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Report on the microbiological and oxidative stability of developed pumpkin fruit pulp formulation

DELIVERABLE 5.1

PulpIng

Developing of **Pu**mpkin Pu**lp** Formulation using a Sustainable **In**te**g**rated Strategy

Index

Document Information

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1. Summary

The PulpIng project aims to create a premium pumpkin pulp product, enriched and preserved with value-added compounds derived from pumpkin by-products (discarded parts of fruit and leaves), while advocating for an integrated and sustainable approach. The main focus of WP5 involves preservation studies and quality assessment during shelf-life. This report refers to WP5 deliverable D5.1 Report on the microbiological and oxidative stability of developed pumpkin fruit pulp formulation. In this deliverable, the first pumpkin pulp was evaluated during 34 days of shelf life.

2. Description of work

Due to the COVID-19 pandemic situation, the beginning of this WP didn't started at the scheduled time. However, ATB performed a meeting with DECORGEL and IPB to outline the tasks that will be performed in WP5, since the entire WP5 is dependent of the WP4. In second technical meeting it was agreed that ATB would produce a pumpkin pulp in order to start the experiments and to reduce the delay of the deliverables.

3. Goal

The main objective with this Task, was to evaluate the stability of the pumpkin pulp (100% pumpkin, not added of any preservative or natural ingredient) processed by High pressure compared with thermal treatment and stored at 5 °C (refrigerated temperature) and 20 °C (room temperature). Through the different temperature of storage, we could identify if the treatments applied would improve the shelf life of the pulp, identifying the best condition for the next steps. For this, analysis of microbiology, color, texture, pH and other quality aspects of the pulp was used for the shelf life evaluation.

4. Methodology

Pumpkin pulp fruit production

The **Figure 1** shows the pumpkin pulp produced in ATB and used for the shelf life experiments.

Figure 1. Pumpkin pulp produced in ATB by using variety *Cucurbita maxima*.

Using a blender (Retsch GM 200, Germany) the pumpkin pulp (100% pulp from *Cucurbita maxima* variety) was produced following the steps showed in the **Figure 1**. The pumpkin pulp yield was 62 % as showed in Table 1. After the production, the pulp was stored at -30°C. The fresh pulp was used in the study of non-thermal processing optimization to improve the safety and quality of the pumpkin pulp. For this, the shelf life was analyzed in a comparative way with thermal treatment, high pressure processing and *in natura* sample (used as the control, no process applied). With the process parameter selected, physical-chemical characterization was conducted, in order to evaluate the effect of the process on the microbiology, color, texture, pH and other quality aspects of the pulp.

Table 1. Pumpkin pulp yield.

Pumpkin pulp yield		
	g	%
Total weight Pumpkin	4872,3	100
Seeds	665,5	13,7
Peel + Fibers	1148,4	23,6
Pulp	3058,4	**62,8**
Waste (Seeds and Peel + Fibers)	1813,9	37,2

According to the meeting and talks performed with DECORGEL, was decided to apply a thermal treatment (90 °C / 3 min) normally used in the industry for the pulping pasteurization. The high pressure process (HPP) conditions were also chosen according to what is industrially applied like cold pasteurization (600 MPa/4 min). The thermal treatment was applied by retort and HPP using a U4000 High pressure system (Unipress, Warsaw, Poland). The processed samples were stored at two different temperatures in order to simulate a refrigerate condition (5 °C) and room temperature condition (20 °C). A *in natura* sample, was used a control with no process applied. The shelf life was conducted up to 34 days and 6 points of analysis were made in total.

Microbiology assays

For the microbiology assays, total aerobic mesophilic were analyzed in plate count agar (Roth, Carl Roth GmbH & Co. KG, Germany) incubated at 37 °C for 48 hours. For *Enterobacteriaceae*, was used Endo agar (Roth, Carl Roth GmbH & Co. KG, Germany), added with fuchsine solution (10 % in 95 % ethanol) which is also a selective agar for *Escherichia coli*, incubated at 37 °C for 48 hours. For molds the Maltodextrose agar (Roth, Carl Roth GmbH & Co. KG, Germany) was used and incubated at 30 °C for 72 hours.

Color measurement

The Color measurement was performed using a white light (6000K) reflector from left and right side to guarantee similar light conditions. Prior taking the pictures the samples were spread (10 g) evenly in a petri dish **(Figure 2)**. The photos were taken with a Nikon camera (COOLPIXP7700) from the top (20 cm). A simple image processing routine implemented in GNU Octave (Version 4.2.1) was used to determine the L*, a*, b* parameters of the thermal treated, untreated and high pressure samples. Besides that was also measured the nearest standard color.

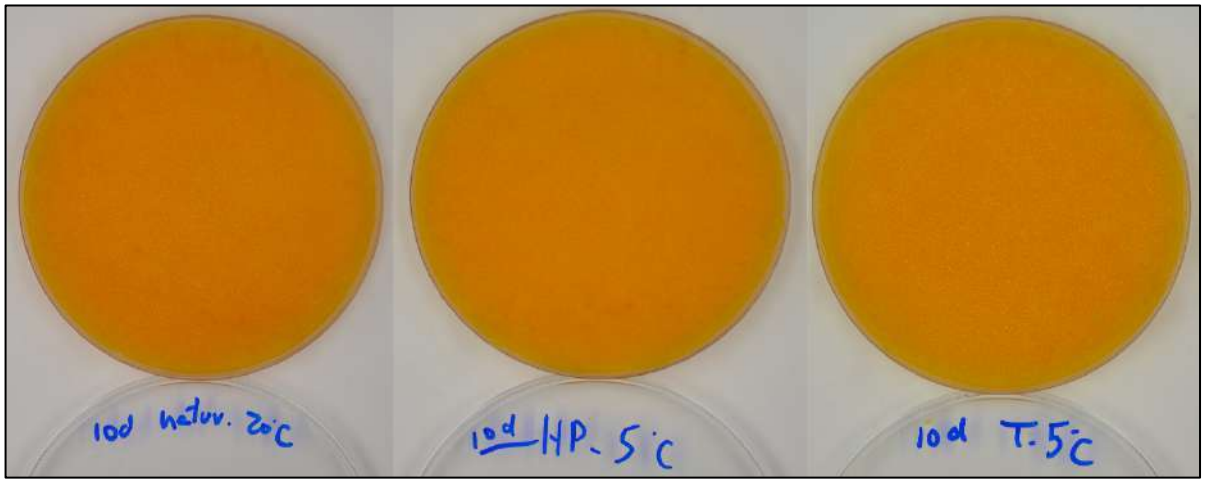


Figure 2. Color measures during shelf life of pumpkin pulp.

The pH was measured by benchtop pH-meter (SI Analytics Lab 850 pH-Meter, SI Analytics GmbH, Mainz, Germany).

Profile Texture Analysis (TPA)

