Current Research in Food Science 9 (2024) 100857

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Review Article

Yogurt enriched with nanoencapsulated anthocyanins: Effects on the modulation of the gut microbiota and its influence on health

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ARTICLE INFO

Handling Editor: Dr. Yeonhwa Park

Keywords:
Dairy products
Phenolic compounds
Functional food
Nanotechnology
Anti-inflammatory activity

ABSTRACT

Anthocyanins are phenolic compounds present in various plant products with interesting functional characteristics studied by science, such as their anti-inflammatory and antioxidant activities, among others. However, anthocyanins are considered unstable to various factors, which can affect their functional capacity. For this reason, some technologies, such as nanoencapsulation, are being applied to ensure their functional capacity effectively. The incorporation of anthocyanins in yogurt has shown various benefits, such as the ability to inhibit pathogenic microorganisms, reduce enzyme activity, and prolong the shelf life of the product. Additionally, the functional effects include their ability to modulate the gut microbiota, generating antioxidant, anti-inflammatory, and even antiproliferative responses, thereby reducing the capacity of tumor progression. For these reasons, this graphic review discussed the functional effects of yogurt enriched with nanoencapsulated anthocyanins on the gut microbiota and its influence on human health.

1. Introduction

Currently, consumers are becoming increasingly demanding regarding their diet and the benefits that food can directly offer to their health (Pinto et al., 2023). Thus, foods known as functional have been gaining more space in the daily lives of many consumers because they can offer much more than the basic function of feeding and nourishing, bringing specific benefits to the body in the medium and long term, such as probiotic and prebiotic effects (Fazilah et al., 2018), anti-inflammatory (Chen et al., 2019), antioxidant (Demirci et al., 2017), among others.

Yogurt, a widely consumed fermented dairy product worldwide, is capable of offering various benefits to consumers' bodies on its own, such as B12 complex vitamins and nutrients of high biological value (Wang et al., 2023). Yogurt is rich in lactic acid bacteria (LAB), which can promote gut microbiota balance, inhibiting the presence of potential pathogenic bacteria, and providing various important nutrients such as calcium, zinc, potassium, B12 complex vitamins, and high biological value proteins (Gullón et al., 2015; Naibaho et al., 2022).

Anthocyanins are phenolic compounds, also known as natural pigments, belonging to the flavonoid group and responsible for a wide variety of colors found in various plant-based products such as fruits, seeds, leaves, and stems (Fig. 1), ranging from red to purple and blue (Hussain et al., 2023). In addition to color, anthocyanins can improve the quality of food by delaying degradation, which can be caused by enzymes and bacteria (de Moura et al., 2024).

Anthocyanins are composed of two benzene rings connected by a three-carbon chain. Their molecular structure can differ based on their source and the degree of hydroxylation and methoxylation in their aromatic rings. These variations result in different types of anthocyanins, such as pelargonidins, delphinidins, cyanidins, peonidins, petunidins, and malvidins. Each of these compounds can have distinct properties and functions, depending on their specific chemical configuration (Rosales et al., 2022).

Due to their functional properties, the enrichment of yogurt with anthocyanins has been studied by various researchers, who seek to understand the technological and functional characteristics of their application in yogurt. These include antioxidant effects, which can help

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https://doi.org/10.1016/j.crfs.2024.100857
Received 19 July 2024; Received in revised form 8 September 2024; Accepted 15 September 2024
Available online 16 September 2024

