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Impact of ultrasonication and pulsed light treatments on phenolics concentration and antioxidant activities of lactic-acid-fermented mulberry juice

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Antiradical activity*Chemical compounds studied in this article:*
Ferulic acid (PubChem CID: 445858)
p-coumaric acid (PubChem CID: 637542)
Quercetin (PubChem CID: 5280343)
Cyanidin-3-O-glucoside (PubChem CID: 12303203)
Sodium acetate (PubChem CID: 517045)
Rutin (PubChem CID: 5280805)
Syringic acid (PubChem CID: 10742)
Acetonitrile (PubChem CID: 6342)
Esculin (PubChem CID: 5281417)
Sodium nitrite (PubChem CID: 23668193)

ABSTRACT

This study sought to assess the effect of ultrasonication (28 kHz, 60 W, 15 min), pulsed light (1.213 $Jcm^{-2}pulse^{-1}$, 360 μs, 3 Hz, 4 s) and their combined usage on the phenolics concentration and antioxidant activities of lactic-acid-fermented mulberry juice (LFMJ). The results showed significant improvement in the total phenolic concentration, total flavonoid concentration, total anthocyanin concentration, antiradical activity against 2,2-diphenyl-1-picrylhydrazyl scavenging activity (DPPH$^{\bullet}$-SA), 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid radical cation scavenging activity ($ABTS^{\bullet+}$-SA) and reducing power capacity (RP-CA) in all the non-thermal treated fermented juice compared to the control sample. Among the individual non-thermal treatments, ultrasonication caused a significant ($p < 0.05$) upsurge in the phenolic and antioxidant properties of the LFMJ compared to the pulsed light treatment. In the case of the combined treatments, the application of pulsed light before ultrasonication (PUT) exhibited the utmost performance. This suggests that PUT technique could successfully be implemented on industrial scale for the processing of LFMJ.

1. Introduction

Conventional thermal pasteurization techniques have been employed to most foods to enhance their safety and prolong their shelflife. To date, the use of conventional thermal pasteurization in fruit juice processing remains the most common method for preservation due to its ability to inactivate microbes and enzymes. However, some studies suggest that thermal treatments have adverse effect on the levels of nutraceutical compounds and sensorial qualities of foods (Chen et al., 2013; Choi, Cheigh, Jeong, Shin, & Chung, 2010; Igual, Contreras, Camacho, & Martínez-Navarrete, 2014). Though some scientists (Lespinard, Bambicha, & Mascheroni, 2012; Miri et al., 2008) have optimized thermal processing conditions to minimize the negative effects, the impact still remains a great challenge in fruit juice processing. To maintain and/or improve food quality and to meet the increasing consumer demands for healthier foods at optimized pasteurization cost, there is the need to shift from conventional thermal treatments to non-thermal preservation techniques. These non-thermal technologies are designed to produce stable and safe food products without the damages induced by thermal pasteurization (Gomez-Lopez, Ragaert, Debevere, & Devlieghere, 2007). Moreover, studies have demonstrated that, the use of non-thermal technologies as food preservation methods do not only maintain the quality of foods but also enhance their functionalities (Chouliara, Georgogianni, Kanellopoulou, & Kontominas, 2010; Rawson et al., 2011).

Among the non-thermal technologies being explored to complement or replace the conventional heat pasteurization are high hydrostatic pressure, pulsed electric fields, pulsed magnetic field, pulsed light and ultrasonication (Adekunte, Tiwari, Cullen, Scannell, & O'Donnell, 2010; Donsì, Ferrari, & Maresca, 2010). Pulsed light (PL) is presently employed as a novel non-thermal technology that inactive microorganisms using short duration (1 μs – 0.1 s) penetrating pulses and intense broad-

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spectra (200–1100 nm) electromagnetic radiation (Oms-Oliu, Aguiló-Aguayo, Martín-Belloso, & Soliva-Fortuny, 2010). There is a growing shift towards the use of ultrasonication (US) processing which relies on acoustic cavitation that causes disruption of microbial cell wall and membrane (Rajasekhar, Fan, Nguyen, & Roddick, 2012) and/or formation of free radicals due to sonochemical reactions (Adekunte et al., 2010; Kadkhodaee & Povey, 2008) to improve on the safety and quality of foods. These non-thermal techniques have also been reported to impact positively on bioactive compounds and antioxidant activities of some foods (Caminiti et al., 2011, 2012). The effect of non-thermal processes on the quality of apple and cranberry juice blend (Caminiti et al., 2011), grapefruit juice (Aadil, Zeng, Han, & Sun, 2013), apple juice (Abid et al., 2014) among others have been reported. Moreover, these non-thermal processes may impact on reactions (co-pigmentation, condensation, polymerization, oxidation among others) which occur during processing and storage, thus affecting some quality properties of beverages (Tchabo et al., 2018).

Mulberry fruit is rich in bioactive compounds such as phenolic acids, flavonols and anthocyanins that characterize its antioxidant properties (Bao et al., 2016; Donno, Cerutti, Prgomet, Mellano, & Beccaro, 2015) and health benefits (Landete, Curiel, Rodríguez, de las Rivas, & Muñoz, 2014; Natić et al., 2015). The fruit contains high amount of bioactive compounds such as morin, quercetin 3-O-rutinoside, catechin, cyanidin 3-O-glucoside, pelargonidin 3-*O*-rutinoside, vanillic acid, caffeic acid, ferulic acid, *p*-coumaric acid, chlorogenic acid, *p*-hydroxybenzoic acid, gallic acid among others (Bao et al., 2016; Donno et al., 2015). Some researchers have demonstrated the possibility of processing mulberry fruit into fermented juice, jam, jelly and wine (Kwaw et al., 2017; Pérez-Gregorio, Regueiro, Alonso-González, Pastrana-Castro, & Simal-Gándara, 2011; Tchabo, Ma, Kwaw, Zhang, & Li, 2017).

