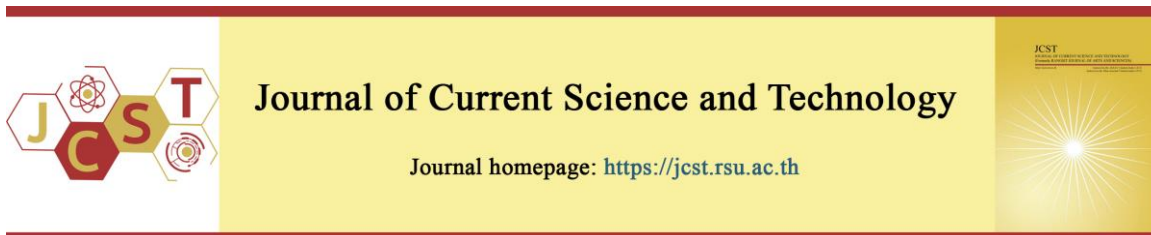


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Effects of thermal processing on cannabidiol degradation in cannabidiol-infused pomegranate juice and evaluation of its antioxidant property

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Abstract

Cannabidiol (CBD) is an active phytocannabinoid widely used in functional foods as well as nutraceutical and medicinal products. In this study, the effects of pasteurization and sterilization on the change of cannabidiol and total phenolic content, including antioxidant capacity in pomegranate juice containing 3.5 and 7.0 mg/L CBD, were evaluated. The CBD solution was prepared in absolute ethanol and added to pomegranate juice to obtain a final CBD concentration of not more than 1.41 mg/package. CBD and its degradation products; Δ^9 -tetrahydrocannabinol (THC) and cannabinal (CBN), after heat treatment were measured using the HPLC-DAD method. After heat treatment, total phenolic content of the juices significantly increased except for the juice containing 7.0 mg/L corresponding to an increase of antioxidant capacity. The results showed that thermal processing decreased CBD content. The higher the temperature, the lower the CBD content remained. Pasteurized pomegranate juice containing 3.5 and 7.0 mg/L CBD decreased by roughly 25%, but the sterilized juice decreased by 85.7% and 92% respectively. In consideration of CBN, a less psychoactive cannabinoid compound was not detected after pasteurization and sterilization. Sterilization increased the considerable THC content in pomegranate juice containing 7.0 mg/L CBD more than that containing 3.0 mg/L CBD. Conversely, only the pomegranate juice which contained 7.0 mg/L of CBD had a slight rise in THC content due to pasteurization. It could be concluded that thermal processing by sterilization had potential effects on CBD degradation. However, the formation of THC did not exceed 1.6 mg/package as specified in the Notification of the Ministry of Public Health No. 427 B.E. 2564 (2021), Thailand.

Keywords: antioxidant; cannabidiol (CBD); pasteurization; pomegranate juice; sterilization; tetrahydrocannabinol (THC)

1. Introduction

Cannabidiol (CBD) is one of phytocannabinoids found in *Cannabis* and several other plants. Cannabinoids can be divided into two main subclasses: phytocannabinoids, which are

natural compounds, and synthetic cannabinoids (Messina, Rosati, Curni, & Marcotullio, 2015). Two major natural cannabinoids that have been studied extensively are delta-9-tetrahydrocannabinol or Δ^9 -tetrahydrocannabinol (Δ^9 -THC, THC), the main

psychoactive compound and CBD, a non-psychoactive compound. The biological properties of these two compounds are associated with their different interactions with the cannabinoid system in humans (Pisanti et al., 2017). The therapeutic potential of CBD has benefits in the treatment of various neuropsychiatric disorders including autism spectrum disorder, anxiety, psychosis, neuropathic pain, cancer pain, HIV, migraine, multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, hypoxic-ischemic injury, and epilepsy (Chayasirisobhon, 2019). Most common adverse events are diarrhea and somnolence (Huestis et al., 2019). CBD also shows significantly low abuse potential and could be applied to treat or prevent COVID-19 and its complications (Peng et al., 2022). In 2018, CBD was approved by the US Food and Drug Administration (FDA) as a medicinal product for the treatment of certain epileptic conditions (Ryan, 2020). In consideration of THC, although it is a psychoactive substance, it also has appetite stimulant, anti-inflammatory, analgesic, and anti-emetic qualities, making it a very promising medication for medical applications (Iftikhar et al., 2021). Both THC and CBD showed antioxidant properties (Hacke et al., 2019) and were demonstrated to be neuroprotective antioxidants which can prevent hydroperoxide-induced oxidative damage better than chemical antioxidants and neuronal cultures (Hampson, Grimaldi, Axelrod, & Wink, 1998). Pure CBD was reported to be more potent DPPH radical scavenger than pure THC. Therapeutic effects of oral THC (4 mg/kg) were found to alleviate parkinsonian symptoms (Van Vliet, Vanwersch, Jongsma, Oliver, & Philippens, 2008) and THC could be a potential therapeutic treatment option for Alzheimer's disease through multiple functions and pathways (Cao et al., 2014). Combination of THC and CBD was reported to increase clinical efficacy with reduced undesirable effects of THC (Russo, & Guy, 2006). World Health Organization (2018) has reported that until now there is no evidence of public health related problems associated with the use of pure CBD.

