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RESEARCH ARTICLE

Synergistic Hurdle Effect of Physical and Chemical Treatments for Non-thermal Pasteurization of Guava (*Psidium guajava*) Juice

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Abstract

Guava (*Psidium guajava*) is commonly known for its food and nutritional values throughout the world. It has high fermentable sugar composition when mature and ripe .It's known for its sweet and tangy flavor and many uses. It is a fruit with good nutritional attributes but has short shelf-life under the ambient conditions. Dimethyl dicarbonate is a microbial control agent inhibitor widely applied in the food industry. Various current and potential applications of ultrasound in the fruit processing have been identified. Consumers prefer to safe, nutritious, high quality guava juices without thermal treatment. In our research, synergistic or hurdle effectiveness by ultrasound combined dimethyl dicarbonate was studied on guava juice. Different parameters of ultrasonic duration (10, 15, 20, 25, 30 min), dimethyl dicarbonate addition (10, 15, 20, 25, 30 µL/100mL) was examined on guava juice in respect of microbial inactivation. Log_{10} reductions of total plate count and *coliform* were evaluated to define the optimal variables. Our results revealed that microbial loads in guava juice would be decreased dramatically by ultrasonic treatment in 25 minutes at frequency 37 kHz with 20 µL/100mL supplementation of dimethyl dicarbonate to create a synergistic hurdle effect for a significant microbial reduction. It could be considered as an excellent non-thermal pasteurization for a better fruit juice quality, not only for guava but also for other tropical fruits to enhance their added value.

Keywords: *Guava juice, Synergistic, Hurdle, Ultrasound, Dimethyl dicarbonate, Microbial reduction.*

Introduction

Guava (*Psidium guajava*) is an evergreen shrub or small tree in the family Myrtaceae grown for its edible fruits. The fruit is prized for its very pleasant, sub acidic and aromatic nature. Guava possesses favourable nutritional characteristics as a source of phenolic compounds, carotenoids and vitamin C, excellent flavour, aroma and colour. Guava fruits are fresh during the harvesting season but perishable under the prevailing conditions of temperature and humidity as well as lack of adequate storage facilities [1].

It is mostly eaten raw (ripe or semi-ripe) or consumed in the form of juice, syrup, ice cream, jams, and jellies [2]. Carotenoids and ascorbic acid were the dominant phytochemicals in fresh fruits. Different pharmacological activities were recorded including antioxidant, antipyretic, antifungal, antimicrobial, hypotensive, analgesic & anti-inflammatory effect [3]. Ultrasound was utilized as an innovative method superior to conventional thermal treatments to pasteurize and sterilize foodstuffs. The introduction of ultrasound into liquid created bubble cavitation owing to pressure fluctuation [4].It caused micro bubbles collapse, internal hot spot and cavitation. The concentrated local energy and high pressure lead to micro sterilization effect without creating any significant macro-thermal accumulation [5]. Ultrasound using low and high energy was applied in food processing, analysis, and quality control.

Low-energy ultrasound had frequencies higher than 100 kHz at intensities below 1 W/cm^2. High-energy ultrasound had intensities higher than 1 W/cm^2 at frequencies between 20 and 500 kHz [6]. Low-power ultrasound enhanced the extraction of bioactive compounds by cell wall disruption [7].Dimethyl dicarbonate is an effective microbial inhibitor by inactivating irreversibly with the amino groups on active sites of cellular enzymes, especially alcohol dehydrogenase and

glyceraldehydes-3-phosphate dehydrogenase, in preventing yeast spoilage in alcoholic beverage and wine [8, 10]. It's quickly decomposed to methanol and carbon dioxide in instantaneous manner [11]. The effectiveness of dimethyl dicarbonate depended on different variables such as species, strain, initial microbial load, chemical component, temperature, procedure of supplementation and homogenization [8, 9].Consumers demand for safe and nutritious juices with high quality leading to the development of innovative processing technologies.

The synergistic strategy involves the combination of processing techniques with the purpose of limiting detrimental impacts on nutritional and organoleptic attributes normally existed in thermal processing. Purpose of our study evaluated the microbial reduction by the synergistic effect of dimethyl dicarbonate combined with ultrasonic wave on guava juice.

