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Effect of ultrasonication on microbial quality, colour and ascorbic acid of passion-fruit juice during storage

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Running title: sonication of passion-fruit juice

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Passion fruit juice (PFJ) has a delicate flavour very susceptible to thermal degradation. This study pursued to test the effect of sonication as non-thermal preservation method on some quality parameters of PFJ. The effect of ultrasound (20 kHz, 263 W, 89.25 μ m) on the indigenous microflora, colour and ascorbic acid content of PFJ was studied. Firstly, the kinetic of microbial inactivation was determined for aerobic mesophilic counts and yeasts counts. Data was fitted to Weibull model, and a treatment time of 8 min was selected for stability studies. To this, untreated and sonicated juice was stored at 4 and 10 °C up to 10 days and microbial quality, instrumental colour, pH and ascorbic acid content were evaluated. In general, ultrasound kept juice microbiologically stable for up to 10 days at 4 °C without markedly affecting other parameters evaluated. Ultrasound seems suitable to stabilize microbiologically PFJ.

Keywords: ultrasound processing, fruit juices, microbial inactivation, preservation.

High power ultrasound refers to pressure waves with a frequency of 20 KHz or more that has the ability to cause cavitation, which inactivates microorganisms. Cavitation micro bubbles are generated due to pressure changes that collapse violently in the succeeding compression cycles of a propagated sonic wave resulting in regions of high localized temperature and high shearing effects. The intense local energy and high pressure bring about a localized sterilization effect (PIYASENA et al., 2003). Sonication is considered a suitable technique for treatment of liquid foods due to the fact that transferring of acoustic energy to food is instantaneous and throughout the whole product, with reduction of the processing time, higher throughput and lower energy consumption (PIYASENA et al., 2003; ZENKER et al., 2003). As a consequence, it has been investigated as a way to provide high quality fruit juices. Sonication may affect key quality parameters such as colour and ascorbic acid, however such effects are generally subtle (TIWARI et al., 2008a). The use of sonication in fruit juice processing is a topic of current interest; recent research has been conducted on juices from the following fruits among others: pomegranate (UYSAL et al., 2015), blueberry (MOHIDEEN et al., 2015) and orange (GUERROUJ et al., 2016); and older studies have been recently revised (ZINOVIADOU et al., 2015).

There is a growing demand for processed fruit pulp which has aroused industry interest to search for novel technologies, this being of particular interest to tropical fruit juice production since the consumption of tropical or “exotic” fruits has increased all over the world (FRESHFEL, 2015). Passion fruit is one of the most exotic fruits. Its juice has a delicate flavour that is very susceptible to thermal degradation. Pasteurization of passion fruit juice (PFJ) at 75 °C/60 s drastically reduces the concentration of its four key volatiles (SANDI et al., 2004). Additionally, thermal processing of passion fruit results in the production of the

toxic compound 5-hydroxymethyl-2-furfural (SOARES et al., 2017) and the degradation of some bioactive compounds; for example, 15 % loss of ascorbic acid and 12 % loss of carotenoids have been reported for PFJ subjected to pasteurization at 90 °C for 60 seconds (FERNANDES et al., 2011). For these reasons, non-thermal processing may be indicated to produce high quality stable PFJ. To the best of our knowledge, sonication of PFJ has not been documented.

Furthermore, there is a trend nowadays to supplement fruit juices with minerals, which is more prominent in the orange juice industry (BERK, 2016). The addition of calcium to exotic fruit juices such as PFJ gives them an additional attractive to the market as well as contribute to consumer's health.

The goal of this study was to evaluate the effects of sonication on the stability of PFJ by determining the evolution of microbial populations, instrumental color, pH and ascorbic acid content during storage at two low temperatures.

1. Materials and methods

1.1. Preparation of PFJ samples

Yellow passion fruits (*Passiflora edulis f. flavicarpa* Degener) were purchased in a local market (Caracas, Venezuela). Unblemished fruits were selected, washed and subjected to an extraction process using a commercial juice extractor (Omega Products, Inc., Harrisburg, PA). The fresh juice was standardized with commercial sucrose to a total soluble solids/total titratable acidity ratio of 5 and added with 425 mg of calcium (Gynopharm, Venezuela) per 100 ml of juice in order to enhance the nutrient content of this juice. Total soluble solids were determined with a manual refractometer (Atago PR-101, Tokyo, Japan) and expressed in

Brix degrees corrected to 25°C (method 932.12, AOAC, 1990) and total titratable acidity by titration with NaOH (method 942.15 B, AOAC, 1990) and expressed as citric acid percent.

