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Review

Alternative Processes for Apple Juice Stabilization and Clarification: A Bibliometric and Comprehensive Review

Lea Nehmé [1], Myriam El Tekle [1], Nathalie Barakat [2], Alexandra El Khoury [1], Samar Azzi-Achkouty [1] and Youssef El Rayess [1,*]

[1] Department of Agriculture and Food Engineering, School of Engineering, Holy Spirit University of Kaslik, Jounieh BP 446, Lebanon; leanehme@usek.edu.lb (L.N.); myriam.eltkle@net.usek.edu.lb (M.E.T.); alexandraelkhoury@outlook.com (A.E.K.); samarazzi@usek.edu.lb (S.A.-A.)

[2] Laboratoire de Génie Chimique, Université de Toulouse, Centre National de Recherche Scientifique, Institut National Polytechnique de Toulouse, Université Paul Sabatier, 31326 Toulouse, France; barakatnathalie@gmail.com

* Correspondence: youssefrayess@usek.edu.lb; Tel.: +961-76643765

Abstract: Apple juice is one of the most consumed fruit juices in the world. Raw apple juice is viscous, turbid, and brown in color and contains several spoilage microorganisms. These are the reasons behind the application of several steps of clarification and stabilization prior to juice commercialization. Thermal pasteurization remains the most used process for apple juice microbial stabilization, but it damages its organoleptic and nutritional characteristics. Juice settling used for clarification does not allow the achievement of the desired level of clarification. Therefore, this article provides a comprehensive and bibliometric review of all the alternative treatments for thermal pasteurization in order to reduce microorganisms and patulin levels such as pulsed electric fields, microwave processing, high hydrostatic pressure, ultrasonication, etc., and their effect on apple juice characteristics as well as the techniques used for apple juice clarification.

Keywords: apple juice; non-thermal pasteurization; clarification; membrane filtration; patulin; enzymes

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1. Introduction

Apple juice ranks as one of the most consumed fruit juices globally, appreciated for its authentic and distinct flavor by both adults and children. Beyond its flavor, it is recognized for its health benefits attributed mostly to its richness in phenolic compounds. These compounds contribute to its demonstrated biological effects including anti-atherosclerotic, anti-inflammatory, and neuroprotective effects [1]. The global market offers various types of apple juice such as clear and cloudy apple juice, juice from concentrate, pure juice, blends, and more.

The production of apple juice begins by sorting the apples to remove damaged or rotten fruits. Subsequently, the apples are processed through a disintegrator, hammer mill, or grating mill in order to ground and crush them. Enzymes are applied to these milled apples to optimize juice extraction, with pectinase being the most frequently employed enzyme. This operation facilitates the further breakdown of plant cells in solution, thus increasing juice yield. The enzymatic breakdown hydrolysis of pectin depends on several factors, including the duration of incubation period, temperature, pH levels, and enzyme concentration. Additionally, enzymatic treatment is used for clarification.

Haze and turbidity in freshly pressed juice primarily result from pectin and other polysaccharides. Transparency and homogeneity stand as the primary characteristics for fruit juices [2].

Fruit juice clarification aligns with international standards (Codex stan 63 and Codex stan 247) and is pivotal for enhanced outcomes. Effective enzymatic clarification not only boosts juice yield but also streamlines juice clarification [3]. Enzymes like pectinases,

amylases, and cellulases reduce fruit juice viscosity and turbidity. Their usage in apple juice production improves yield, clarity, and filterability [4]. The presence of pectin in fruit juice is known to maintain cloud stability, preventing the natural clarification of the juice. However, the use of commercial pectinases allows the degradation of the viscous soluble pectin, leading to the aggregation of cloud particles and the clarification of fruit juice.

Apple juice is susceptible to spoilage by various microorganisms such as yeasts (*Saccharomyces*, *Zygosaccharomyces*, and *Candida* spp.), various bacilli (*Alicyclobacillus* spp. and *Lactobacillus plantarum*), and the heat-resistant fungus *Byssochlamys fulva* [5]. Traditionally, spoilage prevention involves thermal treatments, with pasteurization being a common method. Pasteurization typically employs high-temperature short-time (HTST) treatment (75–90 °C and 20–30 s), effectively reducing the number of viable microorganisms in juices. Despite its advantages, pasteurization results in biochemical and nutritional changes that cannot be avoided. Excessive heat exposure may generate undesirable off-flavors in apple juice production [6].

Despite the availability of alternatives on the market, apple juice maintains its popularity. To meet consumer demands for the organoleptic and preservation characteristics of apple juice, the industry seeks simplified technologies for the quick clarification and stabilization of apple juice [7]. Furthermore, the increasing demand for fresh and natural juices has spurred research into non-thermal processes such as pulsed electric fields, microwave, ultrasound, high hydrostatic pressure, high pressure homogenization, etc. The main aim of the use of these non-thermal processes is to avoid the loss of nutritional components such as phenolic compounds and vitamins [8–10] as well as sensory attributes [11,12]. Moreover, these processes are usually effective against microorganisms, environmentally friendly, requiring less energy, safe devoid of any toxic compounds, and preserve the quality of the juice, minimizing the loss of nutritional compounds and biological activities. Table 1 summarizes the differences between the thermal and non-thermal pasteurization of apple juice.

Table 1. Summary of differences between thermal and non-thermal pasteurization.

Aspect	Thermal Pasteurization	Non-Thermal Pasteurization
Principle	Application of heat to reduce or inactivate microorganisms and enzymes.	Use of pressure, electric fields, ultrasounds, microwaves, or other non-thermal means to achieve pasteurization without significant heat.
Temperature Range	High temperatures, typically above 70 °C.	Lower temperatures depending on the process, avoiding extreme heat, preserving the product's sensory attributes.
Microbial Inactivation	High efficacy in microbial reduction.	Effective against many microorganisms but may not achieve the same level as thermal pasteurization.
Nutrient Retention	May cause nutrient degradation especially of vitamins and antioxidants due to heat sensitivity.	Preserves nutritional quality and flavor due to the usage of lower temperatures.
Equipment and Operating Costs	Generally lower initial costs and use common equipment.	Higher initial costs due to specialized machinery; operational costs may vary.
Processing Time	Shorter processing times.	Longer processing times, especially for non-thermal methods like high-pressure processing.
Sensory Quality	May impact color, flavor, and texture.	Preserves sensory attributes better, resulting in fresher-tasting products.

Table 1. *Cont.*

Aspect	Thermal Pasteurization	Non-Thermal Pasteurization
Shelf-Life	Extended shelf-life due to high microbial reduction.	Shelf-life extension, though may not match the longevity achieved through traditional thermal pasteurization.
Environmental Impact	Uses energy for heating.	Generally, reduced energy consumption. All depending on the process used.

Using a mixed approach, including meta-analysis and systematic review, the primary goal of this review is to explore the principles, key findings, advantages, and disadvantages related to alternative treatments for thermal processing in apple juice production, as well as the technologies employed for apple juice clarification.

2. Scientometrics and Bibliometrics Analysis

2.1. Methodology

This paper collects and analyzes the available research on different strategies and techniques for apple juice clarification and stabilization, resulting in the generation of mapping and the visualization of different parts of bibliometric records.

The Scopus database was used to retrieve the data. The Scopus database was searched for bibliometric data in January 2023 using the following keywords: (apple juice) AND (pulsed electric fields OR microwave OR ultrasonication OR high hydrostatic pressure OR high-pressure homogenization OR patulin removal OR CO_2 treatment OR ultrafiltration OR enzyme clarification). The PRISMA guidelines (www.prisma-statement.org, accessed on 2 February 2023) were used for data refinement where the total number of primary searches was 911; after filtering the documents, the final number of relevant articles was 403. For this review, all titles and abstracts of identified articles were screened by the authors, and the full text was evaluated if appropriate. Additional inclusion criteria were that the article should deal specifically with apple juice stabilization and clarification using non-thermal or novel technology. No temporal restrictions were applied to the literature search.

The resulting documents were stored in CSV format for further assessment to be analyzed using R software (version 4.2.2.) (biblioshiny function) and VOSviewer (version 1.16.19).

2.2. Results

Figure 1 illustrates the trends in the annual publication count and the cumulative document count. It is evident that the number of publications per year exhibited growth, albeit with occasional fluctuations. The highest number of publications, totaling 31, was recorded for the years 2020 and 2022. This pattern signifies a notable surge in research interest surrounding apple juice clarification and stabilization in recent years.

Table 2 provides a ranking of the top 20 most productive institutions and countries based on the analysis of 403 records. According to these records, contributions were made from 370 different institutions with only 40 of them having 10 or more publications. The most productive institution is China Agricultural University, which has produced 83 publications, accounting for 20.59% of the total collected articles.

In term of countries, studies were found from 50 different countries related to apple juice clarification and stabilization. China led the list with a frequency of 516 followed by Spain and the USA. This observation suggests that China actively engages in collaborations with other countries. Additionally, China secured the top spot in terms of the total citations (TC) with 2578 citations. However, when it comes to average article citations (Av. Art. C.), other countries, notably Ireland, Germany, Croatia, and France, demonstrated stronger performances.

