

Review

# Advancing Sustainable Innovations in Mulberry Vinegar Production: A Critical Review on Non-Thermal Pre-Processing Technologies

Turkson Antwi Boasiako <sup>1,2,3,\*</sup>, Isaac Duah Boateng <sup>4</sup> , John-Nelson Ekumah <sup>1,2</sup> ,  
Nana Adwoa Nkuma Johnson <sup>1</sup> , Jeffrey Appiagyei <sup>5</sup>, Mian Shamas Murtaza <sup>1</sup>, Bismillah Mubeen <sup>1</sup>   
and Yongkun Ma <sup>1,\*</sup>

- <sup>1</sup> School of Food and Biological Engineering, Jiangsu University, Zhenjiang 202013, China; nelsonjokum@gmail.com (J.-N.E.); nana.anjohnson@yahoo.com (N.A.N.J.); shamas.murtaza@mnsuam.edu.pk (M.S.M.); bismillah.mubeen@gmail.com (B.M.)
  - <sup>2</sup> College of Basic and Applied Sciences, University of Ghana, Legon P.O. Box LG 134, Ghana
  - <sup>3</sup> Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
  - <sup>4</sup> Certified Group, 199 W Rhapsody Dr, San Antonio, TX 78216, USA; boatengisaacduah@gmail.com
  - <sup>5</sup> College of Agriculture, Food and Natural Resources, University of Missouri, Columbia, MO 65211, USA; boakyejeff@gmail.com
- \* Correspondence: turk.sky92@gmail.com (T.A.B.); mayongkun@ujs.edu.cn (Y.M.)

**Abstract:** Mulberry is renowned for its medicinal properties and bioactive compounds, yet its high moisture content renders it highly perishable and challenging to transport over long distances. This inherent limitation to its shelf life poses sustainability challenges due to potential food waste and the increased carbon footprint associated with transportation. To address this issue sustainably, mulberry vinegar emerges as a biotechnological solution. Utilizing a fermented mixture of crushed mulberries, sugar, and mixed acid, transforms the highly perishable raw material into a more stable product. However, conventional methods of mulberry vinegar production often involve heat-intensive processing, which poses environmental concerns and energy inefficiencies. Recognizing the need for sustainable practices, this review delves into alternative non-thermal technologies (NTTs) that can revolutionize mulberry vinegar production. These technologies, such as ultrasonication, ultra-high-pressure homogenization, pulsed light treatments, enzyme-assisted pretreatment, and membrane filtration, offer eco-friendly alternatives by eliminating the need for excessive heat. NTTs enhance energy efficiency and sustainability in mulberry vinegar production by deactivating the microbes and extending the shelf life, thereby enhancing product stability and quality without using thermal methods. Ultrasonication, for example, plays a pivotal role in improving bioactive compound extraction, contributing to the overall quality enhancement of mulberry juice. Enzyme-assisted pretreatment, specifically with Pectinex Ultra SP-L and Viscozyme L, not only enhances juice quality, but also holds promise for sustainable vinegar production. Furthermore, ultra-high-pressure homogenization and pulsed light treatments positively influence mulberry processing, offering additional sustainable alternatives. Membrane filtration, especially ultrafiltration, not only enhances the phenolic content, but also contributes to stability in mulberry juice, showcasing potential benefits for vinegar production. In conclusion, exploring these NTTs represents a transformative shift from traditional heat treatment methods in mulberry food processing. By providing energy efficient, environmentally friendly, and high-quality alternatives, this review offers valuable insights into sustainable practices, particularly in mulberry vinegar production, thereby contributing to a more sustainable future for the mulberry food industry.

**Keywords:** sustainable; mulberry vinegar; non-thermal technologies; ultrasonication; ultra-high-pressure homogenization; pulsed light treatment; membrane filtration



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## 1. Introduction

The mulberry fruit (Figure 1a), abundantly distributed in Asia, Europe, America, India, and Africa, belongs to the *Moraceae* family [1,2]. Noteworthy species include *Morus alba*, *Morus nigra*, and *Morus rubra*, which are known for their medicinal properties [3–5]. China leads in regard to cultivation, boasting the largest area and production volumes globally [6]. The geographical hotspots for mulberry cultivation in China include Guangdong, Zhejiang, Shanghai, Shaanxi, Xinjiang, Beijing, and other areas [7]. Globally, the primary use of mulberries revolves around their significance in silkworm production [8], as these insects exclusively consume mulberry leaves. China has led in terms of mulberry cultivation [7], boasting the largest area and production volumes of ~700,000 ha and  $1.82 \times 10^7$  tons, respectively [9], followed by India (~280,000 ha). While other countries, such as Thailand [10] and Brazil, maintain some mulberry production, their scales are considerably smaller, for instance, 35,000 ha and 848.6 kg/acre/year [11], respectively.



**Figure 1.** (a) Mulberry fruit and (b) the final product as mulberry vinegar.

Mulberry, a fast-growing, deciduous, or medium-sized, woody perennial tree, adapts well to diverse climates [12], soils (pH: 6.2–6.8), and altitudes of up to 4000 m above sea level [3,11]. Ideal growth conditions include temperatures of 24–28 °C, humidity of 65–80%, and annual rainfall between 600 and 2500 mm. The plant features lobed leaves, yellowish-green blooms, and clusters of small drupes encased in perianth [4]. The nutritional profile is rich in sugar (9–20%) [2], acidity (0.47–2.5%), pectin (0.94%), and mineral materials (0.9%), and mulberries ripen in spring, offering juicy, sweet fruits. Primary products derived from mulberries include juice, beverages, wine, seeds, paste, and anthocyanins [3,12,13]. Anthocyanins contribute to the vivid coloration of mulberries and are associated with potential health benefits. Modern research highlights the bioactive compounds in mulberry fruit, including vitamins, minerals, fiber, amino acids, polysaccharides, polyphenols, flavonols, phenolic acids, and anthocyanins [4,14]. The pharmacological health benefits include anticarcinogenic, anti-cardiovascular, neuroprotective, antihyperglycemic, antioxidative, antidiabetic, and anti-inflammatory effects [3,15–17].

Despite its numerous nutritional and pharmacological benefits, the biotechnological potential of this fruit remains largely untapped because of its high moisture content, which leads to a short shelf life and increased perishability. Transporting highly perishable goods over long distances requires rapid and often energy-intensive means of transportation, contributing to greenhouse gas emissions and the environmental impact [18]. To address mulberries' limited storage duration and susceptibility to spoilage, vinegar production from the fruit has been explored [18,19]. The focus on mulberry vinegar (Figure 1b) as a

biotechnological solution becomes not only a method for stabilizing the product, but also a means to reduce the environmental burden associated with transportation. Mulberry vinegar, with its high acidity, is resistant to spoilage and represents an innovative avenue for utilizing this medicinal fruit. Additionally, overripe mulberries can be transformed into mulberry wine [20], creating a potential gap in mulberry vinegar production. Repurposing overripe mulberries into wine offers an alternative product and highlights mulberries' versatility in different biotechnological applications. This dual approach addresses the challenge of the fruits' limited shelf life and opens up new avenues for utilizing mulberries, showcasing their adaptability and potential in various industries. This review is focused on consolidating acceptable non-thermal pre-processing techniques designed to preserve the nutrients and bioactive compounds in mulberry fruit juice, setting the stage for subsequent steps like fermentation or acetification. By incorporating non-thermal technologies (NTTs), this review not only aims to enhance the quality of mulberry products, but also to align the production process with sustainability goals by eliminating the need for heat-intensive processing. These alternative technologies contribute to product stability without relying on traditional methods, potentially reducing the energy requirements for production and promoting a more environmentally friendly approach. The methodology employed in this review takes a comprehensive literature review approach to provide insights into NTT pretreatments for improving mulberry vinegar quality (Figure 2a–c). The data were sourced from online databases, including the Web of Science and Lens.org, using the search term “mulberry vinegar (lens.org)” to capture all the relevant studies on the topic (Figure 2a–c). Thorough examination of the data involved filtering relevant papers based on both relevance and quality. The inclusion criteria for this review encompassed research papers published until December 2023 that reported the use of non-thermal technology pretreatment to enhance the quality of mulberry vinegar production. This exploration not only holds promise for optimizing the health benefits of mulberry vinegar, but also signifies a technological advancement in the workflow during mulberry fruit operations. By adopting these non-thermal technologies, the review contributes to broader sustainability objectives, promoting energy efficiency, reducing carbon emissions, and fostering environmentally conscious practices within the mulberry food industry. The coherent integration of these methodologies and practices aims to provide a holistic perspective on sustainable mulberry vinegar production, offering valuable insights for future advancements in the field.

