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TECHNICAL NOTES

Critical analysis of the use of preservatives sorbic acid and potassium sorbate in yogurts in light of Brazilian legislation

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Highlights

- Fungal spoilage remains a significant problem for the quality of yogurts
- Cereal or fruit preparations are a source of important fungal contaminants
- Sorbate has a role in the stability of the yogurt and food safety
- The use of sorbate in yogurt is an effective and safe technological practice

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KEYWORDS
Food safety;
Dairy industry;
Fungal spoilage;
Regulatory limits.

Abstract: The purpose of this technical note is to provide support for the dairy industry based on the state-of-the-art and international regulatory situation, concerning the exclusion of the possibility of using sorbic acid and sorbate salts in yogurts according to the Brazilian Health Regulatory Agency's (ANVISA) new Normative Instruction 286 of 2024. Fungal spoilage remains a significant problem for the quality of dairy products, especially for fermented products such as yogurt formulated with fruit/cereal preparations. Ingredients used in yogurt (*e.g.* fruit preparations) are a source of important fungal contaminants, typically psychrotrophic and acidophilic yeasts. For example, *Candida famata* and *Kluyveromyces fragilis* are capable of hydrolyzing casein and producing bitter flavors. Fungal spoilage of yogurt can also cause visible defects such as swelling and superficial mycelium growth. Furthermore, these spoilage deviations represent a significant financial loss to the industry and reduce food safety. We understand that the presence of the preservative sorbate in the final product (due to the principle of transfer via preparations) has a role in the stability of the yogurt and that there is a need to maintain the expected use via preparation transfer. Technologically, due to the acidity characteristic of yogurts, it is necessary, due to the contribution of microbial load by the addition of preparations (*e.g.* fruit; honey; cereals) after fermentation, to maintain the concentration of up to 300 mg/kg (ppm) in the final product. Maintaining sorbate levels within legal limits is imperative for stability and microbial safety, underscoring the need for balanced regulatory and consumer-awareness strategies. The technical note is not intended to be a literature review or deepen the discussion regarding the toxicological implications of preservatives, but rather to generate relevant and regulatory information to support a debate regarding this issue.

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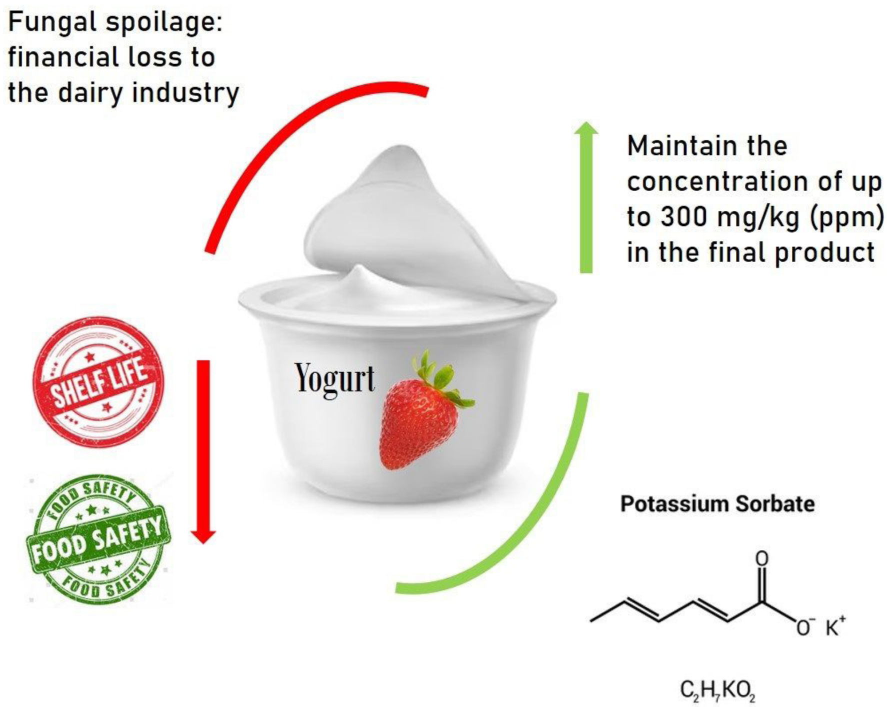
Graphical Abstract



Premises

Number 1: Codex General Standard for Food Additives (GSFA) Online Database; Updated up to the 46th Session of the Codex Alimentarius Commission (2023); Dairy-based desserts (*e.g.* pudding; fruit; flavoured yogurt) (01.7); Sorbates as Sorbic Acid maximum level 1,000 mg/kg.

