PHYTOCHEMICAL CONTENTS AND ANTIOXIDANT ACTIVITIES OF PASTEURIZED *CITRUS AURANTIUM* L. (BITTER ORANGE) JUICE

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Abstract

Bitter orange (*Citrus aurantium* L.) is an ancient therapeutic plant. Normally, peels of bitter orange fruit are used as a food ingredient in Thailand, leaving the pulp as waste. This research aimed to develop pasteurized bitter orange juice by studying the effect of dilution and pH on phytochemical contents and antioxidant activity. The bitter orange juice concentrations were prepared at 50, 75, and 100% w/v by DI water and adjusting pH at 2.5, 3.0, and 3.5 by using potassium chloride. The bitter orange juices were pasteurized at 70°C for 5 min. The result showed that 100% w/v pasteurized bitter orange had the highest contents of titratable acidity, ascorbic acid, and total phenolic, approximately 41.60 to 48.67 g/L, 31.26 to 32.26 mg/100 mL, and 54.80 to 57.08 mg GAE/100 mL of juice, respectively when compared to other concentrations. Likewise, antioxidant activity results (DPPH, ABTS, and FRAP) of 100% w/v pasteurized bitter orange juice gave the highest values about 390.58 to 458.15 GAE/100 mL of sample 546.20 to 503.33 µg Trolox/100 mL of sample, and 525.55 to 422.48 µg Trolox/100 mL of sample.

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The pH variation did not affect the alteration of phytochemical and antioxidant activities in 100% w/v pasteurized bitter orange ($p<0.05$). Conversely, pH adjusting of 50 and 75% w/v pasteurized bitter oranges showed significant differences in the ascorbic acid and total phenolic contents ($p\leq0.05$) but non-significant differences in citric and ascorbic acid contents. All pasteurized bitter orange juice microbiological resulted showed that the total coliform counts was <2.2 MPN/ 100 mL of sample and yeast and molds were not detected initially and after 5 days of storage, confirming the safety of the pasteurization process.

Keywords: *citrus aurantium* l., phytochemical, antioxidant activity

Introduction

Bitter orange or sour orange (*Citrus aurantium* L.) is in the Rutaceae family (Memariani et al., 2020). It is native to Southern and Eastern Asia, Malasia, New Coledonia, and Australia (Reuther et al., 1967, as cited in Benayad et al., 2021), and is the most resistant plant of all Citrus species (Cerdagne, 2004, as cited in Benayad et al., 2021). Bitter orange commonly named in Thai as Som-Sa, is a rare ancient medicinal plant normally used as an ingredient in food (Lualamai, 2021). Bitter orange is also an herb used in China and South America (Xutian et al., 2014; Stohs, 2017). Bitter orange fruit is about 5-8 cm round. The peel is rough and thick; it has an intense aroma. Raw fruit is very green, while ripe fruit is yellow (Figure 1). The flesh has a sweet and sour flavor and is succulent with many seeds (Sustainable Agriculture Foundation, 2020; Lualamai, 2021).

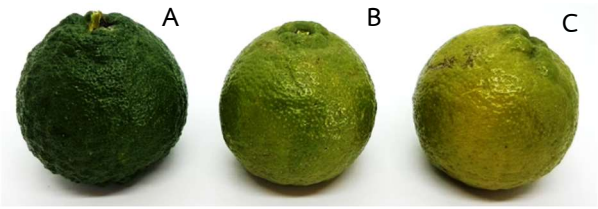

Figure 1 Bitter orange fruit (A) Unripe stage (B) Semi-ripe stage and (C) Ripe stage