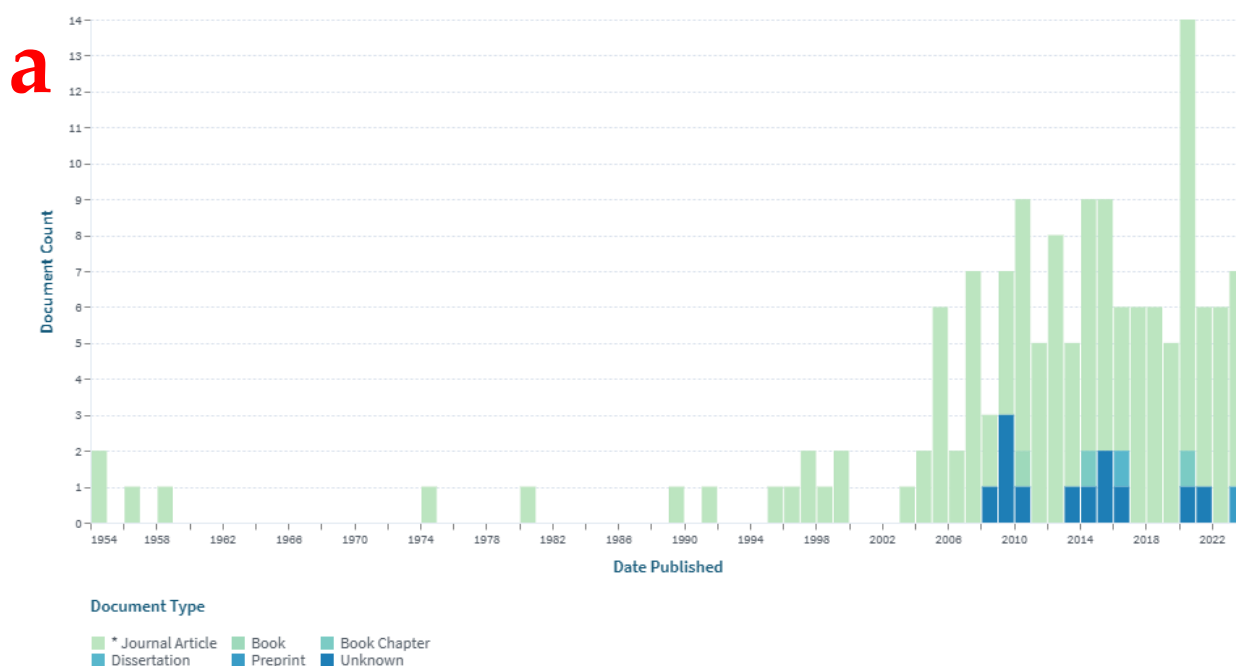


Figure 2. Cont.

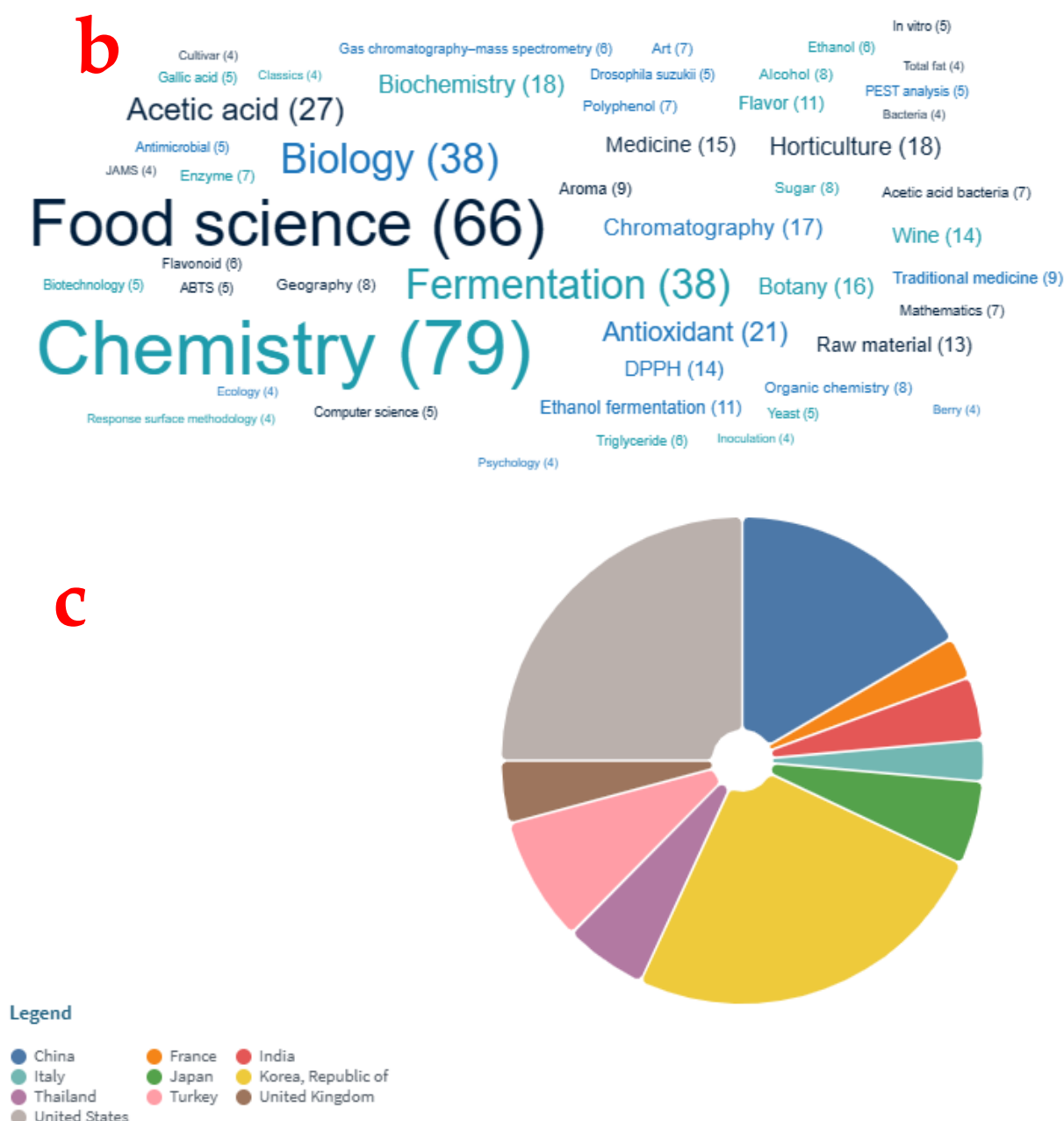


**Figure 2.** (a) Scholarly works on mulberry vinegar processing over time; (b) fields of study; (c) the most active countries that have published research on mulberry vinegar processing. (Data were taken from Lens.org on 3 December 2023).

## 2. Mulberry Vinegar Production: An Overview

The mulberry vinegar production process involves the preparation of a solution using ripe mulberries, with sugar content ranging from 16 to 18 °Brix [21]. This solution undergoes various steps, including the addition of sugar for adjustment, mixed acid for pH regulation, pectinase for fiber breakdown [14], sulfite for bacterial removal [22], and the preparation of an acetic acid culture solution. The fermented mixture is created by combining 33.0 to 45.0% by weight of the prepared solution with crushed mulberries, sugar, purified water, pectinase, and mixed acid. The resulting mulberry liquid has a specific sugar content and acidity [23]. Following this, a yeast inoculation step involves dissolving the yeast in purified water and evenly adding it to the prepared solution. Subsequently, an alcoholic fermentation step occurs in a sealed fermentation vessel at

25 °C for 6 to 10 days, until the specific gravity reaches 1.02 [21]. The wine produced undergoes filtration and dilution to achieve 5–6% alcohol by volume (ABV). The acetic acid fermentation step involves the addition of an acetic acid culture solution [7] and fermentation for 25–35 days at 25–30 °C [21]. The supernatant is then subjected to low-temperature ripening for 3–5 months at 5–10 °C. Sterilization is performed at 120 °C for 20–25 min, followed by bottling and packaging in unit doses. The mulberry vinegar produced by this process has a composition of 58.69% crushed mulberry, 29.34% purified water, 5.87% sugar, 5.87% oligosaccharide, and 0.23% citric acid [21]. This mixture, with a sugar content of 15–16 °Brix and an acidity of pH 3.7 to 3.8, undergoes heat treatment, juicing, and filtering to obtain mulberry juice [21]. A functional beverage is prepared by adding 10–15% vinegar, prepared using the process above, to 85–90% mulberry juice [21]. The sugar content is adjusted to 14–15 °Brix and the acidity to pH 3.6–3.7, followed by sterilization at 80 °C for 10–15 min [21].