Due to its benefits for health, hemp CBD is an excellent choice for future functional food ingredients and the expansion of the market for food products and medicinal CBD-based goods (Pisanti,

et al., 2017; King., 2019; Chen and Pan (2021); Rasera, Ohara, & Soares de Castro, 2021; Kanabus, Bryla, Roszko, Modrzewska, & Pierzgalski, 2021). According to a survey from High Yield Insights (2019), consumers wanted CBD in baked goods (57%), chocolate (45%), beverages (30%), snack foods (29%), capsules (42%), and gummies (37%). In recent years, the improvement of consumer health benefits due to "naturally functional beverages" that contain natural nutrients and bioactive compounds has drawn a lot of attention. These beverages have positive effects on the body's immune defense system, mental energy, cholesterol control, and other advantages related to specific organs like heart, liver, and eyes (Musarra, Jirillo, Rupa, & Vinci, 2019). Compared with other fruits such as grape, blueberry, cranberry, and orange juices, pomegranate juice has the highest antioxidant capacity (Abountiolas, & Nunes, 2017). Pomegranate juice was found to be rich with phytochemicals, which are responsible for its antioxidative and anti-inflammatory potential (Vučić, Grabež, Trchounian, & Arsić, 2019; Tarantino et al., 2021). The antioxidant capacity of pomegranate juice strongly contributes to anthocyanin content (Mena et al., 2013). Several in vivo and in vitro studies have revealed the beneficial physiological activities of pomegranate juice, especially its antioxidative, antimicrobial, and anti-inflammatory properties. Regular consumption of pomegranate fruit, juice, or its compounds added in other food products may protect or improve the course of many diseases like obesity, diabetes, cardiovascular diseases, and some cancer types (Kandylis, & Kokkinomagoulos, 2020). Gliménez-Bastida, Ávila-Gálvez, Espín, and González-Sarías (2021) reported that the positive effects of pomegranate juice and extracts on human health was due to protection from oxidative stress-related and cardiometabolic diseases. In addition, potential mechanisms for the improvement of glucose homeostasis in type 2 diabetes by pomegranate juice has recently been reviewed (Olvera-Sandoval et al., 2022). Therefore, pomegranate juice was chosen as a functional beverage for this study.

The stability of CBD should be considered when developing beverages that contain the component because several cannabinoids are transformed into other cannabinoid derivatives when

exposed to heat or under storage conditions (King, 2019). Stability studies of CBD in the form of solid powder and sunflower oil solution by Kosović, Sýkora, and Kuchař (2021) indicated that various degradation products including cannabinol (CBN) which is much less psychoactive than THC and several oxidation products were formed when keeping the products at 25 °C for 365 days under the defined stability conditions. They also found that CBD powder was significantly more stable than CBD in an oil solution. Additional research (Fraguas-Sánchez, Fernández-Carballido, Martín-Sabroso, & Torres-Suárez, 2020) stated that CBD was thermally unstable and very sensitive to photolytic reaction and oxidation. Solvents also influenced CBD stability, and CBD in an ethanol solution was more stable than in an aqueous solution. Regarding CBD-containing products, concerns are caused by adverse effects reported by anecdotal consumers after consuming the products; these effects are assumed to be induced by hydrolytic conversion of CBD to psychotropic THC in the stomach (Merrick et al., 2016; Lachenmeier et al., 2021). Nevertheless, most studies suggest that CBD was not converted to psychotropic THC under in vivo conditions (Golombek, Müller, Barthlott, Sproll, & Lachenmeier, 2020). The three main cannabinoids in cannabis resin were subjected to a thermal degradation study which revealed that CBD, CBN, and THC reacted more quickly in acidic solutions and at high temperature; the minimum transformation could have occurred by processing the cannabinoids for a short period of time at low temperature and with mild to moderate acidity (Jaidee et al., 2021). Therefore, THC formation in food products containing CBD should be evaluated to control the THC concentration from exceeding the threshold for a physiological response. The objective of this study was to investigate the effects of pasteurization and sterilization on the change of CBD and total phenolic contents including antioxidant capacity in pomegranate juice containing different concentrations of CBD.