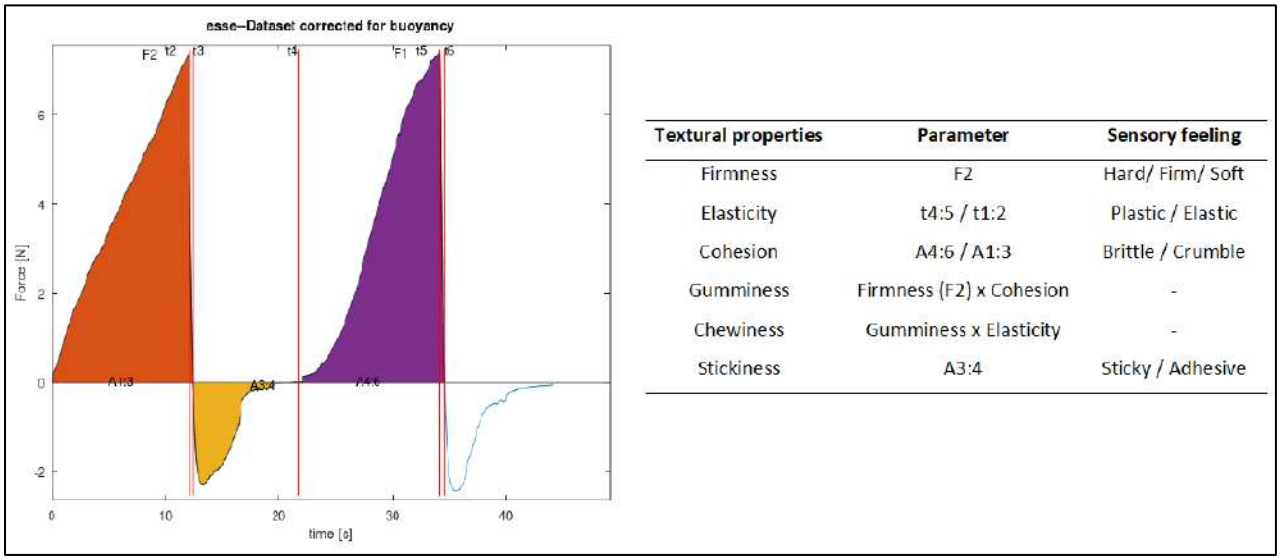
The Profile Texture Analysis of the treated and non treated samples were conducted using a calibrated Texture Analyzer (TA XT Plus, Stable Micro Systems, UK). Texture parameters such as firmness, adhesiveness, cohesiveness, springiness, and gumminess were measured in triplicate. The following instrumental test parameters were used: mode was forced in compression; load cell value was 1 kN; trigger type was 0.1 N; and a cylindrical aluminum probe (25 mm diameter, 40 mm height) was used. An aliquot of each sample (~50 g) was used. The entry depth of the probe was set to 10 mm using a test speed of 50 mm/min and samples were analyzed at room temperature. The force–time curve was further evaluated to determine the texture parameters using an **octave** code that had been programmed according to the interpretation rules for textural profile analysis showed in Table 2 and Figure 21.

Table 2. Measurement conditions for the texture analyzer.

Measurement	Condition
Test Mode	Compression
Speed	50 mm/min
Trigger	0.1 N
Entry depth	10 mm
Beaker	Ø50 mm, Height 105 mm
Sample height	50 mm

Adaptor	Ø25 mm, Height 40 mm
Load cell	1 kN

Textural properties	Parameter	Sensory feeling
Firmness	F2	Hard/ Firm/ Soft
Elasticity	t4:5 / t1:2	Plastic / Elastic
Cohesion	A4:6 / A1:3	Brittle / Crumble
Gumminess	Firmness (F2) x Cohesion	-
Chewiness	Gumminess x Elasticity	-
Stickiness	A3:4	Sticky / Adhesive



Textural properties	Parameter	Sensory feeling
Firmness	F2	Hard/ Firm/ Soft
Elasticity	t4:5 / t1:2	Plastic / Elastic
Cohesion	A4:6 / A1:3	Brittle / Crumble
Gumminess	Firmness (F2) x Cohesion	-
Chewiness	Gumminess x Elasticity	-
Stickiness	A3:4	Sticky / Adhesive

Figure 3. Typical TPA profile (left) and calculation of the textural properties (right) based on the profile.

Oxidative stability

To evaluate oxidative stability of the pumpkin pulp, changes in b-Carotene, Lutein and y-Tocopherol content, phenolic content, and changes in antioxidant capacity were analyzed.

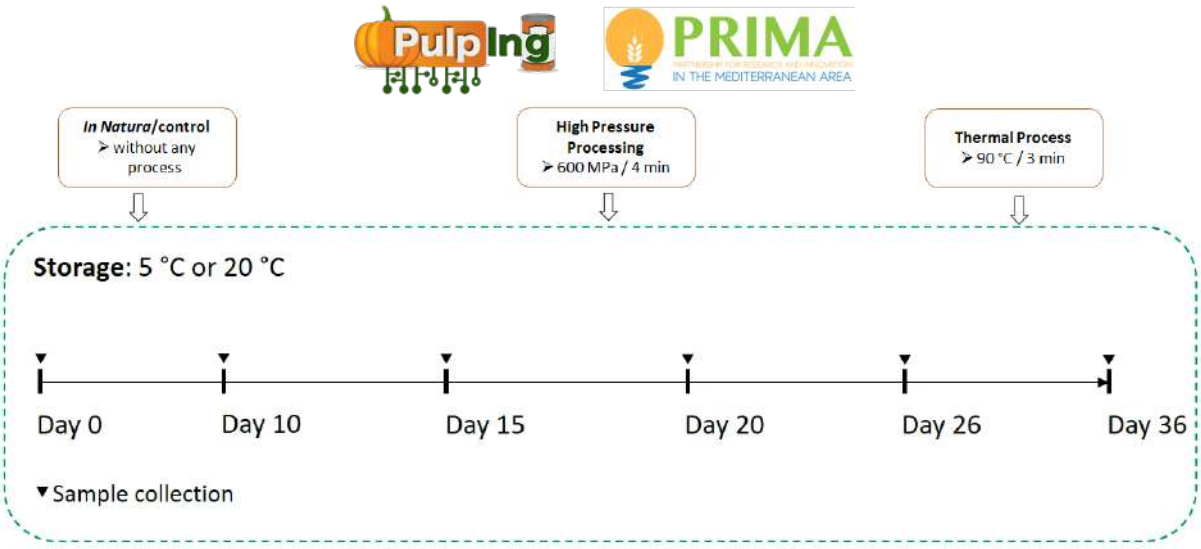


Figure 4. Overview of the design of experiment.

Sample preparation

All samples were freeze-dried prior to mass spectrometric analysis, determination of total phenolic content and the determination of antioxidant capacity. For this purpose, an amount of 1 g of pumpkin pulp was weighed into a 2-mL-reaction tube. The difference between the weight before and after freeze-drying was noted. The results were used to calculate the percentage content of water in pumpkin pulp. Each sample was measured in duplicate.

Extraction of Carotenoids, Tocopherols and Vitamin K1

Extraction of Carotenoids, Tocopherols and Vitamin K1 was performed according to the method of Guzman *et al.* (2012) (dx.doi.org/10.1021/jf302475d) with slight modifications. An amount of 0.01 g of freeze-dried pumpkin pulp was extracted with 500 µL of pure ethanol. The samples were shaken for 20 min in the dark (80 rpm, room temperature (RT)) and then centrifuged (9000 g, 15 min, RT). The supernatant was completely collected and transferred to a new 2-mL-reaction tube. The extraction was performed for another three times (until the pellet was colorless). The solvent was then completely evaporated using a centrifugal evaporator (no heating, no pulse ventilation, approximately 3 hours). Prior to mass spectrometric analysis, the residue was dissolved in 1 mL (for the determination of carotenoids) or in 100 µL isopropanol (for the determination of tocopherols and vitamin K1). To completely dissolve the residue, the extracts were placed in an ultrasonic bath for 5 min. The extracts were stored at -20 °C until further analysis.

HPLC conditions:

Column: Multospher 120-RP18AQ (250 x 4.0 mm x 3 µm)

Injection volume: 5 µL

Gradient:

A = MeOH + 0.1% Formic acid; **B** = Ethyl acetate + 0.1% Formic acid

Table 3. HPLC-MS/MS mobile phase gradient conditions.

Time	A (%)	B (%)	Flow (mL/min)	Max. Pressure Limit (bar)
0.00	96.0	4.0	0.80	500
2.00	96.0	4.0	0.80	500
5.00	20.0	80.0	0.80	500
10.00	20.0	80.0	0.80	500
Postrun			5 min	

Table 4. MS/MS conditions.

Eigenschaft	Bedingungen
Ion source	AJS ESI
Gas	Nitrogen
Gas temperature	200 °C
Gas Flow	11 L/min
Nebulizer	35 psi
Sheath Gas Temp	275 °C
Sheath Gas Flow	11 L/min

Important: Needle must be washed with isopropanol about six times before starting the measurement

Sample preparation for estimation of polyphenols and antioxidant capacity

For the determination of polyphenols and the antioxidant capacity, two different extraction procedure were tested according to Mala *et al.* (2016). Freeze-dried sample (0.01 g) was extracted in either 200 µL methanol or 200 µL methanol/water (80:20, *v/v*). The samples were extracted for 3 hours with agitation (in the dark, 70 rpm, RT) and then centrifuged (10.000 g, 10 min, RT). The supernatant was fully collected and transferred to a new reaction tube. The extracts were stored at - 20 °C until further analysis. Since extraction with methanol/water (80:20, *v/v*) tends to provide higher values for polyphenol content and antioxidant capacity, all results below refer to this extraction method. The analysis was performed in triplicate.