Recently, our team has performed a thorough study on the effect of combined usage of pulsed light and sonication on the microbial safety and organoleptic properties of lactic-acid-fermented mulberry juice (unreported data). However, considering the high nutraceutical composition of mulberry fruit which are highly susceptible to degradation, it is important to assess the influence of preservation methods on its health-related properties. To date, there is paucity of information on the combined usage of pulsed light and ultrasonication treatments on health-related properties of food products. Thus, this study sought to assess the effect of ultrasonication, pulsed light as well as their different combined usage on the phenolic profile and antioxidant activities of lactic-acid-fermented mulberry juice.

2. Materials and method

2.1. Beverage formulation and fermentation

Prior to the formulation of the beverage, the lactic acid bacteria (*L. plantarum* ATCC SD5209) was activated as described by Kwaw et al. (2017). The juice was prepared by crushing (Hurom slow juicer, Roland Products, Los Angeles, California, USA) the commercially matured *Morus nigra* fruit (black color). Thereafter, ascorbic acid (1 g/kg) was added to minimize oxidation of the must. The must was then treated with Pectinex UF enzyme (0.1 mL/kg, Novozymes, Beijing, China) according to the manufacturer's instructions. The juice was centrifuged (Avanti J-25, Beckman Coulter) at 5920 xg for 15 min at 4 °C and subsequently inoculated (1.0% v/v) with the lactic acid bacteria (LAB) before incubating it for 36 h at 37 °C in a rotary incubator (IS-RDD3, Crystal Technology and Industries, Jiangsu, China) at 100 xg. The lactic-acid-fermented mulberry juice (LFMJ) was eventually subjected to the proposed non-thermal treatments. Untreated fermented juice was used as control (CON).

2.2. Non-thermal pasteurization of fermented mulberry juice

2.2.1. Ultrasonic treatment of fermented juice

Ultrasonication treatment (UT) was performed using the method described by Engmann, Ma, Tchabo, and Ma (2015) with some modifications. Briefly, 150 mL of the (LFMJ) was dispensed into 500 mL Erlenmeyer flask and placed in a treatment chamber with internal dimensions (36.1 cm × 29.5 cm x 50.5) and ultrasonicated (Wuxi Fanbo Biological Engineering Co. Ltd, Wuxi, China) for 15 min at a frequency of 28 kHz, constant temperature of 5.0 °C and a power of 60 W generated by an emitter ultrasonic probe (35.3 cm × 28.2 cm x 10.4 cm). The pulsating time intervals was 10 s (on) and 3 s (off). The ultrasound treated fermented mulberry juice (UTFMJ) was centrifuged (Anke GL-20B, Shanghai Anting Scientific, Shanghai, China) at 4 °C for 10 min at 3500 xg.

2.2.2. Pulse light treatment

Pulsed light treatment (PT) was carried out using the method described by Palgan et al. (2011) with few modifications. In sum, the LFMJ was cooled to 3 °C and dispensed into a petri dish (100 mm diameter) to a depth of 1 mm. The dish was positioned at a height of 2.5 cm from the quartz window of the Xenon flash lamp (Model No. RC 747, Xenon Corporation MA, USA) of the RS-3000B Steripulse-XL system (Xenon Corporation, Wilmington, MA, USA). The sample was subjected to light pulses at a pulse width of 360 μs, frequency of 3 Hz and delivering radiant energy of 1.213 $Jcm^{-2}pulse^{-1}$. Exposure time of 4 s to obtain a sub-lethal energy doses of 7.26 J/cm^2 was employed. Thereafter, the pulsed light treated fermented mulberry juice (PTFMJ) was centrifuged (Anke GL-20B, Shanghai Anting Scientific, Shanghai, China) at 3500 xg for 10 min at 4 °C.

2.2.3. Combined ultrasonic and pulse light treatment

The combined treatment was performed by combining the ultrasonication (Engmann et al., 2015) and pulse light (Palgan et al., 2011) treatment methods previously described. In essence, for the ultrasound pulsed light treatment (UPT), the juice was first sonicated and concurrently pulsed light treated. The reverse of the two procedures was used for the pulsed light ultrasound treatment (PUT).

2.3. Phenolics concentration assay

The total phenolic concentration (TPC) was assessed using the Folin-Ciocalteu method described by Kwaw et al. (2017). The TPC was expressed as milligram of gallic acid equivalent per 100 mL of LFMJ.

The total flavonoid concentration (TFC) was measured using the aluminum chloride colorimetric method described by Kwaw et al. (2017). The result was expressed as milligram of rutin equivalents per 100 mL LFMJ.

The pH differential method as described by Tchabo, Ma, Kwaw, Zhang, Li, et al. (2017) was employed in the determination of the total anthocyanin concentration (TAC). The TAC was calculated (equation (1)) and expressed as milligram equivalent of cyanidin 3-gucoside per 100 mL of LFMJ.

$$TAC = [(A_1 - A_2) - (A_3 - A_4)] \times \frac{MW \times DF \times 10^2}{\varepsilon \times L} \quad (1)$$

where ε is the molar extinction coefficient for cyanidin-3-glucoside ($26900\,l\,mol^{-1}\cdot cm^{-1}$); A_1 is the absorbance at 520 nm at pH 1.0, A_2 is the absorbance at 700 nm at pH 1.0, A_3 is the absorbance at 520 nm at pH 4.5, A_4 is the absorbance at 700 nm at pH 4.5, MW is molecular weight of cyanidin-3-glucoside ($449.2\,g\,mol^{-1}$); L is the path length (1 cm) and DF is the dilution factor (100).

2.4. Antioxidant activity assay

The 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

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