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Comparative study on the effect of mild temperature conditions in fractionated sterilization of carrot juice on microbiological stability and sensory properties

Carrot juice is valued for its high vitamin and antioxidant content, necessitating gentle thermal processing to preserve these nutrients. Its slightly acidic pH value requires a two-step heating process, warranting optimization to enhance product quality and resource efficiency. This study investigated the impact of varying the first heating step between 100 and 130 °C on chemical, sensory, and microbiological parameters. While other chemical parameters remained stable, lactic acid content increased significantly from 55 to 1405 mg/L over downtimes, highlighting the influence of external factors that could not be influenced within the investigations. Lower heating temperatures compromised microbiological stability, with spore-forming bacteria (5 colony forming units per 20 mL) detected at just a 10 °C reduction. Sensory quality showed minimal change, with descriptive analysis identifying only 3 respectively 4 significantly different attributes out of 19 across the factors experimental parameter setting and technical repetition. The quality of raw materials had a more pronounced impact on sensory outcomes than the heating temperature. This study concludes that adjusting the first heating temperature has limited benefits for sensory quality but risks microbiological safety. Emphasis should therefore be placed on ensuring high-quality raw materials and consistent raw juice properties to maintain product quality.

Descriptors: fractionated sterilization, carrot juice, descriptive analysis, microbiological stability

1 Introduction

Carrot juice belongs to the product group of vegetable juices and is consumed pure on the one hand, but for the majority of consumers it is probably known and consumed as a component of multivitamin and ACE juices, for example [1]. It is also used in concentrated and processed form as a food ingredient, e.g. as a coloring food or carotenoid additive. Carrots are known for their high vitamin content and antioxidants, including nutritional antioxidants such as vitamin A, C, and E, and non-nutritional antioxidants, like b-carotene and polyphenols [2, 3]. Carrots and carrot products have thus a healthy image, particularly due to its high carotenoid content and mild taste. It is precisely this mild taste, which is caused by a pH value greater than 4.5, that makes it more susceptible to microbiological spoilage by spore formers than fruit juices. In order to produce microbiologically stable carrot juices, the juices are heated two times to inactivate enzymes, vegetative microorganisms and spores. Thermoresistant bacterial endospores place the highest demands on the inactivation process. One method of preservation for this type of product is tyndallization. This involves fractional heating or discontinuous sterilization at temperatures of between 80 and 100 °C with spore rests between the heating steps [4]. The classic tyndallization process consists of three steam sterilization steps with an one-day-incubation period in between [5, 6]. After an initial temperature treatment, the spores are given the conditions to germinate and can then be killed as vegetative cells during the next temperature treatment. In modifications of this, processes used in biotechnological and pharmaceutical processes and as well as for food preservations use milder temperatures to avoid technically complex and product-damaging temperatures of more than 121 °C [7, 8]. As spore-forming bacteria can contain pathogens and spoilage organisms, it has become established practice for low-acid drinks such as carrot juice to be heated in two stages at temperatures above 121 °C with a spore rest period at room temperature in between. This process has evolved over time and has proven its worth. However, as with all nutritive ingredients, there is a risk of loss due to excessive heat treatment [2, 9]. In addition to vitamin degradation and sensory losses, carrot juices have a greater potential to form benzene when heated to over 100 °C due to ingredients such as β-carotene, amino acids or certain flavors. Among other things, benzene has a carcinogenic effect and is therefore harmful to health. Although there are no limit values for benzene in carrot juices, there are guideline values that are based on the limit values for drinking water of 1 µg/L according to the Drinking Water Ordinance. This means that there is also an

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incentive to reduce the thermal load of carrot juices from a health perspective [10, 11].

As with other product groups, many findings have been made in recent years that clearly demonstrate the advantages of product-specific adaptation of the heating parameters [12–17]. For fruit juices and beers, for example, it was found that the conventional formulas for calculating the necessary lethal heat input are very unspecific and include high safety margins, which lead to the quality losses described above [18]. Knowledge of the existing microbiome of a product enables a more specific treatment by determining the D and z values of the most critical bacteria and using them to parameterize the process [19]. The D and z values are measures of the inactivation rate and the temperature dependence of this process. There are already databases with corresponding results for various juices and beers, all of which have different factors influencing the growth of microorganisms, such as the pH value, sugar and alcohol content as well as other protective or inhibiting factors [20]. However, this data is not yet available for many products, including carrot juice, so further research is required. This is preceded by an examination of the microbiome of a product.