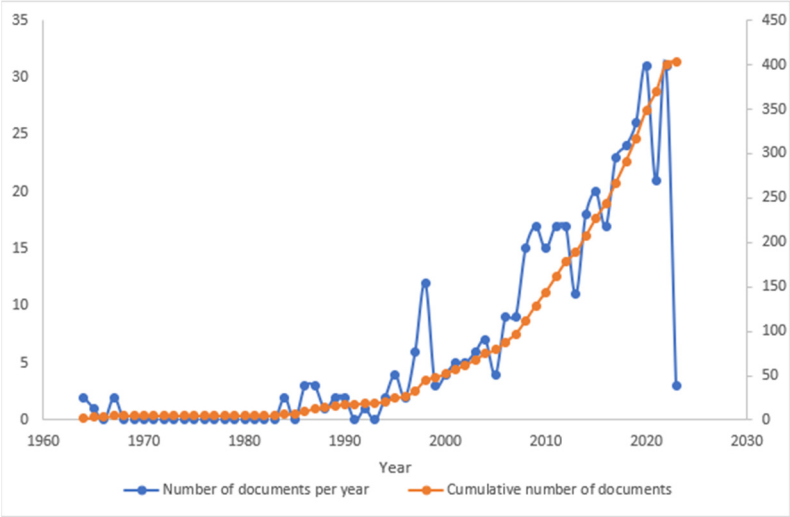

Figure 1. Trends of the number of publications per year and the cumulative number of documents.

Table 2. Top 20 most productive institutions and countries.

Affiliation	Articles	Countries	Freq	TC	Av. Art. C.
CHINA AGRICULTURAL UNIVERSITY	83	CHINA	516	2578(1)	30.69(14)
NORTHWEST AANDF UNIVERSITY	63	SPAIN	191	1113(3)	31.80(12)
NANJING AGRICULTURAL UNIVERSITY	48	USA	112	1509(2)	52.03(7)
JIANGNAN UNIVERSITY	41	ITALY	94	572(8)	33.65(10)
UNIVERSITAT AUTÒNOMA DE BARCELONA	32	BRAZIL	87	521(10)	28.94(15)
HUAZHONG AGRICULTURAL UNIVERSITY	28	TURKEY	85	937(4)	31.23(13)
UNIVERSITY COLLEGE DUBLIN	28	POLAND	79	263(15)	20.23(16)
UNIVERSITY OF ZAGREB	27	INDIA	65	360(14)	18.00(19)
UNIVERSITY OF LLEIDA	26	GERMANY	50	635(7)	79.38(2)
HACETTEPE UNIVERSITY	24	IRELAND	49	726(6)	80.67(1)
HUAIYIN NORMAL UNIVERSITY	22	IRAN	46	262(16)	20.15(17)
NOTREPORTED	20	ARGENTINA	41	453(13)	32.36(11)
UNIVERSITY OF BURGOS	20	PAKISTAN	41	45(20)	11.25(20)
UNIVERSIDAD DE ZARAGOZA	19	FRANCE	39	744(5)	67.64(4)
WASHINGTON STATE UNIVERSITY	19	CANADA	35	535(9)	59.44(5)
MEDICAL RESEARCH COUNCIL	16	CROATIA	33	462(12)	77.00(3)
UNIVERSITY OF OTAGO	15	MEXICO	24	463(11)	57.88(6)
ZHEJIANG UNIVERSITY	15	SOUTH KOREA	24	259(17)	51.80(8)
HOHENHEIM UNIVERSITY	14	PORTUGAL	23	98(19)	19.60(18)
ATATURK UNIVERSITY	13	NEW ZEALAND	22	180(18)	36.00(9)

Out of the collected records, a total of 881 keywords were identified. Notably, only 64 of them appeared more than five times. Figure 2 represents the word cloud featuring the most frequently used keywords. "Apple juice" emerges as the most cited keyword, appearing 150 times, followed by "patulin" and "ultrafiltration".

Figure 3 illustrates the co-occurrence network of the top 64 keywords, each of which has appeared more than five times in the collected records. In this visualization, each circle represents a keyword, while the connecting lines indicate the co-occurrence relationships between these keywords. The keywords are grouped into distinct clusters, each distinguished by a unique color:

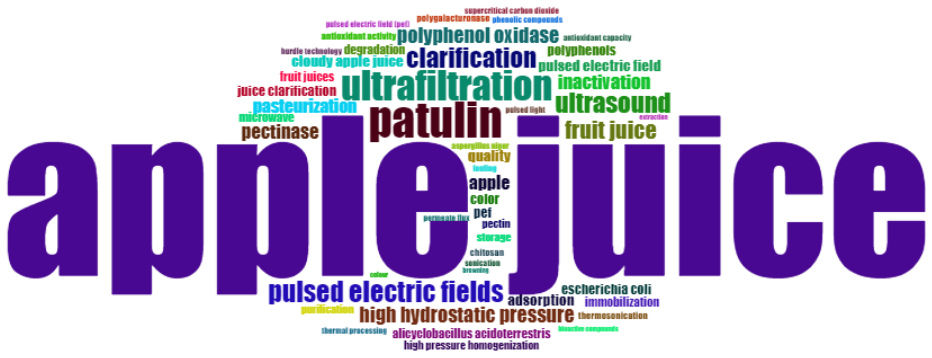

Figure 2. Word cloud of the most used keywords.

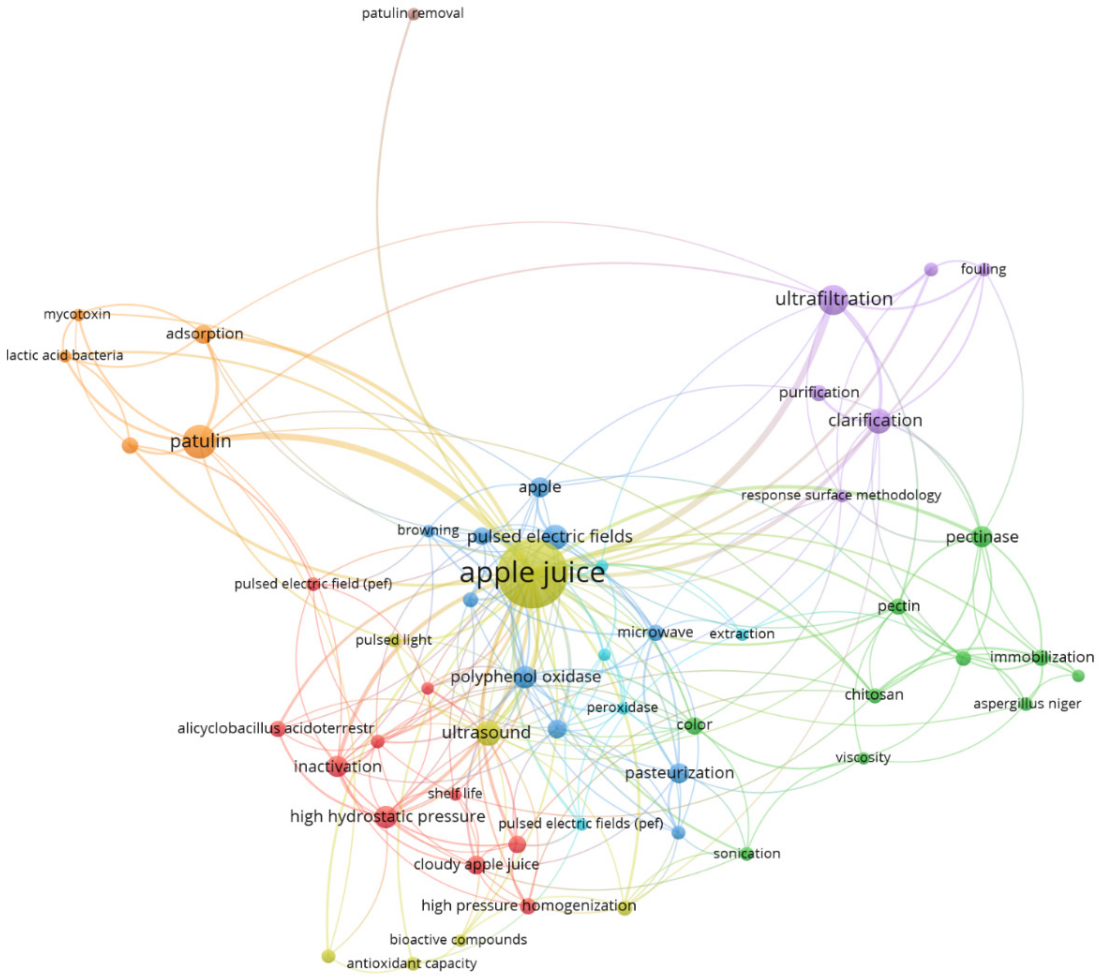

Figure 3. Co-occurrence network of the top 64 keywords.

Cluster 1 (orange): this cluster focuses on the removal or degradation of patulin in apple juice. Keywords within this cluster are "patulin", "patulin removal", "absorption", "degradation", "mycotoxin", and "lactic acid bacteria".

Cluster 2 (purple): this cluster primarily encompasses keywords related to membrane filtration in apple juice, featuring terms such as "ultrafiltration", "clarification", "fouling", "permeate flux", and "purification".

Cluster 3 (green): within this cluster, the keywords revolve around the clarification of apple juice using enzymes, and keywords associated with this cluster include "pectinase", "immobilization", "pectin", "*Aspergillus niger*", "chitosan", "viscosity", and "clarification".

Cluster 4 (red): this cluster is primarily composed of keywords related to the microbial stabilization of apple juice, incorporating terms such as "inactivation", "*Alicyclobacillus acidoterrestris*", "pulsed electric field", "high hydrostatic pressure", "high pressure homogenization", and "shelf life".