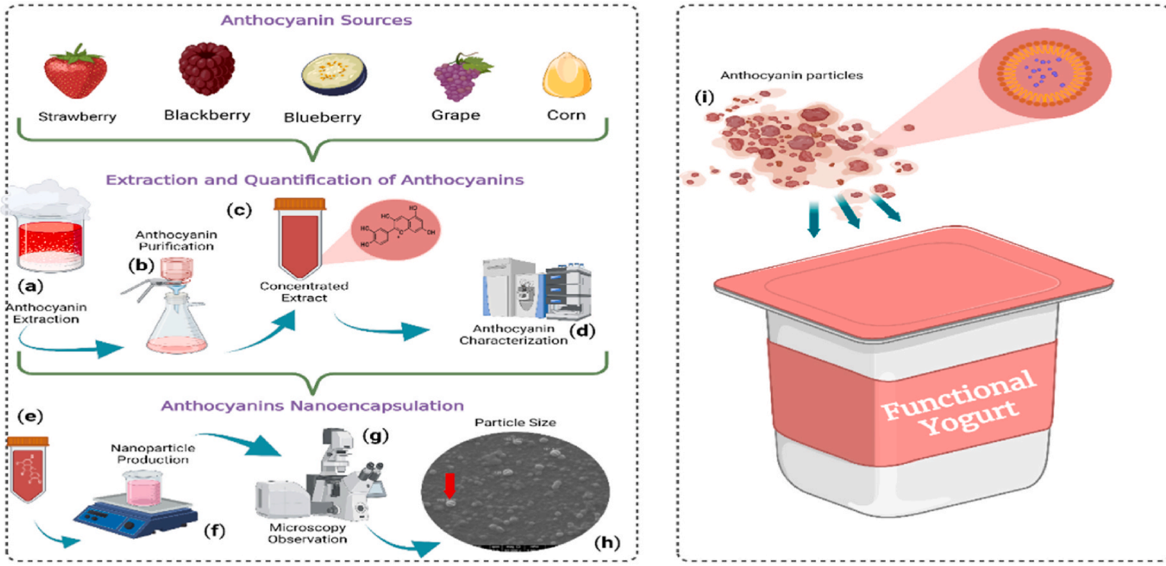

Fig. 1. Extraction, Nanoencapsulation, and Addition of Anthocyanins in Yogurt. Various sources of anthocyanins can be used, such as blackberry and grape, which are the most studied. (a) Example of anthocyanin extraction from blackberry (Rubus spp), with cold sample preparation using liquid nitrogen. (b) Anthocyanin extraction from the sample can be performed using methanol, ethanol, or water, followed by sample purification with a polyamide column. Using water is often more suitable for anthocyanin extraction when the intended application is for food products. (c) Obtaining the anthocyanin-concentrated extract. (d) Identification and quantification of anthocyanins by HPLC-DAD. (e) Concentrated and characterized extract, ready for Nanoencapsulation. (f) Preparation of the wall material with pectin and lysozyme under heating (80 °C/30 min) and subsequent cooling to 4 °C, and addition of the anthocyanin extract under magnetic stirring for 1 h until the formation of the nanoparticle. (g) Scanning electron microscopy (SEM) used to visualize the morphology of the nanoparticle. (h) Example of an anthocyanin nanoparticle observed under SEM in a Quanta 650 FEG microscope, with 60.000× magnification (Rosales et al., 2023). (i) Example of a nanoparticle prepared to be added to yogurt, with amplification showing the double-layer wall with pectin and lysozyme.

extend the product's shelf life, color improvement (as anthocyanins are considered natural colorants), antimicrobial effects, and their impacts on consumers' bodies, such as anti-inflammatory effects (Wang et al., 2023) and modulation of the gut microbiota, which can trigger a series of health benefits (Kumar et al., 2024).

When consumed frequently, anthocyanins can show functional capacity with antiproliferative, neuroprotective and cardioprotective activity (Singh and Yadav, 2022). Some studies have demonstrated various benefits of enriching foods with anthocyanins, making the food a carrier for this compound (Naibaho et al., 2022; Kumar et al., 2024). Yogurt is an excellent example of a food to be enriched with anthocyanins due to its characteristics and widespread acceptance and consumption by all types of consumers. However, anthocyanins can present instability to certain factors such as light, oxygen, and high temperatures (de Moura et al., 2024).