1.2. *Ultrasound treatment*

PFJ was treated by ultrasonication in two kind of experiments. In the first, the inactivation of relevant microbial groups by sonication was followed during 10 minutes by withdrawing juice samples every two minutes. In the second experiment, PFJ was treated under conditions selected according to results of the first experiment and then stored for stability studies.

Ultrasonic treatment was carried out in a refrigerated cylindrical vessel that kept juice temperature at 10°C. Ultrasound at a frequency of 20 kHz was applied continuously to 100 ml of PFJ using an ultrasonic unit with a rated power of 500 W (CPX-500, Cole-Parmer, Chicago, IL) and a 13 mm diameter probe which tip was kept at 4 cm from the vessel bottom (GÓMEZ-LÓPEZ et al., 2010). Treatment time was the only parameter that was changed during the tests; which were extended up to 10 min since this time has been proved to be enough to stabilize other fruit juices treated under similar conditions (GÓMEZ-LÓPEZ et al., 2010). The juice was sonicated at 75% power (89.25 μm wave amplitude), which is the maximum power that can be set for the probe without risking its fracture according to manufacturer's instructions. Supposing a mean transducer efficiency of 70 % (η), the acoustic power (WA) actually supplied into the juice was 263 W according to BEVILACQUA co-workers (2014) equation.

1.3. *Microbial analysis*

Aerobic mesophilic count (AMC) and yeast and moulds count were determined for kinetic modelling as well as for stability during storage. At the time intervals, samples were aseptically taken and serially diluted in 0.1 mg 100g⁻¹ peptone water. AMC was determined by pour plating on PCA (Merck, Darmstadt, Germany), overlaid with the same medium, and

incubation at 35-37 °C for 1-2 days. Yeast and moulds count were determined by spread plating on acidified PDA (Merck) and incubation at 20-25 °C for 3-5 days. Results are means of triplicates.

1.4. *Stability of the sonicated juice*

Untreated (control) and PFJ sonicated for 8 min were aseptically packed in sterile screwed-caps glass bottles and stored at 4 and 10 °C, during 10 days. Samples were taken at time intervals (0, 2, 4, 6, 8 and 10 days) and different quality indicators were evaluated, namely: AMC, yeast and moulds count, instrumental colour, ascorbic acid concentration and pH. Microbial quality was assessed as described before. Instrumental colour was determined in triplicate using the Hunter Lab space system by means of a Macbeth® colour-eye colorimeter Model 2445 (New Windsor, NY) with a CIE Standard Illuminant D65 and a CIE 1931 2° Standard Observer; the colorimeter was calibrated with a white standard tile (L=94.61, a=1.17 and b=2.17). Ascorbic acid was determined by the microfluorometric method 967.22 of AOAC (1990) and fluorescence measured by a spectrofluorophotometer RF-530 IPC (Shimadzu Corporation, Kyoto, Japan). pH was measured by method 945.10 (AOAC, 1990) with a pHmeter HI 9321 (Hanna Instruments, Padova, Italy). All measurements were made in triplicate.

1.5. *Data analysis*

Inactivation curves were fitted to eight models by using GInaFiT (GEERAERD et al., 2005); only the results for Weibull and log-linear + shoulder models are shown because they yielded the best adjust (lowest root mean sum of squared error, RMSE).

The Weibull model reads as follows:

$$\log N = \log N_o - \left(\frac{t}{\delta} \right)^p$$

Herein, N stands for the number of surviving microorganisms, N_0 for the initial number of microorganisms, t is the treatment time, δ (time unit) is a scale parameter and p (dimensionless) a shape parameter.