Number 2: Regulation (European Commission) N. 1333/2008 of 16 December 2008 on food additives; Category Number: 01.4 Flavoured fermented milk products including heat-treated products; E 200-213 Additive Sorbic acid – potassium sorbate; Benzoic acid – benzoates; Maximum level (mg/L or mg/kg as appropriate): 300; Restrictions/exceptions: only non-heat-treated dairy-based desserts.

Number 3: European Food Safety Authority (EFSA) Food and Feed Information Portal Database; Additive Name: Sorbic acid - potassium sorbate; E number 200 – 202; The additive is authorised to be used in the following category: 1.2 Unflavoured fermented milk products; Individual restriction(s)/exception(s): Maximum Level = 1000 mg/L, only curdled milk; Footnotes: The additives may be added individually or in combination; The maximum level is applicable to the sum and the levels are expressed as the free acid; Legislations: Commission Regulation (EU) 2018/98 of 22 January 2018 amending Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards calcium sorbate (E 203). Text with EEA relevance, applicable from 10/02/2018.

Number 4: The most recent EFSA activity concerning sorbate was the evaluation of a reproductive toxicity study at the request of the European Commission, which, however, was published in 2021. In this opinion, the EFSA Panel on Food Additives and Flavourings (FAF Panel) was requested by the European Commission to carry out a scientific evaluation of an extended one-generation reproductive toxicity study (EOGRTS) to determine whether it would allow reconsideration of the temporary group acceptable daily intake (ADI) for sorbic acid (E 200) and potassium sorbate (E 202), established by the Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) in 2015. From the EOGTRS, the FAF Panel identified a lower confidence limit of the benchmark dose (BMDL) of 1,110 mg sorbic acid/kg body weight (bw) per day. By applying a default uncertainty factor of 100, the Panel established a group ADI expressed as 11 mg sorbic acid/kg bw per day for sorbic acid (E 200) and its potassium salt (E 202). In addition, European Commission asked EFSA to review a report on the 'Stability of sorbic acid (E 200) and its potassium salt (E 202) during food processing and storage' provided by industry. No new information was provided in this report, and therefore, in this opinion, there was no re-assessment of the EFSA ANS opinion conclusions from 2015 regarding the stability of sorbates in food.

Number 5: Brazilian Ministry of Agriculture, Cattle and Supplying (MAPA) - Normative Instruction number 46 (2007); Technical regulation on identity and quality of fermented milks; Item 5 on Additives and technology/production aids; 5.1.3. In all cases, the presence of additives transferred through optional ingredients will be admitted in accordance with the principle of transfer of food additives (Resolution GMC 105/94 - Principles of transfer of food additives/ Codex Alimentarius. Volume 1A 1995. Section 5.3.) and its concentration in the final product must not exceed the proportion that corresponds to the maximum concentration allowed in the optional ingredient; when dealing with additives indicated in this Regulation, it must not exceed the maximum limits authorized in this Regulation. In the particular case of fruit pulp aggregate or fruit preparation, both for industrial use, the presence of sorbic acid and its sodium, potassium, and calcium salts in a maximum concentration of 300 mg/kg (expressed as sorbic acid) in the final product.

Number 6: Brazilian Health Regulatory Agency (ANVISA) – Normative Instruction number 286 (2024); Amends Normative instruction 211 (2023), which establishes the technological functions, maximum limits and conditions of use for food additives and technology aids authorized for use in foods; Article 2 - Annex III of Normative Instruction 211 (2023), comes into force with the exclusions contained in Annex II of this Normative Instruction (Table 1): 01.2 Fermented milks; 01.2.2 Fermented milk with addition; Function: Preservative; INS: 200-203; Additives: Sorbic acid and sorbate; Maximum limit: 300 (mg/kg or mg/l); Note: Only in the case of fruit pulp aggregate or fruit preparation for industrial use. Limit expressed as sorbic acid for additives INS 200, 201, 202 and 203 alone or combined.

Technical note

Globally, according to the Food and Agriculture Organization of the United Nations (FAO), an estimated 11 to 25% of dairy products produced are discarded, depending on the region, with a large proportion wasted due to fungal spoilage. Fungal spoilage occurs throughout the product's shelf life and can originate from very low initial contamination levels (ca. 3,0 log CFU/g). Consequently, it is important to understand and predict how different parameters, such as storage temperature and time, and the presence of preservatives, influence product stability and quality.