Some technologies are being applied to ensure the stability of the beneficial effects of anthocyanins, such as microencapsulation (de Moura et al., 2022) or nanoencapsulation, which protect anthocyanins from these factors and can maintain stability during the gastric digestion process, releasing the phenolic content in a controlled manner and ensuring more effective absorption (Rosales et al., 2022).

In this regard, nanoencapsulation offers superior capability compared to microencapsulation, as nanoparticles (1–100 nm) are significantly smaller than microparticles (1–1000 μm), increasing the absorption rate by intestinal cells and enhancing the phenolic content's effect on the body (Rosales et al., 2022) (Fig. 2). Studies suggest that a daily intake of anthocyanins (50 mg) per 100 g of food, such as yogurt, can offer various health benefits, but their bioaccessibility and bioavailability can significantly interfere with the expected effects (Tsuda T, 2012).

For these reasons, and to facilitate the understanding of the benefits of consuming yogurt enriched with anthocyanins, this graphical review aims to summarize the main changes in gut microbiota and the resulting benefits for the body.

2. Gut microbiota modulation

The human intestine is a large and complex organ responsible for hosting various species of microorganisms that live in a symbiotic relationship with their host. These microorganisms colonize this important organ without causing harm to the body, and they are known as gut microbiota (GM) (Gareau et al., 2010). The gut microbiota functions as an ecosystem capable of contributing to nutrition, maintaining homeostasis, and interacting with the immune system and the entire host organism (Mukherjee et al., 2024). The GM is also susceptible to modulations caused by various factors, including the consumption of certain foods, which can beneficially or detrimentally alter the presence of these microorganisms in the intestine (Gareau et al., 2010).

The bacteria present in fermented dairy products like yogurt can offer various benefits to the body by interacting with the gut microbiota. They induce beneficial transformations in segments of the intestine, such as the production of exopolysaccharides, which are long sugar polymers produced outside cells and are associated with modulating the host's immune system and antioxidant activity. These bacteria also inhibit pathogens through nutrient competition, enhance the absorption of phenolic compounds by intestinal epithelial cells, making them available to the circulatory system, and increase anti-inflammatory and antiproliferative responses in the body (Mukherjee et al., 2024) (Fig. 3). This cascade of events in the intestine also allows interaction with other organs, such as increasing the permeability of amino acids and organic acids that provide benefits to the brain, thereby impacting overall health (Fazilah et al., 2018; Mukherjee et al., 2024).

3. Influence on health

The functional mechanism of anthocyanins in the body is complex and interactive, starting when the nanoparticles release anthocyanin molecules into the bloodstream, allowing them to interact freely with all the body's cells. When they cross the cell membrane, the anthocyanins initiate a series of events in the cell's cytoplasm, activating various

