

ALOE SUPPLEMENTS IN FOCUS: INTEGRATING QUALITY MANAGEMENT AND RISK CONTROL FOR MICROBIOLOGICAL SAFETY

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Purpose: The purpose of this paper is to assess the microbiological safety of aloe-based dietary supplements available on the Polish market. The study investigates how the form of the aloe supplement (juice vs. capsules) and the storage time affect microbial contamination levels, emphasizing the importance of quality management and risk management in ensuring the safety of plant-based supplements.

Design/methodology/approach: The study focused on assessing the microbiological stability of 68 aloe-based dietary supplements, including Aloe vera juice and gel-shell capsules, at two key time points: immediately after opening (time 0) and at the final storage period (time K). Microbiological analysis included testing for the presence of aerobic mesophilic bacteria, yeasts, molds, *Staphylococcus aureus*, and *Escherichia coli*, alongside an evaluation of the active acidity and microbiological stability in the juice preparations. The research methodology emphasized a comprehensive laboratory-based approach to evaluate the impact of the supplement form (juice vs. capsules) and storage duration on microbial contamination. By focusing on the end-user stages, the study indirectly addressed risk management by identifying potential contamination risks linked to product form and storage. Additionally, quality management principles were applied through the systematic assessment of supplements' compliance with microbiological safety standards.

Findings: The study found that *S. aureus* and *E. coli* were absent in all samples, with 18% of the samples free from any microbial contamination. Aloe capsules were more prone to fungal contamination compared to juices. Juices demonstrated greater microbiological stability, with approximately 19% stability compared to around 11% for capsules. These findings underscore the importance of rigorous quality control to ensure the microbiological safety of aloe supplements and highlight the need for ongoing post-market monitoring to mitigate potential health risks.

Research limitations/implications: The study focused specifically on aloe supplements from the Polish market, which may limit its generalizability to other types of plant-based supplements. Future research could broaden the scope to include other popular plant supplements, such as young barley, to compare microbiological stability and safety across different categories of plant-based products. This would provide a more comprehensive view of potential risks and quality control measures needed for various supplements.

Practical implications: The research underscores the need for enhanced quality control measures during the production, storage, and distribution of aloe supplements. Manufacturers should implement risk management strategies to reduce contamination risks, particularly in capsule-based products, and improve hygiene practices to meet safety standards. These findings could influence regulatory policies and encourage more rigorous post-market surveillance of dietary supplements.

Social implications: The study highlights the potential risks associated with the uncontrolled consumption of plant-based supplements and emphasizes the importance of ensuring their safety. The findings can contribute to promoting more responsible supplement use and may encourage stricter regulatory frameworks, ultimately improving public health and enhancing consumer protection.

Originality/value: This study provides new insights into the microbiological safety of aloe-based dietary supplements available on the Polish market. It contributes to the fields of quality management and risk management in dietary supplements by offering valuable information for manufacturers, regulators, and health professionals. Additionally, the findings are relevant for consumers, as they highlight the importance of product safety and informed usage.

Keywords: *Aloe vera*, dietary supplements, quality management, risk management, microbiological safety.

Category of the paper: research paper.

1. Introduction

1.1. Regulatory Framework and Consumer Perception of Dietary Supplements

According to the Food and Nutrition Safety Act of 25 August 2006, dietary supplements are foods composed of nutrients, primarily intended to complement the normal diet (Dz.U. 2006, nr 171, poz. 1225). In recent years, there has been a systematic expansion of the dietary supplement market, with products available in various forms (capsules, tablets, dragees, and powder sachets) and compositions (vitamins, minerals, herbs, plant extracts, amino acids). According to a report commissioned by the OSAVI supplement manufacturer, almost 76% of Poles declare regular use of these products, without consulting specialists beforehand (about 45%). Every third person knows, but only to a limited extent, what the difference is between a drug and a supplement (Poles and Dietary Supplements..., 2022). One of the most important differences is the lack of a statutory requirement to monitor the safety of dietary supplements, whereas medicines belong to products subject to control at every stage of manufacture and distribution (Brzezińska, Grembecka, 2021). The results of the report show that almost 41% of the Poles surveyed believe that supplements are tested to the same degree as drugs, while less than 4% believe that these products are not controlled (Poles and Dietary Supplements..., 2022). The manufacturer or importer is primarily responsible for the quality and safety of a dietary supplement placed on the market. The Sanitary Inspectorate conducts inspections of products on the market; however, due to the number of supplements on the market, the activities carried out have a very limited scope (Information on inspection results..., 2021). During the

