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## EFFECTS OF NATURAL BIOACTIVE COMPOUNDS ON MICROBIAL SAFETY AND QUALITY OF DAIRY PRODUCTS

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**Abstract.** Dairy products are susceptible to contamination by foodborne pathogenic and spoilage microorganisms, which can result to a reduced shelf life of products as well as risks to the consumers' health. This determines the possible use of preservatives in the manufacturing process of dairy products. Consumers require healthy food, free of synthetic preservatives, looking for natural alternatives to ensure food safety. Just for this reason, natural ingredients are receiving increasing attention as substitutes for synthetic additives. Currently, research is focusing on the identification of natural antimicrobial agents, especially from plants such as fruits, vegetables, herbs and spices, as they contain significant amounts of compounds with antimicrobial activity. In addition, plants contain bioactive compounds, which could provide health benefits in preventing many diseases. This review aims to discuss the impact of natural antimicrobials on foodborne pathogenic and spoilage microorganisms in products, the antimicrobial efficacy of plant extracts and essential oils and the impact of their incorporation on the sensory characteristics of dairy products such as yogurts, cheeses, butter and ghee.

**Keywords:** *spoilage, foodborne pathogen, natural antimicrobial, plant extracts, essential oil, shelf-life.*

### Introduction

Food safety is one of the main problems in the food industry, and there is always concern about the emergence of foodborne diseases among food producers, inspectors, researchers and consumers. The International Conference on Food Safety held in Addis Ababa in February 2019, and the International Forum on Food Safety and Trade held in Geneva in 2019, reiterated the importance of food safety in achieving the Sustainable Development Goals [1].

Besides contributing to food and nutrition security, a safe food supply also supports national economies, trade and tourism, and stimulates sustainable development. As the world's population grows, the intensification and industrialization of agriculture and animal production to meet increasing demand for food creates both opportunities and challenges for food safety [2]. These challenges place great responsibility on food industry producers to ensure food safety. Over the last decade, serious outbreaks of foodborne illness have emerged. Unsafe food poses a threat to global health, endangering the entire population.

Infants, young children, pregnant women, the elderly and those with a underlying disease are particularly vulnerable. Every year, 220 million children suffer from diarrheal diseases and 96000 die [1].

The situation regarding acute diarrheal diseases in the Republic of Moldova is also considered unfavorable. About 20000 cases of the disease are registered annually, including 10-20 deaths in children under the age of 5. In the structure of morbidity predominate children aged 0 - 17 years (60 - 75%) and in the urban population, it is 1,9 times higher compared to the rural population. The morbidity index due to acute diarrheal diseases in 2017 is 464,4 per 100 thousand population and are increasing compared to 2016 by 9,4% [3].

The main cause of acute diarrheal diseases and food poisoning is the use of food contaminated with microbial pathogens, toxins or radioactive components. Foodborne pathogens (*Clostridium botulinum*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Bacillus cereus*, *Listeria monocytogenes*, *Cryptosporidium*, *Escherichia coli* O157: H7 etc.) are the main food safety concerns [4].

Therefore, the production of safe food is one of the most important priorities in the food industry. Various methods, including heat processing, decreased water activity, various packaging methods, irradiation, high pressure, high intensity pulsed electric field processing and the addition of preservatives have been used to produce safe food [5].

The addition of GRAS preservatives is one of the most widely used methods of ensuring food safety. Among the most used synthetic preservatives are nitrates, benzoates, sulfites and sorbates which, despite the benefits to food, such as safety and quality of products, pose risks to consumers due to the side effects that consumption can cause [6].

Many synthetic preservatives, such as butylhydroxyanizole (BHA) and butylhydroxytoluene (BHT) have been used successfully to prevent food spoilage caused by lipid oxidation. In contrast, synthetic compounds have significant disadvantages, such as the risks of handling and the increase of chemical residues left in food. In addition, these preservatives could have negative consequences on consumers'health, with possible carcinogenic effects. Therefore, the food industry and research institutions are concerned with identifying natural preservatives as an alternative to synthetic ones, in order to meet consumer requirements for safe and healthy foods [7].

### Dairy spoilage microorganisms

Globally, food spoilage caused by microorganisms still widely affects all types of food and causes food waste and loss, even in developed countries. It has been estimated that annual food losses worldwide reach up to 40% due to various factors, including food spoilage under the action of microorganisms [8].