The chemical compounds in bitter orange are sugar, pectin, vitamins, minerals, carotenoids, citric acid, ascorbic acid, and other organic acids, including phytochemicals such as coumarins and flavonoids (Divya et al., 2016). Flavonoids are important bioactive phytochemicals in bitter oranges (Suntar et al., 2018). All parts of bitter orange contain phytochemicals and essential oils. The flowers contain naringin and linalool volatile

compounds, the seeds contain flavonoids, mainly naringin and linoleic acid, and the leaves contain phenols, flavonoids, and linalyl acetate. Peel and juice contain ρ-coumaric and ferulic acids, mostly phenolic compounds, and limonene, an essential oil (Maksoud et al., 2021). Bitter orange juice had phenolic compounds as a component of 86% (Tounsi et al., 2011, as cited in Maksoud et al., 2021). Bitter orange juice had phenolic compounds and flavonoid content of about 295±4 mg GAE/g and 26 mg Eq QE/g (quercetin equivalent per g of fresh juice), respectively. In addition, the juice of C. *aurantium* and *C. maxima* showed better values than *C. clementina*, *C. limon*, and *C. sinensis* (Haraoui et al., 2020). The antioxidant activities of the unripe and ripe bitter orange juice were 60.5-89.5 and 57.7-88.8%, respectively. However, topography, season, and harvesting time affect the phytochemicals and essential oils contents of bitter orange (Maksoud et al., 2021). Divya et al. (2016) reported that phenolic compounds in bitter orange juice using methanol and water as solvents were 60.0±1.4 and 58.0±2.1 mg/g, respectively, while the anti-oxidation activities (DPPH) were about 64.3±1.7% and 31.8±1.7%, respectively. Bitter orange juice is an active antimicrobial against *Salmonella enterica* Typhimurium and *Listeria monocytogenes* (Karabıyıklı et al., 2014). Bitter orange juice can inhibit *Pseudomonas aeruginosa* isolated from burns Infections, although it is not as effective as *C. Limon* (Mohammed et al., 2018). In Thailand, the commercial planting area of bitter orange is in Nonthaburi Province (Lualamai, 2021). Most bitter oranges are purchased to use the peels as ingredients in food, such as sauces and curry paste, leaving the pulp as waste. Pulps may be eaten, but most are considered worthless. Currently, peels are being sold through online platforms. This research aims to develop pasteurized bitter orange juice to take advantage of whole fruit by studying dilution and pH that affecting pasteurized juice's quality. Regardless of whether bitter orange juice has a more beneficial effect on the human body, the fruit's high acidity (pH 2.3) limits its consumption. Thus, the development of pasteurized bitter orange juice by adjusting the pH value from 2.5 to 3.5, similar to the pH of commercial juice and beverages in the market was conducted. The survey of some juices and beverages as commercial products in this research, such as orange juices, passion fruit juices, tomato juices, energy drinks, etc., found that their pH is about 2.5-4.3. The pH control of beverages at a pH lower than 4.6 can increase the efficiency of the pasteurization process (Saeeduddin et

al., 2016). Pasteurization is divided into 4 types: high-temperature long time (HTLT), high-temperature short time (HTST), mild temperature-long time (MTLT), and mild temperature-short time (MTST) (Ağçam et al., 2018). The application of mild temperature slightly changes the nutrition and sensory characteristics of the product (Fellows, 2017). Juice pasteurization typically is between 60°C and 100°C, with the temperature depending on the target microbial species or enzymes that want to be destroyed (Ağçam et al., 2018). Likewise, the pH directly affects the sensory, stability, and shelf life of beverages (Brossard et al., 2019; Singh et al., 2022) and the stability of vitamin C (Yin et al., 2022), including antioxidant activity (Yu et al., 2022).

Materials and methods

Preparation of pasteurized bitter orange

Bitter oranges planted in Muang district, Suphanburi province, were harvested at the ripe stage and without defects in May at 9:00-10:00 a.m. The appearance of bitter orange flesh at the ripe stage shown as in Figure 2.

Figure 2 The appearance of bitter orange flesh at the ripe stage**.**

The fruits were washed with clean water 2 times, drained, peeled out. Squeezed the pulp and filtered it through a colander (125 microns) to extract the juice. The bitter orange was diluted at 50, 75, and 100% w/v by DI water, and the pH was adjusted by 1 M potassium hydroxide (KOH) (Loba Chemie™, India) to 2.5, 3.0, and 3.5, respectively. The juice was pasteurized at 70°C for 5 min. Then it was hot filled into 100 mL sterile bottles at a controlled temperature not exceeding 60°C. The bitter orange juice (the whole bottle) was pasteurized again by boiling for 5 min at 70°C and cooling down in cooled water until temperature decreased to 25-30°C and kept at 4°C. The titratable acidity, ascorbic acid, total phenolic contents, and antioxidant activities were analyzed within 2 days. The microbiological analysis was performed on the initial day and after 5 days of storage.

