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RESEARCH ARTICLE

Efficacy of Ultrasound on Pre-fermentation Maceration in Red Dragon Fruit Winemaking

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Abstract

Ultrasound has recently been applied to evaluate the possible effect on color and flavor extraction at different stages of the winemaking process. Red color and aromatic compounds are localized in the skin cells and their release is facilitated by mechanical actions, disruption of tissues and cells, temperature and alcohol. Red dragon fruit has various nutrients, vitamins and minerals and medicinal values. Our present research focused on the effect of power and time of ultrasound as pretreatment maceration in red dragon fruit winemaing. Results revealed that using ultrasound power 270 w in 20 minutes as a pre-treatment on crushed red dragon fruit for the extraction of polyphenol, aromas. The application of ultrasounds allowed a significant reduction of the maceration duration and to get high-quality wine with high alcohol content, total phenolic, and good sensory characteristics.

Keywords: *Red dragon fruit, Ultrasound, Maceration, Power, Duration, Winemaking.*

Introduction

Red dragon fruit or pitahaya (*Hylocereus*) is a vining, terrestrial or epiphytic cactus with fleshy stem. It grows best in dry, tropical and subtropical climates enduring hot temperature. Its fruit has thin, leathery rind with sweet flavoured red pulp inside. Very small, black coloured edible seeds are embedded in the pulp [1]. The fruit pulp is rich in antioxidants, total soluble phenolics, total ascorbic acid, total dietary fibre and pectin and vitamin C, polyunsaturated fatty acids, vitamin B, carotene, protein and minerals like calcium, iron, potashiun, sodium [2].

Eating fruit is considered beneficial for carbohydrate metabolism, strengthening bones and teethes, heart tissues, healthy blood and tissue formation, strengthening immune system, faster healing of bruises and wounds, respiratory tract infections and even as a mild laxative due to substantial fibre content. It is believed to able to lower cholesterol concentration, to balance blood sugar concentration, to prevent colon cancer, to strengthen kidney function and bone, to strengthen the brain workings, increasing the sharpness of the eyes and even used in cosmetic ingredients. It is eaten as fresh or dried fruit, as a vegetable, as a fodder, as a natural colouring agent in various drinks and beverages, as pectin source [1]. Ultrasound is defined as sound waves with frequencies above 20 kHz. It can be applied directly or indirectly. Direct application of ultrasound (probe ultrasound) refers to direct application to the sample medium. Indirect application (bath ultrasound) refers to the application of ultrasound into a secondary medium (water bath) [3]. Direct ultrasound is usually applied with an ultrasonic probe and has a high localized intensity compared to bath ultrasound.

Indirect ultrasound can be used to process larger volumes of solution at a more consistent intensity [4]. Ultrasound creates alternating low- and high-pressures, making compression and rarefaction replication in the liquid. Rarefaction comes to the cavitations, which implode in compression. This is a frequency function and the oscillation amplitude. The incidental decrease in pressure causes vacuum bubbles or cavities. This is followed by an immediate feedback to the original pressure, which creates the collapse cavition, releasing power, which is then moved to liquid [5]. The intensity of cavitation causes local hot spots and powerful pressures in a short period of time. At low frequencies (20-100 kHz), the mechanical effect caused by unstable cavitation predominates, and as the frequency approaches 20 kHz, the bubbles collapse with increasing violence [6]. At medium frequencies (200-500 kHz), the chemical effect is prevalent, as a greater number of bubbles form that collapse less violently. At high frequencies (>1 MHz), both the chemical and physical effects related to cavitation are minimal, while the effect of the acoustic flow is predominant [7].

Ultrasound has been applied to food technologies due to its mechanical and/or chemical effects on the processes of homogenization, mixing, extraction, filtration, crystallization, dehydration, fermentation, and degassing through its antifoaming actions, reduction of particle sizes, temporary or permanent modifications of viscosity, modulation of the growth of living cells, cell destruction and dispersion of aggregates, inactivation of microorganisms and enzymes, and sterilization of equipment [8].

