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Effect of High Intensity Ultrasound and Pasteurization on Anthocyanin Content in Strawberry Juice

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Summary

The purpose of this investigation is to study the influence of high intensity ultrasound and pasteurization on the stability of anthocyanins and their content in strawberry juice. Different ultrasound process parameters for the treatment of juices are compared to the classical thermal treatments. For ultrasound treatments, three parameters were varied according to the statistical experimental design. Central composite design was used to optimize and design experimental parameters: temperature (25, 40 and 55 °C), amplitude (60, 90 and 120 μm) and time (3, 6, and 9 min). It was found that the anthocyanin content after pasteurization (85 °C for 2 min) was reduced by 5.3 to 5.8 % compared to untreated juices. After treatment with ultrasound (20 °C for 3, 6 or 9 min) or thermosonication (40 °C for 3, 6 or 9 min and 60 °C for 3 or 6 min), the degradation of anthocyanins was generally less intensive and was 0.7–4.4 % compared to the untreated juices. Only in the case of ultrasonic treatment at a temperature of 55 °C and treatment time of 9 min the total content of anthocyanins, compared to untreated juice, was reduced by 5.8 to 7.1 %, and their degradation was greater than that of pasteurized juices. From the results it can be concluded that total anthocyanin content was greater in more than 85 % of the selected ultrasound treatments compared to pasteurized juices. Ultrasound treatment can replace pasteurization in terms of preserving total anthocyanin content. The modelling approaches using response surface methodology (RSM) developed in this study exploit data in order to identify the optimal processing parameters for lowering degradation of anthocyanins in strawberry juice during ultrasound processing.

Key words: high intensity ultrasound, anthocyanins, strawberry juice, response surface methodology

Introduction

Heat, compared to other food preservation methods, has the important advantage of ensuring food safety and long preservation due to its destructive effect on enzymes and microorganisms. However, the non-specific effect of heat can cause reductions in nutritive and sensorial quality of foods and impairs their functional properties. To avoid unwanted effects of heat, many attempts have been made to design alternative procedures for food preservation and sanitation. Ultrasound has been identified as a potential technology to meet the FDA requirement of a 5-log reduction in pertinent microorganisms found in fruit juices (*1*). Sonication technology can improve the

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process through reduced processing time, higher throughput and lower energy consumption (*2–4*). Ultrasonication is a non-thermal method of food preservation that has the advantage of inactivating microorganisms in food without causing the common side-effects associated with conventional heat treatments. Food processing using ultrasound involves the transmission of energy at frequencies higher than 20 kHz. The low-intensity ultrasound, which uses very small power levels (typically less than 1 W/cm^2, with the frequency range of 5–10 MHz), causes no physical or chemical alterations in the properties of the treated material and thus can be used to measure the texture, composition, viscosity or concentration of food. In contrast, the high-intensity ultrasound, which uses much higher power levels (typically in the range of 10–1000 W/cm^2, with the frequency of 20–100 kHz), causes physical disruption of the material to which it is applied and promotes certain chemical reactions (*2*). If ultrasound were to be used in any practical application, it would most likely have to be used in conjunction with pressure treatment (manosonication), heat treatment (thermosonication) or both (manothermosonication). The enhanced mechanical disruption of cells is the reason for the enhanced killing when ultrasound is combined with heat or pressure (*2–4*).

Consumption of fruit juices containing bioactive compounds such as anthocyanins can reduce various degenerative diseases. Strawberries and their products contain an array of health-promoting compounds. Juices prepared from strawberries contain a relatively high content of anthocyanins. Strawberry juice colour is a key factor influencing consumer's perception of quality. The bright red colour of strawberry juice is due to the presence of anthocyanins, water soluble pigments. The most abundant anthocyanins in food are the glucoside forms of cyanidin, malvidin, delphinidin, peonidin, petunidin and pelargonidin. Pelargonidin-3-glucose (P3G) is the major anthocyanin found in strawberry juice. The anthocyanin content of fruits is greatly influenced by various genetic (cultivar), environmental and agronomic factors. Anthocyanins are highly unstable and very susceptible to degradation. Their stability is greatly affected by processing conditions including pH, temperature, light, oxygen, solvents, and the presence of enzymes, flavonoids, proteins and metal ions. Ultrasound processing of juices is reported to have a minimal effect on the degradation of key quality parameters such as colour and anthocyanin content in strawberry and blackberry juices. Sonication is also reported to enhance cloud stability of juice. Applications of ultrasound in food processing have been reviewed by Knorr *et al.* (*5*) and the effects of sonication on fruit juices by Tiwari *et al.* (*6–8*) and Rawson *et al.* (*9*). The effect of ultrasound on anthocyanins was studied for strawberry juice by Tiwari *et al.* (*10,11*). They reported a slight increase (1–2 %) in the P3G content of the juice at lower amplitude levels and treatment times, which may be due to the extraction of bound anthocyanins from the suspended pulp. However, in a study by Tiwari *et al.* (*12*), the anthocyanin content of the juice was degraded when higher amplitude levels were employed, but the maximum observed degradation was <5 %.

