

FORTIFICATION WITH NATURAL PLANT-BASED ADDITIVES IN CHEESE PRODUCTION: SUSTAINABLE CONCEPTS

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Abstract: Fortification of cheese involves the addition of micronutrients, including them in the casein matrix and proper improvement of the cheese, making the final product more attractive to consumers. In the Agri-Food sector, it is possible to enrich cheeses by adding plants secondary metabolites and/or using by-products from the processing plants. Interest in fortification of cheeses with antioxidants and phenolics derived from plant extracts (PE), spices, and plant by-products has been increasing significantly and encourage the development of a sustainable, circular economy and create “greener” environmentally friendly compounds that improve consumer health. Synthetic additives have become undesirable in the food due to potential harmful effects on human health. Consumers are trying to consume healthier or functional foods, safer alternatives to foods with synthetic additives. Numerous plant-based preservatives have been tested for use in the food industry. These natural molecules increase the microbiological stability of food and the levels of bioactive ingredients (BAI), while exhibiting antimicrobial (AM) and antioxidant (AO) effects. Cheeses are generally susceptible to contamination by pathogens and spoilage agents. In the dairy industry (more often on smaller farms), several sources of contamination have been observed: use of raw milk as a raw material, the environment where milk is obtained, stored and processed, or due to the practice of milk curdling with vegetable coagulants. The use of preservatives in cheese production is imperative, but they can degrade sensory properties, which consumers will not accept. This

review discusses an innovative approach, primarily focuses on the frameworks and procedures for cheese fortification.

Key words: natural plant additives, cheese making, functional properties, antimicrobial effects, antioxidative activity, shelf-life

Introduction

When we consider the physiological well-being and biochemical processes in the human body, the consumption of dairy products is of essential importance, as they represent a significant source of essential vitamins (A, D and B₁₂), fatty acids, high-quality protein, minerals (calcium, magnesium, potassium, selenium and zinc) and the primary source of certain BAI that protect and promote health (polar lipids, proteins and peptides, oligosaccharides, live and active cultures of microorganisms in fermented milk products). To address the challenge of lactose intolerance in milk and digestive problems in children/adults that lead to reduced milk consumption, the most appropriate alternatives are lactose-free or lactose-reduced milk products (the most accessible and affordable source of essential nutrients).

Following more modern trends, there is an increase in interest in dairy products fortified with plant PE and plant-based antioxidants, necessary for the neutralization and removal of free radicals, which are constantly generated in the human body. They can damage vitally important molecules and predispose to the occurrence of chronic non-infectious diseases (cardiovascular diseases, diabetes, and cancer). The goals of food fortification are: improving its chemical and nutritional quality and techno-functional properties, increasing the content of essential micronutrients in food, and providing health benefits, with minimal risk or completely no consequences. Fortification of cheeses with dietary herbs, spices, and PE may lead to AM, AO, antimutagenic, anti-inflammatory, and immunomodulatory effects, and contribute to better public health (Picciotti et al., 2021; Kurćubić et al., 2015; Ritota and Manzi, 2020; Kurćubić et al., 2023a; Comerford et al., 2024; Kurćubić et al., 2024; Wazzan, 2024; Ferreira et al., 2025).

The food market is abundant in fortified dairy products (42.9%) (Watson et al., 2006), as they are the most famous carriers of functional BAI and the most widespread nutraceutical foods. Dairy products are of such a composition that they provide a simple way to incorporate the fortifiers into these matrices, and are therefore significantly represented in many diets (Choi et al., 2006). In order not to reduce the acceptability and palatability of the final product by consumers, one of the most important guidelines is that cheese fortification must not degrade the sensory and physicochemical properties of the cheeses (Lacroix et al., 2013). The incorporation of BAI gives cheeses numerous nutritional advantages, as they are a

suitable means for their transport (Kumar, 2015). During ripening and aging, more attention must be paid to the preservation and protection of the activity of BAI in the matrix.