Many researchers have reported on the effectiveness of ultrasound during fermentation to enhance mixing, cell deagglomeration, CO_2 release, mass transfer, bioconversion [9, 16]. A greater percentage of ethanol production may happen due to higher amounts of glucose uptake, a decrease in the ethanol concentration and CO_2 on yeast cell surface [17, 18]. Ultrasound has been recently tested to improve the extraction of phenolic compounds from different materials. Sonication at a controlled temperature can be used to substantially enhance productivity of bioethanol fermentations [19].

Ultrasound could create the hydrodynamic cavitation to improve starch release and improve biofuel ethanol production [20]. Using a mechanical two-stage grinding of lignocellulosic raw materials with ultrasonication increases the efficiency of subsequent enzymatic hydrolysis and fermentation [21].

An investigation was carried out to extract the polyphenols from mango peels by ultrasound-assisted extraction (UAE) and maceration techniques and to assess the antioxidant potential and quantification of phenolic compounds by high performance chromatography [22].

The influence of the maceration techniques such as classic maceration, thermo-maceration, microwave maceration, ultrasound maceration and cryomaceration on the volatile and phenolic compounds content of the wines was demonstrated [23]. The application of high-power ultrasounds to crushed grapes to increase the extraction of phenolic compounds was mentioned [24]. One study optimized the ultrasound-assisted extraction of phenolic compounds from jussara and blueberry fruits.

The optimum extraction conditions for the jussara fruits were: extraction time between 30 and 62 min for total anthocyanins and total phenolics, fruit: solvent ratio of 10% and 6% (w/v) for total anthocyanins and total phenolics, respectively [25]. The effects of ultrasound on mass transfer limitations during fermentation were examined. Direct and indirect ultrasound had negative effects on yeast performance and viability, and reduced the rates of glucose uptake and ethanol production [3].

The application of high power ultrasounds to the crushed grapes to increase the extraction of phenolic compounds was presented [26].During traditional winemaking, grapes are crushed and skin macerated for several days, with pumps overs to facilitate the colour extraction.

To increase this extraction, some chemical (maceration enzymes) or physical technologies (thermovinification, cryomaceration, flash-expansion) can be applied. Maceration is responsible for all the specific characteristics of sight, smell and taste where the volatile compounds (primary aromas and aroma precursors) and phenolic compounds (anthocyanins and tannins) are extracted from skins, seeds and stems [27].

Maceration process is influenced by numerous factors such as temperature, duration, alcohol content, SO_2 where the extraction of the volatile and phenolic compounds varies according to variety, maturation conditions and other factors. Various methods were developed that are available to the winemaker to adjust extraction levels during maceration such as thermo-maceration, microwave and ultrasound maceration [28, 31]. In the present study, we focused on the application of power ultrasound to treat crushed red dragon fruit pulp, to look for an increase of alcohol content, a reduction of the maceration time needed for the extraction of phenolic and volatile compounds in its winemaking.

Materials and Method

Material

Red dragon fruits were collected from Tien Giang province, Vietnam. Ripen and second-grade fruits were utilized to get pulp as substrate for the processing of wine. Maceration and fermentation treatments were performed triplicate. All must samples were come from the same batch. Lab utensils were used including ultrasonic bath, digital balance, glassware, UV-VIS spectrophotometer. Chemical agents were used in this study including sodium carbonate, Folin-Ciocalteu, *Saccharomyces cerevisiae*

Researching Procedure

Effect of Ultrasound Power as Pretreatment (Maceration) to the Red Dragon Fruit Wine Fermentation

500 grams of dragon fruit pulp samples which was added with 500 mL of distilled water in Erlenmeyer flask was placed in ultrasonic bath. An Elmasonic ultrasonic bath was coupled with controlled heating using a cooling coil; connected with a cooling chiller system; and a water pump. Extracts were made at 37 kHz frequency with four ultrasonic power settings (100 w, 180 w, 270 w, 450 w) at bath temperatures of 30°C in 10 minutes.

The treated must would then be fermented by *Saccharomyces cerevisiae* 0.05% in Erlenmeyer flask at 28.0±1.0°C in 14 days as the primary fermentation at 9.0±0.5°C in three months as the aging. These wine would be sampled to analyze alcohol content (%, w/v), total phenolics (mg GAE/g), and sensory score of dragon fruit wine.

