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ULTRASONIC TREATMENT UTILIZATION DURING STRAWBERRY JUICE PROCESSING

KORIŠĆENJE ULTRAZVUČNOG TRETMANA ZA VREME PROIZVODNJE SOKA OD JAGODE

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ABSTRACT

Berry fruits are a rich source of various nutrients, while their products also represent a nutritionally valuable source. Technological processes are developing in the direction of minimal processing techniques, avoiding intensive heat treatments and use of chemical additives.Use of non-invasive treatments such as ultrasounds shows a number of advantages, from increased bioactive compounds content to a significantly shortened time. The aim of study was to determine the effect of different processing methods (thermal and ultrasonic treatment) on the physicochemical properties, specialized metabolites and antioxidant activity of strawberry juice. The highest dry matter content and electrical conductivity was determined in thermal treated juices, while ultrasonic treatment positively affected on the total acids content and total soluble solids. Ultrasonic treated juices had even 51 % higher vitamin C content, 23 % higher total anthocyanin content and also significantly higher antioxidant capacity compared to the thermal treated samples.

Key words*: ultrasonic treatment, thermal treatment, specialized metabolites, antioxidant capacity, strawberry juice*

REZIME

Jagodaste voćne vrste bogat su izvor različitih nutrijenata, a njihovi proizvodi također predstavljaju nutritivno vrijedan izvor različitih biološki aktivnih spojeva visokog antioksidacijksog djelovanja. Jedan od važnih proizvoda ploda jagode je i sok koji tijekom proizvodnje podrazumijeva i toplinsku obradu što značajno negativno utječe na nutritivnu kvalitetu gotovog proizvoda reducirajući sadržaj vrijednih biaktivnih spojeva i fitonutrijenata.U današnje vrijeme tehnologijski procesi u preradi i doradi voća značajno se razvijaju u smjeru tehnika minimalnog procesiranja uz izbjegavanje intenzivnih toplinskih tretmana i upotrebu kemijskih aditiva. Upotreba neinzvazivnih tretmana poput ultrazvuka pokazuje brojne prednosti, od povećanog prinosa bioaktivnih spojeva do značajno reduciranog vremena. Cilj ovog rada bio je utvrditi utjecaj različitih procesnih metoda (toplinski i ultrazvučni tretman) na fizikalno-kemijska svojstva, sadržaj specijaliziranih metabolita i antioksidacijsku aktivnost soka jagode. Analizirana su slijedeća fizikalno-kemijska svojstva soka: ukupna suha tvar, topljiva suha tvar, ukupna kiselost, pH vrijednost i električna provodljivost, dok od bioaktivnih spojeva sadržaj vitamina C, ukupnih fenola (flavonoida i neflavonoida), ukupnih antocijana te antioksidacijski kapacitet. Najviši sadržaj ukupne suhe tvari i električne provodljivosti utvrđen je u toplinski tretiranim sokovima, dok je ultrazvučni tretman pozitivno utjecao na sadržaj ukupnih kiselina i topljivu suhu tvar. Ultrazvučno tretirani sokovi imali su čak 51 % veći sadržaj vitamina C, 23 % veći sadržaj ukupnih antocijana i značajno veći antioksidacijski kapacitet u usporedbi s toplinski tretiranim uzorcima.

Ključne riječi*: ultrazvučni tretman, toplinski tretman, specijalizirani metaboliti, antioksidacijski kapacitet, sok od jagode.*

INTRODUCTION

Minimal processing techniques are considerably developing in the production of foodstuffs. The main goal of mentioned processesis avoiding intensive heat treatments, use of chemical additives and harmful organic solvents and in generalare based on the principles of „green chemistry“ (*Anastas and Eghbali, 2009; Babić, 2014; Đukić-Vuković et al., 2017*). During production of strawberry juice heat treatment is necessary given the demand for microorganism inactivation but also to prolong the shelf-life of final product. Nowadays, the use of various non-invasive treatments such as ultrasounds shows a number of advantages, from significantly shortened time to aincreased yield of different chemical compounds and phytonutrients (*ŠicŽlabur et al., 2017*).Ultrasound used in different food technology processes is characterized by phenomena of transient cavitation and is often called high intensity ultrasound (Knorr et al., 2004).