A large number of studies on natural herbal supplements for the fortification of cheeses and other dairy products are available, aligned with current trends, cost-effective principles and circular economy, and in environmental sustainability and human well-being (“safer and greener alternatives”, which can be easily purchased and incorporated directly to the milk, cheese curd, or final product) (Ansari and Kumar, 2012; El-Aziz et al., 2012; Dwyer et al., 2015; Bukvicki et al., 2018; El-Sayed and Youssef, 2019; Dupas et al., 2020; Ritota and Manzi, 2020; Kumar Paswan et al., 2021; Muñoz-Tebar et al., 2021; El-Loly et al., 2022; Difonzo et al., 2023; El-Aziz et al., 2023; Kandyliari et al., 2023; Silva et al., 2023; Ferreira et al., 2024; Tarchi et al., 2024; Fernandes et al., 2025; Maddaloni et al., 2025).

Aim of our review is to consider the possibilities and potential for further, future research: a) optimization of extraction procedures; b) more precisely determining the concentrations required to achieve the desired health benefits; c) the impact of fortification on the absorption of macronutrients present in cheese (proteins, calcium); d) testing solubility and possible interactions with other milk constituents; e) achieving stability and functionality of cheeses during processing and storage; f) the potential for fortification while preserving the sensory quality of the final product (Wazzan, 2024).

Biopreservation strategy: the use of herbs and spices like source of plant-derived BAI to prevent the growth of pathogenic microorganisms in cheese and improve his functional and nutritional properties

Preparation of herbal supplements for use in food - basic postulates

Fortification of cheeses can be achieved by incorporating plants/spices into the milk before cheesemaking, adding them to curdled cheese, or rolling the cheese in chopped herbs (Dupas et al., 2020). PE as food additives are made from a large number of different plants, using different methodologies and solvents (non-toxic, approved for the industrial production of food ingredients and food products) (EU, Directive 2009/32/EC). To achieve uniform quality of PE, it is necessary to take into account the fact that variations in their chemical composition are contributed by plant genotype, geographical location, environmental conditions and agronomic conditions (Martillanes et al., 2017). The extraction method, temperature, solvent

and pressure affect the chemical composition of the prepared PE, so it is necessary to choose the optimal extraction parameters to obtain the desired compounds and their bioactivity (Sasidharan et al., 2010; Ferreira-Santos et al., 2020). Today, modern extraction techniques are used, which include subcritical water extraction, supercritical fluid extraction, enzyme-assisted extraction, microwave and ultrasound, pulsed electric field and accelerated solvent extraction. Conventional extraction methods (maceration, percolation, infusion, decoction, reflux extraction, Soxhlet extraction and hydrodistillation (steam and water distillation or their combination) are still successfully used (Azwanida, 2015; Zhang et al., 2018; Pisoschi et al., 2018; Cheng et al., 2021). Today, it is top priority to take into account the environmental impact of extraction methods while favoring more sustainable and “greener” processes that optimize the recovery of high-value BAI, minimizing the amount of solvents used and the resulting waste (Ferreira-Santos et al., 2020).

Application of PE as a AM preservative in cheeses

Contamination with various pathogenic bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* species) is a critical point in cheese production, since cheese (especially in artisanal processing) is produced from unpasteurized milk. The unique and consumer-attractive sensory characteristics of artisanal raw milk cheeses are the result of the use of unpasteurized milk. Pasteurization reduces bacterial load and extends the shelf life of milk, but denatures whey proteins and leads to complex interactions with casein micelles, fat globules, and minerals (Singh and Waungana, 2001). Above mentioned bacteria can be treated as a significant health risk, but by producing enzymes that degrade proteins and lipids, they also seriously affect the physicochemical properties of cheese (texture, taste and aroma). The general public is concerned about the production of thermostable enterotoxins by *Staphylococcus aureus* (*S. aureus*), which persist even after cheese ripening, leading to spoilage and potential foodborne diseases (Johler et al., 2018; Oliveira et al., 2022). Controlling the presence of the above-mentioned bacteria in cheeses is essential for preserving their safety and quality (Kyrykbaeva et al., 2025).

