Study on Combined Effects of Acidification and Sonication on Selected Quality Attributes of Carrot Juice during Storage

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Summary: This study evaluated the combined effects of acid blanching and sonication treatments on selected quality parameters of carrot juice stored at 4 ºC for 18 days. Carrots were blanched in acidified water (40g/L citric acid) at 100 ºC for 4 min and the juice was then extracted. Sonication of the juice was done at an amplitude level of 70% and a frequency of 20 kHz for 2 min at 15 ºC, keeping the pulse duration of 5 Sec on and 5 Sec off. As results, the combined treatment of acidification and sonication of carrot juice showed a significant decrease in pH and increase ($P < 0.05$) in acidity which remained stable during storage period. No significant changes were observed in °Brix. Color values (L*, a*, b*) and non enzymatic browning (NEB) influenced significantly in acidified and sonicated carrot juice during storage period. Maximum stability of total phenol, total antioxidant capacity, cloud value and ascorbic acid were also observed in the combined treatment of acidification and sonication. The findings of this study indicated that the combined treatments of acidification and sonication may successfully be utilized for the production of high quality carrot juice with improved stability of total phenol, total antioxidant capacity, cloud value and ascorbic acid during 18 days of storage.

Keywords: Carrot juice, Acidification, Sonication, Storage, Total phenol, Ascorbic acid.

Introduction

Fruits and vegetables are considered an important part of the human diet. They have been playing a significant role in the prevention of many diseases due to the presence of antioxidant compounds such as carotenoids, phenolic compounds and ascorbic acid [1]. Among vegetables, carrot is a very important nutritious root crop containing a considerable quantity of vitamins (B_1, B_2, B_6 and B_{12}) and minerals [2]. It is a major source of carotenoids and depicts strong antioxidant potential due to the presence of ascorbic acid and phenolic compounds [3]. Among human foods, carrot is a richest source of β-carotene, a precursor of vitamin A [4]. These unique characteristics lead to the interest of making carrots as a source of juice. Carrot juice is very nutritious and liked by many people throughout the world especially due to the presence of high amount of β-carotene, an important health promoting component [5]. As a strong antioxidant compound, β-carotene can improve immune system and inhibit the growth of tumor cells [6]. However, carrot juice is difficult to preserve due to its low acidity which provide a favorable environment for microbial growth that spoils the product [7]. Certainly, the shelf life of carrot juice can be improved by increasing acidity either by the addition of citric acid or by fermentation [7].

Color is an important quality parameter of carrot juice. Carotenoids are the major compounds responsible for the characteristic orange color of carrots [8]. But, the condensation of phenolic acids inducing by enzymatic and non enzymatic browning (NEB) reactions during storage would cause discoloration of carrot juice [9]. Therefore, the color of the thermally treated juice can be improved specially by acid blanching prior to juice extraction [10]. Carrot juice also contains ascorbic acid, a powerful antioxidant but it is very much sensitive to heat. Its loss may also be an indicator of some other modifications which effect sensory and nutritional quality of the juice [11].

Traditionally, shelf life of vegetable juices has been improved by using thermal techniques which inactivate enzymes and microorganisms [12]. Thermal processing causes adverse effects on color, flavor and nutrients [13, 14]. Consumers now demand for products of extended shelf life with natural quality characteristics. Importance of vegetable juice lies in its natural taste and freshness [15]. In response to the consumers demand, therefore, food industry is now looking for new techniques that are less aggressive to the food agents responsible for its freshness and nutritional value. Sonication is one of those techniques which are gaining more attraction

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and importance as an alternative to heat treatments. Due to changes in pressure, power ultrasound produces bubble cavitations (basis of ultrasound benefits) in the liquid. The localized temperature of liquid rises up to 5000°C and pressure up to 50000kPa due to violent collapse of bubbles in the next compression cycle of ultrasonic waves resulting high shear forces [16]. A recent study has shown that sonication could effectively improve the quality of apple juice [17]. Keeping in view the demands of manufacturers, consumers and recent innovative use of ultrasound, we investigated the effects of sonication in combination with acidification on the quality of carrot juice in the present study. The objective of this study was to evaluate the combined effects of acid blanching and sonication on selected quality parameters such as pH, titratable acidity, ºBrix, color attributes (L*, a* and b* value), total phenol, total antioxidant capacity, ascorbic acid, cloud value, non-enzymatic browning (NEB) and electrical conductivity of carrot juice during storage period of 18 days.