The objective of this study is to investigate the effect of high intensity ultrasound and pasteurization on the content of anthocyanins in strawberry juice using a frequency of 20 kHz under various conditions (treatment time, temperature and amplitude).

Materials and Methods

Standards and chemicals

Cyanidin-3-glucoside chloride and pelargonidin-3--glucoside chloride were obtained from Fluka (Neu-Ulm, Germany). HPLC-grade methanol, ethanol and formic acids were obtained from Merck (Darmstad, Germany).

Samples

Four different strawberry (*Fragaria×ananassa* Duch. cv. Alba) juices, two cloudy (samples A and C) and two clear (samples B and D) were subjected to ultrasound treatments.

Juice preparation

Each juice was made from approx. 10 kg of strawberries in the small-scale pilot plant. After screening, the removal of stems and shower washing, strawberries were subjected to blanching (85 °C per 1 min) in flow preheater (Pecon, Zagreb, Croatia). For samples A and B, the blanched pulp was cooled and pressed on hydraulic pack press (Euclid, Vinkovci, Croatia). For samples C and D, the blanched pulp was cooled at 50 °C, subjected to maceration by enzymes (Endozym Pectofruit Press, AEB S.p.A, Brescia, Italy) and also pressed on hydraulic pack press. After pressing, samples B and D were subjected to clarification by enzymes and centrifugation (Beckman J-21 B, Coventry, UK). All samples were pasteurized in a flow pasteurizer for 2 min at 85 °C (AP, BP, CP, DP) or by ultrasound (samples 1–16; Table 1).

Ultrasound treatments

Strawberry juice (100 mL) was placed in a round--bottom glass (200 mL), which served as the treatment chamber. An ultrasonic processor (S-4000, Misonix Sonicators, Newtown, CT, USA), set at 600 W, 20 kHz and 12–260 μm with a 12-mm diameter probe, was introduced into the vessel. Ultrasonication was carried out with 60, 90 and 120 μm amplitude. Strawberry juice samples were treated ultrasonically for 3, 6 and 9 min at 25 °C. In the case of thermosonication before ultrasonic treatment, the samples were heated at 40 and 55 °C. Overheating of the samples was prevented by water cooling of the treatment chamber. Each experiment was conducted at least in triplicate. For this study, 16 samples of each juice were ultrasonically treated (Table 1).

The laboratory use of this sonication system includes discontinuous use of ultrasound probe that is immersed in the sample, and which is intended for small scale testing. Possible implementation of this experiment must be performed on the scale-up system (more than 100 L), because the linearity of ultrasound experimental analysis between small scale and large scale is not possible.

Determination of acoustic power

The most widely accepted method for determining the power from an acoustic horn into an aqueous solu-

Table 1. Treatment time, amplitude, temperature, power intensity and added energy during ultrasound treatments of juices

Strawberry juices	X_1 Amplitude/mm	X_2 Time/min	X_3 Temperature/°C	Intensity/(W/cm^2)	Energy/J
A, B, C, D	–	–	–	–	–
AP, BP, CP, DP	–	–	–	–	–
A, B, C, D 1	60	9	25	15.67–15.98	56.79–58.55
A, B, C, D 2	90	3	40	6.01–6.09	15.25–15.52
A, B, C, D 3	120	3	25	6.53–6.64	20.98–21.52
A, B, C, D 4	120	3	55	6.80–6.97	19.11–19.15
A, B, C, D 5	90	6	55	11.78–12.08	37.98–38.14
A, B, C, D 6	60	3	55	6.79–6.86	13.37–13.43
A, B, C, D 7	120	9	55	16.63–16.75	64.00–64.13
A, B, C, D 8	90	6	40	12.71–12.85	36.45–36.84
A, B, C, D 9	60	6	40	12.04–12.13	28.07–28.39
A, B, C, D 10	90	9	40	16.06–16.13	61.98–62.87
A, B, C, D 11	120	9	25	16.34–16.49	64.22–64.35
A, B, C, D 12	90	6	40	12.71–12.85	36.45–36.84
A, B, C, D 13	60	3	25	5.99–6.14	12.65–12.76
A, B, C, D 14	90	6	25	11.05–11.16	36.73–37.01
A, B, C, D 15	120	6	40	12.04–12.16	42.99–43.08
A, B, C, D 16	60	9	55	15.22–15.26	60.07–60.43