2. Materials and Methods

2.1. Raw material

Giffarine Granada 100% Pomegranate juice was purchased from Hap 193 Co., Ltd. (Pattaya, Thailand). The method of pineapple juice canning (Rouweler,

2.2. Chemicals and reagents

CBD powder with a purity of 99.9% was obtained from Medicinal Cannabis Research Institute, Rangsit University. Cannabis cannabinoids (CBD, CBN and THC) were purchased from Fisher Scientific (Pittsburgh, PA, USA). All HPLC grade solvents (ammonium formate, acetonitrile, methanol, and water) including gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were analytical grade.

2.3. Preparation of pomegranate juice containing different concentration of CBD

According to the Notification of the Ministry of Public Health No. 427 B.E. 2564 (2021) issued by Virtue of the Food Act B.E. 2522 (1979) Re: Food products containing some parts of cannabis or hemp as an ingredient, CBD content in the food product containing some parts of cannabis or hemp shall not exceed 1.41 mg/package and THC content shall not exceed 1.6 mg/package. Therefore, in this study, two different concentrations of CBD in pomegranate juice were prepared at 3.5 and 7.0 mg/L to obtain the final CBD concentration in the juice following this Notification. CBD solution of 7.0 mg/mL was prepared by dissolving CBD powder in absolute ethanol. To prepare pomegranate juice containing 3.5 mg/L and 7 mg/L CBD, 0.5 ml and 1 mL of 7 mg/mL CBD solution was added into 1 liter of the juice, respectively.

2.4. Process for pasteurization and sterilization

In general, pasteurization and sterilization are the common thermal treatments in juice processing. Fruit juices with a pH level lower than 4.2 require a few seconds of pasteurization at temperatures ranging from 80 to 93 °C to inactivate the vegetative microorganisms that can grow in them (Featherstone, 2016). In this study, the pomegranate juice containing different concentrations of CBD was pasteurized at 80 °C for 30 seconds following the mild treatment pasteurization of reconstituted pomegranate juice (Kong et al., 2020). According to sterilization treatment, the juice was filled into a can purchased from Hap 193 Co., Ltd. (Pattaya, Thailand), the method of pineapple juice canning (Rouweler,

2015). The experimental runs were performed in 3 batches (10 bottles/batch for pasteurization and 30 cans/batch for sterilization).

For pasteurization, it was conducted in a kitchen lab using an electric stove on medium-high heat. Pomegranate juice with varying different concentrations of CBD was heated to 80°C and held for 30 seconds. The temperature of the juice during the heating process was monitored using a food laboratory RTD digital handheld thermometer (EW-90026-09, Cole-Parmer, USA). After 30 seconds, the juice was transferred into a presterilized stainless steel jar and hot filled into a presterilized 200 mL glass bottle and the glass bottle was closed with metal caps (initial CBD concentrations in the juice before pasteurization were 0.7 and 1.4 mg/package). The pasteurized juice was then cooled in an ice bath for 20 minutes.

For sterilization, the juice was filled into a 180 mL can (initial CBD concentrations in the juice before sterilization was 0.63 and 1.26 mg/package), put on a rack, and loaded into a water spray retort (KM Grand Pack Co. Ltd. Model ML1400). The retort temperature was set at 105 °C with the pressure of 120 kPa (absolute) throughout the heating process time of 15 minutes and cooling cycles. The pasteurized and sterilized juices were kept frozen at – 20 °C until analysis.

2.5 pH and total soluble solid (°Brix) measurement

The pH values of the pomegranate juice containing different concentrations of CBD before and after pasteurization and sterilization were measured at room temperature using a pH meter (OHAUS model Starter 300, U.S.A). Total soluble solid (TSS) of the juices was also measured before and after heat treatment using a hand refractometer (Atago Brix 0-32, Japan).