Table 1

#### Key groups of spoilage bacteria known to contaminate pasteurized milk and their characteristics

Group	Examples of genera/species	Overview of key phenotypic characteristics relevant to fluid milk spoilage
<i>Pseudomonas</i>	<i>P. fluorescens</i> <i>P. fragi</i> <i>P. lundensis</i> <i>P. putida</i>	- many strains can grow at 4°C and below; - many strains can produce extracellular enzymes (e.g., proteases and lipases); - some strains produce pigmented siderophores (e.g., pyoverdin).

Continuation Table 1

Coliforms	<i>Enterobacter</i>	
	<i>Klebsiella</i>	- many strains can grow at 4°C and below;
	<i>Citrobacter</i>	- many strains can produce extracellular enzymes (e.g., proteases and lipases).
	<i>Hafnia</i> <i>Serratia</i>	
Non-coliform, non- <i>Pseudomonas</i> gram-negatives	<i>Aeromonas</i>	
	<i>Flavobacterium</i>	- many strains can grow at 4°C and below;
	<i>Acinetobacter</i>	- many strains can produce extracellular enzymes (e.g., proteases and lipases).
	Non-coliform <i>Enterobacteriaceae</i>	
Gram-positives sporeformers	<i>Bacillus cereus</i>	- some <i>B. cereus</i> group lineages (e.g., <i>B. weihenstephanensis</i> ) and several <i>Paenibacillus</i> strains can grow at temperatures as low as at least 6°C;
		- some strains produce extracellular enzymes (e.g., proteases and lipases).

Alteration of milk is primarily due to the increase in the population of psychrotrophic microorganisms that trigger the lipolysis reactions of fatty acids and proteolysis reactions of milk proteins, even at a temperature of 0°C (the optimal development temperature is 20...30°C) [9]. Lipolysis of milk lipids leads to the accumulation of free fatty acids, short-chain glycerides and to the appearance of unwanted flavoring of milk and other dairy products.

Quality problems and defects associated with the presence of lipolysis in dairy products can be described as rancid, butyric, bitter, soapy and astringent. Once lipolysis produces detectable flavors, it is not possible to remove them from the product [5]. In addition, the hydrolysis of milk proteins under the action of proteases produced by *Pseudomonade*, *Aeromonade*, *Serratia* and *Bacillus spp.* leads to the formation of bitter peptides and amino acids, to the gelation and coagulation of milk [10, 11, 12, 13].

Alteration of milk is mediated by lipases that are naturally present in milk as well as lipases secreted by psychrotrophic bacteria that can contaminate milk during milking, storage and transport [12, 14]. One of the most important properties of these bacterial enzymes is thermostability [10, 15].

Therefore, it is important to develop good practices and strategies to reduce the risks of milk contamination before pasteurization, as the action of residual enzymes during storage will shorten the shelf life of the milk [10].

When making cheese and butter, flavor defects caused by lipolysis may occur before or after manufacture, while yogurt is less susceptible to lipolysis defects due to technological factors such as low pH, low storage temperature and short shelf life [11, 16].

Although various applications of herbs and spices are being studied to capitalize on them as new food ingredients, there is a lack of information on the effect of adding these extracts on the lipolysis or proteolysis process in dairy products.

This should be considered, as some ingredients, such as pepper, promote lipase activity in cheese, leading to the accumulation of fatty acids and the appearance of rancid flavor [5].

### Foodborne pathogens in dairy products

Farm animals represent a major source of pathogens that can be transferred milk [17, 18]. *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157: H7 and *Campylobacter* are the most common potential pathogens associated with milk or dairy products in industrialized countries [19, 20]. Foodborne bacteria can contaminate dairy products at any point along the production chain: milking, storage or packaging [21].

Reports from developed countries indicated that milk and dairy products are implicated in 1...6% of the total bacterial foodborne outbreaks [22], with 39,1% attributed to milk, 53,1% to cheeses and 7,8 % to other dairy products [23].

The severity of food poisoning caused by the consumption of raw milk products depends on several factors, such as the pathogenicity of the bacterial strain, the number of ingested microorganisms, the physiological state of the microorganism and the health of the consumer at the moment of ingestion [24].