tion is the calorimetric technique described by Margulis and Margulis (*13*). This method involves taking a known volume of water and applying ultrasound (for approx. 3 min) while monitoring the change in temperature with time for various ultrasonic amplitudes. The ultrasonic power can be readily determined from the following equations:

$$P = m \cdot C_p \cdot \frac{\partial T}{\partial t} \qquad /1/$$

$$AI = P/A \qquad /2/$$

where P is the ultrasonic power (W), m is the mass of the sample (kg), C_p is the specific heat capacity of strawberry juices (3.368 kJ/(kg·°C)), AI is the ultrasonic intensity (W/cm^2), and A is the surface of the probe (cm^2). Acoustic intensity applied in sonication of juices varied from 5.99 to 16.75 W/cm^2.

Anthocyanin extraction

Anthocyanins were extracted from sonicated and pasteurized samples using 10 mL of a sample and 20 mL of 30 % (by volume) aqueous ethanol. The mixture was extracted for 30 min at 70 °C in ultrasonic bath, filtered through Whatman No. 40 filter paper (Whatman International Ltd., Kent, UK) using a funnel in volumetric flasks. The filtrates were adjusted to 50 mL in volumetric flask with 30 % aqueous ethanol.

Anthocyanin determination

Chromatographic separation and determination of anthocyanins was performed using Varian ProStar HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a ProStar solvent delivery module 230, injector Rheodyne 7125 and ProStar 330 UV-VIS photodiode array detector (230–510 nm). Separation of anthocyanins was performed on a Pinnacle II C-18 (250×4.6 mm i.d., 5 μm) including Pinnacle C-18 guard column (10×4 mm i.d., 5 μm) (Restek, Bellefonte, PA, USA). A volume of 2 mL of the supernatant was filtered through 0.45-μm filter syringe (nylon membranes, Supelco, Inc. Bellefonte, PA, USA) before injection (20 μL) into the HPLC.

The elution was carried out at room temperature using 2.5 % formic acid aqueous solution and methanol solution mixed in various ratios: A – water/methanol 5:95, B – water/methanol 12:88 and C – water/methanol 80:20 with the following gradients: first 150 min 100 % A, from 15 to 35 min 100 % B, from 35 to 50 min 75 % B and 25 % C, from 50 to 52 min 50 % B and 50 % C and from 52 until 60 min 100 % C. Flow rate was 1 mL/min with a UV-VIS detector set at 510 nm. Calculation of the mass fractions was based on a calibration curve made of external standards of the investigated anthocyanins by comparison of their retention times and peak area of standards. Chromatograms were recorded and processed with Varian ProStar chromatography software.

Experimental methodology

Multivariate methods provide advantages over more traditional optimization designs including the fact that a smaller number of experiments produces more information and allows the identification of interactions between variables. Response surface methodology has 4 major steps, which are: experimental design, model fitting, model validation and condition optimization (*14*). Experimental designs such as central composite design (CCD) are useful for response surface methodology (RSM) because they do not require an excessive number of experimental runs. RSM, a statistical method, uses quantitative data from appropriate experiments to determine and simultaneously solve multivariate equations (*15*). It is a collection of statistical techniques for designing experi-

ments, building models, evaluating the effects of factors, and analyzing optimum conditions of factors for desirable responses.

A general factorial design (STATGRAPHICS Centurion, StatPoint Technologies, Inc, VA, USA) consisting of 16 experimental trials was designed and chosen to obtain general observation of the effect of ultrasound treatment on the content of anthocyanins in strawberry juice. In order to determine the influence of each factor on the content of anthocyanins in strawberry juice, CCD and face-centered cubic lattice model were chosen. The ultrasound factors of amplitude (μm), temperature (°C), and treatment time (min) were studied. Analysis of variance (ANOVA) was carried out to determine any significant differences ($p<0.05$) among the applied treatments. The operating variables were considered at three levels, namely low (–1), central (0) and high (1). Accordingly, 16 experiments were conducted organized in a factorial design (including factorial points, axial points and centre point) and one of them was replicated to get a good estimate of experimental error. Repetition experiments were carried out after other experiments followed by the order of runs designed by the program. Response (output) values were total anthocyanins in strawberry juice in mg/kg.

The designs were based on a two-level full factorial design, which were augmented with centre and star points (*16*). The total number of experiments of the designs (N) can be calculated as follows:

$$N=N_i+N_o+N_j \qquad /3/$$

where $N_i=2^n$ is the number of experiments of the two-level full factorial design, N_o is the number of centre points, and $N_j=2\cdot n$ is the number of star points.