Material and Method

Material

Guava fruits were collected from Tien Giang province, Vietnam. After collecting, they were preserved at 8°C in dry containers and quickly conveyed to laboratory for experiments. They were subjected to cleaning by 25 ppm peracetic acid for sanitation. These fruits were mashed and filtered to get juice. Juice was stored in sealed glass bottles ready for experiments.

Researching Method

Effect of Ultrasound Duration in Microbial Reduction

Guava juice bottles were treated in ultrasonic bath at intensity power 250W in frequency 37 kHz for various duration (10, 15, 20, 25, 30 min).After treatments, $\log_{10}$ reductions of total plate count (TPC) and *coliform* were evaluated to define the optimal ultrasonic duration.

Effect of Dimethyl dicarbonate in Microbial Reduction

Guava juice bottles were added with dimethyl dicarbonate in different concentration (10, 15, 20, 25, 30 µL/100mL). After treatments, $\log_{10}$ reductions of total plate count (TPC) and *coliform* were evaluated to define the optimal dimethyl dicarbonate concentration

Synergistic Effect of Ultrasound Combined Dimethyl dicarbonate in Microbial Reduction

Guava juice bottles were added with 20 µL/100mL of dimethyl dicarbonate under ultrasonic wave in 25 minutes at frequency 37 kHz. The control samples were used with guave juice bottles added 20 µL/100mL of dimethyl dicarbonate alone or guave juice bottles treated by ultrasonic wave in 25 minutes at frequency 37 kHz alone. Log_{10} reductions of total plate count (TPC) and *coliform* were also evaluated to demonstrate the best treatment formula.

Microbial and Statistical Analysis

Total plate count (cfu/ml) and *coliform* (cfu/ml) were enumberated by 3M-Petrifilm. The plates were then incubated at 37°C for 24 hours and 48 hours for *coliform* and total plate count respectively. The experiments were run in triplicate with three different lots of samples. Statistical analysis was performed by the Statgraphics Centurion XVI.

Result & Discussion

Effect of Ultrasound Duration in Microbial Reduction

Non-thermal technology gave better fruit juice quality in inactivation of food borne microorganisms [12, 13]. Ultrasound was used to store fruit juices [14-19]. Anti-microbial effects of ultrasound were created by intracellular acoustic cavitations degrading membrane the permeability, losing selectivity, thinning cell membrane, hot-spot heating, emission of free radicals [20].

Cavitation significantly affected to gram-positive bacteria, spores, spherical-shaped, and small round cells [21]. In our research, guava juice bottles were treated in ultrasonic bath at intensity power 250W in frequency 37 kHz for various duration (10, 15, 20, 25, 30 min).Our results revealed that 25 minutes was adequate for microbial reduction (see Table 1). In watermelon juice, ascorbic acid, lycopene, and phenolic contents decreased dramatically at higher amplitude levels and at the maximum processing time [22]. In jamun juice, anthocyanin degradation

increased with increasing amplitude and duration of exposure [6].

In a calcium-added orange juice, the ascorbic acid content decreased with sonication in a time-dependent manner [15].

Table 1: Effect of ultrasound duration (minutes) in microbial reduction

Ultrasound duration (min)	10	15	20	25	30
Log_{10} reduction of TPC (cfu/ml)	1.05 ± 0.00^{c}	1.97 ± 0.02^{b}	2.24 ± 0.00^{ab}	2.51 ± 0.02^{a}	2.54 ± 0.00^{a}
Log_{10} reduction of *coliform* (cfu/ml)	1.23 ± 0.02^{c}	1.48 ± 0.01^{bc}	1.65 ± 0.03^{b}	1.83 ± 0.01^{ab}	1.97 ± 0.02^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Effect of Dimethyl dicarbonate in Microbial Reduction