Determination of total phenolic compounds (TPC) with plate reader

The estimation of total phenolic compounds was performed using Folin-Ciocalteu method. First, 20 µL of standard solution or sample extract was mixed with 150 µL distilled water, 10 µL Folin reagent and 20 µL NaOH (1 M). This was followed by incubation of the samples in the dark at RT for 30 min.

The photometric measurement was performed at a wavelength of 765 nm. The calibration curve was performed using gallic acid at different concentrations ranging from 0 – 100 µg/mL. The results were expressed as mg gallic acid equivalent (GAE)/100 g dry matter (dm). The analysis was performed in triplicate.

Ferric ion reducing antioxidant power assay (FRAP) with plate reader

The antioxidant capacity was measured using FRAP method. The determination involves the reduction of Fe^{3+} to Fe^{2+} ions, resulting in a blue colored iron-tripyridyl-triazine complex with an absorption maximum at 593 nm. For the measurement, 10 µL of sample extract or standard solution were mixed with 150 µL of FRAP reagent (TPTZ:$FeCl_3$ (1:1); 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ in 0.25 M acetic acid) . After incubation in the dark at RT for 6 min, photometric measurement of the samples was performed at 595 nm. Ascorbic acid, dissolved in distilled water, was used as standard substance at different concentrations ranging from 0 – 178 µg/mL. The results were expressed as mg ascorbic acid equivalent (AAE)/100 g dry matter (dm). The analysis was performed in triplicate.

Statistical Analysis

For statistical analysis two-way ANOVA was used, followed by Tukey multiple comparisons test (GraphPad Prism 8, GraphPad Software San Diego, CA, USA). The results were statistically different for *p*-values less than 0.05 (95% confidence interval). All data refer to the dry mass and are presented as mean ± standard deviation.

5. Results

The **Figure 5** and **6** shows the microbiological results of the shelf life analyses up to 34 days. As it can be observed in all evaluated times, the role of the temperature of storage showed influence on the results.

At day 10 the control sample storage at 20 °C showed up to 7.5 log of growing for all microorganisms evaluated, on the other hand the same samples storage at 5 °C showed a 2.12, 2.37 and 4.1 log for mesophilic, *Enterobacteria* and molds respectively. For HPP no growing was observed at 5°C, but at 20 °C 3.68, 3.82 and 5.57 log was observed for mesophilic, *Enterobacteria* and molds respectively, showing again that the effect of the storage temperature had an influence on the microbial counts. The same behavior was observed for TP samples. For all samples at 5 °C a slowly growing behavior was observed, in special for the processed samples. For HPP at 5°C the process was able to hold the growing until 26 days for mesophilic bacteria, 20 days for *Enterobacteria* and 34 days for molds. For TP at 5°C right after 10 days was observed a growing (1.52 log) for mesophilic bacteria and

Enterobacteria and after 20 day for molds (4.11 log). It is also important to mention that thermal process (TP) and HPP showed a similar effect in terms of inactivation when stored at 20 °C.

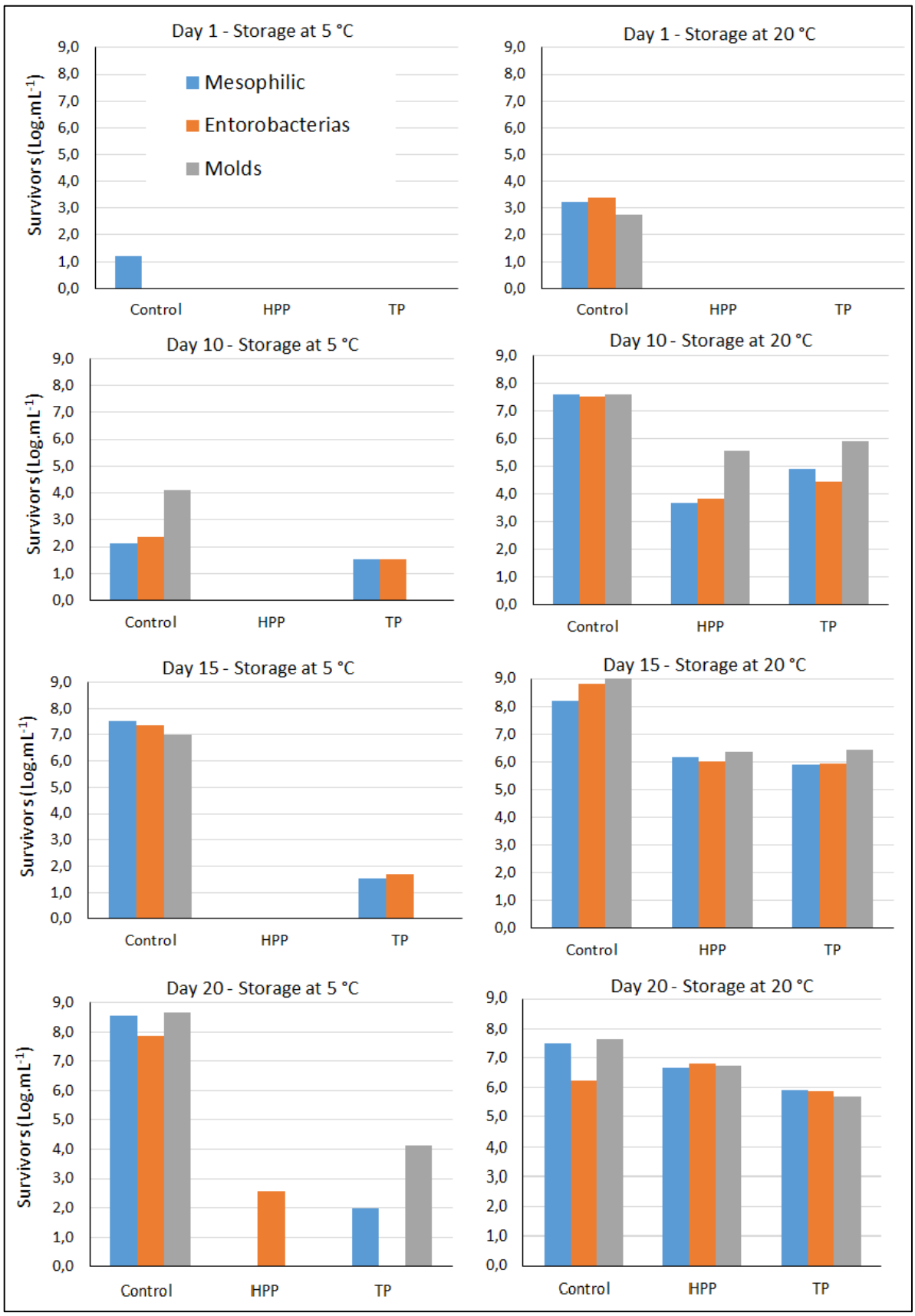


Figure 5. Shelf life of pumpkin pulp at the days 1, 10, 15 and 20 of storage.