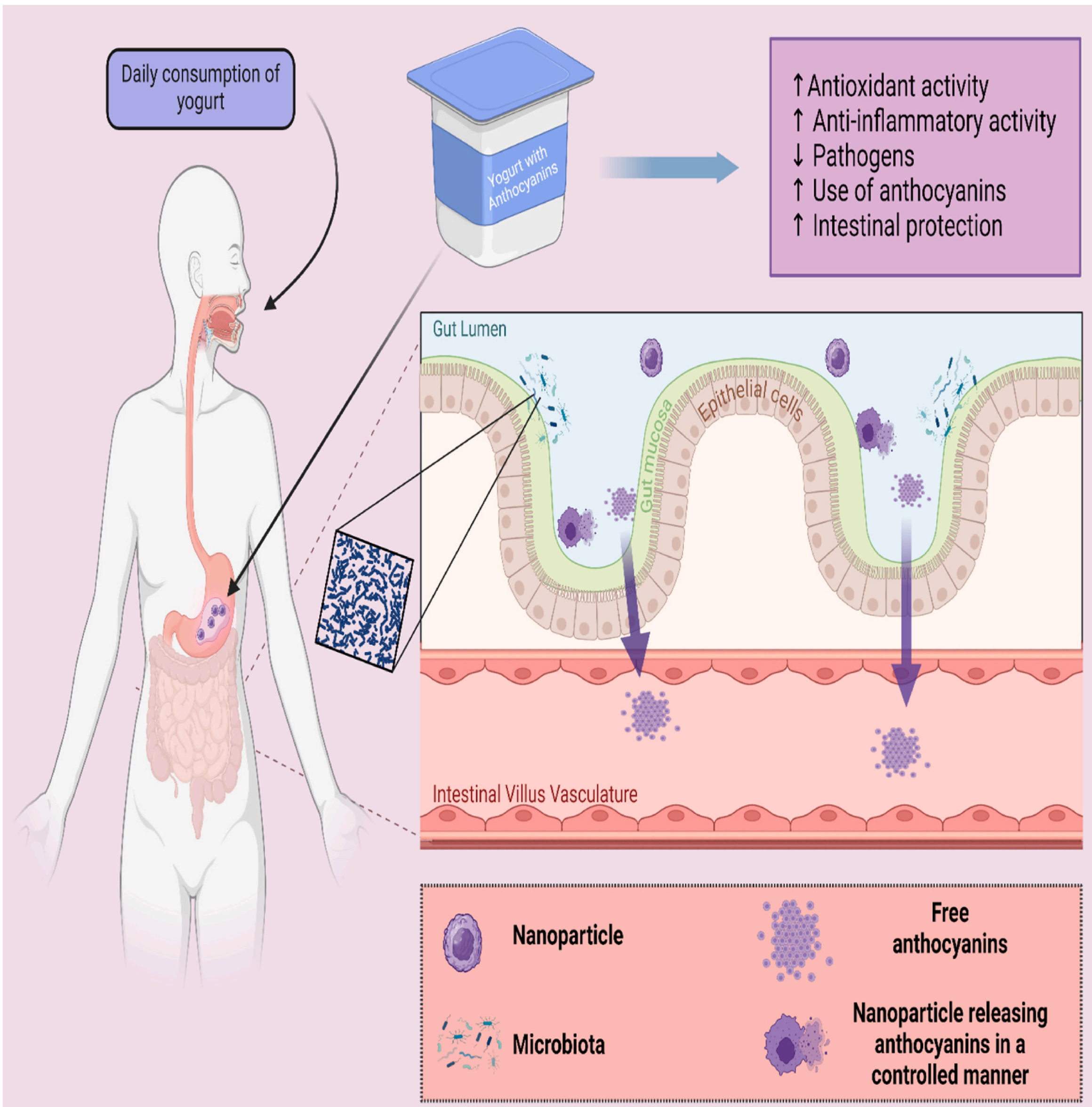

Fig. 2. Intestinal absorption of nanoencapsulated anthocyanins. (a) The presence of probiotic bacteria in the intestine promotes the passage of anthocyanins through the intestinal epithelium, and the presence of anthocyanins in the intestine inhibits the presence of pathogenic bacteria (adapted from Naibaho et al., 2022). (b) Anthocyanins released in a controlled manner into the bloodstream can offer protection to all cells of the body with their antioxidant and anti-inflammatory capacities (adapted from Kumar et al., 2024).