Cluster 5 (blue): keywords in this cluster are mainly related to the physico-chemical and microbial stabilization of apple juice, including terms such as "pulsed electric field", "microwave", "polyphenol oxidase", "peroxidase", "color", "polyphenol", "browning", "pasteurization", and "antioxidant activity".

These clusters and their associated keywords will be further elaborated in the subsequent sections of this review.

To conclude, bibliometric analysis has emerged as a valuable methodology in examining the landscape of food science especially the novel technologies, shedding light on the trajectory of scientific research, its challenges, and future perspectives. This statistical method taps into literature-driven insights, unveiling publication patterns and the potential applications that inform our understanding of the field of alternative processes used for apple juice stabilization and clarification. This analytical approach allowed the identification of five clusters that will be developed lately in this paper.

3. Alternative Treatments for Apple Juice Pasteurization

Currently, thermal processing stands as the prevailing method employed globally for apple juice treatment due to its ability to inactivate spoilage microorganisms and enzymes responsible for browning, thus extending shelf-life [13]. Nevertheless, this approach exerts an impact on the juice's quality, resulting in the alteration in color and the loss of flavor [13–16]. Therefore, alternative treatments to thermal processing have been developed over the years.

3.1. Pasteurization Using Pulsed Electric Fields (PEF) Treatment

PEF represents a non-thermal approach of food preservation, utilizing short- and high-voltage pulses of electricity during a short time to inactivate microorganisms while minimizing the adverse effects on food quality attributes [17]. This process is based on electroporation or electro-permeabilization that creates pores in the microorganism's cell wall by applying high-intensity electrical pulses (Figure 4). During PEF treatment, the application of an electrical field ($E \neq 0$) leads to the accumulation of free charges at inner and outer surfaces of the membrane, generating a transmembrane potential (Vm) and accumulating electro-compressive forces on the membrane surface. This results in the formation of reversible pores. Increasing the electrical field beyond a critical level provokes a dielectric rupture of the membrane and the formation of irreversible pores. The electroporation process induces the leakage of ions and cytoplasmic content as a consequence of osmotic pressure, leading to cell shrinkage and subsequently to cell lysis. A summary of the primary studies regarding apple juice pasteurization using PEF is provided in Table 3.

Table 3. Summary of the main studies dealing with the pasteurization of apple juice by PEF.

Apple Juice	Operating Conditions	Results	Reference
Freshly squeezed juice from Gala variety	Electric field strengths of 12, 24, and 36 kV cm^{-1} and replication rates of 400, 600, and 800 pulses per second.	At the highest strength applied (36 kV cm^{-1}), a reduction of seven logs in *E. coli* was achieved. At this strength with the maximum frequency of 800 pulses per second, up to six log reductions were attained.	[18]
Freshly squeezed juice from golden delicious variety	Electric field strength of 35 kV cm^{-1}, and a frequency of 1200 pulses per second.	*Lactobacillus brevis* and *Saccharomyces cerevisiae* were reduced by 6.3 and 4.2 log, respectively.	[13]

Table 3. *Cont.*

Apple Juice	Operating Conditions	Results	Reference
Apple juice prepared from concentrate	Four different levels of pre-heating temperatures (35–50 °C), electric field strengths (28–40 kV cm^{-1}), and total treatment times (25–100 µs).	8.2 log10 reduction of *Staphyloccocus aureus* obtained at 40 kV cm^{-1}, 100 µs, and 46 °C.	[19]
Freshly squeezed juice from golden delicious variety	Electric field strengths of 30.8 and 38.5 kV/cm, pulse numbers of 150, 200, 250 and 300, and the pulse frequency of 1 Hz with 200 µs pulse duration.	Highest enzyme inactivation (70% of polyphenol oxidase inactivation) attained when 38.5 kV/cm for 300 pps at 50 °C is applied.	[20]
Freshly squeezed apple juice of the Fuji variety	Pulse rise time (PRT) of 2 µs and 0.2 µs, 16 Hz pulse frequency, and electric field strengths of 25, 30, and 35 kV cm^{-1}.	Residual activities of polyphenol oxidase (PPO) and peroxidase (POD) were 1.5 and 5.8%, respectively, at 35 kV cm^{-1} and 2 µs.	[21]
Freshly squeezed apple juice, from golden delicious apple variety	Electric field strengths of 30 and 40 kV cm^{-1} for 50, 100, 150, and 200 pulses after preheating to 40 °C.	No microbial activity occurred in PEF-treated apple juices during the 3-month storage period. PPO activity became completely inactive at 100 or more pulses at 40 kV cm^{-1}.	[22]
Royal Gala apple juice	Electric field strength of 24.8 kV/cm, 60 pulses, 169 ms treatment time, and 53.8 °C.	Increase in the total color difference after treatment. A 19% decrease in the antioxidant activity after 30 days of storage. A decrease of 85% in polyphenol oxidase activity.	[23]
Unclarified, unpasteurized, and unsweetened apple juice	Different number of cycles: 4, 6, and 8 (total 200, 300, and 400 pulses, respectively). Electric field strength was 30 kV cm^{-1}. Each cycle consisted of 50 pulses (one pulse every 30 s).	PEF-treated juice did not show changes in the amount of vitamin C and the total polyphenol content during storage for 72 h under refrigeration. PEF treatment was the effective method for the inactivation of a wide range of the most common food spoilage microorganisms (mesophilic bacteria, microscopic fungi, and yeasts).	[24]
Cloudy apple juice (Belgian cultivars)	Low intensity: electric field strength of 12.5 kV/cm, flow of 27.6 L/h, energy input of 76.4 kJ/L, frequency of 62 Hz, T_{inlet} of 37.6 °C, and T_{outlet} of 59.5 °C. High intensity: electric field strength of 12.3 kV/cm, flow of 24.5 L/h, energy input of 132.5 kJ/L, frequency of 94 Hz, T_{inlet} of 37.3 °C, and T_{outlet} of 72.8–73.8 °C.	Increase in color intensity. Decrease in enzyme activity using the high-intensity PEF. Decrease in Vit C after storage. No significant changes in pH, titratable acidity, organic acid, and sugar content, which also corresponded to a sweet and sour taste.	[25]
Sterile apple juice (Thailand)	Field strength: 10–30 kV/cm, pulse number: 1–300, pulse, and width: 1–2.5 µs.	Higher yeast inactivation (about 4 logs) observed with a treatment at 30 kV/cm and 100 pulses.	[26]

PEF research dates back to 1998 [27,28]. A comparative study was conducted to assess the advantages of pulsed electric fields (PEF) over thermal processing for pasteurizing apple juice, with a focus on preserving the juice's inherent characteristics [18]. Both conventional pasteurization (high temperature-short time (HTST)) and PEF treatments were applied to apple juice samples [18]. The study reported significant color changes in both juices subjected to different treatments, but PEF appeared to better retain the natural product's color. Sulaiman et al. [23] compared thermal and PEF treatments in terms of enzymes activity. Sensory analysis indicated perceptible taste differences between pulsed electric field-treated juices and thermally treated juice. Juice processed via PEF exhibited stability during storage, with consistent pH and soluble solids, and no signs

of fermentation. Polyphenol oxidase remained inactive during storage. The PEF process resulted in a reduction in polyphenol oxidase and peroxidase enzymes [25]. In comparison to thermal processing, fresh juice had the highest vitamin C content, followed by PEF-treated juices. Untargeted volatile profiles revealed an increase in esters after PEF treatment, while thermal processing exhibited ester degradation reactions alongside the development of off-flavors [29]. PEF treatment of whole apple fruit or apple slices increased the juice yield and concurrently elevated the polyphenol content and the antioxidant capacity of the juice [30].

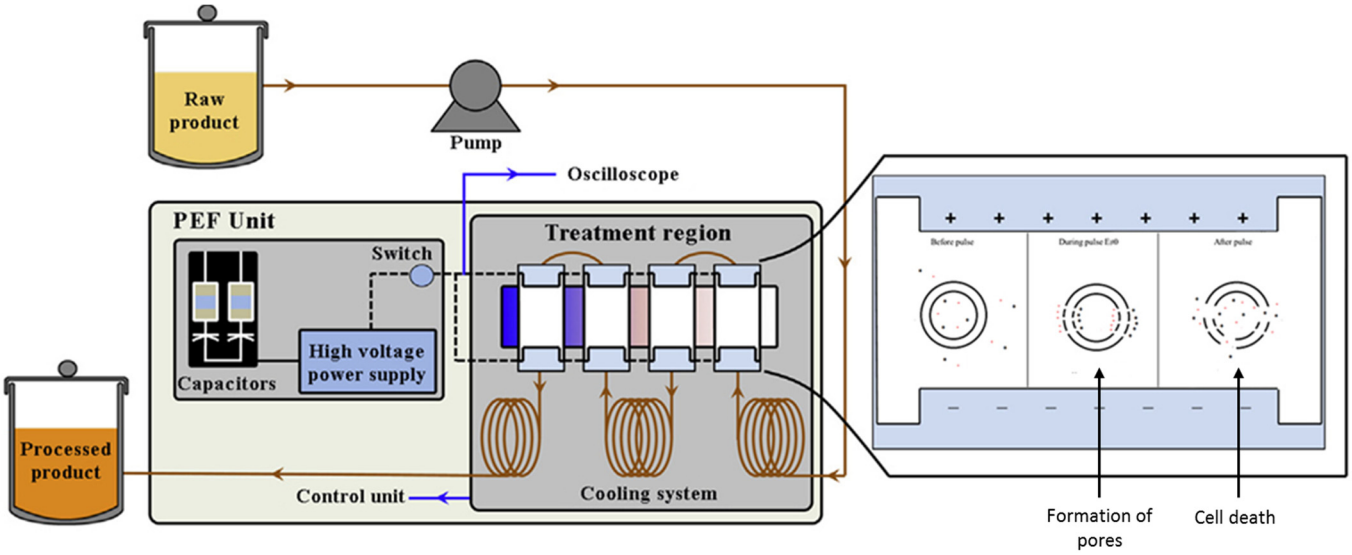

Figure 4. The PEF pasteurization process principle using the electroporation process.