Results and Discussion

Effects of Acidification and Sonication on pH, Titratable Acidity and ºBrix of Carrot Juice during Storage

The pH and acidity are important quality parameters which maintain the shelf life of vegetable juices, and ºBrix value is also a very common quality parameter of juices [18, 19]. The results regarding the effects of acidification and sonication on pH, acidity and ºBrix of carrot juice are mentioned in Table-1. The results of the present study demonstrated that the pH and acidity of acid blanched (AB) and acid blanched and sonicated (ABS) treatments of carrot juice on day 0 were significantly different as compared to control sample. It was also observed that AB and ABS treatments of carrot juice showed higher stability regarding pH and acidity compared to control sample during storage period of 18 days. The observed significant decrease in pH and increase in acidity for AB and ABS treatments on day 0 was due to the addition of citric acid. But we did not notice any significant difference in ºBrix for AB and ABS treatments on day 0 as compared to control, whereas these treatments remained more stable regarding ºBrix compared to control sample which showed significant decrease in ºBrix during storage period of 18 days at 4°C (Table-1). In this study, we found that pH, acidity and ºBrix of acidified and sonicated carrot juice samples remained more stable compared to control untreated carrot juice sample during storage period of 18 days. This would not alter the organoleptic characteristics of juice, which depend largely on the balance among acids, sugars and other compounds present in the juice [19].

Table-1: Effect of acidification and sonication on Brix, pH and titratable acidity of carrot juice during storage period at 4℃.

Treatments	Time (Days)	pH	Titratable acidity	ºBrix
Control	0	5.95 ± 0.04^{ab}	0.10 ± 0.01^{e}	7.00 ± 0.10^{abc}
	3	6.06 ± 0.02^{a}	0.09 ± 0.02^{e}	7.00 ± 0.10^{abc}
	6	5.94 ± 0.05^{ab}	0.10 ± 0.02^{e}	6.80 ± 0.02^{de}
	9	5.80 ± 0.04^{bc}	0.14 ± 0.04^{d}	6.70 ± 0.11^{e}
	12	5.70 ± 0.09^{c}	0.16 ± 0.02^{d}	6.50 ± 0.03^{f}
	15	5.00 ± 0.20^{d}	0.22 ± 0.03^{c}	6.50 ± 0.03^{f}
	18	4.90 ± 0.09^{d}	0.25 ± 0.03^{c}	6.00 ± 0.01^{g}
AB	0	3.79 ± 0.08^{efg}	0.30 ± 0.03^{ab}	7.00 ± 0.10^{abc}
	3	3.88 ± 0.12^{ef}	0.29 ± 0.05^{b}	7.00 ± 0.10^{abc}
	6	3.91 ± 0.10^{e}	0.29 ± 0.02^{b}	7.10 ± 0.20^{a}
	9	3.84 ± 0.09^{efg}	0.29 ± 0.03^{b}	7.10 ± 0.05^{a}
	12	3.74 ± 0.16^{fg}	0.31 ± 0.05^{ab}	7.00 ± 0.10^{abc}
	15	3.70 ± 0.09^{g}	0.32 ± 0.05^{ab}	7.00 ± 0.67^{abc}
	18	3.70 ± 0.11^{g}	0.31 ± 0.04^{ab}	6.90 ± 0.12^{bcd}
ABS	0	3.78 ± 0.15^{efg}	0.32 ± 0.01^{ab}	6.95 ± 0.20^{abcd}
	3	3.90 ± 0.13^{e}	0.31 ± 0.03^{ab}	7.00 ± 0.08^{abc}
	6	3.89 ± 0.08^{ef}	0.31 ± 0.05^{ab}	7.08 ± 0.02^{ab}
	9	3.84 ± 0.12^{efg}	0.32 ± 0.03^{ab}	6.90 ± 0.08^{bcd}
	12	3.82 ± 0.14^{efg}	0.31 ± 0.06^{ab}	6.90 ± 0.13^{bcd}
	15	3.82 ± 0.10^{efg}	0.30 ± 0.01^{ab}	6.90 ± 0.16^{bcd}
	18	3.77 ± 0.16^{efg}	0.33 ± 0.04^{a}	6.85 ± 0.15^{cde}