The use of preservatives in the dairy industry is essential to ensure microbiological safety and increase the shelf life of products. Among the most commonly used preservatives is sorbate (and its salts), which effectively inhibits the growth of fungi (molds and yeasts) and some bacteria. The sorbate addition to yogurts aims to increase the product's shelf life and microbiological safety. The scope of this technical note is to justify the need to maintain sorbate within the current limit of Brazilian legislation to guarantee the safety and quality of yogurt, based on scientific research and technical considerations on microbiological contamination and product shelf life.

Fungal contamination of fermented dairy products can come from the processing environment, through airborne transmission and industrial surfaces, or raw materials added after fermentation, such as preparations and other additives. It is well known that the main source of contamination is from added ingredients, such as fruit preparations and other food additives. These contaminations can compromise product safety and significantly reduce its shelf life.

Using microbial cultures with bioprotective properties offers a strategic and redundant method to increase the effectiveness of the preservative system in mitigating fungal spoilage in fermented dairy products. They can slow the growth of undesirable microorganisms throughout the shelf life by competing for nutrients, producing weak organic acids, and potentially producing other secondary metabolites such as antimicrobial bioactive peptides (*e.g.* bacteriocins) (Buehler et al., 2018; Siedler et al., 2020). In particular, in yogurt, research has focused on specific fungal strain (*e.g.* Molds: *Candida parapsilosis*; *Clavispora lusitaniae*; *Kloeckera apiculate*; *Metschnikowia pulcherrima*; *Penicillium commune; Penicillium brevicompactum*; *Penicillium crustosum*; *Penicillium solitum*. Yeast: *Cryptococcus fragicola*; *Debaryomyces hansenii*; *Rhodotorula mucilaginosa*; *Saccharomyces cerevisiae*; *Torulaspora delbrueckii*) inhibition, using food as a model system. However, there are limited reports on preservative culture use and the results have shown little efficacy.

Traditional methods for controlling fungal spoilage in yogurt include the use of food additives such as potassium sorbate (Davidson et al., 2012). However, the food industry is challenged by the "clean label" consumer's pressure to produce products of the same quality, with the same shelf life, and without the use of traditional food additives. This requirement needs to be extremely well evaluated so that food safety and the economic vitality of the industries are not compromised. Although yeasts are primarily known as beneficial organisms due to their fermentative activity for various foods and beverage production, they also play an important role as specific spoilage organisms and are the main cause of microbial changes in yogurt. The ability to

Table 1. Annex II from the ANVISA Normative Instruction number 286 (2024). Exclusions in the list of authorized food additives for use in foods and their respective technological functions, maximum limits, and conditions of use from Annex III of Normative Instruction 221 (2023).

01.2 Fermented milks
01.2.2 Fermented milk with addition

Function	INS	Additive	Maximum limit (mg/kg or mg/L)	Note
Preservative	200	Sorbic acid	300	Only in the case of added fruit pulp or fruit preparation for industrial use. Limit expressed as sorbic acid for additives INS 200, 201, 202, and 203, alone or combined.
Preservative	202	Potassium sorbate	300	Only in the case of added fruit pulp or fruit preparation for industrial use. Limit expressed as sorbic acid for additives INS 200, 201, 202, and 203, alone or combined.

grow at low temperatures (4°C) and in environments with high sugar concentrations (up to 50-60%) allows yeasts to multiply in yogurt (Moreira et al, 2001; Tirloni et al., 2015).

In yogurts, fungi may be present due to processing, including the addition of preparations, packaging materials, or operations. Filling equipment for yogurt packaging can range from relatively open to ultra-clean, where the product is in an atmosphere of High-Efficiency Particulate Arrestance (HEPA)-filtered air, and the packaging materials are subjected to a decontamination treatment. The filling equipment for yogurt packaging currently used in dairies is very diverse, but it is not capable of guaranteeing an aseptic operation. In the absence of a validated aseptic operation, preservatives such as sorbic acid must be added to the fruit preparations. Even with good control during manufacturing, the shelf life of yogurts can be limited by fungal contamination, especially in open or semi-open filling operations. In these cases, the reliable shelf life of the products will be less than 6 weeks and may be reduced even further if there is greater exposure to fungi.