period 2017-2020, approximately 63,000 notifications about the introduction of, or the intention to introduce, a new dietary supplement were submitted to the General Sanitary Inspectorate (GSI). Only 11% of them were audited by the GSI ((Un)controlled dietary supplements..., 2022). Uncontrolled use of dietary supplements (including plant supplements) can cause health problems, resulting from, among other things: incorrect dosage, interaction with other drugs and/or supplements, falsified composition, presence of contaminants (potentially hazardous substances or microorganisms) (Ratajczak et al., 2020).

1.2. Aloe Vera in contemporary lifestyles: health benefits and diverse applications

A large proportion of Poles use vitamin and mineral supplements (81.1%) and one in five uses supplements containing vegetable extracts (Poles vs Dietary Supplements..., 2022). Natural plant products are a source of bioactive compounds with a broad molecular and functional diversity. The physicochemical properties and activity of these products depend, among other things, on the type of extract used, the species and age of the plant, the conditions of cultivation, and the methods of harvest (Leja, Czaczyk, 2016; Yadeta, 2022). *A. vera*, with its healing and therapeutic properties, has been utilized by humans for many centuries. Moreover, it exhibits immunomodulatory, anticancer, immune-boosting, anti-aging, and antioxidant properties (Hamman, 2008; Tomasin et al., 2015; Maan et al., 2018). In medicine and herbal medicine, the best known and appreciated are *Aloë arboresces* (woody) and *Aloë vera* (ordinary), otherwise called *Aloë barbadensis* and *Aloë ferox* (prickly), respectively (Kukułowicz, Steinka, 2010). *A. vera* is the most popular species among aloes (Maan et al., 2018). The colorless gel obtained from the inner pulp of the leaf is a rich source of bioactive compounds. Many properties of the gel are attributed to its complex polysaccharide composition, particularly acemannan. In addition to being the primary polysaccharide and the most studied, acemannan is used as one of the qualitative markers applied to define aloe products (Hamman, 2008; Ahluwalia et al., 2022). Due to its health benefits, aloe can be added to jellies, jams, juices, candies, soft drinks, yogurts, cottage cheese, and ice cream. It is also used in the food industry as a functional food ingredient. In the cosmetics industry, it is used as a base material for the production of creams, lotions, soaps, shampoos, face wash products, and other products, while in the pharmaceutical industry, its properties are used in the production of ointments and gels, as well as tablets and capsules. Important pharmaceutical properties of *A. vera* gel, as well as whole leaf extract, include the ability to improve the bioavailability of vitamins for humans, which adds value to quality management in the production of aloe-based products (Hamman, 2008; Kukułowicz, Steinka, 2010; Mann et al., 2018; Kamble et al., 2022; Yadeta, 2022).

1.3. Microbial contamination: a risk management perspective on medicinal plants

The market for aloe foods and supplements has grown rapidly in recent years, probably due to increasing consumer awareness of its health benefits (Yadeta, 2022). Medicinal plants, due to their origin, are exposed to the presence of microorganisms. In addition, microbiological contamination may originate from the primary plant microflora, the presence of microbes in the processing plant, the use of contaminated water, and pre-harvest and post-harvest treatments (including processing, storage, and distribution). Unsanitary growing conditions can increase contamination, affect shelf life, and reduce the potential benefit of medicinal plants (Okunlola et al., 2007; Aljaloud et al., 2013; Bhowmik et al., 2017; Ratajczak et al., 2020). Microorganisms and their toxins, when present in contaminated plants, can pose significant health risks, potentially leading to the onset of diseases, highlighting the need for risk management. Elevated temperatures and increased moisture levels are primary contributors to the proliferation of these hazardous microorganisms. Earlier investigations into herbal medicines have unveiled the presence of highly pathogenic microorganisms, including methicillin and vancomycin-resistant *Staphylococcus aureus*, various *Bacillus* species, and multiple fungal species such as *Aspergillus flavus*, *Penicillium viridicatum*, and *Fusarium oxysporum* (Van Vuuren et al., 2014).