$^{a\text{-}g}$ Means followed by the same letter within each column are not significantly different (factorial test, n = 3, $p < 0.05$); AB: acid blanched, ABS: acid blanched and sonicated.

Effects of Acidification and Sonication on Color (L, a* and b* value) of Carrot Juice during Storage*

Color is one of the most important visual quality parameters for the prompt identification and ultimate acceptance of fruit juices. It is one of the sensory characteristics on which the acceptance and satisfaction of consumers is based. Results regarding the effects of acidification and sonication on the color values of carrot juice are mentioned in Table-2. In the present study, we observed a significant increase in all the color values (L*, a* and b*) in AB and ABS treatments of carrot juice compared to control untreated juice samples. Similarly, increases in all color values of carrot juice in AB and ABS treatments were also observed during 18 days storage period at 4°C. The increases in color values in the AB treatments might be due to the improved extraction of carotenoids due to acidification which may cause color improvements. It has already been observed that acidification at lower pH was more effective than that at higher pH [20]. In ABS treatment, sonication caused further improvement in color by accelerating isomerization of carotenoids due to severe physical conditions of temperature and pressure [21]. The actions of air, light and heat during storage can cause oxidation of carotenoids, cis/trans changes and changes in epoxide rings which ultimately, altered the color of the juices [22].

Table-2: Effect of acidification and sonication on color attributes of carrot juice during storage period at 4℃.

Treatments	Time (Days)	L*	a*	b*
Control	0	40.61 ± 0.05^{t}	20.97 ± 0.06^{q}	16.66 ± 0.15^{n}
	3	47.29 ± 0.06^{l}	24.96 ± 0.04^{l}	29.70 ± 0.11^{h}
	6	47.29 ± 0.05^{j}	25.48 ± 0.05^{k}	30.03 ± 0.08^{g}
	9	45.39 ± 0.08^{q}	24.44 ± 0.03^{m}	28.69 ± 0.13^{i}
	12	45.53 ± 0.06^{p}	24.94 ± 0.02^{l}	28.77 ± 0.10^{i}
	15	45.71 ± 0.09^{o}	24.41 ± 0.04^{mn}	27.42 ± 0.12^{j}
	18	45.77 ± 0.07^{n}	24.38 ± 0.08^{n}	26.23 ± 0.13^{k}
AB	0	42.83 ± 0.04^{s}	22.49 ± 0.06^{p}	18.50 ± 0.04^{m}
	3	49.39 ± 0.09^{d}	26.48 ± 0.04^{j}	33.21 ± 0.08^{e}
	6	49.50 ± 0.06^{c}	27.10 ± 0.09^{g}	33.29 ± 0.05^{de}
	9	47.35 ± 0.04^{k}	26.55 ± 0.07^{i}	33.25 ± 0.06^{de}
	12	48.06 ± 0.06^{h}	26.89 ± 0.08^{h}	33.04 ± 0.12^{f}
	15	47.32 ± 0.02^{kl}	27.78 ± 0.05^{d}	33.35 ± 0.05^{de}
	18	47.24 ± 0.07^{m}	27.54 ± 0.04^{e}	33.37 ± 0.12^{d}
ABS	0	43.42 ± 0.07^{r}	24.17 ± 0.06^{o}	19.03 ± 0.07^{l}
	3	49.71 ± 0.05^{a}	28.48 ± 0.08^{b}	35.21 ± 0.08^{ab}
	6	49.67 ± 0.06^{b}	28.55 ± 0.04^{a}	35.25 ± 0.11^{a}
	9	47.77 ± 0.08^{i}	27.52 ± 0.06^{e}	34.77 ± 0.08^{c}
	12	48.94 ± 0.07^{e}	27.41 ± 0.08^{f}	35.10 ± 0.11^{b}
	15	48.10 ± 0.06^{g}	28.36 ± 0.10^{c}	35.07 ± 0.14^{b}
	18	48.19 ± 0.10^{f}	28.45 ± 0.07^{b}	35.11 ± 0.10^{ab}

[a-t] Means followed by the same letter within each column are not significantly different (factorial test, n = 3, $p < 0.05$); AB: acid blanched, ABS: acid blanched and sonicated.

