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The Effect of Ultraviolet and Heat Treatments on Microbial Stability, Antioxidant Activity and Sensory Properties of Ready-to-serve Tropical Almond Drink

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ABSTRACT

There is a growing trend towards the production of ready to use multifunctional foods which has nutritional and medicinal value as well as good sensory properties. On the other hands, microbial safety of those foods is a major concern in food industry. Treatments used to ensure microbial safety of foods can affect the medicinal, nutritional and sensory properties of food. Aim of this study was to evaluate the affect of two such treatments namely UV irradiation and thermal processing on some important properties of ready to serve tropical almond drink. Prepared tropical almond ready to serve drink samples were subjected to UV irradiation and three different heat treatments. Then microbial stability, antioxidant activity and sensory properties of those treated samples were evaluated comparatively to non-treated samples. Two properties tested in this study, namely microbial stability and antioxidant activity was found to be affected by both treatments tested. Sensory properties of the product were found to be affected only by thermal processing. Compared to non-treated and thermal processed tropical almond ready to serve drinks, UV irradiated tropical almond ready to serve drinks were found to be microbiologically more safe and with preserved sensory properties. Hence, UV irradiation was identified as a suitable method to treat tropical almond ready to serve drinks to ensure microbial safety over thermal processing.

Key words: UV irradiation, thermal processing, microbial stability, antioxidant activity, sensory properties

INTRODUCTION

Tropical almond (*Terminaliya catappa*) is primarily a coastal tree belonging to the family combretaceae which commonly forms beach forests from sandy shores in to the forests behind. However, it is also a feature of the landscape in regions even far from the sea. This tree is reported to be indigenous to Andamans and its neighboring islands. It is presently grown in countries such as Brazil, India, Ghana, Malaysia, Myanmar, Papua New Guinea, Mexico, Peru, Puerto Rico, The Philippines, Singapore, Sri Lanka, Taiwan etc. In Sri Lanka it is grown in the lowland areas in various parts of the island, up to an elevation of about 300 m, as a beautification and fruit plant (Gunasena *et al.*, 2007). Tropical almond fruit is reported to be containing antioxidant, anticancer and antidiabetic compounds and hence it is an important medicinal plant (Lall *et al.*, 1999; Nagappa *et al.*, 2003). There is a growing trend towards production of foods with various functions as nutritious as well as medicinal value using medicinally important plants (Muchuweti *et al.*, 2007; Lee *et al.*, 2009). Ready to treat tropical almond drink is such product produced concerning its nutritious as well as medicinal value.

Food can be contaminated by microorganisms at various stages of production, processing, storage and distribution. Some of those microorganisms are pathogenic to man and animals. So, it is safe to assume that food may carry risk of food-borne illness if not properly handled and prepared before consumption (Loaharanu, 1996). The occurrence of illness due to consumption of contaminated unpasteurized fruit juice has led some countries to establish microbial quality standards to control such illnesses (Gabriel and Nakano, 2009; Goodrich *et al.*, 2005). As per these regulations, to ensure the microbial safety, manufacturers of juice products are compelled to subject those juice products to a processing step or combination of processes capable of reducing populations of target pathogens by a given amount (Goodrich *et al.*, 2005). Thermal processing, ultraviolet and gamma irradiation, osmotic dehydration etc., are used by food manufacturers in this regard. However, many factors should be considered in the selection of a suitable method, out of those methods for a particular food, which has minimum effect to the characters of the food. Effect of these microbial control methods to antioxidants activity and sensory properties of food is major concern as they directly effect the medicinal value and consumer preference of the food respectively (Chipurura and Muchuweti, 2010; Chipurura *et al.*, 2010).