Heat treatment of milk (pasteurization or UHT treatment) aims to inactivate pathogenic bacteria. However, inadequate pasteurization or post-pasteurization contamination can lead to recontamination of milk if hygiene measures are not followed at the farm, leading to incidences of food poisoning [25]. Outbreaks of foodborne illness have been mainly linked to the consumption of raw milk or unpasteurised milk products, such as raw milk cheeses, whose consumption is constantly increasing due to a growing demand for natural and unprocessed foods [26, 27].

The number of outbreaks of fresh dairy poisoning due to contamination with pathogens is less common, although some cases have been reported for yogurt and fermented milk. In these products, the acidity of the matrix acts as a barrier in the growth of pathogenic bacteria. However, milk must be pasteurized because some pathogens, such as *E. coli* O157: H7, can also grow in acidic environments [4].

Outbreaks caused by the consumption cheese made from unpasteurized milk are often caused by: *Salmonella* (34%), *Campylobacter* (26%), *Brucella* (13%), *Escherichia coli* (11%) and *Shiga toxin Escherichia coli* (11%) [28]. *Listeria monocytogenes* is inactivated by pasteurization, however worldwide *Listeria monocytogenes* has been implicated in numerous outbreaks caused by the consumption of contaminated milk and dairy products [29, 30]. In 2015, dairy products were identified as the main sources of listeriosis [31, 32]. Raw milk can be contaminated with *Listeria monocytogenes* from unhygienic equipment during milking, storage or transportation to processing enterprises where hygienic control measures are not followed [33]. Due to its high mortality rate, listeriosis is, after salmonellosis, the second most frequent cause of foodborne infection-related deaths in Europe [17, 29]. However, outbreaks from *Listeria monocytogenes* are not common compared with those caused by pathogens such as *Salmonella* [34, 35].

*Staphylococcus aureus* can multiply in milk, especially if its storage temperature is higher than 10°C. *Staphylococcus aureus* can develop staphylococcal enterotoxins in milk, which are responsible for staphylococcal food poisoning [36, 37]. This bacterium is commonly found in a wide variety of mammals and birds and can be transferred to food mainly by dairy animals that have mastitis and by human carriers during food processing [38]. *Staphylococcus aureus* can have a broad occurrence in raw dairy products, with frequencies between 5 and 100% in cheeses [24]. The number of *Staphylococcus aureus* in raw milk or raw milk cheeses needs to be less than 10<sup>4</sup> cfu/g, according to national

regulations [39]. Foods of high protein content such as milk and dairy products, meat, meat products and bakery products favor the growth of bacteria, and these types of foods have been frequently incriminated in outbreaks of *Staphylococcus aureus* [40].

*Staphylococcus aureus* is present in raw materials and food and can be inactivated with heat treatment, but enterotoxins are heat-resistant. In the dairy industry, the following milk pasteurisation regimes are used: 72°C for 15 seconds or 63°C for 30 minutes, to ensure inactivation of bacteria and toxins [10].

*Salmonella* spp. is the most frequent cause of foodborne outbreaks, and human salmonellosis is the second most frequently reported zoonosis in the European Union [41]. Milk is a food with a high chance of contamination by *Salmonella* spp. [42], mainly before leaving the farm, usually due to fecal contamination during the milking process [22]. Additionally, *Salmonella* spp. can be transmitted to humans through the consumption of contaminated dairy products [41], especially unpasteurized or insufficiently pasteurized milk and cheese [22]. The main source of Shiga toxin-producing *Escherichia coli* are ruminants, contaminating milk through subclinical mastitis or feces, and bacteria can persist in milking equipment [17].

One of the most important tasks of the food industry in the field of food safety is to eliminate or at least reduce contamination with foodborne pathogens [43].