Response surface methodology

The experimental results were analyzed by response surface methodology (RSM) using the software STATGRAPHICS Centurion. Calculations were done at 95 % confidence level. The ultrasound factors amplitude – X_1 (μm), treatment time – X_2 (°C) and temperature – X_3 (min) were studied using RSM. In order to optimize the ultrasound treatment and investigate the effects of the above independent variables on the content of anthocyanins in strawberry juice, a central composite rotary design with the variables at three levels was used in the experiments (Table 1). Design matrix for the experiment and the regression model proposed for response is given below (*17*):

$$Y=\beta_0+\sum_{i=1}^{4}\beta_i X_i+\sum_{i=1}^{4}\beta_{ii}X_i^2+\sum_{i<j}^{4}\beta_{ij}X_iX_j \qquad /4/$$

where β_0 is the value of the fixed response at the central point of the experiment which is the point (0, 0, 0); and β_i, β_{ii} and β_{ij} are the linear, quadratic and cross-product coefficients, respectively. While demonstrating the significant effect, 3-dimensional fitted surfaces were drawn (*18*). The model was fitted by multiple linear regressions (MLR). The validity of the quadratic empirical model was tested with analysis of variance (ANOVA). The confidence level used was 95 %.

Results and Discussion

In this study, four strawberry juices were prepared using different technological processes that involve blanching. Patras *et al.* (*19*) found that anthocyanins (cyanindin--3-glucoside and pelargonidin-3-glucoside) in blackberry and strawberry puree were significantly degraded by heat treatment, without blanching inactivation of polyphenol oxidase enzyme in the samples. Immediately after preparation of juices, the composition of anthocyanins and total anthocyanin content were determined, and then the juice was pasteurized (85 °C for 2 min) or treated with ultrasound or thermoultrasound.

The purpose of this investigation was to examine the influence of high intensity ultrasound on the stability of anthocyanin content in strawberry juice. Different process parameters of ultrasound (amplitude, time, temperature) have been compared to the classical thermal treatments. In this work, the content of anthocyanins in strawberry juice after ultrasonic treatment was analyzed by RSM using STATGRAPHICS Centurion software. Calculations were done at 95 % confidence level. In these different ultrasound treatments, the combination effect of amplitude as X_1, treatment time as X_2 and temperature X_3 was studied using RSM. A general factorial design consisting of 16 experimental trials was designed to obtain general observation of ultrasound treatment of anthocyanins in strawberry juice (Table 1).

The anthocyanin content of strawberry juices is listed in Tables 2–5, where concentrations of anthocyanins in untreated, pasteurized and sonicated strawberry juices are shown. Four major strawberry juice anthocyanins were significantly influenced by ultrasound, namely cyanidin--3-*O*-glucoside, cyanidin-3-*O*-rutinoside, pelargonidin-3--*O*-glucoside and pelargonidin-3-*O*-rutinoside. Anthocyanin content during sonication was influenced by three investigated factors, *i.e.* ultrasound amplitude level (μm), sonication time (min) and temperature (°C). Their effects were either individual or interactive.

The contribution of total anthocyanin content in untreated samples varied from 118.915 (sample B) to 121.78 mg/kg (sample C), depending on the technological process applied for their production.

Samples which were pasteurized (marked with P) at 85 °C for 2 min show the lowest levels of anthocyanins, as compared to sonicated or untreated juices, namely because of the thermal treatment, which causes decomposition of anthocyanins. Tiwari *et al.* (*8*) reported a slight increase (1–2 %) in the pelargonidin-3-glucoside content of the juice at lower ultrasound amplitude levels and treatment times, which may be due to the extraction of bound anthocyanins from the suspended pulp. Similarly, weak ultrasonic irradiation is reported to promote an increase in the amount of phenolic compounds found in red wine (*20*). This behaviour is not in agreement with our results. Here, antocyanin content of sonicated samples is between untreated and pasteurized samples. Data show that anthocyanin content decreases with longer ultrasound treatment and increases temperature of sonication. Also, it has been observed that decrease of anthocyanin level is higher when sonication temperature is increased than when sonication period is prolonged. Maximal degradation of anthocyanins in juices in this study was observed after

Table 2. Mass fractions of anthocyanins in untreated, pasteurized and sonicated strawberry juices A

Strawberry juice A	*w*(cyanidin-3-*O*-glucoside)	*w*(cyanidin-3-*O*-rutinoside)	*w*(pelargonidin-3-*O*-glucoside)	*w*(pelargonidin-3-*O*-rutinoside)	Σw_i
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
A	0.843	7.346	104.312	7.551	120.053
AP	0.613	6.554	99.314	6.839	113.321
A1	0.683	7.069	100.647	7.180	115.578
A2	0.683	7.107	102.295	7.300	117.386
A3	0.693	7.140	102.364	7.311	117.508
A4	0.680	6.807	100.750	6.970	115.207
A5	0.679	6.793	100.660	6.978	115.111
A6	0.680	6.827	100.959	6.700	115.460
A7	0.417	6.343	99.216	6.515	112.491
A8	0.714	7.189	101.027	7.197	116.128
A9	0.711	7.109	101.018	7.196	116.035
A10	0.663	6.938	100.390	7.002	114.993
A11	0.653	7.059	100.233	7.171	115.115
A12	0.714	7.189	101.027	7.197	116.128
A13	0.744	7.240	102.586	7.401	117.971
A14	0.733	7.230	102.088	7.387	117.437
A15	0.709	7.100	100.928	7.176	115.913
A16	0.438	6.253	99.606	6.763	113.060