Determination of titratable acidity

Titratable acidity analysis adapted from Desseva et al. (2020). The sample was pipetted 10 mL into an Erlenmeyer flask and mixed with 50 mL of DI water. Few drops of phenolphthalein were added as an indicator. Afterward, the juice samples were titrated with 0.1 M sodium hydroxide (NaOH) (Loba Chemie™, India) until the endpoint was reached (record the volume of 0.1 M NaOH). Titratable acidity is calculated from equation [1], expressed as % citric acid (the gram equivalent weight of citric acid is 64.04 g.).

$$\text{Titratable acidity (g citric acid/L)} = \frac{N \times V_{NaOH} \times 64.04 \times 100}{1000 \times V_{sample}} \quad [1]$$

where, N: NaOH concentration, V_{NaOH}: NaOH volume (mL), V_{sample}: Sample volume (mL)

Determination of ascorbic acid

Ascorbic acid analysis adapted from Arunrung-aree et al. (2005). The 0.01 M iodine was prepared by dissolving 6.35 g of iodine and 10 g of potassium iodide (KI) in 200 mL of DI water, and the solution's volume was adjusted to 500 mL with DI water. Ten mL of the sample was mixed with 50 mL of DI water into an Erlenmeyer flask, and 2% starch (indicator) was added in 2-3 drops. Later, the samples were titrated with an iodine solution until the endpoint was reached (record the iodine solution volume). One g/L of ascorbic acid was used as the standard solution. The ascorbic acid was calculated from equation [2].

$$\text{Ascorbic acid content (mg/100 mL)} = \left(\frac{(V_{iodine} \times 1.76}{V_{juice}} \right) \times 100 \quad [2]$$

where, V_{iodine}: Volume of 0.01 M iodine (mL), V_{juice}: Volume of sample (mL), 1.76: 1 mL of I_2 (0.01 M) = 1.76 mg of ascorbic acid.

Determination of total phenolic contents

The sample or standard solution (gallic acid, Sigma-Aldrich, USA) was 200 µL and mixed with 2.5 mL of DI water. Then 500 µL of 1% Folin & Ciocalteu phenol reagent (Sigma-Aldrich, USA) was added into the sample and keep in the dark for 5 min at room temperature. Afterward, the reaction sample was added with 2 mL of 7.5% sodium carbonate (Na_2CO_3) (Loba Chemie™, India) and kept in the dark for 90 min at room temperature (adapted from Rimlamduan et al., 2024). Then, the absorbance was measured

at 765 nm; total phenolic contents were calculated from the standard curve equation ($y = 2.0246x + 0.1515$, $R^2 = 0.9939$ when y = absorbance, and x = gallic acid concentration).

DPPH radical scavenging (DPPH assay)

The sample or standard solution (Trolox, Sigma-Aldrich, USA) (300 µL) was mixed with 1.5 mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA) in ethanol and kept in the dark for 40 min at room temperature (adapted from Rimlamduan et al., 2024). The absorbance was measured at 517 nm; DPPH radical scavenging was determined from the standard curve equation ($y = -1.9876x + 0.8293$, $R^2 = 0.9907$ when y = absorbance, and x = Trolox concentration).

ABTS radical scavenging activity (ABTS assay)

Mixing 7 mM 2,2 -Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (Sigma-Aldrich, USA) with 2.45 mM potassium persulfate ($K_2S_2O_8$) (Q RëC, New Zealand) at a ratio of 1: 1, kept in the dark and at room temperature for 12 hr. The solution was diluted with 95% methanol, and the solution absorbance at 734 nm was controlled at about 0.700±0.05. The sample or standard solution (Trolox, Sigma-Aldrich, USA) (20 µL) were mixed with 1,980 µL of ABTS solution, stored in the dark for 5 min at room temperature (Rimlamduan et al., 2024). The absorbance was measured at 734 nm, and antioxidant activity was calculated from the standard curve equation ($y = -0.3785x + 1.0377$, $R^2 = 0.9948$ when y = absorbance, and x = Trolox concentration).