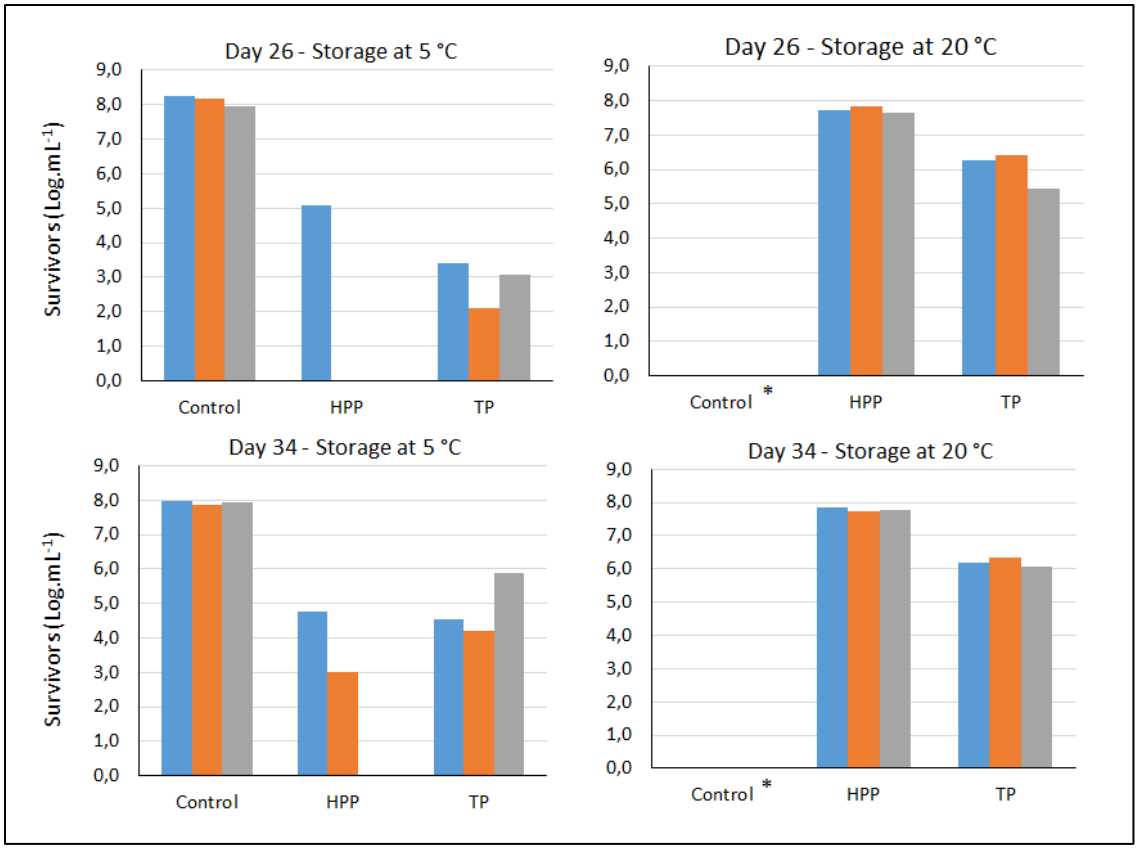


Figure 6. Shelf life of pumpkin pulp at the days 26 and 34 of storage.

* For the control sample stored at 20 °C, the analysis were performed until day 26, once the microorganism growing was intensively elevated (> 8 log). Thus, a comparison with the processed samples (HPTT and TP) would not be fair. The appearance, smell, texture and pH were compatible with a product already in a high stage of decomposition. However, this result was expected, since raw pumpkin pulp is a perishable product with high moisture content and water activity, consequently not suitable to be stored at 20 °C.

The **Figure 7** shows the pH evaluation during the 34 days of shelf life for samples storage at 5 °C and 20 °C.

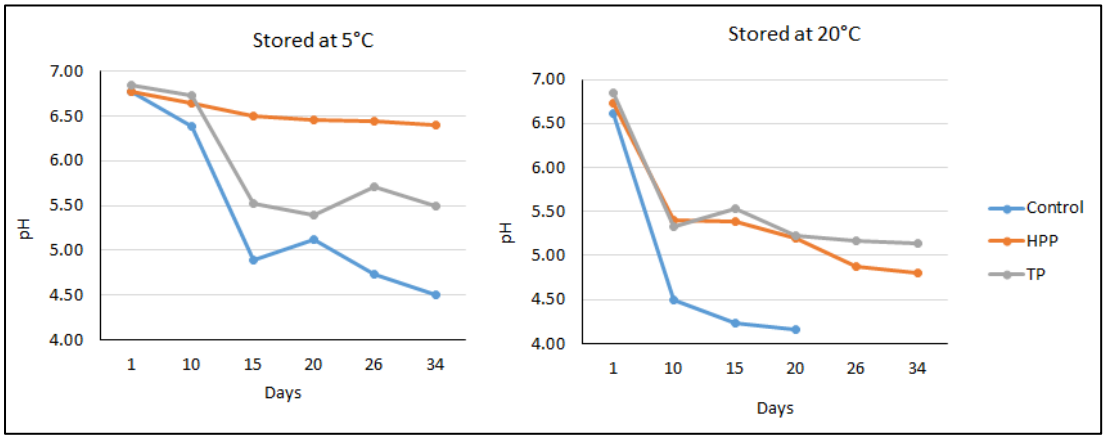


Figure 7. pH evaluation during the pumpkin pulp shelf life.

The pH for all pumpkin pulp samples started the shelf life test with slightly variations ranged between 6.6 to 6.8. In this evaluation, the temperature of storage showed also a considerable impact. Right after 10 days of storage, the control sample showed the biggest pH reduction to 4.5 and continued to decrease until the last day of shelf life evaluated for this sample (pH = 4.16 at day 26). All samples showed the same behavior of reduction during the shelf life, however the HPP sample for both storage conditions (5 °C and 20°C) showed a smaller reduction, especially at 5°C, which showed a highest pH value of 6.44 at day 34. Control sample (at 5°C and 20°C) showed the faster pH reduction, which shows the positive effect of HPP and TP on the pumpkin pulp quality during the shelf life evaluation.

Figure 8 shows the color evaluation during the 34 days of shelf life. In general, it was observed that all samples (control, HPP and TP) showed similar behavior, the biggest difference observed was for the samples stored at 20 °C. On day 1, it was observed that the HPP sample presented the most similar color when compared to the control sample and the TP presented a darker appearance. For the other days, the samples stored at 20 °C were always darker, presenting a lower value of the L* parameter (lightness).

Days		Storage (°C)	L*	a*	b*	**
1	Control	5	63,07	25,58	68,96	
		20	-	-	-	
	HPP	5	63,78	17,13	68,84	
		20	-	-	-	
	TP	5	53,10	26,18	60,50	
		20	-	-	-	
10	Control	5	56,71	28,26	63,82	
		20	55,80	28,00	62,94	
	HPP	5	57,20	25,30	63,90	
		20	61,42	26,05	67,27	
	TP	5	57,80	26,72	64,44	
		20	53,78	24,24	60,83	
15	Control	5	56,31	28,54	63,56	
		20	52,32	30,40	60,29	
	HPP	5	53,63	30,62	61,39	
		20	51,54	30,07	59,51	
	TP	5	53,27	31,58	61,18	
		20	52,29	30,05	60,08	
20	Control	5	58,18	23,58	64,57	
		20	57,90	14,22	63,46	
	HPP	5	55,10	31,33	62,70	
		20	56,35	30,81	63,51	
	TP	5	57,32	26,44	64,14	
		20	55,86	30,77	62,91	
26	Control	5	58,01	24,75	64,41	
		20	-	-	-	-
	HPP	5	58,34	27,84	64,83	
		20	54,49	28,46	60,51	
	TP	5	58,55	29,93	65,10	
		20	55,86	29,13	61,55	
34	Control	5	56,89	26,65	63,86	
		20	-	-	-	-
	HPP	5	54,15	25,54	61,32	
		20	51,63	30,78	59,41	
	TP	5	54,83	25,59	61,92	
		20	54,34	33,63	61,51	

Figure 8. Color evaluation during 34 days of shelf life of pumpkin pulp processed by thermal process and high pressure process.

** Nearest color measured.

In general, the TPA analysis showed improvements for the samples processed by HPP_5°C, as it can be observed on the **Figure 9** for firmness (N). For elasticity, no difference was observed, keeping the same tendency behavior during the shelf life evaluation. For the HPP pulp stored at 5°C the firmness

results showed the same tendency as the in natura samples, on the other hand the pulp processed by thermal treatment showed a reduction on its firmness of 53% compared to the HPP_5°C sample.