2.6 Determination of total phenolic content

Total phenolic content (TPC) of the pomegranate juice containing different concentrations of CBD before and after heat treatment was determined using Folin-Ciocalteu reagent by the modified method proposed by Fang et al. (2009). Folin-Ciocalteu reagent of 0.5 mL and 5 mL of 5% Na₂CO₃ solution were added to 1 mL of sample and shaken vigorously. Then the solution

was immediately diluted to 25 mL with distilled water and mixed thoroughly. The absorbance of sample was measured at 765 nm after incubation in dark at room temperature and was compared to standard curve of gallic acid solution. Total phenolic content was expressed as milligrams of gallic acid equivalents (mg GAE/L).

2.7 Determination of antioxidant capacity

Antioxidant activity of the pomegranate juice containing different concentrations of CBD before and after heat treatment was analyzed by DPPH scavenging assay using a modified method of Kim, Lee, Lee, and Lee (2002). The sample of 0.1 mL was added to 2.9 mL of 1 mM DPPH that was dissolved in 80% aqueous methanol. The mixture covered with aluminum foil was incubated for 30 minutes in the dark. Then the decrease of absorbance was measured at 517 nm. A control consisted of 0.1 mL of 50% aqueous methanol and 2.9 mL of DPPH solution. L-ascorbic acid solution was used as the standard for the analysis by DPPH method. The amount of absorbance reduction of the sample was compared to that of vitamin C standard, and the result was calculated as vitamin C equivalent antioxidant capacity (VCEAC) in mg/L.

2.8 Effect of thermal processing on cannabinoids content in the pomegranate juice

Three main cannabinoids: CBD, CBN, and THC content in the pomegranate juice containing different concentrations of CBD after pasteurization and sterilization was determined following the AOAC Official Method 2018.11 using high performance liquid chromatography-diode array detection (HPLC-DAD) (Vaclavik et al., 2019). The analysis was performed using Agilent G 1956B LC/MSD (Agilent 1100 Series, U.S.A). Chromatographic separation was performed on a XBridge C18 column (3.5 µm, 4.6 × 150 mm) at 25 °C by gradient elution. The mobile phase was composed of 20 mM aqueous ammonium formate, pH 3.2 (A) and acetonitrile (B), a flow rate was set at 1.0 mL/minute. The injection volume was 25 µL, and the quantification wavelength was 240 nm. The juice samples were diluted ten-fold with methanol, mixed by a vortex mixer and centrifuged at 1700 g for 15 minutes at 4 °C before analysis.

2.9 Statistical analysis

A completely randomized block design with two different CBD concentrations serving as a block was applied in this research. The experimental runs were performed in 3 batches and each analysis (pH, TSS, TPC, VCEAC, CBD, and THC) was conducted in triplicate (N = 9 by random sampling). The data were presented as mean $\pm$ standard deviation and a paired t-test was used to calculate the difference between each parameter at $p < 0.05$ using statistical software (SPSS Inc., Chicago, U.S.A) version 20.0.

3. Results and Discussion

3.1 Changes of pH, total soluble solid, total phenolic content, and antioxidant capacity in the pomegranate juice before and after thermal processing

The changes of physicochemical compositions and antioxidant capacity of the pomegranate juice containing different concentrations of CBD before and after pasteurization and sterilization were presented in Table 1 and Table 2.

Table 1 pH, TSS, TPC, and VCEAC in the pomegranate juice containing different concentrations of CBD before and after pasteurization

CBD concentration (mg/L)	Pasteurization	pH	TSS (°Brix)	TPC (mgGAE/L)	VCEAC (mg/L)
3.5	before	2.67 ^{ns} $\pm$ 0.01	14.00 ^b $\pm$ 0.17	736.16 ^b $\pm$ 10.04	909.52 ^{ns} $\pm$ 4.36
	after	2.66 ^{ns} $\pm$ 0.00	14.83 ^a $\pm$ 0.06	781.09 ^a $\pm$ 7.84	915.24 ^{ns} $\pm$ 3.30
7.0	before	2.67 ^{ns} $\pm$ 0.01	14.10 ^b $\pm$ 0.00	766.59 ^{ns} $\pm$ 16.32	889.52 ^b $\pm$ 8.73
	after	2.66 ^{ns} $\pm$ 0.00	14.83 ^a $\pm$ 0.06	785.43 ^{ns} $\pm$ 6.52	950.48 ^a $\pm$ 3.30

