Global Research journal of Natural Science & Technology (GRJNST)

Volume: 04 - Issue 2 (2026), 2052
ISSN P: 2790-7643 ISSN E: 2790-7651
www.grjnst.net
https://doi.org/10.53762/grjnst.04.02.04

Development and Quality Evaluation of Functional Yogurt Enriched with Plant-Based Bioactive Components

Received: 29 December 2025. Accepted: 23 January 2025. Published: 30 March 2026

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***Abstract**: The increasing health sensitive food demand across the world has made functional dairy products especially yogurt take the center stage in the field of nutrition science. Yogurt presents an outstanding vehicle of bioactive compounds as it is palatable, has extensive acceptability and is a potential probiotic. The review examines the production and quality assessment of functional yogurt enriched with the plant-based bioactive compound such as polyphenols, flavonoids, dietary fibers and essential oils found in fruits, vegetables, herbs and spices. The implementation of these natural additives will be done to increase the therapeutic value of yogurt, which focuses on antioxidant, anti-inflammatory, antimicrobial and cardioprotective effects. Nevertheless, introduction of the plant matrices poses serious technological difficulties, affecting the physicochemical characteristics, rheological characteristics, sensory characteristics and microbiological stability of the end product. This paper summarizes the existing literature regarding the choice of plant sources, incorporation technique and the complex effect on yogurt, such as syneresis, viscosity, color and acceptability by the consumer. In addition, it looks into the synergistic effects in combination with starter cultures and the ramifications of the shelf-life extension. The review finds that although plant-based enrichment is a promising concept in functional foods of next generation, careful optimization is necessary to achieve a balance between bioactivity and other attributes such as sensory excellence and stability of the products.*

***Keywords:** Functional yogurt, plant bioactive compounds, polyphenols, antioxidant activity, sensory evaluation, prebiotics.*

I. Introduction

Nutrition paradigm is no longer but satiating the hunger but regulating bodily functions to enhance health and well-being, leading to the emergence of the concept of functional foods (Shahidi and Alasalvar, 2016). Fermented dairy products have an exceptionally privileged niche among these, yogurt being one of the most frequently used and most flexible vehicles of functional ingredients. Yogurt is also naturally functional because it contains live bacterial cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*), which are beneficial, i.e., lactose digestibility, and modulation of gut microbiota (Savaiano and Hutkins, 2020; Champagne *et al.*, 2020).

At the same time, the demand in the use of natural, plant-based products that are safe and healthy increases. There have been a lot of studies on plant-based bioactive compounds, including polyphenols, anthocyanins, carotenoids and dietary fibers, with regard to their antioxidant, antimicrobial and anti-carcinogenic activities (Alves-Silva *et al.*, 2020; Caleja *et al.*, 2017). Accordingly, the strategic fortification of yogurt with such plant matrices can be considered a synergistic method of creating a product with increased nutritional and therapeutic performance - a phenomenon that is commonly known as the super-yogurt (Aswal *et al.*, 2021).

Nevertheless, the creation of such functional products is not that easy. The introduction of vegetable ingredients has the potential to interfere with the sensitive casein micelle network, resulting in a change in the texture, higher syneresis and unwanted color or flavor (Gahruie *et al.*, 2020; Baba *et al.*, 2020). Also, the contact between bioactive compounds and plant bioactives may impact the viability of starter cultures, as well as the bioavailability of bioactive compounds themselves (Kobus-Cisowska *et al.*, 2019; Rashidinejad *et al.*, 2021).

The purpose of the review is to critically assess the existing body of knowledge in the field of the functional development of yogurt fortified with plant-based bioactive compounds. It will address the variety of the sources of plants that are treated, the technology of its implementation, the effect on the physicochemical and rheological and sensory properties and the effects of health benefits. It is aimed at giving a comprehensive picture that will inform further studies and practical applications in the industry in creating stable, palatable, and effective functional yogurts.

2. Plant-Based Bioactive Components: Sources and Functional Properties

The plant material that has been considered to fortify yogurt is very extensive, covering the fruit and vegetables pomace, as well as herb and spice extracts. These plants have bioactive compounds that can be divided into phenolic compounds, carotenoids, dietary fibers and essential oils.