Effects of Acidification and Sonication on Electrical Conductivity Color of Carrot Juice during Storage

Mostly, liquid foods contain water and nutrients like vitamins, minerals, proteins and fatty acids. Normally, these nutrients are electrical conductors [23]. The results regarding the effects of acidification and sonication on electrical conductivity of carrot juice are shown in Fig. 1. In our study, we observed a decrease in electrical conductivity in AB and ABS treatments of carrot juice on day 0 compared to untreated control juice sample. It was also observed that the electrical conductivity in all the AB and ABS treatments decreased during storage period of 18 days but the value of electrical conductivity in ABS treatment on day 18 was very close to the value on day 0 which showed that it was quite stable as compared to control and AB treatment. The decrease in electrical conductivity could be attributed to the reaction of added citric acid with metallic ions [24] and increase in electrical conductivity in ABS treatment could be directly attributed to the cavitations produced during sonication which could produce radicals and also increase in pressure and temperature in the center of bubbles, gas phase of bubbles and in its liquid interface [25]. The decrease in electrical conductivity of carrot juice during the storage period might be due to the loss of nutrients responsible for electrical conductivity.

Effects of Acidification and Sonication on Non-Enzymatic Browning (NEB) of Carrot Juice during Storage

NEB is an important quality parameter of carrot juice. Fig. 2 shows the results regarding the effects of acidification and sonication on NEB of carrot juice during storage period of 18 days. Significant changes in NEB of carrot juice in AB and ABS treatments were observed when compared with the untreated control juice sample. During storage period of 18 days, less increase in NEB was observed in AB and ABS treatments of carrot juice as compared to control which showed more increase in NEB during the storage. Our results regarding the increase in NEB during storage period of carrot juice are in agreement with the observations of sonicated orange juice [26]. Increase of NEB in ABS treatment might be attributed to the breakdown of color pigments due to sonication treatment. Oxidation of carotenoids and condensation of phenolic acid caused browning in the carrot puree [9]. The degradation of ascorbic acid during storage period is also responsible for NEB [27].

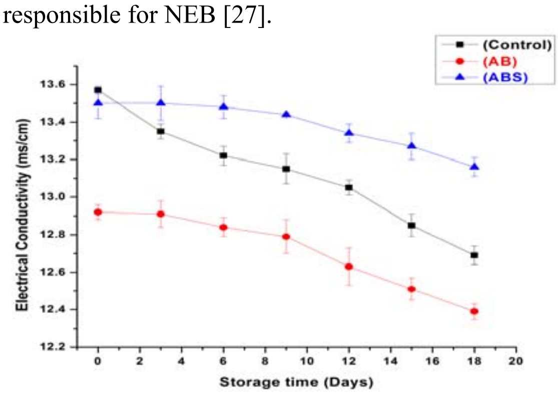

Fig. 1: Effect of acidification and sonication on electrical conductivity of carrot juice during storage at 4℃

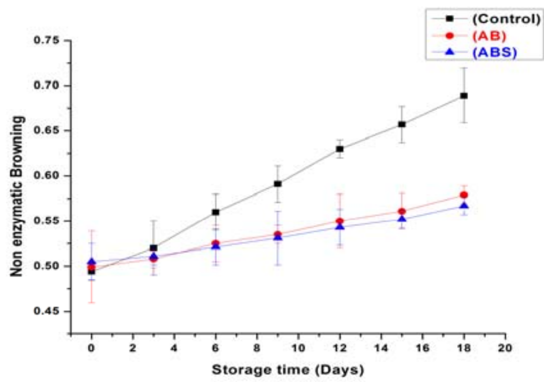

Fig. 2: Effect of acidification and sonication on non-enzymatic browning of carrot juice during storage at 4℃