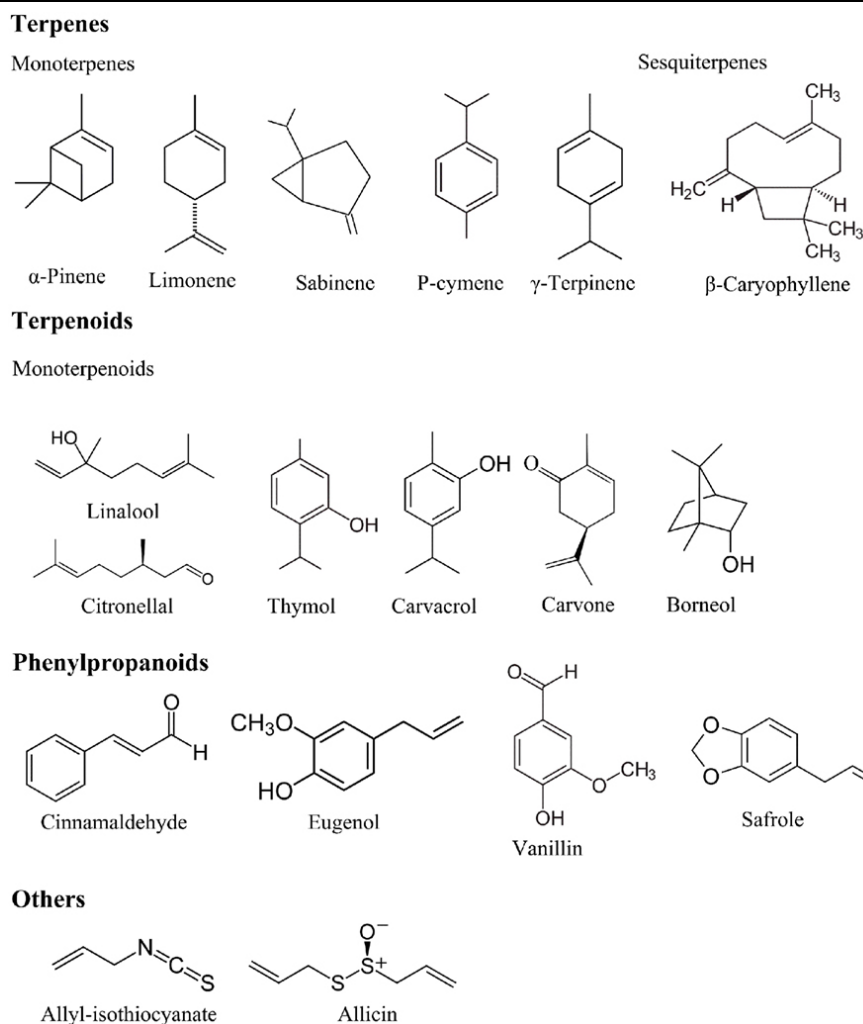
### **Antimicrobial agents and the safety of dairy products**

Food preservatives are food additives that extend the shelf life of food by protecting against damage caused by microorganisms. Different types of preservatives include antimicrobial agents, antifungals, synergistic antimicrobial agents, bacteriophage control agents and fungistatic agents [44]. Currently, research focuses on the identification of natural antimicrobial agents, especially from plants [45] such as fruits, vegetables, herbs and spices [46, 47, 48, 49, 50], because they contain significant amounts of compounds with antimicrobial activity.

As shown by *in vitro* experiments, plants produce secondary metabolites that have high antimicrobial activity [46, 51]. Secondary antimicrobial metabolites of herbs are generally classified into three major classes: phenolic compounds, terpenes and alkaloids (Figure 1). Numerous studies have shown that the antimicrobial activity of herbs is determined by the potential of secondary metabolites to:

- promote cell wall disruption and lysis;
- induce the production of reactive oxygen;
- inhibit biofilm formation;
- inhibit the construction of the cell wall;
- inhibit microbial DNA replication;
- inhibit energy synthesis;
- inhibit bacterial toxins to the host [52, 53,54, 55, 56].

Additionally, these compounds can prevent antibacterial resistance, as well as synergists to antibiotics, which can eventually destroy pathogenic microorganisms [51]. In the Republic of Moldova there is a vast and historical experience in the aromatic and medicinal plant industry. Being an agricultural country with favorable pedoclimatic conditions, the Republic of Moldova has an enormous potential in the cultivation, processing and marketing of aromatic and medicinal plants, which is currently not fully explored [57].



**Figure 1.** The chemical structure of antibacterial bioactive compounds in herbs [56].

### Potential application of herbs in dairy products

Table 2 selects the articles referring to the antimicrobial activity of aromatic plants applied in the production of dairy products.