1.4. Ensuring microbiological safety in Aloe Vera supplements

While aloe preparations are recognized as potentially beneficial additions to a healthy diet, there is a concern about the adequacy of their supervision and control, especially in terms of microbial contamination. The limited scope of supervision for marketed dietary supplements raises questions about the safety and quality of these products. The paper aims to provide valuable insights into the microbiological safety of aloe supplements, elucidating how the form of the product (juice vs. capsules) and the storage time (both immediately after opening and on the last day of storage) can influence microbial stability. Additionally, the findings offer information on the prevalence of microbial contamination in aloe supplements and underscore the significance of post-market monitoring, environmental control, and hygiene procedures in the production and storage of plant-based supplements to ensure quality management and effective risk management.

2. Materials and methods

2.1. Materials

The research material consisted of the following dietary supplements:

- juice (*Aloe vera* juice (from 99.7% to 100%) with or without pulp) sealed in pharmaceutical brown bottles, which limits UV radiation (n = 32);
- gel-coated capsules (freeze-dried *Aloe vera* leaf pulp juice / dry powder / leaf extract / extract) in pharmaceutical brown bottles or white HDPE plastic containers (n = 36).

These forms of sales were chosen for the study, because according to a report commissioned by the supplements manufacturer OSAVI, Poles most often choose preparations in the form of tablets or capsules for swallowing (as much as 71.2%), followed by effervescent tablets (11%) (no *Aloe vera* on sale) and liquids (5.1%) (Poles and Dietary Supplements ..., 2022). The products were purchased in organic food stores or pharmacies in the Tri-City in 2023.

2.2. Methods

Test products were analyzed immediately after opening (time 0; n = 34) and on the last day of storage (time K; n = 34). The expiry time (K) is considered as the expiration date declared by the manufacturer. Once opened, juices were stored at 4±1°C and the capsules were stored in a dry place at room temperature (temp. did not exceed 25°C). Ten grams of each supplement were collected in a laminar airflow chamber and then homogenized together with 90 mL of Ringer's solution using a Stomacher lab-blender 400 (Seward, Worthing, United Kingdom). Homogenates were subjected to further dilution. Bacterial counts were determined using the pour-plate technique. In the dietary supplements analyzed:

- mesophilic aerobic bacteria counts (AMC) on Merck's nutrient agar (incubation at 30°C for 72 hours),
- yeasts and moulds counts (YMC) on Merck's YGC chloramphenicol agar (incubation at 25°C for 120 hours),
- the number of *Staphylococcus aureus* on bioMérieux's Baird Parker + RPF medium (incubation at 37°C for 48 hours),
- the number of *Escherichia coli* on the selective medium Coli ID bioMérieux (incubation at 37°C for 48 hours).

Microbiological analyses were performed using the dilution method, and microorganisms were counted according to PN-EN ISO 7218:2008. Microbiological stability was determined using the formula (1) (Steinka, 2017):

$$S = N_K / N_P < 1, \quad (1)$$

where:

S = microbiological stability

N_K = number of microorganisms after storage

N_P = number of microorganisms prior to storage

Microbial counts were expressed as log cfu/g.

In addition, the active acidity of the juices was measured using a Hanna HI 9321 pH meter. The results of the studies were statistically analyzed using Statistica 13 Software.

3. Results

S. aureus and *E. coli* were not detected in the analyzed aloe supplements (capsules and juices), either immediately after opening or at the end of storage. The lowest mean values of aerobic mesophilic counts (AMC) were observed on the day of opening in capsule supplements (1.06 ± 0.83 log cfu/g) and the highest (1.99 ± 0.80 log cfu/ml) in end-of-life juice formulations. Although the capsules had the lowest mean values of AMC on the day of opening, at the end of storage, their quantity had increased, similarly to aloe juice, by about 0.6 log cycles (Table 1).