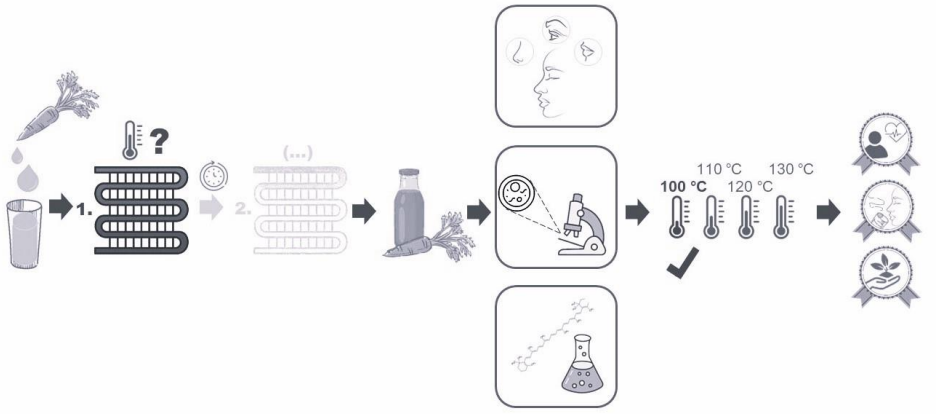

Fig. 1 **Graphical visualization of the content of the research study**

In contrast to beers and fruit juices, vegetable juices have milder pH values (pH > 4.5) and therefore need to be heat treated more intensely, as spore formers in particular pose a greater risk. The F value is used here to describe the heating effect, for which D and z values are also decisive. Clostridium botulinum with a z value of 10 °C is used as the lead germ because it is one of the most dangerous pathogenic microorganisms for human health and one of the most heat-resistant. A F-value of 1 corresponds to 1 min at 121.1 °C [21].

In order to make the process of preserving carrot juice less product- and resource-intensive, a temperature must be found for the first heating stage that results in a safe product and at the same time leads to a nutritious and sensory high-quality product, i.e. minimum thermal stress while ensuring microbiological safety. Moreover, it is necessary to examine the microbiome for potentially harmful germs.

This challenge gives rise to the specific objectives of this reseach work. Firstly, an evaluation of the energetic optimization of the production process of carrot juice is to be carried out, taking into account the first heating stage of the process. These tests are accompanied by microbiological, chemical and human sensory analyses in order to identify correlations and provide a basis for evaluating the pasteurization program. Figure 1 provides a graphical summary of the content of this study.

Table 1 **Overview of experimental set-up of the heating parameters for carrot juice pasteurization with z = 10 °C**

Heating Temperature [°C]	Heat holding time [s]	F value [min]
100	90	0.01
110	90	0.12
120	90	1.19
130	45	5

2 Materials and Methods

2.1 Experimental Samples

For the heating experiments, carrot juice was used which was bottled and delivered at 70 °C directly after pressing by the company riha WeserGold Getränke GmbH & Co. KG. After a delivery time of less than one hour, the carrot juice had a temperature of 60 °C at the time of delivery. Batches of three different production days of fresh carrot juice were delivered to perform a measurement repetition in the form of a technical repetition of three different batches of three test days.

2.2 Pasteurization

The pasteurization experiments were carried out using a HT220 flash pasteurizer from the manufacturer OMVE BV (De Meern, Netherlands). The volume of the heat holding section was 0.42 liters. The temperature levels were 100, 110, 120 and 130 °C. The parameter settings used for each test temperature of the first heating stage are shown in table 1.

After the first heating stage, the samples were filled aseptically in bag-in-box bags, cooled down to 20 °C in a water bath and stored for 24 h at room temperature for a spore rest. After the spore rest, the samples were exposed to a second heating stage at 130 °C with an F value of 5 and aseptically filled again. The experimental procedure is shown in figure 2.

2.3 Chemical Analyses

During pasteurization, samples were taken in sterile containers for chemical analyses before, after the first and after the second heating stage.