2.1. Phenolic Compounds and Polyphenols.

Phenolic acids, flavonoids and tannins are secondary metabolites, which are characterized by their strong antioxidant and anti-inflammatory properties. These compounds occur in sources like the green tea (Camellia sinensis), grape pomace (Vitis vinifera) and pomegranate (Punica granatum). These compounds are able to scavenge the free radicals, decrease the lipid peroxidation as well as may regulate the gut microbiota by promoting the good bacteria in yogurt (Du *et al.*, 2021; Li *et al.*, 2021). Oktay and Gulcin (2019) also proved that phenolic extracts of different plants have good radical-scavenging activity that is well transferable into dairy matrices.

2.2. Dietary Fibers

Fiber of plants, especially fruits (e.g., apple, banana) and cereals (e.g., oats) are prebiotics, which activate the growth and activity of probiotic bacteria. Insoluble fibers may positively affect gastrointestinal flow, whereas soluble fibers (e.g., pectin, b-glucan) may have an effect on the viscosity and texture of yogurt and tend to replace fats (Niamah *et al.*, 2020). Gomez *et al.* (2020) have adequately reported the prebiotic potential of pectin-derived oligosaccharides, which results in improved growth of beneficial microbiota of the gut.

2.3. Carotenoids and Pigments

This is done by adding natural pigments such as b-carotene (carrots), lycopene (tomatoes) and anthocyanins (berries) to fortify the color and antioxidants properties of the yogurts. These substances help to enhance visual quality as well as add to the functional characteristics of the product (Paszczyk *et al.*, 2019; Turgut and Cakmakci, 2020). In their study, Dias *et al.* (2020) have documented that grape juice enrichment offered desirable color, as well as, antioxidant activity to probiotic yogurt.

Table 1: Common Plant Sources, Bioactive Components, and Associated Health Benefits in Yogurt

Plant Source	Bioactive Components	Functional Benefits	References
Pomegranate	Anthocyanins, Tannins, Ellagic acid	Antioxidant, Antihypertensive, Anti-inflammatory	(Aswal *et al.*, 2021; Du *et al.*, 2021)
Green Tea	Catechins (EGCG)	Antioxidant, Antimicrobial, Antidiabetic	(Kobus-Cisowska *et al.*, 2019; Rashidinejad *et al.*, 2021)
Grape Pomace	Resveratrol, Anthocyanins	Antioxidant, Cardioprotective, Prebiotic	(Gahruie *et al.*, 2020; Dias *et al.*, 2020)
Turmeric	Curcuminoids	Anti-inflammatory, Antimicrobial	(Alves-Silva *et al.*, 2020; Giri *et al.*,

			2020)
Oat Fiber	β-glucan	Cholesterol-lowering, Prebiotic, Texture modifier	(Gómez *et al.*, 2020; Niamah *et al.*, 2020)
Carrot	β-carotene	Provitamin A, Antioxidant	(Paszczyk *et al.*, 2019; Turgut and Çakmakçı, 2020)

3. Impact on Yogurt Starter Cultures and Viability

Preservation of viability of starter cultures (and added probiotics) over shelf-life is one of the key factors in the development of functional yogurts. Plant bioactive compounds have a dual effect on the growth of microorganisms, as they are prebiotics or antimicrobial active compounds.

3.1. Stimulatory Effects

Most plant extracts contain oligosaccharides and micronutrients which are growth substrates of lactic acid bacteria (LAB). As an example, addition of fruit fibers has been reported to enhance the stability of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in refrigerated storage, preserving the counts at levels above the log CFU/g recommended therapeutic level (Espirito-Santo *et al.*, 2021; Sah *et al.*, 2020). Low concentrations of phenolic compounds also have the potential to induce the growth of bacteria since they are electron shuttles. Mandal *et al.* (2020) identified that fruit extracts that were underutilized enhanced the growth of probiotic cultures in functional yogurt preparations.

3.2. Inhibitory Effects

On the other hand, LAB growth and fermentation can be disrupted, which is caused by high concentrations of polyphenol and essential oils and the disruption of bacterial cell membrane. The effectiveness of the antimicrobial action is conditional on the kind and the concentration of the plant extract. To illustrate, low concentrations of green tea extracts improve the LAB survival, whereas high concentrations might decrease the number of *Streptococcus thermophilus* because green tea extracts have a bactericidal action (Kobus-Cisowska *et al.*, 2019; Georgakopoulos *et al.*, 2019). Hashemi *et al.* (2019) also established that *Zataria multiflora* essential oil in concentrations of over 0.3% inhibited probiotic bacteria in yogurt significantly. Thus, a fine balance should be maintained to ensure that bioactivity is maximized and fermentation kinetics is not affected (Geci *et al.*, 2021).