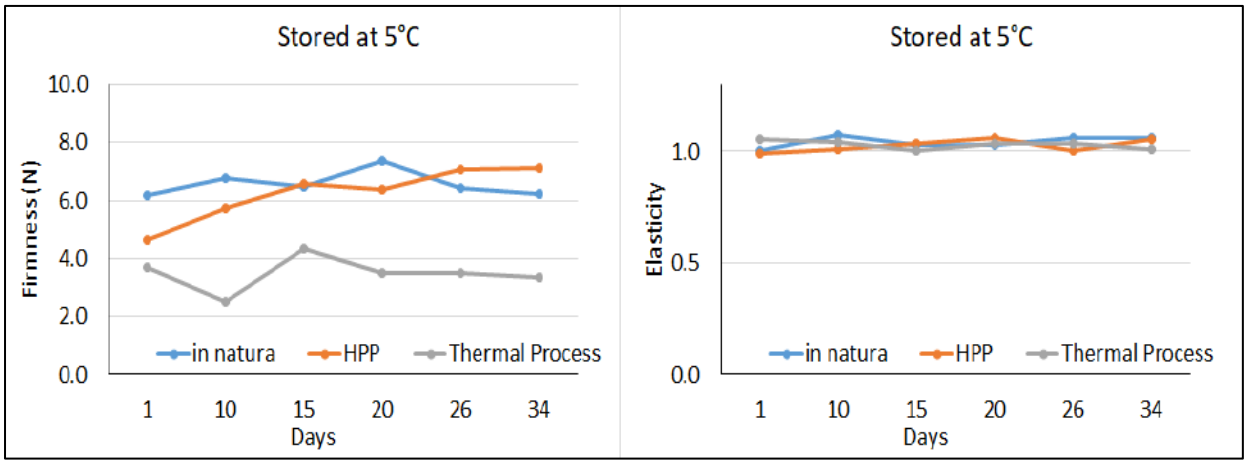


Figure 9. Firmness and elasticity during shelf life of pumpkin pulp fruit processed by HPP and Thermal process.

For all samples at 20 °C, the same trend was observed after 10 days of storage, a phase separation (serum) which made the samples brighter and more fluid characteristic **(Figure 10)**, these conclusions are supported by the texture profile analyses (Firmness).

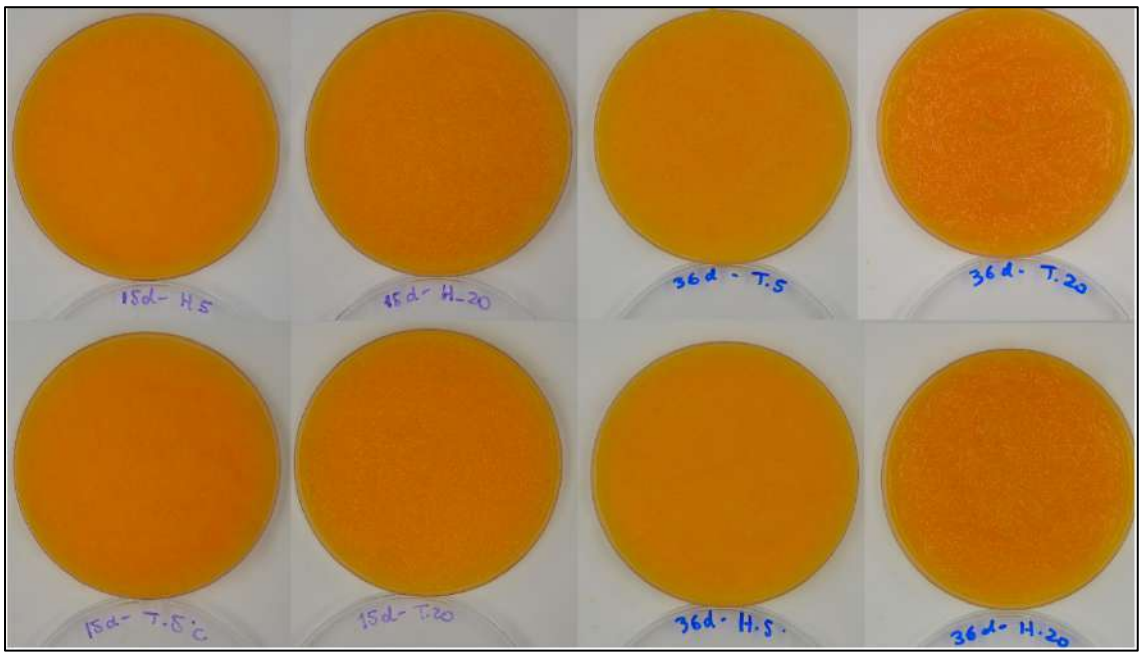


Figure 10. Pumpkin pulp appearance processed by thermal (90°C / 3 min) and high pressure process (600MPa / 4 min) after 15 and 34 days of storage at 5°C and 20°C.

After 15 days of shelf life, it was observed that samples stored at 5°C presented better stability when compared to samples at 20°C. From **Figure 11** it is possible to observe a clearly increase in the production of gas permeated in between the pumpkin pulp. This result is in agreement with the findings

for microbiology at day 15, compared to the samples at 5°C, the TP_20°C samples showed higher microbial growth for all microorganisms evaluated, 4.38, 4.24 and 6.45 log for mesophilic, *Enterobacteria* and molds respectively.

For samples processed by HPP_20°C it is also possible to observe some spots what suggest a gas production, represented by black arrows on the **Figure 12.**

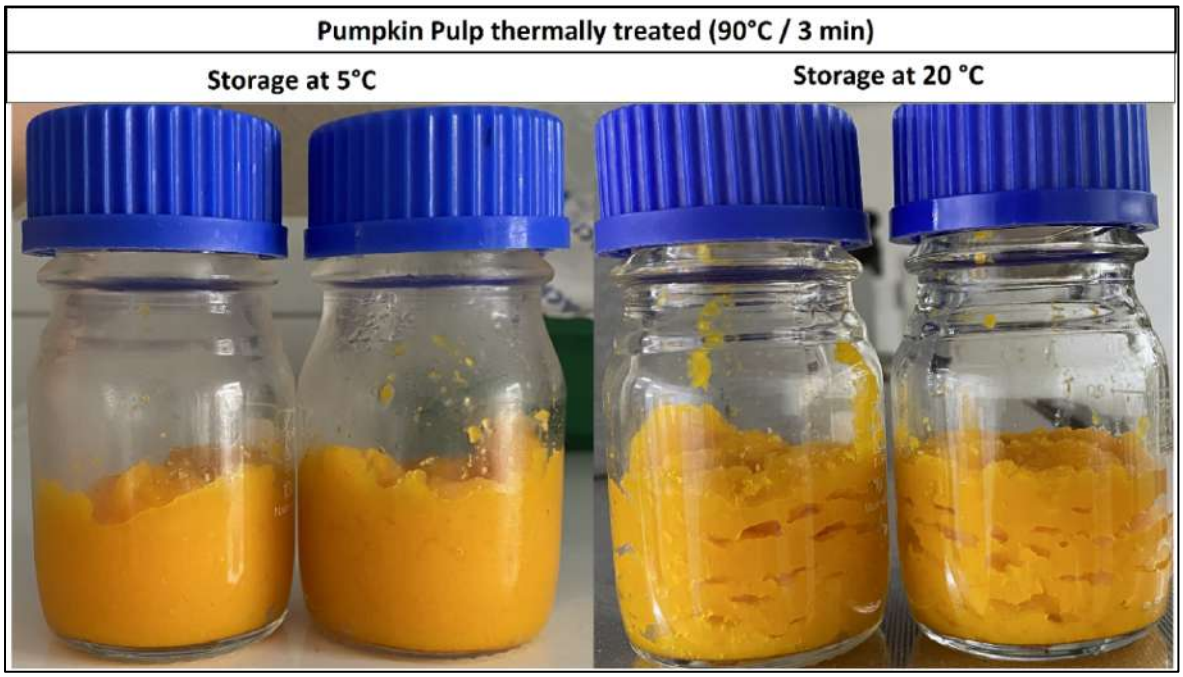


Figure 11. Pumpkin pulp processed by thermal treatment and stored at 5 °C and 20 °C at day 15 of shelf life.