Different letters in superscript of each CBD concentration (before and after pasteurization) indicate a significant difference at $p < 0.05$
TSS: total soluble solid; TPC: total phenolic content; VCEAC: vitamin C equivalent antioxidant capacity

Table 2 pH, TSS, TPC, and VCEAC in the pomegranate juice containing different concentrations of CBD before and after sterilization

CBD concentration (mg/L)	Sterilization	pH	TSS (°Brix)	TPC (mgGAE/L)	VCEAC (mg/L)
3.5	before	2.65 ^{ns} $\pm$ 0.00	14.10 ^b $\pm$ 0.00	712.25 ^b $\pm$ 8.79	885.71 ^b $\pm$ 4.95
	after	2.64 ^{ns} $\pm$ 0.01	14.83 ^a $\pm$ 0.06	801.38 ^a $\pm$ 10.72	932.38 ^a $\pm$ 5.95
7.0	before	2.66 ^{ns} $\pm$ 0.01	14.17 ^b $\pm$ 0.06	738.33 ^b $\pm$ 8.79	893.33 ^{ns} $\pm$ 10.03
	after	2.66 ^{ns} $\pm$ 0.00	14.90 ^a $\pm$ 0.00	778.91 ^a $\pm$ 5.75	892.38 ^{ns} $\pm$ 5.95

Different letters in superscript of each CBD concentration (before and after sterilization) indicate a significant difference at $p < 0.05$
TSS: total soluble solid; TPC: total phenolic content; VCEAC: vitamin C equivalent antioxidant capacity

According to the data, pH of the juice in all treatments did not change which indicated that adding CBD and thermal processing did not affect an acidity of the pomegranate juice. This is in accordance with research by Kong et al. (2020) that showed an insignificant difference in pH, titratable acidity, and total soluble solids of reconstituted pomegranate juice after high-temperature pasteurization at 95 °C and mild-temperature pasteurization at 80 °C for 30 seconds. However,

thermal processing increased the TSS of the juice, which may be due to an increment of total solid solubility during the heating. The significant increase in TPC of the juice containing different concentrations of CBD was observed after thermal processing except the juice containing 7.0 mg/L CBD after pasteurization. The increase in TPC after heat treatment was also observed in reconstituted pomegranate juice (Kong et al., 2020), tomatoe juice (Gahler, Otto, & Böhm, 2003), lychee juice (Su et

al., 2019) and apple juice (Murtaza et al., 2020). The effect of high temperature on releasing the bound polyphenol could be the cause of the increase in TPC after thermal processing. TPC levels in pomegranate juice in this study ranged between 712.25–738.33 mgGAE/L which was similar to 707.25 mgGAE/L reported by Triguerous Wojdylo and Sendra (2014). The pomegranate juice polyphenols varied within the range of 784–1551 mgGAE/L (Gözlekci, Saracoğlu, Onursal, & Özgen, 2011). According to the antioxidative property, the individual phenolic compounds and anthocyanins which are the major group of phenolic compounds may be responsible for this antioxidant activity (Triguerous et al., 2014; Legua, Forner-Giner, Nuncio-Jáuregui, & Hernández, 2016). In addition, the antioxidant activity of CBD and THC were determined by DPPH·scavenging assay (Hacke et al., 2019). Therefore, both phenolic compounds and CBD played an important role in the radical scavenging activity of the pomegranate juice in this study. The antioxidant capacity of the pomegranate juice containing 3.5 and 7.0 mg/L CBD after pasteurization and sterilization tended to increase. Benjamin and Gamrasni (2020) also indicated that TPC and antioxidant activity of pomegranate juice after pasteurization at 55°C, 65°C, and 75 °C for 15 seconds were higher than fresh juice. Since the total phenolic content was highly correlated with

antioxidant activity in pomegranate and other fruit juices (Zaouay, Mena, Garcia-Viguera, & Mars, 2012; Hua, Yi-Fei, & Zhi-qin, 2018; Guofang, Xiaoyan, Xiaoli, Yongling, & Zhibing, 2019; Azman, Azlan, Khoo, & Razman, 2019; Mejia, et al., 2020; Lim & Eom, 2022), the increase in antioxidant capacity after heat treatment could be due to the increase of TPC. However, TPC and antioxidant activity of cloudy pomegranate juice decreased after processing at high temperature for a short time at 110 °C, 8.6 seconds (Chen et al., 2013). Farahmand, Golmakani, Mesbahi, and Farahnaky (2016) found that pasteurization of pomegranate juice with a plate heat exchanger (85-90 °C) for 45 seconds had no significant effect on TPC and antioxidant activity. The different results could be due to the influence of different initial concentrations of TPC as well as different temperatures, and time of the heat treatment.