3.2. Pasteurization Using Microwave Processing

Microwaves are electromagnetic waves characterized by the electrical field and the magnetic field, using frequencies ranging between 300 MHz and 300 GHz. The most used frequency for microbial inactivation in food matrices is 2.45 GHz [31]. Microwaves interact with polar molecules and ions. The polar molecules rotate and collide with each other under an oscillating electric field (Figure 5). In parallel, the ions accelerate, moving along the electric field and colliding with water molecules, which creates friction that releases thermal energy (Figure 6) [32].

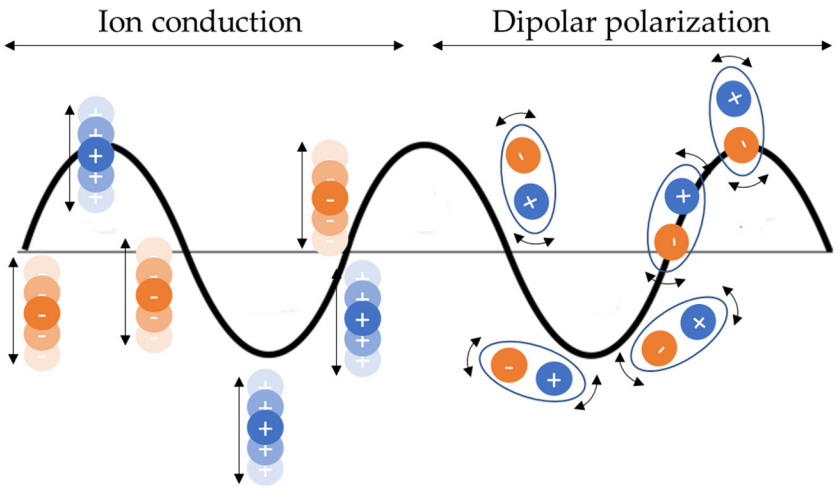

Figure 5. Ion conduction and dipolar polarization phenomena in microwave processing.

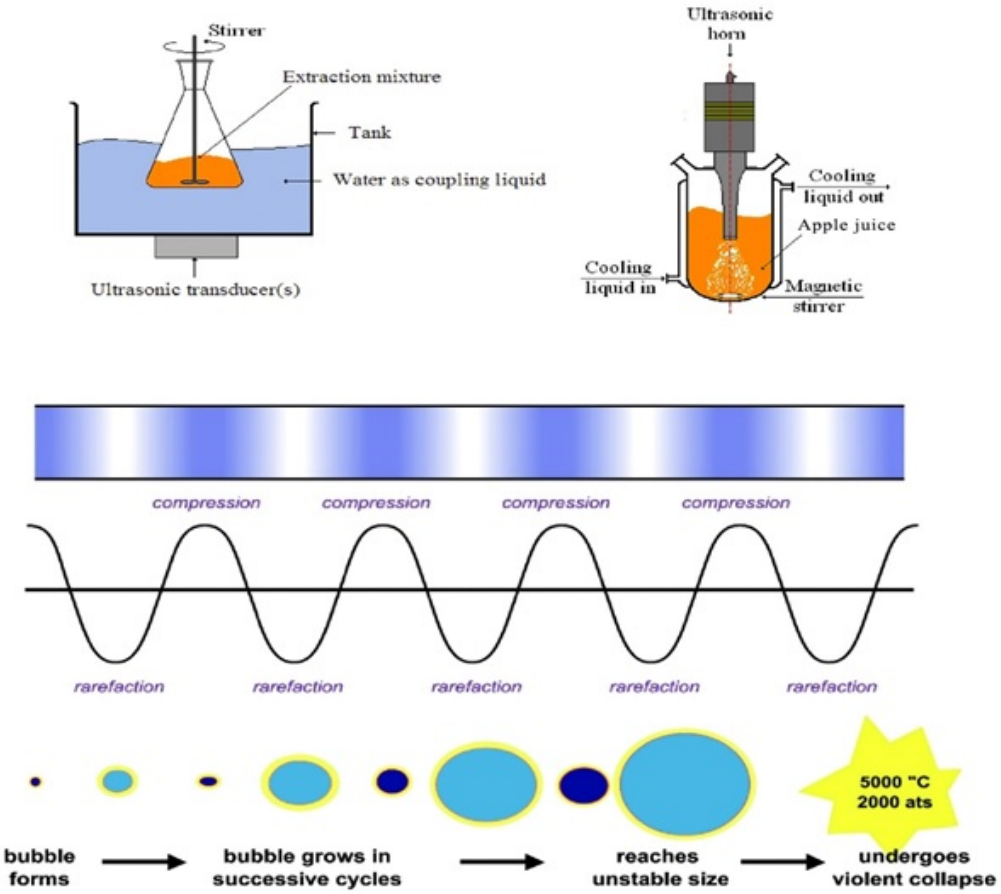

Figure 6. The ultrasound processing instrumentation and the creation of cavitation bubbles and their collapse.

Siguemoto et al. [33] showed that the application of non-thermal microwave to cloudy apple juice did not inactivate pectin methylesterase (PME), polyphenol oxidase (PPO), and peroxidase (POD). In contrast, Gen et al. [34] showed that microwave sterilization, using high temperature, could more effectively inactivate enzymes (PPO and PME), better than microwave pasteurizing, when lower temperatures are applied, while better maintaining the original color of the juice and significantly increasing its polyphenol content and its antioxidant capacity.

The impact of microwave processing on soluble sugars, organic acids, volatile compounds, phenolic contents, and antioxidant capacities of cloudy apple juice was evaluated [35]. Results showed that the alternative method (microwave pasteurization) preserved the juice and offered a better volatile profile compared to juice pasteurized using conventional method [35]. Furthermore, Zhang and Zhang [36] demonstrated that by using the appropriate microwave pretreatment mode for apple raw material, some specific nutrients and antioxidant activities could be enhanced. For instance, when treating apple juice with 900 W microwave through 75 s, the polyphenol content increased by 115% compared to other treatment methods.

On the other hand, several studies measured the efficacity of microwave on microbial populations like *Alicyclobacillus acidoterrestris*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* [33,36–40]. The details of their findings can be found in Table 4.

Table 4. Summary of the main studies dealing with the microwave pasteurization of apple juice.

Apple Juice	Operating Conditions	Results	Reference
Commercial pasteurized apple juice inoculated with the precultures of *S. cerevisiae* and *L. plantarum*	A 700 W and 2450 MHz microwave oven (Model RE620TC). Exit temperatures ranging between 52.5 and 65 °C.	D-values under microwave heating were 4.8, 2.1, and 1.1 s at 52.5, 55, and 57 °C, respectively, for *Saccharomyces cerevisiae*, and 14, 3.8, and 0.79 s at 57.5, 60, and 62.5 °C, respectively, for *Lactobacillus plantarum*, with corresponding z-values of 7 and 4.5.	[39]
Unwaxed Royal Gala red apple	Samples treated at 900, 720, 450, and 270 W with a continuous flow energy for times ranging between 40 and 90 s (defined as zero dwell time).	Decrease in *Escherichia coli* populations in apple juice at 900 and 720 W power levels for 60 and 90 s. Decimal reduction times ranged from 0.42 min at 900 W to 3.88 min at 270 W. 2–4 logs population reduction in apple juice pasteurized at 720–900 W for 60–90 s.	[40]
Juice produced by squeezing apples	Different power modes (90 W, 270 W, 450 W, 720 W, and 900 W) and times (25, 50, 75, 100, and 125 s).	Longer time at 900 W resulted in a more effective prevention against *Alicyclobacillus acidoterrestris*. Weak destruction of *Alicyclobacillus acidoterrestris* in shorter time and lower power.	[36]
Commercial apple juice	Microwave heating at four different power levels, i.e., 400 W, 600 W, 800 W, and 1000 W, with different heating times ranging between 50 and 390 s.	5-log10 reduction of *E. coli* and *L. monocytogenes*, as recommended by the FDA.	[33]
Fresh apple juice	A domestic microwave oven with a 2450 MHz frequency. The treatment time fixed at 20, 40, 60, 80, and 100 s and various power levels at 180, 300, 450, 600, and 900 W.	For the time duration of 80 to 100 s, the deactivation of *E. Coli* cells occurred. The inactivation of yeast cells occurred at 60 s.	[38]
"Golden delicious" apples (Malus domestica Borkh, cv. Golden Delicious)	A customized Panasonic microwave oven (2450 MHz and 1200 W). Different power levels (600 W and 720 W) with different treatment times (5 s, 10 s, 15 s, 20 s, and 25 s).	Reduction of *E. coli* O157:H7 and *Salmonella Typhimurium* by 7-log units with 720 W at 25 s treatment, meeting the FDA guidelines for processed juices.	[37]

3.3. Pasteurization Followed by Ultrasonication (US)

Ultrasonication is a process that uses high-frequency sound energies to disintegrate particle agglomerates through cavitation, a phenomenon involving the creation, expansion, and implosion of bubbles [41]. The major effects of US on extraction in liquid medium are attributed to the cavitation process. This process emerges from the formation of microbubbles formed from gases initially dissolved in the liquid, resulting in various effects, such as surface peeling, erosion, and particle breakdown (Figure 6) [42].