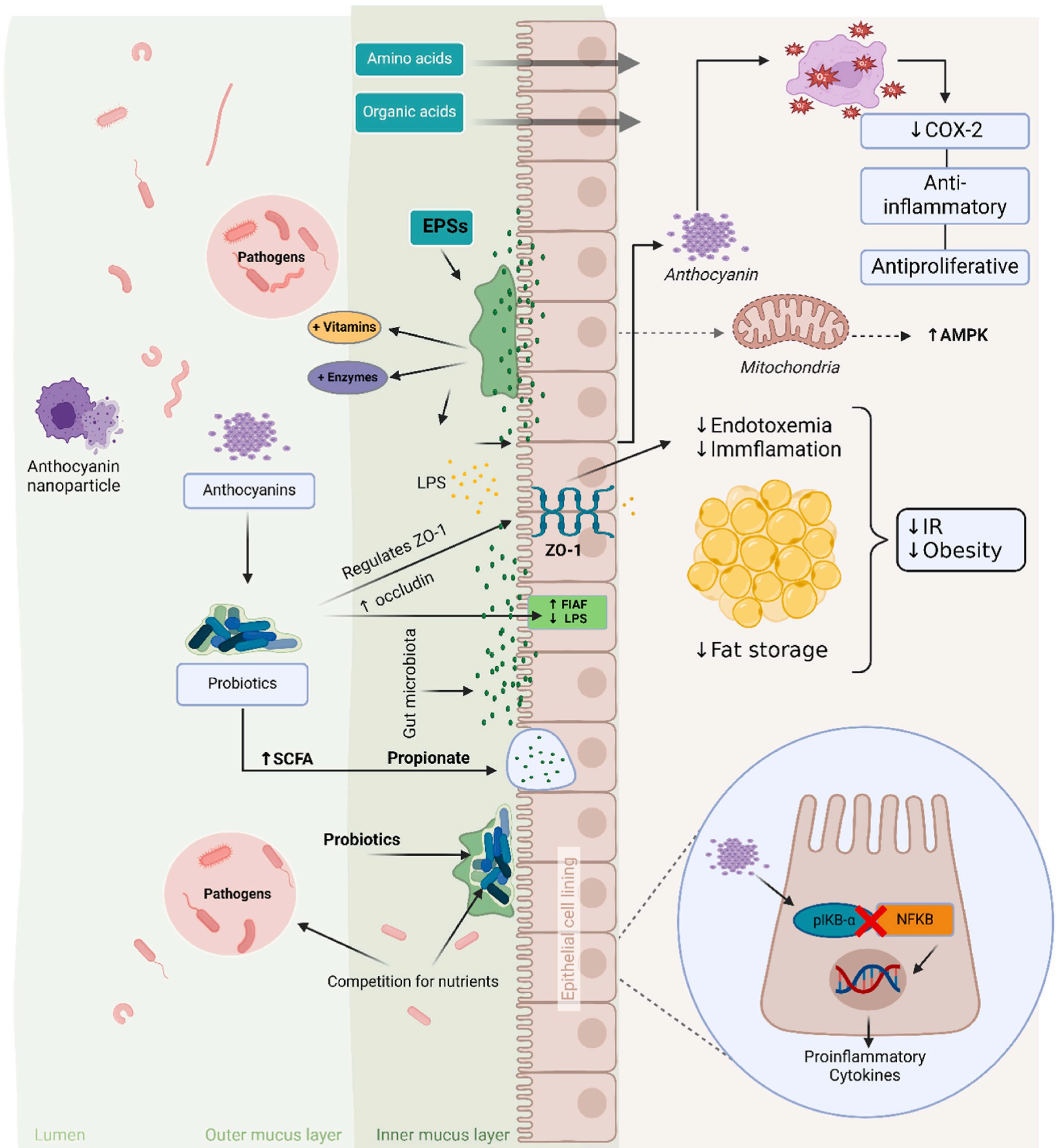

Fig. 3. Yogurt enriched with anthocyanins and its prebiotic potential in modulating the intestinal microbiota and fat metabolism. Exopolysaccharides (EPSs) facilitate the passage of anthocyanins through the epithelium, triggering a cascade of events such as the neutralization of free radicals, the release of vitamins and enzymes and the reduction of antioxidant, anti-inflammatory and anti-proliferative activities. The action of probiotics increases the production of short-chain fatty acids (SCFA) and the control of the occlusion zone (ZO-1), decreasing the passage of lipopolysaccharides (LPS), preventing inflammation and endotoxemia and decreasing fat storage and fasting-induced adipose factor (FIAF), as well as showing better control of insulin release (IR). Probiotic bacteria strengthen the intestinal epithelium, increasing the passage of anthocyanins and competing with pathogenic bacteria for the nutrients available in the intestinal villi. Anthocyanins As stimulate the activation of AMP protein kinase (AMPK), increasing its phosphorylation and reducing lipid metabolism and triglyceride synthesis, as well as increasing fatty acid oxidation and mitochondrial biogenesis.