Effect of Ultrasound Duration as Pretreatment (Maceration) to the Red Dragon Fruit Wine Fermentation

500 grams of dragon fruit pulp samples which was added with 500 mL of distilled water in Erlenmeyer flask was placed in ultrasonic bath. An Elmasonic ultrasonic bath was coupled with controlled heating using a cooling coil; connected with a cooling chiller system; and a water pump. Extracts were made at 37 kHz frequency with four ultrasonic duration settings (10, 15, 20, 25 minutes) in ultrasonic power 270 w at bath temperatures of 30°C. The treated must would then be fermented by *Saccharomyces cerevisiae* 0.05% in Erlenmeyer flask at 28.0±1.0°C in 14 days as the primary fermentation at 9.0±0.5°C in 3 months as the aging. These wine would be sampled to analyze alcohol content (%, w/v), total phenolics (mg GAE/100g), and sensory score of dragon fruit wine.

Physico-chemical and Biological Analysis

Alcohol content (%) was measured by using densimetric measurements with a flow-through infrared sensor [32]. Total phenolic (mg GAE/100 g) was analyzed by the Folin-Ciocalteu assay [33]. Sensory score was evaluated by a group of 12 members using 9 point-Hedonic scale.

Statistical Analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT). Statistical analysis was performed by the Stat graphics Centurion XVI.

Result & Discussion

Effect of Ultrasound Power as Pretreatment (Maceration) to the Red Dragon Fruit Wine Fermentation

Ultrasound was used as pretreatment for de-agglomeration, particle size reduction, dispersion [34, 38], particle separation [39], and corn ethanol and wastewater treatment processes [40, 34]. It has also been used to disrupt cells [41, 42, 43, 9, 44] and cell membranes, allowing the recovery of intracellular components [45, 46] and for the inactivation of microbes [47, 48] Wine color is one of the main organoleptic characteristics influencing its quality.

It is of special interest in red vinifications due to the economic resources that wineries have to invest for the extraction of the phenolic compounds [26]. In this experiment, we examined the maceration of red dragon fruit must under 37 kHz frequencies with four ultrasonic duration settings (10, 15, 20, 25 minutes) in ultrasonic power 270 w at bath temperatures of 30°C. The treated must would then be fermented by *Saccharomyces cerevisiae* 0.05% in Erlenmeyer flask at 28.0±1.0°C in 14 days as the primary fermentation at 9.0±0.5°C in 3 months as the aging. These wine would be sampled to analyze alcohol content (%, w/v), total phenolics (mg GAE/100g), and sensory score of dragon fruit wine. Results were noted in table 1. It's obviously concluded that ultrasound power 270 w was adequate for maceration in winemaking.

Table 1: Effect of ultrasound power as pretreatment (maceration) to the red dragon fruit wine fermentation in respect of alcohol (%, w/v), total phenolic (mg GAE/ 100 g), sensory score

Ultrasound power (w)	Alcohol content (%)	Total phenolic (mg GAE/100 g)	Sensory score
100	3.69±0.01^{b}	39.37±0.02^{b}	6.61±0.03^{b}
180	3.95±0.00^{ab}	41.45±0.01^{ab}	6.92±0.01^{ab}
270	4.32±0.02^{a}	41.78±0.00^{a}	7.14±0.03^{a}
450	4.40±0.01^{a}	41.81±0.03^{a}	7.17±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%)

The application of high-power ultrasounds to crushed grapes to increase the extraction of phenolic compounds was mentioned. Crushed grapes were treated with this non-thermal technology and vinified, with 3, 6 and 8 days of skin maceration time and the results were compared with a control vinification. The wines made with ultrasound-treated grapes showed differences with the control wine, especially regarding total phenol content and tannin content.

The wines elaborated with sonicated grapes and with only three days of skin maceration time presented similar concentration of anthocyanins and twice the concentration of tannins than control wines elaborated with 8 days of skin maceration [24]. The influence of the maceration techniques such as classic maceration, thermo-maceration, microwave maceration, ultrasound maceration and cryomaceration on the volatile and phenolic compounds content of the wines was demonstrated. It was observed that the total polyphenol index and Folin-Ciocalteu index expressed higher values for the wine samples [23].