Ferric reducing antioxidant power assay (FRAP assay)

FRAP solution was prepared by mixing the 300 mM sodium acetate buffer (pH 3.6), 20 mM ferric chloride ($FeCl_3$), and 10 mM 2,3,5-Triphenyltetrazolium chloride (TPTZ) in 40 mM HCl at a ratio of 10: 1: 1 (v/v/v). The sample or standard solution (Trolox, Sigma-Aldrich, USA) was about 150 µL mixed with 1.5 mL of FRAP solution and kept in the dark for 20 min at room temperature (Rimlamduan et al., 2024). The absorbance was measured at 593 nm, and antioxidant activity was calculated from the standard curve equation ($y = 2.9047x + 0.3752$, $R^2 = 0.9936$ when y = absorbance, and x = Trolox concentration).

Microbiological analysis (total coliform counts, and yeasts and molds)

The Juice was sampled on an initial day and 5 days of storage at 25°C. They were diluted through 5 serial dilutions using 0.85% NaOH. Potato dextrose agar (PDA,

Himedia, India) was used as a culture medium with the spread plate technique for yeast and mold analysis. The yeast and mold colonies were counted and reported following the Bacteriological Analytical Manual (2001). Total coliform counts were analyzed using the most probable number (MPN) technique (Bacteriological Analytical Manual, 2020).

Statistics analysis

The experiment was repeated 3 times. Statistical results were analyzed using analysis of variance (ANOVA), and differences between means were examined using Duncan's New Multiple Range Test (DMRT).

Results

Titratable acidity, ascorbic acid, total phenolic contents, and microbiological analysis of pasteurized bitter orange juice

The titratable acidity, ascorbic acid, total phenolic contents, total coliform counts, and yeasts and molds of pasteurized bitter orange juice are shown in Table 1.

Table 1 Titratable acidity, ascorbic acid, total phenolic contents total coliform counts, and yeasts and molds of pasteurized bitter orange juice.

Sample number	Juice conc. (% w/v)	pH	Citric acid (g/L of sample)	Ascorbic acid (mg/100 mL of sample)	TPC (mg GAE/100 mL of sample)	TCC (MPN/100 mL of sample)		Yeasts & Molds (CFU/100 mL of sample)	
						0 day	5 days	0 day	5 days
1	50	2.5	26.47±1.48^{c}	17.90±0.94^{c}	40.36±1.23^{c}	<2.2	<2.2	n.d.	n.d.
2	50	3.0	25.62±0.00^{c}	17.34±0.21^{c}	42.70±1.87^{c}	<2.2	<2.2	n.d.	n.d.
3	50	3.5	26.47±1.48^{c}	18.01±0.87^{c}	40.20±1.56^{c}	<2.2	<2.2	n.d.	n.d.
4	75	2.5	35.86±2.56^{b}	24.43±1.61^{b}	51.90±2.16^{b}	<2.2	<2.2	n.d.	n.d.
5	75	3.0	38.42±0.00^{b}	25.91±0.34^{b}	55.59±0.07^{ab}	<2.2	<2.2	n.d.	n.d.
6	75	3.5	36.72±1.48^{b}	25.14±1.06^{b}	53.64±2.33^{ab}	<2.2	<2.2	n.d.	n.d.
7	100	2.5	42.34±0.84^{b}	31.26±0.99^{a}	54.80±1.78^{ab}	<2.2	<2.2	n.d.	n.d.
8	100	3.0	41.96±0.79^{b}	31.87±0.99^{a}	55.09±3.12^{ab}	<2.2	<2.2	n.d.	n.d.
9	100	3.5	41.60±0.58^{b}	33.02±1.24^{a}	56.02±2.47^{ab}	<2.2	<2.2	n.d.	n.d.
10 (control)	100	2.3	48.67±2.56^{a}	32.26±1.40^{a}	57.08±1.05^{a}	<2.2	<2.2	n.d.	n.d.