The log-linear + shoulder model reads as follows:

$$\log N = \log N_0 \cdot e^{-k_{max} \cdot t} \cdot \left(\frac{e^{k_{max} \cdot S_l}}{1 + (e^{k_{max} \cdot S_l} - 1) \cdot e^{-k_{max} \cdot t}} \right)$$

Herein, k_{max} (units: inverse of time) stands for the first order inactivation constant and S_l (time units) for the shoulder length (GEERAERD et al., 2005).

Statistical analysis was carried out by firstly checking normality by the Kolmogorov-Smirnov test and homoscedasticity by the Levene's test. Parametric comparisons were performed by using ANOVA and Tukey tests at $P=0.05$ in all cases except for the inactivation curve for yeasts. In this case, the Kruskal-Wallis test was used for media comparisons since data did not fulfil homoscedasticity. Statistical analysis was carried out using IBM SPSS Statistics 24 (IBM, Armonk, USA). $P=0.05$ was used in all cases.

2. Results and discussion

2.1. Microbial inactivation

Ultrasound treatment was able to decrease the aerobic mesophilic count (AMC) and yeast count (YC) of PFJ; no moulds were detected. The inactivation curves are shown in fig. 1a for AMC and fig. 1b for YC, and results of modelling parameters are shown in table 1. Weibull and log-linear + shoulder models gave place to identical curves, only curves corresponding to the Weibull model are shown in fig. 1 following the parsimony principle. The Weibull model has been widely used to describe the kinetic of microbial inactivation in

fruit juices by ultrasonication, such as natural microflora in orange juice (GÓMEZ-LÓPEZ et al., 2010) and *Saccharomyces cerevisiae* in pomegranate juice (UYSAL et al., 2015).

<Fig. 1 here>

<Table 1 here>

Fig. 1 shows that both microbial groups exhibited a shoulder followed by an inactivation phase, which means that the damage caused by the ultrasound treatment to both microbial groups is not enough to start their immediate inactivation but a threshold must be reached to start decreasing their viability. The shoulder length was longer for AMC than for YC as it can be observed in fig. 1 and has been quantified (table 1), which means that more energy is required to start the inactivation of microorganisms enumerated by AMC than for yeasts. The inactivation phase of both curves exhibited downward concavity as it can be observed in fig. 1 and a p value (shape value) > 1 (table 1) in the Weibull model, which was more pronounced for AMC inactivation. Values for the other parameters of the inactivation phase, δ and K_{max} , are in line with previous reports (GÓMEZ-LÓPEZ et al., 2010; UYSAL et al., 2015). The lowest microbial counts in the current study were found for the 10 min treatment. However, a shorter time (8 min) was selected for storage studies since counts after 8 min of sonication were statistically equal than those at 10 min ($P > 0.05$).

2.2. Microbiological stability

The evolution of microbial populations during storage at cold temperatures are shown in fig. 2. It can be observed at day 0 that sonication decreased AMC by $1.35 \log \text{CFU ml}^{-1}$ (fig. 2a), and YC by $0.74 \log \text{CFU ml}^{-1}$ (fig. 2b). The results are in line with other reported for indigenous microflora of different fruit juices such as those made from orange (VALERO et

al., 2007; GÓMEZ-LÓPEZ et al., 2010) and blueberry (MOHIDEEN et al., 2015), which are not higher than 2 log CFU ml⁻¹.

<Fig. 2 here>

Overall, microbial populations of sonicated samples were persistently lower than those of non-sonicated samples during refrigerated storage. That occurred specifically at 4 °C, where the differences in microbial counts after 10 days of storage were similar to those initially achieved by the ultrasound treatment, as it has also reported for ultrasound-treated orange juice stored at 4 °C (GÓMEZ-LÓPEZ et al., 2010) and 5 °C (GUERROUJ et al., 2016). This indicates that sonication brought about a PFJ with higher microbiological quality than untreated juice up to 10 days. At 10 °C however, the stability did not last beyond the sixth day due to increase in AMC (fig. 2c), and in spite of the stability of YC (fig. 2d).