Hanifah et al. (2016) added *L. acidophilus* and roselle extract in the manufacture of goat milk based-yoghurt. These yoghurt was characterized by increased antimicrobial activity and extensive selectivity for Gram positive and Gram negative bacteria (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella Typhi*), which might be attributed to the production of higher antimicrobial compounds, including antimicrobial peptides and organic acid [58]. Also, Ghalem and Zouaoui (2013) supplemented yoghurt with *Rosmarinus officinalis* oil at ratio of 0,14; 0,21; 0,29 and 0,36 g/L and kept it up to 21 days. Panelists gave the maximum score for taste, flavor, and texture, to the herbal yoghurt supplemented with 0,14 g/L of essential oil. Addition of *R. officinalis* essential oil improved the qualities of yoghurt by decreasing pH, lactose values and dry matter but raised the titratable acidity, proteins, ash and fat contents [59].

El-Sayed et al. (2017) reported that the supplementation of Labneh with different ratios (100, 150 and 200 mg/ml) of *Moringa oleifera* oil increased the total solid, fat, total volatile fatty acid, DPPH scavenging activity, tocopherols and total lactic acid bacterial counts content of labneh.

The *M. oleifera* oil displayed a great effect against Gram-positive, Gram-negative, yeast and fungal strains with the increase of the ratios of *M. oleifera* oil [60].

Table 2

## Selected publications on the antimicrobial activity plantes in dairy products

Type of dairy products	Type of herbs and spices	Microorganisme type	Reference
<b>Yoghurt</b>			
Goat milk based-yoghurt	Roselle ( <i>Hibiscus sabdariffa</i> L) extract	<i>Bacillus cereus</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> and <i>Salmonella Typhi</i>	[58]
Yoghurt	<i>Rosmarinus officinalis</i> oil (0.14, 0.21, 0.29, and 0.36 g/L)	-	[59]
Labneh	<i>Moringa oleifera</i> oil (100, 150 and 200 mg/ml)	<i>M. oleifera</i> oil displayed well effect against Gram-positive, Gram-negative, yeast and fungal	[60]
<b>Cheese</b>			
Fresh cow Cheese	<i>Satureja hortensis</i> L. (dry plant (0.5...1.5%), alcoholic extract and essential oil (0.1%, 0.25%, 0.5%))	<i>Staphylococcus aureus</i> , total number of germs	[61]
Fresh sheep cheese	<i>T. vulgaris</i> L. and <i>Origanum vulgare</i> L. essential oil (4 and 8 µL/mL)	<i>S. aureus</i>	[62]
Wagashi	<i>Ocimum gratissimum</i> (200, 400, 600, 800, 1000 mg/L)	<i>Aspergillus flavus</i> , <i>Aspergillus tamarii</i> , <i>Fusarium poae</i> , <i>Fusarium verticillioides</i> , <i>Penicillium citrinum</i> and <i>Penicillium griseofulvum</i>	[63]
Domiaty cheese	<i>Nigella sativa</i> oil (1% and 0.2% (w/w))	<i>Staphylococcus aureus</i> , <i>Salmonella enteritidis</i> , <i>Escherichia coli</i> and <i>Listeria monocytogenes</i>	[64]
<b>Butter and ghee</b>			
Ghee	Dry clove and rosemary	-	[65]
Butter	Sage or rosemary (0,2%)	-	[66]
Ghee manufactured by creamery buffalo butter	Arjuna alcoholic extract (7%)	-	[67]

Results of studies conducted by Erselia et al. 2018, have shown that the addition of *Satureja hortensis* L. dry plant and essential oil led to a reduction in the total number of germs, this reduction being more significant when the essential oil was used.

The antimicrobial effect of *Satureja hortensis* L. dry plant in various proportions (0,5-1,5%) and essential oil (0,1%; 0,25%; 0,5%), on fresh cow's cheese, was assessed after 3 and 7 days [61].

In a study by Amatiste et al. (2014), the antimicrobial activity of *T. vulgaris* L. and *Origanum vulgare* L. essential oils against *S. aureus* in fresh sheep cheese was assessed. The determined MIC and MBC of the essential oils were 4 and 8  $\mu\text{L}/\text{mL}$ , respectively. In addition, it was observed the aforementioned essential oils had no effect on *S. aureus* count in cheese samples during 7 days of storage which was attributed to the interaction of active components of essential oils with cheese matrix [62].