Preservatives are food additives that extend the shelf life of food by protecting it from spoilage caused by microorganisms (most commonly as AM and antifungal agents, fungistatic agents, and agents for preventing exponential multiplication of bacteriophages). Waste from the processing of plant raw materials (by-products like grape skins and seeds, grape flour and seed extracts, pineapple peel, pomegranate seeds) have been shown to exhibit strong AM activity, without

detrimental effects on bacterial starter cultures and endogenous bacterial strains, not incorporated into the starter culture (Costa et al., 2018).

The results of the study by Bermudez-Beltrán et al. (2020) showed that the design of Petit Suisse cheese from gooseberries, incorporated into moringa leaf powder and gelatin, created an innovative dairy product (cheese) with desirable physicochemical, microbiological, and bromatological quality. Petit Suisse can become more sensorially acceptable and desirable after the addition of gelatin, which is a significant improvement, especially when considering its specificities (fresh and smooth cheese) and already good acceptance by consumers, especially children but also other age categories. Enrichment with moringa leaf powder reveals higher values for ash, protein, fiber and fat content, i.e. better, improved nutritional value of the fortified cheese.

The aim of the study of Kurćubić et al. (2015) was to assess the impact of cheese enrichment with polyphenols from ethanolic PE of the *Kitaibelia vitifolia* (Pirotski kachkaval) applied by adding to the cheese lump after texturization or by spraying on the surface of the cheese. The number of colonies of aerobic bacteria, coagulase-positive staphylococci, *S. aureus* and anaerobic sulfite-reducing bacteria did not differ significantly ($p > 0.05$) between the control and experimental group samples, as confirmed by microbiological testing. The AO activity of conventional and enriched cheese was assessed using 5 modern methods and revealed a slight increase associated with phenols in enriched cheese samples compared to control samples. Enriched cheese samples showed better sensory quality compared to control one, as assessed by a five-member expert panel. Statistically significant ($p < 0.05$) changes were observed in moisture content and total solids of the control and modified cheese batches, but other parameters did not differ significantly ($p > 0.05$).

The abundance of phenols, dietary fiber (DF) and high AO capacity of the fruit of the *Arbutus unedo* L. plant interested Masmoudi et al. (2020) to evaluate the effect of enriching soft cheese “Sardaigne” with an PE obtained by applying an aqueous decoction. LC-MS analysis of the PE revealed quinic acid and catechin as the dominant phenolic compounds. DPPH radical activity detected an IC_{50} value at the level of ~ 0.31 mg/mL. Fortified cheese showed a higher crude protein content and higher firmness. Fortification at an amount of 0.3 g/L of PE increases cheese yield, improves (after 5 days of storage) DPPH radical scavenging activity, without changing color and sensory properties, which qualifies it as a useful functional ingredient, with potential health benefits.

Solhi et al. (2020) reported recently that asparagus powder (AP) can be a quality source of BAI for the enrichment of processed cheeses (it reduces pH and lipolysis indices, and increases phenolic content, AO power and proteolysis of processed cheeses). The evidence was revealed by investigating the qualitative

properties of enriched processed cheese (PC) with different levels (0.5%, 1% and 1.5% w/w) during the storage period. Technologically, AP contributed to the structure of PC being more elastic and rigidity, with reduced spreadability compared to the control sample (determined by dynamic oscillatory rheometry).

One of the most recent pieces of evidence is that hops represent a promising herbal natural supplement for improving both the microbiological and physicochemical characteristics of cheese, as hop extract effectively inhibits the pathogenic bacteria *S. aureus* and *Listeria monocytogenes* (*L. monocytogenes*) (Kolenc et al., 2022; Plaskova and Mlcek, 2023). Studies are constantly being deepened, considering the balance of antimicrobial (AM) effectiveness and efficiency with sensory acceptability, by examining the interactions of hop extracts with cheese matrix components (lipids and proteins), optimal doses and methods of application, and the impact on sensory properties, texture, and overall quality (Betancur et al., 2023). Hop extract effectively reduced bacterial growth, especially *S. aureus*, while improving the physicochemical properties of cheese (firmness and texture) during a 90-day ripening period. Future trials of the application of hop extract in commercial cheese production are planned to reveal the practical viability and parameters of economic success. In cheeses treated with hop extract, a significant increase in AO activity was demonstrated by the DPPH test. The improved AO profile, along with the high phenolic contribution of hop extract, highlights its candidacy as a functional additive for improving the nutritional value and shelf life of cheese (Kyrykbaeva et al., 2025).