2.3. *Colour and pH*

PFJ has a characteristic intense yellow colour. Ultrasound altered its colour parameters determined instrumentally ($P < 0.05$). The juice became lighter, greener and yellower (fig. 3). Changes were small (from 31.68 to 32.92 for lightness, from -0.81 to -1.30 for “a” and from 35.70 to 37.15 for “b”) and their magnitude comparable with results reported by VALERO co-workers (2007) and TIWARI co-workers (2008b) for sonicated orange juice. After the initial changes caused by the treatment, the colour became stable during storage, with changes between day 0 and 10 that did not exceed -1.4, 0.3 and 2.1 for L, a and b parameters respectively. As consequence, the differences between sonicated and control samples persisted during the 10-day shelf life experiment, with no considerable effect of storage temperature. The increase in lightness of PFJ after sonication could be a result of decreasing the particle size (VALERO et al., 2007) and the further stability of this parameter during

storage could be related to the decrease in ascorbic acid concentration (fig. 4) since the oxidation of this compound has been related to lightness reduction after thermal processing of PFJ (SANDI et al., 2004). Carotenes are major pigments present in yellow fruit juices, and changes in colour parameters a and b might be related to carotenoid isomerization (SANDI et al., 2004).

<Fig. 3 here>

<Fig. 4 here>

pH was in the range of 2.98-3.10 (table 2) and showed statistically different values ($P < 0.05$). The variation of pH was very small and did not show any pattern that could suggest correlation with changes observed in the other studied variables, which is in agreement with results by CHENG co-workers (2007) for guava juice and GÓMEZ-LÓPEZ co-workers (2010).

<Table 2 here>

2.4. *Ascorbic acid content*

Sonication caused a significant ($P < 0.05$) reduction in ascorbic acid content of PFJ (fig. 4). Although this result might look discouraging, TIWARI co-workers (2009) has shown that the degradation of ascorbic acid due to sonication is less than in thermally treated juice. Ascorbic acid may be degraded during sonication either due to thermolysis and combustion during the very short high local temperature increase of the cavitation bubble or reaction with hydroxyl radicals (TIWARI et al., 2009). In spite of this reduction, the concentration of ascorbic acid of all samples reached the same value ($P > 0.05$) from the second day of storage on and declined during storage until similar ($P > 0.05$) concentrations.

3. Conclusions

Ultrasound (20 kHz, 263 W, 89.25 μm) applied to passion fruit was able to decrease its aerobic mesophilic and yeast counts. The kinetic study showed that Weibull and log-linear + shoulder models yielded the best data fit for the inactivation of both microbial groups. The inactivation was not immediate but preceded by a shoulder. Storage studies showed that PFJ sonicated for 8 minutes was microbiologically stable during 10 days at 4 °C but not at 10 °C. Sonication made the juice lighter, greener and yellower, but the change was small; and no considerable variations in colour and pH were observed during storage at 4 °C. Even though ultrasound treatment caused an immediate decrease in ascorbic acid content, the final concentration of this compound was equal to that of the untreated samples after the second day of storage. The microbial stability of sonicated PFJ stored at 4 °C along with the subtle effects of this non-thermal technology on colour and ascorbic acid content during refrigerated storage allows concluding that sonication could be a suitable alternative for PFJ processing.

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FIGURE LEGENDS

Fig. 1. Microbial inactivation of aerobic mesophilic counts (a) and yeast counts (b) of sonicated passion fruit juice. Curves are fitted to Weibull model. ^{abc} Points with different letters are statistically different ($P < 0.05$).

Fig. 2. Microbial stability of untreated and sonicated passion fruit juice stored at two different temperatures. Aerobic mesophilic counts (a) and yeast counts (b) at 4 °C; aerobic mesophilic counts (c) and yeast counts (d) at 10 °C. o: untreated, □: sonicated.

Fig. 3. Colour of untreated and sonicated passion fruit juice stored at 4 and 10 °C. Lightness (a), parameter “a” (b), parameter “b” (c). o: control, 4 °C; ■: sonicated, 4 °C; Δ: control, 10 °C; x: sonicated, 10 °C.

Fig. 4. Changes in ascorbic acid content during storage of untreated and sonicated passion fruit juice. o: control, 4 °C; ■: sonicated, 4 °C; Δ: control, 10 °C; x: sonicated, 10 °C.

Table 1. Parameters of the Weibull and log-linear + shoulder models for mesophilic aerobic counts and yeast counts (log CFU/ml) of sonicated passion fruit juice.