The effect of ultrasound on Gram-positive and Gram-negative bacteria has stirred controversy. Some studies suggest that Gram-positive bacteria exhibit greater resistance than Gram-negative ones, potentially due to the thicker cell wall of Gram-positive bacteria [43]. These studies propose that ultrasound may target the lipopolysaccharide layer of Gram-negative bacteria. Conversely, other studies have reported that ultrasound techniques are effective against both bacterial groups [44]. A summary of the effects of US on various microorganisms can be found in Table 5.

Table 5. Summary of the main studies on the US treatment of apple juice.

Apple Juice	Operating Conditions	Results	Reference
Malus domestica cv. Fuji purchased from a local fruit market, Nanjing, China	Juice treatment with ultrasound (for 0, 30, 60, and 90 min, at 20 °C and 25 kHz frequency).	A significant decrease in the total plate count of samples sonicated for 60 and 90 min. Significant decrease in yeast and mold in all the sonicated samples as compared to those in the control sample.	[45]
Golden delicious, Turkey	Fresh juice treatment parameters: various amplitude levels (50 and 100 µm), ultrasonic pulse durations (50% and 100%), treatment temperatures (40, 50, and 60 °C), and exposure times (5 and 10 min) at a constant frequency of 24 kHz.	The total inactivation of yeasts and molds obtained at 60 °C with an amplitude of 100 µm and a pulse of 100% for 5 min. Similar results obtained at a lower temperature with an increase in treatment duration.	[46]
Fresh apples (M. domestica cv. Fuji) Nanjing, China	Sonication treatment: 70% amplitude, 25 kHz frequency, and 30- and 60-min duration at 20 °C.	After 30 min treatment, significant increase in sugars and polyphenolic compounds was observed. At 60 min, the total carotenoids and mineral elements (Na, K, and Ca) increased. For both treatment times, no changes in the total anthocyanins and the Zn contents.	[47]
Commercial and natural squeezed apple juice	Treatment parameters: 600 W, 20 kHz, and 95.2 µm wave amplitude for 10 or 30 min at 20, 30, or 44 ± 1 °C.	Ultrasound treatment inefficient against the inactivation of *Alicylcobacillus acidoterrestris*. The moderate inactivation of Saccharomyces cerevisiae cells for both juices after 30 min treatment at 44 °C.	[48]
Red apples, North Carolina, USA	The ultrasound device was set at 45% amplitude for the inactivation of *E. coli* and 50% amplitude for *S. aureus*. The generated AED (average acoustic energy density) was about 18.3 W/(mL liquid). The continuous pulse mode was applied, and each pulse comprised 5 s of processing time and 30 s of pause time.	Ultrasound treatment effectiveness related to the pulp content of juice. Treatment was less lethal to *S. aureus*, while it had no significant effect on *E. coli* in high pulp juice. But when apple free pulp juice was processed ultrasonically, the 5-log reduction time was 35 s for *E. coli* at 60 °C and 30 s for *S. aureus* at 62 °C. No effect of the treatment was observed on antioxidant activity, while it increased the total phenolic content.	[49]
Fresh 'Ralls' apples (Malus pumila Mill.), Jinzhou, Liaoning Province, China	They ultrasound-sterilized apple juice for 12 min at 50 °C in a circulating water bath using an JRA-20CQ, Wuxi, China ultrasonic sterilizer. A constant frequency of 20 kHz and a 975 W power output were applied.	Reduction in microbial load to below 1 CFU/log after US treatment. The ultrasound sterilization significantly improved the contents of soluble protein, ascorbic acid, soluble pectin, and the total soluble content of apple juice.	[50]

The literature has presented some conflicting findings regarding the inactivation of microorganisms in apple juice through ultrasonication. Ertugay and Baslar [46] claimed that the US treatment completely inactivated yeasts, while Ferrrario et al. [48] demonstrated a moderate inactivation (2.5 log) of *Saccharomyces cerevisiae* cells during the US treatment of apple juice. These studies highlight that temperature plays a pivotal role in US treatment, with higher temperatures resulting in more effective microbial inactivation. It is worth noting that several studies have suggested that US alone may not be entirely efficient, particularly when dealing with spore inactivation [48,51]. Consequently, different studies have illustrated the effectiveness of combining ultrasonication with other treatment methods [52–54]. The combination of US with high hydrostatic pressure (HHP) was shown to be more effective in microbial inactivation than US treatment alone [55,56]. It was found

that the combination of these processes with HHP functioning at 450 MPa led to the total inactivation of yeasts and molds and the reduction in the total plate counts [56]. The application of nisin-assisted thermosonication (TS) for apple juice pasteurization has also been explored, with results showing that TS + nisin enhances the inactivation of aerobic bacteria (AB) almost by a factor of two in fresh apple juice compared to treatment with nisin + mild heat (MH) or TS alone [57]. The inactivation of naturally occurring microorganisms in fresh apple juice was found to be significantly influenced by temperature.

Ultrasonication has also been tested for enzyme inactivation and the reduction in juice browning. Sun et al. [58] showed that the ultrasound treatment of fresh apple juice can inhibit juice browning. Bot et al. [59] provided evidence of the efficacy of US for enzyme inactivation in apple juice. With temperature control, US led to 90% decrease in polyphenol oxidase at the longest treatment time (45 min), while the total enzyme inactivation was achieved by subjecting samples to 6 min of US without temperature control. The combination of US and HHP proved to be efficient against enzyme inactivation, targeting polyphenol oxidase (79% decrease), peroxidase (67% decrease), and pectin methyl esterase (76% decrease) [56].

The impact of US on apple juice quality has been thoroughly evaluated. US applied to fresh apple juice samples for 0, 30, and 60 min at 20 °C (frequency of 25 kHz and amplitude of 70%) resulted in significant enhancements in sugars, polyphenolic compounds, and the total carotenoids compared to those of an untreated sample [47]. US was shown to significantly improve ascorbic acid, cloud value, phenolic compounds, and antioxidant capacity [45]. Zhu et al. [50] further confirmed that US remarkably improved the content of soluble protein, ascorbic acid, total soluble solid, and soluble pectin in apple juice. Compared to microwave sterilization, ultrahigh pressure sterilization, and conventional pasteurization, US demonstrated its ability to preserve the bioactive compounds of apple juice [60,61].

3.4. High Pressure Homogenization (HPH) Treatment

HPH principle for microbial inactivation in liquid foods is based on shear forces, cavitation, and turbulence that occur where the liquid is flowing through the homogenizing valve gap. The cells that are subjected to high mechanical stress are disrupted and disintegrated. The pressure used in HPH ranged between 100 and 400 MPa. Later, a depressurization phenomenon to 10–20 MPa occurs, leading to an increase in temperature (1.5–3.0 °C for every 10 MPa of pressure) [62]. The principle of this treatment is illustrated in Figure 7.

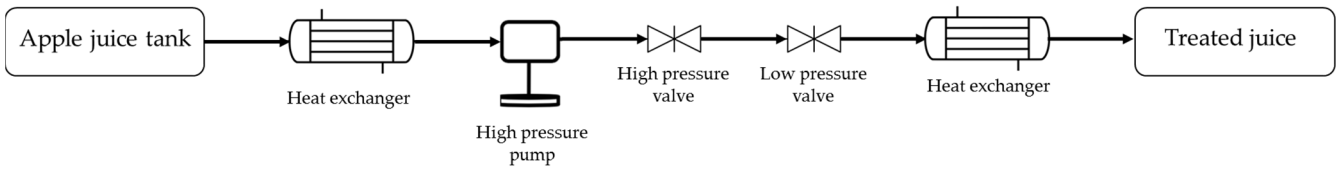

Figure 7. The flow diagram of HPH process.

Mckay [63] showed that the HPH at 300 MPa reduces the spore concentrations of *Saccharomyces cerevisiae*, *Exophiala phaeomuriformis*, and *Aureobasidium pullulans* and also the concentrations of *Penicillium expansum*, *Aspergillus niger*, and *Byssochlamys fulva* conidiospores in apple juice.

On the other hand, apple juice treatment by HPH (100–200 MPa) showed a mild inactivation of microorganisms and enzymes [64]. Also, HPH treatments at 100 MPa and 200 MPa were ineffective in inactivating *Taloramyces macrosporus* and *Neosartaya spinosa* ascospores [65]. However, Sauceda-Gálvez et al. [66] demonstrated that UHPH (ultra high-pressure homogenization) treatment at 300 MPa was efficient in the inactivation *A. acidoterrestris* at 4.8 log10 in apple juice heated at 80 °C.

Bevilacqua et al. [67] investigated the application of natural antimicrobials like limonene and citrus extract in conjunction with high-pressure homogenization (HPH) to manage the

proliferation of *Saccharomyces bayanus* in apple juice. The findings highlighted the efficacy of citrus combined with HPH and limonene with citrus extract. Specifically, the colony count was diminished to undetectable levels. Nevertheless, the utilization of limonene proved inconsistent due to its significant impact on the sensory attributes of apple juice [67].