Strawberry is a rich source of various phytochemicals with significant antioxidant activity, so from health point of view its consummation regardless in fresh state or as processed is strongly recommended (*Huang et al., 2012*). The main chemical compounds which characterize strawberry and are responsible for its recognizable red coloration are anthocyanins, mainly pelargonidin, cyanidin, delphinidin etc. (*Crecente-Campo et al., 2012*;*Voća et al., 2014*). Besides mentioned, strawberry fruits are in general significantly nutritively valuable because of high content of vitamins (vitamin C), polyphenols, flavonoids, phenolic acidsand its derivatives, tannins like ellagitannin as the most common (*Belitz i Grosch, 1999*; *Dai andMumper, 2010; Paredes-López et al., 2010*). Polyphenols gained great popularity among food products by the discovery of their significant antioxidant activity and number of potential benefits on human health like prevention of cancer, cardiovascular diseases, dementia etc. (*Finkel and Holbrook, 2000*; *Carocho and Ferreira, 2013*).

The aim of this study was to determine the effect of different processing methods of strawberry juice (thermal treatment and ultrasonic treatment) on the physicochemical properties, the content of specialized metabolites and antioxidant activity.

MATERIALS AND METHODS

Strawberry juice preparation

Strawberry fruits (*Fragaria x ananassa*Duch.) fromcultivar ‘Clery’ were collected from Fragaria d.o.o. (Croatia) and transported to the laboratory of the Department of Agricultural Technology, Storage and Transport on the University of Zagreb Faculty of Agriculture. Fruits with mechanical damage or visible signs of spoilage were removed. Fruits intended for juice production were washed, packed in polyethylene bags (in each bag average of 1 kg of fruits), frozen at -18 °C and stored for one month. Before juice production, fruits were defrosted at ambient temperature. For the research purposes, strawberry juice was prepared by cold pressing on the centrifugal juicer (Bullet Express, Germany) and was used as a control. Prepared juice was divided for further treatments including: heat treatment at 60 (SjT1) and 80 °C (SjT2) for two time periods (15 and 35 min) and ultrasonic treatments at room temperature (SjUAE) and at 60 (SjUAE1) and 80 °C (SjUAE2) for two time periods (15 and 35 min). The ultrasonic treatments were conducted in ultrasonic bath (Bandelin, Germany) frequency of 35 kHz and maximal nominal output power of the device 140 W. The experiment design of strawberry juice treatments is shown in Table 1.

Table 1. Experiment design of strawberry juice treatment

Sample	Treatment	Temp. (°C)	Time (min)	Ultrasonic bath
SjC	control	-	-	-
SjT1 15	thermal	60	15	-
SjT1 35	thermal	60	35	-
SjT2 15	thermal	80	15	-
SjT2 35	thermal	80	35	-
SjUAE 15	sonication	21.4	15	35 kHz, 140 W
SjUAE 35	sonication	21.4	35	35 kHz, 140 W
SjUAE1 15	sonication	60	15	35 kHz, 140 W
SjUAE1 35	sonication	60	35	35 kHz, 140 W
SjUAE2 15	sonication	80	15	35 kHz, 140 W
SjUAE2 35	sonication	80	35	35 kHz, 140 W

Determination of physicochemical properties of strawberry juice

Physicochemical properties of strawberry juice included determination of: dry matter content (%) by drying at 105 °C till constant mass, total acid content (TA, % dry weight) by potentiometric titration, total soluble solids content (TSS, %) by digital refractometer (Refracto 30 PX, Mettler-Toledo, Switzerland), pH- value by digital pH-meter (Sevenmulti, Mettler Toledo, Switzerland) and electrical conductivity (mS cm^{-1}) by digital conductor (SevenEasy, Mettler-Toledo, Switzerland) all provided according to the standard methods (*AOAC, 1995*).