Parameters	Mesophilic aerobic counts		Yeast counts	
	Log-linear + shoulder	Weibull	Log-linear + shoulder	Weibull
RMSE ^a	0.0458	0.0464	0.0892	0.0895
R ²	0.9830	0.9826	0.9763	0.9761
δ (min) ^b		11.66±0.49		8.94±0.68

p (dimensionless) ^c		2.44±0.46		1.33±0.29
Log (N _o)	5.17±0.03	5.16±0.03	5.08±0.08	5.07±0.08
S _l (min) ^d	7.02±0.58		2.81±1.59	
k _{max} (min ⁻¹) ^e	0.47±0.08		0.37±0.08	

^a RMSE: Root mean sum of squared error. ^b δ : time for the first decimal reduction. ^c p: parameter describing concavity or convexity of the curve. ^d S_l: shoulder length. ^e k_{max}: specific inactivation rate.

Table 2. pH evolution of non-sonicated and sonicated passion fruit juice during storage at two different temperatures.

Storage temperature (°C)	Storage time (days)	Non-sonicated	Sonicated	
--	0	3.04±0.00 ^{ef}	3.05±0.00 ^{de}	
	4	2	3.06±0.01 ^{bcd}	3.01±0.01 ^{gh}
		4	3.05±0.01 ^{de}	2.98±0.01 ⁱ
		6	3.06±0.01 ^{bcd}	3.06±0.00 ^{bcd}
		8	3.02±0.01 ^{gh}	3.00±0.01 ^{hi}
		10	3.02±0.01 ^{fg}	3.00±0.01 ^{gh}
10	2	3.07±0.01 ^{bc}	3.07±0.00 ^{bcd}	
	4	3.10±0.01 ^a	3.08±0.01 ^b	
	6	3.05±0.01 ^{cde}	3.06±0.01 ^{bcd}	
	8	3.07±0.01 ^{bcd}	3.07±0.02 ^{bcd}	
	10	3.07±0.01 ^{bcd}	3.00±0.01 ^{gh}	

Values denoted by different letters differ statistically (P<0.05).

Figure 1.