Since we stated in the Abstract that one of the most important sources of contamination is the environment where milk is obtained, we cite the significant results of El-sawy et al. (2024), who, in their research, determined that four tested ethanol extracts from medicinal plants (cinnamon bark, chamomile flower, calendula flower and sage leaf) exhibit antibacterial and anti-biofilm efficacy (against gram-positive and gram-negative food-borne pathogenic bacteria). Mixtures of the tested low concentrations of extracts exhibited synergy (more effectively inhibited biofilm than each of the extracts individually). In an *in vitro* safety study of pasteurized milk and white soft cheese, the PE cocktail used to control *L. monocytogenes* ATCC7646 biofilms on stainless steel surfaces significantly improved the safety and quality of the cheese produced and achieved a significant reduction in *L. monocytogenes* counts without compromising sensory quality and without showing toxicity (even during cheese storage at cold chain temperatures). From all of the above, it can be concluded that the tested extracts are a quality alternative for mitigating *L. monocytogenes* infections.

As we mentioned in the Introduction chapter, the valorization of agricultural by-products is of great importance, and a good example is the most recent research by Ferreira et al. (2025) on onion peels (OP), a major agricultural

by-product that negatively impacts the environment. The study presents sustainable innovations in food - the development of foods (cream cheeses) enriched with natural compounds that improve their nutritional value and shelf life. The strategy of waste management and reducing their impact on the environment is recognizable. As a solution to the above challenge, phenolic compounds from onion peels, which exhibit AO power, are extracted and added to food, while extending their stability through the microencapsulation process. The extract was found to exhibit antidiabetic effects (significantly inhibits α -amylase and β -glucosidase activities, at levels of 91.9% and 95.8%). Cheese samples enriched with the extract have improved technological properties (excellent water retention and reduced syneresis). A comparison of the effects of encapsulated and free extract indicates the favorable effects of microencapsulation in maintaining stable total phenol content and AO capacity, i.e. the best oxidative stability (controlled release of the extract from microparticles). The incorporation of organic enhancers (OP) in microencapsulated extracts can be a winning combination for prolonging the shelf life and stability of fortified foods.

Muñoz-Tébar et al. (2019) investigated whether oil extracted from the seeds of *Salvia hispanica* L. could be a sustainable source of omega-3 fatty acids for the fortification of sheep cheese. The oil was prepared in two concentrations (3 and 5 g/L) as Ca-caseinate-stabilized emulsions, incorporated into pressed sheep cheese, and comprehensive quality were determined during a 32-day ripening period. Sheep milk coagulation was not degraded by fortification, the ripening process was preserved, with improved fat, dry matter, and α -linolenic acid content. Cheeses enriched with chia oil showed good sensory acceptance by panelists. Fortification positively contributed to the physicochemical quality of the cheeses.

Farrag et al. (2017) investigated the application of hibiscus soft drink on the characteristics and quality of white soft cheese, with AM and hypertensive treatment by adding hibiscus soft drink (prepared by soaking and blanching, at 0, 2, 4, 6, 8 and 10%). Total solids, protein, fat and pH values decreased with increasing hibiscus PE addition. Cheese hardness was reduced, as were gumminess and chewiness, in cheeses where the soft drink was added by soaking, and were even lower in cheese enriched by blanching. Cheese color parameters (L^* , a^* , b^* ,) were higher in cheese samples enriched with blanched hibiscus soft drink compared to those enriched by soaking. Anthocyanin values (polymer color, color density, tannin contribution and total anthocyanin concentration) were lower in blanched hibiscus cheese samples than in other types of cheese. Color was rated as acceptable for those cheese samples that contained up to 6% of soaked or blanched hibiscus soft drink. Cheese with more hibiscus was unacceptable to the evaluators. Total counts of filaments, yeasts and molds were lower in blanched hibiscus cheese samples than in soaked hibiscus cheese, compared to control cheese samples.