### 3. Current Thermal Procedures in Mulberry Vinegar Production

Thermal processes have sustained food preservation studies over the past few decades; however, modern interventions [24] have proven to have significant or minimal effects on food quality, such as a reduction in nutritional value, color change, reduced flavor, textural changes to products, and undesirable by-products [25]. Nevertheless, pasteurization or heat treatment may be applied in commercial or industrial-scale vinegar manufacturing to achieve the dual objectives of sterilization and prolonged shelf life. Throughout the process, meticulous steps are taken to ensure safety, quality, and bioactivity, resulting in a high-quality product with flavorful and nutritionally enriched results. Various thermal technologies have been used to process mulberry juice, including ohmic heating, microwave heating, and osmotic distillation [22,24–28]. Ohmic heating is an emerging sterilization method, based on the high-temperature–short-time (HTST) principle. Darvishi et al. [26] assessed the impact of the ohmic heating method (OHM) on various parameters associated with the concentration process for black mulberry juice and compared their results with those of conventional heating methods (CHM). The total phenolic content (TPC) in the OHM-treated samples was 3–4.5-fold higher than in the CHM-treated samples. Applying a higher voltage gradient to the OHM resulted in modest pH and TPC alterations. Notably, the energy consumption by the OHM (3.33–3.82 MJ/kg water) was 4.6–5.3 times lower than that observed in CHM (17.50 MJ/kg water). The OHM concentration efficiency surpassed that of CHM, by 38–46%. Additionally, the electrical conductivity exhibited a positive correlation with the concentration ratio of the sample, modeled as a function of the process variables, including phase heating, water content, sample temperature, and voltage gradient. Darvishi et al. [26] indicated that the application of the OHM yielded superior quality and substantially reduced the engineering parameters compared to CHM during the concentration process for black mulberry juice, while reducing the energy consumption. These results are consistent with the general concerns of Hardinasinta et al. [27] regarding ohmic heating as a feasible pasteurization technique for mulberry juice, demonstrating that enhanced electrical conductivity and heating rates correlated with the temperature. Moreover, it presents promising outcomes regarding sterilization efficacy and favorable rheological characteristics. This study revealed that the electrical conductivities of fruit juices ranged from 0.128 to 0.430 S.m<sup>−1</sup>, increasing linearly with temperature. The heating rates were 0.57–0.66 °Cs<sup>−1</sup>, and the system performance coefficients varied from 0.64 to 0.81. Overall, ohmic heating was suitable for sterilizing these fruit juices because of the short heating times and high coefficients of performance. However, the designed ohmic heating system appeared more suitable for jambolana juice than mulberry and bignay juice.

Microwave heating has demonstrated superior efficacy compared to conventional methods for preserving the phytochemicals and quality attributes in mulberry juice. This encompasses the retention of color, anthocyanin content, and antioxidant activity [28,29]. They focused on the concentration of black mulberry juice using various heating methods (conventional and microwave heating), across different operational pressures (7.3, 38.5,

and 100 kPa). This study investigated the effects of each heating method on the evaporation rate and quality attributes of concentrated juice. A rotary evaporator obtained a final juice concentration of 42° Brix for 140, 120, and 95 min at 100, 38.5, and 7.3 kPa, respectively [30]. The microwave energy application, notably, reduced the processing time to 115, 95, and 60 min, respectively. This study explored the changes in color, anthocyanin content, antioxidant properties, phenolic content, turbidity, pH, and acidity, during the concentration process. Notably, anthocyanin degradation was more pronounced with the rotary evaporation method than with microwave heating, even though both methods had a degradative effect on the anthocyanin content. The rate constants for the thermal degradation of anthocyanins during microwave heating at 7.3, 38.5, and 100 kPa were 0.034, 0.144, and 0.191 h<sup>-1</sup>, respectively. In contrast, under conventional heating, the rate constants increased to 0.066, 0.173, and 0.348 h<sup>-1</sup> [30]. Additionally, higher phenolic concentrations resulted in lower EC<sub>50</sub> values, indicating elevated antioxidant activity in the mulberry extract. This study provides comprehensive insights into the effect of heating methods on the quality and concentration dynamics of black mulberry juice. Furthermore, Fazaeli et al. [31] extended their investigation to assess the concentration of black mulberry juice using conventional and microwave heating under varying operational pressures (7.3, 12, 38.5, and 100 kPa). This study focused on assessing the impact of each heating method on the phytochemical changes, specifically the total anthocyanin content and antioxidant activity. The concentration process was conducted using a rotary evaporator, resulting in a final juice concentration of 42° Brix for black mulberry, and the attainment of a 42° Brix concentration was accomplished within 140, 120, and 95 min under vacuum pressures of 100, 38.5, and 7.3 kPa, respectively. The application of microwave energy proved to be an effective expedient, reducing the processing time required for black mulberry juice to 115 min, 95 min, and 60 min. Notably, microwave heating significantly reduces the overall processing duration, thereby enhancing the efficiency of the concentration process. Although the concentration process achieved a final juice concentration of 42° Brix for black mulberry using a rotary evaporator and microwave energy, it is crucial to acknowledge the inherent drawbacks associated with thermal technologies in regard to anthocyanin degradation and the subsequent reduction in antioxidant activity using the rotary evaporation method and microwave heating [31].

Osmotic distillation is advantageous for preserving the heat-sensitive constituents of black mulberry juice, resulting in increased anthocyanin content and antioxidant activity [32], as opposed to conventional thermal evaporation. In this study, extended storage duration and elevated storage temperatures led to a decline in the concentrates' anthocyanin content and antioxidant activity. An increase in the polymeric color ratio and turbidity accompanied this decline. Although this effect was not immediately prominent after production, it eventually resulted in the loss of juice flavor and color, nutrient reduction, and formation of mutagens, such as hydroxymethylfurfural (HMF). According to Chottamom et al. [33], whether untreated or osmotically treated with sucrose, sorbitol, or maltose, mulberries were dried in a tray dryer at 60 °C, with an air velocity of 1 ms<sup>-1</sup>. The drying kinetics of the mulberries were explained using the Page model, with R<sup>2</sup> values ranging from 0.985 to 0.993 and E<sub>s</sub> values ranging from 0.031 to 0.091. During air drying, the anthocyanins and phenolics were degraded, following a zero-order reaction, with R<sup>2</sup> values ranging from 0.866 to 0.996. However, osmotic distillation is more advantageous than conventional thermal evaporation methods. It effectively maintained the dried mulberries' phenolic and anthocyanin content, especially when maltose was used as the osmotic solution [33]. During the concentration of fruit juices, osmotic distillation utilizing potassium pyrophosphate as the stripping solution was observed to have minimal impact on the physicochemical characteristics of the final juice [34]. The permeation ranged between 0.56 and 1.45 kg/m<sup>2</sup>h; however, this was dependent on the feed temperature, with no notable impact on the degradation of ascorbic acid or the loss of volatile compounds. The procedure maintained the physicochemical characteristics of the juice, including the color and acidity.

The limitations of using thermal technologies in mulberry juice production include the degradation of heat-sensitive nutrients, potential alterations in flavor and color, significant energy consumption, incomplete microbial control, loss of volatile compounds, challenges in achieving uniform heating, and the formation of undesirable by-products [20,22,25,31,34]. These limitations highlight the need for careful process design, consideration of alternative methods, and implementation of quality control measures to mitigate potential impacts on the nutritional content, sensory qualities, and overall quality of mulberry juice before vinegar production. Alternative non-thermal processes are being utilized to mitigate the adverse effects on food products associated with traditional thermal procedures [30,33,35].