Table 1.

Changes in the number of tested microorganisms and pH values during storage of aloe supplements (mean values \pm SD)

Form of supplement	AMC		YMC		pH	
	log cfu/ml(g)				Time 0	Time K
	Time 0	Time K	Time 0	Time K		
J	1,42 \pm 1,02	1,99 \pm 0,80	0,28 \pm 0,50	0,31 \pm 0,56	3,67 \pm 0,63	3,75 \pm 0,67
C	1,06 \pm 0,83	1,64 \pm 0,83	0,79 \pm 1,08	0,96 \pm 1,15	-	-

time 0: product immediately after opening; time K: last day of storage; J: juice; C: capsules aerobic mesophilic bacteria (AMC), yeasts and moulds (YMC).

Source: own research.

For capsules and supplements in the form of juice (except for time 0), similar values of standard deviation were observed, which indicates a similar concentration of the obtained results around the mean (Table 1). The maximum (3.62 log cfu/ml) AMC was found for aloe juice immediately after opening the package, while for capsules the maximum value (2.56 log cfu/g) was highest at the end of storage. The difference between the baseline total number of microorganisms of all tested aloe supplements and their number at the end of shelf life was statistically significant ($p = 0.0085$). The tested fungi were more likely to contaminate aloe capsules than juices (Figure 1).

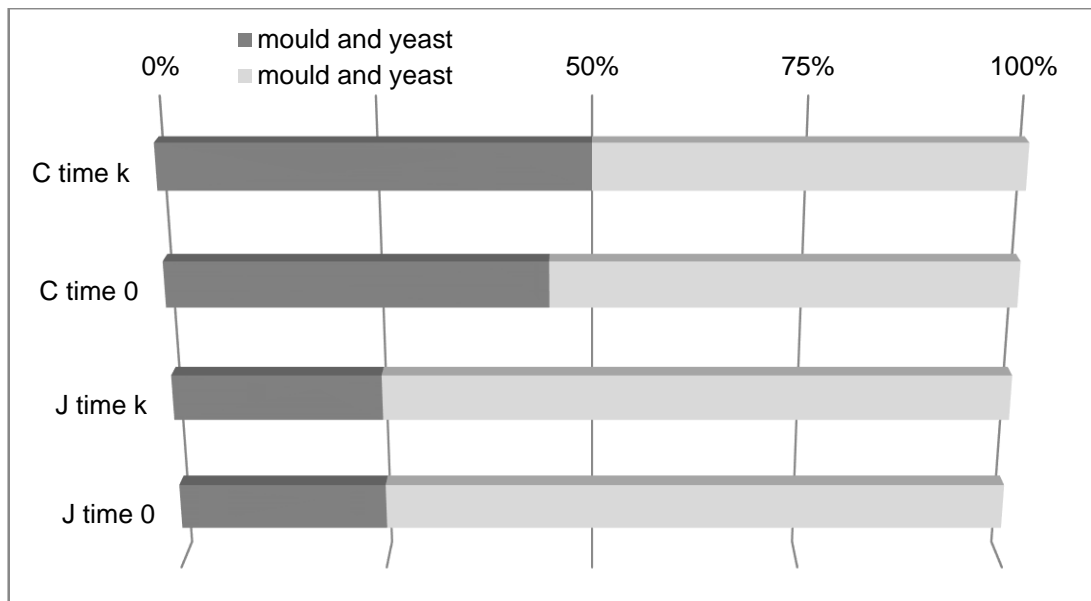


Figure 1. Percentage of samples contaminated with fungi depending on form and storage time, time 0: product immediately after opening; time K: last day of storage; J: juice; C: capsules.

Source: own research.

