Review

**Spirulina** phenolic compounds: natural food additives with antimicrobial properties

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**Abstract**

Food safety is a scientific discipline to ensure consumers’ safety and prevent food-related harms, hazards, or risks along the entire food supply chain. Although fish and seafood products are the best means for securing food and nutrition in a population, they are also highly perishable, being vulnerable to pathogenic bacteria and fungi. This review thus aimed to provide updated scientific information on the role of the phenolic compounds of *Spirulina* as food additives, and their antimicrobial activities in fish and seafood products, in a food safety context. Recent applications of *Spirulina* phenolic compounds showed good results in contaminated fish and seafood products. Different studies have concluded that *Spirulina* phenolic compounds such as polyphenols, C-phycocyanin, γ-linolenic acid, fatty acids, and the combination of lauric and palmitoleic acids have antimicrobial activity in eliminating and/or controlling the growth of pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, and fungi such as *Aspergillus flavus* and *Aspergillus niger*. Overall, *Spirulina* can be considered as an emerging natural food additive with antimicrobial activities against pathogenic fungi and bacteria.

**Abbreviations:**

*A. flavus*, *Aspergillus flavus*; *A. niger*, *Aspergillus niger*; *A. parasiticus*, *Aspergillus parasiticus*; ANSES, French Agency for Food, Environmental and Occupational Health and Safety; *A. sessilis*, *Alternanthera sessilis*; *B. subtilis*, *Bacillus subtilis*; *C. cibarius*, *Cantharellus cibarius*; *C. tora*, *Cassia tora*; *C. versicolor*, *Coriolus versicolor*; *E. coli*, *Escherichia coli*; FAO, Food and Agriculture Organization; GAC, gallic acid equivalents; *L. deliciosus*, *Lactarius deliciosus*; *L. pyrogalus*, *Lactarius pyrogalus*; *M. luteus*, *Micrococcus luteus*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *P. oleaceae*, *Portulaca oleacea*; QE, quercetin equivalents; *S. aureus*, *Staphylococcus aureus*; *S. platensis*, *Spirulina platensis*; *A. platensis*, *Arthrospira platensis*; *S. maxima*, *Spirulina maxima*; TPC, total phenolic content; TFC, total flavonoid content; *V. vulnificus*, *Vibrio vulnificus*; WHO, World Health Organization.

**Keywords**

food safety, fish, seafood, *Spirulina*, phenolic, antimicrobial, additive

**Article history**

Received: 7 July 2020
Received in revised form: 8 April 2021
Accepted: 10 May 2021

**Introduction**

Food safety is a scientific discipline to ensure consumers’ safety and prevent food-related harms, hazards, or risks along the entire food supply chain, and it should be observed “from farm to fork” (WHO, 1997; FDA, 2020). Fish and seafood products are the main sources of healthy nutrition all over the world; more than 4.5 billion of the world’s population get at least 15% of their animal protein intake from fish (Béné et al., 2015; FAO, 2016). However, fish and seafood products are the most perishable foodstuffs. Therefore, monitoring, inspection, and quality control are the key agendas in the fishery industry.

Microbial contamination of fish and seafood products occurs after catching. During processing and postharvest, biochemical changes cause spoilage in these food products very rapidly (Møretrø et al., 2016; Jia et al., 2019; FDA, 2020). In aquaculture, fungal contamination such as *A. flavus* has been reported in fish feed and the fish itself (Mohamed et al., 2017). Another study on smoked fish reported that fungal contamination is due to unhygienic handling, filleting, and storage (Akwuobu et al., 2019). Fish contamination may arise from the culturing environment such as the water used (Hennersdorf et al., 2016). For example, fungi may contaminate the fish through water, soil sediments, or plant remains.
Aspergillus and Penicillium have been reported as the most common fungi related to sediments and biofilms in water bodies (Gonçalves et al., 2006). Foysal and Lisa (2018) reported different Bacillus spp. from soil sediment, whereas other studies reported Vibrio spp., Photobacterium spp., and Flavobacterium spp. from different farmed fish species (Hennersdorf et al., 2016). Vibrio spp., Listeria monocytogenes, Yersinia spp., Salmonella spp., and Clostridium botulinum have also been reported from fish and seafood products (Terentjeva et al., 2015; Novoslavskij et al., 2016; Don et al., 2020).

