely applied in production of jams, fillings, jellies and other types of gel foods (Xu et al. 2019). Low-acetyl gellan gum can form strong gel in the presence of cations, leading to electrostatic shielding and making the connection between the double helices closer, resulting in reducing water loss and preparing steamed cakes with better chewiness and elasticity under microwave (Chen et al. 2022).

### 5.3.3. Biofilms

Taking advantage of the effects of acetyls on crystallization behavior and thermal stability, biofilms with rapid degradation and antibacterial properties were prepared, which can be widely used in food packages (Choo, Lin, and Mustapha 2021). Acetylated glucuronoxylans and glucomannans from Okoume (*Aucoumea klaineana* Pierre) are high-quality sources for food packaging films due to their better biodegradabilities and thermal barrier properties (Moukagni et al. 2021). The mucopolysaccharides in *Aloe vera*, mainly composed of highly acetylated β-1-4-linked polysaccharides, are biocompatible, environmentally friendly and edible making them an exceptional alternative to traditional synthetic plastic materials (Kumar et al. 2022). A composite film, prepared by cellulose acetate as raw material and chitosan and silica as additives, is expected to replace petroleum-based plastic products in food preservation. Its oxygen permeability was reduced by 83.5%, tensile stress and tensile strain were increased by 2.8 and 25.6 times, respectively, with good light transmission and thermal stability (Zhou et al. 2021). The composite membrane with added chitosan had better water resistance and thermal stability under hydrogen bonding and electrostatic interactions, which responded well to lactic acid and amine gases, being usable for freshness testing of milk and pork (Zheng et al. 2022). The optimized hyaluronic acid films can monitor cadmium and lead in honey, leaves and egg whites with a sensitivity of 0.6 μA/nM and 2.6 μA/nM, which is important for heavy metal ions monitored in the food industry (Priya et al. 2020).

### 5.3.4. Food products

Furthermore, changes in mobility and mechanical properties caused by acetyls also add more possibilities to polysaccharides. Acetylated corn starch can be used as a fat replacer in the meat industry, enhancing redness, moisturizing and sensory properties of beef patties, as well as reducing cooking loss, diameter reduction and shape shrinkage (Osman et al. 2022). Slow-digesting noodles were prepared using the influence of acetyl on starch digestion rate, which is beneficial for people suffering from hyperglycemia and their texture qualities including adhesion, chewiness, cohesiveness and resilience were improved (Lin et al. 2019). The addition of 0.5% chitosan reduced content of free carboxymethyllysine and carboxyethyllysine in sponge cakes by 51.68% and 42.43%, respectively, but with no change in hardness, color and moisture content, indicating that chitosan can be used as a potential inhibitor of advanced glycation end products formation in baked products (Wang, Zheng, et al. 2022). The hyaluronic acid-based edible film could promote saliva flow and serve as a palatable green food for people who have difficulty swallowing solid forms in the oral cavity (Kweon and Han 2023).

## 6. Bioacceptability

Acetylation is essentially a process of polysaccharide esterification, and its bioacceptability can be considered both in vivo and in vitro as well. In vitro, the MTT cell viability assay showed that acetylated pectin polysaccharide was not toxic to macrophages at concentrations of 62.5–250 μg/mL with a higher viability than the control at 125 and 250 μg/mL, indicating that the acetylated polysaccharide can increase cell viability and be safe-to-apply (Huang et al. 2021). In vitro, *Cordyceps militaris* exopolysaccharide containing acetyl significantly improved cyclophosphamide-induced weight loss and alleviated spleen and thymus tissue morphological abnormalities in mice without toxic side effects (Yu et al. 2022). In acute toxicity tests, mice did not exhibit any clinical signs of toxicity after 28 days of treatment even at the highest oral dose of 1500 mg/kg bw of AcPPS, indicating that AcPPS is nontoxic (Song et al. 2020). These data suggest that acetylated polysaccharides are generally safe and nontoxic in cellular and animal models. Also, the sensory properties of beef patties prepared by acetylated starches as fat substitute were evaluated and remained highly acceptable after 60 days of cold reading storage, indicating that the acetylation of starch is nontoxic, harmless and edible (Osman et al. 2022). However, acetylation is a chemical modification. Although alkaline method as achieved certain level of green safety, there are still acetylated polysaccharides with high substitution requirements that need to be prepared by acetic anhydride—pyridine method and use organic and chemical reagents. Whether all acetylated polysaccharides and their products are nontoxic and safe remains to be considered, and more clinical data are needed to support the safety of acetylated polysaccharide foods.

## 7. Conclusion and prospect

Different acetylated modification means catalysts and acetylation reagents could be chosen for different types of carbohydrates and preparation targets. Acetic anhydride-pyridine and alkaline acylation are common approaches, and saponification is a typical method for estimating DS. Acetyls can give rise to changes in molecular weight, monosaccharide composition, and spatial structure of polysaccharides, mainly due to hydrogen bonding and polyglucan chain stretching, resulting in differences in physicochemical properties like decreasing surface tension, crystallinity and electron cloud density, but increasing water solubility, viscosity, thermal stability and emulsification. Supported by acetyls, an outcome that polysaccharides exhibit excellent antioxidant and immunological activity, is inextricably linked to the influence of their structural and physicochemical properties.

Although many works about the preparation, physics and biology of aceylated polysaccharides have been done, such as: how to prepare highly substituted acetylated polysaccharides while ensuring green environmental protection; how to catalyze the acetylation process of polysaccharides more efficiently; how to obtain acetylated polysaccharides with exact substitution sites; the mechanism of acetyl groups and their substitution sites in corresponding physical and biological activities of polysaccharides has not been more thoroughly explored and more definite conclusions; how to expand the possibilities of non-homogeneous polysaccharides in the food field, although acetyl polysaccharides already have more applied in vaccines, food auxiliaries, biofilms, etc., with a preference for cellulose, starch, chitosan and hyaluronic acid as bases; how to make the application of acetylated polysaccharides in food and pharmaceutical fields more industrialized and commercialized, and other research limitations are still waiting for more research workers to explore and solve. With the development of biotechnology over the years, structures and mechanics of carbohydrates will be further enlightened. Acetylated polysaccharides will be noticed and developed with a bright future as physically and biologically active potential for food, pharmaceuticals and biomaterials.

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