Various analyses were carried out before heating, after the first

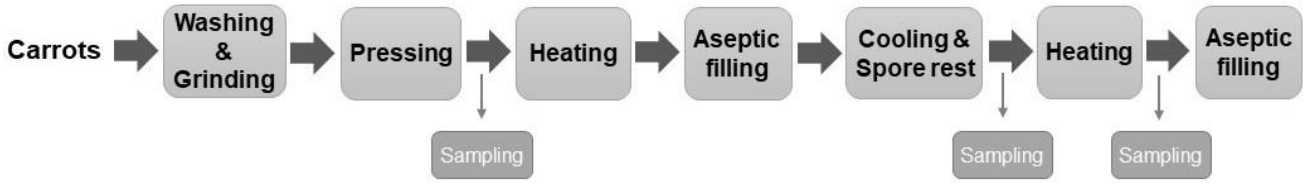

Fig. 2 Scheme of carrot juice production and experimental design with points of sample taking

heating and after final heating. These include the Brix value, pH value, nitrite content, total carotenoid content and color, measured in the CIELAB color space. The methods of the chemical and microbiological tests were validated in round robin tests. A technical repetition was carried out on three different measurement days.

2.4 Total carotenoid content

The total carotenoid content was determined by photometry using a Cadas 100 spectrophotometer (Dr. Lange). For this purpose, the carrot juice was first diluted with water so that the absorbance difference was at least 0.2 and the maximum absorbance did not exceed 1.2 units. A pre-dilution of 1:5 was used for carrot juice. After pre-dilution, 1 mL of the pre-diluted carrot juice was pipetted into a centrifuge tube with 8 mL of water to prepare the actual sample. This was mixed with 0.2 mL each of Carrez reagent I and Carrez reagent II. To sediment the precipitate, it was centrifuged for 5 min at approx. 4,000 rpm and the supernatant was decanted. The sediment was then dissolved with 1 mL tetrahydrofuran and shaken thoroughly. After 2 min extraction time, 3.5 mL acetone was added and shaken again. The volume was then adjusted to 10 mL with acetone and membrane filtered into a glass cuvette. During the photometric measurement of the total carotene content, the absorbance difference was measured against acetone at 450 nm. The measurement was evaluated according to the following formula:

$$\text{Total carotenoids } [\frac{mg}{L}] = \frac{\Delta E \cdot 4 \cdot V_{total} \cdot f}{V_{sample}} \quad \text{(Eq. 1)}$$

with:

$\Delta E = E_{sample} - E_{blank\ value}$

4 = Average extinction coefficient of β-carotene (empiri-cally determined within the scope of a round robin test, see IFU No. 59)

V_{total} = Total volume after sample preparation in glass cuvette (= 10 mL)

f = Dilution factor of the pre-dilution (= 200)

V_{sample} = Sample volume of pre-diluted carrot juice before actual sample preparation [mL]

2.5 Brix value and pH value

The Brix value was measured with a refractometer type J157 Automatic Refractometer (Rudolph Research Analytical, Hackettstown, NJ) by placing a small amount of sample in the measuring chamber. Brix-value was read at a reference temperature of 20 °C. The determination of the pH value was carried out potentiometrically at 20 °C with a pH meter Protos 3400S (Knick Elektronische Messgeräte GmbH & Co. KG).

2.6 Nitrite content

The nitrite content is determined photometrically using a Gallery (Thermo Fisher Scientific) at 520 nm. To prepare the sample, the carrot juice was first decolored so that the sample color would not interfere with the measurement at 520 nm. For this purpose, 3 mL of carrot juice sample was mixed with 2 spatulas of polyvinylpolypyrrolidone (PVPP). After 5 min of reaction time, the mixture was transferred to a centrifuge tube and the PVPP was centrifuged off. The decolorized sample was then transferred to a test tube of the Gallery. For the photometric measurement, the analyzer draws up 100 µL of water and pipettes 90 µL of it into the reaction cuvette. Then 110 µL of sample is drawn up and 100 µL of this is pipetted into the cuvette. After 90 s of incubation, a blank is measured, then 40 µL of Nitric Oxide (NO) color reagent and 10 µL of additional water are pipetted into the cuvette. After a further 180 s, the absorbance is measured at 520 nm against a blank with water.

2.7 CIELAB color space

The color of the sample was measured using the ColorFlex EZ (HunterLab) with a ring and disk set, which is a stamp and spacer ring for the accurate measurement of translucent samples, with a D65 illuminant and 45° illumination and 0° observation. Prior to measurement, calibration was performed with black and white standards. The sample was prepared by diluting it with water in a 1:10 ratio, reducing it from 8 to 0.8 °Brix. Next, 20 mL of the diluted sample was placed in a glass cuvette, and the black ring (spacer) was attached. Measurements were taken against the white stamp as background. Each measurement was repeated three times, with the final result given as an average.