Effects of Acidification and Sonication on Cloud Value of Carrot Juice during Storage

Cloud is concerned with the suspended particles, and the cloud stability in fruit juices is desirable characteristic that plays a role in the enhancement of color and flavor of juice. It is one of the visual quality parameters of cloudy fruit juices for identification and ultimate acceptance of consumer [28]. Fig. 3 shows the results regarding the effects of acidification and sonication on the cloud value of carrot juice during storage. In the present study, significant increases in cloud values for AB and ABS treatments were observed on day 0 as compared to control juice sample. It was also observed that the ABS treatment showed more retention and stability of clouds even after 18 days of storage as compared to control. Acidification and sonication collectively caused a more pronounced improvement and retention in the cloud value of carrot juice. Previously, some studies conducted on sonicated orange juice and guava puree have shown similar results regarding the cloud value [29, 30]. The reason behind is the extraction of juice from the carrots blanched at lower pH due to acid addition that showed a strong effect on pectin esterification which caused lower pectin precipitation and ultimately enhanced the cloud value of the juice [31]. Additionally, ultrasound treatments produced more consistent, more uniform and more homogenized product by reducing the size of suspended particles in the juice due to cavitations. Hence, combination of acidification and sonication could enhance and stabilize clouds in the carrot juice even after storage of 18 days.

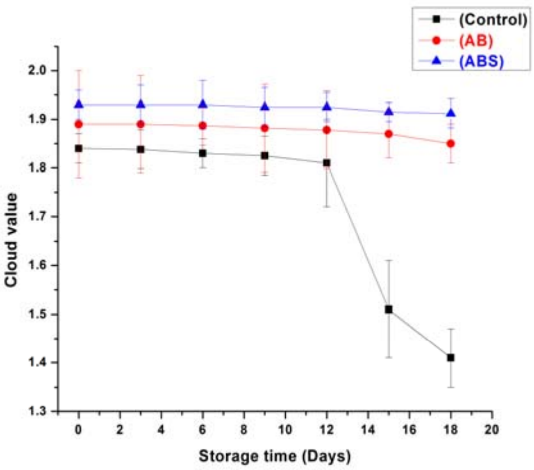

Fig. 3: Effect of acidification and sonication on cloud value retention of carrot juice during storage at 4℃.

Effects of Acidification and Sonication on Ascorbic Acid of Carrot Juice during Storage

Ascorbic acid is an important antioxidant compound which plays a role in protecting our bodies from the risk of many heart and cancer diseases [32]. The stability and retention of ascorbic acid in the juices serve as an indicator of quality and shelf life. Fig. 4 showed the results regarding the effects of acidification and sonication on the contents of ascorbic acid of carrot juice. On day 0, we observed a slight decrease in the contents of ascorbic acid in AB treatment, whereas a significant increase in the contents of ascorbic acid in the ABS treatment of carrot juice samples was observed when compared with untreated control juice samples. During 18 days storage period at 4°C, the ABS treatment showed more stability and retention in contents of ascorbic acid as compared to control. Our results regarding significant enhancement in contents of ascorbic acid in the ABS treatment on day 0 are consistent with the observations of sonicated apple juice [17]. Furthermore, the results regarding more stability and retention of contents of ascorbic acid during storage are in accordance with the observations of sonicated orange juice [26]. The improvement and stability in the contents of ascorbic acid in the ABS treatment of carrot juice could be directly attributed to the sonication technique in which no heat is supplied with the product and further and protection of juice from oxidation of ascorbic acid contents by the removal of oxygen from the juice as a function of sonication [33]. So, sonication could protect the ascorbic acid of carrot juice from both heat and oxygen which are the chief sources of its loss during processing.