The inactivation of enzymes by HPH was evaluated. Bot et al. [59] showed that this technique is not effective against the inactivation of enzymes. However, apple juice treatment with ultrahigh pressure homogenization (300 MPa) showed a totally inactivated polyphenol oxidase and a great decrease in pectin methyl esterase [66].

3.5. High Hydrostatic Pressure (HHP) Treatment

This technology consists of applying high pressure (100 to 800 MPa) to the packaged juice by compressing the surrounding water and transmitting pressure throughout the product uniformly and rapidly as shown in Figure 8 [68]. In the literature, HHP was tested on apple juices for its potential effect on microbial and enzyme inactivation.

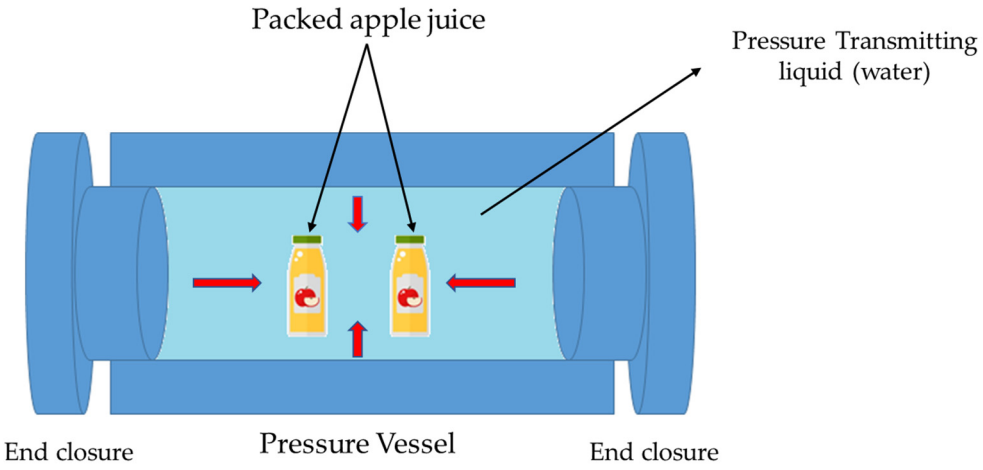

Figure 8. HHP pressure vessel illustration for apple juice treatment.

Concerning microbial stabilization, Mckay et al. [69] evaluated the impact of high hydrostatic pressure (HHP) on the microbial quality of fresh apple juice during storage at an optimal temperature (4 °C). They found that the total aerobic counts significantly decreased after a 1-minute treatment at 500 and 600 MPa, and these counts did not notably increase during storage at 4 °C. Additionally, Bayındırlı et al. [70] demonstrated the total inactivation of *Staphylococcus. aureus* 485, *Escherichia coli* O157:H7, and *Salmonella* Enteritidis at 350 MPa and 40 °C within 5 min. Conversely, Sokołowska et al. [71] indicated that the HHP treatment of apple juice (200 MPa, 50 °C) is not entirely effective against *Alicyclobacillus acidoterrestris*, with its effectiveness linked to the juice's total solid content. However, HPP treatments combined with heat, as shown by Hartyáni et al. [72], can deactivate *A. acidoterrestris*, maintaining microbial stability during storage at 4 °C.

HPP was also tested in combination with TiO_2-UV photocatalysis (TUVP) for the inactivation of microorganisms in commercial apple juice [73]. The combined treatment showed a higher inactivation of microorganisms than HHP alone. A total inactivation of Gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) was obtained, while Gram-negative bacteria, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium, were reduced by 7.1 and 7.2 log CFU/mL, respectively.

Juarez-Enriquez et al. [74] used the ultrahigh hydrostatic pressure (UHHP) for apple juice treatment. Results demonstrated the total inactivation of microorganisms and a reduction in polyphenol oxidase (PPO) and pectin methyl esterase (PME) activity, allowing an extension of juice shelf-life without alterations in the organoleptic characteristics.

The impact of high hydrostatic pressure treatment (HHP) at 500 MPa for 3 min at 25 °C on cloudy apple juice's bioactive compounds, antioxidant properties, immunomod-

ulatory effects, and microbial safety was investigated [75]. Findings indicated that HHP treatment did not significantly alter the vitamin C content, while it increased the total polyphenol content. The antioxidant capacities, assessed through DPPH and nitric oxide (NO) radical scavenging tests, remained unchanged after HHP treatment. Additionally, the immunomodulatory properties of the apple juice were preserved by HHP. Moreover, the HHP-treated juice demonstrated microbiological safety and experienced no physicochemical changes during a 21-day storage period at 4 °C [75].

3.6. Carbone Dioxide Treatment for Apple Juice Preservation

Supercritical carbon dioxide processing (SCCD) is a non-thermal technology that has shown effectiveness in preserving heat-sensitive compounds, decreasing enzymes activity, and inactivating microorganisms in food. SCCD presents a significant advantage when compared to other alternative pasteurization treatments like HPP and HPH because it operates at relatively low pressures and temperatures.

Marszałek et al. [76] studied the impact of SCCD (10–60 MPa/45 °C/30 min) on the enzymatic, physicochemical, and nutritional parameters of apple juice. It was concluded that no significant changes in sugars and the total polyphenol content were observed after SCCD treatment, while a significant degradation was noticed in Vitamin C and the individual polyphenol contents. Concerning the inactivation of oxidative enzymes in apple juice, the inactivation kinetics significantly depended on the SCCD parameters [77]. An increase in the temperature and pressure resulted in an increase in the k-value and a decrease in the D-value for PPO and POD.

Few studies deal with the effect of SCCD on the inactivation of microorganisms in apple juice. Therefore, research on this matter is a must in order to determine the optimum operating conditions. However, the combination of SCCD with HPP showed moderate effectiveness in the inactivation of *A. acidoterrestris* spores in apple juices [78].

High-pressure carbon dioxide (HP-CO_2) is also proposed as an alternative non-thermal pasteurization technique for foods. This technique is a cold pasteurization method affecting the microorganisms' survival and enzymes through the molecular effects of CO_2 maintained at pressures of less than 50 MPa [79]. Manzocco et al. [80] focused on a high-pressure carbon dioxide treatment and its impact on polyphenoloxidase activity and the stability of fresh apple juice when compared with pasteurization. The results showed a strong impact of pressure on the enzymatic activity. The enzyme is strongly denatured while increasing the pressure. However, the HP-CO_2 treatment was not able to reduce browning phenomena during storage. Concerning microbial stabilization, the lactic acid bacteria showed a noticeable sensitivity toward the high-pressure carbon dioxide treatment contrary to yeast [80]. Illera et al. [81] concluded that the HP-CO_2 treatment of apple juice was a valid alternative technology for PPO and PME inactivation. However, Illera et al. [82] showed that apple juice treated with HP-CO_2 at 20 MPa, 45 °C, and 60 min was successful in inactivating only PPO but not PME. Guangsen et al. [83] concluded that HP-CO_2 juice processing technique can be used effectively for processing fruit juices with quality attributes.

3.7. Other Processes

Gialleli et al. [84] evaluated an innovative method to preserve apple juice by using a nano/micro-porous cellulosic material. It was concluded that this technique could be a good substitute for conventional thermal pasteurization treatment. The treated apple juice samples showed an immobilization of both *S. cerevisiae* and *L. plantarum* cells in the pores and tubes of tubular cellulose (TC). No significant changes were observed in polyphenol levels and in malic acid [84]. However, a reduction in the concentration of volatile compounds of the apple juice was observed after the treatment [84]. The proposed process presented a remarkable advantage by being a cost-effective treatment for the microbial stabilization of apple juice.

Cold plasma is another non-thermal alternative technology used in food preservation. The Dielectric Barrier Discharges (DBD) is the most efficient method to produce cold plasma.

Cold plasma consists of ionized gas carrying a mix of electrons, charged ions, excited molecules, free radicals, and UV photons. This technique was evaluated for its capacity to inactivate bacteria and yeasts in apple juice, especially osmophilic yeasts [85]. For this reason, the *Zygosaccharomyces rouxii* strains LB and 1130 were suspended in apple juice after the plasma treatment, and results demonstrated that increasing discharge voltages can reduce the plasma treatment time and achieve the desired microbial inactivation level [85]. The mechanisms of microbial inactivation by cold plasma are not completely understood. The inactivation can be attributed to the generation of highly reactive species, especially the reactive oxygen species (ROS) and the reactive nitrogen species (RNS), which peroxidize membrane lipids, increase membrane permeabilization, denature proteins structures, damage DNA, and change the inner pH of a microorganism [86].

4. Drawbacks of the Non-Thermal Pasteurization Processes

Non-thermal pasteurization processes in apple juice, while offering advantages in terms of preserving the nutritional quality and flavor of the product, also present certain drawbacks. These drawbacks are presented in Table 6.

Table 6. Principal drawbacks associated with the non-thermal pasteurization processes of apple juice.