The highest means for yeast and mould were also observed in capsules (Table 1). At the end of the storage period in this form of supplements, the level of fungi was found to be three times higher than that shown in preparations in the form of juice. The maximum (1.49 log cfu/g) YMC observed at the end of shelf life for aloe juice was twofold lower than the maximum value (3.08 log cfu/g) demonstrated for capsules. It was shown that the form of the supplement significantly altered the mean total number of fungi ($F = 4.29$; $p = 0.0069$). The difference between the initial total number of fungi of all tested aloe supplements and the number at the end of shelf life was not statistically significant ($p = 0.6568$). Tested aloe juice supplements had a pH ranging from 2.92 to 5.06 and from 2.93 to 5.28 for times 0 and K, respectively. Immediately after opening the bottles, the mean pH value of aloe juice supplements was 3.67 ± 0.63 . At the end of the storage period, the pH had increased slightly (Table 1). The difference between the initial and final pH of the juices was not statistically significant ($p = 0.7391$). When the number of microorganisms tested after storage is equal to or less than the baseline level, or equal to the number determined before storage, we can talk about the microbiological stability of the products (Steinka, 2017). It was shown that less than 15% of all tested supplements (both capsules and juices) were characterized by microbiological stability. Taking into account the form of the analyzed products, juices turned out to be more stable (about 19%) than capsules based on aloe (about 11%).

4. Discussion

Although products of natural origin and plants are good ingredients to use, in the case of *Aloe vera*, it should be borne in mind that during long-term supplementation they can cause various ailments. There have been reports of allergic conditions and hypersensitivity to aloe preparations. The use of aloe juice for a long time or at increased doses may cause electrolyte imbalances (including sodium loss, which may result in secondary hyperaldosteronism, while potassium loss may result in hypokalemia, leading to fatigue, muscle weakness, or renal dysfunction). Oral *A. vera* gel may cause symptoms of cramp, abdominal pain, and diarrhea due to the possible contamination of *A. vera* products by anthraquinones. Some components of dietary supplements may interact with drugs and dietary components (Ahlawat, Khatkar, 2011; Brzezińska, Grembecka, 2021; Yadeta, 2022). Medicinal plant materials carry a significant load of bacteria and moulds, often derived from the soil, while simultaneously hosting a diverse range of naturally occurring bacteria and fungi on the surface of herbs. Herbal substances/preparations have the potential to be contaminated with various species of bacteria and fungi, including yeasts and moulds. It is imperative to specify the content of live bacteria, fungi, and their spores in accordance with relevant regulations (Reflection paper on microbiological..., 2015). Currently, there are no acts implemented in Polish law concerning the permissible limits of contamination by microorganisms in dietary supplements. However, as these preparations are legally classified as food, the same rules apply to them as to food. The Commission Regulation (EC) No. 1441/2007 in force in Poland does not distinguish dietary supplements as a separate product group. The seventh edition of the Polish Pharmacopoeia (PP) contains requirements for microbiological purity of the drug divided into four categories (Marczewska, 2013). The tested *A. vera* supplements can be classified as category IV of group B, as herbal medicinal products not treated with boiling water prior to use. According to the microbiological criteria contained in the PP, the acceptable total number of aerobic bacteria (AMC) and the total number of moulds and yeasts (YMC) for these products are 10^5 cfu/g (5.0 log cfu/g) and 10^4 cfu/g (4.0 log cfu/g), respectively (Marczewska, 2013). Our own research revealed that 18% of all *A. vera* supplements tested were microbiologically clean. All tested aloe-based supplements (regardless of the form) both immediately after opening and at the end of the storage period met the requirements of the PP (Table 1). Only one sample immediately after opening and two samples at the end of storage showed AMC and YMC levels of 3.51–3.62 log cfu/g(ml) and 3.08–3.46 log cfu/g(ml), respectively. Okunlola et al. (2007) reported higher levels of aerobic bacteria in capsules than in liquids, which was the opposite of what we found in our own studies (Table 1). In their analysis of samples from dietary supplements containing plant components, Długaszewska et al. (2019) and Ratajczak et al. (2020) demonstrated that the aerobic bacteria content ranged from 1.0 to 6.78 log cfu/g. In studies by de Sousa Lima et al. (2020), a total of 31.8% and 23.5% of the samples exceeded safety limits ($\log \text{cfu/g} \leq 10^5$) for aerobic bacteria