Effect of essential oil from *Ocimum gratissimum* (200, 400, 600, 800 or 1000 mg/L) was assessed against six fungi including *Aspergillus flavus*, *Aspergillus tamarii*, *Fusarium poae*, *Fusarium verticillioides*, *Penicillium citrinum* and *Penicillium griseofulvum* isolated from a traditional cheese named wagashi. It was noted that mycelia growth was reduced by increasing the essential oil level. A significant fungistatic activity against all the examined species was observed with MIC values, ranging from 800 to 1000 mg/L [63]. Mahgoub et al. (2013) studied the effect of adding 0,1% and 0,2% (w/w) of *Nigella sativa* oil to Domiati cheese on the inhibition of foodborne pathogens (*Staphylococcus aureus*, *Salmonella enteritidis*, *Escherichia coli* in addition to *Listeria monocytogenes*) inoculated in cheese during storage. Addition of 0,2% oil showed the most effective antimicrobial potential on pathogens and improved the physicochemical and sensory properties of the cheese [64].

Najgebauer et al. (2009) evaluated the storage stability of butter prepared from sour cream with 2% addition of dried herbs (sage or rosemary). They concluded that the addition of rosemary herb was more effective in delaying lipolysis in butter than sage, but both supplemented products had increased oxidative stability through storage than the control. TBA analyses test exposed that the sage and rosemary butters contained significantly smaller concentrations of secondary oxidative products like malonoaldehyde and ketones than the butter without herbs [66]. Parmar and Khamrui (2017) found that the ghee manufactured by creamery buffalo butter supplemented with 7% arjuna alcoholic extract had maximum phytosterol content with suitable sensorial characteristics [67].

### **Effect of herbs on the sensory properties of dairy products**

Adding herbs as flavor to some varieties of cheese is already a common practice. Therefore, the acceptance of cheese with essential oils and extracts can be facilitated if consumers are already accustomed to this type of product. However, essential oils and extracts from herbs often have a strong aroma even when used in low concentrations, which can cause a negative sensory impact, with a possible rejection of the product, making it a limiting factor in the use of herbs and their components. Acceptance of the product is extremely important because only a high antimicrobial effect is not sufficient if the consumer does not accept it from a sensory point of view. Among the plant extracts and essential oils added to cheeses, oregano, black cumin, green pepper, rosemary, thyme and lemon grass have led to good sensory acceptance [68, 69].

However, the concentration of these substances applied in cheeses should be carefully considered, due to the possible negative impacts on sensory properties. To minimize the possible negative effects of plants and their constituents in the aroma of products, a combination of different extracts and volatile oils was investigated, which can lead to a good antimicrobial effect at lower individual concentrations. These combined compounds may, in some cases, have a higher antimicrobial activity than when added separately, thus having a synergistic effect [51, 60, 61].



The combined use of these natural compounds with emerging technologies such as ultrasound, high pressure and electrical pulses may also allow the use of lower concentrations of essential oils, as these technologies frequently affect the cell membrane, thus facilitating penetration and antimicrobial activity.

Also, the application of essential oils together with other antimicrobial agents and preservatives may be more effective in preventing microbial growth and increasing the safety and quality of cheese [70].

### Conclusions

Plant extracts and essential oils have been shown to be natural preservatives and antioxidants with significant inhibitory activity against foodborne pathogens and spoilage microorganisms, thus helping to extend the shelf life of the dairy product. The main compounds of aromatic plants, including thymol, carvacrol, eugenol, carvone and cinnamaldehyde are mainly responsible for achieving antimicrobial activity through various mechanisms, such as increasing cell permeability and interfering with enzymes involved in energy production that eventually lead to cell death.

However, given the losses during processing and their stability during storage, several studies should be performed to determine the concentration of extracts and essential oils added in dairy products, as well as the action of these plant antimicrobial compounds on lactic acid bacteria.

In addition, there is a need to expand and deepen sensory studies related to the use of essential oils and plant extracts in various dairy products, in order to better understand the possible impacts on consumer acceptance.

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