Process	Drawbacks
PEF	Limited effect on some enzymes and microbial spores High-cost investment Needs a cooling system due to ohmic heating Difficulties in scaling-up Possible contamination of juice due to the erosion of electrodes
Microwave	High energy consumption Cooling systems are needed Non uniform sports resulting in the presence of cold spots
US	Limited antimicrobial effect Can affect juice quality at high doses Production of oxidation products such as free radicals Limited intensity of industrial scale equipment
HHP	Batch functioning mode limiting its industrial use High energy consumption High investment cost Limited effect on spores and some enzymes Only plastic packaging materials can be used
SCCD	High equipment and operation costs Long processing time for turbid juices Needs temperatures higher than 55 °C for enzyme inactivation
UV	Ineffective for turbid juice due to low UV transmittance Limited effect on spores' inactivation Can damage some vitamins and antioxidants Low recognition and acceptability by the consumer
Cold Plasma	High-cost equipment Skilled trained operators needed Oxidation and destruction of vitamins and antioxidants Difficulties in scaling-up

5. Patulin Treatment

Patulin is a mycotoxin produced by fungi (*Penicillium*, *Aspergillus*, and *Byssochlamys*) that has been found in apple juices and nectars, causing health hazards and economic losses worldwide [87]. Treating apple juice to decrease its patulin levels is an interesting factor to consider since patulin levels are regulated in many countries. For example, 50 μg/L is the

maximum allowable level by Food and Drug Administration in the USA and the European Union [87].

Nevertheless, even within countries enforcing regulated standards and despite juice processors' efforts to monitor raw material quality, instances of samples slightly exceeding the permitted maximum levels can be identified in the market. However, since pre-harvest treatments do not consistently guarantee the sufficiently low levels of patulin in juices, various methods have been explored to eliminate or break down this toxin during processing.

Patulin is proven to exhibit high heat resistance in low pH environments; hence, the standard pasteurization method for apple juice (71 °C for 6 s) proves inadequate in eliminating a significant quantity of this mycotoxin [88]. This fact has pushed researchers to test alternative processes and techniques for the thermal elimination of patulin.

Ultraviolet (UV) radiation was studied for patulin reduction in apple juice. All studies showed a significant decrease in patulin levels. Assatarakul et al. [89] showed that UV exposure, ranging from 14.2 mJ/cm^2 (one pass) to 99.4 mJ/cm^2 (seven passes), was successful in reducing patulin levels by 21.43% to 94.86%, respectively. Three UVC wavelengths (222, 254, and 282 nm) were evaluated for patulin degradation in apple juice. A 90% reduction of patulin was obtained when the three wavelengths were combined.

The far UVC (222 nm) possessed the highest efficiency for patulin reduction in apple juice [90]. Diao et al. [91] also revealed that UV irradiation of apple juice significantly decreased patulin from 99.42 µg/L to 16.98 µg/L (82.92% reduction) after exposure for 5 min at UV intensity of 3.8 mW/cm^2. Additionally, it was shown that the treatment of apple juice containing patulin with UV light (254 nm and 450 kJ/cm^2) resulted in more than 98% reduction in patulin and at the same time an inactivation of the spores of *Penicillium expansum* [92].

The effect of pulsed light (PL) was tested in different doses on patulin degradation in artificially contaminated apple juice. The exposure of all samples to PL doses between 2.4 and 35.8 J/cm^2 resulted in a significant decrease in patulin levels [93]. Pulsed light-treated apple juice supplemented with Glutathione and Fe^{2+} showed 97.3% reduction in patulin levels [94]. The same reduction in patulin levels (96.27%) was obtained when PL treatment (40.50 J/cm^2 for 6 min 30 s) was applied [95].

Pulsed high hydrostatic pressure was evaluated for the patulin decontamination of artificially contaminated apple juice. Apple juice samples were treated for 5 min at different pressure treatments (300–500 MPa) in combination with different temperatures (30–50 °C) and pulses (6 pulses × 50 s and 2 pulses × 150 s). Results showed that the levels of patulin can be reduced up to 62.11% when the pressure is applied with mild heat and pulses [96].

Gaseous ozone is a strong oxidizer used in the food industry as an antimicrobial and proven to be useful for mycotoxin decontamination by interacting with its functional groups and changing its molecular structure. This led researchers to test ozone for patulin decontamination in apple juice. Tests showed that the ozone treatment at a concentration of 12 mg/L and flow rate of 3 L/min for 15 min reduces patulin by 75.36% in apple juice [97]. In order to improve the decontamination efficacity of patulin, some parameters should be taken into consideration such as ozone concentration, treatment time, pH level, patulin concentration, and soluble solids [91].

Bioremoval is an effective and inexpensive way of removing and eliminating mycotoxin from juices. The bioremoval of patulin from apple juice has been investigated using the different types of microorganisms such as inactivated *Alicyclobacillus* strains and lactic acid bacteria. Inactivated *Alicyclobacillus* at a concentration of 40 g/L reduces patulin levels in apple juice by a maximum of 88.8% after 24 h of incubation [98]. Furthermore, Sajid et al. [99] demonstrated that heat-inactivated *Alicyclobacillus acidocaldarius* DSM 451 cells and spores showed a high adsorption capacity for patulin at 30 °C and pH 4.0 for 24 h. Lactic acid bacteria have also been studied for the bioremoval of patulin. The application of *Lactobacillus pentosus* DSM 20314, *Lactobacillus plantarum* ATCC 8014, *Lactobacillus kefiranofacien* JKSP109, and *Lactobacillus casei* YZU01 decreased patulin levels in apple juice by 53,14%, 59.74%, 93%, and 95%, respectively [100–103].

6. Apple Juice Clarification

6.1. Membrane Filtration

Membrane filtration technology has become prominent as an alternative to conventional clarification methods in the fruit juice industry because it requires less manpower, and it reduces operating costs. It also preserves the phytonutrients in fruit juices due to its low-temperature functioning. The membrane processes used in the clarification of apple juices are microfiltration and ultrafiltration. The difference between these two processes is the mean pore diameters of the membranes and the operating conditions.

Membranes processes for apple juice clarification began to be tested in the late 1980s and the early 1990s [104]. Both microfiltration and ultrafiltration processes were applied, but the juice processed using microfiltration was retained by the taste panel [105]. Apple juice was filtered by microfiltration and ultrafiltration ceramic membranes. After the optimization of operating conditions (8 m/s, 414 kPa, and 50 °C), ultrafiltration showed higher fluxes and less fouling than the microfiltration membrane [106]. Gökmen et al. [107] showed that the combination of ultrafiltration and the laccase treatment of apple juice is an effective process for polyphenol removal and for obtaining a stable juice in terms of color and clarity. Furthermore, the use of custom-designed membranes crafted from polyethersulfone (PES) and polyvinylpyrrolidone (PVP) in the ultrafiltration process proved more efficient compared to commercially available cellulose membranes. Specifically, it demonstrated greater effectiveness in reducing both polyphenols and yellowish-brown pigments in apple juice [108]. Zárate-Rodríguez et al. [28] concluded that the ultrafiltration process is efficient for apple juice clarification, with the preserving of quality aspects. Youn et al. [109] studied the clarifying process of apple juice using membrane filtration with a filter-aid in order to obtain a stable juice by replacing the treatment with traditional finning agents (gelatin, bentonite, and silica gel) with membrane processes. Two filter-aids were used for this purpose, i.e., bentonite and polyvinylpolypyrrolidone (PVPP) on the one hand and the enzymes, pectinase and amylase, on the other hand [109]. Pretreated apple juice was filtered using a filter-aid and then membrane separation was performed using microfiltration and ultrafiltration. Results showed ultrafiltration with bentonite pretreatment to be an optimal process for apple juice clarification [109]. The pretreatment in gelatin and bentonite during ultrafiltration proved its ability to limit the adverse effects of foulants on the flux performance [110]. The membrane technologies were studied for the clarification and concentration of apple juice [111]. The raw apple juice pretreated with gelatin and bentonite was ultrafiltered through 10 kDa and 100 kDa membranes. Results highlighted the effectiveness of this methodology toward the clarification of apple juice without altering the juice composition.

It is well known that the operating conditions such as transmembrane pressure (TMP), cross-flow velocity (CFV), temperature, and volume concentration ratio (VCR) in the membrane processes and the membrane characteristics influence the permeate fluxes and the performances of these processes [112].

He et al. [113] studied the impact of operation conditions of ultrafiltration on apple juice without using enzymes treatments and pasteurization. They found that increasing the transmembrane pressure (TMP) increased the initial permeate fluxes till 2 bar where a plateau was observed. Increasing the cross-flow velocity and the temperature from 23 to 50 °C led to an improvement in the permeate flux. Also, they showed that the feed and the viscosity were the major factors influencing membrane fouling. The latest findings were also confirmed by De Bruijn et al. [114] where they stated that there were no unique optimum operating conditions while testing the tangential velocity of 2 and 7 m/s and the transmembrane pressure of 150 and 400 kPa. These findings suggested that the feed composition and pretreatments had a major influence on the ultrafiltration process performance of apple juice. The fouling phenomena can be explained by the concentration polarization phenomenon. This phenomenon is due to the accumulation of retained molecules at the membrane surface, which produce a concentration boundary layer along the membrane channel [115]. The latest findings contradict the models predicting zero flux

at infinite time. Furthermore, Zhao et al. [116] showed that pectin plays a significant role in membrane fouling during the microfiltration of apple cider, and the association of pectin with polyphenols and proteins was the major factor inducing membrane fouling.

Fuenmayor et al. [117] tested new Nylon—nanofibrous membranes produced by electrospinning for apple juice filtration. These membranes showed higher performances in terms of permeate flux and regarding turbidity removal, color, and antioxidant capacity than commercial polyamide membranes. Three different commercial polymeric ultrafiltration membranes (polysulphone, polyethersulphone, and regenerated cellulose) with different pore sizes and hydrophobicities were studied during apple juice clarification. Increasing the pore size and the hydrophobicity of the membrane led to reversible fouling while cake formation was more prominent when using membranes with narrower pore size. Concerning the fouling nature, reversible fouling was found to be the main fouling mechanism, while increasing the pore size and the hydrophobicity during cake formation was more prominent for the membranes with narrower pore sizes [118].