2.8 Total lactic acid content

The total lactic acid was determined using an Enzytec™ Liquid # E8240 enzyme kit and a Diskreter Gallery™ Analysator (Thermo Fisher Scientific), which performs an automated determination. Before dosing reagent 1, the needle is washed with perchloric acid (0.7 %). The analyzer then pipettes 60 µL of GLAC R1 reagent into the cuvette for analysis. Subsequently, 5 µL of sample is dispensed. Now pipette 30 µL GLAC R2 into the cuvette. After 600 s of incubation, the absorbance is measured at 340 nm.

2.9 Microbiological Analyses

During pasteurization, samples were taken in sterile containers for microbiological analyses before, after the first and after the second

Table 2 Overview of the attributes determined by the panel in the descriptive analysis of carrot juice and the used standards

Attribute category	Attribute	Reference standard	Scale
Optic	homogeneity	sample with the most and the least homogeneity	homogeneous – inhomogeneous
	particles	sample with the most and the least particles	none – very many
	turbidity	sample with the most and the least turbidity	clear – very cloudy
	sediment	sample with the most and the least sediment	none – very strong
Taste	sweet	aqueous solution with sucrose	not at all – very strong
	sour	aqueous solution with citric acid	not at all – very strong
	salty	aqueous solution with sodium chloride	not at all – very strong
	bitter	aqueous solution with caffeine	not at all – very strong
	umami	aqueous solution with monosodium glutamate monohydrate	not at all – very strong
Mouthfeel	adstringent	aqueous solution with tannic acid	not at all – very strong
	chalky	water starch solution	not at all – very strong
	coating	milk (full fat)	not at all – very strong
	creamy	cream	watery – creamy
Retronasal odor	red vegetables	tomato puree	not at all – very strong
	white vegetables	parsnip puree	not at all – very strong
	carrot like	grated carrots (Fresh), carrot puree (boiled)	not at all – very strong
	fruity	multivitamin juice	not at all – very strong
	earthy	red beet juice	not at all – very strong
	porridge like	porridge	not at all – very strong

heating stage. The total bacterial cell count was determined before pasteurization, after the first heating stage and after the final heating stage, each in duplicate. Furthermore, the spore forming bacteria were sequenced using the samples from the first heating stage in order to achieve a specific identification of the microbiome. The total bacterial count was carried out aerobically and anaerobically at an incubation temperature of 30 °C by means of cultivation on orange serum agar for 72 h, both for the total bacterial count and for the spore formers. Germ identification was carried out as a contract service by a certified testing laboratory using MALDI-TOF mass spectroscopy with prior cultivation on blood agar.

2.10 Sensory evaluation of carrot juices

2.10.1 Descriptive Analysis

The sensory analysis of the carrot juice samples was carried out by means of a descriptive analysis. The panel consisted of 12 participants, student workers and employees of the University of Applied Sciences Ostwestfalen-Lippe, who were between 21 and 59 years old. Four training sessions were completed, three of these were used to develop the attribute list and establish reference standards for the attributes. Due to the timing of the carrot juice production and the shelf life of these samples, the training for the attribute definition and product familiarization was done with seven commercial carrot juices. In order to cover a various range, commercial products from different manufacturers were used as well as carrot juice from riha Wesergold. The latter was served as purchased and also thermally treated twice in order to achieve a sensory variance. In total, there were 9 different carrot juices for the training. The fourth training session was used to train the intensity evaluation on scales.

All attributes were rated on unstructured line scales that were later consisting of 100 units. The ticks at both ends of the scales were labled with "not at all" to "very strong" in most cases. Table 2 provides an overview of the defined attributes resulting from the moderated panel discussion and the reference standards used.

The actual evaluation sessions took place in individual booth according to the DIN EN ISO 8589:2014-10 [22], all equipped with tablets for data collection, water for neutralization. The samples were evaluated in three tasting sessions and presented in randomized order in triplicates following a Williams Latin square design. Hence, there were twelve samples per session, which were coded with random three-digit numbers. The samples were served at room temperature in transparent 50 mL plastic cups. After each sample, the panelists were instructed to neutralize their palate with water and a one-minute break was taken for the next sample. RedJade Software (RedJade Sensory Solutions LLC, Pleasant Hill, CA, USA) was used for data collection.

2.10.2 Ethics approval

The sensory analysis was conducted during the panelists' work time. No ethics committee was required for this sensory study.