Effect of Ultrasound Duration as Pretreatment (Maceration) to the Red Dragon Fruit Wine Fermentation

Various groups of components, such as aromas, polyphenols, organic substances and minerals, have effectively been extracted from a variety of materials using ultrasound [7]. Cavitation causes the cell walls to break and release their elements into the medium [49, 51]. Higher yields are archived in short time with lower processing temperatures. The main benefits are decreases in extraction and processing times, the amount of energy and solvents used unit operations, and CO_2 emissions [52]. Ultrasound extraction releases water and lipid-soluble extracts and is more advantageous than conventional methods because all active components are potentially extracted simultaneously using a solvent mixture, ensuring that the matrix is exhausted.

In this experiment, we examined the maceration of red dragon fruit must under 37 kHz frequencies with four ultrasonic duration settings (10, 15, 20, 25 minutes) in ultrasonic power 270 w at bath temperatures of 30°C. The treated must would then be fermented by *Saccharomyces cerevisiae* 0.05% in Erlenmeyer flask at 28.0±1.0°C in 14 days as the primary fermentation at 9.0±0.5°C in 3 months as the aging. These wine would be sampled to analyze alcohol content (%, w/v), total phenolics (mg GAE/100g), and sensory score of dragon fruit wine. Results were noted in Table 2. It's obviously concluded that ultrasound duration in 20 minutes was adequate for maceration in winemaking.

Table 2: Effect of ultrasound duration as pretreatment (maceration) to the red dragonfruit wine fermentation in respect of alcohol (%, w/v), total phenolic (mg GAE/100 g), sensory score

Ultrasound duration (minutes)	Alcohol content (%)	Total phenolic (mg GAE/100 g)	Sensory score
10	4.32±0.02^{b}	41.78±0.00^{b}	7.14±0.03^{b}
15	4.77±0.00^{ab}	42.01±0.03^{ab}	7.86±0.01^{ab}
20	4.96±0.01^{a}	42.45±0.01^{a}	7.99±0.04^{a}
25	5.00±0.03^{a}	42.53±0.02^{a}	8.03±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%)

During cavitation, the collapse of bubbles destroys the cell walls of plant materials, releasing their phytochemical components such as anthocyanin [53, 54], bioflavonoids [44], polysaccharide extracts [55]. Ultrasound has been used to break the cell walls via cavitation. An increase in the extraction efficiency of phenolic compounds from grape wine was mentioned [54, 56, 57, 58, 59].Ultrasound has also been used in the pretreatment step before fermentation to eliminate contaminated microbials that might hinder the main fermentation process [60]. An investigation was carried out to extract the polyphenols from mango peels by ultrasound-assisted extraction (UAE) and maceration techniques and to assess the antioxidant potential and quantification of phenolic compounds by high performance chromatography.

Results showed that highest extraction yield obtained through solvent methanol at 80% concentration level while ultrasound-assisted extraction was more efficient technique and led to comparatively higher polyphenols content than maceration [22]. In another report, crushed grapes (400 kg) from the 2017 harvest were treated with ultrasound, and three different lengths of skin maceration period (2, 3 or 7 days) and the results were compared with a control vinification, where grapes were not subjected to any treatment and were skin macerated during 7 days. The wine chromatic characteristics and the individual phenolic compounds were followed during all the maceration period, at the end of alcoholic fermentation and after bottle storage.

The wines made with ultrasound treated grapes presented differences with control wine, especially as regard color and total phenol and tannin content, the wines with three days of maceration time presenting similar chromatic characteristics than control wines with 7 days of maceration time [26].

Conclusion

Ultrasound is composed of mechanical sound waves that come from molecular movements that oscillate in a propagation medium. Ultrasound pretreatment of biomass has been shown to improve starch release and ethanol yield. It also helps to extract the phenolic and volatile compounds responsible for wine colour.

These compounds are mainly located inside the skin cell vacuoles. The transfer of phenolic compounds from grapes to must during vinification is essential. Red dragon fruits (*Hylocereus* spp.) are considered as a heavenly fruit with high nutritive and medicinal values. The employment of ultrasound could provide high-quality wines in shorter time, with reduced spoilage organisms and higher content in anthocyanins together with pleasant flavor [61].

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