Thermal processing has very long history as an effective means of juice pasteurization. Moreover, thermal processing has been recognized as an efficient method of pasteurization which ensures microbial safety and enhances the shelf life of fruit juice (Donahue *et al.*, 2004). However, many fruit juice manufacturers elect not to apply thermal processing due to its effect to the nutritional and sensory properties of the product. Therefore, methods such as ultraviolet and gamma irradiation, pulsed electric field application are being considered by fruit juice manufacturers as alternative methods (Noci *et al.*, 2008). In UV irradiation food products are exposed to germicidal light with a wavelength of 220-300 nm which inactivates microbial contaminants. UV irradiation causes the cross-linking of neighboring pyrimidine nucleotide bases in the same DNA strand of microbial cells which eventually cause cell death (Sizer and Balasubramaniam, 1999).

Many studies have been carried out on the effect of thermal processing on different qualities of different foods (Mozolewski *et al.*, 2004; Jeong *et al.*, 2004; Agbede, 2004; Enujiugha and Akanbi, 2005; Terpinc *et al.*, 2011; Chipurura and Muchuweti, 2010). Moreover, research has been carried out to study the effect of UV and gamma irradiation on different qualities of different foods (Lee *et al.*, 2009; Chipurura *et al.*, 2010). However, effect of those to qualities of ready to serve drinks and ability to use UV irradiation to substitute thermal processing has not been studied. This study was conducted to evaluate the ability of thermal processing and UV irradiation to ensure the microbial safety and their effect to antioxidant activity and sensory properties of ready to serve tropical almond drink.

MATERIALS AND METHODS

Preparation of RTS: Ready to serve tropical almond drink was prepared by the following procedure. Fruit flesh (150 g) was blended well and pulp was separated using sieve. Then 1000 mL of water was added into pulp and mixed well. Then 6 g of citric acid 220 g of sugar and 0.3 g of SMS was added and 12° Brix RTS was produced. Then, prepared RTS drink was filled into bottles and sealed.

Preparation of bacterial cultures: Five bacterial species were used in this study namely, *Salmonella enteritidis*, *Salmonella typhimurium*, *Vibrio parahaemolyticus*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Bacterial samples for heat and ultra violet inactivation test

were prepared by following procedure as explained by Lee *et al.* (2009). Individual test bacterium was loop-inoculated from Nutrient Agar slant stock culture into a sterile 10 mL nutrient broth tube and incubated at 37°C. The bacteria were harvested for inactivation studies 18-24 h post incubation. The cells were harvested by spinning 1.0 mL of the Nutrient broth suspension on a bench top centrifuge at 8000 rpm for 5 min. The supernatant liquid was then decanted and the cell pellets were re-suspended in 1.0 mL of prepared RTS drink. The re-suspended cells were allowed to acclimatize in the suspending medium for 15-30 min prior to the inactivation studies.

Ultraviolet irradiation and heat inactivation: RTS samples inoculated with five test bacteria and acclimatized were used for UV irradiation and heat inactivation studies. Prior to UV exposure, 0.5 mL of the acclimatized, re-suspended cells were diluted with 4.5 mL of prepared RTS. Then, another 10-fold dilution was done by mixing 2.5 mL of the resulting suspension with 22.5 mL of the prepared RTS. Eventually, 5 mL of the final suspension were aspirated into sterile 43 mm plastic petri plates where the contained volume had a height of 5.0 mm.

For the UV irradiation studies, each prepared plate were exposed to UV radiation for 0-2 min in a biological safety cabinet with a pair of 15 UV light source at a lamp-to-juice surface distance of 55.0 cm.

For the heat inactivation studies, 9.9 mL of inoculated RTS samples in glass test tubes were heated to reach desired temperatures (55, 65 and 75°C) on a water bath. The medium temperature was measured by inserting a thermometer through the cold point of a control tube. When the cold point temperature reached desired temperature, 0.1 mL of the RTS samples inoculated with five test bacteria and acclimatized were pipetted into each of the tubes and heated up to 2 min while agitated manually. After heat treatments, tubes were immediately immersed in an ice bath and kept until survivor enumerations.