Table 3. Mass fractions of anthocyanins in untreated, pasteurized and sonicated strawberry juices B

Strawberry juice B	*w*(cyanidin-3-*O*-glucoside)	*w*(cyanidin-3-*O*-rutinoside)	*w*(pelargonidin-3-*O*-glucoside)	*w*(pelargonidin-3-*O*-rutinoside)	Σw_i
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
B	0.842	7.234	103.704	7.535	118.915
BP	0.613	6.529	99.119	6.826	113.087
B1	0.670	7.109	100.921	7.201	115.901
B2	0.691	7.133	102.376	7.300	117.500
B3	0.690	6.312	103.834	7.331	118.167
B4	0.681	6.828	100.751	6.999	115.259
B5	0.684	6.818	100.659	6.978	115.139
B6	0.690	6.889	100.960	6.999	115.538
B7	0,475	6,433	99.156	6,533	112.597
B8	0.720	7.209	101.089	7.238	116.256
B9	0.722	7.115	101.075	7.254	116.166
B10	0.616	6.965	101.518	7.007	116.106
B11	0.651	6.081	102.567	7.199	116.498
B12	0.720	7.209	101.089	7.238	116.256
B13	0.754	7.276	102.996	7.454	118.480
B14	0.744	7.278	102.428	7.399	117.849
B15	0.720	7.115	101.324	7.202	116.361
B16	0.461	6.259	99.601	6.691	113.012

ultrasound treatment at the amplitude of 90 or 120 μm for 9 min at 55 °C (samples 7 and 16; Tables 2–4). In another investigation (*12*) after sonication, the content of anthocyanins compared to untreated (control) samples decreased for about 3.2 %. This behaviour is not in agreement with our results, which show that the anthocyanin content after pasteurization was reduced by 5.3 to 5.8 % compared to untreated juices. After treatment with ultrasound or thermoultrasound, degradation of anthocyanins was generally less intensive and was 0.7–4.4 % compared to the pasteurized juices. Only in the case of ultrasonic treatment at 55 °C for 9 min, total content of anthocyanins, compared to untreated juice, was reduced from 5.8 to 7.1 %, and their degradation was

Table 4. Mass fractions of anthocyanins in untreated, pasteurized and sonicated strawberry juices C

Strawberry juice C	*w*(cyanidin-3-*O*-glucoside)	*w*(cyanidin-3-*O*-rutinoside)	*w*(pelargonidin-3-*O*-glucoside)	*w*(pelargonidin-3-*O*-rutinoside)	Σw_i
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
C	0.849	7.487	105.248	7.794	121.278
CP	0.617	6.739	99.894	6.873	114.123
C1	0.697	7.153	101.376	7.300	116.526
C2	0.711	7.353	102.976	7.378	118.418
C3	0.727	7.512	103.740	7.409	119.388
C4	0.689	7.003	101.751	7.105	116.548
C5	0.674	6.881	100.959	7.094	115.608
C6	0.707	7.245	101.962	7.197	117.111
C7	0.516	6.318	99.431	6.512	112.777
C8	0.745	7.147	102.079	7.431	117.402
C9	0.767	7.215	102.095	7.484	117.561
C10	0.731	6.940	101.564	7.018	116.253
C11	0.736	7.055	102.357	7.174	117.322
C12	0.745	7.147	102.079	7.431	117.402
C13	0.741	7.611	103.511	7.589	119.452
C14	0.774	7.286	102.528	7.519	118.107
C15	0.728	7.137	101.990	7.291	117.146
C16	0.561	6.519	99.631	6.702	113.413

Table 5. Mass fractions of anthocyanins in untreated, pasteurized and sonicated strawberry juices D