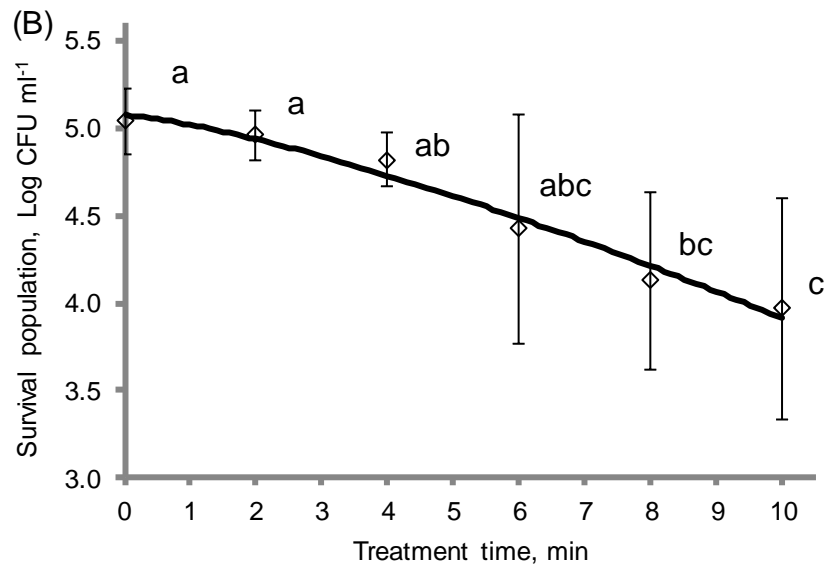
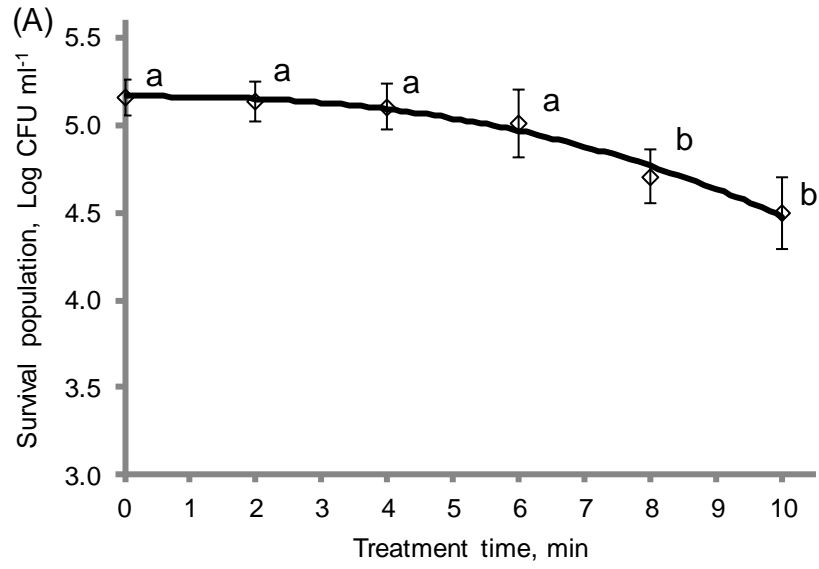
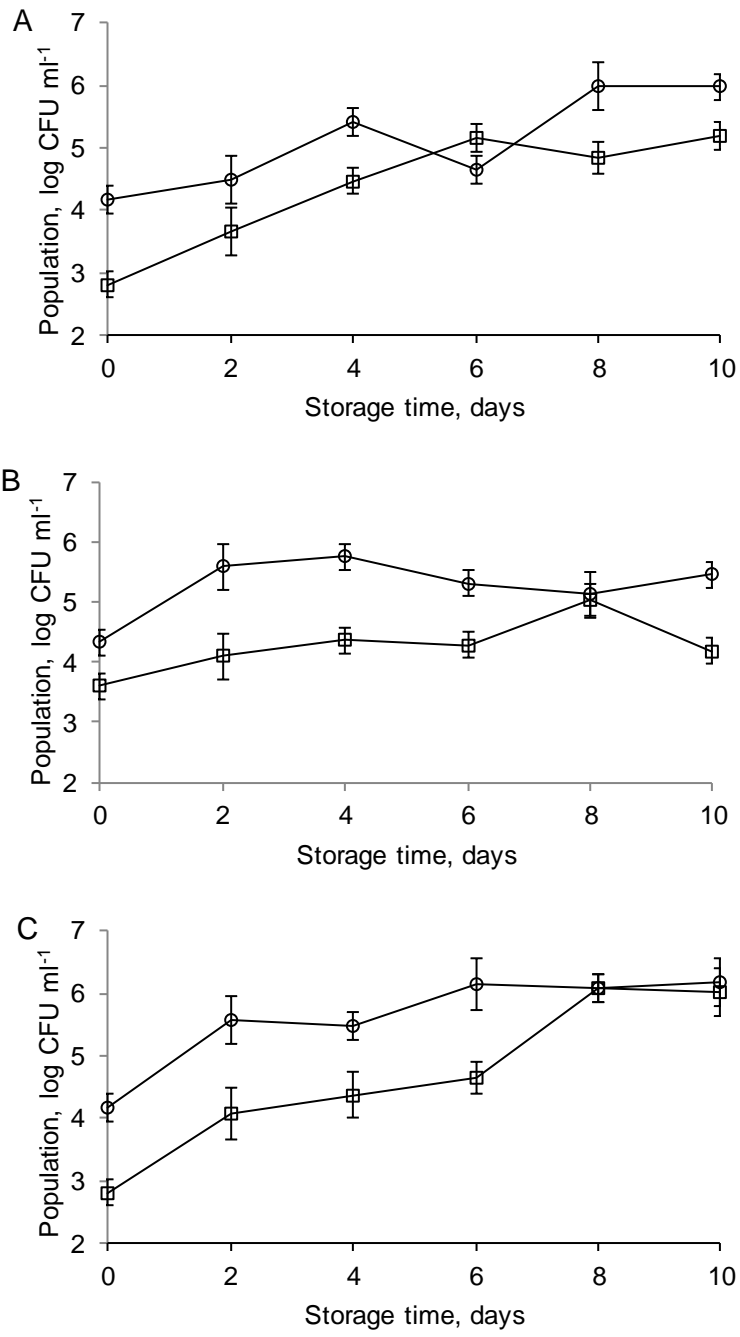