Figure 12. Pumpkin pulp processed by high pressure and stored at 5 °C and 20 °C at day 15 of shelf life.

Oxidative stability:

General characteristics

The average percentage water content was 89 %. The storage of pumpkin pulp for 34 days appeared not to cause loss of water as showed in Table 5.

Table 5. Percentage content of water of pumpkin pulp with different treatments and different storage times. Samples were stored at 5 °C (**A**) or 20 °C (**B**).

A

Treatment	Storage time (d)	Water (%)
In Natura	0	89
	10	87
	15	86
	20	91
	26	93
	36	92
HP	0	86
	10	88
	15	87
	20	87
	26	87
	36	88
TP	0	93
	10	89
	15	88
	20	89
	26	88
	36	89

B

Treatment	Storage time (d)	Water (%)
In Natura	0	89
	10	90
	15	90
	20	89
	26	n.A.
	36	n.A.
HP	0	86
	10	88
	15	88
	20	87
	26	89
	36	89
TP	0	88
	10	89
	15	88
	20	89
	26	91
	36	88

n.A.: not available; HP: High pressure; TP: thermal Process

Carotenoid, Tocopherol, Vitamin K1 and Total Phenolic Content (TPC)

Storage of pumpkin sample at 5 °C resulted in significant reduction of lutein, β-carotene and vitamin K1 in all groups (*In Natura*, Temperature, High Pressure). Only for γ-Tocopherol no significant reduction could be detected after thermal treatment. The content of polyphenols in samples treated thermally or with high pressure remained between 56 mg GAE/100 g dw and 71 mg GAE/100 g dw at the same storage temperature and did not differ significantly from initial conditions. On the other hand, a different effect was seen in the *In Natura* samples. Here, a significant increase in phenolic content over time can be observed. The thermal treatment and also the high pressure treatment seem to have an effect on the vitamin and phenolic content, as the *In Natura* samples show significant higher vitamin and polyphenolic content for the start of experiment (Storage time t = 0) (**Table , A**). Storage of the pumpkin samples at 20 °C also resulted in a significant reduction of vitamin contents. However, similar

to storage at 5 °C, no significant reduction in lutein and γ-Tocopherol content was observed in the thermally treated samples. The phenolic content increased over time for all samples. After a storage period of 36 days, a significant higher phenolic content was detectable in all groups compared to the beginning (t = 0) (**Table , B**).

Carotenoid contents (as a sum of β-Carotene and Lutein) also correlate with antioxidant capacity, with the strongest linear correlation present for the thermally and high pressure treated samples that were stored at 5 °C (Pearsons's r = 0.9290 and r = 0.9968, respectively). Similarly, a positive correlation was found between phenolic content and antioxidant capacity. However, this trend was not observed for the samples treated with high pressure and thermal treatment, which were stored at 20 °C (**Figure** , **Figure**).

Table 6. β-Carotene, Lutein, γ-Tocopherol, Vitamin K1 and total phenolic content (TPC) in pumpkin pulp after different treatments and at different storage times. Samples were stored at 5 °C (**A**) or 20 °C (**B**).

A

Treatment	Storage time (d)	β-Carotene (mg/100 g)	Lutein (mg/100 g)	γ-Tocopherol (µg/100 g)	Vitamin K1 (µg/100 g)	TPC (mg GAE/100 g)
In Natura	0	8.54 ± 0.52^{a}	3.13 ± 0.11^{a}	8.54 ± 1.45^{a}	N.D.	74,25 ± 1.20^{a}
	10	3.90 ± 0.99^{b}	1.06 ± 0.27^{b}	2.29 ± 0.16^{cb}	N.D.	86,05 ± 2.24^{b}
	15	2.15 ± 0.04^{c}	0.47 ± 0.09^{c}	2.19 ± 0.05^{ce}	N.D.	172,33 ± 9.34^{c}
	20	1.54 ± 0.22^{d}	0.36 ± 0.06^{c}	3.21 ± 0.32^{b}	N.D.	161,97 ± 4.98^{d}
	26	5.03 ± 0.37^{e}	1.94 ± 0.63^{e}	4.91 ±0. 22^{d}	N.D.	145,85 ± 2.25^{c}
	36	6.25 ± 0.14^{f}	2.04 ± 0.33^{e}	2.87 ± 0.11^{be}	N.D.	359,12 ± 12.91^{e}
HP	0	6.27 ± 0.07^{a}	3.16 ± 0.43^{a}	5.02 ± 0.33^{a}	N.D.	67,64 ± 0.60^{a}
	10	4.45 ± 0.49^{b}	2.08 ± 0.01^{b}	2.27 ± 0.22^{b}	N.D.	71,70 ± 0.64^{a}
	15	3.55 ± 0.04^{c}	1.26 ± 0.14^{c}	2.26 ± 0.06^{b}	N.D.	68,09 ± 1.64^{a}
	20	2.52 ± 0.15^{d}	0.65 ± 0.05^{d}	2.04 ± 0.09^{b}	N.D.	64,04 ± 1.22^{a}
	26	1.76 ± 0.05^{e}	0.24 ± 0.01^{d}	1.57 ± 0.22^{b}	N.D.	66,61 ± 1.28^{a}
	36	1.50 ± 0.15^{e}	0.24 ± 0.02^{d}	1.35 ± 0.03^{b}	N.D.	64,05 ± 2.89^{a}
Temperature	0	5.49 ± 0.10^{a}	1.96 ± 0.14^{a}	2.79 ± 0.04^{a}	N.D.	65,58 ± 1.88^{ab}
	10	3.23 ± 0.02^{b}	0.82 ± 0.06^{b}	1.73 ± 0.04^{bc}	N.D.	65,75 ± 1.33^{ab}
	15	1.97 ± 0.35^{c}	0.30 ± 0.02^{cb}	1.31 ± 0.00^{c}	N.D.	63,08 ± 1.15^{ab}
	20	3.72 ± 0.06^{bd}	1.54 ± 0.05^{a}	1.37 ± 0.41^{ba}	N.D.	65,67 ± 1.87^{ab}
	26	0.91 ± 0.10^{e}	0.03 ± 0.00^{c}	1.04 ± 0.07^{c}	N.D.	56,69 ± 2.19^{b}
	36	2.34 ± 0.05^{cf}	0.48 ± 0.01^{cb}	1.92 ± 0.29^{abc}	N.D.	67,97 ± 1.05^{a}

B

Treatment	Storage time (d)	β-Carotene (mg/100 g)	Lutein (mg/100 g)	γ-Tocopherol (µg/100 g)	Vitamin K1 (µg/100 g)	TPC (mg GAE/100 g)
In Natura	0	8.44 ± 0.16^{a}	4.20 ± 0.01^{a}	7.94 ± 0.51^{a}	N.D.	88,23 ± 3.33^{a}
	10	3.62 ± 0.30^{b}	1.69 ± 0.54^{b}	3.06 ± 0.79^{b}	N.D.	147,07 ± 2.91^{b}
	15	6.25 ± 0.59^{c}	2.40 ± 0.23^{c}	3.96 ± 0.15^{c}	N.D.	169,74 ± 6.67^{c}
	20	N.D.	N.D.	N.D.	N.D.	101,15 ± 5.98^{d}