and fungi, respectively. Bhowmik et al. (2017) found very high levels of contamination of tested plant products with aerobic microbes (from 4.3 to 8.57 log cfu/g) and fungi (up to 7.0 log cfu/g). In their study, Długaszewska et al. (2019) observed fungal contamination in 86.9% of dietary supplement samples, ranging from less than 1 log cfu /g to 4.84 log cfu /g. In our own studies, 35.3% of the samples were found to be contaminated with fungi in the range of 1-3.46 log cfu/g, with capsules exhibiting a higher prevalence of fungal contamination. High fungal contamination of medicinal plants may indicate the presence of various mycotoxins, which may pose a serious threat to human health. The harmfulness of mycotoxins depends on the toxicity, degree of exposure, age, and nutritional status of a person, and the possible synergistic effects of other chemicals to which people ingesting plant preparations are exposed (Bhowmik et al., 2017; de Sousa Lima et al., 2020; Onodugo et al., 2023). No *S.aureus* or *E.coli* were found in the analyzed supplements, which was in accordance with the limits set in the Polish Pharmacopoeia established for oral drugs (Marczewska, 2013). However, these bacteria were among the most frequently isolated microorganisms in studies conducted by de Sousa Lima et al. (2020). In a study conducted by Okunlola et al. (2007) on the microbiological quality of different medicinal products, they discovered that 47% of the samples were contaminated with *E. coli* bacteria and 71.4% with *S. aureus*. The results of Bhowmik et al. (2017) indicated the presence of *S. aureus* in all 20 herb samples and *E. coli* in 11 samples. However, Aljaloud et al. (2013) found that only 11.2% of dietary supplements were contaminated with coliforms, *E. coli*, *Salmonella*, and *S. aureus*. Pathogenic bacterial species pose a risk of causing infectious diseases or other adverse effects in people taking herbal preparations (Reflection paper on microbiological..., 2015). The microorganisms present in the product may impair its stability (Ratajczak et al., 2020), which has also been observed in our own studies. Only 15% of all tested aloe supplements were characterized by microbiological stability. Growing microbes can cause changes in physical properties, inactivation of active ingredients, and decomposition of supplement components into toxic compounds (Reflection paper on microbiological ..., 2015; Ratajczak et al., 2020). Plant ingredients can be metabolized by microorganisms, leading to undesirable chemical and sensory changes (appearance, smell or taste), and pH changes in supplements. The mean pH value of the analyzed aloe juice was 3.67 ± 0.63 for time 0. At the end of the storage period, the pH increased slightly (by less than 0.1) (Table 1). If the pH changes significantly in herbal preparations/supplements containing a chemically ionized preservative whose effectiveness depends on the pH (e.g., benzoic acid and sorbic acid), then the effectiveness of the preservative may be reduced. A low pH (approx. 3.7) may have affected the reduction of microbial growth in tested aloe supplements (Reflection paper on microbiological..., 2015; Onyeneto et al., 2015). It is common knowledge that herbal medicinal products are a good source of nutrients that microorganisms require for growth and survival. Therefore, these products may contain a diverse population of microbes, including foodborne pathogens. Therefore, herbal medicinal preparations are considered "high-risk drugs" of plant origin (Onodugo et al., 2023). Developed quality management systems (ISO 9000:2000) and

safety systems (HACCP) are used by the industry to guarantee biological activity, sensory stability, and final value (including microbiological purity) of *Aloe vera* - based products. Hence, safety control points include pasteurization and the addition of vitamin C and citric acid, while quality control points include raw material collection, leaf filleting, filtration, sterilization, and storage (Yadeta, 2022).

Conclusions

The conducted studies indicate that the tested aloe supplements showed microbial contamination levels that did not exceed the microbiological criteria established by the Polish Pharmacopoeia. However, the intensity of microbial growth was influenced by the form of the preparation. Although *S. aureus* and *E. coli* were not detected, it is essential to include these pathogens in post-marketing monitoring for risk management purposes. Strict control of environmental conditions and the enhancement of hygiene procedures during the production, processing, and storage of plant-based supplements are crucial to maintaining quality standards. Furthermore, precautions should be taken when administering Aloe vera orally to prevent potential interactions with specific compounds that could pose health risks.

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