Remark "TPC" means total phenolic content.; "TCC" means total coliform counts.; "GAE" means Gallic acid.; The letters "a, b, c, d" in each column indicate a significant difference at p≥0.05.; The letter "n.d." means non-detect.

Antioxidant activities of pasteurized bitter orange juice

The results of antioxidant activities of pasteurized bitter orange juice are shown in Table 2.

Table 2 Antioxidant activities of pasteurized bitter orange juice.

Sample number	Juice conc. (% w/v)	pH	ABTS μg Trolox/100 mL of sample)	DPPH mg GAE/100 mL of sample	FRAP μg Trolox/100 mL of sample)
1	25	2.5	119.20±7.01^{d}	54.34±7.72^{d}	80.07±4.88^{e}
2	25	3.0	120.08±2.68^{d}	53.91±12.45^{d}	81.31±3.77^{e}
3	25	3.5	127.76±3.30^{d}	55.80±11.56^{d}	79.78±1.74^{e}
4	50	2.5	279.99±19.72^{c}	130.13±21.78^{c}	184.37±3.66^{d}
5	50	3.0	296.45±16.07^{c}	159.69±24.95^{c}	202.22±20.71^{d}
6	50	3.5	303.58±22.30^{c}	148.74±12.39^{c}	197.07±11.25^{d}
7	75	2.5	503.33±17.42^{b}	390.58±16.06^{b}	422.48±8.77^{c}
8	75	3.0	513.78±5.28^{b}	406.75±9.53^{b}	456.79±13.61^{b}
9	75	3.5	505.27±8.35^{b}	403.87±19.60^{b}	422.75±7.49^{c}
10 (control)	100	2.3	546.20±10.10^{a}	458.15±25.05^{a}	525.55±15.26^{a}

Remark "GAE" means Gallic acid.; The letters "a, b, c, d" in each column indicate a significant difference at p≥0.05.

The results indicated that 100% w/v pasteurized bitter orange juice had the highest values of DPPH, ABTS, and FRAP assay as 458.15±25.05 mg GAE/100 mL of sample, 546.20±10.10 μg Trolox/100 mL of sample, and 525.55±15.26 μg Trolox/100 mL of sample, respectively. There results also correlated with the titratable acidity, ascorbic acid, and total phenolic contents of pasteurized bitter orange juice.

Discussions

Juice is the source of beneficial components and nutrients (Aadil et al., 2019). Dilution of fruit juice reduces nutrients and components. Moreover, fruit juice also undergoes pasteurization, which is one method used to manage the quality of beverages (Aadil et al., 2019). The amount of nutrients and components of the juice decreased from dilution and pasteurization together. The dilution of bitter orange juice reduced ascorbic acid and total phenolic contents (Table 1). Likewise, the citric acid content decreased proportionally with juice dilution (Cairns et al., 2002). The pasteurized