Strawberry juice D	*w*(cyanidin-3-*O*-glucoside)	*w*(cyanidin-3-*O*-rutinoside)	*w*(pelargonidin-3-*O*-glucoside)	*w*(pelargonidin-3-*O*-rutinoside)	Σw_i
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
D	0.846	7.372	104.699	7.563	120.480
DP	0.614	6.573	99.597	6.843	113.627
D1	0.681	7.093	101.136	7.199	116.109
D2	0.698	7.251	102.786	7.258	117.993
D3	0.739	7.131	103.379	7.359	118.608
D4	0.671	6.933	101.681	7.054	116.339
D5	0.653	6.801	100.839	6.991	115.284
D6	0.694	7.095	101.865	7.077	116.731
D7	0.506	6.298	99.561	6.302	112.667
D8	0.722	7.003	102.019	7.361	117.105
D9	0.743	7.105	102.025	7.334	117.207
D10	0.719	6.967	100.938	7.051	115.675
D11	0.755	7.075	100.359	7.191	115.380
D12	0.722	7.003	102.019	7.361	117.105
D13	0.733	7.611	103.341	7.319	119.004
D14	0.702	7.286	102.298	7.209	117.495
D15	0.696	7.137	101.959	7.031	116.823
D16	0.523	6.319	99.624	6.459	112.925

greater than that in pasteurized juices. Higher degradation of anthocyanins in these samples results from the formation, growth, and rapid collapse of microscopic bubbles. Cavities formed by sonication may be filled with water vapour and gases dissolved in the juice, such as O_2 and N_2, which may be responsible for oxidative degradation of anthocyanins.

After data for anthocyanin content in untreated, pasteurized and sonicated fruit juices were obtained, modelling for optimal conditions of ultrasound treatments were developed. Under optimal conditions, process parameters of ultrasound treatment that cause minimal degradation (anthocyanin content) can be obtained. The modelling approaches developed in this study exploit data

in order to identify the optimal process parameters for lowering degradation of anthocyanins in strawberry juice during ultrasound processing. This means that ultrasound has less influence on anthocyanin degradation than thermal treatment if sonication treatment is well optimized. Response surface methodology (RSM) may be employed to optimize critical processing parameters by estimating interactive and quadratic effects of factors that influence the process. RSM has been successfully employed to optimize food processing operations by several investigators (*9*,*21*).

Analysis of variance for ultrasound treatments of strawberry juices, specific amplitude, treatment time, and temperature are reported in Table 6. From the ANOVA results, there is no significant influence ($p>0.05$) of processing parameters on the anthocyanin content of fruit juices. Regression equation which fits best to the data obtained has been developed for each strawberry juice (A, B, C or D). The equation of the fitted model for sonicated strawberry juices is shown in Table 7. From these polynomial fits, several investigated factors are combined in linear, quadratic and cross-product coefficients. From the fits, anthocyanin values for each desired value of amplitude, treatment time or temperature in ultrasound process can be calculated. The aim of this study was to minimize the degradation of total anthocyanin content during ultrasound process, so optimal conditions for ultrasound treatment were needed to be determined. From Table 8 optimal conditions for ultrasonic treatment using response surface models and total anthocyanin content have been determined. It was determined that at a specified amplitude, temperature and treatment time, for each strawberry juice, values for maximal anthocyanin content were as follows: total anthocyanins at optimal values (mg/kg) 118.775 for juice A, 119.197 for juice B, 120.155 for juice C, and 119.822 for juice D. Response surface plots for total anthocyanin content in sonicated strawberry juices at 40 °C are shown in Fig. 1. For each juice, it can be seen that total anthocyanin content is dependent on the amplitude and treatment time, and that at specified temperature (40 °C) when amplitude and treatment time are increased, there is a decrease in total anthocyanin content. Maximal total anthocyanin content is determined at minimal amplitude used and at shortest treatment time. Temperature at 40 °C was used to graphically describe the influence of process parameters on total anthocyanin content because at this temperature there is no thermal degradation of anthocyanins. Degradation of anthocyanins is primarily caused by oxidation, cleavage of covalent bonds or enhanced oxidation reactions due to thermal processing (*19*).

Also, contour plots that illustrate the effect of amplitude (μm) and sonication time (min) on anthocyanin

Table 6. Analysis of variance for ultrasound treatments of strawberry juices at 95 % confidence level

Source	Juice A			Juice B			Juice C			Juice D		
	F-ratio	p-value	F-critical	F-ratio	p-value	F-critical	F-ratio	p-value	F-critical	F-ratio	p-value	F-critical
X_1 amplitude/mm	2.12	0.1953	2.43	2.07	0.1998	2.43	0.81	0.4018	2.43	1.19	0.3167	2.43
X_2 treatment time/min	1.99	0.2077	2.43	3.38	0.1156	2.43	2.56	0.1604	2.43	1.73	0.2366	2.43
X_3 temperature/°C	0.63	0.4591	2.43	1.05	0.3457	2.43	0.62	0.4603	2.43	0.24	0.6382	2.43

Table 7. Polynomial fit models for total anthocyanin content in sonicated strawberry juices