Some product spoilage during its shelf life, caused by bacterial activity, is inevitable, albeit statistically infrequent. This spoilage results from the ongoing activity of yogurt culture bacteria, which can lead to post-acidification, and from endospore-forming bacteria that survive heat treatment. Modified atmospheres in the headspace of packaging have been used to retard microbial growth and therefore improve shelf life. For pasteurised blended yogurt, microbial spoilage due to moulds should not be a problem if the process and packaging are well controlled. Depending on the pasteurisation conditions, spoilage due to bacterial action is also retarded.

Yeasts are generally more resistant than bacteria to extreme conditions, in particular at low pH. The ability to ferment lactose and sucrose, which are then converted to ethanol and carbon dioxide, and the production of several newly formed secondary metabolic compounds, such as higher alcohols, organic acids (prevalently succinic and acetic acid), esters, aldehydes, and ketogenic substances have been described by many authors as metabolites directly related to spoilage. The production of proteolytic and lipolytic enzymes has also been widely documented for spoilage yeasts (Garnier et al., 2017). Yeast spoilage begins to be evident when microbial loads reach 5,0 log CFU/g. Suriyarachchi & Fleet (1981) characterized yeasts found in retail yogurts and reported that the most frequently isolated species, *Candida famata* and *Kluyveromyces fragilis*, were capable of hydrolyzing casein and producing bitter flavors. The main species related to yogurt spoilage was *D. hansenii*, *Kluyveromyces marxianus*, *K. fragilis*, *Rhodotorula glutinis*, *R. mucilaginosa*, *Rhodotorula rubra*, *S. cerevisiae*, and *Yarrowia lipolytica*. In Brazil, Moreira et al. (2001) identified 577 yeasts from 72 package yogurts. The most abundant yeasts belonged to ten species (*D. hansenii*, *S. cerevisiae*, *Mrakia frigida*, *Hansenula* sp., *C. parapsilosis*, *Debaryomyces castellii*, and *Candida maltose*). Low-level contamination with *Monilia* and *Penicillium* species was found in a few samples. Growth tests suggested that the ability to ferment sucrose, growth at 5°C, and in the presence of 300 mg/kg sorbate, were the three most significant physiological properties to account for these yeasts in yogurts. More recently, Garnier et al. (2017) reviewed the state-of-art diversity of spoilage fungi in dairy products. Fungal spoilage of yogurt can also cause visible defects such as swelling and superficial mycelium growth (Snyder et al., 2016). As previously mentioned, molds and yeasts can be introduced by adding fruits and other preparations to yogurt after fermentation. Commercially, most fruits for mixing with yogurt are supplied pasteurised and in the presence of preservatives. Aseptic "commercially sterile" preparations are also available but are less common. Low-moisture cereals, almonds, and walnuts theoretically do not favor the growth of microorganisms, but walnuts and honey are a potential source of molds and fungal spores, and potentially reduce the shelf life of yogurt by introducing contamination.

In preparations containing fruits, cereals, almonds, and honey, the yogurt industry must ensure that when they are diluted in the yogurt mass after fermentation and pre-packaging, the amount of preservative provided by the preparation maintains the minimum levels necessary for antifungal efficacy in the final product. Several studies highlight the importance of sorbate in controlled concentrations to prevent microbial proliferation, especially in products containing additional ingredients susceptible to contamination, such as fruit preparations (Garnier et al., 2017). Tirloni et al. (2015) demonstrated that sorbate presence in yogurt, especially in those with added fruits, is crucial to slowing down yeast growth, extending the product shelf life, especially under refrigerated storage conditions. In the study, yogurts stored at 4°C were more stable, while under more abusive conditions (>10°C), yeast growth was the limiting factor in the microbiological shelf life of the product. The addition of sorbate in the study, with the aim of inhibiting yeast growth, never exceeded 300 mg/l (ppm). This is the maximum level permitted for flavored fermented dairy products, according to European regulations (European Commission, 2011). Mihyar et al. (1997) reported that the sorbate use at specific concentrations is effective in maintaining low yeast levels in yogurt for up to 21 days. The authors reported that to ensure counts below 5,0 log CFU/g for 14 days at 5°C for the most sensitive yeast tested in the study (*Trichosporon cutaneum*), 100 ppm was required, while 200 ppm was required to ensure the same level for 21 days. For all other species tested in the study (*Pichia farinosa* and *D. hansenii*), higher concentrations (>400 ppm) were required, demonstrating that the effectiveness of sorbate is species/biotype dependent. In this case, it would be advisable to add concentrations above 300 ppm, but taste and toxicological issues would come to light. It is therefore suggested to associate redundancy in the preservative system and work on aspects of a cleaner fill process as previously mentioned.