bitter orange juice indicated that antioxidant activities decreased when the dilution of juice increased, as did the results of titratable acidity, ascorbic acid, and total phenolic contents (Table 1). Phenolic compounds have anti-oxidative effects (Zeb, 2020). Therefore, the decreasing of total phenolic compounds resulted in decrease of antioxidant activities. The pH value highly affects the structure of phenolic compounds in OH groups because OH groups in the first benzene ring of the meta-position cannot oxidize to quinones, resulting in the change of phenolics structure (Friedman & Jurgens, 2000). Quinones are unstable intermediates from plant phenolic oxidation that may react with nucleophiles (Schieber, 2018). Common phenolic compounds comprising an aromatic ring, such as phenol, 2,4-dichlorophenol, and *o*-cresol (Al-Khalid & El-Naas, 2012) are more sensitive to increasing pH than those contained several aromatic rings such as catechin, epigallocatechin, and rutin. The structure of several aromatic ring phenolic compounds is more complex than those of the common phenolic compounds. Additionally, the ionized- and resonance structures of phenolic compounds containing more aromatic rings are more tolerant to pH than monocyclic polyphenolic compounds (Friedman & Jurgens, 2000). In addition, pasteurization decreased the quality attributes or compounds of juices such as color, viscosity, overall quality, anthocyanins, ascorbic acid, carotenoids, flavonoids, antioxidant capacity, aromatic compounds, protein content, soluble solids, and phenolic content (Petruzzi et al., 2017). Furthermore, pasteurization-type MTLT (temperature <80 °C and holding times >30 sec) could increase anthocyanin, phenolic content, enzyme inactivation, and microbial inactivation (Petruzzi et al., 2017). Kowalska et al. (2023) reported that the pH of fresh citrus fruit juices was 4.1–4.4 and that of pasteurized commercial juice was 3.9. The ascorbic acid content was about 30.6-49.1 mg/100 mL of fresh juices and 30.6-49.1 mg/100 mL of pasteurized juices at 72°C. The fresh juice of *C. reticulata* Blanco had titratable acids and ascorbic acid contents in the range of 1.68±0.25 to 3.24±0.26% and 23.28±0.27 to 38.17±0.57 mg/100 g wet weight, respectively (Guo et al., 2023).

All samples of pasteurized bitter orange juice were examined in yeasts and molds, and total coliform counts initially and then again after 5 days of processing for monitor basic safety measurement (Table 1). The results reported that yeasts and molds were lower than 2.2 MPN/100 mL of sample and no of total coliform counts was

not detected that complies with the requirements of Notification of Ministry of Public Health (No. 356) B.E. 2556 (2013) Re: Beverages in Sealed Containers. This research proved that 2-rounds of pasteurization at 70°C for 5 min produced safe pasteurized bitter orange juice that was safe for consumption. Fruit juice or beverages with a pH< 4.6 are classified as high-acid foods (Erkmen & Bozoglu, 2016). All microorganisms had the pH optimum and suitable pH range of growth. In the case of high pH, the microbial environment has a high number of protons (H^+), causing energy use and metabolism of microorganism changes (Erkmen & Bozoglu, 2016). Pathogenic bacteria can grow at a pH of 4.6 to 7.0 (Overstreet et al., 2010). Yeasts have a growth ability in a pH range of 4 to 4.5. Molds can grow at a pH of 2 to 8.5. However, mold favors acidic conditions at pH 3.5 to 5.0 (Erkmen & Bozoglu, 2016). However, pathogens can survive in juice by developing their genetic and physiologic to endure acidic conditions (Petruzzi et al., 2017). In controlling pathogens, which survive in acid (pH 3.5-5.5), they are induced by stress such as heat, cold, osmosis, antibiotics, disinfectants, and non-thermal technology or use combined stresses for pathogen protection, being hurdle technology (Petruzzi et al., 2017; Wu et al., 2022).

The components in juice decreased as the juice concentration decreased, regardless of the pH levels. The pH variation did not impact the juice components and the product safety at the equivalent concentration levels. Notably, the components in 75% w/v juice at pH 3.0-3.5 did not exhibit a significant difference compared with those in 100% w/v juice. Thus, the former concentration was recommended. From the cost reduction perspective, a juice at concentration of 75% w/v and a pH range of 3.0-3.5 were deemed the most appropriate rations.

Conclusions

The suitable concentration of pasteurized bitter orange was 75% w/v with a pH adjustment of 3.0-3.5 by pasteurization condition at 70°C for 5 min, resulting in product safety. The research can be applied to produce pasteurized bitter orange juice to add value to the bitter orange fruit or use the pH adjustment applied in fruit juices or beverages for raw material preparation (standardization process) before entering the pasteurization process.

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References

Aadil RM, Madni GM, Roobab U, ur Rahman U, Zeng X. 1-Quality Control in Beverage Production: An Overview. In Grumezescu AM, Holban AM. (Eds.), Quality Control in the Beverage Industry. Cambridge: Academic Press; 2019:1-38.