#### 4. Non-Thermal Pre-Processing Techniques in Mulberry Vinegar Production

Non-thermal technologies (NTTs) represent innovative food processing and preservation approaches that diverge from conventional heat-based methods, such as pasteurization or sterilization [24]. These techniques, including high-pressure processing, pulsed electric fields, and ultrasound, are employed to deactivate microbes and extend the shelf life of food products [36]. Recognized for its ability to maintain nutritional quality, flavor, and overall sensory attributes, NTTs offer an energy efficient and environmentally friendly alternative to traditional thermal methods [33,35].

Contemporary exploration of non-thermal processes, such as ultrasonication, high-pressure processing, pulsed light (PL) treatments, and cold atmospheric conditions, is a burgeoning field. This approach aims to mitigate the adverse effects associated with traditional methods and thermal procedures on food products [30,36–41].

In mulberry vinegar production, NTTs would eliminate the need for heat application during pasteurization or sterilization [24]. The application of technologies, such as ultrasound (Figure 2a), in mulberry juice production can be optimized individually or in combination to assess their impact on the technological value of mulberry products (powder, wine, or vinegar) [3,42–46]. Whether using NTTs in isolation or combination, these techniques are expected to contribute to our understanding of mulberry vinegar production, enhancing the elucidation and utilization of bioactive compounds. In turn, this has the potential to create superior mulberry vinegar, with enhanced sensory attributes.

##### 4.1. Ultrasound Technology

###### Ultrasonication and Ultrasonic Homogenization

Ultrasonication (Figure 3A), a cutting-edge non-thermal technology (NTT) that utilizes low- and high-frequency sound waves [47–49], has been widely applied in mulberry juice production. It is a very sustainable and efficient method [50]. Its efficacy lies in enhancing the extraction of bioactive compounds, aiding cellular breakdown, and improving the overall process efficiency. Operating at frequencies ranging from 20 kHz to 500 MHz, the power of ultrasound spans from 20–100 kHz, and low-intensity ultrasound falls within 2–10 MHz [51]. Cavitation induced by ultrasound, involving the formation and implosion of bubbles, facilitates the disintegration of cell walls [51], thereby promoting the enhanced extraction of bioactive components, such as anthocyanins and total phenolics [52], without relying on heat-intensive methods.

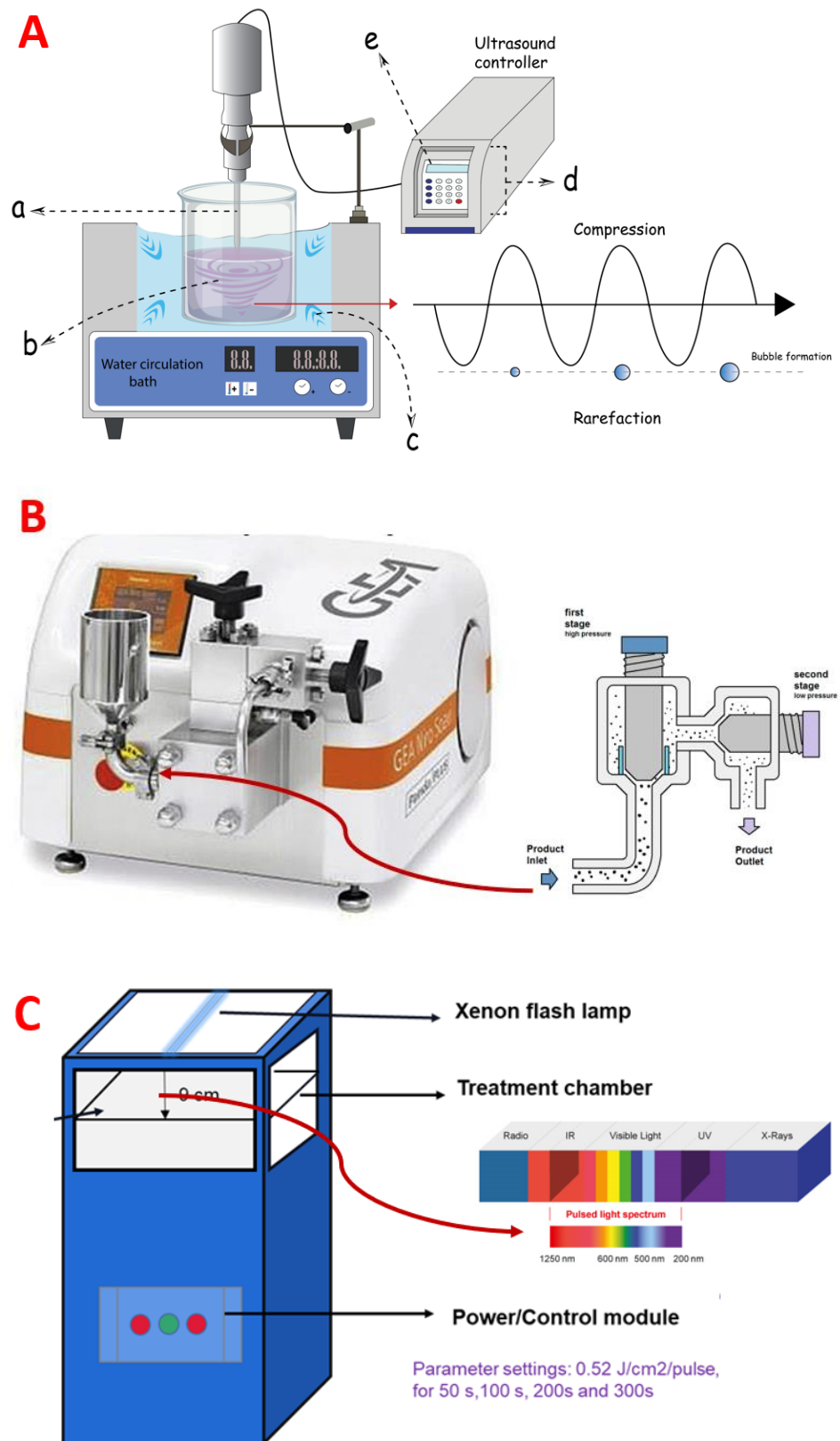


**Figure 3.** Experimental setup for (A) ultrasound, (adapted from Boateng et al. [52] with modifications); (B) ultra-high-pressure homogenization, (adapted from Patrignani et al. [53] with modifications), and (C) pulsed light (adapted from Li et al. [54] with modifications) pretreatment, for mulberry vinegar processing.

Ultrasonication and lactic acid-fermented mulberry juice (LFMJ) present a compelling exploration of non-thermal pasteurization methods, specifically ultrasonication, and their impact on various aspects of juice quality. The findings, as outlined by Kwaw et al. [48], indicate a positive influence of ultrasonication on the physicochemical, volatile, and phytochemical profiles of LFMJ compared to thermal treatments. One notable outcome was the identification of the optimal conditions for ultrasonication, including a frequency of 24 kHz, an exposure period of 10 min, and a power level of 60 W. These conditions, determined through their non-thermal pasteurization approach, enhanced the phenolic and antioxidant properties of LFMJ. Even though the study attributed this improvement largely to the ability of *L. plantarum* to produce  $\beta$ -glucosidase [55] and esterases to significantly increase the phytochemical composition, ultrasonicated juice better impacted these qualities compared to traditional thermally treated mulberry juice (RMJ). This study reports an increase in the total phenolic content (TPC), total antioxidant capacity (TAC), and total flavonoid content (TFC) in sonicated samples, with variations in frequency and exposure duration. This finding aligns with the positive correlation between pasteurization and increased phenolic compounds, while thermal treatment negatively affects the phytochemical properties. Furthermore, this investigation focused on the color properties of LFMJ, noting changes in lightness ( $L^*$ ), redness ( $a^*$ ), yellowness ( $b^*$ ), and chroma ( $C^*$ ), associated with sonication parameters. The impact of the sonication time on the volatile concentration, with varying effects at different frequencies, adds a layer of complexity to the study. The observed increase in the total volatile concentration with low-frequency sonication time and the decrease at a high frequency might be attributed to localized heat exposure during cavitation phenomena. In conclusion, the critical analysis of this study highlights the multifaceted effects of ultrasonication on lactic acid-fermented mulberry juice, offering valuable insights into the potential of non-thermal methods for enhancing juice quality across physicochemical, volatile, and phytochemical profiles.