Determination of specialized metabolites and antioxidant capacity by ABTS assay

Analysis of specialized metabolites included determination of: vitamin C content (g 100 g^{-1}dry weight) by titration with 2,6-dichlorindophenol according to *AOAC (2002)*, total phenol content (TPC),total flavonoids (TFC) and non-flavonoids (TNFC) content spectrophotometrically (Shimadzu UV 1650 PC) in which absorbance of the blue color was measured at 750 nm with distilled water as a blank according to the Ough and Amerine (1988) and determination of total anthocyanin content (TAC,g kg^{-1} dry weight) by method of bisulphite bleaching (*Ough and Amerine, 1988*). For TPC and TFC determination as an external standard gallic acid and catechin was used and the final content of TPC, TFC and TNFC was expressed as mg GAE 100 g^{-1}dry weight.

The antioxidant capacity of the strawberry juice was determined by the ABTS method according to the *Miller et al. (1993)* and *Re et al. (1999)*. As an antioxidant standard Trolox was used. For preparation of stable ABTS radical solution the 5 mL of ABTS solution (7 mM) and 88 µL of potassium persulfate (140 mM) solution were mixed and allowed to stand in the dark at room temperature for 16 h. On the day of analysis 1 % ABTS•1 solution in 96 % EtOH was prepared. A 160 µL of ethanol extract (prepared for the TPC isolation) was directly injected in the cuvette and mixed with 2 mL 1 % ABTS•1. After 5 min the absorbance at 734 nm was measured (Shimadzu 1650 PC, Germany). The final results of the antioxidant capacity was calculated based on calibration curve and expressed as mmol TE L^{-1}. All treatments and chemical analysis were made in triplicate. Obtained data were analyzed using one-way analysis of variance (ANOVA) in *SAS* software package, version 9.3. (2010). Mean values were compared by the t-test (LSD) and were considered significantly different at $p \leq 0.0001$. In tables are shown different letters which indicates significant differences between mean values within each column and also standard deviation (±SD) was expressed.

RESULTS AND DISCUSSION

Physicochemical properties of strawberry juice

High significant statistical differences ($p \leq 0.0001$) of analyzed physicochemical properties of strawberry juice were determined between all varied treatments (Table 2). In general, the highest dry matter content (DM) was determined in heat treated strawberry juices with the highest determined value of 12.80 % in sampleSjT2 15 which was treated by temperature of 80 °C for 15 min. Ultrasonic treatment did not significantly affected on the DM content instrawberry juices even compared to the control sample or to the heat treated samples. On average, in heat treated juices approximately 24 % higher DM content were determined compared to the control sample and ultrasonic treated samples. Ultrasonic treatment positively affected on the total acid content (TA) since on average 11 % higher values was determined compared to the heat treated samples and 5 % higher compared to the control sample. The highest TA content (100.79 % DW) was determined in sample treated by ultrasound at 60 °C for 35 min (SjUAE1 35). In accordance with the highest DM content in heat treated juices, total soluble solids content (TSS) was also higher compared to the control sample (about 12 %) and ultrasonic treated samples (about 11 %). Ultrasonic treated samples had slightly higher TSS content compared to the control sample with the highest determined value of 8.87 % in sample treated by ultrasound at 80 °C for 15 min (SjUAE2 15). Average pH value for heat treated samples amounted 3.39, while for ultrasonic treated 3.35 which according to the statistical analysis are significantly different values. Ultrasonic treatment did not significantly positively influenced on the change of pH values compared both to the control and heat treated samples. Fruits juices are a rich source of chemical compounds which are good conductors such as vitamins, minerals, proteins etc. so higher values of electrical conductivity (EC) suggests a greater content of mentioned compounds. In this research in heat treated samples the highest values of EC was determined, in range from 3.07 mS cm^{-1} (SjT1 15) to 3.45 mS cm^{-1} (SjT2 35). In ultrasonic treated samples EC ranged from 2.20 (SjUAE 15) to 2.88 mS cm^{-1} (SjUAE1 35) which are in general lower values compared to the heat treated samples, while slightly higher values compared to the control sample. Other varied factors in experiment, temperature (60 and 80 °C) and time period of treatments (15 and 35 min) slightly affected on some physicochemical parameters, for example higher temperature in heat treated samples on DM content or longest time period on TA content in sonicated treated samples.