Ağçam E, Akyıldız A, Dündar B. Chapter 17 - Thermal Pasteurization and Microbial Inactivation of Fruit Juices. In Rajauria G, Tiwari BK. (Eds.), Fruit Juices: Extraction, Composition, Quality and Analysis. Cambridge: Academic Press; 2018:309-339.

Al-Khalid T, El-Naas MH. Aerobic Biodegradation of Phenols: A Comprehensive Review. Critical Reviews in Environmental Science and Technology 2012;42:1631-1690.

Arunrung-aree A, Otaka K, Sisot P. Chemistry Laboratory II. Bangkok: Ramkhamhaeng University Press; 2005.

Bacteriological Analytical Manual. (2001). BAM Chapter 18: Yeasts, Molds and Mycotoxins. Available at: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-and-mycotoxins. Accessed April 8, 2024.

Bacteriological Analytical Manual. (2020). BAM Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria. Available at: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria. Accessed April 8, 2024.

Benayad O, Bouhrim M, Tiji S, Kharchoufa L, Addi M, Drouet S, Hano C, et al. Phytochemical Profile, α-Glucosidase, and α-Amylase Inhibition Potential and Toxicity Evaluation of Extracts from *Citrus aurantinum* (L) Peel, a Valuable By-Product from Northeastern Morocco. Biomolecules 2021;11(11):1555.

Brossard N, Fellenberg MA, Ibáñez RA, Muñoz MF, Osorio F, Salazar FN, Vargas-Bello-Pérez E, et al. Influence of milk pH on the chemical, physical and sensory properties of a milk-based alcoholic beverage. Journal of Dairy Research 2019;86(2):248-251.

Cairns AM, Watson M, Creanor SL, Foye RH. The pH and titratable acidity of a range of diluting drinks and their potential effect on dental erosion. Journal of Dentistry 2002;30(7-8):313-317.

Desseva I, Stoyanova M, Petkova N, Mihaylova D. Red Beetroot Juice Phytochemicals Bioaccessibility: an *In Vitro* Approach. Polish Journal of Food and Nutrition Sciences 2020;70(1):45-53.

Divya PJ, Jamuna P, Jyothi LA. Antioxidant properties of fresh and processed *Citrus aurantium* fruit. Cogent Food & Agriculture 2016;2:1184119.

Erkmen O, Bozoglu TF. Food Microbiology: Principles into Practice, 2 Volume Set. New Jersey, NJ: Wiley; 2016.

Fellows PJ. Food Processing Technology. 4th ed. Cambridge: Woodhead Publishing; 2017.

Friedman M, Jurgens HS. Effect of pH on the Stability of Plant Phenolic Compounds. Journal of Agricultural and Food Chemistry 2000;48:2101-2110.

Guo H, Zheng Y, Wu D, Du X, Gao H, Ayyash M, Zeng DG, et al. Quality evaluation of citrus varieties based on phytochemical profiles and nutritional properties. Frontiers in Nutrition 2023;10:1165841.

Haraoui N, Allem R, Chaouche TM, Belouazni A. *In-vitro* Antioxidant and Antimicrobial Activities of Some Varieties Citrus Grown in Algeria. Advances in Traditional Medicine 2020;20:23-34.

Karabıyıklı Ş, Değirmenci H, Karapınar M. Inhibitory Effect of Sour Orange (*Citrus aurantium*) Juice on *Salmonella Typhimurium* and *Listeria monocytogenes.* LWT - Food Science and Technology 2014;55(2):421-425.

Kowalska M, Konopska J, Feszterová M, Zbikowska A, Kowalska B. Quality Assessment of Natural Juices and Consumer Preferences in the Range of Citrus Fruit Juices. Applied Sciences 2023;13:765.

Lualamai K. *"Citrus aurantium var. aurantium"* is a rare ancient medicinal plant used for cooking delicious food. Available at: https://www.technologychaoban.com/agriculturaltechnology/article_71253#google_vignette. Accessed April 8, 2024.