To improve the resistance of polymeric membranes to fouling during the clarification of apple juice, UF (ultrafiltration) membranes consisting of PSF/PEI (20/2 wt%) were enhanced by incorporating TiO_2 (titanium dioxide) and Al_2O_3 (aluminum oxide) nanoparticles via the phase inversion method. All the resulting nanocomposite UF membranes exhibited greater permeate flux compared to that of the PSF/PEI membrane. Among them, the membrane containing 0.01% TiO_2 displayed the highest permeate flux, reaching 44.6 $L/m^2.h$ at a steady state [119].

Despite the contributions made by previous research, little fundamental understanding of the process exists. Although it is accepted that macromolecules such as polysaccharides, proteins, and phenolic compounds as well as microorganisms are involved in membrane clogging, the impact and contribution involved in clogging are not identified. Thus, further research is needed to better understand the fouling phenomena during apple juice clarification by membrane processes in order to enhance their performances.

6.2. Clarifying Agents

Cloudy apple juice is a colloidal suspension encompassing pectin, protein, polyphenols, and cell debris [120], affecting the presentation and taste of this juice. To avoid haze and turbidity in apple juices, the use of finning agents is a common industrial practice for juice clarification. Among these fining agents, bentonite, gelatin, and silica sol were successfully employed for the clarification and stabilization of apple juice.

At a low pH range, gelatin is positively charged and reacts with negatively charged phenolics, while bentonite is used to remove the excess amount of unstable proteins. Studies in the literature dealing with the impact of finning agents on the quality and composition of apple juice are scarce. Oszmiański and Wojdyło [121] investigated the impact of conventional clarification agents using gelatin, bentonite, silica sol, and water-soluble chitosan on the phenolic compounds, antioxidant activity, and color of apple juice. The results showed that the use of chitosan and gelatin treatment decreased the concentration of polymeric procyanidins by 46–63% in apple juices. No significant influence of the chosen clarifying agents on antioxidant capacity was observed. Gökmen et al. [122] observed a decrease in the total phenolics (28%) when using gelatin and bentonite together in the finning step.

Chitosan is a cationic polysaccharide with a high molecular weight that was introduced in the food industry for its antibacterial and antifungal activities [123]. In the apple juice industry, chitosan was also tested for its clarification capacity. Rungsardthong et al. [124] tested fungal and shrimp-originated chitosan for apple juice clarification at different concentrations and incubation temperatures. Apple juice with chitosan treatment at 0.7 g/L and 40 °C reached its maximum clarity. The clarity and color changes in the apple juice correlated closely for both fungal and shrimp chitosan treatments. Abdelmalek et al. [125] showed that the application of chitosan to apple juice increased its transmittance to 91%, and thus, it reduced its turbidity and improved its clarity. In this study, the organic

acids and sugar levels in apple juice remained unaltered with chitosan application. When comparing the application of chitosan to control apple juice, Belgheisi and EsmaeilZadeh Kenari [126] found that chitosan treatment allowed to reach a low cloud point, meaning of clearer apple juice compared to the control one. Taştan and Baysal [127] determined that the best conditions for clarifying apple juice with chitosan involved a concentration of 191.6 mg/100 mL juice, a process temperature of 20 °C, and a duration of 30 min. The molecular weight of chitosan has been shown to influence the clarification capacity of chitosan in apple juice production [128].

Chitosan possesses several intrinsic characteristics that make it an effective coagulant by causing the separation of suspended particles from juices [124,129,130]. Chitosan charge is affected by the solution pH as shown by Hong et al. [131]. At pH $\geq$ 6.5, chitosan lost its electrostatic charge and became insoluble [131]. Under acidic conditions of apple juice, the amino groups of chitosan are protonated, and chitosan is expected to exhibit the typical behavior of a polyelectrolyte [132]. In fact, chitosan is a positively charged compound that can combine with negatively charged components such as pectin and proteins through electrostatic interaction, leading to flocculant precipitation [133].

In the literature, it was shown that chitosan has an antimicrobial ability [134,135]. In fact, reducing the amounts of microorganisms in apple juice can lead to clarity since microorganisms contribute to juice turbidity. Malinowska-Pańczyk et al. [135] showed that chitosan treatment (0.4% w/v) in apple juice production lowered the total counts of bacteria, yeasts, and molds by 0.5–3.0 log (CFU per g) after storage at 5 °C for 15 days. Abdelmalek et al. [125] demonstrated the strong inhibitory effect of chitosan against various microbial strains. Despite this, when applied to the apple juice clarification process, chitosan did not significantly reduce the presence of *Alicyclobacillus acidoterrestris* [130].

6.3. Clarification Using Enzymes

Pectin in juices can be divided into negatively charged rhamnogalacturonans and neutral arabinogalactans. The presence of these compounds increases juice viscosity and form haze when combined with proteins. Also, pectin plays the role of a colloidal protector, which opposes the natural decantation and clarification of colloidal particles. In order to reduce the impact of pectin, pectolytic enzymes are used during juice processing. Several types of pectolytic enzymes have been used and studied such as pectinesterase, pectinelyase, and polygalacturonase [136].

To clarify apple juice, Gomez-Ruiz et al. [137] tested the efficacity of endo-polygalacturonase from *Kluyveromyces fragilis*. The results were compared to commercial pectinase. It was concluded that this produced enzyme could be successfully used in the clarification of apple juice. A complete clarification (85%) of commercially available apple juice was achieved using 15 IU of pectolytic enzyme preparation from *Aspergillus niger* van Tieghem at 45 °C after 6 h of incubation. A viscosity drop of 35% was observed [138].

Saxena et al. [139] showed that 24 units of immobilized polygalacturonase on an activated polyethylene matrix at 45 °C for 1h of incubation time decrease the turbidity of apple juice by 55%.

Sandri et al. [140] tested two crude enzymatic extracts produced by *Aspergillus niger* T0005007-2 (TE1) and *Aspergillus oryzae* IPT 301 (TE2) for apple juice clarification where the reactions were conducted at 30 and 50 °C for 30 and 60 min. The obtained results were compared to those obtained by the commercial preparations of pectinase. It was shown that time increase is positively correlated with improved clarification, whereas temperature increase did not present any clear impact on the clarification rate. Results obtained by the crude preparation TE1 resulted in a similar clarification rate when compared to the commercial products. In 2013, Sandri et al. [3] selected a new strain of *Aspergillus niger LB23* for the production of pectolytic enzymes for the clarification of apple juice. They showed that these enzymes reduce juice viscosity and turbidity because of pectin hydrolysis, leading to the flocculation of pectin–protein complex. The decrease in turbidity was approximately

90%. The phenolic compounds and the antioxidant activity of apple juice were maintained after enzymatic treatment.

Polygalacturonase produced by *Aspergillus awamori* Nakazawa and α-amylase produced from *Aspergillus oryzae* were used for apple juice clarification. In the presence of 1% polygalacturonase and 0.4% α-amylase, a large decrease in turbidity (97%) was observed after 2 h of incubation at 50 °C in the presence of 10 mM $CaCl_2$. Slight changes in the total phenolic content and the antioxidant activity of juice were noted [141].

Rajdeo et al. [142] found that apple juice treated with free pectinase or immobilized pectinase (commercial pectinase from *Aspergillus aculeatus*) showed similar characteristics. In the treated samples compared to untreated samples, clarity was achieved due to the huge decrease in the viscosity and the pectin content measured by the formation of galacturonic acid.

de Oliveira et al. [143] tested the impact of pectinase immobilization in alginate beads on apple juice clarification in a continuous process (packed bed reactor). A flow rate of 10 mL/min and a recirculation time of 10 min were the optimal processing conditions. Results showed a clarification rate of 97.22% and a reduction of viscosity of 20.8%.

The immobilization of pectinases into calcium alginate microspheres for apple juice clarification was studied recently by Deng et al. [144]. The results of the immobilized polygalacturonase-treated apple juice compared to free polygalacturonase showed lower turbidity, color value, and soluble solid content. In addition, the immobilized enzyme had more temperature tolerance, wider pH adaptation range, better thermal stability, and better freeze–thaw stability than the free enzyme.

7. Conclusions

In post-processing, apple juice appears cloudy and contains microorganisms, necessitating both clarification and sterilization. Thermal processes remain widely used for microbial and enzymatic stabilization, yet they adversely impact the composition and quality of the fresh juice.

Non-thermal technologies like PEF, US, HPH, and microwave are well developed at the laboratory scale and effectively reduce the microbial load while minimizing the fresh juice quality losses. However, their industrial application is hindered by the high cost of equipment and treatment. Although optimizing technology for the maximum microbial reduction is crucial, striking a balance to preserve sensory and nutritional quality while reducing operational costs is equally important.

Non-thermal processing requires the meticulous optimization of operating parameters to deactivate vegetative cells or spores. Improper non-thermal processing demands considerable attention for the viable but nonculturable state of pathogenic and spoilage cells (VBNC), posing significant challenges for juice processors.

Prior to implementing non-thermal processes industrially, extensive studies are necessary to optimize these parameters across a wide spectrum of microorganisms and enhance juice quality. Additionally, combining a non-thermal process with membrane filtration for clarification and microbial stabilization shows promise for exploration. Understanding consumer perception of these new technologies is also vital for future adoption. Finally, complete life cycle assessment (LCA) studies are necessarily for designing and evaluating the sustainability of these processes.

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