Fish contaminated with fungi that could produce mycotoxins are harmful to human health (Mohamed et al., 2017; Akwuobu et al., 2019). Due to this, in many countries, fish and seafood products are traditionally processed and preserved to allow for long-term storage without spoilage. Preservation is mainly performed by reducing the water content in the fish, along with high and low temperature treatments, as well as combined measures (Nithin et al., 2020; Tsironi et al., 2020) to control and eliminate toxic bacteria such as E. coli, P. aeruginosa, and B. subtilis, and the growth of moulds and yeasts such as A. niger (El-Sheekh et al., 2014).

Many studies have shown that extracted Spirulina phenolic compounds exhibit good results in controlling certain toxic bacteria and fungi, and could serve as an antimicrobial agent in fish and seafood products (El-Sheekh et al., 2014; Pugazhendhi et al., 2015; Usharani et al., 2015; Pagnussatt et al., 2016). Therefore, this review explores the up-to-date scientific knowledge and future perspectives regarding the antimicrobial role of Spirulina phenolic compounds in fish and seafood products, in a food safety context.

What is Spirulina (Arthrospira)?

Spirulina is a Gram-negative free-floating filamentous cyanobacterium capable of photosynthesis, having robust cell wall, of blue green coloration (hence the name cyano_), and non-toxic (Fedor, 2011; Koru, 2012; Usharani et al., 2012; ANSES, 2017). Prior to 1962, they were considered a eukaryotic alga (blue green algae) until they were reclassified as a prokaryote (domain Bacteria). Taxonomically, two genera are currently accepted; Spirulina (various species) and Arthrospira (important species include A. platensis and A. maxima) (Belay, 2008; ANSES, 2017).

Spirulina can be sourced and harvested from two sources; the first one is natural warm alkaline water or lakes found in African countries (Ethiopia, Chad, and Tunisia), Latin American countries (Mexico and Peru), and Southern Asian countries (India, Sri Lanka, and Thailand) (Belay, 2008; ANSES, 2017; Assaye et al., 2018; Karssa et al., 2018; IOC, 2011), and the second one is artificial aquaculture ponds (Moorehead et al., 2011; ANSES, 2017).

Across the world, Spirulina is primarily known for its high nutritional value, and considered as a miracle / super food (Moorehead et al., 2011; Tidjani et al., 2018; Uddin et al., 2018; Jung et al., 2019), functional food and dietary supplement (ANSES, 2017; Panjar et al., 2017), feed supplement (Yusuf et al., 2016), technofunctional ingredient in meat products to improve meat quality (de Medeiros et al., 2020), and protein and vitamin supplement for fish farming industries (Usharani et al., 2012; Ragaza et al., 2020). Spirulina is rich in micro- and macronutrients (Moorehead et al., 2011; ANSES, 2017), and used as an ingredient in the production of valuable innovative foods in the food industries (Lafarga et al., 2020). It is also considered as a future food for mankind around the globe (Belay, 2008; Soni et al., 2017; Mathur, 2019). Throughout history, the Aztecs in Mexico and Chad communities in tropical Africa have eaten the naturally harvested Spirulina in a dried cake form (Ahsan et al , 2008; Tidjani et al., 2018; Lafarga et al., 2020).

Spirulina contains desirable chemical compositions such as proteins, carbohydrates (e.g., sulphated polysaccharides), essential amino acids, minerals, essential fatty acids (e.g., γ-linoleic acid), vitamins, and pigments (e.g., C-phycocyanin) (Belay, 2008; Thomas, 2010; IOC, 2011; Lafarga et al., 2020).