Maksoud S, Abdel-Massih RM, Rajha HN, Louka N, Chemat F, Barba FJ, Debs E. *Citrus aurantium* L. Active Constituents, Biological Effects and Extraction Methods. An Updated Review. Molecules 2021;26:5832.

Memariani Z, Gorji N, Moeini R, Farzaei MH. Chapter Two - Traditional uses. In Seyed Mohammad Nabavi SM, Suntar I, Barreca D, Khan H. (Eds.), Phytonutrients in Food. Cambridge: Woodhead Publishing; 2020:23-66.

Mohammed BB, Najim, SY, Shatti ZO, Jasim EI, Dari WA. Comparative study of antibacterial activity between the Citrus Limon (Lemon) & *Citrus Aurantium* (Bitter Orange) Extracts on *Pseudomonas Aeruginosa* Isolates from Burns Infections. Mintage Journal of Pharmaceutical & Medical Sciences 2018;7(1):20-23.

Overstreet K, Roy, Heli J. Bacteria (Lesson 22). Available at: https://www.lsuagcenter.com/topics/food_health/education_resources/eatsmart/lessons/bacteria-lesson-22. Accessed April 8, 2024.

Petruzzi L, Campaniello D, Speranza B, Rosaria C, Sinigaglia M, Bevilacqua A. Thermal Treatments for Fruit and Vegetable Juices and Beverages: A Literature Overview. Comprehensive Reviews in Food Science and Food Safety 2017:668-691.

Rimlamduan T, Wechsuwan P, Sompeerapun O, Romsomsa N, Kachenpukdee N, Thaiwong N. Effect of temperature on drying rate, phytochemicals and antioxidant activities in *Vitex glabrata* R. BR. fruit. Life Sciences and Environment Journal 2024;24(1):113-126.

Saeeduddin M, Abid M, Jabbar S, Wu T, Yuan Q, Riaz A, Hu B, Zhou L, Zeng X. Nutritional, microbial and physicochemical changes in pear juice under ultrasound and commercial pasteurization during storage. Journal of Food Processing and Preservation 2017;e13237.

Schieber A. Reactions of Quinones-Mechanisms, Structures, and Prospects for Food Research. Journal of Agricultural and Food Chemistry. 2018;66(50):13051-13055.

Singh R, Rathod G, Meletharayil GH, Kapoor R, Sankarlal VM, Amamcharla JK. Invited review: Shelf-stable dairy protein beverages—Scientific and technological aspects. Journal of Dairy Science 2022;105(12):9327-9346.

Stohs SJ. Safety, Efficacy, and Mechanistic Studies Regarding *Citrus aurantium* (Bitter Orange) Extract and ρ-Synephrine. Phytotherapy Research 2017;31:1463–1474.

Suntar I, Khan H, Patel S, Celano R, Rastrelli L. An Overview on *Citrus aurantium* L.: Its Functions as Food Ingredient and Therapeutic Agent. Oxidative Medicine and Cellular Longevity 2018;7864269.

Sustainable Agriculture Foundation Thailand. *Citrus aurantium* L. Available at: https://sathai.org/?p=2152. Accessed April 8, 2024.

Wu RA, Yuk H, Liu D, Ding T. Recent advances in understanding the effect of acid-adaptation on the cross-protection to food-related stress of common foodborne pathogens. Critical Reviews in Food Science and Nutrition 2022;62(26):7336-7353.

Xutian S, Tai S, Yuan CS. Handbook of Traditional Chinese Medicine. China: World Science Publishers; 2014.

Yin X, Chen K, Cheng H, Chen X, Feng S, Song Y, Liang L. Chemical Stability of Ascorbic Acid Integrated into Commercial Products: A Review on Bioactivity and Delivery Technology. Antioxidants 2022;11(1):153.

Yu L, Wu Y, Liu D, Sheng Z, Liu J, Chen H, Feng W. The kinetic behavior of antioxidant activity and the stability of aqueous and organic polyphenol extracts from navel orange peel. Food Science and Technology 2022;42:e90621.

Zeb A. Concept, mechanism, and applications of phenolic antioxidants in foods. Journal of Food Biochemistry 2020;44(9):e13394.