Survivor enumeration and decimal reduction times calculations: RTS samples obtained at five different time intervals after treatments (0, 0.5, 1.0, 1.5, 2 min) were used for survivor enumeration and decimal reduction time calculations. All UV-irradiated and heat inactivated samples were serially diluted using sterilized distilled water and pour plated on nutrient agar. Plated dilutions were then incubated at 37°C. Emerging colonies were enumerated 24-48 h post incubation. The decimal reduction times (D value) of each of the test sample were then determined by plotting the log₁₀ of the calculated colony-forming units versus irradiation or heating time. The Survivor Curve (SC) was then determined by tracing the best-fitted straight line in the survivor plots. The D value was equivalent to the number of minutes of irradiation or heating that resulted to 90% loss in the viability of the test organism and graphically equivalent to the negative inverse of the slope of the survivor curve (Jay *et al.*, 2005).

Total phenolic contents, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity and Ferric reducing antioxidant power (FRAP): Total phenolic contents of all treated samples and control were measured using the Folin-Ciocalteu colorimetric method as explained by Gao *et al.* (2000). Phenolic contents were expressed as gallic acid equivalents. DPPH free-radical scavenging effect was estimated according to the method proposed by Blois (1958). The FRAP (ferric reducing/antioxidant power) assay was performed as described by Benzie and Strain (1996) using a spectrophotometer. In this assay, reductants in the sample reduce the Fe(III)/tripirydyltriazine complex, present in the stoichiometric excess, to the blue ferrous form, with an increase in the

absorbance at 593 nm wave length . Absorbance readings were taken after 0.5 sec and every 30 sec thereafter during the monitoring period for 5 min and the readings at 4 min were used as the FRAP value (mM g^{-1}) as described by Lee *et al.* (2009).

Sensory evaluation: All RTS samples which were subjected to UV and heat treatments and control sample were evaluated for their sensory properties by a panel of 15 expert members. Seven point hedonic scale ranging from “dislike extremely to “like extremely” was used to evaluate six different sensory properties namely, odor, color, taste, off odor, off taste and overall acceptance. Each sample was labeled with three digit code and about 50 mL of each sample were given individually to the panelists for evaluation. Water was provided to wash the oral cavity after testing of each sample. The sensory test was carried out three times and average values of those were used for the analysis (Meilgaard *et al.*, 1999).

RESULTS AND DISCUSSION

Microbial analysis: The calculated D values per test bacterium in UV-irradiated and heat treated samples are summarized in Table 1. The bacteria *Listeria monocytogenes* were shown to be significantly more resistant than the other organisms in all treatments. The bacteria *Salmonella enteritidis* and *E. coli* O157:H7 were shown to be significantly less resistance to all treatments. These results are in agreement to the results obtained in previous studies using different food matrices. In Gabriel and Nakano (2009) had obtained similar results for inoculated PBS and apple juice samples subjected to UV irradiation. In Beltran and Canovas (2005) also reported that *Listeria* had better resistance to UV irradiation compared to *E. coli* when suspended in apple juice.

When consider heat treatments, the results were not in agreement with the results reported by Gabriel and Nakano (2009) for inoculated apple juice samples and results reported by Sharma *et al.* (2005) for inoculated watermelon juice. In those studies, *E. coli* was reported to be more heat resistant than *Listeria monocytogenes* but in the present study results was vice versa. Mak *et al.* (2001) and Mazzotta (2001) also reported that *E. coli* O157:H7 is more heat resistant than *L. monocytogenes* and *Salmonella* in their studies conducted using inoculated apple juice. However, results of the present study for heat treatments were in agreement with some other studies. In a study conducted by Murphy *et al.* (2004), *L. monocytogenes* had greater resistance than *Salmonella* spp. and *E. coli* O157:H7 when heated at 55°C in ground pork. In another study conducted by Sharma *et al.* (2005), *L. monocytogenes* was found to be relatively more heat resistant than *E. coli* O157:H7 and *Salmonella* spp. when heated at 57°C in cantaloupe juice. Hence an appropriate universal target organism for evaluating the lethality of thermal processes of food can't