Dimethyl dicarbonate has been demonstrated as an effective agent for microbial reduction in fruit juices [23, 24]. Dimethyl dicarbonate was proven for microbiological stabilization of bottled wine; prevention of undesirable yeasts and lactic bacteria proliferation; blocking the fermentation of sweet, semi-sweet and semi-dry wine [25, 26]. In our research, guava juice bottles were added with dimethyl dicarbonate in different concentration (10, 15, 20, 25, 30 µL/100mL).Our result revealed that 20 µL/100mL of dimethyl dicarbonate was adequate to inactivate microorganism (see Table 2). Divol et al [27]. Reported that 20 µL/100 mL dimethyl dicarbonate was very effective against *Zygosaccharomyces bailii* in wine. Supplementation of dimethyl dicarbonate into the UV treated pineapple juice has reduced by 2.61 and 4.87 log CFU/mL, respectively, total plate count and yeasts and molds; dimethyl dicarbonate did not cause any negative effect in the juice [28].

Table 2: Effect of dimethyl dicarbonate concentration (µL/100mL) in microbial reduction

Dimethyl dicarbonate (µL/100mL)	10	15	20	25	30
Log_{10} reduction of TPC (cfu/ml)	1.47 ± 0.00^{c}	1.94 ± 0.01^{bc}	2.46 ± 0.00^{b}	2.61 ± 0.02^{ab}	2.73 ± 0.00^{a}
Log_{10} reduction of *coliform* (cfu/ml)	1.69 ± 0.02^{b}	2.07 ± 0.03^{ab}	2.68 ± 0.02^{a}	2.70 ± 0.01^{a}	2.72 ± 0.03^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Synergistic Effect of Ultrasound Combined Dimethyl dicarbonate in Microbial Reduction

There has a great interest in novel nonthermal processing techniques that minimally modify sensory, nutritional, and functional attributes of fruit and vegetable juices and beverages [29]. In our research, guava juice bottles were added with 20 µL/100mL of dimethyl dicarbonate under ultrasonic wave in 25 minutes at frequency 37 kHz.

The control samples were used with guave juice bottles added 20 µL/100mL of dimethyl dicarbonate alone or guave juice bottles treated by ultrasonic wave in 25 minutes at frequency 37 kHz alone. Our results noticed that physical and chemical combination created better hurdle effect for microbial reduction compared to the solo technique (see Table 3). Antimicrobial agents added to mango nectar before UV treatment enhanced the stability at low temperature storage [30]. Dimethyl dicarbonate added into pitaya juice prior to ultraviolet treatment achieved significantly higher microbial reduction compared to ultraviolet alone, which were 4.12 log 10 and 4.14 log10 reductions for total plate count and for yeast and mold count [31].

The addition of 75 mg/L of dimethyl dicarbonate prior to the combined UV-C light treatment at 55◦C resulted in 5 log10 reductions after only 1.8 min, reducing the treatment time and UV dose of the combined UV-Heat treatment by 44% [32]. The efficacy of high pressure homogenization and dimethyl dicarbonate on microorganism and nutritional compositions of mulberry juice was demonstrated. High pressure homogenization passed at 200 MPa or

supplementation of dimethyl dicarbonate at 250 mg/L significantly inactivated the indigenous microorganisms in mulberry juice [33].

Table 3: Synergistic effect of ultrasound combined dimethyl dicarbonate in microbial reduction

Treatment	Ultrasound alone (25 minutes)	Dimethyl dicarbonate alone (20 µL/100mL)	Ultrasound alone (25 minutes) + Dimethyl dicarbonate alone (20 µL/100mL)
Log_{10} reduction of TPC (cfu/ml)	2.51 ± 0.02^{b}	2.46 ± 0.00^{b}	5.79 ± 0.03^{a}
Log_{10} reduction of *coliform* (cfu/ml)	1.83 ± 0.01^{c}	2.68 ± 0.02^{b}	6.34 ± 0.00^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Conclusion

Guava is an underutilized fruit crop with a high content of important antioxidants and radio-protective ability. A very well-known nutritional benefit of consumption of guava is its rich Vitamin C content performing varied immune functions and protecting the body from free radicals. Efficacy of ultrasound depends both on extrinsic and intrinsic control parameters. In this research, we have successfully demonstrated that ultrasound combined dimethyl dicarbonate created a synergistic effect in microbial inactivation.

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