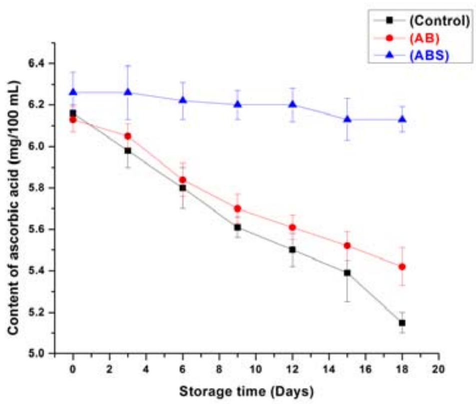

Fig. 4: Effect of acidification and sonication on ascorbic acid retention of carrot juice during storage at 4 ºC

Effects of Acidification and Sonication on Total Phenols and Total Antioxidant Capacity of Carrot Juice during Storage

Phenolic compounds play a significant role in the prevention of arteriosclerosis diseases [34, 35] and also serve as antioxidants and thus reduce the risk of cancer and cardiovascular diseases [36-39]. Results regarding the combined effects of acidification and sonication on total phenolic compounds and total antioxidant capacity of carrot juice during storage period of 18 days are mentioned in Table-3. Significant increases in total phenol in AB and ABS treatments of carrot juice samples were observed on day 0 as compared to control which then, decreased significantly in AB treatment during the storage period while in ABS treatment it remained stable as compared to control. Similarly on day 0, a significant increase in total antioxidant capacity of AB or ABS treatment was observed as compared to control untreated sample. But a decrease in total antioxidant capacity was observed in AB treatment during storage period of 18 days as compared to control while it remained more stable in ABS treatment during this period when compared with control. The addition of citric acid could change the pH and acidity of carrot juice which could affect the total phenolic compounds and total antioxidant capacity of carrot juice. The changes in phenolic compounds of treated carrot juice samples initially and during the storage period may also affect the total antioxidant capacity of the carrot juice accordingly. Our results regarding total antioxidant capacity in AB treatment are in accordance with a previous report [40] which showed a significant increase in total antioxidant capacity of the juice extracted from carrots blanched in citric acid and then decreased over a storage period of 21 days. The decrease in total antioxidant capacity in AB treatment over the storage period may be due to the addition of citric acid which may adversely affect the stability of antioxidant compounds present in the juice [40]. In our present study, initially the improvement and then the stability of total antioxidant capacity in the ABS treatment of the carrot juice during storage might be due to the enhancement and stability of antioxidant compounds like phenolic acids, flavonoids and vitamins in the juice due to sonication treatment. It has already been reported that the total antioxidant capacity of juices is connected with the amount of bioactive compounds like phenolic acids, flavonoids, carotenoids and vitamins [41]. In this study, we found higher stability of phenolic compounds and total antioxidant capacity specially, in the combined treatment of acidification and sonication of carrot juice even after 18 days of storage.

Experimental

Chemicals

Gallic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin Ciocalteu reagent was purchased from Fluka (Buchs, Switzerland). HPLC grade methanol was purchased from Hanbon Science and Technology (Nanjing, China). Sulfuric acid, sodium phosphate, sodium carbonate, sodium hydroxide, citric acid, ammonium molybdate, aluminum and ascorbic acid were obtained from Sinopharm Chemical Regent Co., Ltd (Shanghai, China). All other chemicals and reagents used were of analytical grade.

Table-3: Effect of acidification and sonication on total phenols and total antioxidant capacity of carrot juice during storage period at 4℃