Juices	Models
A	Total anthocyanins=$123.851-0.174\cdot X_1+0.0211\cdot X_2+0.0525\cdot X_3+0.00087\cdot X_1^2+0.0056\cdot X_1\cdot X_2-0.00096\cdot X_1\cdot X_3-0.0034\cdot X_2^2-0.0174\cdot X_2\cdot X_3+0.00144\cdot X_3^2$
B	Total anthocyanins=$123.541-0.163\cdot X_1+0.1043\cdot X_2+0.07485\cdot X_3+0.00092\cdot X_1^2+0.0044\cdot X_1\cdot X_2-0.0013\cdot X_1\cdot X_3-0.0142\cdot X_2^2-0.0154\cdot X_2\cdot X_3+0.00128\cdot X_3^2$
C	Total anthocyanins=$122.199-0.195\cdot X_1+0.487\cdot X_2+0.188\cdot X_3+0.0012\cdot X_1^2+0.0033\cdot X_1\cdot X_2-0.0014\cdot X_1\cdot X_3-0.0217\cdot X_2^2-0.0208\cdot X\cdot X_3+0.00046\cdot X_3^2$
D	Total anthocyanins=$119.353-0.1603\cdot X_1+0.5784\cdot X_2+0.21\cdot X_3+0.00086\cdot X_1^2+0.0048\cdot X_1\cdot X_2-0.0011\cdot X_1\cdot X_3-0.0195\cdot X_2^2-0.0256\cdot X_2\cdot X_3+0.0003\cdot X_3^2$

X_1 – amplitude (μm) X_2 – treatment time (min) X_3 – temperature (°C)

Table 8. Optimal conditions for ultrasonic treatment using response surface models and total anthocyanins at optimal processing parameters

Source	Juice A	Juice B	Juice C	Juice D
X_1 – amplitude/mm	61.58	69.35	60.72	73.52
X_2 – treatment time/min	3.12	3.06	3.24	3.07
X_3 – temperature/°C	52.71	47.92	54.06	44.94
Total anthocyanins at optimal values of *w*/(mg/kg)	118.775	119.197	120.155	119.822

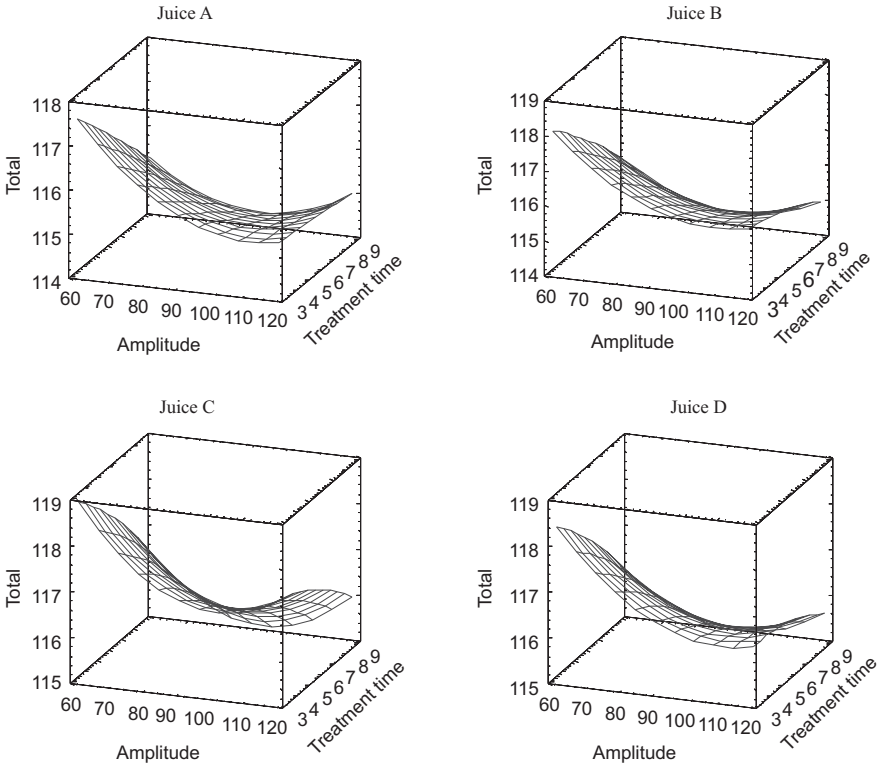

Fig. 1. Response surface plots for total anthocyanin content in sonicated strawberry juices at temperature of 40 °C

content in strawberry juices for processing at 40 °C are shown in Fig 2. From these plots, it is also obvious that values of total anthocyanin content are lower if ultrasound amplitude is higher and treatment time is longer. This is correlated with energy and intensity input of ultrasound probe systems when juices are sonicated (Table 1). When ultrasound amplitude is higher, larger amount of energy and intensity is input in the sonicated system (juice), causing a collapse of cavitation bubbles. Then, vapour and gases from the cavities are dissipated