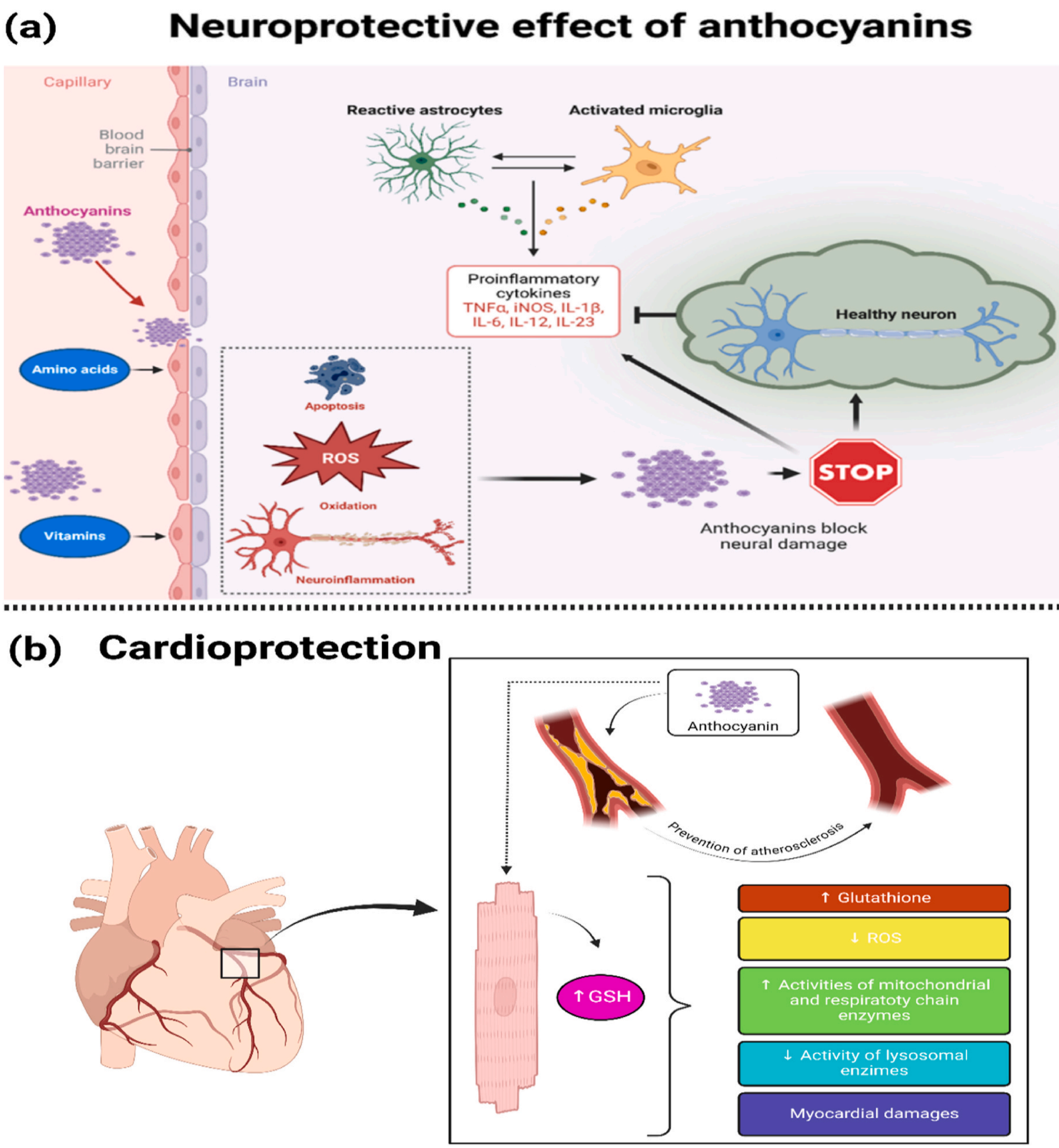

Fig. 4. (a) Anthocyanin's neuroprotective effect: Anthocyanins can block the action of pro-inflammatory cytokines. Additionally, they exhibit anti-apoptotic, antioxidant, and anti-inflammatory effects, providing direct protection to neural cells. This ensures the health and protection of neurons. Their ability to cross the blood-brain barrier facilitates the entry of vitamins and amino acids into the brain. (b) Anthocyanin's cardioprotective effect: Anthocyanins can prevent atherosclerosis, which is the accumulation of fatty plaques in the arteries. Furthermore, they stimulate the production of the hormone GSH, which offers various protective effects to heart cells, such as protection against oxidative stress, increased mitochondrial and respiratory activities, and reduced activity of lysosomal enzymes, thereby decreasing damage to the myocardium (Salehi et al., 2020).

enzymes and signaling pathways. This activation can modulate signaling pathways and generate various responses in the body, such as improved insulin sensitivity and blood glucose regulation, better control of the cell cycle and apoptosis, antiproliferative and anti-inflammatory effects and even control of tumor progression (Bhushan et al., 2024). Thus, anthocyanins can exert effects on various systems in the body, including protection of the circulatory and nervous systems, and can also influence the oral/intestinal microbiota axis (Salehi et al., 2020) (Fig. 4).

Considering that oral bacteria can be ingested during the digestion process, it can disseminate to epithelial tissue throughout the body, being able to achieve areas such as intestine and colon (Olsen and Yamazaki, 2019). This fact is concerning, especially considering that in the intestine, a large amount of *Streptococcus* spp. may be linked to subclinical coronary atherosclerosis (Sayols-Baixeras et al., 2023), indicating significant systemic alterations.

By participating in the process of controlling this dysbiosis, phenolic compounds can inhibit the growth of pathogenic bacteria in the oral cavity, such as Streptococcus mutans, which is associated with dental caries (Nijampatnam et al., 2018). In addition, phenolic compounds can act to reduce intestinal pathogens, suppressing harmful bacteria such as Clostridium spp. and promoting a better environment for the colonization of Lactobacillus (Paturi et al., 2018). In this way, anthocyanins can express their functional characteristics, presenting benefits to the body's various systems (Fig. 5).