	26	n.A.	n.A.	n.A.	N.D.	n.A.
	36	n.A.	n.A.	n.A.	N.D.	n.A.
HP	0	6.80 ± 0.46^{a}	3.75 ± 0.03^{a}	6.46 ± 0.87^{a}	N.D.	$65,97 \pm 3.82^{a}$
	10	7.23 ± 0.59^{a}	3.17 ± 0.22^{ab}	6.19 ± 0.39^{a}	N.D.	$73,92 \pm 0.37^{b}$
	15	7.27 ± 0.92^{a}	3.51 ± 0.18^{a}	4.91 ± 0.32^{b}	N.D.	$77,61 \pm 0.60^{b}$
	20	5.31 ± 0.13^{cb}	2.57 ± 0.38^{bd}	4.38 ± 0.54^{b}	N.D.	$80,76 \pm 2.27^{b}$
	26	4.64 ± 0.61^{b}	2.23 ± 0.05^{c}	4.03 ± 0.03^{b}	N.D.	$97,59 \pm 19.13^{c}$
	36	5.96 ± 0.12^{c}	2.77 ± 0.06^{cd}	4.72 ± 0.36^{b}	N.D.	$104,70 \pm 1.43^{d}$
Temperature	0	5.64 ± 0.21^{a}	2.21 ± 0.25^{a}	2.70 ± 0.23^{a}	N.D.	$66,54 \pm 2.61^{a}$
	10	4.06 ± 0.20^{b}	2.67 ± 0.36^{a}	4.04 ± 0.04^{b}	N.D.	$73,03 \pm 2.66^{a}$
	15	7.73 ± 0.31^{c}	3.90 ± 0.52^{b}	4.11 ± 0.01^{b}	N.D.	$95,14 \pm 0.99^{b}$
	20	7.24 ± 0.20^{c}	3.86 ± 0.52^{b}	6.06 ± 0.31^{c}	N.D.	$100,11 \pm 1.38^{cb}$
	26	7.64 ± 0.36^{c}	3.76 ± 0.40^{b}	4.68 ± 0.86^{b}	N.D.	$103,32 \pm 5.11^{c}$
	36	3.92 ± 0.06^{be}	2.23 ± 0.29^{a}	2.67 ± 0.18^{a}	N.D.	$95,08 \pm 1.73^{bd}$

GAE: Gallic acid equivalent; HP: High Pressure; N.D.: Not detected; N.A.: Not available
Different letters indicate statistically significant differences within a group ($p < 0.05$, Tukey Test)

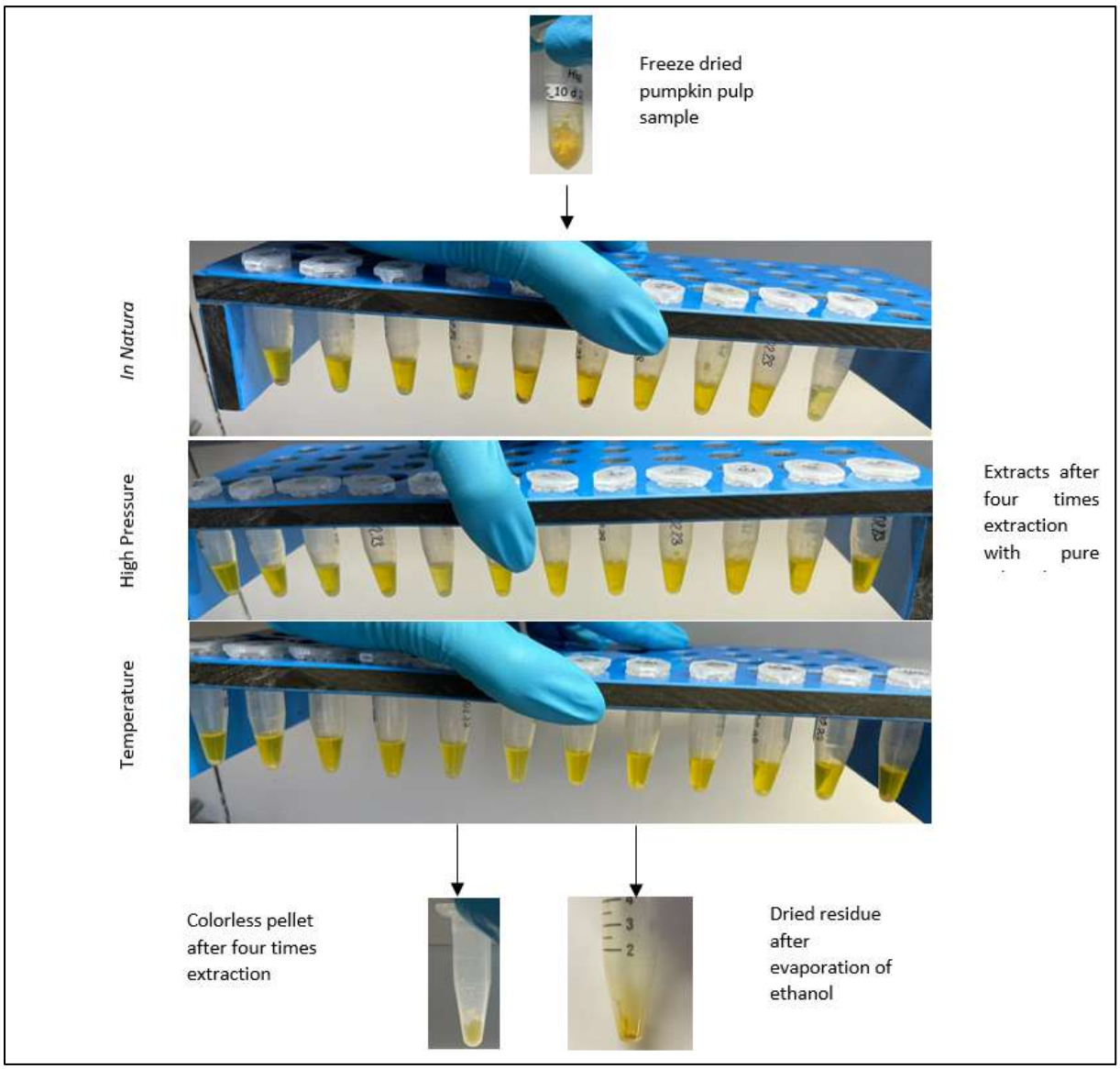


Figure 13. Overview of pumpkin pulp extraction.

Reducing ability (FRAP)

A significant reduction in antioxidative capacity was observed for the high pressure treated samples stored at 5 °C. In addition, the antioxidative capacity for the thermally treated samples decreased, although the difference was not significant. In contrast, a significant increase was observed in the *In Natura* samples. The results for the samples stored at 20 °C were similar. Here, too, the antioxidant capacity of the thermally treated samples tended to decrease, whereas a significant increase was observed in the *In Natura* samples.

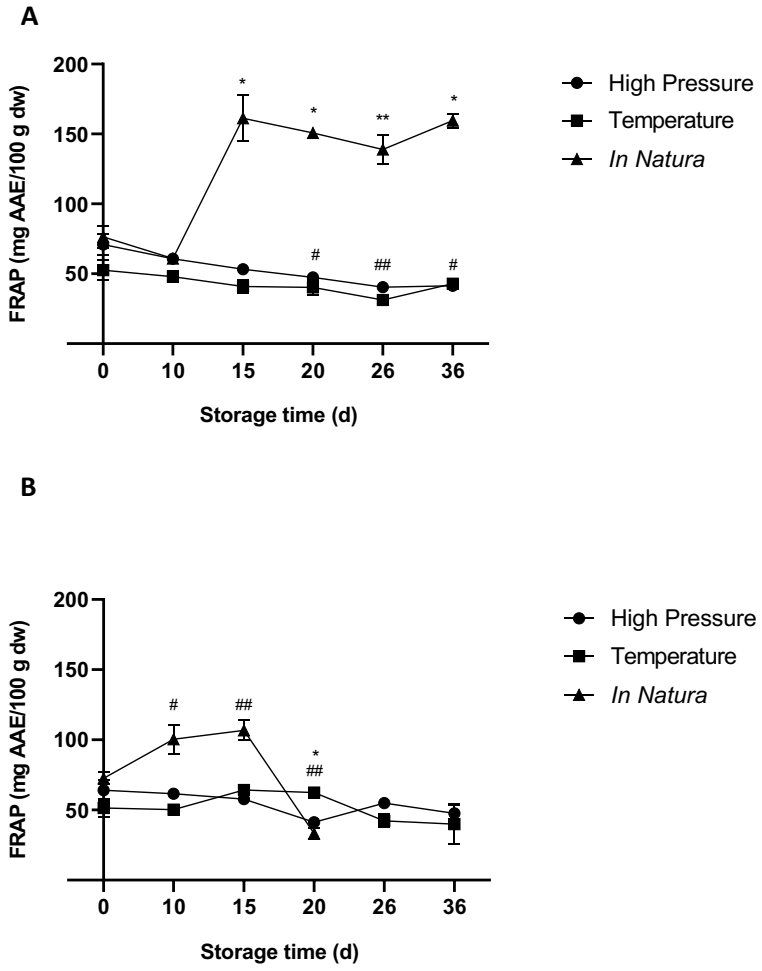


Figure 14. Changes in antioxidant activity of pumpkin pulp at different storage times and different treatments. Samples were stored at 5 °C (**A**) or 20 °C (**B**).