Table 2: Effect of Plant Enrichment on Probiotic Viability in Yogurt

Plant Additive	Concentration	Target Culture	Effect on Viability	Reference
Pomegranate Peel	0.5%	*L. acidophilus*	Increased (prebiotic effect)	(Aswal *et al.*, 2021)
Green Tea Extract	>0.5%	*S. thermophilus*	Decreased (antimicrobial)	(Kobus-Cisowska *et al.*, 2019)
Grape Pomace	2%	*Bifidobacterium spp.*	Maintained > 7 log CFU/g	(Gahruie *et al.*, 2020)
Turmeric	0.1%	Mixed LAB	No significant inhibition	(Alves-Silva *et al.*, 2020)
Apple Fiber	1.5%	*L. casei*	Increased (prebiotic)	(Gomez *et al.*, 2020)
Pineapple Waste	2%	*L. acidophilus*	Increased	(Sah *et al.*, 2020)
Rosehip Pulp	1%	*Bifidobacterium*	Maintained	(Uysal *et al.*, 2021)

4. Physicochemical and Rheological Properties

Plant elements combination is a very important factor that changes the physical structure of yogurt. The main issues are variations in pH, acidity, water holding capacity (WHC), syneresis and viscosity.

4.1. pH and Titratable Acidity

Plant materials may serve as a buffer to the yogurt matrix or affect the post-acidification rate. High buffering capacity compounds like proteins and minerals in seed flours would delay the pH decrease and fermentable sugars would hasten the formation of acid. A small drop in pH during the storage phase has been reported in most studies and the change is usually conditioned by the composition of the additive (Paszczyk *et al.*, 2019; El-Sayed, 2020). According to Yildiz and Ozcan, (2021), seed oil enrichment with black cumin produced low pH changes in storage.

4.2. Water-Holding Capacity and Syneresis.

One of the major textural defects is syneresis, which is the expulsion of whey. Hydrocolloids are plant fibers and polysaccharides, which increase the WHC of the gel network. It has been observed that the addition of fruits with a high pectin content or oat derived b-glucan can greatly decrease syneresis and stabilize the protein network and immobilize free water (Gomez *et al.*, 2020; Niamah et al., 2020). On the other hand, the presence of insoluble particles in raw plant materials may interfere with the casein matrix resulting in the augmentation of whey separation (Sendra *et al.*, 2020).

4.3. Rheology and Viscosity

The rheological behavior that is defined by the parameters like consistency index and thixotropy are important towards consumer acceptance. Plant fibers tend to elevate the apparent viscosity of yogurt as a result of the ability to bind water. But this effect is dependent on the concentration and the size of the particle. Plant extracts that are nano-encapsulated or micro-particulated do not make the gel gritty, whereas large particles can contribute to a more cohesive gel (Gahruie *et al.*, 2020; Rinaldi *et al.*, 2020). Jovanovic *et al.* (2021) have proved that apple fiber had a significant effect on the consistency coefficient of probiotic yogurt.

Table 3: Influence of Plant Enrichment on Physicochemical Properties of Yogurt

Plant Additive	Concentration	Effect on pH	Effect on Syneresis	Effect on Viscosity	Reference
Pomegranate Juice	10%	Slight decrease	Decreased (↑WHC)	Increased	(Aswal *et al.*, 2021)

Grape Pomace	2%	Stable	Decreased	Increased (more shear-thinning)	(Gahruie *et al.*, 2020)
Oat β-glucan	0.5%	Stable	Significant decrease	Significant increase	(Gomez *et al.*, 2020)
Carrot Pulp	5%	Slight decrease	Increased (due to fiber disruption)	Decreased	(Paszczyk *et al.*, 2019)
Turmeric	0.2%	No change	No change	No change	(Alves-Silva *et al.*, 2020)
Black Carrot	5%	Slight decrease	Decreased	Increased	(Turgut and Çakmakçı, 2020)
Psyllium Husk	1%	Stable	Significant decrease	Increased	(Niamah *et al.*, 2020)

5. Sensory Evaluation and Consumer Acceptability

Consumer acceptance is what determines the success of functional yogurt. Although enrichment of plants adds functionality, they frequently add off flavor, bitter taste or unwanted colors that may ruin the traditional yogurt experience.