Figure 2

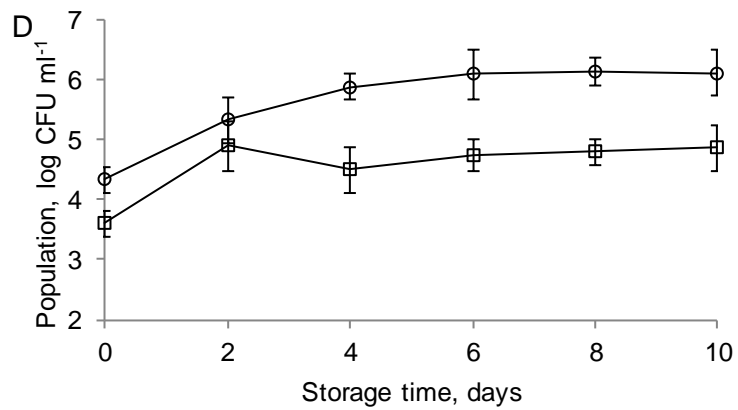
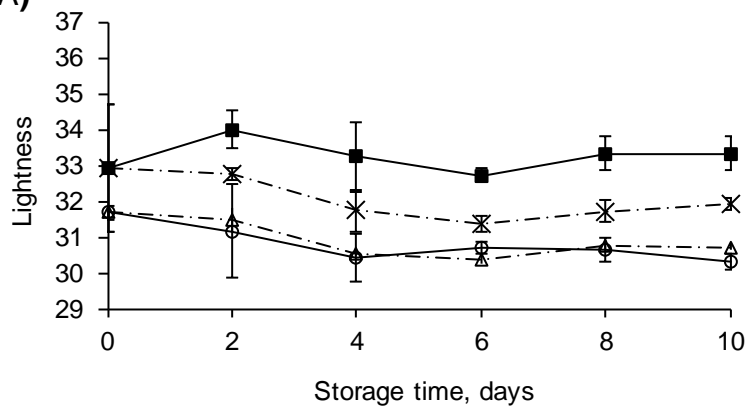
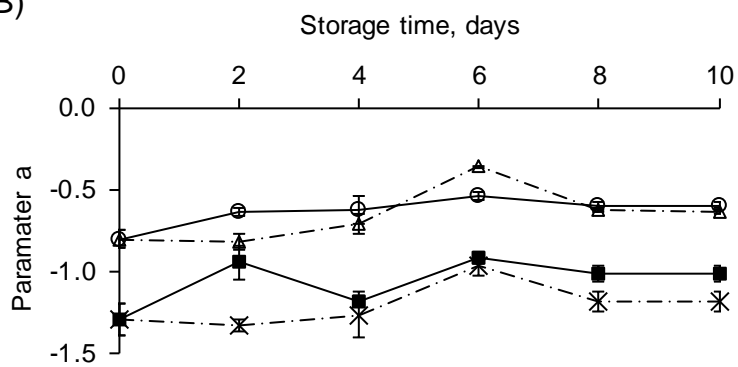


Figure 3

(A)



(B)



(C)

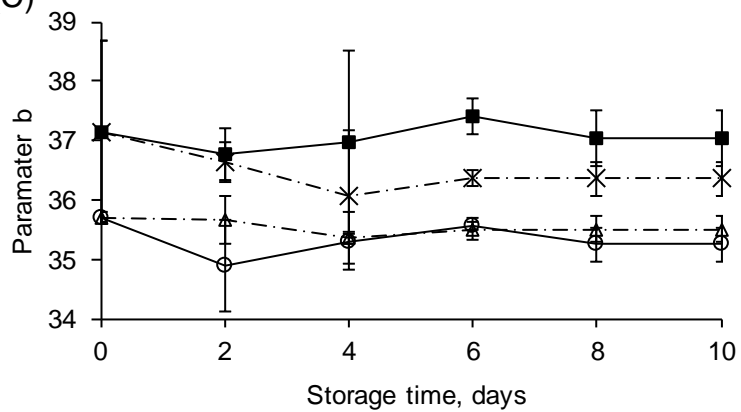


Figure 4