Phenols and polyphenols of Spirulina

In organic chemistry, phenols or polyphenols are defined as compounds that have one or more hydroxy substituents in an aromatic ring, such as structural compounds (e.g., esters, methyl ethers, glycosides) (Lattanzio, 2013). Together, these phenolic compounds range from simple phenols to polyphenols and can be derived from plants and microorganisms including Spirulina (Al-Dhabi and Valan Arasu, 2016; Hussain et al., 2019; Olszewska et al., 2020). Most phenolics have two or more classes of hydroxyl, and are biologically active, having antimicrobial and/or antioxidant properties. Phenolics occur widely in plant sources, and are consumed daily by the global population, as illustrated in Table 1. Polyphenols have different categories based on their food sources, which include flavonoids, phenolic acids, lignans, and stilbenes.
Table 1. Phenolic and flavonoid contents from selected foods.

<table>
<thead>
<tr>
<th>Author</th>
<th>Article title</th>
<th>Studied food</th>
<th>Total phenolic content (mg GAE/g)</th>
<th>Total flavonoid content (µg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>El-Baky et al.</td>
<td>Production of phenolic compounds from <em>Spirulina maxima</em> microalgae and its protective effects</td>
<td><em>Spirulina maxima</em></td>
<td>12.94 ± 0.93</td>
<td>4.65 ± 0.14</td>
</tr>
<tr>
<td>Gurnani et al.</td>
<td>Chemical composition, total phenolic and flavonoid contents, and <em>in vitro</em> antimicrobial and antioxidant activities of crude extracts from red chilli seeds (<em>Capsicum frutescens</em> L.)</td>
<td>Chili seed</td>
<td>7.95 - 26.15</td>
<td>4.64 - 12.84</td>
</tr>
<tr>
<td>Jang et al.</td>
<td>Antioxidant and antimicrobial activities of fresh garlic and aged garlic by-products extracted with different solvents</td>
<td>Garlic</td>
<td>47.58 ± 5.27</td>
<td>338.04 ± 1.60(^a)</td>
</tr>
<tr>
<td>Alexandre et al.</td>
<td>Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction</td>
<td>Pomegranate</td>
<td>219 ± 2.6(^b) and 211 ± 0.7</td>
<td>No report</td>
</tr>
<tr>
<td>Ayvaz et al.</td>
<td>Phenolic profile of three wild edible mushroom extracts from Ordu, Turkey and their antioxidant properties, enzyme inhibitory activities</td>
<td>Mushroom</td>
<td>2.27 to 5.34 ± 0.55</td>
<td>5.02 to 17.54 ± 1.45</td>
</tr>
<tr>
<td>Papalia et al.</td>
<td>Impact of different storage methods on bioactive compounds in <em>Arthrospira platensis</em> biomass</td>
<td><em>Arthrospira platensis</em></td>
<td>15.77 ± 1.10(^b)</td>
<td>20.82 ± 0.08(^c)</td>
</tr>
<tr>
<td>Seghiri et al.</td>
<td>Functional composition, nutritional properties, and biological activities of Moroccan <em>Spirulina</em> microalga</td>
<td><em>Spirulina</em></td>
<td>4.19 ± 0.21</td>
<td>15.60 ± 2.74</td>
</tr>
</tbody>
</table>

\(^a\) aged garlic powder extracted with distilled water; \(^b\) high pressure extraction condition; and \(^c\) total phenol and flavonoid of fresh *A. platensis*.

**Figure 1.** Categories of polyphenols.
Figure 1 illustrates the categories of polyphenols (Becerra-Herrera et al., 2014; de Medina et al., 2015; Papalia et al., 2019).