AAE: Ascorbic acid equivalent; dw: dry weight
indicate significant differences within *In Natura* samples ($p < 0.05$ (#), $p < 0.005$ (##)) compared to t=0
* indicate significant differences within High Pressure samples ($p < 0.05$) compared to t=0

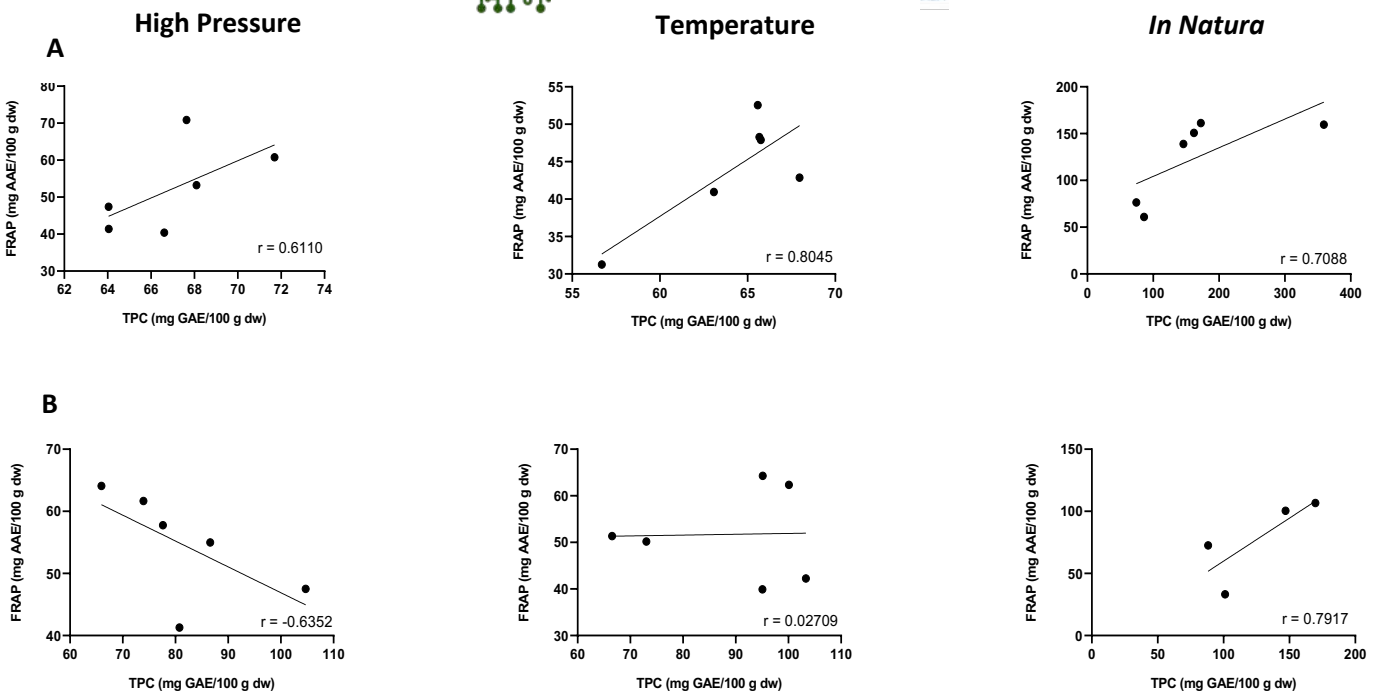


Figure 15. Correlation between Total phenolic compounds (TPC) and antioxidant activity. Samples were stored at 5 °C (**A**) or 20 °C (**B**).

AAE: Ascorbic acid equivalent; GAE: Gallic acid equivalent; dw: dry weight

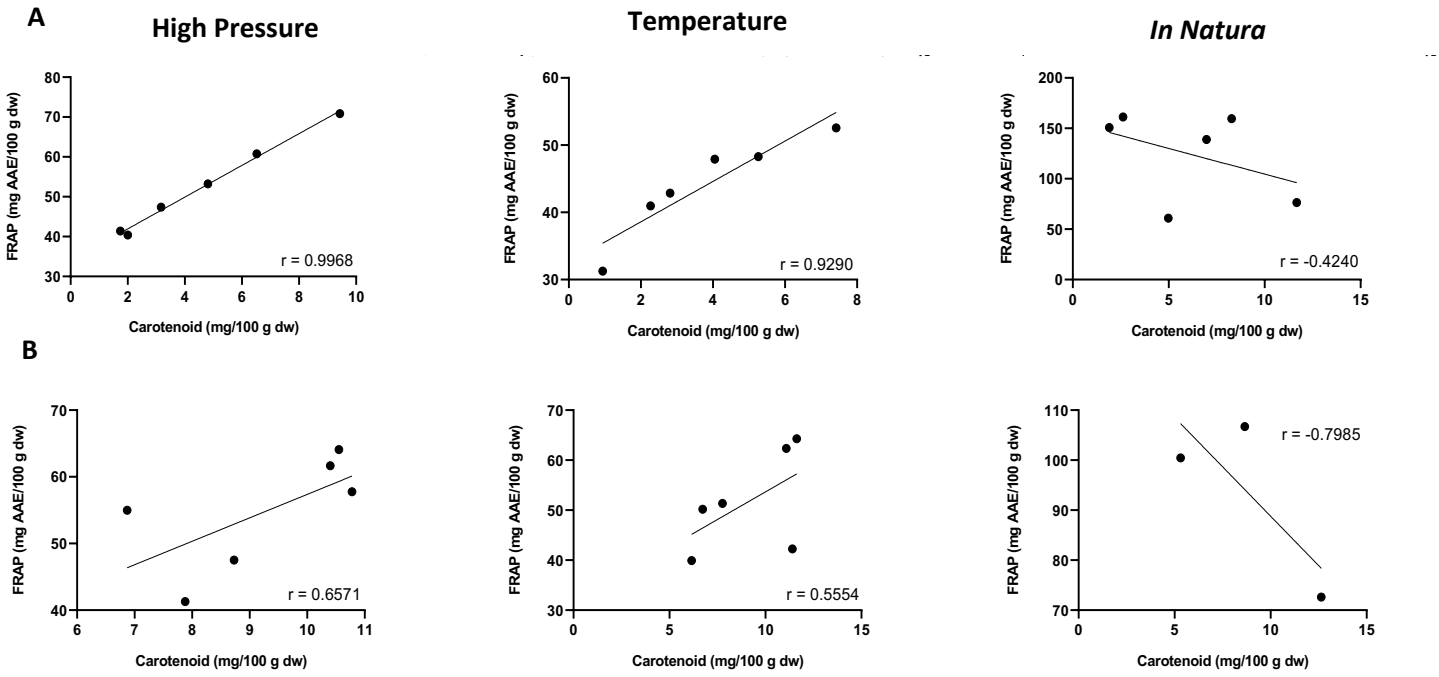


Figure 16. Correlation between Carotenoid content (sum of Lutein and β-Carotene) and antioxidant activity. Samples were stored at 5 °C (**A**) or 20 °C (**B**).

AAE: Ascorbic acid equivalent; dw: dry weight

6. Conclusion

In conclusion, the applied processes showed a positive effect on the quality attributes of pumpkin pulp at the beginning of the shelf life, especially HPP stored at 5°C. However, the pulp stored at 20°C showed poor performance, with an increasing contamination of up to 7.5 log for all microorganisms evaluated, with a considerable drop in pH and other quality attributes losses. Since it is not a sterilized product, the results are justified. However, there is a potential prospect of using the natural pumpkin extract in combination with high pressure as a non-thermal processing to improve the safety and quality of pumpkin fruit pulp stored at refrigeration temperature.