Treatments	Time (Days)	TP (GAE µg/g)	TAOC (AAE µg/g)
Control	0	352.11 ± 0.99^{f}	256.41 ± 0.89^{k}
	3	353.70 ± 0.91^{e}	257.10 ± 0.80^{j}
	6	345.67 ± 0.96^{g}	252.14 ± 0.96^{l}
	9	329.34 ± 0.76^{j}	243.56 ± 0.95^{n}
	12	316.57 ± 0.87^{l}	235.76 ± 0.98^{o}
	15	305.90 ± 0.71^{n}	221.83 ± 0.96^{q}
	18	270.18 ± 0.20^{o}	200.16 ± 0.97^{r}
AB	0	355.12 ± 0.33^{d}	267.21 ± 0.93^{e}
	3	353.56 ± 0.98^{e}	265.34 ± 0.97^{g}
	6	338.76 ± 0.88^{h}	250.45 ± 0.95^{m}
	9	330.09 ± 0.98^{ij}	243.56 ± 0.90^{n}
	12	310.56 ± 0.97^{m}	227.37 ± 0.78^{p}
	15	305.32 ± 0.86^{n}	199.79 ± 0.90^{s}
	18	260.67 ± 0.90^{p}	180.23 ± 0.93^{t}
ABS	0	370.45 ± 0.96^{a}	280.73 ± 0.97^{b}
	3	369.35 ± 0.90^{b}	281.94 ± 0.96^{a}
	6	365.78 ± 0.96^{c}	279.43 ± 0.87^{c}
	9	364.80 ± 0.99^{c}	272.56 ± 0.92^{d}
	12	355.72 ± 0.93^{d}	265.78 ± 0.94^{f}
	15	330.45 ± 0.96^{i}	263.78 ± 0.85^{h}
	18	323.81 ± 0.90^{k}	260.67 ± 0.97^{i}

[a-t] Means followed by the same letter within each column are not significantly different (factorial test, n = 3, $p < 0.05$); AB: acid blanched, ABS: acid blanched and sonicated, TP (GAE µg/g): total phenols (gallic acid equivalent µg/g), TAOC (AAE µg/g): total antioxidant capacity (ascorbic acid equivalent µg/g).

Blanching and Extraction of Carrot Juice

Fresh good quality carrots were purchased from a local vegetable market of Nanjing, China. Carrots were washed with tap water to get rid of external impurities and ½ inch top and bottom were discarded. Then carrots were peeled and sliced manually to 2 cm thickness using stainless steel knife. The slices were divided into two parts. One part for blanching in water (300g carrots/L water) and another for blanching in acidified water (40g citric acid/L water) at 100 ºC for 4 min. After cooling, these slices were pressed to make juice using a Panasonic juice extractor (MJ-M176P, Panasonic Manufacturing Berhad, Malaysia). Clear Juice was obtained by passing through eight layered cheese cloth.

Ultrasound Treatment

Sonication of carrot juice (250 mL in a 500 mL jacketed vessel) was done using ultrasonic processor of 750W (VC 750, Sonics and Materials Inc., Newtown, CT, USA) with 0.5 inch probe at constant temperature of 15 ºC. Juice samples were processed at a frequency of 20 kHz and amplitude level of 70 % for 2 min with pulse duration of 5 Sec on and 5 Sec off. All the treatments were done in triplicate and water blanched juice was selected as a control.

Packaging and Storage

After sonication all the juice samples (90 mL each) were put into 100 mL sterilized polypropylene tubes which were enclosed in aluminum foil to avoid any interference of light with samples. Samples were stored at 4 ºC for 18 days and were analyzed after three day intervals.

Determination of pH, Titratable Acidity and ºBrix

Digital pH meter (Delta 320 pH meter, Metller Toledo Instruments (Shanghai) Co., Ltd, China) was used to determine the pH of all the samples. Ten mL sample was put into a beaker and continuously stirred using magnetic stirrer and pH was measured at 20 ± 0.5 ºC. Buffer solutions of pH 7.0 and 4.0 were used to calibrate the pH meter.

Titratable acidity was determined by AOAC method [42]. The sample was diluted by mixing 90 mL of distilled water and 10 mL of juice sample in a 250 mL beaker. Samples were titrated against 0.1 N NaOH standard solutions to the end point (pH 8.2 ± 0.1) by continuously stirring with magnetic stirrer. Titratable acidity was calculated by using equation given below:

$$\text{TA (\%)} = \frac{\text{V} \times 0.1\ \text{N NaOH} \times 0.067 \times 100}{m}$$

where V is the titer volume of NaOH, and m is the volume of carrot juice (mL).

Hand refractometer (WYT-80, Quanzhou Wander Experimental Instrument Co., Ltd, China) was used to measure ºBrix of all the carrot juice samples at 20 ± 0.5°C. Before measuring the value of next sample, refractometer prism was washed with distilled water. All measurements were carried out in triplicate.