2.10.3 Data analysis

Sensory data were evaluated, applying a multifactorial Analysis of Variance (ANOVA) with all two-way interactions to uncover main and interaction effects of participant, repetition, heating temperature and technical repetition on each sensory attribute with the factor

Table 3 **Contents of nitrite and total carotenoids in correlation to the different heating temperature measured in a threefold; after the first (Vxxx) and after the second heating treatment (Vxxx I xxx)**

		Nitrite content [mg/L]			Total Carotenoids [mg/L]		
	Trial number	**1**	**2**	**3**	**1**	**2**	**3**
Parameter settings	Zero sample	0.0	0.0	0.2	135	139	170
	V100	0.1	0.0	1.2	133	140	168
	V110	0.4	1.0	0.0	136	142	150
	V120	2.9	3.1	0.0	131	136	159
	V130	7.5	4.6	0.0	126	131	160
	V100 I 130	0.0	0.0	0.8	131	143	170
	V110 I 130	0.5	2.1	0.1	135	139	155
	V120 I 130	3.2	4.3	0.0	135	136	160
	V130 I 130	7.6	4.1	0.0	133	133	164

Table 4 **Results of the Brix and pH measurement in a threefold for the several parameter settings of heating treatment; after the first (Vxxx) and after the second heating treatment (Vxxx I xxx)**

		Brix value [°Bx]			pH value [-]		
	Trial number	**1**	**2**	**3**	**1**	**2**	**3**
Parameter settings	Zero sample	8.4	8.6	8.3	5.8	5.8	5.9
	V100	8.4	8.6	8.4	5.8	5.7	5.6
	V110	8.4	8.3	8.3	5.9	5.9	5.6
	V120	8.3	8.0	8.2	5.9	5.8	5.3
	V130	8.3	8.2	8.2	5.8	5.8	4.9
	V100 I 130	8.3	8.8	8.4	5.7	5.7	5.7
	V110 I 130	8.4	8.8	8.3	5.8	5.8	5.6
	V120 I 130	8.4	8.8	8.3	5.8	5.8	5.3
	V130 I 130	8.3	8.6	8.2	5.8	5.8	4.9

Table 5 **Results of the Lab color measurement in a threefold for the several parameter settings of heating treatment; after the first (Vxxx) and after the second heating treatment (Vxxx I xxx)**

		L* value			a* value			b* value		
	Trial number	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Parameter settings	Zero sample	41.8	40.9	42.6	36.12	37.14	37.1	24.92	24.85	25.63
	V100	41.27	41.8	42.65	35.33	36.44	37.86	24.39	25.54	25.75
	V110	40.98	40.7	42.19	35.92	35.28	38.26	24.44	24.56	26.06
	V120	40.69	41.96	42.94	35.54	35.95	37.53	24.44	25.21	26.09
	V130	40.96	40.9	43.21	35.00	34.99	37.45	24.6	24.77	26.47
	V100 I 130	42.15	42.33	42.09	35.16	35.57	37.44	25.29	25.5	25.78
	V110 I 130	40.89	41.64	41.99	35.11	34.97	36.88	24.36	25.13	25.78
	V120 I 130	41.00	41.13	42.10	35.20	34.85	36.59	24.40	25.08	25.95
	V130 I 130	41.03	41.53	42.4	34.78	34.63	36.75	24.60	25.32	26.42

Table 6 **Additional measurement of the absorbance to determine the lactic acid content for the third technical replication; after the first (Vxxx) and after the second heating treatment (Vxxx I xxx)**

		Extinction 420 nm (Serum) [mE]	Total lactic acid [mg/L]
Parameter settings	Zero sample	1072	55
	V100	809	243
	V110	719	292
	V120	608	659
	V130	707	1343
	V100 I 130	900	246
	V110 I 130	833	248
	V120 I 130	734	730
	V130 I 130	690	1405

judges as a random factor. If the effect was significant at α = 0.05 Fisher´s Least Significant Difference (LSD) test was used to identify significantly different means. A principal component analysis (PCA) was calculated based on the covariance table of the mean values for all sensory attributes. All statistical evaluations were carried out using XLSTAT (Version 2024.2.1, Addinsoft, Paris, France).

3 Results

3.1 Chemical Analysis

The nitrite content increases in the first two of the three test runs depending on the heating temperature. In comparison between the second and first heating stage, there is no change in the content. The results are shown in table 3. In contrast, the carotenoid content does not change as a function of the heating temperature.

The Brix value does also not change depending on the heating temperature or the heating stage, nor does the pH value. Only the pH value of the third technical repetition shows a decrease with increasing heating temperature and thus with the length of the standing time until processing. Both results are shown in table 4. As can be seen in table 5, the color also showed no change.