5.1. Color

The first attribute of sensory attribute is color. Although natural pigments (e.g., berries or carrots) can result in nice colors, some unanticipated ones, such as turmeric (yellow) or green tea (greenish-brown) can make the otherwise white or fruit-colored yogurt. Research has shown that consumers may be drawn to novelty, but radical changes in color may result in a backlash (Du *et al.*, 2021; Fazilah *et al.*, 2020). Turgut and Cakmakci (2020) discovered that the carrot concentrate (black) was beneficial in giving a pleasant purple-red hue to the panelists.

5.2. Flavor and Taste

Plant extracts are often used to add a bitter flavor (because of polyphenols), astringency or herbal flavor. To present the bioactive compounds, encapsulation technologies have been utilized to cover ugly tastes. As an illustration, microencapsulated curcumin greatly alters bitter taste of free curcumin, but preserves an antioxidant activity (Alves-Silva *et al.*, 2020; Giri *et al.*, 2020). Balance of sweetness is also essential; natural sweeteners are also usually added to plant fortification to conceal bitterness (Georgakopoulos *et al.*, 2019).

5.3. Texture and Mouthfeel

One of the quality determinants of yogurt is Mouthfeel. Fiber enrichment may result in a perceived grittiness or pastiness in case the particle size is not managed. It has been demonstrated that high-pressure homogenization of plant additives before incorporation enhances the viscosity and general palatability of the end product (Espirito-Santo *et al.*, 2021; Rinaldi *et al.*, 2020). To ensure the acceptable mouthfeel in yogurt, Sendra *et al.* (2020) identified that citrus by-products needed the reduction of particle size.

Table 4: Sensory Characteristics of Functional Yogurt Enriched with Plant Bioactives

Plant Additive	Positive Sensory Attributes	Negative Sensory Attributes	Strategy for Improvement	Reference
Green Tea Extract	Novel, herbal aroma	Bitterness, astringency, grey color	Encapsulation; use of sweeteners	(Kobus-Cisowska *et al.*, 2019)
Turmeric	Appealing yellow color	Earthy, bitter taste	Microencapsulation; combination with fruit	(Alves-Silva *et al.*, 2020)

Grape Pomace	Red/purple hue, fruity notes	Astringency, seed grittiness	Particle size reduction; fiber separation	(Gahruie *et al.*, 2020)
Pomegranate	Pleasant red color, tangy taste	Sourness, low viscosity	Prebiotic fiber addition	(Aswal *et al.*, 2021)
Banana Flour	Creamy texture, sweet	Dough-like flavor	Blending with fruits	(Gómez *et al.*, 2020)
Black Carrot	Attractive purple color	Earthy aftertaste	Sugar addition	(Turgut and Çakmakçı, 2020)
Sweet Potato	Natural sweetness	Slight vegetal note	Blending with spices	(El-Sayed *et al.*, 2020)

6. Functional and Health Benefits

In addition to nutrition, enriched yogurts have a high bioactivity. The functional advantages that have been determined as the main ones are antioxidant capacity and possible therapeutic effects.

6.1. Antioxidant Activity

Plant enrichment increases significantly the total phenolic content (TPC) and antioxidant capacity (measured by DPPH, ABTS or FRAP assays) of yogurt. It has been shown that the antioxidant activity of enriched yogurt can be 2-5 times higher than that of plain yogurt and these characteristics are often preserved even during refrigeration (Du *et al.*, 2021; Rashidinejad *et al.*, 2021). According to Sah *et al.* (2020), pineapple waste powder positively affected TPC and antioxidant activity of yogurt in addition to increasing the viability of the probiotics. The fermentation process can enhance the bioaccessibility of these antioxidants because bacterial enzymes can release bound phenolics in the cell walls of plants (Baba *et al.*, 2020).