Table 1: Decimal reduction values (D) of test pathogens in UV irradiated and heat treated tropical almond ready to serve drinks

| Organisms | D value (min) | | | |
|---------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | UV | 55 | 65 | 75 |
| | | ----- (°C)----- | | |
| <i>Salmonella enteritidis</i> | 0.32±0.03 ^z | 0.82±0.12 ^w | 0.68±0.14 ^{cx} | 0.27±0.04 ^{dz} |
| <i>Salmonella typhimurium</i> | 0.61±0.07 ^y | 1.08±0.32 ^w | 0.81±0.08 ^{cx} | 0.58±0.21 ^{cy} |
| <i>Vibrio parahaemolyticus</i> | 0.45±0.12 ^y | 2.34±0.62 ^{bw} | 1.76±0.13 ^{bx} | 0.52±0.11 ^{cy} |
| <i>Escherichia coli</i> O157:H7 | 0.34±0.05 ^z | 2.42±0.21 ^{bw} | 1.78±0.20 ^{bx} | 0.87±0.38 ^{by} |
| <i>Listeria monocytogenes</i> | 1.16±0.12 ^{xy} | 3.22±0.33 ^{aw} | 2.68±0.16 ^{ax} | 1.08±0.61 ^{ay} |

Values with different letters (a-d) within the same column and values with different letters (w-z) within the same row are significantly different at $p < 0.05$

Table 2: Values of tests, Total phenolic content (TPC), DPPH activity and FRAP assay of UV irradiated, heat treated and non-treated tropical almond ready to serve drink

| Treatment | Tests for phenolic compounds | | |
|-----------|------------------------------|-------------------------|--------------------------|
| | TPC | DPPH | FRAP |
| Control | 44.12±0.01 ^d | 32.37±0.11 ^b | 0.208±0.003 ^c |
| UV | 48.26±0.03 ^c | 36.68±0.12 ^a | 0.358±0.002 ^a |
| 55°C | 51.22±0.01 ^b | 36.42±0.03 ^a | 0.271±0.006 ^b |
| 65°C | 56.30±0.02 ^a | 36.61±0.12 ^a | 0.268±0.003 ^b |
| 75°C | 57.43±0.01 ^a | 36.68±0.08 ^a | 0.279±0.002 ^b |

Values with different letters (a-d) within the same column are differ significantly (p<0.05)

be identified and different studies should carried out to identify suitable organisms for different food matrixes. When compare the results obtained for UV irradiated samples and heat treated samples, UV irradiation found to be significantly effective than heat treatments except samples of *Salmonella typhimurium*, *Vibrio parahaemolyticus* and *Listeria monocytogenes* treated by 75°C. These results confirm the possibility of using UV irradiation to replace heat treatments in food industry to ensure the microbiological safety.

Total phenolic contents, DPPH activity and FRAP assay: Values of total phenolic content, DPPH radical scavenging activity and ferrous reducing antioxidant power assay for UV irradiated, heat treated and non-treated (control) tropical almond ready to serve drink are summarized in Table 2.

Total phenolic contents (TPC): Compared to control sample, significantly high Total Phenolic Contents (TPC) was observed in UV eradiated samples. This significant increase of TPC in irradiated samples may be due to the degradation of larger phenolic compounds into smaller phenolic compounds by irradiation as previously explained by Harrison and Were (2007). When irradiated, radicals such as hydrated electrons, hydroxyl radicals and hydrogen atoms are produced by radiolysis of water in the samples (Fan and Mastovska, 2006). In addition to that, these radicals may break glycosidic bonds in larger phenolic compounds and produce smaller phenolic compounds (Lee *et al.*, 2009). Total phenolic content of the heat treated samples were also significantly higher compared to control sample. Moreover, total phenolic content of heat treated samples were significantly higher than the UV irradiated samples. Total phenolic content was found to be increased with the increase of treatment temperature as previously explained by Jeong *et al.* (2004) and Terpinic *et al.* (2011) for other foods.