The lactic acid content was measured in order to investigate the influence of the standing times from the production of the juice to the thermal preservation. The results for the third technical repetition are shown in table 6 and show a clear increase from the 100 °C sample to 130 °C and thus in correlation with the standing time of the samples.

3.2 Results of microbiological analysis

With regard to the analysis of the total bacterial count measured in colony forming units (CFU) per mL, 0 CFU/mL resulted after the second heating, even with a first heating temperature of 100 °C. However, in the results based on 20 mL, colony-forming units were detected after the second heating stage with a heating temperature of 100 and 120 °C in the first stage. The exact results are shown in table 7.

3.3 Sequencing results

Sample V100 of the first experimental run (trial number 1) was sent to an analytical laboratory for sequencing, where it was cultivated on blood agar. Germs of the genus *Bacillus subtilis* and *Bacillus cereus*, both spore formers, were detected using MALDI-TOF MS germ identification.

3.4 Results of Sensory Analysis

The statistical analysis of the descriptive sensory evaluation was carried out using a multi-factorial ANOVA and subsequently the differences were examined using Fisher´s LSD test. The results of the pairwise comparison of those attributes for which the factor "Experimental parameter settings", respectively the factor "Technical repetition" were significant are shown in table 8 and table 9. The experimental parameter settings showed significant differences in four attributes. In comparison, significant differences were found for three attributes in the factor "Technical repetition".

Looking at the sensory attributes that are significant with regard to the experimental parameter settings, there are distinct deviations

Table 7 **Total bacterial count aerobic/anaerobic in CFU/20 mL as a result of the microbiological analyses of the carrot juice samples before and after the second heating stage**

		Zero sample		V100		V110		V120		V130	
Trial number		TBC OSA	Spore formers	TBC OSA	Spore formers	TBC OSA	Spore formers	TBC OSA	Spore formers	TBC OSA	Spore formers
1	aerobic	2275	6	0	0	0	0	0	0	0	0
	anaerobic	3335	2	0	0	0	0	0	0	0	0
2	aerobic	265	0	3	0	1	0	2	0	0	0
	anaerobic	675	0	3	0	0	0	0	0	0	0
3	aerobic	48000	7	0	0	1	0	0	5	0	0
	anaerobic	65000	0	0	0	0	0	0	0	0	0

TBC total bacteria count; OSA orange serum agar

Table 8 **Results of the Fisher (LSD) analysis with a 95 % confidence interval, containing the mean-value estimate for the experimental parameter settings**

	Experimental parameter settings			
Attribute	**100 °C**	**110 °C**	**120 °C**	**130 °C**
Homogeneity				
Particles				
Turbidity				
Sediment				
Sweet	52.18^{AB}	53.74^{A}	50.01^{B}	44.24^{C}
Sour	14.48^{A}	9.73^{B}	15.42^{A}	26.91^{C}
Bitter				
Umami				
Chalky				
Coating	23.03^{AB}	25.92^{A}	21.89^{AB}	18.78^{B}
Creamy	41.37^{A}	43.13^{A}	41.10^{A}	35.28^{B}
Earthy				

n = 12 judges. Means in the same line showing common letter are not significantly different (p = 0.05); attributes without mean-values did not show any significant differences in the ANOVA analysis

Table 9 **Results of the Fisher (LSD) analysis with a 95 % confidence interval, containing the mean-value estimate for the technical repetition**

	Technical repetition		
Attribute	**1**	**2**	**3**
Homogeneity			
Particles	19.17^{A}	24.61^{B}	32.25^{C}
Turbidity			
Sediment			
Sweet			
Sour	14.08^{A}	11.38^{A}	24.44^{B}
Bitter	14.43^{A}	18.24^{B}	8.63^{C}
Umami			
Chalky			
Coating			
Creamy			
Earthy			

n = 12 judges. Means in the same line showing common letter are not significantly different (p = 0.05) ; attributes without mean-values did not show any significant differences in the ANOVA analysis

and differentiations only with regard to the 130 °C sample. The differences between the three lower temperature levels do not correlate with the temperature level, meaning that no correlation can be identified.

More distinct differences were found between the technical replicates. There were significant differences in both the particle content and the bitterness between all three replicates. For the sour attribute, the third repetition in particular showed significant deviations. When looking at the test conditions as well as at the technical repeatability, it is noticeable that the significant attributes only relate to the visual, tactile and fundamental tastes, but not to the odor-perceptible attributes.