The functions of *Spirulina* polyphenols are not only limited to antimicrobial and antimitogenic activities, but also anticancer, antiaging, anti-inflammation, lung defensive; neuroprotective, cardioprotective, hepatoprotective, and many more as illustrated in Figure 2. Furthermore, different studies have indicated their antimicrobial roles against different foodborne pathogens (Ghomari et al., 2019; Papalia et al., 2019; Polverari et al., 2019), antioxidative roles (Salamatullah, 2014; Irakli et al., 2018; Papalia et al., 2019; Kukharenko et al., 2020), antifungal and antimycotoxin roles (Shishido et al., 2015; Christ-Ribeiro et al., 2019), cardiovascular health promoting properties (Huang et al., 2018), control and elimination of life-threatening viruses (Carrera-González et al., 2013; Martínez-Martos et al., 2014; Mathur, 2019), neural protection and boosting the immune system (Martínez-Martos et al., 2014; Kumar et al., 2017), antidiabetes, antihypertensive, and anti-obesity roles (Hu et al., 2019; Lafarga et al., 2020), anticancer properties (Jung et al., 2019; Marková et al., 2020), and the prevention of retinal neurodegeneration and improvement of eyesight (Okamoto et al., 2019). Another study showed the chemopreventive role of *Spirulina* water extract solution which revealed good results in a human lung cancer A549 cell line with a considerable reduction of cancer cell viability and multiplication (Czerwonka et al., 2018).

The quantity and efficiency of phenols, polyphenols, C-phycocyanin, and other bioactive compounds like γ-linolenic acid and carotenoids depend on the growth medium and condition of *Spirulina* (Sassano et al., 2014; Gupta et al., 2018; Papalia et al., 2019), light intensity (Kepekçı and Saygideger, 2012), storage (Papalia et al., 2019), processing and extraction methods, and the solvents used (Salamatullah, 2014; Papalia et al., 2019). According to Papalia et al. (2019), the highest quantity of phenols, phycocyanin-C, and ascorbic acid was found in fresh *Spirulina* biomass, and the highest total flavonoid content was found in freeze-dried *Spirulina* biomass. On the other hand, the highest antimicrobial activity was found in both fresh and freeze-dried biomass due to the high carotenoid concentration, thus indicating the high concentration of phenolic compounds present in *Spirulina*.

**Antimicrobial effect of Spirulina phenolic compounds**

Antimicrobials are natural or man-made substances that can be used to control, kill, and/or inhibit the development and growth of microorganisms including viruses, bacteria, fungi, and protozoa (de Medina et al., 2015; Sherrard et al., 2014). *Spirulina* is known globally as a dietary supplement for human consumption, but in recent times, it has also been recognised as a good antimicrobial compound due to its ability to control and inhibit the development or growth of foodborne pathogens and

![Figure 2. Polyphenol activities of Spirulina spp.](image-url)
food spoilage microorganisms in the food industry (Duda-Chodak, 2013; Seghiri et al., 2019; Elshouny et al., 2020; Martelli et al., 2020). Different studies reported the antimicrobial effect of *Spirulina* on the growth of different bacteria, yeasts, and moulds including *Alicyclobacillus acidoterrestris*, *Aspergillus flavus*, *Aspergillus niger*, *Bacillus subtilis*, *Cladosporium*, *Geotrichum*, *Micrococcus luteus*, *Penicillium*, and *Rhodotorula* (Souza et al., 2011; Duda-Chodak, 2013).