In general, strong influence of sonication treatment on analyzed physicochemical properties of strawberry fruit were not observed which is in accordance with other similar research studies. For example, *Aadil et al.* (2013) did not determined any changes in the content of TA, TSS and pH value in the sonicated grapefruit juices but also other research studies affirm that sonication did not significantly influence on mentioned parameters (*Bhatet al. 2011; Zou et al., 2016; Zou et al. 2017*).In opposite, some studies reported an increase of EC during sonication of liquid samples (fruit juices) which could be related to the possibility of ultrasound (transient cavitation) to break up the cell walls and release cell nutrients (e.g., vitamins, minerals, proteins, etc.) in solution, therefore to increase a EC of liquid sample (*Aadil et al., 2015; Zou et al., 2016; Zou et al., 2017*). During sonication, temperature increase of system (liquid sample) as a direct result of transient cavitation phenomena is expected and may affect on some physicochemical properties but only significantly when applied ultrasound power (amplitude) is higher such in the ultrasound systems with directly immersed probe (*Knorr et al., 2004*).

Specialized metabolites content and antioxidant capacity of strawberry juice

In Table 3 is shown the content of analyzed specialized metabolites in different treatedstrawberry juice samples. As expected, heat treatment at higher temperature (80 °C) and longer time period (35 min) significantly contributed to a degradation of vitamin C content, so in sample SjT2 35 the lowest vitamin C content (0.224 g 100 g^{-1} DW) was determined. In general, compared to the control sample in all heat treated strawberry juices lower vitamin C content was determined, even 36 % lower values. Contrary results were observed in sonicated juices which in general had higher vitamin C content compared to the control and heat treated samples. The highest vitamin C content (0.677 g 100 g^{-1} DW) was determined in ultrasound treated juice at 80 °C for 35 min. Strawberry juice samples treated with ultrasound without applied temperature in bath did not show a higher vitamin C content compared to the control sample, and a slightly higher value compared to the heat treated samples which suggest that application of ultrasound in combination with higher media temperature positively affected on the content of vitamin C. On average, in ultrasound treated samples of strawberry juice (SjUAE 15- SjUAE2 35) even 51 % higher vitamin C content was determined compared to the heat treated samples (SjT1 15- SjT2 35) and 11 % higher compared to the control sample. Mostly other research studies also suggest an increase of vitamin C content during sonication (*Tiwari et al., 2009; Aadil et al., 2013; ŠicŽlabur et al., 2017*) as obtained in this research.But some research data recorded possibility of slight decrease of vitamin C content during sonication as an result of cavitation phenomena (*Dias et al., 2015; Ordóñez-Santos et al., 2017*).

Bioactive compounds are relatively unstable in food systems primarily because of high temperatures which are exposed during different technological processes. Polyphenolic compounds are also characterized as thermolabile compounds suggesting that higher temperatures strongly affecting on decrease of their content. Results of this study also proved stated, while in juice samples regardless of the treatment method higher temperature application (80 °C) significantly reduced total phenol (TPC), total flavonoid (TFC) and total non-flavonoid (TNFC) content. The highest TPC (2.084 g GAE 100 g^{-1} DW) was determined in control sample, while on average in thermal treated samples 28 % lower and in ultrasound treated juices 15 % lower values. Among ultrasound treated samples the highest TPC content (2.031 g GAE 100 g^{-1} DW) was determined in juice treated by ultrasound without applied additional temperature for 35 min time period (SjUAE 35). Comparing the influence of treatment method on TPC can be concluded that ultrasound significantly contributed to the increase of TPC since in ultrasound treated juices 12 % higher values was determined compared to the thermal treated regardless of the temperature and time period. The same trend as for TPC was also observed for the TNFC during which the highest TNFC was determined in control sample, while ultrasound treated juices had significantly higher TNFC compared to the thermal treated. Available literature data mainly suggest a positive impact of sonication on the content of polyphenolic compounds compared to the conventional processes which is in accordance with the results of this research (*Aadil et al., 2013;Alighourchi et al., 2013; Uribe et al., 2015; ŠicŽlabur et al., 2017*). As mentioned, one of the most important and characteristic chemical compounds for strawberry fruit are anthocyanins, red color pigments from the group of polyphenols. Anthocyanin level strongly depends on the certain environmental factors in technological processes, as the most important: pH value, light, temperature, oxygen etc. According to the results from this study, the highest anthocyanin content (TAC, 2.246g kg^{-1} DW) was determined in juice sample treated by ultrasound for 15 min without additional applied temperature (SjUAE 15) while the lowest (1.45 g kg^{-1} DW) in thermal treated juice (80 °C) for 15 min (SjT2 15). Thermal treatment significantly negatively influenced on TAC and as expected the lowest TAC values were recorded in thermal treated juices regardless of the applied temperature. Also, higher temperature (80 °C) and longer time period in thermal treated juices significantly decreased TAC compared to the lower applied temperature (60 °C) and shortest time period (15 min) in mentioned samples. Ultrasound treatment significantly affected on the TAC in strawberry juices and even 23 % higher values were determined in ultrasound treated samples compared to the thermal treated. Data of TAC content from other research studies are variable since some studies noticed the