Nguyen and Nguyen [47] investigated the impact of ultrasonic treatment on the yield and quality of mulberry juice extraction. The highest juice yield and antioxidant content were achieved under the optimal conditions (45 °C for 60 min). Ultrasonic treatment led to significant increases in various parameters compared to pressing alone: extraction yield (29.6%), total soluble solids (8.7%), titratable acidity (39.3%), L-ascorbic acid content (94.3%), total phenolic content (174.1%), total anthocyanin content (156.9%), and antioxidant capacity (40.7%). The study highlighted a strong positive correlation between the total phenolic content and the antioxidant capacity, emphasizing the pivotal role of phenolic compounds as antioxidants in beverages. Overall, ultrasonic treatment demonstrated a substantial improvement over pressing alone in regard to multiple aspects, including the total soluble solids, L-ascorbic acid content, phenolic content, anthocyanin content, antioxidant capacity, extraction yield, and titratable acidity.

Despite the aforementioned benefits from these studies [44,50–52], a potential decrease in anthocyanins exists probably due to the accompanying heat [56]; however, appropriate treatment can mitigate this effect [57]. Owing to the inefficiencies reported from a single application of ultrasound, new techniques involving ultrasound with accompaniments, such as thermosonication [58] and pressure (manosonication) [59], have been devised to provide food products with a more stable shelf life. Ultrasonication and ultrasonic homogenization use high-frequency acoustic waves [60] for laboratory and industrial applications.

In conclusion, ultrasonication and ultrasonic homogenization are valuable tools for enhancing the efficiency and quality of mulberry fruit and juice processing. Their potential lies in improving the physicochemical properties and extracting valuable bioactive compounds, making them significant contributors to modern food processing, with room for further exploration and innovation in the industry.

#### 4.2. Ultra-High-Pressure Homogenization

Ultra-high-pressure homogenization (Figure 3B) achieves sterilization by pumping fluid at 200–600 MPa [53] through a special valve, causing shear forces and impacting

microorganisms. Ultra-high-pressure homogenization (UHPH) can be applied to various foods to provide sterility even at low temperatures, although temperature management is crucial [61]. This method safeguards the nutritional integrity of foods, while obviating the necessity for excessive heat, thereby making a significant contribution to sustainability. Furthermore, non-thermal processes, such as UHPH, reduce sample handling, enhance repeatability, and increase automation in food production. High hydrostatic pressure (HHP) [62,63] at 400–600 MPa for 600 s effectively eliminates yeast [53,57,59,61] in grape must, but is inefficient against lactic acid bacteria [64].

In mulberry juice processing, UHPH and HHP have notable effects on various aspects. Yu et al. [65] demonstrated that UHPH induces significant changes in the phenolic compounds, antioxidant capacity, and anti-glucosidase activity. Specifically, UHPH processing at 200 MPa for 1–3 passes (inlet temperature of 4 °C) was compared with conventional pasteurization (95 °C, 1 min). In contrast to pasteurization, UHPH resulted in more substantial reductions (10–35% and 30–40% for 1 and 3 UHPH passes at 200 MPa, respectively) in anthocyanins, phenolic acids (gallic, protocatechuic, caffeic, and *p*-coumaric acids, along with unknown hydroxycinnamic acid), and quercetin aglycone contents. This was accompanied by a decrease in the oxygen radical absorbance capacity (ORAC) without ascorbic acid [65]. However, the observed reductions during UHPH were effectively mitigated by adding ascorbic acid to mulberry juice. Additionally, UHPH processing showed no significant change ( $p < 0.05$ ) in the  $\alpha$ -glucosidase inhibitory activity, whereas a 14% reduction was noted in thermally pasteurized mulberry juice. Furthermore, Yu et al. [66] investigated its integration with dimethyl dicarbonate (DMDC), which proved effective in inactivating and inhibiting indigenous microorganisms in mulberry juice, offering an alternative to thermal pasteurization. Assessment of the microbial and nutrient qualities of mulberry juice produced by high-pressure homogenization (HPH) and DMDC revealed that either repeated HPH passes at 200 MPa, or the addition of 250 mg/L DMDC, significantly inactivated indigenous microorganisms ( $p < 0.05$ ). Although some molds (*M. circinelloides*) recovered during storage at 4 °C, the combined treatment of three passes of HPH and 250 mg/L DMDC (HPH–DMDC) reduced the population of surviving microorganisms to the level achieved as a result of heat treatment at 95 °C for 1 min (HT), with no significant increase ( $p < 0.05$ ) in the microorganism population during subsequent storage at 4 °C. This trend suggests that certain components, such as phenolic compounds, in mulberry juice may hinder the repair of total aerobic bacteria (TAB) and lactic acid bacteria (LAB). Despite no significant changes ( $p > 0.05$ ) in the physical attributes, including pH, TSS (°Brix),  $L^*$ ,  $a^*$ , and  $b^*$  values, the HPH–DMDC treatment retained higher levels of TPC and  $\alpha$ -glucosidase inhibitory activity, despite greater losses in cyanidin 3-glucoside, cyanidin 3-rutinoside, and antioxidant capacity. Ultimately, HPH–DMDC treatment has emerged as a viable alternative to conventional thermal pasteurization of mulberry juice. Wang et al. [67] emphasized that HHP, a subtype of UHPH, preserved the elevated levels of bioactive compounds (resveratrol 24.20  $\mu\text{g/mL}$ ) and antioxidant activity (oxygen radical absorbance capacity 481.68  $\mu\text{mol TE/mL}$ ) in mulberry juice compared to conventional thermal processing. This study aimed to compare the effects of HHP and thermal processing on the microbiological quality, bioactive compound content, antioxidant activity, and volatile profile of mulberry juice. HHP at 500 MPa for 10 min reduced the microbial counts to lower levels (2.00 and 1.30 log CFU/mL, respectively, for yeasts and molds and total viable counts), ensuring microbiological safety comparable to thermal processing at 85 °C for 15 min. Additionally, HHP maintained significantly higher levels of total phenolic content (TPC) and total flavonoid content (TFC) (4.27 mg GAE/mL, 7.25 mg RE/mL, respectively) compared to thermal processing (3.15 mg GAE/mL, 5.63 mg RE/mL, respectively). The volatile compound concentrations (alcohols, aldehydes, and ketones) in mulberry juice were enhanced by HHP, whereas thermal processing resulted in reduced concentrations. These findings suggest that HHP processing could be an alternative to conventional thermal methods for producing high-quality mulberry juices. However, it is crucial to note that HHP, particularly when applied at lower pressure levels, may induce alterations in the

anthocyanin composition of juice, leading to the formation of new anthocyanins [68]. This aspect was further elucidated in an investigation of mulberry fruit anthocyanins before and after HHP treatment [68]. The three samples were treated at 200, 400, and 600 MPa for 20 min. The untreated samples primarily contained cyanidin-3-O-glucopyranoside (55.56%) and cyanidin-3-O-coumaroylglucoside (44.44%). After HHP treatment at 200 MPa, two new anthocyanins (pelargonidin-3-O-coumaroylglucoside (0.46%) and delphinidin-3-O-coumaroylglucoside (5.8%)) emerged. At 400 MPa, a new anthocyanin, delphinidin-3-O-coumaroylglucoside (5.38%), was detected. No new anthocyanins have been identified at 600 MPa [68].

In summary, ultra-high-pressure homogenization (UHPH) and high-pressure homogenization (HPH) significantly affected mulberry juice processing. UHPH, as demonstrated by Yu et al. [65], induces noteworthy alterations in the phenolic compounds, antioxidant capacity, and anti-glucosidase activity, resulting in reduced anthocyanins and phenolic acids. Integration with dimethyl dicarbonate (DMDC) is a promising alternative to thermal pasteurization. Similarly, HPH, as studied by Wang et al. [67], maintains heightened levels of bioactive compounds and antioxidant activity, positioning it as a viable substitute for traditional thermal processing. It is important to highlight that low-level high hydrostatic pressure (HHP) applications may influence anthocyanin composition, forming new anthocyanins. Despite these considerations, UHPH and HPH, especially when combined with DMDC, have emerged as effective and innovative methods for crafting high-quality mulberry juice, contributing significantly to contemporary food processing practices.