A principal component analysis was carried out for further evaluation. Figure 3 shows the results in form of a PCA-biplot. It shows the first two principal components with a total variance of 79.01 %. The first principal component contains 59.81 %, the second 19.20 %. As can be seen in the biplot, the attributes "particles", "sediment" and "turbidity" are close to each other. In addition, the attributes "creamy", "porridge-like", "sweet" and "coating" are close to each other. The samples of the first two technical replicates are all located in a group and cannot be differentiated from each other based on the attributes in the biplot. Samples 11, 12 and 13, as well as sample 14, are located away from this. The latter can be differentiated not only along variable 2, but also along variable 1, which correlates significantly with the attribute "sour".

4 Discussion

When looking at the results of the chemical parameters, there were no significant changes in the Brix and pH values or in the color values. Only in the case of the pH value a drop in the pH value with increasing heating temperature can be seen in the third technical repetition. However, this is not due to the temperature, but the external factor of standing times between juice production and thermal preservation. As the test temperature increased, the sample material had a longer stagnation time before processing, which led to lactic acid fermentation. This assumption was confirmed by measuring the lactic acid content. What also suggests an influence of the standing time is the increased nitrite content, which indicates microbiological processes that have progressed during the standing time [23]. Based on the chemical analyses, no influence on the carotenoid content could be determined, i.e. a reduction in the heating temperature had no effect on the chemical and nutritional properties investigated.

When looking at the results of the microbiological analysis, no colony-forming units can be detected for the analyses of 1 mL sample volume, regardless of the heating temperature of the first heating. However, when the sample volume was increased to 20 mL, the presence of germs was occasionally detected at 100 and 120 °C, but not at 110 °C. The detection of germs can therefore not be clearly attributed to the temperature, but may also have been caused by recontamination in the system itself or during filling. Furthermore, it was striking that even in the test repetitions in which spore formers were found in the zero sample, no more germs could be detected after the first heating stage. This could be due to the fact that the spores were young or had already germinated and were therefore easy to inactivate. The current literature also shows gaps in the knowledge of the two spore formers Bacillus cereus and Bacillus subtilis with regard to their spore formation and heat stability. What has been clearly established, however, is that factors such as strain variability, the age of the ascospores, the sporulation conditions (composition of the sporulation me-