Table 2. Physicochemical properties of strawberry juice

Treatment	DM (%)	TA (% DW)	TSS (%)	pH	EC (mS cm^{-1})
SjC	9.42^{ef}±0.06	92.11^{d}±1.98	8.50^{c}±0.1	3.406^{ab}±0.01	2.83^{d}±0.02
SjT1 15	9.80^{d}±0.05	91.91^{d}±0.82	8.50^{c}±0.1	3.41^{a}±0.02	3.07^{c}±0.01
SjT1 35	11.29^{c}±0.19	82.95^{e}±1.84	8.47^{d}±0.81	3.406^{ab}±0.03	3.22^{b}±0.01
SjT2 15	12.80^{a}±0.37	77.97^{f}±2.50	9.43^{b}±0.21	3.39^{ab}±0.01	3.21^{b}±0.03
SjT2 35	12.33^{b}±0.07	96.57^{bc}±0.71	11.73^{a}±0.06	3.39^{ab}±0.01	3.45^{a}±0.03
SjUAE 15	9.43^{ef}±0.08	95.45^{bcd}±1.25	8.53^{c}±0.06	3.37^{bc}±0.01	2.20^{g}±0.06
SjUAE 35	9.50^{de}±0.06	95.09^{bcd}±0.30	8.70^{c}±0.1	3.36^{cd}±0.02	2.61^{e}±0.02
SjUAE1 15	9.37^{ef}±0.04	94.73^{cd}±0.69	8.70^{c}±0.1	3.36^{cd}±0.03	2.48^{f}±0.04
SjUAE1 35	9.00^{g}±0.06	100.79^{a}±2.33	8.67^{c}±0.06	3.33^{cd}±0.02	2.88^{d}±0.05
SjUAE2 15	9.42^{ef}±0.26	95.53^{bcd}±3.34	8.87^{bc}±0.21	3.35^{cd}±0.03	2.84^{d}±0.01
SjUAE2 35	9.11^{fg}±0.06	98.91^{ab}±0.49	8.63^{c}±0.06	3.32^{d}±0.01	2.87^{d}±0.04
Pr≤F	0.0001	0.0001	0.0001	0.0001	0.0001

DM- dry matter content; TA- total acid content; TSS- total soluble solids; EC- electrical conductivity

Table 3. Specialized metabolites content and antioxidant capacity strawberry juice