#### 4.3. Pulsed Light Treatment

Innovations in food processing aim to minimize thermal energy use with pulsed light [69] (Figure 3C), preserving heat-labile substances, such as vitamins and flavors [70]. Pulsed light is commonly applied to food surfaces, equipment, and packaging materials [71], using high-intensity broad-spectrum sources, such as pulsed white light. In this treatment, electromagnetic energy accumulates in a capacitor within seconds and is released in nanoseconds to milliseconds, amplifying the power with minimal energy consumption [72]. Pulsed light setups typically include adjustable xenon lamp units, power units, and high-voltage connections. Broad-spectrum light spans ultraviolet (UV), visible (VIS), and near-infrared (IR) regions [73]. Research has indicated that pulsed light treatment significantly enhances phenolic concentration and antioxidant activity in lactic acid-fermented mulberry juice [74]. This study evaluated the combined effect of ultrasonication (28 kHz, 60 W, 15 min) and pulsed light ( $1.213 \text{ J cm}^{-2} \text{ pulse}^{-1}$ , 360  $\mu\text{s}$ , 3 Hz, 4 s) on these attributes. Non-thermal treatment substantially improved the total phenolic content (TPC), total flavonoid content (TFC), anthocyanins, and antioxidant activities, compared to the control sample. This enhancement could be attributed to the positive outcome of applying pulsed light before ultrasonication, which facilitates the degradation of certain phytonutrients and elevates phenolic acids [74]. The increase in 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH $\bullet$ -SA) can be attributed to the augmentation of TPC and the likely presence of constituents with proton-donating properties engaging in reactions with DPPH radicals. This was supported by a notable positive correlation ( $r = 0.863$ ) between TPC and DPPH $\bullet$ -SA. The results demonstrated a significant positive correlation between DPPH $\bullet$ -SA and TPC in various plant products, possibly owing to the synergistic interplay between PT and UT. The surge in 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (ABTS $\bullet^+$ -SA) is intricately linked to phenolic hydroxyl groups and the delocalization of electrons induced by the resonance between aromatic benzene rings and the free electron pair on the phenolic oxygen. Consequently, the enhancement of ABTS $\bullet^+$ -SA may be attributed to the improved extraction of antioxidant compounds, notably monomeric anthocyanins. Ultrasonication alone showed a significant ( $p < 0.05$ ) increase in the phenolic and antioxidant properties compared to pulsed light treatment, while the combined treatment (PUT) exhibited the highest performance, suggesting industrial viability for processing lactic acid-fermented mulberry juice (LFMJ). This combined

treatment effectively reduced the microbial load, without affecting the physicochemical properties [74]. Moreover, when applied individually, pulsed light (PL) treatment with exposure times of 2, 4, and 8 s significantly reduced the microbial load to acceptable levels ( $1.02 \log_{10}$  CFU/mL), without impacting the physicochemical properties. Although an 8 s exposure caused a slight decrease in anthocyanin concentration, overall, PL treatment improved the quality of fermented juice compared to thermal treatment [74]. However, when combined with ultrasonic treatment, PL treatment enhances juice quality, resulting in a superior product with an extended shelf life at the recommended storage temperature of 5 °C for 6 months [74].

In conclusion, these studies demonstrated that combining ultrasonication and pulsed light treatment enhances phenolic concentration and antioxidant activity in lactic acid-fermented mulberry juice. This non-thermal approach positively affects the quality parameters, particularly when pulsed light precedes ultrasonication. The results suggest industrial viability for processing lactic acid-fermented mulberry juice, offering improved microbial safety without compromising the physicochemical properties. The combined treatment presented a superior product with an extended shelf life, highlighting its potential in the food processing industry, with an extension to mulberry vinegar.

#### 4.4. Enzymatic Pretreatment

The insights from studies on enzyme pretreatment in mulberry juice [70,74] and related products [75] provide a valuable foundation for extending these advancements to mulberry vinegar production. Generally, using enzymes for pretreatment processes can improve the efficiency of various operations, such as extraction and clarification, without the need for high temperatures, thereby reducing energy consumption [75,76]. The application of commercial enzymes, particularly Pectinex Ultra SP-L and Viscozyme L, has substantially enhanced the quality attributes of mulberry juice, encompassing the extraction yield, total phenolic content, and antioxidant capacity [77]. This knowledge serves as a stepping stone toward a comprehensive investigation into the influence of commercial enzymes on the extraction yield and quality of mulberry vinegar. In mulberry juice production, using Pectinex Ultra SP-L and Viscozyme L, individually applied to mulberry mashes, has shown promising results. The study incorporated varied incubation periods, ranging from 60 to 240 min, with a detailed analysis of parameters, such as the juice yield, total phenolic content, total anthocyanin content, antioxidant capacity, L-ascorbic acid content, total soluble solids, and titratable acidity. The comparison with non-enzymatically treated juice revealed a significant elevation in the quality attributes for both Pectinex Ultra SP-L and Viscozyme L-treated juices. The study also revealed that the enzymes significantly impacted the yield of mulberry juice, with the highest extraction yield observed at 60–180 min for Pectinex Ultra SP-L and Viscozyme L, with the extraction yield ranging from 84.3 to 87.1% and 82.3 to 83.4% respectively. However, the juice yield decreases with increasing incubation time. It was found that beyond 180 min, the juice yield was  $84.9 \pm 0.19\%$  and even lower at 240 min ( $81.4 \pm 0.26\%$ ). The promising aspect of this study was its alienation from previous studies on asparagus by Sun et al. [78], emphasizing the potency of enzymes in extracting bioactives from mulberry juice before it is made into vinegar. Perhaps, a possible explanation of this effect could be explained in the report by Nguyen and Nguyen [77], that the enzyme treatment reduces pectin's water-holding capacity, releasing more juice, and increasing the juice yield, even though this contrasts with previous studies on enzyme treatment of plum, peach, pear, and apricot, with juice yields ranging from 52% to 78%. However, an extension of the incubation period beyond 180 min led to a decline in the antioxidant values, except for the total anthocyanin content. The application of enzymes at 50 °C significantly increased the total phenolic and anthocyanin contents in mulberry juice, compared to non-enzyme-treated juice. This was due to pectinases degrading the pectin in the middle lamella of the fruit tissue and enhancing the antioxidants [79]. Samples treated with Pectinex Ultra SP-L showed higher levels of total phenolics and anthocyanins than those treated with Viscozyme L and could emphasize the greater degradative ability of

Pectinex Ultra SP-L on the cell wall for the exudation of more polyphenolic compounds into the cytoplasm of the cell. The reduction in the total phenolics, anthocyanins, and DPPH scavenging in some samples may be due to pectinase hydrolysis, resulting in less oxidation-stable polyphenols. Pectinase preparation may contain enzymes like rutinase, quercetinase, and laccase [77], which can cause a loss of antioxidant capacity in any juice. It is also possible to assert that the reduction in antioxidant potential could result from potentially binding phenolic acids to plant cell walls, eventually affecting their extractability [80]. The two-way analysis emphasized the inseparable impacts of enzyme type and treatment duration on mulberry juice and would be a suitable pretreatment in the acetification of mulberry juice. The optimization model by Qadir et al. [81] of phenolic recovery involving *Morus alba* leaves (MAL) could inform the design of enzyme-assisted extraction processes for mulberry vinegar, where the enzyme concentration and pH were identified as crucial parameters. The utilization of enzyme complexes, including kemzyme dry-plus, natuzyme, and zympex-014, showcased the effectiveness of zympex-014 in extracting phenolic bioactives from MAL [81]. This aligns with enhancing the phenolic profile of mulberry juice, which was already mentioned by Nguyen and Nguyen [47]. The model designed provided insights into possible bioconversion efficiencies in juice extraction using kemzyme dry-plus, natuzyme, and zympex-014. Although all the other conditions remained constant (5% enzyme concentration, 8.5 pH, 70 °C temperature, and 45 min duration), zympex-014 was found to have a higher response (extract yield) than both natuzyme (24.50 g/100 g) and kemzyme dry-plus (25.00 g/100 g), with a common maximum extraction yield of 30.00 g/100 g. Additionally, the exploration by Yang et al. [82], focusing on the efficient extraction of mulberry leaf polysaccharides, adds another dimension to the potential improvements in mulberry vinegar production. The ongoing efforts to enhance extraction processes for mulberry products, with a focus on improvement of the overall quality, can be seamlessly extended to the context of mulberry vinegar, offering opportunities for advancements in both the extraction efficiency and product quality.