Therefore, the application of hibiscus addition as an AM agent and in the treatment of hypertension in the consumption of fortified white soft cheese can be analyzed commercially, for the benefit of producers and consumers.

Pomegranate peel and grape seeds, as vegetable by-products from the Agri-Food sector, are proven effective natural preservatives against *L. monocytogenes*, *S. aureus* and *Salmonella enterica* in cheese (Jayathilakan et al., 2012). Their polyphenols have AM effects - changes that lead to bacterial death are achieved by penetrating the cell walls with membrane rupture, damaging proteins and membrane enzymes (Cowan -M.-M. 1999; Daglia, 2012).

The study by Kandyliari et al. (2023) focused on the analysis of dairy products fortified with aqueous PE from plant-based by-products (*Citrus aurantium* peel, *Citrus limon* peel and *Rosa canina* seeds) and herbs (*Sideritis* spp., *Hypericum perforatum*, *Origanum dictamnus*, *Mentha pulegium* L., *Melissa officinallis*, *Mentha spicata* L. and *Lavandula angustifolia*). Fortified cream cheese, kefir, yoghurt and vegan yoghurt were tested for AO content, phenolic profile and sensory characteristics. Fortification of cream cheese with St. John's wort extract contributed to higher antioxidant and phenolic content (0.53 ± 0.16 mmol Fe^{2+} /L and 56.36 ± 7.13 mg GAE/g, respectively) than when fortified with BAI from other prepared and tested plant materials. AO capacity and phenolic content also differed statistically significantly ($p < 0.05$). Overall acceptability ranged from 4.0 ± 1.1 for the control group to 2.6 ± 1.0 (the lowest value, for cream cheese enriched with mountain tea extract). A statistically significant difference ($p < 0.05$) was found between the samples for texture, taste and overall acceptability, but not for color and aroma ($p > 0.05$). The use of phytocomplex cocktails from PE originating from different plants leads to higher bioavailability of BAI (Malongane et al., 2017). In line with the findings of this study, in study of Kandyliari et al. (2023), the cocktail of plants and/or processing by-products also showed higher concentrations of antioxidants and total phenols before and after *in vitro* digestion.

Mahajan et al. (2016) investigated and concluded that aqueous extracts of pine needles (*Cedrus deodara* (Roxb.) Loud.) improve the oxidative stability of lipids in low-fat *Kalari* cheese, a typical Indian hard and dry cheese, as well as the microbial load, due to the AO and AM properties of pine needle phytochemicals.

Ansari and Kumar (2012) in their review report on cheese as a quality and suitable medium for the delivery of oil-soluble natural colorants and phytochemicals (carotenoids, bixin, lutein, omega 3-fatty acids, docosahexanoic acid and eicosapentanoic acid).

Possibilities for improving the sensory properties of cheese with natural herbal additives

The addition of plant extracts to dairy products must result in products that are accepted by consumers. Therefore, attention must be paid to the sensory evaluation of the quality of the fortified products. Attention must be given to the types of plants from which the extracts are derived, the concentrations of the applied extracts, as well as the stages at which the extracts are incorporated during the cheese production process (Ritota and Manzi 2020).

The concentration of added green tea aqueous extract in cottage cheese plays an important role in its sensory acceptability. Panelists noted that concentrations of 8% and 9% resulted in a pleasant cheese taste and flavor, while concentrations above 10% led to a bitter taste, which became more pronounced with increasing extract concentration (Evstigneeva et al., 2016). Foda et al. (2010) investigated the sensory properties of white cheese enriched with spearmint essential oil. The best results were observed in cheeses fortified with spearmint essential oil at concentrations of 0.5, 0.75, and 1.0 mL/kg retentate, respectively. On the other hand, cheeses with higher concentrations of spearmint essential oil (1.5, 2.0, and 2.5 mL/kg of retentate) received significantly lower scores from the panelists. According to the study by Tarchi et al. (2024) the addition of varying concentrations of olive leaf extract to Cantal cheese resulted in noticeable color changes. These changes were directly dependent on the concentration of the added extract, leading to alterations in the L^* , a^* , and b^* values. The fortified cheese appeared darker, redder, and more yellow compared to the control sample. The hardness and cohesiveness of Cantal cheese increased significantly ($p < 0.05$) after 28 days of storage, in correlation with the increasing concentration of olive leaf extract. On the other hand, in the study conducted by Licón et al. (2012), the addition of saffron to ewe milk cheese resulted in a darker, less red, and more yellow product. The tendency toward a yellow tone became especially pronounced with increasing concentrations of added saffron.