DPPH activity: Number of research have been carried out to investigate the effect of UV and gamma irradiation on the DPPH radical scavenging activity. However results of those studies are not consistence. In some of those research irradiation found to be effecting to increase the DPPH radical scavenging activity (Ahn *et al.*, 2004; Variyar *et al.*, 2004; Jo *et al.*, 2003). In some of those studies irradiation found to be effecting to decrease the DPPH radical scavenging activity (Ahn *et al.*, 2005; Suhaj *et al.*, 2006). In some cases, no significant changes of the radical scavenging abilities were observed (Byun *et al.*, 2002; Byun *et al.*, 1999). In the present study DPPH radical scavenging activity of irradiated samples was found to be significantly increased compared to fresh samples. When consider heat treatments, as previously explained by some other

Table 3: Sensory values of UV irradiated and heat treated tropical almond ready to serve drink

| Parameter | Treatments | | | |
|--------------------|------------------------|------------------------|------------------------|------------------------|
| | UV | 55 | 65 | 75 |
| Color | 4.25±0.12 ^a | 3.18±0.11 ^b | 3.10±0.10 ^b | 2.46±0.14 ^d |
| Odor | 4.36±0.08 ^a | 3.27±0.14 ^b | 3.41±0.07 ^b | 3.12±0.08 ^c |
| Taste | 4.89±0.23 ^a | 3.22±0.09 ^b | 3.36±0.12 ^b | 3.08±0.10 ^c |
| Off odors | 1.22±0.22 ^c | 3.23±0.17 ^b | 3.31±0.09 ^b | 4.81±0.15 ^a |
| Off tastes | 1.03±0.04 ^c | 3.33±0.12 ^b | 3.41±0.14 ^b | 4.63±0.09 ^a |
| Overall acceptance | 4.82±0.21 ^a | 3.35±0.03 ^b | 3.43±0.06 ^b | 3.06±0.11 ^c |

Values with different letters (a-d) within the same row are significantly different at (p<0.05)

authors for some other foods (Jeong *et al.*, 2004; Terpinic *et al.*, 2011), DPPH radical scavenging activity was found to be significantly increased by the heat treatment compared to the control. However, significant difference of DPPH radical scavenging activity was not observed UV irradiated samples with heat treated samples or in different heat treatments.

FRAP assay: The FRAP (ferrous reducing antioxidant power) assay is commonly used for assessing antioxidant activity, since it has high sensitivity and is rapid and inexpensive. In this assay, inactivation of oxidant by the reductants (antioxidants in the sample) is used as the principle to assess the antioxidants. In the present investigation, significantly high antioxidant activity was observed in UV and heat treated samples compared to the control. However, no significant difference was observed in antioxidant activity of different heat treated samples. The antioxidant activity of UV irradiated samples was significantly higher than all other samples tested. This could be due to formation of Maillard reaction products by irradiation which have the ability to scavenge hydroxyl radical and superoxide as explained by Chawla *et al.* (2007).

Sensory evaluation: Table 3, shows the sensory evaluation results of odor, color, taste, overall acceptance, off odor and off taste. A significant difference in the sensory scores was observed in the irradiated ready to use tropical almond drink when compared to that of heat treated samples. These samples were highly preferred by the sensory panel. The sensory data for the samples heat treated by 55 and 65°C were not significantly different. Sensory data of the sample heat treated by 75°C were significantly different from the other and were least preferred by the sensory panel. These results indicate that irradiation by UV treatments helps to maintain sensory qualities compared to heat treatments. Moreover, the results shows that sensory qualities are highly affect when high temperature conditions are used. Some comparative studies have been carried out to compare the sensory qualities of different fresh and irradiated foods (Song *et al.*, 2007) and no significant changes were observed. Those results are supportive to the results obtained in this study. However, the present study is the first study which compares the effect of heat and UV treatments to the sensory qualities of ready to serve drinks.

CONCLUSIONS

Thermal processing was found to be affecting all parameters tested namely, microbial stability, antioxidant activity and sensory properties of tropical almond ready to serve drink. UV irradiation found to be effecting two of those parameters namely, microbial stability and antioxidant activity.

Compared to thermal processed tropical almond ready to serve drinks, UV irradiated tropical almond ready to serve drinks were found to be with preserved sensory properties and microbiologically more safe. In conclusion, UV irradiation was identified as a substitute for thermal processing in tropical almond ready to serve drink production.

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