Determination of Color Attributes

Minolta Colorimeter (Chroma Meter CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) was used to measure the color values of carrot juice samples. Before determination, the colorimeter was calibrated using white reference tile. Color values were shown as CIE L* (whiteness or brightness/darkness), a* (redness/greenness) and b* (yellowness/blueness) system. All measurements were carried out in triplicate.

Determination of Electrical Conductivity, NEB and Cloud Value

Conductivity meter (Mettler Toledo LE703, Anton Paar, China) was used to measure the electrical conductivity of all the samples at 20 ºC.

NEB was determined by a method stated by Meydav *et al*. [43] with some modifications. Five mL carrot juice samples and 5 mL ethyl alcohol were mixed and centrifuged at 5000 rpm (TGL-16G, Anke Shanghai Anting Scientific Instrument Factory, China) for 20 min. Absorbance of supernatant was measured at 420 nm by using a spectrophotometer (722S Visible Spectrophotometer, Shanghai Jinghua Science & Technology Instruments Co., Ltd, China).

Cloud value was determined by using a method reported by Versteeg *et al*. [44] with some modifications. Briefly, 5 mL carrot juice sample was centrifuged at 5000 rpm for 15 min. And then absorbance was measured using spectrophotometer at 660 nm with distilled water used as blank.

Determination of Ascorbic Acid

Ascorbic acid was measured by a method described by Lee and Coates [45] with slight modifications. HPLC of Agilent 1100 series (Agilent Technologies, USA) consisted of a model G1311A pump, G1379A degasser, G1316A column oven and G1315B diode array detector with Tskgel ODS-100Z column (4.6 × 150 μm, 5 mm, Tosoh, Japan) was used. Mobile phase of methanol 30% and flow rate of 1.0 mL/min with detector wavelength of 280 nm was used. Twenty μL sample was injected after passing through a syringe filter of 0.45 μm. Proper concentrations of standard ascorbic acid were used to make a suitable calibration curve and final results were expressed as mg ascorbic acid/100 mL of carrot juice.

Determination of Contents of Total Phenolics

Total phenol was determined by the Folin-Ciocalteu colorimetric method [46] with some modifications. Juice sample (500 μL) was taken in a vial and 1000 μL of 10 % Folin-Ciocalteu reagent was added in it. After 6 min, 2000 μL of 20% sodium carbonate was mixed. The mixture was then allowed to stand for 60 min in a water bath at 30 ºC. The absorbance was measured at 760 nm. Standard solution of gallic acid was used to make a suitable curve and the amount of total phenols was shown as μg gallic acid equivalent per gram of sample.

Determination of Total Antioxidant Capacity

Total antioxidant capacity was assessed by using a method developed by Prieto *et al*. [47]. A known sample (400 μL, 250 μg/mL in methanol) was taken in a vial and then 4000 μL reagent solution was added. Reagent solution contained 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The mixture was then kept for 90 min in a water bath at 95°C. The blank solution contained 4000 μL of reagent solution and 400 μL of methanol. Absorbance was determined at 695 nm by using a spectrophotometer against blank after cooling to the room temperature. Total antioxidant capacity was shown as μg ascorbic acid/g of sample using ascorbic acid as standard.

Statistical Analysis

For statistical analysis, a completely randomized design was used with a factorial experiment design and significant differences between mean values were determined by LSD pair-wise comparison test at a significance level of $p < 0.05$. Statistical analyses were conducted using Statistix 9.0 software (Analytical Software, Tallahassee FL, USA).

Conclusions

Our study showed that acidification of carrots prior to juice extraction in combination with sonication treatment could produce high quality carrot juice with higher stability of total phenols, total antioxidant capacity, cloud value and ascorbic acid during storage period of 18 days. In combined treatments of acidification and sonication, pH, acidity and °Brix also remained stable during storage period. On the basis of results obtained from this study, evaluation of sensory characteristics and coloring pigments including β-carotene value, lutein and lycopene also warrants further investigations. Based on the present study, we suggest that the acid blanching and sonication in combination may be implemented on a commercial scale for the production of carrot juice with improved quality and stability during storage to get more benefits.

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