One of the most significant impacts of PE on the sensory attributes of fortified cheeses is the color change, which most commonly tends to shift slightly or more noticeably toward a green tone (Setyawardani et al., 2023). Such changes were observed in study by Miwada et al. (2023) samples containing up to 4% moringa leaf extract were considered acceptable, showing no significant differences compared to the control, whereas those containing 6% and 8% were rated as undesirable by the sensory panel.

The use of agricultural by-products, which represent an excellent source of bioactive compounds with beneficial effects on human health, plays a significant role in the sustainable development of agriculture when applied in cheese

production (Difonzo et al., 2023). In a study of white soft cheese enriched with varying concentrations of celery leaves (5%, 10%, and 15%), Awda et al. (2019) reported improved flavor and greater overall acceptability for cheeses containing 5% and 10% celery leaves. The addition of *Allium roseum* paste and powder to double cream cheese led to an improvement in sensory properties, particularly in samples containing 6% paste and those with 0.8% powder (Gliguem et al., 2019). Barbaccia et al. (2021) investigated the properties of stretched sheep cheese with the addition of 1% (w/w) grape pomace. The results indicate that, in addition to a high polyphenol content in the cheese, there was a positive impact on sensory attributes, with the biggest impact observed in odour and aroma.

The future of cheese fortification and enrichment with additives of plant origin: how to make a successful and sustainable step forward?

The purpose of this review was to consider the AM and AO activity of natural molecules of plant origin applied in cheese production, as well as their impact on the overall quality of cheese (chemical, nutritional and sensory characteristics of the product, as well as extending the shelf life of cheese). Herbs and spices are multifunctional in the dairy sector and cheese manufacturing as antioxidants, AM agents and flavorings, enhancing the attractiveness of fortified foods. Food production is extremely complex because it affects technological parameters, economic, environmental, and health aspects. Fortification of food makes it healthier/functional, "preventive" or "therapeutic", and address various nutritional deficiencies and disorders. The interaction of the food matrix with the AM mechanism of natural additives requires further knowledge because they can reduce the AM effect. The dose in which PE or other plant-based additives are incorporated into the cheese matrix is also important. The price of natural preservatives is often high, which discourages food manufacturers from using them in the production process, so it must be reasonable compared to synthetic compounds that have the same or stronger effect. Also, the legislation on natural additives is often limited and confusing, and government approvals are required for their use in food. Definitely, additional studies of plant-based ingredients in cheese production processes would be valuable, especially *in vivo* studies, which are otherwise scarce.

Herbs and spices can be used as substitutes for less desirable ingredients, and have the potential to be significant in the dairy sector as partial or complete replacements for salt, one of the main ingredients of various cheeses. Salt adds flavor to cheese, helps in drying the curd, is necessary for the development of a

good rind and has an AM effect. Milk and dairy products are among the most consumed foods by adults in Europe, with a high average cheese consumption of 34.2 g/day. Reducing salt intake to less than 5 g/day (as recommended by the WHO), represents an important challenge to reduce the development of diseases, such as hypertension and cardiovascular diseases, associated with excessive salt consumption. There is no research on partial or complete replacement of salt with natural preservatives, and they should be encouraged.

The results of the research provide valuable information on the strategic potential of using PE and agricultural by-product extracts to enrich a wide range of dairy products. This strategy is a key step in creating innovative, consumer-oriented, dairy products that are in line with the evolving preferences of health-conscious consumers, with environmental benefits through the reduction of by-products (waste) from the agricultural and food industries. The use of by-products from the processing of plant raw materials promotes a circular economy, along with eco-friendly sustainable production of natural food supplements and healthier, fortified foods. Further research into sensory properties is necessary, as their optimization is of paramount importance for consumer acceptance of cheeses and other dairy products.

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Conflict of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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