In summary, enzyme-assisted extraction studies, particularly using Pectinex Ultra SP-L and Viscozyme L, provide a basis for improving mulberry vinegar production. These enzymes enhance the quality attributes in mulberry juice, showing an increased extraction yield and phenolic content. Optimizing enzyme applications and exploring efficient extraction methods offer opportunities for advancing mulberry product processing and quality.

#### 4.5. Membrane Filtration

Membrane filtration is crucial in modern food processing, preserving nutritional quality and flavor. Advancements in microfiltration, ultrafiltration, nanofiltration, and reverse osmosis improve efficiency, reduce energy consumption, and ensure high-quality food and beverages. Studies show its applications in dairy processing and in regard to fruit juice quality. Techniques like ultrafiltration and microfiltration (Table 1) use membranes to separate components in a solution based on their size [83,84]. These processes can be energy efficient and contribute to the production of high-quality products, with a reduced thermal impact [83–85].

**Table 1.** Overview of non-thermal technologies involved in mulberry juice processing.

| Non-Thermal Technologies | Treatment Conditions              | Effects and Outcomes          | Key Findings                                 | References |
|--------------------------|-----------------------------------|-------------------------------|----------------------------------------------|------------|
| Ultrasonication          | Operating frequencies: 20–500 MHz | Bioactive compound extraction | Enhancement of bioactive compound extraction | [51]       |

Table 1. Cont.

| Non-Thermal Technologies           | Treatment Conditions                                                                                                                                                    | Effects and Outcomes                                                                                  | Key Findings                                                                                                                                                                                                                                                                 | References |
|------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
|                                    | Optimal conditions for LAFMJ (low-temperature ultrasonic-assisted fermentation of mulberry juice)                                                                       | Cellular breakdown and efficiency improvement                                                         | Study on LAFMJ: sonication at 24 kHz, 10 min, and 60 W increased a* (redness) and $\Delta E$ (total color difference) values<br>Improved phytochemical properties of fermented juice                                                                                         | [48]       |
|                                    | Optimal conditions for ultrasonic treatment vs. pressing on juice yield and quality: 45 °C for 60 min                                                                   | Impact on color and phytochemical properties<br>Comparison of ultrasonic treatment vs. pressing alone | Ultrasonic treatment (UT) vs. pressing alone: extraction yield from UT increased total soluble solids (8.7%), titratable acidity (39.3%), L-Ascorbic acid content (94.3%), total phenolic content (174.1%), total anthocyanin content (156.9%), antioxidant capacity (40.7%) | [47]       |
| Ultra-high-pressure homogenization | UHPH                                                                                                                                                                    | Inactivation of microorganisms<br>Bioactive compounds and antioxidant activity                        | Impact on molds ( <i>M. circinelloides</i> )<br>Elevated levels of TPC and $\alpha$ -glucosidase inhibitory activity                                                                                                                                                         | [66]       |
|                                    | HPH and DMDC (HPH passes at 200 MPa or the addition of 250 mg/L DMDC)                                                                                                   | Total plate count and $\alpha$ -glucosidase inhibitory activity                                       | Reduction in microbial counts (log CFU/mL)<br>Specific levels of bioactive compounds (resveratrol 24.20 $\mu\text{g/mL}$ ) and antioxidant activity (oxygen radical absorbance capacity 481.68 $\mu\text{mol TE/mL}$ )                                                       | [66]       |
|                                    | HPH: 500 MPa for 10 min                                                                                                                                                 | Total phenolic content (TPC)<br>Total flavonoid content (TFC)                                         | Maintenance of TPC and TFC levels (4.27 mg GAE/mL and 7.25 mg RE/mL, respectively)                                                                                                                                                                                           | [67]       |
|                                    | HHP: 200, 400, and 600 MPa for 20 min                                                                                                                                   | Volatile compound concentrations<br>Anthocyanin detection and composition                             | Enhancement of volatile compound concentrations (alcohols, aldehydes, and ketones)<br>Detection of new anthocyanins under HHP treatment at different pressure levels                                                                                                         | [68]       |
| Pulsed light treatment             | UT (ultrasonic treatment) and PL (pulsed light treatment)<br><br>parameters:<br>1.213 $\text{Jcm}^{-2}\text{pulse}^{-1}$ ,<br>360 $\mu\text{s}$ , 3 Hz, 4 s             | Microbial load reduction<br><br>Extended shelf life (6 months at 5 °C)                                | Viable microbial count:<br>1.02 $\pm$ 0.04 $\log_{10}$ CFU/mL                                                                                                                                                                                                                | [48]       |
| Enzymatic pretreatment             | Enzymes used: kemzyme dry-plus, natuzyme, zympex-014<br>Incubation periods (60 to 240 min), enzyme concentration (5%), pH (8.5), temperature (70 °C), duration (45 min) | Comparison of MAL extract yield from enzymes: zympex-014 vs. natuzyme vs. kemzyme dry-plus            | Extraction yield at different time intervals:<br>Pectinex Ultra SP-L (60–180 min): 84.3% to 87.1%<br>Viscozyme L (60–180 min): 82.3% to 83.4%<br>Beyond 180 min: 84.9 $\pm$ 0.19%<br>Common maximum extraction yield: 30.00 g/100 g                                          | [81]       |

Table 1. Cont.

| Non-Thermal Technologies       | Treatment Conditions                                                                  | Effects and Outcomes                                              | Key Findings                                                               | References |
|--------------------------------|---------------------------------------------------------------------------------------|-------------------------------------------------------------------|----------------------------------------------------------------------------|------------|
| Membrane filtration techniques | MF (microfiltration) and UF (ultrafiltration)                                         | Particle permeation and $R_f$ values:                             | Initial polyphenol content: 809 mg per 100 mL juice                        | [29]       |
|                                | Membrane: MCE (mixed cellulose ester)                                                 | MF pore size 0.1 $\mu\text{m}$ to MF pore size 0.22 $\mu\text{m}$ | Polyphenol content after membrane clarification: 396.3 mg per 100 mL juice | [29]       |
|                                | Pore sizes investigated: 0.1 $\mu\text{m}$ , 0.22 $\mu\text{m}$ , 0.025 $\mu\text{m}$ | UF pore size 0.025 $\mu\text{m}$                                  |                                                                            |            |

Note: MHz—megahertz; LAFMJ—lactic acid-fermented mulberry juice; UHPH—ultra-high-pressure homogenization; HPH—high-pressure homogenization; DMDC—dimethyl dicarbonate; mg/L—milligram per liter; MPa—mega Pascal; UT—ultrasonic treatment; PL—pulsed light treatment; kHz—kilohertz; W—watts;  $\mu\text{s}$ —microsecond; MF—microfiltration; MCE—mixed cellulose ester;  $\mu\text{m}$ —micrometer; TPC—total phenolic content; TFC—total flavonoid content; MAL—*Morus alba* leaves,  $R_f$ —fouling resistance; UF—ultrafiltration; CFU—colony forming unit;  $\mu\text{g}$ —microgram; mL—milliliter; TE—trolox equivalent; GAE—gallic acid equivalent.