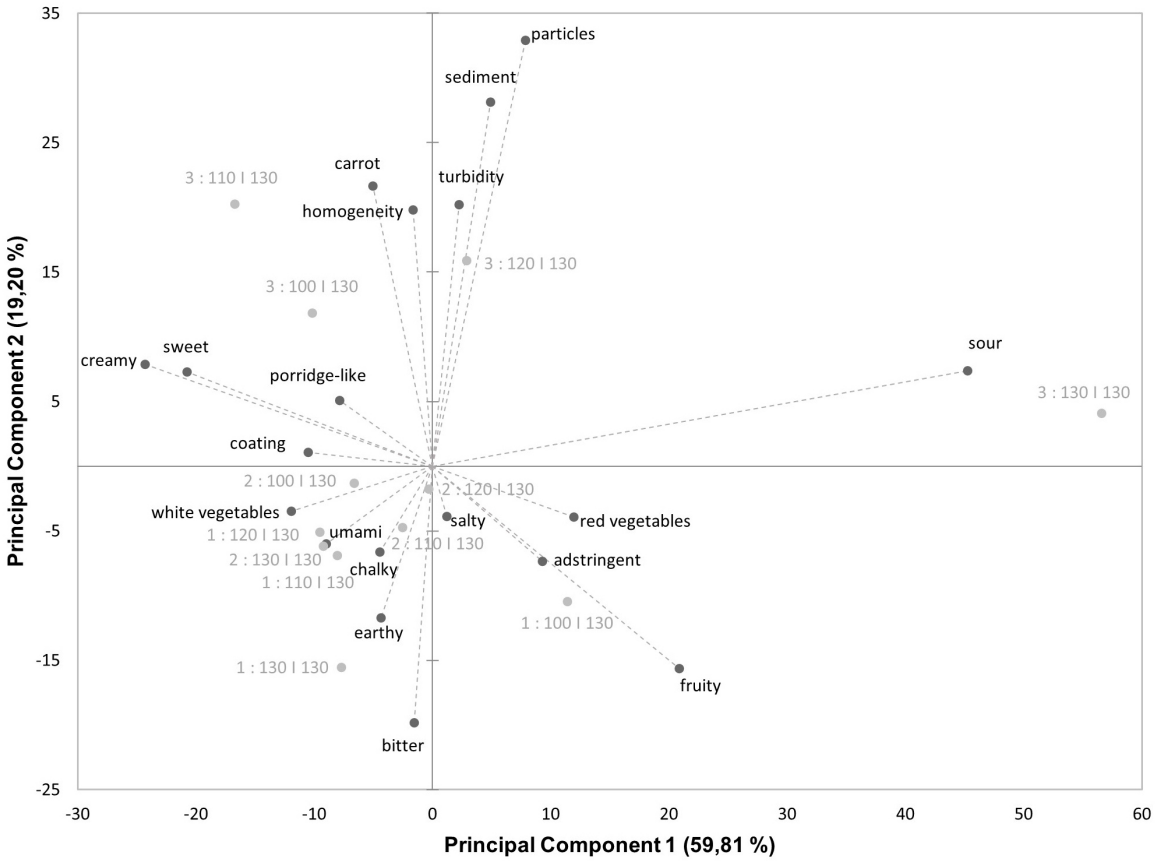

Fig. 3 **PCA-Biplot of principal components 1 and 2, including 79.01 % of variance; dark gray dots represent variables, light gray dots represent observations**

dium, pH value, temperature) and factors in the product such as the pH value have a significant influence on the heat resistance of the bacteria and their spores. In addition, the factors themselves and their interactions with each other are not clear with regard to their influence on heat resistance. All these findings are a possible explanation for the ambiguous microbiological results within this study [24].

In the analysis of the sensory tests, a total of three significant attributes were identified for the technical repetition, and four attributes for the experimental settings. For the factor of technical repetition, the three repetitions deviated significantly from each other. In particular, the 130 °C sample, which was always processed last, proved to be more acidic. This was also confirmed by the results of the lactic acid measurement, indicating lactic acid fermentation as a result of an excessively long downtime and delay in further processing. This shows that the conditions and circumstances in production with regard to resting times and standing times as well as the properties of the raw juice have a significantly influence on the results. Furthermore, the type of attributes that showed significance showed that the sensory attributes of the odor do not depend on the temperature of the first heating stage, at least not if a second heating stage at 130 °C is performed. An improvement in sensory quality could therefore not be demonstrated by lowering the temperature of the first heating stage. Finally, the results of the ANOVA are also reflected in the principal component analysis, where the individual samples are not clustered according to the test temperatures, but according to the technical repetitions. The influence of the raw material, for example the variety, the time of harvest, downtimes, etc., affect the product quality to a greater extent than the heating temperature of the first heating stage. An energetic analysis would be worthwhile here. However, the microbiological studies show that the microbiological stability of the product must not be disregarded when considering reducing the temperature of the first heating stage.

5 Conclusion

In summary, this study demonstrates that the initial heating phase in carrot juice pasteurization influences both microbiological stability and sensory properties, albeit in a nuanced manner. A temperature reduction of just 10 °C in the heating phase compromised microbiological safety, resulting in 5 CFU/20 mL spore formers under the tested conditions. Conversely, even a larger temperature reduction to 100 °C failed to yield measurable improvements in sensory attributes or nutrient retention. Variations in sensory properties and chemical parameters were attributed to factors such as sample handling, standing times, and natural product variability, rather than changes in pasteurization temperature. Consequently, the potential to enhance quality through process optimization is limited, as maintaining microbiological safety must remain the top priority. Exploring alternative methods to improve microbial control could help achieve a balance between energy efficiency and product quality without compromising product integrity.

This study contributes to the field of research by exploring resource conservation and quality improvement through the re-

design of conventional manufacturing processes while ensuring microbiological safety. The approach used in this study offers a low-investment strategy for process re-evaluation, helping to meet growing demands for resource efficiency without compromising product quality. In the food industry, where many processes are based on long-standing practices, this approach could be widely applicable to other processes as well.

One limitation of the study is that only a single parameter, the temperature, was varied. Potential interactions that might arise due to broader variability in raw materials, carrot juice preparation methods, or alternative heating profiles were not fully explored.

For future research, exploring more complex heating profiles with varying temperatures and holding times is recommended. A thorough examination of chemical parameters, coupled with a long-term sensory analysis, would also provide deeper insights into product stability and quality under real storage conditions. These findings highlight the potential for gentle yet safe food processing methods while emphasizing the importance of further studies to refine applications for real-world production scenarios.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

Ethical Statements

Oral informed consent was obtained from all study participants. This study did not fall under the requirements of a committee examination in accordance with the university's guidelines.

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