The need to produce safe foods has encouraged the exploration of natural ingredients that possess antimicrobial activity (Souza et al., 2011; Jung et al., 2019; Martelli et al., 2020). Some of these natural ingredients can be obtained from *Spirulina*. *Spirulina* phenolic compound extracts have shown excellent antimicrobial activities (Sun et al., 2016; Swain et al., 2017; Jung et al., 2019). *Spirulina platensis* methanolic extract (1.15 mg phenolic compound per gram of *S. platensis* biomass) has shown antifungal effect against *A. flavus* at 54 µg/mL minimum inhibitory concentration (MIC), four times lower than the control (Souza et al., 2011). Parisi et al. (2009) reported that *S. platensis* phenolic compound extract inhibited *S. aureus* at 47.5 mg/mL MIC. Scaglioni et al. (2019) reported 50% inhibition using 33.9 µg/mL *S. platensis* phenolic compound extract against *Fusarium*. Other studies reported good inhibition results of *S. platensis* phenolic compound extract against *E. coli*, *P. aeruginosa*, *B. subtilis*, *V. vulnificus*, *A. flavus*, and *A. niger* (El-Sheekh et al., 2014; Rajasekar et al., 2019; Seghiri et al., 2019). C-phycocyanin from *S. platensis* inhibited drug-resistant bacteria such as *E. coli*, *K. pneumonia*, *P. aeruginosa*, and *S. aureus* (Sarada et al., 2011).

Another study regarding the inhibition success of *Spirulina* phenolic compound extract against fungus was carried out, and the application of 8% liposomal phenolic compound was found more effective, with 90% inhibition, than the free extract solution, with 74% inhibition (Pugazhendhi et al., 2015). Seghiri et al. (2019) reported that the methanolic extract of *Spirulina* phenolic compound exhibited a good result against pathogenic bacteria and fungi. It was assumed that the cell concentrate of *S. platensis* has the suitable ability to control the development and growth of pathogenic bacteria and fungi including *B. subtilis*, *S. aureus*, *E. coli*, *M. luteus*, *A. flavus*, *A. niger*, and *C. versicolor* (Usharani et al., 2015; Pugazhendhi et al., 2015; Seghiri et al., 2019).

Other researchers reported that the extract of *S. platensis* is active for the control and inhibition of fungi and bacteria, and serves as a good source of antimicrobial activity due to the bioactive compounds like γ-linolenic acid, fatty acids, and interaction of lauric and palmitoleic acids (Mendiola et al., 2007; Ramadan et al., 2008; El-Sheekh et al., 2014; Yang et al., 2019). Other studies reported that the antibacterial activity of C-phycocyanin from *S. platensis* was amplified at higher concentrations, specifically 400 µL/disk and above (Safari et al., 2019). This is possibly due to the resistance of those bacterial strains to the extract of C-phycocyanin differing from one species to another. As concluded in a study conducted by Safari et al. (2019), *Listeria* and *Streptococcus* were the most susceptible and resistant bacterial isolates to C-phycocyanin, respectively. The researchers’ finding also highlighted that C-phycocyanin from *S. platensis* has excellent antimicrobial activity, and it can be used as a natural antimicrobial in different food commodities, mainly foods with high lipids, as well as in cosmetic products. Both antioxidant and antibacterial activities of C-phycocyanin decrease in long-term storage at -18°C, most likely due to the effect of the freezing temperature on the protein structure of C-phycocyanin (Safari et al., 2019; Usharani et al., 2015).

**Conclusion**

Fish and seafood products are the best means of food and nutrition security. At the same time, however, they are highly perishable, being vulnerable to pathogenic microorganisms including *E. coli*, *P. aeruginosa*, *B. subtilis*, *A. flavus*, and *A. niger*. Currently, *Spirulina* is known as a dietary supplement, and its bioactive compound extracts are known for their antimicrobial activity towards pathogenic bacteria and fungi. Promoting this emerging natural compound in fish and fish products will save both our food and the public from food-related risks. *Spirulina* bioactive compounds include polyphenols, phycocyanins, and fatty acids. Flavonoids and phenolic acids from the polyphenol category could be promising. Future perspectives of *Spirulina* should include issues such as multifunctional study of polyphenols including antimicrobial agent development, drug development, and application in animal and fish feed production. Implications for climate mitigation measures should also be further elucidated and studied.

**Acknowledgement**

Special thanks to Dr. Amha Belay, a senior algal researcher and resource person on *Spirulina*, for the technical assistance rendered.
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