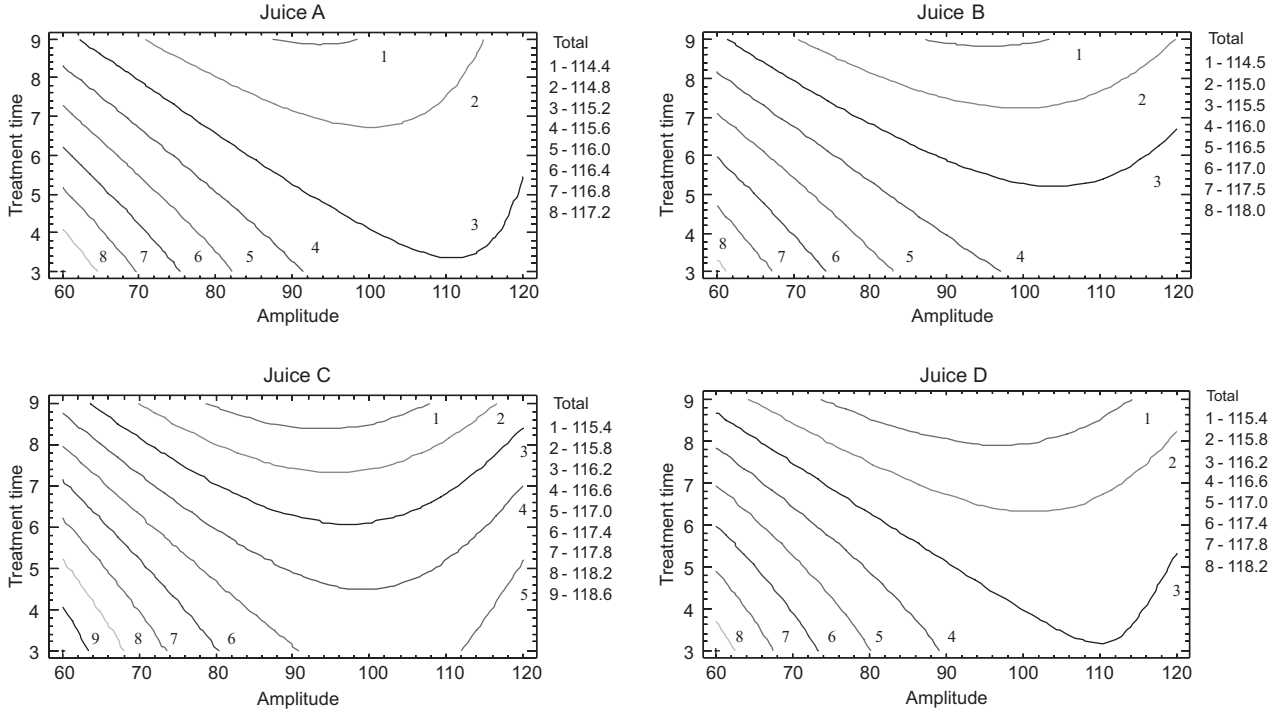

Fig. 2. Response surface plots illustrating the effect of amplitude (μm) and sonication time (min) on the anthocyanin content in strawberry juices processed at 40 °C

in the systems, causing several chemical reactions. Also, anthocyanin degradation during ultrasonic processing could be related to oxidation reactions, promoted by the interaction of free radicals such as hydroxyl (·OH) formed during sonication following the reaction ($H_2O \rightarrow {}^{\cdot}OH + H^{\cdot}$), which leads to chemical decomposition by opening of rings and formation of chalcone (*22*). The major reaction path for the degradation of polar compounds is pyrolysis within cavitation bubbles in the liquid or gas pockets trapped in the crevices of the solid boundaries in the liquid medium (*9*).

Conclusion

The purpose of the simultaneous use of the combined effect of ultrasound and temperature on the content of total anthocyanins in strawberry juices was to reduce the temperature and/or time of thermal processing. Results of this investigation on the effects of combined ultrasound and heat treatment *versus* thermal processing alone on bioactive compounds in juices also clearly indicate the improved retention levels by the combined treatment. Only in the case of ultrasonic treatment at a higher temperature and longer treatment time, the degradation of bioactive compounds was greater than that of pasteurized juices.

The modelling approaches by response surface methodology (RSM) developed in this study exploit data in order to identify the optimal processing parameters for lowering the degradation of anthocyanins in strawberry juice during ultrasound processing. This work demonstrates that thermosonication influences the content of antocyanins in strawberry juices and that RSM may be used to optimise critical process parameters to obtain juices with high retention levels of bioactive compounds.

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