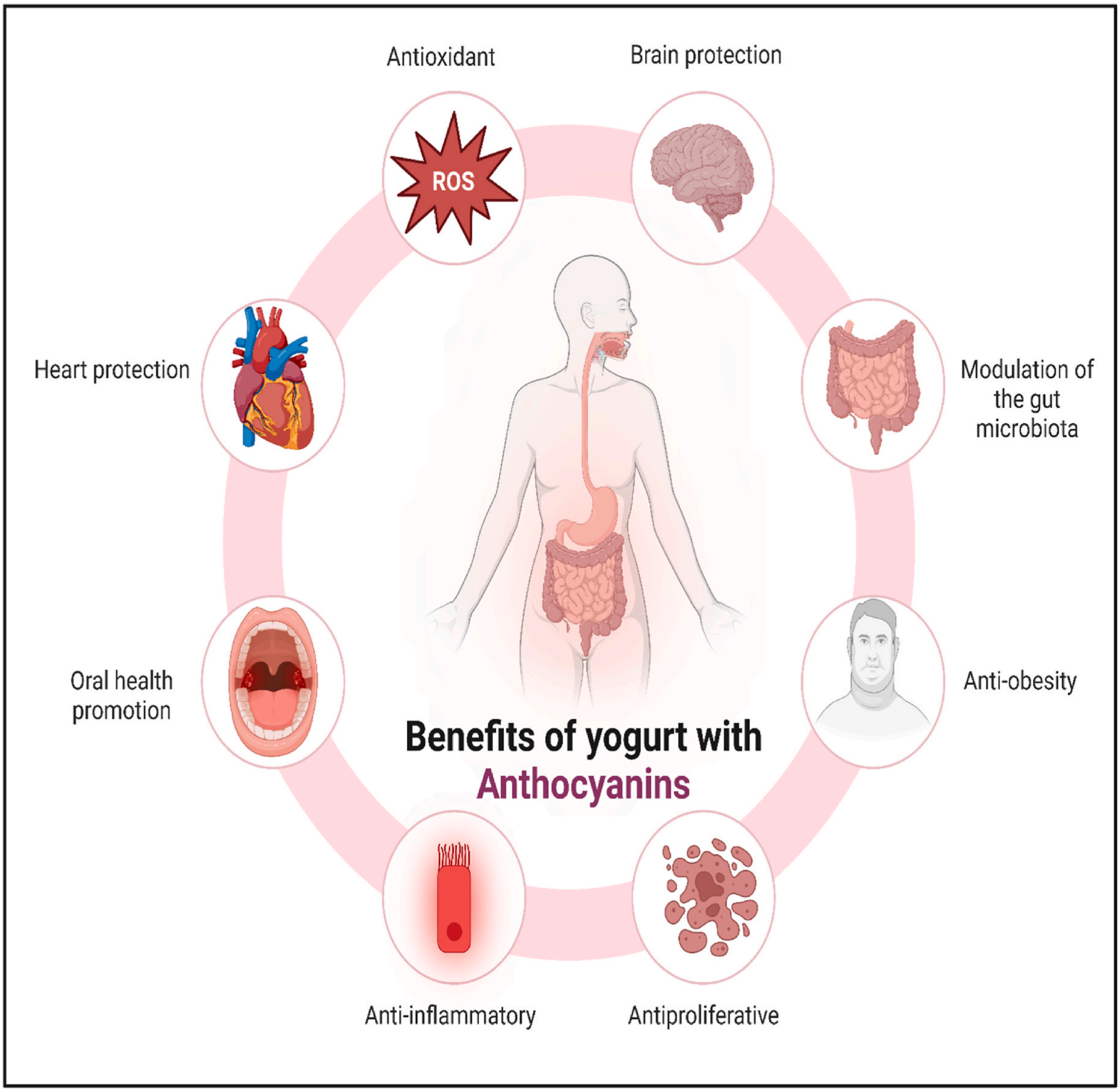

Fig. 5. Benefits of consuming yogurt with anthocyanins for the body.

4. Final considerations

Anthocyanins are powerful phenolic compounds capable of offering various health benefits when routinely included in the diet. Therefore, the enrichment of yogurts with anthocyanins can help encourage and increase their consumption by the population, as they not only improve the sensory and technological characteristics of yogurt but also enhance its health benefits. The modulation of the gut microbiota can be considered the most important aspect of these benefits, as it has the ability to positively interact with the entire organism.

This review discussed the effects of consuming yogurt with anthocyanins on the modulation of the gut microbiota and its health impacts resulting from this consumption. Most studies report these occurrences in *in vitro* models, and future studies are needed to investigate the effects of anthocyanins on metabolic pathways *in vivo*, indicating their consequences for the human body.

Availability of data and material

Not applicable.

Code availability

Not applicable.

CRediT authorship contribution statement

Carlos Eduardo Cardoso de Aguiar Freire: Conceptualization, Writing – original draft. **Samuel Ferreira Gonçalves:** Writing – review & editing. **Carolina de Souza Moreira:** Writing – review & editing. **Ranam Moreira Reis:** Writing – review & editing. **Severino Matias de Alencar:** Conceptualization, Supervision, Writing – review & editing. **Aline Silva Mello Cesar:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

Carlos Eduardo Cardoso de Aguiar Freire acknowledges the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance code (001) for his Ph.D. fellowship grant. The figures were created with BioRender.com.

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