6.2. Anti-inflammatory and Anti-hypertensive Action.

In vitro and animal model research has demonstrated that functional yogurt supplemented with polyphenol rich extracts has the capacity to prevent pro-inflammatory cytokines and angiotensin-converting enzyme (ACE). As an example, pomegranate peel extract used as a yogurt supplement showed great ACE-inhibitory effect, which indicates possible antihypertensive effect (Aswal *et al.*, 2021; Beltran-Barrientos *et al.*, 2018). Giri *et al.* (2020) conducted a review on the immunomodulatory effects of curcumin in functional food and reported that it can be used in yogurt recipes.

6.3. Antimicrobial Activity

LAB metabolites (bacteriocins, organic acids) can be combined with plant antimicrobials (e.g., essential oils), which will result in a synergistic hurdle effect against foodborne pathogens like *Escherichia coli* and *Listeria monocytogenes* and enhance safety and shelf-life (Alves-Silva *et al.*, 2020; Sakkas *et al.*, 2021). Georgakopoulos *et al.* (2019) proved that the oregano essential oil in yogurt was a suitable way to inhibit the *Listeria monocytogenes* growth throughout the storage. The same results were found by Hashemi *et al.* (2019), who demonstrated the antimicrobial synergy between the *Zataria multiflori* essential oil and LAB cultures.

7. Challenges and Future Perspectives

Although the prospect has good prognosis, there are a number of obstacles that impede the commercialization of plant enriched functional yogurt.

7.1. Bioavailability and Bio accessibility

One of the major questions is whether the bioactive compounds are bioavailable upon processing and gastrointestinal digestion. Dairy matrix has the ability to stabilize polyphenols and also contains polyphenols, which can change the absorption. It is advised that future studies address in vitro models of digestion to determine the actual bioaccessibility of such compounds (Espirito-Santo *et al.*, 2021; Baba *et al.*, 2020). Mohammadi *et al.* (2021) stressed the necessity of harmonized procedures to assess the bioaccessibility of dairy products with functional purposes.

7.2. Standardization

The challenge to standardization is the variability of phytochemical composition because of geographical origin, time of harvesting and processing procedures. It is critical to develop standardized extracts or concentrates that have reproducible bioactivity profiles to provide reproducible functional action (Shahidi and Alasalvar, 2016; Caleja *et al.*, 2017). Champagne *et al.* (2020) emphasized quality control as a key attribute in probiotic and plant-enriched dairy products.

7.3. New Processing Technologies.

Microencapsulation, nano emulsions and high-pressure processing (HPP) are emerging technologies that reduce sensory flaws and preserve the bioactive compounds. Off flavors can be covered, release can be regulated and sensitive compounds are more stable to fermentation and storage when encapsulated (Kobus-Cisowska *et al.*, 2019; Rinaldi *et al.*, 2020). Reviewing the use of plant essential oils in dairy products, Karimi *et al.* (2020) find out that encapsulation technologies could greatly enhance their stability and activity.

7.4. Sustainability

Circular economy principles are in line with the use of agri-food by-products (e.g., fruit peels, seed pomace) as sources of bioactives. This will not only decrease waste but also decrease the product cost, which will make them more affordable (Gahruie *et al.*, 2020; Sah *et al.*, 2020). Sendra *et al.* (2020) showed that citrus by-products can be incorporated into yogurt with the purpose of functionality and sustainability.

8. Conclusion

Commercialization of functional yogurt supplemented with plant-based bioactive ingredients is an important development in the dairy industry as it fills the gap between nutrition and therapeutics. The addition of polyphenols, fiber and natural pigments to yogurt through strategic fortification (using pomegranate, green tea, grape pomace and oats) is reported to increase antioxidant activity, gut microbiota homeostasy and may have cardioprotective and anti-inflammatory properties. Nonetheless, to make such products commercially successful, a delicate balance is required. The technological issues, such as the effects on the viability of starter cultures, changes in texture and rheology (syneresis, viscosity) and the sensory issues vital to the product (flavor, color, mouthfeel) should be carefully considered. Future studies need to aim at maximizing incorporation techniques using emerging technologies such as encapsulation and nano-emulsification in order to maintain the bioactivity and at the same time be consumer friendly. Moreover, agri-food by-products can be used as a source of these bioactives, which provides a sustainable future. Finally, a multidisciplinary method involving food science, nutrition and sensory analysis is needed to develop a stable, palatable and genuinely functional yogurt that is able to satisfy the needs of the health-conscious consumer.

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