Research on membrane filtration in mulberry juice has consistently demonstrated its significant impact on enhancing various properties. The investigation by Li et al. [86] specifically focused on ultrafiltration using a 100 kDa molar weight cut-off (MWCO) membrane, revealing notable improvements in the phenolic compounds, antioxidant activity, and storage stability of mulberry juice. The study compared the effects of ultrafiltration membranes with 100 or 18 kDa MWCO on the clarification process and subsequent storage, highlighting the superior outcomes with the 100 kDa MWCO membrane. This membrane increased the total polyphenols, monomeric anthocyanins, phenolic acids, and flavonoids, enhancing the antioxidant activity,  $\alpha$ -glucosidase inhibitory rate, and redness. Conversely, the 18 kDa MWCO membrane led to a sharp reduction in these attributes. Furthermore, mulberry juice treated with the 100 kDa MWCO membrane exhibited superior storage stability. In practical terms, the 100 kDa MWCO ultrafiltration membrane is recommended for mulberry juice processing, promising improved phenolic enrichment, enhanced bioactive activities, and improved sensory quality. Building on this, Xiong et al. [87] demonstrated the positive impact of microfiltration on the clarity and stability of mulberry wine. The mulberry wine samples (samples included those taken immediately after stabilization that pertained to naturally clarified samples, while the others were associated with the permeate from microfiltration) exhibited minimal differences in terms of the alcoholic strength, total sugar, total acid, pH, and total soluble solids (TSS). Microfiltration led to decreased chroma and total phenol, a substantially decreased turbidity (<2 NTU from 372 NTU), and higher polydispersity in microfiltered samples than naturally clarified ones. Membrane differences were noted: 0.22  $\mu\text{m}$  membranes produced smaller particles with lower polydispersity than 0.45  $\mu\text{m}$  membranes. Headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC-MS) analysis identified 58 volatile compounds, predominantly esters and alcohols [87]. After microfiltration, the total volatile compound content decreased, with PVDF0.45 and PES0.22 membranes preserving the compounds effectively. Notably, membrane performance did not strictly correlate with material type or pore size, but reflected a combination of both [87]. While the investigation was intriguing, Hojjatpanah et al. [29] delved into the fouling mechanisms in membrane clarification of black mulberry juice, utilizing mixed cellulose ester (MCE) flat membranes. This investigation examined the effects of the membrane pore size, transmembrane pressure, and cross-flow velocity on membrane fouling, revealing that a larger pore size and pressure increased fouling, while a higher velocity decreased fouling. Analysis of the resistance emphasized the role of reversible and irreversible fouling resistances, with no observed cake resistance. A distinction between ultrafiltration (UF) and microfiltration (MF) was evaluated by treating mulberry juice with MCE membranes at three different pore sizes (0.1, 0.22, 0.025  $\mu\text{m}$ ). In their analysis, the fouling resistance ( $R_f$ ) in MF with pore sizes

of 0.22 and 0.1  $\mu\text{m}$  increased with an increasing pore size (from  $1.9 \times 10^{10} \text{ m}^{-1}$  for MCE 0.1  $\mu\text{m}$ , to  $4.4 \times 10^{10} \text{ m}^{-1}$  for MCE 0.22  $\mu\text{m}$ ). The basis for this trend could be explained by the membrane permeation, which tends to move more solid particles easily [29]. These results were, however, in contrast to UF (pore size: 0.025  $\mu\text{m}$ ); smaller pore sizes blocked more particle permeation, with the eventual fouling resistance increase depicted by a value of  $6.0 \times 10^{10} \text{ m}^{-1}$ . The investigation noted increased reversible and irreversible fouling resistances in MCE 0.22  $\mu\text{m}$  in contrast to MCE 0.1  $\mu\text{m}$ , highlighting the predominant influence of reversible fouling resistance. Transcending these physics into the bioactivity of black mulberry juice before and after clarification showed that the membrane filtration strategies adopted had a significant impact on the polyphenol content (decreasing from 809 mg per 100 mL juice to 396.3 mg per 100 mL juice after membrane clarification). The fouling in this process was primarily attributed to the presence of large particles. Further, scanning electron microscopy (SEM) illustrated intermediate blocking as the dominant fouling mechanism in MCE 0.025  $\mu\text{m}$  and standard blocking in MCE 0.1 and 0.22  $\mu\text{m}$ .

In conclusion, the collective research on membrane filtration's positive impact on mulberry juice properties lays the groundwork for exploring similar applications in mulberry vinegar production. The potential enhancements to the phenolic content, clarity, and stability demonstrated in these studies suggest that membrane filtration techniques could improve the overall quality of mulberry-derived products, extending their applications beyond juice to items like mulberry vinegar. In the context of the above information, the limitations include focusing on specific membranes and parameters, insufficient strategies for fouling mitigation, and a lack of consideration of the scale-up challenges. Enhancements should involve comprehensive membrane comparisons, broader parameter investigations, effective fouling mitigation strategies, and scale-up studies for practical implementation in mulberry juice and vinegar processing.

## 5. Conclusions

Thermal technologies employed in mulberry juice production exhibit limitations, including nutrient degradation, alterations in flavor and color, high energy consumption, and incomplete microbial control. In response to these challenges, researchers are exploring alternative non-thermal technologies (NTTs). Noticeably, ultrasonication and ultrasonic homogenization prove to be valuable tools, enhancing the physicochemical properties and facilitating the extraction of bioactive compounds. Both ultra-high-pressure homogenization (UHPH) and high-pressure homogenization (HPH) have demonstrated significant impacts on mulberry juice processing. UHPH induces alterations in the phenolic compounds, while HPH maintains heightened levels of bioactive compounds. Enzyme-assisted pretreatment, specifically employing Pectinex Ultra SP-L and Viscozyme L, shows promise in enhancing mulberry vinegar production. Meanwhile, membrane filtration techniques have the potential to elevate the overall quality of mulberry-derived products, expanding its application beyond juice to include items like mulberry vinegar. However, challenges persist, including a focus on specific membranes and parameters, inadequate fouling mitigation strategies, and insufficient consideration of the scale-up challenges. Pulsed light treatment emerges as a particularly effective and attractive method within emerging non-thermal technologies. Its distinguishing features include a brief treatment duration and the capacity to counteract the effects of heat treatment, setting it apart from other technologies. Collectively, these innovative approaches emphasize their potential to revolutionize mulberry vinegar processing, enhancing product quality and providing sustainable alternatives to conventional methods.

## 6. Future Studies

In future studies on mulberry vinegar production, the exploration of non-thermal technologies holds untapped potential. The focus should center on refining individual parameters to create optimal conditions for preserving bioactive compounds. Conducting comparative analyses across a spectrum of non-thermal methods will provide valuable

insights into identifying the most effective and sustainable approaches. Understanding consumer perceptions is pivotal; unraveling the sensory attributes that significantly impact market acceptance and guide product development. Long-term stability assessments are essential to ensure the quality of non-thermal mulberry vinegar during extended storage.

Furthermore, environmental impact evaluations will contribute to eco-friendly practices, scrutinizing energy efficiency and waste reduction. A deeper investigation into the microbial dynamics promises enhanced safety protocols for fermentation under non-thermal conditions. The exploration of synergistic combinations of different non-thermal technologies holds the potential to unlock unprecedented efficiency and quality improvements. Economic viability studies play a crucial role in ascertaining the feasibility of large-scale adoption, considering both initial investments and long-term economic benefits. Understanding the effects of non-thermal treatments on health-promoting compounds emphasizes the potential health advantages of mulberry vinegar. Adapting these technologies to different mulberry varieties is key, shedding light on varietal influences and paving the way for widespread application and innovation in the field. These suggested further studies collectively aim to deepen our understanding of the intricacies surrounding the application of non-thermal technologies in mulberry vinegar production. By addressing specific areas, they contribute to the scientific knowledge and offer practical insights for implementation in the food industry.

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## Abbreviations

|                        |                                                                                     |
|------------------------|-------------------------------------------------------------------------------------|
| ABTS <sup>•+</sup> -SA | 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity |
| CHM                    | conventional heating methods                                                        |
| DMDC                   | dimethyl dicarbonate                                                                |
| DPPH <sup>•</sup> -SA  | 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity                          |
| ESI/MS                 | electrospray ionization–mass spectrometry                                           |
| HHP                    | high hydrostatic pressure                                                           |
| HPLC                   | high-performance liquid chromatography                                              |
| HS-SPME-GC–MS          | headspace solid-phase microextraction gas chromatography–mass spectrometry          |
| HTST                   | high-temperature–short-time                                                         |
| LFMJ                   | lactic acid-fermented mulberry juice                                                |
| MAL                    | <i>Morus alba</i> leaves                                                            |
| MCE                    | mixed cellulose ester                                                               |
| MWCO                   | molar weight cut-off                                                                |
| NTTs                   | non-thermal technologies                                                            |
| OHM                    | ohmic heating method                                                                |
| ORAC                   | oxygen radical absorbance capacity                                                  |
| PEF                    | pulsed electric field                                                               |
| PL                     | pulsed light treatment                                                              |

|      |                                    |
|------|------------------------------------|
| PME  | pectin methylesterase              |
| PMF  | pulsed magnetic fields             |
| POD  | peroxidase                         |
| PPO  | polyphenol oxidase                 |
| UHPH | ultra-high-pressure homogenization |
| TFC  | total flavonoid content            |
| TPC  | total phenolic content             |

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