Furthermore, sorbate helps to extend the shelf life of yogurts, especially those undergoing long logistics chains and prolonged storage. This is crucial to avoid economic losses and to ensure product quality and safety until consumption. Sorbic acid and potassium sorbate are frequently used for other food products and dairies (*e.g.* non-heat-treated dairy-based desserts; cheeses; cheese products; curdled milk) preservation as well. Although it has greater inhibitory activity against fungi than against bacteria, sorbate has been used to inhibit, for example, *Escherichia coli* and

Staphylococcus aureus from dairies (Sahan and Golge, 2005; Zhang et al., 2024).

Although not numerous, outbreaks related to the yogurt-contaminated consumption with potentially pathogenic bacteria (enterohemorrhagic *E. coli*, *Salmonella* Typhimurium, and *Cl. botulinum*) have been reported in the literature. Furthermore, *Listeria monocytogenes*, a psychrotrophic pathogen present in dairy production environments capable of adapting to environmental stress, is recognized as a post-processing contaminant of great concern in dairy products, such as yogurt. In the study by Tirloni et al. (2015), potassium sorbate in strawberry yogurt provided antimicrobial action and was considered one of the main protective factors for yogurt. The acidic environmental conditions provided by the product allowed the survival of *E. coli* and especially *L. monocytogenes* in samples at both initial inoculum concentrations. The strong acid resistance evidenced by *L. monocytogenes* resulted in the presence of the viable pathogen in both types of yogurts (with and without sorbate) throughout the period considered. The environmental persistence of this pathogen is a major concern for the dairy industry and should be considered in the risk assessment.

Other studies have demonstrated that sorbate effectively inhibits the growth of several potentially pathogenic bacteria, including *S. aureus*, *Campylobacter jejuni*, *Salmonella* sp., *Shigella* sp., *L. monocytogenes*, and *E. coli* O157:H7. These bacteria were added to yogurt in challenge tests, simulating conditions of ineffective heat treatment or post-contamination, and were unable to survive or multiply.

The strong antimycotic activity combined with the resistance of lactic acid bacteria (LAB; *e.g. Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) to benzoic and sorbic acid (Lück & Jager 1997; Sofos, 1997; Stopforth et al., 2005) may be responsible, at least in part, for their use by the yogurt industry to control fungal growth. The use of sorbate to extend the shelf life of yogurt has been reported by many other authors, in addition to those cited above throughout the text, in varying concentrations, without interfering with the count and viability of the LAB used in the fermentation process (Freitas et al. 2024; Küçükçetin et al. 2004; Lai & Tang 2024; Tfouni & Toledo 2002). Sorbate is particularly active at higher pH levels, where it interferes with cellular functions such as nutrient absorption. Sorbate is effective at concentrations that do not normally significantly affect LAB. LAB normally ferment under acidic conditions (such as in yogurt), and in an acidic environment, sorbate activity is reduced, which improves the situation for LAB metabolism.

Conclusions

The safety evidence at typical consumption levels applied for decades (generations) on the effects of consuming the preservative sorbic acid and its salts justifies the lack of need to change the current regulations for use in yogurts in Brazil. The Codex Alimentarius maintains the safety of use, and the EFSA regulates the use in the European community with limits of 0.1% (1000 mg/kg) in yogurt. The EFSA assessments were based on scientific studies on the compound safety, including its toxicity and possible effects on human health. Furthermore, the US FDA classifies potassium sorbate as a food-grade additive based on updated scientific evidence. Potassium sorbate use in yogurts is therefore an effective and safe practice.

The use of sorbate in yogurts presents a series of clear benefits, including product shelf life extending and maintaining its quality and microbiological safety. The combination of safety and consumer sensitivity may lead to improved acceptance and understanding of the use of sorbate as an effective preservative in dairy products. Although there are challenges related to consumer perception, its application is justified for technical, toxicological, and economic reasons.

The exclusion of the possibility of using sorbic acid and sorbate salts in yogurts according to the Brazilian Health Regulatory Agency's (ANVISA) new Normative Instruction 286 of 2024 is an issue that needs to be revisited. Maintaining sorbate levels within legal limits is imperative for stability and microbial safety, underscoring the need for balanced regulatory and consumer-awareness strategies.

Conflict of interests

The authors have no competing interests to declare that are relevant to the content of this technical note.

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