Treatment	**VIT C (g 100 g^{-1} DW)**	**TPC (g GAE 100 g^{-1} DW)**	**TFC (g GAE 100 g^{-1} DW)**	**TNFC (g GAE 100 g^{-1} DW)**	**TAC (g kg^{-1} DW)**	**ABTS radical assay (mmol TE L^{-1})**
SjC	$0.439^{cd}\pm0.64$	$2.084^{a}\pm0.06$	$0.804^{bcd}\pm0.15$	$1.280^{a}\pm0.12$	$2.091^{b}\pm0.006$	$2.28^{b}\pm0.05$
SjT1 15	$0.407^{cd}\pm0.86$	$1.849^{c}\pm0.004$	$0.857^{ab}\pm0.13$	$0.992^{d}\pm0.11$	$1.966^{bc}\pm0.13$	$2.285^{b}\pm0.001$
SjT1 35	$0.375^{de}\pm0.93$	$1.575^{e}\pm0.02$	$0.7^{f}\pm0.16$	$0.875^{e}\pm0.09$	$1.693^{d}\pm0.46$	$2.275^{c}\pm0.04$
SjT2 15	$0.285^{ef}\pm0.182$	$1.504^{f}\pm0.04$	$0.716^{ef}\pm0.21$	$0.787^{f}\pm0.19$	$1.450^{e}\pm0.52$	$2.275^{c}\pm0.01$
SjT2 35	$0.224^{f}\pm0.08$	$1.568^{e}\pm0.005$	$0.777^{de}\pm0.009$	$0.791^{f}\pm0.004$	$1.596^{de}\pm0.05$	$2.285^{b}\pm0.003$
SjUAE 15	$0.350^{de}\pm0.23$	$1.839^{c}\pm0.013$	$0.918^{a}\pm0.69$	$0.922^{e}\pm0.56$	$2.246^{a}\pm0.20$	$2.272^{de}\pm0.05$
SjUAE 35	$0.287^{ef}\pm0.35$	$2.031^{ab}\pm0.13$	$0.854^{abc}\pm0.13$	$1.177^{b}\pm0.19$	$1.980^{bc}\pm0.48$	$2.269^{e}\pm0.001$
SjUAE1 15	$0.552^{b}\pm0.89$	$2.013^{b}\pm0.12$	$0.915^{a}\pm0.13$	$1.098^{c}\pm0.21$	$1.938^{c}\pm0.17$	$2.271^{e}\pm0.003$
SjUAE1 35	$0.497^{bc}\pm0.25$	$1.576^{e}\pm0.13$	$0.652^{fg}\pm0.16$	$0.924^{e}\pm0.13$	$2.032^{bc}\pm0.02$	$2.274^{cd}\pm0.05$
SjUAE2 15	$0.558^{b}\pm0.81$	$1.811^{c}\pm0.61$	$0.783^{cde}\pm0.63$	$1.028^{d}\pm0.41$	$2.072^{bc}\pm0.63$	$2.272^{de}\pm0.03$
SjUAE2 35	$0.677^{a}\pm0.55$	$1.635^{d}\pm0.08$	$0.598^{g}\pm0.009$	$1.037^{d}\pm0.08$	$2.109^{ab}\pm0.25$	$2.288^{a}\pm0.03$
Pr≤F	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

VIT C- vitamin C content; TPC- total phenol content; TFC- total flaovnoid content; TNFC- total non-flavonoid content; TAC- total anthocyanin content

negative effect of ultrasound on TAC content (*Tiwari et al., 2008a; Tiwari et al., 2008b*), while some suggest positive impact of ultrasound on TAC content (*Galvan et al., 2012; Mane et al., 2015; Šic Žlabur et al., 2017*).

Antioxidant capacity of all analyzed strawberry juice samples regardless of the treatment is high (Table 3), suggesting a strong possibility of juice samples for inactivation of free radicals and oxidation process. Ultrasound treated samples exhibit on average the highest antioxidant capacity, even 17 % higher compared to the thermal treated and control sample. The highest antioxidant capacity (2.288 mmol TE L^{-1}) was determined in ultrasound treated sample with additional applied temperature (80 °C) in time period of 35 min(SjUAE2 35).

CONCLUSIONS

Ultrasound treatment did not significantly influenced on the most analyzed physicochemical properties of strawberry juice with the exception of total acids content and slight increase of total soluble solids during sonication. Thermal treatment positively affected on the dry matter content and electrical conductivity. From group of specialized metabolites ultrasound treatment influenced on the increase of vitamin C content, total phenols, anthocyanins and antioxidant capacity. In general, during sonication additional temperature apply is not necessary with the aim of increasing the content of bioactive compounds. But further research of ultrasonic treatment areindispensable first of all in the terms of microbial inactivation and stability of final product during longer time period.

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