3.2 Changes of CBD, CBN, and THC in the pomegranate juice before and after thermal processing

Cannabidiol and its potential degradation products were separated on a C18 column by gradient elution. The content of CBD, CBN, and THC in CBD-infused pomegranate juice after pasteurization and sterilization were quantified as shown in Table 3.

Table 3 CBD, CBN, and THC contents in the pomegranate juice containing different concentration of CBD after pasteurization and sterilization

CBD concentration (mg/L)	Heat Treatment	CBD (mg/L)	CBN (mg/L)	THC (mg/L)
3.5	Pasteurization	2.62 ± 0.05 ^a	nd	nd
	Sterilization	< 0.15 ^b	nd	0.31 ± 0.09 (0.06 mg/package)
7.0	Pasteurization	5.30 ± 0.04 ^a	nd	0.15 ± 0.05 ^a (0.03 mg/package)
	Sterilization	0.56 ± 0.07 ^b	nd	1.53 ± 0.07 ^b (0.27 mg/package)

Different letters in superscript of each CBD concentration (pasteurization and sterilization) indicate a significant difference at $p < 0.05$
nd: not detected

The data indicated that, after thermal processing, CBD in pomegranate juice decreased 25% by pasteurization, 85.7 and 92% by sterilization respectively, but CBN was not detected. Formation of THC was observed slightly in the juice containing 3.5 mg/L CBD after sterilization. However, for the juice containing 7.0 mg/L of CBD, the THC content in the juice after sterilization was significantly higher than after pasteurization. In addition, the higher the temperature of thermal processing, the higher THC content. The results highlighted the instability of CBD in pomegranate juice containing CBD concentration of 3.5 – 7.0 mg/L. As reported, CBD is highly unstable (Fraguas-Sánchez et al., 2020), and the heating of CBD in some acid solutions changed CBD to THC (Gaoni, & Mechoulam, 1966). According to CBD-infused foods and beverages, a content of THC must be adhered to for consumer health because of its psychotropic effects. The European Food Safety Authority (EFSA) determined the Lowest Observed Adverse Effect Level (LOAEL) of THC on the central nervous system as 2.5 mg/day (Beitzke, & Pate, 2021). A single oral dose of 20 mg THC resulted in symptoms such as tachycardia, conjunctival irritation, “high sensation” or dysphoria in adults within one to four hours. However, the amount and psychotropic observed effect of THC depended on the individual (Lachenmeier et al., 2021). A survey of THC in alcoholic beverages from the Italian market was found to be in the range of 0.014 – 1.48 mg/L, which is below the maximum limits for total THC content at 2.0 mg/kg (Pisciottano, Guadagnuolo, Soprano, Esposito, & Gallo, 2021). In this research, the THC content in pomegranate juice containing different concentrations of CBD was below 1.6 mg/package after pasteurization and sterilization as specified by Notification of the Ministry of Public Health No. 427 B.E. 2564 (2021), Thailand.

4. Conclusions

Thermal pasteurization and sterilization are most widely used in fruit juice processing. The processes increased TPC and antioxidant capacity of CBD infused pomegranate juice with a concentration of 3.5-7.0 mg/L. The concentration of CBD in pomegranate juice significantly decreased during thermal processing, 25% by pasteurization, 85.7 and

92% by sterilization. However, CBN was not detected. In addition, sterilization caused more THC formation than pasteurization. After sterilization, the formation of THC in the juice with 7.0 mg/L CBD was much higher than that in the juice with 3.5 mg/L CBD. Since a high THC content causes an adverse effect on consumers, ongoing quality control of the THC content is needed to maintain a low THC level in thermally processed beverages especially acidic beverage products. Moreover, the stability of the products during shelf life should be evaluated.

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6. Conflict of interest

The authors declare that they have no conflict of interest.

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